UNIVERSITY OF AGRONOMIC SCIENCES AND VETERINARY MEDICINE OF BUCHAREST
FACULTY OF AGRICULTURE

SCIENTIFIC PAPERS
SERIES A. AGRONOMY

VOLUME LXVI, NO. 1

2023
BUCHAREST

SCIENTIFIC PAPERS

SERIES A. AGRONOMY

Volume LXVI, No. 1, 2023

University of Agronomic Sciences
and Veterinary Medicine of Bucharest
Faculty of Agriculture

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Bucharest

PUBLISHER: University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Agriculture, Romania
Address: 59 Mărăști Blvd, District 1, 011464, Bucharest, Romania
Phone/Fax: + 40 213 318-0466; E-mail: journal@agro-bucuresti.ro; Webpage: http://agronomyjournal.usamv.ro

CERES Publishing House
Address: 29 Oastei Street, District 1, Bucharest, Romania; Phone: + 40 21 317 90 23, E-mail: edituraceres@yahoo.com

To be cited: Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023

ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

International Database Indexing: Web of Science Core Collection (Emerging Sources Citation Index), CABI, Index Copernicus, Google Scholar, CNCSIS B+, Ulrich's Periodicals Directory, Research Bible, Scipio, Scientific Indexing Service, PBN (Polish Scholarly Bibliography), OCLC (WorldCat)

SUMMARY

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MISCELLANEOUS

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON EARTHWORM COMMUNITY IN MAIZE CROP IN DOBROGEA PLATEAU

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Abstract

Earthworms play an important role in soil formation contributing to the composition and functioning of its ecosystem. By their activity in the soil, earthworms offer many benefits: increased nutrient availability, better drainage, and a more stable soil structure, all of which help improve farm productivity. The purpose of this study was to assess the presence of earthworm species in maize crops in Dobrogea Plateau over the years 2020-2022. The sampling consisted of 15 pits of 25 x 25 x 40 cm. Five earthworm species belonging to three genera Aporrectodea, Allolobophora and Proctodrilus were identified. he most abundant species was Aporrectodea caliginosa nocturna and Aporrectodea caliginosa. This study reports the first data on earthworm fauna for the Dobrogea Plateau.

Key words*: earthworms, Lumbricidae, diversity of populations, agriculture, maize.*

INTRODUCTION

Earthworm has caught imagination of philosophers like Pascal and Thoreau. Yet its role in the nutrition of agricultural fields has attracted attention of researchers worldwide only in recent decades (Adhikary, 2012)

Soil is the most precious natural resource and is the greatest inheritance of mankind. Our connection with soil is based upon the cultivation of soil throughout human history and led to the success of civilizations. During the Green Revolution, an extensive quantity of chemical pesticides and fertilizers were used to boost up crop yield from agricultural land (Datta et al., 2016) which resulted in good yield and productivity. Excessive use of chemical pesticides and pesticide fertilization and loss determine negative effects on the environment, soil and food pollution with waste, degradation of soil quality (Ju et al., 2009) and agricultural biodiversity (Minuto et al., 2006; Gill and Garg, 2014). According to Fred (1991), agriculture has had both positive and negative effects, and the use of synthetic fertilizers leads to the loss of the soil's natural nutrients when used on its surface. Earthworms are one of the most significant soil organisms having a real potential to maintain the fertility of the soil and thus play a key role in agriculture sustainability. They are also acknowledged as farmer’s friend, ecological engineers, biological indicators, intestines of the earth and plowman of the field. Earthworms are extremely important in soil formation, principally through activities in consuming organic matter, fragmenting and mixing it intimately with soil mineral particles to form water stable aggregates (Amuza et al., 2020)

Earthworm activity makes a significant contribution to soil aeration (Kretzschma, 1978) by creating channels, particularly in heavy soils, that allow air to penetrate into deeper layers of soil, minimizing the incidence of anaerobic layers. The carbon: nitrogen (C: N) ratio in the organic matter falls progressively during feeding of earthworms. Moreover, most of the nitrogen is converted into the ammonium or nitrate form; phosphorus and potassium are converted into a form available to plants.

Earthworm populations are generally lower in arable land comparative to undisturbed habitats (Chan, 2001). Direct mortality level depends on the severity and frequency of soil disturbance. Cuendet (1983) estimated that 5 to 10% of the earthworm biomass was brought to the surface by plowing, with about 25% of these earthworms mortally wounded. Rotary cultivation can reduce numbers by 60 to 70% (Boström, 1988).

MATERIALS AND METHODS

Study area. The soil sampling was performed in maize crops in the experimental field from Beidaud town, over the years 2020-2022. The town of Beidaud is located in the south-estern part of Tulcea (N 44°42′35, 9″, E 28°35′42,6″). The soil is argilloiluvial chernozem. The climate of the Beidaud area is characteristic of the semi-arid steppe region, with two types of microclimate, a silvo-steppe near the forest and a dry steppe. The climate is temperate, with a pronounced continental character, manifested by hot summers, cold winters, often marked by blizzards and low precipitation. Average annual precipitation totals between 359 mm. The average temperature being 23°C.

Earthworm sampling. Earthworms were collected in March-May and September 2020-2022. The soil was extracted using a spade and was put into a high sided tray in order to prevent earthworm escape. The extracted soil was hand-sorted for living earthworms. It was made it 15 pit soil and each soil pit had sides of 25 cm x 25 cm and 40 depth; the distance between pit soil was 10-30 cm on the crop row. The adult specimens were fixed in 70% ethanol, analysed under a stereomicroscope and identify to the species level. Juveniles are kept in the soil in the lab conditions to obtain adult stage.

Agricultural techniques. The field was treated with 42.9% nicosulfuron + 10.7% rimsulfuron (herbicid) 250 ml/ha, lambda-cihalothrin 5% (insecticid) 250 ml/ha, piraclostrobin 200 g/l (fungicid) 1 l/ha, the soil was fertilized with 180 kg nitrogen/ha, soil work: weeding, autumn plowing at a depth of 30 cm, discussed. In 2020 the maize was irrigated and in 2021-2022 maize was grown without irrigation.

RESULTS AND DISCUSSIONS

According to Table 1, we observe a much higher density of both adults and juveniles in 2020 because the land was irrigated, and the earthworms had optimal development conditions compared to the other years when irrigation was abandoned. In 2020, a total of 57 individuals of adult and juvenile Lumbricidae were collected, in 2021, 30 individuals were collected and identified, and in the 2022 study year, 15 were collected.

Table 1. The number of adult and juvenile Lumbricidae

Year of sampling	The number of adult and juvenile Lumbricidae			
	March	April	May	September
2020	15	20	12	10
2021	10	12	3	5
2022	3	5	5	2
Total	28	37	21	17

According to Table 1, the populations of earthworms are much more numerous in the month of April, because the soil temperature and humidity are favorable for them.

Table 2. The number of adult

Year of sampling	The number of adult			
	March	April	May	September
2020	5	8	5	3
2021	3	5	1	2
2022	1	2	1	0
Total	9	15	7	5

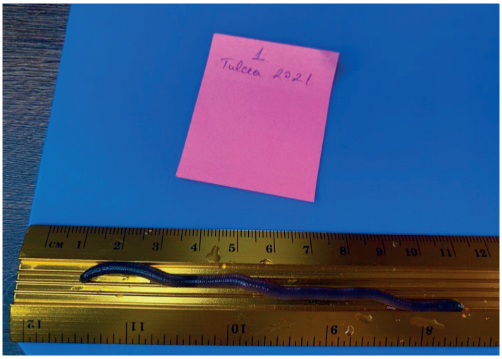
In the Table 2 we notice that the year 2022 was the most suitable for the development of earthworms, the irrigation system having a great impact on their development. In 2020, 21 adult individuals were collected, in 2021, 11 adults were collected and in 2022, in all 4 months of the study, only 4 adult individuals were collected.

Table 3. The number of juveniles

Year of sampling	The number of juveniles			
	March	April	May	September
2020	10	12	8	7
2021	7	7	2	3
2022	2	3	4	2
Total	19	22	14	12

In Tables 4, 5 and 6 we observe the fluctuations of earthworm populations from one month to another. In this area we identified 6 species of Lumbricidae: *Aporrectodea trapezoids, Aporrectodea longa, Aporrectodea smaragdina, Aporrectodea caliginosa, Aporrectodea caliginosa nocturna, Allolobophora chlorotica* (Picture 3).

Were found six different species, only one species is part of the anecic group, reespectively *Aporrectodea caliginosa nocturna* (Picture1), the other five species are part of the edogeic ecological group. In all three years, the *Aporrectodea caliginosa* (Picture 2) species resists the environmental conditions best, finding a total of 16 individuals. Autumn plowing destroys in very large proportions the populations of earthworms, in general the epigeic ones that stay on the surface of the soil and that feed mostly on organic matter.



Picture 1. *Aporrectodea smaragdina*

Table 4. Lumbricidae species in 2020

Species	March	April	May	September
Aporrectodea trapezoides	3	3	-	-
Aporrectodea longa	2	-	2	-
Aporrectodea smaragdina	-	3	-	-
Allolobophora chlorotica	-	2	-	-
Aporrectodea caliginosa nocturna	-	-	-	2
Aporrectodea caliginosa			3	1

Table 5. Lumbricidae species in 2021

Species	March	April	May	September
Aporrectodea trapezoides	-	2	-	-
Aporrectodea longa	-	-	-	-
Aporrectodea smaragdina	-	-	-	-
Allolobophora chlorotica	-	-	-	-
Aporrectodea caliginosa nocturna	-	1	-	-
Aporrectodea caliginosa	3	2	1	2

Table 6. Lumbricidae species in 2022

Species	March	April	May	September
Aporrectodea trapezoides	-	2	-	-
Aporrectodea longa	-	-	-	-
Aporrectodea smaragdina	-	-	-	-
Allolobophora chlorotica	-	-	-	-
Aporrectodea caliginosa nocturna	1	-	-	-
Aporrectodea caliginosa	0	2	1	-

Table 7. Average monthly minimum temperatures

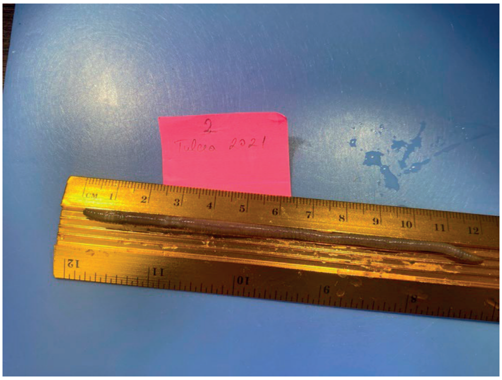
Average monthly minimum temperatures												
Year	January	February	March	April	May	June	July	August	September	October	November	December
2020	-2.0	0.3	3.2	2.7	9.6	14.7	16.7	16.3	13	10.7	2.5	1.7
2021	0.9	-1.5	0.1	4.2	10.4	15.3	17.3	16.8	10.6	5.8	3.8	0.2
2022	-2.4	-1	-2	5.1	9.8	15.3	17.2	19	12	6.3	3.7	0.2

Table 8. Average monthly maximum temperatures

Average monthly maximum temperatures												
Year	January	February	March	April	May	June	July	August	September	October	November	December
2020	6.6	11.2	15.1	18.9	22.4	28.6	31.1	30.1	27.4	21.8	10.4	7
2021	6.4	7.2	10.1	15.2	22.7	26.1	31.3	30.6	23.7	16.3	13.4	7.5
2022	6.2	10.6	9.4	18.3	24.5	28.6	31.5	32	25.2	20.6	13.4	8.3

Table 9. Average monthly precipitation

Average monthly precipitation												
Year	January	February	March	April	May	June	July	August	September	October	November	December
2020	0.03	6.02	0.02	0.08	0.94	0.39	0.48	0	0.51	0.72	1	1
2021	1.85	0.2	0.38	0.38	0.25	1.97	0.41	0.41	0.35	0.92	0.09	1.15
2022	0.24	0.25	0.19	0.41	0.98	1.47	0.35	0.20	0.46	0.29	0.58	0.59



Picture 2. *Aporrectodea caliginosa*

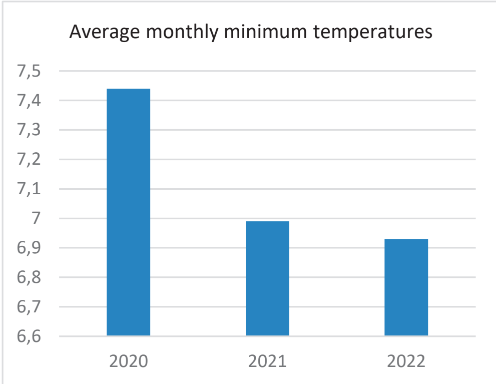


Figure 1. Average monthly minimum temperatures

In 2020 the average of the minimum annual temperatures according to the Figure 1, are much higher (the average 7.44) compared to the two years, respectively 2021 (the average 6.99) and 2022 (the average 6.93) where much lower temperatures were recorded, favoring the development of the earthworm populations compared to the last two years of study, as they are very sensitive to low temperatures.

In 2021 and 2022, the average minimum temperatures are similar, respectively the average of 83.9 for the year 2021 and the average of 83.2.

According to the Figure 2, the average maximum temperatures are recorded in 2022, the average being 18.5 in comparison with the other two years of study, respectively the average of 2021 is 17.54 and the average of 2020 being 18.51.

Picture 3. *Allolobophora chlorotica*

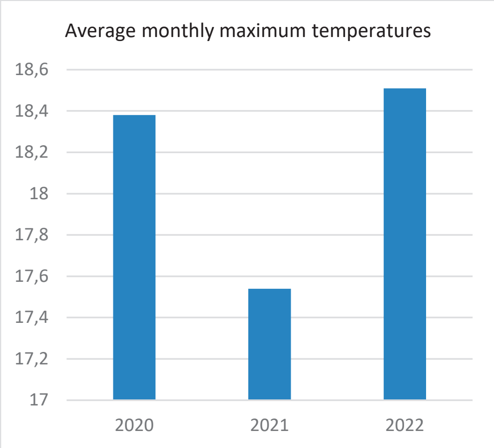


Figure 2. Average monthly minimum temperatures

The highest precipitation average, according to the Figure 3 was recorded in 2021, being 0.69, compared to the two years, 2020 recording the lowest precipitation average of 0.47.

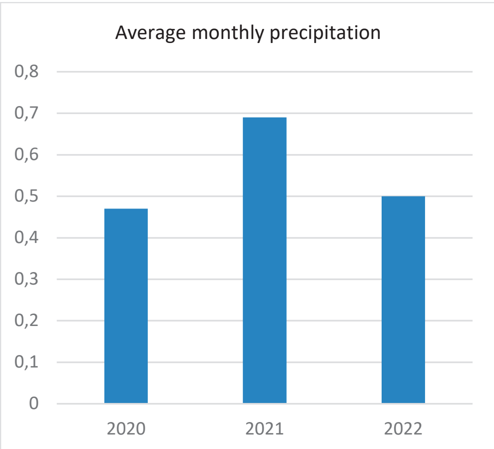


Figure 3. Average monthly precipitation

CONCLUSIONS

Our data from 2020-2022 showed presence of six species of earthworms *Aporrectodea trapezoids, Aporrectodea longa, Aporrectodea smaragdina, Aporrectodea caliginosa, Aporrectodea caliginosa nocturna, Allolobophora chlorotica.*

The most earthworms (adults + juveniles) were found in maize crop especially in the 2020 in April. The impact of the irrigation system on earthworm populations is quite large, which is also evident from tables 7, 8, 9, in 2020 although the average rainfall was the lowest with soil moisture and a milder winter compared to other years, earthworm populations could develop much better without being stressed by the level of soil moisture. The most abundant species was *Aporrectodea caliginosa* with a total of 15 adult individuals found in the 3 years of study and just in 2021 it was found 8 idividuals. The next species with the most adults is *Aporrectodea trapezoids* with 10 adult individuals and the species with the fewest specimens being *Allolobophora chlorotica* with 2 adult individuals. In this three years of study, it appears that the april month is the most favorable for the development and activity of earthworms.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF THE MORPHOMETRIC PARAMETERS OF THE CUBOLTA RIVER BASIN WITH THE GIS SUPPORT

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Abstract

The morphometric parameters of a territory are an important part in the research carried out in order to have a complete picture of its relief. Morphometry represents the main elements through which it is possible to achieve a complete description of geomorphological conditions within a given territory. This article presents aspects regarding the morphometric parameters that are achieved with the help of GIS technology. With the help of the digital terrain model, a series of morphometric parameters were generated: altitude, slope inclination, relief fragmentation. Subsequently, these data can be used in the complex and detailed geomorphological and pedological analysis of the basin territory.

Key words*: morphometric parameters, GIS technology, geomorphological conditions.*

INTRODUCTION

The morphometric parameters of the relief represent an important potential in the development of geomorphological processes. For example slope declivity, through the value of the slope angle, together with other parameters of the relief, can condition the triggering of torrential and gravitational processes on the slope.

The morphometric parameters (length, slope, shape of the slope, etc.), act as determining factors in combination with other factors, type and intensity of the process. The morphometric characteristics can be evaluated as a result of the dynamics of the factors in question as well as a consequence of subsequent changes in the landscape. This method gives a complex characteristic of the relief of the researched territory based on the topographical material: the characteristic of the altitudes (hypsometry), the angle of the slope, the depth and the density of the fragmentation of the relief, etc.

The relief of the basin, sculpted by the numerous flowing waters, forms alluvial beds, slopes, interfluvial ridges, etc., characteristic of the fluvial relief type (Donisă et al., 1994). The slopes of the valleys of the Cubolta tributaries that are part of the basin have altitudes between 50-100 m, and the length of the slopes often does not exceed the length of 1000 meters.

MATERIALS AND METHODS

Due to the possibility of applying informatics in geography and by use of the Geographical Information Systems (GIS) it was possible to map the morphometric parameters of the researched basin and make their graphic representation through maps and graphs. Topographic maps at a scale 1: 25,000 represented the cartographic support of the Geographical Information System. For the morphological and morphographic analysis of the studied region, a specialized software was used that allows the development of high-precision maps within the limits of the topographic base taken as a starting point. All developed maps were made with the help of MapInfo 9 and ArcGIS 9.3 programs. Thus, basing on the interpolation of the obtained level curves the main thematic maps (altitude, slope, exposure, etc.) were automatically created.

RESULTS AND DISCUSSIONS

The digital terrain model was created for the evaluation of the morphometric indicators (Juc et al., 1995; Rudraiah et al., 2008; Gustavsson et al., 2006). Thus, from the morphometric point of view, the relief within the boundaries of the Cubolta basin is relatively fragmented (Figure 8). It is characterized by

altitudes between 85-280 m, with an average altitude of 221 m. The maximum altitude values are 280 m, and the minimum 85 m.
The hypsometric map highlights 6 altitudinal steps (Figure 1) present on the studied territory. The histogram of surfaces by hypsometric classes (Figure 2) represents their share from the total area of the territory.

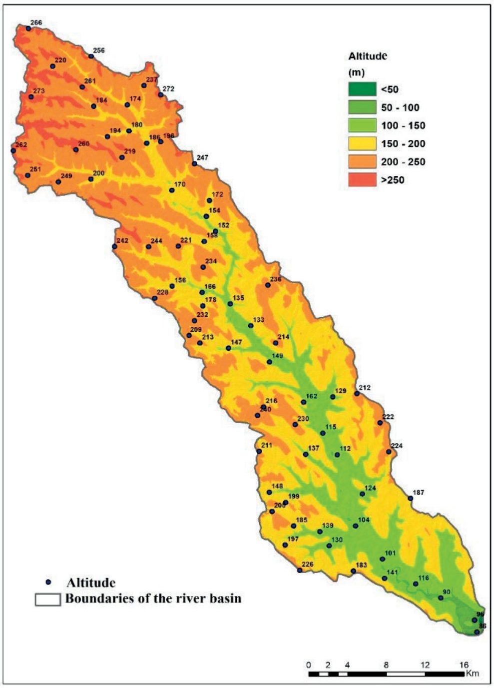

Figure 1. Hypsometric map of the Cubolta basin

As a whole, altitudes between 150-200 meters (41.19% of the area) occupy the highest share in the area of the basin. The altitude range of 200-250 m makes up 35.27% and the range of 100-150 m is represented by 17.27% of the area. The lowest values, which do not even reach 2% of the total area of the basin, are represented by the altitudinal ranges up to 50 m and 50-100 m, reaching a share of 0.33% and 1.51%, respectively. Surfaces that exceed the altitude of more than 250 m, occupy 4.43% of territory and are located in the upper course of the basin.
Slope is a very important feature in relief analysis. It is one of the essential factors in the development of some geomorphological processes, especially the processes related to the movements on the slope.

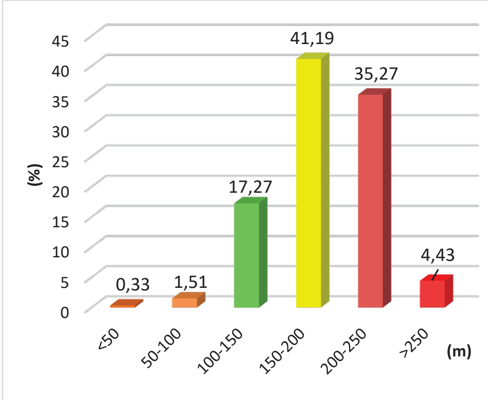

Figure 2. Histogram of surfaces by hypsometric classes (m)

The slope map was generated from Digital Terrain Model (DTM). Within the investigated territory, the slope values were classified into 5 classes (Figure 3).

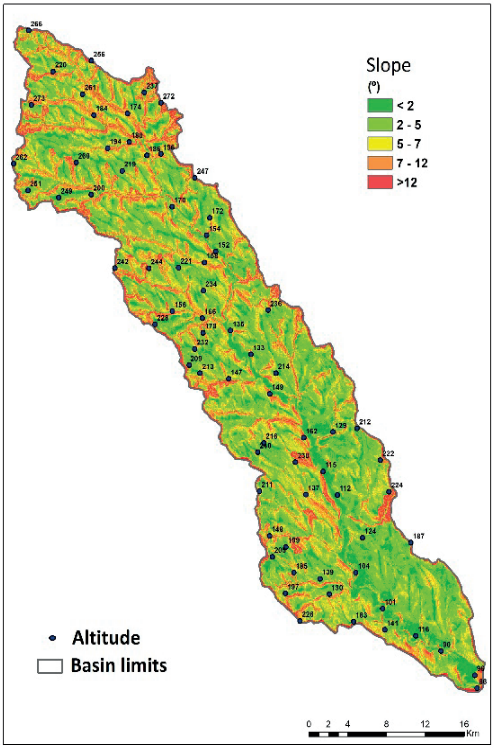

Figure 3. Slope map in the Cubolta basin

Analysing the histogram of the slopes (Figure 4), it can be noticed that slopes below 2° makes 13.19% of the territory and are spread in the river meadows and on interfluvial peaks.

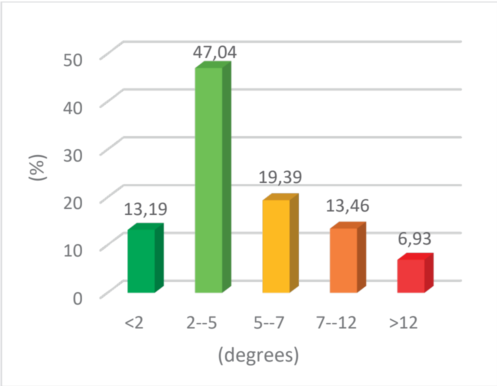

Figure 4. Histogram of slope classes in the Cubolta Basin

The class of slopes between 2-5º recorded the largest share, which constitutes 47.04% of the total area of the territory. That makes practically half of the studied area.

The next category of slopes is the class of slopes between 5-7º, which is almost two times inferior to the previous class, occupying 19.39% of the basin surface and is spread on the slopes of small rivers (tributaries of the Cubolta).

Slopes between 7-12º occupy roughly the same area as slopes lower than 2º and makes 13.46% of the total area of the basin. The lowest share have the slopes higher than 12º and in most cases they are spread at the bottom of the slopes.

A no less important characteristic is site orientation or the exposure of the slopes (Condorachi, 2000; Haidu, 1998). The analysis of the site orientation map (Figure 5) made possible to quantify the share of slopes with different exposure which determine variations in the thermal regime. The thermal regime of slopes have significant influences on the triggering of some slope processes. Slopes with northern orientation are shaded, and, as a result, they are wetter and colder. In conditions of a substrate represented by clay-sand rocks, they are more prone to trigger negative exogenic processes such as landslides, torrential erosion and solifluction.

Thus, according to Figure 6, the north-east and south-west oriented sites dominate in the studied area in comparison with north-west, north and south-east slopes, exceeding the values of the latter practically twice, in places triple.

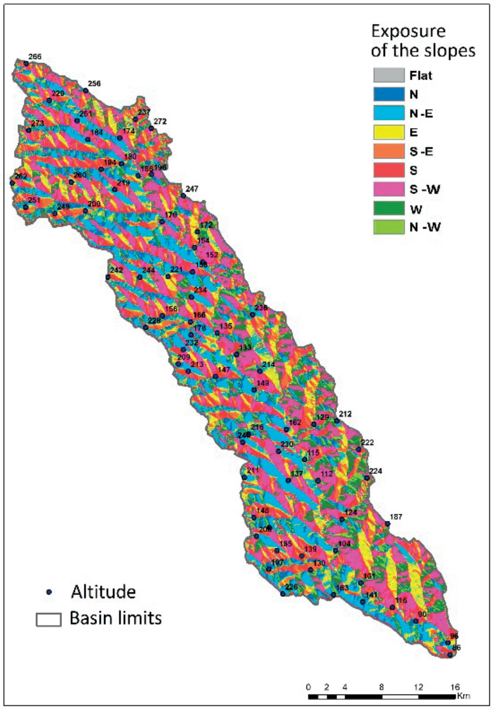

Figure 5. Exposure of the slopes in the Cubolta basin

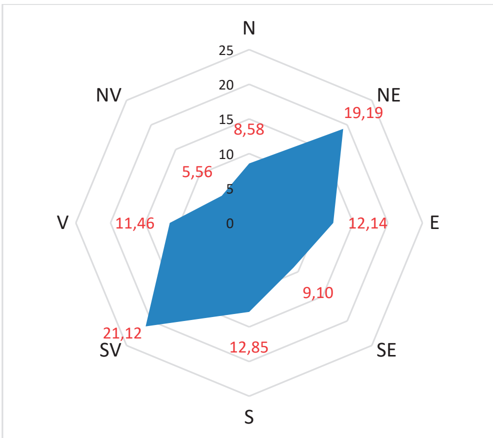

Figure 6. Exposure of the slopes in the Cubolta Basin

The north-east and south-west oriented lands have the largest share and occupy 19.19% and 21.12% respectively of the total area of the territory. The lands with eastern exposure occupy surfaces of the relatively gentle slopes with lengths of over 1000 m, and the lands with southwest orientation occupy the short and steep surfaces of the slopes.

The western, eastern and southern slopes are represented with relatively equal values. They have a share of 11.46%; 12.14% and 12.85% respectively. The western ones are predominantly located on the left side of the Cubolta river, and the southern ones on its right. The lowest values are presented by the northwestern and northern slopes, which constitute 5.56% and 8.58%, respectively. The slopes with north-west exposure are represented by short slopes and in most cases with a slope higher than 5º, and the northern ones occupy surfaces of relatively gentle slopes with slopes between 5-7º.

Two other morphometric parameters with appreciable potential in triggering external geomorphological processes are vertical fragmentation or relief energy (Figure 7) and relief fragmentation density (Figure 8) or horizontal fragmentation (Popuşoi et al., 2012; Canţîr, 2012).

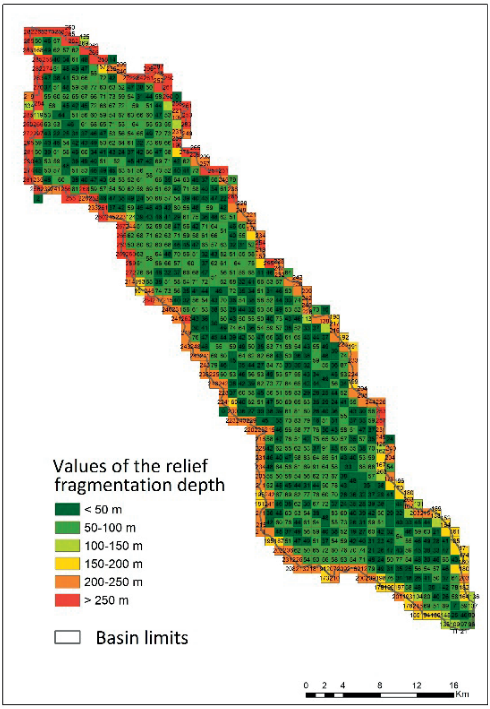

Figure 7. Relief fragmentation depth

The vertical fragmentation or relief energy represents the difference of level between the maximum and minimum elevation within a square with a side of 1 km. The values thus calculated for each individual square were later interpolated, being then divided into 6 classes (Figure 9).

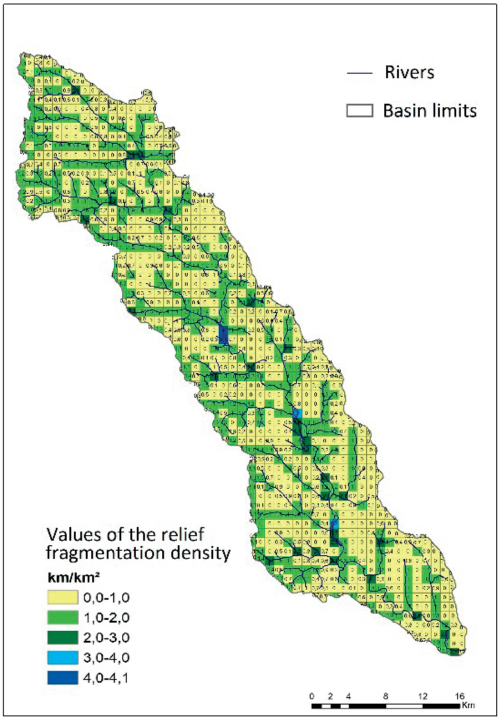

Figure 8. The density of relief fragmentation

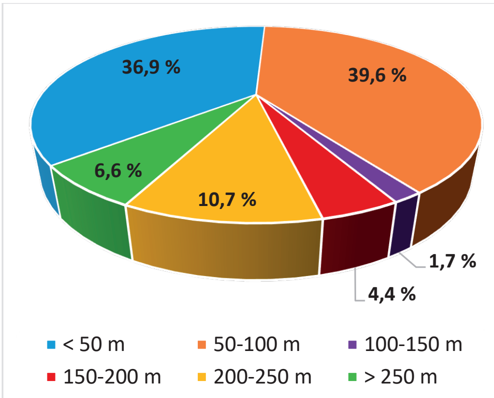

Figure 9. The share of surfaces by classes of vertical fragmentation of the relief

The average value of the vertical fragmentation of the relief is 151.0 m. About 36.9% of the territory's surface is fragmented (<50 m), and the surfaces with relief fragmentation between

50-100 m occupy roughly the same area as the previous class and constitutes 39.6% of the total area of the basin. The average values (150-200 m) occupy only 4.4%. The higher values (200-250 m and >250 m) have the share of 10.7% and 6.6% of the total basin.
The density of relief fragmentation map (Figure 8) was made by the cartogram method, using the ArcGis program. The fragmentation density or the horizontal fragmentation of the relief is defined as the total length of the hydrographic network in relation to the total surface of the territory, hence the unit of measure was km/km². To create this map, it was necessary to create the hydrographic network on the base of information from the topographical map. It was created a grid in kilometres and the grid was cut according to the limit of the study area. The lengths of all the rivers in each square within the grid were determined and finally the layout part was made (Crăciun & Bejan, 2019).
It was found that the territories that have the largest share of the total surface of the basin (62.9%) are the surfaces with a relief fragmentation density that does not exceed 1 km/km² (Figure 10).

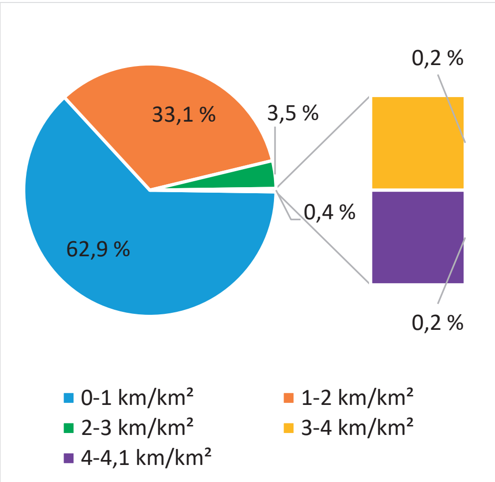

Figure 10. Relief fragmentation density histogram

These are followed by lands with a density of fragmentation between 1.0-2.0 km/km² with a share of 33.1%. The density between 2.0-3.0 km/km² occupies 3.5% of the entire territory, and the surfaces with a density exceeding 3.0 km/km² and 4.0 km/km² barely reach 0.2% of total area.

CONCLUSIONS

Digital terrain modelling is the initial stage in performing relief analysis of a territory. These modelings are impossible to accomplish without the help of the Geographical Information System. The use of GIS techniques presents numerous advantages, by monitoring, verifying and permanently supplementing a field monitoring database. With the help of these systems it is possible to process a large amount of information with the possibility of supplementing this database with new data. In the present case, the analysis of the database resulted in the cartographic visualization of the morphometric parameters of the relief (hypsometry, orientation, slope, etc.) within the limits of the Cubolta basin.
The morphometric parameters provide information with reference to the territories likely to be affected by geomorphological processes. The relief of the Cubolta basin is characterized by altitudes between 85-280 m, with an average altitude of 221 m. The maximum values of the altitude are 280 m, and the minimum of 85 m with a less pronounced slope, the surfaces between 2-5º occupying a weight of 47.04% of the total area of the territory. On the territory of the basin, the southwest-oriented slopes predominate, and the most extensive depth interval of relief fragmentation is 50-100 m (39.6%) of the total area of the basin.

ACKNOWLEDGEMENTS

This research work was carried out within the State project 20.80009.7007.08 "Spatio-temporal modeling of abiotic environmental factors for estimating the ecological stability of landscapes"

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE STATE AND PROTECTION OF LAND RESOURCES AND DEPOSITS IN THE LEOVA DISTRICT

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Abstract

The protection of the environment is a national priority, which directly aims at the living conditions and health of the population, the achievement of economic and social-human interests, as well as the sustainable development capacities of society in the future. The purpose of the research was to evaluate the state of land resources and deposits located on the territory of the Leova district, as well as to identify the factors that contribute to the ecological state of land resources. Leova district is located in the southwest of the Republic of Moldova, on the border with Romania, along the left bank of the Prut river. The district has an area of 764.7 km², representing 2.26% of the total area of the country. The natural resources of the district are forests, rivers and ponds. The underground natural wealth is represented by deposits of sand, clay, clay, bentonite, mineral waters. Of the total area of the district of 76.5 thousand ha, the agricultural land constitutes 57.3 thousand ha, and the non-agricultural land constitutes 19.2 thousand ha.

Key words: *environmental protection, deposits, land resources, Leova district.*

INTRODUCTION

Under the conditions of the reform of land ownership on the territory of the Republic of Moldova and the forms of management, the direct attitude towards the organization, use and evaluation of land resources has changed radically in recent years. In this process, large areas of valuable agricultural land were transferred to private ownership, on the basis of which peasant households, private households with collective property in common and cooperatives were organized, in which the organization of the use of land resources reached a new level with everything different. At the same time, the problem of developing effective approaches to the rational use of land resources in a market economy requires an urgent solution to increase the efficiency of not only the use of land resources, but also to create conditions for obtaining ecological products from each hectare of land.

The rural area of the Republic of Moldova in 2011 was in a deep crisis, marked by the mass exodus of the population abroad, the ruin of agriculture, the serious damage to the social and production infrastructure, access roads, etc. The anti-ecological utilization of agricultural land and the accumulation of unauthorized garbage, the negligence of the population and local public authorities, of the bodies of supervision and control of land resources have a particular impact. The most frequent form of impact on land resources is erosion. The accelerated increase of the surfaces affected by erosion and its intensity is conditioned by the non-compliance with the ecological requirements of the lands at the time of granting agricultural land quotas in private ownership, by the inefficient exercise of the main functions of managing land resources, as well as by the superficial involvement of local public authorities. At an inefficient level, the functions of assessment and control, regulation and coordination of the use and supervision of land resources are exercised (Bacal et al., 2011).

The soil as a producer of goods, a means of production, can be exploited over millennia, provided that degradation processes are excluded. The current state of the soil cover of the Republic of Moldova is worrying. It is necessary to urgently implement a system of measures aimed at efficient use, protection, preservation of the quality and diversity of soils. The Republic of Moldova enjoys a favourable climate and fertile land. The soils of Moldova have about one billion tons of humus, 50 million tons of nitrogen, 60 million tons of phosphorus, 700 million tons of potassium, according to the Republican Center for Applied

Pedology. The maximum weight in the economy is held by the agricultural sector and the basis on which half of the exports of the Republic of Moldova are produced. The average credit rating per country is 63 points and is reduced annually depending on degradation activities. Thus, weak erosion reduces the productive potential (and therefore the quality of the soil) by 20%, medium - by 40%, strong - by 60-80%.

The current condition of the soils, as well as the efficiency of the use of soil resources, cannot be considered satisfactory for the following main reasons: the parceling of the land fund and the deterioration of the regional anti-erosion systems; lack of anti-erosion organization of agricultural land and soil conservation measures; insufficient amounts of fertilizers incorporated into the soil; the lack of pastures, perennial grasses and the predominance of cultivation on the slopes of grazing crops. (IPM Yearbook, 2022. p. 130).

The total area of the land fund of the Republic of Moldova is 3,384.72 thousand ha, including 2,091.9 ha - agricultural land. The surface of publicly owned lands of the state constitutes 783.9 thousand ha (23.1%), the surface of publicly owned lands of administrative-territorial units - 700.4 thousand ha (20.7%) and the surface of privately owned lands (56.2%) constitute 1,900.4 thousand ha (IPM Yearbook, 2022).

A major influence on the reduced efficiency of the functions of managing the impact on land resources is also the superficial implementation of the mechanism of administrative sanctions. Although soils are declared the main asset of our nation, the number of fines for unauthorized use of land and causing various forms of degradation was very low (up to 100 lei). The absolute majority of fines were applied for the illegitimate occupation of land and for actions causing soil pollution. Very rarely were administrative sanctions applied for the destruction of the fertile soil layer, failure to take measures to prevent soil erosion, the lifting of the upper fertile soil layer from the lands of the forest fund, for falsifying and concealing information about the condition of the lands (Bacal et al., 2011).

Currently, the policies in the field of land improvements and land fund are elaborated and promoted by the Ministry of Agriculture and Food Industry in accordance with the provisions of point 6, subsection 12 of the Regulation on the organization and functioning of the Ministry of Agriculture and Food Industry, approved by Government Decision no. 695/2017 (IPM Yearbook, 2022).

In the Republic of Moldova (Bacal, 2016), the normative basis for the application of land taxes is the Law for the application of Title VI of the Fiscal Code (the Law for the implementation of Title VI of the Fiscal Code, 2000) regarding the taxation of real estate, the Land Code (Land Code, 1991) and the annual State Land Cadaster (Bacal, 2014).

The specificity of the Republic of Moldova in terms of mineral resources consists in the fact that it has a comparatively modest space in terms of size with limited resources. The continuous development of the mineral raw material base, the rational use and protection of useful mineral resources are factors that decisively ensure the economic security of the state, the sustainable development of society. Most of the mineral resources of the Republic of Moldova are exploited through quarries and only some varieties of limestone through galleries. The exploitation of resources through quarries usually causes the destruction of soils, vegetation, leads to the accumulation of mining waste, which is later spread by air and water currents, producing imbalances in the ecological balance.

Currently, over 400 deposits of useful mineral substances (limestone, clay, sand, sandstone, gypsum, granite, diatomite) are registered on the territory of the country by the prospecting bodies. Approximately 40% of the total number are exploited, of which approximately 130 deposits are exploited by daily exploitation, and approximately 50 deposits are in underground exploitation (IPM Yearbook, 2022).

The most frequently used underground minerals are carbonate, siliceous, clay rocks, sands and gravels, and less used - caustobiolite rocks (oil, gas, brown coal) because their quantities are insignificant. Deposits of non-metallic mineral substances are represented by granite and gabbronorite (for finishing slabs and gravel of different fractions), sandstone (for finishing slabs and blocks), gypsum (for the building materials industry, medicine, export), chalk, limestones (for cutting limestone blocks of different sizes, for sugar factories, for cement factories, for its disaggregation into different fractions of gravel), clays (for the production of cement, ceramics, tiles, pipes, cheramsite, brick, terracotta, in the chemical industry and food for cleaning wines, juices, oils - "bentonite", etc.), sands (for glass, mortar, and for forming - "glue sand"), sand-crushing (for construction), tripol (diatomite) and marl, which are the basis of providing the building materials industry and construction sites with raw material. As a result of the violations, the total amount of calculated damage caused to the basement for the year 2021 is 733,668,354 lei (Bacal, 2015; IPM Yearbook, 2022).

In recent years, it has been found that one of the factors that significantly influence the soil is the non-compliance with the legal provisions in the exploitation of land resources, including the exploitation of useful mineral substances. Thus, areas of agricultural land are excluded from the agricultural circuit. Also, the authors of the Yearbook mention that "unauthorized mining is a pressing problem. Following the assessment of the situation on the ground in 2020 regarding the state of abandoned quarries and the unauthorized exploitation of useful mineral substances, 385 illegally exploited sectors were detected, the activity of which resulted in the degradation of land with a total area of 736 ha. The total amount of the calculated damage caused to soil resources is 7,530,092 lei. The damages paid for the basement and land resources amount to 220,334 lei. For these reasons, there is a risk of causing enormous damages to the agricultural field and the environment, as follows: pollution of the environment; non-reclamation of land sectors degraded by mining works, storage of household and industrial waste on adjacent lands, etc. (IPM Yearbook, 2021).

During the year 2021, the fertile soil stored and preserved from construction and quarries constitutes 37,183 thousand m^3, of which 32,890 thousand m^3 were used. The current condition of the soils, as well as the efficiency of the use of soil resources, cannot be considered satisfactory for the following main reasons: the parceling of the land fund and the deterioration of the regional anti-erosion systems; lack of anti-erosion organization of agricultural land and soil conservation measures; insufficient amounts of fertilizers incorporated into the soil; the lack of pastures, perennial grasses and the predominance of cultivation on the slopes of grazing crops (IPM Yearbook, 2022).

MATERIALS AND METHODS

In the paper, scientific research methods and various investigations were used using: data analysis and synthesis groups, evaluation methods regarding the condition and protection of land resources and deposits. The data provided by the Environmental Protection Inspectorate of the Republic of Moldova, the Land Cadastre, Laws, various scientific publications for different years, as well as the IPM Yearbook at the 2022 year of the Leova district were used as research materials.

In our investigations, the Leova district, in the south of the Republic of Moldova, was selected as a research object (Figure 1).

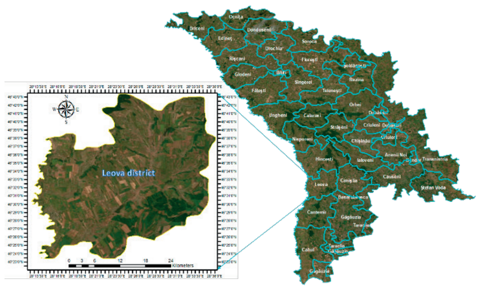

Figure 1. Representation of the Leova district, in the south of the Republic of Moldova map.
Source: Created by authors

Leova district is located in the southwest of the Republic of Moldova, on the border with Romania, along the left bank of the Prut river. The district has an area of 764.7 km², representing 2.26% of the total area of the country (IPM Yearbook Leova, 2022).

The Leova district was created based on the Law on administrative-territorial reform no. XIV-764 of 27.12.2001 and from 23.05.2003 the district is a distinct administrative-territorial unit with legal personality.

The Leova district borders several territorial administrative units of level II: - to the North with Hâncești district; - to the South with the Cantemir district; - to the West with the county of Vaslui in Romania; - to the East with Cimişlia district and UTA Gagauzia.

From an administrative point of view, the Leova district has 25 town halls. The district includes 40 localities, of which 2 cities (Leova, Iargara), 24 communes and 14 villages (components of the communes). The seat of the district is the city of Leova.

RESULTS AND DISCUSSIONS

The Republic of Moldova has a rich pedological resource that, to a large extent, ensures economic activity. About 3/4 of the surface of agricultural land is occupied by chernozems, which are considered the most productive soils. However, the productive potential of soils in the conditions of current land relations is not used adequately, on the arable background the degradation processes continue and accelerate.

If we refer to the analysis of the Leova district (the research object) as a location - the zonal relief is part of the hilly - undulating plain of Southern Moldova. The elements of the relief are very inhomogeneous exposed on narrow water basins that generally stretch from north to south, slopes with different inclinations, deep and long ravines and valleys, the amplitude varies within the limits of 50-230 m, the lowest being located in the meadow of the Prut and Sărata. The elevated plains of the district, dismembered in the downward slope from North to South, are grouped into 4 large units:

- The Prut meadow plain;
- Central Plateau - Moldavian;
- The elevated plain of Grâneţ;
- The Tigheci Plateau with a maximum altitude of 294 m.

The climate is temperate continental with a homogeneous regime, as a result of the non-uniformity of the plain relief, it is characterized by very hot summers (drought prevails 3-4 years out of 10 years) and cold winters. The climate is distinguished by the particularities of periodic droughts, intensive torrential rains and sudden temperature changes. The territory of the district belongs to an area with insufficient moisture. The annual average of atmospheric deposition is 400-500 mm, most of it falls in the form of torrential rains. The average annual temperature is +9.6°C, absolute maximum +40°C, absolute minimum +33°C. The coldest month is January with values of -4.97°C. The number of days with precipitation (≥ 1.0 mm) comprises 101.72 days (27.87%), and days without rain around 263.28 days (72.13%).

The natural resources of the district are forests, rivers and ponds. The underground natural wealth is represented by deposits of sand, clay, clay, bentonite, mineral waters. There are 2 quarries for the acquisition of sand on a total area of 3.6 ha, of which 2 ha are located in the outskirts of Sărata Răzeşi village and 1.6 ha in the outskirts of Hănăsenii Noi village. The forest fund occupies 13.3% of the territory of the district with an average age of 32-45 years.

According to the Land Cadastre according to the situation on 01.01.2022 of the total area of the district Leova are of 76.5 thousand ha, the agricultural land constitutes 57.3 thousand ha, and the non-agricultural land constitutes 19.2 thousand ha (Figures 2-3).

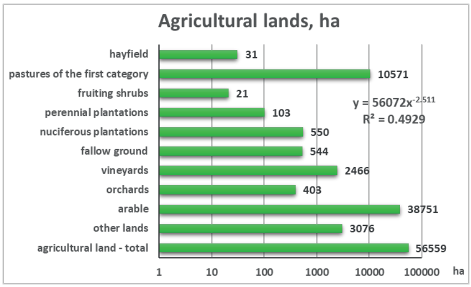

Figure 2. Representation of the agricultural lands in the Leova district
Source: Created by authors

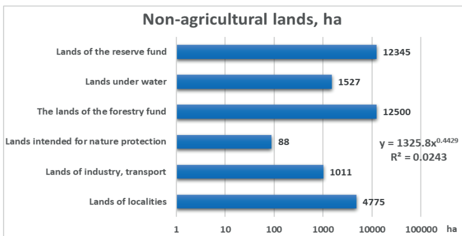

Figure 3. Representation of the non-agricultural land in the Leova district
Source: Created by authors

As a result of the examination and analysis of the data of agricultural land, represented in Figure 2, we notice that the degree of plowing is quite high, constituting 73.9%, and the degree of afforestation is small, constituting 16.3% of the total area of the Leova district.

During the year 2021-2022 or allocated for permanent use to various enterprises and organizations for non-agricultural purposes, land on a total area of - 11.71 ha, namely: - in Tomai town hall for the location of water tanks, on the area of - 1.0 ha; - in Leova town hall for the construction of the water pool and the restoration of the town beach 10.71 ha.

The work of uncovering the fertile soil on the lands allocated for construction in 2022 did not take place. Fertile soil in the volume of - 2.5 thousand m^3 uncovered in previous years, is stored on the land allocated for the construction of schools in the city of Iargara.

During 2022, cases of non-discovery of mixing with the parent rock of the fertile soil layer were not detected. However, there were cases of illegitimate land occupation being detected - 16 cases, namely, from the urban areas - 2 cases on the surface of - 0.05 ha; in the municipalities of Sărata-Noua and Tochile-Răducani, from the outskirts of the localities, 14 cases were detected: on the total area of - 2.8 ha (pastures) in the municipalities of Leova, Colibabovca, Tomai, Ceadîr, Borogani, Baiuș.

The structure of agricultural land according to the degree of erosion constitutes total eroded - 27822.4 ha, of which (Figure 4):

- weakly eroded on slopes of $1-5^0$ - 14216 ha, or - 18.59% of the total area;
- moderately eroded on slopes of $5-8^0$ - 9195 ha, or - 12.03% of the total area;
- strongly eroded on the slope of $8-25^0$ - 4411.35 ha, or - 5.77% of the total area.

The area of landslides constitutes - 182.0 ha, or - 0.23% of the total area, of landslides is - 232.0 ha, or - 0.30% of the total area.

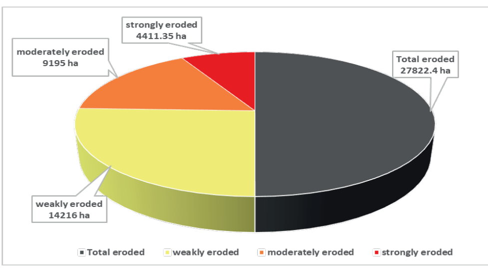

Figure 4. Qualitative characteristics of land according to the degree of erosion in the Leova district
Source: Created by authors

The average soil quality according to the quality of the soils in the Leova district is - 57 points.

The highest soil quality is in Baiuș town halls - 71 points; Tomaiul-Nou - 69 points; Iargara - 68 points; Colibabovca - 65 points; Borogani - 63 points; The carpet - 60 points; Ceadîr - 60 points.

The lowest soil quality is in Sărăteni town halls - 50 points; Sărata-Răzeși - 51 points and Orac - 52 points.

The soils covered with chernozem occupy an area of 44033 ha, and the thickness of the humus layer is 50-70 cm, and those subject to erosion have an area of 27822.4 ha. The total area of land affected by erosion represents about 50% of the total area of agricultural land. In recent years, the area of land affected by erosion has increased significantly, occupying about 40% of the total area of agricultural land. This is also due to the fact that the agricultural lands are insufficiently protected by the existing network of forest curtains to protect the fields.

According to the degree of manifestation of the unfavorable properties, the following soil classes are highlighted:

- non-degraded, the productivity of the soils corresponding to their natural fertility, the possible deviation of the property values in an unfavorable direction being up to 5%;
- slightly degraded, soils whose productivity has decreased by 5-25%;
- moderately degraded, soils whose productivity has decreased by 25-50%;
- strongly degraded, their productive capacity being reduced by 50-75%;
- very strongly degraded, their productive capacity being reduced by more than 75%.

Soils are in a continuous process of degradation, at an accelerated rate, which inevitably leads to the decrease of soil fertility, of the degree of supply of the soil with the main nutrients: nitrogen, phosphorus, potassium, it being known that the nutrients in the soil, lost through erosion, cannot be restored to their original form by applying fertilizers.

Soil erosion worsens the water regime of the soil, the drainage conditions and the hydrological situation of the territory; exerts an extremely negative influence on the soil biota - the totality and number of bacteria, fungi, small animals, which populate the soil and which, through their joint activity, determine the productivity of the soil. Already with a weak manifestation of the phenomenon of erosion, the production of field crops decreases by 10-20%, with a moderate manifestation - by 30-40% and with a strong one - by 50-60% and more.

The factors that contribute to the ecological state of land resources remain the water, wind and biological erosion conditioned by the insufficient administration of organic fertilizers, the non-application of agrotechnical, phyto-ameliorative, hydrotechnical anti-erosion measures to agricultural crops, without taking into account the relief, the variety of soils, the processing of hill land in the valley, on the sloping lands.

As a result of the heavy rains in the spring, erosion processes took place on most of the agricultural fields on the slopes, more significant on the agricultural lands in the municipalities of Tigheci, Filipeni, Sărăteni, Ceadîr, Cneazevca, Covurlui, Tomai, Vozniseni, Cazangic, Sărata-Nouă.

In order to prevent the burning of stubble, straw, dry grass in accordance with the provisions of art. 62 of Law No. 1515 regarding the protection of the environment, town halls, economic agents owning agricultural land were informed by warning letters about the prohibition of burning stubble, dry grass, plant residues, waste, etc.

However, following the harvesting of the spiky cases of stubble burning took place on the lands of two peasant households in the municipalities of Orac and Sîrma on an area of 2 ha, the observed damage to land resources amounted to - 500 lei.

There were cases of dry grass burning that took place on a total area of 20.9 h in the following districts: Sîrma, Hănăsenii-Noi, Tomai, Borogani, Iargara, Sărata-Răzeși, Leova, Sărata-Nouă, Baiuș.

The area of pastures in the Leova district constitutes - 10571 ha. The livestock decreased compared to the previous year, being - 21,900 heads, returning to an average of - 0.48 ha of pasture per head of population, which is in accordance with grazing norms.

The lands in private ownership constitute the surface of 45442.16 ha, of which agricultural lands comprise 44962.6 ha and non-agricultural lands 479.56 ha.

The total area of irrigated land in the district is 2950 ha.

During the year 2021-2022, the irrigation of agricultural land was carried out on the total area of - 148.0 ha, with the pumping of water from the Prut river, in the town halls: - Tochile; - Răducani (SRL FructAgroPrut - 140 ha) growing legumes - 30 ha, wheat - 110 ha and Filipeni (SRL Vicfilvas - 8.0 ha) growing legumes - 8 ha.

The area of land with functional sanitation networks is - 398 ha.

The creation of riparian zones for the protection of rivers and water basins was carried out in the municipalities of Baiuș and Filipeni on a total area of - 0.50 ha.

At the same time, cases of processing riparian protection sheets were not detected. However, there were cases of waste storage in the riparian water protection sheets that were detected in the municipalities of Sărata-Nouă, Hănășenii-Noi and Tomai, and minutes were drawn up against natural persons guilty of violating the waste management rules.

The application of chemical fertilizers under the fruit of the year 2022, was carried out on an area of - 34500 ha, being as additional food to agricultural crops during the sowing period, as well as during the vegetation period, being used - 1901000 kg, there were - 55 kg for 1 ha. Organic fertilizers were not used.

For the purpose of improving degraded lands and soil protection or spent silvotechnical work in the forest fund, planted with forest crops - 15.3 ha of land in the territorial-administrative borders of Baiuș and Sărăteni municipalities.

Riparian forest cover was planted to protect the banks of the streams on a total area of - 0.5 ha, in the mayor's office of Baiuș and Filipeni.

The work of planting a scuar on the surface of - 2.0 ha took place on the field where the work of consolidating the turnip in the center of the village of Covurlui was carried out.

Capital investments foreseen for the protection of soils and the implementation of projects in this field, including funded ones, regrettably did not take place.

Damages caused to soil resources were found as a result of stubble burning in the amount of -

500 lei. We believe it is too small a sum for these violations.

The sown area of agricultural crops under the harvest of 2020 in agricultural enterprises and peasant (farmer) households with an area of 10 ha and over constituted in 2020 about 28443 ha, with 1002 ha or 3.7% more compared to 2019. In 2021, the sown area was 30325 ha, 6.6% more than in 2020, and in 2022 it was 31043 ha, 2.4% more than in 2019. Thus, in the period 2019-2022 the sowing area increased by 4602 ha.

In 2020, of the total sown area, 57.1% are cereals and pulses for grains, 42.3% are technical crops, 0.4% potatoes, vegetables and food crops, and crops for fodder constitute 0.2%.

In 2021, of the total sown area, 58.6% represent cereals and pulses for grains, 40.9% are technical crops, 0.4% are potatoes, vegetables and food crops, and crops for fodder constitute 0.1%.

In 2022 compared to 20120, there is a 2.9% increase in the areas sown with cereals and legumes for grains and a 2.8% decrease in the areas with technical crops. The area of fodder crops also decreased by 38 hectares compared to 2020.

In 2020, compared to 2019, animal production in agricultural enterprises and peasant households (farmers), which have animals on the balance sheet, recorded a decrease in cattle production (in live mass) by 23.8%. In 2022, compared to 2021, there was a decrease of the respective indicator by 19.0%.

In the Leova district, the mineral deposit available for exploitation is clay and sand for construction.

The sand is extracted openly, at the authorized quarry, which is managed by SRL Iulautocomplex, located in the territorial-administrative boundaries of Sărata-Răzeși town hall, Leova district.

The surface of the mining perimeter projection, indicated on the topographical plan of the land surface, is - 5.5 ha.

The land with cadastral number 5732104328 allocated for lease for sand extraction according to the lease agreement and Local Council Decision no. 01/07 of 12.03.2019 with an area of - 2.0 ha, is ready for exploitation. Land recultivation works are not carried out.

The activity of extracting the deposit was legalized by license series A MMII no. 050524 from 28.12.2015, but it was valid until 27.12.2020.

According to the informative note presented, during the year 2022 sand was extracted in the amount of - 66.5 thousand m^3.

Violations of the method of protection and use of the basement were detected in: the Sărata-Nouă town hall, the unauthorized extraction and use of clay by natural persons in a volume of 60 m^3 from pasture land (former clay quarry), which was transported to be used for the repair of a dike at the local pond.

SA Vinal-Coci was detected in the town hall of Tigheci commune and the village of Cuporani, where 3.0 m^3 of clay was extracted unauthorized from agricultural land as pasture for the needs of the wine factory.

In the Covurlui town hall, the unauthorized extraction of sand from a ravine formed after the rains was detected, from where 1.0 m^3 of sand was excavated by an individual from the village of Covurlui, Leova district, for his own needs.

In the Tomaiul-Nou town hall, the unauthorized extraction of clayey sand in a volume of 60 m^3 from the forest fund was detected, at the direction of the mayor of the locality, which was used to backfill a local road in the village.

For the examination of the given case, a request was submitted to the Leova District Prosecutor's Office.

The number of fines applied is 16,500 lei, paid - 8,250 lei.

The amount of damage caused to the environment following the illegitimate extraction of useful mineral substances was calculated to be - 49184.17 lei, of which only - 1509.07 lei were paid.

Remedial recommendations for the agricultural sector in Leova district are as follows: Consolidation of investments in agriculture by: - promoting high-performance technologies in agriculture (No till - Mini till, vegetable cultivation on protected lands, intensive and super-intensive multi-year plantations); - identifying and promoting opportunities for the development of intensive agriculture with the use of drip irrigation; - promoting advanced technologies for processing, preserving, packaging and marketing the agro-food production in the district; - informing agricultural producers about various internal and external financing opportunities, and providing assistance for attracting investments; - promotion of modern drip irrigation methods; - identification of options for creating water sources for irrigation; - improving soil quality by informing farmers about the consequences of non-compliance with crop rotation and incorrect soil processing; - the record of agricultural surfaces subject to erosion and the taking of protection measures against erosion; - afforestation of degraded lands; - planting trees and shrubs on slopes on degraded lands, repairing field protection sheets.

CONCLUSIONS

The soils covered with chernozem occupy an area of 44033 ha, and the thickness of the humus layer is 50-70 cm, and those subject to erosion have an area of 27822.4 ha. This is also due to the fact that the agricultural lands are insufficiently protected by the existing network of forest curtains to protect the fields. The area of landslides constitutes - 182.0 ha, or - 0.23% of the total area, of landslides is - 232.0 ha, or - 0.30% of the total area.

Soils are in a continuous process of degradation, at an accelerated rate, which inevitably leads to the decrease of soil fertility, of the degree of supply of the soil with the main nutrients: nitrogen, phosphorus, potassium, it being known that the nutrients in the soil , lost through erosion, cannot be restored to their original form by applying fertilizers.

Soil erosion worsens the water regime of the soil, the drainage conditions and the hydrological situation of the territory; exerts an extremely negative influence on the soil biota - the totality and number of bacteria, fungi, small animals, which populate the soil and which, through their joint activity, determine the productivity of the soil.

As a result of the heavy rains in the spring, erosion processes took place on most of the agricultural fields on the slopes, more significant on the agricultural lands. The factors that contribute to the ecological state of land resources remain the water, wind and biological erosion conditioned by the insufficient administration of organic fertilizers, the non-application of agrotechnical, phyto-ameliorative, hydrotechnical anti-erosion measures to agricultural crops, without taking into account the relief, the variety of soils, the processing of hill land in the valley, on the sloping lands. The structure of agricultural land according to the degree of erosion constitutes total eroded - 27822.4 ha

The lands in private ownership constitute the surface of 45442.16 ha, of which agricultural lands comprise 44962.6 ha and non-agricultural lands 479.56 ha. The area of pastures in the Leova district constitutes - 10571 ha.

The total area of irrigated land in the district is 2950 ha. During the year 2021-2022, the irrigation of agricultural land was carried out on the total area of - 148.0 ha, with the pumping of water from the Prut river.

The area of land with functional sanitation networks is - 398 ha.

In the Leova district, the mineral deposit available for exploitation is clay and sand for construction.

Capital investments foreseen for the protection of soils and the implementation of projects in this field, including funded ones, regrettably did not take place.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

Lumbricidae FAMILY IN BROWN FOREST SOILS: ABUNDANCE, BIOMASS, PROFILE DISTRIBUTION

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Abstract

The Lumbricidae family of brown forest soils in natural and agricultural ecosystems located in the central zones of the Republic of Moldova has been investigated in May, 2021 and 2022. Earthworms sampling was carried out from test cuts by manual sampling of soil layers to the depth of soil fauna occurrence. The number and biomass of Lumbricidae family in natural brown soils are greater by 3.8-17.9 times and by 2.6-16.5 times compared to brown soils of agricultural ecosystems. The highest values of abundance and biomass of earthworms were registered in the typical brown forest soils under natural vegetation and the smallest values - in arable luvic brown forest soils. A characteristic feature of the natural brown soils is the high concentration of invertebrates and Lumbricidae family in the upper layers of soils. The species Lumbricus terrestris, Lumbricus rubellus, Aporrectodea caliginosa and Aporrectodea longa and other were identified in the faunal samples from the natural brown forest soils.

***Key words**: Lumbricidae family, brown forest soil, natural and agricultural ecosystems.*

INTRODUCTION

Lumbricidae family belong to the phylum *Annelida*, class *Clitellata,* subclass *Oligochaeta*, order *Opisthopora,* superfamily *Lumbrcoidea*.

Charles Darwin was the first scientist to draw attention to the important role of earthworms in soil formation processes (Darwin, 1881). The structure-forming role of earthworms attracts attention of many scientists (Lee & Foster, 1991; Edwards & Bohlen, 1996; Jouquet et al., 2008; Lapied et al., 2009). The activity of earthworms produces a significant effect, not just on the structure, but also on the chemical composition of the soil, since a large part of the organic matter ingested by earthworms returns to the soil in the form easily used by plants. Earthworms contribute nutrients to the soil and improve porosity, tilth, and root development (Fragoso et al., 1997).

Earthworms are the dominant macrofauna and a functionally significant component of the soil. *Lumbricidae* family are recognized as a key factor to the functioning of many terrestrial ecosystems (Bartlett et al., 2010). Earthworms can serve as indicators of anthropogenic land use, soil quality and a factor for soil biological site classification (Martinez-Salgado et al., 2010; Römbke et al., 2005; State of Knowledge of Soil Biodiversity. Status, challenges and potentialities. Report 2020. Retrieved from Report Soil Biodiversity.pdf).

In the 1960s, nine species of earthworms were identified in the soils of Moldova (Perel, 1962; Prokhina, 1965; 1968; Striganova, 1968). The edaphic species of fauna, unique only to brown soils, especially from *Lumbricidae* family, have been discovered (Gilyarov, 1965).

Aporrectodea rosea predominated in most soils in the 1990s, *Aporrectodea caliginosa, Aporrectodea trapezoides, Octalazion transpadanum, Aporrectodea jassiensis* and *Allolobophora leoni* are much less common (Cherevatov, 1991).

In this context, the purpose of the research was to investigate the abundance, biodiversity and profile distribution of *Lumbricidae* family in brown soils of natural and agricultural ecosystems for the biodiversity conservation and development of the monitoring system for the status of useful edaphic fauna.

MATERIALS AND METHODS

Experimental sites are located in central zones of the Republic of Moldova. The content of *Lumbricidae* family in brown forest soils with the normal profile in the condition of the long-term use in agricultural production were investigated in comparison with the undisturbed soils in natural ecosystems of forest. Two experimental sites have been tested.

Experimental sites with typical and luvic brown soil are located in the central zone of the Republic of Moldova, in the wooded steppe of the central - Moldovan forest province, in the district No. 8 of brown, gray forest soils and leached chernozems of the wooded steppe of hilly Kodru Forests.

The site with typical brown soil (profile 1 under forest; profile 2 under arable) is situated in the Tuzara village and Gorodische com., Kalarash region (Figure 1).

Figure 1. Site with the typical brown forest soil in the Tuzara village and Gorodische com., Kalarash region

The site with luvic brown soil (profile 5 under forest; profile 6 under arable) is located in the Dolna com., Strasheni region (Figure 2).

Figure 2. Site with the luvic brown forest soil in the Dolna com., Strasheni region

Status of invertebrates and Lumbricidae family. Testing of semi-profiles in the amount of 3 units was carried out around the main test cut at a distance of 5-10 m. The state of invertebrates and *Lumbricidae* family was determined from test cuts by manual sampling of soil layers to the depth of soil fauna occurrence with application of Gilyarov and Striganova's method (1987). At the same time, earthworms were also selected from the litter layer and on the soil surface. Ethyl alcohol was used to immobilize (destroy) the earthworms. The taxonomic studies used during the identification were accomplished according to Vsevolodova-Perel T.S. (1997).

The studies were carried out in May of 2021 and 2022.

RESULTS AND DISCUSSIONS

The number and biomass of edaphic fauna including *Lumbricidae* family in brown forest soils of natural ecosystems are characterized by the higher values of these indicators in comparison with arable brown forest soils (Table 1). Number of invertebrates and *Lumbricidae* family in the typical brown forest soil decreases on average for 2 years (2021-2022) from 330.7 to 65.3 ex m^{-2} and from 152.0 to 61.4 ex m^{-2}, in the luvic brown forest soil - from 274.7 to 5.4 ex m^{-2} and from 140.0 to 4.0 ex m^{-2} accordingly. Similar changes were observed in the total biomass of the edaphic fauna and *Lumbricidae* family.

The share of earthworms in the total number of invertebrates in the typical brown forest soil of natural ecosystems constitutes of 51.8% in 2021 and 38.3% in 2022, in the luvic brown forest soil - 51.5% and 50.7% accordingly. Their contribution to the total biomass is 82.5% in 2021 and 51.0% in 2022 in the typical brown forest soil. The proportion of *Lumbricidae* family in the total biomass of the edaphic fauna is 89.3% and 81.6% in the the natural luvic brown forest soil in studied years.

The share of *Lumbricidae* family in the total abundance of invertebrates in arable forest soils constitutes 94.0% in the typical brown forest soil, and 74.1% in the luvic brown forest soil on average for 2 years of research. The contribution of earthworms to the total biomass is 99.1% and 92.1% accordingly.

Table 1. Number and biomass of invertebrates and *Lumbricidae* family in brown soils under forest and arable land (n = 3 for each profile)

Soil	Land use	Semi-profile	Number, ex m^{-2}		Biomass, g m^{-2}	
			total	*Lumbricidae* fam.	total	*Lumbricidae* fam.
2021						
Typical brown soil	forest	45	408.0	152.0	73.6	58.4
		46	352.0	144.0	60.0	38.4
		47	368.0	288.0	131.2	121.6
		mean values	376.0	194.7	88.3	72.8
	arable	48	144.0	144.0	68.0	68.0
		49	40.0	40.0	16.8	16.8
		50	96.0	80.0	29.0	28.4
		mean values	93.3	88.0	37.9	37.7
Luvic brown soil	forest	51	96.0	24.0	28.6	24.8
		52	240.0	152.0	100.8	96.0
		53	208.0	104.0	52.2	54.0
		mean values	181.3	93.3	60.5	54.0
	arable	54	8.0	0	1.6	0
		55	24.0	24.0	20.8	20.8
		56	0	0	0	0
		mean values	10.7	8.0	7.5	6.9
2022						
Typical brown soil	forest	99	416.0	152.0	117.0	47.2
		100	264.0	112.0	92.8	58.4
		101	176.0	64.0	41.4	22.4
		mean values	285.3	109.3	83.7	42.7
	arable	102	64.0	56.0	12.2	12.0
		103	40.0	40.0	9.6	9.6
		104	8.0	8.0	0.4	0.4
		mean values	37.3	34.7	7.4	7.3
Luvic brown soil	forest	93	208.0	72.0	34.0	25.4
		94	424.0	176.0	72.0	54.0
		95	472.0	312.0	120.8	105.8
		mean values	368.0	186.7	75.6	61.7
	arable	96	0	0	0	0
		97	0	0	0	0
		98	0	0	0	0
		mean values	0	0	0	0
Average value for 2021-2022						
Typical brown soil	forest	mean values	330.7	152.0	86.0	57.8
	arable		65.3	61.4	22.7	22.5
Luvic brown soil	forest		274.7	140.0	68.1	57.9
	arable		5.4	4.0	3.8	3.5

The average weight of one exemplar of earthworms in the natural typical brown forest soil constitutes 0.38 g, in the natural luvic brown forest soil - 0.41 g. The average weight of a specimen of the *Lumbricidae* family constitutes 0.37 g in the typical brown soil in conditions of agricultural ecosystems, and 0.88 g - in the arable luvic brown forest soil.

The base mass of fauna in the brown soils under the forest is located in the litter layer and 0-10 cm layer: in the typical brown soil - 90.8% and 95.3%, in luvic brown soil - 91.2% and 90.6% in the first and second years of research (Figure 3). The accumulation of edaphic fauna in the arable typical brown soil was registered in the 0-30 cm layer, in the arable luvic brown soil - in the 0-10 cm layer in 2021. The number of invertebrates index decreases in natural soil profiles to a depth of 40 cm, in arable soils - to a depth of 20-30 cm.

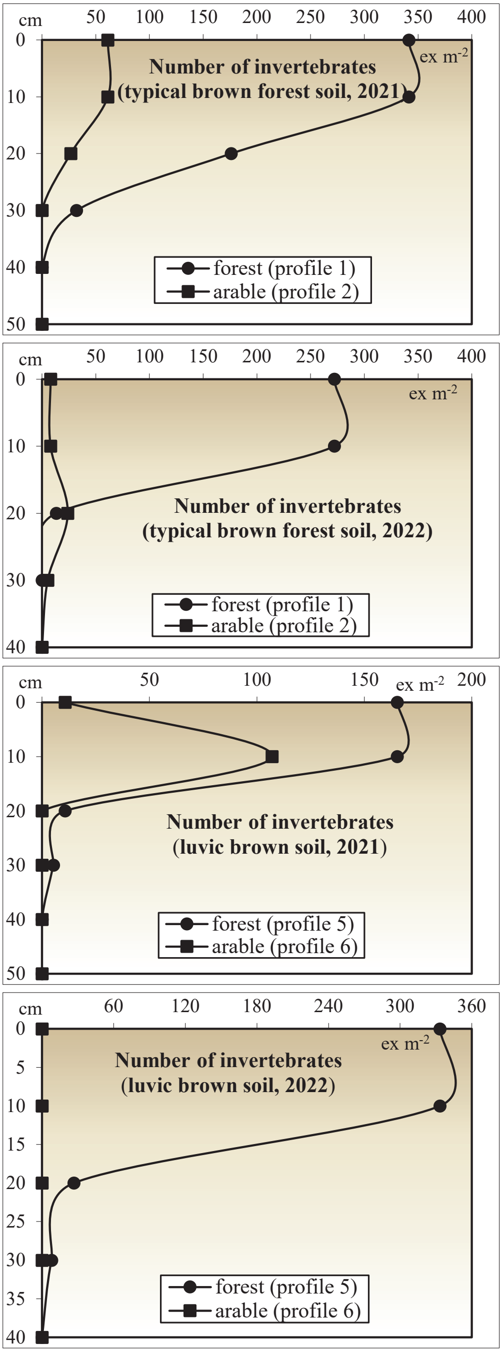

Figure 3. The profile distribution of invertebrates in brown forest soils of natural and agricultural ecosystems

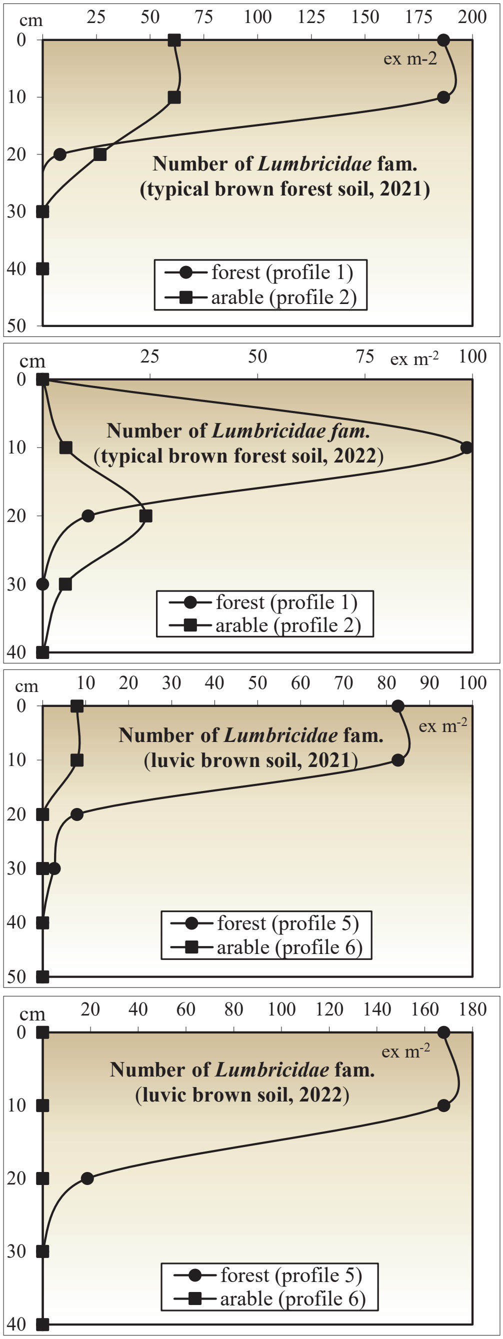

Figure 4. The profile distribution of *Lumbricidae* family in brown forest soils of natural and agricultural ecosystems

Lumbricidae family in the natural brown soils is located in the litter layer and 0-10 cm layer, amounting to 95.2% typical soil and 89.3% luvic soil in the total number (Figure 4).

The largest number of earthworms in the arable typical brown soil was found in the 0-30 cm layer, in the arable luvic brown soil - in the 0-10 cm. In the second year of research, earthworms were absent in faunal semi-profiles.

Natural brown forest soils are characterized by a high diversity of invertebrates and *Lumbricidae* family compared to arable brown forest soils. There are 16 families of invertebrates in the natural typical brown soil and 15 families of edaphic fauna - in the natural luvic brown soil.

The long-term use of plowing leads to the considerable decrease of the invertebrates' biodiversity. Arable brown forest soils contain only 2 families of invertebrates in this period of fauna selection.

The species *Lumbricus terrestris, Lumbricus rubellus, Aporrectodea caliginosa* and *Aporrectodea longa* and other were identified in the faunal samples from the natural brown forest soils (Table 2). The greatest species biodiversity of *Lumbricidae* was in the forest ecosystems, which is inhabited by representatives of 4-5 species. The lowest species richness of earthworms was observed in the arable soils of agricultural ecosystems, where 1-3 species are widespread.

The epi-endogeic group in the natural typical forest soil is represented primarily by *Lumbricus rubellus*, the number of which in 2021 was especially high (41.1% of the total number). The saturation of the species composition of worms with anecic species *Aporrectodea longa* and *Lumbricus terrestris,* and also endogeic specie *Aporrectodea caliginosa* was also at a rather high level. *Aporrectodea caliginosa* has been also dominated in the *Lumbricidae* species composition in 2022 (70.8% of the total number). On average, over 2 years of research, 46.8% of earthworms in the natural typical forest soil belong to endogeic species, 24.3% - to anecic species and 20.5% - to epi-endogeic species.

Part of the earthworm's species in the natural luvic brown soil, living in the litter and in the upper soil layer, are surface-dwelling or litter-dwelling species (epi-endogeic earthworms). For example, the specific contribution of *Lumbricus rubellus* to the total number of *Lumbricidae* is 25.7% in 2021 and 27.2% in 2022.

A significant percentage of anecic species, such as *Lumbricus terrestris*, which were found in 2021 (34.3%), and endogeic species - *Aporrectodea caliginosa*, which were recorded in 2022 (48.6%), have been identified in the luvic brown forest soil of the natural ecosystem.

The number of *Aporrectodea longa* amounted to 14.3-15.7% in this soil. The epi-endogeic species *Aporrectodea trapezoides* was found in single specimens.

Endogeic species account for 32.9%, epi-endogeic species - 30.8% and anecic species - 20.7% of the total abundance of earthworms on average over 2 years of research.

Aporrectodea rosea (endogeic species) dominated in the arable typical brown forest soils in 2021, accounting for 63.6% of the total number of earthworms. Among the *Lumbricidae* family, the endogeic species *Aporrectodea caliginosa* prevailed (77.0%) in this soil in 2022.

Aporrectodea rosea has been found in the arable luvic brown forest soil in small quantities.

Table 2. Biodiversity of the *Lumbricidae* family (ex m^{-2}) at the species level in brown soils of natural and agricultural ecosystems

Lumbricidae family species	Typical brown soil, forest (P1)		Typical brown soil, arable (P2)		Luvic brown soil, forest (P5)		Luvic brown soil, arable (P6)	
	2021	2022	2021	2022	2021	2022	2021	2022
Lumbricus terrestris	21.3	5.3	0	0	32.0	13.3	0	0
Lumbricus rubellus	80.0	0	0	0	24.0	50.7	0	0
Aporrectodea caliginosa	29.3	77.4	21.3	26.7	5.3	90.7	0	0
Aporrectodea longa	58.7	21.3	0	0	13.3	29.3	0	0
Aporrectodea rosea	0	0	56.0	8.0	10.7	0	8.0	0
Octolasion lacteum	5.4	5.3	10.7	0	0	0	0	0
Aporrectodea trapezoides	0	0	0	0	8.0	0	0	0
Species unidentified	0	0	0	0	0	2.7	0	0
In total	194.7	109.3	88.0	34.7	93.3	186.7	8.0	0

The earthworms complex accounts 65.8-73.2% in the natural typical brown forest soil and 50.7-81.3% in the natural luvic brown forest soil from the total number of saprophagous. In arable soils their share is 97.0% and 100.0%.

CONCLUSIONS

Lumbricidae family is an important trophic level in the ecological chain nutrition of the ecosystem. Earthworms has a great importance for biological processes in soil, increase the fertility and humus formation by mechanical decomposition of plant residues and the formation of water-stable soil structure. Earthworms are indicators of soil quality, anthropogenic land use and a factor for soil biological classification. The highest values of abundance and biomass of earthworms were registered in the natural typical brown forest soils and the smallest values - in arable luvic brown forest soils. The number and biomass of *Lumbricidae* family in natural brown soils are greater by 3.8-17.9 times and by 2.6-16.5 times compared to brown soils of agricultural ecosystems. A characteristic feature of the natural brown soils is the high concentration of invertebrates and earthworms in the upper layers of soils. The species *Lumbricus terrestris, Lumbricus rubellus, Aporrectodea caliginosa, Aporrectodea longa* and other were identified in the faunal samples from the natural brown forest soils. *Aporrectodea rosea* and *Aporrectodea caliginosa* species is the most typical representative of the *Lumbricidae* in the arable brown forest soils.

ACKNOWLEDGEMENTS

This research work was carried out in the framework of the institutional project "Evaluation of the soil state of the Republic of Moldova in the agrocenosis conditions, improvement of the classifier and the soil rating system, elaboration of the methodological-informational framework for monitoring and enlarged fertility reproduction" (project code 20.80009.7007.17) in 2021-2022.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ABUNDANCE AND DIVERSITY OF *Carabidae* FAMILY IN SOILS OF THE REPUBLIC OF MOLDOVA

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Abstract

The family of Carabidae in soils of natural and agricultural ecosystems located in the different zones of the Republic of Moldova has been investigated in May and August, 2021. Carabids sampling was carried out from test cuts by manual sampling of soil layers to the depth of soil fauna occurrence. Abundance of Carabidae family in natural soils is much greater compared to soils of agricultural ecosystems. Carabids were practically absent in arable soils at the time of sampling. The share of Carabidae family in the total abundance of invertebrates in forest soils of natural ecosystems constitutes of 2.1-9.6%, in chernozems - 3.0-10.2%. The base mass of Carabidae family in soils under natural vegetation is located in the litter and 0-10 cm layer. Species of the Carabus nemoralis, Carabus granulatus, Harpalus affinis, Harpalus rufipes, Calosoma inquisitor and others were identified in the faunal samples from soils in natural ecosystems.

Key words: *Carabidae family, forest soil, chernozem, natural and agricultural ecosystems.*

INTRODUCTION

The family of *Carabidae* is a part of the largest insect families, with about 40,000 species worldwide (Lövei & Sunderland, 1996; Kromp, 1999). The summary list of ground beetles (*Carabidae*) from the Republic of Moldova at the beginning of the 1990s constituted 466 species (Neculiseanu, 1991). Rare and endangered carabid's species in this area have been investigated (Neculiseanu et al., 1992; 2002; 2003). Review of the carabid's fauna "Catalogue of beetles of the Republic of Moldova", published in Bulgaria, includes 497 species belonging to 89 genera (Neculiseanu & Matalin, 2000). Later there were identified 505 species of carabids belonging to 88 genera, 15 sub-tribes, 33 tribes, 18 supra-tribes and 4 sub-families (Neculiseanu, 2003-2004).

Carabidae family plays a major role in reducing weeds and insect pests in agricultural fields. They have a wide range of nutrition. Many species are considered generalist predators, meaning they feed on a wide range of pests. For example, the species *Calosoma sycophanta* is an effective exterminator of forest pests. Species of the genera *Carabus* and *Cychrus* play a significant role as destroyers of harmful mollusks (Bagirova, 2014). In addition to being predators, many species are omnivorous, feeding on weed seeds and can have a serious impact on weed management on the arable land (Blubaugh & Kaplan, 2016). Many species are known to have a seasonal diet shift based on food availability, allowing the predators to exploit different resources throughout the year (Symondson et al., 2002; Honek et al., 2006).

Thus, being one of the most important components of natural and anthropogenic ecosystems, ground beetles (*Carabidae*) play an important role in the natural abundance of many invertebrates.

Carabids represent one of the main groups of insects that is used for indication of character and directions of soil and vegetation changes. They play an important role in the diagnosis of soil types in deciduous forests. So, some species of the *Carabidae* family are unique and characteristic for the brown forest soils of Moldova (Gilyarov, 1965). This group of coleopterans is recommended to be used as indicator species and one of the main ones in soil monitoring studies (Baban, 2006).

The purpose of the research was to investigate the abundance and diversity of the *Carabidae* family in soils of natural and agricultural ecosystems for the biodiversity conservation and development of the national soil quality standards.

MATERIALS AND METHODS

Experimental sites are located in central and northern zones of the Republic of Moldova (Figure 1). The content of *Carabidae* family in soils with the normal profile in the condition of the long-term use in agricultural production were investigated in comparison with the undisturbed soils in natural ecosystems (Figure 2). Six experimental sites have been tested.

Figure 1. Fragments of natural and agricultural landscapes located in the central and northern zone of the Republic of Moldova

Figure 2. Fragments of forest ecosystems

Experimental sites with typical and luvic brown soil are located in the central zone of the Republic of Moldova, in the wooded steppe of the central - Moldovan forest province, in the district No. 8 of brown, gray forest soils and leached chernozems of the wooded steppe of hilly Kodru Forests.

The site with typical brown soil is situated in the Tuzara village and Gorodische com., Kalarash region.

The site with luvic brown soil is located in the Dolna com., Strasheni region.

The site with molic gray forest soil is situated near the Grozeshti village, Nisporeni region. According to pedogeographic zoning, this site is located in the Central Plateau of Kodru Forests, in the region V of Kodru' Plateau, in the district No. 8 of brown, gray forest soils and leached chernozems.

The site with typical gray forest soil is also located in the hilly forest steppe zone of the Northern Plain (I), the forest-steppe hill of Rezina district No. 5 of gray forest soils and argillaceous chernozems near the Raspopeni village, Sholdaneshti region.

The site with albic gray forest soil falls within the hilly forest-steppe zone of the Northern Plain (I), in the forest-steppe of the Northern Plateau, in the district No. 1 of gray forest soils and clay-alluvial chernozems. Nearby is located Terebna village, Edinets region.

The site with podzolic chernozem are located in the zone of the hilly wooded steppe of the Northern Plain (1), in the district of wooded steppe of the middle Prut (2) with gray forest soils, podzolic and leached chernozems, in the Shaptebani village, Ryshkani region.

Status of invertebrates. Testing of semi-profiles in the amount of 3 units was carried out around the main test cut at a distance of 5-10 m. The state of invertebrates and *Carabidae* family was determined from test cuts by manual sampling of soil layers to the depth of soil fauna occurrence with application of Gilyarov and Striganova's method (1987).

At the same time, carabids were also selected from the litter layer and on the soil surface. Imago and larvae have been collected. Ethyl alcohol was used to immobilize (destroy) the carabids. The species identification was carried out according to the external morphological features. The taxonomic studies used during the identification were accomplished according to Kryzhanovsky (1983). Carabids larvae were identified according to Gilyarov M.S. (1964).

The studies were carried out in May and August of 2021.

RESULTS AND DISCUSSIONS

The abundance of the edaphic fauna in soils of natural ecosystems are characterized by higher values in comparison with the arable soils (Table 1). The typical brown forest soil contains invertebrates on average 376.0 ex. m^{-2}, luvic brown forest soil - 181.3 ex. m^{-2}; molic gray forest soil - 194.7 ex. m^{-2}, albic gray forest soil - 192.0 ex m^{-2}, typical gray forest soil - 290.7 ex. m^{-2}. A significant number of invertebrates was also found in the podzolic chernozem - 352.0 ex. m^{-2}.

The natural molic gray forest soil and natural luvic brown forest soil are characterized by the high content of carabid beetles, reaching 18.6 ex. m^{-2} and 16.0 ex. m^{-2}. The natural albic gray forest soil, typical gray forest soil and podzolic chernozem contained species of carabids in the amount of 13.3; 10.6 and 10.7 ex. m^{-2} respectively. The typical gray forest soil in a natural forest ecosystem contained the lowest content of carabid beetles - 8.0 ex. m^{-2}.

The share of *Carabidae* family in the total abundance of invertebrates in forest soils of natural ecosystems in our research constitutes of 2.1-9.6%, in the podzolic chernozems - 3.0%. According to Prokhina N.A. (1968), the share of carabids in the total composition of the edaphic fauna in the calcareous chernozem was 7.1%, in the ordinary chernozem - 8.2%, in the leached chernozem - 6.7% and in the typical chernozem - 10.2%. In general, it can be assumed that the contribution of *Carabidae* family in the total amount of the edaphic fauna in natural soils does not exceed 10.0-11.0%.

The long-term use of arable land with pesticides leads to a considerable decrease in the number of invertebrates and to the degradation of the soil faunal complex in general. The number of invertebrates in arable forest soils decreased by 1.3-16.9 times, in the podzolic chernozem - by 66.4 times respectively in comparison with natural soils. *Carabidae* family reduced to zero values in soils under agricultural conditions. Only a few of the most mobile species of *Amara aenea* have been occasionally found.

Table 1. Number of invertebrates and *Carabidae* family (imago + larvae) in soils in conditions of natural and agricultural ecosystems (n = 3 for each profile)

Soil	Land use	Number, ex. m^{-2}	
		total	*Carabidae* fam.
Typical brown forest soil	forest	376.0	8.0
	arable land	93.3	0
Luvic brown forest soil	forest	181.3	16.0
	arable land	10.7	0
Molic gray forest soil	forest	194.7	18.6
	arable land	146.7	0
Albic gray forest soil	forest	192.0	13.3
	arable land	24.0	0
Typical gray forest soil	forest	290.7	10.6
	arable land	18.7	0
Podzolic chernozem	virgin land	352.0	10.7
	arable land	5.3	0
Calcareous chernozem*	virgin land	171.0	12.1
	arable land	38.0	1.4
Ordinary chernozem*	virgin land	232.0	19.0
	arable land	47.0	3.1
Leached chernozem*	virgin land	174.3	11.7
	arable land	71.0	7.8
Typical chernozem*	virgin land	166.6	17.0
	arable land	64.0	4.0

*Cited from Prokhina N.A., 1968

The base mass of edaphic fauna and carabids in the natural soils is located in the 0-10 cm layer, in the litter and in the mix of litter with soil.

In total, 13 species of carabid beetles from 7 genuses (*Bembidion*, *Calosoma*, *Carabus, Harpalus, Lebia, Ophonus* and *Panagaeus*), 5 tribes (*Carabini, Bembidiini, Harpalini, Lebiini and Panagaeini*) and from subfamily *Carabinae* were found and identified in the studied soils. At the time of sampling, forest soils in natural ecosystems contained 11 species from the *Carabidae* family. There are 5 species of carabid beetles in the natural luvic brown soil, 4 species - in the natural molic gray forest soil and 3 species of carabids in each - in the natural typical brown soil, albic and typical gray forest soils.

Species of *Bembidion obtusum, Calosoma inquisitor, Carabus granulatus, Carabus nemoralis, Harpalus affinis, Harpalus rufipes, Ophonus sabulicola,* have been identified in the brown forest soils of natural ecosystems (Table 2). In the natural gray forest soils species of *Bembidion obtusum, Calosoma auropunctatum, Carabus nemoralis, Harpalus affinis, Harpalus griseus, Harpalus progrediens, Harpalus rufipes* and *Panagaeus cruxmajor* have been found.

Species of *Calosoma inquisitor, Carabus coriaceus* and *Lebia cruxminor* have been reported in the chernozem under natural vegetation.

The life form analysis of carabids indicated four life form types according to the mode of nutrition. Groups of mixo-phytophagous and zoophagous predominate in the composition of the carabid beetles (Figure 3).

Their contribution to the total number of carabids is quite significant and constitutes 36.2% and 34.7%, respectively (Figure 3). Species of carabids, which belong to phytophagous, account for 15.5% of the total abundance of *Carabidae* family. The share of predators in the total number of carabid beetles in the natural soils was not so significant in terms of the specific contribution, their share constituted 13.6%.

Table 2. Biodiversity of the *Carabidae* family (ex. m^{-2}) at the species level in brown soils in natural and agricultural ecosystems

Carabidae family species	Typical brown forest soil	Luvic brown forest soil	Molic gray forest soil	Albic gray forest soil	Typical gray forest soil	Podzolic chernozem
	forest	forest	forest	forest	forest	virgin
Bembidion obtusum	2.7	0	0	2.7	0	0
Calosoma auropunctatum	0	0	5.3	0	0	0
Calosoma inquisitor	2.7	0	0	0	0	2.7
Carabus coriaceus	0	0	0	0	0	5.3
Carabus granulatus	0	2.7	0	0	0	0
Carabus nemoralis	0	5.2	2.7	0	0	0
Harpalus affinis	0	2.7	5.3	0	4.0	0
Harpalus griseus	0	0	0	2.6	0	0
Harpalus progrediens	0	0	0	8.0	0	0
Harpalus rufipes	0	2.7	5.3	0	4.0	0
Lebia cruxminor	0	0	0	0	0	2.7
Ophonus sabulicola	2.6	2.7	0	0	0	0
Panagaeus cruxmajor	0	0	0	0	2.6	0
Total	8.0	16.0	18.6	13.3	10.6	10.7

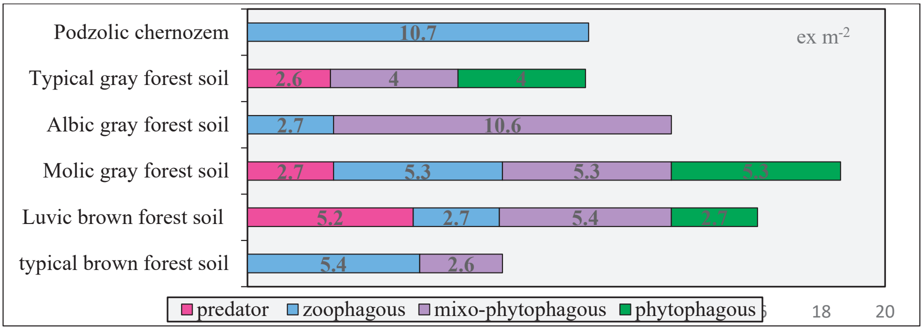

Figure 3. Composition of *Carabidae* family according to the mode of nutrition in soils in natural ecosystems

All four types of nutrition have been identified in carabid beetles living in the molic gray forest soil and luvic brown forest soil under forest.
Three groups of carabids (predators, mixo-phytophagous and zoophagous) were registered in the natural typical gray forest soil.
The typical brown forest soil and albic gray forest soil had two groups of representatives from the *Carabidae* family.

Only one group of zoophagous has been registered in the podzolic chernozem under virgin land.
According to zoogeographical analyses, carabids species belong to 7 zoogeographic elements with a predominance of Trans Palaearctic (19.8%), followed by Palearctic (17.4%), European elements (13.3%), which is 65.4% of the collected species (Figure 4).

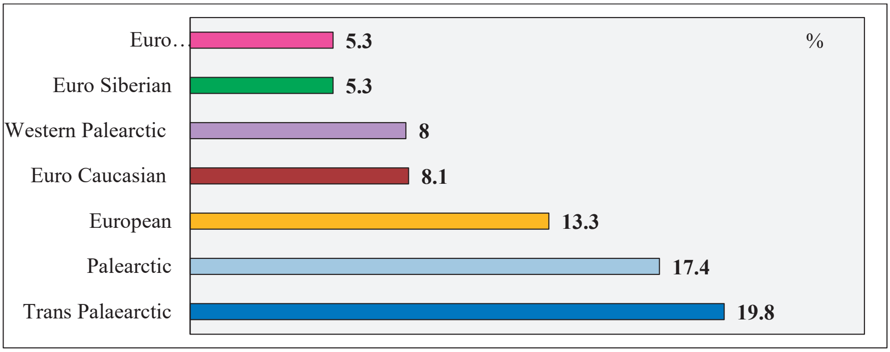

Figure 4. Geographical distribution of carabids selected from the studied soils

A smaller number of species are represented by Euro Caucasian (8.1%), Western Palearctic elements (8.0%), Euro-Siberian (5.3%) and Euro Mediterranean elements (5.3%).

CONCLUSIONS

Ground beetles (*Carabidae* family) are one of the most important components of the edaphic fauna of natural and anthropogenic ecosystems. The practical importance of carabids is due to their biodiversity and variety of mode of nutrition. Carabids, as a good indicator, may become one of the main objects in solving cadastral problems, conducting soil-ecological monitoring and developing soil quality standards.

Abundance of the edaphic fauna and *Carabidae* family in natural soils is much greater compared to soils of agricultural ecosystems. Their number is 8.0-18.6 ex. m^{-2}. The share of *Carabidae* family in the total abundance of invertebrates in forest soils of natural ecosystems constitutes of 2.1-9.6%, in chernozems - 3.0-10.2%. The base mass of *Carabidae* family in soils under natural vegetation is located in the litter and 0-10 cm layer.
13 species of carabid beetles from 7 genuses, 5 tribes and 1 subfamily *Carabinae* were found

and identified in the studied soils. Species of the *Carabus nemoralis, Carabus granulatus, Harpalus affinis, Harpalus rufipes, Calosoma inquisitor* and others have been detected in the faunal samples from soils in natural ecosystems. Groups of mixo-phytophagous and zoophagous predominate in the composition of the carabid beetles. Trans Palearctic, Palearctic and European species predominate in the range, accounting for 65.4% of the total number of carabids.

A characteristic consequence of the long-term use of soils for arable land is the destruction of the population of carabids beetles. The negative effect on the edaphic fauna and *Carabidae* family is observed as a result of long-term land management practices with the non-standard application of chemicals. The state of carabids fauna in the old-arable soil is characterized by the significant reduction in the number and diversity in comparison with the level of the 1960s and with soil's standards that are in conditions of natural ecosystems. Carabids were practically absent in arable soils at the time of sampling.

The problem of impoverishment and destruction of the *Carabidae* fauna is caused by the fragmentation and degradation of biocenoses, violation of the rules for the application of pesticides and environmental pollution.

ACKNOWLEDGEMENTS

This research work was carried out in the framework of the institutional project "Evaluation of the soil state of the Republic of Moldova in the agrocenosis conditions, improvement of the classifier and the soil rating system, elaboration of the methodological-informational framework for monitoring and enlarged fertility reproduction" (project code 20.80009.7007.17) in 2021.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

FORMATION OF SOIL STRUCTURE OF CHERNOZEMS IN DIFFERENT AGROECOSYSTEMS

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Abstract

In order to preserve the satisfactory functioning of agroecosystems of the Left Bank of Ukraine in the context of global warming, based on the results of soil structure formation studies, proposals can be made for the reproduction of fertility and protection of chernozem soils and rational land use. Soil organic matter and soil biota have a leading influence in soil structure formation. It is established that the best structured variants of agroecosystems with the addition of organic and organo-mineral fertilizers. Such agrochernozems have the highest content of organic soil and the highest biogenicity indicators. The biogenicity index is calculated by the percentage of the number of ecological and trophic groups of microorganisms.

Key words*: soil structure, chernozem, organic part of soil, biological activity of soil.*

INTRODUCTION

Under the action of the soil-forming process, physical, chemical, physico-chemical, and biological characteristics of soils are formed, which largely determine the level of fertility of specific soils. Among them, physical indicators stand out: structural condition, soil density, solid phase density, etc.

The use of typical chernozems in conditions of deficiency of organic substances and biophilic elements in comparison with virgin analogues, according to Nosco (2006), weakens the chernozem process of soil formation and reduces soil fertility.

Even in the early works of Dokuchaev, Sibirtsev, Kostychev noted that during the plowing of virgin chernozems, the amount of humus decreases, the granular structure loosens (destroys), that is, plowing soils causes their “ploughing”. Dokuchaev compared arable soils with bricks made from natural clay, and virgin (natural) chernozems were formed on clay.

Of particular importance in the life of plants is the structural state of the soil, which creates better conditions for seed germination, growth and development of plant roots, due to which the yield of crops grown on structural soils is usually high and guaranteed to be stable. Therefore, the study of the structural state of soils is constantly relevant.

In the works of Dokuchaev, Kostychev the main aspects of the role of the structure in the formation of the agronomic qualities of the soil were noted. The role of structure in soil fertility was studied in detail by Williams. Further research by Gedroits (1912; 1922), Sokolovskij (1941), Hrinchenko (1973), Medvedev (2017) and others laid the foundation for studying the physical properties of soils.

According to Nosco (2006), in particular, studied chernozems should differ from other soils by a high level of potential fertility, the most favorable structure and water regime for plants.

In the course of agricultural development and subsequent use of soils, there is a change in the water resistance of structural aggregates, the density and density of the solid phase, water permeability and general porosity, and the formation of a surface crust. This fact is confirmed by the research of Medvedev (2013; 2009; 2004), Tikhonenko (2014), Nosko (2006), Mucha (2004) and others.

It is known that the plowing of chernozems leads to a decrease in the amount of humus (increases mineralization), the supply of nutrients and exchangeable calcium. All this together determines the deterioration of the structural condition of chernozems and its physical indicators (Dehtiariov, 2011).

Microbiological activity (Geisseler, 2014) of chernozems under various phytocenoses is highest in the upper (0-40 cm) soil layers, where soil formation processes under the influence of vegetation, leaf litter, and soil organisms occur more intensively.

Medvedev (2015) points out that the structure and density of soils are the main parameters that determine their physical properties and modes, which strongly affects the yield of agricultural plants. Mucha et al. (2006) notes that one of the leading mechanisms of the negative effect of soil degradation is a change in the structural-aggregate composition and density of the soil structure during the growing season.

Factors that lead to a significant deterioration of the agrophysical condition of soils in agronomic terms include: excessively intensive cultivation, the compacting effect of agricultural machinery, uncontrolled irrigation, violations of crop rotation. However, it is heavy equipment that causes the greatest deformations of the soil (Medvedev, 2013).

The aim of the work: to investigate the change in the structural state of chernozems in different agroecosystems.

MATERIALS AND METHODS

Two objects were chosen to solve the tasks. The first of them is the territory of “Mykhailivska virgin land” of the Ukrainian Steppe Nature Reserve (USNR) - Sumy Region. The second is the educational-scientific-production center “Experimental field” of the State Biotechnological University - Kharkiv region.

The studied area is typical for all indicators (including soil) for the Left Bank of the Forest-Steppe of Ukraine, where typical medium and heavy loam chernozems were formed on loess-like loams.

Four cuts were made within the “Mykhailivska virgin land”.

Cut 1 (50.747344, 34.192909) was laid on a flat area of the local watershed within the absolute wilderness where a typical deep medium-humus (structural) medium-loam chernozem was formed on loess-like loam. Cereal and herbaceous vegetation grows here, covering 100% of the soil surface. Above-ground mass of herbs (phytomass) reaches 20 t/ha.

Cut 2 (50.748104, 34.176687) is laid within the mowed fallow. Grasses are mowed once every two years. Cut 2 is located in the flat area of the local watershed. Projective coverage of the soil surface - 100%. The association is forbaceous, thin-legged and reedy. Grasses dominate: forbs - 65%, cereals - 25%, legumes - 5%, sedges - 5%.

Cut 3 (50.750548, 34.173361) was laid next to cut 2 in the fodder crop rotation field, where zoned crops were grown for more than 120 years. The reserve of terrestrial phytomass is 9 t/ha, the saturation of crop rotation with row crops is 60%. During 1996-2019, an average of 8.7 kg of nitrogenous, 9.0 kg of phosphoric and 3.3 kg of potash mineral fertilizers and 1.7 t/ha of manure of the crop rotation area were applied to the soil per year. A typical deep, low-humus (weakly structured) medium-loam agrochernozem was formed here on loess-like loam.

Cut 4 (49.900221, 36.448754) is located on an area (0.52 ha) of fallow land that has been plowed for more than 100 years. Since 1946, the site has been overgrown with grass. Over 70 years of fallow, a grass cover was formed, which covers 100% of the soil surface. Association of grasses: forb-bluegrass. Stock of above-ground phytomass is 11-12 t/ha. Under the grasses of the fallow, a profile of typical deep, medium-humus (structural) heavy loamy chernozem on loess-like loam was formed.

The 5 cut (49.903918, 36.439602) is laid on the experimental field, where for more than 100 years zoned agricultural crops of field rotation have been grown: winter wheat, corn, barley, vetch-oats, sugar beet, sunflower, etc. Stock of above-ground phytomass in crop rotation fields is 8-9 t/ha.

The water resistance of the macrostructure was determined by the method of Savinov - dry and wet sieving (Petrenko et al., 2013).

RESULTS AND DISCUSSIONS

Structural state of the soil. After conducting analyzes of soil samples of agrochernozems and typical chernozems under different phytocenoses, results were obtained regarding the structural condition and water resistance of soils depending on humus content and biogenicity.

Depending on the type of soil-forming process, different types of structural aggregates are distinguished in soils: granular, prismatic, nut-like, blocky, etc. Chernozems are characterized by a granular structure, or lumpy-granular with a clear predominance of 1-3 mm aggregates (Table 1).
The granular structure determines the good density of the structure, soil porosity, air, heat and nutrient regimes. The best quantitative indicators are characteristic of virgin chernozems, and the worst - arable (agrogenic) soils. 70 years of fallow use improves the structural condition and almost reaches the level of completely virgin soils. The most agronomically valuable aggregates (from 0.25 to 10 mm) within the humus-accumulative horizon are quantitatively represented as follows: chernozem virgin soil - 82%, arable - 70-75%, fallow - 75-80%.

Table 1. The content of structural aggregates in typical chernozems, %

Cut number	Genetic horizon. Index, cm	Content of structural aggregates, mm, %								
		>10	10-7	7-5	5-3	3-2	2-1	1-0.5	0.5-0.25	<0.25
1	Ak 0-41	4.7	7.0	11.5	21.9	16.3	17.3	2.4	5.6	13.3
	B_1k 41-73	7.0	5.9	7.7	13.2	13.3	17.8	3.4	7.5	24.2
	B_2k 73-120	9.0	7.1	8.0	11.7	12.2	17.6	4.1	7.7	22.7
	BCk 120-160	2.7	4.3	6.1	11.1	12.8	18.4	3.5	9.1	32.1
2	A 0-35	5.5	7.0	9.1	16.1	13.5	16.6	2.5	7.5	22.2
	B_1k 35-52	1.8	4.8	6.6	13.4	13.0	20.0	3.1	9.2	28.2
	B_2k 52-85	5.9	7.5	10.6	16.8	14.8	17.6	3.3	7.1	16.6
	B_3k 85-121	4.0	6.2	7.2	13.3	13.2	18.8	3.1	8.9	25.4
	BCk 121-152	6.1	8.1	8.1	13.1	13.0	18.6	3.7	8.9	20.5
	Ck 152-189	6.5	5.9	7.5	11.7	12.4	18.4	3.7	11.1	22.9
3	A 0-23	8.1	7.7	8.1	12.3	10.5	18.0	3.1	10.9	21.4
	B_1k 23-42	3.1	6.1	7.9	15.5	16.5	21.8	3.0	9.1	17.1
	B_2k 42-64	2.6	4.5	7.9	17.7	17.7	21.8	3.5	8.1	16.2
	B_3k 64-85	4.1	6.6	7.5	12.7	14.7	21.1	2.8	9.5	21.0
	B_4k 85-111	4.3	4.6	6.8	12.0	13.8	19.6	3.4	10.4	25.2
	BCk 111-169	6.6	7.6	7.7	12.8	12.8	20.3	3.8	10.4	18.0
	Ck 169-230	5.9	8.7	8.9	14.1	12.3	18.9	4.5	12.5	14.3
4	A 0-45	20.3	19.6	17.6	19.6	10.7	8.4	1.4	1.6	0.8
	B_1k 45-72	10.7	10.7	12.8	22.7	17.9	15.6	2.2	4.3	3.1
	B_2k 72-94	9.4	9.5	9.6	19.1	16.1	19.1	3.9	7.8	5.5
	BCk 94-115	15.4	8.3	8.6	14.2	14.0	20.3	2.9	9.2	7.1
	Ck 115-153	14.3	7.7	8.7	14.6	12.6	19.6	3.5	10.4	8.6
5	A_1 0-20	12.7	8.1	7.2	9.7	10.1	24.6	6.2	11.7	9.7
	A_2 20-45	19.0	17.2	13.7	17.6	13.7	13.2	2.4	2.3	0.9
	Bk 45-73	10.7	13.4	16.9	22.8	16.5	13.0	2.0	3.0	1.7
	BCk 73-102	16.9	12.6	11.1	16.8	14.3	16.8	2.6	6.2	2.7
	Ck 102-130	17.0	12.0	10.3	17.5	13.5	16.1	2.9	6.4	4.3
LSD_{05}		2.1	1.3	1.4	1.9	1.6	2.1	0.8	1.2	2.4

In this case, let us prove that organic substances have an influence on the formation of structural aggregates. The degree of soil structure is expressed by the coefficient of structure (Table 2).
Its indicators in typical chernozems are as follows: virgin soils - 4.6, arable - 2.4-3.4, fallow - 2.6-3.8, which also distinguishes arable soils.
Water resistance of structural units. The content of water-resistant aggregates undergoes the most significant changes as a result of soil cultivation. In addition, water resistance is an important agronomic property of the soil structure, which is manifested by the ability of structural aggregates to resist the destructive action of water flow for a long time. The stability of soil aggregates, first of all, depends on the quality of humic substances, which are the main "glue" material. Therefore, in all variants, without exception, the percentage content of aggregates greater than 0.25 mm prevailed (Table 3).
Among the agronomically valuable (water-resistant) aggregates of 0.5-0.25 mm, aggregates with a size of 7 to 0.25 mm were evenly distributed along the profile on the fallow variant of the "Experimental Field".
A somewhat different situation is observed in the completely virgin and fallow chernozem of the "Mykhailivska virgin land", where aggregates of 2-1 mm were dominant, but also with insignificant fluctuations in the profile. On arable land variants, the content of the 0.5-0.25 mm fraction is the largest, and water-resistant aggregates are unevenly distributed.

Table 2. Indicators of the structural state of typical chernozems

Cut number	Genetic horizon. Index, cm	Coefficient of structure	Σ aggregates 0.25-10 mm	Assessment of structural condition
1	Ak 0-41	4.5	82	excellent
	B_1k 41-73	2.2	69	good
	B_2k 73-120	2.1	68	good
	BCk 120-160	1.9	65	good
2	A 0-35	2.6	72	good
	B_1k 35-52	2.3	70	good
	B_2k 52-85	3.5	78	good
	B_3k 85-121	2.4	71	good
	BCk 121-152	2.8	73	good
	Ck 152-189	2.4	72	good
3	A 0-23	2.4	70	good
	B_1k 23-42	4.0	80	good
	B_2k 42-64	4.4	81	excellent
	B_3k 64-85	3.0	75	good
	B_4k 85-111	2.4	71	good
	BCk 111-169	3.0	70	good
	Ck 169-230	4.0	80	good
4	A 0-45	23.8	79	good
	B_1k 45-72	12.5	86	excellent
	B_2k 72-94	10.3	85	excellent
	BCk 94-115	6.9	78	good
	Ck 115-153	8.6	77	good
5	A_1 0-20	12.6	78	good
	A_2 20-45	26.7	79	good
	Bk 45-73	11.5	88	excellent
	BCk 73-102	8.9	80	excellent
	Ck 102-130	12.8	79	good

Table 3. The content of water-resistant aggregates in typical chernozems, %

Cut number	Genetic horizon. Index, cm	Content of waterproof aggregates, mm, %						
		7-5	3-5	3-2	2-1	1-0.5	0.5-0.25	<0.25
1	Ak 0-41	11.2	14.8	14.0	15.4	8.8	8.5	27.3
	B_1k 41-73	11.1	8.0	7.9	14.4	6.8	9.3	42.5
	B_2k 73-120	8.5	5.9	6.8	10.9	7.4	10.2	50.2
	BCk 120-160	1.9	2.5	4.1	6.8	6.6	11.3	66.8
2	A 0-35	10.6	13.5	11.4	17.0	6.4	9.1	32.0
	B_1k 35-52	11.1	8.3	11.9	14.8	8.1	10.3	35.5
	B_2k 52-85	11.3	10.2	11.6	14.5	11.5	10.6	30.4
	B_3k 85-121	3.4	5.2	8.1	12.8	9.2	13.5	47.7
	BCk 121-152	3.2	3.2	4.7	10.0	7.2	14.3	57.4
	Ck 152-189	0.5	0.8	0.6	1.0	2.5	9.2	85.5
3	A 0-23	1.6	2.4	2.6	4.5	9.5	24.6	54.8
	B_1k 23-42	0.5	2.4	6.0	15.2	12.7	18.6	44.5
	B_2k 42-64	1.1	6.7	12.2	17.8	9.6	13.5	39.0
	B_3k 64-85	3.0	7.2	8.4	15.1	8.3	10.8	47.1
	B_4k 85-111	0.8	4.5	6.1	11.3	7.4	11.9	58.0
	BCk 111-169	4.8	2.1	3.2	6.6	6.1	11.5	65.6
	Ck 169-230	0.0	0.0	0.4	0.2	2.9	7.3	89.2
4	A 0-45	1.5	4.8	9.0	23.6	11.1	23.6	26.4
	B_1k 45-72	0.0	0.9	3.3	15.3	13.5	29.8	37.2
	B_2k 72-94	0.0	0.6	3.0	12.4	13.0	30.9	40.0
	BCk 94-115	0.4	0.7	1.8	6.6	8.6	24.3	57.6
	Ck 115-153	0.0	0.2	0.5	2.6	6.5	28.8	61.4
5	A_1 0-20	0.5	2.2	6.1	28.2	13.9	21.4	27.8
	A_2 20-45	0.0	0.3	1.3	8.5	14.6	39.5	35.7
	Bk 45-73	0.0	0.3	1.0	6.9	12.0	34.2	45.6
	BCk 73-102	0.0	0.4	1.6	7.8	11.8	32.4	45.9
	Ck 102-130	0.0	0.6	1.8	10.4	12.4	28.3	46.4
LSD_{05}		1.1	0.5	0.9	1.2	1.1	0.7	-

The coefficient of water resistance of virgin soils is about 0.90 in the upper humus-accumulative horizon and gradually decreases with the depth of the profile (Table 4).

The fallow variants are characterized by even a slight increase in the coefficient of water resistance - 0.93-0.94, and arable soils, on the contrary, have a decrease from 0.93 to 0.64. Evaluation of the studied soils by the sum of water-resistant aggregates greater than 0.25 mm shows a decrease in their number down the profile (Table 4).

The best water resistance is present in the variants with the introduction of a fallow regime (excellent rating), and also, naturally, in the variant with completely virgin soil due to the presence of a significant number of aggregates of agronomically valuable sizes of structural elements (the sum of water-resistant aggregates is more than 0.25 mm). Arable soils have a good assessment of water resistance.

Table 4. Indicators of water resistance of typical chernozems

Cut number	Genetic horizon. Index, cm	Coefficient of water resistance	Σ aggregates > 0.25	Assessment of water resistance
1	Ak 0-41	0.89	73	excellent
	B_1k 41-73	0.84	58	good
	B_2k 73-120	0.73	50	good
	BCk 120-160	0.51	33	satisfactory
2	A 0-35	0.94	68	excellent
	B_1k 35-52	0.92	65	excellent
	B_2k 52-85	0.90	70	excellent
	B_3k 85-121	0.74	52	good
	BCk 121-152	0.58	43	good
	Ck 152-189	0.21	15	unsatisfactory
3	A 0-23	0.64	45	good
	B_1k 23-42	0.69	56	good
	B_2k 42-64	0.75	61	excellent
	B_3k 64-85	0.71	53	good
	B_4k 85-111	0.60	42	good
	BCk 111-169	0.46	34	satisfactory
	Ck 169-230	0.14	11	unsatisfactory
4	A 0-45	0.93	74	excellent
	B_1k 45-72	0.73	63	excellent
	B_2k 72-94	0.70	60	good
	BCk 94-115	0.55	42	good
	Ck 115-153	0.50	39	satisfactory
5	A_1 0-20	0.93	72	excellent
	A_2 20-45	0.80	64	excellent
	Bk 45-73	0.62	54	good
	BCk 73-102	0.67	54	good
	Ck 102-130	0.68	54	good

The highest percentage of humus is characteristic of virgin chernozems - up to 10.1% (humus reserve - 190 t/ha), fallows and chernozems under the forest belt - up to 7.5-8.5% (172-181 t/ha), and arable soils have a maximum of up to 5-6% (138-139 t/ha).

Arable (agrogenic) chernozems have the highest biogenicity - in the upper genetic horizon - 6.88, and 7.07 million colony-forming embryos in 1 g of completely dry soil on the “Experimental field” and “Mykhailivska virgin land” arable land variants. Post-agrogenic use leads to a significant decrease in biogenicity under all the studied variants. At the same time, the values of the variants of fallow and absolute virgin land fluctuate insignificantly within the range of 5.63-5.91 million colony-forming embryos in 1 g of completely dry soil. The lowest biogenicity is observed in chernozems of post-agrogenic use.

As for the correlation between the structure factor and the humus content, we have a weak direct relationship - 0.23. Speaking of the correlation between the water resistance coefficient and the content of humic substances, here we have a strong direct relationship - 0.81. Therefore, it is the humic substances that act as a binding material under the mechanical action of water on the soil and prevent structural aggregates from collapsing.

CONCLUSIONS

Humus content and biogenicity play an important role in forming the structure of soils in general and chernozems typical under various phytocenoses in particular. Thus, the peptizing effect is caused by the presence of ammonium ions, and calcium, on the contrary, acts as a coagulant. The humus-accumulative process of soil formation determines the dominant role of exchangeable calcium, which, together with other factors, contributes to the good structure of soils. In arable soils (agrochernozems), the amount of exchangeable calcium decreases, at the same time, cultivation leads to deterioration of the structural condition of the soils.

Thus, fallow and virgin chernozems typically have an excellent structural condition and have the highest water resistance. Arable soils (agrochernozems) are characterized by structural deterioration.

According to the indicators of the structural state, the studied soils can be divided into three groups: the first - virgin soils, with the best indicators of structure and water resistance; the second - fallow, with values close to virgin; the third - arable soils (agrochernozems), with the smallest and significantly different structure values from the previous variants.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON THE INFLUENCE OF THE CONSERVATIVE TILLAGE SYSTEM ON MAIZE CULTURE, AN AGROTECHNICAL AND ECONOMIC ALTERNATIVE FOR SUSTAINABLE AGRICULTURE, UNDER THE CONDITIONS OF A.R.D.S. PITESTI - ALBOTA

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Abstract

The research was carried out in the experimental field in the ARDS Pitesti area on the typical luvosol soil. The paper contains results obtained in 2022 regarding the effect of pedo-ameliorative and basic soil works - classic and unconventional system direct sowing - on agricultural maize crops. The yields were influenced by the factors studied (scarified, nonscarified; the working depth of the basic soil works), but also by the climatic conditions recorded during the research period. The average grain maize yield in 2022 was 7707 kg/ha in the conventional system with the scarified soil variant and 6681 kg/ha in the conventional system with the nonscarified soil variant with a difference of 1026 kg/ha in the advantage of the scarified soil variants. From an economic point of view, the most efficient soil tillage system, in the ARDS Pitesti-Albota area, for maize culture is the conventional deep plowing scarified system that ensures superior profitability compared to the conservative system sown directly because of the achieved yield.

Key words: *direct sowing, economic efficiency, maize yield, tillage system.*

INTRODUCTION

Currently, the conservative (unconventional) works of the soil define extremely varied processes, from direct sowing (no-tillage, direct drill) in unprocessed soil to deep loosening without turning the furrow. Between these two extremes there are variants such as: reduced works (classically rationalized), minimum works (with coverage below 30%, minimum tillage), minimum works with vegetal mulch (with coverage over 30%, tillage mulch), sowing on balls (ridge tillage), partial works or in strips (strip till, till areas), works with protective layer (cover crops, catch crops) etc. This terminology highlights the specific character that defines that process applied at a certain time, in a certain area, in accordance with the local specificity (Griffith et al., 1992; Horn and Arvidsson, 2000; Moroizumi and Horino, 2002; Guş et al., 2003). Maize is an important agricultural crop, both in Romania and globally, which is evident from the extensive areas on which it is cultivated and the place it occupies in the structure of agricultural crops. Soil work has been a fundamental part of agriculture since the beginning and served several important purposes: preparing the germination bed, reducing soil compaction in order to increase aeration and better development of the plant root system, reducing the degree of weeding, incorporating fertilizers and amendments, managing plant debris (Niţă, 2007; Niţă et al., 2018, Niţă et al., 2019).

These conservative technologies have significantly contributed to strengthening and improvement of soil fertility and productivity and consequently other environmental resources (Guș, 1997).

For reducing fuel consumption and to avoid increased soil subsidence through repeated machines passing on the ground that lead to the worsening of the physical properties of soil, it acts in the direction of the number of soil works or to perform, in a single pass, a multiple operations through minimum system works (Șimon et al., 2013).

Changing yield technologies, through the use of performance machines, as we all know, require extensive research to understand their long-term effects on the physical, chemical and biological properties of soils (Mihalache, 2012; 2015; Marin, 2015).
In the concept of sustainable agriculture development, it is unanimously accepted that there is no valid universal tillage system due to local differences, especially climate and soil, but also due to the technical level of equipment. Soil conservation systems in different areas must have specific characteristics in relation to the ecological particularities of the place and the technological characteristics of the cultivated plants, so that differentiation becomes mandatory (Canarache, 1999; Guş et al., 2004).
The maize, being a species with a high diversity of varieties, with many genetic, morphological and physiological differences, is considered a plant with high ecological plasticity, respectively easily adaptable to environmental conditions (Carena, 2010; Tritean, 2015).
According to the research carried out, it shows that maize is picky about tillage, soil type, climatic conditions, yield results confirming this (Chetan et al., 2014).
The research carried out confirms that the work which include plowing have a major impact on soil structure and fertility (El Titi, 2003), bare soil is exposed to the precipitation and the wind that accentuates erosion processes, productivity reduction and quality (Lal et al., 2007).
The main characteristic of minimum tillage systems is soil conservation, maintaining a proper soil organic matter ratio and amelioration the activity of microorganisms (Ulich et al., 2006; Sarauskis et al., 2009).

MATERIALS AND METHODS

The paper analyzes and presents the results of the research carried out in the year 2022, on some systems and variants of soil works for the maize yield, under the conditions of the ARDS Pitesti-Albota and their correlation with the obtained yields.
The final purpose of these experiences is the establishment of the best tillage system, in the pedoclimatic conditions of ARDS Pitesti-Albota, following the yield results obtained.
The experience had a stationary character and was located in the experimental field of ARDS Pitesti, located at an altitude of 287 m, latitude of 44°51'30", 24°52'30″ in the year 2022, in a three-year crop rotation: 1. weeding plants (maize); 2. annual leguminous plants (peas); 3. grains (wheat).
The soil on which the research was carried out is a typical luvosol, with a clay texture (clay content 46%), poorly supplied with nitrogen and phosphorus (Nt = 0.130 %mg, PAL 33 mg/kg), moderately supplied with potassium (KAL = 89 mg./kg) with a humus content in the arable horizon of 2.26% and pH = 5.3.
The experimental scheme used (Table 1) was that of subdivided plots laid out according to the method of completely randomized blocks in four repetitions.

Table 1.The experimental scheme

SPECIFICATION	Soil tillage variant (B)
A1 SCARIFIED SOIL	b1- deep plowing 28-30 cm b2- normal plowing 23-25 cm b3-disc b4- direct sowing
A2 NONSARIFIED SOIL	b1-deep plowing 28-30 cm; b2- normal plowing 23-25 cm b3-disc b4- direct sowing

The main plots were assigned both in scarified soil and in nonscarified land, and the subplots, for tillage systems, contain four plots each with the gradations: (deep plowing, normal plowing, disc and direct sowing).
The surface of an individual plot was 560 m^2 (5.6 x 100 m).
The influence of tillage systems was determined under loosening and no-tilling conditions, work carried out only at the establishment of the experiment, in the fall of 2021 with the scarifier MAS 5 at a depth of 40-45 cm, the maize crop benefiting from the effects of loosening in the first year of culture.
The vegetable remains of the preceding crop (straw) were chopped and spread evenly on the land, simultaneously with its harvesting. In the spring, two weeks before sowing, a total weeding with glyphosate active substance (1 kg/ha) was applied to the uncultivated plots to control weeds.

Sowing in the conventional system was carried out on the date 15.04.2022, with the SPC8 sowing machine, at a sowing depth of 4-5 cm, using the Olt maize hybrid with good adaptability to the climatic conditions in the area, with a norm of seed of 20 kg/ha, corresponding to a sowing density of 58.000 germinating seeds/ha, and in the non-conventional no-till system sown directly in the stubble, the sowing was also carried out on the date 15.04.2022, with the Mzuri Pro-Til 3T Select sowing machine of at ETU-Farm, observing the same strict technological conditions as in the conventional system.

Before sowing, $N_{16}P_{16}K_{16}$ complex fertilizers were applied, 60 kg s.a./ha.

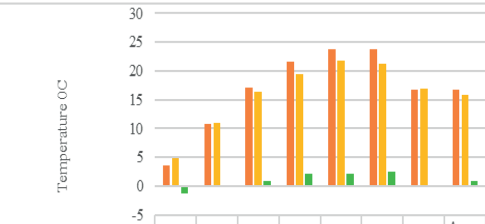
Immediately after sowing, the entire surface was herbicided with Dual Gold, 1.3 l/ha and Gardoprim, 4l/ha. The crop harvesting was carried out on the date 16.09.2022.

The experimental data were processed through variance analysis and the establishment of limit differences (Anova test).

From the climate point of view, ARDS Pitesti is located in an area with a temperate continental climate, with a multiannual average temperature of the last 50 years of 10.7°C.

Temperatures and rainfall registered during the agricultural year (March-September) were monitored for maize, in order to follow the influence of environmental factors on the evolution of plants from the first phases of vegetation to harvesting.

Climatic data were registered at the meteorological station of ARDS Pitesti Albota, located about 750 m from the experimental field. The climatic conditions of the research years 2022 are presented in Figure 1.

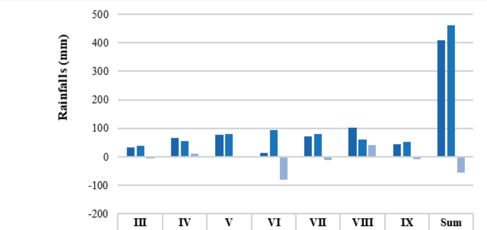


	III	IV	V	VI	VII	VIII	IX	Average
Monthly average	3.61	10.77	17.1	21.6	23.77	23.73	16.69	16.75
Multiannual average	4.8	10.9	16.3	19.5	21.7	21.3	16.9	15.91
Deviation	-1.19	-0.13	0.8	2.1	2.07	2.43	-0.21	0.84

Figure 1. The monthly average temperature registered in the period March-September 2022

The climate data of the 7 months characterizes the year 2022 as hot and dry, an atypical year, with low temperatures in March and April (with negative deviation from the multiannual average of: -1.19°C in March and April and with positive deviation in the months May (0.8°C), June (2.1°C), July (2.07°C) and August (2.43°C), with large day-night temperature alternations, but also from day to day and with quantitatively lower precipitation than multiannual average (Figure 1).

In the 2022 agricultural year, the average annual temperature was 16.75°C, exceeding the multiannual average temperature of 15.91°C by 0.84°C (Figure 1).



	III	IV	V	VI	VII	VIII	IX	Sum
Monthly sum	32.4	65.7	77.6	14.3	71	101.1	45.6	407.7
Multiannual sum	38	55.9	80.5	93.6	81.1	59.7	52.9	461.7
Deviation	-5.6	9.8	-2.9	-79.3	-10.1	41.4	-7.3	-54

Figure 2. The monthly sum rainfall registered in the period March-September 2022

The multiannual sum rainfalls are 683.1 mm. It should be emphasized that their distribution is totally uneven, both from one year to another and within a year.

The rainfalls sum registered in the period March - September 2022 was 407.7, with a deficit of -54 mm, than the multiannual sum of 461.7 mm (Figure 2).

RESULTS AND DISCUSSIONS

The increase in the average annual temperature, as well as the uneven distribution of precipitation, inevitably leads to the approach of unconventional tillage options that facilitate the accumulation and preservation of water in the soil (Popa et al., 2019; Chețan, 2020; Chețan et al., 2021).

The basic soil works carried out in the two systems: classic system with plowing and conservative direct seeding system experienced in the Agrotechnics-Soil Works laboratory at the ARDS Pitesti-Albota, influenced the yields.

The yields were influenced by the factors studied (scarified, not scarified; the working depth of the basic soil works), but also by the climatic conditions recorded during the research period.

The influence of the tillage system on the average height of the maize crop

In the version sown directly in the stubble due to the presence of plant residues on the surface, a lower temperature, a higher relative humidity, a lower development of the root system is recorded in the soil, which causes the phenomenon of slow plant growth, in the first part of the vegetation period. As the plants advance in vegetation, the gap in their growth and development gradually diminishes, so that until the flowering and maturity of the plants, the differences between the tillage variants are reduced (Figure 3).

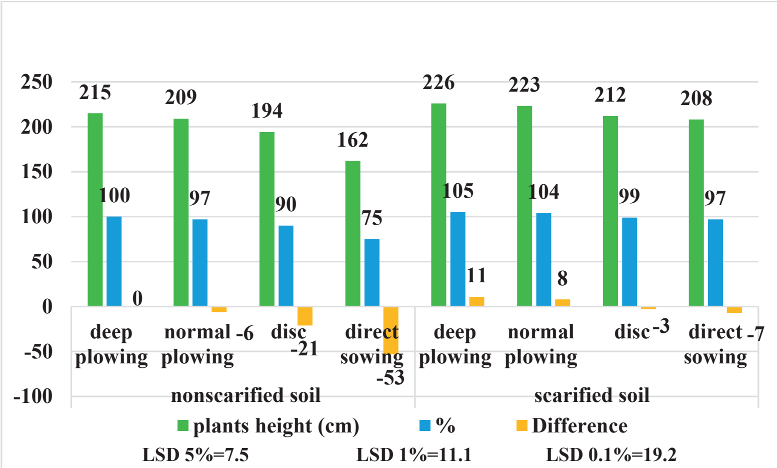


Figure 3. The influence of the tillage system on the average height of the maize crop

The influence of the pedoameliorative and basic soil work system on the maize crop

In Figure 4 shows results regarding the effect of applying the pedoameliorative and basic soil work system to the maize crop.

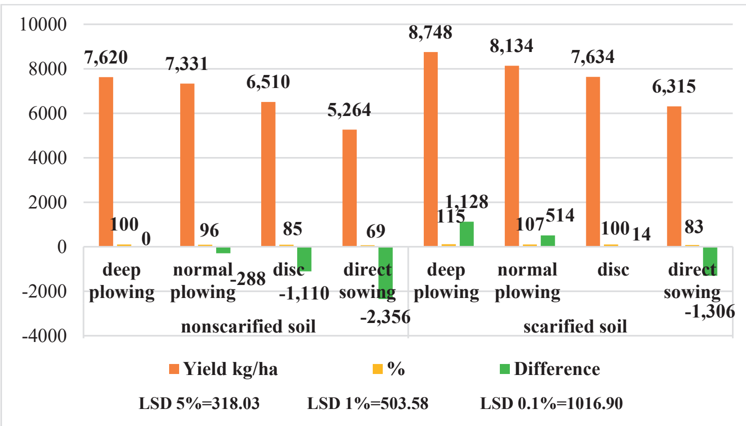


Figure 4. Grain maize yield (kg/ha)

The pedoameliorative work (scarification) of the soil carried out in 2021 brought increases in yield in both tillage systems in the experimental field.

The average grain yield in 2022 was 7,707 kg/ha in the scarified soil variant and 6,681 kg/ha in the nonscarified soil variant with a difference of 1,026 kg/ha in favor of the scarified soil variants.

The average grain maize yield in 2022 with the highest value was recorded in the conventional system, the scarified soil variant, deep plowed with a value of 8,748 kg/ha, followed by the normal plowed scarified soil variant with 8,134 kg/ha, discussed with 7,634 kg/ha and directly sown with 6,315 kg/ha and as yield with the lowest value was registered in the conservative system nonscarified soil directly sown with only 5,264 kg/ha.

From the results obtained, it is found that the value of grain maize yield, in the conventional tillage system, is higher than in the conservative direct sowing system. Comparing the two tillage systems, a difference of 2,356 kg/ha is observed, in the variants with nonscarified soil and a difference of 1,306 kg/ha in the variants with scarified soil.

The influence of the pedoameliorative and basic soil work system on total biomass yield

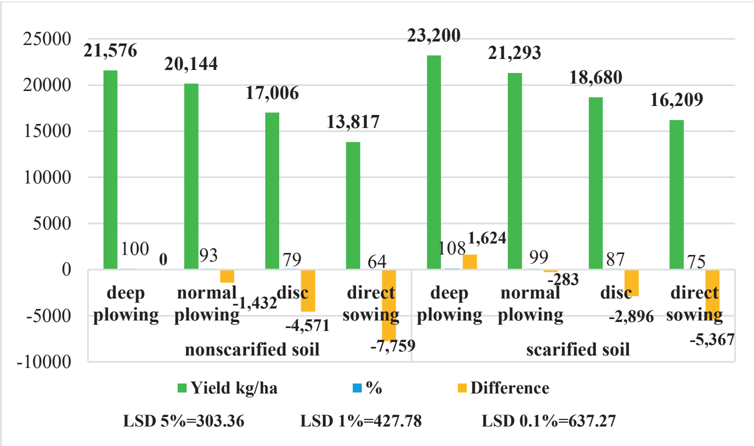


Figure 5. Maize Biomass yield (kg/ha)

Following the influence of the pedoameliorative and basic soil works system on the total biomass yield in the nonscarified soil variant, it can be observed, from the data presented in Figure 5, that in the period of 2022, the highest yield in the experimental area was recorded in the deep plowed variant (28-30 cm), with a value of 21,576 kg/ha, followed by the normal plow variant with a yield of 20,144 kg/ha, disc with a yield of 17,006 kg/ha, respectively direct sowing 13,817 kg/ha.

In the scarified soil variant, the highest biomass yield value of the Olt hybrid maize was also recorded in the deep plowed variant, 23,200 kg/ha, followed by the normal plowed variant, 21,293 kg/ha, disk 18,680 kg/ha and direct sowing 16,209 kg/ha.

The conservative system registers very significantly negative differences compared to the conventional system with values of 7,759 kg/ha, in the nonscarified soil variant and of 5,367 kg/ha in the scarified soil variant.

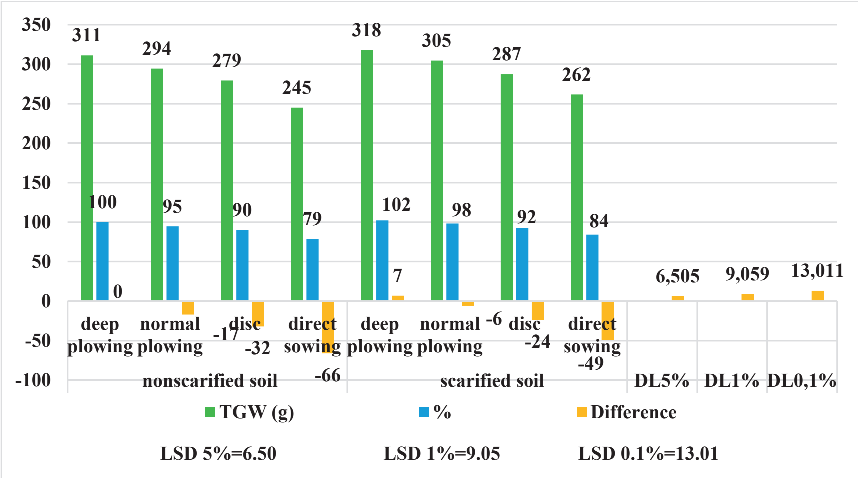


Figure 6. The influence of the system of pedoameliorative and basic soil works on the absolute mass of TGW

Influenced by the tillage system, the average thousand grain weight of the maize Olt hybrid varies with statistically assured differences in the conventional system versus the conservative system. Thus, compared to the conventional system, in the direct sown conservative system, the thousand grain weight (TGW) was -66 g lower in the direct sown nonscarified soil variant and -49 g lower in the scarified soil variant, both differences being very significantly negative from a statistical point of view (Figure 6).

Economic efficiency of maize crop, comparison between soil tillage (lei/ha)

The main characteristic of conservative systems refers to the reduction of fuel consumption by reducing or eliminating the number of technological works to reduce costs in order to achieve agricultural yield. In addition to the fuel economy achieved following the application of conservative tillage systems, the degree of soil degradation and environmental pollution with CO_2 is reduced. In order to introduce some tillage variants in a technology of cultivated plants, it is necessary to know the level of suitability of the soil for different processing methods.

The main agrotechnical factors (soil work, fertilization, plant protection and harvesting) are the biggest energy consumers, soil work and crop fertilization being technological stages at which, by introducing conservative systems, the necessary costs to apply an optimal technology to agricultural crops can be reduced. In the conservative system, on nonscarified soil, the fuel consumption was 38.6 l/ha, while in the classic system the fuel consumption was 66.4 l/ha. Comparing the two tillage systems shows a higher fuel consumption in the classic system by 27.8 l/ha.

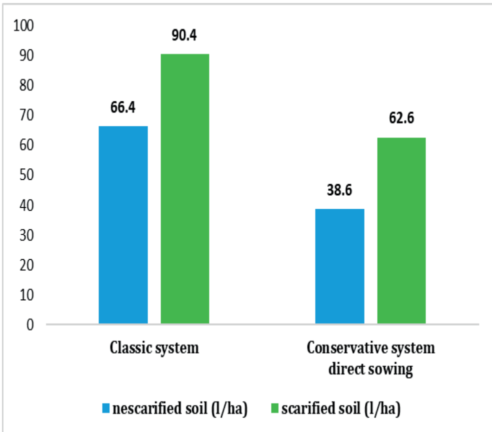


Figure 7. Total diesel consumption (l/ha)

On the scarified soil, in the conservative tillage system, the fuel consumption was 62.6 l/ha, while in the classic system the fuel consumption was 90.4 l/ha (Figure 7).

The fuel consumption was influenced by the tillage method. Scarification, soil improvement work and ploughing, as a basic work, consume the largest amount of fuel in the total of a culture system (24 l/ha and 22 l/ha respectively).

The average grain maize yield in 2022 was 8748 kg/ha in the conventional system with the scarified soil variant and 7260 kg/ha in the conventional system with the nonscarified soil variant with a difference of 1488 kg/ha in favor of the scarified soil variants (Figures 7, 8). In the conservative system, the average grain maize yield in 2022 was 6315 kg/ha in the scarified soil variant and 5264 kg/ha in the nonscarified soil variant, with a difference of 1051 kg/ha in favor of the scarified soil variants.

From the analysis of the presented data, it follows that the scarification work brings a significant increase in yield to both soil work systems, achieving a profit of 1858 lei/ha in the conventional system and 1159 lei/ha in the conservative system.

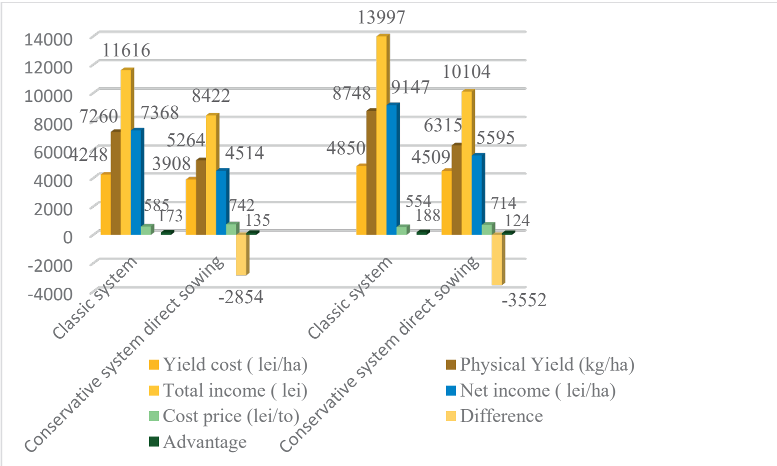


Figure 8. Economic efficiency of maize crop, comparison between soil tillage systems - nonscarified soil and scarified soil

Although the scarification work has a high cost price (523 lei/ha), I recommend that it be executed, because it has a high economic efficiency and a number of advantages by changing the physico-chemical and biological conditions in the soil, as well as a regime favorable aerohydric, which positively influences plant development and current yields. Referring to the total expenses for the achievement of a work system, we observe from table 2 that in the classic system the cost is 4248 lei/ha, and when is applied the conservative system (direct sowing) the cost is reduced by 8.1%, 3908 lei/ha, in the nonscarified soil.

The cost of the necessary materials (seed, pesticides, etc.) is the same in the two tillage systems and represents the largest part of the total technology, reaching up to 2085 lei/ha.

The highest profit value was recorded in the conventional system, the scarified soil variant (9147 lei) with a higher yield compared to the value recorded in the conservative system.

In the results presented in Table 2, the conventional system with nonscarified soil generates a yield of 1.73 lei per 1 leu of operating expenses, while the direct seeded system generates 1.15 lei of profit per 1 leu of operating expenses.

In the conventional system, in the scarified soil variant, the yield is 1.88 lei per 1 leu of operating expenses compared to the conservative, direct-sown system where the yield was 1.24 lei profit per 1 leu of operating expenses.

From an economic point of view, the most profitable type of tillage was in the classic deep plowed scarified system (28-30 cm), because of average operating costs of 4850 lei/ha, and through a good yield of 8748 kg/ha, this generating an average profit of 9147 lei/ha and a yield of 1,88 lei profit per 1 leu spent on

establishing, maintaining and harvesting the crop (Table 2).
At the opposite pole, the lowest average profit was recorded for the work in the direct sown conservative system, with a value of 4514 lei/ha and a yield of 1.15 lei, recording exploitation costs lower than 3908 lei/ha, but also a yield lower than 5425 kg/ha.

Table 2. Economic efficiency of maize crop, comparison between soil tillage systems

Soil tillage systems		Yield Cost lei/ha	Earned income lei/ha	Advantage/ profit lei/ha	Randament* lei
Conventional system	nonscarified	4248	11616	7368	1.73
	scarified	4850	13997	9147	1.88
Conservativ system direct sowing	nonscarified	3908	8422	4514	1.15
	scarified	4509	10099	5590	1.24

*The yield per surface unit (hectare), representing the amount of net profit obtained per 1 leu of allocated operating expenses

CONCLUSIONS

The yields were influenced by the factors studied (scarified, nonscarified; the working depth of the basic soil works), but also by the climatic conditions recorded during the research time.
The average grain yield of the Olt maize hybrid obtained in 2022, under the influence of the soil tillage system in the ARDS Pitesti-Albota area, was very significantly reduced under the conditions of applying the minimum soil tillage system directly sown both in the variant scarified soil, as well as in the variant with nonscarified soil.
The average grain maize yield in the direct sown conservative system was 5264 kg/ha in the nonscarified version, -3484 kg/ha lower than the yield value recorded in the conventional system, in the nonscarified soil version. In the conservative system directly sown scarified soil variant, the average yield of the Olt hybrid was 6314 kg/ha, -2434 kg/ha lower than in the conventional deeply plowed scarified system where the value is 8748 kg/ha.
Through the influence of the type of tillage on the yield of grains obtained in 2022, the highest yields are recorded for deep plowing (28-30 cm), for all the researched variants, with values between 7620 kg/ha (nonscarified version) and 8748 kg/ha (scarified version). The increases in yield obtained with the deep plowed variant compared to the other variants were statistically ensured, with values between: -615 kg/ha (normal plowed variant); -1114 kg/ha (discussed version); and -2434 kg/ha (direct sowing variant).
The lowest yields, with very significantly negative differences compared to the deep plowed variant, were recorded for the direct seeding operation, both in scarified and nonscarified soil conditions.
The influence of the tillage system on the thousand grain weight (TGW) obtained in 2022 is manifested by its significant reduction under the conditions of the application of the direct sown conservative system both in the scarified soil and in the nonscarified soil variant.
The average TGW of maize for which specific works were applied to the direct sown conservative system was 245 g in the nonscarified soil variant, -66 g lower than the value recorded in the conventional system, in the nonscarified soil variant. In the scarified soil variant, the thousand grain weight in the direct sown conservative system was 262 g, -56 g less than in the conventional system.
The influence of the type of tillage on the thousand grain weight is distinguished by recording the highest values of the indicator for deep plowing, both in the nonscarified soil and in the one with scarified soil. For this type of work, the TGW values were between 311 g, the variant with nonscarified soil and 318 g, the variant with scarified soil.
The classic system with the deep plowing variant recorded the highest TGW values between 311g of nonscarified soil and 318 g of scarified soil.
The lowest values of TGW were obtained in the direct sown conservative system of 245 g in the nonscarified soil variant and 262 g in the scarified soil variant.
The scarification work brings a significant increase in yield to both soil work systems, making a profit of 1858 lei/ha in the conventional system and 1159 lei/ha in the conservative system.
The most profitable tillage system, in the area of ARDS Pitesti-Albota, is the conventional deep plowing scarified type with an average profit of 9147 lei/ha and a yield of 1.88 lei profit per 1 leu spent on establishing, maintaining and harvesting the crop. At the opposite pole, the lowest average profit was

recorded in the conservative direct sown system with a value of 4514 lei/ha and a yield of 1.15 lei.

The highest profit was obtained by the conventional system in the deeply plowed scarified soil work variant, with a value of 9147 lei/ha and a yield of 1.88 lei.

The lowest profit value, of 4514 lei/ha, was obtained by the conservative direct sowing system, with a yield of 1.15 lei.

From an economic point of view, the most efficient soil tillage system, in the ARDS Pitesti Albota area, for maize culture is the conventional deep plowing scarified system that ensures superior profitability, the conservative system sown directly because of the achieved yield.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

DEVELOPMENT OF A BIOFERTILIZATION TECHNOLOGY BASED ON MYCHORIZAE

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Abstract

The importance of mycorrhiza for the plant is not limited to the absorption of water and nutrients from the soil. Symbiotic plants are often more competitive and more tolerant to environmental stress than those without mycorrhizae. Here we can give an example of osmotic stress, mycorrhizae make plants more resistant to cold weather (below 15 degrees). Arbuscular mycorrhizal fungi colonize the roots of most monocotyledons and dicotyledons despite their different root architecture and cell patterning. Key Result Large lateral roots are preferentially colonized, and fine lateral roots are immune to arbuscular mycorrhizal colonization. Fungal preference for large lateral roots also occurred in sym mutants that block colonization of the root beyond rhizodermal penetration.

Key words*: biotechnology, mycorizae, wheat, roots.*

INTRODUCTION

Farmers and agricultural experts are under pressure to increase crop yield due to the rising demand for food and the shrinking amount of arable land. To increase agricultural output, this has resulted in an overuse of chemical pesticides and fertilizers. The symbiotic relationship between rooted plants and arbuscular mycorrhiza (AM) is one of the earliest known symbiotic relationships in nature. About 80% of the species of terrestrial plants are colonized by a fungus called arbuscular mycorrhiza. In exchange for carbon sources, these plants take nutrients from the earth and transfer them to hosts plants. Indeed, AM fungi play a significant role in sustainable agriculture by protecting plants from biotic and abiotic stressors. Due to the negative effects of chemical-based fertilizers, the AM fungi are crucial for biofertilizers because of these characteristics.

Arbuscular mycorrhizal fungi (AMF or AM fungi) are an important link between plants and the mineral nutrients in the soil. As a result, interest in them as natural fertilizers is growing. According to Schüßler et al. (2001), AMF are obligatory symbionts that are members of the phylum Glomeromycota and develop mutualistic symbioses with roughly 80% of land plant species, including a number of commercial crops. They exchange water and mineral nutrients for photosynthetic byproducts from the host plant (Smith and Read, 2008). When the AMF mycelium emerges from the root system, it can access portions of the soil where roots cannot get to get nutrition (Smith et al., 2000). Additionally, fungal hyphae are significantly thinner than roots and can therefore pierce smaller pores because of this (Allen, 2011). Afterward, carbohydrates and mineral nutrients

Mycorrhizae are fungi that form mutualistic symbioses with plant roots, providing them with nutrients and food necessary for growth and development. Mycorrhiza-based products are used in intensive agriculture to improve soil quality and increase plant productivity.

Arbuscular Mycorrhizal Fungi (AMF) are obligate root biotrophs that trade mutually beneficial effects with roughly 80% of plants. Since they give the host water, nutrients, and protection from pathogens in return for photosynthetic products, they are regarded as natural biofertilizers. AMF are hence essential biotic soil components that, when lacking or depleted, might result in an ecosystem working less effectively. In order to promote sustainable agriculture, the process of restoring the natural level of AMF richness can be a viable substitute for traditional fertilization techniques. Direct reinoculation of AMF propagules (inoculum) into a target soil is the major tactic that can be used to accomplish this purpose. Initially, AMF were proposed as agriculturally acceptable since they were widely considered as lacking host- and niche-specificity.

The fungi of the genus *Glomus*, including *Glomus intradiceps*, form mutualistic symbioses with plant roots by means of mycorrhizal hyphae, which penetrate the roots and extend through them. In this process, fungi use enzymes to fix nitrogen from the air in the form of ammonia (NH3) and nitrites (NO2-), which are then converted into organic compounds, such as nitrates (NO3-), used by plants as sources of nitrogen. In this way, *Glomus intradiceps* mushrooms provide plants with the nitrogen necessary for growth and development, in exchange for carbohydrates and other nutrients provided by plants. This symbiosis is beneficial for both plants and fungi, which are protected by the growth and development of plant roots and by access to sources of energy and nutrients. It is important to mention that the process of nitrogen fixation is dependent on many factors, including soil pH, the availability of other essential nutrients, such as phosphorus and potassium, and climatic conditions. Therefore, it is important to monitor these factors and to control them adequately to ensure an efficient symbiosis between *Glomus intradiceps* and the host plants.

MATERIALS AND METHODS

The soil taken into analysis from the geographical area of Muntenia, Romanian Plain, more precisely Berceni commune, Ilfov county is located within the physical body 150 noted as soil profile P1. The soil profile P1 framed as argic cherniosome presents a profile of the type Amp-Am-AB-Bt-Ck, being formed by loessoid deposits. The groundwater is located at a depth of over 5 m. The soil was formed by an illuviation process of the clay from the upper horizon.

Experimental work was carried out both on the field of the experimental field and in the collaborating laboratories of Agricola Berceni SRL. It was used to multiply the *Glomus intraradices* fungus, on a nutrient broth type solid culture medium. After Multiplication, they were stabilized in suitable solutions, later arriving on the sample land cultivated with the wheat host plant. In the experimental field, two experimental wheat plots were established. One plot was cultivated as a control, while the second plot was cultivated and treated with mycorrhizae. For the establishment of the wheat culture, the technology according to this culture was executed, without producing a rebate from any operation.

The abiotic factors that have an impact on mycorrhiza were studied. The light. The energy source of the symbiont fungus is in the plant and depends directly on the way it carries out its photosynthesis process and on its ability to translocate the products of photosynthesis to the root (Varma, 2008). The lack of the light source produces a restriction for the development of the fungus, so its evolutionary process is slowed down, sporulation no longer occurs, and the expansion of the mycelium in the soil and in the root is reduced.

Temperature. From the point of view of the processes of spore germination, root penetration by hyphae and their proliferation inside the cortical cells, temperature can be a factor with a limiting effect (Gavito et al., 2005) soil pH. The efficiency of the fungus-plant association is determined by the adaptability of the fungal partner to a certain soil pH level. The pH affects both spore germination and their development. The relationship between soil pH and the effects of mycorrhizae depends on the host species, the type of soil, the forms of phosphorus and the species of fungi involved Salinity. In the case of high salinity, a decrease in the production of propagation structures (propagules) and in the colonization of vesicular-arbuscular fungi was observed (Pfeiffer and Bloss, 1988).

A well-developed root system means a good capacity for the absorption of nutrients from the soil followed by the sustained development of the aerial part of the plant, the increase of the vegetative mass and the final increase of the quality of the harvested vegetables. The addition of phytohormonal solutions can add to the growth of the plant.

In most types of mycorrhizae, the movement of carbohydrates, produced during photosynthesis, is done from the host plant (autotrophic partner) to the symbiotic fungus (heterotrophic partner). In the case of absorption of nutrients from the soil, the transfer has an inverse direction, from the fungus to the host plant (Jakobsen, 1999). The contribution of vesicular-arbuscular fungi to the assimilation of nutrients is the absorption of nutrients (especially phosphorus) from the soil, with the help of extraradicular hyphae - especially from those parts of the soil to which the plant did not have access. The hyphae of the fungus act similarly to the absorbent hairs on the root of the plant; After comparing the diameter of the absorbent hairs (5-20 μm) with that of the mushroom hyphae (3-7 μm), the absorbent hairs would gain the cause, but comparing the length and density of the mushroom hyphae with that of the absorbent hairs - the fungus would be, because it exceeds the possibilities of expansion of the plant by 10 to 100 times more.

Variant 1. Mixture based on mycorrhizae: with a rooting effect was used to fertilize the crop. The fertilizer was added to the surface of the culture substrate when the wheat crop is in the needle phase. Fertilizer was added in one pass. The same treatment options were applied for the three analyzed varieties.

The mycorrhizal fertilizer was added in order to determine the rapid development of the root system and to process food from the soil with the help of fungi. A well-developed root system means a good capacity to absorb nutrients from the soil followed by the sustained development of the aerial part of the plant, the growth of the vegetative mass and finally the increase in the quality of the harvested grains.

Variant 2. At sowing, it was fertilized by incorporating NPK complex (15-15-15 active substance) N 45 kg active substance, P_2O_5 45 kg active substance, K_2O 45 kg active substance. Straw cereals have average specific consumption of nutrients (Cs = kg N, P_2O_5, K_2O/1 t production), but they are extremely demanding on fertilization, given the fact that they have a poorly developed root system and have a low capacity to solubilize substances nutrients from soil reserves, especially wheat.

RESULTS AND DISCUSSIONS

The need to fertilize crops and the principles of rational fertilization are summarized in the fertilization plan, which represents the control and management tool for fertilizers. The fertilization plan is based on a foundation made up of the combination of the following parameters: crop rotation, the genetic production potential of the crop, the availability of the soil nutrient reserve, the water resource and the dose of applied fertilizers. The dose of applied fertilizers is the result obtained from the calculation of the system made up of three equations: the genetic production potential of the crop, the availability of the soil nutrient reserve and the water resource.

Basic fertilization is carried out with organic fertilizers and complex chemical fertilizers that provide the plants with the necessary nutrients, which they need for the desaturation of the vegetative cycle with a satisfactory result.

The expected productions for sustainable agriculture cannot be based on the nutrients provided by the environment. Fertilization technology is based on two classes of nutrients: macroelements and microelements, both showing the same degree of importance in obtaining a sustainable production. The macronutrient class has 3 major nutrients and the micronutrient class consists of 6 major elements, which plants need to go through a healthy vegetative cycle.

The terminology of macroelements and microelements, respectively, does not represent the nutritional importance to the plant, but strictly the quantities needed for a plant to produce the maximum. Both macroelements and microelements are equally important, being key elements in the biochemical processes carried out in the plant growth cycle. Macroelements are N (nitrogen), P (phosphorus), K (potassium), and microelements are Ca (Calcium), Mg (Magnesium), S (Sulfur), Fe (Iron), Mn (Manganese) and Zn (Zinc), adding another series of microelements: Ni (Nickel), Mo (Molybdenum), Co (Cobalt), Cu (Copper), B (Boron), etc. which plants need in relatively small quantities, but which are essential to life. These elements are part of the composition of many enzymes that catalyze biochemical processes.

The advantage brought by the existence of mycorrhizal symbioses for plant nutrition, highlighted the influence that this association has on the growth and development of plants. The existence of mycorrhizal fungi was demonstrated about 400 million years ago, the first discoveries being the fossils of Aglaophyton major plants that showed traces of shrubs, these being considered edifying transfer structures for the vesicular-arbuscular endomycorrhizal type.

Mycorrhizae are present in mature ecosystems, ecosystems that show a cyclical and unitary evolution of the components between the biotic and abiotic unit, when mycorrhizal associations have the role of regulating the assimilation of food resources for the plants with which they are associated.

In this association, hyphae play an important role in the nutrient cycle, having the function of stopping losses in the ecosystem

From the data analysis, it is observed that in the variant treated with phytohormone fertilizer, the highest gluten accumulation was recorded for all 3 varieties of wheat, the differences between the varieties being characteristic of the genetic potential (Table1).

Table 1. Determination of gluten from the obtained production

No	R	V.M (g %)		V. 1 (g %)		V. 2 (g %)	
1. Glosa	1.1	24.02	24.06	29.01	29.03	28.02	27.97
	1.2	24.05		28.98		27.98	
	1.3	24.1		29.1		27.91	
2. Apache	2.1	22.97	22.99	27.41	27.32	26.8	26.91
	2.2	23.01		26.98		27.02	
	2.3	22.99		27.56		26.91	
3. Arnold	3.1	32.01	32.07	38.92	38.94	38.74	38.83
	3.2	32.11		39.01		38.95	
	3.3	32.09		38.89		38.81	

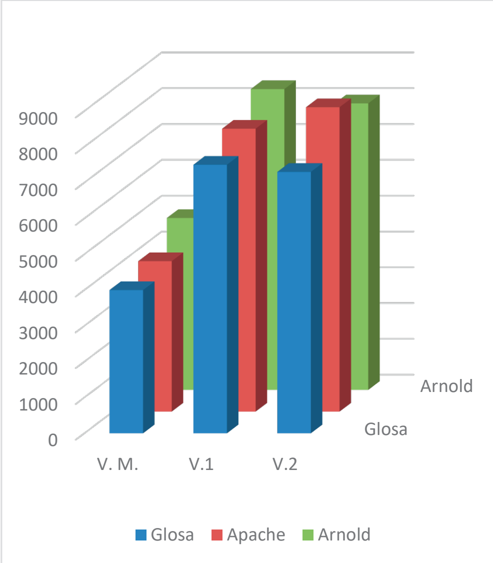

Figure 1. Influence on production

Analysis of the results revealed variations in protein content between the two fertilization options for all 3 wheat varieties. The results obtained are presented in Figure 1. Increases in protein content are observed for the variants treated with fertilizers compared to the control variant with differences between 0.8 and 2.88%.

In conclusion, the mycorrhizal fertilizer determined the most pronounced increase in terms of protein synthesis. In terms of protein content, the leading variety is Arnold followed by Glosa at a difference of 2.47% (Table 2).

In the case of the Glosa wheat variety, the ratio between the protein content and the production achieved is directly proportional. In the case of the first variant of fertilization with mycorrhizae, which had a rooting effect, they stimulated the increase in the protein content of the production obtained by 1.06% compared to the second variant of fertilization, which has both a lower protein content and a lower production.

Thus differentiating the two variants, a fact that highlights that a plant with a well-developed root system, a system that maintains the plant throughout the vegetative cycle, can exploit the genetically coded productivity characteristics much more efficiently.

Table 2. Determination of wheat proteins

No.	R	V.M (g %)		V. 1 (g %)		V. 2 (g %)	
1.Glosa	1.1	12.23		13.85		13.12	
	1.2	12.17	12.17	13.91	13.91	13.25	13.18
	1.3	12.11		13.98		13.17	
2.Apache	2.1	11.98		12.54		12.01	
	2.2	11.52	11.75	12.46	12.55	12.11	12.07
	2.3	11.74		12.65		12.09	
3.Arnold	3.1	13.76		16.41		15.84	
	3.2	13.23	13.50	16.35	16.38	15.91	15.82
	3.3	13.52		16.39		15.71	

Increasing protein content has two associated components, namely quantity and quality. Both are directly related to the amount of nitrogen assimilated by the plant in accordance with the vegetative cycle. A high yield is believed to decrease the grain protein content, so it is essential to determine the expected yield as the crop approaches the grain filling phase. When protein quality is desired, mainly to mimic high molecular weight, long chain gluten proteins. Gluten proteins (gliadin, glutenin, albumin and globulin) give wheat products unique extensibility and processing properties.

CONCLUSIONS

The need to use AMF as a biofertilizer to create sustainable agriculture is becoming more and more critical since proper management of these simbiotic fungs may lessen the need for agrochemicals. Inoculating AMF propagules in a concentrated solvent is the primary approach taken to achieve this goal.
Unfortunately, AMF are required to be symbiotic and cannot be grown in pure cultures without the use of their host plants' gasd. This limiting characteristic makes producing a long scare of AMF inoculation very challenging and complex.
There are three primary types of AMF vaccinations. First off, because it contains colonized fragments, AMF spores, and hife in a typical manner, soil from an AMF-hosting plant's zone may be used as an immunization.
The general conclusion of the research is to identify the intake of nutrients brought by mycorrhiza for wheat cultivation. The mycorrhizal relationship leads to the solubilization of minerals, the production of plant growth stimulants and the control of pathogens.
The cumulative benefit brought to the plant leads to a high production by substituting chemical fertilizers
It is concluded that it brings a major benefit in the transport of nutrients for plants. In most types of mycorrhizae, the movement of carbohydrates, produced during photosynthesis, is done from the host plant (autotrophic partner) to the symbiotic fungus (heterotrophic partner). In the case of absorption of nutrients from the soil, the transfer has an inverse direction, from the fungus to the host plant (Jakobsen 1999). The contribution of vesicular-arbuscular fungi to the assimilation of nutrients is the absorption of nutrients (especially phosphorus) from the soil, with the help of extraradicular hyphae - especially from those parts of the soil to which the plant did not have access.
The nutritional variants applied differentially influenced the three varieties of wheat used in the experiment, so that both the accumulation of all biocomponents, expressed as protein, and the particular accumulation of gluten was made according to the variety and according to the treatment.
Here are some of the benefits that wheat can get from symbiosis with *Glomus intraradices*:
1. Increasing nutrient absorption: *Glomus intraradices* can increase the absorption surface of wheat roots and help more efficient absorption of nutrients such as nitrogen, phosphorus and potassium. This can improve plant health and increase crop yield.
2. Stress tolerance: *Glomus intraradices* can help increase the wheat plant's tolerance to abiotic stress such as drought, high temperature or nutrient poor soils. It can help increase the plant's resistance to harsh environmental conditions and improve the plant's survival under such conditions.

3. Disease Protection: *Glomus intraradices* can help protect the wheat plant against diseases and fungal infections. It can increase the immunity of the plant and help reduce the incidence of diseases.
4. Improving soil quality: *Glomus intraradices* can help improve soil structure and fertility. This can help reduce soil erosion, improve the soil's ability to retain water and nutrients, and increase soil microbial biodiversity.

The symbiotic relationship between *Glomus intradiceps* and wheat is manifested by the formation of a mycorrhizal collet between the wheat roots and the fungal filaments. In this collet, the fungi provide the plants with nutrients, including nitrogen, phosphorus and other minerals, obtained from the soil, while the plants provide the fungi with carbohydrates produced through photosynthesis.

This mycorrhizal collet can grow and develop in the wheat roots, thus improving its ability to absorb nutrients from the soil. In addition, mushrooms help to improve the structure of the soil, reducing its compaction and increasing the permeability of water and air. Therefore, the symbiotic relationship between *Glomus intradiceps* and wheat can lead to better growth of wheat, with higher productivity and increased resistance to water stress and pests.

Nitrogen in the air is fixed by fungi and cyanobacteria, transforming it into nitrogen compounds, such as nitrates and ammonia.

Plants absorb these nitrogen compounds from the soil and use them to build proteins and other substances necessary for growth. Animals eat plants and accumulate proteins and other nitrogenous substances in their bodies. When animals defecate or die, organic residues with nitrogen are released into the soil. Fungi in the soil break down organic residues and release nitrogen in the form of ammonia, which can be fixed again and used by plants.

The process continues, allowing the transfer of nitrogen between different components of the ecosystem. It is important to mention that this natural circuit can be disturbed by human activities, such as the excessive use of synthetic fertilizers or soil pollution. This can have negative effects on the health of ecosystems and on agricultural productivity.

ACKNOWLEDGEMENTS

The work of Dragota Marina Alina was supported by the project "PROINVENT", Contract no. 62487/03.06.2022 - POCU/993/6/13 - Code 153299, financed by The Human Capital Operational Programme 2014–2020 (POCU), Romania.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INFLUENCE OF BIODESTRUCTOR ON DECOMPOSITION OF CROP RESIDUES AND HUMUS CONDITION OF TYPICAL CHERNOZEM

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Abstract

The study examined the composting of agricultural plant residues during the summer period of 2021 in typical chernozem soil. The mass of plant residues decreased during composting, with the use of a destructor resulting in a greater loss of plant material. Different rates of decomposition were observed for residues of different crops, and surface residues generally decomposed more rapidly than root residues. Changes in total humus content were also observed, with an increase in the humus content during composting of wheat and barley residues without a destructor. However, most variants with a destructor showed negative changes in the content of total humus. The study found that humification processes took place only in the version with winter wheat and spring barley, and the use of a destructor led to a decrease in the intensity of humification processes or to the passage of dehumification processes. Overall, the study highlights the importance of considering the impact of crop residues and the use of a destructor on soil quality and nutrient cycling.

Key words: *typical chernozem, crop residues, stubble destructor, winter wheat, spring barley, maize, sunflower.*

INTRODUCTION

Organic matter and the processes of its transformation play a significant role in the formation of soil, its most important properties and features. Thus, organic matter participates in the nutrition of plants, creates favorable water and physical indicators of the soil, promotes the migration of elements in the soil and the biosphere.

Potential sources of organic matter in the soil are aerial and root remains of woody and herbaceous plants, biomass of invertebrates and microorganisms. The biomass of green plants (phytomass) and its annual increase (primary production) is several dozen, or even hundreds of times higher than the biomass of invertebrates and microorganisms (Tomashivskyi et al., 2020).

Above-ground and below-ground phytomass of groups depends on their composition, biological features of the dominant, meteorological conditions and rockiness of the substrate. On the example of the "Steppe Tract" plant group, the highest values of above-ground and underground mass are 330.0-735.0 and 1025.0-1310.0 g/m^2, respectively (Shevchuk, 2019). According to the data, the average accumulation of raw and dry above-ground mass of winter wheat varies from 3644 g/m^2 to 5900 g/m^2 for raw mass and from 1500 g/m^2 to 2200 g/m^2 for dry above-ground mass (Lytovchenko, 2015).

Preservation of plant remains helps to enrich the chemical composition of the soil with organic matter, in contrast to their traditional burning, and serves to feed and activate the activity of biota. The most active role in this is played by microorganisms capable of producing enzymes that destroy lignin, cellulose, fiber, and proteins of plant residues (Kovalenko, 2021). In addition, microorganisms actively convert soil minerals into soluble form: phosphorus, magnesium, calcium, sulfur, iron, boron, molybdenum, zinc, etc. Along with the processes of decomposition of organic residues in the soil, the process of synthesis of humic substances takes place.

The action of destructors is aimed at accelerated decomposition of plant residues and their transformation into humus substances, that is, at improving soil fertility and, as a result, increasing the yield of cultivated crops (Sidyakina, 2021; Korsun et al., 2017; Serhiieva, 2017; Kovalenko, 2022).

The analysis of publications allows us to state that in the experiments using stubble destructors, either microbiological processes during the introduction of destructor drugs, or its effect on the yield of the next crop are largely investigated, while not paying attention to the humus condition of the soil.

Given the critical scale of human impact on soils, today approximately 33% of global soil resources are degraded. Dehumification ranks first among soil degradation phenomena. Loss of humus by soils causes deterioration of their physical properties, reduction of nutrient content, intensification of erosion processes, etc. And therefore, soils lose their fertility. Therefore, the topic chosen by us to study the processes of humification of agricultural crop residues in the modern conditions of the introduction of agriculture using a stubble destructor is relevant in terms of developing directions for restoring the content of humus in arable soils.

MATERIALS AND METHODS

The research was conducted on the territory of the state enterprise of educational and research farm "Dokuchaevske" within the training, research and production center "Experimental Field" of State Biotechnological University. The soil was used for research: chernozem typical heavy loam on loess-like loam. Plant raw materials - surface post-harvest remains of winter wheat, corn, sunflower, barley.

Soil samples of typical chernozem were taken from the arable layer with a depth of 0-30 cm.

Dry matter and moisture by weight were determined. Gravimetric method. Soil samples are dried to constant weight at (105 ± 5) °C.

Determination of total humus by the Turin method. The method is based on the oxidation of soil organic matter by a solution of potassium dichromate in sulfuric acid, followed by determination of organic carbon content through the determination of potassium dichromate after oxidation by titrometry.

Calculation and preparation of plant material and soil for composting was performed according to the method of Chesnyak. Plant residues are selected a week before harvest. The rests are selected manually, the crop rests are crushed to the size of 0.5 cm and in a shade bring to an air-dry condition. The weight of the soil in terms of a completely dry sample for each repetition is equal to 100 g. The selected general soil sample to grind it better with plant residues is ground, sifted through a sieve and visible plant remains are selected. Two days before the start of the experiment in the general soil sample determine the moisture content and the content of total humus.

After the preparation of plant material and soil, each sample was placed in a kapron bag, the volume of which corresponds to the volume of the soil (6*10 cm). The samples are at a depth of 14-16 cm in the arable layer of the soil. To determine the influence of the stubble destructor on the humification of agricultural crop residues, the prepared soil and plant material were treated with the "Ekostern" stubble destructor, produced by "BTU-Center", according to the recommendations: corn, sunflower - 1.2-2.0 l/ha, cereals - 1.0-1.5 l/ha.

In field conditions "composting" of plant samples with soil was carried out in the summer of 2021 for 3 months - June, July, August at a depth of 15 cm.

RESULTS AND DISCUSSIONS

In the territory where the research was conducted, the weather conditions according to the average multi-year data collected at the weather station of the KhNAU (Obraztsova, 2001) are characterized as follows (Table 1): the average multi-year precipitation is 529 mm, according to the average multi-year data of the same weather station, the wettest month is July with 71 mm of precipitation. The amount of precipitation in June and August, according to consolidated long-term data, is slightly lower and amounts to 59 mm and 56 mm, respectively. The average annual temperature is +7.2°C. July is the warmest with a temperature of +20.5°C, the air temperature in June reaches a mark of +19.2°C, and in August the temperature drops to +19.6°C.

Table 1. The long-term average data for monthly air temperature and precipitation levels according to the KhNAU weather station data

Month	VI	VII	VIII	Annual
Average air temperature, °C	19.2	20.5	19.6	7.2
Amount of precipitation, mm	59	71	56	529

In the year of the study (Table 2), in comparison with long-term data, an increase in the average monthly temperature is observed in each of the months. Thus, in June, the average air temperature was higher than the long-term data by 1.6°C and amounted to +20.8°C. July was 4.6°C warmer compared to long-term data, the average monthly temperature in 2021 reached +25.1°C. The largest temperature difference compared to long-term data was observed in August, so in 2021 the air temperature reached +24.7°C, which is 5.1°C more than the average long-term observations. The distribution of precipitation in the summer months of 2021 was as follows: in June, the amount of precipitation was 82 mm, which is 23 mm more than the average of long-term observations. In July, in comparison with long-term data, a slight decrease in the amount of precipitation to 65 mm was found. A significant difference between the average multi-year data and the data of 2021 was found in the month of August, the amount of precipitation decreased by 44 mm to 12 mm (Table 2).

Table 2. The average monthly air temperature and precipitation levels in 2021 according to the KhNAU weather station data

Month	VI	VII	VIII	Annual
Average air temperature, °C	20.8	25.1	24.7	9.6
Amount of precipitation, mm	82	65	12	520

All in all in 2021, the monthly temperature increased compared to long-term data, particularly in August, and there was less precipitation in August than the long-term average.

The amount of plant material laid down for composting and the content of total humus in typical chernozem before the start of the experiment are given in the Table 3.

Table 3. The content of general humus in chernozem typical and the mass of plant material when setting up the experiment

Agricultural crop	The content of total humus in the soil, %	Mass of plant material, g	
		surface plant residues	root residues
Winter Wheat	5.8	8.52	8.52
Spring Barley	5.8	7.94	7.94
Sunflower	5.9	9.19	9.19
Maize	5.7	8.28	8.28

The highest content of total humus before laying the experiment was recorded in the sunflower variant at 5.9%. The content of total humus in the soil of other variants did not exceed 6%. The lowest humus content was found in the corn variant at 5.7%.

During three summer months of composting with winter wheat, the following changes occurred: the content of total humus in the variant ranged from 6.4% to 6.6%. The highest amount of humus was found in the variant with the addition of surface plant residues without the use of a stubble destructor, at 6.6%. The lowest amount of humus, 6.4%, was observed when using the stubble destructor with both surface and root crop residues. The largest increase in newly formed humus in this variant was 0.8% (Figure 1).

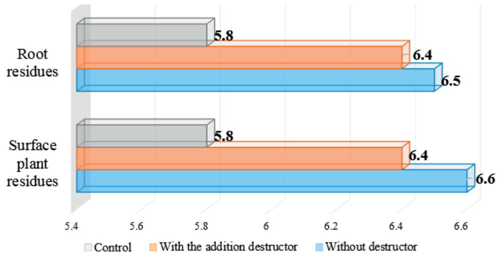

Figure 1. Content of total humus in the version with winter wheat

The variation in mass fluctuation of plant residues with winter wheat was distributed as follows over the same period of time: a uniform decrease in mass was observed in the variant without adding the destructor, while the average decrease for both types of plant residue input was 3.0 g. When adding the destructor, there was an uneven manifestation of plant material reduction, with the mass of root plant residues decreasing by half to 4.26 g, which was slightly more than 1 g less than the previous variant. In the variant with the adding of the stubble destructor, there is an uncharacteristic effect of weakening the transformation of the stubble in comparison with a similar option, but without the adding of the stubble destructor. The mass of this variant was smaller than the control by 1.69 g and larger than the variant without adding the destructor by 1.33 g (Figure 2).

In the variant with spring barley, the following situation was observed. When root residues were added without adding the destructor, the increase in newly formed humus was 0.6%, whereas in the variant where the destructor was implemented, the amount of total humus decreased by 0.7% to a value of 5.1%, indicating the prevalence of the mineralization process over humification (Figure 3).

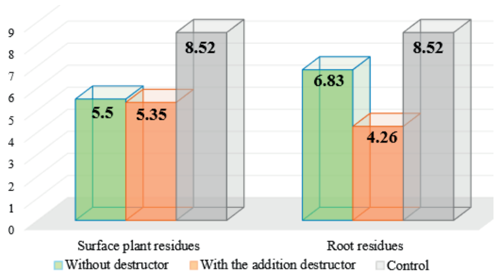

Figure 2. Change in the weight of surface and root residues in the version with winter wheat

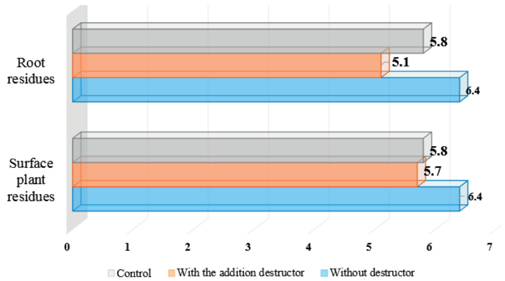

Figure 3. Total humus content in the variant with spring barley

From the presented diagram, we can see that in the variant with spring barley and addition of straw destructor, the weight loss was 2.55 g for surface harvest residues and 2.53 g for root residues (Figure 4). In contrast, in the variant without straw destructor, the weight loss was 0.85 g for surface harvest residues, which is 1.70 g less than in the corresponding variant with straw destructor. The weight loss for root residues without the addition of straw destructor was 0.87 g.

Regardless of the experimental variant, a decrease in the total humus content was observed by 0.4% compared to the control after 90 days of composting.

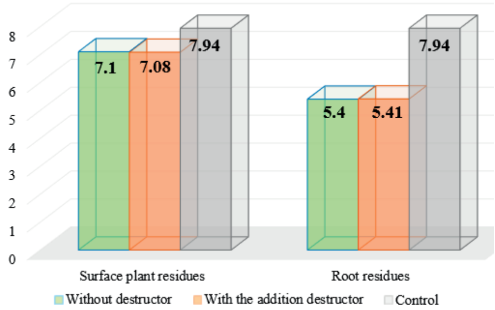

Figure 4. Change in the weight of harvest residues in the variant with spring barley

The total humus content decreased to 5.3%. Therefore, we can assume that despite the introduction of plant residues, mineralization processes prevailed over humification processes, leading to a decrease in humus content in the typical chernozem arable layer (Figure 5).

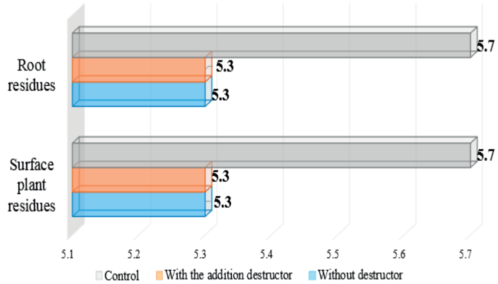

Figure 5. Content of total humus in the version with maize

In the version with maize, a trend similar to the version with barley can be traced after 90 days of composting. Thus, with the addition of a stubble destructor, the weight decreases by 2.68 g and amounts to 5.60 g with the addition of surface root residues, and with the addition of roots, the weight decreases to 5.01 g, i.e., it decreases by 3.27 g. Without the addition of the stubble destructor with the introduction of surface harvest residues of corn, the weight decreases to 7.33 g, which is 0.95 g less than the control, and with the introduction of root harvest residues of corn, the weight decreases by 1.41 g to 6.87 g (Figure 6).

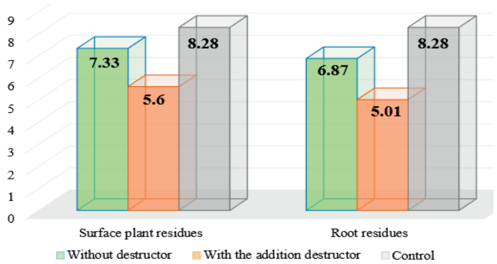

Figure 6. Change in the weight of plant material in the version with maize

The overall humus content in the variant with sunflower underwent the following changes after 90 days of summer composting. Only in the variant with the addition of surface plant residues without the addition of straw destructor, there was an increase in newly formed humus, but not significantly - by 0.1%. The opposite situation occurred when adding straw destructor and when adding root harvest residues, in this variant, there was a decrease in overall humus by 0.1%. Therefore, we can say that the processes of mineralization-synthesis in this variant were in balance (Figure 7).

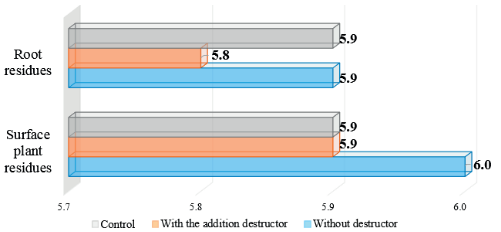

Figure 7. The content of total humus in the version with sunflower

Moreover, the change in the weight of the residues during this period of time according to the options is not of a similar nature. For example, in the variant with the application of surface residues, the weight differs as follows: with the addition of the destructor, it decreased by 4.24 g compared to the control, and without its action, by 2.73 g. Therefore, we can conclude that the action of the destructor accelerated the processes of conversion of plant mass. When comparing the experiment variants with the addition of root residues, we see the following: the decrease in weight due to the action of the destructor exceeds the same variant without its application by 0.54 g and amounts to 6.85 g (Figure 8).

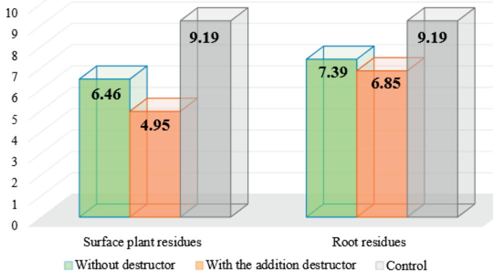

Figure 8. Weight change in the version with sunflower

So, the majority of plant material, whether above-ground or root mass, decomposes on average one and a half times faster under the action of the destructor. However, this does not necessarily mean that when plant residues decompose in this way, they will necessarily be fixed as newly formed humus.

CONCLUSIONS

During the composting of plant residues in chernozem typical agrocenosis, which lasted during the summer period of 2021, there was a change in the mass of both root and surface residues of grain and industrial crops.

In the control variant without a destructor, the smallest decomposition of plant material on average is observed in variants with corn residues, at around 17%. Taking into account the decomposition of surface and root residues, it is found that in variants with wheat and sunflower, the decomposition of surface residues occurs more intensively than the decomposition of root residues. The weight loss of surface residues is 30-35% compared to 20% for root residues. Depending on their morphological affiliation, the residues of barley and corn also had different rates of decomposition. In this case, the intensity of decomposition of root residues (loss of 21-47%) prevails over the intensity of decomposition of surface residues (loss of 12-13%). The most intensive weight loss among surface residues was observed for winter wheat residues, and among root residues - for barley.

When using a destructor, a greater loss of plant material is observed during the summer composting period, and only in the variant with barley does the loss of plant material mass equal or is less than that of the control. The residues of winter wheat decomposed most intensively, with an average weight loss of plant material of around 43%. In most variants, under the action of the destructor, root residues underwent greater decomposition. In the variant with sunflower, the intensity of decomposition of surface residues was almost twice as high as that of root residues. The loss of plant material for corn root residues was 39% and for surface residues was 32%. Surface residues of sunflower and root residues of winter wheat were most intensively transformed.

During the composting of agricultural plant residues in typical chernozem soil during June-August 2021, changes in the total humus content were observed.

In the variants without the addition of a destructor, an increase in the total humus content was found during the composting of wheat and barley residues. In the variant with winter wheat, the composting of surface residues resulted in a 14% increase in humus content, while for root residues, it was a 12% increase. Composting barley residues led to a 17% increase in humus content, regardless of the morphological features of the residues. Composting of technical crops residues did not result in an increase in the humus content of typical chernozem soil. In the variant with sunflower, no changes in the total humus content were detected, while in the variant with maize, a process of humus loss was observed - by 7%.

When using the destructor of crop residues, most of the variants showed negative changes in the content of total humus. In the variant with spring barley, no change in humus content was detected when composting surface residues, but with root residues, the humus content decreased by 12%. In the variant with sunflower, the humus content remained unchanged. The incorporation of corn residues into the soil with the use of the destructor, as well as in the control variant, led to a decrease in the content of total humus by 7%. Only in the variant with winter wheat did the humus increase, with an increase of 10% for both surface and root residues.

So, in the conditions of the summer period of 2021, the composting of the remains of agricultural plants (winter wheat, spring barley, sunflower, corn) in the arable layer of 0-30 cm of typical chernozem led to a decrease in their mass, and the mass of the remains was lost more intensively under the conditions of using a stubble destructor. It was found that humification processes took place only in the version with winter wheat and spring barley. Moreover, the use of a stubble destructor led either to a decrease in the intensity of humification processes, or to the passage of dehumification processes.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INFLUENCE OF CROP SPECIES ON SOIL MICROBIAL ABUNDANCE AND DIVERSITY

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Abstract

Agricultural crop species can influence the soil microbial population through the influence of root exudates, which compounds released by plant roots into the soil. Some crop species exude compounds that are more beneficial for certain groups of microbes, while others exude compounds that inhibit the growth of certain groups. Additionally, different crop species have different root systems, which can affect the physical structure of the soil and the availability of water and nutrients, which in turn can influence the soil microbial population. Moreover, crop species can also influence the soil microbial population by changing the soil pH and the level of organic matter. Crop species that produce large amounts of biomass can increase the amount of organic matter in the soil, which can support a greater diversity of microorganisms. Maize and soybean are some of the most important agricultural crop globally. Both corn and soybean are important crop species for the global food system and also for the economy of many countries, including Romania. A study was conducted to determine the composition of the soil microbial community in the rhizosphere of maize (Zea mays L.) and soybean (Glycine max (L.) Merrill.) at different phenological stages and to determine whether the two crops influence the microbiological abundance and diversity in the soil.

Key words: *soil microbial communities, maize, soybean.*

INTRODUCTION

Global demand for food is currently projected to increase by approximately 70% by 2050 due to the need to feed the ever-growing human population (Wise, 2013; FAO, 2009). However, under current conditions, it is highly unlikely that agricultural practices will be able to fulfil this requirement, faced with continued land degradation, declining soil fertility, climate change and increased frequency of extreme weather events. In addition to these changes, the agricultural sector also faces important problems due to: a] economic growth, such as high production costs (i.e. the increase in the prices of agricultural inputs: seeds, fertilizers, pesticides, fuel, etc.) which leads to the registration of a low margins of profitability, b] the social situation, the lack of labor, the demands of the population for good quality food and without residues of chemical substances; c] environmental issues, avoiding or minimizing soil, water and air pollution; d] political, such as the ecological agreement of the European Union, through the application of which the concomitant promotion of crop productivity, but also environmental sustainability is desired (Knez et al., 2022; Igliński et al., 2022). To deal with these situations, knowledge of soil microbiota and its role can complement conventional agricultural practices to ensure sustainable food supply (Tian et al., 2020).

Microorganisms play an essential role in sustaining life on Earth (Turner et al., 2013). However, so far we have been able to understand very little about the microorganisms that colonize environments such as ocean water, the atmosphere, soils, etc. Techniques based on the growth of microorganisms isolated from various environments on culture media have allowed them to be studied in detail. The exploitation of microorganisms associated with cultivated plants represents one of the solutions that is shown to be among the most promising in the long term, facing the challenges encountered on the road to ensuring food security, while also supporting the sustainability of the environment (Singh et al., 2020). Microorganisms are responsible for many processes that take place in the soil. As a result of these processes, nutrients, arrive in

forms accessible to plants, plant health can be promoted by means of microorganisms. Microorganisms also help plants improve resistance to biotic and abiotic stress (Tkacz et al., 2020).
The microbiota represents the totality of microorganisms that are naturally present in a given environment that is studied together with the host and the surrounding environment, such as the plant-soil complex. Microorganisms are in a very close relationship with the environment in which they live, but the changes that occur in the environment are able to produce changes on the microbiota.
Crops, widely grown in fields, for human consumption, animal feed, raw materials for various industries (i.e. wheat, corn, rice, soybean) have gone through important changes in cultivation technologies, along with industrialization and development of human society, due to the increased use of agricultural machinery, chemical fertilizers and pesticides. For these reasons, the composition of the microbiota associated with crop plants has been strongly modified (Lamb et al., 2011). Increasing the productivity of agricultural crops has been an objective pursued throughout the last centuries, but the current situation, represented by globalization, rising prices and climate change, requires finding solutions that allow the production of more food in an ecologically and socially sustainable way (Chaparro et al., 2012). To achieve this goal, agriculture must be based on the relationships that are established between plants and microorganisms, aiming to promote soil microbial diversity.
Current research shows the importance of plant-microorganisms relationships on the productivity and health of agricultural crops (Howard et al., 2020; Berg & Smalla, 2009; Morrissey et al., 2004).
As discussed above, the agricultural sector must be aware of the role that soil microorganisms have on agricultural crops, which is why the present study aims to present the influence that two of the most important and cultivated agricultural crops (corn and soybean) have on the abundance and diversity of bacteria and filamentous fungi in the soil.

MATERIALS AND METHODS

To highlight the microbiological activity carried out at the soil level, in the case of corn and soybean crops, and to determine the total number of bacteria (Gram-positive bacteria and Gram-negative bacteria) and filamentous fungi per gram of soil, the plating into nutritive media method was used.
The soil that was microbiologically analyzed in this study was taken from the northeastern region of Moldova, Romania. In the studied area, the climate is temperate continental. The average annual air temperature is ~9.5°C, with extremes between 40.0°C and -35.0°C. The sum of the average annual precipitation is ~520 mm. Significant deviations from the long-term average of precipitation and temperature have been observed over the past 10 years. The soil texture is primary clay loam, and the natural vegetation of the site is silvo-steppe (Gafencu, 2019).
The analyzed soil type was represented by chernozem cultivated with corn and soybean under conventional farming conditions, using synthetic fertilizers and chemical pesticides.
Soil samples were collected for each cultivated species, 6 times during the vegetation period, between May and October 2022.
For each individual crop, 10 points were randomly chosen on the surface of the plot so that the entire surface was covered. Before each sampling, organic debris present on the soil surface was removed. Soil samples were taken from a depth of approximately 7-10 cm, using sterile instruments. Soil samples were collected in paper bags and transported to the microbiology laboratory for further analysis. The samples were kept overnight at 4°C, and the next day they were left to dry at room temperature. Any organic residue was removed from the soil mass, and then the soil was homogenized using a mortar and pestle (sterilized in advance to avoid contamination of the soil samples with other microorganisms).
After the preparation of soil samples for microbiological analyses, the total number of bacterial colony-forming units (CFU) was determined by serial dilutions and plating in nutritive media.
One gram of dry soil was introduced into a first tube (sterile 15 ml Falcon tube) containing 9 ml of sterile water and homogenized very well

(stirred for 5 minutes using a MX-RD-E Vertical Rotator), obtaining the dilution 10^{-1}. Then, from the 10^{-1} dilution, one millilitre of the suspension was transferred using a sterile Pasteur pipette into a second tube, thus obtainning the 10^{-2} dilution. After a series of successive dilutions, dilutions from 10^{-2} to 10^{-6} were prepared. From each dilution obtained, one millilitre of the suspension was spread on nutrient media to evaluate the number of bacteria and filamentous fungi. For an easy identifycation of the colonies, different culture media were used, specific for each group of microorganisms. Thus, to determine the total number of bacteria, simple PDA medium (Potato Dextrose Agar) was used, to determine the number of Gram-positive bacteria (G+), was used PDA medium with the addition of streptomycin, 35 mg L^{-1}, added after sterilization when the temperature of the medium was around 46-48°C, and to determine the number of filamentous fungi, the PDA medium with the addition of Rose Bengal, 33 mg L^{-1} was used. After inoculation the Petri dishes were incubated in the thermostat at a temperature of 28°C. The number of bacterial colonies was determined after 24 hours, and the fungal colonies after 120 hours. The determination of the number of bacterial colonies was performed using an Interscience Colony Counter Scan 1200 automatic colony counter.

The data obtained in the experiments were statistically evaluated using the SPSS program (IBM SPSS Statistics 26). and the result with $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSIONS

The analysis of microbiota from the soil samples showed that there are differences in the abundance and structure of the microbial communities in the soil, being influenced by both, the crop plant and the phenophase in which the plants are found (seasonal variation). Similar results were obtained by other authors (Jat et al., 2021; Longley et al., 2020; Peralta et al., 2018), their results indicate that the growth stage and management system have significant effects on the corn and soybean soil microbiome.

A close examination of the microbiota in the corn and soybean rhizosphere shows us the presence of a high variability in the abundance activity and structure of the microbial communities.

The results show us that the crop influences the total number of bacteria in the soil (Figure 1). Even if plots cultivated with corn and soybean are found in the same area, the soil has the same physical and chemical properties, under the influence of the cultivated plant, changes occur at the level of the soil microbiome.

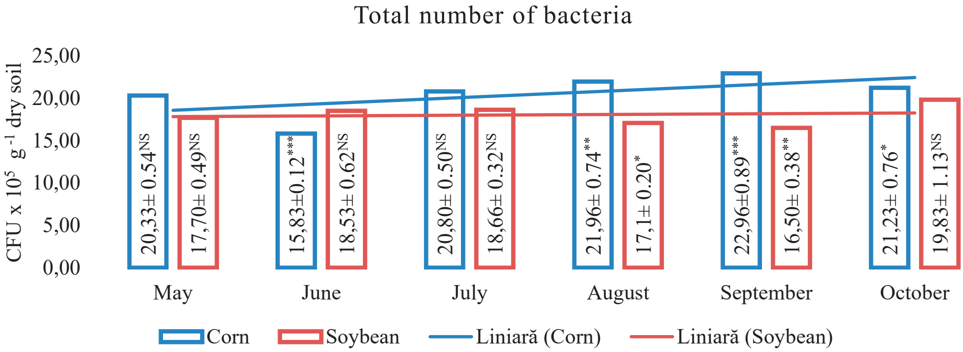

Figure 1. Evolution of total soil bacteria during the vegetation period of corn and soybean crops
[NS = not significant; *not significant; **significant; ***distinctly significant]

The results show that in the case of the corn crop, the total number of bacteria increased from May to October. The highest values were recorded in September, when a count of 22.96 ± 0.89 CFU x 10^5 g^{-1} dry soil was recorded. If in the case of the corn crop the trend is upward, in terms of the total number of bacteria, in the soybean crop it can be observed that the trend was linear. Even though from May to October different values were recorded at each time when samples were taken, it can be seen that the values obtained were somewhat similar.

Gram-negative bacteria (G^-) represent the most important group of microorganisms in the studied soil. This particular group of bacteria represents about 70-75% of the total number of microorganisms that can be cultivated on the culture medium. In the case of Gram-negative (G^-) bacteria, the numerical evolution was similar to that recorded for the total number of bacteria. In the case of the soil taken from the corn crop, it can be observed that from May to October, during the studied period, the number of Gram-negative bacteria (G^-) followed an upward direction. The highest value was obtained in September, 21.47±1.07 CFU x 10^5 g^{-1} dry soil. In soybean crop, the numerical density of Gram-negative bacteria (G^-) remained unchanged throughout the vegetation period, except for October, when the highest value was recorded, 18.30±1.12 CFU x 10^5 g^{-1} dry soil (Figure 2).

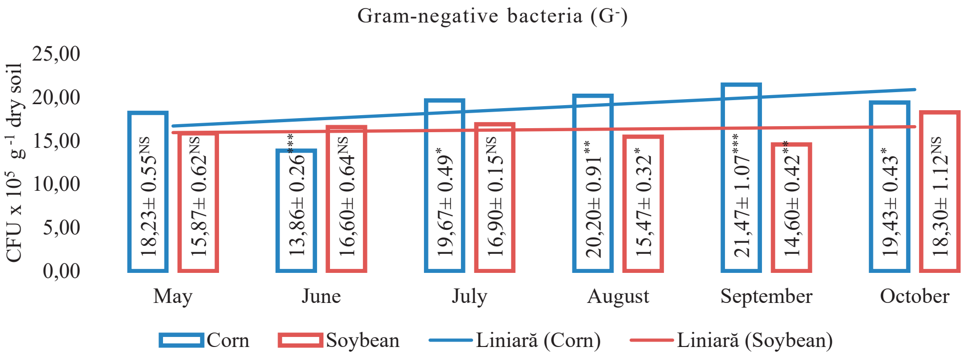

Figure 2. Evolution of total soil Gram-negative bacteria (G^-) during the vegetation period of corn and soybean crops [NS = not significant; *not significant; **significant; ***distinctly significant]

Gram-positive bacteria (G^+) represent the particular group of microorganisms that was the least represented during the study (Figure 3). The results show that the numerical density of Gram-positive bacteria (G^+) was not influenced by the plant crop. In both cases, corn and soybean, the trend was the same. The numerical density of Gram-positive bacteria (G^+) decreased from May until October. The highest values, 1.93±0.03 and 1.90±0.06 CFU x 10^5 g^{-1}, respectively, were recorded in the soybean crop, in July and September.

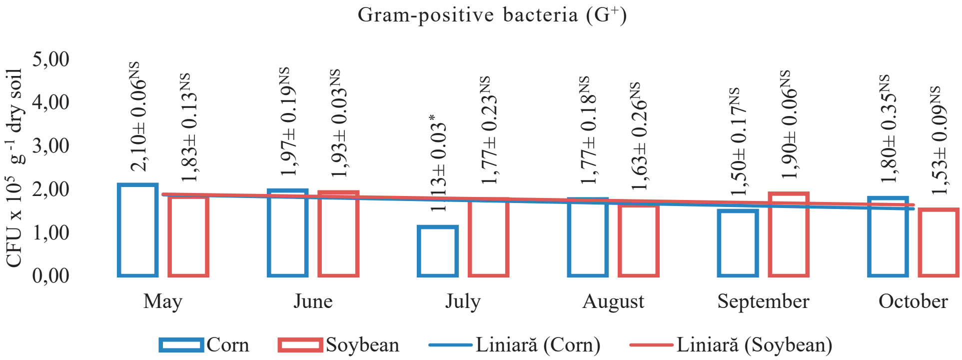

Figure 3. Evolution of total soil Gram-positive bacteria (G^+) during the vegetation period of corn and soybean crops [NS = not significant; *not significant; **significant; ***distinctly significant]

The results obtained from the microbiological analysis regarding the abundance and structure of the communities of filamentous fungi in the soil indicate the presence of saprophytic and parasitic fungal genera. The determined fungal genera and their structure are shown in Figure 4.

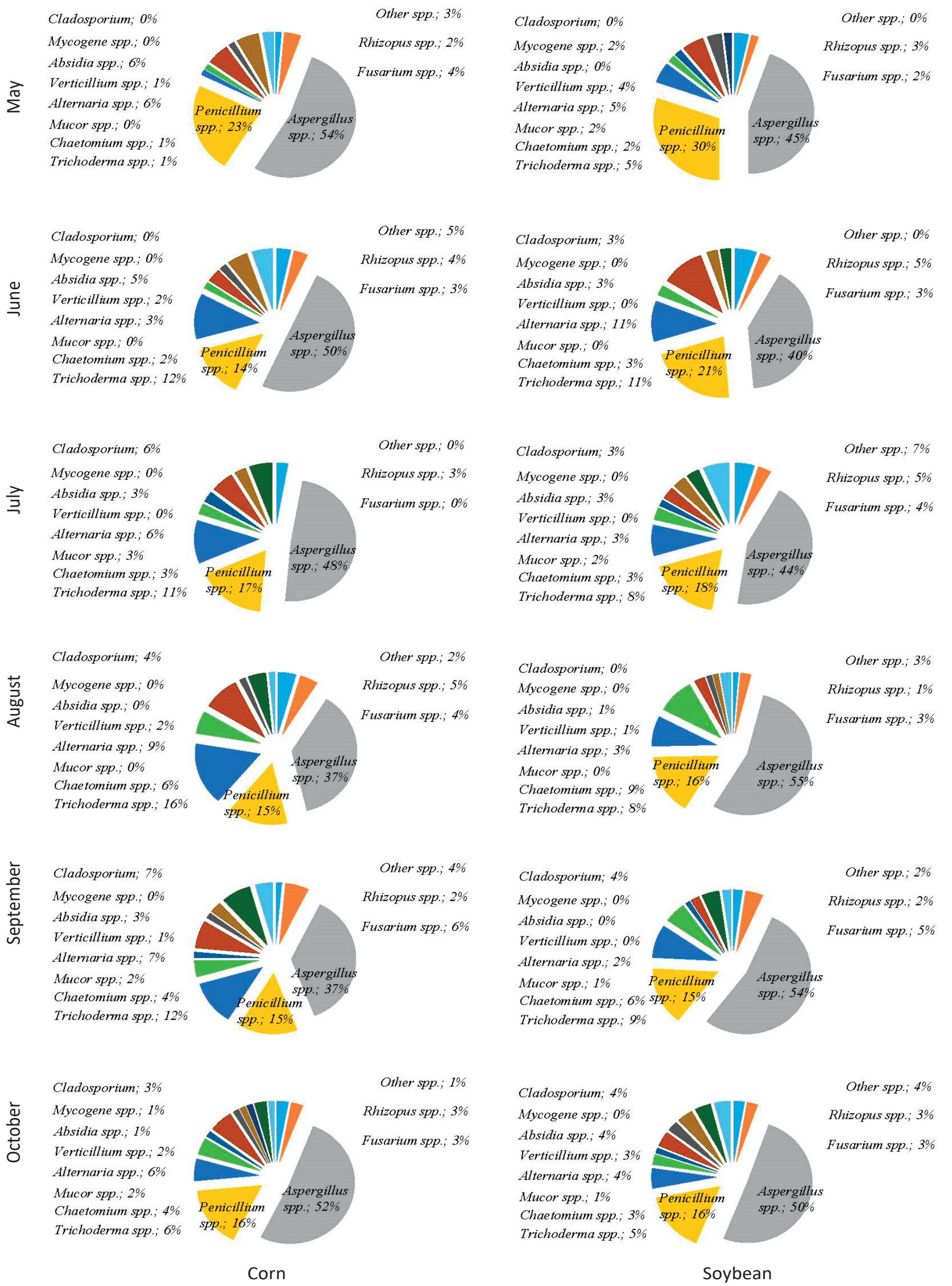

Figure 4. Frequency of isolated filamentous fungi during the vegetation period of corn and soybean crops

As a result of the research, we observed that the number of isolated filamentous fungi genera were different from one culture to another and, in the case of the same culture, throughout the vegetation period. The species of filamentous fungi culturable on artificial medium belonged to 12 genera of micromycetes: *Cladosporium, Mycogene, Absidia, Verticillium, Alternaria, Mucor, Chaetomium, Trichoderma, Rhizopus, Fusarium, Penicillium* and *Aspergillus*. In addition to these genera that were identified based on morphological characters, other filamentous fungi were also developed, in the case of which the identification could not be 100% certain, which is why they were included in a separate group, *Other* spp.

Among the micromycetes determined, in all cases, the genus *Aspergillus* stood out due to the large number of colonies it formed on the culture medium, the isolation of this genus being represented by a rate between 37 and 55% of the total genera identified. Another genus of micromycetes frequently identified was represented by *Penicillium*, with a rate between 14 and 30%. The genus *Fusarium* was also frequently isolated, this could be related to the fact that on the plots analyzed, maize monoculture was practiced for a long period of time.

CONCLUSIONS

Our observations on the total number of microorganisms/g (CFU g^{-1} dry soil) in the sampled soils showed differences in the microbiological activity carried out in the rhizosphere of corn and soybean crops. Also, during the vegetation period, the abundance and structure of microbial communities experienced changes, generally showing a positive trend.

Finally, in the soil layer (up to a depth of 10 cm) intense biological activity takes place due to the presence of microorganisms (Gram-positive and Gram-negative bacteria and micromycetes).

ACKNOWLEDGEMENTS

This research was supported by the project "PROINVENT", Contract no. 62487/ 03.06.2022-POCU/993/6/13-Code 153299, financed by The Human Capital Operational Programme 2014–2020 (POCU), Romania.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

CHARACTERISTICS OF SOME NEW VARIETIES AND LINES OF WHEAT UNDER THE YEAR 2021-2022 CONDITIONS

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Abstract

And in the conditions of luvic soils, a series of new characters were obtained in winter wheat lines. Compared to the control varieties, Trivale and Ursita, the 4 new lines obtained showed a number of improved characteristics. Thus, lines A4-10 and A57-14 approached the size of the Trivale variety, and in culture all these lines showed a very good uniformity. In terms of the ear formed, the new lines have surpassed those of the Trivale variety in length. Spike weight was superior to A4-10 and A57-14. The number of grains in a spike was higher in the A57-14 line (42.0 grains). Under the same conditions, the weight of the grains in one ear in the lines A4-10 and A57-14 again exceeded the Trivale variety. Line A57-14 slightly outperformed the new Ursita wheat variety. Positive correlations were obtained between grain yield and crude protein (CP%) and between PB and wet gluten (WG%). From the results obtained with the new winter wheat lines, a genetic progress was found, which recommends the future steps for homologation.

Key words*: luvic soil, morphology, new wheat lines, production and quality.*

INTRODUCTION

Winter wheat is now widespread (Black & Halmer, 2006), due to high yields (Bonjean & William, 2001) and superior quality (Day et al., 2006). Varieties and especially new lines fall within these parameters due to ongoing breeding activity (Tester & Langridge, 2010). The areas where the plant grows and develops, the optimal parameters are the temperate ones. Because of this the plants develop in a moderately short season. Under these conditions, the grains form high-quality flour widely used in baking (Li et al., 2014). And the varieties that are part of the present study have a grain content in diverse and balanced nutritional elements, being very important in food human. Over time, the plant has evolved through the most important characters desired by the breeder, so from a genetic point of view, today wheat is considered one of the most genetically diverse cereals. For example, there are wheat types of autumn and spring, with dressed and naked grains. The studied varieties are part of the *erythrospermum* Korn variety. They have white spikes, awned with glabrous glumes and red grain. At the same time, these new varieties have in their composition also a reduced waist gene, thanks to which they become more productive, because they support fertilization at higher levels and mechanized harvesting. From a botanical point of view, the spike has a terminal position, is distich, 4-18 cm long. The spikelets that form them are sessile, they attach singly to the rachis in a zigzag pattern. The grain (caryopsis), is ellipsoidal in shape, with a central channel on one side, having a length of 4-12 mm and a thickness of 1.5-4 mm. The TGW is between 15-60 g. The height of the plants is between 50-150 cm. The research undertaken to establish the variation of some characters in the new wheat lines referred to: the length of the straw, the length and weight of the ear, the number and weight of grains in an ear.

MATERIALS AND METHODS

The experimental variants included two varieties: Trivale (Pitești) and Ursita (Fundulea), as well as 4 lines A4-10, A57-14, A44-13 and A95-13. The experiment was set up according to the balanced square method, where the variants were 8.4 sq m in four repetitions. The technology used was within the parameters developed by the resort. At full maturity the two varieties and the new wheat lines were harvested and several determinations were made. In this way, the height, ear length, ear weight, number of grains in the ear, the weight of grains per ear and the average yield per ha were determined for the plants. From the point of view of grain quality, crude protein and wet gluten were determined using the PERTEN apparatus. In the statistical calculation, the Anova test was used to establish the 3 types of limit difference: LSD 5%, LSD 1% and LSD 0.1%. The test was applied both to the morphological characteristics of the wheat and to their quality. Simple correlations were established between the obtained values, in order to observe existing trends between the characters. The significance of the correlation coefficient was made by comparing it with the r_{max} value for the 5%, 1% and 0.1% levels of transgression probabilities.

RESULTS AND DISCUSSIONS

The influence of climatic factors on wheat plants. Regarding the monthly averages, two influences were found. Thus, in the period after sowing (October - December) (Table 1), the average of the period was 6.4°C with 0.6°C more than the multi-year average. In the second period (January - June), only in the month of June were found average values that exceeded the multi-year average. This proves that there was a technical nuance to the rise in temperatures due to global warming (Fang & Xiong, 2015). Regarding the precipitation regime, it was found for the first period in autumn, an increase of 1.6 mm, in the second analyzed period, deficiencies were found (January - March), after which the months (April - May), the rains were at the level of multiannual values. In the month of June, there was an accentuated deficit of rains. For wheat cultivated in the period 2021-2022, the water regime had a lack of 133.0 mm. This aspect obviously disadvantaged the wheat plants and especially the deposition of dry matter in the grains. From the point of view of the favorability of wheat for the Pitesti area, it is found that at the multiannual average of 338.3 mm, the water need of wheat is close to this (Etp = 378 mm).

Table 1. Climate factors evolution from winter wheat vegetation

Month	Temperatures, tn^0C		Precipitations, mm		Etp, mm
	Multi.	2022	Multi.	2022	
Oct.- Dec.	5.8	6.4	46.8	52.9	25
	±	+0.6	±	+6.1	
Jan	-1.1	1.4	39.7	6.6	28
Feb	0.7	3.7	38.0	10.4	28
Mar	4.8	3.6	36.5	31.0	40
Apr	11.0	10.8	53.6	66.4	72
May	16.3	17.1	79.5	76.6	96
Jun	19.5	21.6	91.0	14.3	114
Mean Sum	8.53	9.70	338.3	205.3	378
	±	**+1.17**	±	**-133.0**	

Evolution of morphological characters in wheat varieties and lines. Regarding the height of the plants, it was found that compared to the Trivale variety (check), both in Ursita and in the 4 lines, the average values exceeded them. Spike length was below 8 cm Trivale and A95-13, while in the other varieties, the values were very close (Table 2). Spike weight, specific experienced a significant increase in Ursita variety and lines A4-10 and A57-14. The number of grains in the ear was between 30.0 and 42.0, but without significant differences. The weight of these grains in one ear was between 1.05 and 1.49 g, also without significant differences. The same elements of statistical processing are found in the case of Table 3 of dispersion analysis for the wheat varieties studied.

Table 2. Morphological characters of winter wheat lines and varieties

Varieties, line	Plant high, cm	Ear length, cm	Ear weight, g	No. grains/ ear	Grains weight/ ear, g
Trivale	77	7.4	1.65	33.6	1.33
Ursita	114	**8.3**	**2.12**	39.1	1.47
A4-10	81	**8.4**	**1.88**	39.9	1.38
A57-14	83	**8.5**	**2.33**	42.0	1.49
A44-13	108	**8.4**	1.50	30.0	1.05
A95-13	**167**	7.3	1.55	34.4	1.24
LSD 5 % =	*68.6*	*0.24*	*0.223*	*2.45*	*0.233*
LSD 1 % =	*94.9*	*0.33*	*0.309*	*3.43*	*O.322*
LSD 0.1 % =	*131.2*	*0.46*	*0.427*	*4.74*	*0.445*

Table 3. Dispersion analysis of wheat plants morphology

Character		Plant high, cm			Ear length, cm			Ear weight, g			No. grains/ ear			Grain weight/ ear, g		
Variability c	LD	SP	S^2	F	SP	S^2	F	SP	S^2	F	SP	S^2	F	SP	S^2	F
Repetition	3	6160			0.75			0.08			6.23			0.04		
Variance	5	23291	4658	2.24	6.12	1.22	61***	2.20	0.44	22***	414.58	82.92	30.6***	0.54	0.11	5.5
Error	15	31122	2075	(2.90)	0.37	0.02		0.33	0.02		40.59	2.71		0.36	0.02	
Total	23	60573		(4.56)	7.24			2.61			461.41			0.94		

Correlations studied within morphological characters of wheat. Between the 5 morphological characters, determined in the two varieties and 4 new lines of wheat, both negative influences and positive characteristics were found. Regarding the height of the plants, with the other determinations (Figures 1-4), a positive aspect was highlighted, between the height of the plants and the length of the ear and 3 negative situations, respectively with the weight of the ear, the number of grains and the weight of the grains in an ear.

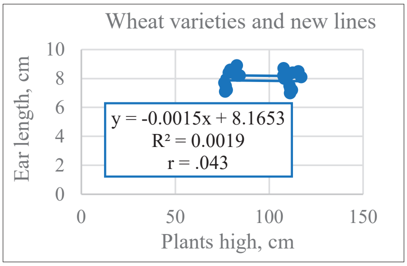

Figure 1. Correlation plants high x ear length

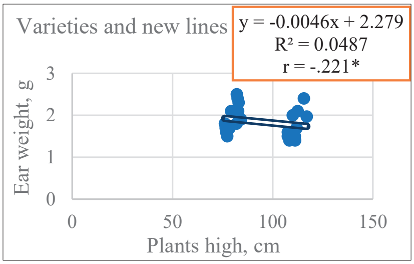

Figure 2. Correlation of plants high x ear weight

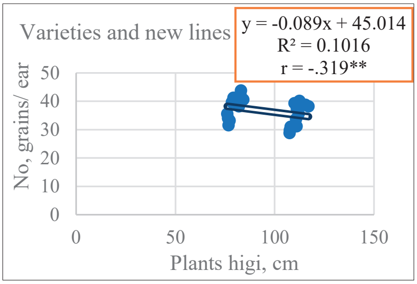

Figure 3. Correlation plants high x no. grains/ ear

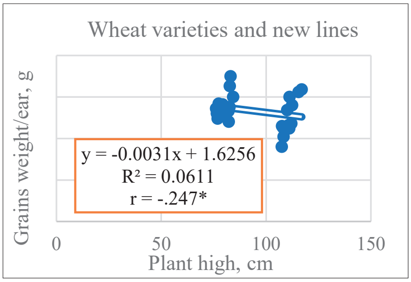

Figure 4. Correlation plants high x grains weight/ear

The explanation lies in the fact that at the time of the deposition of dry weight, there was an accentuated period of drought in the grains (Farooq et al., 2014). Spike length correlated with each other 3 characters out of one. From a significant point of view, ear length had favorable valences with ear weight and the number of grains in the ear. Only the grain weight in the ear was insignificant with the weight of the grain in the ear. The other correlations (Figures 5-10) being in a direct and highly significant relationship (Table 4).

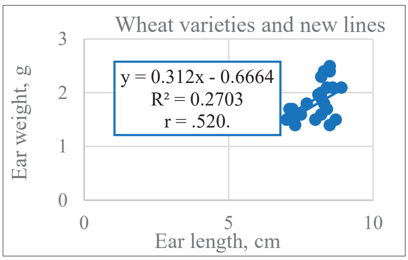

Figure 5. Correlation ear length x ear weight

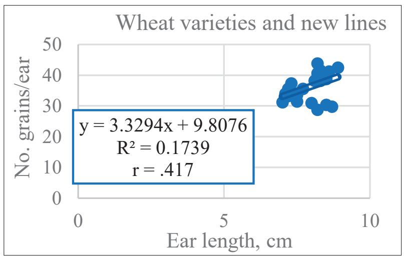

Figure 6. Correlation ear length x no. grains/ear

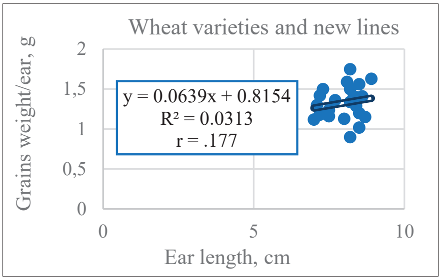

Figure 7. Correlation ear length x grains weight/ear

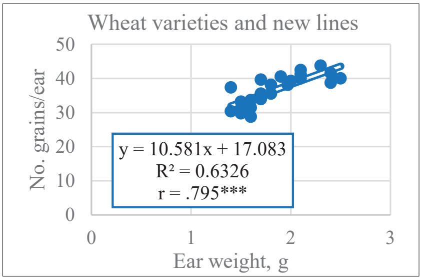

Figure 8. Correlation ear weight x no. grains/ear

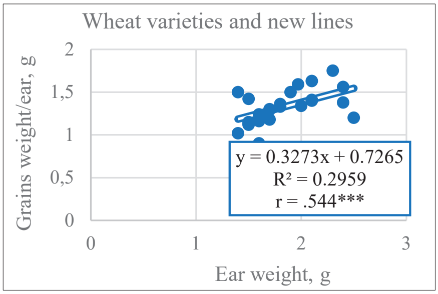

Figure 9. Correlation ear weight x grains weight/ear

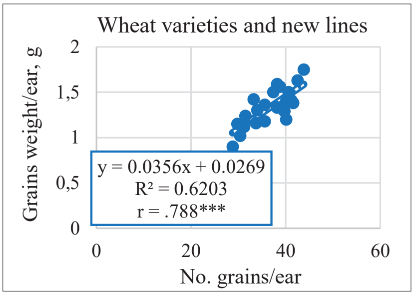

Figure 10. Correlation no. grains/ear x grains weight/ear

Table 4. Correlations between the main morphological elements

Characters	Ear length, cm	Ear weight, g	Number grains / ear	Grain weight/ ear, g
Plant high, cm	.043	**-.221**	**-.319**	**-.247**
Ear length, cm	1	**.520**	**.417**	.177
Ear weight, g		1	**.795**	**.544**
Number grains/ear			1	**.788**
	LSD 5 % = .190	*LSD 1 % = .250*	*LSD 0.1 % = .320*	

Evolution of wheat grain quality. Considering the obtained grain production of 5,451-7,570 t/ha, the lower lines than the control can be found in all 4 studied lines (Table 5). The protein content ranged between 10.05 (Ursita) and 13.90 (Trivale) and the crude protein of the lines was lower than mt. Moist gluten was 18.15% (A4-10) and (30.30%) A95-13. The obtained data are highlighted by dispersion analysis (Table 6).

Table 5. Aspects of grains quality

Variety, line	Grain yields, t.ha^{-1}	Crude protein, CP %	Wet gluten, WG%
Trivale	7,462	13.90	22.48
Ursita	7,570	10.05	21.90
A4-10	6,748	10.38	18.15
A57-14	5,451	10.90	24.60
A44-13	6,654	10.95	28.28
A95-13	6,388	**11.33**	**30.30**
LSD 5 % = *LSD 1 % =* *LSD 0.1 % =*	*0.459* *0.635* *0.878*	*0.683* *0.944* *1,305*	*5,255* *7,268* *10,044*

Table 6. Dispersion analysis wheat grains quality

Character	LD	Grain yields, t/ha			Crude protein, %			Wet gluten, %		
Variability c.		Sum sq.	S^2	F	Sum sq.	S^2	F	Sum sq.	S^2	F
Repetition	3	24.55			1.30			44.02		
Variance	5	11986.90	2397.4	25.8***	37.78	7.56	36.82***	619.04	123.81	10.18***
Error	15	1393.72	92.91	(2.90)	3.08	0.21		182.45	12.16	
Total	23	13405.2		(4.56)	42.16			845.51		

Correlations obtained between wheat quality analyses. Grain production was positively correlated with crude protein (Figure 11), demonstrating genetic gain in the quality of the wheat lines.

Regarding the correlation between grains and wet gluten, the trend was negative (Figure 12), which means that climatic factors were resilient to this character.

Between crude protein and wet gluten (Figure 13), a positive but insignificant relationship is found. This means that the new wheat lines also demonstrated high baking capacity (Figures 14, 15, 16), the intensity of the correlations in Table 7.

Table 7. Correlations between the first main quality elements

Character	Yields, t/ha	Crude protein, %	Wet gluten, %
Grain yield	1	**.208**	**-.343**
CP, %		1	.066
WG, %			1
LSD 5 % = .190	LSD 1% = .250	LSD 0.1 % = .320	

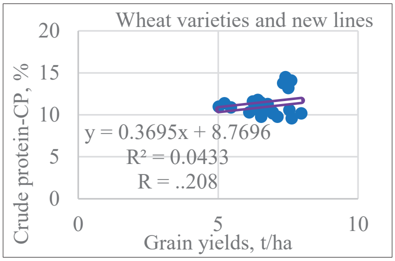

Figure 11. Correlation grain yields x CP %

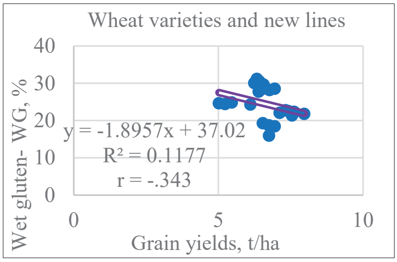

Figure 12. Correlation grain yields x WG %

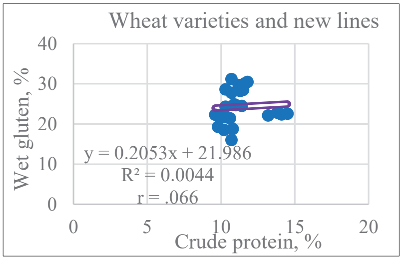

Figure 13. Correlation Crude protein x wet gluten

Figure 14. Ursita grains aspect

Figure 15. Ursita, the new wheat variety

Figure 16. A4-10, new wheat line

CONCLUSIONS

The new wheat lines studied, compared to two methods recommended at farm level, demonstrated both positive and negative characters. The climatic regime in which the wheat varieties grew this year experienced a warming of 1.17 °C throughout the vegetation period and a -133.0 mm of precipitation. Compared to the multiannual average but also to the ETP vegetation water requirement, obvious drought conditions were encountered. Due to drought, wheat could not manifest its characteristics in optimal perimeters (Farooq et al., 2014). From the analysis of the morphology of the plants, the new wheat lines showed the average height, the long length of the ear, the weight of the ear, but with the number of grains and their weight in an ear, at insignificant values.

Plant height was negatively correlated with the other characteristics, highlighting the genetic character of plant growth reduction. Positive and highly significant correlations were established between the ear characters: length, weight, grain number and grain weight.

The relationship between crude protein and gluten was non-significant but positive, showing increased quality within the wheat lines.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE CHARACTERISTICS OF THE ADSORPTIVE COMPLEX AND THE REACTION OF SOILS SUBJECTED TO HIGH ANTHROPOGENIC PRESSURE FROM THE COPȘA MICĂ AREA

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Abstract

Anthropogenic pollution triggers a series of processes that also affect the bioavailability of nutrients, the soil being a key element in food security and sustaining biodiversity. The purpose of this research is to identify the influence of the soil reaction and the values of the adsorptive complex indices (SH, SB, CEC, V) on the nutritional status of the soils around the town of Copșa Mică in the context of over 70 years of pollution). Soil pH is a reliable indicator of chemical transformations and a predictor of possible deficiencies or toxicity of elements necessary for plant physiology. The adsorptive complex indices have a diagnostic and prognostic role on the health of the soils, reflecting the degree of nutrient supply. In the 13 sample areas, the soil reaction varies from very strongly acidic to weakly alkaline, an important role being attributed to the influence of the orography of the land and the local pollutant-dispersing microclimate. The dynamics of the determined or calculated values of the adsorptive complex indices vary depending on the sampling depth and the investigated SP.

Key words*: adsorptive complex indices, soil reaction, Copșa Mică area.*

INTRODUCTION

Soil reaction is the result of multiple chemical transformations with prognostic role on the danger of nutritional deficiencies or potential toxicity (Slattery et al., 1999; McKenzie et al., 2004). In agronomy, acidity is an indirect indicator of soil fertility through its impact on root exudates (Stoltz & Greger, 2002; Pansu & Gautheyrou, 2006), acting as a limiting factor on plant growth (especially roots), nutrient uptake due to the release of toxic Al^{3+} in susceptible plants (Cronan et al., 1995; Lindsay & Walthall, 1996). Islam et al. (1980) highlight the corrosive action of low pH on root membranes. Bessho & Bell (1992), Menzies et al. (1994) support the idea that in soils with similar mineralogy and highly degraded soils, pH can accurately predict Al toxicity. Humic substances in the composition of organic matter can buffer the *pH*. Spurgeon et al. (2006), Rooney et al. (2006) indicate industrial pollution as a determining factor in influencing soil *pH* with an immediate effect on the bioavailability and toxicity of metal pollutants on the soil biocenosis. Anthropogenic acid discharges have effects similar to natural soil acidification (Kennedy, 1992; Saha, 2017) and affect soil fertility by removing essential basic cations, thus increasing the toxicity of Al and Mn (Rhode et al., 1995), and the toxic action of metals heavy impacts mineralization, nitrification, symbiotic relationships, and decreases *pH* value and P bioavailability (Foy, 1984; Al-Fredan, 2011; Bach et al., 2020). Heavy metals such as Pb, Cd, Cu, Ni or Zn shows a mathematical correlation with the concentration in the soil solution, with *pH* being the most critical factor whose increase favours the absorption of these metals (Basta & Tabatabai, 1992; McBride, 1994). In "virtually unaffected" pollution areas, soil acidification occurs as a result of the accumulation of H^+ from the biogeochemical circuit of C, N and S (Ulrich, 1991).

Changes in *pH* due to the action of anthropogenic pollutants depend on the type and chemical composition of the pollutant emissions. SO_2 rich emissions from non-ferrous smelters increase soil acidity, and the presence of particulate matter containing metal oxides cannot buffer the SO_2 impact on *pH*. In forest soils, both wet deposition (via acid rain) and dry deposition (gases, vapours, particulate matter, tailings) accelerate soil acidification (Davidson, 1990; Legge, 1990; Lorenz, 1995). Research has not revealed the dependence of pollutant type or pH with soil depth. (Kozlov et al., 2009). The increase in soil *pH* triggers a decrease in the solubility of most elements, thus limiting their flow into the soil solution. The mobility and bioavailability of some elements at high *pH* is due to the formation of complexes with soil organic fractions (Kabata Pendias, 2011; Bravo et al., 2017; Solly et al., 2019). Forest soils are more acidic than those intended for crop plants, the input of H^+ being also added to that of N and heavy metals, and trees can accumulate significant amounts of Cd and other heavy metals (Mayer, 1993; Li & Li, 2005).

Adsorptive complex indices and their role in soil health and trophicity

Cation exchange capacity (CEC) represents the sum of the exchangeable cations ŐCa^{2+}, Mg^{2+}, K^+, Na^+ (in Al^{3+} acid soils), adsorbed by the soil, with metals such as Mn, Fe, Cu, Zn having insignificant contribution (Osman, 2013; Hazelton & Murphy, 2016). From the perspective of agronomists and ecologists, exchangeable cations and CEC are intrinsic indicators of the health, fertility, physical and chemical status of soils, the retention capacity of nutrients, pollutants or, as the case may be, of fertilizers. Hazelton & Murphy, (2016); Mukhopadhyay et al. (2019), highlights the role of CEC in buffering *pH* fluctuations, Ca^{2+} content and soil structural changes. Revil & Leroy (2004) show the major role of CEC on plant growth, fertility and soil transport processes. Among soil colloids, organic matter presents a high CEC, between 100 and 300 cmol/kg, being influenced by clays and organic matter (Fooladmand, 2008; Sulieman et al., 2018). The adsorptive complex that regulates the degree of storage, distribution, mobility and bioavailability (Dalal & Moloney, 2000; Pansu & Gautheyrou, 2006; Ross et al., 2008) impacts potentially toxic cations from anthropogenic polluting sources (Pb).

Studying the impact of carbon black on nutrient retention in anthrosols of Amazonian Brazil, Liang et al. (2006) highlights that soils with a high carbon black load show a high CEC (i.e. *pH* 7). CEC value depends on the type of soil, fertile soils showing values between 8 and 10 meq/100 g soil, the sandy ones <10 meq/100 g soil, and the clayey ones between 10-15 meq/100 g soil. A moderate CEC value i.e. >12 indicates a good nutrient retention capacity (Botta, 2015; Ghorbani et al., 2015). Soils with low CEC values are vulnerable lacking essential cations that are washed out of the soil.

The degree of saturation with bases (V, %) represents a proportion of CEC occupied by bases (Osman, 2013), showing a close correlation with *pH* in acidic or neutral soils, ranging between 20 and 60% at pH ≥ 5. This index may be impacted by clays and it shows how close nutritional status is to potential fertility (Rengasamy & Churchman, 1999). Many forest soils in Central Europe have a low saturation with bases, being depleted in exchangeable cations (BML, 1996). Soils with V, % < 50 are dystrophic, poorly or moderately fertile. Lukac & Godbold (2011) show that soils with high V, % buffer soil acidity more effectively by controlling the release of toxic elements, while a low V, % enhances the sensitivity of soils to acid deposition.

The characteristics of the edaphotopes of the Copșa Mică area by the time of research

According to Untaru et al. (2000), with regard *pH*, the systematic edaphic unit and the degree of pollution cause an acidification of the soil in the range of extremely acidic to neutral or weakly alkaline reaction (6.0-8.0). The negative effects of pollutant emissions on soils, observed by Alexa et al. (2004) are as follows: increased acidity and base saturation loss in adsorptive complex. In the Târnăvioara Improvement Perimeter, the slope is affected by pollution, artificial terracing and erosion. Because of the mass displacements, steep detachment ravines appear alternating with landslides as effect of slope breaks (Alexa et al., 2004). Based on data collected between 1973-1999 Cotârlea et al. (2001) cited by Alexa et al. (2004), fully covers the forestry real estate from management unit (MU) II Micăsasa, MU III Târnava within forest district (FD) Mediaș in zone I of very strong pollution and 40% of forests within zone I and 60% in zone II on the territory of MU I Șeica Mică. It is worth mentioning that the sample areas-SP (delimited for this study) SPs 2, 6, 7 East, 7

West, 8 9 (MU III Târnava/FD Mediaș); SPs 12, 13, 14, 15 and 16 (MU I Șeica Mică/FD Mediaș) are within the pollution zones I and II (Alexa et al., 2004).

The multi-decade aggression on the soils within the Copşa Mică area was also due to acid rains and fine powders containing Pb, Cu, Cd, Zn, and Fe. Ianculescu et al. (1994) observe that the *pH* in the horizons of the upper part of soil decreases by 0.6-2.4 units, the degree of saturation with bases (V, %) shows a reduction of 20-30%, the exchangeable A1 content ranging between 700-800 ppm. The same author finds a high acidity (*pH*=4) in the soil Ao horizon, which is a characteristic present throughout the depth of the profiles, with some exceptions, the *pH* having values below 6.0 in the deeper horizons of the soil. Between 1985-1994, some plots showed a slightly decreasing trend of the soil reaction in the upper horizons. After reviewing the data obtained from 1996 and 2001, Alexa et al. (2003) conclude that the soil surface horizon is generally acidic, while at depths of more than 40 cm the reaction becomes weakly acidic or neutral. A recent research work by Iordache et al. (2020) finds a slightly acidic soil reaction at the depths of 0-20 cm and 20-40 cm.

The research on the physical and chemical characteristics of the edaphotopes within the Copșa Mică area was carried out in the year when the cessation of activity of the main polluter i.e. the company S.C. Sometra S.A. from Copșa Mică (26.01-31.03.2009) took place, the other polluter, i.e. the company Carbosin S.A. being decommissioned permanently since 1995.

MATERIALS AND METHODS

Soil sampling took place between 15 November and 4 December 2009 in accordance with the sampling norms proposed by the National Research-Development Institute for Pedology, Agro-chemistry and Environmental Protection (ICPA) Bucharest (xxx, 1981). Sampling depths were as follows: 0-5 cm, 10-15 cm, and 30-35 cm. The experimental device includes 14 sample areas (see Figure 1) abbreviated SP1 (MU 1 Veseuș/FD Aiud); SPs 2, 6, 7 East, 7 West, 8 9 (MU III Târnava/O.S. Mediaș); SP 10 (MU VII Moșna/FD Mediaș); SAs 12, 13, 14, 15, 16 17 (MU I Șeica Mică/FD Mediaș). SP 1 represents the control sample area located westward from the city of Blaj, 26.36 km away from the main polluter i.e. S.C. Sometra S.A.

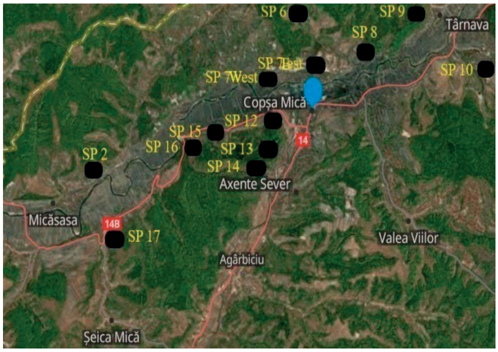

Figure 1. Location and nomenclature of the sample surfaces (SPs) in the Copșa Mică town area subject to multidecadal pollution

The following types of soil characterise the SAs taken into consideration: typical preluvosoil (SP 1-MU 1 Veseuș, FD Aiud), typical luvosoil (SP 2-MU II Micăsasa; SP 7West, SA 8, MU III Târnava); calcareous regosol (SP 6 MU III Târnava, SPs 15, 16 MU I Șeica Mică); stagnant luvosoil (SP 7East-MU III Târnava); typical brown luvic soil (SP 10, MU VII Moșna); marnic phaeozems soil (SP 9 - MU III Târnava; SPs 12, 13, 14 - MU I Șeica Mică, FD Mediaș) (***2008; ***2018; ***2020; Florea & Munteanu, 2003). Basic samples sampled from four different points were mixed resulting in an average sample, the laboratory samples being obtained by the four quarters method (Dean, 2002). The drying, conditioning and processing of the soil samples was carried out according to the protocols issued by ICPA Bucharest (***1981) and the undecomposed plant remains, and gravel and foreign materials were selected and removed. The soil samples thus obtained were dried, ground with a lab mill and sieved through a 2 mm mesh sieve. Afterwards, the resulting fine soil was stored and labelled in hermetically sealed plastic boxes. In order to determine the *pH*, and of the indices of the adsorptive complex (SH, SB, CEC, V, %) we used the working methodology indicated by Târziu and Spârchez (1987).

Determination of soil *pH*

Bélanger et al. (2006) show that forest soils tend to have the same ionic strength throughout the year and indicate the determination of soil *pH* in soil-water suspension. Couchense et al. (1995) draw attention to the shock induced by both the heat treatment and the soil sampling in terms of affecting *pH* values. The principle of the research method involves the measurement of H^+ activity using a double glass calomel electrode, immersed in the solution to be analysed. The soil suspension in H_2O has a 1:2.5 mass/ volume ratio. The results are expressed in *pH* units, with an accuracy of 0.05 (in *pH* units). As a result, we considered the average of the last two measurements, the value of which does not differ by more than 0.1 units of *pH* (Târziu & Spârchez, 1987).

Determination of total exchangeable soil acidity (SH)

KC1 exchangeable acidity in agronomy is an important indicator of exchangeable phytotoxic A1 (Pansu & Gautheyrou, 2006). Exchangeable acidity was determined by the Kappen's method, which entails treating the soil with a neutral KC1 1 N solution (Thomas, 1982) that activates the extraction (H^+ and Al^{3+}, $Al(OH)^{2+}$, $AL(OH)_2^+$) and the volumetric titration of the excess HC1 formed with a NaOH solution in the presence of phenolphthalein until the appearance of a faint orange colour. Drying the soil sample at 105°C is necessary for soils rich in oxides and hydroxides (the presence of Fe oxides causing colouring errors). Pratt & Bair (1961) and Espiau & Pedro (1980) show that the KC 1 method is the most efficient, being often used in comparison with the $BaCl_2$ based method.

Determination of the basic cation exchange capacity (SB) (Kappen's method)

It entails treating the soil sample with a known or excess amount of 0.1 N HC1, resulting in the cation exchange of the soil adsorptive complex with H^+ ions of HC1. The excess of HC1 was titrated with 0.1 N NaOH solution until the colour changed from blue-violet to persistent green for two minutes, and the amount of HC1 consumed was calculated by the remainder. A control sample was also titrated separately. (Târziu & Spârchez, 1987).

The total cation exchange capacity (CEC) was determined by calculation, i.e. by summing the exchangeable bases and the total exchangeable acidity, according to the formula: CEC $(_{m.eq\ /100\ g\ soil})$ = SB + SH (1) where: SB is the sum exchangeable bases, in m.eq. and SH the total exchangeable acidity in m.eq. /100 g soil.

The degree of saturation with bases (V, %) was determined by the ratio between the sum of exchangeable bases (SB) and the total cation exchange capacity (CEC), expressed in % according to formulas 2 and 3 below: V%=SB/(CEC)x100 (2) or V%=SB/(SB+SH)x 100 (3) (Târziu & Spârchez, 1987).

Data analysis

The charts showing the variation of the analytical values of the researched parameters were developed in MS Excel 2019, and the dependence between the sum of cation exchangeable bases and CEC/V(%) was subjected to research using linear regression type y=ax+b.

RESULTS AND DISCUSSIONS

The range of variation in soil acidity at the proposed sampling depths encompasses a generous *pH* range with very strongly acidic reaction minima and moderately or weakly alkaline reaction maxima (ICPA, 1987). Maximum acidity values that fall into the slightly alkaline reaction class were also determined by Szanto et al. (2012) at sampling depths of 0-10 cm, 10-20 cm and 20-30 cm in areas partially overlapping with the SPs of this survey. The relative difference of 372% between the maximum and minimum value expresses a wide variation of *pH* values in the surveyed soils. The sample from the control sample area (SP 1) shows a slightly acidic reaction at all three sampling depths. To facilitate the reading of the charts of the statistical processing of the *pH* values and the adsorptive complex indices, the sampling depths of the soil samples at 0-5 cm, 10-15 cm and 30-35 cm were equated with sampling depths 1, 2 and 3.

In the case of the first sampling depth, the *pH* values are between 4.35 (very strongly acidic reaction in SP 2), and a maximum value of 8.65 (moderately alkaline reaction in SP 7West). At a depth of 10-15 cm, the minimum value is 4.31 (very strongly acidic reaction) and is registered in SP 16 (i.e. the Curmătura Perimeter additionally polluted by the emissions of vehicles passing through National Road DN 14B), and the maximum value is 8.6 (moderate alkaline reaction), in SP 7East. With regard the acidity at the sampling depth 3, the minimum value of 4.61 (strongly acidic reaction) was also determined in SP 16, and the maximum value of 8.48 (weakly alkaline reaction) was found in SP 9 with intensely humic soil at the sampling depth 1. The strongly and very strongly acidic reaction determined in SPs 2, 16, 17 (located at a distance of approx. 8km West away from the polluting industrial platform) is due to the Târnava Mare depression corridor that imprints the direction and the channelling of the dominant winds carrying the pollutants over a long distance).

To determine the dominant *pH* classes, the clustering tendency of the determined values was reviewed. Thus, about 36% of the values are within the *pH* range of 8.0-9.0 (conclusions similar to Iordache (2009) who conducted studies in the Copșa Mică area) which shows the preponderance of the slightly to moderately alkaline reaction of the soils at the time of the analytical determinations in the surveyed SPs (Figure 2). The soil reaction affects the activity and diversity of microorganisms living in the soil. Bacteria, the most useful microorganisms for the soil, are present in the weakly acidic to weakly alkaline reaction range. Descriptive statistics applied did not reveal the correlation between the *pH* and the distance or distance classes between the major polluter in the area and SA.

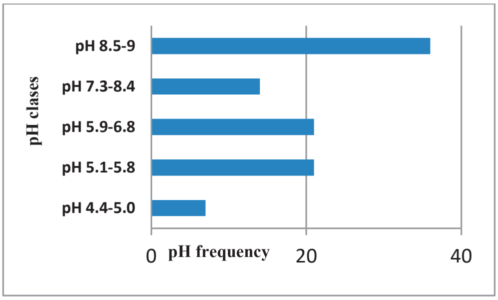

Figure 2. Weighted averages distribution frequency of *pH* for sampling depth 0-35 cm

The ratio of exchangeable bases (SB) is a valuable indicator in assessing soil fertility. The maximum values for the three sampling depths were determined in SPs 9, 14 and 15, and SP 16 obtained minimum values at all stages of soil sampling (Figure 3).

The statistical processing highlighted the fact that the sum of exchange cations did not showed a mathematical correlation with the distance between the main polluter of the surveyed area and the SA within the experimental device.

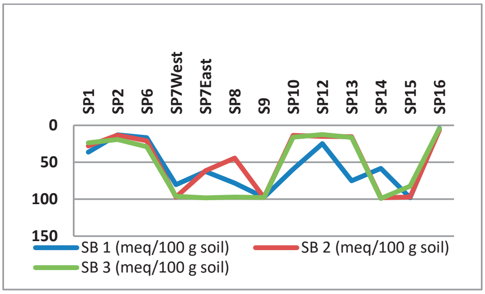

Figure 3. Variation with SPs and sampling depth of the sum of base exchange cations (SB)

Since no SB value exceeded 100, CEC was calculated by summing the base exchange cations (SB) and total exchangeable acidity (SH). CEC varies discontinuously with sampling depth. The high level of humus found in SP 8 and SP 14 correspond to high CEC values (Figure 4). In terms of CEC, SP 15 and 7East recorded maximum values and SP 16 recorded minimum values at the three sampling depths.

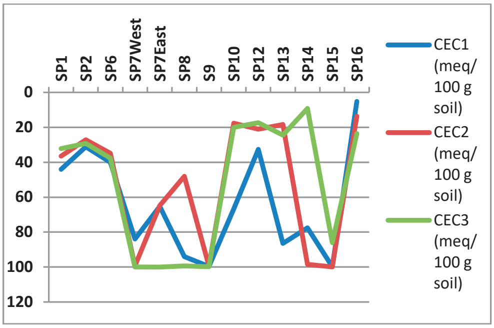

Figure 4. Dynamics of the CEC values calculated by sample plots in the experimental device for the three sampling depths considered

The downward trend of total exchange acidity SH is present in SPs 2, 6, 8, 10, 12, 13, 14 while for SAs 15 and 16, the determined values of SH increase with the sampling depth (Figure 5). Determination of SH is an important indicator since it features the presence of H^+ in the adsorptive complex of the soil, the proportion of which increases with the intensification of soil base saturation loss processes. The maximum values of SH were determined in SPs 2, 6, 16 while in SP 14 minimum values were found for the sampling depths 2 and 3.

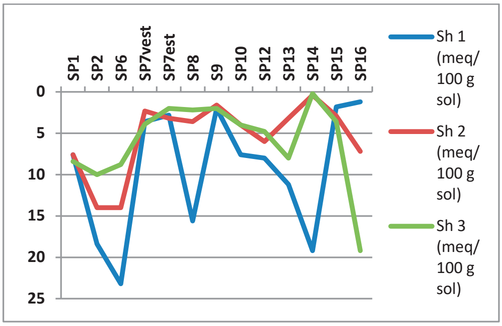

Figure 5.Variation of total exchangeable acidity (SH) by sample area and sampling depth

As in the case of the sum of exchangeable bases, the total exchangeable acidity did show a correlation with the distance between the point pollutant of the area and the SPs surveyed.

As one can notice from figure 6, and according to Metson (1961), the level of saturation with bases (V, %) is very high regardless of the SP considered or the sampling depth applied. According to the saturation with bases (V, %) calculated, the soil from the first sampling depth from SPs 2 and 6 is moderately washed by the bases, the other SPs being very weakly washed according to the classification given by Metson (1961). With regard extreme values, SPs 14 and 16 are clearly distinctive for the sampling depths 2 and 3.

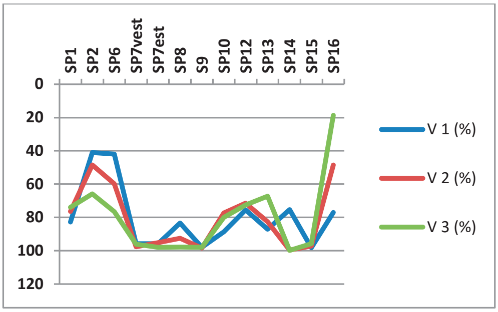

Figure 6. Fluctuation of the values of V (%) assigned to the sampling areas in the experimental device for the three sampling depths

Application of the statistical test resulted in correlation between SB and CEC, as well as between SB and V, % at all three sampling depths according to Table 1 and Figures 7 and 8.

Table 1. The value of the correlation coefficients between the studied variables

Correlation coefficient SB1-CEC 1	0.973041	**Correlation coefficient SB1-V%1**	0.771107
t_{exp}	14.61504	**t_{exp}**	4.195336
t_{table}	2.179	**t_{table}**	2.179
Correlation coefficient SB2-CEC 2	0.995543	**Correlation coefficient SB2-V%2**	0.828759
t_{exp}	36.5666	**t_{exp}**	5.130234
t_{table}	2.179	**t_{table}**	2.179
Correlation coefficient SB3-CEC 3	0.765227	**Correlation coefficient SB3-V%3**	0.809682
t_{exp}	4.117716	**t_{exp}**	4.779303
t_{table}	2.179	**t_{table}**	2.179

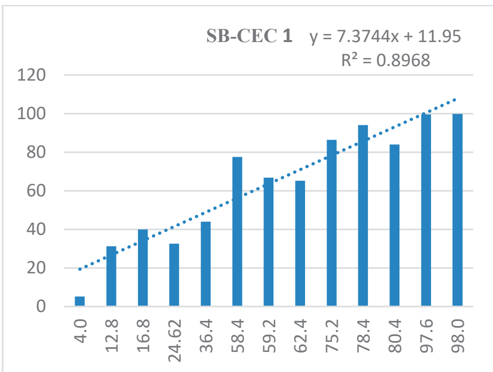

Figure 7. Correlation between SB and CEC at the soil sampling depth 1

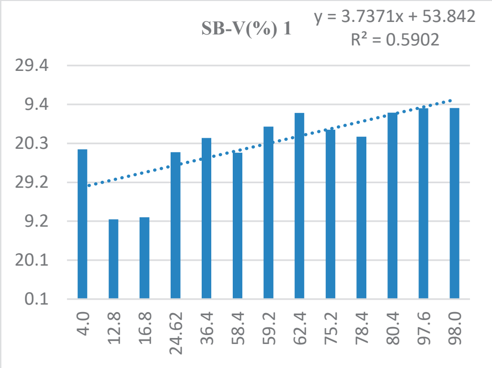

Figure 8. Statistical relationship between SB and V (%) at soil sampling depth 1

CONCLUSIONS

Through the multilateral and in-depth analysis of the physical and chemical characteristics of soils, it is possible to objectively establish the properties they possess and make available to cultivated plants. The *pH* of the surveyed soils indicates the dominance of weak-acidic to moderate-alkaline chemical reaction classes, an aspect also captured by other research conducted within the area suggesting a reduced mobilization of some potentially toxic heavy metals from the soil. Changes in terms of *pH* due to the action of anthropogenic pollutants depend on the type and chemical composition of pollutant emissions. SO_x-rich emissions from non-ferrous smelters increase soil acidity, and the presence of particles containing metal oxides cannot buffer the effect of acidic pollutants on soil *pH*.

The level of saturation with bases (V, %) of the surveyed soils is very high regardless of the SP or the sampling depth applied. The soil sampling depth 1 in SPs 2 and 6 is moderately base-washed, the other SPs being weakly base-washed. CEC varies discontinuously with sampling depth, and the high level of humus in SPs 8 and 14 corresponds to high values of CEC. The correlation coefficient (r) between Sb and CEC (r ranging 0.7652-0.9955) and between Sb and V% (r between 0.7711-0.8287) for the three sampling depths, indicates a very strong correlation between the variables subjected to research. Further studies may also research the influence of soil texture and organic matter content on CEC.

It is only through detailed research works that we can find the factors with optimal regimes, respectively the limiting factors by insufficiency or excess that determine both the nature of the species that can be cultivated (especially in the environmental restoring of the Copșa Mică area) and the level of the productivity thereof. The richness in humus and nutritious mineral substances in accessible forms, the depth of the physiological layer, the good loosening widen the range of species that can be selected and cultivated successfully. On the contrary, a marked friability or compactness, acidity or accentuated basicity of the soil is a limiting factor in terms of diversity of species that can be capitalised without requiring additional interventions that entails additional costs.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

AGRO-CLIMATOGENIC NEOHYDROMORPHISM: PLACE IN THE EVOLUTION OF ARABLE CHERNOZEMS

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Abstract

The evolution of chernozems within the current phase of anthropo-natural pedogenesis is influenced by the agro-climatogenic neohydromorphism whose development is determined by the modification of the atmospheric precipitation regime caused by climate change and the physical degradation of soils induced by agrogenesis. The latter manifests itself in the reduction of water permeability, the hydraulic conductivity of soils and the overwetting of their upper and middle segments during periods of heavy rainfall. As a result, within the space with an advanced degree of physical degradation, the automorphic-non-percolative hydric regime is replaced by the stagnant-non-percolative hydric regime (ephemeral, periodic, permanent) and the derno-chernosiomic pedogenesis is replaced by the hydrometamorphic one. Within it, the evolution of soils is determined by the processes induced by overwetting: gleyzation, montmorillonitization, metastructuring.

Key words*: non-percolating stagnant water regime, gleyzation, montmorillonitization, structure degradation, differential porosity.*

INTRODUCTION

The genesis of chernozems in the Pridanubian area is the product of typogenetic processes (formation and accumulation of humus, migration of carbonates, aggregation-structuring of the soil mass, biogenic accumulation) determined by the evolution and dynamics on the pedological scale of time, climate and other pedogenetic factors (biological, geomorphological) determined directly from this. As a result, the chernozems in the region have a high capacity to buffer the impact of various natural and anthropogenic factors, to adapt to the newly formed conditions and to preserve the characteristics and regimes of the soils, thus ensuring the stability and reproduction of the steppe landscapes. The substitution of the latter with agro-landscapes attracted significant changes in the pedogenesis of chernozems that led to the destabilization of agro-landscapes and the disturbance of the balance between their components established on the pedological scale of time. This led to the establishment in soils of a complex of interdetermined and interdependent processes and reactions materialized in the neohydromorphization of automorphic soils. The development of neohydromorphism gained momentum at the beginning of the seventies of the last century, the surface of neohydromorphized soils increasing from 30 thousand ha in 1972 to 50 thousand ha in 1980, 80 thousand ha at the end of the nineties, 120 thousand ha at the beginning of the current century. According to more recent calculations, their surface in 2020 was about 180 thousand ha. In our opinion, the development of neohydromorphism overlapped, over time, with the intensification of the processes of physical soil degradation and the differentiation of the soil profile into two distinct functional layers: a) agrogenic and b) underagrogenic.

Previous research on this subject was limited to: a) establishing the phenomenon; b) recording, through the lens of fertility factors, the quantitative and qualitative changes in soil characteristics and regimes; c) establishing the place in the classification system; d) development of procedures and measures for improvement and utilization. The genetic aspects were reduced only to the stagnation of water on the surface of the impermeable clay layer for water (Сувак, 1977; 1986).

In this paper, the neohydromorphism of chernozems is examined through the prism of the unity of the pedogenetic process within the genetic-evolutionary chain "factors ↔ regimes ↔ processes ↔ properties ↔ soil" taking into account the place of the soil in the evolution of pedogenetic factors. Through this prism of ideas, the soil cover is considered a factor with a decisive role in the current climate trend on a global and regional scale.

MATERIALS AND METHODS

In the framework of the working concept for evaluating the genesis of neohydromorphism, we start from the principle of the priority role of the degradation of physical properties and regimes in determining the meaning and intensity of cernoziomic typogenetic processes within the current phase of anthropo-natural cernoziomic pedogenesis. The disaggregation-destructuring of the chernozemic structure (3-1 mm), the reduction of aggregate stability, the overcompaction of the arable and sub-arable layer, the degradation of the pore space led to the reduction of water permeability and hydraulic conductivity, thus creating premises for the stagnation of water from atmospheric precipitation in the agrogenic layer of arable chernozems. At the same time, against the background of an increase in the average amount of precipitation by 8% in the cold semester and a reduction by 2.5% in the warm semester of the years 1991-2020 compared to 1961-1990, their seasonal regime changed in the sense of increasing the amount in the autumn months and decreasing in the summer months.
According to the data of the State Hydrometeorological Service, in about 40% of the years, in some periods of the year, the amount of atmospheric precipitation can exceed the amount of precipitation during the cold period of the year (1.XI-1.IV).
Against the background of the general decrease in the number of days with precipitation ≥0.1 mm, the frequency of the number of days with extremely abundant precipitation ≥50 mm has increased greatly.
The number of days with precipitation >20 mm is increasing by 11.7%, those with precipitation >30 mm by 27.4% and those with precipitation >50 mm is increasing by 132%.
In addition, in some years the deviation of the amount of precipitation from the multiannual average value can constitute ± 200-300 mm. In about 40% of the years, the deviation from the multiannual norm is ± 100 mm.
In conditions of an advanced degree of physical degradation in years/periods with abundant atmospheric precipitation, water stagnates in the upper and middle segment of the soil profile and the processes induced by overwetting start.
According to our more recent research, two distinct types are clearly outlined within the neohydromorphization process (Jigău et al., 2017).
The development of the first type is determined by the increase in the degree of continentality of the climate materialized in regional manifestations of humidification and aridification of soils that determine the imbalance of relations between the components of agro-landscapes and their degradation. In this sense, we consider that neohydromorphism is a response reaction of chernozems to the cumulative impact of the entire complex of anthropo-natural factors and one of the main causes that favors it is physical degradation. In this sense, research has shown that in years with abundant spring precipitation (2017), gravity water drains from the soil profile only in the second half of June.
In such years, the state of overwetting in the active pedogenetic layer of arable chernozems is maintained for a long period of time (April-June) (Jigău et al., 2017).
The second type of neohydromorphism is caused by the cumulative effect of humidification of the water regime of arable chernozems under conditions of agrophytocenoses as a result of the change in the mode of water consumption from the soil to evapo-transpiration.
The phenological rhythm of operation and the particularities of the root system of cultivated plants cause the reduction of water consumption from the lower horizons of the soil profile. The shorter the vegetation period of agrophytocenoses compared to biocenoses also contributes to this. At the same time, in arable chernozems, the porous space is divided

into two substrates, with the formation of an anisotropic-discontinuous space. Therefore, during vegetation, water reserves from the agrogenic layer are consumed more intensively, including physical evaporation. As a result, there is a slow cumulative increase of water reserves in the lower horizons with the start of the processes of loosening the structural aggregates and clogging the pores, creating a predominantly reducing aerohydric regime.

In these conditions, the processes of quasi-gleyzation and local gleyzation start in the soils with the subsequent intensification of the phenomenon of changing the way of placement and packing of the solid components, and as a result, of the ratio between the mass and the volume of the soil phases in favor of the solid phase materialized in increasing the degree of compaction and reduction of the volume of the porous space.

Therefore, over time, the thickness of the layer available for wetting is reduced, so that even in the years with the amount of precipitation corresponding to the multiannual average, in the early phases of the vegetation period, in soils with an advanced degree of physical degradation, there is an excess of moisture, which implies a change in the meaning and the intensity of the chernozemic typogenetic processes and favors the hydrometamorphization processes (Jigău et al., 2017).

Starting from the working concept, the research included the evaluation of the changes in typogetic processes within a “chain” of soils with different degrees of development of neohydromorphism. The research involved applications in the field and in the laboratory. In the field were studied 6 soil profiles that characterize soils with different degrees of physical degradation and hydrometamorphism. Within the evaluated chain of soils the degree of physical degradation and hydrometamorphism increases as follows: typical chernozem with low humus content (forest strip) → typical chernozem with low humus content (arable land) → typical cvasi-gleyied chernozemlike soil with low humus content (arable land) → typical gleyied chernozemlike soil with low humus content → typical compacted chernozemlike soil with low humus content → gleyied hardsetted chernozemlike soil with low humus content. In order to evaluate the quantitative changes in the evolution processes of the mineralogical component, the mineralogical composition of the fine clay fraction was evaluated with the X-ray diffraction method (the analyzes were carried out in the Institute of Geology and Seismology, Chisinau). The humus composition was evaluated with the Kononova-Belicikova method (Kaurichev, 1980). The structural-aggregate composition was evaluated with the Savvinov method. The differential porosity was evaluated based on the suction curve (Jigău & Nagacevschi, 2006).

RESULTS AND DISCUSSIONS

The agro-climatogenic neohydromorphization of chernozems involves several genetic-evolutionary stages: a) seasonal - substitution of the non-percolating automorphic water regime with the stagnant-non-percolative ephemeral water regime; b) transitional - the establishment in soils of the periodic stagnant-non-percolative water regime; c) the development and deepening of the permanent stagnant-non-percolative water regime. Within the mentioned genetic-evolutionary stages, the derno-chernozemic pedogenesis is replaced by the hydrometamorphic pedogenesis, which involves the modification of the composition of all soil phases, including the living one, and materializes in the modification of the direction and intensity of the humus formation and accumulation process, the montmorillonitization of the finely dispersed fraction, the metastructuring of the soil mass, the restructuring of the porous space (Jigău et al., 2017; Jigău & Leşanu, 2021).

The evolution of the mineralogical composition of the fine clay fraction (<0.001 mm) under conditions of neohydromorphism is determined by its montmorillonitization as a result of illite-hydromicas (Table 1).

The data presented in Table 1 shows that the mineralogical composition of the <0.001 mm fraction of the automorphic chernozems, within the investigated chain, is characterized by illite-montmorillonite composition and relatively uniform distribution of illite and montmorillonite on the profile. Changing the water regime, already in the initial phase, leads to the start of the montmorillonitization process and the increase of the montmorillonite content up to 25.1%. With the increase in the degree of hydrometamorphism, the intensity of the montmorillonitization process intensifies with the formation of compacted gleyied and hardsetted gleyied profiles.

Table 1. The mineralogical composition of the fine clay fraction (<0.001 mm) of soils with different degrees of hydrometamorphism

Soil	Depth, cm	Mineral content, %				Finely dispersed quartz
		Montmorillonite	Illite	Chlorite	Kaolinite	
Typical chernozem with low humus content (forest strip)	0-10	40.8	44.3	5.9	9.0	13.9
	20-30	44.3	40.0	5.3	10.4	
	40-50	43.8	45.5	4.5	6.2	
Typical cvasi-gleyied chernozemlike soil with low humus content (arable land)	0-10	40.8	44.3	5.3	9.6	13.9
	20-30	44.3	40.8	8.5	6.4	6.9
	40-50	59.6	25.1	10.9	4.4	7.6
Typical gleyied chernozemlike soil with low humus content	0-10	37.2	45.6	9.8	7.4	17.8
	20-30	55.4	29.7	7.9	7.0	6.9
	40-50	73.9	15.3	4.5	6.3	5.3
Typical compacted gleyied chernozemlike soil with low humus content	0-10	44.3	46.1	6.9	2.7	12.2
	20-30	62.7	25.0	7.3	10.0	10.1
	40-50	72.4	17.5	6.1	4.0	10.1
Gleyied hardsetted chernozemlike soil with low humus content	0-10	37.8	45.3	8.4	8.5	18.7
	20-30	69.7	18.4	8.8	3.1	16.1
	40-50	69.3	19.9	6.4	2.3	7.6

Increasing the degree of hydrometamorphism leads to increasing the share of the humification process and reducing the share of the mineralization process within the decomposition-transformation process of organic matter in the soil, manifested in the increase of the organic carbon content. At the same time, however, as the hydrometamorphization process intensifies, the active humifer profile of the soils is clearly divided into two layers with different organic carbon content. The 0-30 cm layer in which the row organic matter is mainly stored is characterized by a unidirectional trend of increasing the organic carbon content. In the 30-50 cm layer, the organic carbon content remains practically unchanged. At the same time, the distribution of the fraction of mobile humic substances (extracted with 0.1 NaOH solution) in the active humic layer of chernozemlike soils indicates a more intensive humification process in the 0-30 cm layer.

The process of humification under conditions of neohydromorphism proceeds with the formation, predominantly of humic acids, and is manifested in the increase of Cha: Cfa values in the 0-30 cm layer of chernozemlike soils (Table 2). The changes that can be seen in the content and profile distribution of the carbon of the non-hydrolyzed residue allow us to consider that the particularities of the humic system of the studied soils are determined by the hydrometamorphism of the pedogenetic process.

The changes in the mineralogical composition of the fine clay fraction and in the composition of the humic system of the soils led to the establishment of the metaaggregation process in the chernozimoid soils, which led to a significant change in the structural-aggregate composition of the chernozemlike soils (Table 3).

This implies the increase, in chernozemlike soils compared to chernozems, of the content of aggregates >10 mm and the reduction by 10-15% of the content of agronomically valuable aggregates (10-0.25 mm).

Table 2. The composition of the humic system
of chernozemlike soils with different degrees of hydrometamorphism

Soil	Depth, cm	Carbon content, %	The composition of the humic system, % of the total C content				Cha/ Cfa
			C extracted with 0.1 NaOH	Cha	Cfa	C unhydrolyzed residue	
Typical chernozem with low humus content (forest strip)	0-10	2.86	5.8	36.1	18.9	39.2	1.91
	10-20	2.93	6.7	37.6	18.8	36.9	2.00
	20-30	2.32	7.7	34.4	19.7	38.2	1.75
	30-40	1.98	7.3	33.8	21.1	38.8	1.60
	40-50	1.72	6.1	33.1	21.9	38.4	1.51
Typical chernozem with low humus content (arable land)	0-10	2.31	9.6	31.7	20.6	38.1	1.54
	10-20	2.16	9.3	34.2	19.1	37.4	1.79
	20-30	1.87	8.4	35.8	20.3	35.0	1.76
	30-40	1.71	5.8	32.4	21.5	40.3	1.51
	40-50	1.67	4.7	32.7	22.1	40.5	1.47
Typical cvasi-gleyied chernozemlike soil with low humus content (arable land)	0-10	2.77	10.2	34.9	18.9	36.0	1.85
	10-20	2.42	10.0	36.2	18.9	34.9	1.92
	20-30	1.90	7.1	33.2	19.4	40.3	1.71
	30-40	1.74	5.4	33.8	20.7	40.1	1.63
	40-50	1.65	4.8	32.6	21.1	41.5	1.55
Typical gleyied chernozemlike soil with low humus content	0-10	2.81	11.8	38.2	19.1	30.9	2.00
	10-20	2.63	11.3	37.7	19.4	31.6	1.94
	20-30	2.55	10.0	35.5	20.3	34.2	1.75
	30-40	1.83	7.9	33.8	20.9	37.4	1.62
	40-50	1.66	7.7	31.5	21.7	39.1	1.45
Typical compacted gleyied chernozemlike soil with low humus content	0-10	2.91	11.5	39.1	19.8	29.6	1.97
	10-20	2.71	10.7	38.3	20.3	30.7	1.89
	20-30	2.27	6.3	36.8	20.9	36.0	1.76
	30-40	1.76	5.1	34.0	21.6	39.3	1.57
	40-50	1.58	4.1	33.1	21.8	41.0	1.52
Gleyied hardsetted chernozemlike soil	0-10	2.86	11.7	38.9	19.4	30.0	2.01
	10-20	2.67	11.0	37.9	19.9	31.2	1.90
	20-30	2.44	9.9	36.2	20.3	33.6	1.78
	30-40	1.80	5.3	33.7	20.9	40.1	1.61
	40-50	1.60	4.4	31.4	21.7	42.7	1.45

The content of 5-1 mm aggregates and waterstable aggregates undergo less noticeable changes.

In our opinion, the "relative conservation" of the chernozems structure (5-1 mm) and its waterstability is due to the permanent reproduction of aggregates <5 mm with the participation of newly formed humic substances, the aggregation process being supported by the predominance of calcium cation (Ca^{2+}) in the composition of the adsorptive complex of chernozemlike soils (Jigău & Leşanu, 2021).

The changes in the structural-functional organization of chernozemlike soils led to the restructuring of their porous space. From Table 4 we can see that as the degree of hydrometamorphism intensifies, the total pore volume decreases significantly from 62-63% to 47-52% in the 0-30 cm layer and from 61-62% to 43-45% in the 30-50 cm layer.

The specified changes are made on account of interaggregate pores (aeration pores) whose volume is much (about 1.3-2 times) below the limit of critical values. As the soil mass is reorganized in the compact and slitted chernozemlike soils, a slight reduction in the capillary pore volume can also be seen. The changes in the composition of the porous space allow us to consider that the agro-climatogenic neophydromorphism established in chernozems with an advanced degree of physical degradation has an irreversible unidirectional character.

Table 3. Aggregate structural composition of chernozem soils
with different degree of hydrometamorphism

Soil	Depth, cm	Aggregate size, mm Content of aggregates, %				Waterstable aggregates
		>10	Σ10-0.25	5-1	<0.25	
Typical chernozem with low humus content (forest strip)	0-10	9.6	87.2	57.4	3.2	50.5
	10-20	10.9	85.9	59.8	3.2	49.8
	20-30	11.3	85.8	61.3	2.9	48.8
	30-40	12.8	84.5	57.9	2.7	48.2
	40-50	13.4	83.9	56.6	2.7	47.8
Typical chernozem with low humus content (arable land)	0-10	16.9	80.2	52.1	3.9	46.5
	10-20	16.7	79.5	53.6	3.8	49.7
	20-30	19.9	77.0	53.2	3.1	47.7
	30-40	13.3	83.8	54.7	2.9	51.3
	40-50	13.8	83.3	52.0	2.9	50.8
Typical cvasi-gleyied chernozemlike soil with low humus content (arable land)	0-10	24.6	71.6	50.7	3.8	46.5
	10-20	28.9	68.6	48.4	2.5	49.6
	20-30	36.2	62.0	48.4	1.8	48.6
	30-40	29.6	68.6	49.1	1.8	49.4
	40-50	28.4	69.9	48.9	1.7	48.9
Typical gleyied chernozemlike soil with low humus content	0-10	26.1	69.7	52.5	4.2	47.4
	10-20	28.4	68.3	49.8	3.3	47.8
	20-30	38.9	59.7	51.6	1.4	50.3
	30-40	32.7	65.6	49.0	1.6	46.1
	40-50	32.0	66.9	48.3	1.1	48.4
Typical compacted gleyied chernozemlike soil with low humus content	0-10	26.3	69.2	53.6	4.5	49.8
	10-20	31.1	67.0	46.3	2.9	49.6
	20-30	39.1	59.1	43.2	1.0	48.3
	30-40	39.8	59.4	41.6	0.8	49.8
	40-50	35.3	63.5	44.9	1.2	45.7
Gleyied hardsetted chernozemlike soil with low humus content	0-10	30.7	67.4	48.5	1.9	50.2
	10-20	32.5	66.2	46.7	1.3	47.7
	20-30	36.9	62.4	43.1	0.8	44.9
	30-40	39.1	59.9	35.9	1.0	40.7
	40-50	38.0	61.0	36.6	1.0	39.6

Table 4. The differential porosity of chernozemic soils with different degrees of hydrometamorphism

Soil	Depth, cm	Porosity, (Ɛ), %					
		Ɛt	Ɛw	Ɛase	Ɛall	Ɛk	Ɛaer
Typical chernozem with low humus content (forest strip)	0-10	62.5	31.4	8.7	5.1	17.6	31.1
	10-20	62.7	34.8	8.4	5.4	21.0	27.9
	20-30	62.3	33.6	8.4	5.6	19.6	28.7
	30-40	61.6	30.4	8.1	5.5	16.8	31.2
	40-50	58.3	29.9	8.3	5.7	15.9	28.4
Typical chernozem with low humus content (arable land)	0-10	57.8	33.7	8.5	5.3	19.9	24.1
	10-20	56.1	35.3	8.9	5.5	20.9	20.8
	20-30	54.8	36.0	9.1	5.7	21.2	18.8
	30-40	53.5	36.4	9.3	5.7	21.4	17.1
	40-50	52.7	38.3	9.8	5.9	22.6	14.4
Typical cvasi-gleyied chernozemlike soil with low humus content (arable land)	0-10	55.3	44.0	9.1	5.6	29.3	11.3
	10-20	55.3	44.3	9.0	5.4	29.9	11.5
	20-30	53.7	42.8	9.4	5.6	27.8	10.9
	30-40	52.1	43.2	9.7	6.0	27.5	8.9
	40-50	50.9	44.8	10.4	6.2	28.2	6.1
Typical gleyied chernozemlike soil with low humus content	0-10	52.7	44.3	9.3	6.3	28.7	8.4
	10-20	53.4	44.8	9.3	6.4	29.1	8.6
	20-30	51.2	43.0	9.8	6.0	26.2	8.2
	30-40	49.7	42.2	9.8	5.9	26.5	7.5
	40-50	49.3	42.0	10.1	5.9	26.0	7.3
Typical compacted gleyied chernozemlike soil with low humus content	0-10	51.8	43.7	9.2	6.0	28.5	8.6
	10-20	49.4	42.0	9.4	5.7	26.9	7.4
	20-30	47.8	41.6	9.6	5.7	26.3	6.2
	30-40	45.3	40.8	9.6	5.4	25.8	5.3
	40-50	45.9	40.9	9.4	5.9	25.6	5.0
Gleyied hardsetted chernozemlike soil with low humus content	0-10	50.1	41.9	9.3	5.7	25.9	8.2
	10-20	47.3	40.2	9.3	5.7	24.2	7.1
	20-30	45.0	40.0	9.6	5.4	25.0	5.0
	30-40	43.7	38.2	9.6	5.4	22.2	5.5
	40-50	43.4	38.8	9.8	5.3	23.7	4.6

CONCLUSIONS

The development of agro-climatogenic neophydromorphism is favored by changes in the atmospheric precipitation regime induced by the current trend of regional climate conditions and by the advanced degree of physical degradation of arable chernozems. Within it, the derno-chernosiomic pedogenesis process is replaced by the hydrometamorphic pedogenesis process materialized in the montmorillonitization of the mineralogical composition of the fine clay, the intensification of the humification process with the predominant formation of humic substances and the metaaggregation of the soil mass with the formation of aggregates >10 mm. The evolution of the volume and structure of the porous space indicates that the processes induced by neohydromorphism are unidirectional and irreversible, materialized in a new form of chernozems degradation - the hydrological one.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

REDOX REGIME OF ALLUVIAL SOILS IN THE SIVERSKY DONETS BASIN

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Abstract

Alluvial soils are a special product of pedolithogenesis, which takes place in floodplain conditions. Their formation is associated with the passage of floodplain and flood processes. This leads to the formation of soils that have maximum biogenicity and fertility levels. Changes in the conditions of soil formation during the year (changes in the level and composition of groundwater, the presence or absence of floods, features of the activity of the biological factor) cause changes in the physical and chemical parameters of soils, the intensity of the passage of biochemical processes, which is necessarily reflected in redox processes. This relationship is not only direct, but also reverse. The indicators of the redox potential of alluvial soils of the floodplains of the rivers of the Siversky Donets basin are given in the article. They were determined by the seasons of the year in layers at depths from 0 to 40 cm. The ORP level was determined in the field using the potentiometric method. Both for soil layers and for the seasons of the year, the indicators of the redox potential indicate the passage of reduction processes, and only in summer these processes can have the character of low-intensity oxidation. The restoration processes in the soil are most intense in spring. The bog alluvial soils of the near-Teras depression are characterized by more pronounced processes of reduction, as evidenced by the minimum values of the redox potential.

Key words: *alluvial soil, floodplain, redox potential.*

INTRODUCTION

The Siversky Donets River is one of the largest rivers in Ukraine and the main water artery in the east of the country. More than 64% of the territory of the east of Ukraine is the drainage basin of the Siversky Donets. This is the area from which atmospheric water is collected in the channel of this river. The basin occupies 9.1% of the territory in relation to the area of the country and is the fourth by largest-54,500 km^2.

There are 3,112 rivers at this area, of which 118 are small and medium-sized. There are 106 such rivers on the territory of the Kharkiv region, which belong to the Siversky Donets basin (Vasenko at al., 2006).

Floodplains are areas that are inundated by river waters during a flood and are components of a river valley. Alluvial soils, which are unique in their genesis and properties, were formed on floodplains. Gleyic Fluvisol, Calcari-Gleyic Fluvisol and Humi-Gleyic Fluvisol prevail among them (Vepraskas & Craft, 2017). The area of soils with weak waterlogging within Kharkiv region is 23,000 hectares, and with strong waterlogging is 770 hectares. A significant area of soils with waterlogging is alluvial soil (Fluvisol) (Ecological, 2021).

The relevance of the study of floodplain soils is beyond doubt, neither from a purely scientific point of view or from a practical point of view.

The floodplain alluvial soils occupy a special place among the great variety of soils. They differ from watershed soils in genesis, composition indicators, and economic use. These soils are formed in complex biogeochemical conditions due to the heterogeneity of the floodplain process. It is related to the flood regime, features of the granulometric and mineralogical composition, physic-chemical and physical properties of alluvial deposits in different parts of the floodplain, features of the composition and regime of the groundwater level, the specifics of the species composition of plants, and the microclimatic conditions of the river valley. These soils are in a pronounced development and transformation. This is due to flooding processes, the location of this territory at the point of intersection of several geochemical pathways of substances and the peculiarities of variegated changes in the physical, chemical and physic-chemical parameters of soils both during the day and according to the seasons of the year. The combined effect of these factors causes the formation of soils of complex genesis and with specific properties.

Floodplain soils were distinguished by a higher level of fertility compared to soils of flat interfluve. A valuable natural sources of cheap and biologically complete feed for animals were located here (Fageria et al., 2002; Lóránt & Podmaniczky, 2015).

The alluvial soils of the floodplains of the Siversky Donets basin, in contrast to the zonal soils located in the adjacent territories, are occupying a relatively small area. They are almost not studied from the point of view of the properties and mode of the level of oxidation-reduction processes in natural conditions. A more thorough assessment of the ecological state of the functioning of alluvial soils and a clearer understanding of their genesis will be provided when studying this issue. The purpose of the research: to investigate the level of redox potential during the growing season in field conditions of alluvial soils of small and medium-sized rivers of the Siversky Donets basin.

Each type of soil is distinguished by a unique level of redox potential and its dynamics. Changes in the conditions of soil formation during the year (changes in the level and composition of groundwater, the presence or absence of floods, the peculiarity of the activity of biological factors) cause changes in the physical and chemical parameters of the soil, the intensity of biochemical processes. As a result, redox processes are change. This connection is not only direct, but also inverse. Aeration and chemical composition of the soil affects the size of the redox potential. The above-mentioned indicator affects the transformation of soil components (Davidson et al., 2015; Denef & Lehmann, 2016; Nunan & Boivin, 2016; Zak & Tilman, 2018; Bragato et al., 2019).

There is the most contrasting dynamics of the redox potential in soils with a close occurrence of groundwater and a variegated biological factor, which is associated with significant changes in the water-air regime of the soil and large fluctuations in soil temperature. These types of soils include alluvial (Fluvisol) soils (Chen et al., 2017; Herrmann & Werban, 2017; Fuentes-Rodriguez et al., 2018).

There is heterogeneity of redox potential not only in time, but also for each genetic horizon separately and even for microzones within one genetic horizon for the prevailing majority of alluvial (Fluvisol) soils. A lower indicators of redox potential exist in overmoistened glial horizons of the hydromorphic series of soils. According to scientists, this is due to the heterogeneity of microbiological activity and aeration in the soil profile (Zhelezova et al., 2013; Komatsu & Sato, 2021).

I. Kaurichev separated into independent groups semi-hydromorphic and hydromorphic soils, which also include alluvial (Fluvisol) soils, according to OVP indicators. The first group has seasonal waterlogging in the upper or lower horizons or their entire profile. The contrast indicators of ORP are typical for the soils of this group. Another group has rewetting throughout the soil profile throughout the year (Kaurichev, 1982).

Redox potential (ORP or redox potential) determines the redox conditions in the soil, that is, the degree of aeration and the level of biogenicity in it (McDaniel et al., 2006; Belyaeva et al., 2017).

The value of ORP at the level of 600-750 mV is typical for maximally aerobic conditions, 400-600 mV - for normally aerated conditions, 300-400 mV - for conditions with moderate waterlogging, where aeration is difficult, 200-300 mV - for conditions with medium waterlogging, where aeration is significantly complicated, and less than 200 mV - for conditions with a predominance of recovery processes, sludge formation actively occurs in the soil and compounds toxic to plants accumulate (Khtryan, 1976).

MATERIALS AND METHODS

The studies were carried out within the following territories: the central and terraced parts of the floodplain of the Mokryi Izyumets River and the riverbed, central and terraced parts of the floodplain of the Voloskaya Balakleyka River, Izyumsky District, Kharkov Region.

Mesophilic and hygrophilic herbaceous vegetation grows on the territory of floodplains. The territories are used as natural hayfields and pastures.
The soil covering the flood of the river Mokriy Izyumets and Voloska Balakliyka to the basin of the Siversky Dintsya is to be folded and formed from different taxonomic units.
On the territory of the central part of the floodplain of the Mokriy Izyumets river, Izyumsky district, Kharkov region, Humi-Gleyic Fluvisol (N 49°24'03,0", E 37°19'39,2") studied.
On the territory of the near-terrace part of the floodplain of the Mokriy Izyumets river, Izyumsky district, Kharkov region, Humi-Gleyic Fluvisol (N 49°24'00,7", E 37°19'47,5") studied.
On the territory of the near-channel part of the floodplain of the Voloska Balakliyka river, Izyumsky district, Kharkov region, Alluvial Gleysol (N 49°32'43,2", E 37°02'36,4") studied.
On the territory of the central part of the floodplain of the Voloska Balakliyka river, Izyumsky district, Kharkov region, Alluvial Gleysol (N 49°32'41,1", E 37°02'35,8") studied.
On the territory of the near-terrace part of the floodplain of the Voloska Balakliyka river, Izyumsky district, Kharkov region, Alluvial Gleysol (N 49°32'37,9', E 37°02'39,9") studied.
Determination of ORP was carried out in soil layers 0-10, 10-20, 20-30 and 30-40 cm in field conditions immediately after the opening of the horizons according to the seasons of the growing season - spring, summer and autumn.
The EMF was measured (in mV) between the EPV-1 thin-layer platinum electrode (Figure 1) and the EVL-1M auxiliary silver chloride electrode (Figure 2), which were lowered into the soil ball, to determine the soil redox potential. The electrodes were connected to a portable millivoltmeter pH-150 (Figure 3). The temperature compensation was performed before each measurement according to the readings of an electronic temperature sensor. ORP was measured after 15 min after the installation of the electrodes – during this period, equilibrium is established between the soil and the electrodes. For the final calculation of the ORP index, 201 was added to the obtained figure to take into account the value of the potential of the auxiliary electrode with silver chloride.

Figure 1. Platinum electrode EPV-1

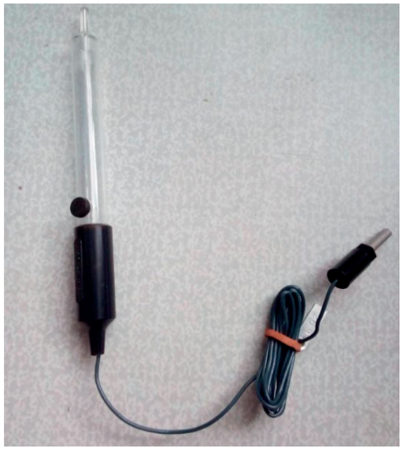

Figure 2. Auxiliary silver chloride electrode EVL-1M

Figure 3. Portable millivoltmeter pH-150

A mixture of salts (3.8018 g $K_4[Fe(CN_6)]$ and 13.5001 g $K_3[Fe(CN_6)]$) in 1 liter of distilled water is used as a reference solution for ORP measurements. The EMF of the circuit at 20°C±1°C relative to the auxiliary electrode with silver chloride is 272 mV±5 mV. A check against the reference solution is carried out before each new test point.

RESULTS AND DISCUSSIONS

As mentioned earlier, alluvial soils have quite specific properties. This originality is also manifested in the dynamics of the redox potential (Table 1).

The floodplain of the Voloska Balakliyka river is monotonous in terms of soil cover. There is Alluvial Gleysol irrespective of the part of the floodplain. At the same time, regardless of the homogeneity of the soil, the indicators of redox potential is differ depending on the part of the floodplain.

The ORP of the Alluvial Gleysol of the near-channel part of the floodplain of the Voloska Balakliyka river, which is somewhat elevated above other parts of the floodplain, is differs both with depth and with the seasons of the year. The ORP index indicates the weakly reducing nature of redox processes in the spring in the 0-10 cm soil layer. Deeper, the degree of reduction processes increases and reaches a maximum in the 20-30 cm layer, where the ORP is equal to 238 mV. In the summer, the ORP indicator changes dramatically in this soil in almost all the studied layers. Moderately reducing conditions are created in the 0-10 cm layer, which change sharply to weakly oxidizing conditions with increasing depth. This is indicated by the ORP indicators in the 10-20 cm soil layer is 438 mV and in 20-30 cm - 404 mV. Moderately reducing conditions are created at a depth of 30-40 cm - 282 mV. This digital does not differ significantly from the indicator recorded in the spring.

Table 1. Content and profile distribution of ORP index in floodplain soils of Siverskyi Donets basin

Rivers	Part of floodplain	Soils	Depth, cm	ORP, mV Selection period Spring	Summer	Autumn
Mokriy Izyumets	Central	Humi-Gleyic Fluvisol	0-10	324	464	339
			10-20	339	176	361
			20-30	280	351	305
			30-40	300	129	329
	Near-terrace	Humi-Gleyic Fluvisol	0-10	300	255	303
			10-20	291	449	327
			20-30	330	440	334
			30-40	304	417	315
Voloska Balakliyka	Near-channel	Alluvial Gleysol	0-10	306	239	323
			10-20	253	428	280
			20-30	238	404	263
			30-40	280	282	307
	Central	Alluvial Gleysol	0-10	321	271	333
			10-20	295	342	316
			20-30	311	252	318
			30-40	300	202	302
	Near-terrace	Alluvial Gleysol	0-10	283	369	295
			10-20	258	160	267
			20-30	315	423	326
			30-40	279	198	288

In autumn, the pattern of the profile distribution of the potential repeats the spring distribution with a slight increase in the level. Weakly reducing conditions are formed in the upper soil layer (0-10 cm - 323 mV) and the deepest layer under investigation (30-40 cm - 307 mV). At a depth of 10-30 cm, the ORP indicator does not exceed 300 mV, which indicates moderately reducing conditions.

With regard to the redox conditions of the Alluvial Gleysol of the central part of the floodplain of the Voloska Balakliyka river, it is possible to assert, based on the OVP indicators, that the restoration processes are intensified compared to the conditions in the soil of the near-channel part of the floodplain. In the spring, in the layers of 10-20 cm and 30-40 cm, the indicators of redox potential differ by only 5 mV and are equal to 295 mV and 300 mV. These indicators indicate that the intensity of recovery processes is on the border between weak and moderate intensity. Between the 0-10 cm and 20-30 cm layers, the difference in the ORP level is somewhat larger - 10 mV. In these layers, restoration processes begin to manifest themselves well. In summer, the intensity of recovery conditions increases almost throughout the profile. On the contrary, only at a depth of 10-20 cm, the intensity of regeneration processes is suppressed compared to spring. Here the ORP level exceeds the mark of 300 mV and is equal to 342 mV. In autumn, with increasing depth, there is a clear tendency to decrease the ORP indicator from 333 mV in the 0-10 cm layer to 302 mV in the 30-40 cm layer. According to the indicators studied, the soil layers of 10-20 cm and 20-30 cm are almost the same. In general, regeneration processes take place here with moderate activity.

The ORP indicators of the Alluvial Gleysol of the near-terrace part of the floodplain of the Voloska Balakliyka river, which is lower relative to the level of the central floodplain, indicate that this soil is more moist than the previous ones. In spring, the maximum value of ORP is fixed at a depth of 20-30 cm - 315 mV. In this part of the soil profile, the nature of the processes acquires the manifestation of weak recovery. Higher and deeper, recovery processes is intensify and has reach maximum development for a part of the profile up to a depth of 40 cm in a layer of 10-20 cm (258 mV). In the summer, there was a sharp differentiation of the layers of the Alluvial Gleysol according to the nature of oxidation-reduction processes. In addition to reduction processes, weak oxidation processes also appear here. As evidenced by the ORP indicator in the 20-30 cm layer - 423 mV. Intensive recovery processes that take place in the soil layers of 10-20 cm (160 mV) and 30-40 cm (198 mV) create an unfavorable nutrition regime for plants. In the upper part of the soil, 0-10 cm, weakly reducing conditions are created, which is indicated by the ORP of 369 mV. In autumn, the variability of the ORP level decreases with depth and is within 200-300 mV in most soil layers. Moderate recovery processes are underway here. The intensity of these processes decreases somewhat at a depth of 20-30 cm, where the redox potential rises to 326 mV.

The soil cover of the Mokryi Izyumets floodplain is represented by one type of soil - Humi-Gleyic Fluvisol. Compared to Alluvial Gleysol of the Voloska Balakliika river floodplain, the redox potential index is on average higher, which indicates a lower moisture content of the Humi-Gleyic Fluvisol and a decrease in the intensity of recovery processes.

At the spring, the redox potential of the Humi-Gleyic Fluvisol of the central floodplain was within 280-339 mV. With depth, there is a general tendency to decrease this indicator. The nature of the processes is variable and weakly reversible. In summer, there is a significant change in indicators compared to spring and autumn, and a sharp change in them is recorded by soil layers. Three groups of indicators are distinguished here. The first group - those that are less than 200 mV. They were found in layers of 10-20 cm - 176 mV and 30-40 cm - 129 mV. In these layers, the intensive recovery processes take place, which, as mentioned earlier, have a negative effect on plant nutrition. The second group of indicators, which are within 300-400 mV. Such an indicator is in the layer of 20-30 cm - 351 mV. In this layer, the intensity of recovery processes is very weak. The last group of indicators - in the range of 400-500 mV - is in the 0-10 cm layer - 464 mV, where weak oxidation processes take place, which are favorable for the formation of nitrate nitrogen during the nitrification process. In autumn, the ORP level stabilizes and no sharp changes are recorded. With depth, there is a general tendency to decrease the level of ORP, which indicates an increase in the intensity of recovery processes, which is associated with an increase in soil moisture and a decrease in its aeration.

The ORP indicator of the Humi-Gleyic Fluvisol of the soil differs from the indicators of the previous soil. In the spring, a general tendency to increase the ORP level is observed with increase of depth. The lowest indicator was found in the 10-20 cm soil layer - 291 mV, and the highest - 330 mV in the 20-30 cm layer. These indicators indicate that recovery processes are weak and moderate in nature. At the summer, the level of redox potential in most soil layers increased sharply - to 417-449 mV. Which indicates the change of reduction processes to oxidation processes. The fluctuation of the potential here is not large and is in the range of 303-334 mV and slightly increases with depth.

CONCLUSIONS

The obtained data testify to the specificity of the oxidation-reduction potential and the nature of the oxidation-reduction processes of the alluvial soils of the river floodplains of the Siverskyi Donets basin.

In most cases, both by soil layers and by seasons, indicators of redox potential indicate the passage of redox processes, and only in summer these processes can change to the opposite, namely, oxidation processes. Oxidation processes are characterized by weak intensity. According to the averaged data, the lowest indicators of redox potential are observed in spring. They tend to grow seasonally from spring to fall. This indicates the greatest intensity of soil recovery processes in the spring.

Alluvial Gleysol soils, especially those of near-terrace subsidence, are characterized by more pronounced recovery processes, which is evidenced by the minimum indicators of redox potential.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

CONTENT OF MACRONUTRIENTS OF MALTING BARLEY IN DEPENDENCE OF NITROGEN NUTRITION LEVEL

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Abstract

*Macronutrients content of five barley varieties was studied at three levels of nitrogen 0, 200, and 400 mg N/kg soil. It was found that the Kristi variety had a low grain protein content of 8.8% on average. Obzor, Krami and Kaskadior varieties increased the crude protein in the grain to 12.3-13.0% at the N_{400} level. Strong positive correlation was proven between the soil nitrogen and grain protein (r=0.993**). Added nitrogen (N_{400}) increased the straw nitrogen content from 0.51% N_0 to 0.74% N. The Krami variety showed a higher concentration of grain phosphorus 0.87% P_2O_5, on average. The grain potassium content slightly depended on the variety. Varieties grown at N_{400} level had a higher concentration of potassium at maturity and of phosphorus in the grain than the plants at N_0. Calcium content of grain was higher in Emon and Kristi varieties (0.30 and 0.29% CaO, respectively), and in straw in Kristi and Kaskadior (1.10 and 1.14% CaO, respectively). Nitrogen level had a little effect on the calcium and magnesium contents. Their average concentrations of barley grain were 0.28% CaO and 0.12% MgO at N_{400} variant.*

Key words: *mineral composition, malting barley, nitrogen.*

INTRODUCTION

Barley is the main source of raw material for the brewing industry as well as for feeding animals. Its intensive cultivation is due to high-yielding varieties, possessing high ecological plasticity and yield stability. The genotypic specificity of mineral nutrition is a complex problem and depends on a number of interacting factors (Rogers et al., 2022). The main task is the creation of varieties with high efficiency in the use of both natural soil fertility and mineral fertilizers. In this way, the obtaining of sustainably high, qualitative and economical yields is ensured. Genetic specificity in mineral nutrition is judged by responsiveness to the level of fertilization, and one of the main indicators of research is the content of nutritional elements in the grain and straw of barley (Below, 1995).

Higher yields of modern barley varieties have been found under a wide range of nitrogen fertilization (Abeledo et al., 2003a). Despite significant genetic improvements in grain yield, their impact on grain mineral content may be different. Regarding grain nitrogen, it is important how the selection process has affected the balance of utilization of the two elements nitrogen and carbon in plants (Acuñaet al., 2005; Rogers et al., 2019). Quality barley for malting industry should contain more than 60% of starch and less than 12% of total proteins (Popovich et al., 2011). The effect of nitrogen level on barley characteristics depends on applied nitrogen rates and climate characteristics during the growing period. Sample nitrogen rates of 80 to 120 kg N/ha are most often indicated for malting barley (Koteva, 2001). High nitrogen rates increase the productivity of barley and, in parallel, the crude protein content increases, which, above certain limits, adversely affects its brewing-technological qualities (Rogers et al., 2022; Peev and Krasteva, 1989).

A number of factors influence the total nitrogen in the barley grain, respectively the crude protein. During grain filling, nitrogen is redistributed from the vegetative parts to the grain. Roots remained active during the grain filling, in which high mobile nitrogen content in the soil at the end of the growing season could account for a higher percentage of nitrogen in the grain. Barley can absorb up to 35 kg N/ha from the soil after flowering (Gastal & Lemaire, 2002). High nitrogen content in the grain may be due to a high input of nitrogen to the grain late in the growing season. This gain may be the result of soil uptake, enhanced reutilization by vegetative parts, or poor plant carbohydrate stores (Abeledo et al., 2008). Other causes of high nitrogen content in the barley grain are drought, lodging, disease, all of which reduce yield without affecting the redistribution of nitrogen to the grain (Stevens et al., 2015). The effect of barley fertilization can influence in a different direction from the purpose of selection. Research on grain nitrogen concentration in new barley genotypes is necessary for breeding technology, brewing industry and future selection. Cultivars must maintain grain nitrogen at appropriate values for beer production so that changes in plant nitrogen status are narrower than carbon for a stable NHI/GHI ratio (Lawlor, 2002). The chemical composition of barley depends on the variety and the level of mineral nutrition, and on the specifics of the course of phenological phases (Verma et al., 2003).

The aim of the present study was to investigate the effect of the level of nitrogen nutrition on the content of the macronutrients nitrogen, phosphorus, potassium, calcium and magnesium in the grain and straw of Bulgarian varieties of malting barley.

MATERIALS AND METHODS

Five Bulgarian varieties of malting barley, registered in the Official Varietal List of the country, well known and used in the country, were included in the present study. Obzor and Emon are long-standing standards for winter double-row barley, created at the Institute of Agriculture - Karnobat. They have a distinct winter-spring type of development, medium-tall stems with medium-thick, spiky spikes. Krami and Krispi varieties were selected at the Agricultural University of Plovdiv. They have very good brewing technological qualities and are suitable for cultivation in the dry regions of the country, especially southern Bulgaria (Lukipudis, 2008). Stems are lower, with shorter spikes. These two varieties are characterized by high productivity due to better productive tillering. The Kaskadyor variety is part of the selection achievements of the Agricultural Institute. It is distinguished by a taller stem and long spikes, as well as good productivity under the conditions of Northern Bulgaria.

The plants were grown in plastic containers with a volume of 5 L (10 plants per container). The barley varieties were studied at three levels of nitrogen nutrition: 0, 200 and 400 mg N/kg soil. Each variant was run in four replicates. The nitrogen levels were created by NH_4NO_3 fertilization. The soil used has pH water = 7.2 and contains mineral nitrogen 43.7 mg N/kg, mobile phosphorus (Egner-Riem) and absorbable potassium (2N HCl) 20.9 and 45 mg/100 g, respectively.

Barley grain and straw were analyzed at maturity. The plants were previously dried at 60°C to constant weight and weighed. The dry plant samples were ground and an aliquot part of them was mineralized with concentrated sulfuric acid under a hydrogen peroxide catalyst, after which the content of total nitrogen, phosphorus (colorimetrically on a spectrophotometer model Camspec M105) and potassium (on a flame photometer model PFP-7) was determined.

The calcium and magnesium content of barley grain and straw was determined in concentrated hydrochloric and nitric acids (ratio 3: 1) on an AAS model Perkin-Elmer 200 Analyst after microwave mineralization on a Milestone 2000 mega furnace. The crude protein content of the barley grain was calculated from the percent of nitrogen content multiplied by a factor of 5.7 (N% x 5.7).

Analysis of variance (ANOVA) for two-factor experiments and Duncan's (1955) multivariate comparison test were used for mathematical processing of the obtained results. Only differences at α=0.95 were accepted as proven.

RESULTS AND DISCUSSIONS

An improvement in barley productivity is associated with a decrease in nitrogen concentration in the grain (Abeledo et al., 2003b). The percentage of grain nitrogen depends on the genotype (Cox et al., 1985; Emebiri & Moody, 2004; Przulij & Momcilovic, 2001), growing conditions during the vegetation period (Boonchoo et al., 1998; Paynter & Young, 2004) and their interaction (Bertholdsson, 1999). Crude protein content of the barley grain is only one of the characteristics related to malting quality, but it is the most important indicator for the brewing industry, varying between 8.5% and 12.5% (Gali & Brown, 2000). In England, malting barley varieties are used for beer production if 70% of the production contains total grain nitrogen within the range of 1.55% to 1.85% or 8.8% to 10.5% expressed as crude protein (Collen, 2006).

Table 1. Crude protein concentration of barley grain, %

N level / Variety	N_0	N_{200}	N_{400}	Average variety
Obzor	8.6ab*	10.2a	12.3ab	10.4ns***
Emon	8.3b	10.7a	11.9b	10.3
Krami	7.9bc	9.3b	12.5a	9.9
Kristi	7.5c	8.9b	10.1c	8.8
Kaskadior	9.1a	10.2a	13.0a	10.8
Average N level	8.3C**	9.9B	12.0A	

*Values in each column followed by the same lowercase letters are not significantly different at $p<0.05$
**Values in the row followed by the same uppercase letters are not significantly different at $p<0.05$
***Not significantly different at $p<0.05$

The average content of crude protein in the grain of the five Bulgarian varieties grown at three levels of nitrogen was 10.1% (Table 1). It varied within the limits of 7.5% (Kristi variety at N0) to 13% (Kaskadior variety at N_{400}). Our results confirmed that nitrogen fertilization increased the crude protein content of the grain. This indicator increased from 8.3% on average for unfertilized plants to 12.0% for varieties fertilized with 400 mg N/kg soil. The Kristi variety was characterized by a low content of crude protein in the grain. This was found at all three nitrogen levels. Varietal response averaged over the three nitrogen levels was unproven. Increased nitrogen fertilization N_{400} leaded to high values of grain protein concentrations (12.3-13.0%) of Obzor, Krami and Kaskadior varieties and it reduced the quality of grain for beer production. Strong positive correlation was found between the level of nitrogen nutrition and the percentage of crude protein in the grain (r=0.993**).

Table 2. Nitrogen concentration of the barley straw, (N %)

N level / Variety	N_0	N_{200}	N_{400}	Average variety
Obzor	0.49b	0.57ns	0.72ab	0.59ns
Emon	0.53ab	0.58	0.74ab	0.62
Krami	0.57a	0.64	0.79ab	0.67
Kristi	0.43b	0.55	0.63b	0.54
Kaskadior	0.55a	0.68	0.83a	0.69
Average N level	0.51C	0.60B	0.74A	

The average concentration of nitrogen in the straw of the studied barley varieties increased in parallel with the level of nitrogen nutrition from 0.51% at the N_0 to 0.74% at the N_{400} level (Table 2). The content of nitrogen in the straw was the lowest in Kristi variety 0.43% at N_0 and the highest in Kaskadior variety grown at N_{400} level. The differences in the concentration of nitrogen in the straw depending on the variety of the plants grown at the N_{400}, as well as in the average of the three nitrogen levels, were insignificant.

Table 3. Phosphorus grain concentration of barley, (P_2O_5%)

N level / Variety	N_0	N_{200}	N_{400}	Average variety
Obzor	0.72ab	0.77b	0.84a	0.78ab
Emon	0.67b	0.73b	0.79b	0.73b
Krami	0.81a	0.87a	0.92a	0.87a
Kristi	0.69b	0.76b	0.82ab	0.76ab
Kaskadior	0.63b	0.70b	0.75b	0.69b
Average N level	0.70B	0.77aAB	0.82A	

The average content of phosphorus in the grain of the studied barley varieties grown at nitrogen levels N_0, N_{200} and N_{400} was 0.76% P_2O_5 (Table 3). It changed from 0.63% P_2O_5 in unfertilized plants of the Kaskadior variety to 0.92% P_2O_5 in the Krami variety at the N_{400} level. The level of nitrogen in the soil increased the phosphorus content of the barley grain, but proven differences were found between the level of N_{400} and the cultivars grown without nitrogen addition. The Krami variety was distinguished by a higher concentration of phosphorus in the grain at all three levels of nitrogen. Varietal response was demonstrated regardless of the level of applied nitrogen.

Table 4. Phosphorus concentrations of barley straw, (P_2O_5 %)

N level / Variety	N_0	N_{200}	N_{400}	Average variety
Obzor	0.43ns	0.46ns	0.50ns	0.46b
Emon	0.39	0.43	0.45	0.42ab
Krami	0.47	0.50	0.53	0.50a
Kristi	0.42	0.48	0.51	0.47ab
Kaskadior	0.45	0.51	0.55	0.50a
Average N level	0.43ns	0.48	0.51	

The Krami variety was characterized by a high concentration of phosphorus in the grain (0.89% P_2O_5) compared to Emon (0.73% P_2O_5) and Kaskadior (0.69% P_2O_5) varieties.

The concentration of phosphorus in barley straw had similar values and was 0.47% P_2O_5

on average for the present experiment (Table 4). Proven varietal differences at the three levels separately were not observed. However, the average values of N_0, N_{200} and N_{400} levels indicated Obzor variety with lower grain phosphorus content compared to Krami and Kaskadior cultivars. The levels of nitrogen nutrition had little influence on the average phosphorus content of barley straw.

Table 5. Potassium grain concentrations of barley, (K_2O, %)

N level / Variety	N_0	N_{200}	N_{400}	Average variety
Obzor	0.64ns	0.77ns	0.82ns	0.74ns
Emon	0.55	0.65	0.73	0.64
Krami	0.59	0.66	0.74	0.66
Kristi	0.62	0.69	0.75	0.69
Kaskadior	0.61	0.71	0.78	0.70
Average N level	0.60B	0.70A	0.76A	

The studied barley varieties contained an average of 0.69% K_2O in the grain (Table 5). The values of this indicator varied within relatively narrow limits from 0.55% K_2O (Emon variety at N_0) to 0.82% K_2O (Obzor variety at N_{400}). Varietal response of barley in grain potassium concentration was weak and unproven. This was observed regardless of nitrogen level. The potassium content of the barley grain increased as a result of the applied nitrogen. Unfertilized plants contained an average of 0.60% K_2O.

Table 6. Potassium concentrations of barley straw, (K_2O %)

N level / Variety	N_0	N_{200}	N_{400}	Average variety
Obzor	1.24c	1.29c	1.36c	1.30b
Emon	1.33bc	1.42b	1.51b	1.42b
Krami	1.42ab	1.51ab	1.63ab	1.52a
Kristi	1.51a	1.60a	1.67a	1.59a
Kaskadior	1.47a	1.61a	1.71a	1.60a
Average N level	1.39B	1.49AB	1.58A	

Average grain potassium concentration increased with soil nitrogen level to 0.70% K_2O (N_{200}) and 0.76% K_2O (N_{400}), but the difference between the two nitrogen levels was not proven.

The content of potassium in the straw varied from 1.24% K_2O (Obzor variety at N_0) to 1.71% K_2O (Kaskadior variety at N_{400}) (Table 6). Kristi and Kaskadior varieties were characterized by higher concentrations of potassium in the straw (1.59-1.60% K_2O). Obzor variety had the lowest content 1.30% K_2O on average of the studied nitrogen levels. Straw potassium content of barley cultivars increased with nitrogen fertilization, but the difference between unfertilized plants and the N_{200} level was unproven. The cultivars grown at the N_{400} contained an average of 1.58% K_2O in the straw, which was 13.7% higher than that of the unfertilized plants.

Table 7. Calcium concentrations of barley grain, (CaO %)

N level / Variety	N_0	N_{200}	N_{400}	Average variety
Obzor	0.21ns	0.23b	0.24ns	0.23b
Emon	0.29	0.31a	0.31	0.30a
Krami	0.25	0.26ab	0.28	0.26ab
Kristi	0.26	0.29a	0.31	0.29a
Kaskadior	0.23	0.25ab	0.27	0.25b
Average N level	0.25ns	0.27	0.28	

The level of nitrogen slightly affected the percent of calcium in the grain and straw of the barley varieties (Table 7 and Table 8).

Table 8. Calcium concentrations of barley straw, (CaO %)

N level / Variety	N_0	N_{200}	N_{400}	Average variety
Obzor	0.79c	0.81b	0.85b	0.82d
Emon	0.87c	0.88b	0.93ab	0.89c
Krami	0.99b	1.02ab	1.05a	1.02b
Kristi	1.05b	1.11a	1.13a	1.10a
Kaskadior	1.18a	1.11a	1.14a	1.14a
Average N level	0.98ns	0.99	1.02	

The average values within the trial were 0.27% CaO (grain) and 0.99% CaO (straw). The grain of Emon and Kristi varieties had proven higher concentrations of calcium (0.30-0.29% CaO on average) compared to Obzor and Kaskadior varieties. A varietal response was demonstrated with regard to calcium content of straw. Straw calcium concentration was higher (1.10-1.14% CaO) in Kristi and Kaskadior varieties. The Obzor variety was showed low average concentration of calcium in the straw of 0.82% CaO.

The effect of nitrogen level and variety on the magnesium content of barley grain and straw was weak (Table 9 and Table 10). The magnesium concentrations of barley grain were close values (0.11-0.15% MgO) and proven differences were not observed.

Table 9. Magnesium concentrations of barley grain, (MgO %)

N level / Variety	N_0	N_{200}	N_{400}	Average variety
Obzor	0.11ns	0.12ns	0.11ns	0.11ns
Emon	0.12	0.13	0.12	0.12
Krami	0.11	0.12	0.12	0.12
Kristi	0.10	0.12	0.12	0.11
Kaskadior	0.14	0.15	0.14	0.14
Average N level	0.12ns	0.13	0.12	

Table 10. Magnesium concentrations of barley straw, (MgO %)

N level / Variety	N_0	N_{200}	N_{400}	Average variety
Obzor	0.09ns	0.11ns	0.10ns	0.10ns
Emon	0.07	0.08	0.09	0.08
Krami	0.06	0.07	0.07	0.07
Kristi	0.07	0.07	0.08	0.07
Kaskadior	0.08	0.09	0.11	0.09
Average N level	0.07ns	0.08	0.09	

The magnesium content of barley straw varies within narrow limits from 0.06% MgO (Krami variety at N_0) to 0.11% MgO (Obzor variety at N_{200} and Kaskadior variety at N_{400}).

CONCLUSIONS

Kristi variety had a low grain protein content of 8.8% on average. Obzor, Krami and Kaskadior varieties increased the crude protein in the grain to 12.3%–13.0% at the N_{400} level. Strong positive correlation was proven between the soil nitrogen and grain protein (r=0.993**). Added nitrogen (N_{400}) increased the straw nitrogen content from 0.51% (N_0) to 0.74% N. The Krami variety showed a higher concentration of grain phosphorus 0.87% P_2O_5, on average. The grain potassium content slightly depended on the variety. Varieties grown at N_{400} level had a higher potassium concentration in maturity and grain phosphorus than the plants at N_0. Calcium content of grain was higher in Emon and Kristi varieties (0.30-0.29% CaO), and in straw in Kristi and Kaskadior (1.10-1.14% CaO). Nitrogen level had a little effect on the calcium and magnesium contents. Their average concentrations of barley grain were 0.28% CaO and 0.12% MgO at N_{400}.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Agricultural University of Plovdiv, Bulgaria also was financed from Project 17-12 "Support of the publication activity of the teachers from Agricultural University of Plovdiv".

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE EFFECTS OF THE APPLICATION OF ORGANIC AND MINERAL FERTILIZERS IN LONG-TERM EXPERIMENTS

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Abstract

The paper presents the results of soil samples obtained from a long-term experience at Livada (Satu Mare County). The experiment carried out was of bifactorial type with variants fertilized with manure and mineral fertilizers with nitrogen and phosphorus. Manure was applied once every 5 years in 4 doses (0 t/ha, 20 t/ha, 40 t/ha and 60 t/ha) and mineral fertilizers in 4 doses (N0P0, N50P0, N50P50, N100P100 kg/ha) annually. The obtained results showed that in the variants fertilized only with manure, the content of mobile potassium increased very significantly for all applied doses, from 162 mg/kg in the control variant to 239 mg/kg in the variant fertilized with 60t/ha. Fertilization with nitrogen and phosphorus determined significant and very significant accumulations of nitrogen, mobile phosphorus, the potassium content having a decreasing trend. The humus content of the soil increased significantly when applying 40 t/ha and 60 t/ha. Productions were reduced in all experimental variants, the year 2022 being a very dry year.

***Key words**: organic matter, mineral fertilization, long-term experience.*

INTRODUCTION

The agriculture has to face a large number of challenges imposed by increasing population and diets change, climate change, decreasing resources, geostrategic changes, economic gaps, as well as by the obligation to minimize the impact on the environment as much as possible.

Agricultural production is dependent on environmental resources such as soil, water and air and is vulnerable to climate change, including floods and drought.

Current levels of consumption and production are not sustainable and risk diminishing the planet's ability to provide food for the increasing population. The production and consumption systems must be rethought in order to allow the production of the same amount of products with fewer resources and constantly considering measures to significant and quantifiable improve the quality of the environment.

Soil erosion and contamination, as well as the soil organic matter reduction, reduce its resilience, or its ability to absorb the changes to which it is exposed.

Good nutrient management using a balanced long-term approach is part of a sustainable agricultural system that is resilient to climate and economic change.

Organic nutrient sources are limited and fertilizer preparation requires energy, and recycling nutrients from organic materials and improving nutrient accessibility from well-structured biologically active soils leads to better resource use and economic sense.

The long-term use of chemical fertilizers to increase crop productivity has often negatively affected the complex system of biogeochemical cycles (Perrott et al, 1992; Steinshamn et al., 2004). To increase crop productivity and ensure sustainable agriculture, the use of organic and inorganic sources of nutrients is recommended along with other complementary measures. Organic fertilizers have favorable effects on soil structure and texture and facilitate greater and faster availability of nutrients for plants (Avnimelech, 1986).

The instantaneous assimilation capacity of the soil system is closely related to the ability to aerobically degrade organic matter, thus avoiding problems resulting from septic soil conditions (odors and damage to plants). This capacity is dependent on the state of soil aeration (determined by the texture, structure and moisture of the soil), temperature and organic strength (resistance to mineralization of zootechnical residues). The organic strength of residues is measured in 5-day biological oxygen consumption and/or chemical oxygen consumption, which are appropriate for various residues (Carton and Magette, 1994).

To improve the quality of the soil as well as to increase the yield of crops, an effective way is the application of manure in normal or high doses of amendment, combined with chemical fertilizers (Duan et al., 2011; He et al., 2015; Maltas et al., 2018). However, to date, the response of soil organic carbon stability to fertilization is highly uncertain because the mechanisms controlling its accumulation are not fully understood. Therefore, understanding and characterizing the effect of fertilization on organic carbon accumulation is important for long-term sustainable agriculture management and development (Zhou et. al., 2022).

MATERIALS AND METHODS

The experimental fields were located in the northwestern area of Transylvania, the area that falls within the climatic province Cfbx (according to Köppen), characterized by a moderate temperate-continental climate. The multiannual average temperature recorded at the weather station S.C.D.A. The orchard for 60 years is 9.9°C. In the period 1961-2021, at Livada the multiannual average of the amount of precipitation was 753.2 mm (Figure 1).

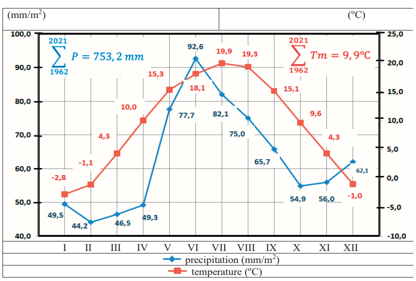

Figure 1. Evolution of monthly multi-annual average of temperatures and mean precipitation sum recorded in the period 1962-2021 at SCDA Livada

In the year 2022, the most severe drought in the history of the existence of these experiences was recorded. Practically in the months of May, June, July, which are decisive for the corn crop, only 65 mm of precipitation was recorded, being much lower than the multiannual average (252.4 mm) (Figure 2).

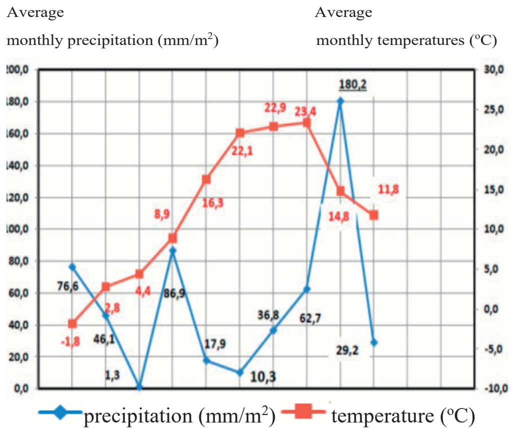

Figure 2. The evolution of average monthly temperatures and precipitation in 2022 recorded at the meteorological station S.C.D.A. Livada

In the same time sequence, precipitation was associated with excessive temperatures. As a result, the productions obtained were the lowest in the last 61 years. The drought period was followed by heavy rainfall that greatly extended the maize growing season.

The stationary experiments with the application of manure and chemical fertilizers (NP) were located in 1967, according to the method of subdivided plots, in a bifactorial manner. The manure was applied once every 5 years in variants of 0, 20, 40, 60 t/ha and annually mineral fertilization with 4 gradations N0P0, N5P0, N50P50 and N100P100 kg/ha. The samples were collected after the maize crop.

Soil samples were collected from the topsoil, at a depth of 0-20 cm, and soil analyzes were performed by the following methods:

- total nitrogen (N%): Kjeldahl method, disintegration with H_2SO_4 at 350°C, potassium sulphate and copper sulphate as catalyst - SR ISO 11261: 2000;
- available phosphorus (mobile): according to the Egner-Riehm-Domingo method and dosed colorimetric with molybdenum blue, according to the Murphy-Riley method (reduction with ascorbic acid);

- total phosphorus, colorimetric method. ICPA Methodology 1986, chap. 8, point 2, PT 2.

RESULTS AND DISCUSSIONS

Production - The data presented in Figures 3, 4 and 5 highlight a very low level and a high variability of productions under the influence of the accentuated drought from the year 2022. Figure 1 highlights the increase of productions under the influence of mineral fertilization. The highest level of production, significantly different from the unfertilized control variant, was obtained following the administration of the highest dose ($N_{100}P_{100}$). The contribution of phosphorus to the increase in production can be observed, which can be attributed to the fact that it is required especially in the first phases of vegetation when it has a significant influence on the growth of the root system, therefore also on resistance to drought and diseases.

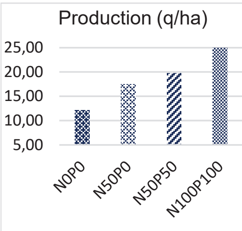

Figure 3. The influence of NP fertilization on production

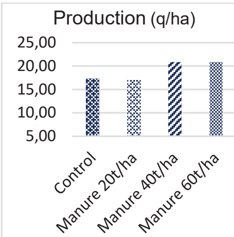

Figure 4. The influence of manure fertilization on production

Although administered only once every 5 years, manure fertilization is useful because it ensures less variability of productions due to decomposition over time and longer-term provision of the nutrients needed by the plants, increases the plants' resistance to drought, provides an intake of organic matter in the soil, with all the advantages it offers. Organic fertilization with cattle manure, with doses of 20-60 t/ha, did not ensure statistically guaranteed increases in the corn crop in 2022 (Figure 4). Statistically assured production increases are only achieved in years 1 and 2 of administration.

Figure 5 shows the results that highlight the impact of organic and mineral fertilization on production. The data show significant yield increases over N_0P_0 where doses of $N_{50}P_{50}$ or $N_{100}P_{100}$ + 40-60 t/ha manure were applied.

The results obtained require the recommendation to increase the doses of fertilizers applied above the level of $N_{100}P_{100}$ and to apply organic fertilizers every year at least once every 2 years.

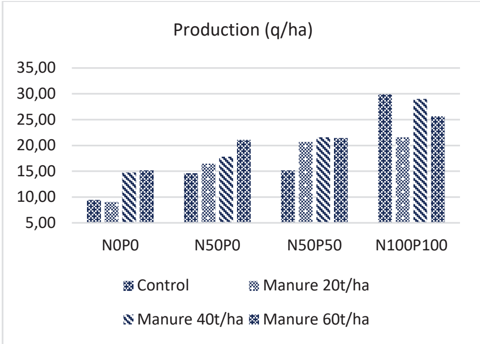

Figure 5. The influence of NP and manure fertilization on production

pH evolution - one of the most extensive soil degradation processes at SCDA Livada is acidification, which covers over 60-65% of the area. This process has increased in the last 50 years, on the one hand as a result of industrial development (acid rain, deposits with acid reaction), and on the other hand as a result of the large-scale use of fertilizers with physiological acid reaction (Henzsel et al., 2012).

The pH values determined after 55 years are presented in Figures 6, 7, 8. Analyzing the evolution of the obtained values, it was found that the most pronounced acidification was generated by the application of nitrogen in doses of 50 kg/ha on a background of P0 and $N_{100}P_{100}$, doses at which soil acidification was very significant from a value of 5.90 in the unfertilized control to 5.43 in the N50P0 variant, respectively 5.13 for $N_{100}P_{100}$, values to which the application of amendments is required. When $N_{50}P_{50}$ doses were applied, the growth was significant, the phosphorus intake stimulated plan.

The application of manure contributed considerably to the fading of the acidity generated by mineral fertilization, achieving a slight increase from 5.41 in the control variant to 5.54 when applying 60 t/ha of manure, a fact that recommends mineral fertilization together with the organic one even once every 5 years.

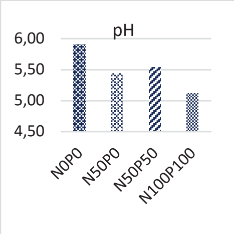

Figure 6. The influence of NP fertilization upon soil pH

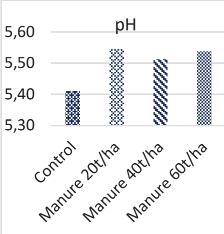

Figure 7. The influence of manure fertilization upon soil pH

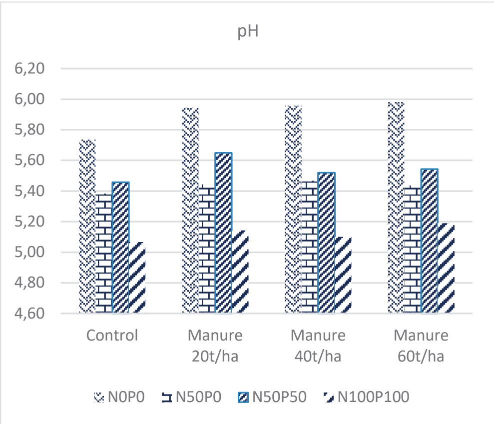

Figure 8. The influence of NP and manure fertilization upon soil pH

Humus - intensive agriculture causes land degradation and other environmental problems, such as: pollution, soil erosion, loss of fertility, decrease in biodiversity and greenhouse gas emissions, which exacerbate climate change (Francaviglia et al., 2023).

Humus is considered to be a sensitive indicator of assessing the effects of different agricultural practices on soil fertility and the soil organic carbon pool (Jin et al., 2023).

Soil organic carbon plays an important role in soil nutrient supply, energy flow and material transformation (Wu et al., 2023). Some previous studies suggested that long-term application of organic fertilizers alone (Wei et al., 2016) or combined with inorganic fertilizers (Su et al., 2006) can significantly increase organic carbon content in large aggregates; for example, Zhang et al. (2019) found that organic carbon was significantly fixed at 0.25–2 mm aggregates; Zhang et al. (2017) found that organic carbon content in aggregates >2 mm increased significantly. However, some studies suggested that inside the microaggregates the content was higher (Huang et al., 2010); for example, Zhang et al. (2018) found that in aggregate of 0.01-0.1 mm, the organic carbon content of the total aggregates is exceeded by 50%.

Soil aggregates are the most basic structural units and are important components of soil. About 90% of organic carbon on the soil surface is fixed in aggregates, and the "hierarchical structure" between aggregates of different particle sizes leads to the transformation of soil matter and energy, which promotes nutrient cycling and soil supply (Su et al., 2006).

Isolation and quantification of soil organic matter pools under the influence of management practices is necessary to assess changes in soil fertility, long-term manure + inorganic fertilization is crucial for improving C and N sequestration by changing the size and response of soil organic matter pools (Mustafa, et al., 2022). Guo et al. (2017) found that long-term addition of nitrogen (126-252 $kg \cdot ha^{-1}$) and phosphorus (140-280 $kg \cdot ha^{-1}$) significantly reduced mineralization rate and cumulative C fluxes by increasing the aromaticity of organic carbon substrates.

The results obtained in the experimental variants presented in Figures 9, 10, 11, demonstrated that the application of mineral fertilizers with nitrogen and phosphorus significantly and very significantly increased the humus content of the soil from 1.38% in the control variant to 1.61% in application of N100P100. For the variants of N50P0 and N50P50 the humus value was approximately equal, which means that the phosphorus input of 50kg/ha did not influence the humus content of the soil.

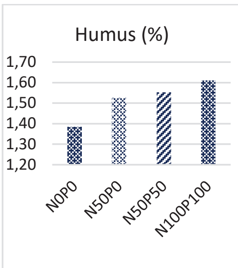

Figure 9. The influence of NP fertilization upon humus content

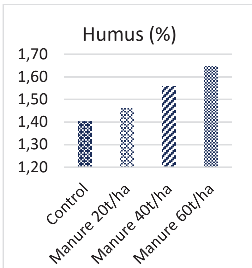

Figure 10. The influence of manure fertilization upon humus content

Although small doses of mineral fertilizers were applied, they were sufficient to achieve the low productions caused by the drought which diminished the process of mineralization of organic matter in the soil. The fact that the soil samples were collected in the fifth year after the application of manure, at doses of 20t/ha, its effect was insignificant. The application of 40 and 60 t/ha even in the fifth year increased the humus content of the soil very significantly (by 11.1% when applying 40t/ha and by 17.2% at 60 t/ha).

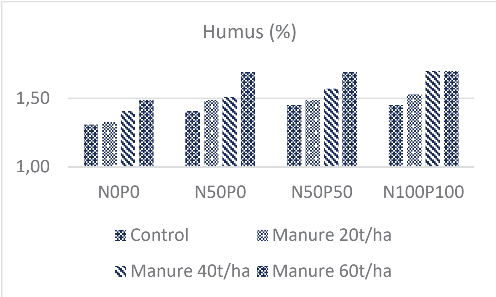

Figure 11. The influence of NP and manure fertilization upon humus content

Nitrogen - Among the nutrients, nitrogen has the greatest effect on plant growth, production and production quality. Appropriate nitrogen management is required because excess nitrogen leads to reduced yields, financial losses, delays ripening, reduces resistance to fall and drought, increases sensitivity to diseases and pests, changes the ratio between straw and grain, reduces storage resistance, etc. The utilization of manure requires controlling the moment of application, knowing the nitrogen load, reducing losses through ammonium volatilization, denitrification and washing, increasing the degree of utilization of the contained nutrients.

Nitrogen is a major driver of N_2O emissions, but also the limiting nutrient for crop production and yield (Linquist et al., 2012). 70% of the global N_2O emission comes from the processes of nitrification (conversion of ammonium into nitrate) – denitrification (conversion of NO_3^- into atmospheric dinitrogen) (Ussiri, 2013).

In order to reduce nitrogen losses, it is necessary to apply doses of fertilizers in accordance with the requirements of the plants, the reserve in the soil, the preceding plant, the time and method of application. The effect of different doses of nitrogen on production and soil characteristics will be researched.

The results obtained in the long-term experiment (Figures 12, 13, 14) demonstrated a significant increase in the total nitrogen content in the soil from 0.096% in the unfertilized version to 0.104% in the versions in which doses of 50 kg N/ha.

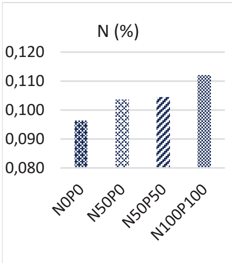

Figure 12. The influence of NP fertilization upon nitrogen content

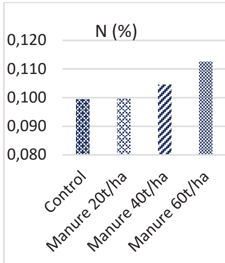

Figure 13. The influence of manure fertilization upon nitrogen content

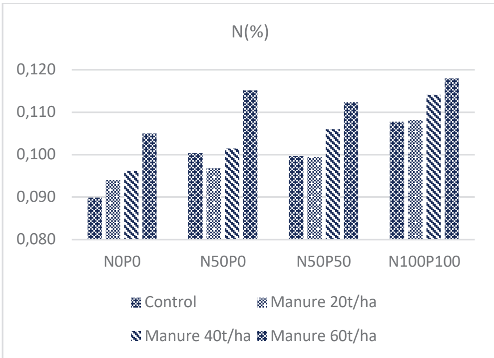

Figure 14. The influence of NP and manure fertilization upon nitrogen content

The application of phosphorus did not cause changes in the total nitrogen content, a fact also demonstrated by the results obtained at SCDA Teleorman after 39 years of experience (Mărin & Negrilă, 2022). When applying N100P100, the soil nitrogen concentration increased significantly from 0.096% in the unfertilized version to 0.112%. This increase in the nitrogen content of the soil can be largely due to the deficient rainfall regime that determined small agricultural productions, therefore a reduced consumption of nutrients, but also a decrease in losses through leaching on the soil profile. In the fifth year after application, the manure brought an input of 5.03% at doses of 40t/ha and 13.2% of nitrogen in the soil at doses of 60t/ha.

Phosphorus - Globally, phosphate rock reserves are decreasing, while the amount of P-containing organic waste is constantly increasing. The use of such biowastes as organic amendments in agriculture is the ultimate disposal goal of environmental and agricultural agencies worldwide (Baram et al., 2023).

In soil, phosphorus comes from the parent rock on which the soil formed and evolved. Sedimentary rocks contain 0.05-0.3% P_2O_5, crystalline ones 0.2-0.7% P_2O_5, and igneous ones 0.3-1% P_2O5 (Sala, 2007). Of the total phosphorus content of the soil, only 0.5-1% is accessible to plants, its mobility in the soil being low. In soil organic matter, the C: N: P ratio is 100: 10: 1. If the C: N ratio is 200: 1 or lower, then the mineralization of organic matter and the replenishment of the soil solution with phosphate ions occurs, and if the ratio is 300:1 or higher, the insolubilization of phosphorus occurs (Mărin et al., 2022) .

Analyzing the results from the long-term experience presented in Figures 15, 16, 17, it is found that the application of phosphorus fertilizers contributed to very significant increases in mobile phosphorus in the soil (from 20 mg/kg P in the control variant to 62 mg/kg P when applying 50 kg P/ha, respectively 120 mg/kg P when applying doses of 100 kg P/ha. As with nitrogen, drought conditions and low production led to accumulations of this element in the soil. Manure did not generated statistically guaranteed increases in mobile phosphorus in the soil.

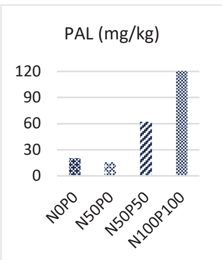

Figure 15. The influence of NP fertilization upon phosphorus content

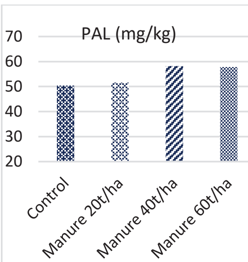

Figure 16. The influence of manure fertilization upon phosphorus content

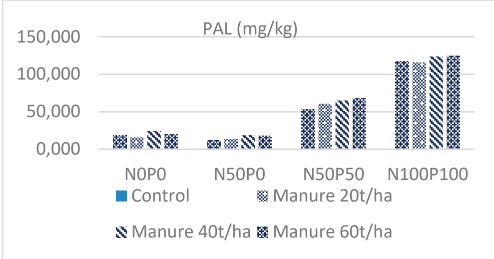

Figure 17. The influence of NP and manure fertilization upon phosphorus content

Excessive uptake of phosphorus (P) by maize can lead to a decrease in grain yield. P uptake indirectly influences the uptake of other nutrients and their translocation within the plant. Consumption of luxury P beyond the point corresponding to maximum grain yield significantly decreases N, S, Fe, Cu and Zn (Penn, et. al., 2023).

Potassium - in the variants fertilized with NP, a decrease in the mobile potassium in the soil was found, not statistically guaranteed, very significant increases were determined when the manure was added to all three applied doses (Figures 18, 19, 20).

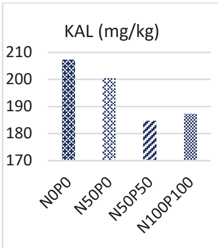

Figure 18. The influence of NP fertilization upon potassium content

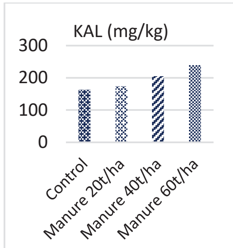

Figure 19. The influence of manure fertilization upon potassium content

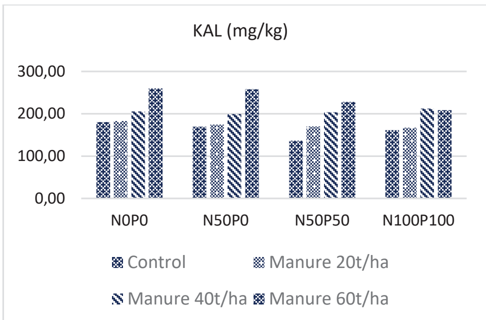

Figure 20. The influence of NP and manure fertilization upon potassium content

CONCLUSIONS

Although the year was very dry, fertilization with high doses of nitrogen and phosphorus brought the biggest increases in production.

Long-term fertilization with nitrogen ensures an increase in the nitrogen content of the soil both at doses of 100 kg N/ha and 50 kg N/ha, accumulations to which the very low amount of precipitation and implicitly low production also contributed, but it causes an acidification pronounced soil, it is recommended to apply calcareous amendments.

Manure has an important role in the supply of nutrients to the soil, and through the buffering effect it contributes to the fading of soil acidity generated by mineral fertilization with nitrogen. The application of 40 and 60 t/ha even in the fifth year increased the humus content of the soil very significantly, the application of 20 t/ha of manure after 5 years had insignificant effects on the production and fertility of the soil.

ACKNOWLEDGEMENTS

This work was supported by two grants of the Romanian Ministry of Research, Innovation and Digitization, project number PN 23 29 02 01 (New organic fertilizer products for sustainable agriculture in the context of efficient use of natural resources) and project number 44 PFE /2021, Program 1 - Development of national research-development system, Subprogram 1.2 - Institutional performance - RDI Excellence Financing Projects.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

BIOFORTIFICATION OF MAIZE WITH ZINC

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Abstract

The article presents the experimental results obtained in the greenhouse to demonstrate the efficacy of administering zinc to the soil, plant, and seed in order to reduce the incidence of zinc deficiency in maize. For the experimentation in vegetation pots, a late hybrid FAO 430 and an organo-mineral material constituted from topsoil originating from a soil of the calcaric chernozem type, characteristic of the south-eastern region of Romanian Plain which received different treatments with $CaCO_3$, KH_2PO_4 and $ZnSO_4 \cdot 7H_2O$, were used. At 3 to10 days intervals, phenological observations were made on maize plants, and at the end of the experiment, soil and plant samples were collected and analysed in the laboratory. According to obtained data, the most effective method for biofortification of plants with zinc, among the three methods of administration, was the application of zinc to seed. According to an analysis of the absorption of nutritional elements by maize plants, it can be stated that the administration of the microelement both in soil and on seed are optimal methods for biofortifying maize plant with zinc, with practically identical performance.

Key words*: zinc, maize, biofortification, soil, greenhouse.*

INTRODUCTION

Zn deficiency in maize (*Zea mays*) occurs during the well-developed 4-7 leaves phenophase. The phenomenon is typically observed in plants grown in soils with a neutral-alkaline reaction, which are well and extremely well supplied with P but have low Zn levels. Zn deficiency is manifested by reduced plant height, short internodes and a thick appearance, yellowing of young leaves and a parchment paper appearance in the form of whitish bands present along the length of the leaf, parallel to the midrib, when the plants are in an advanced stage of deficiency (Lăcătușu, 2016).

Lately, to reduce the incidence of this phenomenon, in specialized literature the notion of biofortification is used, which involves a process of increasing the contents of micronutrients, in the basic crops consumed on a large scale, through conventional breeding techniques, agronomic practices or genetic modifications (Lividini et al., 2017). Agronomic biofortification is an effective and practical method that provides a quick solution to the problem of maintaining sufficient amount of available Zn in the soil, maximizing plant uptake and adequate transport of Zn to the grain (Cakmak, 2008). In this study, the methods of biofortification (increasing Zn content) in maize plants suffering from this deficiency are presented. In agricultural practice, there are three methods to reduce the occurrence of zinc deficiency or to biofortify plants susceptible to this phenomenon with zinc: (I) application of zinc to the soil; (II) application of zinc to the plant; and (III) application of zinc to the seed prior to its introduction into the soil.

From the research carried out so far, the specialized literature does not present conclusive results regarding the maximum effectiveness brought by one or another of the three stated methods. Therefore, Borlan et al. (1994), recommends with priority the repeated administration of Zn on the plant, at intervals of 4-6 days, with solutions of 0.25-0.40% $ZnSO_4 \cdot 7H_2O$, when the first morphological symptoms of Zn deficiency manifestation appear. It is recommended to apply Zn in the soil, once every 4-5 years in different doses from 4 to 15 kg Zn/ha, depending on the natural supply level of the soil and the nature of

the plant to be cultivated (Alloway, 2008; Graham et al., 1992). Zn application on the seed is also practiced. Farooq et al. (2012), carried out a synthesis study regarding the application of microelements (Zn, B, Mo, Mn, Cu, Co) on the seed of different plants (wheat, rice, oats, beans, maize, sunflower, etc.). Regarding Zn, the effect of the treatment on bean, beetroot and maize seeds is highlighted. To these, Harris et al. (2007), obtained a 27% increase in production in plants grown from Zn treated seed compared to the production obtained in untreated plants. Likewise, a 19% increase in production was obtained for chickpeas grown from seeds treated with Zn (Harris et al., 2008).
In light of the fact that no study was found that compared the three methods of applying Zn under identical experimental conditions, we conducted an experiment in which we compared the development of maize plants during the first part of the vegetation period, depending on the method of application, implying the biofortification of maize with Zn.

MATERIALS AND METHODS

The experiment was carried out under greenhouse conditions, and a 12 hours light and 12 hours darkness schedule was maintained throughout the experiment. A constant temperature of 22.3 ± 2.0°C was maintained during the light period, an average luminosity of 4949 ± 1100 lux and an air humidity value of RH equal to 30.6 ± 2.4%. Experiments were carried out with a soil material (0-20 cm) from the surface horizon, the calcareous chernozem characteristic of the South-East Romanian Plain with the following characteristics: pH H_2O (1: 2.5): 7.83; $CaCO_3$: 0.7%; organic matter: 3.50%; N: 0.169%; N-NO_3: 18 mg·kg^{-1}; P_{AL}: 34 mg·kg^{-1}; K_{AL}: 177 mg·kg^{-1}; Zn: 60 mg·kg^{-1}; Cu: 27 mg·kg^{-1}; Mn: 686 mg·kg^{-1}; Co: 8 mg·kg^{-1}; Ni: 31 mg·kg^{-1}; Cr: 31 mg·kg^{-1}; Cd: 0.17 mg·kg^{-1}; Pb: 13 mg·kg^{-1}; Fe: 2.43%. The soil material was loaded into 15 vegetation pots, with 6.0 kg each, and the treatments were carried out according to the data in Figure 1. 4 seeds of late hybrid FAO 430/pot were seeded. During the growing season, soil humidity was maintained at 70% water capacity of the soil in the pot. Phenological observations were made, following the height of the plants at time intervals between 3 and 10 days. Maize plant samples were collected at two times: 44 days after seeding, two plants (aerial part); 58 days after seeding, when the experiment ended, the other two plants (root and aerial part).

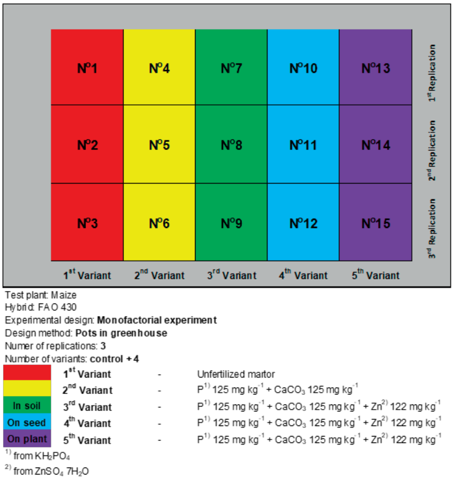

Figure 1. The treatments performed on the soil in the vegetation pots

Along with these, soil samples were collected from the entire depth of the pot.
For the soil samples, the following analyzes were performed: pH H_2O (1: 2.5), potentiometric measurements, using a combined glass - calomel electrode; the $CaCO_3$ content, using the volumetric gas method (Scheibler); N content, using Kjeldahl method; NO_3^- content using potentiometric measurements with ion selectiv electrode; the mobile content of P and K were determined by spectrophotometry, respectively flame spectrometry, using extraction with ammonium acetate-lactate solution at pH 3.7, Egnèr-Riehm-Domingo method. The contents of trace elements (Zn, Cu, Fe, Mn), mobile forms, were determined in CH_3COONH_4-EDTA at pH 7.0 (Lăcătuşu et al., 1987) and total forms (Zn, Cu, Fe, Mn), were passed into solution with a mixture of concentrated mineral acids (HNO_3, $HClO_4$, HCl) and hydrogen peroxide. Measurements were performed by atomic absorption spectrophotometry in an air-acetylene flame.
The collected plant material samples (root and aerial part) were analyzed from the point of

view of the chemical composition of macro- and microelements. N was determined according to the Kjeldahl method, P was determined by spectrophotometry, K and Ca by flame photometry and Mg, Zn, Cu, Fe and Mn were determined by atomic absorption spectrophotometry in the clorhydric solution obtained after solubilization of ash plant at a temperature of 450°C.
All methods used are technical procedures certified in the ISO and STAS systems. The analytical data obtained were statistically calculated, using ANOVA SPSS 14, Duncan's test.

RESULTS AND DISCUSSIONS

The evolution of average height of maize plants during the vegetation period

During the 58 days that the vegetation period lasted, the plants reached an average height between 59.7 cm in the control variant and 66.3 cm in the variant in which Zn was applied on the seed (Table 1).

Table 1. Average height of maize plants (cm) during the vegetation period

Measurement date / Variant	10 days after seeding	17 days after seeding	23 days after seeding	29 days after seeding	38 days after seeding	48 days after seeding	58 days after seeding
Control	10.6	31.3	43.3 a	47.6 a	50.0 a	56.7 a	59.7 a
0 Zn	10.7	32.0	45.0 ab	47.0 ab	55.0 b	61.7 c	64.0 b
Zn in soil	11.0	31.3	46.3 bc	49.0 ab	57.1 cd	64.7 d	66.1 b
Zn on seed	11.3	33.7	48.0 c	49.7 ab	58.0 c	62.7 c	66.3 b
Zn on plant	10.7	31.7	46.7 bc	48.7 b	55.2 bc	59.7 b	61.3 a

It was observed that the maximum effect of Zn application occurred when the microelement was applied on the seed, compared on the soil application variant. The heights of the plants in these two variants were comparable, as shown by the data in Table 1, Figures 2 and 3 in decreasing order of the plant height, depending on the place where the microelement was applied.
Next is the variant with Zn applied to the plant through foliar application with $ZnSO_4 \cdot 7H_2O$ solution, applied three times at one-day intervals. At each size measurement, the lowest values were recorded in control plants and then in those that did not receive Zn, but received P. The separation of the size of the plants according to the method of Zn application, was evident and statistically significantly ensured after 23 days after seeding, when the plants had developed 3-4 pairs of leaves.

Figure 2. 44 days after seeding - Four plants per pot. MT1 - control; 4 - soil without Zn addition; 9 - Zn applied to the soil; 10 - Zn applied to the seed; 15 - Zn applied to the plant

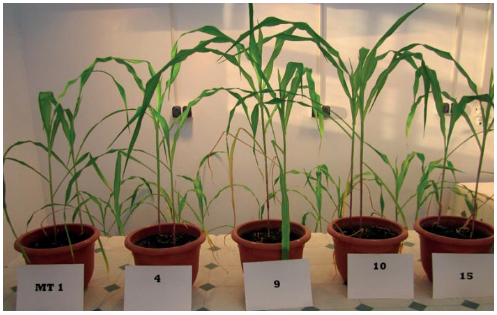

Figure 3. 44 days after seeding - two plants per pot. MT1 - control; 4 - soil without Zn addition; 9 - Zn applied to the soil; 10 - Zn applied to the seed; 15 - Zn applied to the plant

The statistical calculation of all mass values, both green and dry (Table 2) clearly demonstrates the beneficial effect of applying the micronutrient to the soil and the seed, as compared to its application to the plant.

Table 2. Green and dry mass (g) obtained in the experimental variants

Variant	Green mass		Dry mass	
	aerial part	root	aerial part	root
Control	25.0 a	1.1 a	4.0 a	0.8 a
0 Zn	38.7 b	2.7 ab	5.7 a	1.6 ab
Zn in soil	56.0 c	4.3 c	9.0 b	2.5 c
Zn on seed	52.3 c	3.9 c	9.0 b	2.3 c
Zn on plant	34.0 ab	2.7 ab	5.7 a	1.8 ab

The first morphological symptoms of Zn deficiency appeared in the last part of the vegetation period, in the plants to which no Zn was applied (Figure 4) and prior to the application of Zn to the plant (Figure 5).
The addition of zinc to the soil resulted in a greater microelement distribution area and a more uniformly distributed plant nutrient source.

Figure 4. 26 days after seeding - Morphological symptoms of deficiency occurrence in plants in pots without Zn application

Figure 5. 26 days after seeding - Early deficiency symptoms in plants treated with Zn on plant

In an experiment carried out in the greenhouse, Vasconcelos et al. (2011), obtained a 5% increase in the development of maize plants from the variants in which Zn was applied in the soil compared to its application on the plant.

The effect of applied treatments on some agrochemical properties of the soil from the vegetation pots

Following the cessation of plant growth, agrochemical analyzes were conducted on soil samples collected from vegetation pots. Tables 3 and 4 present analytical data that have been statistically calculated.

After $CaCO_3$ application, soil pH increased by 0.32 units, from 7.97 in the control sample to 8.29 in the sample where zinc was applied on plant. But the reaction domain was the same, low-alkaline. In parallel, the $CaCO_3$ content also increased by 0.77%, from 0.73% in the control sample to 1.50 in the sample where zinc was applied on plant.

The different $CaCO_3$ concentrations must be attributed to the differential degradation of the applied $CaCO_3$ based on the size of the plant's development. $CaCO_3$ dissolution was most intense in the variants where Zn was applied to the soil, where the plants grew best.

Total N content is relatively constant in all variants with a decrease of up to 0.022% in the variant where Zn was applied on plant. The non-application of any amount of nitrogen, on a medium natural background of assurance with this essential macroelement, contributed to the slight fluctuation of the total and mobile content between the variants.

Table 3. Reaction, content of $CaCO_3$ and macroelements in the soil material present of the vegetation pots at the end of the experiment

Variant	pH_{H2O}	$CaCO_3$	N_t	$N\text{-}NO_3$	P_{AL}	K_{AL}
		%		mg·kg^{-1}		
Control	7.97 a	0.73 a	0.176 a	9.13 b	32.7 a	192 a
0 Zn	8.13 ab	1.43 a	0.157 a	7.10 ab	59.3 c	255 b
Zn in soil	8.15 ab	1.37 a	0.162 a	6.43 a	54.0 bc	251 b
Zn on seed	8.20 b	1.43 a	0.153 a	5.93 a	48.3 b	241 b
Zn on plant	8.29 b	1.50 a	0.152 a	6.73 ab	49.7 bc	252 c

Table 4. The total and mobile content of microelements (mg·kg^{-1}) in the soil material present in the vegetation pots at the end of the experiment

Variant	Zn		Cu		Fe		Mn	
	total	mobile	total	mobile	total	mobile	total	mobile
Control	65.1 b	1.47 b	27.7 b	3.13 b	2.65 c	8.33 b	717 b	8.33 b
0 Zn	60.8 a	1.00 a	25.8 a	2.47 a	2.54 c	3.23 a	673 ab	2.27 a
Zn in soil	62.8 ab	2.73 c	25.5 a	2.47 a	2.56 c	3.23 a	705 ab	2.30 a
Zn on seed	60.5 a	1.10 a	25.7 a	2.40 a	2.41 b	2.47 a	652 ab	1.80 a
Zn on plant	61.3 a	1.10 a	26.4 ab	2.47 a	2.27 a	3.00 a	636 a	2.10 a

On the contrary, the application of P in the soil material of the vegetation pots led to obtaining, after ending the experiment, a high content of mobile P. Even under these conditions a differentiation of up to 11 mg·kg^{-1} was obtained between the variant that did not receive Zn and the one in which Zn was applied on the seed. The situation is similar regard to K mobile, whose content was also in the high range of supply with a difference of 14 mg·kg^{-1} between the variant with zero zinc applied and the variant where zinc was applied on the seed.

Also interesting are the values of microelements contents, both total and mobile, which without exception are higher, in the control variant than in the variants treated according to the presented scheme. The fact that the lowest concentrations of these elements were found in the variant in which Zn was applied to the seed, where the maximum green and dry plant masses were obtained, indicates that the plants absorbed these elements more efficiently.

The evolution of the chemical composition of maize plants according to the method of Zn application

The results of the statistical processing of the chemical analyzes performed regarding the content of macro- and microelements in the aerial part and the root part of the plants are presented in Tables 5-8.

Although the control plants, collected at 44 days after seeding, had a higher N content than the plants grown with Zn intake, the differences were not statistically ensured. However, in the plants collected at 58 days after seeding, the trend was maintained, even at lower content values, being this time statistically ensured. Considering also the values of the N content in the roots, where the highest concentration was in the roots from the control variant, it can be stated that the impact with P and Zn in the other variants contributed to the reduction of N absorption both in the roots and in the aerial part of plants.

In the case of P, the phenomenon was the opposite, the maximum absorption of the macroelement occurred in the plants of the variants treated with P and Zn, both in the roots and in the aerial part. Of course, the amount of 125 mg P applied per kg of soil material in the pot also contributed to this phenomenon. It is observed (Tables 5 and 6) that only the differences from plants collected at 44 days after seeding are statistically ensured.

And in the case of K, higher contents were registered in the plants of the variants treated with P and Zn both in the aerial part, at the two harvests (Table 5) and in the roots (Table 6). The higher mobility of K led to the more intense uptake in the aerial part of the plant and especially in younger plants.

Table 5. Macroelements content (%) of maize plants (aerial part)

Collect date	Variant	N	P	K	Ca	Mg
44 days after seeding	Control	1.92 a	0.24 a	3.19 a	0.73 b	0.57 b
	0 Zn	1.61 a	0.33 b	5.60 b	0.31 a	0.28 a
	Zn in soil	1.40 a	0.29 b	5.39 b	0.31 a	0.28 a
	Zn on seed	1.72 a	0.30 b	4.22 ab	0.33 a	0.27 a
	Zn on plant	1.57 a	0.32 b	4.87 b	0.34 a	0.30 a
58 days after seeding	Control	1.73 b	0.16 a	2.34 a	0.59 b	0.48 b
	0 Zn	1.16 a	0.24 a	3.58 c	0.26 a	0.22 a
	Zn in soil	0.93 a	0.24 a	3.27 bc	0.22 a	0.18 a
	Zn on seed	0.95 a	0.22 a	3.06 b	0.22 a	0.20 a
	Zn on plant	1.11 a	0.24 a	3.39 bc	0.24 a	0.20 a

The two macroelements Ca and Mg analyzed had, as in the case of N, a more intense accumulation in the control plants, both in the aerial part and in the roots. The content differences being statistically ensured, with one exception, the Mg content in the roots (Table 6).

Table 6. Macroelements content (%) of maize plants (root part)

Collect date	Variant	N	P	K	Ca	Mg
58 days after seeding	Control	1.32 b	0.19 a	0.51 a	0.94 c	0.27 a
	0 Zn	1.08 ab	0.21 a	1.94 b	0.65 a	0.25 a
	Zn in soil	0.89 a	0.20 a	2.33 c	0.64 a	0.19 a
	Zn on seed	0.90 a	0.19 a	2.32 c	0.85 bc	0.19 a
	Zn on plant	1.19 b	0.22 a	1.87 b	0.73 ab	0.21 a

Regarding the content of microelements (Tables 7 and 8), differences were observed for Zn depending on the method of microelement application, namely, in the aerial part, values ranging from 8.5 to 17.0 $mg \cdot kg^{-1}$ in the case of variants in which Zn was applied in the soil or on the seed, and high values ranging from 66.6 to 112.6 $mg \cdot kg^{-1}$ in the case of variants in which Zn was applied on the plant. The phenomenon was also similar in the case of roots, with close values between the control and the variants of application of Zn in the soil or on the seed. The fact that the highest content was registered in the roots of plants sprayed with Zn demonstrates that the trace element also circulated from the leaves to the roots.

Tabele 7. Microelements content ($mg \cdot kg^{-1}$) of maize plants (aerial part)

Collect date	Variant	Zn	Cu	Fe	Mn
44 days after seeding	Control	30.1 c	5.53 b	61.56 a	65.47 a
	0 Zn	12.5 a	3.80 a	62.53 a	57.53 a
	Zn in soil	21.6 b	3.17 a	60.57 a	38.76 a
	Zn on seed	12.4 a	3.10 a	62.93 a	50.43 a
	Zn on plant	112.6 d	4.00 a	61.87 a	53.43 a
58 days after seeding	Control	26.0 b	4.73 b	71.4 a	65.16 a
	0 Zn	8.56 a	3.06 a	70.5 a	38.47 a
	Zn in soil	17.0 ab	2.57 a	63.23 a	37.50 a
	Zn on seed	9.13 a	2.63 a	63.60 a	37.07 a
	Zn on plant	66.6 c	2.63 a	68.60 a	41.63 a

The phenomenon described in the absorption of Zn in plants also occurred in the case of Cu, but at a lower intensity. Both in the aerial part and in the roots, the highest Cu content was determined in the control plants. Differences in content between the control and the other variants are statistically ensured. Although there were no statistically significant differences between the contents of Fe and Mn in the aerial part of the plants, the phenomenon of the decrease of microelements in the variants trated with P and Zn is also preserved in this case on a smaller scale.

Table 8. Microelements content (mg·kg^{-1}) of maize plants (root part)

Collect date	Variant	Zn	Cu	Fe	Mn
58 days after seeding	Control	30.67 a	26.97 b	934 a	79.03 ab
	0 Zn	27.23 a	15.40 a	3193 b	113.7 c
	Zn in soil	28.60 a	17.10 ab	2250 b	75.2 ab
	Zn on seed	27.20 a	17.10 ab	2212 b	55.5 a
	Zn on plant	49.20 b	19.1 ab	961 a	106.5 bc

Instead, in the root there was a significant accumulation of Fe in the plants from the variants treated with P and Zn and at the same time a significant decrease in the Mn content.

CONCLUSIONS

For the biofortification of plants with Zn, the application of Zn on the seed was the most effective in terms of plant development (height), while the application of Zn in the soil was statistically the most effective in terms of green and dry mass, with values comparable to those of the application variant of the microelement on the seed.

The treatments applied to the soil material in the vegetation pots consisted of the administration of $CaCO_3$ and P. Zn was administered both in the soil, on the seed and on the plant in the different experimental variants. At the conclusion of the vegetation period, the treatments resulted in an increase in soil reaction and $CaCO_3$ content and a decrease in total and mobile Nt, $N\text{-}NO_3$, P_{AL}, K_{AL}, and microelement (Zn, Cu, Fe, Mn) contents due to their absorption by plants.

A different absorption of nutrients in the plant was recorded in the variants treated with $CaCO_3$, P and Zn applied to the soil, in the sense of a decrease in the absorption of N, Ca, Mg, Cu, Fe, Mn, partially and Zn and an increase in the absorption of P and K. The highest contents of macro- and micronutrients were recorded both in the aerial part and in the roots, in variant where Zn was applied directly on the plant.

After completing the study, it can be concluded that the administration of the microelement both in the soil and on the seed are the optimal methods for biofortifying maize plant with zinc, with nearly equal performance.

ACKNOWLEDGEMENTS

This research work was carried out with the support of two projects of the Romanian Ministry of Research, Innovation and Digitization-National Authority for Scientific Research and Innovation project: PN 23 29 05 01/2023 (Development of indicators on the role of soil microbiota biodiversity and functionality in providing ecosystem services, improving soil health and increasing resilience to climate change); PN 16 07 02 06/2016 (Research on zinc biofortification of maize) and project No. 44 PFE/2021, Program 1 - Development of national research-development system, Subprogram 1.2 - Institutional performance - RDI Excellence Financing Projects.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INFLUENCE OF APPLIED TECHNOLOGIES ON THE PHYSICO-CHEMICAL PROPERTIES OF SOILS IN PERIȘORU AREA, CĂLĂRAȘI COUNTY

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Abstract

The studied area is located the north-eastern part of Călărași County, belonging to the cadastral territory Perişoru, currently used as arable. The pedological mapping was carried out with the purpose of identifying the zonal soil, by assessing its fertility as well as the influence of applied technologies on the physico-chemical properties of the soil. A soil profile and several surveys were opened from which soil samples were collected in natural and modified settlement, for morphological, physical and chemical analysis. The soil type identified is represented by typical chernozem, vermic with undifferentiated loamy texture on the profile. The main physical characteristics (bulk density, total porosity and compaction degree), chemical (soil reaction, humus content, nitrogen, phosphorus, potassium) and hydrophysical indices were determined by indirect methods. Applying high-performance culture technologies, they highlighted the improvement of the aforementioned characteristics, by creating a favorable aerohydric regime and implicitly important production increases.

Key words*: bulk density, total porosity, compaction degree, typical chernozem, performance technologies.*

INTRODUCTION

Soil work can directly or indirectly influence its physical characteristics, both on the mobilized depth and below this limit. The main physical characteristics of the soil that can be influenced as a result of these works are: structure, total porosity and aeration, bulk density, penetration resistance, permeability, which in turn can influence the physical-mechanical characteristics of the soil (consistency, adhesiveness, plasticity, plowing resistance, etc.) (Cârciu et al., 2019; Coronavski, 2010).

The structure of the soil is often damaged by incorrect execution of soil work and inappropriate moisture content or by repeated passes without justification. Soil moisture at the time of execution of the work has a very important role because, in the case of a soil that is too dry, plowing results in clumped soil, it is performed with high energy consumption (fuel) and requires additional work for the soil to be shredded (Mihalache, 2014).

By plowing, the upper horizon is covered by bringing to the surface the soil layer restored and sometimes enriched in nutrients, achieving favorable conditions for germination and plant growth. At the same time, when a loosening of the soil is effectuated, its volume is increased by at least 25%, hydrostable aggregates are created especially at bulk density values of 1.1-1.3 g/cm^3.

This volume disappears over time, by natural laying, when preparing the seedbed or during the growing season (Dumitru et al., 2011; Mihalache & Ilie, 2008).

Total porosity and aeration are variable characteristics, depending on the texture and soil works, specific to each type of soil from the clay (vertosols) to the sandy (alluvial soils or psamosols). Both characteristics have unfavorable consequences on the growth and development of plants, when their values are low because the root system develops hard, infiltration is reduced, so is the activity of aerobic microorganisms.

The optimal limits of total porosity for plants are 48-58% of soil volume, capillary porosity of 30-36% and aeration porosity of 18-24%.

Through the agrotechnical works applied to the soil, its physical characteristics and implicitly the aerohydric regime are modified, thus conferring favorable conditions for the activity of microorganisms. Changes due to soil work have direct implications also on the chemical and biological characteristics of the soil, by increasing porosity, reducing the bulk density value and changing the temperature regime.

As a result of these works, aerobiosis conditions are created in the soil, favoring microflora (bacteria, fungi and actinomycetes), with a role in the decomposition of organic cellulosic substances. Due to the fact that fresh organic substances decompose intensively, the coefficient of their transformation into humus is greatly reduced.

By creating the conditions of aerobiosis, the mineralization of organic debris is intensified and, as a result, a deficit of energy material is created in the soil and bacteria are forced to decompose humus. This phenomenon can be reduced by avoiding excessive loosening work.

After Munteanu and Florea (2009), in löess soil the nitrification process is more intense, reaching the maximum intensity when the volumetric weight values are between 1.11 and 1.15 g/cm^3. Due to nitrification, the favorable processes by which phosphorus, potassium, calcium and other nutrients pass from hardly soluble forms into forms easily accessible to plants are emphasized. The typical example, is the solubilization of phosphorus (Budoi & Penescu, 1996).

Compaction leads to the degradation of the soil structure, which causes damage to its other physical properties (Richard et al., 2001).

An indicator of compaction is the bulk density, which represents the most accurate possibility of assessing soil horizons, being also a determining factor of the other physical and chemical properties. When applying organic fertilization, the bulk density has the lowest values, between 1.09 g/cm^3 and 1.23 g/cm^3, for a cambic chernozem in Western Romania. In this case, the highest values were in variants in which NPK was applied in doses of $N_{150}P_{100}K_{50}$, which resulted in values between 1.46 g/cm^3 and 1.48 g/cm^3 (Mihuț & Rusu, 2007).

Figure 1. Perișoru, Călărași County

Soil structure can be expressed by the stability of soil structural aggregates (the ability of soil aggregates to resist decay by water).

Based on the experiments carried out, it was found that organic fertilization had a positive impact on the stability of structural aggregates, the aeration porosity being significantly higher at organic fertilization (9.6% on average), compared to unfertilized treatments (8.8 %).

The bulk density was significantly influenced by fertilization, according to the research conducted by Stehlik et al. (2019), where the bulk density recorded in fertilized plots was 1.31 g/cm^3, compared to 1.35 g/cm^3 in unfertilized plots.

The bulk density or volumetric weight correlates very well with the total porosity and compaction degree of the soil, being conditioned by the texture, content in organic matter and the agrotechnical works performed (Canarache, 1990). According to the literature, the root system develops optimally at bulk density values between 1.0-1.4 g/cm^3.

MATERIALS AND METHODS

The experiment was conducted in the Southeastern part of Romania, in Perișoru area, Călărași County, on a typical chernozem (Figure 1).

The placement of the soil profiles was made according to the complexity of the terrain and the soil cover, according to MESP, 1987 vol. I (Collection and systematization of pedological data).

It is a pedological study of Type B, category I of complexity. The density of soil profiles was established according to the current methodology (INCDPA Bucharest), but also according to the research purpose (technology applied for each studied area).

The description of each soil profile was carried out according to the guide for the field description of the soil profile and the specific environmental conditions, INCDPA Bucharest, (Munteanu & Florea, 2009).

Two series of profiles were carried out in the period April-May, 2019 (Figure 2) and in the period August-September, 2021 (Figure 3), after harvesting the crops.

Soil analysis

The samples were analyzed in the INCDPA Bucharest laboratories. Soil samples were dried at room temperature; soil subsamples were homogenized, milled, and sieved through a 250 μm sieve.

The following analytical methods were used to determine the chemical properties:

– organic matter (humus): volumetric determination, (Walkley-Black humidification method, STAS 7184/21-82);
– $CaCO_3$ (carbonates): gasometrical method (Scheibler calcimeter, SR ISO 10693: 1998, %);
– the nitrogen content, by calculation, based on the humus content and the degree of saturation with bases (IN = humus x V/100);
– mobile phosphorus content (Egner-Riehm-Domingo method and colorimetric molybdenum blue, Murphy-Riley method ascorbic acid reduction);
– mobile potassium content (Egner-Riehm-Domingo extraction and flame photometry);
– pH (potentiometric method in aqueous suspension at soil/water ratio of 1/2.5 - SR 7184 /13-2001);
– hydrolytic acidity, extraction with sodium acetate at pH 8.2;
– base saturation degree, V% (Kappen Schoffield method).

The following physical characteristics were determined:

– determination of granulometric fractions: pipette method, for fractions ≤ 0.002 mm; wet grinding method for fractions of 0.002-0.2 mm and dry grinding method for fractions > 0.2 mm.

The results are expressed as a percentage of the material remaining after pretreatment.

– bulk density (BD): The known volume of metal cylinders (100 cm^3) at the instant soil moisture (g/cm^3);
– total porosity (TP): by calculation (% by volume - % v/v);
– aeration porosity (PA): by calculation (% volume -% v/v);
– degree of compaction (DC): by calculation (% by volume - % v/v), where: PMN - minimum required porosity, clay of the sample is calculated with the formula PMN = 45 + 0.163 A (% by volume -% v/v); PT = total porosity (% v/v); A - clay content (% w/w),
– hygroscopicity coefficient (HC): drying at 105°C of a pre-moistened soil sample at equilibrium with a saturated atmosphere with water vapor (in the presence of 10% H_2SO_4 solution) - % by weight (% w/g);
– wilting coefficient (WC, %, g/g), calculated based on hygroscopicity coefficient;
– field water capacity (FWC, % w/w), calculated based on Dumitru et al. (2009) formula, considering clay content (%), silt content (%), bulk density (g/cm^3), and layer depth (cm);
– useful water capacity (UWC, % w/w) is calculated as the difference between field capacity (% w/w) and wilting coefficient (% w/w);
– total water capacity (TC, % w/w) is determined as the ratio between total porosity (% v/v) and bulk density (g/cm^3).

RESULTS AND DISCUSSIONS

Profile 1 - Typical chernozem

Coordinates: *$44^0$453 252 - N & $27^0$501 395 - E*
Landscape: plain
Use: arable
Parent material: loessoid deposits
Groundwater: >10 m

Figure 2. Typical chernozem (2019)

Morphological characterization
Horizon Am (0-32 cm), dusty clay, dark brown, (10 YR 2/1 to wet and 10 YR 3/2 to dry) moderately developed glomerular structure, porous, permeable, frequent fine roots from cultivated vegetation, weak effervescence, gradual transition to the lower horizon;
Horizon AC (32-57 cm), medium clay, yellowish-brown, (10 YR 4/3 to wet and 10 YR 5/4 to dry), poorly developed glomerular structure in the upper half of the transition horizon, slightly friable, loose, accumulations of carbonates in the form of pseudomycelia, moderate effervescence;
Horizon Cca (>57 cm), dusty sandy clay, yellowish (10 YR 5/4 in wet and 10 YR 6/4 in dry) unstructured, friable, loose, with accumulations of carbonates in the form of pseudomycelia and small crumbly concretions, strong effervescence.

Profile 2 - Typical chernozem
Coordinates: *44^0 26' 05" - N & 27^0 30' 03" - E*
Landscape: plain
Use: arable
Parent material: loessoid deposits
Groundwater: >10 m

Figure 3. Typical chernozem, CL (2021)

Morphological characterization
Horizon Am (0-34 cm), dusty clay, dark brown (10 YR 2/1 to wet and 10 YR 3/3 to dry), well-formed glomerular structure, rough, porous, loose, non-plastic, non-adhesive, frequent fine roots, does not effervescence, wavy gradual transition;
Horizon AC (34-62 cm), medium clay, light brown (10 YR 3/3 to wet and 10 YR 4/4 to dry), moderately developed glomerular structure, rough, porous, loose, non-plastic, non-adhesive, frequent fine roots, weak effervescence, wavy gradual transition;
Horizon Cca (62-115 cm), sandy clay loam, yellowish (10 YR 4/3 to wet and 10 YR 5/4 to dry), poorly structured in the upper half, very friable, porous, loose, non-plastic, non-adhesive, rare fine roots, strong effervescence, clear straight passage.
Variation of chemical characteristics (pH and humus content) for this type of soil is shown in Figure 4. Based on the graph, it can be seen that the value of the soil reaction is 7.8 (weakly alkaline) at the surface and it increases to over 8.5 at the base of the profile. The humus content varies inversely to the soil reaction, from 4.6 in the bioaccumulative horizon to 1.8 at the base of the soil profile.

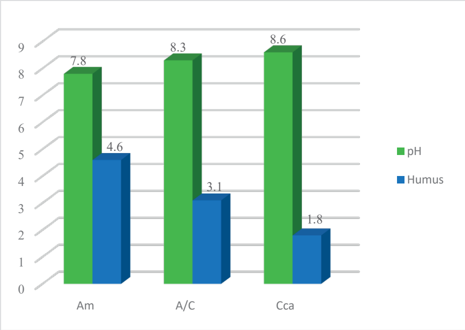


Figure 4. Variation of chemical characteristics

On the typical chernozem from Perişoru, due to the medium and undifferentiated texture on the depth of the profile, but also due to the good quality agrotechnical works, the bulk density values are the most favorable during the three years, 2019-2021 (Figure 5).

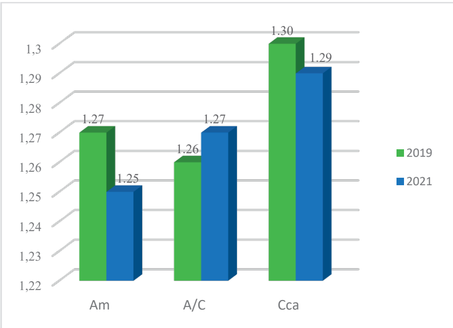


Figure 5. Bulk density (2019-2021)

The total porosity values correlate with the bulk density, due to the agrotechnical works performed, in conjunction with the clay texture and moderately developed soil structure (Figure 6).

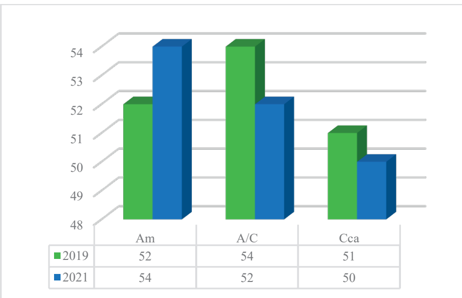


Figure 6. Total porosity (2019-2021)

The perfect correlation between total porosity and bulk density is reflected on the degree of subsidence, with almost parallel values between 2019 and 2021 (Figure 7).

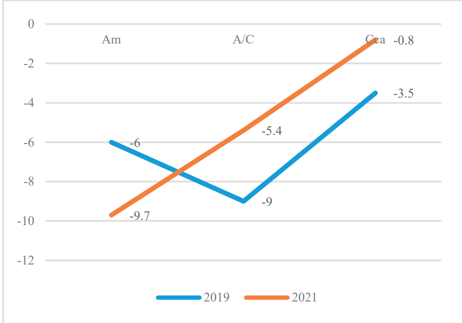


Figure 7. Compaction degree (2019-2021)

The results obtained show the most favorable characteristics, usually encountered in soils with loamy texture (medium), undifferentiated on the profile but also due to the applied technology.

The studied land is classified in the second grade of quality for arable lands, with 66 points, due to the poor climatic conditions and the groundwater level below 10 m. The graphic representation of the land evaluation marks, by crops is shown in Figure 8.

The economic efficiency by crops, during the three years of research (2019-2021) is presented in Figures 9, 10 and 11.

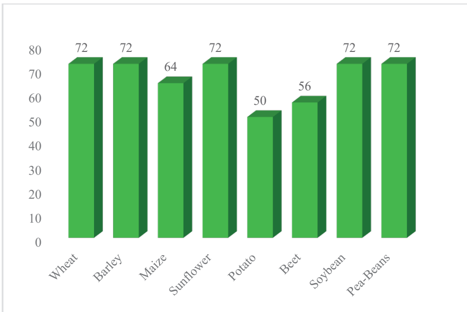
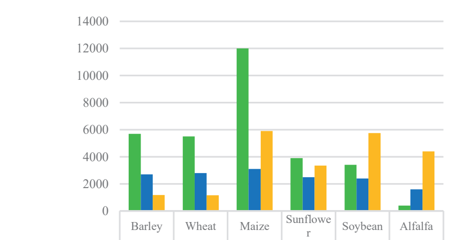


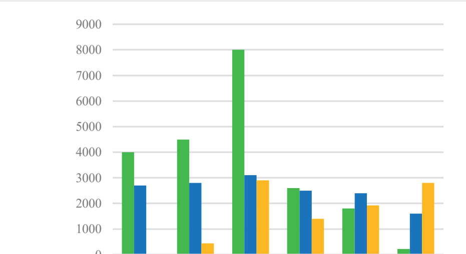
Figure 8. Land evaluation marks, by crops



	Barley	Wheat	Maize	Sunflower	Soybean	Alfalfa
Yield (kg/ha)	5700	5500	12000	3900	3400	400
Costs (Ro/ha)	2700	2800	3100	2500	2400	1600
Profit/ha	1176	1160	5900	3350	5760	4400

Figure 9. Economic efficiency (2018-2019)

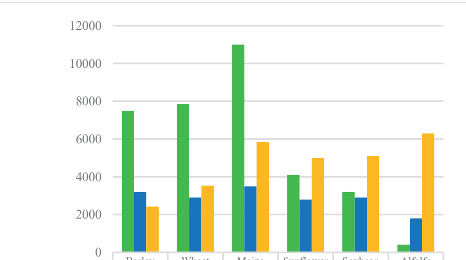
Based on the graphic results, it can be shown that in the first year of research, profit was obtained for all crops in rotation, as a result of the superior technology applied, but also due to the productive potential of the soil.



	Barley	Wheat	Maize	Sunflower	Soybean	Alfalfa
Yield (kg/ha)	4000	4500	8000	2600	1800	220
Costs (Ro/ha)	2700	2800	3100	2500	2400	1600
Profit/ha	20	440	2900	1400	1920	2800

Figure 10. Economic efficiency (2019-2020)

In the second year of research, the economic situation is repeated, but with a lower profit, obtained on the basis of the resulting productions. The technology was the same, but there were less favorable climatic conditions.



	Barley	Wheat	Maize	Sunflower	Soybean	Alfalfa
Yield (kg/ha)	7500	7850	11000	4100	3200	405
Costs (Ro/ha)	3200	2900	3500	2800	2900	1800
Profit/ha	2425	3537	5850	4990	5100	6300

Figure 11. Economic efficiency (2020-2021**)**

In the third year of research, the economic situation was the most beneficial, both in terms of climate and in terms of soil potential and applied superior technology.

CONCLUSIONS

In the conditions of the typical, vermic chernozem, formed in the steppe zone, on loessoid deposits, with groundwater found at over 10 m, in temperate climate, the following were found:

- the soil works were applied in a controlled, systematized way, at adequate humidity, were of good quality and alternated the classical system with the conservative one;
- under these conditions, the yields obtained were very good, with a substantial profit being obtained, both due to the potential of the soil and the applied technology;
- a large part of the area is equipped for irrigation, especially for the maize and soybean crops, and 3-4 waterings were applied with norms of 500 m^3/ha;
- application of chemical root fertilizers, was performed according to a rigorous plan, since agrochemical mapping is carried out once every 4 years.
- the production obtained for the main crops in the investigated period was at an average level, with relatively low expenses and with a substantial profit for each crop;
- in the case of maize for grains, the production reached the level of 12000 kg/ha, primarily due to the type of soil well-supplied with nutrients, with favorable physico-chemical characteristics, to which the applied technology is associated;
- due to the high productive potential of the soil type and the top technology applied, the soybean crop recorded a production of about 3.5 tons/ha, thus generating a profit of over 5700 lei/ha.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INTRA-FIELD SPATIAL HETEROGENEITY PREDICTION FOR THE PURPOSES OF PRECISION FARMING: COMPARISON OF FREQUENCY RATIO AND SHANNON'S ENTROPY MODELS

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Abstract

This paper provides the results of assessing the possibility of using frequency ratio (FR) and Shannon's entropy (SE) models to predict the intra-field spatial heterogeneity zones (IFHZ) which are taken into account when performing various technological processes in precision farming. The studies were carried out in 2021-2022 in the Radomyshl community of Zhytomyr raion (Zhytomyr oblast, Ukraine) in an area of 6.602 km². To determine IFHZ, nine soil parameters were used, the suitability of which for prediction was determined by multicollinear analysis. These data include the hydrolytic acidity, nitrogen, phosphorus and potassium content, the soil buffer balance index, and B, Mo, Cu, and Zn content. The area under the receiver operating characteristic (AUROC) method has been utilised to validate both FR and SE models. The research suggests that the AUROC curve for SE (0.84) was better than that for FR (0.82). Hence, the SE model predicts IFHZ more accurately than the multivariate statistical model FR in the study area.

Key words*: intra-field heterogeneity, forecasting; frequency ratio, Shannon's entropy, model comparison.*

INTRODUCTION

Precision farming technology provides a viable solution for managing profitability and reducing production costs in agriculture, especially in the face of dwindling farmland and increasing energy and raw material costs for mineral fertilizers (Myslyva et al., 2021; Hrynevych et al., 2022). This assertion is supported by the expected growth of the precision farming market to $11.54 billion by 2026, with a CAGR of 14.3% (ReportLinker, 2022).

Ukraine is an important global agricultural producer, accounting for 41% of the country's total exports in 2021, and is a top exporter of sunflower meal and oil, corn, and wheat (USDA, 2022). Precision farming technology has already gained significant traction in Ukraine, with an average usage percentage of 51.2% (UCAB, 2021). Its relevance to the Ukrainian agrarian sector is particularly noteworthy, given the shift in the main grain production regions from the country's south and southeast to its less fertile northern and western regions, including the 100,000 km² Ukrainian Polissia.

It is widely understood that the successful adoption of precision farming requires the identification and demarcation of spatially diverse areas within a field known as intra-field spatial heterogeneity zones (ISHZ). These zones are taken into consideration when carrying out various technological operations in crop production, as highlighted by research conducted by Córdoba et al. (2016) and Méndez-Vázqueza et al. (2019).

Considering the specific nature of land use and land tenure in Ukraine, as well as the specialization of agricultural enterprises, the most effective method for identifying intra-field heterogeneity zones is an approach that takes into account multiple soil characteristics. However, regardless of the specific approach and parameters used, Geographic Information Systems (GIS) and mathematical modelling methods such as Frequency Ratio (FR) and Shannon's Entropy (SE) provide a universal tool for identifying ISHZs. Although these methods have been extensively and effectively utilized for flood prediction (Arabameri et al., 2019; Arora et al., 2021), landslide susceptibility (Shano et al., 2020; Wubalem, 2021), and forecasting groundwater resources distribution (Al-Ruzouq et al., 2019; Chatterjee et al., 2020), their use in identifying of intra-field spatial heterogeneity zones remains relatively scarce.

Considering all of the above, the objective of this study is threefold: (1) to process initial data on soil parameters and generate thematic layers containing relevant attribute information; (2) to identify and map ISHZs with distinct land quality, based on a combination of soil parameters, using the FR and SE modelling techniques; and (3) to compare the outcomes of both methods to determine which one is most effective in detecting ISHZs.

MATERIALS AND METHODS

The studies were carried out in 2021-2022 in the Menkivka starostynsky okrug Radomyshl community of Zhytomyr raion (Zhytomyr oblast, Ukraine). The study area is a part of the Zhytomyr physical-geographical region of the Ukrainian Polissia and located between 50°37′ to 50°3′ N and 29°08′ to 29°12′ E and spreads in an area of 6.59 km^2 (31.8% of total arable land) (Figure 1).

The climate of the study area is temperate (Dfb due to Köppen-Geiger climate classification).

The soil cover of the study area is represented by Dystric Leptosols (0.28 km^2), Anthric Luvisols (3.44 km^2), Anthric Retisols (0.23 km^2) and Umbric Gleysols (2.65 km^2) (according to the international soil classification system (WRB, 2014) and has a predominantly sandy-loamy texture.

A total of 145 geo-referenced representative surface soil samples at 0-20 cm depth were collected within the territory of interest. The selection was carried out on an irregular grid. The samples were crushed, air-dried in shade at room temperature (~ 25°C), and passed through a 2 mm sieve for further analysis.

Figure1. Location of the study area

The following parameters were determined in each soil sample: humus content (Hu) - according to NSTU 4289:2004; pH$_{KCl}$ (pH) - according to GOST 26483-85; cation exchange capacity (CEC) - according to ISO 11260:1994; calcium (Ca) and magnesium (Mg) - according to GOST 26487-85; hydrolytic acidity (Ha) - according to GOST 26212-91; nitrogen (N) - according to NSTU 7863:2015; phosphorus (P) and potassium (K) - according to NSTU

4405:2005; molybdenum (Mo) - according to GOST 50689-94; boron (B) - according to GOST 50688-94. The acid soluble (1N HCl extractant) forms of lead (Zn) and copper (Cu) were determined through the method of atomic and absorption spectrometry on the SOLAAR MkII-M6 Double Beam AAS device. The soil buffer balance index (SB) was determined by the Nadtochy method (Nadtochy, 1993).

To determine which soil parameter is best suited for identifying ISHZs, a multicollinearity analysis was conducted. The analysis utilized variance inflation factor (VIF) and tolerance (TOL) to assess whether the explanatory variables used in the modelling are highly dependent on one another.

The integration of thematic layers and their corresponding percentages was utilized to determine the spatial distribution of intra-field spatial heterogeneity through overlay analysis in an ArcGIS 10.8 environment. To prepare a spatial variability map for each soil variable, both geostatistical and deterministic methods were used for interpolation techniques. Geostatistical methods were used for hydrolytic acidity, the content of B and Mo, and the soil buffer balance index.

Cross-validation analysis was conducted to evaluate the accuracy of interpolation utilizing methods.

The optimal number of gradations of heterogeneity zones within the study area was established by the Principal Component Analysis (PCA) technique (Zeraatpisheh et al., 2020).

FR is defined as (Guru et al., 2017) (1):

$$FR = (W/TW)/(CP/TP) \quad (1)$$

where FR is a frequency ratio of the class of each soil parameter, W is the number of pixels of the most fertile and least fertile soil locations for each class of thematic maps; TW is the number of total pixels of the most fertile and least fertile soil locations in the study area; CP is the number of pixels in each thematic class and the TP is the total number of pixels in the study area. In the FR model, the FR value of each class in the thematic layer was considered as the weight of that particular class in thematic parameters to determine intra-field heterogeneity.

The SE for all the explanatory variables to prioritize the susceptibility of the individual explanatory variable to intra-field spatial heterogeneity forming and subsequent final susceptibility mapping has been calculated using the following equations (2-6):

$$P_{ij} = b/a \quad (2)$$

$$(P_{ij}) = \frac{P_{ij}}{\sum_{j=1}^{S_j} P_{ij}} \quad (3)$$

$$H_{ij} = -\sum_{j=1}^{S_j} (P_{ij}) \log_2 (P_{ij}),\ j = 1, \ldots, n \quad (4)$$

$$I_j = \frac{H_{jmax} - H_{ji}}{H_{jmax}}, I = (0,1), j = 1, \ldots, n \quad (5)$$

$$W_j = I_j \cdot P_{ij} \quad (6)$$

where *a* is the class area of the independent variable and *b* is the area of the most fertile and least fertile soil locations falling within the class, expressed as a percentage, (P*ij*) is the probability of density, H_j and H_{jmax} represent entropy values, I_j is the information coefficient. W_j represents the resultant weight value for the factor as a whole.

Microsoft Excel program was used in the calculation of the FR and SE of the total input factors and the spatial analyst module of ArcGIS 10.8 has been used to reclassify and the final intra-field spatial heterogeneity zones maps were produced using a raster calculator.

Receiver Operating Characteristic (ROC) was chosen as the method for estimating model performance. In this method, for every possible cut-off value, false positive rates (FPR) and true positive rates (TPR) were plotted on the x-axis and y-axis, respectively (7), (8):

$$FPR = FP/(FP+TN) \quad (7)$$

$$TPR = TP/(TP+FN) \quad (8)$$

where FP is the number of false positive cases, TN is the number of true negative cases, TP is the number of true positive cases, and FN is the number of false negative cases. The FPR also termed sensitivity (Arabameri et al., 2019), is the probability a test will render an intra-field spatial heterogeneity when it exists and the TPR, which is also known by the name

“1-specificity” indicates one minus the probability a test will be negative in case of actually non-occurrence of intra-field spatial heterogeneity. Hence, in ROC, the sensitivity is plotted as a function of the false positive rate for various levels of cut-off points meaning thereby, each point on ROC is a sensitivity/specificity pair connected to a particular decision threshold (Arora et al., 2021).

The area under the curve (AUC) represents the discriminatory power of a model with which it accurately predicts the occurrence or non-occurrence of intra-field spatial heterogeneity. AUC values of <0.6, 0.6-0.7, 0.7-0.8, 0.8-0.9, and >0.9 indicate, respectively, poor, moderate, good, very good, and excellent model performance (Nhu V.H. et al., 2020).

The ROC curve and the AUC were calculated using the ROC Tool of ArcSDM.

RESULTS AND DISCUSSIONS

At the outset, the study planned to utilize 14 soil parameters. However, after conducting the multicollinearity analysis, it was found that variables like humus content, $pH_{(KCl)}$, cation exchange capacity (CEC), Ca, and Mg content had high multicollinearity with VIF>10 and TOL <0.1, and were excluded from the analysis. The VIF and TOL values for all the variables ranged from 1.60 to 8.13 and 0.121 to 0.62, respectively, as shown in Figures 2 and 3.

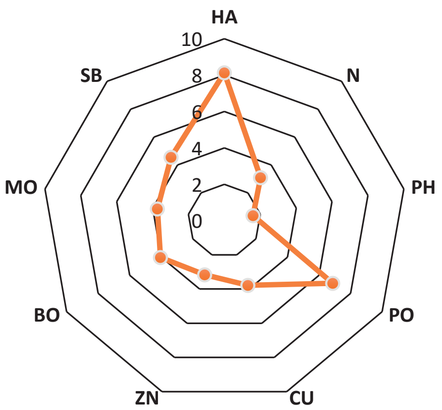

Figure. 2. Multicollinearity analysis of the soil parameters (VIF)

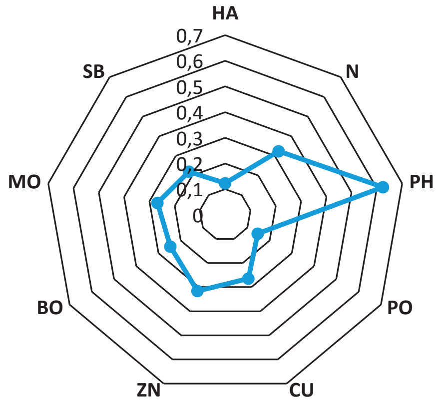

Figure. 3. Multicollinearity analysis of the soil parameters (TOL)

Then deterministic and geostatistical interpolation methods were used to visualize the spatial distribution of nine soil parameters. Thus, nine raster images were generated as a result and then were utilized as the fundamental geospatial data to carry out the forecasting of intra-field spatial heterogeneity (Figures 4-12).

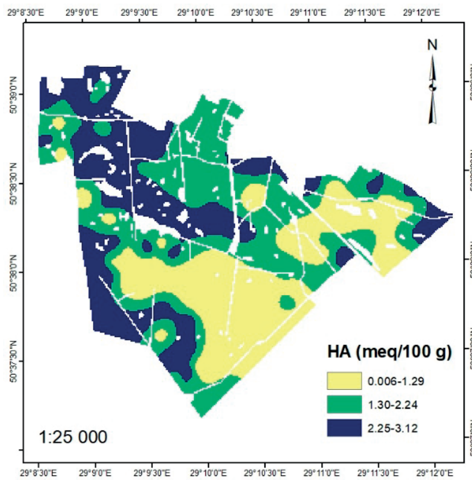

Figure 4. Intra-field spatial heterogeneity factors (soil parameters) used in this study: hydrolytic acidity

It is important to point out that the southeastern and eastern areas of the study area exhibit the highest values for all of the soil parameters examined, whereas the land parcels with lower values are primarily concentrated in the northwestern and western areas.

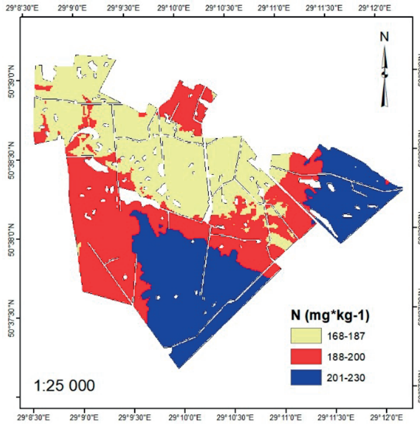

Figure 5. Intra-field spatial heterogeneity factors (soil parameters) used in this study: nitrogen

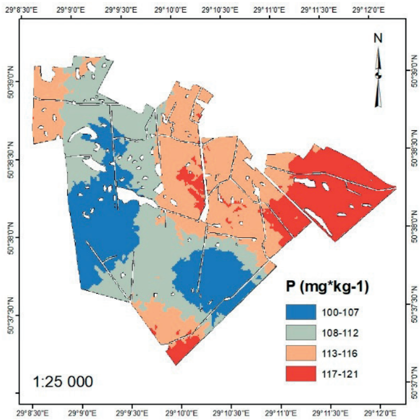

Figure 6. Intra-field spatial heterogeneity factors (soil parameters) used in this study: phosphorus

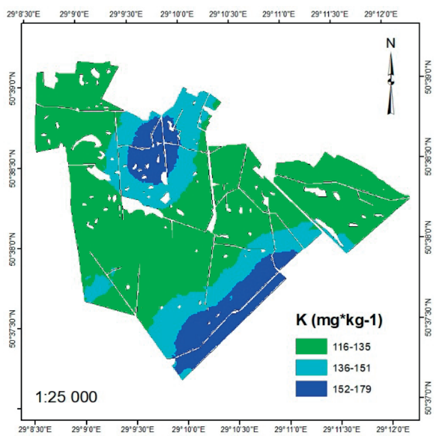

Figure 7. Intra-field spatial heterogeneity factors (soil parameters) used in this study: potassium

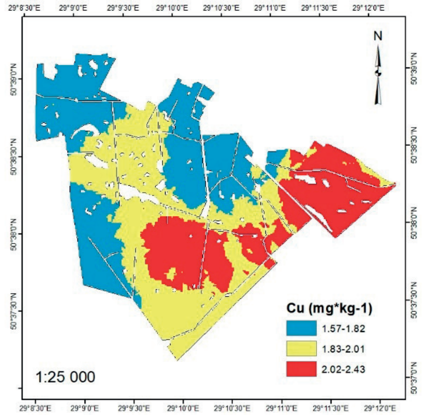

Figure 8. Intra-field spatial heterogeneity factors (soil parameters) used in this study: copper

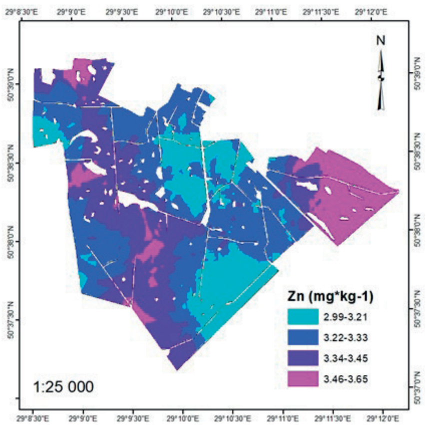

Figure 9. Intra-field spatial heterogeneity factors (soil parameters) used in this study: lead

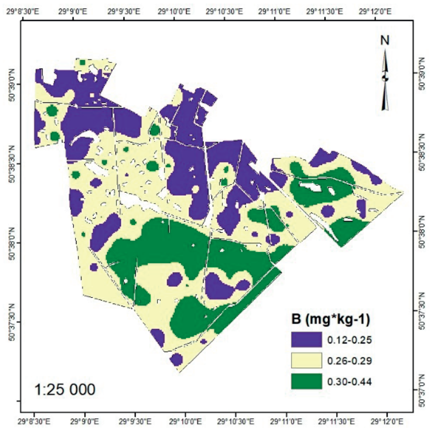

Figure 10. Intra-field spatial heterogeneity factors (soil parameters) used in this study: boron

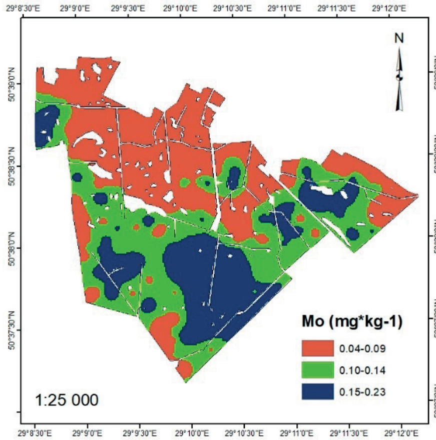

Figure 11. Intra-field spatial heterogeneity factors (soil parameters) used in this study: molybdenum

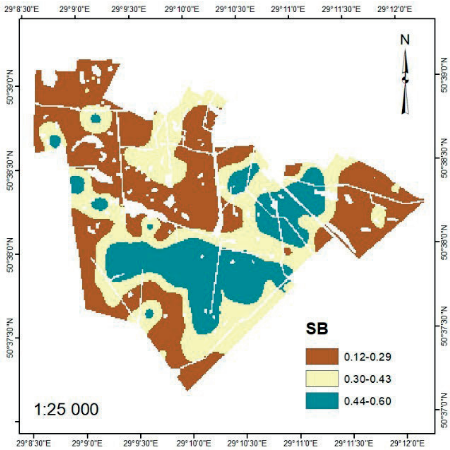

Figure 12. Intra-field spatial heterogeneity factors (soil parameters) used in this study: soil buffer balance index

This variability is largely attributed to the diverse soil cover, which comprises four different types of soil based on the FAO classification and eleven different types of soil according to the national classification system used in Ukraine.

To determine the appropriate number of categories for the intra-field spatial heterogeneity zones, PCA was applied. The principal components (PCs) with eigenvalues greater than 1 and accumulative contributions exceeding 60% were chosen (Oldoni et al., 2019; Srinivasan et al., 2022). Only the first three PCs were selected based on this criterion, which explained a total variability of 76.21% (Table 1).

Table 1. Principal component analysis for soil parameters

Principal compo nents	Eigenvalues	Component loadings (%)	Cumulative loadings, (%)
PC1	3.98	44.20	44.20
PC2	1.92	21.32	65.52
PC3	1.19	10.69	76.21
PC4	0.82	9.09	85.31
PC5	0.52	5.73	91.03
PC6	0.37	4.15	95.18
PC7	0.24	2.67	97.85
PC8	0.14	1.57	99.42
PC9	0.05	0.58	100.0

Frequency ratio is a susceptibility model that is commonly used and operates on the premise that the ratio of two event frequencies is a more effective predictor of the likelihood of those events occurring than the frequencies alone. In cases where the FR value for a soil parameter is higher, it suggests a strong correlation between the dependent variable (intra-field spatial heterogeneity zone) and the independent variable (the soil parameter in question). Conversely, FR values less than 1 indicate a weak relationship between the two variables (Tehrany et al., 2013).

The levels of phosphorus and zinc in the study area were categorized into four different classes, while the other soil parameters were divided into three classes, as shown in Table 2.

The highest frequency ratio values for indicators such as potassium, boron, copper, molybdenum, and soil buffer balance index were found in the upper categories (class 3). On the other hand, the maximum frequency ratio values for phosphorus, zinc, and hydrolytic acidity in the soil were observed in the lower categories (class 1), while the content of nitrogen showed the highest frequency ratio in the medium categories (class 2).

The highest prediction rate (5.667) was achieved for the phosphorus content, while the lowest prediction rate (2.423) was obtained for the zinc content. Based on these prediction rates, the studied soil parameters can be arranged in descending order as follows: P > SB > K > Mo > N > B > Ha > Cu > Zn.

Table 2. Intra-field spatial heterogeneity zones, computed using frequency ratio (FR)

Soil parameter	Class of soil parameter	Frequency Ratio (FR)	Prediction Rate (PR)
B	0.12–0.25	0.460	3.525
	0.26–0.29	2.400	
	0.30–0.44	3.452	
Cu	1.57–1.82	0.877	2.692
	1.83–2.01	0.721	
	2.02–2.43	1.147	
Mo	0.04–0.09	0.486	4.549
	0.10–0.14	1.182	
	0.15–0.23	3.413	
Ha	0.006–1.29	2.667	3.345
	1.30–2.24	0.584	
	2.25–3.12	0.527	
SB	0.12–0.29	0.141	5.483
	0.30–0.43	1.607	
	0.44–0.60	1.888	
N	168–187	0.489	4.328
	188–200	0.571	
	201–230	0.489	
P	100–107	1.733	5.667
	108–112	1.512	
	113–116	0.869	
	117–121	0.494	
K	116–135	0.379	4.652
	136–151	1.127	
	152–179	8.596	
Zn	2.99–3.21	4.265	2.423
	3.22–3.33	0.692	
	3.34–3.45	0.647	
	3.46–3.65	0.305	

To identify the intra-field spatial heterogeneity zones using Shannon's entropy modelling, each of the nine soil parameters was assigned a weight (W_j) based on Equation (6) of the SE model. The highest weight was assigned to the soil phosphorus content (5.456), while the lowest weight was assigned to copper (0.046) (Table 3).

The maximum probability density (P_{ij}) was determined for the lower categories (class 1) based on the levels of phosphorus, zinc, and hydrolytic acidity in the soil, while the remaining parameters showed the highest probability density in the upper categories (class 3). Based on the assigned weights, the soil parameters can be arranged in descending order as follows: K >N > Zn > Mo > B > Ha > SB > P > Cu.

Table 3. Intra-field spatial heterogeneity zones, computed using Shannon's entropy (SE)

Soil parameter	(P_{ij})	H_j for each	H_j (total)	H_{jmax}	I_j	P_{ij} (all)	W_j
B	0.07	0.28	1.28	1.59	0.19	6.31	1.21
	0.38	0.53					
	0.55	0.48					
Cu	0.32	0.53	1.56	1.59	0.02	2.75	0.05
	0.26	0.51					
	0.42	0.53					
Mo	0.10	0.32	1.20	1.59	0.24	5.08	1.24
	0.23	0.49					
	0.67	0.39					
Ha	0.71	0.36	1.17	1.59	0.26	3.78	0.99
	0.16	0.42					
	0.14	0.40					
SB	0.04	0.18	1.19	1.59	0.25	3.64	0.90
	0.44	0.52					
	0.52	0.49					
N	0.06	0.23	0.65	1.59	0.59	8.78	5.17
	0.07	0.26					
	0.88	0.16					
P	0.38	0.53	1.86	2.00	0.07	4.61	0.33
	0.33	0.53					
	0.19	0.45					
	0.11	0.35					
K	0.04	0.18	0.73	1.59	0.54	10.10	5.46
	0.11	0.35					
	0.85	0.20					
Zn	0.72	0.34	1.27	2.00	0.36	5.91	2.15
	0.12	0.36					
	0.11	0.35					
	0.05	0.22					

While FR model is typically utilized for evaluating landslide susceptibility (Abdo et al., 2022; Babitha et al., 2022), flood hazard assessment (Pawar et al., 2022; Isiaka et al., 2023), or identifying potential groundwater areas (Guru et al., 2017; Olajide et al., 2022), in the current study, it exhibited strong performance for detecting ISHZs (Figure 13) with an accuracy rate of up to 82% (as demonstrated in Figure 14).

To implement Shannon's entropy model for ISHZ detection, it is necessary to compute the entropy value for each site in the study area based on the selected soil parameters determined by the multicollinear analysis. Parcels with the highest entropy value will display the greatest discrepancies in soil parameters, while parcels with the lowest entropy will show the greatest uniformity in soil parameters.

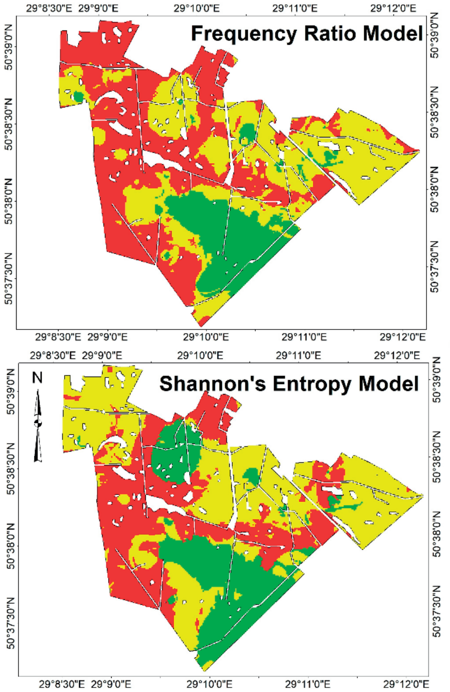

Figure 13. Zones of intra-field spatial heterogeneity with corresponding soil quality (red – low quality; yellow – moderate quality; green – high quality)

Despite the fact that Shannon's entropy model is often utilized for predicting groundwater levels (Razzagh et al., 2021), forecasting flood-prone areas (Haghizadeh et al., 2017), and assessing the degree of urban expansion in various regions (Das & Angadi, 2020), it can be effectively employed to predict the presence of ISHZs with up to 84% precision, as shown in Figure 15.

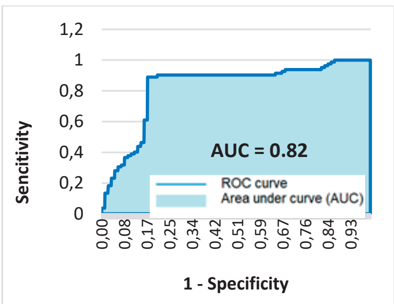

Figure 14. Performance of the model for the spatial prediction of intra-field heterogeneity zones using the ROC curve technique and AUC for FR model

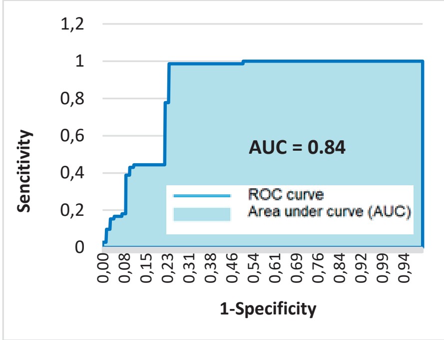

Figure 15. Performance of the model for the spatial prediction of intra-field heterogeneity zones using the ROC curve technique and AUC for SE model

As a result of the performed studies, it was found that the efficiency of the FR model is lower than the efficiency of the SE. However, given its ease of use as well as its robustness to small sample sizes, the FR model can be used to pre-detect the presence of ISHZs and is the best choice for analyzing datasets with a limited number of observations.

As previously mentioned, there is currently no universal method for identifying intra-field spatial heterogeneity zones, and different techniques have been proposed by researchers (Oshunsanya et al., 2017; Kutsayeva & Myslyva, 2020). This study focused solely on an approach based on the chemical properties of the soil, and the list of soil parameters used is not exhaustive and can be expanded depending on the availability of geospatial data on soil properties and the requirements for identifying ISHZs.

CONCLUSIONS

While both the SE and FR models demonstrated high accuracy in identifying intra-field heterogeneity zones, the SE model performed better and has significant potential for mapping these zones not only within Ukraine's Polissia region but also in neighbouring countries with similar soil cover parameters. Therefore, we recommend using the SE model for identifying intra-field heterogeneity zones as a tool to enable agricultural enterprises of different ownership structures to implement precision farming practices, including variable rate (VR) technologies, more effectively.

Future studies should prioritize carrying out field trials with crops using a crop rotation approach to validate the obtained outcomes and to provide more clarity on the established limits of the identified intra-field spatial heterogeneity zones.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SEARCHING POSSIBLE PGPR FROM NATURAL ECOSYSTEM

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Abstract

The utilization of Plant Growing Promoting Bacteria (PGPR) holds significant importance in agricultural systems, especially as a biofertilizer. This study aimed to select effective PGPR for maize to improve yield and nutrient content in a greenhouse pot experiment. Forty-five bacteria were isolated from three different ecosystems as forest, organic farm site, and pasture. The results indicated that PGPR application increased macro nutrients ranging from 12.5% to 50% compared to the control. With the PGPR isolated from forest application, the micronutrient content of Fe, Zn, Mn, and Cu in maize increased around 100%, 20%, 60%, and 100%, respectively. In terms of physiological parameters such as fresh and dry biomass weight, plant height and stem diameter in maize plants were statistically significant than the control treatment. The results proved that PGPR isolated from various ecosystem applications had a more stimulating impact on macro micronutrient content and physiological parameters in maize plants than non-PGPR applications. In general, organic farming sites would be the more promising starting point for PGPR isolation.

***Key words**: PGPR, ecosystem, nutrient, yield, maize.*

INTRODUCTION

Mazie is a widely cultivated crop plant around the world. One of the most widely utilized grains in the world, maize is used for a variety of purposes, including feed and biofuel. The top five countries producing maize worldwide are the United States of America, China, Brazil, Argentine, and Ukraine, in that order (FAO, 2023). While Turkey's maize cultivation areas were in the range of 6.6-5.9 million hectares between 2014-2018, it increased by 8% compared to the previous period and reached 6.4 million hectares in 2019. The production of maize increased from 6.5 million tons in 2020-2021 to 6.75 million tons in 2021-2022, an 3.84% rise (BÜGEM, 2022). However, one of the biggest costs in maize production is chemical fertilizer. Chemical fertilizers not only cost a large amount of money, but they also have a negative impact on the environment and human health (Sigua et al., 2005). Promoting the use of biofertilizers to reduce the usage of chemical fertilizer applications is the recent option for sustainable maize cultivation.

The rhizosphere is home to a group of bacteria known as plant growth-promoting rhizobacteria (PGPR) (Rodriguez & Fraga, 1999). The phrase 'plant growth promoting bacteria' refers to bacteria that colonize the roots of plants (rhizosphere) that enhance plant growth. Rhizosphere is the soil environment where the plant root is accessible and is a zone of maximum microbial activity resulting in a confined nutrient pool in which essential macro- and micronutrients are extracted. PGPR plays an important role in enhancing plant growth through a wide variety of mechanisms. Some examples of these mechanisms are nitrogen fixation (Montanez et al., 2009; Arruda et al., 2013), regulation of plant growth as well as phosphorus solubilisation (Perez et al., 2007), the ability to produce phytohormones Egamberdiyeva (2007) and the production of siderophores (Ahmad et al., 2006). Since PGPR has been used as one of the indicators of the quality of the soil, it could be attractive to evaluate some macro and micronutrient contents in maize to isolate bacteria from the soil in the different ecosystems. Interests in the beneficial rhizobacteria associated with the crops have increased recently and several studies clearly showed the positive and beneficial effects of PGPR on the growth and yield of different crops, especially maize in different environment under variable ecological conditions. Some indigenous bacteria such as *Bacillus* spp. (Zakry et al., 2012) and *Pseudomonas* spp. (Piromyou et al., 2011) from the rhizosphere has been qualified as PGPR to maize through phosphate solubilization and phytohormone production. Waday et al. (2022) reported that fresh weight (1.4 g), dry weight (0.45 g), and length (9.9 cm) of shoot maize plant inoculated with bacterial strain (JEC4) was significantly higher than control (0.6 g, 0.1 g, 6.8 cm). Similarly, Pande et al. (2017) studied the impact of some phosphate solubilizing bacteria on the growth of maize in a greenhouse pot experiment and found three isolated species showed a significant stimulating effect on maize growth in shoot height, fresh and dry weight compared to the control. The impact of the PGPR on maize production with six different bacteria in non-sterile and sterile pot experiments was reported by Gholami et al. (2009). As a result, non-sterile soil was found to have a greater stimulating effect on plant development than sterile soils. Previous research has assessed the impact of some PGPR bacteria on the growth and yield of several crops. The researchers have concentrated their efforts in recent decades on gathering PGPR from the intensive agricultural farm site. It was not fully determined how PGPR isolated from different ecosystems will affect growth and the number of macro-micro-nutrients. The main objective of this study was to isolate plant growth-promoting bacteria from different ecosystems such as forests, pastures, and organic farm sites using morphological characteristic features to increase yield and macro-micro nutrient content in maize plants.

MATERIALS AND METHODS

Soil sample

The soil for the pot experiment was transferred from International Agricultural Research and Traning Center's (IARTC) farm site. Soil samples were analyzed for pH, EC, lime and organic matter contents, and phosphorus and potassium concentrations. Results are shown in Table 1. Considering the soil analysis report, 150 ppm N fertilizer was applied using ammonium sulfate. Phosphorus and potassium were not applied due to their adequate abundance in the soil (Table 1). Through the experiment, herbicides or pesticides were not required. Maize (Tarex Albayrak) was sowed on 22 July 2022, 69 days after the seedling, and on 29 September 2022 plants were harvested. The study was arranged as a completely randomized design (CRD) with three replicate pots per treatment. Of the 45 bacteria isolated from different ecosystems, in particular, 17 isolates were from the forest, 16 isolate from organic farm sites, and 12 isolate from pasture. One positive and one negative control were also added to the experiment.

Bacteria application was done 10 days after corn planting. For each isolate, 1 ml of PGPR bacteria solutions was applied to the surface of the soil, just after irrigation was done. As a positive control, 1 ml sterile TSB medium solution was applied.

Sample collection and isolation of bacteria

The soil samples were collected from three different ecosystems as pastures, organic farm sites, and forest land around the West-Aegean region in Türkiye. Two composite samples were taken from pasture and five composite samples from the forest and organic farm site from 0 to 20 cm depth and each sample was mixed thoroughly. In total 13 samples were transferred to the laboratory to ensure uniformity and stored at 4°C before use. The serial dilution method was used to isolate PGPR. The first step in the dilution process was the addition of 10 g of soil to 90 ml of the extraction solution (0.85% saline solution) resulted in a 10^{-1} weight by volume dilution and repeated five more times (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}). Starting from 10^{-3} dilution, 1 ml of soil dilution was transferred to replicate agar plates (Tryptic Soy Broth Agar). Next, agar plates inoculated were placed in the incubator at 28 °C for 24 hours. After the incubation process, different bacterial isolates with distinct colony morphology were selected from each of three (10^{-3}, 10^{-4}, 10^{-5}) dilutions, and pure cultures were obtained by streaking on TSB agar plates. Forty-five bacterial isolates were chosen randomly considering the different sizes and shapes of the colonies for the greenhouse experiment. To measure plants' physiological parameters, before harvesting plants' height and diameters (2 dimensions) were measured. Then to determine biomass fresh weight (BFW), plants were harvested from each pot and weighed. Thereafter, samples were placed in an oven (65°C) until there was no change in the biomass weight. To determine macro and micronutrient analysis, oven-dried maize samples were milled with the grinder. Then sample masses of 400 mg substrates were microwave-assisted digested using 9 ml of 1 molar HNO_3 and 1 ml of perchloric acid. The program was performed in three steps: (1) 25 min to reach from 25°C to 180°C, (2) 15 min to hold 180°C, and (3) 15 min to cool down to room temperature. After cooling, the vessels were opened and transferred to a 50 mL volumetric flask. The final volume was made up to 50 mL with distilled water. P, K, Ca, Mg, B, Fe, Cu, Zn, and Mn, were determined in these solutions.

Table 1. Some of the soil properties

pH	EC ($dS \cdot m^{-1}$)	Lime (%)	O.M. Content (%)	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})
7.88	630	4	1.5	138	1360
alkaline		Low	Low	Medium	High

Statistical analysis

A one-way analysis of variance (ANOVA) was performed to evaluate the effect of the PGPR application. JMP software version pro16 was used to analyze the experimental data. Tukey Honestly Significant Difference (HSD) test at the 5% level of significance ($p \leq 0.05$) was used for the comparisons of means.

RESULTS AND DISCUSSIONS

Macronutrients

In this study, average phosphorus (P) nutrients with PGPR treatments were as follows: isolated from pasture (0.189%), and organic farm site (0.186%) (Table 2). These Phosphorus nutrient rates were significantly higher than both positive (0.141%) and negative (0.144%) control treatments.

The phosphorus nutrient concentration was 0.161% higher in forest-isolated bacteria treatment which was significantly higher than the negative control (0.144%). This is an agreement with Kumar et al. (2014) who conducted an experiment on the influence of the PGPR on growth, yield and nutrient content in wheat. Phosphorus nutrient content in grain and straw with PGPR treatment was 1.81-fold and 1.72-fold higher than non-PGPR treatment. The second sampling point in the pasture and the fourth sampling point in the organic farm site had the highest average values of phosphorus nutrients in maize plants, respectively.

Additionally, PGPR treatments isolated from pasture in potassium nutrient content (3.82%) were the highest value compared to all other treatments. No statistical differences were observed for potassium nutrient content as inoculated by bacteria from the forest, organic farm site, and both negative and positive control. The first sampling points in the pasture had the highest average values of potassium nutrients in maize plants.

PGPR treatments isolated from all ecosystems in calcium (Ca) nutrient content in maize was significantly higher than the both positive and negative control treatment. Whereas the first sampling point in the organic farm site had the highest average values of calcium nutrients in maize plants, the first sampling point in the pasture was the lowest. While PGPR treatments isolated from organic farm sites and pasture in Mg nutrients content in maize were significantly higher than the positive control, no statistical differences were observed from the forest (0.205%) both negative (0.200%) and positive control (0.170%). The third sampling point in the organic farm site had the highest average values of magnesium nutrients in maize plants.

Our results showed that macronutrient content such as P, K, and Mg in maize under PGPR treatments isolated from different ecosystems was significantly higher than in negative control treatments. Similarly (Karthikeyan et al., 2010) used some PGPR such as *Azotobacter*, *Bacillus* and *Pseudomonas* separately or in combination to assess their impact on *Catharantus roseus*.

Table 2. Selected macronutrient contents

Ecosystem	Sampling point	Isolate	P (%)	K (%)	Ca (%)	Mg (%)
Forest	1	1	0.164	3.44	0.437	0.230
		2	0.166	3.54	0.412	0.212
		3	0.158	3.51	0.441	0.212
	2	1	0.161	3.58	0.483	0.213
		2	0.150	3.32	0.444	0.196
		3	0.145	3.48	0.441	0.200
	3	1	0.171	3.15	0.524	0.218
		2	0.158	3.43	0.488	0.201
		3	0.148	2.98	0.461	0.186
	4	1	0.185	3.79	0.392	0.229
		2	0.170	3.25	0.461	0.180
		3	0.160	3.35	0.464	0.205
		4	0.154	3.08	0.492	0.192
	5	1	0.184	3.76	0.446	0.237
		2	0.148	3.17	0.470	0.192
		3	0.155	3.84	0.431	0.194
		4	0.161	3.07	0.435	0.187
	Mean		*0.161 B*	*3.40 AB*	*0.454 A*	*0.205 BC*
Organic	1	1	0.180	3.48	0.421	0.229
		2	0.191	3.56	0.425	0.241
		3	0.195	3.69	0.441	0.247
	2	1	0.183	3.77	0.533	0.265
		2	0.193	3.30	0.427	0.232
		3	0.191	3.30	0.439	0.229
	3	1	0.194	3.53	0.482	0.249
		2	0.187	3.55	0.494	0.260
		3	0.186	3.81	0.454	0.251
	4	1	0.182	3.83	0.575	0.267
		2	0.201	3.85	0.438	0.248
		3	0.195	3.63	0.430	0.240
	5	1	0.166	3.33	0.495	0.239
		2	0.172	3.81	0.525	0.277
		3	0.190	3.60	0.406	0.252
		4	0.172	3.41	0.400	0.230
	Mean		0.186 *A*	3.59 *AB*	0.462 *A*	0.247 *A*
Pasture	1	1	0.184	4.39	0.447	0.263
		2	0.194	4.27	0.387	0.233
		3	0.176	4.30	0.439	0.243
		4	0.195	3.43	0.484	0.243
		5	0.197	3.67	0.431	0.249
		6	0.171	3.42	0.414	0.231
	2	1	0.188	4.02	0.485	0.262
		2	0.194	3.87	0.389	0.233
		3	0.188	3.88	0.406	0.249
		4	0.180	3.42	0.491	0.262
		5	0.196	3.67	0.417	0.253
		6	0.200	3.51	0.422	0.237
	Mean		0.189 *A*	3.82 *A*	0.434 *A*	0.247 *AB*
Control-		0	0.144 *C*	3.03 *B*	0.314 *B*	0.200 *ABC*
Control+		0	0.141 *BC*	3.05 *B*	0.232 *B*	0.177 *C*

Most of isolates showed all nutrient contents (N, P, K, Ca and Mg) increase compared to the control. However, only Ca content in maize was significantly higher than the both positive and negative control.

Micronutrients

PGPR treatments isolated from different ecosystems that were applied in the rhizosphere of maize plants resulted in a micronutrient content increased for all tested treatments (Table 3). The results for micronutrients such as Fe, Cu, Zn, Mn, and B were evaluated individually. iron (Fe) nutrients in maize with PGPR treatments isolated from the forest and organic farm site were significantly higher than the both positive and negative control. While iron (Fe) nutrients in maize with PGPR treatments isolated from the pasture were significantly higher than the positive control. The third sampling point in the forest and the fourth sampling point in the organic farm site had the highest average values of iron nutrients in maize plants, respectively. Rahimi et al. (2020) studied on the impact of PGPR on improving the acquisition of iron content in quince seedling. Their result showed that iron concentration increased 1.5-fold by PGPR application. Sharma et al. (2013) suggested that PGPR application can be affirmative strategy to solve the issue of iron deficiency in rice cultivation. The highest zinc (Zn) nutrients in maize were presented by PGPR treatments isolated from organic farm sites (53.2 ppm) followed by forest (52 ppm) and pasture (48 ppm). All PGPR treatments in terms of Zinc (Zn) nutrients in maize were significantly higher than the positive control. However, Zinc (Zn) nutrients in maize were significantly greater in organic farm sites than in both the positive and the negative control.

In comparison to both positive and negative controls, manganese (Mn) nutrients in maize were significantly higher in organic farm sites and forests. Manganese (Mn) elements in maize with PGPR treatments isolated from pasture were significantly greater than the positive control. The third sampling point in the forest and the fourth sampling point in the organic farm site had the highest average values of manganese nutrients in maize plants, respectively.

Copper (Cu) nutrients in maize with PGPR treatments isolated from all ecosystems were significantly higher than the both positive and negative control. While the highest value of copper nutrients in maize was PGPR treatments isolated from the forest (12.6 ppm), the lowest value was PGPR treatments isolated from organic (11.5 ppm). PGPR treatments isolated from all ecosystems in boron (B) nutrient content in maize was significantly higher than the both positive and negative control treatment. The second sampling point in the organic farm site had the highest average values of boron nutrients in maize plants.

In terms of micronutrient contents, while the highest values for Fe, and Mn in maize plants were noted in PGPR treatments isolated from forests followed by organic farm sites, the highest value of Cu content in maize plants was noted in the forest followed by pasture. In this study, micronutrient content (Fe, Zn, Mn) in maize under PGPR treatments isolated from different ecosystems was significantly higher than the negative control treatments. According to Rana et al. (2012) an enhancement of 28-60% in micronutrient content was recorded in treatments on wheat plant receiving the mix PGPR application with 2/3 recommended dose of NPK, as compared to full dose of fertilizer application. However, copper (Cu) and boron (B) were statistically significant than both negative control and positive control. The negative control had a larger micronutrient content than the positive control in the maize plant, even though there was no statistically significant difference between the control treatments.

Physiological parameters

The impact of different PGPR treatments on the physiological parameters in maize such as fresh and dry biomass weight, height, and shoot diameter is demonstrated in Table 4. Fresh biomass in maize ranged from 70.4 g per plant to 30.3 g per plant. Unexpectedly, positive control of fresh biomass with 70.4 g per plant performed the best result followed by PGPR treatment isolated from pasture with 66.7 g per plant and organic with 59.5 g per plant.

Table 3. Selected micronutrient contents

Ecosystem	Sampling point	Isolate	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})
Forest	1	1	318	51.0	54.8	12.2	18.8
		2	259	43.1	51.4	12.1	14.5
		3	328	55.9	52.0	11.9	16.0
	2	1	235	41.1	56.9	12.3	14.0
		2	319	48.5	59.3	13.7	28.4
		3	325	49.7	50.6	12.2	24.6
	3	1	302	53.7	66.0	13.0	31.8
		2	526	57.5	66.0	13.2	28.4
		3	430	60.8	55.3	12.7	29.8
	4	1	261	46.3	45.0	11.2	23.6
		2	367	46.4	56.5	12.6	27.4
		3	308	51.7	49.4	13.1	24.0
		4	474	66.0	62.9	13.7	30.9
	5	1	235	51.2	43.9	11.2	26.0
		2	288	50.7	49.6	12.4	25.8
		3	292	50.6	46.7	12.5	24.7
		4	416	62.0	54.5	14.0	28.7
	Mean		*334 A*	*52.1 AB*	*54.2 A*	*12.6 A*	*24.6 A*
Organic	1	1	326	51.5	49.6	11.5	25.2
		2	292	50.1	46.3	11.6	24.8
		3	312	55.3	51.2	12.0	27.9
	2	1	301	51.7	50.4	12.6	26.4
		2	332	52.5	51.5	12.1	26.7
		3	348	49.6	54.1	11.1	30.2
	3	1	306	56.6	56.8	11.6	27.6
		2	346	53.8	55.7	10.9	25.7
		3	352	62.6	45.4	10.4	23.6
	4	1	423	58.0	59.5	12.0	26.5
		2	214	46.9	51.0	11.6	25.1
		3	406	58.4	52.0	11.7	30.6
	5	1	393	53.5	63.7	12.2	29.1
		2	402	54.9	57.5	11.5	21.1
		3	236	44.6	48.5	11.0	14.5
		4	288	47.8	44.1	10.5	15.8
	Mean		330 *A*	*53.2 A*	*52.2 A*	*11.5 B*	*25.4 A*
Pasture	1	1	238	42.3	47.8	13.2	26.8
		2	231	42.1	44.8	11.8	24.8
		3	284	49.7	44.6	11.4	26.7
		4	387	54.0	58.0	12.0	17.5
		5	289	47.7	49.6	12.0	17.3
		6	270	48.6	44.3	12.0	25.1
	2	1	301	51.6	51.8	13.1	24.8
		2	249	46.0	46.0	11.8	25.2
		3	303	46.7	42.8	11.0	25.8
		4	362	56.5	57.4	13.2	28.7
		5	233	49.8	47.3	12.8	22.4
		6	265	52.0	48.6	12.8	26.3
	Mean		284 *AB*	48.9 *AB*	*48.6 AB*	*12.3 AB*	*24.3 A*
Control-		0	164 *BC*	39.7 *BC*	34.7 *BC*	5.9 *C*	5.1 *B*
Control+		0	112 *C*	29.1 *C*	28.3 *C*	5.1 *C*	3.0 *B*

Table 4. Plant weight, height, and diameter at harvest

Ecosystem	Sampling point	Isolate	Fresh weight (g)		Dry weight (g)		Plant height (cm)		Stem diameter (mm)	
Forest	1	1	52.8		6.73		134		7.59	
		2	45.5		6.13		129		8.07	
		3	50.2		6.83		135		8.07	
	2	1	47.0		6.33		136		7.52	
		2	34.1		5.10		132		7.51	
		3	48.7		6.67		133		7.30	
	3	1	36.7		5.27		131		6.64	
		2	39.1		5.65		132		6.73	
		3	45.0		6.07		130		7.42	
	4	1	66.1		9.20		135		8.60	
		2	44.2		6.20		127		7.24	
		3	42.2		5.97		134		7.23	
		4	37.7		5.33		122		6.76	
	5	1	71.7		10.33		145		8.09	
		2	54.3		7.73		133		7.52	
		3	46.8		6.90		131		7.85	
		4	42.2		6.17		128		7.23	
	Mean		*46.9*	*B*	*6.50*	*B*	*132*	*A*	*7.49*	*C*
Organic	1	1	60.1		8.13		127		8.53	
		2	70.9		9.63		138		8.88	
		3	67.6		10.43		147		8.22	
	2	1	50.9		6.73		131		7.63	
		2	67.0		9.60		144		8.45	
		3	70.6		10.93		140		8.99	
	3	1	55.4		7.37		130		7.88	
		2	57.0		8.27		141		8.20	
		3	64.0		9.70		142		7.98	
	4	1	57.2		7.57		135		8.66	
		2	56.4		7.77		139		8.00	
		3	61.0		9.33		141		7.82	
	5	1	50.9		7.63		118		8.13	
		2	52.5		7.03		140		7.03	
		3	46.9		6.60		131		7.72	
		4	55.1		7.95		141		8.30	
	Mean		*59.5*	*A*	*8.50*	*A*	*137*	*A*	*8.20*	*B*
Pasture	1	1	62.9		9.60		110		10.26	
		2	67.6		9.77		111		10.74	
		3	76.0		12.63		134		9.57	
		4	56.9		7.77		117		9.09	
		5	66.4		9.17		128		7.95	
		6	71.7		10.97		140		8.68	
	2	1	59.0		8.43		113		9.55	
		2	70.1		9.60		121		9.51	
		3	75.6		11.77		137		9.94	
		4	51.6		7.20		115		8.15	
		5	72.3		9.60		134		8.74	
		6	69.9		10.37		137		8.11	
	Mean		*66.7*	*A*	*9.70*	*A*	*125*	*B*	*9.20*	*A*
Control-		0	30.3	*B*	4.70	*B*	110	*C*	5.42	*D*
Control+		0	70.4	*A*	11.27	*A*	117	*BC*	8.67	*ABC*

Whereas there were no statistical differences between the PGPR treatments isolated from the forest and the negative control for the fresh biomass weight of maize, they were statistically significant compared to the organic farm site and pasture, respectively. Sandini et al. (2019) reported that seed inoculation by *Pseudomonas fluorescens* to maize increased grain yield and biomass accumulation of maize plants.

Our result showed that the plant height in maize was also statistically significant by PGPR treatments isolated from different ecosystems. The height of the maize plants varied from 137 cm to 125 cm. In terms of stem diameter in maize, all PGPR treatments were statistically significant than the negative control. The highest stem diameter was presented by pasture at 9.2 mm followed by organic farm site at 8.2 mm, and forest at 7.5 mm.

CONCLUSIONS

This study aimed to investigate the impacts of PGPR practices isolated from various ecosystems on the nutrient content of macro-micro and yield quality in maize plants in the greenhouse condition. Of the factors analyzed, PGPR practices had a positive impact on macro-micro nutrient content and physiological parameters in maize. Even though the results from PGPR treatments isolated from forests and organic farm sites did not show statistically significant effects on the potassium content in maize compared to the control, they nevertheless contributed to higher potassium content in maize. Our results suggest that PGPR isolated from organic farm sites and PGPR isolated from pasture are the most effective PGPR treatments for positively influencing macronutrient content in maize plants in the greenhouse condition. PGPR applications isolated from organic farm sites are the most effective treatments for maize plants when evaluated for their macro-micronutrient composition.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (TAGEM), and also was funded by Project No. 6056/2022.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

MODERN APPROACHES FOR THE IMPLEMENTATION OF BACKGROUND SOIL MONITORING

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Abstract

FAO's conclusions on the rate of soil degradation in the light of climate changes and the necessity of global soil monitoring actualize improvement of the methodology for research of reference or virgin soils. This is especially relevant for Ukraine, which is characterized by a high level of agricultural land plowing. Results of developing the fundamentals of multilevel thematic processing of satellite imagery data for diagnostics of virgin soil heterogeneity as the groundwork for establishing an information support system for soil research, as well as automated monitoring systems for agricultural land, are presented on the example of the soil studies of the Mikhailovskaya virgin land which is the only preserved area of virgin meadow steppe in the Forest-Steppe zone of Ukraine. Parameterization of soil properties and vegetation indices in classes derived from decoding of satellite imagery data and presented as a power-law probability distribution or geostatistical indicators are shown to provide a quantitative description of soil heterogeneity and are recommended for the purpose of comparison multitemporal satellite images as a high-sensitivity method to determine changes in their condition due anthropogenic influences.

Key words: *heterogeneity, geostatistical analysis, soil monitoring, virgin lands, remote sensing.*

INTRODUCTION

Global climate change and the intensification of soil degradation processes (FAO, 2015) highlight the consistent improvement of the methodological framework for background (reference) monitoring (Israel, 1982), including soils (Medvedev, 2012), which is an integral and important part of the global monitoring system of environment and is necessary for the development of a unified state system of environmental monitoring in Ukraine. It should be reminded that the main task of background (reference) soil monitoring is to provide data on the input (initial or zero) assessment of soil properties and soil cover in the natural state. This allows determining the direction and intensity of anthropogenic transformation of soils and soil cover by comparing with soil data on agricultural lands (Monitoring…, 2008).

Analysis of world experience in establishing modern soil monitoring systems shows its focus on creating and periodically updating cartographic and analytical materials on soil state, the functioning of automated information system for forecasting soil changes depending on the type and intensity of anthropogenic activity; substantiation of soil protection measures, as well as evidence of the variety of used methods and approaches (Proposal for an European soil monitoring…, 2001; Medvedev et al., 2012; Kibblewhite et al., 2008; King et al., 1995; Soil monitoring in Europe, 2021). At the 13th meeting of the Parties to the United Nations Convention to Combat Desertification in Ordos, China, the countries emphasized the importance of involving space scanning data as the latest source of objective information on the state of the Earth's surface in existing systems on monitoring the state of the environment and environmental management (UNCCD documents…, 2017). The use of high-resolution multispectral satellite images, which typically have geographical compliance, continuity, and are regularly updated, seems to be the promising approach to ensuring compliance to high requirements for the accuracy and impartiality of the data on the national soil resources. Additionally, satellite images, as up-to-date digital materials, in conjunction with modern geographic information systems, provide means of precise determining the soil heterogeneity, both in detailed and large-scale surveys.

In connection with the above, the purpose of the study is to determine the fundamental possibility and methodological basis for the use of space scanning data for background monitoring of soils. The main tasks of the research include analysis and generalization of existing theoretical and methodological principles of background monitoring in Ukraine, improvement of soil monitoring based on the results of thematic decoding of space scanning data, as well as the definition and development of methodological approaches to combining the results of decoding space images and sample ground research to determine the dynamics of changes in the state of the Earth's surface within protected areas.

MATERIALS AND METHODS

A practicing of a creation of system for using high-resolution multispectral space scanning data to research of reference or virgin soils based on space scanning data was carried out on the example of the Mykhailivska virgin land. This polygon occupies 50 ha in Sumy region.

The test polygon is located in one of the northeast physical and geographical areas of the Livoberezhno-Pridneprovsky Territory of the Forest-Steppe Zone of Ukraine (National Atlas of Ukraine, 2007), which has the predominantly flat broad-wavy relief. Annual rainfall in this territory is from 550 to 450 mm, the average annual air temperature is 6-7 C (Marinich & Shishchenko, 2005). On the territory of the Mykhailivska virgin lands it is preserved a forb-fescue meadow steppe with northern and southern species of steppe plants, in the development of which seasonal changes are well expressed.

We tested developed technologies for determination of the Earth's surface state using Landsat-8 satellite data that provides digital images of the Earth’s surface in the multispectral bands. It should be recalled that the Landsat 8 satellite payload consists of two science instruments - the Operational Land Imager (OLI) and the Thermal Infrared Sensor (TIRS). These two sensors provide seasonal coverage of the global landmass at a spatial resolution of 30 meters (visible, NIR, SWIR); 100 meters (thermal); and 15 meters (panchromatic). USGS leads post-launch calibration activities, satellite operations, data product generation, and data archiving at the Earth Resources Observation and Science (EROS) center.

Research included: statistical analysis and clasification of multitemporal images of virgin lands, analysis of archival data of field investigation of virgin soils and laboratory analysis of soil samples, expert assessment of image complexity and analytical results as the basis for image classification and soil-cover models, parameterization and geo-statistical analysis of the spatial variation of soil indicators, and extrapolation procedures based on interpretation of spectral signatures.

Using a GPS, a regular grid of elementary sites was established (100 × 100 m) for 48 soil sampling were collected from the 0-30 cm layer, and 2 soil cuts were dug to characterize the soils (morphological structure of the soil profile, depth of humus profile, spatial configuration of plow layer) in the field. Samples were collected according to Soil Survey Standards of Ukraine (ISO 10694:1995, DSTU 4287:2004, DSTU 4728:2007, DSTU 4730:2007). Also in the field it was investigated the physical soil properties (bulk density, soil penetration resistance - according to DSTU 5096:2008). At the laboratory-analytical stage of the research, it was determined: total humus content (DSTU 4289:2004); pH (DSTU ISO 10390:2007); granulometric composition by the method of pipetting in the modification by N.A. Kaczynski (DSTU 4730:2007); the content of mobile compounds of phosphorus and potassium by Chirikov’s modified methods (DSTU 4115:2002).

Statistical and data processing methods used ENVI and TNT programs for pre-processing of space images, NDVI calculation, primary image processing, transformation, general statistical analysis and image classification; and STATISTICA 10 for variance, correlation and regression analysis.

RESULTS AND DISCUSSIONS

The concept of background monitoring was introduced and developed in the scientific works of Yu.A. Israel (1974; 1978; 1984). According to modern ideas, background monitoring is a spatio-temporal observation of components of the natural environment or factors that lead to their change, in given spatio-temporal intervals to assess variations in their quality and quantity V.V. Medvedev (2012). Despite significant theoretical elaboration of the issues of creating a system of integrated background monitoring (Israel et al., 1980; Gromov et al., 2015), it should be recognized that background monitoring in Ukraine as a system of constant monitoring changes in the properties of soils in space and time, which have state status and reflect natural diversity, as well as all types of economic use of soils, is at the stage of initial restoration.

One of the main factors is the lack of clear reference criteria, in particular for the assessment of soil parameters. However, there are developments on this issue in Ukraine. Thus, V.V. Medvedev (2012) substantiated the idea of multiple standards. For example, in the study of ancient arable soils, it is recommended to use as standard the soil parameters obtained at the beginning of observations, including materials of large-scale soil survey of Ukraine 1957-1961 and the results of the first round of observations for agrochemical land certification (Bylugin et al., 2019).

Scientists also allow the use of optimal parameters of physical, physicochemical (pH), agrochemical (humus and nutrients) properties of soils, which are known for most crops grown in Ukraine (Medvedev, 2012; Optimal parameters…, 1984; Medvedev, 1988; Nadtochiy et al., 2003; Nadtochiy et al., 2019; Nadtochiy, 2013) and indicators of particularly valuable soils, which are characterized by optimal or close to them parameters of morphological, physical, chemical, physicochemical and technological characteristics that allow to realize the potential of climate, plants to develop accurate agricultural technologies (Medvedev et al., 2015).

In our opinion, the best standard is the parameters of virgin soils, which are not subject to intense anthropogenic impact and have purely natural cycles of substance transformation and soil genesis, are characterized by high stability of textural and structural components, moisture balance and chemicals. Therefore, the comparison of such soil with the developed one is able to provide useful information about the change of soils and soil cover in the conditions of economic activity. In Ukraine, which is characterized by a high level of plowed land, the use of fallow lands that have not been cultivated for at least 20-25 years can be considered an acceptable standard. In general, at present, in Ukraine, background monitoring has not been introduced in any department, and there is no uniform methodology for its conduct and there is a lack of accumulated data on background indicators of virgin soils.

Two main trends in diagnostics and evaluation of soil cover state were determined by addressing the multivariateness of the problem of soil monitoring and considering the specifics of satellite imagery digital data. The first one is determination and evaluation of the estimated heterogeneity of the Earth's surface image, which involves spatial and structural, abstract mapping of units, as well as representation of their relative spatial position and their spatial structure with a pre-set geometric and topological similarity. The second trend comprises a quantitative evaluation of a soil heterogeneity by certain characteristics of soils using methods of statistics and geostatistics. The primary outcome of a soil lateral heterogeneity evaluation is a range of geostatistical parameters, which confirm the predictability of spatial variation of soil characteristics; while intermediate outcomes include digital soil maps.

Both directions of using space survey data to determine the heterogeneity of the Earth's surface and soil cover are well developed on arable soils of Ukraine. However, background soil monitoring is a more complex case for space-based observations, as the soil surface is always covered with vegetation or residues, making it impossible to determine soil properties and soil peds by direct decoding features. In this regard, a possible way to observe the state of virgin soils is to determine the system of vegetation indices within virgin lands, construction of different time cartographic materials based on them and conducting periodic ground surveys during which soil samples are taken to determine a wide range of indicators, pedotransfer modeling and soil state assessment. At the same time, the prerequisites for effective background monitoring of soils and soil cover are a regular network of main sampling points, identification of permanent sites for detailed geobotanical description and use of landscape indication methods, equidistant observations, a wide range of indicators, in situ and on- line, cartographic identification of areas characterized by the greatest changes in the state.

The testing of methodological bases of using multispectral space scanning data for background monitoring was performed on the example of the soil studies of the Mykhailivska virgin land which is the only preserved area of virgin meadow steppe in the Forest-Steppe zone of Ukraine. The soil cover of the reserve is mainly represented by typical medium loam chernozems, which are confined to placors and low slopes. These soils are characterized by sufficiently high natural fertility, high humus content, significant reserves of nutrients, saturation of the soil colloidal complex with metabolic bases and, especially, calcium, neutral (or close to it) reaction of soil solution.

A more detailed description of typical chernozems of the Mykhailivska virgin land is given in the works of O.A. Chesnyak et al. (1970), N.M. Breus (1968), N.I. Laktionov (1974), V.V. Degtyarev (2011). Some average parameters of virgin soil are presented in Table 1.

Table 1. Average parameters of virgin typical medium loam chernozem of Sumy region (Mykhailivska virgin reserve) (Medvedev, Plisko, 2005)

Indicator, units of measurement	Value	Indicator, units of measurement	Value
Total humus content, % mass	6.3	Content of agronomically valuable aggregates (10-0.25 mm), %	80-85
Equilibrium structure density, g/cm^3	1.1-1.2	Clusters of blocks (>10 mm), %	5-7
Water permeability at equilibrium density, mm/year for 6 hours	65-70	Coefficient of water resistance of the structure	0.7-0.8
Dispersion factor	4.0	Dust content (<0.25 mm), %	8-10
Suction coefficient	1.1-1.2	Coefficient of water resistance of the structure	0.7-0.8

Since the cosmic image of virgin lands is determined mainly by natural associations of plants, we also analyzed information about the vegetation of the meadow steppe of Mykhailivska virgin land, which is highlighted in detail in the scientific works by Ye.M. Lavrenko and I.G. Zoza (1928), S.S. Kharkevich (1956), G.I. Bilyk and V.S. Tkachenko (1972; 1973) and confirmed by ground surveys. In particular, it is determined that the characteristic formations for this meadow steppe are (Geobotanical zoning of the Ukrainian SSR, 1977):

1. *Cytiseta ruthenici* with the association *Cytisus ruthenicus*, *Stipa capillata* + *Poa angustifolia* + motley grass;
2. *Stipeta pennatae* with the association *Stipa pennata* + *Poa angustifolia* + *Salvia pratensis*, *Fillipendula hexapetala*;
3. *Stipeta capillatae* with the association *Stipa capillata* + *Festuca sulcata* + *Carex humilis* + *Salvia nutans*, *Fillipendula hexapetala*, *Carex nutans*;
4. *Helyctotrichoneta pubescontis*.

The westernmost locations of *Zerna riparia* Nevski and *Phlomis pungens* Willd (Geobotanical Zoning) are also marked on the Mykhailivska virgin land.

Since soil quality can be considered as a measure of how soil functions (it is the capacity of a soil to sustain its functions, including biomass production, storage, filtering, buffering and transformations of natural and anthropogenic produced substances, a biological habitat and gene reservoir and a sink for carbon (Schjønning et al., 2004) so the use of vegetation indices obtained by satellite imagery data can be considered as indirect indicators to the integral assessment of soil quality and state of soil cover.

The state of natural vegetation quite often reflects changes in the physical properties and regimes of the soil, which are determined by both anthropogenic human activity and climate change.

With this approach, significant changes in soil regimes or properties will affect the optical characteristics of the leaf surface of natural plants, especially in the initial stages of its vegetation, as the area, number of leaves and their sizes are important phytometric indicators that depend on plant growth conditions and play an important role in their photosynthetic activities.

We selected two images of Landsat 8 (shooting dates 07.05.2014 and 10.05.2019), which were taken in the absence of clouds, had a complete set of metadata, which allowed us to develop a complete algorithm for thematic decoding, which involved radiometric and atmospheric image correction, NDVI calculation, its statistical analysis and classification.

Comparative analysis of algorithms of uncontrolled classification of space scan data showed greater efficiency for determining the contours of a small area of the ISODATA method compared to the K-means algorithm, which implements only local minimization of the functional.

Statistical analysis of the data showed that the classification of NDVI values from the 2014 space image allowed us to distinguish the virgin land and establish the confinement of classes to certain areas of the surface that differed in steepness and solar exposure (Figure 1).

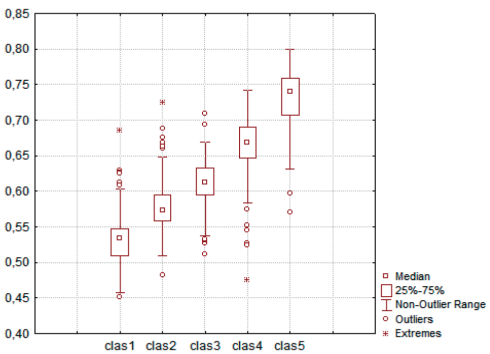


Figure 1. Statistical analysis of NDVI values (from the 2014 space image) allowed to classification the virgin land

Geostatistical modeling of sample field data was carried out on a complex of soil indicators for interpretation and spatial analysis of the constructed NDVI cartogram.

The research also used the method of dynamic mapping, which consists in the creation and analysis of differential spectral images obtained from different time space images in digital format.

In our case, the use of difference images was in finding differences between NDVI cartograms obtained during image processing in 2014 and 2019 and was achieved by calculating the difference between NDVI values to determine areas of the Earth's surface that did not change much over time and were very contrasting in comparison with sites that significantly changed their optical characteristics and NDVI values.

Comparison of cartograms of soil properties obtained by geostatistical modeling, as well as difference images revealed that the largest changes in the optical characteristics of vegetation were characteristic of virgin areas in the upper part of the slope and had the lowest values of total humus content (about 6.5-7.0%).

Also in our opinion, the results of this study make it possible to use probabilistic-statistical modeling of the lateral heterogeneity of the soil and vegetation cover based on satellite imagery for monitoring purposes. It is determined that the representation of variation of NDVI and soil properties within the highlighted contours in the form of a probability distribution law completely solves the task of quantitative description of soil heterogeneity, which is recommended for comparing the results of decoding of different temporal images of the virgin land.

Comparative analysis of the results of decoding of different-time space images of the Mykhailivska virgin land was carried out on a quantitative assessment of the complexity of spatial differentiation, which is known from the theory of geographical science. In particular, the information model of territorial differentiation of Mykhailivska virgin land was calculated by calculating several information indicators of complexity. It should be reminded that the indicator of complexity in the information model of territorial differentiation is the information (entropic) diversity function, which is based on the probabilistic formalization of territorial dismemberment, which is represented as the ratio of the area of each contour (S_i) to the total area ($S_з$). (Gerenchuk et al., 1975):

$$p_i = S_i / S_з,$$

where:

p_i - probability of the i-th element of territorial division;

s_i - area of each contour, m^2;

s_3 - total area, m^2.

Spatial systems, the elements of which can be given by probabilities with the prerequisite that the sum of all probabilities is 1.0, have an information index of diversity or complexity of morphological division, which is calculated by the K. Shannon's formula:

$$H = -\sum p_i \log_2 p_i ,$$

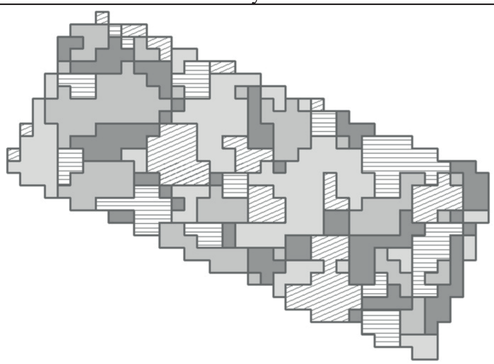
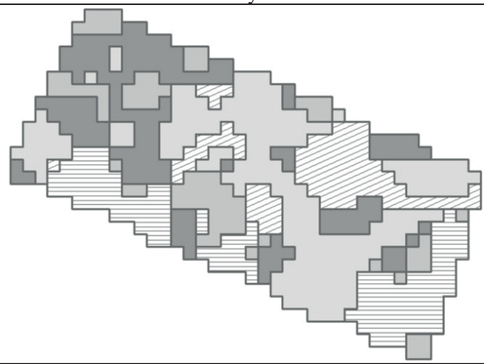
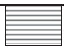
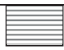
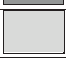
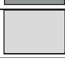
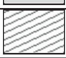
where:

H - information indicator of diversity;

p_i - probability of the i-th element of territorial differentiation.

Let's illustrate its calculation on the example of the results of the classification of NDVI values within the Mykhailivska virgin land by different time space images (2014 and 2019). Information modeling of this landfill was carried out in several stages. Firstly, the area of each of the Si areas selected as a result of image classification was determined using GIS. Secondly, for each area the value of the probability pi is set, according to which the value of the function - $p_i log_2 p_i$ is found (Table 2). Thirdly, these values were summed up to determine the information indicator of the complexity of the territorial division of the landfill, which amounted to 5.26 in 2014 and 3.99 in 2019.

Table 2. Comparative analysis of the results of the classification of NDVI values within the Mykhailivska virgin land, conducted by the method of Isodata at the level of 5 classes

Cartographic results of NDVI value classification					
2014 year			2019 year		
					
Class number, symbol on the map	General characteristics of the contours		Class number, symbol on the map	General characteristics of the contours	
	Number of contours	Area (m^2)		Number of contours	Area (m^2)
1 	12	82800	1 	4	94000
2 	14	120600	2 	13	65050
3 	12	99000	3 	10	114354
4 	13	118800	4 	6	158696
5 	12	77400	5 	5	66490
The total area of virgin land - 498600 m^2					

Indicators of the information model of territorial differentiation		
2014	Indicators of the information model	2019
5.26	Information complexity indicator - H	3.99
5.98	Theoretical the maximum possible degree of complexity of dismemberment for this division - $Hmax$	5.04
0.72	Indicator of imbalance - $\Delta H = Hmax-H$	1.06
0.12	Indicator of relative imbalance - $I = \Delta H/ Hmax$	0.21

Correct interpretation of this indicator is that a single degree of complexity (1 bit) has the simplest structure, which represents the division of the territory into two equal, and therefore equally likely parts (Gerenchuk et al., 1975). Undifferentiated territory, represented by one whole contour, has zero degree of complexity, because its probability is 1.0. An increase in the number of elements of territorial division or a change in the ratio of their areas is recorded by the corresponding changes in the information indicator of complexity (Gerenchuk et al., 1975). Thus, the established parameters show how the complexity of territorial differentiation depends on the number of elements of territorial differentiation and how much on the ratio of their areas.

If the components of the territorial division were absolutely equal (equally probable), the informational degree of complexity of the division could depend only on the number of units of division and would be the maximum possible for this differentiation (Gerenchuk et al., 1975):

$$H_{max} = log_2 n,$$

where:

H_{max} - the maximum possible level of information complexity;

n - number of units of territorial differentiation.

Thus, to assess the results of the classification of images of Mykhailivska virgin land, taken in 2014, there are two informational estimates of complexity: real (equal to 5.26) and theoretical, i.e. the maximum possible for division into 63 areas, which is 5.98. Since the second (maximum possible) complexity indicator is calculated under the condition of absolutely equal dismemberment, it is clear that the difference between the actual and maximum estimates exists due to the deviation of the actual areas of territorial division from the theoretical equal. This difference is an indicator of the imbalance of territorial differentiation is calculated by the following formula (ΔH) (Gerenchuk et al., 1975):

$$\Delta H = H_{max} - H$$

Thus, the information index of complexity increases rapidly with increasing number of units and decreases with increasing fluctuations in their size. The imbalance index (H_{max}) is an absolute value that characterizes the imbalance of spatial differentiation as a whole. The ratio of this value to the maximum degree of complexity is an indicator of the relative imbalance of territorial differentiation (I) (Gerenchuk et al., 1975):

$$I = \frac{\Delta H}{H_{\max}} = \frac{H_{\max} - H}{H_{\max}}$$

The simplicity of the presented approach allows us to conclude about the prospects of its use in generalizations and comparative analysis of the results of processing different time space scanning data and the development of automated cartographic data analysis systems for remote monitoring of land resources. Since the complexity indicator takes into account the dependence of the complexity of dismemberment both on the number of constituent parts of the territorial division and the ratio of areas of its elements, therefore, this approach can be effective in quantifying changes in the complexity of the image of territorial objects due to degradation processes in the context of increasing anthropogenic pressure or global climate change.

However, it should be noted that the use of the above approach is appropriate only if you use images taken in similar conditions of the soil surface, as well as using the same methods and settings of image classification, which allows comparing results of contour decoding of the Earth's cover at different times of space images.

The analysis of the indicators of the information model of Mykhailivska virgin land allows us to conclude that there is a pronounced tendency to reduce the information indicator of the complexity of territorial division (more than 1.0) in five years, as well as a significant increase in imbalance and relative imbalance. In our opinion, this may indirectly indicate a decrease in the natural diversity of virgin areas and justify the need for terrestrial, detailed geobotanical and soil research in the near future. The obtained cartographic materials also allow us not only to plan ground research, but also to significantly optimize it, which will have a positive impact on the cost of field work.

CONCLUSIONS

Academic novelties in the results presented herein, include justification and development in the fundamentals of practical methodology for thematic processing of satellite imagery data for diagnostics of the state of virgin soils as a basis for creating a modern system of information support for background monitoring of soils.

We propose to establish the exact identification of components of soil evaluation system based upon the geoinformatics principle, including the number of samples and the distance between sampling points, involving a geostatistical analysis of optical characteristics of virgin lands according to the spatial imagery data. It ensures the identification of a predictable component within a variation of soil properties and its spatial direction (anisotropy) within a certain territory. The basic requirement for this approach to organizing surveys is the need for accurate georeferencing of sampling points and sites of soil profiles using GPS devices, as well as need for taking the number of entirely individual soil samples sufficient for the planned analytical research.

The results obtained from contour decoding of satellite imagery data for estimating the virgin land heterogeneity, justify the possibility of information modeling of soils, utilizing the entropy function of diversity. The simplicity of such approach allows us to recommend it for developing the automated cartographic systems designed for analyzing the data of remote monitoring of country's lands. Since the complexity factor accounts for dependence of territorial division on a number of components, as well as on a proportion of their areas, this approach appears to be preferable for rapid quantitative estimation of temporal changes in virgin soils, considering the increasing human impact or climate changes. Application of this approach is presumed to be appropriate only for images taken in similar conditions of Earth's surface, and processed by the same methods and settings for image classification, which ensures accurate comparison of results of decoding from satellite images taken in different times.

ACKNOWLEDGEMENTS

We gratefully acknowledge the specialists of United States Geological Survey and Center for Earth Resources Observation and Science for providing the satellite imagery free of charge, researcher of Soil Geoecophysics Laboratory named after academician of the National Academy of Sciences of Ukraine Vitaliy Medvedev, Svitlana Nakisko and a leading engineer of Soil Erosion Control Laboratory and Remote Sensing Alexander Sherstyuk of National Scientific Center "Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky" for their assistance during the field and analytical research.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE DEGREE OF DEPENDENCY OF SOIL ECOSYSTEM SERVICES ON THE SOIL MICROBIOTA ACTIVITY

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Abstract

To reveal the degree of dependency of soil ecosystem services on soil biota activity, the detailed investigations at macroscopic - microscopic level had been used. At macroscopic level, the physical data showed a low to medium bulk density and consequently a high to medium porosity. The poral space quantified by the image analysis at microscopic level showed the dominance of the pores in the size classes of 100-300 µm (equivalent pore diameter), the elongated pores being dominant. Among them the fine fissures delimitating the biological pedofeatures by the surrounding matrix were also included and represent the path for water and air circulation, creating thus hospitable conditions for the microorganisms developed on the biogenic pedofeatures surfaces. The micromorphological investigation showed textural differences in the macrofauna coprolites: many areas with skeleton grains concentrations depleted of plasmic material. But their further ingestion by the soil mezofauna resulted in the re-mixing of soil constituents, mezofauna proving to have an active role in the textural soil matrix restoration. Soil biota covered all the web food needs for the "factory fertility": the main soil ecosystem service.

Key words*: micromorphology, image analysis, soil fauna, Chernozem, porosity.*

INTRODUCTION

One of the most important functions of soil is that of habitat for soil biota. But when soil and its biota are studied, it is difficult to separate the habitat function of the soil from the food provider function, due to the complex activity of biota which „built" its habitat (by burrowing and create casts) and feeding on soil (ingesting it, modelling it, and then enriching it with mucilaginous secretions). Even if difficult, both functions/activities match together and evolve simultaneously. In this respect, soil biota is the best and most complete soil indicator of soil status.

The micromorphological method of study is a complex and complete technique to investigate soils, the main ecosystem services provider. The study of the soil at micromorphological scale could bring important information concerning the architectural structure of the soil as a complex edifice that provides ecosystem services which support all the above-ground biota.

Micromorphology has been for long a useful tool for characterizing the interaction between pedofauna and soil physical properties, thus, the study of soil thin sections provides the opportunity for investigating fauna-soil relationships since evidence of animal activities such as burrowing and deposition of excrement (fecal pellets) can be identified and quantified (Gargiulo et al., 2011). Micromorphological techniques are also commonly used to characterise void space and soil structure (Bruneau et al., 2004).

Most of the global environmental sustainability issues of today, such as food, water and energy security, climate change, and biodiversity protection require that the knowledge acquired in the last few decades by soil science is fully exploited and shared with all the other relevant disciplines (McBratney et al., 2014; Calzolari et al., 2016).

Considering only the inherent soil properties, it is likely that soils rich in organic matter and not compacted are potentially capable to host a relatively higher biodiversity pool (Gardi et al., 2013).

The assessment and monitoring of soil life and soil health can be used to encourage the development and adaptation by farmers of more sustainable and productive farming systems, especially were backed up by appropriate technical support and incentives (Bunning and Jimenez, 2003).

Soil organisms provide important ecosystem services (Jeffery et al., 2010). These include the storing and cycling of nutrients and pollutants, the decomposition and cycling of soil organic matter, the biocontrol of pests. Among soil organisms, soil microfauna has been used as indicator of soil quality; its role includes litter fragmentation, macropores formation, bioturbation (Calzolari et al., 2016).

Only by knowing soil in all its complexity, while maintaining its functionality and quality through actions aimed at protecting its properties, and acknowledging the importance it assumes in the quality of life worldwide, can we embark on a truly sustainable use of soil perceived as a resource and build a proper Man/Soil relationship to be left to future generations (Menta, 2012).

Soil fauna is an important reservoir of biodiversity and plays an essential role in several soil ecosystem functions; furthermore, it is often used to provide soil quality indicators (Menta, 2012).

A few large invertebrates (mainly earthworms) and social insects (ants and termites) can efficiently dig the soil and produce organo-mineral structures (casts and organo-mineral pellets that are resistant macro aggregates, mounds and nests) and a large variety of pores (galleries, chambers and voids resulting from an uncompleted backfilling of galleries) (Lavelle, 1996).

In what concerning the soil mesofauna, an index for assessing the biological quality of soil, is based on the number of microarthropod groups adapted to the soil habitat (Parisi et al., 2005), thus, the underlying concept is that the higher the soil quality, the higher the number of microarthropod groups adapted to the soil habitat (Parisi et al., 2005).

The sum of structures produced by a population or community of invertebrate engineers creates a specific environment“ defined by Lavelle (2002) as a functional domain which is characterized by (i) the nature and spatial array of the biogenic structures, solid aggregates, mounds or constructs and pores of different shapes or sizes; (ii) the specific communities of smaller organisms from the meso- and microfauna and microorganisms that they host; and (iii) the spatial and temporal scales at which soil processes operate.

Despite the formation of casts, earthworms generally change the soil structure by the formation of macropores when penetrating the soil; the burrowing activity leads to complex burrow systems (Emmerling et al., 2002).

The soil biological communities are characterized by a higher diversity, by several orders of magnitude, compared to aboveground biomass, and therefore, this environmental compartment has become one of the last great frontiers in the study of biodiversity (Gargiulo et al., 2011).

The shape and size of voids are expected to be influenced by faunal activities (Bruneau et al., 2004). The role of structures created by these organisms may be highly significant in the ecosystem functions since the often are privileged sites for all basic soil processes (Lavelle, 1996).

The image analysis techniques allow direct investigations of the soil pore system and provide valid tools to quantitatively analyse both shape and size distribution of pores (Gargiulo et al., 2011).

The soil invertebrates communities can be used as assessment and prediction tools of ecosystem services; many species of invertebrates being important in soil fertility and playing a vital role in the production and maintenance of healthy soils (Chiriac & Murariu, 2021).

The paper goal was to emphasize the dependency degree of a soil ecosystem services on the soil microbiota activity, studying the pedofeatures generated by the biota activity (coprolites) as well as the bio-poral space, by the aim of the physical data, the micromorphological investigation and the image analysis quantification on soil thin sections.

MATERIALS AND METHODS

The researches had been performed in a site located in the Eastern part of the Romanian Plain, in Southern Bărăgan Plain, with a temperate continental climate and a steppe bioclimate. The soil is Typic Chernozem

(according to SRTS-2012 and Vermic Chernozem according to WRB-SR-2014).
The average annual temperature is 10.8°C, and the average annual rainfall is 480 mm, while the evapotranspiration reaches 700 mm. The global drainage is good. The water table is at > 10 m.
The soil was sampled both undisturbed (in metal cylinders for physical analysis and in micromorphological boxes for image analysis and micromorphological investigations) and disturbed (for physical and chemical analysis) from each pedogenetic soil horizon. The soil sampled and data interpretations were made according to ICPA- Methodology (1987).
The granulometry was determined by the aim of the pipette method while for the bulk density (g cm^{-3}) the cylindrical core method has been used, and the aeration porosity has been calculated.
For the micromorphological investigation, the undisturbed soil blocks (after air dried and impregnated with epoxy-resins) were used to prepare oriented thin section (having 25-30 µm thick). Each thin section has been studied with the Documator (20 X) and the optical microscope (50-100 X) in PPL (plain polarized light) and XPL (crossed polarized light). The terminology used for the micromorphological description was according to Bullock et al. (1985).
The porosity was quantified by the aim of image analysis, in order to characterize, at micromorphological level, the pore space. The image analysis has been performed on soil thin sections with the help of an image-analyzing computer (PC-IMAGE software produced by Foster Findlay Associates - London). The instrument was adjusted to measure pores greater than 50 µm. The pores have been measured by their shape, which is expressed by the shape factor ($perimeter^2/4\pi$*aria).

RESULTS AND DISCUSSIONS

At macroscopic level, the physical analyses pointed out the presence of a relatively uniform medium (medium loam) texture in the soil profile, which emphasized a balanced distribution of the granulometric fractions in all the pedogenetic horizons of the soil profile.
On this general background, of a soil with loamy texture, the bulk density has been low (1.28 g/cm^{-3}) in the top horizon, and increasing to medium (1.37–1.45 g/cm^{-3}) in the underlying horizons, while the total porosity values were, consequently, high in the upper horizons (51.9 %v/v) and slowly decreased to medium (ranging between 45.5-48.3% v/v) in the deeper horizons.
At microscopic level, the image analysis quantification of the pores (according to their shape and size) showed that the total porosity (<100 - >1000 µm) was 0.27 m^2m^{-2} (Figure 1). According to the micromorphometric method, a soil is considered compact when the total macroporosity is less than 0.10 m^2m^{-2}, moderately porous when the porosity ranges from 0.10 to 0.25 m^2m^{-2}, porous when the porosity ranges from 0.25 m^2m^{-2} to 0.40 m^2m^{-2}, and extremely porous over 0.40 m^2m^{-2} (Pagliai, 1988).
In this respect the obtained data emphasised that studied soil was porous. Further, the image analysis data of the pores (according to their size) showed the dominance of the pores in the size classes of 100-300 µm equivalent pore diameter (Figure 1).

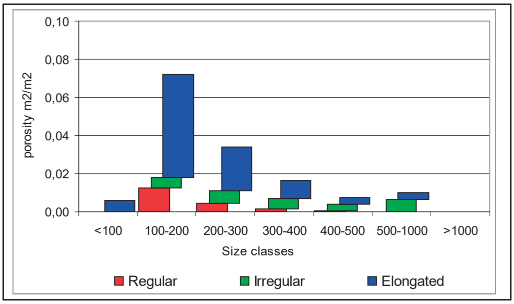

Figure 1. The pore size distribution according to their shapes

Regarding the pore distribution, characterized by their shape, there have been defined three categories: regular pores (more or less rounded); irregular pores (with irregular shape); elongated pores (mainly fissure). The pore size distribution is expressed as equivalent pore diameter, for regular pores and as width for elongated pores.
The regular pores (in the studied soil) are better represented in the classes of 100-200 µm equivalent pore diameter, decreasing slowly in

the classes of 200 to 400 μm equivalent pore diameter. This type of porosity is generated mainly by the biological activity (fauna and plant roots).

The irregular pores are more frequent and became dominant in the class of 100-200 μm equivalent pore diameter, decreasing in the higher value classes.

The irregular pores are most common in the soil, being mainly generated by the physico-mechanical processes, the pores opening along the less resistant directions. This type of porosity included also the pores generated by the collapse of the biogenic channels and/or chambers.

The elongated pores were the most frequent, being also dominant in the classes ranging between 100 to 400 μm (equivalent pore diameter).

Among the elongated pores there are also included the fine fissures that delimitated the rounded or ellipsoidal biological pedofeatures by the surrounding soil matrix. These fine fissures are the path for the water drainage as well as for the air, creating vital conditions for the microorganisms which populated the coprolite and pedotubules surfaces.

In the case of the size class of 100-200 μm (equivalent pore diameter), the proportion of the different type of pores (regular, irregular, and elongated) are best represented.

The poral space constantly renewed under both biological activity and physico-mechanical processes (due to the soil wetting - drying events).

At microscopic level, the micromorphological investigation on soil thin sections showed a complex structure with mainly granular and crumby structural aggregates while local the structure is spongy.

The structural aggregates are mainly biogenic (Figures 2 and 3) as a result of the high biological activity.

The porosity is represented by the fine cracks, biogenic channels, packing and interconnected voids, and is relatively high. Packing voids also appear inside the structural elements.

The soil matrix shows a certain non-uniformity due to the presence of a high number of zoo-aggregates (coprolites) and pedotubules with different compositions (with soil material brought by the fauna from different horizons of the soil profile) generated by a high pedoturbation process.

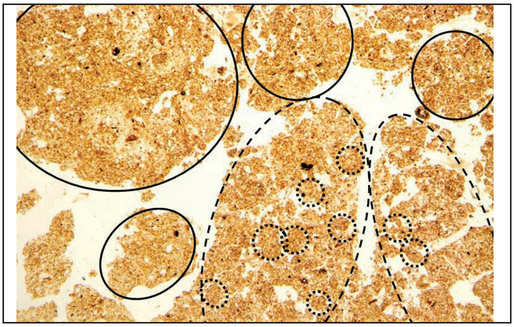

Figure 2. Biogenic pedofeatures: macrofauna coprolites ◯; and pedotubules ; macrofauna coprolites .

Many lumbric pedotubules are rich in small plant fragments (Figure 3).

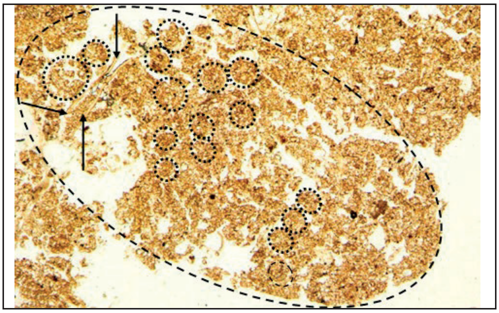

Figure 3. Pedotubul consumed by the mesofauna and replaced by the small coprolites ; vegetal fragment →

Locally, it has been observed many areas (reduced as surfaces), in which the skeleton grains (of the fine sand and loam sizes) appear concentrated and depleted of the plasmic material.

The soil matrix plasma is clayey-humico-Fe, dark brown due to the strong pigmentation with humons. The coarse fraction (soil skeleton) is composed of subangular–subrounded mineral grains predominantly of fine sand and loam size of: plagioclase feldspar, K-feldspars, muscovite, biotite, chlorite, green hornblende, calcite, glauconite, garnet, epidote, rutile, sphene, and opaque mineral. The chloritization process affected some feldspars grains and mica flakes.

The organic matter is represented mainly by the humified constituents and less by the plant

residues, located both in bio-voids and in soil matrix. The pedofeatures were mainly biogenic, being represented mostly by coprolites and podotubules, as well as depleted small areas. The big coprolites and pedotubules had been partially consumed by coprophagous mesofauna and replaced by the small coprolites (Figure 2).
The soil texture is one of the most stabile soil characteristics, its changing could appear under drastic threats as flooding, erosion, landslides, etc.
The detailed micromorphological investigation located the area with depleted skeleton grains into the lumbric biogenic pedofeatures (Figures 2-4). In these areas the skeleton grains were partially or totally depleted of plasmic material (Figure 4), and from their more or less lax spatial distribution, a porous micro-space had been resulted, which further favours the soil solution circulation, and consequently creates furthermore leaching conditions for soil plasma.

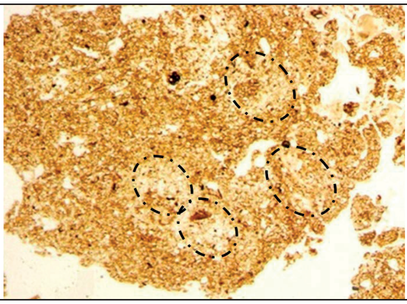

Figure 4. Many areas in the lumbric biogenic pedofeature (coprolite) with depleted skeleton grains concentrations

Thus, the secondary enrichment in the depleted skeleton grains (which appear in many areas of the coprolites) could be considered textural changes. These textural changes had been generated at a microscopic scale and only locally.
But the further evolution of the coprolites is their ingestion by mesofauna.
In this respect, in the lumbric biogenic pedofeatures consumed by the soil mezofauna, the soil matrix is again reorganized: the plasmic material and the skeleton grains being mixed again (Figures 2 and 3).
It seems that mesofauna had an active role of restoring the soil matrix composition (at textural level), by mixing the soil constituents and strengthening the structural edifice of the soil.
The soil biota covered all the web food (it doesn't matter how harsh the environmental conditions are), covering all the needs of the "factory fertility" and driving the soil organic matter transformation from fresh vegetal remains (Figure 3) to the humified organic matter and accomplish the main soil ecosystem service: soil fertility. Consequently, the soil is the mirror of its biodiversity.
Besides the organic substances transformations by both macrofauna, the higher „soil architects", and mesofauna, the biota restored the soil conditions (also the textural organization).
The combination of the macroscopic (physical data) - microscopic investigations (image analysis quantification and micromorphological observations on soil thin sections) allowed to emphasizing the high degree of dependency of the soil ecosystem services on the soil biota activity: from the micro-textural fabric of the soil to the 3D architectural organization of the soil aggregates and adjacent poral system.

CONCLUSIONS

At macroscopic level, the physical analyzes pointed out that on the general background of a soil with loamy texture, the bulk density has been low to medium, and consequently the porosity values were high to medium.
At microscopic level, the image analysis quantification of the pores (according to their shape and size) showed that studied soil was porous, and pores prevail in the size classes of 100-300 µm (equivalent pore diameter).
Concerning the pore distribution (characterized by their shape), the elongated pores were dominant, followed by the irregular and regular pores respectively.
Among the elongated pores the fine fissures delineating the biological pedofeatures by the surrounding soil matrix were also included, and represent the path for water and air, creating thus vital conditions for the microorganisms that developed on the biogenic pedofeatures surfaces.

At microscopic level, the micromorphological investigation on soil thin sections showed a high activity of the soil fauna, emphasized by the high number of zoo-aggregates (coprolites) and pedotubules.

In many lumbric coprolites skeleton grains concentrations (depleted of plasmic material) appeared, but mezofauna consuming these pedofeatures mix again the soil constituents, having thus an active role in the soil matrix composition restoration (at textural level).

The combination of the macroscopic – microscopic investigations throughout image analysis quantification and micromorphological observations on soil thin sections, allowed to emphasizing the high degree of dependency of the soil ecosystem services on the soil biota activity.

ACKNOWLEDGEMENTS

This work was supported by the Romanian Ministry of Research, Innovation and Digitization, through the Project number PN 23 29 05 01; and the Project number PN 23 29 06 01. Financial support for the publication of this study was jointly provided by the Romanian Ministry of Research, Innovation and Digitization, trough the Project number 44 PFE/2021, Program 1 - "Development of national research-development system, Subprogramme 1.2 - Institutional performance - RDI Excellence Financing Projects".

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

MICROBIAL BIOMASS IN FOREST SOILS OF NATURAL AND AGRICULTURAL ECOSYSTEMS

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Abstract

The profile of vertical distributions of microbial biomass and its correlation with organic carbon in the brown and gray forest soils of the Republic of Moldova have been investigated in natural and agricultural ecosystems. Sampling was carried out in 10 profiles per soil horizons to a depth of 170-200 cm. Microbial biomass constituted in brown forest natural soils 367.5-643.1 µg C g^{-1} soil and in gray forest natural soils 322.7-828.6 µg C g^{-1} soil in the top layer. The negative effects on soil microbial biomass were observed as a result of long-term land management practices. Microbial biomass decreased in brown forest arable soils to the level of 91.6-116.4 µg C g^{-1} of soil and in gray forest arable soils - to 117.1-283.0 µg C g^{-1} of soil in the arable layer. Microbial biomass was connected with the humus content. Correlation coefficients constituted 0.98-0.99 in brown forest soils and 0.82-0.91 in gray forest soils. The link effect between microbial biomass and humus content decreased from virgin to arable soils. A negative link has been established with profile depth.

***Key words**: microbial biomass, forest soils, organic carbon, humus.*

INTRODUCTION

The soil matrix is the surface of soil particles with active centers, around which layers of adsorbed substances (organic, mineral substances, microorganisms, gases, ions, molecules) are formed in a certain way. The soil matrix is an active part of the soil, capable of reproducing a complex of cations, a film of sorbed water, an organic matrix on the surface of soil particles. It includes three matrices: mineral, organic, and organo-mineral. Organo-mineral matrix includes both mineral and organic matrix areas, as well as immobilized enzymes and microorganisms (Zubkova & Karpachevsky, 2001).

On the other hand, microbial materials are an important constituent of stable soil organic matter. Soil microbes produce chemically diverse, stable soil organic matter. It has been shown that soil organic matter accumulation is driven by distinct microbial communities more than clay mineralogy, where microbial-derived soil organic matter accumulation is greatest in soils with higher fungal abundances and more efficient microbial biomass production (Kallenbach et al., 2016).

On the whole, the soil microbial biomass represents both the living part of the total soil organic matter and the active part of the soil matrix, constantly synthesizing organic matter in the form of humic substances.

Many microbiological indicators in the matrix are related to organic matter and are linked to nutrient cycling and the biodiversity and productivity functions. Determination of the soil microbiological biomass content is one of the basic parameters for monitoring soil changes. Soil microbial biomass carbon is important in regulating soil organic carbon dynamics along soil profiles by mediating the decomposition and formation of soil organic carbon (Tingting Suna et al., 2020). The importance of studying the contribution of soil microorganisms to total organic carbon is determined by their exceptional role in the formation of soil quality, understood as "the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health" (Doran & Parkin, 1994; 1996; Doran & Safley, 1997; Anderson & Domsch, 1989). At the same time, it has been shown that increase in the share of microbial carbon in organic carbon by more than 20% in crop rotation plots and in mixed forests is an indicator of the growth of soil biodiversity (Anderson Traute-Heidi, 2003).

Soil management has a large impact on the size of the biomass pool of microorganisms (Von Lützow et al., 2002; Nima Tshering Lepcha & Bijayalaxmi Devi, 2020). Numerous studies have shown that microbial biomass and microbial turnover is much lower in deeper soil layers compared to surface layers (Eilers et al., 2012; Spohn et al., 2016; Young et al., 2019). However, the land management can influence this trend. One study showed that while in natural ecosystems (forests, grasslands) microbial biomass decreased with depth, in an arable field there was no significant change (Van Leeuwen et al., 2017). This was probably a consequence of the homogenizing influence of agricultural activities. Conversely, it had been shown that in soil environments that are mostly unfavorable in the surface layer biomass levels increase with depth, because subsoil communities are protected from surface stresses such as radiation or desiccation (Mueller et al., 2015). Despite this, the subsoil represents a significant reservoir of microbial biomass. Estimation the share of the total microbial biomass contained in the subsoil ranges from 30% (Fierer et al., 2003; Van Leeuwen et al., 2017) to 58% (Schütz et al, 2010) or more.

The importance of microbial carbon analysis of deeper soils in soil research is justified (Naylor et al., 2022) as an indicator of changes in the quality of soil organic matter.

Spatial patterning of soil microorganisms can occur both horizontally and vertically, through the soil profile. Most studies focus on processes occurring in the upper A horizons because so much of the short-term dynamics occurs there (Raubuch & Beese, 1995). Our research were focused on vertical distributions of microbial biomass densities in natural soils and those that exposedto to the long-term agricultural use.

Thus, the study of the microbial carbon gradient in soils of contrasting ecosystems is a necessary step for assessing the architectonics of the soil organoprofile and understanding carbon fluxes in soil layers at different depths.

MATERIALS AND METHODS

Our comparative study has been conducted in central and northern zones of the Republic of Moldova (Figures 1, 2). Five experimental sites have been tested. The content and profile distributions of microbial biomass of arable forest soils with the normal profile were investigated in comparison with the undisturbed forest soils in natural ecosystems. The research foresees the use of the profile method - "unplowed (natural) - plowed" pairs. Investigations were performed on the brown and gray forest soil. Sampling was carried out in 10 profiles per soil horizons to a depth of 150-240 cm.

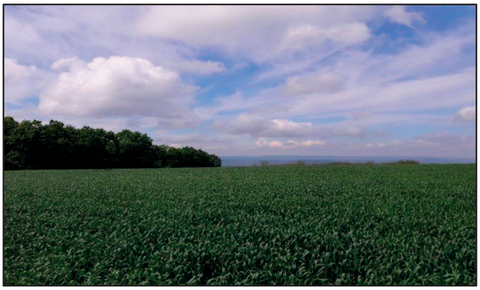

Figure 1. Fragments of natural and agricultural landscapes located in the central zone of the Republic of Moldova

Figure 2. Fragments of natural and agricultural landscapes located in the northern zone of the Republic of Moldova

Experimental sites with brown forest soils are located in the central zone of the Republic of Moldova, in the wooded steppe of the central - Moldovan forest province, in the district No. 8 of brown, gray forest soils and leached chernozems of the wooded steppe of hilly Kodru Forests.

The plot with *typical brown soil* (profile 1 under forest; profile 2 under arable) is situated in the Tuzara village and Gorodische com., Kalarash region (Figure 3).

The plot with *luvic brown forest soil* (profile 5 under forest; profile 6 under arable) is located in the Dolna com., Strasheni region (Figure 4).

Profile 1 Profile 2

Figure 3. Profiles of virgin typical brown forest soil (profile 1) and arable typical brown forest soil (profile 2)

Profile 5 Profile 6

Figure 4. Profiles of virgin luvic brown forest soil (profile 5) and arable luvic brown forest soil (profile 6)

Investigations were also conducted on the molic, albic and typical gray forest soils (classification of soils by Ursu A. (2001, 2016). The site with *molic gray forest soil* (profile 3 under forest; profile 4 under arable) is situated near the Grozeshti village, Nisporeni region (Figure 5). According to pedogeographic zoning, this site is located in the Central Plateau of Kodru Forests, in the region V of Kodru' Plateau, in the district No. 8 of brown, gray forest soils and leached chernozems.

The site with *albic gray forest soil* (profile 7 under forest; profile 8 under arable) falls within the hilly forest-steppe zone of the Northern Plain (I), in the forest-steppe of the Northern Plateau, in the district No. 1 of gray forest soils and clay-alluvial chernozems. Nearby is situating Terebna village, Edinets region (Figure 6).

The site with *typical gray forest soil* (profile 9 under forest; profile 10 under arable) is also located in the hilly forest steppe zone of the Northern Plain (I), the forest-steppe hill of Rezina district No. 5 of gray forest soils and argillaceous chernozems near the Raspopeni village, Sholdaneshti region (Figure 7).

Profile 3 Profile 4

Figure 5. Profiles of virgin molic gray forest soil (profile 3) and arable molic gray forest soil (profile 4)

Profile 7 Profile 8

Figure 6. Profiles of virgin albic gray forest soil (profile 7) and arable albic gray forest soil (profile 8)

Profile 9 Profile 10

Figure 7. Profiles of virgin typical gray forest soil (profile 9) and arable typical gray forest soil (profile 10)

The microbial biomass C was measured by the rehydratation method based on the difference between C extracted with 0.5 M K_2SO_4 from dried soil at 65-70°C within 24 h and fresh soil samples with K_c coefficient of 0.25 (Blagodatsky, Blagodatskaya et al., 1987). K_2SO_4 - extractable organic C concentrations in the dried and fresh soil samples were simultaneously measured by dichromate oxidation. The ratio between microbial and organic carbon was determined according to Kennedy & Papendick (1995). The quantity of K_2SO_4 - extractable C was determined at 590 nm with spectrophotometer.

Organic C was analyzed by the dichromate oxidation method (Arinushkina, 1970). The humus content was calculated using the coefficient of 1.724. The microbial biomass index and humus content was evaluated statistically by the correlation analysis.

RESULTS AND DISCUSSIONS

Soil microbial biomass decreases with soil depth in all land-use types of forest soils. Maximum content of microbial biomass in topsoil in natural forest soils is obesrved due to the availability of a larger amount of organic matter from trees. The presence of trees continuously adds litter to the top layer and increases root turnover. Organic matter in the litter layer in tree-based forest systems increases the amount of organic carbon in the soil profile, thereby helping in the restoration of better soil quality. As a result, microorganisms of natural soils exist in conditions of high supply of the organic matter and its conservation within the limits of the ecosystem.

The distribution of microbial biomass on the genetic horizons in soils of natural and anthropogenic ecosystems is sharply different. The highest level of the microbial biomass in natural brown forest soils have been determined in the layer 0-19 cm (A_1 genetic horizon), these constituting 367.5 µg C g^{-1} soil in the typical brown forest soil and 643.1 µg C g^{-1} soil in the luvic brown forest soil (Figure 8).

The quantity of the microbial biomass reaches in the natural molic gray forest soil 322.7 µg C g^{-1} soil in the layer 0-19 cm (Ad genetic horizon), in the albic gray forest soil – 802.5 µg C g^{-1} soil (Ao genetic horizon, layer 0-7 cm), in the typical gray forest soil - 828.6 µg C g^{-1} sol in A_1d genetic horizon in layer 0-30 cm (Figure 9).

The biomass index decreases in the soil profile of natural brown forest soils to a depth of 65-175 cm, in the soil profile of natural gray forest soils - to a depth of 122-190 cm.

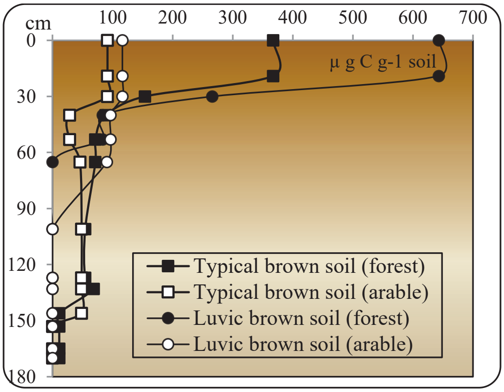

Figure 8. The profile distribution of microbial biomass in natural and arable brown forest soils

Thus, microbial biomass in natural soils is concentrated in the A genetic horizon, in brown forest soils - in the amount of 44.4-56.9%, in gray forest soils - in the amount of 45.0-64.7% from the profile total biomass. The profile distribution of microbial biomass in natural soils is associated with the concentration of leaf litter and the deposition of soil organic carbon in the upper layers.

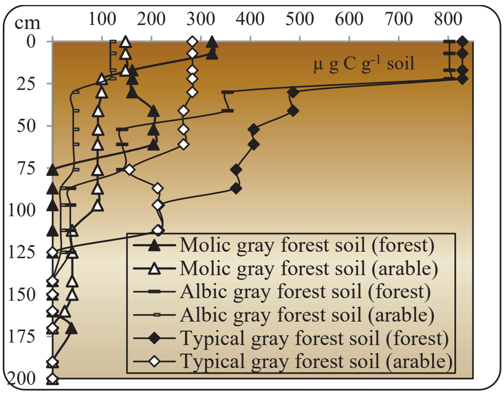

Figure 9. The profile distribution of microbial biomass in natural and arable gray forest soils

A similar trend has been noticed in the distribution of organic carbon content in natural soils, the value of which in the upper layers (Ad genetic horizon, layer 0-19 cm) in the typical brown forest soil constitutes 1.67%, in the luvic brown forest soil - 2.08% (Table 1). The organic carbon content in natural molic gray forest soil constitutes 3.16% (Ad genetic horizon, layer 0-17 cm), albic gray forest soil - 1.70% (Ao genetic horizon, layer 0-7 cm) and typical gray forest soil - 2.20% in the A1d genetic horizon, layer 0-30 cm (Table 2).

The share of microbial carbon from the organic carbon content (C_{MB}/C_{org}) in the A genetic horizon of natural brown forest soils constitutes 1.74-2.20% and natural luvic brown forest soil - 3.09-3.10%. The share of microbial carbon from the organic carbon content in gray forest soils under the forest constitutes in the molic gray forest soil - 1.02% in the soil layer 0-17 cm (genetic horizon Ad), in the albic gray forest soil - 4.72% and 3.05% in the soil layer 0-7 cm and 7-30 cm (genetic horizon Ao and A1), in the typical gray forest soil - 3.77% in the soil layer 0-30 cm (genetic horizon A1d).

Increasing the contribution of microbial carbon to the content of organic matter in the lower layers of horizon B is associated with growth of the abundance of anaerobic microorganisms in these layers.

The long-term use of arable management leads to the decrease of the content of microbial biomass in arable chernozems both in the upper horizons, and in the soil profile in general. The low content of microorganisms in the upper layers is characteristic of arable forest soils. The long-term use of forest soils leads to the reduction of the microbial biomass in the arable layer by several times.

Table 1. The profile distribution of the organic carbon content and share of microbial carbon on genetic horizons in brown forest soils of natural and agricultural ecosystems

Soil, profile	Horizon index	Depth, cm	C_{org}, %	C_{MB} / C_{org}, %
Typical brown forest soil (forest), P1	Ad	0-19	1.67	2.20
	A1	19-37	0.85	1.81
	A2	37-53	0.51	1.74
	B1	53-101	0.33	2.17
	B2	101-133	0.24	2.28
	BC	133-146	0.20	3.45
	C	146-175	0.13	0.87
Typical brown forest soil (arable), P2	A ar	0-34	0.45	2.04
	A1A2	34-62	0.27	1.06
	B1	62-83	0.19	2.41
	BC	83-153	0.14	3.47
	C	153-175	0.14	0
Luvic brown forest soil (forest), P5	Ad	0-19	2.08	3.09
	A1	19-30	0.86	3.10
	B1	30-65	1.00	0.84
	B2	65-105	0.21	0
	BC	105-128	0.16	0
	C	128-170	0.12	0
Luvic brown forest soil (arable), P6	A ar	0-40	0.46	2.53
	B1	40-65	0.34	2.85
	B2	65-102	0.20	4.54
	BC	102-127	0.18	0
	C	127-165	0.10	0

Microbial biomass index gradually decreases in the profile of arable soil. In arable brown forest soils, some species can be detected at a depth of 102-153 cm, in arable gray forest soils – at the depth of 115-240 cm. The profile distribution of microbial carbon in arable forest soils corresponds to the distribution of organic carbon. The share of microbial carbon in the organic carbon content constitutes 2.04% in the arable typical brown forest soil (layer 0-34 cm) and 2.53 % in the arable luvic brown forest soil (layer 0-40 cm). The share of microbial carbon in the organic carbon content constitutes 0.70% in the soil layer 0-22 cm (genetic horizon A1ar) of the molic gray forest soil, 1.14% in the soil layer 0-30 cm (genetic horizon A1ar) of the albic gray forest soil and 2.26% in the soil layer 0-32 cm (genetic horizon A1B1ar) of the typical gray forest soil.

Thus, the share of microbial carbon in its organic content in arable forest soils is lower than in natural ones.

Because of the long-term use of arable land, homogenization of the arable layer and decrease in the reserves of microbial carbon and humus, the natural stability of chernozems reduces.

The vertical distribution of microorganisms in forest soils is inextricably linked with the distribution of organic matter along the soil profile and, accordingly, with its depth. The analysis of the interdependence between the microbial biomass indicator, on the one hand, and the humus content, on the other hand, demonstrated their close positive connection (Tables 3, 4). The correlation coefficient (R^2) between the biomass of microorganisms and humus content in the typical brown soil constitutes R^2 = 0.98 (n=12) and in the luvic brown soil - R^2 = 0.99 (n=11). The correlation coefficient (R^2) between the biomass of microorganisms and humus content in the molic gray forest soil R^2 = 0.82 (n=12), in the albic gray forest soil - R^2 = 0.91 (n=12) and in the typical gray forest soil - R^2 = 0.82 (n=11).

Table 2. The profile distribution of the organic carbon content and share of microbial carbon on genetic horizons in gray forest soils of natural and agricultural ecosystems

Soil, profile	Horizon index	Depth, cm	C_{org}, %	C_{MB} / C_{org}, %
Molic gray forest soil (forest), P3	Ad	0-17	3.16	1.02
	A1	17-41	1.87	0.86
	B1	41-75	0.82	2.49
	B2	75-125	0.69	0
	BCk	125-170	0.49	0
	Ck	170-190	0.39	0.98
Molic gray forest soil (arable), P4	A1 ar	0-22	2.11	0.70
	A1	22-33	1.91	0.52
	B1	33-61	0.96	0.96
	B2	61-112	0.62	1.47
	BCk	112-158	0.35	1.15
	Ck	158-170	0.22	1.13
Albic gray forest soil (forest), P7	Ao	0-7	1.70	4.72
	A1	7-30	1.16	3.05
	A2	30-52	0.51	2.77
	Bt1	52-87	0.41	1.88
	Bt2	87-142	0.34	1.06
	BC	142-211	0.64	0
	C	211-240	0.60	0
Albic gray forest soil (arable), P8	A1ar	0-30	1.03	1.14
	B1	30-87	0.41	1.02
	Bt2	87-142	0.48	0.35
	BC	142-211	0.52	0
	C	211-240	0.49	0.66
Typical gray forest soil (forest), P9	A1d	0-30	2.20	3.77
	B1	30-43	0.85	5.72
	Bt2	43-76	0.50	8.14
	Bt3	76-97	0.44	8.43
	BC	97-122	0.41	5.25
	C	122-160	0.31	0
Typical gray forest soil (arable), P10	A1B1ar	0-32	1.25	2.26
	Bt2	32-66	0.37	7.16
	Bt3	66-77	0.37	4.21
	BC	77-115	0.33	6.44
	C	115-150	0.26	0

Table 3. Correlation between microbial biomass and humus content in forest soils

Soil	Land use	Correlation coefficient (R^2)
Typical brown forest soil	forest	0.98
	arable	
Luvic brown forest soil	forest	0.99
	arable	
Molic gray forest soil	forest	0.82
	arable	
Albic gray forest soil	forest	0.91
	arable	
Typical gray forest soil	forest	0.82
	arable	

Table 4. Correlation between microbial biomass and humus content in forest soils

Soil	Land use	Correlation coefficient (R^2)
Brown forest soil	forest	0.97
	arable	0.79
Gray forest soil	forest	0.56
	arable	0.29

The correlation coefficient between the microbial biomass and humus content in brown forest soils under natural vegetation is R^2 = 0.97 (n=13); in arable soils - R^2 = 0.79 (n=10). The correlation coefficient between the microbial biomass and humus content in natural gray forest soils constitutes R^2 = 0.56 (n=19); in arable soils - R^2 = 0.29 (n=16).

Since the abundance of microorganisms decreases with the depth of the soil profile, the correlation between these values is negative. The correlation coefficient (R^2) between the biomass of microorganisms and soil profile depth in brown forest soils constitutes R^2 = -0.66 (n=23) and in gray forest soils - R2 = -0.64 (n=35). The correlation coefficient between the microbial biomass and soil profile depth in brown forest soils under natural vegetation constitutes R^2 = -0.74 (n=13); in arable soils - R^2 = -0.76 (n=10). The correlation coefficient between the microbial biomass and soil profile depth in natural gray forest soils amounts R^2 = -0.74 (n=19); in arable soils - R^2 = -0.65 (n=16).

CONCLUSIONS

Soil microbial biomass represents both the living part of the total soil organic matter and the active part of the soil matrix. The results of the study demonstrated that land use affect have a significant impact on the microbial biomass carbon. In its turn, the profile distribution of organic carbon of the soil also affects this indicator. The content of organic and microbial carbon depends on the depth of the soil profile.

Evaluation of microbial biomass resources in forest soils in conditions of natural and agricultural ecosystems showed significant differences between these soils.

Undisturbed forest soils in conditions of the natural ecosystems are characterized by a higher biomass of soil microorganisms in comparison with arable soils and concentrated in the A genetic horizon. Microbial biomass constituted 367.5-643.1 μg C g^{-1} soil in brown forest natural soils and in 322.7-828.6 μg C g^{-1} soil gray forest natural soils in the top layer. The differences between brown and gray natural forest soils are manifested in a higher specific concentration of microbial biomass in the A genetic horizon of gray forest soils (45.0-64.7% of the total biomass in gray soils versus 44.4-56.9% in brown soils) and in a greater depth of microbial distribution along the soil profile (up to depths of 122-190 cm) in gray forest soils, while in brown forest soils microorganisms were found to a depth of 65-175 cm.

The long-term arable use of forest soils led to the reduction of microbial potential and contributed to the degradation and decrease of soil stability. In the conditions of arable land microbial biomass was reduced to the level of 91.6-116.4 in brown forest soils and to 117.1-283.0 μg C g^{-1} of soil in gray forest soils in the arable layer. The share of microbial carbon in its organic composition in arable forest soils is reduced compared with those under natural vegetation. At the same time profiles of the arable forest soils are covered by the degradation process in general. The negative effects on soil microorganisms were observed as a result of enhanced mineralization process, the content of organic carbon in arable soils has been significantly reduced.

The microbial biomass, being a part of the labile organic matter, was connected with the soil organic carbon content. The vertical distribution of microorganisms in forest soils is closely related with the distribution of organic matter and with its depth. The interaction between microbial components and humus status is closer in soils of natural ecosystems. Correlation coefficients constituted 0.98-0.99 in brown forest soils and 0.82-0.91 in gray forest soils. The link effect between microbial biomass and humus content decreased from natural to arable soils. A negative link has been established with the profile depth.

ACKNOWLEDGEMENTS

This research work was carried out in the framework of the institutional project "Evaluation of the soil state of the Republic of Moldova in the agrocenosis conditions, improvement of the classifier and the soil rating system, elaboration of the methodological-informational framework for monitoring and enlarged fertility reproduction" (project code 20.80009.7007.17) in 2021.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SPATIAL DISTRIBUTION OF PHOSPHORUS ON THE SOIL CATENA OF CHROMIC CAMBISOLS COMPLEX

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Abstract

The present study aims to establish the influence of the processes of secondary pedogenesis on the content and distribution of mobile forms of phosphorus in Chromic cambisols complex formed on a silicate base. Phosphorus is not a major nutrient in pedogenesis and is not clear its natural redistribution in the range of soils with general geological origin of terrigenous materials, but located differently in terms of their eluvial-deluvial transfer within a common long soil catena. The size of the total sample is 15 soil sampling points, and at each point samples are taken from the layer 0-25 and 25-50 cm. The sampling points are selected in the middle of a characteristic slope, without manifestation of local linear erosion forms or accumulation zones. Based on the study, it was found that the content of P_2O_5 in the top soil layer did not depend on the location, and the deeper horizon should be considered as diagnostic one in terms of its distribution.

Key words: *soil catena, phosphorus, topographic factors, variogram.*

INTRODUCTION

The complex of Chromic cambisols in Bulgaria is very heterogeneous in terms of genesis, composition and properties. This is due to the true genetic heterogeneity of this group of soils for which in the Bulgarian classification, based mainly on their color and zonal affiliation, a common genetic taxon has been determined at the of the soil type level. Nevertheless, they occupy common areas and in terms of their distribution on Bulgarian territory are zonal, despite the fact that their "zonation" is considered on one side from the European moderately continental and Atlantic soil zones, and on another side from the soil zones in the Mediterranean. In this sense, they are specific without being homogeneous in their range. Their internal type diversity is based on the factors and conditions of soil formation, but covers not only the main type determining pedogenesis (climate, macro relief, vegetation, etc.), but also much more modern conditions, such as denudation or erosion in general, contemporary vegetation, humidification conditions and others. The second group of factors strongly influences the composition and properties of the soil complex, but at the same time they are not "genetic", in the sense that they are not soil-forming at the level of the soil type. The influence of these factors is logically predictable with respect to some components of the soil composition. For example, those accepted at some taxonomic level as eroded or shallow Chromic cambisols have the expectation to establish a lower content of organic carbon and nitrogen, lighter soil texture, weakly manifested biogenic accumulation processes on the surface of the profile. For other elements of the soil composition these dependencies are not obvious, and at the same time some of them are important for the general functionality and for the agricultural suitability of the soil complex. These conditions are met by the content of absorb phosphorus and its distribution in the active part of the soil profile.

As all terrestrial ecosystems, temperate forest ecosystems rely on the availability of phosphorus from the soil, which is related to site parameters as precipitation, bedrock, soil pH, material, and stage of pedogenesis. Many soils in central Europe are young, the phosphorus status of forests is often low or insufficient (Ilg et al., 2009; Jonard et al., 2015). Soil acidification, intensified by anthropogenic deposits, additionally reduces plant available phosphorus (Mohren et al., 1986).

Strong phosphorus sorption, onto humic-mineral-complexes, by Al and Fe oxides and hydroxides, or clay minerals, increased formation of short-range order Al or Fe phosphate, as well as decreased mineralization of organic phosphorus may limit forest growth in many ecosystems (Gerke & Hermann, 1992; Violante & Pigna, 2002; Richardson et al., 2004; Sims & Pierzynski, 2005; Laliberté et al., 2012).

Humic mineral phosphate complexes may increase plant available P and can account for 50-80% of the phosphorus in solution (Gerke, 2010).

Studying phosphorus availability in soil therefore implies accounting for the distribution of different P proportions, in addition to the total P status in soil, as it is assessed by wet-chemical fractionation of soil, and by advanced spectroscopic techniques (Kruse et al., 2015).

Different phosphorus fractions exhibit different properties in soils and chemical P resources in soils change during pedogenesis (Walker & Syers, 1976; Sims & Pierzynski, 2005). The soils accumulate organic P compounds during the first 500 years of pedogenesis (Turner et al., 2007; Prietzel et al., 2013). The geosequence is characterized by a P status gradient, resulting from different bedrock and soil age. In addition to the phosphorus binding form, spatial heterogeneity of P has been identified as an important factor controlling P availability for plants and for an alpine treeline (Jackson & Caldwell, 1993; Liptzin et al., 2013). Phosphorus depth distributions in most cases were assessed only unidimensional (Ferro Vázquez et al., 2014).

Phosphorus is not an essential nutrient in pedogenesis, but at the same time it is very important from an economic point of view. It is not clear its natural redistribution in the area of soils with common geological origin of terrigenous materials, but located differently in terms of their eluvial-deluvial transport within a common long soil catena.

According to Ibrahim et al. (2021) excessive input of phosphorus (P) in agricultural production and its finite resources is becoming a global concern for sustainable P management and more research is needed about the main factors influence on P available.

This study aims to establish the influence of the processes of secondary pedogenesis on the content and distribution of mobile forms of phosphorus in Chromic cambisols complex formed on a silicate base.

MATERIALS AND METHODS

As a basis for the study was used an area of leached, eroded and accumulated (but not meadow) Chromic cambisols in the Eastern slopes of Sakar mountain and in the conditions of dissected hilly terrain. The study was conducted in the period 2019-2021. Soil samples with a sufficiently well-developed contemporary profile were collected and at the same time some of them were strongly eroded. The soil samples were located on the sloping part of a long soil catena, as the height of the sampling points varied from 65 to 200 m above sea level, and the sampling points are grouped in a total of three zones. In each of the altitude zones more than one soil samples were taken at depths of 0-25 and 25-50 cm.

The coordinates of the wide soil catena are in the Universal Transversal Mercator N35 system, with a horizontal resolution of 5 m and a vertical resolution of 1 m, using a height correction from a ground station. The topographic conditions are distinguished by three indicators - altitude with an accuracy of 1 m. In the Baltic altitude system, meter latitude and longitude by Mercator, slope of the terrain in degrees of azimuth and in rhombuses in 90° distributions. It is assumed that in the conditions of the limited area of the soil catena, the climatic conditions and the vegetation are constant factors. The size of the total sample is 15 soil sampling points, and at each point samples are taken from the layer 0-25 and 25-50 cm. The sampling points are selected in the middle of a characteristic slope, without manifestation of local linear erosion forms or accumulation zones. On the basis of the soil samples collected in this way, the soil texture was determined by the photosedimentographic method (Trendafilov et al., 2017) and the total content of mobile phosphorus by the double-lactate method of Egner-Reim, (GOST 26209-91/01.07.93).

The statistical methods used in the study were Pearson correlation analysis, Test of normality - Shapiro-Wilk, Package program - SPSS Statistics.

RESULTS AND DISCUSSIONS

The surveyed soil differences are represented by strongly, moderately and slightly eroded to poorly accumulated profiles. Genetically, the soils have a differentiated profile, but with strongly and moderately eroded differences, the two-membered character of the profile is "masked" not only due to the erosive pedoturbation of the surface horizon with lighter layers lying above and below it.

Studies of phosphorus transformations have mainly focused on P cycling processes resulting in the transformation of original P to other forms. In the early stages of soil development, parent materials contain mainly inorganic phosphate (Cole & Heil, 1981; Smeck, 1973; 1985; Stewart & Tiessen, 1987). The relative proportion of different P fractions is determined by the activities of calcium, iron and aluminum in the soil which, in turn, are greatly influenced by weathering processes (Williams & Walker, l969a; 1969b).

In the described diversity in terms of soil genetic factors (basic and contemporary), was assumed that a relatively stable and reproducible starting point of the modern characteristic could fulfill the topographic factor. Moreover, that it was fundamental to the erosion process, and the latter in the given case determined the modern pedogenesis not only in the specific studied area but also in the area of distribution of the complex eroded, non-eroded and accumulated Chromic cambisols with a common geological basis. The location of the soil sampling points on the topographic basis of the locality is shown in Figure 1.

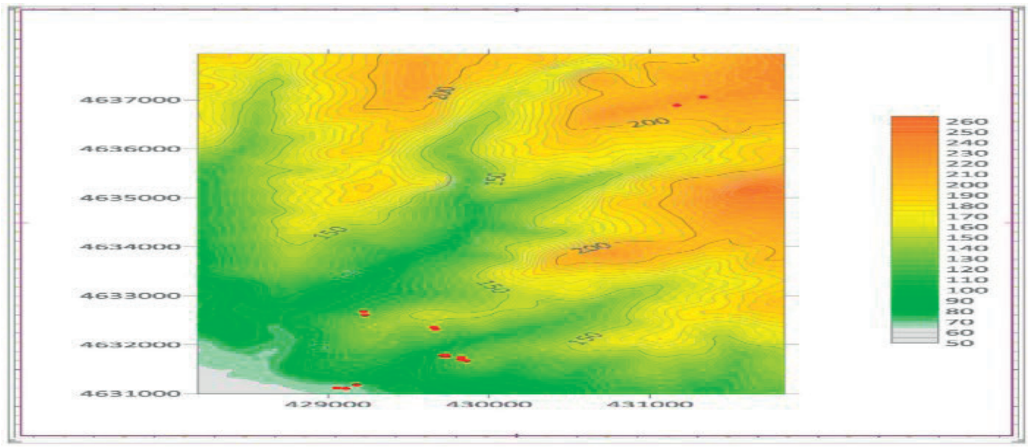

Figure 1. Location of soil sampling points

Total phosphorus content and distribution

The organic and inorganic forms and distribution of phosphorus in the soil vary with different processes as: natural processes that determine soil mineralogy and phosphorus sorption characteristics, as well as human-controlled processes such as the application and timing of phosphorus containing fertilizers, lime and organic material (Batjes, 2010). Under natural conditions, the weathering and dissolution of rocks andrelatively insoluble phosphorus containing minerals is a slow process. This weathering is only capable of supporting slow-growing vegetation and crops adapted to low phosphorus availability. In acid soils, various forms of iron, aluminum and manganese oxides strongly bind phosphorus, while in calcareous soils phosphorus is mainly found in the form of calcium compounds of varying solubility (Dabin, 1980; Fairhurst et al., 1999; Ryan & Rashid, 2006). Clay mineralogy and clay content directly affect phosphorus retention (Sanchez, 1976). The form of phosphorus in the soil will influence phosphorus availability to the plant. Phosphorus uptake is determined by soil water conditions, temperature, crop type and growth rate, root morphology and plant specific characteristics to extract soil phosphorus through excretion of exudates (Hoffland et al., 1992).

Mycorrhizal fungi may also be important in this respect (Smith et al., 2003; Li et al., 2006).

The average P_2O_5 content of the test sample for both depths was 12.9 ± 0.6 mg/kg, and ranged from 8.8 to 21.2 mg/kg. P_2O_5 levels were most commonly found in the range of 10 to 12 mg/kg. In the surface layers of the profile the content of P_2O_5 was on average 14.4 mg/kg, and in the subsoil 11.7 mg/kg. Depends on their location in the soil catena and taking into account the morphology of the soil profile, soil samples were divided into five groups. The characteristics of the soil differences in the individual groups are given in Table 1.

Table 1. Characteristics of the location, the degree of erosion and the soil texture of the soils in the survey groups formed by location

Soil group	Degree of erosion	Soil texture (%)	Elevation zone (m)
1	**Non-eroded and slightly accumulated Chromic cambisols, undifferentiated**	Surface horizon 51.4 Subsoil horizon 43.6	65
2	**Slightly eroded and non-eroded Chromic cambisols, undifferentiated**	Surface horizon 46.6 Subsoil horizon 44.6	117
3	**Moderately eroded Chromic cambisols, undifferentiated**	Surface horizon 35.0 Subsoil horizon 32.0	125
4	**Strongly eroded Chromic cambisols, differentiated**	Surface horizon 40.3 Subsoil horizon 47.3	127
5	**Strongly eroded Chromic cambisols, undifferentiated**	Surface horizon 47.4 Subsoil horizon 45.9	212

In the soils of all groups, the content of P_2O_5 in the surface horizons exceeded that one in the subsoil. In groups 1 and 5, i.e. in non-eroded to slightly accumulated Chromic cambisols, as well as in the Strongly eroded and shallow Chromic cambisols, the difference in the content of P_2O_5 on the soil surface and in depths was statistically proven (Figure 2). At the same time, no statistically proven difference in terms of P_2O_5 content in the total sample was observed.

The mobility of phosphate anions in soil depend on the nature of the mineral surfaces and oxide coatings since phosphate anions are strongly adsorbed by mineral constituents such as sesquioxides and clays (Parfitt, 1978). Organic phosphate compounds differ greatly in their form and in the number of reactive phosphate groups which they possess. Some of theme has the potential for movement, especially mono-phosphate esters (Rolston et al., 1975).

Phosphorus may move with other nutrients in surface flow, as run off, particularly in areas where snow melts before the surface soil thaws and water could infiltrate (Timmons et al., 1977).

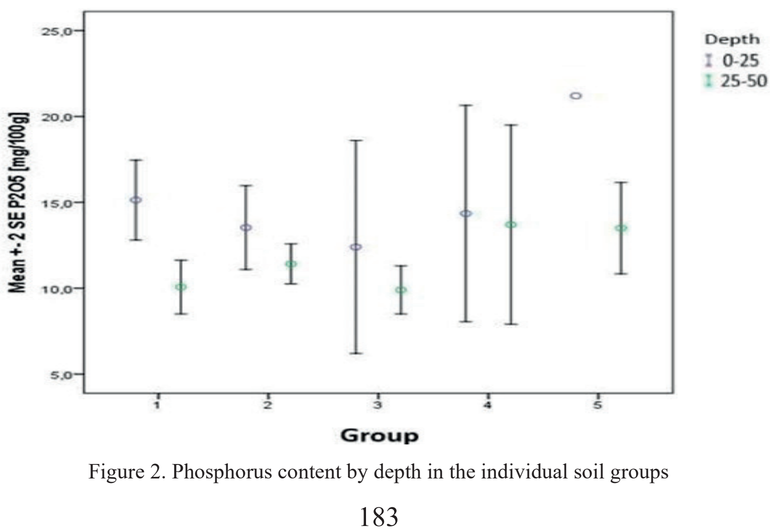

Figure 2. Phosphorus content by depth in the individual soil groups

Influence of the topographic factors of the terrain on the distribution of P_2O_5 in the soil

The influence of the topographic factor was studied by altitude, slope and exposure (location of the soil sampling point).

The influence of altitude within the boundaries of the soil catena is expressed in meters above sea level and was established by regression analysis (Goovaerts, 1992). A tendency to exceed the P_2O_5 content in the total statistical sample is considered to be proven at a probability level of 95% (F. 6.9), as the sampling depth normalizes nearly a quarter (24.2%) of P_2O_5 variation into the studied soils in total. This was the reason for further study in the dynamics of the main studied feature, the samples collected from different depths to be considered as separate statistical subsamples. In order to determine the representativeness of the subsamples normalized from the soil samples, collected at a depth of 0-25 and 25-50 cm, it was necessary to study their frequency distribution, as a preliminary mathematical expectation was that they were distributed in the normal frequency range. The results are shown as frequency histograms in Figure 3.

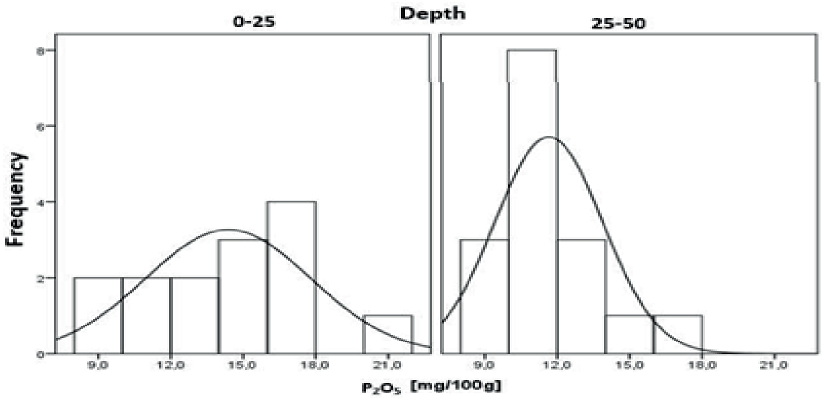

Figure 3. Histogram of the frequency distribution of phosphorus in the individual depths

Although the histogram for the frequency distribution of the amount of P_2O_5 at a depth of 50 cm showed more convincingly the approximation of the experimentally established frequency distribution to a normal frequency distribution, according to the results of the nonparametric K-S test, both distributions in the subsamples at depth 0-25 and 25-50 cm were reliably approximated to normal frequency distributions with a probability level of 95%.

Table 2. Pearson Correlation analisys - groupped mean for depth 0-25 cm

Correlations[a]

		P_2O_5 [mg/100 g]	OZ	OX	OY
Pearson Correlation	**P_2O_5 [mg/100 g]**	1.000	0.623	0.579	0.660
	OZ	0.623	1.000	0.875	0.940
	OX	0.579	0.875	1.000	0.859
	OY	0.660	0.940	0.859	1.000
Sig. (1-tailed)	**P_2O_5 [mg/100 g]**	.	0.009	0.015	0.005
	OZ	0.009	.	0.000	0.000
	OX	0.015	0.000	.	0.000
	OY	0.005	0.000	0.000	.
N	**P_2O_5 [mg/100 g]**	14	14	14	14
	OZ	14	14	14	14
	OX	14	14	14	14
	OY	14	14	14	14

a. Weighted Least Squares Regression - Weighted by Group

Table 3. Pearson Correlation analisys - ungroupped mean for depth 0-25 cm

Correlations

		P_2O_5 [mg/100 g]	OZ	OX	OY
Pearson Correlation	**P_2O_5 [mg/100 g]**	1.000	.348	.354	.487
	OZ	0.348	1.000	0.866	0.886
	OX	0.354	0.866	1.000	0.812
	OY	0.487	0.886	0.812	1.000
Sig. (1-tailed)	**P_2O_5 [mg/100 g]**	.	0.111	0.107	0.039
	OZ	0.111	.	0.000	0.000
	OX	0.107	0.000	.	0.000
	OY	0.039	0.000	0.000	.
N	**P_2O_5 [mg/100 g]**	14	14	14	14
	OZ	14	14	14	14
	OX	14	14	14	14
	OY	14	14	14	14

Table 4. Pearson Correlation analisys - groupped mean for depth 25-50 cm

Correlations[a]

		P_2O_5 [mg/100 g]	OZ	OX	OY
Pearson Correlation	**P_2O_5 [mg/100 g]**	1.000	0.465	0.289	0.415
	OZ	0.465	1.000	0.940	0.970
	OX	0.289	0.940	1.000	0.940
	OY	0.415	0.970	0.940	1.000
Sig. (1-tailed)	**P_2O_5 [mg/100 g]**	.	0.035	0.139	0.055
	OZ	0.035	.	0.000	0.000
	OX	0.139	0.000	.	0.000
	OY	0.055	0.000	0.000	.
N	**P_2O_5 [mg/100 g]**	16	16	16	16
	OZ	16	16	16	16
	OX	16	16	16	16
	OY	16	16	16	16

a. Weighted Least Squares Regression - Weighted by Group

Table 5. Pearson Correlation analisys - ungroupped mean for depth 25-50 cm

Correlations

		P_2O_5 [mg/100 g]	OZ	OX	OY
Pearson Correlation	**P_2O_5 [mg/100 g]**	1.000	0.509	0.355	0.460
	OZ	0.509	1.000	0.931	0.939
	OX	0.355	0.931	1.000	0.923
	OY	0.460	0.939	0.923	1.000
Sig. (1-tailed)	**P_2O_5 [mg/100 g]**	.	0.022	0.088	0.037
	OZ	0.022	.	0.000	0.000
	OX	0.088	0.000	.	0.000
	OY	0.037	0.000	0.000	.
N	**P_2O_5 [mg/100 g]**	16	16	16	16
	OZ	16	16	16	16
	OX	16	16	16	16
	OY	16	16	16	16

Dependence of P_2O_5 content on the location of the sampling points was determined by stepwise linear regression, in which the meter coordinates by OH (East-West), OU (North-South) and OZ (height) were successively entered as steps of the regression analysis (Cressie, 1993).
Measurements of the linear square grid as a result of the design of the mercator were neglected due to the small area of the object (Yost et al., 1982).
The statistical scattering of the sampling points along the OX axis was 1021 m, and along the OU-2867 m axis.

The Pearson correlation coefficients are shown in Tables 2-5. The complex influence of the location of the sampling point on the value of the studied indicator was studied with the help of variograms (Oliver & Webster 1986; Webster & Oliver, 1992), as the direction of the axis of the variogram coincides with the direction of the soil catena on the three axes.
The resultant direction of the soil catena was 150° in azimuth and it was set on the variograms of the studied P_2O_5. The results for the surface and subsoil horizons are shown in Figure 4 and Figure 5.

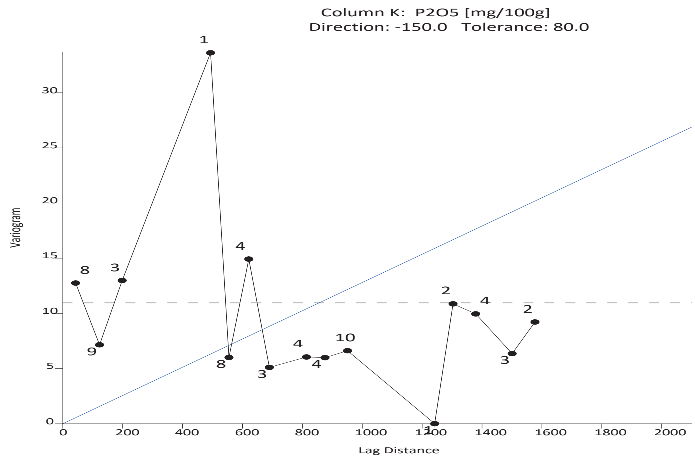

Figure 4. Variogram of the phosphorus content in the surface horizon

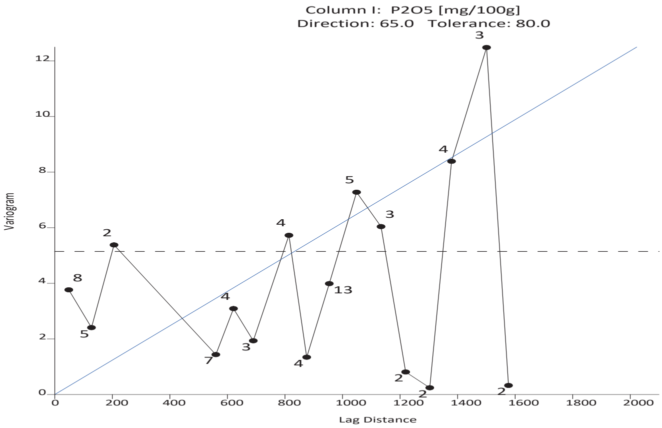

Figure 5. Variogram of the phosphorus content in the subsoil horizon

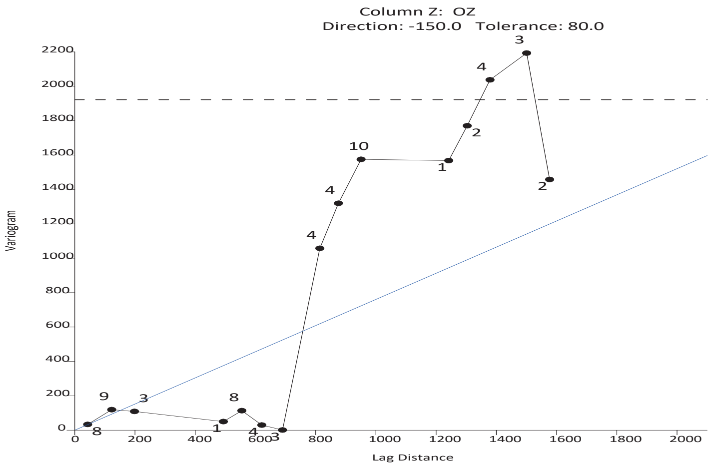

Figure 6. Variogram of the relief from the sampling points

Figure 6 shows the variogram of the relief, as along the Y axis is plotted the altitude at the sampling points and on average for the cluster groups. The increase of the variogram was interpreted as Gaussian, but with the increased of the sampling points it was more probable to be approximated to linear, as within the limits of X-Y there was no equalization of the function.

The distribution of P_2O_5 content within the same X-Y limits also showed a significant dispersion, but in all its other characteristics differs from the distribution of the hyxometric levels. It was studied at two depths 0-25 and 25-50 cm. The only thing between the variograms for the distribution of P_2O_5 in the two studied depths was the length of the log. It was the same for both variograms, about 800 m and represented about 1/3 of the length of the long axis of the X-Y polygon, in which the studied soil catena fits. In their other characteristics, the variograms for the distribution of P_2O_5 differ. The variogram of Figure 4 for the distribution of P_2O_5 in the surface soil layer showed angiotropicity and a certain tendency for cyclical distribution, which however was not proven. It can be argued with the greatest certainty that the content of P_2O_5 in the surface layer did not depend on the location, but at the same time it changes strongly, probably due to other factors. In the deeper layer, regardless of the high dispersion, closer results were found at closer points at a total length of the logs up to 800 m. At a total log over 800 m the total dispersion increased, but some observations were not in accordance with this conclusion, saturation was not established. Probably, with the increase in the number of observations, the variogram for the deep soil layer will be able to be approximated as Gaussian and thus to be identified by type with a variogram showing the change of the hyxometric levels. The influence of the topographic factor within the soil catena was studied by multiple stepwise linear regression, as independent variables were introduced the height, the slope (in any direction expressed as əh/ə (x, y), at the point of sampling and exposure in degrees around the azimuth circle. Significance for the change in P_2O_5 content was only the height of the terrain from which the sample was taken, at a level of extrapolability to the limits of the soil catena. The data did not differ for the surface and the subsoil horizon. The results are presented in Table 6.

Table 6. Linear regressions for the change of phosphorus content depending on the height, slope and exposure in the surface and subsoil horizon

Model Summary[b,c]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics: R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	0.623[a]	0.388	0.337	5.0246	0.388	7.607	1	12	0.017	1.993

a. Predictors: (Constant), OZ
b. Dependent Variable: P_2O_5 [mg/100 g]
c. Weighted Least Squares Regression - Weighted by Group

ANOVA[a,b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	192.056	1	192.056	7.607	0.017[c]
	Residual	302.963	12	25.247		
	Total	495.019	13			

a. Dependent Variable: P_2O_5 [mg/100 g]
b. Weighted Least Squares Regression - Weighted by Group
c. Predictors: (Constant), OZ

CONCLUSIONS

Based on the study, the main conclusions that can be formulated were that the content of P_2O_5 in the surface layer did not depend on the location, but at the same time changes greatly under the influence of a number of other factors. As diagnostic in terms of the distribution of naturally assimilate phosphorus in the soil should be considered the deep subsoil horizon, in which the geological distribution of P_2O_5 was not affected by its biological dynamics. In order to build a reliable distribution model, soil samples must be taken in a geographical network at equal distances along the X-Y axis and taking into account the height of the sampling point.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

PRELIMINARY RESEARCH ON SOIL MICROFLORA AND MACROFAUNA IN THE EXPERIMENTAL FIELD MOARA DOMNEASCĂ

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Abstract

Microorganisms and insects that living in the soil are an important component of it. These components play an important role in supporting and growing plant communities. Microflora and macrofauna exercise processes that influence the physical-chemical, biological and agricultural characteristics of the soil. In order to determine the species of microorganisms per 1 g of soil by the Petri dish culture method on different culture medium: potato-glucose-agar (PGA), dichloran-rose bengal chloramphenicol (DRBC) agar and dichloran 18% glycerol (DG18) agar decimal dilutions were performed beforehand. The results were compared with those obtained by the soil washing method. Bacteria from the genus Pseudomonas, yeasts, fungi from the genera Penicillium, Fusarium, Rhizopus, Aspergillus and Sclerotinia and one species each of coleoptera and lepidoptera were detected along with Lumbricus terrestris.

Key words: *soil microorganisms, bacteria, fungi, culture medium, macrofauna.*

INTRODUCTION

Healthy soils are essential for sustainable agriculture. Agriculture has a recognized impact on soil microorganisms. Soils constitute the vital environment for numerous microorganisms. Microbial activity is very important to improve soil health (Toor and Adnan, 2020).

In the soil there are both microorganisms, which improve the state of soil fertility and contribute to the growth of plants, microorganisms called biofertilizers (nitrogen-fixing bacteria), as well as pathogenic microorganisms for plants that can infect plants through the roots. Ecosystem functioning is largely regulated by soil microbial dynamics (Kennedy and Smith, 1995).

Soil holds millions of microbes which takes part in the improvement of soil fertility and favors plant growth (Gougoulias et al., 2014).

Soil physical and chemical properties depend on quantity and quality of soil organic matter, pH and conditions of redox potential. All of these significantly influence the structure and dynamics of the soil microorganisms (Lombard et al., 2011).

Also, the composition of microorganisms is strongly influenced by various environmental factors, such as climate, soil properties and water (Lau and Lennon, 2012; Wipf et al., 2021; Cotuna et al., 2022).

There are many microorganisms that help plants grow, through which they cope with climate change and agricultural land degradation (Antoszewski et al., 2022).

Among bacteria: *Acinetobacter* sp. and *Pseudomonas putida* ensure to corn increased tolerance to Cu, enhanced chlorophyll content and increased Cu concentration in tissues through IAA (the phytohormone indole-3-acetic acid) synthesis, production of siderophores and solubilization of Cu and P (Rojas-Tapias et al., 2014); *Arthrobacter* sp., and *Bacillus megaterium* to tomato enhanced seed germination ratio, seedling length, and dry and fresh weight under salt stress (Fan et al., 2016); Also to tomatoes, *Pseudomonas putida* ensure increased plant height, stem diameter, radical volume, dry biomass, and fruit yield through production of IAA (Hernández-Montiel et al., 2017) and *Burkholderia tropica* increased yield through N-fixation and P solubilization (Bernabeu et al., 2015); *Azospirillum lipoferum* to wheat improved germination, plant growth, higher chlorophyll content, and improved membrane stability under salt stress; increased production of soluble protein, and sugars under salt stress;

Serratia proteamaculans, *Pseudomonas putida* and *Pseudomonas aeruginosa* reduction the effect of salt stress and ensures enhanced plant height, root length, and yield, and higher chlorophyll content through ACC (1-aminocyclopropane-1-carboxylate) deaminase (an immediate precursor of ethylene) production (Mazhar et al., 2015); *Streptomyces* sp. to alfalfa ensures protection against root-lesion nematode-*Pratylenchus penetrans* (Samac and Kinkel, 2001). Among fungus: *Alternaria solani* IA300 to sweet bell peppers ensure enhanced number of leaves, flowers, dry, and fresh weight (Mauricio-Castillo et al., 2020); *Aspergillus niger* 9-p to bean ensure increased biomass through production of IAA, ACC deaminase, siderophores, protease, amylase, pectinase, xylanase, and P solubilization (Galeano et al., 2021); *Aspergillus fumigatus* to soybean reduction the effect of salt stress and ensures enhanced biomass, leaf area, chlorophyll content, and photosynthetic rate, increased isoflavones, proline, SA (salicylic acid), and JA (jasmonic acid) content and lower ABA (abscisic acid) content through GAs (Gibberellins-diterpenoid phytohormones) production (Khan et al., 2011); Also to soybean, *Fusarium verticillioides* and *Humicola* sp. reduction the effect of salt stress and ensures increased shoot length, protein content, carotenoid, salicylic acid, and enhanced SOD (superoxide dismutase) activity, decreased ABA level and lipid peroxidation (Radhakrishnan et al., 2015); *Penicillium bilaii* to pea ensure increased root dry weight, length, and P content in the shoot (Vessey and Heisinger, 2011); *Penicillium* sp. to hop clover (*Medicago lupulina*), lentil and wheat ensure enhanced shoot growth and dry weight, and increased P uptake (Wakelin et al., 2007); *Trichoderma hamatum*, *T. harzianum* and *T. viride* to common freesia ensure accelerated flowering and enhanced development of lateral inflorescence shoots, increased K, Fe, Mn, and Zn uptake (Janowska et al., 2020); *Trichoderma viride* to rapeseed ensure enhanced biomass, lateral roots development, germination ratio and changes in microbial composition (Znajewska et al., 2018).

Insects make up the most numerous group of organisms on earth, around 66% of all animal species (Zhang, 2011).

Herbivorous insects damage 18% of world agricultural production (Losey and Vaughan, 2006).

Despite this damage less than 0.5 percentage of the total number of the known insect species are considered pests (Kim, 1993).

Although insects are mostly perceived as pests, they are the key components in diverse ecosystems. Of these, the coleoptera it contributes to the loosening of the soil, through the galleries created, and to the shredding of plant material; increase nitrogen, phosphorous and humus from the soil (Zală, 2015).

Insects larvae clean up dead plant matter and break it down for further decomposition by microbes (Jankielsohn, 2018).

MATERIALS AND METHODS

The soil samples were collected from experimental field Moara Domnească-Ilfov (44°29′33″N 26°15′20″E) in early November 2021 from a plot sown with winter wheat, which had maize as the preceding crop The experimental fields are located on preluvosol-reddish soft (reddish-brown) type soil.

The reddish preluvosol (according to the Romanian Soil Taxonomy System) is part of the luvisols class, which presents a mollic A horizon (Am) followed by an intermediate argic horizon (Bt) having colors with values above 3.5 (wet) at least on the faces of the structural aggregates, starting from the upper part and degree of saturation in bases (V) over 53% (Mihalache, 2006).

The climate in the Moara Domnească Farm area falls under the Köppen-Geiger classification system (Peel et al., 2007) in the formula D.f.a.x.

For the determination of microorganisms, were taken 5 samples from the cultivated plot (one from each of the 4 edges and one from the center), with the help of the agrochemical probe. At each point, a sample was taken from the depth of 0-20 cm. Samples were collected in sterile plastic bags. For the agrochemical characterization, 2 soil/parcel samples were taken at a depth of 0-20 cm.

The determination of the species of microorganisms per 1 g of soil was carried out by the culture method in Petri plates on different culture media: potato-glucose-agar

(PGA), dichloran-rose bengal chloramphenicol (DRBC) agar and dichloran 18% glycerol (DG18) agar. To determine the microorganisms species we used decimal dilutions 10^{-1} … 10^{-7} (Waksman, 1927 cited by Zală, 2021; Constantinescu, 1974, cited by Manole and Ciocoiu, 2011) (Figure 1).

Figure 1. Preparation of decimal dilutions 10^{-1} …10^{-7}

From the dilutions performed, seeding were made in Petri plates with a diameter of 7 mm (Figure 2.).

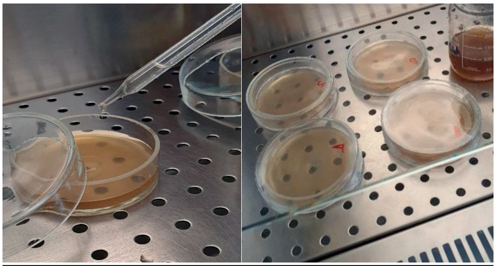

Figure 2. Inoculation of samples on PGA culture medium

Isolation of fungi from the soil was carried out by the soil washing method, which consisted of placing one gram of soil in an Erlenmayer beaker containing 200 ml of sterile water, shaking, tilting the flask at an angle of 45 degrees for one minute to settle the particles, it replaces the water, and the operation is repeated several times until the soil particles are dispersed in the sterile water and inoculated on the culture medium (Watson, 1960, cited by Constantinescu, 1974).
The simple microscopic preparation was performed with the help of a scalpel and consisted of detaching a small amount of the fruiting bodies formed on the surface of the culture media, placing it in the drop of water on the blade and lamella coating (Figure 3.).

Figure 3. Making the microscopic preparation from the white-fluffy area and the yeast area

The microscopic preparations were visualized with a Panthera S (Motic) microscope.
Soil pH was determined in a 1:2.5 aqueous suspension (Figure 4.), by the potentiometric method, with a Hanna pH-meter (Madjar et al., 2019).

Figure 4. Indication of the pH values of the soil samples

The pits for determining the numerical density of pests in the soil had a square shape, with a side of 0.5 m (0.5 x 0.5 = 0.25 sq m) and a depth of 20 cm. The equal distribution of the 5 gopis was in the form of a checkerboard. The soil from a pit was washed away and the insects were placed in a jar (Istrate and Roşca, 2009).

RESULTS AND DISCUSSIONS

On the potato-glucose-agar (PGA) culture medium, in the Petri dishes where samples were taken from the washed soil, more and more varied colonies of microorganisms developed (Figure 5.), unlike the dishes in which there were samples resulting from dilutions (Figure 6.).
The fungi were predominant in the first 20 cm of the soil.

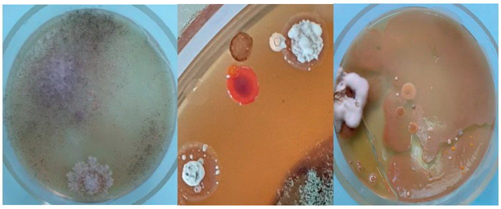

Figure 5. Highlighting the colonies of microorganisms on the PGA culture medium, in the Petri dishes in which samples were taken from the washed soil

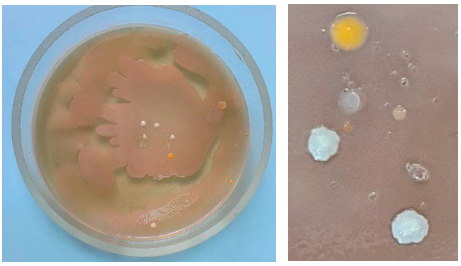

Figure 6. Highlighting the colonies of microorganisms on the PGA culture medium, in the Petri dishes in which the samples resulting from the dilutions were taken

It is known that the optimal pH values for the growth and development of fungi are: 4.5-6.5. (Child et al., 1973).

We identified fungi from 3 genera: *Rhizopus* (rarefied cotton-like mycelium-to be observed in Figure 5., the Petri dish on the left; and the presence of sporangiophores - Figure 7, left), *Fusarium* (fungal colony, white and dense - to be observed in Figure 3., the Petri dish on the left side; and the presence of mycelial hyphae, micro- and macroconidia - Figure 7, right) and *Penicillium* (dense greenish mycelial mass - to be observed in Figure 5, in the right corner of the central Petri dish).

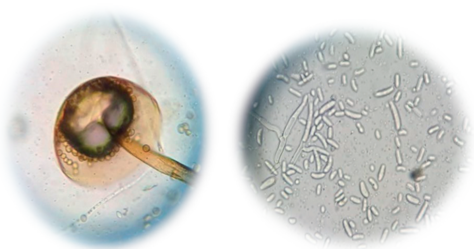

Figure 7. *Rhizopus* spp: sporangiophore, sporangium and sporangiospores (left); *Fusarium* spp: mycelial hyphae, micro- and macroconidia (right) (40x)

On PGA (Figure 3, the Petri dish on the right side), DRBC and DG18 (Figure 8) culture mediums colonies of yeasts of the genus *Saccharomyces* are flat, smooth, moist, shiny and cream-colored.

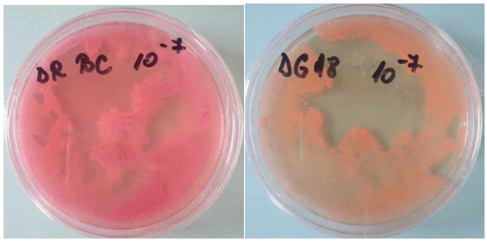

Figure 8. Colonies of *Saccharomyces* spp.

On DRBC and DG18 culture media in the Petri dishes with samples from the washed soil numerous colonies of some species of fungi from the genera *Fusarium* and *Aspergillus* have developed (Figure 9.). As stated by Jackson (1975) cited by Rangaswami and Bagyaraj (2005) the fungi of these genera are part of the mycotic microorganisms most frequently found in the soil.

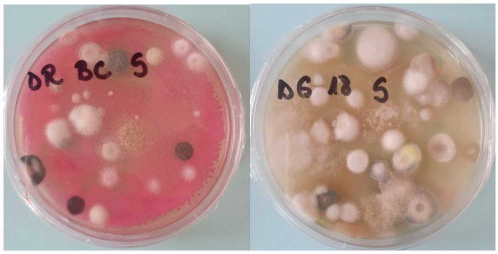

Figure 9. Colonies of *Fusarium* and *Aspergillus* spp.

The presence of bacteria on the PGA culture medium (Figures 5 and 6) was evidenced by the appearance of pigmented colonies in yellow: from the genus *Xanthomonas* and orange-red: from the genus *Corynebacterium* and white: from the genus *Streptomyces* (Schaad et al., 2001).

Regarding the number of colonies grown on the three culture media, in the case of the readings of the dishes with washed soil (Table 1), we note that *Fusarium* spp. recorded the highest number of colonies (13.5) on the DG18 culture media; *Aspergillus* spp. had the highest number of colonies (5.75) on DRBC culture medium; fungi of the genera *Rhizopus* and *Penicillium*, as well as bacteria of the genera *Xanthomonas* and *Corynebacterium* were reported only on the CGA culture medium. Yeasts of the genus

Saccharomyces and bacteria of the genus *Streptomyces* were reported on all three culture media; *Streptomyces* being found in greater numbers (2.5) on the DRBC culture medium. Regarding the number of colonies, in the case of the readings of the dishes with 10^{-7} dilutions, we found the presence of species from the genera *Fusarium* and *Aspergillus* only on the PGA culture medium, while *Saccharomyces* spp. was reported in all culture media.

Table 1. Colony count obtained from wash (W) soil or 10^{-7} dilutions/culture medium

Species	Culture medium					
	PGA		DRBC		DG18	
	W	10^{-7}	W	10^{-7}	W	10^{-7}
Fusarium spp.	5.25	1.25	11.5	-	13.5	-
Aspergillus spp.	2.25	0.5	5.75	-	5.5	-
Penicillium spp.	3.75	-	-	-	-	-
Rhizopus spp.	6.25	-	-	-	-	-
Saccharomyces spp.	+	+	+	+	+	+
Xanthomonas spp.	1.75	-	-	-	-	-
Corynebacterium spp.	1.25	-	-	-	-	-
Streptomyces spp.	1.75	-	2.5	-	0.75	-

Were also detected 2 sclerotia of the *Sclerotinia sclerotiorum* fungus.

After carrying out the five soil surveys, 19 specimens of the common frame-*Lumbricus terrestris* were also detected. They had an average length of 20.7 cm and an average number of 141 segments.

The insect species detected in the 20 cm deep soil layer were: black maize beetle (*Pentodon idiota*) and corn earworm (*Helicoverpa armigera*). Regarding the numerical density of the *Pentodon idiota* species, we identified 6 larvae/m^2 and 1.6 adults/m^2. Regarding the species *Helicoverpa armigera*, we detected a number of 3.2 pupae/m^2. The captured insect species were detected in the Moara Domnească fields and in the context of other research (Roșca and Istrate, 2004).

CONCLUSIONS

The soil is populated by various groups of living organisms. Among them, fungi and bacteria belong to the soil microflora.

The surface layer of the soil contains a large number of microorganisms, because it is well supplied with oxygen and nutrients.

The growth of microorganisms is influenced by the culture medium,

the largest number of species (8) being recorded on the PGA culture medium. Only 4 species develop on DRBC and DG 18 culture media.

Only two species of insects have been detected, a coleoptera: black maize beetle, whose adults feed on stems right at the surface of the soil while the larvae feed on the underground portions of the plant, including the roots; and a lepidopteran: the corn earworm, whose larvae feed on the silk and grains in the milk-wax phase.

ACKNOWLEDGEMENTS

This article was financed by the Faculty of Agriculture, University of Agronomic Sciences and Veterinary Medicine of Bucharest.

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CROP SCIENCES

Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

PRODUCTIVITY OF SOME ROMANIAN POTATO VARIETIES IN THE AGROCLIMATIC CONTEXT OF THE BÂRSA COUNTRY

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Abstract

A highly economic important crop, potato is grown in more than 100 countries and is the fourth consumed food in the world. Twelve Romanian potato varieties were investigated for their growth parameters and yield to determine suitability for production. Experiments were conducted to National Institute of Research and Development for Potato and Sugar Beet Brasov using a randomised block design with four replications. Determination of the tuber number and their mass was done in each repetition. The potato yield was determined in each elementary plot and the yield per hectare was calculated. Darilena produced the tallest plants (90.5 cm) and Castrum. produced the shortest (58.88 cm). The number of stems per hill ranged from 2.5 cm (Foresta cv.) to 9.25 cm (Marvis). Tuber weight average per hill ranged from 2055 g (Sarmis) to 500 g (Castrum). Ervant (37.44 t/ha) and Azaria (37.94 t/ha) records superior productivity and Asinaria and Darilena showed adaptability to climate conditions and suitability for culture under high economic efficiency. Specific technologies must be established for each variety to reach the maximum potential in periods of stress that occur due to climatic variations.

***Key words**: climatic conditions, cultivars, growth parameters, potato, yield.*

INTRODUCTION

Potato is now the world's third most important food crop in terms of human consumption, after wheat and rice (FAOSTAT, 2019) despite the large proportion of potato produce used for seed and as animal feed. Consumption of fresh potatoes accounts for approximately two-thirds of the harvest and around 1.3 billion people eat potatoes as a staple food (more than 50 kg per person per year) including regions of India and China.

A large majority (73.1%) of the EU's harvested production of potatoes in 2020 came from just five Member States; these were Belgium, Germany, France, the Netherlands and Poland. These five countries accounted for a slightly smaller majority (66.8% in 2020) of the area planted to potatoes in the EU, with Romania accounting for an additional 10.0% (EUROSTAT, 2022).

Potato produces the highest amount of energy per unit area and has the highest dry matter yield which may be 74.5% compared to wheat and 58% compared to rice (Ahmed et al., 2017). Besides consumption of fresh potatoes for cooking (boiled, baked, fried, etc.), potato tubers are used in a wide spectrum of applications, like alcohol production, dehydrated and frozen food products, animal feed, commercial starch (Devaux et al., 2021).

None of the currently used varieties or cultivars has potential for production in all environments and for all uses (Bradshaw, 2007), since agroecologies vary with respect to soil type, moisture and temperature regimes, fertility condition and the onset, intensity and duration of rain as well as availability of irrigation facilities (Gebremedhin et al., 2008; Fantaw et al., 2018).

The most desired type of cultivar is one that combines high yield with stability in a dynamic (or agronomic) sense (Piepho 1996; Flis et al., 2014).

Potato has high climate requirements. The availability and amount of water at specific growing stages effect the potato quality and yield. Potato is a water-stress crop, and a long-term lack of water is the main abiotic factor that limits yield (Cantorea et al., 2014). Water deficit decreases number of leaves, stem height, tuber growth and yield per plant. Also high temperature, drought, soil salinity and nutrient stresses adversely affect assimilation and translocation of the photosynthetic end product to the tubers and substantially curtail plant growth, tuberization, tuber bulking, and hence tuber yield and quality (Minhas, 2012; Dahal et al., 2019).

Average tuber weight, stems/plant, tubers/plant and tuber weight/plant are the most important components in potato improvement for increasing tuber yield (Islam et al., 2002; Arslan, 2007).

The aim of present the study was to investigate the performance and stability of some Romanian potato varieties regarding the yield in years with different climatic conditions.

MATERIALS AND METHODS

The experiment was conducted at the Laboratory of Technology and good agricultural practices from National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania, in years 2020-2021. The soil was a cambic chernozeum with pH 6.7. The pre-crop was wheat and for current fertilizer was used 1000 kg/ha N:P:K:15:15:15+S. The size of the plots was 9 m^2, the repetition was four-fold, the planting scheme was 75 cm × 30 cm, having 4 rows with 10 plants each one. Potatoes were planted manually on 6 April 2020 and 3 May 2021.

Twelve potato cultivars were analyzed: Asinaria, Azaria, Cezarina, Castrum, Foresta, Darilena Ervant, Marvis, Sarmis, Sevastia, Christian and Brasovia (control).

Were applied pre- and post-emergent treatments for weeds control, two treatments for Colorado beetle and eight treatments for late blight control in each year. Were collected data regarding the quantitative traits (plant height, haulms, aerial plant weight, number and tubers weight). Potato tubers were harvested semi-mechanized on September 20, 2020 and, respectively, October 4, 2021.

Weather conditions during the experiment are summarized in Figures 1 and 2. Rainfalls from growing season totaled 463.8 mm in 2020 with more 6.4 mm than the multiannual average and 429.7 mm in 2021 with 27.7 mm below multiannual average, respectively.

Temperatures were over multiannual average in both growing season, +1.0°C in 2020 and +0.9°C, respectively.

Results were subjected to statistical analysis, using one-way analysis of variance (ANOVA). Statistical differences with P-values under 0.05 were considered significant and means were compared by Duncan Multiple Range.

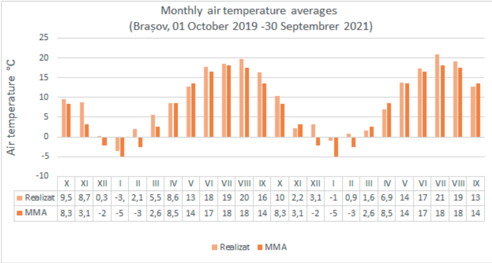

Figure 1. Temperature average
(Brașov, October 2019- September 2021)

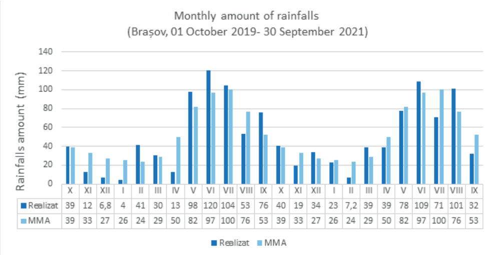

Figure 2. Rainfalls amount
(Brașov, October 2019- September 2021)

RESULTS AND DISCUSSIONS

Main haulm

Its obviously that the variety affect the number of haulms/plant. An increase in absorption of solar radiation can ensure a higher photosynthesis potential and promote synthesis and accumulation of reserve carbohydrates in the potato tuber which has a positive effect on the final tuber yield (White et al., 2007). But under specific climatic conditions the haulms number vary greatly between varieties and in the same variety in different developmental stages.

The control cultivar (Brasovia) presented a relatively high number of haulms in both years taken in the study and was significantly surpassed by the Marvis cv. and significantly distinct from the Christian cv at the first assessment of the year 2020.

Table 1. Main haulm number in different cultivars (2020-2021)

Variety	25.06.2020		30.07.2020		06.06.2021		26.07.2021	
	Main haulm no.	Sign.	Main haulm no.	Sign.	Main haulm no.	Sign.	Main haulm no.	Sign.
Sevastia	6.00	ns	3.13	ooo	4.13	oo	4.63	ns
Marvis	9.25	*	5.75	o	8.13	ns	7.63	ns
Castrum	3.38	oo	3.63	ooo	3.50	ooo	3.13	o
Asinaria	5.38	ns	7.63	ns	5.13	o	4.75	ns
Sarmis	8.25	ns	7.00	ns	6.75	ns	6.00	ns
Cezarina	4.50	ns	4.88	oo	4.88	o	4.13	ns
Christian	10.50	**	6.25	ns	5.75	ns	6.25	Ns
Ervant	4.25	o	5.25	oo	3.50	ooo	3.75	O
Azaria	5.13	ns	5.50	o	5.00	o	5.88	Ns
Foresta	2.50	ooo	2.88	ooo	2.25	ooo	2.00	ooo
Darilena	4.75	ns	4.25	ooo	3.13	ooo	4.88	Ns
Brasovia (control)	6.63	-	7.63	-	6.88	-	6.00	-

In the year 2021, the values were much tighter, even existing the situation some negative differences compared to the control at first assessment for Foresta, Darilena, Ervant and Castrum cultivars (Table 1).

Plant height

Its generally know that the variety affected plant heights.

In 2020 the highest plant height was observed to Darilena cv. (90.50 cm at the first assessment, respectively 109.50 cm at the second assessment) and the lowest to Castrum (58.99 at the first assessment, respectively 59.38 cm at the second assessment) and in 2021 to Ervant cv. (69.00 cm at the first assessment, respectively 73.63 cm at the second assessment) riched the heighest plants compared with the control cultivar.

In 2021 Ervant cv. continue to present the highest plant to the both assessment (69.0 cm, respectively 73.63 cm) (Table 2).

Table 2. Plant height in different cultivars (2020-2021)

Cultivar	Plant height (cm)	Sign.	Plant height (cm)	Sign.	Plant height (cm)	Sign.	Plant height (cm)	Sign.
	25.06.2020		30.07.2020		06.06.2021		26.07.2021	
Sevastia	73.63	o	76.50	ns	54.88	ns	64.00	*
Marvis	69.25	oo	61.38	ooo	52.25	ns	51.88	ns
Castrum	58.88	ooo	59.38	ooo	43.63	O	48.13	ns
Asinaria	75.88	ns	73.13	Oo	56.50	ns	65.25	*
Sarmis	90.25	ns	103.88	*	64.75	**	63.63	*
Cezarina	75.50	ns	91.25	os	55.00	ns	60.25	ns
Christian	79.75	ns	73.50	o	66.88	**	71.25	**
Ervant	66.00	oo	62.38	ooo	69.00	***	73.63	***
Azaria	80.88	ns	95.25	ns	61.75	ns	63.63	*
Foresta	71.88	o	77.75	ns	52.75	ns	56.38	ns
Darilena	90.50	ns	109.50	**	57.88	ns	62.38	ns
Brasovia (control)	84.25	-	89.63	-	53.75	-	51.00	-

Aerial plant weight

The development of the canopy, studied through the green mass of the aerial part of the potato plants, shows that it was influenced by the climatic conditions. The green mass of the potato plants varied a lot in the two years. In 2020, at the first assessment, a maximum of 991 g/plant was recorded for the Sarmis cv. and a minimum of 304.13 g for the Castrum cv., and a decrease in foliage for all varieties at the second assessment, the highest values being for the Sarmis cv. (680.38 g), and the lowest in the Ervant cv. (98.63 g).In 2021, the recorded values were lower than in 2020 for all varieties. At the first assessment, a maximum of 507.88 g/plant was recorded in the Christian cv. and a minimum of 213.63 g in the Castrum cv. and a decrease in foliage in all varieties at the second assessment, the highest values being in the Asinaria cv. (413.13 g), and the lowest in the Marvis cv. (249.50 g).

Brasovia (control) cv. presented a significant decrease in values at the second assessment (413.38 g) in 2020 compared to the first, when the plants were very well developed, a situation that was not repeated in 2021 when the values were within the limits determined by climatic conditions (Table 3).

Table 3. Aerial plant weight in different cultivars (2020-2021)

Cultivar	25.06.2020		30.07.2020		06.06.2021		26.07.2021	
	Aerial plant weight (g)	Sign.	Aerial plant weight (g)	Sign.	Aerial plant weight (g)	Sign.	Aerial plant weight (g)	Sign.
Sevastia	508.75	o	288.13	ns	265.38	ns	280.88	ns
Marvis	529.63	o	237.25	o	335.25	ns	249.50	ns
Castrum	304.13	ooo	244.63	o	213.63	o	323.38	o
Asinaria	432.88	oo	226.50	oo	301.63	ns	413.13	ns
Sarmis	991.25	ns	680.38	***	414,00	ns	312.63	ns
Cezarina	492.13	o	492.75	ns	321.50	ns	309.00	ns
Christian	903.25	ns	169.13	ooo	507.88	*	364.88	ns
Ervant	398.38	oo	98.63	ooo	346.75	ns	291.13	o
Azaria	650.88	ns	526.88	ns	430.88	ns	276.50	ns
Foresta	603.75	ns	340.50	ns	204.50	o	283.50	ooo
Darilena	858.75	ns	450.38	ns	270.63	ns	340.25	ns
Brasovia (control)	852.38	-	413.38	-	364.13	-	271.25	-

Cultivars differed in the duration the canopy was maintained and had infuence on tuber number and weight, and finally on the yield.

Tuber number

Tuber number per plant ranged from 5.63 tubers (cv. Castrum) to 24.75 tubers (cv. Christian) to the first assesment and from 6.88 tubers (cv. Castrum) to 22.75 tubers (cv. Ervant) to the second assesment in 2020 and from 7.13 tubers (cv. Foresta) to 17.25 tubers (cv. Christian) to the first assesment and from 7.25 tubers (cv. Castrum) to 16.75 tubers (cv. Marvis) to the second assesment in 2021 (Table 4).

Table 4. Tuber number in different cultivars (2020-2021)

Cultivar	25.06.2020		30.07.20		06.06.21		26.07.21	
	Tub. no./hill	Sign.	Tub. no./hill	Sign.	Tub. no./hill	Sign.	Tub. no./hill	Sign.
Sevastia	7.13	oo	10.38	Oo	10.50	ns	13.50	ns
Marvis	11.88	ns	15.00	Ns	19.38	ns	16.75	ns
Castrum	5.63	ooo	6.88	Ooo	7.75	o	7.25	oo
Asinaria	12.13	ns	18.50	ns	12.13	ns	16.38	ns
Sarmis	11.38	ns	16.63	ns	12.50	ns	10.63	ns
Cezarina	12.00	ns	22.75	ns	17.13	ns	14.63	ns
Christian	24.75	***	18.63	ns	17.25	ns	16.38	ns
Ervant	11.00	ns	16.13	ns	15.25	ns	13.88	ns
Azaria	11.75	ns	16.50	ns	14.63	ns	13.63	ns
Foresta	8.38	o	11.38	oo	7.13	o	7.25	oo
Darilena	9.38	o	13.50	o	8.25	o	12.75	ns
Brasovia (control)	13.63	-	20.75	-	14.63	-	14.25	-

The capping on the number of tubers in a plant cannot be explained by the lack of genetic tuberization capacity, but by the existence of physiological imbalances, nutritional or hormonal, under the direct influence of the ecological conditions of a year.

A gradual decrease in tuber number was observed with a gradual increase of water stress. As other authors have found, the number of tubers is significantly influenced by drought (Deblonde and Ladent, 2001; Al-Mahmoud et al., 2014).

Tuber weight

The weight of the tubers is in accordance with their number. At the first assessment in 2020, the Sevastia cv. recorded the lowest weight (197.25 g), and cv. Sarmis (795.88 g) the biggest one.

At the second assessment cv. Sarmis (2055.13 g) continued to record the highest weight, with a distinctly significant difference compared to the control, while cv. Castrum (513.00 g) presented the lowest weight. In 2021, for all varieties, the weight of tubers was lower than in 2020.

Climatic conditions negatively influenced the degree of accumulation of tubers, only cv. Ervant (9385.25 g, respectively 789.88 g) had at both assessments (distinctly significant difference) weight greater than the Brasovia cv. (204.38 g, respectively 436.25 g) (Table 5).

Table 5. Tuber weight in different cultivars (2020-2021)

Cultivar	25.06.2020		30.07.2020		06.06.2021		26.07.2021	
	Tub. weight/hill	Sign.	Tub. weight/hill	Sign.	Tub. weight/hill	Sign.	Tub. weight/hill	Sign.
Sevastia	197.25	o	788.50	oo	99.25	ns	476.63	ns
Marvis	460.50	ns	1289.75	ns	274.63	ns	524.25	ns
Castrum	180.50	oo	500.13	ooo	95.25	o	259.88	ns
Asinaria	438.38	ns	982.00	ns	118.13	ns	613.75	ns
Sarmis	795.88	ns	2055.13	**	283.88	ns	567.50	ns
Cezarina	554.88	ns	1340.75	ns	193.00	ns	482.88	ns
Christian	762.00	ns	1009.25	ns	265.88	ns	671.00	*
Ervant	756.75	ns	1259.75	ns	385.25	**	789.88	**
Azaria	584.38	ns	1440.38	ns	272.75	ns	455.88	ns
Foresta	465.50	ns	877.38	o	78.00	o	399.50	ns
Darilena	486.25	ns	1158.50	ns	110.88	ns	600.25	ns
Brasovia Mt	544.75	-	1382.88	-	204.38	-	436.25	-

Yield

Understanding the stress-related physiological, biochemical, and molecular processes is crucial to develop the screening procedures for selecting potato cultivars that can better adapt to drought. The elucidation of such processes may offer new insights into the identification of specific characteristics that may be useful in breeding new cultivars aimed at maintaining or even enhancing potato yield under the changing climate (Gervais et al., 2021).

The productions obtained in 2020 were very good. They were between 37.06 t/ha for the Castrum cv. and over 61 t/ha for the Darilena and Sevastia cultivars. In 2021, production was between 37.97 t/ha for the Azaria cv. and 13.11 t/ha for the Foresta cv. The control cultivar (Brasovia) presented relatively high productions in both years (43.49 t/ha, respectively 30.09 t/ha), being stable under the influence of adverse climatic conditions (Table 6).

We have to mention a significant decrease in the production of all cultivars in 2021 compared to the previous year. The drought at the time of tuberization and the uneven distribution of precipitation caused the accumulation to be deficient, none of the varieties being able to reach their maximum potential.

Table 6. Total tuber yields (Braşov, 2020-2021)

Cultivar	Total yield (t/ha)	Dif. (t/ha)	Sign.	Total yield (t/ha)	Dif. (t/ha)	Sign.	Mean yield of the two years
	2020			2021			
Ervant	59.20	15.71	**	37.44	7.34	ns	48.32
Castrum	37.06	-6.43	Ns	19.57	-10.53	o	28.32
Marvis	54.05	10.56	Ns	29.87	-0.22	ns	41.96
Azaria	60.66	17.17	**	37.97	7.88	ns	49.32
Christian	58.59	15.10	**	30.86	0.77	ns	44.73
Asinaria	44.40	0.91	Ns	29.10	-0.99	ns	36.75
Foresta	55.92	12.43	*	13.11	-16.99	ooo	34.52
Sevastia	61.12	17.63	**	25.90	-4.19	ns	43.51
Darilena	61.13	17.64	**	28.38	-1.71	ns	44.76
Cezarina	59.73	16.24	**	24.04	-6.05	ns	41.89
Sarmis	53.68	10.19	Ns	32.35	2.26	ns	43.02
Brasovia (control)	43.49	-	-	30.09	-	-	36.79
DL 5%	10.82	-	-	8.42	-	-	
DL 1%	14.50	-	-	11.28	-	-	
DL 0.1%	19.14	-	-	14.89	-	-	

Among the varieties, the mean yield of the two years was the highest in Azaria cv. (49.32 tons) which was followed by Ervant cv. (48.32 tons) while Catrum cv. produced the lowest (28.32 tons).

CONCLUSIONS

The tolerant varieties showed comparatively less reduction in plant height, plant mass, tuber number per plant and yield. The lowest reduction in tuber yield was found in Ervant and Azaria cv. followed by Sarmis cv. The mean production was higher in 2020.

The number of tubers and haulm to tubers weight ratio turned out to be less sensitive to changes in the growing regime.

Yield per ha showed significant variation and ranged from 37.06 ton/ha (Castrum cv.) to 61.13 tons/ha (Darilena cv.) in 2020 and from 37.97 tons/ha (Christian cv.) to 13.11 tons/ha (Foresta cv.) in 2021.

In both years Ervant (59.20 t/ha, respectively 37.44 t/ha) and Azaria (60.66 t/ha, respectively 37.94 t/ha) records superior productivity and Asinaria and Darilena showed adaptability to climate conditions and suitability for culture under high economic efficiency. Specific technologies must be established for each variety to reach the maximum potential in periods of stress that occur due to climatic variations.

The results of this field study confirm that weather conditions do influence potato canopy development and subsequent tuber yield. The weather conditions leads to the significant differences in the potato varieties productivity, indicated that higher temperatures lowered potato yields, less precipitation hindered the yields and growth of potato cultivation.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SUNFLOWER GENOTYPES IN FIELD INFESTED WITH BROOMRAPE IN BRAILA LOCATION, IN YEAR 2022

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Abstract

In Romania, sunflower represent the main oil crop and occupies the third place after wheat and maize. Orobanche cumana Wallr (sunflower broomrape) is a parasitic plant who infested sunflower plants and causes low seed yield. In this paper we present behaviour of sunflower genotypes in field natural infested with broomrape, in Braila location, in year 2022. We tested for resistance/tolerance at parasite Orobanche cumana, 21 experimental sunflower hybrids, belonging to NARDI Fundulea and 10 differential genotypes and one additional for determination of broomrape races. Degree attack (GA) of broomrape was between 0.05 at sunflower hybrid H1 and 6.2 at H10 and infested sunflower plants (I %) was between 5% at H1 and 100% at H3 and from H6 to H21. All sunflower differential genotypes was infested with broomrape from 25% at KA41 (gene Or_1) to 100% at AD66 (no Or gene), Record (gene Or_3), S1358, LC 1002 (gene Or_4), P1380, LC 1003 (gene Or_5), P96 (gene Or_6, Or_7). Only one sunflower genotype (additional differential genotype), Neagra de Cluj (accession PI 650368), was resistant at broomrape race H_{RO} or I_{RO}.

Key words: *broomrape, sunflower, race, gene Or, resistance/tolerance.*

INTRODUCTION

The most aggressive populations of sunflower broomrape with high virulence (*Orobanche cumana* Wallr) is located in Turkey, Ukraine, Romania (Braila, Tulcea) and Republic of Moldova (Clapco, 2021). In Romania, in the counties Braila, Tulcea, Constanta is present the most virulent populations of broomrape, more than race H. (Škorić et al., 2020; Duca et al., 2022). In more countries such as Romania, Ukraine, Turkey, Republic of Moldova, Bulgaria, Spain, China, Rusia, race G and H of broomrape was reported (Duca et al., 2022; Antonova et al., 2022, Ryzhenko et al., 2021; Onisan et al., 2018) and race E of broomrape in Serbia (Cvejić et al., 2020).

Sources of broomrape resistance is represented by wild sunflower species, open pollinated varieties, gene pool of cultivated sunflower (Clapco et al., 2020; Cvejić et al., 2020; Kaya, 2022; Seiler, 2019; Seiler et al., 2017).

In Romania in last years (Table 1), area cultivated with sunflower was over one million hectares (Radu et al., 2019; Georgescu et al., 2022; Manole et al., 2022).

Table 1. Area cultivated with sunflower in Romania in years 2015-2020

Year	Area cultivated with sunflowers (ha)	Average seed yield (kg/ha)	Total production (t)
2015	1.011.527	1.765	1.758.771
2016	1.039.823	1.955	2.032.340
2017	998.415	2.917	2.912.743
2018	1.006.994	3.041	3.062.690
2019	1.282.697	2.783	3.569.150
2020	1.170.372	1.883	2.204.312

Source: https://www.madr.ro/culturi-de-camp/plante-tehnice/floarea-soarelui.html

Orobanche cumana Wallr can be controlled by herbicide Pulsar 40 (active substance imazamox 40 g/l) in system Clearfield and herbicide Pulsar Plus (active substance imazamox 25 g/l) in system Clearfield Plus (Škorić et al., 2020; Manole et al., 2018; 2019).

MATERIALS AND METHODS

In field natural infested with broomrape, in Braila location, in year 2022, we tested for resistance/tolerance at parasite *Orobanche cumana*, 21 experimental sunflower hybrids belonging to NARDI Fundulea, from which 15 derived from interspecific hybridization with wild sunflower species (from H1 until H15), one sunflower hybrid in system Sun Express (H16E), one sunflower hybrid in system Clearfield (H17CL), four sunflower hybrids in system Clerfield Plus (from H18CLP until H21CLP) and 10 differential genotypes and one additional for determination of broomrape races.

Interspecific hybridization between cultivated sunflower (lines B, lines with cytoplasmic androsterility, fertility maintainer and C lines, fertility restorer lines) and wild sunflower species (annual and perennial) was made in year 2014 in Fundulea at NARDI Fundulea.

Hybridizations between mother lines (A- sterile line) and father lines (C- restorer line) was made in year 2021 in Fundulea.

Sunflower hybrids H16E (with resistance at sulfonilureea herbicide-Express 50SG), H17CL (with resistance at imidazolinone herbicide-Pulsar 40), H18CLP, H19CLP, H20CLP, H21CLP (with resistance at imidazolinone herbicide-Pulsar Plus) were not treated with herbicide to observe the genetic resistance to the *Orobanche cumana* parasite. The sunflower genotypes were sown in micro plots (9.8 m^2), on two rows, 7 meters long, in three repetition/genotype, in Braila on April 22, 2022 and harvested on September 20, 2022 and sown in Fundulea, on April 21, 2022 and harvested on September 29, 2022 (Table 2).

Table 2. Sunflower genotypes tested for resistance/tolerance at broomrape, in year 2022, in Braila, in field infested natural with parasite *Orobanche cumana* Wallr and in Fundulea in no infested field

No.	Sunflower genotype
H1	(**Line A1** x *Helianthus maximiliani P14Max)* x (**Line C1** x *Helianthus maximiliani P14Max)*
H2	(**Line A1** x *Helianthus maximiliani P14Max*) x (**Line C2** x *Helianthus maximiliani P3Max*)
H3	(**Line A1** x *Helianthus maximiliani P14Max*) x (**Line C3** x *Helianthus maximiliani P14Max*)
H4	(**Line A1** x *Helianthus maximiliani P14Max*) x (**Line C4** x *Helianthus molis P9Mo*)
H5	(**Line A1** x *Helianthus maximiliani P14Max*) x (**Line C5** x *Helianthus maximiliani P3Max*)
H6	(**Line A1** x *Helianthus maximiliani P14Max*) x (**Line C6**x *Helianthus molis P9Mo*)
H7	(**Line A1** x *Helianthus maximiliani P14Max*) x (**Line C7x** *Helianthus molis P9Mo*)
H8	(**Line A1** x *Helianthus maximiliani P14Max)* x (**Line C8** x *Helianthus maximiliani P14Max)*
H9	(**Line A2** x *Helianthus maximiliani P5Max x Helianthus neglectus A1Ne x Helianthus argophyllus A13Arg*) x (Line C9 x *Helianthus divaricatus P12Div*)
H10	(**Line A2** x *Helianthus maximiliani P5Max x Helianthus neglectus A1Ne x Helianthus argophyllus A13Arg*) x (**Line C10** x *Helianthus petiolaris A7Pe A x Helianthus praecox A12Pr*)
H11	(**Line A2** x *Helianthus maximiliani P5Max x Helianthus neglectus A1Ne x Helianthus argophyllus A13Arg*) x (**Line C11** x *Helianthus maximiliani P14Max*)
H12	(**Line A2** x *Helianthus maximiliani P5Max x Helianthus neglectus A1Ne x Helianthus argophyllus A13Arg*) x (**Line C12** x *Helianthus maximiliani P14Max*)
H13	(Line A3 x *Helianthus argophyllus A13Arg*) x (**Line C13** x *Helianthus argophyllus A13Arg*)
H14	(**Line A4** x *Helianthus argophyllus A13Arg*) x (**Line C14** x *Helianthus argophyllus A13Arg*)
H15	(**Line A5** x *Helianthus argophyllus A13Arg*) x (**Line C15** x *Helianthus argophyllus A13Arg x Helianthus maximiliani P3Max*)
H16E	**Line A6E**x **Line C16E**
H17CL	**Line A7CL** x **Line C17CL**
H18CLP	**Line A8CLP** x **Line C18CLP**
H19CLP	**Line A9 CLP** x **Line C19CLP**
H20CLP	**Line A10CLP** x **Line C20CLP**
H21CLP	**Line A11CLP** x **Line C21CLP**

In flowering time of sunflower, we make notations about broomrape attack. For identification of broomrape race present in Braila, in year 2022, we used 10 differential genotypes and Neagra de Cluj (Table 3), an old Romanian sunflower variety (Bran and Ion, 2018).

Table 3. Differential genotypes used for identification of broomrape races, present in Braila, in year 2022, in field infested natural with parasite *Orobanche cumana* Wallr

Sunflower genotype	Gene *Or*	Differential genotype for broomrape race
KA-411 (Pl 251902)*	Or_1	A
J 8281 (Pl 265100)*	Or_2	B
Record (Pl650343)*	Or_3	C
LC1002	Or_4	D
S1358	Or_4	D
LC1003	Or_5	E
P1380	Or_5	E
LC1093	Or_6	F
P96 (Pl 633614)*	$Or_{6,7}$	F,G
0305CxP9Mo	$or_{Mo}or_{Mo}$	G+
AD66	No *Or* gene	
Neagra de Cluj (Pl 650368)*	*Or????*	

*provided from gene bank North Central Regional Plant Introduction Station (NCRPIS), part of United States National Plant Germplasm System (NPGS)

RESULTS AND DISCUSSIONS

All sunflower differential genotypes was infested with broomrape from 25% at KA41 (gene Or_1) to 100% at AD66 (no *Or* gene), Record (gene Or_3), S1358, LC 1002 (gene Or_4), P1380, LC 1003 (gene Or_5), P96 (gene Or_6, Or_7). Only one sunflower genotype (additional differential genotype), Neagra de Cluj (accession PI 650368) was resistant at broomrape race H_{RO}, I_{RO} or more (Table 4, Figures 1 and 2).

Table 4. Observations regarding behaviour of differential genotypes in Braila, in year 2022, in field infested natural with parasite *Orobanche cumana* Wallr

Sunflower genotype	Number of broomrape/ number of sunflower plants	Infested sunflower plants with broomrape (I %)	Degree of attack (GA) with broomrape (average number of broomrape stalks per sunflower plant)
KA-41	3/12	25	0.25
J 8281	7/12	58.3	0.58
Record	66/33	100	1.93
LC1002	89/34	100	2.61
S1358	65/20	100	3.25
LC1003	47/41	100	1.14
P1380	66/11	100	6
LC1093	7/26	26.9	0.26
P96	8/5	100	1.6
0305CxP9Mo	26/29	89.65	0.89
AD66	40/25	100	1.6
Neagra de Cluj	**0/27**	**0**	**0**

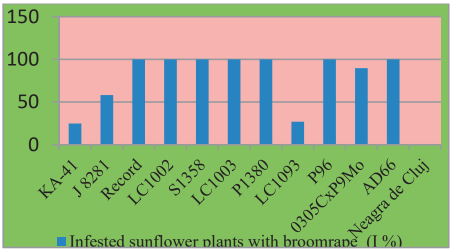

Figure 1. Infested differential sunflower genotypes with broomrape (I %) in Braila, in year 2022

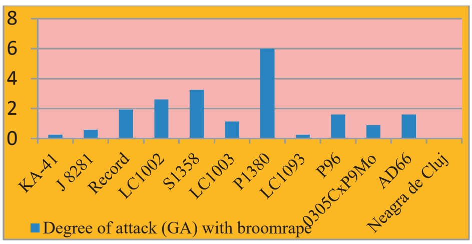

Figure 2. Degree of attack with broomrape on differential sunflower genotypes in Braila, in year 2022

In Braila location, in year 2022, is present broomrape races A, B, C, D, E, F, G, H, I or more. Degree attack (GA) of broomrape was between 0.05 at sunflower experimental hybrid H1 and 6.2 at H10 and infested sunflower plants (I %) was between 5% at H1 and 100% at H3 and from H6 to H21CLP (Table 5, Figures 3 and 4).

Table 5. Observations regarding behaviour of sunflower experimental hybrids at broomrape, in Braila, in year 2022, in field infested natural with parasite *Orobanche cumana* Wallr

Sunflower genotype	Number of broomrape/number of sunflower plants	Infested sunflower plants with broomrape (I %)	Degree of attack (GA) with broomrape (average number of broomrape stalks per sunflower plant)
H1	2/40	5	0.05
H2	113/26	100	4.3
H3	285/371	76.8	0.76
H4	74/118	62.7	0.62
H5	26/48	54.1	0.54
H6	583/123	100	4.7
H7	151/33	100	4.5
H8	166/40	100	4.1
H9	178/67	100	2.6
H10	443/71	100	6.2
H11	245/68	100	3.6
H12	169/79	100	2.1
H13	213/97	100	2.1
H14	108/20	100	5.4
H15	315/85	100	3.7
H16E	256/101	100	2.5
H17CL	178/70	100	2.5
H18CLP	246/41	100	6
H19CLP	284/47	100	6
H20CLP	205/53	100	3.8
H21CLP	150/49	100	3

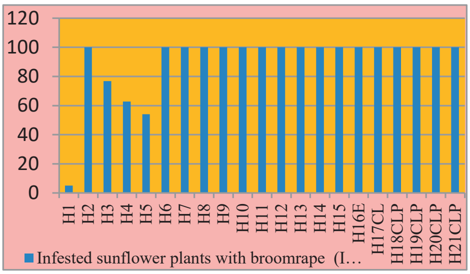

Figure 3. Infested sunflower experimental hybrids with broomrape (I %) in Braila, in year 2022

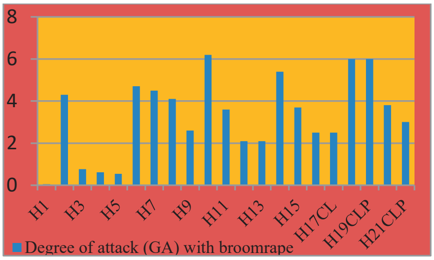

Figure 4. Degree of attack with broomrape on sunflower experimental hybrids in Braila, in year 2022

From all sunflower experimental hybrids tested in Braila, in year 2022, H1 was more tolerant\resistant then other at parasite broomrape and perennial sunflower specie *Helianthus maximiliani P14Max* is in mother line (sunflower line A) and father line (sunflower line C).

In Fundulea in year 2022 with total rainfalls of 258.4 mm, was the most driest year of last 60 years (average on 60 years, 584.3 mm), with a high temperature in every month than average on 60 years (excepting month March). In Braila was registered a total rainfalls of 238 mm (Table 6, Figures 5 and 6).

Table 6. Temperature and rainfalls registered, in Braila and Fundulea, in year 2022

Temperature (°C)			
Locality area / Month	Braila	Fundulea	Average of 60 years (Fundulea)
January	1.3	2.1	-2.4
February	4.1	4.7	-0.4
March	3.8	4.4	4.9
April	11.9	12.1	11.3
May	18	17.9	17
June	22.7	22.6	20.8
July	24.8	25	22.7
August	24.9	25.6	22.3
September	17.9	18.6	17.5
October	13	13.5	11.3
November	8.1	9	5.4
December	2.9	3.5	0
Rainfalls (mm)			
Locality area / Month	Braila	Fundulea	Average of 60 years (Fundulea)
January	6.5	4.8	35.1
February	11.1	5.4	32
March	13.8	12.3	37.4
April	25.1	47.6	45.1
May	24.3	30.1	62.5
June	33.3	59.6	74.9
July	8.9	29.2	71.1
August	26.9	14.4	49.7
September	31.8	35.4	48.5
October	6.1	5.2	42.3
November	30.7	19.6	42
December	19.9	21.8	43.7
Total (mm)	238.4	285.4	584.3

In month July, 2022, in the phenophase of seed filling, in Braila rainfalls registered was 8.9 mm and in Fundulea was 29.2 mm (average rainfalls of 60 years in Fundulea was 1.1 mm).

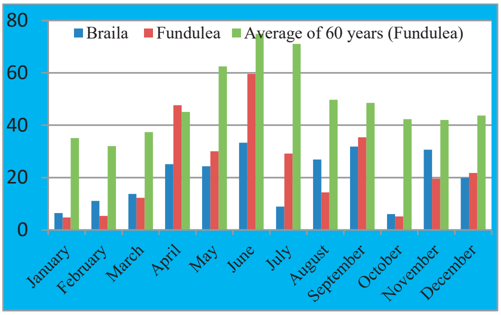

Figure 5. Rainfall (mm) registered in Braila and Fundulea, in year 2022

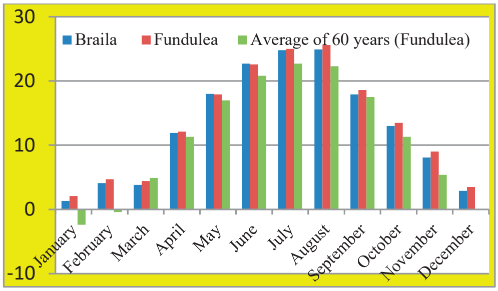

Figure 6. Temperature (°C) registered in Braila and Fundulea, in year 2022

Hectolitre weight, was between 33 kg/hl at sunflower experimental hybrid H3 and 47 kg/hl at sunflower experimental hybrid H15 (Table 7).

One thousand weight (TSW), was between 34 g at sunflower experimental hybrids H2, H11 and 55 g at sunflower experimental hybrid H15 (Table 7).

In Braila, in year 2022, seed yield (kg/ha) was between 357 kg/ha at sunflower experimental hybrid H17CL and 2684 Kg at sunflower experimental hybrid H18CLP (Table 7).

In Braila in field infested with the most aggressive broomrape races, in year 2022, the best seed yield was registered at sunflower experimental hybrids H18CLP (2684 kg/ha) with and H19CLP (2242 kg/ha) although the attack degree was 6 and the percentage of infested plants with parasite *Orobanche cumana* was 100%.

Table 7. Agronomic traits of sunflower experimental hybrids, in Braila, in year 2022, in field infested natural with parasite *Orobanche cumana* Wallr

Sunflower genotype	Hectolitre weight (kg/hl)	TSW (one thousand weight) (g)	Seed yield (kg/ha)
H1	41	45	978
H2	42	34	944
H3	39	41	1374
H4	42	43	1608
H5	43	51	1180
H6	43	48	1530
H7	43	43	962
H8	44	44	1690
H9	43	44	684
H10	41	40	596
H11	39	34	681
H12	38	35	1200
H13	42	42	859
H14	43	44	489
H15	47	55	1259
H16E	37	44	553
H17CL	41	39	357
H18CLP	45	43	**2684**
H19CLP	46	41	2242
H20CLP	45	39	1871
H21CLP	45	38	1595

In Fundulea, in field without broomrape, in year 2022, seed yield (kg/ha) was between 1487 kg/ha at sunflower experimental hybrid H10 and 4053 kg/ha at sunflower experimental hybrid H6 (Table 8). Hectolitre weight, was between 51 kg/hl at sunflower experimental hybrid H9 and 67 kg/hl at sunflower experimental hybrid H12 (Table7). One thousand weight (TSW), was between 34 g at sunflower experimental hybrids H2, H11 and 67 g at sunflower experimental hybrid H6 (Table 7).

Table 8. Agronomic traits of sunflower experimental hybrids, in Fundulea, in year 2022, in field no infested with parasite *Orobanche cumana* Wallr

No.	Average hectolitre weight (kg/hl)	Average TSW (one thousand weight) (g)	Average seed yield (kg/ha)
H1	60	56	3818
H2	54	45	2693
H3	57	48	2198
H4	66	49	2400
H5	66	59	3474
H6	60	67	**4053**
H7	61	56	3084
H8	61	53	2443
H9	51	49	1675
H10	55	54	1487
H11	66	52	1783
H12	67	49	2686
H13	58	53	2385
H14	64	59	3139
H15	55	57	2234
H16E	62	66	4030
H17CL	52	51	3054
H18CLP	55	49	2691
H19CLP	64	56	2695
H20CLP	58	56	3312
H21CLP	61	53	2829

Low rainfalls in phenophase of seed filling, combined with the attack of the parasite *Orobanche cumana* in Braila, led to lower seed yields of experimental sunflower hybrids then in Fundulea where is no infested field with broomrape (Figure 7). Dunăreanu and Radu (2020) reported that yield and yield components were affected by water stress.

Interaction between genotype and environment (G x E), is visible in Figure 7 at all sunflower hybrids tested in booth areas and only sunflower hybrid H18CLP is stabile regarding seed yield.

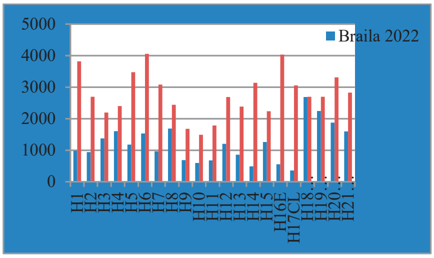

Figure 7. Average seed yield (kg/ha) of experimental sunflower hybrids, in Braila and Fundulea, in year 2022

Average hectolitre weight (kg/hl), of sunflower experimental hybrid, was lower in Braila then in Fundulea, in year 2022 (Figure 8).

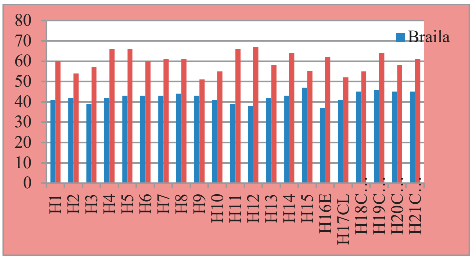

Figure 8. Average hectolitre weight (kg/hl) of experimental sunflower hybrids, in Braila and Fundulea, in year 2022

Average one thousand weight (TSW), was of sunflower experimental hybrid, was lower in Braila then in Fundulea, in year 2022 (Figure 9).

According to Borleanu and Bonea (2020), hectolitre weight was negatively and significantly associated with thousand seed weight.

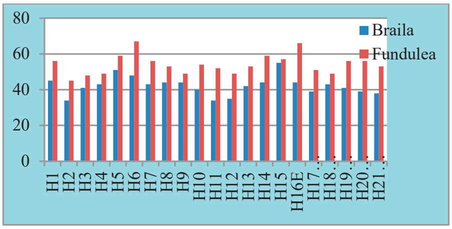

Figure 9. Average one thousand weight (TSW), of experimental sunflower hybrids, in Braila and Fundulea, in year 2022

CONCLUSIONS

In Fundulea, 2022 was the driest agricultural year from past years and this allow us to see behaviour of sunflower genotypes in these conditions.

One thousand weight (TSW) and hectolitre weight (kg/hl), influenced seed yield of sunflower genotypes tested in booth area together with presence/absence of parasite *Orobanche cumana* and lack of precipitation during the seed filling period.

In Braila, in year 2022, we identified a new source of resistance at broomrape at races G, H, I or more and this is old Romanian variety named Neagra de Cluj.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INFLUENCE OF $CaCO_3$ WORDINGS BY NP DOSES ON SOYBEAN YIELDS

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Abstract

Following recent research, the positive influence of calcium on soybean plants, especially on varieties with improved genetic qualities, was found. The results obtained on the plants were significant both on the total biomass of the plants and its components. In our ecological conditions with stagnant white luvic soil (pH = 5.0-5.5) different types of $CaCO_3$ were used to primarily improve the chemistry of the plant culture medium. In the case of the variety Isa TD (group 00), the interaction between $CaCO_3$ and NP fertilizers contributed to a maximum increase of 5.48 t/ha total biomass, 3.44 t/ha pod biomass and 1.47 t/ha of biomass grains. Of the two factors, the new formulations of $CaCO_3$ had the greatest influence, and the interaction with NP was in all cases negative. The correlations obtained between the studied characters were significantly positive, except for the mass of one thousand grains (MTG). The obtained results invite the promotion of soybean crop technology, including the amendment system regarding this type of soil.

Key words: *acid soils, biomass, Ca^{2+}, Isa TD, NP.*

INTRODUCTION

Soybean [*Glycine max*. (L) Merrill, pro syn. *G. angustifolia* Miq., *G. gracilis* Skvort., *G. hispida* (Moench) Maxim] is one of the most important oil plants worldwide (Corke & Wrigley, 2004). Being considered one of the most important sources of vegetable protein, for the prevention and combating of malnutrition, it is aimed to increase the production of soybean crop by up to 2.4%, until the year 2050 (Cramer et al., 2009; Martinez-Ballesta et al., 2010). The plant offers several major advantages in sustainable cropping systems including the ability to fix atmospheric nitrogen (N_2) and therefore mitigate the need to apply large amounts of nitrogen fertilizers. Due to the fact that it is part of the category of leguminous plants, soybean contributes to improving the level of soil fertility (Martinez-Ballesta et al., 2010). The Isa TD soybean variety belongs to the early maturity group (00), has an erect habit, compact bush, determined growth, gray pubescence, purple flowers and gray hilum color. In the first year of cultivation, the morphological characteristics of the variety were as follows: waist 71 cm, number of branches 3-4, number of pods 70, number of grains 111, and mass of one thousand grains (MTG) 180-220 g. The vegetation period was 161 days.

Calcium (Ca) is an essential macronutrient for plants, having the role of improving soil properties and various physiological processes in plants (White & Broadley, 2003; Hepler & Winship, 2010). The amounts of Ca present in the stagnant white luvic soil are insufficient for plant growth. Therefore, it does not show enough potential to maintain the right degrees of base saturation of the soil colloids. In these conditions, the exchangeable aluminum ions Al^{3+} dominate the exchange sites of the clay, contributing to the excessive acidity of the soil. Mobile (soluble) aluminum becomes toxic to the most of plants. Due to the lack of calcium and the excess presence of aluminum, the soil becomes strongly acidic, an environment in which plant development is reduced, which leads to the impairment of production (Milivojevic & Stojanovic, 2003). By applying $CaCO_3$, the following properties are improved: structure, permeability to water and air, development of microorganisms and intake as fertilizer in plant food. Calcium absorbed as Ca^{2+} ions is an essential element for fruit growth and development. The resistance of plants to diseases is also of particular importance (Villegas-Torres et al., 2007) due to the way in which the cell wall is protected.

The roles of Ca in plants are as follows:
- helps the growth and development of plants;
- confers resistance to diseases by protecting the cell wall;
- activation of some enzyme systems;
- stabilizes the membrane and cell integrity (White & Broadley, 2003).

Ca^{2+} is absorbed from the soil solution by the root system and translocated to the shoots through the xylem. The root must balance calcium input to the xylem (White, 2001) to help individual root cells use $[Ca^{2+}]_{cyt}$ for intracellular signaling. At the level of growth tips and leaves, where photosynthesis takes place, Ca activates either alone or together with Mg^{2+} and other chemical elements, such as: Ca, $n.10^{-1}$ in the form of Ca^{2+}; Mg, $n.10^{2}$, in the form of Mg^{2+} and Mg ATP^{2-}; K, 1.6, as K^{+}; Na, $n.10^{-2}$, in the form of Na^{+}; Cl, $n.10^{-2}$, in the form of Cl^{-}. Due to this fact, the leaves of soybean plants have an intense green color. The way calcium enters the plant (Cosgrove, 2005; Franceschi & Nakata, 2005) is passive with the flow of water and other nutrients. Its circulation through woody vessels is favored by plant evapotranspiration (ETP) and root pressure (Holdaway-Clarke & Heppler, 2003; Dayod et al., 2010). Being a macroelement totally different from the others, the permanent application of Ca ions is necessary. In this sense, different formulations of calcium carbonate were used, with or without the addition of magnesium. As a result of the research carried out, the very good effect of the application of amendments and doses of chemical fertilizers was found.

MATERIALS AND METHODS

The experience took place during the year 2022 and was located in the experimental field of the Fertilizer Laboratory, from S.C.D.A. Soybean variety Isa TD (00) was cultivated in non-irrigated culture. The placement was done in a subdivided plot with 3 repetitions each, and the biological material was sown mechanically, in rows 10 m long, 3 m wide, so that each variant had a size of 30 m². Between the rows the distance was 50 cm. The experience is bifactorial and has the following graduations:

-Factor A: -a_1: unamended (0 t/ha);
- a_2: Agrocalcium powder (2.5 t/ha), 93.6% $CaCO_3$;
- a_3: Doloflor powder (2.5 t/ha), 58.87% $CaCO_3$ and 38.24% MgO;
- a_4: Doloflor granules (2.5 t/ha), 58.87% $CaCO_3$ and 38.24% MgO;
- a_5: Neutrosol 9 powder (2.5 t/ha), 97.5% $CaCO_3$;

-Factor B: - b_1: unfertilized;
- b_2: $N_{40}P_{40}$;
- b_3: $N_{80}P_{80}$.

Table 1. The proper soil characteristics

Soil characteristics	Stagnic white luvic soil	
	Values	Interpretation
Humus, %	2.26	low fertility
Total nitrogen-tN, %	0.130	poorly supplied
P_2O_5, ppm	41.68	poorly supplied
K_2O, ppm	89.00	medium supplied
pH	5.12	high acidity

The technology used was the one recommended by the station. The soil in which the experimental field was located is a stagnant white luvic soil, acid, with a low humus content, being a weakly fertile soil, little supplied with nitrogen, phosphorus and moderately supplied with potassium, in accessible forms to the plants (Table 1). Variants harvesting were done manually with the metric frame in all 3 repetitions. The samples were kept for several days in the laboratory at room temperature to bring them to the same humidity required for the determinations. Soybean samples were analyzed for: total biomass, pod biomass, grain biomass and mass of 1000 grains (MTG). The obtained data were processed statistically by the variance analysis method (anova test) and by studying the correlations expressed by excel graphs. Correlations included the correlation coefficient of determination (D%) and correlation coefficient (r) values. The obtained values were compared with the transgression probabilities for the thresholds of 5%, 1% and 0.1%.

RESULTS AND DISCUSSIONS

The influence of climatic factors on soybean products. From a climatic point of view, A.R.D.S. Pitesti is located in an area with a specific climate. Temperatures and precipitations were recorded from May to September 2022 to track their influence on soybean plants. The climatic conditions during the experiment period (Table 2) were differentiated both in terms of temperatures, but especially in the amount of precipitation recorded from one month to another. During the first period of vegetation, the temperatures were close to normal, after which, during the summer, the recorded values were 2-3ºC higher.

The temperatures exceeded the multiannual values recorded by 1.4ºC compared to normal (N) one. During the soybean vegetation period, 1538.8ºC was accumulated, active temperatures (Σtn >10ºC).

Regarding the precipitation regime, it was found that the values in May, July and September were close to normal.

Table 2. Evolution of climatic factors in soybean vegetation

Month	Temperatures, tn^0C				Precipitations, mm			ETP**
	N*	2022	±	$\Sigma tn^0 > 10^0C$	N*	2022	±	mm
May	16.3	17.1	0.8	1538.8	81	77	-4	33
Jun.	19.5	21.6	2.1		94	14	-80	74
Jul.	21.7	23.8	2.1		81	71	-10	141
Aug.	21.3	23.7	2.4		60	105	45	176
Sep.	16.9	16.5	-0.4	Veg. Per.	53	46	-7	66
±	19.14	20.54	1.4	138 zile	369	313	-56	490

Although 56 mm less was recorded, nevertheless the volume of rains that fell mostly covered the needs of the plants. In the month of June, the precipitation was well below the normal limit, and in the month of August, the amount of precipitation recorded exceeded the normal by 45 mm. The additional rains in the second summer month led to the extension of the vegetation period of the plants. Making a comparison between the plant's need at an optimal level expressed by potential evapotranspiration (ETP) and the climatic factors recorded in the Isa TD variety, a deficit of it is found at a sufficiently high level.

Soybean productions obtained within the experiment. Regarding the variants without chemical fertilizers (Table 3), the productions obtained according to the new calcium formulations were between 3.51 t/ha in the control variant and 7.67 t/ha on the Neutrosol 9 amendment.

Table 3. The total soybean biomass formation (t/ha)

Ca/NP	$N_0 P_0$	$N_{40} P_{40}$	$N_{80} P_{80}$	Factors influnce
Check	3.51	4.43	5.59	Maximum growth, 5.48 t/ha, 100%
A-Ca powder	4.29	5.14	8.10	
D-Ca powder	6.37	4.56	6.62	Ca 4.16 t/ha, 76%
D-Ca granule	6.31	8.99	7.06	
N-Ca powder	7.67	7.24	5.70	NP 2.08 t/ha, 37%
	Ca	*NP*	*Ca x NP*	
DL 5 % = *DL 1 % =* *DL 0.1 %=*	*1.03* *1.49* *2.24*	*1.60* *2.19* *2.96*	*3.45* *4.76* *6.56*	Ca x NP -0.76 t/ha, -13%

In the same conditions, in the variants that were fertilized with $N_{40}P_{40}$, the productions obtained were between 4.43 t/ha and 7.24 t/ha, and in the variant with $N_{80}P_{80}$, the levels were between 5.59 t/ha and 5.70 t/ha.

Considering the influences of the 2 factors, the maximum increase in biomass obtained was 5.48 t/ha (100%). Ca contributed to this with 4.16 t/ha (76%), and NP chemical fertilizers brought an extra 2.08 t/ha (37%). Analyzing the interaction between the 2 factors, a negative increase of 0.76 t/ha (-13%) is found.

The causes of the negative interaction of total soybean biomass was due to a possible antagonistic interaction between cations (Table 3).

Table 4. The total pod biomass formation (t/ha)

Ca/NP	$N_0 P_0$	$N_{40} P_{40}$	$N_{80} P_{80}$	Factors influnce
Check	2.55	2.82	3.66	Maximum growth, 3.44 t/ha, 100%
A-Ca powder	2.90	3.37	5.21	
D-Ca powder	4.38	3.16	4.51	Ca 2.87 t/ha, 83%
D-Ca granule	4.43	5.99	4.62	
N-Ca powder	5.42	4.81	3.72	NP 1.11 t/ha, 32%
	Ca	*NP*	*Ca x NP*	
DL 5 % = *DL 1 % =* *DL 0.1 %=*	*1.01* *1.47* *2.21*	*0.66* *0.89* *1.21*	*0.91* *1.28* *1.84*	Ca x NP -0.54 t/ha, -15%

Taking into account the non-chemically fertilized variants (Table 4), the pod productions obtained according to the new calcium formulations were between 2.55 t/ha in the control variant and 5.42 t/ha on the agrofund with Neutrosol 9. In the variants which were fertilized with $N_{40}P_{40}$, the obtained yields were between 2.82 t/ha and 4.81 t/ha. In the same way, the productions obtained in the variants fertilized with $N_{80}P_{80}$, the yields were between 3.66 t/ha and 3.72 t/ha. The maximum increase obtained was 3.44 t/ha (100%), and Ca contributes with 2.87 t/ha (83%), and NP with 1.11 t/ha (32%). If we analyze the interaction between the 2 factors, there is also a negative increase of 0.54 t/ha (-15%). It is possible that this negative range is due to the same relative antagonism between calcium and phosphorus.

Table 5. The soybean grain biomass formation (t/ha)

Ca/NP	$N_0 P_0$	$N_{40} P_{40}$	$N_{80} P_{80}$	Factors influence
Check	1.23	1.25	1.53	Maximum growth, 1.47 t/ha, 100%
A-Ca powder	1.17	1.79	2.79	
D-Ca powder	2.10	1.47	2.13	Ca 1.41 t/ha, 96%
D-Ca granule	2.22	2.64	2.12	
N-Ca powder	2.58	2.20	1.73	NP 0.30 t/ha, 20%
	Ca	*NP*	*Ca x NP*	
DL 5 % = *DL 1 % =* *DL 0.1 %=*	*0.47* *0.68* *1.02*	*0.29* *0.39* *0.53*	*0.41* *0.56* *0.76*	Ca x NP -0.24 t/ha, -16%

The grain yields obtained in the non-chemically fertilized variants (Table 5), were between 1.23 t/ha in the control variant and 2.58 t/ha in the variant fertilized with Neutrosol 9. In the variants where fertilizers of the type were administered $N_{40}P_{40}$ and different types of amendments, the obtained productions were between 1.25 t/ha and 2.20 t/ha. The productions obtained in the variants fertilized with $N_{80}P_{80}$, depending on the new calcium formulations, were between 1.53 t/ha in the control variant and 2.79 t/ha in the variant fertilized with agrocalcium powder. The maximum increase resulted was 1.47 t/ha, Ca contributing 1.41 t/ha, and the NP fertilizer dose 0.30 t/ha, and in this case their interaction is negative: -0.24 t/ha.

In the case of the mass of one thousand grains (MTG) the interaction between the two factors was insignificantly positive at the level of 1 g (3%) (table 6). Under the same conditions, the MTG was between 185 and 216 g, resulting in a maximum increase of 31 g (100%). Calcium contributed 19 g (61%), and NP chemical fertilizer with 11 g (36%) (Table 6).

Table 6. Mass formation of a thousand grains - MTG (g)

Ca/NP	N_0 P_0	N_{40} P_{40}	N_{80} P_{80}	Factors influnce
Check	203	209	214	Maximum growth,
A-Ca powder	196	201	206	31 g, 100%
D-Ca powder	204	196	212	Ca
D-Ca granule	196	216	206	19 g, 61%
N-Ca powder	185	199	211	NP
	Ca	*NP*	*Ca x NP*	11 g, 36%
DL 5 % = *DL 1 % =* *DL 0.1 %=*	*15* *23* *34*	*15* *21* *28*	*27* *37* *52*	Ca x NP 1 g, 3%

Table 7. Scatter analysis of soybean yield formation

Cause of variability	Sq. total				LG	Variance, S^2				F test			
	d.w.	pods	grains	MTG		d.w.	pods	grains	MTG	d.w.	pods	grains	MTG
Rep.	4.80	2.31	0.55	907	2								
A Fact.	45.66	21.73	5.11	579	4	11.41	5.43	1.276	144.7	12.8*	6.26*	6.94*	0.70
A Er.	7.12	6.94	1.47	1648	8	0.89	0.868	0.184	206.0				
Big Fact.	57.58	30.98	7.13	3134	12								
B Fact.	7.31	1.36	0.382	1241	2	3.65	0.68	0.191	620.7	1.37	1.53	0.022	2.56
A x B	48.78	21.70	6.137	1015	8	6.10	2.71	0.767	126.9	2.30	6.09*	0.090	0.52
B Er.	53.14	8.90	170.5	4846	20	2.66	0.445	8.526	242.3				
Small Fact.	109.2	31.96	117.1	7102	30								
Total	166.8	62.94	184.2	10236	44								

In the case of A factor, through the dispersion analysis of the data (Table 7), it emerges that the differences are significantly positive in all determinations (total biomass, pod biomass and grain biomass). In the case of factor B, differences are recorded, but without significance, and in the case of interactions between the two factors, significant positive differences are recorded only in the case of pod biomass. The following figures (Figures 1-4) show images of the soybean crop, both in full vegetation and as a grain appearance.

Figure 2. The period of formation of pods

Figure 1. The variety Isa at the beginning of flowering

Figure 3. The Isa TD variety towards maturity

Figure 4. The grains of the Isa TD variety

The correlations between the production elements (biomass) are highly significant positive which means that the variety Isa TD expressed high values in the experiment (Table 8).
Among the two types of correlations, the determination values were between 67% and 98% regarding plant biomass and between 0.1% and 4.2% in the case of the mass of one thousand grains (MTG).

Table 8. Correlations between the main determinations of soybean plants, variety Isa

Indices	Total d.w.	Pod number	Pod d.w.	Grain number	Grain d.w.	MTG
Total d.w.	1	84.6* **.920****	98.1 **.970**	83.4 **.913**	90.8 **.953**	1.68 .130
Pod number		1	82.8 **.910**	66.3 **.814**	71.1 **.844**	2.39 .155
Pod d.w.			1	88.3 **.940**	92.2 **.960**	0.20 .045
Grain number				1	95.0 **.975**	**4.16 -.204**
Grain d.w.					1	0.10 -.032
MTG						1
	DL 5%= .190, DL 1%= .250, DL 0.1%= .320					

*Correlation coefficient of determination (D%), **correlation coefficient (r)

The obtained data demonstrate that this variety showed characteristics with a certain high degree of adaptability in the conditions at the station.
Correlations were obtained from the mean values from each repetition. The correlation between the number of grains and the number of pods is highly significant positive (Figure 5).
With regard to the technological elements used, the plant manages to produce as large a number of pods as possible and to the same extent a higher number of grains was formed.
The correlation between the number of grains and the MTG is significantly negative (Figure 6), which demonstrates that at a higher number of grains, the MTG keeps its relative uniformity even if it can decrease. This phenomenon is due to the existence of a genetic factor that is not very easily changed from a technological point of view.
From the analysis of the obtained data of the 4 types of biomass, new and valuable aspects of the Isa TD soybean variety are found (Table 9).
Considering the interaction between the two experimental factors, the total biomass varied between 3.51 t/ha in the control and 8.99 t/ha in the variant with doloflor granules + $N_{40}P_{40}$.

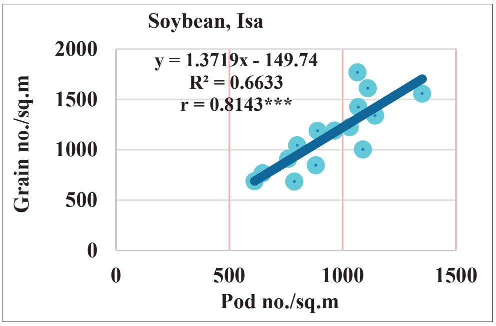

Figure 5. Correlation between pod no. x grain no./sq.m

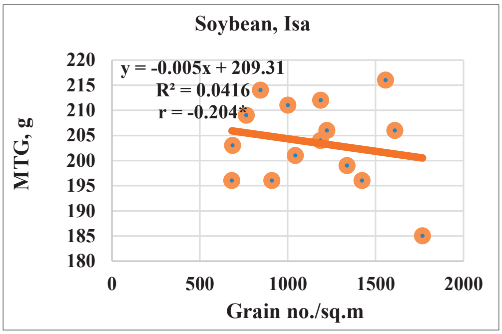

Figure 6. Correlation between grain no./sq.m x MTG

Very significant positive values were found in the variants with: agrocalcium powder + $N_{80}P_{80}$; doloflor granules + $N_{40}P_{40}$; doloflor granules + $N_{80}P_{80}$; neutrosol powder; neutrosol powder + $N_{40}P_{40}$. The pod biomass formed was between 2.55 t/ha in the control version and 5.99 t/ha in the version with doloflor granules + $N_{40}P_{40}$. On average, the biomass of the grains was 1.93 t/ha. Thus, from a percentage point of view, at the level of the whole experiment, the pods represented 67.22%, the grains 31.59%, and in the case of the MTG, the statistical difference was insignificant.

Table 9. Biomass structure of soybean plants, variety Isa TD, depending on the experimental factors

$CaCO_3$	NP	TOTAL d.w. t/ha	Pod d.w. t/ha	%/ total	Grain d.w. t/ha	%/ total	MTG g
0	N_0 P_0	3.51	2.55	72.65	1.23	35.04	203
	N_{40} P_{40}	4.43	2.82	80.06	1.25	28.22	209
	N_{80} P_{80}	5.59	**3.66**	65.47	1.53	27.37	214
A-Ca pw	N_0 P_0	4.29	2.90	67.60	1.17	27.27	196
	N_{40} P_{40}	5.14	3.37	65.56	**1.79**	34.82	201
	N_{80} P_{80}	**8.10**	**5.21**	64.32	**2.79**	34.44	206
D-Ca pw	N_0 P_0	6.37	**4.38**	68.76	**2.10**	32.97	204
	N_{40} P_{40}	4.56	3.16	69.30	1.47	32.24	196
	N_{80} P_{80}	6.62	**4.51**	68.13	**2.13**	32.18	212
D-Ca gr	N_0 P_0	6.31	**4.43**	70.21	**2.22**	35.18	196
	N_{40} P_{40}	**8.99**	**5.99**	66.63	**2.62**	29.14	216
	N_{80} P_{80}	**7.06**	**4.62**	65.44	**2.12**	30.03	206
N-Ca pw	N_0 P_0	**7.67**	**5.32**	70.66	**2.58**	33.64	185
	N_{40} P_{40}	**7.24**	**4.81**	66.44	**2.20**	30.39	199
	N_{80} P_{80}	5.70	**3.72**	65.26	**1.73**	30.35	211
	MEAN	6.10	4.10	67.22	1.93	31.59	203.60
DL 5 % DL 1 % DL 0.1%		3.454 4.759 6.558	0.909 1.282 1.837		0.409 0.557 0.756		26.7 37.1 52.1

CONCLUSIONS

1. The experiment consisted in improving soil acidity by promoting new formulations with different but high percentages of calcium. At the 3 agrofunds of chemical fertilizers of the NP type: 0 kg/ha, 40 kg/ha and 80 kg/ha per amendment fund, both a development of the root system and of the plant biomass was found.
2. The total biomass of the Isa TD soybean plant was 5.48 t/ha to which calcium contributed 76% and NP fertilizer 37%. Regarding pod biomass, the maximum increase was 3.44 t/ha, with the influence of Ca being 83% and NP 32%. In the case of grain biomass, a maximum increase of 1.47 t/ha was recorded, with Ca contributing 96% and NP 20%.
3. The causes of the negative interaction of the two factors, in all three determinations, was due to a possible interaction between the cations.
4. At the mean level, the MTG was 203.6 g with no significance due to the fact that this trait is genetically controlled and therefore less variable.
5. Through the dispersion analysis, significant aspects were demonstrated, which invites the promotion of new soybean varieties under the amendment conditions. The correlation obtained between the production elements: the number of pods/m^2 and the number of grains/m^2, the values show positive and significant links.
6. From the results obtained this year, the Isa TD variety reacted very favorably due to the improvement of both soil and plant physiology in the calcium element.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SELECTIVITY AND STABILITY OF SOME HERBICIDES FOR ANNUAL GRAMINACEOUS WEED CONTROL APPLIED DURING STEM ELONGATION STAGE OF DURUM WHEAT (*Triticum durum* Desf.)

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Abstract

The experiment was carried out during 2018-2020 in the experimental field of Field Crops Institute - Chirpan with durum wheat cultivar Progress (Triticum durum Desf.). The influence of 6 antigraminaceous herbicides was tested - Palace 75 WG (piroxulam) - 250 g/ha; Axial 050 EC (pinoxaden) - 900 ml/ha; Traxos 045 EC (pinoxaden + clodinafop) – 1.3 ml/ha; Topic 080 EC (clodinafop) - 450 ml/ha; Puma super 7.5 EB (phenoxyprop-ethyl) - 1 ml/ha; Hussar max WG (mesosulfuron + iodosulfuron) - 250 g/ha. Herbicides were applied at the beginning of stem elongation stage - during 1st, 2nd and 3rd stem node stage of durum wheat. The highest grain yield was obtained by treatment of herbicide Topic during 1st stem node stage of durum wheat, followed by herbicides Puma super, Axial and Hussar max. During 2nd stem node stage of durum wheat, the highest grain yield was obtained by treatment of herbicide Puma super, followed by herbicides Traxos, Axial and Topic. During 3rd stem node stage of durum wheat, the highest grain yield was obtained by treatment of herbicide Puma super, followed by herbicides Topic and Traxos. Technologically, the most valuable are herbicides Puma super, Hussar max, Traxos and Palace, applied during 1st stem node stage; herbicides Hussar max, Topic and Puma super applied during 2nd stem node stage and herbicides Hussar max, Puma super, Topic and Traxos applied during 3rd stem node stage.

Key words*: durum wheat, herbicides, selectivity, stability, grain yield.*

INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is a traditional material for pasta production and is indispensable in this respect compared to common wheat (*Triticum aestivum* L.).
An important factor that significantly affects the quantity and quality of durum wheat yield is weeds (Delchev, 2016; 2016a; 2017; 2018; 2018a; 2020). If they are not combated, the decrease in crop yields due to weed infestation is on average between 10% and 50%. A decisive role in this regard is done by the chemical method of weed control (Buczek et al., 2007; Nakayama et al., 2010; Gupta et al., 2011; Lobkov et al., 2012; Delchev, 2019; 2019a; 2021; 2022). This method has established itself as an important and fundamental unit in this control.
Chemical weed control in durum wheat is mainly carried out through tillering stage. Many studies have been conducted with herbicides, herbicide combinations and herbicide tank mixtures during this stage of crop development (Callens et al., 1996; Imanat, 2002; Campagna and Rueegg, 2006; Tityanov, 2009; Petit et al., 2010; Zhelyazkova et al., 2016). Some of them have been found to exhibit a phytotoxic effect on durum wheat grain yield (Kumar et al., 1997; Korres et al., 1999; Holm et al., 2000; Cacak-Pietrzak et al., 2008; Delchev and Georgiev, 2015; 2015a; Georgiev and Delchev, 2016).
Due to various reasons (meteorological, financial, organizational), chemical weed control may be delayed and it may be necessary to conduct it during stem elongation stage of durum wheat (Delchev, 2006). In the presence of secondary weed infestation, especially in seed crops, it is necessary to apply herbicides also during this later stage. There is insufficient data regarding the impact of herbicides applied late in the growing season of durum wheat on its productivity. Their influence on grain yield requires further investigation. This will lead to improvements in weed control in durum wheat, particularly secondary weed infestation in this crop.

The aim of the present study is to investigate the selectivity and stability of the actions of some antigraminaceous herbicides applied during the stem elongation stage of durum wheat.

MATERIALS AND METHODS

The experimental work was carried out during 2018-2020 under non-irrigated conditions of the leached vertisol soil type on the experimental field of Field Crops Institute - Chirpan. The experiment was carried out with durum wheat cultivar Progress (*Triticum durum* Desf.). It was carried out according to the block method (Dimova et al., 1999), in 4 repetitions with a harvest plot size of 15 m^2.

The influence of 6 antigraminaceous herbicides was tested. The active substances and doses of the studied herbicides are shown in Table 1. Due to their poor adhesion, the herbicides Palace and Hussar max were applied together with the adjuvants Dasoil - 500 ml/ha and Biopower - 250 ml/ha, respectively.

Table 1. Investigated variants

№	Herbicides	Active substances	Doses
1	Control	-	-
2	Palace 75 WG	piroxulam	250 g/ha
3	Axial 050 EC	pinoxaden	900 ml/ha
4	Traxos 045 EC	pinoxaden + clodinafop-propargyl	1.30 l/ha
5	Topic 080 EC	clodinafop-propargyl	450 ml/ha
6	Puma super 7.5 EB	fenoxaprop-ethyl	1 l/ha
7	Hussar max WG	mesosulfuron + iodosulfuron	250 g/ha

Herbicides were applied at the beginning of stem node stage - during 1^{st}, 2^{nd} and 3^{rd} stem node stage of durum wheat.

The selectivity of herbicides has been established through their influence on grain yield. The math processing of the data was done according to the method of analyses of variance (Shanin, 1977; Barov, 1982; Lidanski, 1988). The stability of herbicides for grain yield with relation to years was estimated using the stability variances σ_i^2 and S_i^2 of Shukla (1972), the ecovalence W_i of Wricke (1962) and the stability criterion YS_i of Kang (1993).

RESULTS AND DISCUSSIONS

The grain yields are a result of the combined effect of weather conditions over the years and the selectivity of the studied herbicides (Table 2). The highest grain yield on mean for the period is obtained by treatment with herbicide Topic during 1^{st} stem node stage of durum wheat - 4637 kg/ha, followed by herbicides Puma super - 4570 kg/ha, Axial - 4547 kg/ha and Hussar max - 4503 kg/ha. During 2^{nd} stem node stage of durum wheat, the highest grain yield is obtained by treatment with herbicide Puma super - 4470 kg/ha, followed by herbicides Traxos - 4200 kg/ha, Axial - 4157 kg/ha and Topic - 4157 kg/ha. During 3^{rd} stem node stage of durum wheat, the highest grain yield is obtained by treatment with herbicide Puma super - 4557 kg/ha, followed by the herbicides Topic - 4487 kg/ha and Traxos - 4353 kg/ha. The lowest grain yields are obtained with the herbicide Palace, especially by treatment during 3^{rd} stem node stage - 3787 kg/ha.

When using the herbicides Palace, Axial, Traxos, Topic, Puma super and Hussar max during 1^{st} stem node stage of durum wheat, the mean yield increase is 118.8% over the control. When using these herbicides during 2^{nd} stem node stage of durum wheat, the mean increase in yield is 112.8% over the control. When using the six herbicides during 3^{rd} stem node stage of durum wheat, the mean increase in yield was 114.7% over the control.

This means that by treatment during stem elongation stage, the selectivity of the herbicides in regard to durum wheat is highest during the 1^{st} stem node stage, followed by the 3^{rd} stem node stage. The selectivity is lowest by treatment during 2^{nd} stem node stage. This fact is important when, in the presence of secondary weed infestation, it is necessary to done a late treatment with herbicides of durum wheat seed crops.

Table 2. Influence of some herbicides on grain yield of durum wheat during stem elongation stage (2018-2020)

Treatment stages	Herbicides	2018		2019		2020		Mean Factor B	
		kg/ha	%	kg/ha	%	kg/ha	%	kg/ha	%
Control		6140	100	3400	100	1570	100	3703	100.0
1st stem node stage	Palace	4740	77.2	4320	127.0	3480	221.7	4180	112.9
	Axial	6130	99.8	3710	109.0	3800	242.0	4547	122.8
	Traxos	5180	84.4	3290	96.8	3420	217.8	3963	107.0
	Topic	6120	99.7	4110	121.0	3680	234.4	4637	125.2
	Puma super	5330	86.8	4420	130.0	3960	252.2	4570	123.4
	Hussar max	5310	83.6	4390	129.0	3810	242.7	4503	121.6
2nd stem node stage	Palace	5220	85.0	4680	138.0	2450	156.1	4117	111.2
	Axial	5540	90.2	3260	95.9	3670	233.4	4157	112.3
	Traxos	5480	89.3	3930	116.0	3190	203.2	4200	113.4
	Topic	5470	89.1	4010	118.0	2990	190.4	4157	112.3
	Puma super	5350	87.1	4130	121.5	3930	250.3	4470	120.7
	Hussar max	5110	83.2	3700	108.8	3040	193.6	3950	106.7
3rd stem node stage	Palace	5650	92.0	4140	121.8	1570	100.0	3787	102.3
	Axial	5550	90.4	3610	106.2	3250	207.0	4137	111.7
	Traxos	6250	101.8	3710	109.1	3100	197.5	4353	117.6
	Topic	6180	100.7	4140	121.8	3140	200.0	4487	121.6
	Puma super	5450	88.8	3830	112.6	4390	279.6	4557	123.1
	Hussar max	5820	94.8	3690	108.5	2970	189.2	4160	112.3
Mean Factor A		5594	-	3906	-	3333	-	-	

LSD, kg/ha:

F.A	p≤5%=81	p≤1%=107	p≤0.1%=138
F.B	p≤5%=204	p≤1%=204	p≤0.1%=348
AxB	p≤5%=353	p≤1%=486	p≤0.1%=603

Analysis of variance for grain yield (Table 3) shows that the years have the highest influence on grain yield - 80.5% on the variants. It is determined by the unequal reaction of the variants to the change in the environmental conditions. The reason is the large differences in the meteorological conditions during the three years of investigation. The strength of influence of herbicides is 3.9%. The influence of years and herbicides is very well proven at $p \leq 0.1\%$. There is an interaction between herbicides and meteorological conditions of years (A x B) - 12.5%. It is very well proven at $p \leq 0.1\%$. The influence of tract of land, i.e. of soil fertility is 0.3%. It is well proven at $p \leq 1\%$.

Table 3. Analysis of variance for grain yield

Source of variation	Degrees of freedom	Sum of squares	Influence of factor, %	Mean squares	Fisher's criterion	Level of significance
Total	170	1956478	100	-	-	-
Tract of land	2	6390	0.3	3195.0	6.6	**
Variants	56	1896644	96.9	33868.6	70.9	***
Factor A - Years	2	1575208	80.5	787604.0	1650.0	***
Factor B - Herbicides	18	77666	3.9	4314.8	9.0	***
A x B	36	343770	12.5	7471.4	14.2	***
Pooled error	112	53444	2.7	477.2	-	-

*p≤5%; **p≤1%; ***p≤0.1%.

Based on proven herbicide x year interaction, it was evaluated stability parameters for each herbicide for grain yield of durum wheat with relation to years (Table 4). It was calculated the stability variances σ_i^2 and S_i^2 of Shukla, the ecovalence W_i of Wricke and the stability criterion YS_i of Kang.

Table 4. Stability parameters of some herbicides for grain yield with relation to years

Treatment stages	Herbicides	$\bar{x}$	σ_i^2	S_i^2	W_i	YS_i
Control		3703	15067.7**	-118.5	27676.3	-7
1st stem node stage	Palace	4180	6091.1**	429.8	11613.1	8+
	Axial	4547	14181.6**	6179.8**	26806.6	1
	Traxos	3963	5026.1**	9202.7**	9707.2	10+
	Topic	4637	6060.0**	6617.9**	7942.6	-4
	Puma super	4570	2109.1*	2944.5*	4487.2	16+
	Hussar max	4503	7473.8**	-107.4	14087.4	13+
2nd stem node stage	Palace	4117	265.5	0.5	1286.3	-1
	Axial	4157	23808.4**	48010.5**	43317.6	-5
	Traxos	4200	7827.3**	15818.1**	14702.0	-1
	Topic	4157	-141.8	141.3	459.2	10+
	Puma super	4470	1288.5	2827.0	3018.9	6+
	Hussar max	3950	1478.6*	121.5	3359.1	13+
3rd stem node stage	Palace	3787	2740.1**	-368.2	5616.4	0
	Axial	4137	5176.5**	12625.7**	17134.0	-4
	Traxos	4353	236.4	674.0	1136.2	5+
	Topic	4487	8081.5**	368.7	15175.4	5+
	Puma super	4557	4711.2**	686.2	9143.7	7+
	Hussar max	4160	14865.0**	13922.3**	27313.5	11+
Mean		4278				4.2
LSD (p = 0.05)		171				

Stability variances (σ_i^2 and S_i^2) of Shukla, which recorded respectively linear and nonlinear interactions, unidirectional evaluate the stability of the variants. These variants which showed lower values are considered to be more stable because they interact less with the environmental conditions. Negative values of the indicators σ_i^2 and S_i^2 are considered 0. At high values of either of the two parameters - σ_i^2 and S_i^2, the variant are regarded as unstable. At the ecovalence W_i of Wricke, the higher are the values of the index, the more unstable is the variant.

On this basis, using the first three parameters of stability, it is found that by treatment during 1st stem node stage, the six tested herbicides exhibited high instability. Instability is mainly due to the significant differences in grain yields in these variants over the years of experience, as the weather conditions are most affected. In herbicides Axial, Traxos, Topic and Puma super there is instability of linear and non-linear type - proven values of σ_i^2 and S_i^2. At them, the values of stability variances σ_i^2 and S_i^2 according to Shukla and of equivalence of Wricke W_i are high and mathematically proven. In herbicides Palace and Hussar max there is only linear type instability - the value of σ_i^2 is proven and the values of S_i^2 are unproven.

By treatment during 2nd stem node stage high instability exhibits herbicides Axial, Traxos and Hussar max. In herbicides Axial and Traxos there is instability of linear and non-linear type - proven values of σ_i^2 and S_i^2. In herbicide Hussar max there is only linear type instability - the value of σ_i^2 is proven and the values of S_i^2 are unproven. Herbicides Palace, Topic and Puma super are stable by treatment during 2nd stem node stage of durum wheat.

By treatment during 3rd stem node stage high instability exhibits herbicides Palace, Axial, Topic, Puma super and Hussar max. In herbicides Axial and Hussar max there is instability of linear and non-linear type - proven values of σ_i^2 and S_i^2. In herbicide Palace, Topic and Puma super there is only linear type instability - the value of σ_i^2 is proven and the values of S_i^2 are unproven. Herbicide Traxos are stable by treatment during 3rd stem node stage of durum wheat.

To evaluate the complete efficacy of each herbicide should be considered as their effect on grain yield of durum wheat and their stability - the reaction of durum wheat to this herbicide during the years. Valuable information about the value of technologic value of the variant give the stability criterion YS_i of Kang for simultaneous assessment of yield and stability, based on the reliability of the differences in yield and variance of interaction with the environment. The value of this criterion is experienced that using nonparametric methods and warranted statistical differences we get a summary assessment aligning variants in descending order according to their economic value.

Generalized stability criterion YSi of Kang, taking into accounts both the stability and value of yields gives high assessments of herbicides Puma super, Hussar max, Traxos and Palace, applied during 1st stem node stage; of herbicides Hussar max, Topic and Puma super, applied during 2nd stem node stage and of herbicides Hussar max, Puma super, Topic and Traxos applied during 3rd stem node stage. These variants are the most valuable from the viewpoint of durum wheat cultivation technology. They combine high values of grain yield and relatively good stability of this indicator in different years.

Negative assessments are given to the control, herbicide Topic applied during 1st stem node stage; herbicides Palace, Axial and Traxos applied during 2nd stem node stage and herbicide Axial applied during 3rd stem node stage. These variants combine lower values of grain yield and low stability of this indicator in different years.

CONCLUSIONS

The highest grain yield was obtained by treatment of herbicide Topic during 1st stem node stage of durum wheat, followed by herbicides Puma super, Axial and Hussar max.

During 2nd stem node stage of durum wheat, the highest grain yield was obtained by treatment of herbicide Puma super, followed by herbicides Traxos, Axial and Topic.

During 3rd stem node stage of durum wheat, the highest grain yield was obtained by treatment of herbicide Puma super, followed by herbicides Topic and Traxos.

Technologically, the most valuable are herbicides Puma super, Hussar max, Traxos and Palace, applied during 1st stem node stage; herbicides Hussar max, Topic and Puma super applied during 2nd stem node stage and herbicides Hussar max, Puma super, Topic and Traxos applied during 3rd stem node stage.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON TESTING NEW REMEDIES WITH SYSTEMIC FUNGICIDAL ACTION IN THE CHEMICAL MANAGEMENT OF WINTER WHEAT, CENTRAL AREA, REPUBLIC OF MOLDOVA

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Abstract

Wheat plants are subject to the impact of a complex of pathogenic agents, which include over ten species of diverse etiological and pathological nature, as key objects of economic importance, that annually cause serious damage, such as: Ustilago tritici; Tilletia caries; T. foetida; Puccinia recondita; P. glumarum; P. graminis; Erysiphe graminis; Fusarium graminearum; Septoria tritici, S. graminum; Helminthosporium tritici-repentis. The fungicidal remedies Camporo 25 EC, Custodia 320 SC and Sizaro ES fungicides have been tested against the diseases detected in winter wheat plantations. The efficiency of these new remedies with anti-fungal action was proved depending on the doses applied and the severity of the disease, as compared with the standard control. Camporo 25 EC and Custodia 320 SC, Sizaro ES fungicides are recommended as efficient chemical products in the integrated protection system of winter wheat. The frequency and intensity of the attack of pathogens, in 2021-2022, ranged between 10 and 45%, depending on environmental factors, and the biological efficiency of the new remedies tested on experimental plots as fungicides was 90-93%, at the level of the standard variant, comparing the variants and doses applied.

Key words: *wheat grain; fungicides; disease¸ integrated protection system, biological control.*

INTRODUCTION

The exploration of autumn cereal agroecosystems includes various objectives with indisputable value, to ensure the high productivity and quality of caryopses, cultivated in various agricultural production systems, on areas specialized for the cultivation of field crops, which can be affected by the instability of climatic conditions of the Republic of Moldova. Annually, various damages, in terms of phytosanitary indices, and losses of biological and agricultural production caused by the influence of the favourable impact of weather conditions on the development of complexes of harmful organisms, as parasitic agents, are reported for these crops. Special attention should be paid and detailed research should be conducted on cereal crops in impact with pathogenic agents, which cause specific diseases, with various pathological etiologies and serious consequences on wheat plants, detected practically in all phenological stages of growth and development (Moraru, 2007; Starodub & Gheorghiev, 2008; Starodub et al., 2013).

Of particular importance in the cultivation of winter wheat are the key diseases, which appear consecutively throughout the growing season of plants caused by environmental conditions, some agrotechnological neglect and the sensitivity of wheat varieties and cultivars. Overall, the consequences of the severity of attack of pathogens causing different diseases on the vegetative organs, seriously favour the premature drying of the foliage, resulting in the reduction of photosynthesis, the retardation of growth, the formation of tiny, underdeveloped caryopses, ultimately causing lower productivity of the crop. Therefore, the foliage and the generative organs of cereal plants are subject to the attack of a complex of pathogenic agents, which includes several dozen species of diverse etiological and pathological nature, noted as key invasive objects of economic importance that cause great damage to various cereal crops including and winter wheat, such as: *Ustilago tritici, Tilletia caries, T. contraversa, T. indica*, *Puccinia recondita, P. glumarum*, *P. anomala, Blumeria graminis* f. sp. *tritici* and *hordei, Septoria tritici, Septoria graminum* and *Septoria nodorum, Helminthosporium gramineum, Helminthosporium teres* etc. (Oroian & Florian, 2006; Bădărău, 2009; 2010).

The complex of protection measures for winter wheat provides for the use of all the available technological means to create unfavourable conditions for the development of pathogenic agents. Chemical treatments applied during the growing season are also very important in controlling the diseases that affect leaves and ears of wheat. The correct use of approved fungicides, by applying them at the optimal time and in doses tested during a detailed research, ensures a high effectiveness in combating the mentioned diseases (Bădărău et al., 2013; Bădărău & Nicolaesc, 2013).

Based on these facts, **the purpose and objectives of the research** carried out in 2021-2022 estimate the comparative studies on some associations of pathogenic agents in the winter wheat crop that trigger serious diseases in order to develop and implement new chemical treatments in the integrated plant protection system. New phytosanitary products, with a complex range of fungicidal action, were researched and tested to establish the biological efficiency of the products, as new remedies in combating pathogens, such as: *Erysiphe graminis, Puccinia recondita, Septoria tritici, Dreschlera tritici-repentis, Fusarium graminearum* and *Cladosporium herbarum,* under the conditions of cereal agrocenoses on productive sectors of the "Răzagro-Prim" Production Association, the central area of the Republic of Moldova, Răzeni commune, Ialoveni district.

MATERIALS AND METHODS

The success of the chemical control of diseases in autumn cereals, within the framework of intensive cultivation technologies, calls for the correct determination of the dates for applying treatments by using highly effective fungicides, the widening of the assortment of approved chemical preparations, which are harmless to the environment. Since it is necessary to minimize productivity losses, it becomes necessary to develop effective crop protection measures that would reduce the intensity of diseases and the damage caused, using a minimum number of chemical treatments and doses of remedies. Thus, the testing of the biological efficiency of some new phytosanitary products with fungicidal action on the key diseases of winter wheat were the reason behind the goal and the main objective of the research carried out by us (Gulii Pamujac, 1992; Bădărău, 2010; Bădărău et al., 2013).

The research carried out as part of the State Testing Program of Skway Xpro control-standard preparations in comparison with the new product Cezaro ES, Camporo 25 EC and Custodia 320 SC on the pathogenic fungi, *Puccinia recondita, Erysiphe graminis, Septoria tritici, Dreschlera tritici-repentis, Fusarium graminearum* and *Cladosporium herbarum* was conducted on the territory of "Răzagro-Prim" Production Association, Ialoveni district, during the growing seasons of 2019-2021-2022. Territorially, this association owns an area of over 720 ha of arable land. According to the humidity and temperature conditions, the territory of the "Răzagro-Prim" Agricultural Production Association is part of the 2nd agroclimatic district of the Republic of Moldova and is characterized by a sum of 3200-3300°C, and the hydrothermal coefficient is 0.7-0.9. The average monthly positive air temperature is maintained during 9 months (03-11). According to multiannual data, late frosts may occur until the middle of May. Average daytime temperatures higher than 10°C are recorded over about 180 days. The amount of precipitation is 340-440 mm, of which 70 mm falls in June.

The results of the surveys carried out in several years have proven that, on the territory of "Răzagro-Prim" P.A., the conditions are very favourable both for the cultivation of winter wheat and for the development of a complex of pathogenic fungi with serious impact, such as: loose smut - *Ustilago tritici*; bunt - *Tilletia caries, Tilletia foetida*; brown rust - *Puccinia recondita*; yellow rust - *Puccinia glumarum*; black rust - *Puccinia graminis*; powdery mildew - *Erysiphe graminis*; fusarium head blight -

Fusarium graminearum; blotch - *Septoria tritici, Septoria graminum*; yellow spot - *Drechslera tritici-repentis* and cladosporiosis - *Cladosporium herbarum.*

Table 1. The scheme of the experiment for testing the biological efficiency of the fungicide Sizaro EC in combating fungal diseases in winter wheat

№	Variants of the experiment	Active ingredient	Harmful organisms	Method of use
1.	Untreated (control)	Treated with water	1. *Erysiphe graminis;* 2. *Puccinia recondite;* 3. *Septoria tritici;* 4. *Pyrenophora tritici-repentis;* 5. *Fusarium graminearum;* 6. *Cladosporium herbarum.*	Two treatments per growing season
2.	Standard Skway Xpro - 1.25 l/ha	tebuconazole, 100 g/l + prothioconazole, 100 g/l + bixafen, 75 g/l		
3.	Sizaro - 0.8 l/ha	prothioconazole, 125 g/l + tebuconazole, 125 g/l		
4.	Sizaro - 1.0 l/ha			
5.	Standard Nativo Pro 325 SC - 0.7 l/ha	Treated with water	1. *Erysiphe graminis;* 2. *Puccinia recondita ;* 3. *Septoria tritici;* 4. *Pyrenophora tritici repentis.*	Two treatments per growing season
6.	Custodia 320 SC - 0.6 l/ha	prothioconazole, 175 g/l trifloxystrobin, 150 g/l		
7.	Camporo 25 EC - 0.8 l/ha	pyraclostrobin, 170 g/l + prothioconazole, 170 g/l		

Figure 1. The experimental area with separate plots for testing the new fungicides, "Vatra-Răzășească" P.A., Ialoveni District, 2021-2022

Figure 2. Two treatments carried out consecutively in various phenological stages on experimental plots "Vatra-Răzășească" P.A., 2021-2022

Figure 3. Phytosanitary surveys and collecting samples for laboratory analyses, 2021-2022

Phytosanitary surveys in the areas planted with winter wheat were carried out from the fall of 2019 to 2022, where we found that in the investigated experimental sector of the "Răzagro-Prim" P.A., significant reserves of infection were detected in the soil, for the primary and secondary inoculation on plant organs, and the environmental conditions had favourable impact on them. For cereals planted in autumn, including wheat, the treatments, during the growing season, are applied according to prognoses and warnings, but more frequently they are used according to the phenological criteria and the severity of attack by pathogens and symptoms of the detected diseases.

The experiments made in the framework of the state testing of the fungicides Cezaro EC, Camporo 25 EC and Custodia 320 SC were carried out on the winter wheat cultivar Odesscaia-269, which is vulnerable practically to all the key diseases of cereal plants. The experiment was started on 09.04.2021, according to the Latin rectangle method (Figure 1). Each variant included four replications. Plot sizes for each replication were 25 x 2 m (50 m^2). The experimental plots were separated by paths of 0.4 m width, to avoid overlapping of the tested solution from one variant to another. The numbers of variants and replications were indicated with paint on boards installed in front of each plot. The plants were treated by spraying the solution with a portable sprinkler at windless morning hours (Table 1, Figures 1-3). On the experimental sector, two treatments were successively applied at an interval of one month (April 26-May 25) in the stem elongation and heading stages. The phenological observations and the phytosanitary surveys of the development of the diseases detected in the plants grown on the experimental sector, as well as the determination of the biological efficiency of the treatments, with the remedies mentioned in Table 1, in different variants and recommended doses, were carried out periodically according to the requirements indicated by: "Methodological guidelines for testing chemical and biological plant protection products against pests, diseases and weeds in the Republic of Moldova" (2002) and "Methodological guidelines for state testing of chemical and biological products for the protection and growth stimulation of agricultural and forestry plants in the Republic of Moldova" (2012). The determination of diseases was carried out by visual and microscopic analysis, according to classic and new guidelines. The surveys to determine the severity of infection with powdery mildew, brown rust, blotch, blight and reticulated leaf spot were carried out according to unanimously accepted methods (Bădărău & Bivol, 2009; Bădărău et al., 2013). Samples were taken 3 times during the growing season, collecting by ten plants from each individual plot, gathering them in labeled bundles, which were then analyzed in the laboratory (https://date.gov.md/ro/system/files/resources/2014-06/Catalog_2021.pdf.)

To determine the severity of diseases by variants and repetitions, plant samples were collected and then analysed in the laboratory. The phytopathological research revealed the causes of the diseases, the frequency (**F%**) and the intensity of development (**I%**). Disease frequency (F%) represents the relative value of the number of affected plants or organs **(n)** per total number of analysed plants or organs **(N)** and is determined according to the formula:

$$F\% = \frac{n \times 100}{N}$$

Disease intensity (I%), which indicates the percentage to what extent a plant or organ was affected. To calculate the qualitative index of the disease intensity, the following formula was used:

$$I\% = \frac{(n_1 \cdot 1) + (n_2 \cdot 2) + (n_3 \cdot 3) + (n_4 \cdot 4)}{N \times 4},$$

where: n_1, n_2, n_3, n_4 - the number of plants or organs affected at the respective grade; N - the total number of examined plants or organs; 4 - the maximum grade of the scale.

Different scales are used to render the intensity. In our experiments, the scale with 4 grades was used to denote the disease intensity, which corresponds to certain intervals that express the percentage of the affected area, namely: 0 - visible symptoms are absent; 1 - the affected area is up to 10% of the leaf blade; 2 - the affected area is from 10 to 25%; 3 - the affected surface is from 25 to 50%; 4 - more than 50% of the surface of the organ is affected.

The biological efficiency of the tested fungicides on leaf diseases of winter wheat was calculated according to the formula:

$$B.i.\% = \frac{I.d. - I.e.}{I.d.} \times 100$$

where: **B.i**. - biological efficiency; **I.d.** - the intensity of disease development in the control; **I.e.** - the intensity of disease development in the experimental variants;

RESULTS AND DISCUSSIONS

The reduction of the damage caused by diseases in winter wheat plantations is achieved by applying a complex system of measures, which involves the use of all possibilities to prevent the attack of pathogens, starting with crop rotation, the use of healthy seeds, the correct application of soil cultivation techniques, sowing at the recommended timing and depth, the rational use of fertilizers, weed control and phytosanitary treatments with approved phytosanitary products, aimed at reducing the degree of attack by numerous pathogens, such as: *Blumeria graminis* f. sp. *tritici, Puccinia recondita, Pyrenophora tritici-repentis, Septoria tritici, Septoria graminum*, *Septoria nodorum, Cladosporium herbarum Fusarium graminearum* etc.

The agroclimatic conditions in the Central area of the Republic of Moldova during the period of research and active growth, 2020-2022, were extremely favourable for the primary infection and the active dynamic evolution of the manifestation and expansion of powdery mildew, blotch, rusts and helminthosporiosis on the leaves and spikelets of wheat autumn and other associated diseases, which seriously affected the plants throughout the growing season, under the favourable influence of environmental factors.

The results of the preliminary and current phytosanitary surveys carried out on the winter wheat sectors indicated that, in the investigated experimental sector of the "Răzagro-Prim" P.A., significant reserves of primary and secondary inocula caused by fungal infections were detected, which caused the development of diseases throughout the growing season of the wheat crop, and the respective diseases with certain values of frequency and intensity of the attack of phytopathogenic agents are reported in Table 2, Figure 4. From the spectrum of diseases detected according to the frequency and intensity of the attack on wheat plants, especially on the vegetative organs, powdery mildew - *Erysiphe graminis* was the most common, being followed by blotch - *Septoria tritici, Septoria graminum*; rust - *Puccinia recondita, Puccinia anomala*; yellow-spot - *Pyrenophora tritici-repentis*; fusarium head blight - *Fusarium graminearum*; cladosporiosis - *Cladosporium herbarum*, mentioned in consecutive descending order according to the values of the impact indices reported during the testing period. In this context, testing the biological efficiency of the preparations Cezaro EC, Camporo 25 EC and Custodia 320 SC, with complex antifungal action against the main diseases affecting winter wheat, was the main goal and objective of the research carried out by us, together with the preventive determination of the phytosanitary status and of the economic damage threshold. The environmental conditions also contributed to a faster growth of winter wheat as well as the outbreak of the estimated specific diseases. According to the research-testing program, comparing variants and doses, individually for each disease, treatments were applied by spraying, at the same time intervals.

The comparative results of the experimental values obtained regarding the testing of the biological efficiency of the products Sizaro EC Camporo 25 EC and Custodia 320 SC, as fungicides against the pathogenic fungi *Erysiphe graminis, Puccinia recondita, Septoria tritici, Dreschlera tritici-repentis, Fusarium graminearum* and *Cladosporium herbarum* are estimated in Table 2 and Figure 4.

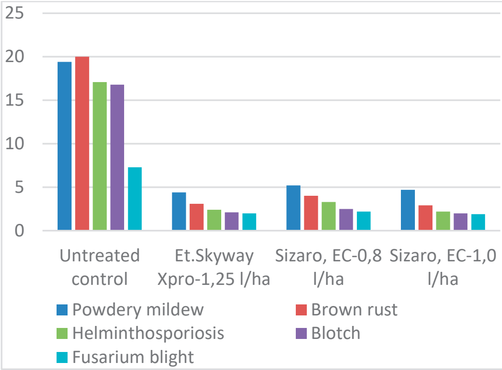

Figure 4. Comparative attack frequency by variants: untreated control, standard variant and the new tested fungicide Sizaro EC (as example)

Table 2. The results of the survey on the severity of diseases in winter wheat, central area of R. Moldova 2021-2022

Disease	Pathogen	F %	I %
Powdery mildew	*Erysiphe graminis*	44.1	28.5
Blotch	*Septoria tritici,*	38.4	20.7
Brown rust	*Puccinia recondita*	34.5	14.0
Yellow spot	*Dreschlera tritici-repentis,*	26.4	14.7
Fusarium blight	*Fusarium graminearum*	22.4	12.2
Cladosporiosis – black spot	*Cladosporium herbarum*	20.1	10.7

Note: F% - frequency, I% - intensity

The indices of the untreated control, where the frequency of the pathogenic attack and the intensity of the development of the diseases are indicated individually, present the following results: the frequency of the attack varied from 7.3% - fusarium blight to 22% - brown rust, and the intensity of the development of the diseases from 4.4% - fusarium blight up to 20.3% - brown rust, thus, all of them advanced within the limits from 4% to 20%. Based on the indices of the untreated control, in the treated variants, the above-mentioned fungicides were applied by spraying, as well as the standard fungicide Skway Xpro - to evaluate comparatively the biological efficiency in winter wheat. Practically all the tested remedies reached the same values as the standard variant 92.5% (Table 3, Figure 5).

As a result of the treatment of the experimental plots with the fungicides Cezaro EC Camporo 25 EC and Custodia 320 SC, the intensity of development of powdery mildew - *Erysiphe graminis* decreased from 10.7% in the untreated control to 1.0% after applying the first dose and 0.8% -after the second, and 0.7% after applying the standard fungicide (Skway Xpro EC 275 - 1.25 l/ha), and the biological efficiency of fungicides in combating the fungus *Blumeria graminis* f. sp. *tritici* in winter wheat was 90.6-92.5%, in comparison with the standard - 93.5%.

Table 3. Establishing the comparative biological effectiveness of the fungicides Sizaro EC, Camporo 25 EC and Custodia 320 SC, comparatively, by doses, in combating pathogenic fungi on the experimental sector planted with winter wheat, "Vatra-Răzășească" P.A., 2021-2022

No.	Variants of the experiment	Frequency of attack, %	Intensity of development of diseases, %	Biological efficiency, %
Powdery mildew - *Erysiphe graminis*				
1	Untreated control	19.4	10.7	0.0
2	Et. Skway Xpro – 1.25 l/ha	4.4	0.7	93.5
3	Sizaro EC – 0.8 l/ha	5.2	1.0	90.6
4	Sizaro EC – 1.0 l//ha	4.7	0.8	92.5
5	Camporo 25 EC – 1.0 l//ha	4.6	1.0	91.8
6	Custodia 320 SC -1.0 l//ha	4.7	0.9	92.6
Brown rust - *Puccinia recondita*				
1	Untreated control	20.0	14.3	0.0
2	Et. Skway Xpro – 1.25 l/ha	3.1	1.3	90.9
3	Sizaro EC – 0.8 l/ha	4.0	1.6	88.8
4	Sizaro EC – 1.0 l//ha	2.9	1.1	92.3
5	Camporo 25 EC – 1.0 l//ha	3.2	1.2	90.6
6	Custodia 320 SC – 1.0 l//ha	2.9	1.0	89.7
Helminthosporiosis - *Pyrenophora tritici-repentis*				
1	Untreated control	17.1	11.5	0.0
2	Et. Skway Xpro – 1.25 l/ha	2.4	1.0	91.3
3	Sizaro EC – 0.8 l/ha	3.3	1.3	88.7
4	Sizaro EC – 1.0 l//ha	2.2	0.9	92.2
5	Camporo 25 EC - \ 1.0 l//ha -	2.5	1.2	91.6
6	Custodia 320 SC – 1.0 l//ha	2.3	1.1	90.8
Blotch - *Septoria* spp.				
1	Untreated control	16.8	9.1	0.0
2	Et. Skway Xpro – 1.25 l/ha	2.1	0.8	91.2
3	Sizaro EC – 0.8 l/ha	2.5	1.0	89.0
4	Sizaro EC – 1.0 l//ha	2.0	0.7	92.3
5	Camporo 25 EC – 1.0 l//ha	2.2	1.1	90.8
6	Custodia 320 SC – 1.0 l//ha	2.4	0.9	91.4
Fusarium blight - *Fusarium graminearum*				
1	Untreated control	7.3	4.4	0.0
2	Et. Skway Xpro – 1.25 l/ha	2.0	0.6	86.4
3	Sizaro EC – 0.8 l/ha	2.2	0.7	84.1
4	Sizaro EC – 1.0 l//ha	1.9	0.5	88.6
5	Camporo 25 EC – 1.0 l//ha	2.3	0.8	87.3
6	Custodia 320 SC – 1.0 l//ha	2.2	0.7	86.6

As for the fungus *Puccinia recondita* the highest values were recorded in the untreated control, with the frequency of the attack of 20.0%, and the intensity of development of the disease of 14.3%. Among the treated variants, the frequency of the attack of brown rust was 3.1% in the standard variant (Skway Xpro- 1.25 l/ha), 4.0% after applying the first dose and 2.9% - after the second. The biological efficiency of the use of new preparations as fungicides in combating the fungus *Puccinia recondite* in winter wheat was 88.8% and 92.3%, respectively, and 90.9% if applying the standard fungicide.

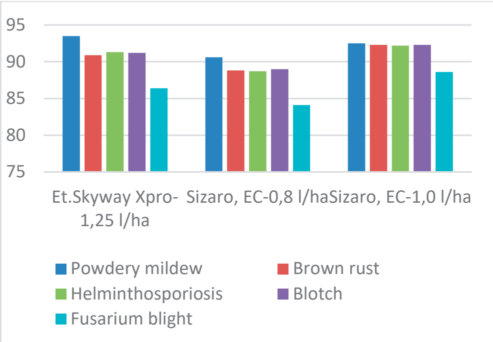

Figure 5. The comparative biological efficiency of the standard and the variant treated with the fungicide Sizaro EC for all the detected diseases

In the treated variants, the frequency of attack of helminthosporiosis was 2.4% in the standard variant - 2.2% and 3.3% in the variants treated with the new tested fungicides, and the biological efficiency of the fungicidal preparations against the fungus *Dreschlera tritici-repentis* was high, representing 91.3% in the standard variant, 88.7% and 92.2% in the tested variants. As a result of the treatments with the new remedies, the intensity of development of blotch in winter wheat decreased from 9.1% to 0.7%, as compared with 0.8% in the standard variant, and the biological efficiency of the treatments with these preparations applied to combat blotch was high, constituting 89.0-92.3% in comparison with the standard variant - 91.2%. The values obtained regarding the biological efficiency of the treatments with the tested preparations against *Fusarium graminearum* and *Cladosporium herbarum* indicated that in 2022, in the experimental sector, they developed more poorly. In the absence of the typical symptom of reddening and blackening of the wheat ears, the basic survey was carried out at the end of June (29.06.22) on the background of the semi-hidden development of the disease, where the frequency of attack was only 7.3%, at the intensity of disease development was 4.4%. In the experimental variants, the frequency of fusarium blight was 2.2% in the variants treated with the new preparations, as compared with 2.0% in the standard variant. The intensity of the development of fusarium blight and the blackening of the wheat ears was reduced by the applied treatments, constituting 0.6% in the standard variant and 0.5-0.7% in the variants treated with the new preparations, and the biological efficiency of the treatments against the fungus *Fusarium graminearum* was 84.1% and 88.6%, respectively, as compared with 86.4% in the standard variant (Skway Xpro EC 275 - 1.25 l/ha).

The statistical processing of the obtained results shows that the biological efficiency of the treatments with the tested preparations in combating all the diseases detected in the researched winter wheat plantation, was at the level of the standard variant in both doses tested and with high values of biological efficiency, individually per disease and dose applied.

CONCLUSIONS

1. The weather conditions in the autumn of 2020 and spring-summer in the growing season of the years 2021-2022, with the exception of May and the first days of June, were unfavourable for the growth and development of winter wheat, but favourable for the primary infection and the later evolution of powdery mildew - *Erysiphe graminis*, brown rust - *Puccinia recondita,* blotch - *Septoria tritici,* helminthosporiosis - *Dreschlera tritici-repentis*, fusarium blight - *Fusarium graminearum*, cladosporiosis -*Cladosporium herbarum* and other infectious diseases detected in winter wheat, cultivated in the central area of the Republic of Moldova.
2. The comparative results of the research conducted to establish the biological efficiency of the treatments with the new remedies such as: Cezaro EC, Camporo 25 EC and Custodia 320 SC as new fungicides used for winter wheat, with a wide spectrum anti-fungal action, indicate a high efficiency of 90-93%, analysed

comparatively by doses and variants, which were at the level of the control variant - applying a standard fungicide.

3. Based on the obtained experimental results, it was proposed to include the tested preparations: Sizaro EC, Camporo 25 EC and Custodia 320 SC in the integrated protection system for winter wheat and in the State Register of phytosanitary products and fertilizers, to be applied in doses of 0.8-1.0 l/ha, two treatments per growing season, in the critical phases of contamination, according to the economic damage threshold.

ACKNOWLEDGEMENTS

This research has been carried out with the support of the National Agency for Research and Development, in the framework of the projects 20.80009.7007.12 and 20.80009.5107.02.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

CRUDE PROTEIN YIELD AND ENERGY NUTRITIONAL VALUE OF FODDER OF PERENNIAL GRASS MIXTURES

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Abstract

The aim of the study was to determine the amount of crude protein yield and the yield of feed units in the fodder biomass of six variants of two-component legume-grass mixtures (Lotus corniculatus - Festuca rubra; Trifolium repens - Lolium perenne; Trifolium repens - Poa pratensis; Trifolium pretense - Phleum pretense; Medicago sativa - Dactylis glomerata; Trifolium pretense - Festuca pratensis) that would provide high-quality fodder in the conditions of the Central Balkan Mountain. The highest crude protein yield is for the mixtures Tr. pratense L. + F. pratensis L. and Tr. repens L. + P. pratensis L. The legume crop predominated in the regrowths of both variants during the entire experimental period from 7.4% to 48.4%, respectively, and the ratio of legume:grass in the mixed grass stands was 47.7:40.3% (Tr. pratense L. - F. pratensis L.) and 62.3:13.9% (Tr. repens L.- P. pratensis L.). A proven difference in crude protein yield was found between Tr. repens L. - P. pratensis L. and M. sativa L. - D. glomerata L. mixtures. The highest yield of feed units for milk and growth was found in the forage mixture of Tr. pratense L. with Ph. pratense L.

Key words: *crude protein; feed unit for milk; feed unit for growth; grass-legume mixtures.*

INTRODUCTION

The plant development and the relationships between species in fodder crops within mixed crops are a significant factor in the composition and quality of the grass mass, and the agro-climatic conditions in the growing region affect their productive capabilities (Das et al., 2016; Gasiev et al., 2019). In this sense, the species selection, the morphological and physiological features, and the biochemical processes related to them are essential for the nutritional value of the obtained fodder (Pavlov, 1996).
Cultivation of perennial legume and grass crops with high adaptive and productive potential leads to a permanent increase (by 1.5-2.0 times more) in the quality and quantity of obtained fodder (Kurgak, 2010).
On the other hand, multicomponent mixed grass stands use soil resources more efficiently (Lipińska et al., 2018; Butenko et al., 2019; Churkova and Churkova, 2020) through the improved vertical arrangement of the root mass in the soil horizons, which allows a more complete assimilation of nutrients and formation of longer lasting and highly productive grass stands (Vasileva and Vasilev, 2012; Luscher et al., 2014; Kusvuran et al., 2014).
Perennial grass-legume and grass associations are a source of environmentally friendly fodder mass to meet animal food requirements (Chand et al., 2022). The composition of the grass stands, the digestibility of the crude protein, the fat, the amount of the fiber fraction and the nitrogen-free extractives are decisive for the energy nutritional value of the dry fodder mass. The inclusion of legume components in mixed grass stands is an effective method to increase the values of crude protein in dry matter (Ding et al., 2014; Cordeau et al., 2017; Churkova and Churkova, 2021; Naydenova et al., 2022) which are involved in the equations for predicting the energy nutritional value of fodder. The precise establishment of energy nutrition, which is the main criterion for modern evaluation of the quality of fodder determined by the fodder units for milk and growth (Todorov, 2010) is essential in the evaluation of fodder.
The aim of the study was to determine the amount of crude protein yield and the yield of feed units for milk and growth in the fodder biomass of six variants of two-component

grass-legume mixtures that would provide high-quality fodder in the conditions of the Central Balkan Mountain (Bulgaria).

MATERIALS AND METHODS

The study was conducted at the Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Bulgaria), in the period 2014-2016, on light gray, pseudopodzolic soils, with $pH_{KCL} = 4.3$.

In the first experimental year (2014), the average precipitation amounts for the period of March-October were significantly higher (1046 mm) than normal for the region (664 mm), and in the third year (2016) by 10.4% lower. The spring regrowths of the first and third experimental years were well supplied with a sufficient moisture amount. In the second experimental year (2015), the growth of grass and legume crops took place under significantly drier conditions and at a higher average monthly air temperature for the period of March-October (15.9°C) compared to the values of the indicator for the first and third experimental year (14.8°C).

Experimental variants were:

1. *Lotus corniculatus* L. (cv. Leo) - *Festuca rubra* L. (cv. Ryder);
2. *Trifolium repens* L. (cv. Huia) - *Lolium perenne* L. (cv. Belida);
3. *Trifolium repens* L. (cv. Huia) - *Poa pratensis* L. (cv. Sobra);
4. *Trifolium pratense* L. (cv. Altaswede) - *Phleum pratense* L. (cv. Erecta);
5. *Medicago sativa* L. (local population) - *Dactylis glomerata* L. (cv. Loke)
6. *Trifolium pratense* L. (cv. Altaswede) - *Festuca pratensis* L. (cv. Laura).

The experiment was based on the block method, in 4 replicates with a plot size of 5 m^2. Sowing was done manually, in a scattered way, with a sowing rate consistent with that of the species in a pure crop at a ratio of 50: 50. Cultivation of the mixtures was without fertilizing under nonirrigated conditions. The grass stands were mowed at the beginning of the blossoming phase of legumes and tasseling/ear formation for the grasses. In total, six regrowths were harvested during the experimental period.

The yield of crude protein (CP, kg/da) and the yield of feed units (feed unit for milk - FUM and feed unit for growth - FUG) were established by the yield of dry matter (kg/da) and the content of the indicators in 1 kg of dry matter.

Statistical processing of the obtained results was performed with ANOVA, LSD test for statistical significance of differences, standard deviation and coefficient according to the variational statistical method (Lidanski, 1988).

RESULTS AND DISCUSSIONS

Competition among species is an essential factor for the optimal performance of fodder crops in the grass stand. In this case, the mixture of *Lotus corniculatus* L. with *Festuca rubra* L. gives a clear idea of a good balance between the components, which is a prerequisite for the formation of a highly productive fodder mass (Figure 1).

Regarding the cenotic activity of the components in the *Medicago sativa* L.-*Dactylis glomerata* L. mixture, the grass crop prevailed in the total yield by more than 75% compared to the legume, which affected the nutritional value of the grass mass.

According to Bozhanska (2017), the fodder from the mixture has a low crude protein content, a high concentration of fibrous components in the cell walls and the lowest *in vitro* digestibility of dry matter (from 0.3% to 13%) compared to the digestibility of the remaining mixtures included in the experiment.

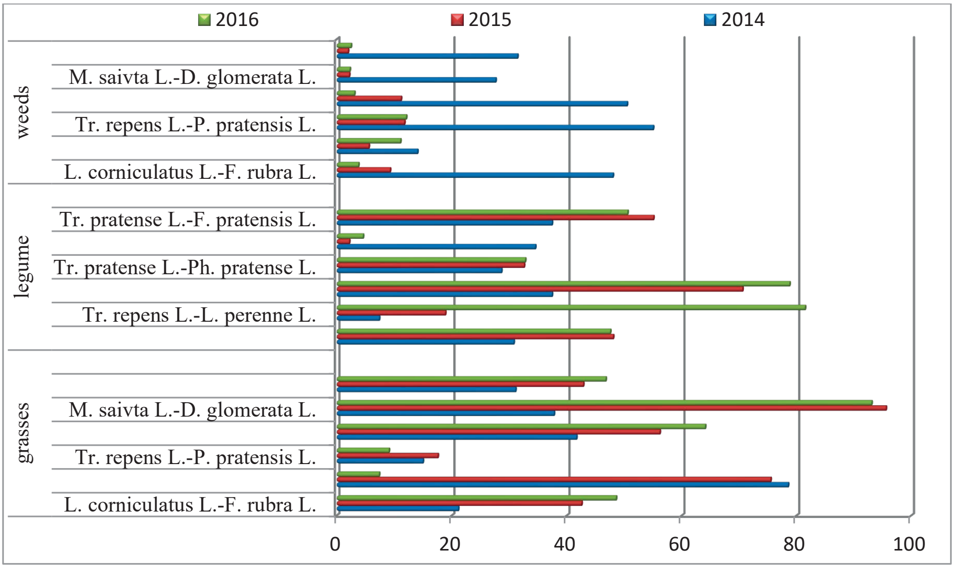

Figure 1. Botanical composition (percentage of components in the grass stand) of perennial grass-legume mixtures, over the years (%)

Lotus corniculatus L. and *Trifolium repens* L. are legume crops of high fodder significance for the Central Balkan Mountain region (Mihovski and Goranova, 2006; Churkova, 2019), whereas *Festuca rubra* L. and *Poa pratensis* L. are the main structure-determining grass components in the composition of mountain grass stands (Mitev and Goranova, 2008; Mitev et al., 2010).

The share of fodder crops in the composition of perennial mixtures changes with increasing the age of the grass stand, which affects the quality and biochemical characteristics of fodder.

The mixed grass stands of *Trifolium repens* L. and *Trifolium pratense* L. with *Poa pratensis* L. and *Festuca pratensis* L., respectively, were the only ones in which the legume component predominated in all regrowths during the three-year experimental period. The ratio of legume and grass crops in the mixed grass stands of *Trifolium repens* L. and *Poa pratensis* L. was 62.3: 13.9%, and in those of *Trifolium pratense* L. and *Festuca pratensis* L., respectively - 47.7: 40.3%.

The development and composition of perennial grasses and grass mixtures is related to a complex of conditions (soil, climate, agrotechnical, biological, etc.) that have an impact on their bioproductivity and quality characteristics. Grass-legume mixtures are preferred over pure grass stands throughout the world because they often increase the total yields of fodder and protein and offer balanced nutrition (Albayrak et al., 2011).

In the year of sowing, crude protein yield in the mixed two-component grass stands ranged from 86.72 kg/da (*Trifolium pratense* L. - *Festuca pratensis* L.) to 114.13 kg/da (*Lotus corniculatus* L. -*Festuca rubra* L.) (Table 1). The excess in the values of the indicator compared to the control is from 1.75% to 18.7%. In the second vegetation (2015), when legume crops reached their maximum development, mixtures of *Trifolium pratense* L., as well as those of *Trifolium repens* L. with *Poa pratensis* L., and *Lotus corniculatus* L. with *Festuca rubra* L., registered from 1.2% to 20.3% higher yield of crude protein compared to the average value of the indicator (71.65 kg/da). In the third vegetation, the highest yield of crude protein was found in the mixed grass stands of *Trifolium repens* L. with *Poa pratensis* L. (139.47 kg/da) followed by *Lotus corniculatus* L. with *Festuca rubra* L. (118.11 kg/da), *Trifolium repens* L. with *Lolium perenne* L. (117.07 kg/da) and *Trifolium pratense* L. with *Festuca pratensis* L. (115.02 kg/da). The excess in the values of the indicator compared to the control is from 4.8% to 26.8%.

Table 1. Yield of crude protein (CP) in the biomass of perennial two-component mixtures, over the years and average for the period 2014-2016 (kg/da)

Variants	**2014**	**2015**	**2016**	**2014-2016**
Lotus corniculatus L.-*Festuca rubra* L.	114.13	72.53	118.11	102.97
Trifolium repens L.-*Lolium perenne* L.	97.83	56.20	117.07	88.71
Trifolium repens L.-*Poa pratensis* L.	94.75	82.48	139.47	105.30
Trifolium pratense L.-*Phleum pratense* L.	93.77	80.00	95.70	90.14
Medicago saivta L.-*Dactylis glomerata* L.	89.68	52.50	74.54	72.45
Trifolium pratense L.-*Festuca pratensis* L.	86.72	86.21	115.02	95.94
Average	***96.15***	***71.65***	***109.99***	***92.59***
Min	**86.72**	**52.50**	**74.54**	**72.45**
Max	***114.13***	***86.21***	***139.47***	***105.30***
CV	***10.0***	***19.8***	***20.2***	***12.8***
SD	***9.64***	***14.18***	***22.23***	***11.89***
$LSD_{0.05}$				***17.6***

The highest average yield of crude protein, for the experimental period, was found in the mixtures - *Trifolium repens* L. - *Poa pratensis* L. (105.30 kg/da), *Lotus corniculatus* L. - *Festuca rubra* L. (102.97 kg/da) and *Trifolium pratense* L. - *Festuca pratensis* L. (95.94 kg/da) (in the conditions of the Central Balkan Mountain).

The excess in the values of the indicator compared to the control is from 13.7%, 11.2% and 3.6%. The lowest yield values for crude protein in the second (52.50 kg/da) and third (74.54 kg/da) experimental year, as well as the average for the period (72.45 kg/da) was registered in the mixture of *Medicago sativa* L. with *Dactylis glomerata* L. The values of the trait are lower by 20.8% compared to the average for a three-year period (92.59 kg/da) and by 31.2% compared to the highest average value of the indicator.

A proven difference in the yield of crude protein (*P < 0.05*) was found between the *Medicago saivta* L. - *Dactylis glomerata* L. mixture and all the other mixed grasses included in the study (except for the *Trifolium repens* L. - *Lolium perenne* L. mixture).

There is also a proven difference in the yield of crude protein between the two-component mixtures of *Trifolium repens* L.

The amount of the trait in the grass stand with *Poa pratensis* L. (105.30 kg/da) is higher than that in the mixtures with *Lolium perenne* L. (88.71 kg/da) and is proportionally dependent on the higher share of the bean component in the volume of the grass mass.

The mixtures of *Trifolium pratense* L. have close average values of the indicator for the study period. With more distinct differences in the yield of crude protein are the variants of the legume crop in the third growing season. The crude protein content of the biomass of *Trifolium pratense* L. -*Festuca pratensis* L. mixture was 20.2% higher compared to that of *Trifolium pratense* L. - *Phleum pratense* L. (95.70 kg/da).

The soil profile in the experimental area does not meet the requirements of plants of the *Medicago saivta* L species. This is one of the main reasons for the unsustainable participation of the leguminous component (a decrease in the percentage share of the crop was registered by year) in the mixed with *Dactylis glomerata* L. and the formation of forage mass with the lowest values of crude protein.

The energy value of the fodder mass, expressed in terms of feed units for milk and growth, is the main criterion for modern assessment of fodder quality (Todorov, 1997).

In the first and second experimental years, as well as on average over the study period, the fodder mixture of *Trifolium pratense* L. with *Phleum pratense* L. had the highest yield of feed units for milk and growth (Table 2). Values of the indicators exceeded the averages respectively by:

- 8.27% (FUM) and 8.59% (FUG) - in the first experimental year;
- 24.3% (FUM) and 25.9% (FUG) - in the second experimental year;
- 9.5% (FUM) and 11.0% (FUG) - on average for the period 2020-2022.

For the study period, the yield of fodder units in the fodder mass of the two-component mixtures - *Lotus corniculatus* L.- *Festuca rubra* L., *Trifolium pratense* L.- *Festuca pratensis* L. and *Trifolium repens* L.- *Poa* *pratensis* L. also exceeded the average values of the indicator by 8.7%, 1.9% and 0.7% (for FUM) and 8.1%, 1.2% and 0.2% (for FUG), respectively.

Table 2. Yield of feed units (FUM and FUG) in the biomass of perennial two-component mixtures, over the years and average for the period 2014-2016 (MJ/da)

Variants	**2014**		**2015**		**2016**		**2014-2016**	
	FUM	FUG	FUM	FUG	FUM	FUG	FUM	FUG
L. corniculatus L.-*F. rubra* L.	469.80	428.30	402.90	368.90	673.20	613.80	517.10	472.10
Tr. repens L.-*L. perenne* L.	409.40	373.30	363.10	331.50	528.90	493.10	430.00	399.30
Tr. repens L.-*P. pratensis* L.	456.70	415.80	384.70	358.40	586.20	525.00	479.20	437.60
Tr. pratense L.-*Ph. pratense* L.	476.00	435.20	519.70	483.10	576.90	529.50	521.20	484.50
M. saivta L.-*D. glomerata* L.	413.80	376.80	353.20	316.30	500.40	455.60	422.50	384.60
Tr. pratense L.-*F. pratensis* L.	412.10	375.20	485.90	444.20	563.90	514.10	484.70	441.90
Average	***439.63***	***400.77***	***418.25***	***383.73***	***571.58***	***521.85***	***475.78***	***436.67***
Min	***409.40***	***373.30***	***353.20***	***316.30***	***500.40***	***455.60***	***422.50***	***384.60***
Max	***476.00***	***435.20***	***519.70***	***483.10***	***673.20***	***613.80***	***521.20***	***484.50***
CV	***7.1***	***7.2***	***16.4***	***17.2***	***10.4***	***10.1***	***8.8***	***9.0***
SD	***31.19***	***28.82***	***68.56***	***65.83***	***59.19***	***52.53***	***41.94***	***39.19***
$LSD_{0.05}$							***57.5***	***52.8***

In the first experimental year, higher content of fodder units were registred in comparison with the average values of the indicators in the dry matter of the two-component mixtures of *Lotus corniculatus* L. - *Festuca rubra* L. (469.80 - FUM and 428.30 - FUG) and *Trifolium repens* L. -*Poa pratensis* L. (456.70 - FUM and 415.80 - FUG). The careful and purposeful approach among them is a prerequisite for better distribution of components in plant communities and production of feed biomass with high economic value (Vîntu et al., 2011; Fattahi and Ildoromi, 2011; Zziwa et al., 2012; Iliev et al., 2022). In the second experimental year, an excess in the values of the studied indicators was registered in the mixed crops of *Trifolium pratense* L. with *Festuca pratensis* L. (485.90 - FUM and 444.20 - FUG), whereas in the third experimental year, higher values were found in the legume-grass grass stands of *Trifolium repens* L. with *Poa pratensis* L. (586.20 - FUM and 525.00 - FUG) and *Trifolium pratense* L. with *Phleum pratense* L. (576.90 - FUM and 529.50 - FUG).

A proven difference (*P <0.05*) was found in the yield of feed unit for milk and growth between the fodder mass of *Trifolium pratense* L. - *Phleum pratense* L. and that of the two-component mixtures - *Medicago saivta* L. - *Dactylis glomerata* L. (with 23.4% for FUM and 26.0% for FUG) and *Trifolium repens* L. - *Lolium perenne* L. (with 21.2% for FUM and 21.3% for FUG).

Species diversity in the composition of grass crops affects the quality and nutritional value of the fodder mass (Belesky et al., 1999; Sanderson et al., 2005; Foster et al., 2019). The percentage share of legume and grass components in grass mixtures is a significant factor for the formation of highly productive biomass to meet the needs of the livestock sector (Vasylenko et al., 2020).

In the present study, the the ratio of species (legume and grass meadow crops) in the mixed grass stands accounted for a significant share of the factorial variance in the studied traits (Table 3).

Table 3. Degree and significance of the factorial influences on the yield of CP, FUM and FUG in mixed grass stands of legume and grass meadow crops

Sources of variation / Traits	**Year and age of grass stand**		**Type of grass stand**	
	η^2 (%)	**P**	**η^2 (%)**	**P**
CP Yield	24.38	ns	53.4	P <0.01
Yield of FUM	20.15	ns	64.3	P <0.001
Yield of FUG	21.16	ns	63.2	P <0.001

Significance of differences at *P <0.05*, *P <0.01* and *P <0.001*; ns - not significant; η^2 - correlation ratio of factorial to total variance

The factor - type of grass stand had a significant impact (*P < 0.05 - P < 0.001*) on the values of the indicators: yield of crude protein (η^2 - 53.4%), yield of feed units for milk (η^2 - 64.3%) and feed units for growth (η^2 - 63.2%).

The analyzed data show also that the agro-ecological characteristics (temperature and precipitation in the area of the experiment) and the age of the examined grass stands did not significantly affect the values of the indicators - CP, FUM and FUG.

CONCLUSIONS

Crude protein yield in the investigated mixed grass stands exceeded the average value of the indicator (92.59 kg/da) by 3.6% (*Trifolium pratense* L. - *Festuca pratensis* L.) to 13.7% (*Trifolium repens* L. - *Poa pratensis* L.). The indicated variants are the only ones in which the legume component prevailed in the regrowth biomass throughout the experimental period in a ratio of legume and grass crops of 47.7:40.3% respectively (for *Trifolium pratense* L. - *Festuca pratensis* L.) and 62.3:13.9% (for *Trifolium repens* L. - *Poa pratensis* L.). A proven difference (P < 0.05) in crude protein yield was found only between *Trifolium repens* L. - *Poa pratensis* L. and *Medicago sativa* L. - *Dactylis glomerata* L. mixtures.

The fodder mass of the mixture *Trifolium pratense* L. with *Phleum pratense* L. had the highest yield of feed units for milk and growth. The values of both indicators exceeded the average (FUM - 475.78 MJ/da and FUG - 436.67 MJ/da) respectively with 9.5% (FUM) and 11.0% (FUG). There is a proven difference (*P < 0.05*) in the yield of feed units for milk and growth between the fodder mass of *Trifolium pratense* L.-*Phleum pratense* L. (FUM - 521.20 MJ/da and FUG - 484.50 MJ/da) and that of the two-component mixtures - *Medicago sativa* L. - *Dactylis glomerata* L. (FUM - 422.50 MJ/da and FUG - 384.60 MJ/da) and *Trifolium repens* L.-*Lolium perenne* L. (FUM - 430.00 MJ/da and FUG - 399.30 MJ/da) .

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

IMPACT OF CONVENTIONAL AND ORGANIC FERTILIZATION ON THE QUALITY AND NUTRITIONAL VALUE OF DEGRADED MOUNTAIN PASTURES

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Abstract

It was found that the dry mass of grasslands with organic fertilizing was by 2.0% higher in vitro digestibility of dry matter, higher content of CP (by 0.1%), CFr (by 1.7%) and N (by 0.1%), and a lower concentration of cellulose (by 0.3%) compared with of the mineral fertilizing variants, which led to a more in the concentration of NFE (by 1.2%), NDF (by 2.9%), hemicellulose (by 2.8%), Ca (by 0.3%) and P (0.2%). The energy nutritional value of fodder with of the mineral fertilizing of exceeded that of control with 0.4-2.8% and at organic fertilizing with 0.5-0.8%. A high correlation was found between the nitrogen content and CP (r = 1.0) of the grasslands with mineral fertilizing. The theoretical regression line and the equation of the regression dependence between the values of the indicators show y = 7.2224x -0.4841 at a high coefficient of determination (R^2 = 0.9988). For the variants with manure, the concentration of CP registered proven correlation (r = 1.0) and regression dependence with the nitrogen content: y = 6.9861x - 0.0815 at R^2 = 0.9999 ($P<0.05$).

***Key words**: mineral fertilizing, natural grassland, chemical composition.*

INTRODUCTION

Natural and semi-natural ecosystems are integrated communities in which through ecological continuity they lead to optimal stability and development of plant species (grasses, legumes, motley grasses). They are the main source of food for farm animals in Bulgaria and their condition significantly affects the productivity and nutritional value of the formed grassland.

Highly efficient agrotechnologies and systems improve the biological diversity of meadow phytocenoses and limit the spread of harmful invasive species. Desired changes in the individual groups and species of the botanical composition of the pastures were observed (Iliev et al., 2021). Changes in the botanical composition of grassland affect fodder yield and quality (Oelmann et al., 2011; Melnyk, 2014; Veklenko et al., 2015, Naydenova & Vasileva, 2019).

Mineral fertilizing provides a good nutritional balance of plants and significantly affects the dry matter yield in the composition of meadow and pasture grasslands (Chintala et al., 2012; Kacorzyk & Głąb, 2017). Nitrogen and phosphorus fertilizing stimulates growth and increases the percentage of legumes, and potassium improves biodiversity in the composition of natural grasslands (Liebisch et al., 2013). A high regression dependence (R^2 = 0.80-0.95) was found between the yield and the applied nitrogen fertilizing in the natural grasslands (Valkama et al., 2016). The generalized multifactor analysis shows a relatively high regression dependence (R^2 = 0.7-0.8) between the productivity of natural biomass with applied fertilizing and the climatic conditions of the region (April - July are critical for Bulgaria), which allows for an approximate forecast of fodder yield (Iliev et al., 2020). Annual fertilizing of natural meadows with the combination N_6P_6 leads to a proven increase in the amount of crude protein, crude fat, minerals and phosphorus in the dry matter of the harvested grassland. The values of the indicators exceed the untreated control by 52.3% (CP), 31.1% (CF), 3.1% (Ash) and 81.2% (P), respectively (Iliev et al., 2019).

Replacing mineral fertilizers with microbiological ones is one of the good practices set in the European programs and strategies for development in the field of environment and agriculture. In mountain regions, the use of organic fertilizers is an economically driven practice (as a source of nutrients in the soil) for the production of organic products (Demyanchik, 2012; Kurgak, 2013; Vasileva, 2015; Hynes et al., 2018). Fertilizing with organic fertilizer increases the productivity of dry matter and positively affects the botanical composition (significantly increases the share of grasses and motley grasses, reduces that of legumes) in natural grasslands (Iliev et al., 2017; Iliev, 2018). The ecological balance and the rich floristic composition of the natural grasslands contribute to the formation of an above-ground mass with higher nutritional value, rich in protein, energy, minerals (P, K, Ca, Mg) and with high digestibility of dry matter (Lemaire & Belanger, 2020; Churkova & Churkova, 2020; Churkova & Churkova, 2021).

The aim of the present study is to establish the impact of the annual mineral organic surface fertilizing on the quality and nutritional value of degraded natural grassland in the region of the Central Balkan Mountain.

MATERIALS AND METHODS

The experiment was conducted at the Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Bulgaria) in the period 2016-2019, on degraded grassland, in the conditions of the Central Balkan Mountain. The experiment was based on the block method, in 4 replications with the size of the harvest plot - 5 m^2. Variants in the study were:

Mineral fertilizing

1. Control (untreated)
2. N_8P_8 (annual combined fertilizing - from March 20 to April 10);
3. $N_{10}P_{10}$. (annual combined fertilizing - from March 20 to April 10);
4. N_8P_8 (annual combined fertilizing - from March 20 to April 10);
5. N_7/I P_7/II P_7/III/N_7/IV (individual fertilizing with N_7 - first and fourth experimental years and individual fertilizing with P_7 - second and third experimental years);
6. P_6/I N_6/II P_6/III/ N_6/IV (individual fertilizing with P_6 - first and third experimental years and individual fertilizing with N_6 - second and fourth experimental years).

For variants 5 and 6, fertilizing with double superphosphate and ammonium nitrate was performed once in autumn (September-October) and spring (April).

***Legend:** I -first experimental year; II - second experimental year; III - third trial year and IV - fourth trial year.

Fertilizing with manure

1. Control (untreated)
2. 10 t ha^{-1};
3. 20 t ha^{-1};
4. 30 t ha^{-1};
5. 40 t ha^{-1}.

The manure treatment was applied annually and once, every year, manually, by spraying, before the onset of active vegetation in the grass cover. The experimental areas were harvested in the phenophase of tasseling/ear formation (for grass species) until the beginning of the phenophase of flowering.

Studied indicators

- Crude protein (CP,%) according to Kjeldahl (according to BDS/ISO-5983); Crude fiber (CFr, %); Crude fats (CF, %) (according to BDS/ISO-6492) - by extraction in an extractor type Soxhlet; Ash (%) - (according to BDS/ISO-5984) decomposition of organic matter by gradual combustion of the sample in a muffle furnace at 550°C; Dry matter (DM, %) - empirically calculated from % of moisture; NFE (%, DM) = 100 - (CP, % + CFr, % + CF, % + Ash, % + Moisture, %); Calcium (Ca,%) - complexometrically and Phosphorus (P,%) - with vanadate-molybdate reagent - spectophotometer (Agilent 8453 UV - visible Spectroscopy System), measuring in the range 425 ηm.
- Neutral detergent fibers (NDF, %); Acid detergent fibers (ADF, %) and Acid detergent lignin (ADL, %) according to the detergent analysis of Van Soest & Robertson (1979).
- *In vitro* dry matter digestibility (IVDMD, %) bythe two-stage pepsin-cellulase method of Aufrere (1982), which includes two stages: I - Pre-treatment with pepsin / 200 FIB-U g-1 /, Merck 7190, in 1 N hydrochloric acid for 24 hours (for protein digestion) and II - Treatment in acidic medium with the enzyme cellulase

"Onozuka R-10" isolated from *Trihoderma viride* /Endo-1.4 -β-glucanase; 1.4- (1.3:1.4) - β-D glucan-4-glucan hydrolase / with enzymatic activity 1.2 U g-1, 1 g 1L in 0.05 M acetate buffer pH 4.6 for 24 hours at 40°C (for cellulose digestion).

- Empirical calculation of: Hemicellulose (%) = NDF - ADF and Cellulose (%) = ADF - ADL and the degree of lignification (coefficient) - percentage of ADL and ADF (Akin & Chesson, 1990).
- The nutritional value of the fodder was assessed by the Bulgarian system as Feed Unit for Milk (FUM) and Feed Unit for Growth (FUG) and calculated on the basis of equations according to the experimental values of CP, CF, Cft and NFE, recalculated by the coefficients for digestibility by Todorov (2010): Gross energy (GE, MJ/kg) = 0.0242*CP + 0.0366*Cft + 0.0209*CF + 0.017*NFE – 0.0007*Zx and Exchangeable energy (EE, MJ/kg) = 0.0152*DP (Digestible protein) + 0.0342*Dft (Digestible fat) + 0.0128*DF (Digestible fibers) + 0.0159*DNFE (Digestible Nitrogen-free extractable substances)-0.0007*Zx.

The results were analyzed by the method of analyzing the variance of a single-factorial trial (ANOVA) using the SPSS 4.5 software. The significance of differences in mean values of the treatments was tested by the LSD test.

RESULTS AND DISCUSSIONS

Quality and nutritional value of degraded grass feed after applied mineral and organic fertilization

Mineral fertilizing

Productivity and quality composition of grassland in natural meadows and pastures are related to environmental conditions, use regime, type and norms of applied fertilizing (Kesting et al., 2009; Pasho et al., 2011; Sîrbu et al., 2012).

In the variants with mineral fertilizing (exception is P_6/I N_6/ II P_6/III / N_6/IV) the content of crude protein exceeds the control by from 5.2% ($N_{10}P_{10}$) to 28.4% (N_8P_8) (Table 1). The annual import of the combination of $N_{12}P_{12}$ increased the highest concentrations of calcium (3.2%) and phosphorus (0.5%). The excess in the values of the indicators compared to the untreated control was by 2.1% (Ca) and 0.3% (P), respectively. The variants with alternative alternation of nitrogen and phosphorus (N_7/I P_7/II P_7/III / N_7/IV) registered the lowest content of crude fiber (34.1%) and the highest concentration of nitrogen-free extractable substances (34.1%) in the dry matter. The harvested biomass from the variant with $N_{12}P_{12}$ had a maximum content of dry matter (90.1%), crude fiber (44.9%) and the lowest concentration of NFE (22.1%).

Table 1. Basic chemical composition (%) of dry matter of degraded mountain grassland, after mineral and organic fertilizing

Variants	DM	CP	CF	CFr	Ash	NFE	Ca	P	N
Mineral fertilizing									
Control	90.0	11.6	3.1	37.3	6.9	31.1	1.1	0.2	1.7
$N_{12}P_{12}$	90.1	13.6	3.0	44.9	6.6	22.1	3.2	0.5	2.0
$N_{10}P_{10}$	90.0	12.2	2.7	40.5	7.2	27.3	1.7	0.4	1.8
N_8P_8	88.9	14.9	2.2	42.3	6.6	22.8	1.9	0.4	2.1
N_7/I P_7/IIP_7/III/ N_7/IV	89.9	13.4	1.8	34.1	6.7	34.1	1.8	0.3	1.9
P_6/I N_6/IIP_6/III/ N_6/IV	90.0	11.6	1.9	42.0	6.7	27.8	1.8	0.3	1.7
Average	***89.8***	***12.9***	***2.5***	***40.2***	***6.8***	***27.5***	***1.9***	***0.4***	***1.8***
SD	***0.5***	***1.3***	***0.6***	***3.9***	***0.2***	***4.6***	***0.7***	***0.1***	***0.2***
$LSD_{0.05}$	***0.9***	***2.2***	***1.1***	***7.2***	***0.4***	***8.4***	***3.2***	***0.1***	***0.3***
Organic fertilizing									
Control	89.9	12.5	2.3	38.8	6.5	29.7	1.6	0.2	1.8
10t ha^{-1}	90.1	12.0	2.4	42.8	6.8	26.0	1.8	0.2	1.7
20t ha^{-1}	90.0	14.1	2.0	41.3	6.6	26.1	1.5	0.1	2.0
30t ha^{-1}	90.2	12.0	1.4	44.4	7.0	25.5	1.6	0.1	1.7
40t ha^{-1}	90.0	14.4	2.3	42.0	7.2	24.1	1.6	0.1	2.1
Average	***90.0***	***13.0***	***2.1***	***41.9***	***6.8***	***26.3***	***1.6***	***0.2***	***1.9***
SD	***0.1***	***1.1***	***0.4***	***2.0***	***0.3***	***2.1***	***0.1***	***0.1***	***0.2***
$LSD_{0.05}$	***0.3***	***0.7***	***0.7***	***4.4***	***0.5***	***4.5***	***0.3***	***0.1***	***0.4***

Organic fertilizing

The dry matter content (90.0-90.2%) in the stands with applied manure was higher by 0.1-0.3% compared to the control. The annual treatment of the natural mass with a dose of 40 t ha^{-1} most significantly affects the content of: crude protein (14.4%), minerals (7.2%) and nitrogen (2.1%).

The values of the indicator exceeded the control by 1.9% (CP), 0.7% (Ash) and 0.3% (N), respectively. With the lowest concentration of the protein fraction (12.0%) and respectively the highest fiber are the grasslands in the variants with imported 10 t ha^{-1}(CFr - 42.8%) and 30 t ha^{-1} (CFr - 44.4%) manure. The content of the macroelement Ca in the treated grasses varies from 1.5% (20 t ha^{-1}) to 1.8% (10 t ha^{-1}).

On average for the period, the dry mass of stands with organic fertilization has a higher content of crude protein (13.0%), crude fiber (41.9%) and nitrogen (1.9%) compared to those with mineral fertilization. The excess in the average values of the indicators by 0.1%, 1.7% and 0.1%, respecttively. Feeding the grassland with N and P (annually combined and successively alternating) increased the concentration of carbohydrate components (to 27.8%) and the content of macronutrients Ca (to 1.9%) and P (to 0.4%). Compared to the variants with imported manure, the excess in the average values of the traits is respectively by 1.2%, 0.3% and 0.2%.

Floristic composition influences the assessment of the nutritional value in the pastures. The dynamics of development during the vegetation, changes in the chemical composition and digestibility of individual plant species are indicators related to the uptake and assimilation of feed by ruminants (Andueza et al., 2010; Dale et al., 2012).

Grasslands with applied mineral fertilization are characterized by a higher content of neutral and acid-detergent fibers compared to the untreated control (Table 2). The excess in the values of the indicators varies from 3.1% to 7.2% (for NDF) and from 0.4% to 3.4% (for ADF). The data from the study show that the dry mass of the variants with annual combined fertilization and self-fertilization with N_7 (first and fourth experimental years) and P_7 (second and third experimental years) has a high concentration of acid-detergent lignin. The excess over the control was 1.2% ($N_{12}P_{12}$), 3.4% ($N_{10}P_{10}$) and 10.5% (N_7/I P_7/II P_7/III N_7/IV).

Table 2. Main structural fiber components (%) of cell walls and in vitro digestibility of dry matter of degraded mountain grassland, after applied mineral and organic fertilization

Variants	NDF	ADF	ADL	Hemicel	Cellul	IVDMD
Mineral fertilizing						
Control	59.0	39.4	12.2	19.6	27.2	58.8
$N_{12}P_{12}$	66.2	42.8	13.4	23.4	29.4	43.3
$N_{10}P_{10}$	62.8	39.8	15.6	22.9	24.3	58.7
N_8P_8	62.1	40.5	10.2	21.6	30.3	58.2
N_7/IP_7/IIP_7/III/ N_7/IV	62.7	41.4	22.7	21.3	18.7	57.8
P_6/I N_6/IIP_6/III/ N_6/IV	65.3	41.9	10.9	23.4	31.0	56.7
Average	***63.0***	***41.0***	***14.2***	***22.0***	***26.8***	***55.6***
SD	***2.6***	***1.3***	***4.6***	***1.5***	***4.7***	***6.1***
$LSD_{0.05}$	***6.9***	***3.8***	***9.5***	***4.1***	***7.8***	***3.0***
Organic fertilizing						
Control	56.9	39.0	12.5	17.9	26.5	59.1
10t ha^{-1}	56.1	38.4	11.8	17.8	26.6	60.1
20t ha^{-1}	56.7	34.3	10.1	24.4	24.2	63.2
30t ha^{-1}	62.3	41.7	22.3	20.6	19.4	57.3
40t ha^{-1}	68.5	53.0	25.9	15.5	27.1	48.5
Average	***60.1***	***41.2***	***16.5***	***19.2***	***24.7***	***57.6***
SD	***5.1***	***7.1***	***7.1***	***3.4***	***3.2***	***5.5***
$LSD_{0.05}$	***10.2***	***13.1***	***12.1***	***6.5***	***5.3***	***5.9***

The values of the indicator are lower in the variants treated with reduced doses of macronutrients, imported in combination (N_8P_8 -10.2%) and alone (P_6/I N_6/II P_6/III N_6/IV - 10.9%). The amount of fully digestible by farm animals polioside - hemicellulose is higher in all treated grasses compared to that of untreated. The excess in the values of the attribute is from

1.7% (N_7/I P_7/II P_7/III N_7/IV) to 3.8% ($N_{12}P_{12}$ and P_6/I N_6/II P_6/III N_6/IV) compared to that of the control (19.6%). The dry matter of self-fertilized stands of N_7 (first and fourth years) and P_7 (second and third experimental years), and those with self-fertilization of P_6 (first and third years) and N_6 (second and fourth years) is respectively with the most low (18.7%) and highest (31.0%) cellulose content, at a control (27.2%). The analyzed data show lower *in vitro* digestibility (43.4-58.7%) of the stands formed in the variants with mineral fertilization at a standard control (58.8%).

The variants with lower doses of manure (10 and 20 t ha^{-1}) are characterized by reduced content of neutral detergent fibers (by 0.2-0.8%), acid detergent fibers (by 0.6-4.7%) and acid detergent lignin (by 0.7-2.4%), which determines the sufficiently high *in vitro* digestibility of dry matter for obtaining quality fodder for ruminants (60.1% and 63.2%). The one-time application of manure (dose 20 and 30 t ha^{-1}) before the onset of active vegetation for the species in the natural grasslands had a positive effect on the concentration of hemicellulose in the dry matter. The excess in the values of the indicator compared to the control is from 2.7% to 6.5%. Grasslands treated with higher doses of organic fertilizer (30 and 40 t ha^{-1}) showed a higher fiber content of basic structural fiber components (NDF, ADF and ADL) in the cell walls and lower digestibility of the dry matter. Empirically calculated values of cellulose varied from 19.4% (30 t ha^{-1}) to 27.1% (40 t ha^{-1}) in the control variant - 26.5%.

On average for the period, the grasslands of the variants with organic fertilizing were characterized by 2.0% higher *in vitro* digestibility of the dry matter and 2.1% lower concentration of cellulose (polyoside - partially digestible by animals) compared to the variants treated with different doses of N and P. The dry mass of the variants with mineral fertilizing has a higher content of neutral detergent fibers (by 0.7%) and hemicellulose (by 2.8%) compared to that of the variants fertilized with manure.

The quality of plant matter is closely dependent on the biological process of lignification - a major factor limiting the nutritional value of fodder and inhibiting digestibility (Casler & Jung, 2006). The lignification coefficient in the studied degraded mountain grassland, after applied mineral fertilizing varied from 13.8 ($N_{12}P_{12}$) to 32.9 (N_7/I P_7/II P_7/IIIN_7/IV) (Figure 1). Variants with $N_{12}P_{12}$, N_8P_8 and P_6/I N_6/II P_6/III N_6/IV had a lower degree of lignification compared to the control by 6.8%, 4.1% and 3.8%, respectively.

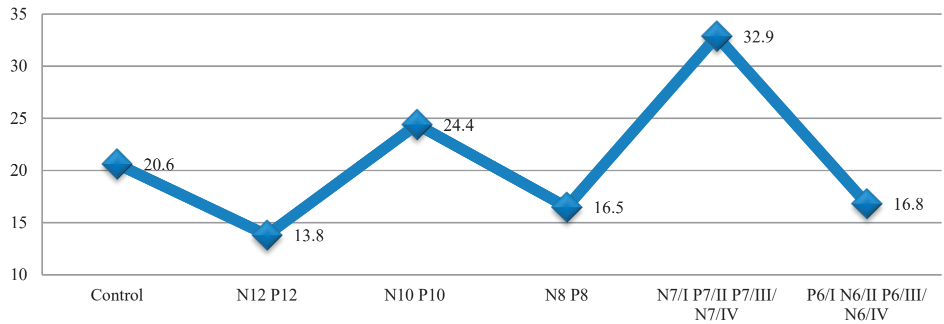

Figure 1. Degree of lignification of degraded mountain grassland after mineral fertilizing (coefficient)

In the variants with organic fertilizing (10 and 20 t ha^{-1}) the values of the lignin coefficient were reduced by 0.9 to 4.7% (Figure 2). The grasslands with higher doses (30 and 40 t ha^{-1}) of organic fertilizer register higher lignification coefficient (35.8 and 37.8) compared to the control (21.9).

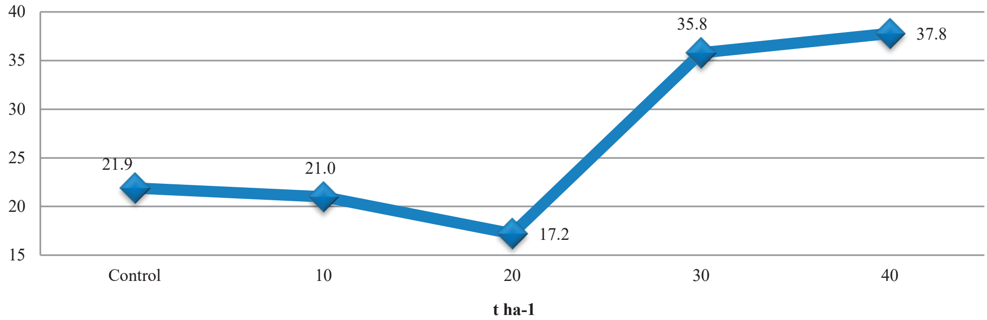

Figure 2. Degree of lignification of degraded mountain grassland after applied organic fertilizing (coefficient)

The biological value of fodder largely depends on the energy nutritional value and some other characteristics that affect the productivity of animals. The diversity and changes in the botanical composition of natural grasslands, as a result of the agrotechnical events, affect the amount of gross and metabolic energy, as well as the number of forage units in the dry matter of the grass matter treated with mineral and organic fertilizers.

The total energy value of the plant matter from the variants with annual combined fertilizing of N and P exceeds that of the untreated control by 0.4% ($N_{10}P_{10}$), 2.1% (N_8P_8) and 2.8% ($N_{12}P_{12}$) (Table 3).

Only the individual fertilizing with N_7 (first and fourth years) and P_7 (second and third years) registered higher values of exchange energy (8.04 MJ/kg DM) and feed units (FUM - 0.74 pieces in DM and FUG - 0.67 pieces in DM) compared to the control.

Table 3. Potential energy nutritional value of degraded mountain grassland, after applied mineral and organic fertilizing

Variants	GE	EE	FUM	FUG
Mineral fertilizing				
Control	18.91	7.86	0.72	0.64
$N_{12}P_{12}$	19.44	7.47	0.67	0.59
$N_{10}P_{10}$	18.98	7.63	0.69	0.61
N_8P_8	19.30	7.56	0.68	0.60
N_7/IP_7/IIP_7/III/ N_7/IV	18.67	8.04	0.74	0.67
P_6/I N_6/IIP_6/III/ N_6/IV	18.90	7.56	0.69	0.61
Average	***19.03***	***7.69***	***0.70***	***0.62***
SD	***0.28***	***0.22***	***0.02***	***0.03***
Organic fertilizing				
Control	18.96	7.79	0.71	0.63
10t ha^{-1}	19.06	7.53	0.68	0.60
20t ha^{-1}	19.09	7.64	0.69	0.61
30t ha^{-1}	18.86	7.38	0.67	0.58
40t ha^{-1}	19.11	7.54	0.68	0.60
Average	***19.02***	***7.58***	***0.69***	***0.61***
SD	***0.10***	***0.15***	***0.02***	***0.02***

GE - Gross energy (MJ/kg DM); ME - Metabolizable energy (MJ/kg DM); FUM - Feed unit for milk (pieces in DM); FUG - Feed units for growth (pieces in DM); ($P<0.05$)

In grasslands with organic fertilizing, a more significant effect of the applied treatment was observed on the amount of gross energy. The values of the indicator exceed the untreated control by 0.5% (10 t ha^{-1}), 0.7% (20 t ha^{-1}) and 0.8% (40 t ha^{-1}), respectively. The effect of manure does not lead to significant changes in the empirically calculated content of metabolic energy and feed units in the dry matter composition.

Correlation and regression dependences between basic qualitative indicators of natural grasslands treated with mineral and organic fertilizers

A high correlation dependence was found (Table 4) between the nitrogen content and the amount of crude protein (r = 1.0) in the dry matter of the grasslands with mineral fertilizing. The theoretical regression line and the equation of the regression dependence between the values of the indicators are shown in Figure 3, where $y = 7.2224x - 0.4841$ at high coefficient of determination - $R^2 = 0.9988$ (P <0.05).

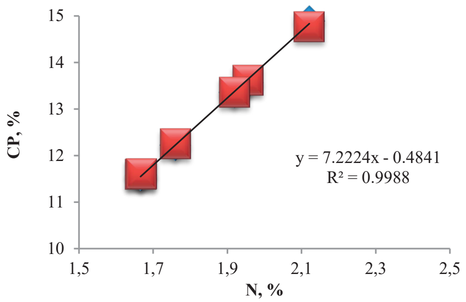

Figure 3. Regression dependence between the amount of nitrogen (%) and the content of crude protein (%) in dry matter of degraded mountain grassland, after applied mineral fertilizing

The effective utilization of natural grasslands and their nutritional value are closely related to the analysis of the basic chemical composition and the composition of the components of the cell walls. The amount of crude fiber is very well correlated with the empirically calculated values of hemicellulose (r = 0.7) cellulose (r = 0.9), and the content of nitrogen-free extracts and acid-detergent lignin with indicators characterizing the energy nutritional value (r = 0.9 for EE, FUM and FUG) of mountain grassland. Correlation dependences of the above features are completely opposite for the variants with organic fertilizing (Table 5). The concentration of the fiber fraction (CFr) strongly correlates with the values of hemicellulose (r = -0.2), cellulose (r = -0.7), gross energy (r = -0.9), exchange energy, FUM and FUG (r = -1.0). This tendency is maintained with regard to the dependence of lignin with the energy indicators of the dry fodder mass (r = from -0.6 to -0.3). In the variants with imported manure, the concentration of crude protein is in a very good correlation (r = 1.0) and regression dependence with the percentage of the macroelement nitrogen at a high coefficient of determination (R^2 = 0.9999) and a statistically proven equation: $y = 6.9861x - 0.0815$ (P <0.05) (Figure 4).

Table 4. Correlation dependences among main qualitative indicators of natural grasslands, treated with mineral fertilizers

	DM	CP	CF	CFr	NFE	Ca	P	N	NDF	ADF	ADL	Hemi	Cellul	GE	EE	FUM	FUG
DM	1																
CP	-0.8	1															
CF	0.2	0.1	1														
CFr	-0.1	0.1	0.7	1													
NFE	0.3	-0.4	-0.7	-0.9	1												
Ca	0.2	0.3	0.6	0.6	-0.6	1											
P	0.1	0.1	**1.0***	0.7	-0.7	0.5	1										
N	-0.7	**1.0***	0.1	0.1	-0.4	0.3	0.1	1									
NDF	0.6	-0.4	0.4	0.6	-0.3	0.7	0.2	-0.4	1								
ADF	0.4	-0.1	0.1	0.3	-0.2	0.8	-0.2	0.0	**0.9***	1							
ADL	0.4	-0.1	-0.3	-0.9	0.8	-0.2	-0.4	0.0	-0.3	-0.1	1						
Hemicellulose	0.6	-0.6	0.5	0.7	-0.4	0.4	0.5	-0.6	0.8	0.4	-0.5	1					
Cellulose	-0.3	0.1	0.3	**0.9***	-0.8	0.3	0.3	0.0	0.5	0.3	-1.0	0.5	1				
GE	-0.3	0.5	0.7	**0.9***	-1.0	0.7	0.7	0.5	0.3	0.3	-0.7	0.3	0.7	1			
EE	0.1	0.0	-0.6	-1.0	**0.9***	-0.5	-0.7	0.0	-0.5	-0.2	**0.9***	-0.7	-0.9	-0.8	1		
FUM	0.1	0.0	-0.7	-1.0	**0.9***	-0.5	-0.7	0.0	-0.5	-0.2	**0.9***	-0.7	-0.9	-0.8	**1.0***	1	
FUG	0.1	-0.1	-0.7	-1.0	**0.9***	-0.5	-0.7	-0.1	-0.5	-0.2	**0.9***	-0.7	-0.9	-0.9	**1.0***	**1.0***	1

(P<0.05)

Table 5. Correlation dependences among main qualitative indicators of natural grasslands, treated with organic fertilizers

	DM	CP	CF	CFr	Ash	NFE	Ca	P	N	NDF	ADF	ADL	Hemi	Cellu	DMD	GE	EE	FUM	FUG
DM	1																		
CP	-0.9	1																	
CF	-0.6	0.3	1																
CFr	**1.0***	-0.8	-0.6	1															
Ash	0.0	0.2	0.1	0.3	1														
NFE	0.3	-0.5	-0.2	0.0	-0.9	1													
Ca	0.3	-0.5	0.5	0.3	0.2	0.0	1												
P	0.1	-0.5	0.6	0.0	-0.2	0.4	**0.9***	1											
N	-0.9	**1.0***	0.3	-0.8	0.2	-0.5	-0.5	-0.5	1										
NDF	-0.3	0.5	-0.1	0.0	**0.9***	-1.0	-0.3	-0.6	0.5	1									
ADF	-0.2	0.4	0.2	0.1	1.0	-1.0	0.2	-0.3	0.4	**0.9***	1								
ADL	0.1	0.2	-0.3	0.4	**0.9***	-0.9	0.0	-0.5	0.2	**0.9***	**0.9***	1							
Hemicell	0.0	0.0	-0.5	-0.2	-0.8	0.7	-0.7	-0.3	0.0	-0.5	-0.8	-0.6	1						
Cellulose	-0.7	0.5	**1.0***	-0.7	0.2	-0.3	0.4	0.5	0.5	0.1	0.3	-0.1	-0.5	1					
DMD	0.2	-0.4	-0.2	-0.1	-1.0	**1.0***	-0.2	0.3	-0.4	-0.9	-1.0	-0.9	0.8	-0.3	1				
GE	-0.9	0.7	**0.9***	-0.9	-0.1	-0.2	0.1	0.2	0.7	0.0	0.1	-0.3	-0.2	**0.9***	-0.1	1			
EE	-0.9	0.7	0.6	-1.0	-0.4	0.2	-0.2	0.1	0.7	-0.2	-0.3	-0.6	0.3	0.6	0.3	**0.9***	1		
FUM	-0.9	0.7	0.6	-1.0	-0.5	0.2	-0.3	0.1	0.7	-0.2	-0.3	-0.6	0.3	0.6	0.3	**0.9***	**1.0***	1	
FUG	-0.9	0.7	0.6	-1.0	-0.5	0.2	-0.3	0.1	0.7	-0.2	-0.3	-0.6	0.4	0.6	0.3	**0.9***	**1.0***	**1.0***	1

(P<0.05)

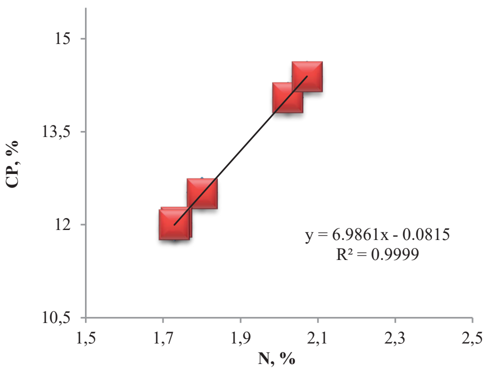

Figure 4. Regression dependence between the amount of nitrogen (%) and the content of crude protein (%) in dry matter of degraded mountain grassland, after applied organic fertilizing

A high correlation was found between the dry matter content in the fodder (treated with manure) and the amount of crude fiber (r = 1.0) and strongly negative with the content of GE, EE, FUM, FUG, crude protein and nitrogen (r = -0.9).

The values of the indicators (GE, EE, FUM and FUG) influencing the potential energy nutritional value of the grass mass are in good correlation (r = 0.7) with the concentration of crude protein and nitrogen.

The amounts of fully digestible polyoside - hemicellulose and partially digestible polyoside - cellulose are positively correlated respectively with the digestibility of the dry matter (r = 0.8) and the total energy value (r= 0.9) of the biomass treated with bovine manure.

CONCLUSIONS

On average for the period, the dry matter of the grasslands with organic fertilizing had a lower concentration of cellulose (by 0.3%), higher content of CP (by 0.1%), CFr (by 1.7%), N (by 0.1%) and higher *in vitro* dry matter digestibility (by 2.0%) compared to the variants with mineral fertilizing. Increase in the concentration of carbohydrate components (by 1.2%), neutral detergent fibers (by 2.9%), hemicellulose (by 2.8%), Ca (by 0.3%) and P (0.2%) was observed in the dry matter of the stands with annual combined and alternating fertilizing with nitrogen and phosphorus.

The energy nutritional value of the fodder, after annual combined fertilizing with N and P, exceeded that of the untreated control by 0.4% ($N_{10}P_{10}$), 2.1% (N_8P_8) and 2.8% ($N_{12}P_{12}$). The organic fertilizing had a greater effect on the amount of gross energy. The values of the indicator exceeded the untreated control by 0.5% (10 t ha^{-1}), 0.7% (20 t ha^{-1}) and 0.8% (40 t ha^{-1}), respectively.

In the stands with mineral fertilization and organic fertilization, the content of N and CP are in proven high correlation (r = 1.0) and regression (R^2 = 0.9988-0.9999) dependence.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ANTIFUNGAL AND ANTI-MYCOTIC PROPERTIES OF ESSENTIAL OILS EXTRACTED FROM DIFFERENT PLANTS ON PATHOGENIC FUNGI THAT BIOSYNTHESIZE MYCOTOXINS

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Abstract

The current scientific paper presents a review of essential oils with antifungal effect, the mechanism of action of the main components of essential oils and the possible synergistic actions between them on pathogens. Essential oils of oregano, cinnamon, fennel, mint and dill in various concentrations have demonstrated effects on ergosterol biosynthesis, specifically reducing the amount of ergosterol. Thus, a significant change in ergosterol biosynthesis will inhibit the growth of fungi and cause their death. An advantage of using essential oils over synthetic ones is the use of small amounts of essential oils to achieve strong fungistatic and fungicidal effects. In conclusion, through this work we tried to make: an enumeration / revision of volatile oils with bio pesticidal potential, highlighting the effects produced on fungi from the Aspergillus, Fusarium and Penicillium families, but also on the mycotoxins biosynthesized by them.

Key words: *agricultural products, antifungal effect, antimycotoxin effect, essential oil.*

INTRODUCTION

Losses of agricultural products due to fungal contamination create big problems in both underdeveloped and developed countries. The problems created by fungal contamination occur throughout the food chain. These problems resulting from the contamination are of an economic nature (quantitative and qualitative losses of the obtained products), ecological (pollution of the environment with toxic residues following the use of synthetic fungicides) and sanitary one (biosynthesis of numerous mycotoxins such as aflatoxins, fumonisines, ochratoxins, zearalenones, etc.) (El Khoury et al., 2016; Lai et al., 2021).

About 25-40% of the cereals consumed worldwide are contaminated with mycotoxins. Of the mycotoxins, the aflatoxins produced mainly by Aspergillus flavus are the most dangerous, and about 4.5 billion people in underdeveloped countries are exposed to aflatoxicosis (Střelková et al., 2021).

Direct exposure of consumers to secondary metabolites/mycotoxins leads to serious health problems due to their carcinogenic, immunosuppressive, nephrotoxic, teratogenic and mutagenic attributes; Aflatoxin B1, among the most common mycotoxins, is classified in group 1 carcinogen by the International Agency for Research on Cancer. The major classes of mycotoxins of the highest agro-economic importance are aflatoxins, ochratoxins, fumonisins, trichothecenes, fusarium emerging mycotoxins, ennantines, ergot alkaloids, alternaria toxins and patulin (Agriopoulou et al., 2020; Bennett & Klich, 2003; Omotayo et al., 2019; Ponzilacqua et al., 2018).

Due to its effectiveness and ease of application, the main approach currently used to control pathogenic fungi, harmful insects, etc., in agriculture is the use of synthetic chemicals (based on: carbamates and dithiocarbonates, benzimidazoles, imidazole and triazoles, morpholines, phenylpyrrole, etc.). They have negative effects on the environment (soil, water and air pollution) but also on consumers, due to the chemical residues resulting from their use; chemical residues that will bioaccumulate in soil, water and in living organisms (organs, muscle tissue, adipose tissue, milk) and that cause toxic, carcinogenic, allergenic efficiencies, negative effects on the endorin, reproductive, gastrointestinal, respiratory and neurological systems of consumers (Aimad et al., 2022; Aktar et al., 2009; Li et al., 2022; Nicolopoulou-Stamati et al., 2016; Regulation (EC) No 1185/2009 of the European Parliament and of the Council of 25 November 2009 on statistics on pesticides, n.d.).

Annually, about 2 million tons of pesticides are used worldwide. Of the 2 million tons of pesticides, 17.5% are fungicides. China is the largest contributing country, followed by the USA, Argentina, Thailand, Brazil, Italy, France, Canada, Japan and India. However, by 2020, global pesticide use has been estimated to increase by up to 3.5 million tons per year (Kumar et al., 2021; Sharma et al., 2019). The share of global pesticide consumption from 2015 to 2018 was 52.2% for Asia, 32.4% for the US, 11.8% for Europe and 2% for Asia (Kumar et al., 2021). In Romania, between 2007 and 2020, the surfaces on which chemicals were applied increased: by 42.9% in the case of insecticides; by 52.2% in the case of fungicides and by 31.1% in the case of herbicides (Popescu et al., 2021).

Substances of natural origin, such as essential oils, obtained from various medicinal plants can represent, nowadays, an important ecological reservoir. The use of volatile oils, as biopesticides, in agriculture, especially in post-harvest practices, can be a more sustainable solution for protecting crops, the environment and last but not least protecting the health of people and animals/consumers. Many studies demonstrate the antifungal and antimicrobial properties of the main bioactive compounds of essential oils. Biopesticides, such as volatile oils, are highly effective in small quantities and decompose quickly without leaving potentially toxic residues in the environment (Achimón et al., 2021; Agriopoulou et al., 2020; Bota et al., 2022; El Khoury et al., 2016; Esper et al., 2014; Gwiazdowska et al., 2022; Han et al., 2022; Hua et al., 2014; Kalagatur et al., 2020; Kumar et al., 2021; Oliveira et al., 2020; Ozcakmak et al., 2017; Perczak et al., 2019; Střelková et al., 2021; Sumalan et al., 2013; Y. Wang et al., 2018).

The purpose of this review is to present the mode of action of essential oils with effects on pathogenic fungi in agriculture. It also highlights the necessity and importance of using essential oils as future biopesticides on pathogenic fungi that cause economic, ecological and sanitary damage in agriculture.

CHEMICAL COMPOSITION AND PHYSICO-CHEMICAL PROPERTIES OF ESSENTIAL OILS

Essential oils are mixtures of volatile compounds (sometimes with over 300 different compounds), which are secondary metabolites of plants. These secondary metabolites, together with the mineral elements, play an important role in the defence system of higher plants (Beicu et al., 2021; Dhifi et al., 2016; Ebani & Mancianti, 2020; Imbrea et al., 2016; Karpiński, 2020).

From a structural point of view, the chemical constituents identified in the composition of essential oils extracted from different parts of plants or even the whole plant, can be classified into four groups: terpenes, terpenoids, phenylpropanoids and other constituents (amino acids, lipids, sulphur derivatives, etc. (Masyita et al., 2022).

Terpenes (isoprenesides) - are the major constituents found in the composition of essential oils. From the chemical point of view terpenes are molecular structures that contain 2-methylbuta-1,3-diennial carbon skeletons (isoprene units) that can be rearranged in cyclic structures, depending on the number of isoprene units that make up the terpenes, there are: hemiterpenes - are formed by a unit of isoprene (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30) and tetraterpenes (C40) (Agriopoulou et al., 2020; Aktar et al., 2009; El Khoury et al., 2016; Lai et al., 2021; Li et al., 2022; Ponzilacqua et al., 2018).

Terpenoids - these are another type of terpenes that contain oxygen molecules. Terpenoids can be divided into alcohols, aldehydes, esters, ethers, epoxides, ketones and phenols. Examples of terpenoids most often identified in the composition of essential oils are: carvacrol, citronellal, geraniol, linalool, linalool acetate, piperitone, menthol and thymol. The compounds listed above are those that provide the important biological property of essential oils (Beicu et al., 2021; Bhavaniramya et al., 2019; Chouhan et al., 2017; Dhifi et al., 2016; Masyita et al., 2022; Nazzaro et al., 2013;

Rassem et al., 2016; Sánchez-González et al., 2011).

In the group of **phenylpropanoids** there are aromatic compounds such as: anethole, cinnamaldehyde, eugenol, isoeugenol, myristicin, safrole and vanillin. According to recent research on these components, their antibacterial, antitumor, antifungal, antiproliferative, antidiabetic, analgesic, anti-inflammatory, etc. effect has been highlighted (Chouhan et al., 2017; Masyita et al., 2022; Nazzaro et al., 2013; Sánchez-González et al., 2011). As a percentage, essential oils contain 85-99% volatile components and 1-15% non-volatile components. Volatile constituents are a mixture of terpenes, terpenoids and other aromatic and aliphatic constituents (Ciobotaru et al., 2017; Sadgrove et al., 2021; Sánchez-González et al., 2011).

In terms of solubility, essential oils are soluble in alcohol, ether and other oils, but insoluble in water (Bhavaniramya et al., 2019; Dhifi et al., 2016; Rassem et al., 2016).

ESSENTIAL OILS' ANTIFUNGAL AND ANTIMICOTOXINIC MECHANISM OF ACTION

The effect of essential oils on pathogenic fungi can be observed both at the macromorphological level (inhibition of the mycelium) and at the cellular level. Some of the macromorphological changes are: lack of sporulation or pigmentation, change in the number of conidia, increased branching of hyphae or change in their size. These changes are the consequence of the activities of the components of oils on the enzymatic reaction of the synthesis of the cell wall, which affects the growth of mold and morphogenesis; also, as a result of the pulling back of the cytoplasm from the hyphae, the death of the mycelium occurs (Carmo et al., 2008; Cotuna et al., 2016; Kiraly et al., 2017; Mătăsărean et al., 2017; Plavsic et al., 2017).

The actions of essential oils and their components list the following effects at the fungal cell level (Bennett & Klich, 2003; Chouhan et al., 2017; Dhifi et al., 2016; Karpiński, 2020; Maurya et al., 2021; Mothana, 2012; Nazzaro et al., 2013; Popescu et al., 2021; Ramsey et al., n.d.; Rezende et al., 2021; Tian et al., 2012; Y. Wang et al., 2018): cell wall degradation; reduction of ergosterol production, see Table 1; damage to cytoplasmic membranes; impairment of membrane ionic channels - > decreased pH - > apoptosis and necrosis of fungal cells; deterioration and disturbance of the activity of some cellular organelles with a vital role for the cell-mitochondria; coagulation of the cytoplasm; changes in the amounts of membrane fatty acids; accumulation in the fungal cell of free radicals; decreases in membrane potential; damage to membrane proteins; increasing the permeability of the cell membrane that leads to the outflow of the cellular content; decrease in the synthesis of ATP; actions on enzymes such as ATPase, histidine carboxylase, amylase and protease; changes in the expression of genes involved in mycotoxin biosynthesis, see Table 2. Given the effects produced by the synergistic action of essential oil components on fungal cells, but also on bacterial cells, there are studies that evaluate the toxic potential of essential oils (Bota et al., 2022; Gurita et al., n.d.). For example, the antifungal activity of cinnamon oil at the concentration of 0.25 mg L^{-1} on *Penicillium expansum* (CGMDD3.3703) caused a decrease in the germination rate of *P. expansum* spores. After 12 hours of treatment, the spores germination rate was below 20% compared to the control, where the spores germination rate was above 75% (Lai et al., 2021).

Table 1. Essential oils that reduce ergosterol production

Essential oils	Reduction of ergosterol to:	Percentage of reduction [%]:	References:
Thymus vulgaris	*Aspergillus flavus*	-	(Karpiński, 2020)
Anethum graveolens		79.4%	
Oregano	*Fusarium graminearum*	99.97%	(Perczak et al., 2019)
	F. culmorum	99.98%	
Cinnamon	*F. graminearum*	99.89%	(Perczak et al., 2019)
	F. culmorum	99.81%	
Orange	*F. culmorum*	68.13%	(Perczak et al., 2019)
Mixture of cinnamaldehyde and citral	*Penicillium expansum*	39.40%	(Y. Wang et al., 2018)

Table 2. Essential oils that modulate gene expression

Essential oils:	Effects on genes responsible for mycotoxin biosynthesis:	References:
Rosemary, Fennel, Anise, Chamomile, Cardamom, Celery	The concentration of 1.0 µl/ml essential oil reduces the expression of genes: acOTApks, laeA, acOTAnrps, acpks and veA involved in the biosynthesis of ochratoxin A in *A. carbonarius*	(El Khoury et al., 2016; Maurya et al., 2021)
Turmeric	Dose-dependent reduction of aflR transcription factor expression (5 days of treatment at 2, 4 and 8 µl/ml) Downregulates the relative expression of the genes: aflO, aflP, aflM, aflD and aflQ, determining the inhibition of aflatoxin B1 production	(Maurya et al., 2021)
Mixture of 3 essential oils (cinnamon, oregano and lemongrass in a ratio of 1:5:48)	At 0.6 µL/disc essential oil, it modulates the gene expression of the aflT, aflD, aflP, aflM and aflS genes, thus causing a disruption of aflatoxin biosynthesis in the case of *Aspergillus flavus* At 1.0 µL/disc (58.04%) there was a significant reduction of the aflR gene.	(Xiang et al., 2020)

Table 3. Essential oils with antifungal effects on *Penicillium* spp.

Fungal species:	Essential oil of:	The major components of the essential oil:	Result:	References:
Penicillium ochrochloron	*Thymus vulgaris* -vapor	thymol (58%), p-cymene (22%), and linalool (3%)	MIC 62.5 µL/L	(Střelková et al., 2021)
	Origanum vulgare - vapor phase	carvacrol (70%), followed by p-cymene (11%) and thymol (3%)	MIC 62.5 µL/L	
	Cymbopogon citratus- vapor phase	geranial (42%) and neral (28%), and in smaller quantities geraniol (5%) and geranyl acetate (4%)	MIC 62.5 µL/L	
	Syzygium aromaticum - vapor phase	eugenol (80%), eugenol acetate (7%), and caryophyllene (7%)	MIC 62.5 µL/L	
Penicillium verucosum Dierckx (D-99756)	*Salvia officinalis*	borneol (15.67%), 1,8 cineole (21.12%), tyranton (13.30%)	MIC 65 µL/ml	(Ozcakmak et al., 2017)
	Mentha x piperita	menthol (42.55%), menthone (30.51%), neo-menthol (13.19%)	MIC 65 µL/ml	
	Allium sativum	allyl disulfide (60.23%), allyl sulfide (19.68%)	MIC 65 µL/ml	
	Origanum onites	carvacrol (78.10%), borneol (2.85%), cymene (7.2%)	MIC 65 µL/ml	
Penicillium verrucosum	*Curcuma longa*	ar-turmerone (46.13%) and ar- curcumene (8.33%)	MIC 1329 µl/ml MFC 1771 µl/ml	(Kalagatur et al., 2020)
	Ocimum basilicum	eugenol (36.58%) and linalool (10.83%)	MIC 1006 µl/ml MFC 1512 µl/ml	
	Zingiber officinale	geraniol isobutanoate (10.41%), geranyl acetone (11.05%), geranyl acetate (14.59%), geranyl proprionate (18.93%), thymol (10.86%) and limonene (10.88%)	MIC 1255 µl/ml MFC 1442 µl/ml	
	Cymbopogon martini	terpinen-4-ol (11.52%) and geranyl acetate (14.88%)	MIC 964 µl/ml MFC 1221 µl/ml	
	Cinnamomum zeylanicum	limonene (10.54%), (E)-cinamaldehyde (35.81%) and eugenol (12.41%)	MIC 837 µl/ml MFC 1441 µl/ml	
Penicillium aurantiogriseum	*Origanum vulgare*	trans-caryophyllene 30.729%, sabinene-18.16%, caryophyllene oxide-8.635% and germacrene-D-8.159%	MIC 0.5 mg·L^{-1} MFC 5 mg·L^{-1}	(Rus et al., n.d.)

MIC- mimimum inhibitory concentration, MFC-minimal fungicital concentration

EFFECTS OF ESSENTIAL OILS ON FUNGI AND THEIR MYCOTOXINS

According to reports in the specialized literature, it seems that essential oils containing monoterpenes: α-pinene, β-pinene, p-cimena, γ-terpinene, linalool and citral (neral and geranial); compounds with phenolic characteristics (carvacrol and thymol); and phenylpropanoids (eugenol) are considered by some authors, the main compounds responsible for the antifungal and antimycotoxinic actions (see Table 3) of essential oils (Beicu et al., 2021; Chouhan et al., 2017; Maurya et al., 2021; Rezende et al., 2021; L. Wang et al., 2019).

Citral (3,7-dimethyl-2-6-octadienal) is the name given to a mixture of two geometric isomers: geranial (trans-citral, citral A) and neral (cis-citral, citral B). These unsaturated monoterpene β-aldehydes are found naturally in many citrus essential oils and other herbs or spice (Leite et al., 2014). It has broad-spectrum inhibitory effects against various pathogens such as: *Alternaria alternata, A. solani, Penicillium italicum, P. expansum, A. flavus, Fusarium moniliform, Candida albicas*. The main effects of citrate on fungi are: destroying the integrity of the cell membrane, releasing cellular components, inhibiting mycelium growth through a mechanism of cell membrane damage, compromising its integrity and permeability, negatively affecting the germination of spores, inhibiting the formation of pseudo hives and chlamydia, inducing disruption of oxidative balance, resulting in disruption of cell integrity. It also reduces the expression of genes involved in the biosynthesis of alternariol and its alternariol derivative monomethyl ether, including pksI and omtI (Leite et al., 2014; L. Wang et al., 2019).

Phenolics, such as eugenol, chavicol and 4-allyl-2-6-dimethoxyphenol, have higher antifungal properties, compared to cinnamic and hydro cinnamic acids; antifungal activity decreases depending on the type of chemical groups as follows: phenols→ alcohols cinnamic aldehydes→ aldehydes →ketones → ethers → hydrocarbs. Thus, the antifungal efficacy of volatile oils extracted by different methods from various plants is influenced by the presence of various active constituents, such as: monoterpenes, sesquiterpenes, phenols, aldehydes and ketones. Constituents such as: terpenoids, alcohols and phenolic terpenes in an oxygenated form increase the antifungal activity of volatile oils (Maurya et al., 2021; Saad et al., 2013).

There are studies that have shown the possibility of using essential oils in the form of vapors, in various concentrations to control the contamination of stored wheat with deposit fungi and also the prevention of contamination with mycotoxins such as mycotoxin deoxynivalenol, a secondary metabolite of Fusarium, see Tables 3, 4, 5, 6 (Bota et al., 2022).

The essential oil extracted from the leaves of *Origanum compactum* (oregano), composed mainly of Carvacrol (38%) and Timol (31.46%), has been tested on *Aspergillus flavus*, *A. niger* and *Fusarium oxysporum*. Inhibition of *A. niger* at a minimum concentration of 3,125 μg/ml was observed. In the case of *A. flavus* and *F. oxysporum*, concentrations of 6.25 and 12.5 μg/mL were required for inhibition, respectively, compared to the controls. Also in this study, a comparison was made between the fungal cultures subjected to the essential oil of oregano and those subjected to fluconazole treatment. The obtained results indicate lower values of the minimum inhibitory concentration following the application of the essential oil compared to the tested fungal strains, compared to the Fluconazole standard (Aimad et al., 2022).

Another study that aimed to highlight the antifungal potential, antimicrobial and phytotoxic effect of essential oils of: *Origanum vulgare*, *Thymus vulgaris* and *Coriandrum sativum*, confirmed that the treatment of cereals with the 3 essential oils listed above, leads to a significant decrease in mycotoxin levels, namely the level of deoxynivalenol in wheat samples, depending on the type of volatile oil, concentration and time of fumigation. The maximum inhibition percentage was obtained 21 days after the application of volatile oils in the form of vapors at a concentration of 0.2%. Inhibition of the development of mycotoxin deoxynivalenol also occurred after 7 days of application of the essential oils of *Origanum vulgare* and *Thymus vulgaris* as vapors (Bota et al., 2022).

Tabel 4. Essential oils with antifungal effects on *Fusarium* spp.

Fungal species:	**Essential oil of:**	**The major components of the essential oil:**	**Result:**	**References:**
Fusarium sporotrichioides and *Fusarium solani*	*Thymus vulgaris* - vapor	thymol (58%), p-cymene (22%) and linalool (3%)	MIC 62.5 µL/L MFC 125 µL/L	(Střelková et al., 2021)
	Origanum vulgare - vapor phase	carvacrol (70%), p-cymene (11%) and thymol (3%).	MIC 62.5 µL/L MFC 125 µL/L	
	Cymbopogon citratus - vapor phase	geranial (42%) and neral (28%), geraniol (5%) and geranyl acetate (4%)	MIC 62.5 µL/L MFC 125 µL/L	
	Syzygium aromaticum - vapor phase	eugenol (80%), eugenol acetate (7%), and caryophyllene (7%)	MIC 62.5 µL/L MFC 125 µL/L	
Fusarium graminearum KZF-1	*Cinnamomum zeylanicum*	cinnamic aldehyde ≤ 70%, eugenol ≤ 4.4%, linalool ≤ 2.6%, limonene ≤ 1.1%, benzyl benzoate ≤ 1.1%, benzaldehyde 0.5%, cinnamic alcohol ≤ 0.4%, and cuminaldehyde ≤ 0.2%	5% essential oil concentration reduces micelle growth by 90%	(Gwiazdowska et al., 2022)
	Cymbopogon martini	geraniol 85%, linalool 2-3%, limonene 1% and citral 1%;	5% essential oil concentration reduces micelle growth by 1%	
	Thymus hiemalis	citral 42% and limonene 40%	5% essential oil concentration reduces micelle growth by 68%	
Fusarium graminearum	*Coriandrum sativum*	linalool	CMFs 0.5%	(Alexa et al., 2022)
	Thymus vulgaris	thymol and o-cymene	CMFs 0.1% CMFg 0.6%	
	Origanum vulgare	carvacrol and o-cymene	CMFs 0.06% CMFg 0.2%	
	A mixture of thyme and oregano essential oil	o-cymene (33.25%), thymol (21.04%) and carvacrol (30.33%)	complete inhibition of the micelle after 28 days	
	A mixture of thyme and coriander	o-cymene (29.33%), β-linalool (28.87%) and thymol (26.18%)	complete inhibition of the micelle after 28 days	
	A mixture of oregano and coriander	o-cymene (24.35%), β-linalool (28.22%) and carvacrol (33.72%)	complete inhibition of the micelle after 28 days	
	A mixture of thyme, oregano and coriander	o-cymene (14.59%), β-linalool (21.68%) and thymol (17.22%)	complete inhibition of the micelle after 28 days	
Fusarium verticillioides M3125	*Curcuma longa*	α-turmerone (44.70%), β-turmerone (20.67%), and Ar-turmerone (17.27%)	125 ppm, inhibition of radial growth was 38.8%	(Achimón et al., 2021)
	Pimenta dioica	methyl eugenol (53.09%), eugenol (16.70%), and β-myrcene (12.80%)	125 ppm, inhibition of radial growth was 20.8%	
	Syzygium aromaticum	eugenol (88.70%), and β-caryophyllene (6.55%)	125 ppm, inhibition of radial growth was 57.4%	
	Rosmarinus officinalis	1,8-cineole (53.48%), α-pinene (15.65%), and (−)-camphor (9.57%)	125 ppm, inhibition of radial growth was 13.3%	
Fusarium avenaceum	*Ammoides pusilla* (lot 1)	Thymol (34.70%), γ-terpinen (27.03%), p-cymene (19.89%) and thymol methyl ether (9.18%)	MIC 0.5 $\mu L \cdot mL^{-1}$ produces a 99.2% inhibition of fungal growth after 7 days of treatment	(Chakroun et al., 2021)
	Ammoides pusilla (lot 2)	Thymol (53.55%), γ-terpinen (16.82%), p-cymene (14.59%) and thymol methyl ether (8.07%)	MIC 0.25 $\mu L \cdot mL^{-1}$	
Fusarium oxysporum (MTCC 9913)	*Nigella sativa*	O-cymene (37.82%), carvacrol (17.68%), α-pinen (10.09%), trans-sabinene hydrat (9.90%) and terpinen-4-ol (7.15%)	MIC 2.69 µg/mL	(Zouirech et al., 2022)

MIC- mimimum inhibitory concentration, MFC-minimal fungicital concentration, CMFs- minimum concentration with fungistatic effect

Table 5. Essential oils with antifungal effects on *Aspergillius* spp.

Fungal species:	Essential oil of:	The major components of the essential oil:	Result:	References:
Aspergillus niger	*Thymus vulgaris*- vapors	thymol (58%), p-cymene (22%), and linalool (3%)	MIC 62.5 µL/L	(Střelková et al., 2021)
	Origanum vulgare- vapor phase	carvacrol (70%), p-cymene (11%) and thymol (3%).	MIC 62.5 µL/L	
	Cymbopogon citratus- vapor phase	geranial (42%), neral (28%), geraniol (5%) and geranyl acetate (4%)	MIC 62.5 µL/L	
	Syzygium aromaticum- vapor phase	eugenol (80%), eugenol acetate (7%), and caryophyllene (7%)	MIC 62.5 µL/L	
	Origanum vulgare- vapor phase	carvacrol (70%), p-cimen (11%) și timol (3%).	MIC 62.5 µL/L	
	Origanum vulgare	4-terpineol (44.11%), Linalool (15.22%), α-terpineol (5.96%)	5.0 µL of essential oil produces a 40.93% inhibition of fungal growth	(Esper et al., 2014)
	Ageratum conyzoides	Dimetoxi ageratocromene (96.53%)	5.0 µL of essential oil produces a 88.37% inhibition of fungal growth	
	Cymbopogon citratus - vapors	geranial (42%) and neral (28%), geraniol (5%) and geranyl acetate (4%)	MIC 62.5 µL/L	(El-Soud et al., 2012;
	Syzygium aromaticum- vapors	eugenol (80%), eugenol acetate (7%), and caryophyllene (7%)	MIC 62.5 µL/L	Střelková et al., 2021;
	Carum carvi	carvone (70.1%); γ -terpinene (12.6%); limonene (5.1%)	At 1000 ppm complete inhibition of fungal growth	Zhao et al., 2016)
	Coriandrum sativum	γ- terpinene (10.6%); linalool (40.9%); geranyl acetate (12.8%)	At 1000 ppm complete inhibition of fungal growth	
	Foeniculum vulgare	estragole (50.1%); limonene (20.2%); fenchone (10.6%)	At 1000 ppm, 50% inhibition of fungal growth	
Aspergillus flavus tulpina MC11	*Eugenia caryophyllata*	eugenol (82.52%)	0.1 and 0.25 µL mL^{-1} suppress mycelial growth by 75 and 85%, respectively	(Oliveira et al., 2020)
	Rosmarinus officinalis chemotip camfor	α-pinen (22.65%)	0.1 and 0.25 µL mL^{-1} do not suppress mycelial growth	
Aspergillus flavus NRRL 3357	*Cinnamomum verum*	cinnamaldehyde (89.33%), (E)-2-methoxycinnamaldehyde (4.66%) and carveol (2.20%)	0.25µl/disc completely inhibits fungal growth	(Xiang et al., 2020)
	Origanum vulgare	carvacrol (84.96%) and thymol (13.26%)	2.50 µl/disc completely inhibits fungal growth	
	Cymbopogon citratus	(Z)-citral (43.66%) and (E)-citral (43.55%)	6.00 µl/disc completely inhibits fungal growth	
Aspergillus ochraceus (CCDCA 10506), Aspergillus flavus (CCDCA 10508)	*Satureja montana*	borneol (36.18%), carvacrol (11.07%), camphene (5.35%), γ -terpineol (12.66%) and p-cymene (9.57%)	MFC for A. ochraceus 3.91 µl/ml, and for *A. flavus* is 0.98 µl/ml	(Rezende et al., 2021)
	Myristica fragrans	limonene (10.15%), sabinene (49.23%), terpinen-4-ol (4.99%), α-pinene (13.81%) and β-pinene (10.75%)	MFC for *A. ochraceus* and *A. flavus* is 15.62 µl/ml	
	Cymbopogon flexuosus	geranial (59 .66%) and general (38.98%)	MFC for *A. ochraceus* and *A. flavus* 0.98 µl/ml	
Aspergillus parasiticus tulpina CECT 2682	Lavandin Grosso	linalool (31.65%), linalyl acetate (24.98%), 1,8-cineole (8.69%), camphor (6.96%), and terpinen-4-ol (3.10%)	MIC and MFC 3µl/ml essential oil	(Lorán et al., 2022)
	Lavandin Abrial	linalool (31.04%), linalyl acetate (19.57%), 1,8-cineole (10.46%), camphor (8.86%), and (E)-β-ocimene (3.50%)	MIC 3µml/ml essential oils, MFC 5µl/ml	
	Origanum virens	carvacrolul (28.71%), p-cimen (9.55%), Ɣ-terpinen (5.22%), α-terpinen (3.00%), mircen (2.05%) and thymol (1.78%)	MIC and MFC 0.6 µl/ml essential oil	
Aspergillus parasiticus tulpina CGC34 (Ap) Aspergillus ochraceus tulpina CGC87 (Ao)	chemotip linalool -*Tymus zygis* - vapor	p–Cymen (39.10%), linalool (32%), thymol (12.11%) and α–pinene (2.5%)	Ap fungal inhibition: 47.78% Ao fungal inhibition: 48.33%	(Hlebová et al., 2021)
	chemotip thymol -*Tymus vulgaris* - vapor phase	p–Cymen (18.36%), thymol (53.40%), γ-terpinene (6.44%), linalool (5.10%) şi carvacrol (2.56%)	Ap fungal inhibition: 48.33% Ao fungal inhibition:37.22%	
	Eucalyptus globulus – vapor phase	Cineol (80.01%) α–pinene (2.88%), p-cimen (6.30%), limonene (6%), γ-terpinene (2.45%)	Ap fungal inhibition: 43.33% Ao fungal inhibition: 33.33%	
	Lavandula angustifolia - vapor phase	Linalool (40.59%), Linalyl anthranilate (10.15%), cineol (12.01%), borneol (7.43%)	Ap fungal inhibition: 65.00% Ao fungal inhibition: 37.78%	
	Mentha piperita - vapori	Menthol (41.84%), citronellal (22.09%), borneol (8.40%), p–Cimen (6.3%), 2-undecanone (5.72%) and β-cariofilene (3.44%)	Ap fungal inhibition: 40.00% Ao fungal inhibition: 26.67%	
	Prunbus dulcis - vapor	Benzaldehide (98.20%)	Ap fungal inhibition: 86.67% Ao fungal inhibition: 88.33%	
	Cinnamomum zeylanicum - vapors	Cinnamaldehyde (65.30%), eugenol (21.03%), β-cariofilene (4.16%)	Ap fungal inhibition: 88.33% Ao fungal inhibition:83.89%	
	Litsea cubeba - vapor phase	Limonene (11.50%), β-citral (29.27%), α-citral (37.15%)	Ap fungal inhibition: 88.89% Ao fungal inhibition: 90.00%	

Table 5. Essential oils with antifungal effects on *Aspergillius* spp. - continuation

Fungal species:	Essential oil of:	The major components of the essential oil:	Result:	References:
Aspergillus parasiticus tulpina CGC34 (Ap) *Aspergillus ochraceus tulpina CGC87 (Ao)*	*Cympogon citrati* - vapor phase	Limonene (14.50%), β-citral (33.37%), α-citral (39%)	Ap fungal inhibition: 87.78% Ao fungal inhibition: 87.78%	(Hlebová et al., 2021)
	Zingiber officinalis - vapor phase	Camphene (3.49%), limonene (3.76%), α-curcumene (14.20%), Zingiberen (44.36%), α-farnesen (12.40%) and Sesquiphellandren (11.41%)	Ap fungal inhibition: 46.67% Ao fungal inhibition: 33.44%	
Aspergillus ochraceus	*Cinnamomum zeylanicum*	limonene (10.54 %), (E)-cinnamaldehyde (35.81 %), and eugenol (12.41 %)	MIC 1106 µl/ml MFC 1430 µl/ml	(Kalagatur et al., 2020)
	Curcuma longa	ar-turmerone (46.13%) and ar- curcumene (8.33%)	MIC 1608 µl/ml MFC 2140 µl/ml	
	Cymbopogon martini	terpinen-4-ol (11.52 %) and geranyl acetate (14.88 %)	MIC 1308 µl/ml MFC 1430 µl/ml	
	Zingiber officinale	geraniol isobutanoate (10.41 %), geranyl acetone (11.05 %), geranyl acetate (14.59 %), geranyl proprionate (18.93 %), thymol (10.86 %), and limonene (10.88 %)	MIC 1898 µl/ml MFC 1756 µl/ml	
	Ocimum basilicum	eugenol (36.58%) and linalool (10.83%)	MIC 1791 µl/ml and MFC 2255 µl/ml	

MIC- mimimum inhibitory concentration, MFC-minimal fungicital concentration

Tabel 6. Essential oils with an effect on the biosynthesis of mycotoxins

Mycotoxins:	The effects of essential oils:	References:
Aflatoxin B1, synthesized by *Aspergillus flavus*	***Carum carvi*** - completely suppresses the production of aflatoxin B1 at the concentration of 1000 ppm essential oil; ***Coriandrum sativum*** - completely suppresses the production of aflatoxin B1 at concentrations of 500, 750 and 1000 ppm essential oil; ***Ageratum conyzoides*** - the volumes of 50, 30 and 15 µL of the essential oil inhibited the production of aflatoxin B1 in maize by 93.70, 34.15 and 15.45%, respectively, and in the case of soybeans, the same volumes of oil essentially inhibited mycotoxin production over 75%; ***Oreganum vulgare*** - volumes of 10, 50, 100 and 200 µL showed inhibitory effect on aflatoxin production in soybeans at 54.4; 88.68; 86.94 and 88.16%; ***Eugenia caryophyllata*** - after 7 days of incubation at a concentration of 0.5 $\mu L\ mL^{-1}$ essential oil, a 100% inhibition of aflatoxin B1 was observed; ***Rosmarinus officinalis*** camphor chemotype – produced an inhibition of 81.4% after 7 days of incubation at a concentration of 5 µl ml-1 essential oil; Mixture of essential oil of cinnamon, oregano and lemongrass in a ratio of 1:5:48 - at 0.6 µl/disc of essential oil, a 67.53% inhibition of aflatoxin B1 is obtained, and at a concentration of 1.0 µl/disc 72.68%.	(El-Soud et al., 2012; Esper et al., 2014; Oliveira et al., 2020; Xiang et al., 2020)
Aflatoxin B1 and **G1**, synthesized by *A. parasiticus*	***Litsea cubeba*** (vapors) significantly affected mycotoxin production at a concentration of 15.625 µl/l of air; ***Cympogon citrati*** (vapors) significantly affected mycotoxin production at a concentration of 15.625 µl/l of air;	(Hlebová et al., 2021)
Aflatoxin B1 and **B2** synthesized by *A. flavus (CCDCA 10508)*	***Satureja montana*** - very low level of aflatoxins at the concentration of 0.015 µl/ml essential oil, and at a higher concentration of essential oil the inhibition of aflatoxins is 100%; ***Myristica fragrans*** - very low level of aflatoxins at the concentration of 0.015 µl/ml essential oil, and at a higher concentration of essential oil the inhibition of aflatoxins is 100%; ***Cymbopogon flexuosus*** **-** very low level of aflatoxins at a concentration of 0.015 µl/ml essential oil, and at a higher concentration of essential oil the inhibition of aflatoxins is 100%;	(Rezende et al., 2021)
Aflatoxins B1, B2, G1 and **G2** synthesized by *Aspergillus parasiticus tulpina CECT 2682*	***Rosmarinus officinalis*** - almost completely inhibits aflatoxin production as follows: over 89% for AFG2 and over 99% for the other aflatoxins (B1, B2 and G1); Lavandin Grosso – significantly inhibited the synthesis of aflatoxins (B1, B2, G1 and G2) in a concentration-dependent manner; ***Origanum virens***- significantly inhibited the synthesis of aflatoxins (B1, B2, G1 and G2) in a concentration-dependent manner; Lavandin Abrial – stimulated the production of aflatoxins B1 and G1 at concentrations of 0.8 µl/ml and 1µl/ml essential oil, also at these concentrations it significantly reduced the percentage of aflatoxins B2 and G2;	(Lorán et al., 2022)
Fumonisins synthesized by *Fusarium* spp.	***Melissa officinalis*** - 2000ppm essential oil produced a 79.67% inhibition of the mycotoxin; ***Salvia officinalis*** **-** 2000ppm essential oil produced a 96.6% inhibition of the mycotoxin; ***Coriandrum sativum*** - 2000ppm essential oil produced a 94.64% inhibition of the mycotoxin; ***Thymus vulgaris*** - 500ppm essential oil produced a 97.32% inhibition of the mycotoxin; ***Mentha x piperita*** - 2000ppm essential oil produced a 95.77% inhibition of the mycotoxin; ***Cinnamomum zeylanicum*** - 500 ppm essential oil produced a 97.32% inhibition of the mycotoxin;	(Sumalan et al., 2013)
Deoxynivalenol (DON), synthesized by *Fusarium* spp.	***Origanum vulgare*** (vapors) - at the concentration of 0.2% essential oil, after 28 days of treatment, DON was inhibited by a percentage of 55.35%, and at a concentration of 0.4%, also under the same conditions, the inhibition was at a percentage of 43.88%; ***Thymus vulgaris*** (vapors) - at the concentration of 0.2% essential oil, after 28 days of treatment, DON was inhibited by a percentage of 64.65%, and at a concentration of 0.4%, also under the same conditions, the inhibition was at a percentage of 39.84%;	(Bota et al., 2022; Gwiazdowska et al., 2022; Perczak et al., 2019)

Tabel 6. Essential oils with an effect on the biosynthesis of mycotoxins - continuation

Mycotoxins:	The effects of essential oils:	References:
Deoxynivalenol (DON), synthesized by *Fusarium* spp	***Coriandrum sativum*** (vapors) - at the concentration of 0.2% essential oil, after 28 days of treatment, DON was inhibited in percentage of 44.03%, and at a concentration of 0.4%, after 14 days of fumigation, the inhibition was in percentage of 57.05%; Concentrations of 20% essential oil of: ***Cinnamomum zeylanicum, Origanum vulgare, Cymbopogon martini, Thymus hiemalis, Mentha viridis, Foeniculum vulgare dulce, Aniba rosaeodora*** *and* ***Citrus aurantium*** produced over 99% inhibition of mycotoxin secretion in the process; Concentrations of 1% essential oil of: ***Cinnamomum zeylanicum, Cymbopogon martini*** *and* ***Thymus hiemalis****, reduce mycotoxin levels by 100%;*	(Bota et al., 2022; Gwiazdowska et al., 2022; Perczak et al., 2019)
Ochratoxin A (OTA) synthesized by *Aspergillus carbonarius* S402	Chamomile - OTA reduction by 67.5% at a concentration of 1µl/ml; Celery- reduction of OTA by 68.5% at a concentration of 1µl/ml essential oil; Rosemary- reduction of OTA by 57.3% at a concentration of 1µl/ml; Anise- reduction of OTA by 76.6% at a concentration of 1µl/ml; Cardamon- reduction of OTA by 74.2% at a concentration of 1µl/ml; Fennel - reduction of OTA by 88.9% at a concentration of 5µl/ml essential oil;	(El Khoury et al., 2016)
Ochratoxin (OTA) synthesized by *Aspergillus ochraceus*	***Satureja montana***- at the concentration of 0.015µl/ml essential oil, the mycotoxin was inhibited in a percentage of 34.35%, and at the concentration of 0.24µl/ml essential oil, the complete inhibition of the mycotoxin was obtained (100%); ***Myristica fragrans***- at the concentration of 0.015µl/ml essential oil, the mycotoxin was inhibited by a percentage of 31.24%, and at the concentration of 0.24µl/ml essential oil, the complete inhibition of the mycotoxin was obtained (100%); ***Cymbopogon flexuosus***- at the concentration of 0.015µl/ml essential oil, mycotoxin inhibition was achieved in a percentage of 24.52%, and at the concentration of 0.24µl/ml essential oil, the inhibition was achieved in a percentage of 93.72%; ***Prunbus dulcis*** – vapors- at the concentration of 15.625µl/l of essential oil air, mycotoxin inhibition is over 50%;	(Hlebová et al., 2021; Rezende et al., 2021)
Ochratoxin A synthesized by *Penicillium verucosum*	***Salvia officinalis, Mentha x piperita***- in the treatments with concentrations of 500, 250, 125 and 65 µL/ml essential oil, the mycotoxin level was reduced; ***Allium sativum, Origanum onites*** - in treatments with concentrations of 250 and 500 µL/ml essential oil, the mycotoxin was no longer detected;	(Ozcakmak et al., 2017)
Zearalenone (ZEA), synthesized by *Fusarium graminearum*	***Cinnamomum zeylanicum, Origanum vulgare, Cymbopogon martini, Thymus hiemalis Mentha viridis, Foeniculum vulgare dulce*** *and* ***Aniba rosaeodora*** (20% essential oil concentration) reduces the amount of ZEA by 100%; ***Citrus aurantium*** (20% essential oil concentration) reduces the amount of ZEA by 99.99%; Concentrations of 1% essential oil of ***Cinnamomum zeylanicum***, ***Cymbopogon martini*** and ***Thymus hiemalis*** reduce mycotoxin levels by over 84%;	(Gwiazdowska et al., 2022; Perczak et al., 2019)
Zearalenone, synthesized by *Fusarium culmorum*	***Cinnamomum zeylanicum, Origanum vulgare, Cymbopogon martini, Thymus hiemalis, Mentha viridis, Foeniculum vulgare dulce*** and ***Aniba rosaeodora*** (in a concentration of 20% essential oil) reduce the amount of mycotoxin in a percentage between 99.08-99.99%; ***Citrus aurantium*** (20% essential oil concentration) reduces the amount of mycotoxin by 68.33%	(Perczak et al., 2019)
Ennantine (ENN) synthesized by *F. avenaceum*	***Ammoides pusilla*** (batch 1)-ENN accumulation was inhibited by 65.76 and 100% at concentrations of 0.1, 0.25 and 0.5 $L\mu mL^{-1}$ essential oil, respectively, and **Ammoides pusilla** (batch 2) - causes a 92% reduction of ENN at the concentration of 0.15 $L\ \mu mL^{-1}$.	(Chakroun et al., 2021)

CONCLUSIONS

The use of essential oils in agriculture as biopesticides can be a sustainable alternative to protecting stored crops, agricultural products, including the environment and the health of the consumer. In addition to the biopesticide wheel, volatile oils can be used to reduce the level of mycotoxins of cereals in the deposits, so these natural products reduce the economic losses caused by contamination and most importantly the risk of poisoning and other pathologies resulting from the consumption of contaminated products. Also, volatile oils, due to the synergistic mode of action between the components, can successfully replace the synthetic preservatives used in the preservation of vegetables and fruits. The preservative effect of oils extracted from plants is due to chemical compounds that have proven antibacterial, antifungal and antioxidant effects.

Most of the pathogens of agricultural crops become resistant to the chemical treatments used. Thus, most of the times, the dose of chemicals is used to be increased in order to succeed in combating them. The increase in the quantity of synthetic substances leads to a direct proportional increase in the negative effects created by the substances used and their residues on the environment but also on the health of consumers, be they of human or animal nature.

The replacement of synthetic substances with eco-friendly substances, such as essential oils extracted from various plants, can be a durable solution in combating the resistance of phytopathogens, due to their vast chemical composition and synergistic effects between the main constituents.

A plus of the use of essential oils at the expense of synthetic substances is the use of small amounts of ethereal oils to achieve strong fungistatic and fungicidal effects. They also decomposes quickly without leaving toxic residues in the environment.

In conclusion, through this scientific research we tried to make: an enumeration of volatile oils with the potential of biopesticides, a highlighting of the effects and the antifungal mode of action of the major components identified in their composition, on pathogenic fungi and biosynthesized mycotoxins.

ACKNOWLEDGEMENTS

Support was also received by the project Horizon Europe (HORIZON) 101071300 - Sustainable Horizons - European Universities designing the horizons of sustainability (SHEs).

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF TUBER YIELD AND CULINARY QUALITY FOR TRUE POTATO SEED GENOTYPES GROWN UNDER DROUGHT STRESS FIELD CONDITIONS

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Abstract

The main objective of this study was to evaluate under field conditions potato genotypes derived from true seeds, that showed tolerance to in vitro induced water stress. The biological material was represented by 3 genotypes: GIL19-03-07, ZIL19-02-43 and GIL19-03-29, for which tuber number, yield and culinary quality were determined. The genotype GIL19-03-07 obtained the best results in terms of total number of tubers (647.20 thousand/ha), surpassing the variety Cosiana (control). In a dry growing year, in terms of total tuber production, the results obtained point to the genotype ZIL19-02-43 which obtained a production of 27.19 t/ha, followed by the genotype GIL19-03-07 with a production of 21.21 t/ha. The results of the culinary and technological quality analyses suggest that genotypes GIL19-03-07 and ZIL19-02-43 are suitable as raw material for chips, while genotype GIL19-03-29 is suitable for salads and other culinary preparations due to its pleasant taste and pulp texture.

***Key words**: culinary quality, field conditions, potato, true potato seed, tuber yield.*

INTRODUCTION

Potato is a cool climate-loving crop and does not perform well at high temperatures. The modern potato is considered a drought-sensitive crop, and it is susceptible to yield loss because of drought stress. Unfortunately, drought severity, frequency and extent have been increasing around the globe because of climate change (Nasir & Toth, 2022).

The increase in drought stress threatens the global agriculture production and food availability. Drought is a major environmental factor that determines the growth, productivity and distribution of plants (Rukundo et al., 2012).

The negative effects of drought conditions are continuously accentuated by the explosive increase of world population, continuous deterioration of arable land, lack of fresh water, and the current climate change.

Drought is considered to be one of the abiotic stressors affecting plant growth and development, food security and causing the greatest crop losses (Anjum et al., 2011; Zaki & Radwan, 2022). Therefore, the sustainability of production will depend on the identification and development of new drought tolerant varieties (Cochard et al., 2008, cited by Rukundo et al., 2012).

Drought is one of the major constrains facing agricultural production worldwide and its impact is expected to increase in future. The world population is also expected to increase and this will require more safe, nutritious, and healthy foods. The cultivated potato (*Solanum tuberosum*) is an important crop that has the potential of feeding the world. However, it is highly susceptible to drought owing to its shallow root network that occupies the upper 0.3 m of the soil layer (Okayo, 2015).

Water is vital for plant growth and development, and many physiological processes are affected when this element is restricted. In the case of potato, water deficit at the tuber stage causes the most significant yield losses compared to other stages of plant development (Anithakumari et al., 2011). In terms of reproduction, the potato can be propagated by two distinct methods: sexually (by botanical seed) or vegetatively (by tubers). Like all other botanical seeds, TPS has the potential to grow into a full plant, but every plant is genetically different, thus making TPS population heterogeneous. TPS has many advantages over potato seed tubers. The major ones are disease - and pest - free planting material, easy storage and transportation, and a highly reduced seed rate (about 150 g/ha as against 2.5-3.0 t/ha of seed tubers) (Buckseth et al., 2022; Muthoni et al., 2013; Ozturk & Dumanoglu, 2021). True potato seed (TPS) offers an alternative technology of crop production for the regions where resource poor farmers can have quality-planting material at a reasonable cost (Adhikari, 2010). Traditionally potatoes are grown in cool climates. The major potato production is now in the northern latitudes and in the highlands of the tropics. With the use of true potato seed (TPS), potatoes can grow in warm, non-traditional potato growing areas (Tuku, 1994).

In 2019, at the National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania, a study was initiated as part of a research project that aimed to identify in populations derived from botanical seed, characterized by a high degree of uniformity (isogenic lines), one or more prospective genotypes in terms of productivity, tolerance to abiotic and biotic stress factors as well as their culinary quality. The main objective was to assess the possibility of producing an alternative planting material from botanical seed to supplement the potato requirement.

MATERIALS AND METHODS

Aspects of field work. The study was carried out in the experimental field of the National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania. In order to follow the evolution of the genotypes derived from botanical seed under open field conditions, the biological material obtained in a protected area ("insect-proof" space) in 2021, consisting in potato minitubers (Prebase), was planted on 14 April 2022 in the clonal field of NIRDPSB Brasov, located in the traditional potato growing area of our country, which is also considered a closed area. The present study evaluated the total tuber yield (t/ha), total tuber number (thousand/ha), number and production of tubers by size fractions and culinary quality from 3 potato genotypes (GIL19-03-07, ZIL19-02-43 and GIL19-03-29). The Cosiana variety was used as control. Minitubers were planted 25 cm apart and 75 cm between rows. The experience was arranged in a randomized complete block design with three replications.

From a climatic point of view, the production season of 2021/2022 in Brasov was a dry one (Table 1).

Table 1. Average monthly temperatures (ºC), rainfall (mm) and MAA during the production season 2021/2022 in Brasov

Year/Month		Average air temperature (ºC)			Rainfall (mm)		
		Recorded	MAA	Deviations	Recorded	MAA	Deviations
2021	October	7.2	8.3	-1.1	30	38.9	-8.9
	November	4.6	3.1	1.5	27.5	32.8	-5.3
	December	1.3	-2.2	3,5	45.5	27	18.5
2022	January	-1.0	-5	4	21.6	25.5	-3.9
	February	0.8	-2.5	3.3	5.6	23.9	-18.3
	March	0.8	2.6	-1.8	24.8	28.9	-4.1
	April	8.3	8.5	-0.2	64.8	50	14.8
	May	14.8	13.6	1.2	48.3	82	-33.7
	June	19	16.5	2.5	37.5	96.7	-65.1
	July	20.6	18.1	2.5	50.1	99.8	-49.7
	August	20.2	17.5	2.7	50.3	76.4	-26
	September	13.6	13.6	0	65.6	52.5	13.1
Winter period (X - III)		2.3	0.7	1.6	25.8	29.6	-3.7
Growing period (IV - IX)		16.01	14.8	1.45	52.77	76.24	-24.4
Production season 2021/2022 (oct. 2021 - sept. 2022)		9.2	7.7	1.52	42.52	52.92	-10.4

Source: Weather Station Ghimbav, Brasov County

In the year 2022 late spring and summer air temperature regime were characterized by a generally higher than usual. In June, July and August the average temperature deviated by more than 2.5°C from the multiannual average (MAA). Also in the period before the potato crop was established (i.e. October to March), the average temperature (2.3°C) was much higher than the MAA value (0.7°C). As regards the precipitation level, the winter period was characterised by a much lower volume than the MAA value (Table 1). Also, in May, June, July and August the low rainfall and the high percentage of warm days were not favourable for the development of potato plants. The amount of rainfall during both winter and the growing season was very low compared to the multiannual average for Brasov and a good water supply for the soil was not ensured.

Pre-planting activities, cultivation and maintenance was in line with current good agricultural practice (Table 2).

Table 2. Calendar of activities performed in experimental field (NIRDPSB Brasov, 2022)

Activities	Date
Autumn plowing (35 cm deep)	11.11.2021
NPK fertilization (15:15:15) - 1000 kg/ha	5.04.2022
Land preparation	8.04.2022
Planting (manually) Planting distance: 25 cm between plants/row and 75 cm between rows	14.04.2022
Hilling	04.05.2022
Weed control	
Sencor - 0.9 l/ha Lontrel 300 - 0.3 l/ha	09.05.2022 27.05.2022
Disease control	
Carial Flex - 0.6 l/ha Carial Star - 0.6 l/ha Lieto - 450 g/ha Shirlane - 400 ml/ha	31.05.2022 15.06.2022 29.07.2022 18.08.2022
Pest control	
Cyperguard – 0.08 l/ha Decis 25 WG - 30 g/ha Coragen - 50 ml/ha Mospilan 20 SG - 0.1 kg/ha	03.06.2022 15.06.2022 08.07.2022 18.07.2022
Haulm killing (mechanically)	30.08.2022
Harvesting	27.09.2022

As regards the application of chemical treatments during the growing season of the potato crop, they were mainly aimed to control the potato late blight (*Phytophthora infestans*), aphids and Colorado potato beetle (*Leptinotarsa decemlineata* Say).

To establish the phytosanitary quality of the material, the viral testing of the seed potato has a decisive role. It is a measure applied to obtain a material with the lowest degree of viral infections. On June 21, leaf samples were taken from the field, for all three potato genotypes: GIL 19-03-07, ZIL 19-02-43 and GIL 19-03-29. The ideal time to harvest the leaves is when the plant is young, or at the latest before the flowering period. Viral testing was performed (using the ELISA technique) for 6 potato viruses: Potato leaf roll virus (PLRV), Potato Virus Y (PVY), Potato Virus A (PVA), Potato Virus X (PVX), Potato Virus S (PVS) and Potato Virus M (PVM). Following the obtained results, the tested material proved to be healthy.

Assessment of the culinary and technological quality of tubers. Culinary quality was determined based on the organoleptic evaluations carried out on boiled tubers. Overall appearance, crushing on boiling, consistency of the pulp, mealiness, moisture, structure of starch granules, taste and after-cooking blackening are appreciated. For the evaluation of enzymic browning one center slice from three tubers has been taken. These thin slices (3 mm thick) with skin are kept on a glass plate for 4 hours.

a. The color of the cooked pulp after 24 hours

b. Enzymic browning after 4 hours

Figure 1. Aspects in the appreciation of potato quality

Both after-cooking blackening (Figure 1a) and enzymic browning (Figure 1b) are undesirable aspects in the appreciation of potato quality.

For a potato genotype to be appreciated in terms of quality, the color of the pulp must remain unchanged or change slightly.

Polikeit balance was used for the determination of starch. From the total weight of the tuber, water represents 65-87%, the remaining 13-35% being the dry substance, of which starch has the largest share. For ware potatoes a starch content of 12-16% is considered optimal, while for industrial processing, varieties with a higher starch content (over 17%) are preferred.

The three genotypes of potato derived from botanical seed were also evaluated regarding the suitability for obtaining chips. For each genotype, a sample of 3 tubers with skin was weighed. Then the tubers were peeled and weighed again, after which they are sliced lengthwise with the help of a grater (the whole tuber) to obtain rounds (Figure 2).

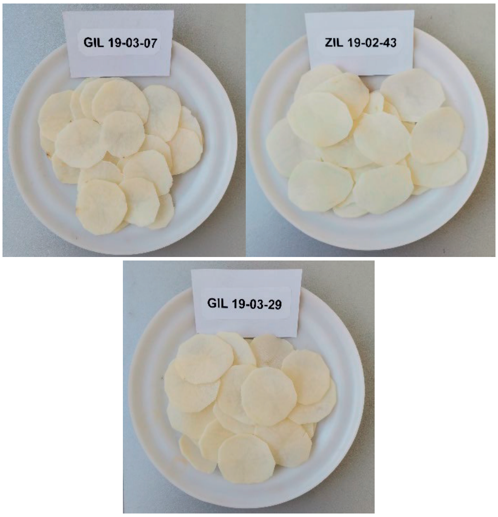

Figure 2. Potato slices before frying

The round potato slices are fried in oil bath, at a temperature of 180ºC. When the slices are crispy, dry (have lost water), they are removed from the oil, placed on a paper towel and weighed. To assess the color of the chip, a standardized scale is used, in the form of cards marked from 1 to 9 (Figure 3). In some countries, light-colored chips are preferred, and in others, slightly more colorful. The color of the chips is directly influenced by the amount of reducing sugars in the tuber. These, present in a higher percentage, give a brown color, rejected by consumers.

Figure 3. Assessment of the chips color

RESULTS AND DISCUSSIONS

Results regarding tuber number and yield

After harvesting, in addition to the total yield (t/ha), the number of tubers and their yield by size fractions were determined. Also, the weight of tubers by size fractions (large, medium and small) was determined for each individual genotype.

The results recorded after harvesting the tubers from the experimental field were processed by variance analysis (Săulescu and Săulescu, 1967).

Regarding the number of large tubers, genotype GIL 19-03-29 came closest to the control variety (Cosiana), compared to which it recorded a negative but insignificant difference (-31.32 thousand/ha), being followed by the genotype ZIL 19-02-43 (-38.52 thousand/ha). Regarding the number of medium tubers, the genotypes GIL 19-03-07 (327.41 thousand/ha) and ZIL 19-02-43 (321.06 thousand/ha) were noted, the differences compared to the control being insignificant (Table 3). The largest number of small tubers/ha was obtained at the GIL 19-03-07 genotype (310.90 thousand/ha), which recorded a positive difference (116.19 thousand/ha) compared to the control variety.

Comparing the three genotypes derived from botanical potato seed with the Cosiana variety in terms of the total number of tubers/ha, it can be seen that GIL 19-03-07 obtained the highest value (647.20 thousand/ha), surpassing the control, compared to which it registered a positive difference of 7.83 thousand/ha. It is followed by ZIL 19-02-43 with a total number of tubers of 525.08 thousand/ha.

Analyzing the influence of the genotype on the distribution of tubers in different size classes, it can be seen in Figure 4 that in all three potato genotypes derived from true seed, the highest percentage was the medium size tubers and then the tubers from the small fraction, exception making the genotype GIL 19-03-29 where the share of tubers from the large fraction (23%) exceeded that of small-sized tubers (16%). The results obtained regarding the distribution of tubers by size category suggest that the genotypes GIL 19-03-29 and ZIL 19-02-43 are competitive from the point of view of marketing the production, which can be used both for consumption and for seed.

Table 3. Effect of genotype on the number of tubers by size fractions and total number of tubers (thousands/ha) (experimental field NIRDPSB Brasov, 2022)

Genotype	Number of large tubers/ha (thousands)	Diff./Sign.	Number of medium tubers/ha (thousands)	Diff./Sign.	Number of small tubers/ha (thousands)	Diff./Sign.	Total number of tubers/ha (thousands)	Diff./Sign.
GIL 19-03-07	8.89	-74.50 oo	327.41	-33.86 ns	310.90	116.19 ns	647.20	7.83 ns
ZIL 19-02-43	44.87	-38.52 ns	321.06	-40.21 ns	159.15	-35.56 ns	525.08	-114.29 ns
GIL 19-03-29	52.06	-31.32 ns	133.76	-227.51 o	34.50	-160.21 o	220.32	-419.05 oo
COSIANA (Ct)	83.39	-	361.27	-	194.71	-	639.37	-
		LSD 5%=40.12 LSD 1%=60.75 LSD 0.1%=97.59		LSD 5%=207.16 LSD 1%=313.70 LSD 0.1%=503.95		LSD 5%=137.70 LSD 1%=208.51 LSD 0.1%=334.97		LSD 5%=270.55 LSD 1%=409.70 LSD 0.1%=658.16

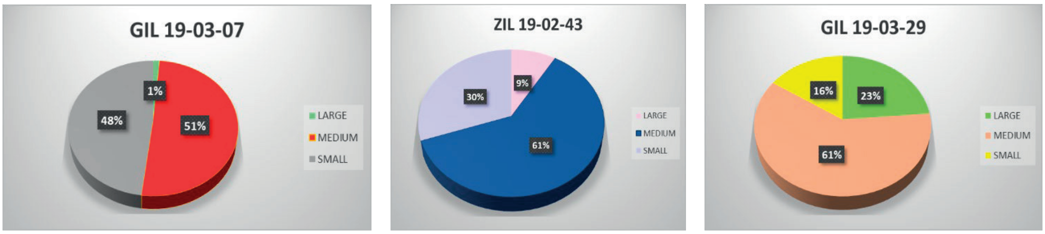

Figure 4. Distribution of tubers by size fractions (%) according to genotype

Examining the data presented in Table 4, regarding the yield of large tubers/ha, the obtained results highlight the GIL 19-03-29 genotype (8.54 t/ha), which came closest to the Cosiana variety (14.20 t/ha), being followed by ZIL 19-02-43 (6.87 t/ha). Regarding the yield of medium tubers, the genotype ZIL 19-02-43 (18.37 t/ha) stands out, surpassing the control (16.18 t/ha) compared to which it recorded a positive difference of 2.20 t/ha. The genotype GIL 19-03-07 obtained the best results regarding the yield of small tubers (4.49 t/ha) recording a difference of 1.90 t/ha compared to the control variety.

Table 4. Effect of genotype on the tuber yield by size fractions and total tuber yield (t/ha) (experimental field NIRDPSB Brasov, 2022)

Genotype	Yield of large tubers (t/ha)	Diff./Sign.	Yield of medium tubers (t/ha)	Diff./Sign.	Yield of small tubers (t/ha)	Diff./Sign.	Total yield (t/ha)	Diff./Sign.
GIL 19-03-07	1.16	-13.05 oo	15.56	-0.62 ns	4.49	1.90 ns	21.21	-11.76 ns
ZIL 19-02-43	6.87	-7.33 o	18.37	2.20 ns	1.95	-0.64 ns	27.19	-5.77 ns
GIL 19-03-29	8.54	-5.66 ns	8.00	-8.17 ns	0.39	-2.19 o	16.94	-16.03 o
COSIANA (Ct)	14.20	-	16.18	-	2.59	-	32.97	-
		LSD 5%=6.80 LSD 1%=10.29 LSD 0.1%=16.53		LSD 5%=8.78 LSD 1%=13.29 LSD 0.1%=21.36		LSD 5%=1.94 LSD 1%=2.93 LSD 0.1%=4.71		LSD 5%=13.11 LSD 1%=19.86 LSD 0.1%=31.90

Regarding the total tuber yield, the obtained results highlighted the genotype ZIL 19-02-43 which obtained 27.19 t/ha, followed by the genotype GIL 19-03-07 with 21.21 t/ha, the differences compared to the control being insignificant. The two potato genotypes derived from botanical seed demonstrated a good production capacity, over 20 t/ha in the conditions of a dry crop year.

Results regarding the evaluation of culinary quality characteristics

Analyzing the results obtained regarding the starch content of potato tubers (Table 5), it can be observed that the genotype GIL 19-03-29 recorded the lowest percentage of starch (12.50%), and GIL 19-03-07 had the richest starch content (17.17%). ZIL 19-02-43 recorded a starch content of 14.17%. Thus, the genotypes GIL 19-03-29 and ZIL 19-02-43 are more suitable for fresh consumption, while GIL 19-03-07 lends itself to industrialization.

Table 5. Starch content (%) of potato genotypes derived from true seed

Genotype	Starch content/replication (%)			Average
GIL 19-03-07	17.25	16.75	17.50	17.17
ZIL 19-02-43	14.25	14.50	13.75	14.17
GIL 19-03-29	12.50	12.25	12.75	12.50

From the point of view of culinary and technological quality, potato genotypes derived from true seed were evaluated in terms of: boiling behavior, dry matter and starch content, flesh color (raw and cooked) and suitability for chips. Table 6 presents mean values for culinary quality traits. By the sum of crushing on boiling, consistency, mealiness, moisture and starch granulation a cooking type index for the studied potato genotypes was obtained. Thus, the GIL 19-03-29 genotype belongs to class A/B, being recommended for salads and other culinary preparations where consistent tubers are needed, which do not crumble, or crumble a little during cooking. The genotypes ZIL 19-02-43 and GIL 19-03-07 belong to class B. Due to the good taste and multiple directions of use, potatoes in this class are highly demanded by consumers, being suitable for most culinary preparations.

Table 7 shows the results regarding the color change of the cooked pulp after 24 hours. Thus, it can be seen that the genotype ZIL 19-02-43 received the best grade, because the pulp color remained almost unchanged, being followed by the genotype GIL 19-03-07, while the genotype GIL 19-03- 29 the color of the pulp has changed compared to the initial appearance, receiving a lower grade.

Regarding the degree of raw pulp enzymic browning, the obtained results (Table 7) highlight the genotype ZIL 19-02-43, which received the best mark (1.5), the color of the fresh pulp remaining almost unchanged (after 4 hours at room temperature) compared to the initial color. This was followed by genotype GIL 19-03-07 (1.83) and genotype GIL 19-03-29 (2.67).

In the industrial process, it is important to know what the peeling losses are and what is the weight difference between the tuber without skin and the sliced one (Table 8). It is desirable that a large amount of the total tuber weight can be used, in order to obtain the final product. In this sense, the genotype GIL 19-03-29 obtained very good results, the losses being the lowest. In order for the losses due to peeling to be as low as possible, the tubers must have a thin skin and the eyes as shallow as possible.

Table 8. Determinations of suitability for chips

1 sample = 3 tubers	Genotype		
	GIL 19-03-07	ZIL 19-02-43	GIL 19-03-29
Tubers weight with skin (g) W_1	402	426	438
Tubers weight without skin (g) W_2	326	358	364
Difference (g) $W_1 - W_2$	76	68	74
Weight of round slices (g) W_3	306	340	352
Difference (g) $W_2 - W_3$	20	18	12

When using potato as a raw material to obtain chips, it is important to determine how many chips were obtained from a certain amount of potatoes. Thus, it is calculated (Table 7) what percentage of the total weight of the tuber with skin was used as chips. Regarding this aspect, the genotype GIL 19-03-07 obtained the best results, having a total efficiency of 31.34%. For a potato genotype to be suitable for this purpose, 1 kg of chips must be obtained from 4 kg of potatoes. From this point of view, all 3 studied genotypes fall within this ratio.

Table 6. Apreciation on culinary quality of boiled potato tubers

Genotype	Average grades for culinary quality traits									Cooking
	Appearance	Taste	Interior color	Crushing on boiling	Consistency	Mealiness	Moisture	Starch granulation	Cooking type index	
ZIL19-02-43	1.00	3.75	2.25	1.00	1.75	2.50	2.63	2.38	10.26	B
GIL19-03-29	1.63	1.75	3.00	1.13	1.38	2.00	2.63	1.63	8.77	A/B
GIL19-03-07	2.50	2.50	2.75	1.50	1.88	2.25	2.13	2.38	10.14	B

Table 7. Culinary quality assessment results (chips, enzymic browning and after-cooking blackening)

Genotype	Dry matter content (%)	Starch content (%)	Enzymic browning	After-cooking blackening	Peeling efficiency (%)	Mechanical processing efficiency (%)	Total efficiency (%)	Chips color
ZIL19-02-43	23.55	14.17	1.50	1.3	84.04	79.81	26.29	7.7
GIL19-03-29	22.17	12.50	2.67	2.7	83.11	80.37	25.11	6.7
GIL19-03-07	24.15	17.17	1.83	2.0	81.09	76.12	31.34	9.0

Regarding the color of the chips, all three genotypes presented a beautiful, golden color, appreciated by the tasters. The best grade was given to the genotype GIL19-03-07 (9), followed by ZIL 19-02-43 and GIL 19-03-29 (Table 7).
When we refer to the potato intended for industrial processing, a high content of dry matter (24% or higher) ensures a better production of the finished product, since the reduced amount of water evaporates faster during frying, and the amount of oil taken up by to the potato slices is smaller. The highest dry matter content was recorded in the genotype GIL 19-03-07 (24.15%).
Potato genotypes with a starch content between 17-19% are preferred for chips. A low starch content in the tubers leads to the absorption of a large amount of oil during frying and the reduction of the product's shelf life. And from this point of view, GIL 19-03-07 also stands out with a percentage of 17.17% (Table 7).

CONCLUSIONS

Three potato genotypes derived from true seed, which showed tolerance to water stress induced *in vitro*, were also evaluated under open field conditions.
Regarding the total number of tubers, the GIL 19-03-07 genotype obtained the best results (647.20 thousand/ha), surpassing the Cosiana variety (control), compared to which a positive difference of 7.83 thousand/ha was recorded.
Analyzing the results regarding the influence of genotype on the distribution of tubers in different size fractions, it emerged that in all three potato genotypes, the highest percentage had medium-sized tubers and then tubers from the small fraction, with the exception of the genotype GIL 19-03-29 where the proportion of tubers from the large fraction (23%) exceeded that of small-sized tubers (16%).
Regarding the total tuber yield, the obtained results draw our attention to the genotype ZIL 19-02-43 which obtained 27.19 t/ha, followed by the genotype GIL 19-03-07 with a production of 21.21 t/ha, the differences compared to the control variety being insignificant. It should be mentioned that these productions were obtained in the climatic conditions of a crop year which in the summer period was characterized by average temperatures higher than 2.5°C compared to MAA, and the level of precipitation was very low compared to the multiannual average for Brasov, both in winter and in the growing season.
Based on the culinary and technological quality analyses, the characterization of the three genotypes from botanical seed potato is as follows:

– **GIL 19-03-07:** the tubers maintain their firmness during cooking, in general the enzymes that cause the blackening of the pulp are missing and have an excellent color when fried. Tubers have a higher dry matter content and a higher percentage of starch. As a priority direction of use this genotype is suitable as a raw material for obtaining chips.
– **ZIL 19-02-43:** after boiling, the tubers remain intact. The enzymes that cause the blackening of the pulp are missing and the tubers are very attractive. The color of the pulp remains unchanged after boiling or frying. It also meets all the requirements to obtain potato chips.
– **GIL 03-19-29:** The tubers do not crush when boiled, are not floury, have a consistent pulp, they are moist, and the starch structure is fine. The taste is very good and the starch content is lower. Due to the pleasant taste and the texture of the pulp, it is suitable for salads and other culinary dishes.

It is recommended to use the genotypes ZIL 19-02-43 and GIL 19-03-07 which have demonstrated a good production capacity under the conditions of a dry year. Also, as a priority direction of use, the two potato genotypes derived from true seed are suitable as raw material for obtaining chips.

ACKNOWLEDGEMENTS

This work was supported by the project PN19-32-01-03, "The use of true potato seed lines in order to identify perspective genotypes in the context of global climate change", contract number 37 N/2019.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE EFFICACY OF A FUNGICIDE MIXTURE IN CONTROLLING BLACK LEG AND STEM ROT IN WINTER OILSEED RAPE

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Abstract

Lately, winter oilseed rape has become more and more grown by farmers in Romania, being a rewarding and versatile crop. Black leg (Leptosphaeria maculans) and stem rot (Sclerotinia sclerotiorum) continue to cause significant yield losses in Romania in winter oilseed rape, despite widespread use of fungicides. A series of three trials were conducted in Călăraşi County in 2021 to quantify the contribution of a mixture of Azoxystrobin 20% and Difenoconazole 12.5% at three different rates and of cultivar resistance. In the field, assessments were performed to conclude the frequency (F%) and intensity (I%) of the attack, in order to calculate the degree of attack (DA%). In the end, the effectiveness of the treatment scheme was calculated. Significant effectiveness and yield differences were recorded between cultivars and fungicides dose rates in the fields. Fungicides higher rates gave better control of diseases than lower ones and contributed to disease control and to yield responses to a greater extent than cultivar resistance. DA% of black leg infection was lower than of stem rot.

Key words*: effectiveness, Leptosphaeria maculans, Sclerotinia sclerotiorum, winter oilseed rape.*

INTRODUCTION

Due to its use both for nutritional and industrial needs, winter oilseed rape or canola (*Brassica napus* var. *oleifera* M. Delile) continues to increase in popularity among farmers. It is considered the second most important oilseed crop of the world after soybean (*Glycine max* L. Merrill) (Friedt et al., 2018; Zheng et al., 2020). Nowadays it has a current share of approx. 15% of world production (https://apps.fas.usda.gov), which shows the high value of the seeds as a source for oil and animal feed. The importance of winter oilseed rape has increased in recent years among consumers since provide a health market niche, having the less level of saturated fat of all edible oils and a very rich level of mono-unsaturated fat (Butkute et al., 2006). In Romania production of winter oilseed rape has increased very fast over the last 20 years. According to FAO 2022 data, in the last 20 years, the areas cultivated in our country with this crop have expanded at least 5 times, currently it is cultivated on approx. 500,000 ha. Consequently, the average annual production per hectare tripled during this period (https://www.fao.org/faostat/en/#data) due to the modern technology and to the occurrence of new hybrids that have high requirements for production increases.

Winter oilseed rape is a crop with multiple uses, and nowadays his oil is more desirable than ever. The main sense for this are the widespread use of his oil in a lot of fields, which include human nutrition, raw material in the chemical industry, an alternative regenerative fuel but also as ecological lubricating oil used for various purposes.

One of the major reasons that causes great losses to winter oilseed rape crop are pathogenic fungi, so that without phytosanitary protection measures there is a great risk to limit crop production or in the worst case yields will be lost. Winter oilseed rape may be infected by plant pathogens such as phoma stem canker, stem rot, black spot, grey mould, powdery mildew and others.

Black leg or phoma stem canker, *Leptosphaeria maculans* (Tode) Desmazières, is a widespread important disease of winter oilseed rape which causing severe losses on crops in all European countries (Fitt et al., 2006). This disease has gained an increasingly large scale in recent years in cultures from Romania but also from all over Europe. Having a world-wide distribution is probably due to the transmission of pathogen through the seeds of many others Brassica crops (West et al., 2001). According to Fitt et al. (2006), there is concrete evidence of an eastward spread of *L. maculans* from Western Europe. The pathogen infects cotyledons, leaves and pods in the form of brownish spots, further causes canker on stems and root crowns and lesions on upper stems (Mitrović & Marinković, 2007). Blackening of the stem base is dangerous if it settles during flowering, pods formation and seed maturation. Most of the time, spots at the base of the stem may coalesce, after which they break forming dry rots. This type of manifestation is considered the most harmful (Padmathilake & Fernando, 2022). Despite the deployment of resistant variety, the winter oilseed rape growing in Romania continues to suffer serious losses from phoma stem canker.

Stem rot, *Sclerotinia sclerotiorum* (Libert) de Bary, is one of the main harmfull fungal diseases of winter oilseed rape worldwide, as almost of the crop could die when lesions spread onto the principal stems. It has a wide range of hosts and can infest more than 600 plant species, including member family of the *Brassicaceae*, *Fabaceae*, *Solanaceae*, *Asteraceae*, *Apiaceae* and *Amaranthaceae* causing huge economic losses every year (Ding et al., 2021). Severe yield losses are reported in winter oilseed rape due to the lack of effective control measures. In some regions it causes damage every year starting from 10-20% and where the disease is severe it can reach 80% (Ding et al., 2021). Yield losses caused by stem rot are mainly significant when infestations occur at the early stage of flowering under humidity conditions (Baicu & Șesan, 1996). Ascospore of *S. sclerotiorum* initially infects petals, which fall onto leaf surfaces, resulting in infection of those organs (Jamaux et al., 1995). Early detection of the fungus on petals demands fungicide applications in cases of substantial disease risk.

The management of this stem rot disease is difficult due to a lack of genetic resistance in most commercially grown winter oilseed rape cultivars (Ni & Punja, 2020). The recommended strategies for management are crop rotations and fungicides. One of the most efficient ways to prevent and control crop diseases is the use of chemical fungicides with different chemical structures and modes of action. However, resistant of winter oilseed rape cultivars are unavailable, chemical control is the most important way to control *S. sclerotiorum* (Ma et al., 2016).

The active ingredients Azoxystrobin 20% + Difenoconazole 12.5% SC, a methoxy-acrylate and a triazole respectively, have different modes of action that complement each other. The active substance Azoxystrobin belongs to the chemical class of strobilurin fungicides and is active as a systemic broad-spectrum, translaminar fungicide with protective, curative and eradicate action. Difenoconazole is a broad-spectrum fungicide that controls a wide variety of fungi. It acts as a seed treatment, foliar spray and systemic fungicide. It is taken up through the surface of the infected plant and is translocate to all parts of the plant. It has a curative effect and a preventative effect (Henegar et al., 2019).

The aim of this study was to analize the efficacy of three rates of a fungicide mixture (Azoxystrobin 20% + Difenoconazole 12.5% SC) applied to control stem diseases at the most appropriate stages.

The efficacy of fungicide applications is dependent on being able to predict when fungal ascospores are first present on floral tissue. Failure to apply fungicides at the most effective time can result in an economic cost to the grower.

MATERIALS AND METHODS

Three field trials were carried out in three locations at Ileana, at Marsilieni and at Dâlga (Figure 1) from Southern Romania in Călărași County on chernozem soils, over the 2021-2022 growing seasons. Winter oilseed rape crops were sown in September 2021 according to local practices (Table 1). The trials were organized in a randomized complete block design, in agreement with the EPPO method 1/152(4) (EPPO Standards, 2012). Individual treatment plot size was of 24 m^2 and four replicates per each treatment were conducted.

Table 1. Agrotechnical measures in the field experiments

Location	Ileana	Marsilieni	Dâlga
Preceding crop	Winter wheat	Winter barley	Winter wheat
Sowing date	Sep-1-2021	Sep-15-2021	Sep-15-2021
Emergence date	Sep-23-2021	Oct-10-2021	Oct-7-2021
Variety	SY Iowa	Phoenix CL	SY Floretta
Density (plants/ha)	500000	450000	500000
Fertilisation	Complex NP-200 kg/ha Ammonium Nitrate-200 kg/ha	Ammonium Nitrate-200 Kg/ha Wuxal Sulphur (3 l/ha)	Ammonium Nitrate-200 Kg/ha Wuxal Sulphur (3 l/ha)
Treatment date	Apr-14-2022	Apr-15-2022	Apr-20-2022
Weeds control	Centurion plus (0.8 l/ha)	Pantera 40 EC (1.5 l/ha)	Pantera 40 EC (1.5 l/ha) BRASAN (2 l/ha)
Pest control	Sumi alpha 5 EC (0.2 l/ha) Karate Zeon (0.15 l/ha)	Lamdex Extra (0.3 kg/ha)	Lamdex Extra (0.3 kg/ha) Scatto (0.2 l/ha)

Assessments were conducted in fields looking for *L. maculans* and *S. sclerotiorums* stem infection, checking and comparing the samples taken from the field with the help of the phytopathological atlas (Ză, 2008).

Samples were analysed in laboratory by microscope too.

On winter oilseed rape, the mixtures of fungicides Azoxystrobin 20% + Difenoconazole 12.5% SC was sprayed at the rate of 0.6, 0.8 and 1.0 l/ha. So, at Ileana, treatments were applied at BBCH 50 (stage of flowers buds present, still enclosed by leaves), at Marsilieni on BBCH 51 (flowers buds visible from above, “green bud” stage) and at Dâlga during BBCH 53 (flowers buds raised above the youngest leaves).

The fungicides were applied by a knapsack sprayer (Solo 425, Germany) to imitate practical application by farmers (water volume: 400 litters/ha). Three assessments were conducted at BBCH 81 (10% of pods ripe, seeds dark and hard), at BBCH 83 (30% of pods ripe, seeds dark and hard) and at BBCH 85 (50% of pods ripe, seeds dark and hard) according to EPPO method no. PP 1/78(3) (EPPO Standards, 2021). A number of 50 randomly chosen stems per plot were analysed, assessed as percentage infected plants. Statistical data - processing of the assessments was made on the analysis of ARM-9 software (P = .05, Student-Newman-Keuls).

The values of the frequency and intensity of the diseases were determined according to the formulas: Frequency (F%) = n x 100/N, where N means number of plants observed (%) and n means number of plants specific symptoms (%). The intensity was evaluated in percentages and calculated according to the formula: Intensity (I%) = Σ (ixf)/n (%), where I means percentage given, f means number of plants/organs with the respective percentage and n is total number of attacked plants/ organs.

Based on the data obtained by evaluating the frequency and intensity, the degree of attack was calculated: DA = F x I/100 (%), where DA means attack degree (%), F means frequency (%) and I means intensity (%).

The effectiveness of the treatments was determined according to the formula: E = [Gam-Gav/Gam] x 100 (%) (Abbott 'formulas), in which: Gam- degree of attack on the control sample, Gav- degree of attack on the treated check (Iosub et al., 2022).

Figure 1. Trial location of Dâlga

RESULTS AND DISCUSSIONS

A high amount of rainfall (litters on square meters) occurred at trail fields in the first half of April as follows: 50.3 at Ileana, 28.8 at Marsilieni and 20.1 at Dâlga. This fact created specific conditions for the outbreak of winter oilseed rape diseases so that *L. maculans* and *S. sclerotiorum* infection damaged the plants. In untreated checks due to weather conditions and lack of treatments, frequency and intensity had an upward evolution and increased at every assessments (Tables 2 and 3). Consequently, the degree of attack reached high values.

Thus, for *L. maculans* at winter oilseed rape, the DA% on stem increased in the untreated plots from 6 to 14.3% at Ileana, from 8 to 16.8% at Marsilieni and from 3.6 to 8.4% at Dâlga (Table 2).

Table 2. Evolution of *L. maculans* in the trials

Location/ Hybrid	Samples	BBCH 81			BBCH 83			BBCH 85		
		F%	I%	DA%	F%	I%	DA%	F%	I%	DA%
Ileana/ SY Iowa	Untreated	24a	25a	6a	29a	30a	8.7a	36a	40a	14.3a
	Azoxystrobin + Difenoconazole 0.6 l/ha	12b	15b	1.8b	15b	17.5b	2.6b	18b	25b	4.5b
	Azoxystrobin + Difenoconazole 0.8 l/ha	8c	12.5c	1c	10c	15b	1.5c	14c	17.5b	2.4c
	Azoxystrobin + Difenoconazole 1.0 l/ha	5d	5d	0.25d	8c	5c	0.4d	9d	7.5c	0.67d
	LSD (P=.05)	2.416	2.870	0.762	4.502	4.114	0.977	4.882	8.940	3.172
Marsilieni/ Phoenix CL	**Samples**	**BBCH 81**			**BBCH 83**			**BBCH 85**		
		F%	**I%**	**DA%**	**F%**	**I%**	**DA%**	**F%**	**I%**	**DA%**
	Untreated	27a	30a	8a	33a	35a	11.6a	42a	40a	16.8a
	Azoxystrobin + Difenoconazole 0.6 l/ha	18b	15b	2.7b	20b	20b	4b	24b	25b	6b
	Azoxystrobin + Difenoconazole 0.8 l/ha	12c	10c	1.20c	14c	15b	2c	17c	20b	3.4c
	Azoxystrobin + Difenoconazole 1.0 l/ha	6d	5d	0.3d	8d	10c	0.8d	12d	10c	1.2d
	LSD (P=.05)	5.211	6.216	2.171	4.208	6.765	2.645	3.343	9.130	3.898
Dâlga/ SY Floretta	**Samples**	**BBCH 81**			**BBCH 83**			**BBCH 85**		
		F%	**I%**	**DA%**	**F%**	**I%**	**DA%**	**F%**	**I%**	**DA%**
	Untreated	18a	20a	3.6a	23a	25a	5.75a	28a	30a	8.4a
	Azoxystrobin + Difenoconazole 0.6 l/ha	12ab	10b	1.2b	16b	12.5b	2b	20b	15b	3b
	Azoxystrobin + Difenoconazole 0.8 l/ha	9b	7.4b	0.67b	12c	7.5c	0.9c	16b	10b	1.6c
	Azoxystrobin + Difenoconazole 1.0 l/ha	3c	1.83c	0.1c	7d	2.85d	0.2d	9c	5c	0.45d
	LSD (P=.05)	6.941	6.802	1.250	4.593	5.661	1.740	6.568	7.382	2.349

Regarding the infection of *S. sclerotiorum* evolved in the untreated plots from 8.4 to 17.6% at Ileana, from 11.2 to 22% at Marsilieni and from 5.25 to 14.4% at Dâlga (Table 3). During the assessments from BBCH 81 to 85 diseased plants showed symptoms of yellowing and wilting. Wet weather formed a white surface, the mycelium of the fungus, in which sclerotia were later formed. When the disease progressed, sclerotia of the fungus were observed inside the stem in the medullary tissue. After completing the lifecycle, the fungus produced sclerotia or long-lived resting bodies which remained active, emphasizing even more the problem of this disease. In these infestation conditions in experimental fields, the fungicidal mixture of Azoxystrobin 20% + Difenoconazole 12.5% SC had a good efficacy in controlling of diseases at all doses tested, the best results being obtained at higher rates.

On consequence, at Ileana, the efficacy on BBCH 85 for black leg at rate of 0.6 l/ha was 68.11, at 0.8 l/ha was 82.17 and at 1.0 l/ha was 95.23% (Figure 2).

In Marsilieni trial the efficacy value at 0.6 l/ha was 61.54, at 0.8 l/ha was 79.3 and at 1.0 l/ha was 92.63% (Figure 3).

As far as concerns Dâlga experiment, efficacy was at 0.6 l/ha was 62.38, at 0.8 l/ha was 79.42 and at 1.0 l/ha was 93.58% (Figure 4).

Table 3. Evolution of *S. sclerotiorum* in the trials

Location/ Hybrid	Samples	BBCH 81			BBCH 83			BBCH 85		
		F%	I%	DA%	F%	I%	DA%	F%	I%	DA%
Ileana/ SY Iowa	Untreated	28a	30a	8.4a	39a	35a	13.7a	44a	40a	17.6a
	Azoxystrobin + Difenoconazole 0.6 l/ha	16b	20b	3.2b	24b	22.5b	5.4b	27b	25b	6.7b
	Azoxystrobin + Difenoconazole 0.8 l/ha	12c	15c	1.8c	17c	17.5b	3c	19c	20c	3.8c
	Azoxystrobin + Difenoconazole 1.0 l/ha	7d	5d	0.35d	12d	10c	1.2d	13d	12.5d	1.6d
	LSD (P=.05)	4.57	4.29	1.993	4.688	6.412	3.131	6.53	5.336	3.962
Marsilieni /Phoenix CL	Samples	BBCH 81			BBCH 83			BBCH 85		
		F%	I%	DA%	F%	I%	DA%	F%	I%	DA%
	Untreated	32a	35a	11.2a	36a	40a	14.4a	44a	50a	22a
	Azoxystrobin + Difenoconazole 0.6 l/ha	21b	22.5b	4.72b	23b	25b	5.75b	30b	30b	9b
	Azoxystrobin + Difenoconazole 0.8 l/ha	16c	17.5c	2.8c	17c	20c	3.4c	22c	22.5c	4.95c
	Azoxystrobin + Difenoconazole 1.0 l/ha	9d	10d	0.9d	12d	10d	1.2d	13d	12.5d	1.63d
	LSD (P=.05)	4.39	4.624	2.126	3.499	4.815	2.186	4.76	7.849	3.933
Dâlga/ SY Floretta	Samples	BBCH 81			BBCH 83			BBCH 85		
		F%	I%	DA%	F%	I%	DA%	F%	I%	DA%
	Untreated	21a	25a	5.25a	29a	35a	10.2a	36a	40a	14.4a
	Azoxystrobin + Difenoconazole 0.6 l/ha	15b	15b	2.25b	20b	20b	4b	24b	25b	6b
	Azoxystrobin + Difenoconazole 0.8 l/ha	10c	10bc	1c	16b	15b	2.4b	18c	17.5c	3.2c
	Azoxystrobin + Difenoconazole 1.0 l/ha	7c	7.3c	0.51c	9c	10c	0.9c	11d	10d	1.1d
	LSD (P=.05)	4.69	6.850	1.769	5.651	6.730	2.90	6.62	7.21	3.604

The best result were recorded at Ileana trial with an efficacy of 95.23 % at 1.0 l/ha rate and the worst was 61.54 at Marsilieni when 50% of pods ripped and seeds were dark and hard.

No phytotoxicity symptoms have been shown in the experimental plots. No symptoms of chlorosis, necrosis, leaf deformation, height reduction, distortion and delay at flowering in plots treated with fungicidal mixture of Azoxystrobin + Difenoconazole were seen. In experimental plots of the three trials symptoms of stem root were detected at every assessments time. As a result of treatment applied, the efficacy on BBCH 85 for *S. sclerotiorum* at rate of 0.6 l/ha was 59.42, at 0.8 l/ha was 77.36 and at 1.0 l/ha was 90.56%, at Ileana (Figure 5). In plots from Marsilieni, the efficacy of 0.6 l/ha mixture was 58.89, of 0.8 l/ha was 77.22 and of 1.0 l/ha was 92.29 % (Figure 6). Regarding Dâlga trial, efficacy for 0.6 l/ha was 58.42, for 0.8 l/ha was 76.88 and for 1.0 l/ha was 91.41% (Figure 7).

The highest performance was registered at Marsilieni trial with an efficacy of 92.29 % at 1.0 l/ha rate and the lowest was 59.42% at 0.6 l/ha in Dâlga trial when 50% of pods ripped and seeds were dark and hard.

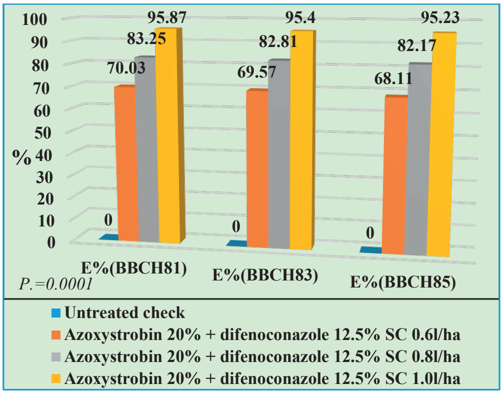

Figure 2. Efficacy of treatments for *L. maculans* at Ileana

The mixture of two active substance with protective and curative action applied at the first stage of infection are a possible and efficient way to control *S. sclerotiorum* and *L. maculans*.

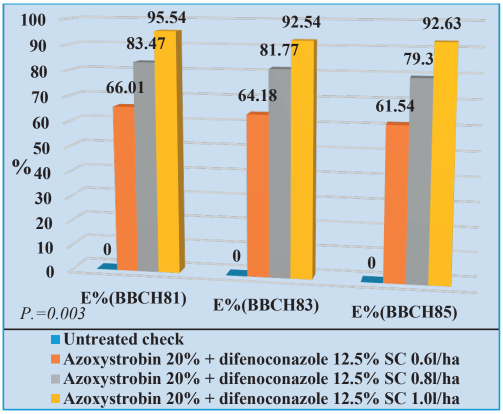

Figure 3. Efficacy of treatments for *L. maculans* at Marsilieni

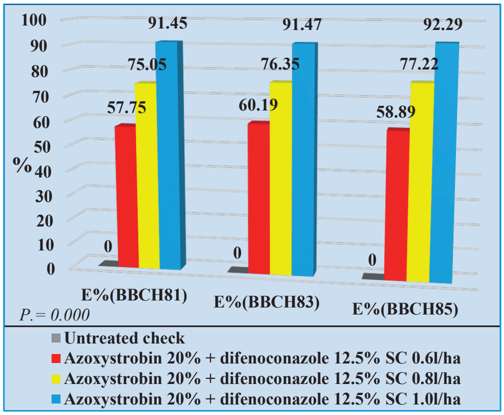

Figure 6. Efficacy of treatments for *S. sclerotiorum* at Marsilieni

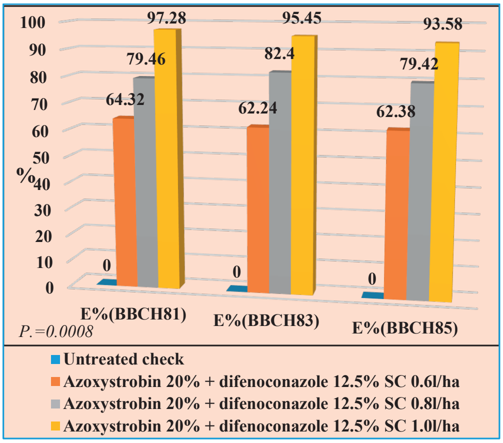

Figure 4. Efficacy of treatments for *L. maculans* at Dâlga

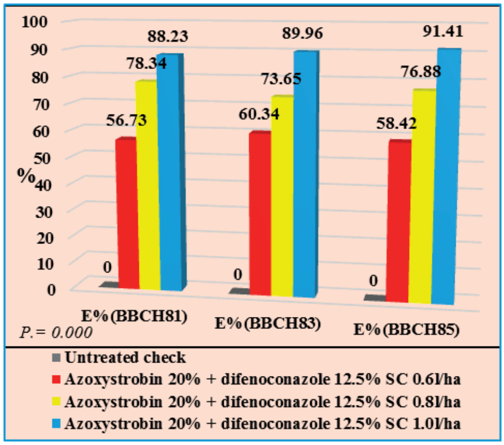

Figure 7. Efficacy of treatments for *S. sclerotiorum* at Dâlga

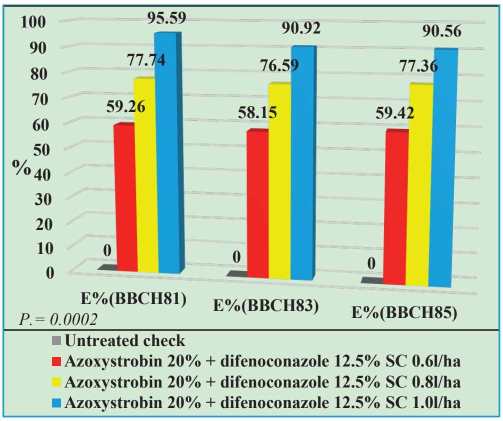

Figure 5. Efficacy of treatments for *S. sclerotiorum* at Ileana

CONCLUSIONS

There was a gradual increase of the degree of attack of the two diseases studied from late spring to the beggining of summer in every location. It was noted that due to low rainfall the degree of the attack of the two diseases was weaker in the location of Dâlga.

For the control of the *L. maculans* disease, by applying the treatments at the stage of flowers buds present, a good effectiveness was ensured which reached 95.23% in Ileana location.

Also, the fungicide mixture applied proved very useful in the control of *S. sclerotiorum* so that the effectiveness was 92.29% in Marsilieni location, at the final observations.

The application of the treatment with Azoxystrobin 20% + Difenoconazole 12.5%

SC at reduced doses is not economically justified because at 0.6 l/ha it only reached 61.54% for black leg and 58.42% for stem root when 50% of pods ripped and seeds were dark and hard.

The most effective was the combination Azoxystrobin 20% + Difenoconazole 12.5% SC applied at the rate of 1.0 l/ha at winter oilseed rape, which reached over 90.0% efficacy.

L. maculans and *S. sclerotiorum* control assays by fungicides on *B. napus* could be limited to a single treatment with a mixture of Azoxystrobin + Difenoconazole applied at 1.0 l/ha dose rate when flowers buds are present.

ACKNOWLEDGEMENTS

Words of gratitude to S.C. Ghinea Prod. S.R.L. and Agronomic Elis S.R.L. the farms who supported us and made our study possible.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ASSESSMENT OF THE SPECIFIC DISEASES IN *Reynoutria sachalinensis* (F. Schmidt) Nakai UNDER THE INFLUENCE OF ENVIRONMENTAL CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

A major risk for the species Reynoutria sachalinensis, as a non-traditional honey, fodder and bioenergy crop, in the Republic of Moldova, are the invasive diseases that affect the plants in the first growing season, once the assimilation organs are formed. It is an early-emerging species, subjected to the influence of stress factors, including diseases that affect the growth potential of plants. The research carried out in 2015-2022 by complex phytosanitary monitoring highlighted the spread of some pathogens that affect the plants of R. sachalinensis annually or periodically, being also favoured by environmental factors. We assessed the most significant diseases detected throughout the growing season, caused by pathogens such as: Peronospora fagopyri, Erysiphe communis f. polygoni, Septoria ascophylli, Ascochyta fagopyri, Phyllosticta polygonorum, Botrytis cinerea, Sclerotinia sclerotiorum, Fusarium gibossus, F. oxysporum f. sp. spinacia, Cucumis virus, Tabacco mosaic virus (TMV). A total of 11 diseases were recorded, 9 of which were of fungal origin and 2 of viral origin, causing various pathologies of etiological nature, of various frequency and significant intensity of damage caused to R. sachalinensis plants.

Key words: *Reynoutria sachalinensis, pathogens, phytosanitary monitoring, pathogens, diseases.*

INTRODUCTION

The major significance of the non-traditional fodder crop, introduced in the Republic of Moldova, *Reynoutria sachalinensis* (F. Schmidt) Nakai, common names - giant knotweed or Sakhalin knotweed, lies in its multiple uses and advantages for the local flora, being attributed to the plants with high estimated potential as honey, energy and ornamental crops (Teleuță et al., 2013; Țîței & Teleuță, 2014).

This species, taxonomically, falls in the order Polygonales, family Polygonaceae Juss., which comprises over 1000 species of herbaceous, annual and perennial plants, which are present worldwide, with some exceptions such as New Zealand and Antarctica (Hrjanovski & Ponomarenko, 1993). *R. sachalinensis* is native to East Asia - northern Japan, China, Korea, including the far east of the Russian Federation, and was later introduced to Western Europe (1855) (Nakai, 1926; Mandak et al., 2004). Later it spread to large areas of the American continent, particularly in USA, where it was grown to explore its bioecological and forage potential, and for the first time it was described back in 1859, in the book "Primitise Floral Amurensis" (Bailey & Conolly, 2012), and then, successively, over time, it was described in various specialized bibliographic sources by many botanists, focusing on its biomorphological and ecological characteristics, as a species used for various purposes: fodder, honey, medicinal, energy, food, ornamental, ecological (Vavilov & Condratiev, 1975; Schnitzler & Muller, 1998; Kawai et al., 2004; Țîței & Teleuță, 2014; Ivanov, 2015; Calalb et al., 2017). The possibilities of using this species as source of fodder and bioenergy are of particular importance for the agro-industrial branch of the Republic of Moldova. The above-mentioned advantages were important reasons of introducing this species in the Republic of Moldova (in 1982); the initial material

(rhizomes) was brought by Teleuță A., PhD, from the Agricultural Institute of Vladikavkaz (The Republic of North Ossetia - Alania, Russian Federation).
In terms of morphological aspects, it is a perennial, herbaceous plant, with erect stems, hollow at the internodes and simple, petiolate leaves, with broad-ovate blade, 20-38 cm long and 15-20 cm wide, with acuminate tip and slightly wavy margin. The leaves have dense hairs on the lower epidermis and are alternately positioned on the stem. The flowers are small, actinomorphic, with simple perianth, produced in panicle inflorescences. The fruits are 3-sided achenes, 2.8-4.5 mm long and 1.1-1.8 mm wide (Fuentes et al., 2011; Cîrlig, 2019; Țîței & Roșca, 2021).
The fast progress of science and technology imposes the objectives of continuously obtaining agricultural productions by means of all plant species with high estimated potential as energy and feed crops, for domestic and wild animals; however, the favourable environmental conditions are advantageous not only to plants but also to various complexes of harmful organisms that act as a limiting factor in increasing the productivity of plants, that is why, as part of an extensive study on plants, some research on biological and phytosanitary control, including the diseases associated with the application of some remedies to regulate the pathological conditions in plants during the cultivation process, is also needed (Bobeș et al., 1972; Baicu & Șăvescu, 1986; Docea & Severin, 1990; Popescu, 2005; Volosciuc, 2009;). The harmful impact of diseases on the productivity of agricultural crops can be enormous, being also facilitated by the current unstable environmental conditions, because of which, science is currently progressing towards the creation of new plant cultivars, characterized by resistance and tolerance to the action of pathogens that triggers invasive diseases with considerable consequences for the agriculture, animal husbandry, forestry and socioeconomic sector (Semencova & Socolova, 1992, Bădărău & Bivol, 2007; 2013). This species, being a physiologically precocious plant, with long growing season, abundant vegetative and generative growth, with fast regeneration of the green mass, has multiple indisputable qualities, but there are also significant manifestations of the consequences caused by phytopathogenic agents, which cause specific diseases, including those acquired through specializations and adaptations of other pathogenic infections, detected practically throughout the growing season, with adaptations facilitated by the favourable environmental conditions of the Republic of Moldova, a fact that motivated us to carry out complex research on the diagnosis of diseases in this species, their etiological features, establishing the frequency and severity of these diseases in *R. sachalinensis* plants. Because of the topicality of the addressed issues, the goal of our research was: the phytopathogenic monitoring of the species *R. sachalinensis,* in correlation with the pedo-climatic conditions of the Republic of Moldova. We set the following *objectives*: keeping biological and phytosanitary control records of the establishment of pathogen complexes and the diseases triggered in the *R. sachalinensis* species, grown under experimental conditions at the "Alexandru Ciubotaru" National Botanical Garden (Institute); establishing the comparative values characterizing the indices of frequency, intensity and severity of damage caused by the phytopathological impact of the detected diseases in this plant species.

MATERIALS AND METHODS

The cultivar 'Gigant' of Sakhalin knotweed, *Reynoutria sachalinensis* (F. Schmidt) Nakai (Figure 1), patented and included in the Register of Plant Varieties of the Republic of Moldova in 2012, served as research subject.

A B

Figure 1. *R. sachalinensis* in the collection of "Alexandru Ciubotaru" National Botanical Garden (Institute): A - vegetative phase; B - generative phase

Complex phytopathological monitoring was carried out at the "Alexandru Ciubotaru" National Botanical Garden (Institute), in the experimental sector of the “Plant Resources” Laboratory, on an area of 0.25 ha, planted with *R. sachalinensis* aged 10 years.

The planting scheme on the experimental sector was: 70 x 70 cm and 70 x 100 cm distance between rows and between plants, on a flat land area located between poplar shelterbelts and the collection of *Junglans* sp. The phenological observations were carried out through periodic surveys, according to the requirements of the methodological guidelines for the detection of harmful organisms in cultivated plants and elements of integrated protection in the Republic of Moldova, 2002 (Bădărău, 2009).

The research activity was carried out by comparing the annual evolution, in 2015-2022. The essential role in the evolution of pathogenic infections affecting *R. sachalinensis* plants was played by the environmental factors characterized annually by high amounts of precipitation, more frequently recorded in April-May, and moderate air temperatures. These conditions were favourable for the fast growth and development of *R. sachalinensis* plants, as well as some phytopathogenic agents, which attacked the plants from the end of May, in the stage of formation of young shoots and foliage until the seed ripening stage, in October.

We conducted special surveys on the evolution of diseases, by assessing the level of invasive impact. Samples were taken at equal distances, a total of 50 plants on the diagonal of the experimental sector (rectangular shape) for each monthly survey, focusing on newly formed shoots, leaves, inflorescences, stems, roots, especially those visibly affected, in the dynamics of the development phases, by carefully highlighting the etiological and symptomatic aspects - the macroscopic field method.

The affected organs were collected and later analysed in the laboratory, applying the humidity chamber method (Figure 2), for 48 hours, and then studied under a microscope, consulting the key identification guidelines of cultivated plants and spontaneous flora according to: Hohreacova et al., 1984; Docea & Severin, 1990; Bădărău & Bivol, 2007; Bădărău, 2009. Later, by calculations, the parameters characterizing the resistance of plants to infectious pathogens were established, determining the degree of attack, using the percentage formula:

$$G_a = \frac{F\% \times I\%}{100}$$

where: *F%* - frequency, and *I%* - intensity.

Preventively, for the estimation of these values, disease frequency (F%) was calculated, representing the relative value of the number of affected plants or organs **(n)** per total number of analysed plants or organs **(N)**.

$$F\% = \frac{n \times 100}{N}$$

Another significant parameter is disease intensity *(I%)*, which indicates the intensity of the development of a disease as a percentage, that is, to what extent a plant or organ was affected. To calculate the qualitative index of the disease intensity, the following formula was used:

$$I\% = \frac{(n_1 . 1) + (n_2 . 2) + (n_3 . 3) + (n_4 . 4)}{N \times 4},$$

where: n_1, n_2, n_3, n_4 - the number of plants or organs affected at the respective grade; *N* - the total number of examined plants or organs; 5 - the maximum grade of the scale.

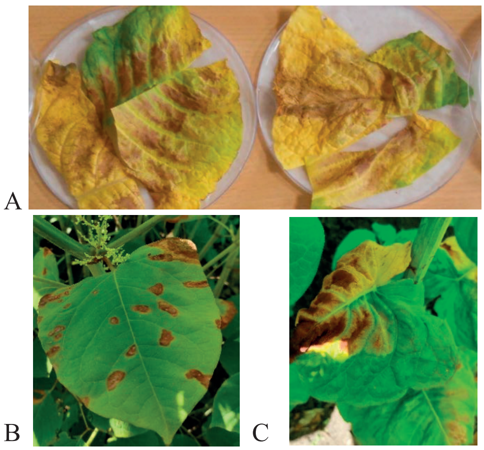

Figure 2. A - establishing the diagnosis on leaves with pathological conditions exposed in humidity chambers (Petri dishes); B - leaves affected by *Septoria ascophylli*; C - leaves affected by *Phyllosticta polygonorum*

In our experiments, the scale with 5 grades was used to denote the disease intensity, which corresponds to certain intervals that express the percentage of the affected area, namely: 0 - visible symptoms are absent; 1 - the affected surface is up to 10%; 2 - the affected surface is from 10 to 25%; 3 - the affected surface is from 25 to 50%; 4 - more than 50% of the surface of the organ is affected. The obtained results were integrated in tables, diagrams and conclusive analyses.

RESULTS AND DISCUSSIONS

R. sachalinensis is an herbaceous perennial with hollow, erect stems at maturity, which, under the climatic conditions of the Republic of Moldova, reach a maximum height of 5-6 m. Underground organs are creeping rhizomes with thin adventitious roots. The flowers are small, cream-white, grouped in panicles. The fruits are 3-sided, brown achenes.

In the researched plants, during the growing season, the symptoms of the infectious process caused by pathogens were specific morpho-anatomical and physiological changes. For the most part, the specific symptoms that accumulated, became visible, and based on them, the specific external symptoms of the disease and the clinical picture of affected plants were established. Obviously, through the evolution of the vegetative stages under the influence of the environmental factors, certain changes in the aspect of the plants occurred and differentiated the forms of manifestation of the detected diseases. Based on the surveys and analyses, the symptoms of specific diseases, signalled by local staining of the tissues on different organs, wilting, chlorosis, browning, mosaic spots, covered with efflorescence (a fine white, grey, cream, pink down) on the upper epidermis and lower, young shoots, stems at internodes and nodes, it was possible to establish the diagnosis and preliminary pathogen, and later through laboratory analyses followed by microscopic analyses, the pathogen and its taxonomic affiliation were determined. But as some diseases such as downy mildew, powdery mildew, septoria, forms of white, grey and dry rot, through evolutionary changes, led to the death of the affected organs. And certain parameters characterizing the frequency and intensity of the disease, related to the degree of damage were reflected in Table 1. For the first time, 11 diseases with pathological impact were diagnosed in *R. sachalinensis* plants, under the conditions of the Republic of Moldova, among which, two are of viral origin and 9 of fungal origin. The diseases detected during the active growth period, the stages of shoot formation - full ripening of seeds (May - October) and the estimation of the taxonomic affiliation of the pathogen by making records and pathological diagnosis are presented below.

Table 1. The etiological composition of the diseases and pathogens detected in the species *R. sachalinensis*

№	Name of the disease	Pathogen	Taxonomic affiliation
1	Downy mildew	*Peronospora fagopyri* Elenev (Berk) de By	Cl. Oomycetes Ord. Pythiales
2	Powdery mildew	*Erysiphe communis* (Wall) Grev. f. *polygoni*	Cl. Ascomycetes Ord. Erysiphales
3	Septoria leaf spot	*Septoria ascophylli* Melnic & M. Petrov	Cl. Deuteromycetes Ord. Capnodiales
4	Ascochyta blight	*Ascochyta fagopyri* Bres	Cl. Deuteromycetes Ord. Pleosporales
5	Phyllosticta leaf spot	*Phyllosticta polygonorum* Sacc.	Cl. Deuteromycetes Ord. Botryosphaeriales
6	Grey mould	*Botrytis cinerea* Fr., pv. *fagopyri*	Cl. Deuteromycetes Ord. Hyphales
7	White mould	*Sclerotinia sclerotiorum* (de By) Korf. Et Dumont.	Cl. Ascomycetes Ord. Helotiales
8	Stem rot	*Fusarium gibbosus*	Cl. Deuteromycetes Ord. Hyphales
9	Root and stem rot	*F. oxysporum* f. sp. *spinacia* W.C. Snyder & H.N. Hansen	Cl. Deuteromycetes Ord. Hyphales
10	Mosaic chlorosis	*Cucumis virus* I Smith	Cl. Eucristallinea Ord. Insectophiles
11	Tobacco mosaic disease	*Tobacco mosaic virus* (TMV)	Cl. Alsuviricetes Ord. Martellivirales

Simultaneously with the pathographic, pathogenic and epidemiological research on Sakhalin knotweed plants, the biological control regarding the qualitative and quantitative aspects of the invasive impact of viral and fungal agents, detected on various plant organs, was carried out.

The field analyses on the researched plants highlighted the manner of manifestation and extent of the diseases through certain specific symptoms, taking into account the individual contact with each phytopathogen, causing diseases of different severity, characterized by: discolouration, chlorosis, spots, browning, followed by organ deformation, partial or total necrosis of the foliage and stems. These symptoms facilitated the determination of the diseases that affected this plant species, which, under the conditions of the Republic of Moldova, was contaminated by downy mildew, Phyllosticta leaf spot, Ascochyta blight, powdery mildew, grey mould, white mould, root and stem rot, mosaic chlorosis, reported more abundantly in ascending aspect on all plant organs.

These associations of pathogens drew the attention of researchers, who frequently conduct phytosanitary surveys, which are included in research programs aimed at introducing traditional and non-traditional fodder, honey and energy crops characterized by resistance and tolerance to environmental stress factors.

The research carried out was completed with records and analyzes related to parasitic impact indices characterizing the degree of individual attack of each detected pathogen, with qualitative and quantitative impact indices.

Resulting from the instability of the climatic factors of the last decade, in the Republic of Moldova, there is an alternation of periods with high amounts of precipitation and diurnal temperatures, annually, between April and May, a fact that conditioned the spread of the following specific diseases that initially appear in the period of foliage formation, such as: downy mildew *Perenospora fagopyri* Elenev, with the highest frequency of 60 % on leaves, followed by powdery mildew *Erysiphe communis* (Wall) Grev. f. *polygoni*, which was detected later, in summer drought periods, with 50 % frequency, which associated in the middle of summer with Septoria leaf spot *Septoria ascophylli* Melnic & M. Petrov - 40%, Ascochyta blight *Ascochyta fagopyri* Bres - 30% and *Phyllosticta* leaf spot *Phyllosticta polygonorum* Sacc. - 24% (Table 2).

Table 2. The etiological composition and the level of invasive impact with the diseases detected in *R. sachalinensis* (2015-2022)

Disease	Frequency (%)	Intensity (%)	Degree of attack (%)	Affected organs
Perenospora fagopyri	60	0.28	0.17	leaves, young shoots
Erysiphe communis f. *polygoni*	50	0.27	0.14	leaves, young shoots
Septoria ascophylli	40	0.20	0.08	leaves
Ascochyta fagopyri	30	0.14	0.04	shoots
Phyllosticta polygonorum	24	0.11	0.03	leaves, shoots
Botrytis cinerea pv. *fagopyri*	18	0.10	0.02	the base of stems
Sclerotinia sclerotiorum	28	0.11	0.03	internodes, nodes
Fusarium gibbosus	16	0.08	0.01	Mature stems
F. oxysporum f. sp. *spinacia*	12	0.05	0.006	stems, old roots
Cucumis virus	12	0.06	0.007	young leaves
Tobacco mosaic virus (TMV).	10	0.05	0.005	mature leaves

All diseases have significant impact, causing severe damage to annual leaves and shoots in the first growing season. In the second growing season, under the influence of environmental factors, other diseases were also detected, such as: forms of grey and white mould, fusarium rot, favoured by the amount of precipitation and diurnal temperature alternations.

At the same time, two diseases of viral origin were detected: *Cucumis virus* I Smith and *Tobacco mosaic virus* (TMV), with and incidence of 12% and 10%, with symptoms such as mottling and distortion (curling) of mature leaves, being caused by vector insects such as cicadas and phytophagous aphids. The analysis of the values related to the intensity and degree of damage were established in the following sequence reflected in Figure 3, where the curve of the comparative values of the detected diseases according to the parameters of frequency and intensity of the damage is estimated. According to these indices, downy mildew and powdery mildew stand out, followed by Septoria leaf spot *Septoria ascophylli* and *Ascochyta* blight *Ascochyta fagopyri* (Figure 3). So, we can mention the consequent damage caused by the above-mentioned diseases, which have a more severe impact on the plants both in the first and in the following growing seasons, annually and

seasonally. Other detected diseases are grey and white mould, root and stem rot. Viral diseases are frequently associated with other diseases, but occur more rarely, therefore, they are considered as facultative diseases that also infest Sakhalin knotweed, with a lower agro-economic importance.
Resulting from the great regenerative capacities of the plant, the affected leaves detach from the stems and the plants continuously generate new structures throughout the growing season, possessing particular tolerance and resistance to the attack of the detected pathogens.

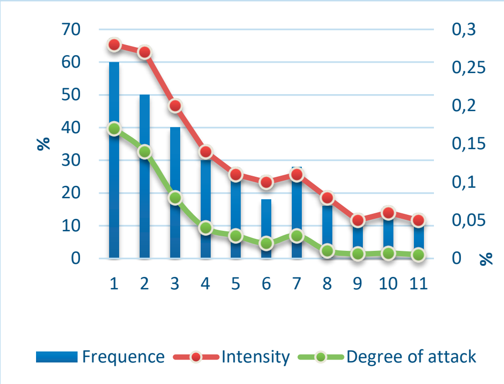

Figure 3. Graphic representation
of the phytopathological parameters analysed

Note: 1 - *Perenospora fagopyri*; 2 - *Erysiphe communis*; 3 - *Septoria ascophylli*; 4 - *Ascochyta fagopyri*; 5 - *Phyllosticta polygonorum*; 6 - *Botrytis cinerea* pv. *fagopyri*; 7 - *Sclerotinia sclerotiorum*; 8 - *Fusarium gibbosus*; 9 - *Fusarium oxysporum* f. sp. *spinacia*; 10 - *Cucumis virus*; 11 - *Tobacco mosaic virus (TMV)*

CONCLUSIONS

The species Sakhalin knotweed has acclimatized and adapted very well to the pedoclimatic conditions of the Republic of Moldova, in terms of morpho-structural, physiological aspects and tolerance, in response to the environmental factors of the region, this claim being proven by such features as fast growth, vitality, longevity, regenerative capacity and rich content of valuable biochemical compounds.
As a result of the biological and phytosanitary monitoring, 11 diseases of significant pathological impact, detected for the first time in this species, under the conditions of the Republic of Moldova, were identified, such as: *Perenospora fagopyri* Elenev (Berk) de By; *Erysiphe communis* (Wall) Grev. f. polygoni; *Septoria ascophylli* Melnic & M. Petrov; *Ascochyta fagopyri* Bres; *Phyllosticta polygonorum* Sacc.; *Botrytis cinerea* Fr., pv. *fagopyri*; *Sclerotinia sclerotiorum* (de By) Korf. Et Dumont.; *Fusarium gibbosus*; *F. oxysporum* f. sp. *spinacia* W.C. Snyder & H.N. Hansen; *Cucumis virus* I Smith; *Tobacco mosaic virus (TMV)*.
The comparative values of the indices of frequency, intensity and degree of damage of pathological interaction with the plants were established individually for each estimated disease and the consecutiveness of the values in descending order is as follows: downy mildew *Perenospora fagopyri* Elenev, with the highest frequency on leaves (60%), followed by powdery mildew *Erysiphe communis* (Wall) Grev. f. *polygoni*, which occurs later, during periods of summer droughts (50%), in the middle of summer, they associate with leaf spot *Septoria ascophylli* Melnic & M. Petrov (40%), *Ascochyta* blight *Ascochyta fagopyri* Bres (30%) and *Phyllosticta* leaf spot *Phyllosticta polygonorum* Sacc. (24%).
However, the species *R. sachalinensis* possesses very high reproduction and regeneration capabilities, especially its foliage. Thus, the affected leaves detach from the plant and it continues to grow new ones, being characterized by tolerance, resistance, high degree of adaptability and ability to maintain high productivity despite the attack of the detected pathogens.

ACKNOWLEDGEMENTS

This research has been carried out with the support of the National Agency for Research and Development, in the framework of the projects 20.80009.5107.02 and 20.80009.7007.12.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON THE ATTACK OF FOLIAR DISEASES IN ALFALFA, MURIGHIOL LOCATION, TULCEA COUNTY

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Abstract

The aim of the research was to monitor the attack of some pathogens specific to alfalfa crops, in the period 2020-2022, in the Murighiol area, Tulcea county. An attack was detected by Pseudopeziza medicaginis ([Lib.] Sacc), responsible for the production of common leaf spot of lucerne or leaf spot of lucerne and Peronospora trifoliorum (de Bary) f. sp. medicaginis-sativae (Thuem.), a pseudofungus that produces downy mildew of alfalfa. The leaf spotting attack was higher during the observation period, reaching 10.3% in the control variant in 2021. The downy mildew attack was lower in 2022. The application of the treatments reduced the impact of the monitored pathogens, ensuring more than 60% effectiveness in the case of the attack of downy mildew and over 70% in the case of the attack of common leaf spot.

Key words: *alfalfa, pathogens, diseases, degree of attack.*

INTRODUCTION

Considered the oldest fodder plant, alfalfa is an important plant for the agriculture of some countries, being widely spread in all countries where this plant is cultivated (Rădulescu et al., 1971). In some countries it is the most important forage legume (Barbetii et al., 2006; Al- Askar et al., 2012). Lucerne, bright high quality fodder, known as the royal fodder (Sun et al., 2012) is also considered an important nitrogen-fixing plant in the soil (Yang et al., 2008). The areas cultivated with alfalfa are also important in our country, alfalfa occupying 45.01% of the area cultivated with fodder plants (Schitea et al., 2020). Alfalfa diseases cause large losses of leaf mass and reduce the nutritional value of forage (Nutter & Guan, 2002) and seed production (Hwang et al., 2006). The diversity of alfalfa pathogens and the co-infection with several pathogens have resulted in a high cost of disease management and control difficulties (Fang et al., 2021; Tollenaere et al., 2016). Also, alfalfa seed can be affected by an important number of pathogens with implications in the quality of the harvest (Cîrstea et al., 2022). *Pseudopeziza medicaginis* ([Lib.] Sacc. [*Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht)] (gd.eppo.int/taxon/PSPZTS) attacks alfalfa plants with different frequencies, but especially on leaves that are strongly illuminated by the sun (Rădulescu et al., 1971). The characteristic attack is on the leaves, on which small spots appear, at first yellow then brown with apothecia-type fruiting in the center of the spot (Meyer & Lutrell, 1986). As the disease progresses, the leaves turn yellow and fall (Cristea, 2005). The attack causes the reduction of dry matter production, and the infection of 15% of the leaf surface reduced the digestibility by 14% and the crude protein content by 16%. Infection caused estrogenic activity in green alfalfa (Morgan & Parbery, 1980). The infection with micromyceta delays and possibly reduces flowering of affected plants (Morgan & Parbery, 1970).

Peronospora trifoliorum (de Bary) f. sp. *medicaginis-sativae* (Thuem.) (*Peronosporaae stivalis* Sydow) causes downy mildew producing serous losses of green mass and seeds (Nan, 2001). Alfalfa downy mildew affects all aerial organs of the plant, the specific attack being observed on the leaves. On the upper side of the leaves appear yellow spots that evolve into brown and on their lower side, next to the spots, the asexual fruits of the pseudofungus (sporangia and sporangiophore) develop, the internodes are shorter (Yu et al., 2022). In favorable conditions of temperature and humidity, severe infections occur, affecting the quantity and quality of alfalfa production (Xue, 2008).

MATERIALS AND METHODS

The aim of the research was to identify and establish the attack of foliar diseases in alfalfa with incidence in the Murighiol area, Tulcea county, in the period 2020-2022. During the experimental period, a leaf spotting attack was detected, caused by the micromycete *Pseudopeziza medicaginis* and pseudofungus *Peronospora trifoliorum* f. sp. *medicaginis-sativae* that produces downy mildew of lucerne. The frequency and intensity of the attack was monitored and, based on the data obtained, the degree of the attack was calculated. The formulas were used: Frequency (F%) = n x 100/N, where: N - number of plants observed (%), n - number of plants characteristic symptoms (%). The intensity was noted in percentages and calculated according to the formula: Intensity (I %) = Σ (i x f)/n (%), where: i - percentage given; f - number of plants/organs with the respective percentage; n - total number of attacked plants/organs. GA=F x I/100 (%), where: GA - attack degree (%); F - frequency (%); I - intensity (%). The biological material was represented by the Romanian variety Dobrogea. When the culture was established, the seed was treated with the biopreparation Nitragin with Rhizobium in a dose of 0.04 l/kg. The vegetation was treated with Faster 0.1 l/ha, the fungicide Probalance 1 l/ha and a biostimulator, Bioactivate in a dose of 0.6 l/ha. The control variant was not treated.

RESULTS AND DISCUSSIONS

In period 2020-2022 (Figures 1 and 2) in the alfalfa crop experimentation area, the common leaf spot or leaf spot attack produced by the pathogen *Pseudopeziza medicaginis* was observed and monitored and downy mildew caused by the pseudofungus *Peronospora trifoliorum* f. sp. *medicaginis-sativae* (*P. aestivalis* Syd). Observations were made on the clinical picture of the diseases induced by the detected pathogens and the attack on the leaves was noted. In the case of the common leaf spot attack, small brown spots were observed on the leaves with a darker central point, representing the fruiting of the fungus - apothecia (Figure 3 a). The leaves dried up and the plants showed defoliation. The attack of downy mildew was manifested by discoloration spots on the upper side of the leaves (Figures 3 b and 4 a), and the sporangiophores and sporangia specific to the genus *Peronospora* were observed on the lower side (Figure 4 b).

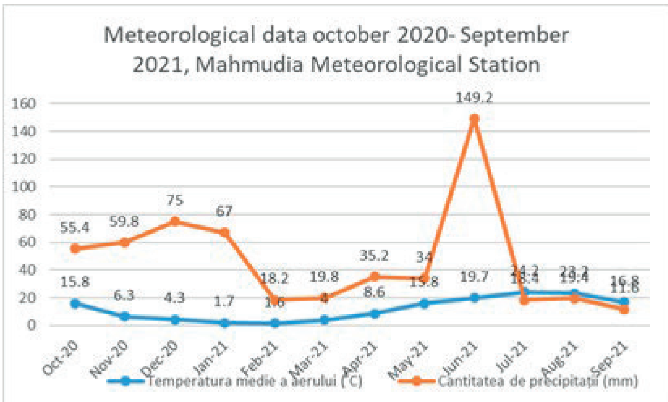


Figure 1. Meteorological data October 2020 - September 2021, Mahmudia Meteorological Station, Tulcea County

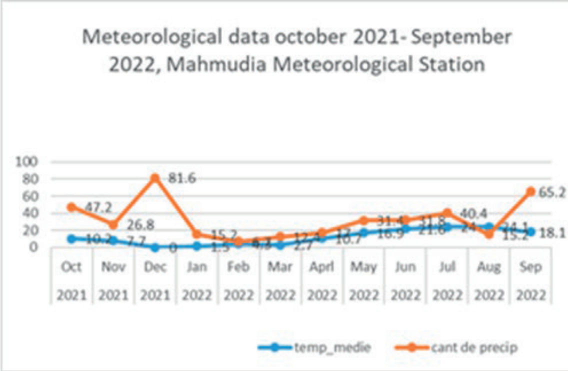
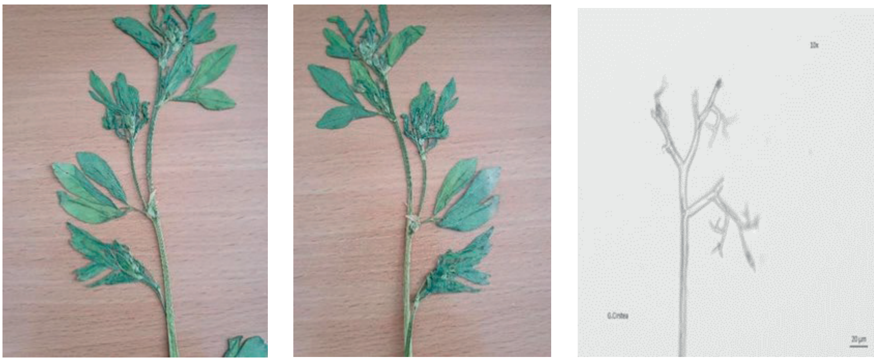


Figure 2. Meteorological data October 2021 - September 2022, Mahmudia Meteorological Station, Tulcea County

a) b)

Figure 3. Common Leaf spot (a) and downy mildew (b) attack in alfalfa



a) b)

Figure 4. Downy mildew attack of alfalfa (a) and sporangiophore (b)

The data in Table 1 show that in 2020-2021 the attack of common leaf spot in alfalfa had a frequency of 68% in the control variant and an intensity of 15%, resulting in a value of the degree of attack of 10.2%. The application of the treatment reduced the attack to 3.5%, with an effect in terms of both the incidence and the intensity of the attack. The attack of downy mildew on the leaves recorded incident values of 23.5% with an intensity of 6.5% in the control variant and compared to F = 12.5% and I = 5% in the treated variant. The value of the degree of attack was sub-unit in the treated variant and GA = 1.5% for the control variant.

Table 1. Attack of common leaf spot and downy mildew on alfalfa, 2020-2022 period, Sarinasuf-Murighiol location, Tulcea County

Variety	Year 2020-2022	Variant Trait/Control	Pathogen / Disease: *Pseudopeziza medicaginis/* leaf spot of lucerne			*Peronospora trifoliorum* f. sp. *medicaginis-sativae/* downy mildew		
			F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)
Dobrogea	2020/2021	Trait	34	8.5	3.0	12.5	5	0.6
		Control	68	15	10.2	23.5	6.5	1.5
	2021/2022	Trait	28	6.5	1.8	10.5	3.5	0.4
		Control	62	11	6.8	21.5	5.5	1.2

In the conditions of 2021-2022, a frequency of 62% was recorded in the case of the attack of common leaf spot in alfalfa, the intensity of the attack was 11%, resulting in GA = 6.8%. With regard to the attack of downy mildew, it had significantly lower values than in the previous year, due to the lower intensity at the time of the assessment of the attack. The frequency of the disease was 21.5% in the control variant and 10.5% in the treated variant.

Table 2. Efficacy of the treatment on the leaf spot and downy mildew attack of alfalfa (2020-2022) location Sarinasuf - Murighiol, Tulcea county

Variety	Year 2020-2022	Variant Trait/Control	Pathogen / Disease: *Pseudopeziza medicaginis/* leaf spot of lucerne		*Peronospora trifoliorum* f. sp. *medicaginis-sativae/*downy mildew	
			GA (%)	E (%)	GA (%)	E (%)
Dobrogea	2020/2021	Trait	3.0	70.5	0.6	60
		Control	10.2	-	1.5	-
	2021/2022	Trait	1.8	73.5	0.4	66.6
		Control	6.8	-	1.2	-

The effectiveness of the application of the treatment was also calculated and its values of 70.5% were obtained in the year 2020-2021 on the attack of *Pseudopeziza medicaginis* and 60% in combating the pathogen *Peronospora trifoliorum* f. sp. *medicaginis-sativae*. In 2021-2022, the effectiveness had values higher than 73.5% against the attack of common spot leaf in alfalfa and 66.6% in combating the attack of downy mildew in alfalfa (Table 2). The application of treatments is an effective means of combating diseases of cultivated plants, being integrated in the schemes for combating plant diseases (Alexandru et al., 2019; Buzatu et al., 2018; Totk & Cristea, 2020; Chiriac & Cristea, 2021). The application of the treatments ensures a good control, based on the assessment of the percentage of defoliation, on alfalfa diseases (Nutter et al., 2022). Treatment application reduced diseases incidence in alfalfa (Wilconxson & Bielenberg, 1972) and the management control at foliar diseases can benefit alfalfa seed production (Hwang et al., 2006)

CONCLUSIONS

In the period 2020-2022, an attack of common spot leaf in alfalfa and downy mildew was detected. Higher values of the attack were calculated in the case of the micromycete responsible for the appearance of brown spotting of alfalfa leaves. The application of the treatment was effective in the condition of the monitored diseases on alfalfa.

ACKNOWLEDGEMENTS

We wish to thank “Dima Ancuta” P.F.A, Tulcea County, for technical support.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

COMPARATIVE STUDY OF PRODUCTIVE AND QUALITY INDICATORS OF WHEAT VARIETIES IN NORTH-EASTERN BULGARIAN REGION

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Abstract

The field experiment was carried out in the selected area of Pristoe village, Shumen area in the period 2017-2019. The 2test was set by the block-plot design method in four replications with a plot size of 15 m^2*, after sunflower predecessor. The purpose of the study was to establish the productivity and quality of some common wheat varieties, grown in North-Eastern Bulgarian region. The varieties 'Avenue' 'Joker' 'Apache' and 'Neven' were tested. The indices; length of spike (cm), number of spikelets per spike number of grains per spike, grain yield (kg/ha), thousand kernel (grain) weight (g), test weight (kg), vitreousness (%), wet gluten content (%), gluten deformation index (mm) were reported. The results showed that the highest grain yield was obtained from Avenue variety - 7900 kg/ha, followed by Joker - 7400 kg/ha and the lowest one - from Neven variety 6600 kg/ha. The highest values of test weight and the vitreousness content was reported for Neven (84.7 kg and 89.6%) respectively. Joker variety show the best values of the investigated technological properties of the grain among the tested varieties wheat.*

Key words*: wheat, yield, thousand kernel (grain) weight, test weight, gluten.*

INTRODUCTION

Taking into account the market conditions nowadays, as well as the main possibilities for increasing and improving wheat yield and quality, it is crucial to create and introduce new high productive varieties and to develop effective technologies for their cultivation (Georgieva & Kirchev, 2020; Chamurliiski, 2019; Matev & Kirchev, 2010; Mitkov et al., 2019; Zhelyazkov et al., 2017; Tityanov et al., 2020).

Wheat is one of the most distributed crop worldwide, as it is of primary importance for people`s living. There is a great diversity of wheat varieties possessing different biological properties. The present-day selection aims at creating and introducing new lines and varieties combining tolerance towards the biotic and abiotic environmental stress factors, high productivity and grain quality in order to meet market needs (Manilov, 2022; Uhr, 2015; Uhr et al., 2021; Chipilski et al., 2022).

The factor *variety* along with its genetic makings has crucial importance for yield productivity and quality. Values of these indicators are genetically determined. Nevertheless, they are influenced by the applied agro-machinery, the climatic factors during vegetation and the specific agro-ecological conditions of the region (Atanasov et al., 2020; Tsenov et al., 2020; Mitkov et al., 2009; Mitkov et al., 2018; Yanev et al., 2021). In this relation, in recent years there have been developed varieties with high genetic potential and have been examined regarding their productivity, grain quality and adaptability towards environmental conditions (Dimitrov et al., 2016; Ilieva 2011; Kirchev & Delibaltova, 2016; Tsenov et al., 2022). The constant introduction of new wheat varieties and their examination in separate micro-regions of the country is always of present interest. A number of studies have reported that wheat varieties reveal their productive possibilities differently in particular agro-ecological regions of the country (Ilieva, 2011; Ivanova et al., 2010; Tonev et al., 2018).

Therefore, studies related to the cultivation of wheat varieties in different country regions have particular scientific and practical significance.

The present research study aims at establishing the productive possibilities and quality of some common wheat varieties cultivated in the region of north-eastern Bulgaria.

MATERIALS AND METHODS

The study was performed in the period 2016-2019 in the region of the village of Pristoe - north-eastern Bulgaria. The experiment was set by the block method in 4 repetitions, with size of the crop plot - 15 m^2. There were examined four common wheat varieties - Avenue, Apash, Joker and Neven, which were sowed after a predecessor sunflower. Plants were cultivated under standard technology. The pre-sowing soil tillage was performed with 2-3 times of disking. The sowing was conducted within optimum terms - with 550-590 g.s. m^2 at a sowing norm of 25-28 kg/ha. The Phosphorus and Potassium fertilizers were applied after the first tillage, as well as 1/3 of the Nitrogen fertilizer (N_{16}). In the early spring the soil was fed two times with the rest of the Nitrogen fertilizer. In the end of the tillering stage herbicide spraying was applied for weed control: against broad-leaved weeds - Sekator 100 ml/ha, and ag ainst wheat weeds - Tsiklop 700 ml/ha. Soligor fungicide 700 ml/ha was used for disease control, and Proteus insecticide in a doze 650 ml/ha - for pest control. Spraying with Kelpak - 2000 ml/ha was applied aiming at growth improvement. For the purpose of the present study the following indicators were examined: grain yield (kg/ha) spike (ear) length (cm), number of spikelets and grains in a spike (ear), as well as the qualitative indicators -TGW (g), hectolitre mass (kg), vitreousness (glassiness) (%), wet gluten content (%) and gluten deformation index (mm).
The received data related to the values of yield structural elements, grain yield and the qualitative indicators were mathematically processed via the method of the dispersion analysis. The differences between options were established via Duncan`s multiple range test.
The examined varieties were grouped via a hierarchical cluster analysis. The method of intergroup connection was used (Ward, 1963). The Euclidean intergroup distance was used as a measure of similarity:

$$D(x,y)=\sqrt{\sum_{i=1}^{n}(x_i-y_i)^2}$$

A dendrogram was built in order to present graphically the clusters. The dotted horizontal line of the dengrogram showed the rescaled distance with which the clusters were formed.
A correlation analysis was carried out aiming at establishing the presence of statistically significant correlations between the examined indicators.
Data processing was performed via SPSS 26 Statistical Program.
The main climatic factors determining growth and productivity of wheat plants are air temperature, sum of precipitations, their combination and their distribution during the vegetation period.
Analysing these factors showed that the values of average monthly temperatures during the experimental years did not differ considerably than those during a long-term period. These factors completely met the requirements of common wheat from germination and sprouting to ripening (Figure 1).

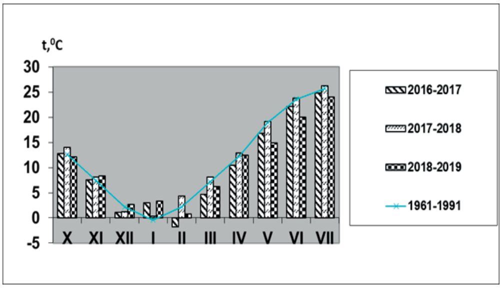

Figure 1. Average monthly air temperature, ^{0}C

Significant differences were observed in the quantity of precipitations during the separate crop years (Figure 2).
The sum of precipitations in the period October-July 2016-2017 was 675 mm. These amounts were distributed equally and were completely sufficient for securing plants with moisture during the whole vegetation period.

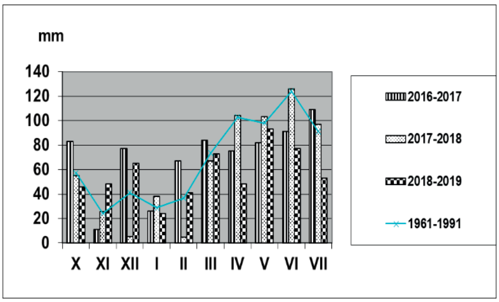

Figure 2. Rainfall, mm

Precipitations, especially during the critical stages of wheat vegetation, had a positive effect on the productive possibilities of plants.

In 2017-2018 the largest quantity of precipitations was reported, i.e. in the second experimental year (705 mm). Precipitations during wheat vegetation were unequally distributed an insufficient, especially in the stages of jointing-heading, precipitations during ripening were abundant.

In 2018-2019 the sum of precipitations during plant vegetation was 655 mm. The precipitation quantity during the vegetation period was equally distributed.

Among the three experimental years the most favorable for plant growth was the first year (2016-2017), followed by the year 2018-2019 and not so favorable - the second year (2017-2018), which affected growth, yield and quality of wheat varieties.

RESULTS AND DISCUSSIONS

Results related to the examined structural elements of yield showed that these indicators changed under the influence of the meteorological factors during the experimental years. The highest values of yield main structural elements were registered in 2017, followed by 2019, and the lowest values - in 2018 (Table 1).

Ear length had the highest values in the crop year 2016-2017 for Joker variety - 11.6 cm, followed by Avenue - 11.2 cm and Apache - 10.6 cm., and the lowest values had Neven variety - 9.2 cm.

Table1. Structural elements of the yield

Indices	Varieties	Years of study			
		2016-2017	2017-2018	2019-2020	Average for the period
Length of the spike, cm	Avenue	11.2 [c]	10.0 [b]	10.6 [c]	10.6
	Apache	10.6 [b]	10.2 [b]	9.8 [b]	10.2
	Joker	11.6 [d]	10.6 [c]	11.0 [c]	11.0
	Neven	9.2 [a]	7.8 [a]	8.4 [a]	8.4
Number of spikeletts per spike	Avenue	25.0 [c]	21.0 [c]	23.0 [c]	23.0
	Apache	21.0 [b]	19.0 [b]	20.0 [b]	20.0
	Joker	24.0 [c]	22.0 [c]	21.0 [b]	22.3
	Neven	19.0 [a]	17.0 [a]	18.0 [a]	18.0
Number of grains per spike	Avenue	68.0 [c]	63.0 [d]	66.0 [c]	65.6
	Apache	64.0 [b]	61.0 [b]	63.0 [b]	62.6
	Joker	71.0 [d]	62.0 [c]	67.0 [d]	66.6
	Neven	59.0 [a]	53.0 [a]	56.0 [a]	56.0

*Means within columns followed by different lowercase letters are significantly different ($P<0.05$) according to the LSD test

The received data were mathematically significant. In the second experimental years ear length of the examined varieties was within the bounds from 7.8 to 10.6 cm, and in the crop year 2018-2019 values of these indicators were slightly higher. It was mathematically proven that averagely for the experimental period (2016-2019) the longest ear had Joker variety – 11,0 cm., which exceeded Avenue, Apache and Neven varieties with 3.7%, 7.8%, and 30.9%, respectively.

Wheat plants formed the smallest number of ears - from 17.0 (Neven variety) to 22.0 (Joker variety) in 2017-2018 compared to 2016-2017 and 2018-2019.

The biggest number of earlets per ear was formed in the experimental year 2016-2017, which was due to the favorable combination of temperature and moisture during the vegetation period. The values of this indicator were within the bounds from 19.0 for Neven variety to 25,00 for Avenue variety. Differences between varieties were mathematically significant, as Joker variety domineered over Apache variety with 3.0 pieces of ears. In the third experimental year the number of earlets per ear varied from 18.0 to 23.0, as the differences between the tested varieties were statistically proven.

Averagely for the experimental period (2016-2019) Avenue variety had the greatest number of earlets per ear - 23.0, followed by Joker variety - 22.3 and Apash and Neven varieties - 20.0 and 18.0, correspondingly.

The greatest number of grains per ear was reported for Joker variety - from 62.0 to 71.0, followed by Avenue variety - from 63.0 to 68.0, and the smallest number of grains was reported for Neven variety - from 53.0 to 59.0.

Differences between the tested varieties were statistically proven.

The dispersion analysis (Table 2) showed that the genotype, as well as the years with their specific climatic conditions had statistically proven influence on yield structural elements.

The strongest influence of these indicators was established for the number of grains per ears, followed by the number of earlets and the ear length.

Table 2. Analysis of variance ANOVA

	Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Length of the spike	Year*	6,24875	2	3,124375	4,938639	0,01	3,259446
	Varieties *	46,0075	3	15,33583	24,24105	0,00	2,866266
	Interaction ^{ns}	2,88125	6	0,480208	0,759056	0,61	2,363751
	Within	22,775	36	0,632639			
Number of spikeletts per spike	Year*	43,875	2	21,9375	12,29183	0,00	3,259446
	Varieties *	157,2292	3	52,40972	29,36576	0,00	2,866266
	Interaction*	33,45833	6	5,576389	3,124514	0,01	2,363751
	Within	64,25	36	1,784722			
Number of grains per spike	Year*	278,7917	2	139,3958	20,88762	0,00	3,259446
	Varieties **	848,7292	3	282,9097	42,3923	0,00	2,866266
	Interaction ^{ns}	40,70833	6	6,784722	1,016649	0,43	2,363751
	Within	240,25	36	6,673611			

*F-test significant at P<0.05; ** F-test significant at P<0.01; ns non-significant

The interaction between the tested factors was significant only for the number of earlets per ear. It was not proven for the ear length and the number of grains per ear.

Data in Table 3 showed that averagely for the experimental period Avenue variety domineered over the rest of the varieties included in the experiment.

Table 3. Grain yield - kg/ha

Varieties	Years of study			Average for the period (kg/ha)
	2016-2017 (kg/ha)	2017-2018 (kg/ha)	2018-2019 (kg/ha)	
Avenue	8300 ^{d}	7500 ^{d}	7900 ^{d}	7900
Apache	7500 ^{b}	6800 ^{b}	7100 ^{b}	7133
Joker	7800 ^{c}	7100 ^{c}	7300 ^{c}	7400
Neven	6800 ^{a}	6400 ^{a}	6600 ^{a}	6600

*Means within columns followed by different lowercase letters are significantly different (P<0.05) according to the LSD test

As a result of the higher moisture that plants were secured with, as well as the equal distribution of precipitations, higher yields were received in 2016-2017 in comparison to 2017-2018 and 2018-2019.

For the conditions of the first year they varied from 6800 kg/ha for Neven variety to 8300 kg/ha for Avenue variety. Joker, Apache and Neven varieties received yields, which were 500 and 800 and 1500 kg/ha lower compared to Avenue variety.

Differences between all varieties were statistically proven.

The experimental year 2017-2018 was characterized with greater amount of precipitations during the vegetation period - 705,0 mm, but they were unequally distributed. Thus, it led to lower yields of the tested varieties compared to 2016-2017 and 2018-2019. The received data related to grain yield varied from 6400 to 7500 kg/ha, and in the third experimental year they were within the bounds from 6600 kg/ha for Neven variety to 7900 kg/ha for Avenue variety.

Averagely for the three experimental years Avenue variety received grain yield 7900 kg/ha, which domineered over Joker, Apache and Neven with 6.7%, 10.7% and 19.6 kg/ha, correspondingly.

The dispersion analysis related to the effect of Variety and Year factors, as well as their interaction on grain yield, showed reliable influence of the studied factors and insignificant interaction between them (Table 4).

Table 4. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Year*	3758750	2	1879375	78,63951	0,00	3,259446
Variety*	10831666,7	3	3610556	151,078	0,00	2,866266
Interaction[ns]	264583,333	6	44097,22	1,845179	0,12	2,363751
Within	860350	36	23898,61			

*F-test significant at P<0.05; **F-test significant at P<0.01; ns non-significant

Table 5 presented data related to the physical and technological properties of grain of common wheat varieties included in the study.

In the first experimental year the mass of 100 grains had the highest values for Neven variety - 52.0 g. Differences between varieties were statistically proven. In 2017-2018 the mass of 1000 grains was within the bounds from 41.0 to 48.0 g, while in 2018-2019 the registered mass of 1000 grans varied from 45 g for Joker variety to 50 g for Neven variety. Differences were mathematically significant. Averagely for the period (2016-2019) Neven variety was registered with the biggest grain - 50.0 g, followed by Avenue - 45.6 g, and the smallest grain was established for Apache and Joker varieties - 43.6 g.

Table 5. Physical and technological properties of the grain

		Thousand kernel (grain) weight (g)	Test weight (kg)	Vitreousness (%)	Wet gluten content (%)	Index deformation gruten (mm)
Years	2016-2017	46.8 b	80.0 b	87.3 c	28.3 a	10.4 a
(A)	2017-2018	43.7 a	77.0 a	84.5 a	27.4 a	9.6 a
	2018-2019	46.7 b	80.3 b	86.0 b	28.0 a	10.1 a
Variety	Avenue	45.6	77.6	85.6	25.5	10.4
(B)	Apash	43.6	74.6	82.7	25.3	11.3
	Joker	43.6	79.3	85.6	31.0	9.3
	Neven	50.0	84.7	89.6	29.6	9.0
2016-2017	Avenue	47.0 b.	79.0 b	86.0 b	27.0 b	10.5 b
	Apash	44.0 a	75.0 a	84.0 a	25.0 a	12.5 c
	Joker	44.0 a	81.0 c	88.0 c	31.0 d	10.0 b
	Neven	52.0 c	85.0 d	91.0 d	30.0 c	8.5 a
2017-2018	Avenue	44.0 c	74.0 b	85,0 c	24.5 a	9.8 b
	Apash	41.0 a	73.0 a	81,0 a	26.0 b	10.0 b
	Joker	42.0 b	77.0 c	84,0 b	30.0 d	9.4 b
	Neven	48.0 d	84.0 d	88,0 d	29.0 c	9.0 a
2018-2019	Avenue	46.0 b	80.0 b	86,0 c	25,0 a	11.0 c
	Apash	46.0 b	76.0 a	83,0 a	25.0 a	11.5 c
	Joker	45.0 a	80.0 b	85,0 b	32.0 c	8.5 a
	Neven	50.0 c	85.0 c	90,0 d	30.0 b	9.5 b
Anova	A	*	*	*	n.s	n.s
	B	*	*	*	*	*
	AB	n.s	n.s	n.s	n.s	*

*Means within columns followed by different lowercase letters are significantly different (P<0.05) according to the LSD test
*F-test significant at P<0.05; ** F-test significant at P<0.01; ns non-significant

The hectoliter mass of the examined varieties in the experimental year 2016-2017 varied from 75.0 kg. for Apache variety to 85.0 kg. for Neven variety. Differences were statistically proven.

In the second and third experimental years the values of the hectoliter mass were within the bounds from 73.0 to 84.0 kg and from 76.0 to 85.0 kg, for 2017-2018 and 2018-2019, correspondingly.

Averagely for the experimental year the hectoliter mass of Neven variety had the highest value - 84.7 kg., followed by Joker variety - 79.3; Avenue variety - 77.6 kg, and the lowest value was reported for Apache variety - 74.6 kg.

The common vitreousness of grain is an important indicator showing the quality of wheat grain. Lower values of this indicator were reported in the crop year 2017-2018, which could be explain with the presence of larger amount of precipitations in June and July - the stage of ripening. The per cent of vitreousness (glassiness) of the varieties was from 81.0% for Apash to 88.0% for Neven.

Taking into account the whole experimental period, the highest per cent of vitreousness was reported for Neven variety - from 88.0% to 91.0%, which statistically proven domineered over the rest from 4.7% to 8.7 %.

Results related to wet gluten content in the tested varieties showed that this indicator was influenced to a great extent by the genotype and did not depend on the climatic conditions of the crop years. Averagely for the period 2016-2019 the per cent of wet gluten in grain varied from 25.3 to 31.0. The highest content of wet gluten was reported for Joker variety - 31.0%, followed by Neven variety - 29.6%, and the lowest content had Avenue and Apache varieties - with 25.5% and 25.3%, correspondingly.

With relation to gluten allocation, the received data showed that Joker and Neven varieties were characterized with the highest values possessing strong gluten, while Avenue and Apache varieties had values over 10 mm, where gluten was determined as weak.

Results of the cluster analysis were presented graphically via a dendrogram. It showed that the tested varieties were grouped in one main cluster (Figure 3). The cluster was more homogenous and included Avenue and Joker varieties, which were similar in the following indicators: ear length, number of earlets per ear, number of grains per ear, mass of 1000 grains, vitreousness and gluten allocation. Later Apache variety joined the cluster with close indicators as follows: length of ear and mass of 1000 grains.

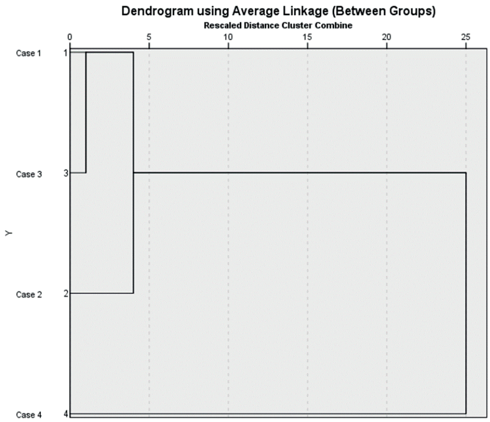

Figure. 3. Dendrogram rescaled distance cluster combine

The correlation coefficients showing the interrelation between the studied indicators were given in the correlation matrix (Table 6)

A strong positive correlation was established between the ears length and the number of grains per ear r = 0.993. A weaker correlation dependence was established between the grain yield and the number of earlets per ear r = 0.963, the number of grains and the number of earlets per ear r = 0.951, the hectoliter mass and the vitreousness r = 0.982. All given correlation coefficients were statistically proven at a level of significance $\alpha = 0.001$.

Table 6. Values of the coefficient of corelatiom

	Grain yield	Length of spike	Number of spikelets per spike	Number of grains per spike	Thousand kernel (grain) weight	Test weight	Vitreousness	Wet gluten content	Allocation of gluten
Grain yield	1	0.834	0.963*	0.880	-0.597	-0.589	-0.457	-0.404	0.389
Length of spike		1	0.910	0.993**	-0.911	-0.723	-0.690	-0.158	0.385
Number of spikelets per spike			1	0.951*	-0.666	-0.520	-0.420	-0.167	0.221
Number of grains per spike				1	-0.857	-0.669	-0.618	-0.145	0.331
Thousand kernel (grain) weight					1	0.867	0.892	0.249	-0.586
Test weight						1	0.982*	0.693	-0.909
Vitreousness							1	0.596	-0.877
Wet gluten content								1	-0.898
Allocation of gluten									1

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

CONCLUSIONS

Averagely for the experimental period (2016-2019) the highest grain yield was performed by Avenue variety - 7900 kg/ha, followed by Joker variety - 7400 kg/ha and Apache - 7133 kg/ha., and the lowest yield had Neven variety - 6600 kg/ha. The higher productivity of this variety was due to the higher values of yield structural elements.

The examined common wheat varieties in the region of north-eastern Bulgaria formed grain with the highest values of the indicators hectoliter mass and vitreousness (glassiness) for Neven variety (84.7 kg and 89.6%).

Taking into account the examined technological properties of grain - wet gluten - the highest indicators had Joker variety - 31.0%, while gluten allocation was the lowest for Neven variety - 9.0 mm. Gluten allocation for Joker and Neven varieties was under 10 mm, which led to the presence of strong gluten.

For the production of forage and alcohol in the region of North-eastern Bulgaria we recommend the cultivation of Avenue and Apache varieties, and for the production of bread - Neven (it has lower yield characterizing with good indicators) and Joker varieties.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY OF THE ELEMENTS OF THE PRODUCTIVITY OF OLD COMMON WINTER WHEAT VARIETIES UNDER CHANGING ENVIRONMENTAL CONDITIONS

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Abstract

The experiment was conducted in the experimental field of IPGR - Sadovo in the period 2018-2020. Biometric analysis was performed on the sixteen old varieties of common winter wheat created in IPGR. The following traits were taken: yield, productive tillering, central spike length, spikelets number in centaral spike, grains number in central spike, grain weight in central spike, grains number in other spikes, grains weight in other spikes, grains number of 1 plant and grains weight of 1 plant. The data are processed by statistical methods – variance (ANOVA), variation and principal component analysis. The results show that the influence of the genotype and the interaction of the genotype x environment was proved in all the monitored traits. In terms of traits, the influence of the environment is unproven only in 3 traits – grains weight in 1 plant, grains weight in the other spikes and spikelets number in the central spike. The aim of the study is to test the effect of climate change on the structural elements of the yield of old varieties of common winter wheat, as the main food crop.

Key words: *common winter wheat, old varieties, climate change, structural elements.*

INTRODUCTION

The productive potential of cereals is very variable depending on the specific growing conditions. Agro-ecological and climatic conditions in certain regions of the country affect the growth, development and productivity of wheat (Bazitov, 2000; Tsenov et al., 2004; Delibaltova & Ivanova, 2006). The variety is one of the main components in the technological solutions of any crop. The construction of a proper varietal structure, depending on the specific agro-ecological conditions of the region can significantly increase yields and product quality (Ilieva, 2011). The successful implementation and use of each wheat variety is related to its behavior in different environmental conditions (Van Ittersum et al., 2013). Unlike other field crops, wheat varieties are characterized by relatively low ecological plasticity, which necessitates the creation of new varieties adapted to individual agro-ecological areas and therefore the correct selection of varieties is crucial for yield and quality of production. The specific conditions in our country are a challenge to the selection of productivity in this crop (Panayotov & Rachinski, 2002).

The aim of the study was to test the effect of climate change on the structural elements of the yield of old varieties of common winter wheat, as a major food crop.

MATERIALS AND METHODS

The experiment was conducted in the experimental field of IPGR - Sadovo in the period 2018-2020. The structural elements of the productivity of the sixteen old varieties of common winter wheat, created in IPGR, were studied. Varietal experiments were performed on a block diagram in four replications, with a size of the experimental plot of 10 m^2 according to the cultivation technology adopted in IPGR.

Biometric measurements were made on the following productivity traits: grain yield - kg/da, total tillering (TT), productive tillering (PT), central spike length (CSL) - cm, spikelets number in central spike (SNCS), grains number in central spike (GNCS), grains weight in central spike (GWCS) - g, grains number in other spikes (GNOS), grains weight in other spikes (GWOS), grains number per plant (GN1P) and grains weight per plant (GW1P). The percentage ratio of grain weight in the central spike to the grain weight of 1 plant (GWCS/GW1P) was calculated. The degree of variation of each of the traits of productivity is determined by calculating a coefficient of variation.

The level of variation has been assumed to be weak if the coefficient of variation is up to 10%, on average - when it is greater than 10%, and less than 20% and strong - when it is over 20% (Dimova & Marinkov, 1999). Mathematical data processing is performed by applying variation, variance and analysis of the main components. The programs SPSS 19 and Microsoft excel for Windows were used. The general statistical assessment of the presence or absence of differences between the variants was determined by the ANOVA method (Dimova & Marinkov, 1999).

RESULTS AND DISCUSSIONS

The agro-climatic conditions during the study are represented by the main meteorological factors for the growth and development of the crop: average monthly air temperature (Figure 1) and average values of the amount of precipitation (Figure 2).

The years in which the survey was conducted (2018/2019 - 2019/2020) are different in terms of climate. There is a variation of climatic factors, both by months and by years, which affects the yield and elements of crop productivity.

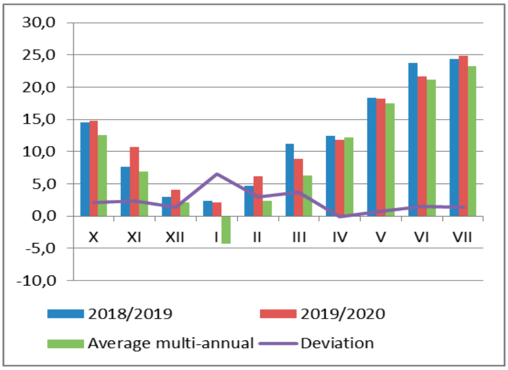

Figure 1. Average temperature sum T°C of months during vegetation years 2018/2019-2019/2020

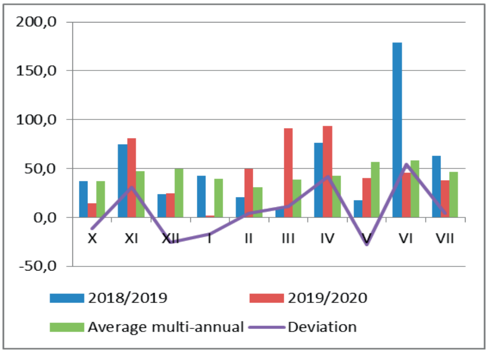

Figure 2. Sums of month rainfall (mm) during vegetation years 2018/2019-2019/2020

For the vegetation year 2018/2019, we can summarize that the average monthly air temperatures are higher than the multi-year values, and the amount of precipitation is unevenly distributed in the individual months of crop development. The precipitation in November was higher than the multi-year values and favored the development of wheat. There is a secondary weeding of crops in June, due to the large amount of rainfall that month.

The meteorological conditions during the vegetation year 2019-2020 differ from those in the previous year. Conditions during the period from sowing to the end of March were not the most favorable for the development of wheat due to the snowfall in late March and early April. On average, the monthly air temperatures were higher than the multi-year values, with the only exception having been observed in April.

Moisture deficiency was not observed during the stem elongation, heading and grain filling phases. The rainfall in April led to the formation of a higher stem compared to the typical height of the varieties. The second growing season of the study can be defined as more favorable for winter common wheat. Confirmation of this was the higher yields obtained compared to the first growing season.

The results of the biometric measurements of the structural elements of the productivity of the studied wheat varieties are presented in Tables 1, 2 and 3. The size of the yield is closely related to the variety, the level of applied agricultural techniques and the soil-climatic conditions of the region (Delibaltova & Ivanova, 2006; Tsenov et al., 2006).

Table 1. Results of the biometric measurements of the structural elements of the productivity of common winter wheat varieties for the period 2018-2020

N°	VARIETY	Grain yield, kg/da			TT, number			PT, number			CSL, cm		
		$\bar{x}$	± D	Sig.	$\bar{x}$	± D	Sig.	$\bar{x}$	± D	Sig.	$\bar{x}$	± D	Sig.
1	Sadovo 1-st	431.5			3.5			3.7			11.6		
2	Bononia	527.6	96.0	+++	4.2	0.7	+	3.5	-0.2	n.s.	11.2	-0.4	n.s.
3	Niky	531.0	99.5	+++	3.5	0.0	n.s.	3.5	-0.2	n.s.	12.2	0.6	n.s.
4	Lucille	619.7	188.2	+++	3.8	0.3	n.s.	3.3	-0.3	n.s.	11.3	-0.3	n.s.
5	Sad. Belya 1	573.1	141.6	+++	3.2	-0.3	n.s.	3.5	-0.2	n.s.	11.4	-0.2	n.s.
6	Tsarevets	526.3	94.8	+++	3.5	0.0	n.s.	4.3	0.7	+	11.4	-0.2	n.s.
7	Pobeda	515.2	83.6	+++	4.3	0.8	++	3.5	-0.2	n.s.	11.5	-0.1	n.s.
8	Mustang	607.2	175.6	+++	3.8	0.3	n.s.	3.3	-0.3	n.s.	12.2	0.6	n.s.
9	Geya 1	604.7	173.1	+++	3.3	-0.2	n.s.	3.8	0.2	n.s.	11.3	-0.3	n.s.
10	Diamond	613.1	181.6	+++	3.8	0.3	n.s.	3.8	0.2	n.s.	11.0	-0.6	n.s.
11	Murgavets	594.9	163.3	+++	3.8	0.3	n.s.	4.0	0.3	n.s.	12.3	0.7	n.s.
12	Sadovo 552	532.4	100.8	+++	4.0	0.5	n.s.	4.0	0.3	n.s.	12.7	1.1	+
13	Sadovo 772	527.4	95.9	+++	4.0	0.5	n.s.	3.8	0.2	n.s.	12.1	0.5	n.s.
14	Guinness	612.0	180.5	+++	3.8	0.3	n.s.	3.8	0.2	n.s.	11.1	-0.5	n.s.
15	Yoana	651.5	219.9	+++	3.8	0.3	n.s.	3.3	-0.3	n.s.	12.8	1.2	+
16	KM 135	628.8	197.3	+++	3.2	-0.3	n.s.	3.2	-0.5	n.s.	11.1	-0.5	n.s.
Mean		568.5			3.7			3.7			11.7		
Minimum		431.5			3.2			3.2			11.0		
Maximum		651.5			4.3			4.3			12.8		
Std. dev.		57.7			0.3			0.3			0.6		
Coef. Var.		10.2			9.1			8.6			4.9		
Standard error		14.4			0.1			0.1			0.1		
GD 5.0%		11.0			0.6			0.7			0.9		
GD 1.0%		14.6			0.8			0.9			1.3		
GD 0.1%		18.9			1.0			1.1			1.6		

+ -,+ + - -,+ + + - - -, significant at GD 5.0 %, GD 1.0 % GD 0.1 %; n.s. - non-significant

Table 2. Results of the biometric measurements of the structural elements of the productivity of common winter wheat varieties for the period 2018-2020

N°	VARIETY	SNCS, number			GNCS, number			GWCS, g			GNOS, number		
		$\bar{x}$	± D	Sig.	$\bar{x}$	± D	Sig.	$\bar{x}$	± D	Sig.	$\bar{x}$	± D	Sig.
1	Sadovo 1-st	20.0			52.8			2.73	0.00		96.2		
2	Bononia	19.8	-0.2	n.s.	44.5	-8.3	n.s.	1.75	-0.98	---	84.8	-11.3	n.s.
3	Niky	19.5	-0.5	n.s.	52.0	-0.8	n.s.	2.46	-0.26	n.s.	89.8	-6.3	n.s.
4	Lucille	19.0	-1.0	n.s.	52.2	-0.7	n.s.	2.33	-0.40	n.s.	102.0	5.8	n.s.
5	Sad. Belya 1	21.2	1.2	n.s.	60.0	7.2	n.s.	1.65	-1.08	+++	92.2	-4.0	n.s.
6	Tsarevets	20.0	0.0	n.s.	63.5	10.7	+	2.54	-0.19	n.s.	120.2	24.0	n.s.
7	Pobeda	19.0	-1.0	n.s.	45.5	-7.3	n.s.	1.99	-0.74	-	99.2	3.0	n.s.
8	Mustang	21.7	1.7	+	52.8	0.0	n.s.	2.48	-0.25	n.s.	116.2	20.0	n.s.
9	Geya 1	18.8	-1.3	n.s.	50.5	-2.3	n.s.	2.42	-0.30	n.s.	85.7	-10.5	n.s.
10	Diamond	20.6	0.6	n.s.	46.7	-6.2	n.s.	1.85	-0.88	--	90.8	-5.3	n.s.
11	Murgavets	20.9	0.9	n.s.	61.5	8.7	n.s.	2.90	0.18	n.s.	142.5	46.3	++
12	Sadovo 552	20.8	0.8	n.s.	45.7	-7.2	n.s.	2.05	-0.67	-	104.0	7.8	n.s.
13	Sadovo 772	20.7	0.7	n.s.	52.0	-0.8	n.s.	1.75	-0.98	--	114.0	17.8	n.s.
14	Guinness	20.2	0.2	n.s.	47.5	-5.3	n.s.	1.63	-1.10	---	102.7	6.5	n.s.
15	Yoana	21.8	1.8	+	50.7	-2.2	n.s.	2.11	-0.62	-	98.2	2.0	n.s.
16	KM 135	19.7	-0.3	n.s.	50.8	-2.0	n.s.	2.38	-0.35	n.s.	84.7	-11.5	n.s.
Mean		20.2			51.8			2.19			101.4		
Minimum		18.8			44.5			1.63			84.7		
Maximum		21.8			63.5			2.90			142.5		
Std. dev.		0.9			5.7			0.40			15.5		
Coef. var.		4.6			10.9			18.17			15.3		
Standard error		0.2			1.4			0.10			3.9		
GD 5.0%		1.7			10.3			0.59			27.9		
GD 1.0%		2.2			13.7			0.78			37.1		
GD 0.1%		2.8			17.8			1.01			48.2		

+ -,+ + - -,+ + + - - -, significant at GD 5.0 %, GD 1.0 % GD 0.1 %; n.s. - non-significant

Table 3. Results of the biometric measurements of the structural elements of the productivity of common winter wheat varieties for the period 2018-2020

N°	VARIETY	GWOS, g			GN1P, number			GW1P, g			GWCS/GW1P
		$\bar{x}$	± D	Sig.	$\bar{x}$	± D	Sig.	$\bar{x}$	± D	Sig.	$\bar{x}$
1	Sadovo 1-st	5.11			149.0			7.84			34.8
2	Bononia	2.69	-2.43	---	129.3	-19.7	n.s.	4.43	-3.41	---	39.4
3	Niky	4.34	-0.78	n.s.	141.8	-7.2	n.s.	6.80	-1.04	n.s.	36.2
4	Lucille	4.69	-0.42	n.s.	154.2	5.2	n.s.	7.02	-0.81	n.s.	33.2
5	Sad. Belya 1	3.21	-1.90	--	152.2	3.2	n.s.	4.86	-2.98	---	33.9
6	Tsarevets	4.22	-0.89	n.s.	183.7	34.7	+	6.76	-1.08	n.s.	37.5
7	Pobeda	3.57	-1.54	-	144.7	-4.3	n.s.	5.56	-2.28	--	35.8
8	Mustang	4.52	-0.60	n.s.	169.0	20.0	n.s.	6.99	-0.85	n.s.	35.4
9	Geya 1	4.47	-0.65	n.s.	136.2	-12.8	n.s.	6.89	-0.95	n.s.	35.2
10	Diamond	4.06	-1.05	n.s.	137.5	-11.5	n.s.	5.91	-1.93	-	31.3
11	Murgavets	5.85	0.73	n.s.	204.0	55.0	++	8.75	0.91	n.s.	33.2
12	Sadovo 552	4.85	-0.26	n.s.	149.7	0.7	n.s.	6.90	-0.93	n.s.	29.7
13	Sadovo 772	3.48	-1.63	-	166.0	17.0	n.s.	5.23	-2.61	--	33.4
14	Guinness	3.29	-1.82	--	122.8	-26.2	n.s.	4.92	-2.92	---	33.1
15	Yoana	3.07	-2.04	--	148.8	-0.2	n.s.	5.18	-2.66	--	40.7
16	KM 135	4.00	-1.11	n.s.	135.5	-13.5	n.s.	6.38	-1.46	n.s.	37.3
Mean		4.09			151.5			6.28			35.0
Minimum		2.69			122.8			4.43			29.7
Maximum		5.85			204.0			8.75			40.7
Std. dev.		0.84			20.8			1.19			
Coef. var.		20.51			13.7			18.90			
Standard error		0.21			5.2			0.30			
GD 5.0%		1.34			34.0			1.59			
GD 1.0%		1.78			45.2			2.12			
GD 0.1%		2.31			58.8			2.75			

For this reason, in order to make the most efficient use of the productive potential of the variety, the correct choice of suitable varieties for each individual agro-ecological region is of great importance as a factor for obtaining a high yield. In the case of grain yield, the data show that the highest average yield was reported for the varieties Joana (651.5 kg/da), KM 135 (628.8 kg/da) and Lucille (619.7 kg/da), and the lowest yield met the standard. Geya 1 (431.5 kg/da). For seven varieties of wheat, the reported average yield is over 600 kg/da, and their difference with the standard is mathematically proven.

Tillering as a biological feature of cereals is determined by the hereditary qualities of the varieties, but it is also strongly influenced by the growing conditions. It has been established that when sowing in the optimal time, a large part of the tillers are formed in the autumn, and when sowing late, the fraternization takes place exclusively during the winter-spring period (Kasimov, 1976). The total tillering of the varieties varies from 3.2 (KM 135, Sadovska Belia 1) to 4.3 number (Bononia). Ten samples fall above the level of the standard, and in two of them the difference with Sadovo 1 is mathematically supported.In addition to the formation of the tillers, it is important for the realization of grain production to turn them into productive ones. According to Tsenov et al. (2009) the appropriate combination between more classes per unit area in combination with a larger number of grains is a prerequisite for increasing the productivity of wheat varieties.

On the other hand, greater productive tillering leads to increased plant tolerance to drought. The cultivars Tsarevets (4.3), Murgavets (4.0) and Sadovo 552 (4.0) are characterized by the largest number of productive tillers, and the smallest number of productive tillers was reported to the variety KM 135 (3.2).

The spike, as a symbol of yield, has always been a major organ of wheat morphology, subject to selective influence. Spike size is not only a morphological trait, but is also one of the factors for increased photosynthesis. In our study, the length of the central spike ranged from 11.0 (Diamant) to 12.8 cm (Joanna). A higher length of the central spike compared to the standard was reported for six varieties of common wheat. The number of spikeles in a spike is a factor that determines the spike density. In the particular case they vary from

18.8 (Gaia 1) to 21.8 (Joanna). Exceeding the trait compared to the standard is observed in eight varieties.

The increase of the grains number in the central spike is directly related to the increase in yield. In general, varieties with more grains have a higher breeding value (Tsenov & Tsenova, 2004). The largest grains number in the central spike was reported for the cultivars Tsarevets (63.5), Murgavets (61.5) and Sadovska Belia 1 (60.0), and the smallest grains number in the cultivars Bononia (44.5) and Pobeda (45.5). On nine genotypes the reported grains number is in the range of 50-60 numbers.

Biometric data reflecting the grains weight in the central spike, as a direct component of the yield show that the highest values of the trait were reported for the following varieties: Murgavets (2.90 g), Sadovo1 - st. (2.73 g) and Nicky (2.46 g). At the last place for this trait are Guinness (1.63 g) and Sadovska Belia 1 (1.65 g). Fourteen wheat genotypes are below the standard level, and in seven of them the difference with Sadovo 1 is mathematically supported.

The grains number in the other spikes has a minimum of 84.7 (Bononia) and a maximum of 142.5 (Murgavets). In seven varieties the reported grains number is over 100, and exceeding the trait compared to the standard is observed in nine wheat varieties . The best results in terms of grain weight in other spikes were shown by the varieties Murgavets (5.85 g) and the standard Sadovo 1 (5.11 g). In six genotypes, the value of the studied trait was below 4.0 g, with the lowest value obtained in the Bononia variety (2.69 g).

The grains number in one plant is in the range from 122.8 (Guinness) to 204.0 (Murgavets). Exceeding the trait compared to the standard was reported in five wheat varieties , and only in two (Murgavets and Tsarevets) of them the difference with Sadovo 1 is mathematically significant. The grain weight of a plant was determined by a number of authors (McMaster et al., 1987; Fufa et al., 2005; Leilah & Al-Khateeb, 2005) as one of the most important breeding traits in the selection of breeding materials. In our study, the grain weight of one plant varies from 4.43 g (Guinness) to 8.75 g (Murgavets). Only the Murgavets variety falls above the level of Sadovo 1 according to this trait.

Table 3 presents the results of the percentage ratio between the grains weight in the central spike and the grains weight from a plant. The data show that the largest share of the central spike in the formation of the grains weight from one plant in the varieties Joanna (40.7%), Bononia (39.4%) and Tsarevets (37.5%). The lowest percentage ratio between these two traits was found in Sadovo 552 (29.7%), Diamant (31.3%) and Guinness (31.3%).

To determine the degree of variation of the studied traits, a coefficient of variation was calculated on the basis of average values for the study period. Tables 1, 2 and 3 present the standard deviation (Std. dev.) and the coefficient of variation (CV) for the different performance elements. According to the importance of CV, the variation of the studied traits is from weak to strong. There is little variation of the spikelets number in the central spikes (C.v. = 4.6%), length of the central spike (C.v. = 4.9%), productive tillering (C.v =8.6%) and total tillering (C.v. = 9.1%). The variation of the yield (C.v. = 10.2%), grains number in the central spike (C.v. = 10.9%), grains number in one plant (C.v. = 13.7%), grains number in the other spikes (C.v. = 15.3%) are estimated as average, grains weight in central spikes (C.v. = 18.2%) and grains weight per plant (C.v. = 18.9%). The most variable is the trait grain weight in the other spikes (C.v. = 20.5%).

To determine whether the variability of the trait depends more on genetic factors or on enviromental conditions, a two-way analysis of variance was applied. It assessed the strength of the influence of the sources of variation - genotype, environment and genotype environment (Table 4). The successful introduction of wheat varieties is related to its behavior in different environmental conditions (Van Itteresum et al., 2013). According to Annicchiarico (2002), the analysis of the genotype x environment interaction is very important for the correct determination of the ecotype for the region. The presented results show that there is a proven influence of genotype, environment and their interaction on the studied indicators.

Table 4. Influence of the sources of variation on the studied traits

Traits	Sources of variation	SS	df	MS	F exp.	F tab.	ŋ, %	Sig.
Yield	Genotype - factor A	300003.5	15	20000.2	443.5	3.0	10.1	***
	Environment - factor B	2426022.1	1	2426022.1	53793.1	11.9	82.0	***
	Interaction - AxB	230675.8	15	15378.4	341.0	3.0	7.8	***
	Error	2886.3	64	45.1			0.1	
	Total	2959587.7	95				100.0	
TT	Genotype - factor A	10.3	15	0.7	5.5	3.0	38.2	***
	Environment - factor B	1.5	1	1.5	12.0	11.9	5.6	***
	Interaction - AxB	7.2	15	0.5	3.8	3.0	26.6	***
	Error	8.0	64	0.1			29.7	
	Total	27.0	95				100.0	
PT	Genotype - factor A	7.5	15	0.5	3.0	1.8	25.8	***
	Environment - factor B	3.8	1	3.8	22.6	4.0	13.0	***
	Interaction - AxB	7.1	15	0.5	2.8	1.8	24.4	***
	Error	10.7	64	0.2			36.8	
	Total	29.0	95				100.0	
CSL	Genotype - factor A	29.3	15	2.0	5.8	1.8	20.4	*
	Environment - factor B	38.1	1	38.1	113.6	11.9	26.6	***
	Interaction - AxB	54.7	15	3.6	10.9	3.0	38.1	***
	Error	21.5	64	0.3			15.0	
	Total	143.6	95				100.0	
SNCS	Genotype - factor A	78.0	15	5.2	5.1	3.0	34.7	***
	Environment - factor B	0.3	1	0.3	0.3	4.0	0.1	n.s.
	Interaction - AxB	81.1	15	5.4	5.3	3.0	36.1	***
	Error	65.5	64	1.0			29.1	
	Total	224.9	95				100.0	
GNCS	Genotype - factor A	2879.5	15	192.0	4.8	3.0	27.3	***
	Environment - factor B	294.0	1	294.0	7.3	7.0	2.8	**
	Interaction - AxB	4791.7	15	319.4	8.0	3.0	45.5	***
	Error	2568.7	64	40.1			24.4	
	Total	10533.8	95				100.0	
GWCS	Genotype - factor A	14.2	15	0.9	7.4	3.0	34.6	***
	Environment - factor B	0.6	1	0.6	5.0	4.0	1.6	*
	Interaction - AxB	17.9	15	1.2	9.3	3.0	43.7	***
	Error	8.2	64	0.1			20.1	
	Total	41.0	95				100.0	
GNOS	Genotype - factor A	21614.6	15	1441.0	4.9	3.0	30.0	***
	Environment - factor B	1190.0	1	1190.0	4.1	4.0	1.6	*
	Interaction - AxB	30595.0	15	2039.7	7.0	3.0	42.4	***
	Error	18746.0	64	292.9			26.0	
	Total	72145.6	95				100.0	
GWOS	Genotype - factor A	63.3	15	4.2	6.3	3.0	31.7	***
	Environment - factor B	2.5	1	2.5	3.7	4.0	1.3	n.s.
	Interaction - AxB	91.0	15	6.1	9.0	3.0	45.5	***
	Error	43.1	64	0.7			21.6	
	Total	199.9	95				100.0	
GN1P	Genotype - factor A	38832.6	15	2588.8	5.9	3.0	33.2	***
	Environment - factor B	240.7	1	240.7	0.6	4.0	0.2	n.s.
	Interaction - AxB	50006.0	15	3333.7	7.7	3.0	42.8	***
	Error	27862.7	64	435.4			23.8	
	Total	116942.0	95				100.0	
GW1P	Genotype - factor A	126.7	15	8.4	8.9	3.0	34.8	***
	Environment - factor B	5.7	1	5.7	6.0	4.0	1.6	*
	Interaction - AxB	170.6	15	11.4	11.9	3.0	46.9	***
	Error	61.0	64	1.0			16.8	
	Total	363.9	95				100.0	

SS - sum of squares; gf - degrees of freedom; MS - variance; F exp. - F experimental; F tab. - F tabular; ŋ - force of influence of the factor (%); * - significant at α = 0.05;** - significant at α = 0.01, *** - significant at α = 0.001; n.s. - non-significant

The only exception is observed in SNCS, GWOS and GN1P, where the influence of growing conditions is unproven. The strongest influence of the genotype was found in the total (ŋ=38.2%) and productive tillering (ŋ=28.5 %). Growing conditions are crucial for yield (ŋ=80.1 %). The interaction of the two factors has a leading role on the indicators GW1P (ŋ=46.9 %), GWOS (ŋ=45.5 %), GNCS (ŋ=45.5 %), GWCS (ŋ = 43.7 %), GN1P (ŋ=42.8 %), GNOS (ŋ=42.4 %), CSL (ŋ = 38.1 %) and SNCS (ŋ=36.1 %).

Table 5 presents the results of the performed PC-analysis. The data in the table show that the four main components PC 1, PC 2, PC 3 and PC 4 explain 84.3% of the total variation of all traits, which is large enough. The first component contains 41.2% of the total variation, the second - 59.7%, the third - 73.3% and the fourth - 84.3%.

Table 6 shows that five features are strongly associated with the first component and relate positively to it: GNCS (0.720), GWCS (0.802), GNOS (0.807), GWOS (0.834), GN1P (0.894) and GW1P (0.858). The second component is strongly, positively associated with two traits: CSL (0.595) and SNCS (0.768). The third component is strongly, positively related to the trait Yield (0.699) and negatively to total tillering (-0.653). The fourth component includes PT (-0.593), which is in a negative relation towards it.

The studied wheat varieties relate differently to the four main components (Table 7). The first main component includes seven genotypes, four of which are positive with PC 1 (Murgavets, Sadovo 1, Lucille and Niki), and the other three are negative (Guinness, Bononia and Diamant). In the positive values of PC 2 are the varieties Joanna (1.671) and Mustang (1.394), and in the negative values of PC 2 is the variety Geya 1 (-1.453). The smallest number of samples of studied materials are related to PC 3. These include Tsarevets (2.132) and Sadovo 772 (1.038), located in the positive values of the component. The fourth main component is represented by three varieties, as Sadovska Belia 1 (1.988) is positively connected to PC 4, and Pobeda (-1.621) and Sadovo 552 (-1.561) fall into the negative values of the component. The varieties Tsarevets (2.132), Sadovska Belya 1 (1.988) and Murgavets (1.988), which are the most remote, can be mentioned as sources of the greatest variation in order to create a variety of starting material and enrichment of the gene pool in ordinary winter wheat, compared to other wheat varieties.

Table 5. Component analysis of the variance in the studied traits

Comp.	Total	% of Variance	Cumulative (%)
1	4.5	41.2	41.2
2	2.0	18.6	59.7
3	1.5	13.5	73.3
4	1.2	11.0	84.3
5	0.8	7.0	91.3
6	0.5	4.6	96.0
7	0.3	2.3	98.3
8	0.1	1.0	99.3
9	0.1	0.5	99.8
10	0.0	0.2	100.0

Table 6. Explained significant components by traits

N°	Traits	Component			
		1	2	3	4
1	Grain yield	-0.134	0.146	0.699	0.210
2	TT	-0.153	0.583	-0.653	0.264
3	PT	0.450	0.107	-0.444	-0.593
4	CSL	0.437	0.595	-0.004	0.460
5	SNCS	0.221	0.768	0.408	0.032
6	GNCS	0.720	-0.049	0.413	-0.486
7	GWCS	0.802	-0.426	0.042	0.303
8	GNOS	0.807	0.446	-0.096	-0.135
9	GWOS	0.834	-0.339	-0.143	0.277
10	GN1P	0.894	0.288	0.05	-0.161
11	GW1P	0.858	-0.382	-0.087	0.297

Table 7. Explained significant components by variety

N°	Variety	Component			
		1	2	3	4
1	Sadovo 1st	1.376	-1.002	0.620	-0.219
2	Bononia	-1.336	-0.746	0.235	-1.090
3	Niky	0.778	-0.606	-0.494	-0.208
4	Lucille	0.814	-0.572	-0.672	-0.067
5	Sadovska Belya 1	-1.470	0.305	-0.220	1.988
6	Tsarevets	0.052	0.219	2.132	1.625
7	Pobeda	-0.258	-0.700	0.562	-1.621
8	Mustang	0.614	1.394	-1.043	-0.189
9	Geya 1	0.588	-1.453	-0.208	0.638
10	Diamond	-0.645	-0.381	-0.005	-0.052
11	Murgavets	1.767	1.694	0.798	0.479
12	Sadovo 552	0.417	0.614	0.414	-1.561
13	Sadovo 772	-0.961	0.879	1.038	-0.446
14	Guinness	-1.366	-0.314	0.268	0.006
15	Yoana	-0.700	1.671	-1.724	-0.324
16	KM 135	0.332	-1.002	-1.700	1.040

CONCLUSIONS

The highest average yield for the studied period was found in the varieties Joana and KM 135. The highest value of the indicators grain mass in the central class and grain mass per plant is characterized by the variety Murgavets, and the highest productive brotherhood and the biggest number of grains in the central class was reported for Tsarevets.

Slight variation of the studied traits was found in the number of spikelets in the central class and the length of the central, and the most variable is the sign of grain mass in the residual classes

The most significant and proven is the influence of growing conditions on yield. The genotype factor is of a paramount importance on the general and productive brotherhood, and the interaction between the two genotype factors is crucial for the mass of grains in the central class and the mass of grains from a plant.

The varieties Tsarevets, Sadovska Belya 1 and Murgavets are distinguished by great variation.

From a practical point of view, the most valuable varieties are Joanna, Km 135, Murgavets, Tsarevets and Sadovska Belya 1. The local farmers can be supplied with seeds from those varieties. These varieties can be used as parent pairs in the breeding and improvement work of winter wheat to create new and highly productive varieties.

ACKNOWLEDGEMENTS

This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018".

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INHERITANCE AND COMBINING ABILITY FOR FIBER LENGTH IN F_1 DIALLELCOTTON CROSSES (*Gossypium hirsutum* L.)

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Abstract

The aim of this study was by means of diallel analysis of fiber length of F_1 cotton hybrids to establish some genetic parameters and inheritance indexes necessary for specifying the breeding strategy by this character and breeding value of parental forms. The hybrid populations of two diallel combinations, each involving 6 parental components, were studied. A half diallel crossing scheme was used including the parents and one set of F_1 hybrids from direct crosses. Each diallel combination was tested in replicated trials in two consecutive years. It was found that additive and non-additive gene effects participated in the genetic control of fiber length. The main component of genetic variance was of non-additive type (dominance and epistasis). The varieties Darmi, Mytra and Dorina, from the 1st diallel combination, and Natalia, from the 2nd diallel combination, were identified as good general combiners for this trait.

***Key words**: cotton, G. hirsutum L., diallel analysis, fiber length, genetic variances, combining ability.*

INTRODUCTION

Bulgarian cotton production is based exclusively on Bulgarian varieties, foreign varieties late in maturing and do not realize their productive potential and fiber properties. In our country cotton is grown under non-irrigated conditions with very limited temperatures and rainfall for this crop. In cotton selection the greatest attention is paid to earliness, productivity and fiber quality, especially its length. At this stage cotton selection is mainly aimed at improving the productivity and quality of the fiber, while preserving earliness.

In Europe cotton is grown in Spain, Greece, more limited in Portugal and Bulgaria. In Spain and Greece cotton is grown under irrigated conditions and yields are much higher. Varieties grown in these countries have a longer growing season, higher productivity and a better quality fiber.

In recent years as a result of selection many new cotton varieties have been created: Sirius (Valkova, 2017), Tsvetelina (Koleva & Valkova, 2019), Aida, Anabel, Tiara and Melani (Dimitrova, 2022a; 2022b; 2022c). All these varieties are early ripening and high yielding, Anabel and Melani have improved fiber quality characteristics. Selection results in terms of productivity and fiber quality of the European countries and a number of other cotton-producing countries are much higher and set higher criteria in front of our selection. In cotton breeding the genetic plasm exchange is very limited, and in addition, genetically modified cotton is grown in the countries outside of Europe.

From the specialized literature it can be seen that various selection methods and techniques are used for genetic improvement of traits and genotypes. Gene action and genetic variation are some of the most important criteria for any breeding program. Additivity, epistasis, dominance, overdominance, heterosis, heterobeltiosis, heritability, general combining ability (GCA) and specific combining ability (SCA) are some of the most important statistical approaches for choosing a selection strategy (Sajjad, 2015). GCA is a criterion for selection of parental forms, while hybrids are selected based mainly on SCA effects. Additive gene action in control of fiber quality properties was reported by Akiskan & Gencher (2014), Carvalho et al. (2018). However, other researchers (Bõlek et al., 2014; Khan et al., 2017) found a non-additive type of gene action. An additive type of gene action with partial dominance for fineness, staple length and strength was reported by Rasheed et al. (2014). In the studies of Nasimi et al. (2016) additive action with partial dominance and absence of non-allelic interactions determined the inheritance of fiber length, strength and fineness under normal and drought conditions. In the studies of Khokhar et al. (2018) non-additive gene effects were essential for fiber length and strength.

In the selection of cotton in our country the genetic control of traits has been insufficiently studied both in intra- and inter-species crossing. The gene action controlling the fiber quality properties has been insufficiently studied. There is limited information on the selection value of modern varieties of Bulgarian and foreign selection as parental components for crossing, on the genes they contain and their effects on the expression of traits. The genetic potential of hybrid populations obtained through intra- and inter-specific crosses is insufficiently appreciated.

Quantitative traits are largely influenced by environmental conditions and modification of their genetic parameters is also insufficiently studied. The use of diallel analysis will help to obtain the necessary information for selection programs and to expand the theoretical basis of cotton selection in our country.

The aim of this study was by means of diallel analysis of fiber length of F_1 cotton hybrids to establish some genetic parameters and indexes of inheritance necessary for specifying the breeding strategy by this character and breeding value of parental forms.

MATERIALS AND METHODS

The hybrid populations of two diallel crosses were studied. First one included six (four Bulgarian and two foreign) upland cotton varieties: Beli Iskar - P_1; Barut 2005 (Turkish) - P_2; Darmi - P_3; Mytra (Greek) - P_4; Helius - P_5 and Dorina - P_6. Second diallel cross included varieties: Chirpan-539 - P_1; Helius – P_2; Rumi - P_3; Boyana - P_4; Natalia - P_5 and Nelina - P_6 (Bulgarian selection).

A half diallel crossing scheme was used including the parents and one set of F_1 hybrids from direct crosses. The study was carried out at the Field Crops Institute in Chirpan and each diallel combination was tested in two consecutive years, in three replicates. The parents and their F_1 hybrids were sown in 2 rows of 2.4 m in a 60×20×1 sowing scheme. Ten plants from each replicate were accounted.

Dispersion and diallel analyses adequate of diallel scheme were applied to the data processing (Mather & Jinks, 1982). Adequacy of data for diallel analysis was assessed by the regression coefficient *b* and *t (Wr - Vr)* (Mather & Jinks, 1982).

The following components of genotypic variation along with their standard errors were calculated: *D* - additive variance; H_1 and H_2 - dominance variances; *F* - shows the ratio of dominant to recessive genes in parents; *E* - variability due to the influence of environmental conditions.

Based on the above components the following indicators were calculated: $(H_1/D)^{1/2}$ - average degree of dominance in each locus; $H_2/4H_1$ - ratio of the positive and negative alleles in the loci showing dominance in the parents; K_D/D_R $[(4DH_1)^{1/2} + F/(4DH_1)^{1/2} - F]$ - the ratio of dominant to recessive genes in parents; $K=(h^2/H_2)$ - number of effective factors; H^2 and h^2 - coefficients of heritability in a broad and in a narrow sense, calculated by Mather & Jinks (1982).

RESULTS AND DISCUSSIONS

Fiber length is a genetically stable characteristic, but it is also influenced by environmental conditions (the years). Due to their specific response to the years conditions, parents changed their order by years, but parents with high and low values of this trait generally maintained their positions.

The preliminary analysis of variance of this trait showed significant differences between crosses from the two diallel combinations (data are not given here).

The statistical analysis to verify the main hypotheses for the representativeness of the results of the diallel analysis (Table 1) reveals that in three of the four experimental studies - 1st DC - 2nd year, 2nd DC - 1st year and 2nd year, the regression coefficient *b* was significantly less than 1 and indicated the presence of complementary epistasis. Only in 1st DC - 1st year the regression coefficient *b* did not significantly differ from 1, which indicates that a regression line can be constructed that reliably reflects the genetic dependencies in the diallel combination.

Table 1. Additive-dominant model test of fiber length in two diallel combinations carried out in two consecutive years 1st DC - 1st and 2nd years and 2nd DC - 1st and 2nd years

Diallel cross, Year	Excluded parent	$b_{Wr/Vr} \pm b$	0 > b >0 b ≠ 0	1 > b >1 b ≠ 1	$t_{(Wr - Vr)}$
1st DC - 1st year	-	0.815 ± 0.127	6.420++	1.469	1.245
	P_4	0.876 ± 0.245	3.581+	0.505	0.123
	P_5	0.881 ± 0.184	4.778+	0.646	0.382
1st DC - 2nd year	-	0.656 ± 0.127	5.172++	2.697	2.287
	P_1	0.840 ± 0.091	9.215++	1.797	1.748
2nd DC - 1st year	-	0.710 ± 0.239	2.965+	1.217	0.649
	P_1	0.852 ± 0.371	2.287ns	0.407	0.209
	P_5	0.829 ± 0.247	3.364+	0.690	0.301
2nd DC - 2nd year	-	0.399 ± 0.465	0.857	1.292	0.237
	P_5	1.134 ± 0.263	4.316+	-0.509	0.930

The diallel scheme (1st DC - 1st year) from which one of the parents - P_4 (Mytra variety) or P_5 (Helius variety) was excluded, provided more constant values of the *Wr* - *Vr* differences. There was an insignificant but considerably variation of the parental rows according to the *Wr* - *Vr* parameter for 1st DC – 2nd year, which can be assumed to be due to the influence of non-allelic interactions. The diallel scheme, from which P_1 (Beli Iskar variety) was excluded, provided more constant values of *Wr* - *Vr* differences and, therefore, a better fit of the additive-dominant model. Variation of parental rows according to the *Wr* - *Vr* criterion for 2nd DC – 1st and 2nd years was statistically insignificant and did not indicate the presence of non-allelic interactions. However, the regression coefficients were low, especially for 2nd DC - 2nd year, and were not significantly different from 0, and it was considered that the regression lines in the diallel graphs did not adequately represent the relationship between the covariances (*Wr*) and variances (*Vr*) of the diallel combinations. After excluding P_1 (Chirpan-539 variety) or P_5 (Natalia variety) from the diallel scheme of 2nd DC - 1st year, and P_5 (Natalia variety) from the diallel scheme of 2nd DC - 2nd year, the resulting set of parents satisfied the requirements for diallel analysis.

Ali et al. (2010) noted that staple fiber length data were unsuitable for further diallel analysis. In our study the fiber length data showed partial or incomplete compliance for genetic interpretation. According to Jinks (1954), Rood & Major (1981) in some situations the exclusion of one or more of the parents of the diallel scheme can improve the fit of the data to the additive-dominant model.

In Figure 1, A-C and Figure 2, A-B the data from the analysis for 1st DC - 1st and 2nd years were graphically presented. From the graphs of the parental forms of the F_1 populations the following characteristics can be made: the inheritance of the studied trait in the populations was overdominant, the regression line crossed the ordinate below the zero point; there was a displacement of the points when the environmental conditions changed; after excluding parents considered to be carriers of non-allelic interactions from the diallel analysis the remaining parents retained their location. After excluding some parents (P_5 - Helius variety from 1st DC - 1st year) the others noticeably moved to the more dominant part of the diallel graph, which means inclusion of more dominant genes in the trait expression (fig 1, C). Darmi variety (P_3) having longer fiber (1st DC) had high dominance possessing the most dominant genes for fiber length during the first year and moderately high dominance during the second year (Figure 2, A and Figure 2, B) Cultivar Mytra (P_4) had high to medium high dominance during the first year and high recessiveness with the most recessive genes during the second year. Beli Iskar variety (1st DC) with most recessive genes (during the first year) and with most dominant genes (during the second year) has shown inconsistent dominance/recessiveness during the two years of the study. Helius variety, having the shortest fiber, was of constant medium-high dominance for fiber length in both years of the study. Dorina variety, having the longest fiber, had relatively high dominance (during the first year) and moderately high dominance (during the second year).

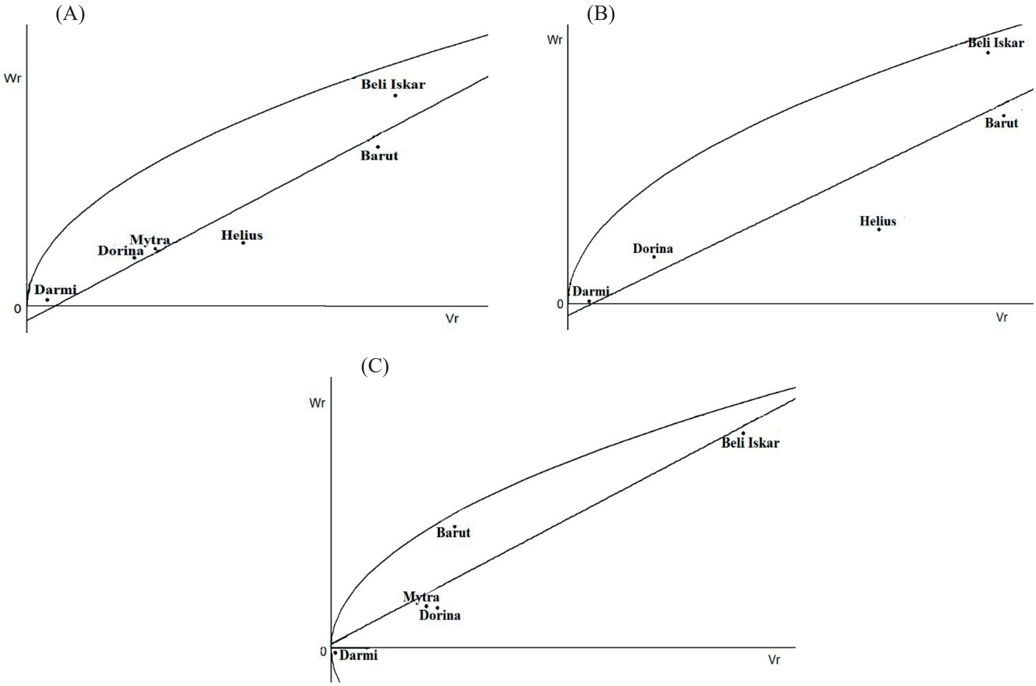

Figure 1. Graphical diallel analysis of fiber length for the first diallel combination tested for first year (1st DC - 1st year). (A) - all parents; (B) - excluded P_4 - Mytra variety; (C) - excluded P_5 - Helius variety

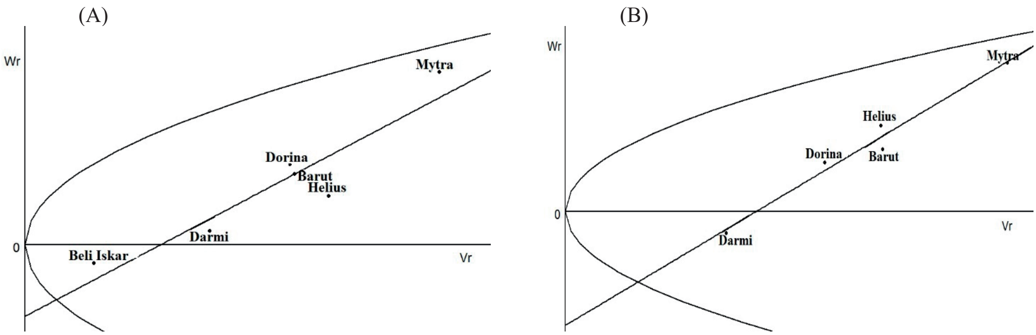

Figure 2. Graphical diallel analysis of fiber length for the first diallel combination tested for second year (1st DC - 2nd year). (A) - all parents; (B) - excluded P_1- Beli Iskar variety

In Figure 3, A the diallel graph of 2nd DC - 1st year without exclusion of parents was constructed, and in Figure 3, B - with excluded P_1 - cultivar Chirpan-539. From the graph with the full set of parents it can be seen that the regression line crossed the ordinate below the zero point and expressed overdominance. Helius variety (having short fiber) had high dominance possessing most dominant genes, while Nelina variety (having the longest fiber) was with most recessive genes. The remaining varieties were located in the dominant part of the diallel graph, which means a preponderance of dominant alleles over recessive ones.

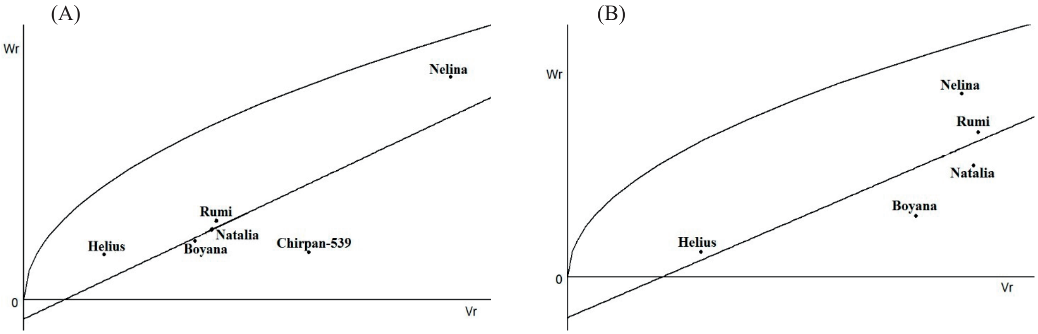

Figure 3. Graphical diallel analysis of fiber length for the second diallel combination tested for first year (2nd DC - 1st year). (A) - all parents; (B) - excluded P_1- Chirpan-539 variety

Compared to 2nd DC – 1st year, in 2nd DC – 2nd year (Figure 4, A) there was a slight movement of parents under the influence of the year conditions. Rumi, Boyana and Chirpan-539 varieties retained their high dominance, while Helius and Nataliya varieties with high dominance in the 1st year had medium high dominance in the 2nd year, their points were shifted towards the middle of the regression line. There was a noticeable reshuffling of the parental forms after exclusion of P_5 (Natalia variety) (Figure 4, B), which was likely due to intraparental interactions.

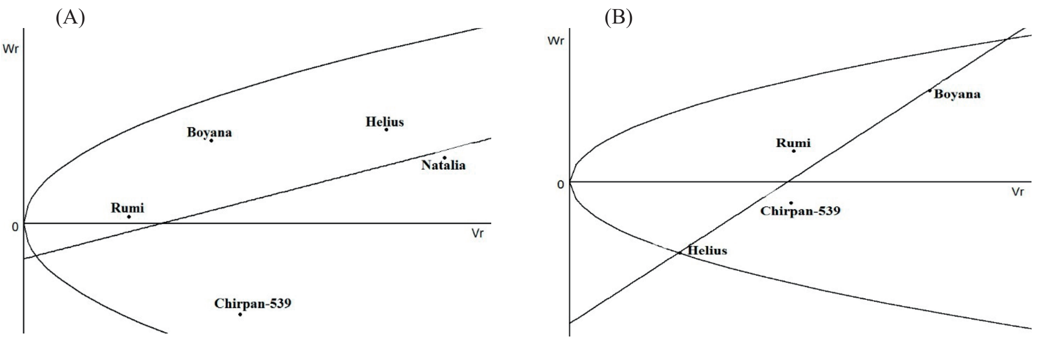

Figure 4. Graphical diallel analysis of fiber length for the second diallel combination tested for second year (2nd DC - 2nd year). (A) - all parents; (B) - excluded P_5 - Natalia variety

Inheritance in 2nd DC - 1st and 2nd years was overdominant with manifestations of complementary epistasis. The regression lines deviated significantly from the normal line and intersected the ordinate below zero. The genetic components from the performed diallel analysis are presented in Table 2. The additive parameter *D* and the dominant parameters H_1 and H_2 were statistically significant. In 1st DC - 1st year the dominant gene action slightly exceeded the additive (*D*) or both variance components had equal participation - with P_5 - Helius variety excluded, while in 1st DC – 2nd year and 2nd DC - 1st and 2nd years the dominant gene action was much more pronounced than the additive one showing the great importance of the dominant variance in the trait variation.

The H_1/D ratio was consistent with the significance of both variances showing overdominance, with the exception of 1st DC - 1st year after excluding P_5 - Helius variety, where complete dominance was observed. Mean dominance in loci expressed as $H_1/D^{1/2}$ was overdominance and complete dominance, respectively. The M_{L1} - M_{L0} indicator showed that the dominance was in the direction of increasing fiber length in both diallelic combinations. The two dominant parameters - H_1 and H_2 in most cases had close values, but the ratio $H_2/4H_1$ showed an unequal distribution of positive and negative alleles showing dominance, with the exception of 1st DC - 2nd year with full set of parents.

The zero value of the parameter $F^2/4D(H_1 - H_2)^{1/2}$ indicated the presence of highly varying dominance by loci in the crosses. The average value of the parameter F in most cases had a positive sign and it can be assumed that dominant alleles prevailed over recessive ones. The growing importance of the dominant alleles was confirmed by the positive and significant values of the parameter h^2 with the exception of 1^{st} DC - 1^{st} year without P_4 - Mytra variety (data are not given).

The parameter F had a negative sign for 1^{st} DC - 2^{nd} year, but it was insignificant for the complete set of parents and it can be assumed that the dominant and recessive alleles in the population had the same frequency. The ratio K_D/K_R reflected superiority of recessive alleles, while for all other experimental settings (1^{st} DC - 1^{st} year and 2^{nd} DC - 1^{st} and 2^{nd} years) it showed superiority of the dominant alleles.

Ali et al. (2009) found that dominant genes outperformed recessive ones, while Igbal et al. (2011) noted a predominance of recessive alleles.

The correlation between the mean values of the parental forms and the total covariance-variance values was negative and high for 1^{st} DC - 1^{st} year, which means that dominant genes having unidirectional, trait-enhancing effect, were acting in the population. For 1^{st} DC-2nd year, this relationship was very weakly negative to weakly positive indicating that genes with multidirectional action were acting in the populations. With such a relationship, it is difficult to assess the nature of the dominant determination of the studied trait in the diallel crosses.

The high and positive correlation in 2^{nd} DC - 1^{st} and 2^{nd} years was an indication that the parents with maximum recessive genes were responsible for increasing fiber length. The high W_R+V_R values for 2^{nd} DC - 1^{st} and 2^{nd} years were in a positive correlation with the parental values, and for 1^{st} DC - 1^{st} year - in a negative correlation. This means that in 2^{nd} DC - 1^{st} and 2^{nd} years parents with high fiber length indices had most recessive genes, while in 1^{st} DC– 1^{st} year parents with a longer fiber had most dominant genes acting to increase the trait. The values of k - number of effective factors indicated a group of genes that were responsible for the trait manifestation.

Heritability coefficients in the broad sense (H^2) in most cases were high, in the narrow sense (h^2) they were low to moderately high and effective selection for fiber length should be conducted in the later hybrid generations F_3-F_4.

Aziz et al. (2014), Srinivas et al. (2014), Ahsan et al. (2015), Wagar et al. (2016), Nizamani et al. (2017), Khan et al. (2017) reported moderate to high heritability, which revealed that this trait was influenced by additive gene effects.

The parents were ranked by mean value and by presence of dominant/recessive genes expressed by the sum of covariances and variances (W_r + V_r) (data are not given hire).

As for 1^{st} DC - 1^{st} year, parental forms with high mean values for fiber length (Dorina, Darmi, Mytra) had low $Wr+Vr$ values, i. e. they had high dominance (data are not given here). Their high dominance makes them very valuable for the selection of a longer fiber with them the dominant genes acted in a positive direction to increase the trait values. For 2^{nd} DC – 1^{st} and 2^{nd} years, Nelina and Natalia varieties, with high average values for fiber length, had high Wr + Vr values, i. e. these had high recessiveness (data are not given hier). The remaining varieties, which had a shorter fiber (Helius, Chirpan-539, Rumi and Boyana) had low Wr + Vr values and had high dominance, but in these the dominant genes acted to reduce the fiber length. The rxp $(Wr + Vr)$ values were positive and medium high to very high for 2^{nd} DC – 1^{st} year without P_1 - cultivar Chirpan-539 carrier of dominant genes for shorter fiber.

The assessment of the degree of dominance of the parental varieties from the graphic analysis was supplemented by the data in the Tables 3 and 4. In 1^{st} DC - 1^{st} and 2^{nd} years constant dominance in different ecological environments was found for Barut 2005 variety. Unstable dominance was found for the varieties Beli Iskar (very high in the first year and low in the second year), Mytra, Helius, Darmi and Dorina (low in the first year and high in the second year).

Table 2. Genetic components and indicators of inheritance of fiber length in the first and second diallel combinations, first and second years (1st DC – 1st and 2nd years and 2nd DC – 1st and 2nd years)

Genetic components	1st DC F_1 - 1st year		1st DC F_1 - 2nd year		2nd DC F_1 - 1st year		2nd DC F_1 - 2nd year	
	Parameters							
	All parents b = 0.815	Excluded P_5 - Helius b = 0.881	All parents b = 0.656	Excluded P_1 - B. Iskar b = 0.840	Full set of parents b = 0.710	Excluded P_1 Chirpan-539 b = 0.852	Full set of parents b = 0.399	Excluded P_1 Chirpan-539 b = 1.134
D	1.272±0.087	1.538±0.118	0.242±0.088	0.192±0.040	1.231±0.135	1.052±0.094	0.434±0.184	0.155±0.034
F	0.562±0.212	0.926±0.295	-0.352±0.214	-0.630±0.099	0.581±0.329	0.385±0.234	0.427±0.460	0.301±0.087
H_1	1.925±0.220	1.506±0.319	2.135±0.223	2.577±0.108	2.412±0.342	1.990±0.253	2.481±0.497	1.418±0.099
H_2	1.349±0.197	1.180±0.289	2.144 ±0.199	2.471±0.977	1.992±0.305	1.797±0.230	2.327±0.451	0.938±0.091
h^2	0.275±0.133	0.834±0.195	0.131±0.134	0.158±0.066	0.306±0.206	0.433±0.155	0.147±0.304	-0.004±0.062
E	0.130±0.033	0.132±0.048	0.245 ±0.033	0.272±0.016	0.141±0.051	0.133±0.038	0.068±0.075	0.070±0.015
	Indicators							
H_1/D	1.513	0.979	8.822	13.422	1.959	1.892	5.716	9.148
$H_1/D^{1/2}$	1.230	0.990	2.970	3.664	1.400	1.375	2.391	3.025
$M_{L1} - M_{L0}$	0.087	0.010	0.067	0.083	0.096	0.130	0.048	0.020
$F^2/4D.(H_1-H_2)^{1/2}$	0.184	0.606	0.00	0.415	0.235	0.165	0.267	0.212
$H_2/4H_1$	0.175	0.193	0.251	0.230	0.207	0.226	0.234	0.165
K_D/K_R	1.438	1.568	0.606	0.381	1.406	1.307	1.518	1.946
r xp (Wr+Vr)	-0.710	-0.738	0.486	-0.048	0.619	0.960	0.503	0.752
k	0.204	0.707	0.061	0.064	0.154	0.241	0.063	-0.004
	Heritability, %							
H^2	0.883	0.853	0.773	0.799	0.800	0.869	0.907	0.825
h^2	0.579	0.523	0.273	0.343	0.455	0.425	0.110	0.354
	Prediction of the most dominant/recessive parent							
YD	29.11	29.12	26.67	27.50	24.0	22.82	26.66	26.58
YR	25.94	25.65	27.91	27.38	27.6	29.69	28.20	27.81

In 2nd DC - 1st and 2nd years Chirpan-539, Boyana, Helius and Natalia varieties had non-permanent dominance.

In the 1st diallel combination (1st DC) 4 crosses showed constant and positive heterosis manifestations conditioned by positive overdominance during the two studed years (data are not given here).

Table 3. Mean values of the Fr parameter for each parental row for fiber length in the first diallel combination during the first and the second years (1st DC - 1st and 2nd years)

1st DC - 1st year							1st DC- 2nd years				
Parents	Complete set of parents b = 0.815		Excluded P_4 – Mytra variety b = 0.876		Excluded P_5– Helius variety b = 0.881		Parents	Complete set of parents b = 0.656		Excluded P_1– B. Iskar variety b = 0.840	
	Fr	R	Fr	R	Fr	R		Fr	R	Fr	R
P_1-B. Iskar	-2.057	6	-1.751	5	-3.046	5	P_1-B. Iskar	1.494	1	-	-
P_2-Barut	-1.345	5	-1.156	4	0.593	4	P_2-Barut	-0.548	3	-0.728	3
P_3-Darmi	2.846	1	3.444	1	3.305	1	P_3-Darmi	0.470	2	1.133	1
P_4-Mytra	1.463	3	-	-	1.925	2	P_4-Mitra	-2.330	6	-2.399	5
P_5-Helius	0.747	4	0.873	3	-	-	P_5-Helius	-0.595	4	-0.945	4
P_6-Dorina	1.719	2	2.558	2	1.852	3	P_6-Dorina	-0.606	5	-0.213	2

Table 4. Mean values of the Fr parameter for each parental row for fiber length in the second diallel combination during the first and the second years (2nd DC – 1st and 2nd years)

2nd DC – 1st year							2nd DC – 2nd year				
Parents	Complete set of parents b=0.710		Excluded P_1 - Chirpan-539 b=0.852		Excluded P_5 – Natalia b=0.829		Parents	Complete set of parents b=0.399		Excluded P_5 –Natalia b=1.136	
	Fr	R	Fr	R	Fr	R		Fr	R	Fr	R
P_1-Chirpan-539	0.575	5	-	-	1.750	4	P_1-Chirpan-539	1.604	1	0.481	2
P_2- Helius	2.140	1	1.987	1	2.147	2	P_2-Helius	-0.597	4	1.110	1
P_3- Rumi	0.911	4	-0.275	4	2.092	3	P_3-Rumi	1.409	2	0.189	3
P_4- Boyana	1.304	2	0.725	2	2.539	1	P_4-Boyana	0.391	3	-0.574	4
P_5-Natalia	1.041	3	0.031	3	-	-	P_5-Natalia	-0.671	5	-	-
P_6-Nelina	-2.482	6	-0.542	5	-3.620	5					

The heterosis effect was the highest in the Beli Iskar × Helius cross – 3.2%, with a fiber length of 27.6 mm. In this cross, both parents had short fiber. The longest fibers were found for the crosses Mytra × Dorina (29.5 mm), Darmi × Mytra (28.8 mm) and Barut × Darmi (28.7 mm) with overdominant inheritance in both years and heterobeltiosis 2.1-2.7%. In these crosses both parental forms had longer fiber than the other parents (data are not given hire).

Overdominant inheritance of this trait has been found by many other researchers (Karademir et al., 2010; Patel et al., 2014). Usharani et al. (2015) noted maximum values - 34.82% relative heterosis and 27.41% heterobeltiosis.

In the second diallel combination (2nd DC) positive overdominance for fiber length was found for two crosses during the first year and for three crosses during the second year. Crosses showed negative overdominance (26.6% in the first year and 33.3% in the second year) and incomplete dominance of the parent with the lower value predominated confirming the results of diallel analysis that the dominance was in direction of reducing the trait meanings.

From the data in Table 5 it can be seen that there were significant differences between the tested crosses in terms of GCA and SCA. In the first diallel combination (1st DC) GCA effects were significant in the first year and insignificant in the second year showing that for the fiber length inheritance under the conditions of second year non-additive gene effects were mainly important. The SCA effects were significant in both years (1st DC - 1st and 2nd years). In the second diallel combination the variances of the general (GCA) and specific (SCA) combining ability were highly significant indicating that additive and non-additive gene effects were important for the inheritance of studied trait. The ratio of variances GCA/SCA was in favor of GCA, but tested with the F-criterion to SCA was insignificant, which did not confirm a greater importance of additive gene effects.

Table 5. Analysis of GCA and SCA variances for fiber length in the first and second diallel combinations, each one tested two years (1st DC – 1st and 2nd years and 2nd DC – 1st and 2nd years)

Dialel combination, year	Source of variation	Degrees of freedom	Mean squares	F-exp.
1st DC - 1st year	Crosses	20	2.999	7.77++
	GCA	5	7.596	19.68++ /5.18++ *
	SCA	15	1.466	3.80++
	Errors	40	0.386	
1st DC– 2nd year	Crosses	20	2.440	3.69++
	GCA	5	3.561	5.39ns/1.72ns *
	SCA	15	2.066	3.13++
	Errors	40	0.661	
2nd DC – 1st year	Crosses	20	2.903	6.60++
	GCA	5	5.582	12.69+ /2.78ns *
	SCA	15	2.010	4.57++
	Errors	40	0.440	
2nd DC – 2nd year	Crosses	14	1.759	8.84++
	GCA	4	1.254	6.30++ /0.64ns *
	SCA	10	1.962	9.86++
	Errors	28	0.199	

*tested with the F-criterion to SCA

The effects of GCA are indicated in Tables 6 and 7. Varieties Darmi, Mytra, Dorina, from the 1st diallel combination, and Natalia, from the 2nd diallel combination, showed a positive and high GCA in different environments (in both years of the study). Since GCA is determined by additively acting genes, it can be considered that these cultivars possessed the largest number of additive genes.

Zhang et al. (2016) reported that GCA effects were closely related to parental values for yield and fiber quality, which was confirmed in our study. Parents having longer fiber had higher GCA values.

Table 6. Mean values in mm (x) and GCA of the parents for fiber length in first diallel combination tested two years (1st DC - 1st and 2nd years)

	1st DC - 1st year		1st DC - 2nd year		Average	
Parent	x	GCA	x	GCA	x	GCA
P_1- B. Iskar	26.0	-0.489	26.6	-0.410	26.3	-0.449
P_2- Barut 2005	27.9	-0.360	26.9	-0.297	27.4	-0.328
P_3- Darmi	28.2	0.124	27.6	0.436	27.9	0.280
P_4- Mytra	28.2	0.453	27.9	0.303	28.1	0.378
P_5- Helius	27.3	-0.555	26.5	-0.335	26.9	-0.445
P_6- Dorina	29.6	0.828	28.2	0.303	28.9	0.565
Standard error	1.01	0.179	1.34	0.235	1.18	

Table 7. Mean values in mm (x) and GCA of the parents for fiber length in second diallel combination tested two years (2nd DC - 1st and 2nd years)

	2nd DC – 1st year		2nd DC – 2nd year		Average	
Parent	x	GCA	x	GCA	x	GCA
P_1- Chirpan-539	24.1	-0.514	26.9	-0.139	25.5	-0.326
P_2- Helius	24.0	-0.639	26.9	-0.205	25.5	-0.422
P_3- Rumi	25.9	0.211	27.0	-0.961	26.5	-0.375
P_4- Boyana	25.4	0.194	27.0	0.032	26.2	0.113
P_5- Natalia	26.4	0.628	27.4	0.408	26.9	0.518
P_6- Nelina	26.8	-0.119	-	-	-	-
GD 5 %	1.09		0.68			
Standard error		0.191		0.138		

SCA data varied more strongly depending on environmental conditions. There were 5 out of 15 crosses from the 1st diallel combination and 2 crosses from the 2nd diallel combination with significant positive SCA in both years (data are not given hier). These crosses have a high selection value. It is believed that they exhibit dominant and epistatic interactions, causing transgressive variability in the next F_2 generation. Estimates of the variance components for the GCA and SCA are presented in Table 8.

Table 8. Variance components for fiber length in the first and second diallel combinations tested in replicated trials in two years (1st DC - 1st and 2nd years and 2nd DC - 1st and 2nd years)

Sources of variation	Variance ± standard error			
	1st DC - 1st year	1st DC - 2nd year	2ndDC - 1st year	2nd DC- 2nd year
Crosses	0.871^{++}±0.317	0.593^{+}±0.261	0.821^{++}±0.308	0.520^{+}±0.222
GCA	0.255± 0.201ns	0.062± 0.099 ns	0.148±0.150 ns	0.034±0.059 ns
SCA	0.360^{+}±0.181	0.468± 0.256	0.523^{+}±0.246	0.587^{+}±0.293
Errors	0.386^{+++}±0.086	0.661^{+++}±0.147	0.440^{+++}±0.117	$0.198±0.053^{+++}$

Their values confirmed the results of the diallel analysis that the non-additive (for the 1st and 2nd diallel combinations) gene effects were important for inheritance. The results obtained were consistent with those reported by other authors (Hussain et al., 2010; Singh et al., 2010; Bölek et al., 2014; Khan et al., 2017) that non-additive gene effects were more important for fiber length. Akiskan & Gencer (2014), Ekinci & Basbag (2018), Carvalho et al. (2018) noted a predominance of additive gene effects. Nasimi et al. (2016) found an additive type of gene action with partial dominance in the inheritance of this trait.

From the research results it can be concluded that additive and non-additive gene effects participated in the inheritance of fiber length. Non-additive gene action predominated over additive and reveals that fiber length was more strongly influenced by genes with non-additive action. The varieties Dorina, Mytra, Darmi (1st diallel combination) and Natalia (2nd diallel combination) exhibited the longist fibers and the highest GCA appered to be the best general combiners and can be used in future breeding programs to enhance this trait. The low to medium-high heritability coefficients in the narrow sense and the high contribution of non-additive genetic variation reveal that effective selection should be conducted in the later hybrid generations - F_3-F_4.

CONCLUSIONS

Additive and non-additive gene effects were important for the inheritance of fiber length. In both diallel crosses the non-additive gene action predominated and selection by this trait should be conducted in the later hybrid generations - F_3-F_4.

Cultivars having longer fibers possessed dominant genes that acted to increase fiber length, while in cultivars having short fiber dominant genes acted to decrease this trait.

The varieties Darmi, Mytra, Dorina, from the 1st diallel combination, and Nataliya, from the 2nd diallel combination, were identified as good general combiners for this trait. These varieties had longer fiber and high GCA effects in different environments.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

BREEDING OF THE FIRST BULGARIAN VARIETY OF SWEET SORGHUM `SHUMEN SWEET`

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Abstract

On the background of the increased interest in the organic plant production in in the Agricultural Institute - Shumen was created the first for the last 50 years Bulgarian variety of sweet sorghum. Shume Sweet is developped after many years studies of local populations from the North-Eastern region of Bulgaria. Assessment and selection of perspective progenies were carried out for consolidation of stabilized population with high productivity of stem mass in technical maturity as a row material for extraction of juice with 11-15% of sugars - sucrose, fructose and glucose. The preliminary tests in comparative variety trials confirm the high and stable productive potential for extraction of juice with optimal qualities for production of concentrated sweet syrops. After tests in the system of IASAS the Shoumen Sweet variety has been certified by the agency in 2020.

Key words*: sweet sorghum, variety, breeding, selection.*

INTRODUCTION

The production potential of sweet sorghum have been discussed by many scientists (Houze 1995; Mukabane et al., 2014). The crop offers great potential for multi-purpose uses, mainly for syrop, forage and ethanol production. Plant breeders in the United States, India and China have developed high - brix cultivars to increase the potential of this crop for syrup production (Abdalbagi and Mohammed, 2020). Sweet sorghum is known to be one of the most efficient temperate crops for biomass accumulation under conditions of extreme drought and global warming (Rooney, 2000).

The sweet sorghum, also known in Bulgaria as sugar broom and reed, has been used here for more than a century to extract sweet syrups by pressing (Iliev 1921; Pavlov, 1938).

Despite its displacement by crystal sugar, it continues to be grown on small areas for own use (Kostov et al., 1950; Stefanov, 1991). Due to the favorable ratio of sucrose and monosaccharides such as fructose and glucose, its increased demand as a healthy food and for feeding bees (Varbanov and Hristova, 1996) is noted.

All cultivated forms of sweet sorghum are subspecies of sorghum bicolor. To this day, many of the varieties are local populations obtained as a result of the natural and artificial selection (Bantalian et al., 2004). Modern forms of sweet sorghum include various hybrids with Sudan grass, technical and grain forms of sorghum. They have a highly pronounced heterosis effect and an optimal combination of high productivity, multiple undergrowth, carbohydrate, protein and cellulose content and are used for fodder production (Smith and Frederiksen, 2000). The selection with the classical methods of hybridization and selection for height, duration of vegetation, qualities of the raw material, is combined with heterosis and use of cytoplasmic male sterility (Stack and Pedersen, 2003).

The publication demonstrates the results of the selection and productivity testing of sugar sorghum populations from the Breeding Program of Agricultural Institute-Shumen. After selection of a stabilized population and testing, the Shumensko Sladko variety was recognized by the State Variety Trials Commission in 2020, and in 2021 a certificate was issued by the Bulgarian Patent Bureau.

MATERIALS AND METHODS

The study was conducted in Experimental fields of Agriculture Institute - Shumen, Bulgaria in the period 2010-2020. The soil is carbonate chernozem, on a flat terrain. The period is characterized by frequent deviations from the agro-climatic norms for north-eastern Bulgaria. The sowing was carried out at a soil temperature of 10-12°C, which is usual for the area after 20-th of April. Test trials were made by the long plot method, with 3 replicates. The experimental plot of 12.6 sq m is three-row with 0.7 m inter-row spacing and a seeding density of 20 plants per sq m. Sowing material was not treated with pesticides and fertilizing.

The individual progenies tested were selected after evaluation from local populations. As a result of recurrent selection, 5 prospective stabilized populations were consolidated - SZT, SZW, SZM, SZA, SZC. The sweet sorghum varieties Yantar, Stavropolskaya, Galia, Endje and Super Sweet, which are hybrids of sweet sorghum and Sudan grass, were used in the comparative trials.

The plants of plots were cut and counted, the panicles and leaves were removed and the cane was recorded and then passed through hand driven two-roller mill. The juice quantity was recorded and kept in closed plastic bottles for analysis.

The following juice quality traits were made:

Brix (%) - total soluble solids were recorded using refractometer.

Pol (%) - sucrose percentage of the juice was estimated following dry lead acetate clarification method using polarimeter.

Redusing sugar (%) - invert sugars were estimated using dinitro-salicid acid method.

Acids, Vitamin C and Poliphenols were estimated in standard analysis method using spectroscopy analysis with the use of limon, follic and gallic acids standard.

The data collected were subjected to analysis of variance ANOVA following the standard procedure of analyzing GD design for productivity and least significant differences LSD procedure for juice quality analysis using GenStat statistical package (2009).

RESULTS AND DISCUSSIONS

In the period 2011-2015, a selection of elite plants was conducted in local populations with testing of their offspring for productivity at technical maturity stage of the seeds (Table 1).

Table 1. Shows the results of the distribution of the tested offspring in terms of productivity to a group standard - an average of three standard varieties - Endje, Super sweet and Yantar

Variants	Endje, t/ha	Yantar, t/ha	Super Sweet, t/ha	Distribution of individual progenies towards the group Standard		
				< 90 %	90-110%	110% <
2011	40.69	47.14	43.57	29	64	7
2012	53.04	55.18	55.89	21	56	12
2013	55.36	73.23	56.44	20	69	11
2014	56.90	56.62	55.17	36	56	8
2015	52.00	68.28	64.15	9	48	5

For each selection cycle, plants are selected from the progenies with values above 110% of the standard.

In 2015, the seeds from the five offspring with over 110% of the standard were harvested to form 5 populations - SZT, SZW, SZM, SZA, SZC. They were tested in 2016 and 2017 in comparative trials together with the standard varieties Yantar, Stavropolskaya, Galia, Enje and Super Sweet.

In the phase of technical maturity of the grain, the weight and dry content of the plants and the proportion of stems were measured. The results are shown in Table 2.

Table 2. Results for productivity dry matter content and part of stems of varieties and elite populations of sweet sorghum for 2016-2017

Variants	2016			2017		
	Yield, t/ha	Dry matter, %	Stems part, %	Yield, t/ha	Dry matter, %	Part of the stems, %
Endje	63.7	60.3	74.9	72.4	57.0	87.4
Super Sweet	57.1	59.0	79.6	63.6	53.8	75.4
Yantar	53.6	66.3	78.1	73.8	52.1	84.5
Stavropolska	55.9	65.5	84.5	70.0	52.9	85.8
Galiya	55,2	65,5	84,5	59,3	56,5	89,9
SZT	64.0	62.1	87.3	74.9	55.1	87.4
SZM	55.2	61.8	88.4	67.1	51.7	78.8
SZW	58.6	63.3	85.7	70.5	49.4	73.8
SZA	61.0	64.1	89.5	72.9	58.7	78.5
SZC	51.1	63.2	86.1	68.6	58.8	86.0
Mean	57.3	63.4	81.6	70.5	53.7	82.2
GD 1%	8.72			11.2		
P %	3.16			3.62		

The productivity in 2016 varied from 51.1 to 64 tons per hectare with an average of 57.3 t/ha. For 2017, as a result of the greater ammount of precipitation, the productivity is higher - varying from 63.6 to 74.9 t/ha with an average of 70.5 t/ha. In both years, the SZT population had the highest yield. The dry matter content of the tested variants varied little between years, with the two year averages differing by almost 10% depending on the wetter meteorological conditions in 2017.

The average values for the share of the stems in the total productivity in both years are practically equal, and the values of the tested variants vary slightly from 73.5-89%.

As a result of the preliminary tests, the SZT population was selected as promising for inclusion as a candidate for a new variety. The average results of a three-year competition test for productivity and qualities of the raw material are indicated in Table 3.

The values for the total productivity and that of the stems correspond to the preliminary tests. The Shumensko Sladko variety exceeds Endje and Super Sweet in terms of productivity and is equal to Yantar, Stavropolska and Galia. The share of stems determining the yield of juice for the new variety has been stable over the years of testing and with 52.9 t/ha is falling back only to Stavropolska.

The most important economic parameter for sweet sorghum is the yield of juice pressed from the stems. It strongly depends on the harvesting period and the genotype of the variety. As seed ripening progresses, the dry content of the vegetative mass increases and the amount of sap in the stems decreases. But the later pressed juice has a higher sugar content, which makes it necessary to calculate the most suitable period of mowing. The average results for the yield of pressed juice and the content of soluble substances, determined refractometrically, demonstrate the high qualities of the new variety when compared to the varieties used in Bulgaria.

Table 3. Average results for productivity and raw material qualities of sweet sorghum varieties, mean of 2018-2020

Variant	Parameters			
	Total yield t/ha	Stems yield, t/ha	Pressed juice, l/ha	Dry matter of juice, %
1. Shumensko Sladko - SZT	67.8	52.9	10600	17.2
2. Yantar	66.2	51.2	10200	16.5
3. Endje	60.0	48.5	9570	14.6
4. Super Sweet	52.5	45.6	9200	15.6
5. Stavropolska	75.5	53.6	10800	17.2
6. Galia	67.5	45.5	9200	15.8
GD 1%	6.88	3.67	736	
P %	4.21	3.48	4.18	

In 2021, samples of pressed juice of 6 varieties were analyzed for qualities such as soluble substances, reducing sugars, sucrose, acids, vitamin C and polyphenoles (Table 4). The ratio and balance between them determine the calloric and taste qualities of the juice to obtain sweet syrups or for alcohol fermentation. The ratio and balance between them determine the caloric and taste qualities of the juice for obtaining sweet syrups or for alcohol fermentation.

The higher content of invert sugar or glucose and fructose in Super sweet, Galia and Stavropolskaya makes an impression. For Shumensko Sladko at a high level of total sugars, the ratio of reduced sugars and disaccharide sucrose is at an average level for the varieties tested.

The ratio of sugars and acids determines the taste of the juice and for the new variety the acidity index is balanced. The content of vitamin C and polyphenols enhance the beneficial properties of the pressed juice.

Table 4 also shows the qualities of syrup thickened up to a quarter by evaporation from the initial volume of juice from Shumensko Sladko. The content of polyphenoles increases the most, sucrose three times, vitamin C twice and invert sugar and acids by 50% each.

Table 4. Qualities of pressed juice from the stems of sugar sorghum varieties, 2021

Variety	Dry matter, (Re) %	Inverted sugar, (%)	Sugar, (%)	Acids, (%)	Vit. C, (mg %)	Total Poliphenols, (mg %)
Endje	14.6	11.4	17.58	0.68	12.32	74.39
Yantar	16.5	15.4	11.97	0.80	12.32	57.50
Super sweet	15.0	25.6	5,13	0.68	12.32	78.19
Galia	16.5	27.3	7.13	0.68	14.08	42.54
Stavropolsk a	17.2	35.8	2.00	0.54	10.56	68.61
Shumensko Sladko-	17.2	22.2	10.64	0.68	12.32	51.49
Syrup	61.2	34.8	35.82	0.94	24.64	431.9
LSD (0.05)	2.71	1.42	3.481	0.084	3.688	34.89

The presented results for the productivity and qualities of the raw material from sweet sorghum are comparable with the data from older studies (Stefanov, 1991; Varbanov, Hristova, 1996). and more recent publications from Africa and Asia (Abdalbagi and Mohammed, 2020). Rajvannshi et al. (2006) note that excellent syrup can be made from sweet sorghum when brix of raw juice is greater than 15%. Syrup yelding ranging 800-1900 l/ha have been reported from Mukabane et al (2014). The negative correlation of the yield and the concentration of the pressed juice with the ripening of the seeds raises the question of the time of harvesting the stems. The simultaneous use of the vegetative mass and the seeds after enzymatic hydrolysis would make the use of the new Shumensko Sladko variety highly efficient for the production of raw material for spirit distillation.

CONCLUSIONS

The new Bulgarian sweet sorghum variety Shumensko Sladko does not fall back the varieties used in practice in terms of productivity and qualities of the pressed juice from the stems. With a yield of 50-55 t/ha of stems in the stage of technical maturity of the seeds, 10,000-12,000 liters of pressed juice are obtained per hectare.

With over 17% soluble substances of the juice and with thickening up to a quarter of the initial volume, up to 2500-3000 l/ha of sweet syrup is obtained, which has a balanced content of reduced sugars and sucrose, acids, vitamin C and polyphenols.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

PRELIMINARY STUDIES REGARDING THE POTENTIALLY EFFECT OF EXTRACTS FROM *Citrullus lanatus* PEELS ON SOME CEREAL`S AND FRUIT'S PATHOGENS

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Abstract

The attack of phytopathogens specific to the main cereal types must be limited because it can lead to increasing mycotoxins levels in the plants and grains, above the limit allowed by the legislation in force. In this regard, at the present, there are intense concerns regarding the use of specific product types for plant protection, in favor of natural (green technologies) products, to avoid the use of fungicidal products obtained by chemical synthesis. In this regard, the present study investigated the effect generated in vitro by some dried peel (ethanolic) extracts, obtained from different varieties of Citrullus lanatus, on pathogens specific to Triticum aestivum and Zea mays. The bioproducts obtained from these varieties were tested on the pathogenic microorganisms for cereals such as Fusarium culmorum, Fusarium verticillioides, Fusarium graminearum, and also on the pathogenic microorganisms for fruits such as Penicillium expansum, and/or Penicillium digitatum. The results performed in vitro revealed that the tested bioproducts exhibit local/moderate antifungal activities, recommending them as potential candidates for obtaining natural bioproducts with antifungal activities.

Key words: *Citrullus lanatus peels extracts, antifungal effect, cereals, fruits.*

INTRODUCTION

It is estimated that 25% of plant production globally and about 20% in the EU is contaminated with mycotoxins. Most are produced by fungi of the genus *Fusarium*, *Aspergillus*, *Penicillium,* and *Alternaria* (Kępińska-Pacelik, 2021). *Fusarium* species are among the most dangerous phytopathogenic microorganisms because they contaminate cereal grains with high toxicological potential mycotoxins (Mielniczuk & Skwarylo-Bednarz, 2020). Among the mycotoxins produced by *Fusarium*, deoxynivalenol (DON) - a mycotoxin from the trichothecene group is found most in cereal grains. Its action is manifested at the cellular level, where its presence induces the signal to start the process of programmed cell death for normal cells (Mielniczuk & Skwaryło-Bednarz, 2020). Fumonisins (FB) (produced by *Fusarium verticillioides*) are the most toxic mycotoxins produced by Fusarium strains, being considered neurotoxins, because they affect the nervous tissues and the brain. Zearalenone (ZEA), another toxic compound is produced by *Fusarium culmorum* and *Fusarium graminearum*; it can also be called estrogenic mycotoxin due to its similarity to the natural structure of estrogens. The presence of this compound can cause changes in the production of hormones and respectively in the normal functioning of the reproductive system. The compound named deoxynivalenol (vomitoxin), represents one of the most powerful toxins because it can cause digestive problems, hemotoxicity, leukocytes, or even death. Patulin and penicillic acid, although not considered strong toxins, can cause cytotoxicity, immunotoxicity and even cancer after repeated exposure. Most commonly mycotoxins found in cereals (wheat, corn) are deoxynivalenol, fumonisin B1 and B2, and zearalenone (Otero et al., 2020; Kępińska-Pacelik, 2021; Palou, 2014). Phytopathogenic species of the genus *Penicillium* and other produce patulin and citrinin, mycotoxins with moderate toxicity. They act especially on kidney cells and can cause the destruction of DNA molecules in the brain, liver, and/or kidneys of laboratory animals (Altomare et al., 2021; Radu et al., 2012). The strategy to reduce mycotoxins generated by the presence of phytopathogenic agents consists in the application of different treatments for the protection of plants during vegetation. Protection from the attack of the phytopathogens can be achieved by biological methods (natural pests or bioproducts obtained from microorganisms of the *Trichoderma* type), the use of synthetic fungicides (triazole, metaconazole, tebuconazole, cyproconazole) or the use of plant protection agents obtained from natural sources. From this point of view, polyphenolic compounds are important, which most of the time can be obtained from residues from the food industry - respectively cucubitaceae peels. The family Cucurbitaceae is a large group of plants, with over 800 known species worldwide. Vegetables in this family have been used for centuries, not only for consumption, but also for their medicinal value. The species belonging to this family are rich in carotenoids, terpenoids, saponins, and other phytochemical compounds (Rolnic et al., 2020). These are non-nutritive compounds and occur naturally in plants. There are numerous phytochemical compounds with antimicrobial activities such as polyphenolic compounds, alkaloids, anthocyanins, carotenoids, tannins, monoterpenes, triterpenes, and saponins (Harith et al., 2017; Badr et al., 2015), the main antimicrobial effects reported by specialized literature being presented in Table 1. An important class of triterpenoid compounds is represented by cucurbitacins, which have the role of defense and appear in the peels of different curcubitaceae species, including *Citrullus* sp. (Kim et al., 2018). In vitro research demonstrated the cucurbitacin compounds identified in *Citrullus lanatus* peels (structure presented in Figure 1), have the antitumor effect, and their mechanism of action is based on the activation of the p53 gene (tumor suppressor gene), arrest the cell cycle in the presynthetic stage (G1) and apoptosis (Duangmano et al., 2012; Ge et al., 2018; Mohammed et al., 2019).

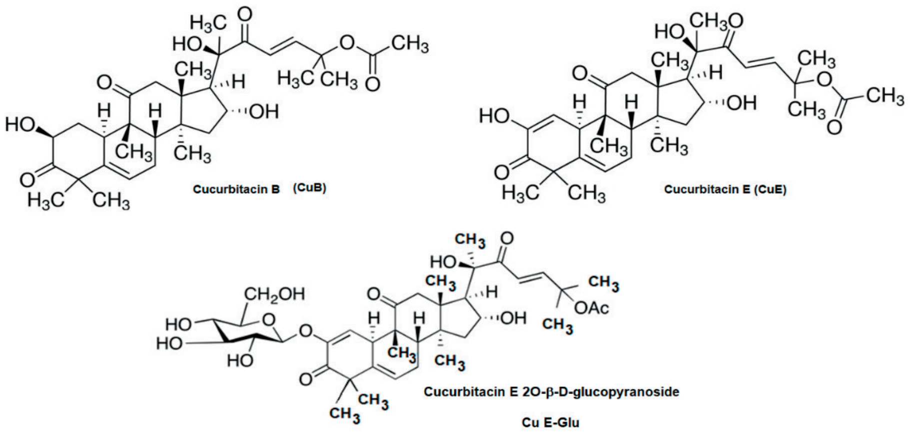

Figure 1. The general structure of compounds from the class of cucurbitacins highlighted in the peels of *Citrullus lanatus*

In recent times, more attention has been paid to the effects that occur after the use of synthetic pesticides, attention due to the associated environmental pollution, and due to the phenomena of resistance to them. The bioproducts with pesticidal effects obtained from natural sources (microorganisms, plants, or animals) are biodegradable and do not produce adverse effects. In organic but also in conventional agriculture, there is a fairly high demand for bioproducts with pesticidal action obtained from natural resources (natural extracts), especially from plants (Lakshmeesha et al., 2019). The mechanisms involved in the antimicrobial effects are not yet known, but studies have shown that the whole extract works better than a single isolated compound (Salehi et al., 2019).

The aim of the work was to test the antifungal effect of some ethanolic extracts obtained from the dried peels of indigenous varieties of *Citrullus lanatus* on *Fusarium culmorum, Fusarium verticillioides, Fusarium graminearum, Penicillium expansum,* and/or *Penicillium digitatum*.

Tabel 1. The antimicrobial and antifungal effect of different extracts of the *Citrullus lanatus* Thunb

Citrullus species	Extract type	Microorganisms	References
Citrullus lanatus (dried peel)	Alcoholic extract (methanol; ethanol)	*S. albus; S. aureus; E. faecalis; P. fluorescens; E. coli; B. subtilis; M. luteus; L.innocua; K. oxytoca; S. enterica; S. sonnei; S. thermophilus; S.typhi; E.coli; K. pneumoniae; P. aeruginosa; B. subtilis; C. albicans; A. niger; P.chrysogenum; T. bepelli.*	Neglo et al., 2021 ; Egbuonu, 2015 ; Mohammed et al., 2020
Citrullus lanatus, Citrullus vulgaris, Citrullus colocynthis (dried peel)	Alcoholic extract (ethanol)	*F.oxysporum; P.chrysogenum; A. niger.* % of inhibition growth (% IG) in the case of *F. oxysporum* can be determined the with relation: *% IG = 22,025 ln(x)-82,283* % of inhibition growth in the case of *A. niger* can be determined with relation: *% IG= 19,845 ln(x)-67,219* *x= extract concentration, µg/mL*	Elonsary et al., 2020; Thirunavukkarasu et al., 2010; Shah et al., 2022

MATERIALS AND METHODS

Sources of polyphenolic compounds

The peels from 3 varieties of *Citrullus lanatus* Thunb (Figure 2 a, b, c) acquired from a local producer in Fundulea, Călărași), were used as the sources of polyphenolic compounds, namely:

- peels from the fruits of *Citrullus lanatus* Tropical variety, a watermelon with dark green peel and yellow pulp (Figure 2 a);
- peels from the fruits of *Citrullus lanatus* Huelva variety, a watermelon with dark green peel and red pulp (Figure 2 b);
- peels from the fruits of *Citrullus lanatus* Lusiana variety, a watermelon with a red core, whose peel shows both dark green and light green stripes (Figure 2 c).

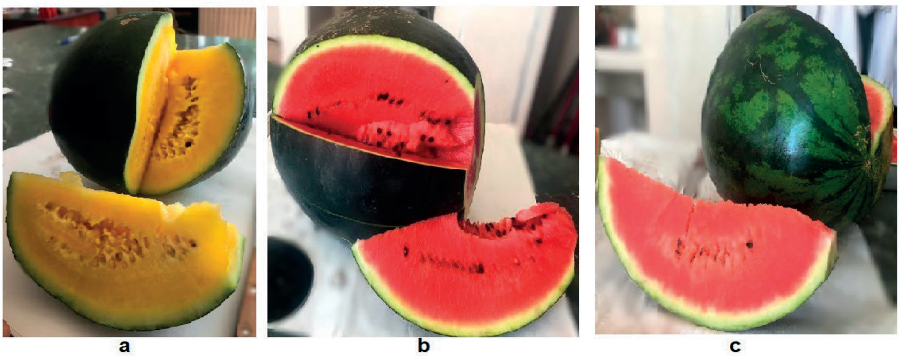

Figure 2. *Citrullus lanatus* varieties used for obtaining the polyphenolic products:
a) Tropical variety; b) Huelva variety; c) Lusiana variety

Strains of phytopathogenic fungi used in *in vitro* studies

The microorganisms used in the in vitro studies were the following: *Penicillium expansum* DSM 62841, *Penicillium digitatum* DSM 2731, *Fusarium verticillioides* DSM 62264, and three aggressive strains of *Fusarium*, isolated from the *Triticum aestivum* plants, respectively *Fusarium culmorum* 46 (a gift from Prof. Thomas Miedaner, University Hohenheim

Germany), *Fusarium graminearum* 96, *Fusarium culmorum* 1056 and *Fusarium culmorum* 1471 (Ittu et al., 2010).
The tested *Fusarium* species are aggressive and were previously characterized through molecular analysis (Cornea et al., 2013), confirming the genus and species of each one, after which were included in the NARDI Fundulea collection.

Obtaining polyphenolic extracts
The peels from three varieties of *Citrullus lanatus* were washed with distilled water, cut into small pieces, and dried at 40°C in a thermostated oven. After drying, the peels were soaked in ethanol 70% (dry plant material:solvent ratio = 1: 5), in dark glass containers, in the dark for 1 month, after which they were filtered and the resulting supernatant was kept in dark color containers. The alcoholic extracts obtained from the peels of the Huelva variety were called CL-100, the alcoholic extract from the Lusiana variety was called CL-101, and the extract obtained from the Tropical variety was named CL-103. All extracts obtained were standardized to 5 mg GAE/ml extract, by dilution with 70% ethanol.

Evaluation of the antifungal effect
Spores from each fungal species were inoculated with a sterile cotton swab on Petri plates with 4 compartments, with PDA (Potato Dextrose Agar) culture medium. After 30 minutes, on the surface of each inoculated Petri plate were put 3 cellulose discs with a diameter of 6 mm, previously soaked in the alcoholic extract. Additionally, were made an inoculation with a spot of 10 microliters of each extract, in a fourth compartment of each Petri plate. As a control sample, a 70% ethanol solution was used.
The effectiveness of the treatment was evaluated after incubation at 24°C for 48 h and measuring the inhibition diameter. The obtained results were presented in the form of the arithmetic mean of three determinations. The evaluation of the antifungal activity of each extract was carried out according to a random scale, established as follows:

- average *inhibition* diameter = 0 mm: extract without antifungal effect;
- 1 mm ≤ average diameter of inhibition ≤ 10 mm: extract with local antifungal effect;
- 10 mm < average diameter of inhibition ≤ 20 mm: extract with moderate antifungal effect
- average diameter of inhibition > 20 cm: extract with significant antifungal effect.

RESULTS AND DISCUSSION

The results obtained after processing the experimental data are presented in figures 3÷6 and reveal the following:

- In the case of CL-100 extract, the best results were obtained for *F. verticillioides* (average diameter of inhibition = 12.67 mm), *P.* expansum (average diameter of inhibition obtained = 11.33 mm), and *F. graminearum* 96 (inhibition diameter obtained = 10.33 mm), the antifungal effect obtained in the case of these micromycetes being a moderate one (Figure 3, Figure 6). For *P. digitatum*, *F. culmorum* 46 and *F. culmorum* 1056 obtained a local antifungal effect, much weaker in the case of the two strains of *F. culmorum* (Figure 3 and the Figures 6 a1, a5, a7).
- The extract CL -101 has a moderate antifungal effect in the case of the following fungal strains: *P. expansum* (inhibition diameter 11.7 mm), *F. graminearum* 96, and *F. verticillioides* (inhibition diameter achieved in the case of the two species of *Fusarium* = 11 mm) (Figure 4, Figure 6 a2, a3, a4). In the case of the other microorganisms studied, the antifungal effect of CL-101 extract is local, except the *F. culmorum* 46, where this extract has no effect.
- The results obtained at exposure of the studied microorganisms at the CL-103 (Figure 5, Figures 6, a6, a8) showed that an inhibition diameter of 15.3 mm is obtained in the case of *P. expansum*, the antifungal effect of the extract on this microorganism being a moderate one. Exposure of *F. graminearum* 96 and *P. digitatum* at the CL-103 has no effect. In the case of the other microorganisms studied, the antifungal effect is local, the diameters of inhibition obtained being less or equal to 10 mm. The results obtained in this study confirm the antifungal effect of the alcoholic extracts of *Citrullus sp*. and are similar to conclusions obtained by Shah and team (Shah and collab., 2022).

• Similar inhibition diameters were obtained for *A. niger* (20 mm) and *F. oxysporum* (13.5 mm), in experiments carried out by El ZAwi et al., by exposing several species of microorganisms to alcoholic extracts of *C. lanatus*. They highlighted a local antifungal activity for *F. oxysporum*, in the case of an alcoholic extract of *C. lanatus* that contained 20 mg crude extract/mL (El Zawai et al., 2015). Other authors (Harith et al., 2018) in their studies regarding the antimicrobial activity of methanolic extracts of *C. lanatus*, found that these extracts inhibit the development of dermatophytes fungi such as *T. mentagrophytes*, the results obtained being better than those obtained with nystatin. Analytical determinations made by Kim and collab. (Kim et al., 2018) using HPLC and LC-MS techniques, with three commercial standards of cucurbitacin (CuB; CuE, CuE-Glu) showed that the alcoholic extracts obtained from dry peels of *C. lanatus* or *C. colocynthis* contain up to 170 µg/g CuE; 50 µg/g CuE-Glu and respectively 7 µg/mL CuB. They found that the cucurbitacins concentration found in *C. colocynthis* is higher than in *C. lanatus*..

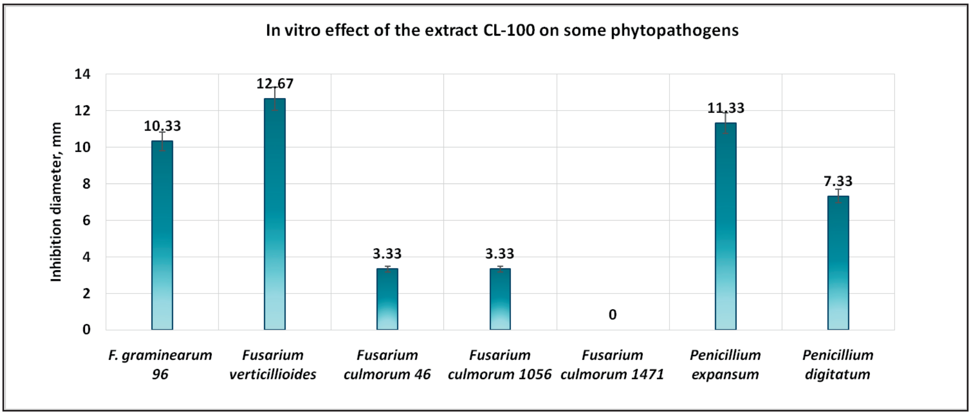

Figure 3. Antifungal effect of CL-100

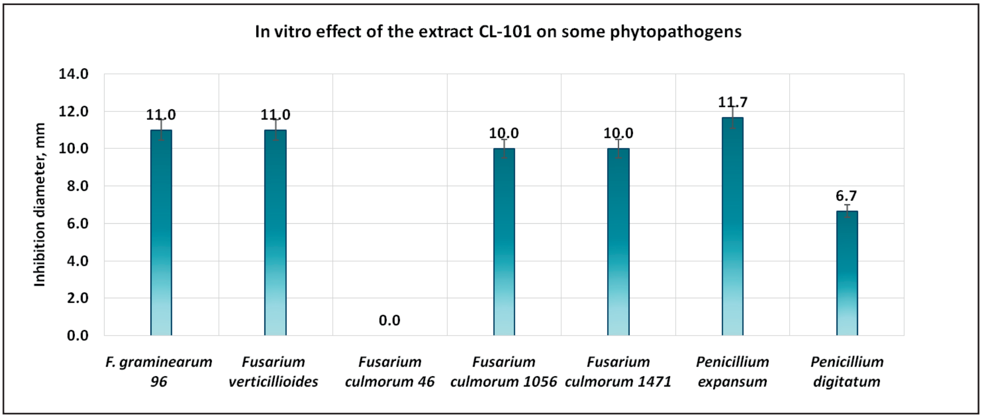

Figure 4. Antifungal effect of CL-101

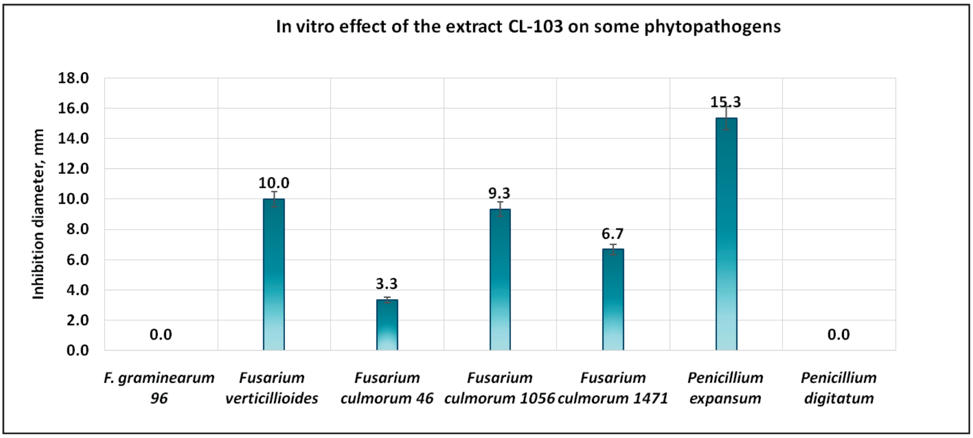

Figure 5. Antifungal effect of CL-103

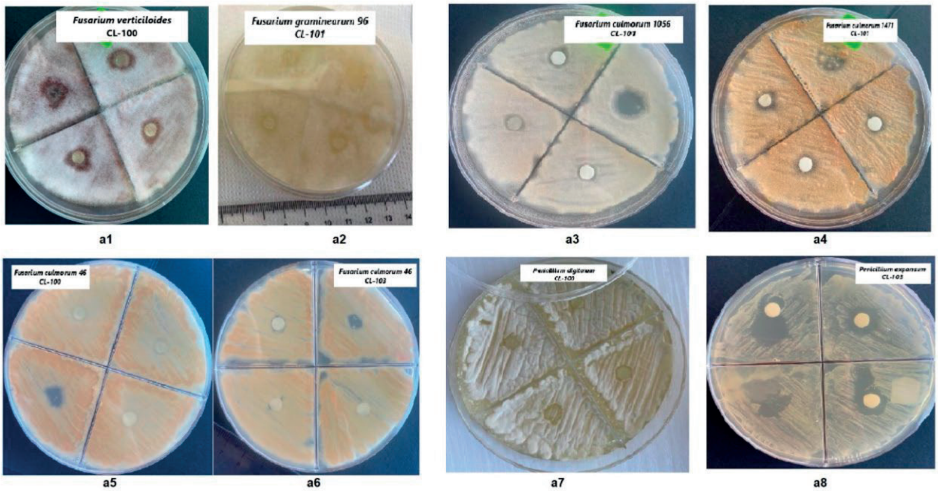

Figure 6. Susceptibility of tested phytopathogens to ethanolic extracts of *C. lanatus*

CONCLUSIONS

Following the carried-out studies, a moderate antifungal effect was highlighted for the peel ethanolic extracts obtained from different indigenous varieties of *Citrullus lanatus*. For the first time, it was highlighted the antifungal effect of *Citrullus lanatus* peel ethanolic extracts on *Fusarium graminearum* type micromycetes and *Fusarium verticiloides*, phytopathogens fungi that cause great damage of cereals production from *Zea mays* and *Triticum aestivum*. In addition, the performed tests highlighted a moderate antifungal effect against *Penicillium expansum*, a phytopathogen that generally causes damage to citrus fruits, and respective of the indigenous fruits of apple, pear or cherry. The results obtained from the in vitro studies have highlighted the antifungal effect of the alcoholic extracts obtained from the biomass of *Citrullus lanatus* (peels), making of this ethanolic peel extract a potential candidate in obtaining new types of natural biopreparations intended the protection of plants grown for cereals and respectively intended for the protection of stored fruits in closed spaces.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

IMPACT OF CLIMATE CHANGE ON MAIZE PRODUCTION IN THE PEDOCLIMATIC CONDITIONS AT ARDS BRAILA

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Abstract

During the agricultural years 2020-2022 at SCDA Braila, experiments with maize hybrids have been placed in which the growth and development processes as well as the productivity of Felix, Iezer and F423 maize hybrids have been followed. In the climatic context of 2020, the growing cycle was staggered over a period of 159 days (10.04.2020-16.09.2020), during which time precipitation totalled 162.4 mm, accumulating a useful temperature of 3254°C. In 2021, the growing cycle was staggered over a period of 147 days (05.05.2021-29.09. 2021), during which period rainfall totalled 335.5 mm, accumulating a useful temperature of 3038.6°C, and under the conditions of this year, 2022, maize sown on 28.04.2022, covered 151 days to reach harvest maturity on 26.09.2022 under the conditions of accumulating a useful temperature required to cover the growth and development phenotypes of 3270°C. Yields recorded in the three crop years for the three hybrids ranged from 7.24 t/ha to 11.66 t/ha for hybrid Felix, from 7.48 t/ha to 9.95 t/ha for hybrid Iezer and from 8.96 t/ha to 11.80 t/ha for hybrid F423.

Key words*: maize, climatic conditions, precipitation, temperature, yields.*

INTRODUCTION

The therm "climate change" is defined differently by various parties, despite the fact that the backdrop is the same. The Intergovernmental Panel on Climate Change (IPCC, 2007) defines climate change as a change in the state of the climate that is identifiable by changes in the mean and/or variability of its attributes and that continues for an extended period, generally decades or longer.

Climate change, as defined by the United Nations Framework Convention on Climate Change (UNFCCC), is a change in climate that is attributed directly or indirectly to human activity that alters the composition of the global atmosphere, in addition to the natural climate variability observed over comparable time periods (IPCC, 2013).

The effects of climate change differ based on a region's level of development. For instance, suggests that rising temperatures and shifting precipitation rates will likely hinder the success of rain-fed agriculture in the majority of emerging nations (IPCC, 2021)

The importance of maize in global agri-food systems, both direct food consumption and indirect feed pathways for animal-sourced commodities, is growing. It is a versatile crop with multiple uses; although predominantly used as feed, it remains a significant food crop and has several non-food uses worldwide. In recent decades, global maize output has increased due to a mix of rising demand, yield gains, and land expansion. The global demand for maize will continue to increase. It is the top cereal in terms of production volume and is projected to become the most extensively cultivated crop in terms of area in the next decade (Erenstein et al., 2022).

In Romania, maize is one of the most significant crops. Around 2.5 million hectares were used to cultivate maize, which remained the most important crop with a share of 47.6% of the area cultivated for cereals and 30% of the overall area cultivated. Between 2011 to

2021, maize yields averaged 5,802 kg/ha. (Ghiorghe & Turek-Ravoveanu, 2022)

Aggressive temperatures in conditions of rainfall deficits during growth phases with maximum water requirements have a significant impact on yield components, regardless of the hybrid chosen, even when using high-performance crop technologies for maize cultivation, (Ion et al., 2013)

The objective of the present paper is to present the results obtained at 3 maize hybrids studied in the field conditions of the ARDS Braila, during three years (2020, 2021 and 2022) and evolution of temperatures and precipitations compared to the multiannual average of the region.

MATERIALS AND METHODS

The study was carried out during the period 2020-2022. The field experiment was located in Agricultural Research and Development Station (ARDS) Braila-Chiscani Experimental Center. Soil was a vermic chernozem with a medium humus content (2.4-3.1%) in the upper horizons and only 1.6% in the transition horizon.

The area where the tests were carried out is in the North Baragan region under the annual average climate for the period 1900-2014 is characterized by the following parameters: annual rainfall (agricultural year) - 445 mm, annual average air temperature - 11°C, potential evapotranspiration (after Thornthwaite) - 715 mm, and climatic deficit water annual average - 272 mm. It forecasts a decline in annual precipitation from 445 mm to 440 mm and an increase in annual average temperatures from 11°C to 11.3°C through 2025. (Visinescu et al., 2014).

The experimental material included three maize Hybrids: Felix, Iezer and F423, developed at the National Institute for Agricultural Research and Development in Fundulea. The experiment was located in the field according to the randomized blocks method, in three replications product after winter wheat predecessor. Soil tillage was done by plowing in autumn at a depth of 25 cm and seedbed preparation was carried out in spring at a depth of 8-10 cm.

The sowing was carried out on 10.04.2020, 05.05.2021 and 28.04.2022, respectively, at a depth of approximately 5-6 cm, at a density of 65,000 germinating grains/ha. The trial has been fertilized by complex fertilizers 200 kg/ha (NPK 18: 46: 0) before sowed and by urea 150 kg/ha, in vegetation. When suitable, pesticides were used to control weeds, diseases, and pests.

Harvesting was carried out in the second decade September 2020, and in the last decade of September 2021 and 2022.

At harvesting in each of the three years, determination and analyses were performed regarding the grain yield, yield per hectare adjusted to 14% moisture, and quality traits (hectoliter weight - kg/hl), thousands of grain weight). The hectoliter mass was determined using the Pfeuffer granulomas. The weight data of seed grains was determined using the ML-C1 automatic seed counter and the electronic balance, and then the average for each hybrid was calculated.

The variability presence in the hybrids was estimated by coefficient of variations (CV).

RESULTS AND DISCUSSIONS

Climatic aspects

The monthly precipitation, average temperatures and multiannual data for 2020, 2021 and 2022 are presented in Table 1.

Temperature and rainfall distribution throughout the growing season is the main climatic elements influencing maize growth and production. Analysis of these characteristics reveals that during the three years of study (2020-2022), monthly average temperatures recorded positive deviations from the multi-year monthly average in most months, and the amount and distribution of precipitation vary between years.

The year 2020 was characterized as an unfavorable year for maize with severe drought stress in April, June and August. Precipitation in the April-September period was 185 mm, the deficit reaching -77 mm compared to the multiannual average. The rainfall deficiency was largely pronounced in May (-30.4 mm), June (-21.1 mm) and August (-35.9 mm). Regarding the thermal regime, between April

and September, the recorded values show that the average monthly temperatures were higher than the multiannual average, with positive deviations ranging from 0.7°C in April to 2.6°C in August and 3°C in September, respectively.
In 2021, the climatic conditions were considered as being favourable for maize crop, during vegetative period, rainfalls of 390 mm were recorded, with a pozitive difference of 128 mm from the multiannual average. The highest rainfall values were recorded in June 173.8 mm. The mean monthly temperatures were over the multiannual average in July (+1.9°C) and August (+1.4°C).
The year 2022 was characterized as an unfavorable year for maize crop with severe drought stress in May, June and July. For the growing period of maize the rainfall was significant less than multiannual average value, deficit reaching -112 mm. The largest positive deviations of the average monthly temperatures as compared to the multiannual average are registered in the months: June (+ 1.8°C), July (+1.9°C) and August (+2.9°C).
Based on the minimum and maximum temperatures recorded daily, the sum of the degrees of temperatures useful for plant development from sowing to harvesting. The sum temperatures for the studied agricultural years was as follows: in the year 2020 3254°C, in the year 2021 3038.6°C and 3270°C in the year 2022, respectively.

Table 1. Monthly and growing season temperature and precipitation at ARDS Braila 2020-2022

Months	Temperature (°C)				Precipitation (mm)			
	2020	2021	2022	Multiannual average	2020	2021	2022	Multiannual average
October	13.2	15.1	10.2	11.5	30.6	26.5	33.1	30
November	10.2	5.7	8.1	5.6	8.7	24.5	27.1	33
December	3.9	4.7	2.5	0.6	14.3	67.7	43.8	36
January	0.9	2.2	1.3	-2.1	3.8	41.2	6.5	28
February	4.6	2.4	4.1	-0.2	28.1	7.4	11.1	27
March	8.7	4.7	3.8	4.7	2.6	31.4	13.8	26
April	11.9	9.4	11.9	11.2	4.6	53.3	25.1	35
May	16.4	16.7	18	16.7	45.8	75.8	24.3	48
June	22	20.2	22.7	20.9	36.9	173.8	33.3	62
July	24.4	24.8	24.8	22.9	54.8	40.4	8.9	46
August	24.6	23.4	24.9	22	3.1	36.7	26.9	39
September	20.3	16.9	17.9	17.3	39.5	10.4	31.8	32
Average/Total	**13.4**	**12.2**	**12.5**	**10.9**	**273**	**589**	**286**	**442**

Source: Meteorological Stations Braila

Grain yield
The studied hybrids have recorded relatively highest yields, the average amount being 9,564 kg/ha in the research period of three years (Table 2) but looking at the average yield of the maize genotypes investigated over the course of three years, there are evident variations. This indicates that the year had a major impact on the grain yield in each of the three years.
Grain yield is one of the complex traits regulated by multiple genotypic and environmental factors interacting with one another. Few yield components are less complex, more heritable, and less responsive to environmental changes (Kashiani & Saleh, 2010).
Of the three years of study, the most favorable for maize was 2021 (11137 kg/ha), followed by 2020 (9650 kg/ha), and the least in 2022 (7907 kg/ha).
In 2020, rainfall during the growing season of maize recorded a deficit of 77 mm, and in 2022, the deficit recorded was 112 mm, which is reflected in the yields obtained. In contrast, in 2021, the rainfall distribution was irregular, but overall it was a favorable year for maize cultivation.

The highest yield in 2020 was achieved by the hybrid F423 (10230 kg/ha), and in 2021 was achieved by the hybrids F423 (11800 kg/ha) and Felix (11660 kg/ha). In the year 2022, the average grain yield was 7907 kg/ha and the highest yield was 8996 kg/ha for the hybrid F423.

The highest yield stability was registered at hybrid Iezer, with a coefficient of variation (CV%) less than 10%, and a respective value of 3.64%, but this hybrid in all three years has achieved small yields.

Table 2. The grain yield (kg/ha) and maize hybrids stability (%)

No.	Genotype	Yield (kg/ha)			Average 2020-2022	C.V. %
		2020	2021	2022		
1	Felix	9360	11660	7240	9420	23.47
2	Iezer	9360	9950	7484	8931	3.64
3	F423	10230	11800	8996	10342	13.59
Average		9650	11137	7907	9564	13.57

Table 3. Thousand seed weight (g) and maize hybrids stability (%)

No.	Genotype	Thousand seed weight (g)			Average 2020-2022	C.V. %
		2020	2021	2022		
1	Felix	210.47	347.10	364.32	307.30	27.43
2	Iezer	252.32	333.40	359.10	314.94	13.14
3	F423	225.82	356.40	342.22	307.15	23.25
Average		229.54	345.63	355.21	309.79	21.27

Table 4. Hectolitre mass (kg/hl) and maize hybrids stability (%)

No.	Genotype	Hectolitre mass (kg/hl)			Average 2020-2022	C.V. %
		2020	2021	2022		
1	Felix	71.55	72.3	66.5	70.11	4.50
2	Iezer	68.55	71.43	65.30	68.43	2.11
3	F423	70.05	74.20	66.90	70.38	5.20
Average		70.05	72.64	66.23	69.64	3.93

Thousand seed weight (TSW)

Thousand seed weight is based on the characteristics of the hybrid, the growing conditions, the presence of limiting factors, and the moisture level of the grain.

The average TSW for the research period (2020, 2021, and 2022) was 309.79 g (Table 3), but there were no significant differences between the three hybrids.

The highest TSW values were recorded in 2022 (average 355.21 g) as follows: Felix (364.32g), Iezer (359.10 g), and F423 (342.22g). The lowest results were recorded in 2020, with an average of 229.54 g.

For this trait, genotype Iezer had the best stability (coefficient of variation was 13.14%) and the hybrid Felix registeres the highest variability (coefficient of variation of 27.43%).

Hectolitre mass

The average hectolitre mass for the researching period (2020-2022) have been 69.64 kg/hl (Table 4). On average, significantly higher values were recorded for hybrid F423 (70.38 kg/hl) and hybrid Felix (70.11 kg/hl).

The highest average value of the hectolitre mass was registered in 2021, with 72.64 kg/hl and the lowest in 2022, with 66.23 kg/hl, these results are influenced by the hydroclimatic conditions of the study years.

Lower values of the variation coefficient for the average hectolitre mass ranged from 2.11% (Iezer) to 5.23% (F423), emphasizing the excellent stability of this characteristic.

CONCLUSIONS

The average yield for all examined hybrids was 9564 kg/ha, the average thousand seed weight was 309.79 g and the average hectolitre mass was 69.64 kg/hl.

On average during the period of the study (2020-2022), the highest grain yield was obtained from F423 - 10342 kg/ha, followed by Felix - 9420 kg/ha and the lowest - from Iezer - 8931 kg/ha. The highest weight of thousand seed weight was obtained from Iezer- 314.94 g and hectolitre mass was registered for F423 hybrid- 70.38 kg/hl.

Thus, following the study, it can be appreciated that the three maize hybrids are suitable for the area of influence of the ARDS Braila, with the F423 hybrid for high yields and the Iezer hybrid for stability under different climatic conditions in the three years of cultivation.

Despite 2021 being a favorable year for maize crop in the area where the field experiment was conducted, the studied maize hybrids responded differently to growing conditions. In order for maize growers in Romania to achieve high and profitable yields under drought conditions, it is imperative that they select the correct maize hybrid based on the region's specific growing, hydroclimatic conditions and technological conditions.

ACKNOWLEDGEMENTS

This research was financed by the Agricultural Research Development Station Braila.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EFFECTS OF FOLIAR FERTILISATION IN THE PRODUCTION OF HYBRID SEED MAIZE

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Abstract

Maize is one of the most widely cultivated crops across the world due to its high importance in animal feeding, human nutrition, and many industrial processes. Fertilization is a key component of any crop production system and it is one of the technological elements where improvement is always searched. Foliar fertilization is an effective way to treat nutrient deficiency, to improve plant nutrition status and to help plants surpass stress periods. Foliar biostimulators are important for giving the possibility to stimulate plant metabolism and enhance plant protection mechanisms. Starting from these ideas, the aim of this paper is to present the results of the research focused on studying the effect of different combinations of classic macro & micro nutrient foliar fertilizers (Basfoliar 36 Extra, Foliar Extra, Vertex Hi-N 34, Maize Extra, Manzinc SC), or of one classic foliar fertilizer combined with one algae based biostimulator (Seamaxx SL) under different base fertilization conditions in irrigated hybrid seed maize production. The research has been conducted for 3 years in irrigated field plots in the pedo-climatic conditions of NE Romania. The obtained results brought to attention the positive effects of foliar fertilizers in hybrid seed production: increased plant height, better foliage development, better tassel development in terms of size and longer pollen shedding time, and finally a yield enhancement.

Key words: *maize, hybrid seed, nutrients, foliar fertilizer, biostimulator.*

INTRODUCTION

Maize is the second most cultivated crop across the world since 2007, when for the first time it exceeded rice. FAO data shows that global maize area exceeded for the first time 200 M ha in 2021 (it being of 205.87 M ha), approaching wheat, which was cultivated on 220.75 M ha (FAOSTAT). Its ecological plasticity and high investment in research makes it a crop which is increasingly important.

To sustain such a wide cultivated area of maize, it is necessary a wide range of seed, especially hybrid seed. The seed quality is the most important and primary factor that impacts the success of maize growth (Bahtiar et al., 2022). Technologically hybrid seed maize production is a challenge for many farmers and only the most experienced and well prepared ones are able to multiply it. Hybrid seed maize field are often at the peak of technological and financial investment and such there is a continuous battle in order to increase yield by any possible means.

Better management practices and seed development programs together have significantly improved maize grain yield during the last century (Ray et al., 2020). High maize yield is possible by providing sufficient water and nutrients during the short growing stage (Koca, 2016). Fertilization is a key component of any crop production system and it is one of the technological elements where improvement is always searched.

Excepting classical synthetic granulated fertilizers, a product increasingly pushed and recommended is foliar fertilizer. Foliar fertilizers have been used since 1980's, at the beginning in horticulture, and nowadays more and more in field crops such as maize (Floratine, 2023).

The soil and foliar application are the main methods of nutrient application but the application of macronutrients is effective only when soil application is provided, while the best method for the micronutrient application is through foliar application (Kashyap et al., 2022).

The foliar fertilization improves the amount of the yield, one being unanimously considered that it is stimulating and corrective for mineral nutrition (Jakab-Gábor, 2017). The content of macro and micronutrients in foliar fertilizers cannot become a substitute for nutrition provided through classical base fertilization, but they can be an enhancer of this fertilization or it can help plants surpass stress or blockages (Racz, 2021).

Even though they require a very small amount of micronutrients, plants cannot grow without them. When micronutrients become a limiting factor, then water, fertilizer and other high-energy production inputs may be wasted, since a plant will only grow and develop to the extent that its most limiting growth factor will allow (Mengel, n.a.).

The availability of micronutrients is limited by the characteristics of the soil, as well as the agricultural practices, therefore an additional supply for plants is required (Luță et al., 2022). Studies proved that micronutrients deficiencies can be overcome through the use of foliar fertilizers (Oldham, 2019). In some situations, foliar-applied micronutrients are more readily available to the plant than soil-applied micronutrients, but foliar applications do not provide continuous nutrition as do soil applications (Vitosh et al., 2006).

The soil parent material and soil formation process over time along with the effects of soil moisture, aeration, and temperature can significantly impact the amount of plant-available micronutrients (Mallarino et al., 2014). Also, some soil conditions such as high soil pH and low organic matter may contribute to decrease the supply of micronutrients to crops (Mueller & Ruiz Diaz, 2011). The total concentration of a micronutrient in the soil is usually a poor indicator of its availability to the maize plant (Johnston & Dowbenko, 2004). Several blockages or immobilisation of these elements can occur in the soil which makes them impossible for absorption through the root system. This imposes for maize production, especially for sensitive inbred lines used in seed production, the development of a management system which needs to take into account conditions that impact microelement availability, most common of these being: soil pH, temperature, interaction between nutrients.

The aim of this paper is to present the results of the research focused on studying the effect of different combinations of classic macro&micro nutrient foliar fertilizers (Basfoliar 34 Extra, Foliar Extra, Vertex Hi-N 34, Maize Extra, Manzinc SC), or of one classic foliar fertilizer combined with one algae based biostimulator (Seamaxx SL) under different base fertilization conditions in irrigated hybrid seed maize production.

MATHERIALS AND METHODS

Field experiments were conducted in 2020, 2021 and 2022 on a chernozem soil in the north-eastern part of Romania, referenced exactly in the pedo-climatic conditions of the Moldavian Plain near the Prut River (NE Romania: 47.5188° N, 27.4490° E) in Bivolari commune, Iasi County.

The experimental variants were based on 4 different foliar fertilization programmes with different macro and micronutrient content applied on the basis of the classical fertilization scheme used by the farmer and quality checked by the parent company that created the inbred lines.

The field experiments were organised using the method of subdivided plots with 4 replications being of type 2 x 5 with the following experimental factors (Table 1):

- Factor A - base fertilization, with 2 graduations:
  - a1 = fertilization only at seedbed preparation;
  - a2 = fertilization at seedbed preparation + microgranulated starter + nitrogen application in the growth stage of the maize plants BBCH 16.
- Factor B - foliar fertilization, with 5 graduations:
  - b0 = no foliar fertilization;
  - b1 = Basfoliar 36 Extra (2 l/ha) + Basfoliar 36 Extra (2 l/ha);
  - b2 = Manzinc SC (0.7 l/ha) + Seamaxx SL (1.5 l/ha);
  - b3 = Maize Extra (1 l/ha) + Vertex Hi-N 34 (3 l/ha);
  - b4 = Foliar Extra (2 l/ha) + Seamaxx SL (1 l/ha).

The experimental variant a1 consisted of fertilization only at seedbed preparation with the following fertilizer rates: 100 kg/ha of ammonium nitrate applied in the autumn of the preceding year; 300 kg/ha of complex fertiliser 14: 14: 14 + 7SO_3 + 4MgO applied at seedbed preparation; 150 kg/ha of complex fertiliser 20: 20: 0 + 0.5 Zn applied at seedbed preparation. These fertilisers assured the following rates of nutrients: 105.5 kg/ha N; 72 kg/ha P_2O_5; 42 kg/ha K_2O; 21 kg/ha SO_3; 12 kg/ha MgO; 0.75 kg/ha Zn.

The experimental variant a2 consisted of fertilization at seedbed preparation as in the case of variant a1, but having an extra microgranulated starter Physiostart applied in a rate of 20 kg/ha during sowing and having an application of ammonium nitrate in the growth stage BBCH 16 in a rate of 200 kg/ha. All these fertilisers assured the following rates of nutrients: 174.1 kg/ha N; 77.6 kg/ha P_2O_5; 42 kg/ha K_2O; 25.6 kg/ha SO_3; 12 kg/ha MgO; 2.8 kg CaO; 1.15 kg/ha Zn.

Physiostart is a microgranulated fertilizer used as a starter, this containing: 8% ammonium nitrogen; 28% P_2O_5; 14% CaO; 23% SO_3; 2% Zn.

If the experimental variant a1 assures good nutritional conditions for maize plants, then the variant a2 was intended to assure very good nutritional conditions especially concerning the nitrogen nutrition, the aim being to see the effect of different foliar fertilizers under different combinations under these good and very good base fertilisation conditions.

The foliar fertilizers combinations used in the field experiments were applied separately in a 2 pass program: one at the critical phase of 3 fully unfolded leaves (BBCH 13) where ear initiation starts and any deficiency can lead to limited production potential (Nielsen, 2007) and one pass before flowering (before detasseling), the intention being to obtain better development of the tassel of the male maize plants to ensure better pollination of the female plants. Each foliar fertilizer combination used contains macro and microelements in order to ensure a balanced fertilization.

Table 1. Presentation of the experimental factors

Experimental variants	Fertilizer products	Fertilizer application period	Fertilizer rate (kg or l/ha)	Nutrient rate (kg/ha)						
				N	P_2O_5	K_2O	SO_3	MgO	CaO	Zn
a1b0 Control 1 (C1)	Ammonium nitrate 14:14:14+7SO_3+4MgO 20:20:0+0.5Zn	Autumn (preceding year) Seedbed preparation Seedbed preparation	100 300 150	33.5 42 30	0 42 30	0 42 0	0 21 0	0 12 0	0 0 0	0 0 0.75
	Total Control 1			*105.5*	*72*	*42*	*21*	*12*	*0*	*0.75*
a1b1	C1 + Basfoliar + Basfoliar	BBCH-13 & BBCH-51	2+2	1.08	0	0	0	0.12	0	0.0004
	Total C1 + Basfoliar + Basfoliar			*106.9*	*72*	*42*	*21*	*12.12*	*0*	*0.7504*
a1b2	C1 + Manzinc + Seamaxx	BBCH-13 & BBCH-51	0.7+1.5	0.06	0.03	0.05	0	0	0	0.25
	Total C1 + Manzinc + Seamaxx			*105.56*	*72.03*	*42.05*	*21*	*12*	*0*	*1.0*
a1b3	C1 + Maize Extra + Vertex	BBCH-13 & BBCH-51	1+3	0.98	0.35	0.05	0	0.11	0	0.09
	Total C1 + Maize Extra + Vertex			*106.48*	*72.35*	*42.05*	*21*	*12.11*	*0*	*0.84*
a1b4	C1 + Foliar Extra + Seamaxx	BBCH-13 & BBCH-51	2+1	0.28	0.18	0.10	0	0.04	0	0.00
	Total C1+Foliar Extra+Seamaxx			*105.78*	*72.18*	*42.10*	*21*	*12.04*	*0*	*0.75*
a2b0 Control 2 (C2)	Ammonium nitrate 14:14:14+7SO_3+4MgO 20:20:0+0.5Zn Physiostart Ammonium nitrate	Autumn (preceding year) Seedbed preparation Seedbed preparation Sowing BBCH 16	100 300 150 20 200	33.5 42 30 1.6 67	0 42 30 5.6 0	0 42 0 0 0	0 21 0 4.6 0	0 12 0 0 0	0 0 0 2.8 0	0 0 0.75 0.4 0
	Total Control 2			*174.1*	*77.6*	*42*	*25.6*	*12*	*2.8*	*1.15*
a2b1	C2 + Basfoliar + Basfoliar	BBCH-13 & BBCH-51	2+2	1.08	0	0	0	0.12	0	0.0004
	Total C2 + Basfoliar +Basfoliar			*175.18*	*77.6*	*42.0*	*25.6*	*12.12*	*2.8*	*1.1504*
a2b2	C2 + Manzinc + Seamaxx	BBCH-13 & BBCH-51	0.7+1.5	0.06	0.03	0.05	0	0	0	0.25
	Total C2+ Manzinc + Seamaxx			*174.16*	*77.63*	*42.05*	*25.6*	*12*	*2.8*	*1.40*
a2b3	C2 + Maize Extra + Vertex	BBCH-13 & BBCH-51	1+3	0.98	0.35	0.05	0	0.11	0	0.09
	Total C2+ Maize Extra + Vertex			*175.08*	*77.95*	*42.05*	*25.6*	*12.11*	*2.8*	*1.24*
a2b4	C2 + Foliar Extra + Seamaxx	BBCH-13 & BBCH-51	2+1	0.28	0.18	0.10	0	0.04	0	0
	Total C2+ Foliar Extra + Seamaxx			*174.38*	*77.78*	*42.10*	*25.6*	*12.04*	*2.8*	*1.15*

Basfoliar 36 Extra is a complex foliar fertilizer which contains: 27.0% nitrogen of which: 3.6% ammonium nitrogen, 4.7% nitric nitrogen, and 18.7% ureic nitrogen; 3.0% MgO; 0.02% B; 0.2% Cu; 0.02% Fe; 1.0% Mn; 0.005% Mo, 0.01% Zn.

Seamaxx SL is a foliar fertilizer enhanced with a biostimulating extract from algae *Ascophyllum nodosum* (200 g/l) which contains: 38 g/l N; 17.5 g/l P_2O_5; 30 g/l K_2O; 0.2 g/l Mn; 0.1 g/l Fe; 0.1 g/l Zn; 36.4 mg/l Cu; 142 mg/l B; 8.7 mg/l Mo.

Manzinc SC is a dedicated foliar fertilizer targeted on Manganese and Zinc deficiencies which are most common in maize crop production, this containing 250 g/l Mn and 352 g/l Zn.

Maize Extra is a foliar fertilizer which contains: 35% P_2O_5; 5% K_20; 8.6% Zn.

Vertex Hi-N 34 is a high nitrogen content foliar fertilizer designed to be a booster especially during intense growth period starting from 8 leaves to tasselling (BBCH-18 onwards), this containing: 328 g/l nitrogen out of which 68 g/l ammonium nitrogen, 99 g/l nitric nitrogen, and 161 g/l ureic nitrogen; 35 g/l MgO; 9 g/l Mn; 5 g/l Cu.

Foliar Extra is a foliar fertilizer which can be used on most types of crops, this containing: 120 g/l nitrogen, out of which 17 g/l nitric nitrogen and 103 g/l ureic nitrogen; 80 g/l P_2O_5; 36 g/l K_2O; 22 g/l MgO; 2.1 g/l Mn.

The field experiments were performed each year under irrigation conditions aiming to assure optimum water supply of maize plants of the inbred lines of hybrid P9889, a FAO 350 hybrid with stay green technology which is well adapted for the climate and soil conditions found in the Moldavian plain. In each experimental year there were applied 5 irrigation passes of 30 mm at the following growth stages: 3-4 leaves, 8 leaves, 12 leaves, after detasselling and at grain fill.

The planting instructions were provided by the parent seed company, respectively a parity of female and male rows of 6:2 with 60 cm between rows. The sowing was performed firstly for the 6 female rows and one row of male (male 1) and then for the second male row (male 2) after 33 GDD (Growing Degree Days) in order to ensure a better flowering time nicking and a longer pollen coverage time. The 6: 2 ratios of female to male rows are less complicated and less costly to handle with conventional planters and harvesters (Wright, 1980).

Each experimental variant had 192 m^2 in size which consisted of 8 rows of plants (6 female rows and 2 male rows) with 60 cm between rows resulting in a width of 4.8 m and 40 m of length.

The sowing distance between seeds per row was of 18.3 cm, which helps ensure high female crop density characteristic of seed production fields.

From a climatic point of view, 2020 has been a warm and dry year, with an average temperature of 12.5ºC which was one of the highest registered ever for the area where the experiments were located, and with 479.9 mm of total rainfall unevenly distributed: 1.8 mm in April and 5.8 mm in August, but an impressive 132 mm in May and 90.4 mm in June which helped in the initial phases of vegetation but late drought seriously impacted yields.

The year 2021 can be characterised as being a favourable one for maize crop, with an average temperature of 10.6ºC and 564.6 mm rainfall among which 390.3 mm in April-August.

The year 2022 is characterised by average temperatures of 11.8°C. Precipitations were the least recorded in history for the area where the field experiments were located with 399.7 mm, the first time under 400 mm and far away from averages of 510 mm for the area. At the same time the dispersion of precipitations was the least favourable for maize production as total precipitation for the period May-July was 85 mm (Weatherspark, 2022).

The evaluation of the foliar fertilizer effects on the maize plants aimed the following:

- Plant height at detasseling (cm);
- Number of leaves per plant at detasseling;
- The flowering time gap between male inbred and the female inbred (days);
- Pollen shedding time (days);
- Tassel length (cm);
- Number of grains per cob;
- Hybrid seed yield (kg/ha).

For statistical analysis of the primary data there was used ANOVA analysis as well as linear regression in order to determine the relationships between studied traits. At the same time the specific tests such as Fisher's test, Tukey's test and Dunnett's were also used to evaluate the studied variants compared to each other and to the 2 control variants.

RESULTS AND DISCUSSIONS

The obtained results highlight that there is a low direct correlation between foliar fertilizer application and final yield as R^2 is 0.195 (Table 2).

Table 2. R squared value of regression

Goodness of fit statistics (Average yield):	
Observations	40
Sum of weights	40
DF	30
R^2	0.195

Besides this one can observe the plus of yield for foliar fertilized variants which for seed crops especially have high economic value: 564 kg/ha increase in seed yield, averaged over 3 years, for Foliar Extra + Seamaxx SL variant (Table 3). This shows us a consistent capacity even under different weather conditions for foliar fertilizers to generate superior yields. By looking at the yield distribution of the variants one can see that there are several ones that vastly outperform average yield of the control variants (Figure 1).

By testing variants between each other to see if there are significant difference regarding yield using the Tukey test one can say that using foliar fertilizers did not decrease yield, but also did not increase it significantly (Table 4). Similar formulations of the treatment plan mean that it is expected that there will not be big differences in yield between them but increases compared to control variants.

The Dunnett test confirmed that foliar fertilizer does not represent a determining factor in final yield obtained compared to the control variants, even though quantitative increases which increase profitability can be observed especially for variants with higher manganese content combined with biostimulator such as Foliar Extra + Seamaxx SL (Table 5).

Observations made at key phases of phenotypical development have shown that there is a consistent increase in plant height for variants with foliar fertilization compared to control by 1 to 20 cm in difference (Table 6). Application of biostimulators before tassel aparition (BBCH-51) stage such as *Ascophyllum nodosum* from Seamaxx has determined the highest difference in plant height at detasseling (20 cm).

Regarding the foliar development, one can observe that foliar fertilizer treatments have supported a better development of the foliar apparatus with peak performance identified for variants Maize Extra + Vertex and Foliar Extra + Seamaxx SL which on average they had one extra leaf at detaselling time (Table 6).

Table 3. Hybrid seed yield results

Experimental variants	Fertilizer products	Hybrid seed yield (kg/ha)				Difference to Control 1	Difference to Control 2
		2020	2021	2022	Average		
Control 1 (C1)	Fertilization at seedbed preparation	8405	8745	8625	8592	Control	-
Control 2 (C2)	Fertilization at seedbed preparation + microgranulated starter + nitrogen application at BBCH 16	9163	9465	9393	9340	749	Control
a1b1	C1 + Basfoliar + Basfoliar	9013	9305	9232	9183	592	-157
a1b2	C1 + Manzinc + Seamaxx	9063	9330	9292	9228	637	-112
a1b3	C1 + Maize Extra + Vertex	8963	9204	9182	9116	525	-224
a1b4	C1 + Foliar Extra + Seamaxx	9275	9610	9505	9463	872	123
a2b1	C2 + Basfoliar + Basfoliar	9075	9280	9295	9217	625	-124
a2b2	C2 + Manzinc + Seamaxx	9463	10070	9692	9742	1150	401
a2b3	C2 + Maize Extra + Vertex	9550	10105	9770	9808	1217	468
a2b4	C2 + Foliar Extra + Seamaxx	9625	10234	9855	9905	1313	564
Average		*9159.5*	*9534.8*	*9384.1*	*9359.4*	-	-

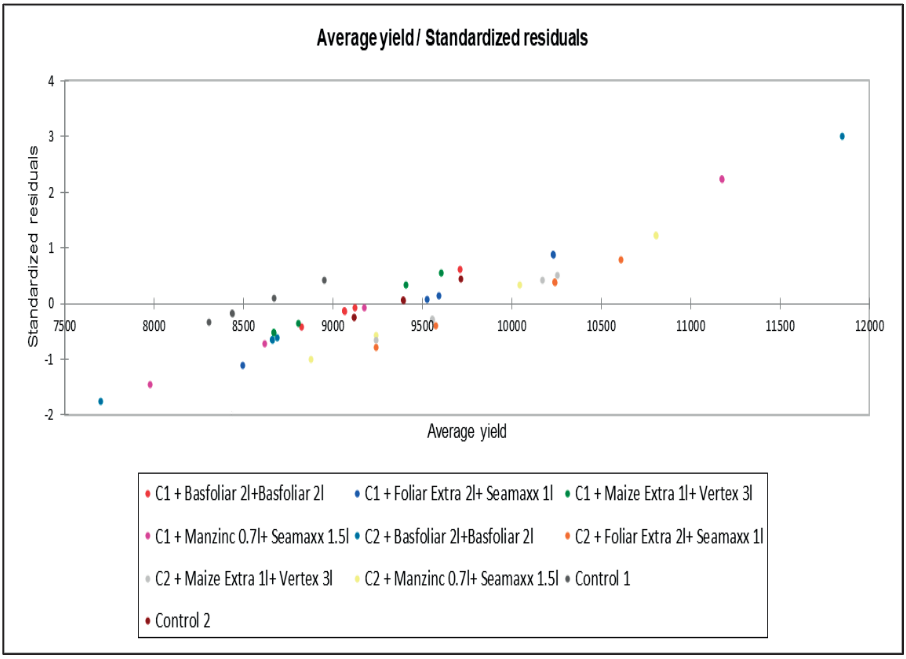

Figure 1. Hybrid seed yield distribution of variants

Table 4. Tukey Test groupings

Category	LS Means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
C2 + Foliar Extra + Seamaxx	9918.333	435.176	9028.585	10807.081	A
C2 + Maize Extra + Vertex	9806.667	435.176	8917.919	10695.415	A
C2 + Manzinc + Seamaxx	9743.333	435.176	8854.585	10632.081	A
C1 + Foliar Extra + Seamaxx	9462.500	435.176	8573.752	10351.248	A
Control 2	9336.667	435.176	8447.919	10225.415	A
C1 + Manzinc + Seamaxx	9237.500	435.176	8348.752	10126.248	A
C2 + Basfoliar + Basfoliar	9226.667	435.176	8337.919	10115.415	A
C1 + Basfoliar + Basfoliar	9182.500	435.176	8293.752	10071.248	A
C1 + Maize Extra + Vertex	9123.333	435.176	8234.585	10012.081	A
Control 1	8593.333	435.176	7704.585	9482.081	A

Table 5. Dunnett Test significance results

Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control 2 vs C2 + Foliar Extra + Seamaxx	-581.667	-0.945	2.856	1757.447	0.925	No
Control 2 vs C2 + Maize Extra + Vertex	-470.000	-0.764	2.856	1757.447	0.977	No
Control 2 vs C2 + Manzinc + Seamaxx	-406.667	-0.661	2.856	1757.447	0.991	No
Control 2 vs C1 + Foliar Extra + Seamaxx	-125.833	-0.204	2.856	1757.447	1.000	No
Control 2 vs Control 1	743.333	1.208	2.856	1757.447	0.786	No
Control 2 vs C1 + Maize Extra + Vertex	213.333	0.347	2.856	1757.447	1.000	No
Control 2 vs C1 + Basfoliar + Basfoliar	154.167	0.251	2.856	1757.447	1.000	No
Control 2 vs C2 + Basfoliar + Basfoliar	110.000	0.179	2.856	1757.447	1.000	No
Control 2 vs C1 + Manzinc + Seamaxx	99.167	0.161	2.856	1757.447	1.000	No

Table 6. Results regarding plant height and foliar development

Experimental variants	Fertilizer products	Plant height at detasseling (cm)				Number of leaves at detasseling			
		2020	2021	2022	Average	2020	2021	2022	Average
Control 1 (C1)	Fertilization at seedbed preparation	181	188	176	182	15	16	14	15
Control 2 (C2)	Fertilization at seedbed preparation + microgranulated starter + nitrogen application at BBCH 16	182	190	177	183	15	16	14	15
a1b1	C1 + Basfoliar + Basfoliar	184	192	177	184	15	16	15	15
a1b2	C1 + Manzinc + Seamaxx	186	194	178	186	15	17	14	15
a1b3	C1 + Maize Extra + Vertex	186	195	178	186	16	17	15	16
a1b4	C1 + Foliar Extra + Seamaxx	189	197	179	188	16	17	14	16
a2b1	C2 + Basfoliar + Basfoliar	187	195	180	187	16	17	15	16
a2b2	C2 + Manzinc + Seamaxx	196	203	189	196	16	18	15	16
a2b3	C2 + Maize Extra + Vertex	198	206	190	198	17	18	16	17
a2b4	C2 + Foliar Extra + Seamaxx	203	210	192	202	17	18	16	17
Average		*189.2*	*197.0*	*181.6*	*189.2*	*15.8*	*17.0*	*14.8*	*15.8*

A parameter that is constantly monitored, this being critical for obtaining good pollination time synchronization between male and female inbreds is the flowering gap between the two. The obtained results showed that foliar fertilization had no negative impact on the flowering gap between male tassel and female stigmata appearing out of the husks. Average flowering gap remained constant, respectively 3-4 days which is consistent with maize's propensity to protandrous flowering. Moreover, one can observe a decrease of flowering gap for the variants Maize Extra + Vertex and Foliar Extra + Seamaxx SL on a better nitrogen supply through base fertilisation (Table 7).

Regarding the length of the tassel one founds that foliar fertilized variants, especially ones with biostimulants and with high nitrogen content applied at BBCH 51 show a better development in terms of length of the tassel which has a potential positive effect on pollination (Table 7).

An important parameter at flowering time is total pollen shedding time by male anthers of the tassel. One can observe that throughout the 3 years of study, foliar fertilized variants, especially ones with biostimulants (Seamaxx) on a base fertilisation at seedbed preparation and that with Basfoliar at a base fertilisation with higher nitrogen supply have led to increased pollen shedding with 1-2 extra days of pollination (Table 8). All these developments in terms of generative elements of the plant have a positive correlation with the final number of grains per cob which again is higher for variants with biostimulator and with high manganese content such as Maize Extra + Vertex and Foliar Extra + Seamaxx SL (Table 8).

Thus, even though the results of the statistical analysis performed showed weak direct correlations regarding yield for each type of foliar fertilizers, one can see that for studied elements especially regarding tassel length, pollen shedding time, number of grains per cob, variants with foliar fertilization containing manganese combined with biostimulator have better results compared to the control variants. One can say that a better development of the tassel due to application of micronutrients such as manganese combined with biostimulator has led to a better pattern of behaviour of the plant at flowering time and better pollination results.

The performed research resulted in un efficiently highlighting and observing all the qualitative and quantitative components that have an impact on plant development and generative growth such as plant height, foliar development, flowering gap between male and female inbreds, tassel length, pollen shedding and number of grains per cob and showing that in certain situations foliar fertilization can have a positive effect

Table 7. Results regarding flowering gap and tassel length

Experimental variants	Fertilizer products	Flowering gape (days)				Tassel length (cm)			
		2020	2021	2022	Average	2020	2021	2022	Average
Control 1 (C1)	Fertilization at seedbed preparation	4	3	4	4	20	22	20	21
Control 2 (C2)	Fertilization at seedbed preparation + microgranulated starter + nitrogen application at BBCH 16	4	4	4	4	21	22	20	21
a1b1	C1 + Basfoliar + Basfoliar	4	4	5	4	21	23	20	21
a1b2	C1 + Manzinc + Seamaxx	4	4	5	4	21	24	20	22
a1b3	C1 + Maize Extra + Vertex	4	4	5	4	21	24	20	22
a1b4	C1 + Foliar Extra + Seamaxx	4	4	5	4	21	24	20	22
a2b1	C2 + Basfoliar + Basfoliar	4	4	5	4	21	23	21	22
a2b2	C2 + Manzinc + Seamaxx	4	3	4	4	25	24	21	23
a2b3	C2 + Maize Extra + Vertex	3	3	4	3	23	25	22	23
a2b4	C2 + Foliar Extra + Seamaxx	3	3	4	3	23	25	22	23
Average		*3.8*	*3.6*	*4.5*	*3.8*	*21.7*	*23.6*	*20.6*	*22.0*

Table 8. Results regarding pollen shedding time and number of grains per cob

Experimental variants	Fertilizer products	Pollen shedding time (days)				Number of grains per cob			
		2020	2021	2022	Average	2020	2021	2022	Average
Control 1 (C1)	Fertilization at seedbed preparation	5	4	5	5	240	272	240	256
Control 2 (C2)	Fertilization at seedbed preparation + microgranulated starter + nitrogen application at BBCH 16	6	5	6	6	256	288	240	256
a1b1	C1 + Basfoliar + Basfoliar	5	5	6	5	256	288	240	224
a1b2	C1 + Manzinc + Seamaxx	7	7	6	7	256	304	240	272
a1b3	C1 + Maize Extra + Vertex	6	6	5	6	256	304	240	272
a1b4	C1 + Foliar Extra + Seamaxx	7	7	6	7	256	304	240	272
a2b1	C2 + Basfoliar + Basfoliar	7	8	6	7	256	288	256	272
a2b2	C2 + Manzinc + Seamaxx	6	5	5	5	272	304	256	272
a2b3	C2 + Maize Extra + Vertex	6	7	6	6	288	320	272	288
a2b4	C2 + Foliar Extra + Seamaxx	6	5	7	6	288	320	272	288
Average		*6.1*	*5.9*	*5.8*	*6.0*	*262.4*	*299.2*	*249*	*270.4*

CONCLUSIONS

The results of performed research show that even though foliar fertilization is not a substitute to classical soil applied fertilization there are several enhancements. Plant height at detasseling was larger for foliar fertilized variants compared to control ones and there was a better foliar development through foliar fertilisation.

Foliar fertilizers containing biostimulator (extract from algae *Ascophyllum nodosum*) and with manganese content applied before flowering resulted in better tassel development in terms of size and the same time a longer pollen shedding time due to better development of the tassel.

The obtained results showed that foliar fertilization had no negative impact on the flowering gap between male tassel and female stigmata appearing out of the husks.

The best foliar fertilizer program in terms of general plant development, generative element development and economical yield enhancement has been the one with Foliar Extra in a rate of 2 l/ha applied at 3 leaves stage (BBCH 31) and Seamaxx SL in a rate of 1 l/ha applied before flowering, respectively before detaselling (BBCH 51) under both base fertilization conditions assuring good and very good nutrition conditions for the maize plants.

AKNOWLEDGEMENTS

This research paper has been supported by the Doctoral School of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. We would like to extend acknowledgement of their work to Mr. Liviu Zbant, PhD agronomist engineer, Mr. Liviu Nicorici, PhD student and agronomist engineer, and Mr. Alexandru Baghiu, agronomist engineer, for all their support in organising and co-supervising the field experience.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON THE INFLUENCE OF PROCAINE TREATMENT ON POTATO PRODUCTION AND QUALITY

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Abstract

The response of agricultural crops to the application of biostimulators depends on the climatic conditions, the type of soil and cultivated variety. While knowing the role of biostimulators on the growth and development of crops, as well as on quantitative and qualitative increases in the scientific field, the aim of this research is to follow the influence of procaine treatments on the level of production and quality of two potato varieties (Armonia and Gared), depending on planting density (55 X 10^3 and 70 X 10^3 tubers/ha). The tubers were treated with procaine by immersion, for two hours, prior to planting, in a 10 mg/l solution. The research was carried out in the period 2019-2022, in the pedoclimatic conditions of the Almaj Depression, on a semi carbonate eutric alluvial type soil, located in the meadow area, with loamy-sandy texture, slightly alkaline soil reaction, pH 7.67, and a medium humus content (2.72%). The researched area has a moderate continental temperate climate, with Mediterranean and oceanic influences, which give a special nuance to the depression, with multiannual average of atmospheric precipitation varying between 670-750 mm. Fertilization of the crop was achieved by incorporating under ploughing 25 t/ha of manure and 600 kg/ha of 15:15:15 complex fertilizers. The results obtained highlight the positive effect of the treatment with procaine on the tubers before planting, on average on the two tested varieties and the two densities, expressed in production increases of 7%.

Key words: *procaine, potato variety, crop planting density.*

INTRODUCTION

Due to its nutritious properties and tastiness, potatoes are used as a basic food in the diet of many peoples (Bende, 1991).

To meet the increasing demand for food products due to the rapid growth of the population, there is a need for sustainable, ecologically safe agricultural systems that can only be achieved through the application of innovative technological solutions (Marshall, 2015; Clark & Tilman, 2017; Jones, 2018; Gonçalves, 2021).

Among these innovative solutions, a common practice is the use of biostimulants, in order to stimulate physiological processes, the absorption of nutritional elements and increase resistance to water stress (Gu et al., 2014; du Jardin, 2015; Craggs, 2017).

The studies carried out so far present the beneficial effects of stimulating the tubers before planting depending on the climatic conditions, the type of soil, the cultivated variety (Cachita and Ardelean, 1996; Berindei 1995; 1996; 1998). Procaine treatments were initiated based on the favorable effect reported by Cachita and Ardelean (1996).

Thus, in the experiments carried out by Cachita and her team by treating the tubers for two hours (by immersion) prior to planting with procaine 10 mg/l, a 13% yield increase was obtained. The same authors mention that in Tisem, Satu Mare County, by treating the tubers with procaine 10 mg/l, the plants grew in height by approx. 12 cm, and the production increased by 10.7% when unsprouted potatoes were used for planting and by 31.3% when sprouted tubers were used for planting.

In Braşov, by bathing the tubers with 10 mg/l, bathing for two hours, they determined a stimulation of plant growth, namely the number of stems and their height increased by 14%, the surface of the leaves increased by 4%, and the number of large tubers was increased by 8.46%.

MATERIALS AND METHODS

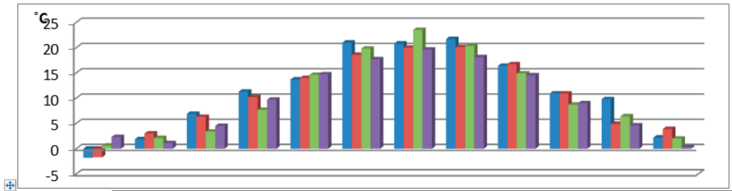
The research was carried out in the period 2019-2022, in the pedoclimatic conditions of the Almaj Depression, on a semicarbonate eutric alluvial soil, located in the meadow area, with loamy-sandy texture, slightly alkaline soil reaction, pH 7.67, and the humus content is medium (2.72%).

The researched area, according to the data recorded at the Bozovici Meteorological Station, belongs to the temperate continental climate, with Mediterranean and oceanic influences, which give a special nuance to the depression, the multiannual average of atmospheric precipitation varying between 670-750 mm (Figures 1 and 2).

In order to follow the effect of the treatment with procaine, a trifactorial experiment was organized, according to the method of subdivided plots with three repetitions, with the following gradations of the factors:

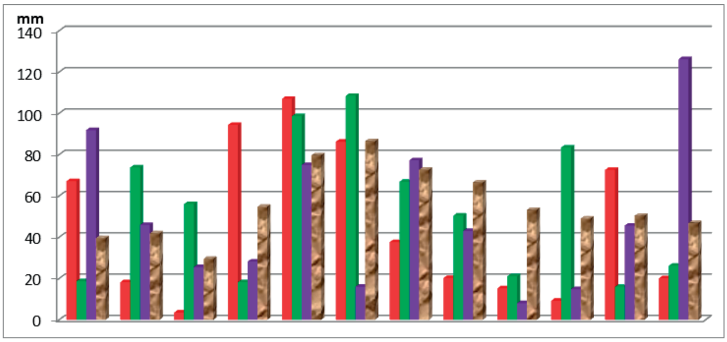
- factor A - the cultivated variety, with two grades: of1 - Harmony; of2 - Garage;
- factor B - treatment of tubers with procaine: b1 - untreated; b2 - treaty;
- factor C - plant density: c1 - 55000 tubers/ha; c2 - 70000 tubers/ha.

The biological material used in the experiment was the varieties Armonia and Gared from the group of late varieties.



	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2019	-1,8	1,9	6,9	11,3	13,7	21,0	20,8	21,7	16,4	10,9	9,8	2,2
2020	-1,7	3,0	6,3	10,2	14,0	18,6	20,0	20,1	16,7	10,9	4,9	3,9
2021	0,6	2,1	3,4	7,7	14,6	19,8	23,5	20,3	14,9	8,7	6,4	2,0
Temperaturi multianuale	2,3	1,1	4,5	9,7	14,7	17,7	19,6	18,1	14,5	9,0	4,6	0,3

Figure 1. Monthly average temperatures recorded at Bozovici meteorological station in the period 2019-2021, compared with multiannual values



	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*
2019	*67,1*	*18,2*	*3,5*	*94,4*	*107,0*	*86,2*	*37,6*	*20,2*	*15,2*	*9,2*	*72,6*	*20,1*
2020	*18,6*	*73,7*	*56,0*	*18,2*	*98,6*	*108,4*	*66,8*	*50,4*	*21,0*	*83,4*	*15,9*	*26,1*
2021	*91,8*	*45,9*	*25,5*	*28,2*	*75,0*	*16,0*	*77,2*	*43,0*	*8,0*	*14,8*	*45,6*	*126,2*
Temperaturi multianuale	*39,5*	*41,8*	*29,5*	*54,7*	*79,6*	*86,5*	*72,7*	*66,5*	*53,2*	*49,0*	*50,3*	*46,8*

Figure 2. Monthly precipitation recorded at Bozovici meteorological station in the period 2019-2021, compared with multiannual values

Soil work consisted of autumn plowing at a depth of 28-30 cm. Land preparation in the spring consisted of a disc harrow work and a combine work before planting. Planting was done in the last decade of March. The tubers were treated with procaine by immersion, for two hours, prior to planting, in a 10 mg/l solution. Fertilization of the crop was achieved by incorporating under the basic plowing 25 t/ha of manure and 600 kg/ha of complex fertilizers of the 15:15:15 type.

Herbicide was carried out with Dual Gold 960 EC 1 (S-metolaclor 960 g/l) l/ha + Sencor 600 SC (Metribuzin 600 g/l), 1 l/ha. The vegetation was treated against diseases and pests. Harvesting was done at the time the tubers matured, when the pods were dry, the tubers were easily detached from the stolons, and the peel was suberified.

RESULTS AND DISCUSSIONS

Table 1. shows the harvests obtained in the 2019-2022 experimental cycle.

It is found that on the set of factors b and c, respectively the treatment of the tubers and the planting density of the GARED variety, an 11% higher yield was obtained than the yield recorded with the ARMONIA variety. The yield difference of 4,465 kg/ha was statistically assured as distinctly significant.

The treatment with procaine applied to the tubers, on average for the two varieties and the two densities, increased the yield by 7%. The yield difference of 2,705 kg/ha was statistically assured as very significant.

The increase in yield inthe variants treated with procaine is lower than that reported in other research carried out in different experiences in the country. The explanation can be found in the fact that they worked with other varieties and in other pedoclimatic conditions, compared to those in which carried out the respective research, which entitles us to the conclusion that the varieties react differently to this treatment.

By increasing the density from 55,000 to 70,000 tubers per hectare, an average increase of 3% was obtained, respectively a distinctly significant difference in yield of 1367 kg/ha.

In the Armonia variety, at both planting densities (55,000 and respectively 70,000 tubers/ha), the treatment with procaine determined yield increases around 3000 kg/ha.

In the GARED variety, at a density of 55,000 tubers/ha in the version treated with procaine, yield increased by over 3,000 kg/ha, whereas at a density of 70,000 tubers/ha, the increase in yield in the treated version was only 1,000 kg/ha.

Table 2 shows the starch content. On average for the experimental factors, in the Gared variety the starch content was higher than the content recorded in the Armonia variety by 1.1%.

Table 1. Summary of production results

Factor A Variety	Factor B Treatment of tubers	Factor C – Planting density		Average factor A (varieties)			
		55000	70000	Crop (t/ha)	%	Difference (kg/ha)	Significance
Harmony	B1- Untreated	35621	40253	39560	100		
	B2 - Treated	39176	43191				
Garage	B1- Untreated	43162	42721	44025	111	4465	20th
	B2 - Treatedt	46479	43741				

DL 5% = 723 kg/ha; DL 1% = 1685 kg/ha; DL 0.1% = 4953 kg/ha

Average factor B Average factor C

Specification	50000	70000
Crop (kg/ha)	41109	42476
%	100	103
Difference (kg/ha)		1367
Significance		20th

Specification	B1- Untreated	B2 - Treated
Crop (kg/ha)	40439	43146
%	100	107
Difference (kg/ha)		2705
Significance		XXX

DL 5% = 575 kg/ha; DL 1% = 10210 kg/ha; DL 0.1% = 1723 kg/ha. DL 5% = 452 kg/ha; DL 1% = 763 kg/ha; DL 0.1% = 1121 kg/ha

Table 2. Starch content

Variety	Tubers not treated with procaine		Tubers treated with procaine		Average varieties
	55000	70000	55000	70000	
Harmony	15.2	15.3	14.7	14.9	15.02
Garage	16.45	16.4	16.05	15.95	16.01
Average of planting treatments and densities	15.82	15.85	15.37	15.42	
Influence of procaine treatment	15.83		15.39		

Increasing the density from 50,000 to 70,000 tubers per hectare did not differentiate the variants in terms of starch content.

Treating tubers with procaine led to a slight decrease in starch content, on average over the two experimental years, the two varieties and the two densities, by 0.44%.

In both varieties, starch production was higher in the variants with a density of 70,000 tubers/ha, compared to 55,000 plants/ha, a more obvious fact in the Armonia variety where the difference was 321 kg/ha, compared to the Gared variety, where the difference was only 101 kg/ha.

In the variants treated with procaine, starch production was 253 kg/ha higher, compared to the variant in which the tubers were not treated, before planting.

In conclusion, the highest amounts of starch per hectare are obtained in the Gared variety, when tubers treated with procaine are used for planting. The increase in density from 55,000 to 70,000 tubers/ha favorably influenced starch production, more evident in the Armonia variety, as a result of the increase in tuber production.

Table 3 shows starch production according to the factors studied. The production of starch in the Gared variety was superior to the production recorded in the Armonia variety by 1,182 kg/ha. This fact is explained both by the higher tuber production and by the higher starch content in the Gared variety. In both varieties, starch production was higher in the variants with a density of 70,000 tubers/ha, compared to 55,000 plants/ha, a more obvious fact in the Armonia variety where the difference was 321 kg/ha, compared to the Gared variety, where the difference was only 101 kg/ha.

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Table 4 shows the protein content of the two studied varieties (Armonia and Gared), depending on the planting density and Armonia, the protein content was higher than that determined in the Gared variety by 0.28%.

The density did not influence the protein content in the Armonia variety, but it was slightly higher at the density of 55,000, compared to the one of 70,000, in the Gared variety. Treating the tubers with procaine before planting favorably influenced the protein content by 0.2%.

Table 3. Starch production depending on variety, density and treatment with procaine

Variety	not treated with procaine		Tubers treated with procaine		Average varieties
	55000 tubers per hectare	70000 tubers per hectare	55000 tubers per hectare	70000 tubers per hectare	
Harmony	5258	5999	5609	6283	5787
Garage	6932	6834	7294	6816	6969
of planting treatments and densities	6095	6416	6451	6553	
Influence of procaine treatment	6255		6508		

Table 4. Protein content (%)

Variety	not treated with procaine		Tubers treated with procaine		Average varieties
	55000 tubers per hectare	70000 tubers per hectare	55000 tubers per hectare	70000 tubers per hectare	
Harmony	2.2	2.1	2.45	2.25	2.25
Garage	1.85	1.9	2.15	2.0	1.97
of planting treatments and densities	2.02	2.0	2,3	2.12	
Influence of procaine treatment	2.01		2.21		

The protein production is presented in Table 5. It is found that protein production was influenced by both protein content and tuber production.

Among the varieties, Armonia stands out, with protein production of 857 kg/ha, compared to 839 kg/ha recorded for the Gared variety.

By increasing the density from 55,000 to 70,000 tubers per hectare, protein production increased in tubers not treated with procaine from 769 kg/ha to 807 kg/ha. In the tubers treated with procaine before planting, the amount of protein was higher in the variants with the density of 55,000 tubers/ha. On average over the experimental cycle, the two varieties and the two planting densities, the amount of protein per hectare was higher in the variants in which the tubers used for planting were treated with procaine, by 120 kg/ha.

In conclusion, it can be found that the protein percentage and protein production was influenced by the variety, the annual climatic conditions and the procaine treatment performed on the tuber.

Table 5. Protein production by density and procaine tratment

Variety	not treated with procaine		Tubers treated with procaine		Average varieties
	55000 tubers per hectare	70000 tubers per hectare	55000 tubers per hectare	70000 tubers per hectare	
Harmony	759	823	934	915	857
Garage	779	792	931	854	839
of planting treatments and densities	769	807	932	884	
Influence of procaine treatment	788		908		

CONCLUSIONS

The research carried out in the 2019-2022 experimental cycle in the Bozovici Depression basin regarding the influence of planting density and procaine treatments carried out on the tubers on the harvest and its quality, in the Armonia and Gared potato varieties, revealed important conclusions from the point of view scientific and practical.

1. On the set of experimental factors, the production recorded in the Gared variety was higher by 11%, i.e. by over 4,000 kg/ha, compared to the one recorded in the Armonia variety.
2. Treating the tubers with procaine before planting increased the yield by 7% on average for the two varieties and two planting densities, returning a yield difference of over 2,700 kg/ha.
3. Increasing the density from 55,000 to 70,000 tubers/ha increased the yield of the Armonia variety by 11%.

In the Gared variety, by increasing the density from 55,000 to 70,000 tubers/ha, production decreased by 4%.

4. The starch content was higher in the Gared variety (16.21%), compared to the Armonia variety (15.02%).

5. Increasing the planting density from 55,000 to 70,000 tubers/ha did not obviously influence the starch content.
6. Treating tubers with procaine led to a slight decrease in starch content, on average over the two experimental years, the two densities and the two varieties by 0.44%,
7. The amount of starch per hectare was influenced by both the starch content and the tuber harvest. The highest amounts of starch/ha were obtained in the Gared variety, when tubers treated with procaine were used for planting. Increasing the density from 55,000 to 70,000 tubers/ha favorably influenced the production of starch in the more obvious variety Armonia as a result of the increase in the production of tubers.
8. The protein content was higher in the Armonia variety than that determined in the Gared variety, by 0.28%.
9. Treating the tubers with procaine, before planting, favorably influenced the protein content by 0.2%.
10. Protein production was influenced by both protein content and protein production.
11. Among the varieties, the Armonia variety stood out, with a protein production of 857 kg/ha, compared to 839 kg/ha for the Gared variety.
12. On average over the experimental cycle, the two varieties and the two planting densities, the amount of protein/ha was higher by 120 kg/ha in the variants in which the tubers used for planting were treated with procaine.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ANALYSIS OF THE AGRONOMIC TRAITS OF 15 MAIZE HYBRIDS CULTIVATED IN THE WESTERN PART OF ROMANIA

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Abstract

This paper presents the analysis of 15 cultivated maize hybrids for further selection of the hybrids that present a high yield potential and favourable agronomical traits, in the context of actual climate change. The analysis and interpretation of the obtained results was performed with the help of the IBM Spss statistical software. Descriptive characteristics were calculated: average, median, standard deviation, minimum values and maximum values. In order to analyse the significant differences, the ANOVA and Duncan statistical test was applied, respectively Kruskal Wallis and Mann-Whitney. For the study of the links between the variables, the Pearson correlation coefficients and the regression lines were determined. The results are indicating the existence of significant differences between the analysed parameters Also, the results are corelated to the meteorological data, registered during the corresponding development phase of the plants.

Key words*: maize hybrids, agronomic characteristics, climate change.*

INTRODUCTION

The climate changes of recent years require seed producers to change their strategies to ensure that their maize hybrids can withstand a changing environment (Haș, 2000; Sarca, 2004; Șuteu et al., 2013 In this regard, genetic selection plays a very important role. Seed producers use genetic selection to develop hybrids that are more tolerant to high temperatures, droughts, floods, or diseases. (Duvick, 1984; Nagy, 2004) In this way, more resistant hybrids to climate change are obtained. Another approach is the development of specially adapted hybrids (Grecu and Has, 2001). Seed producers develop hybrids specifically adapted to certain geographic regions or climatic conditions to achieve better yields (Has et al., 1999; Nagy, 2004; Musteață, 2005). Increasing genetic diversity in the maize breeding process can help reduce the risks associated with climate change, such as drought or the emergence of new diseases (Tătaru, 1974; Tătaru, 1978).

Additionally, advanced agronomic technologies, including irrigation and soil fertility systems, can help increase the productivity of maize hybrids in drier or less rainy environments (Roman et al., 1973; Troyer 1999; Troyer et al., 2000). Adopting better agronomic practices can also help conserve soil and reduce nutrient loss. The use of early detection technologies, such as satellites and sensors mounted on drones, can detect temperature and humidity changes more quickly, allowing producers to make faster decisions and adjust their agronomic practices. In general, efforts to adapt maize hybrids to the current climate context are ongoing and aim to obtain more resistant and adaptable hybrids to climate change to ensure food security and the continuity of agricultural production in the future

MATERIALS AND METHODS

The biological material subjected to research consisted of 15 maize hybrids grown in the western region of Romania. Testing was carried out at Lovrin Agricultural Research and Development Station, on a cambic chernozem soil, with a shallow water table, moist, with weak salinization below 100 cm, moderate alkalinization, slightly decarbonated, on sandy loam with a parent rock composed of sand and with the water table at a depth of 2-5 m. The experience was a comparative culture type, and production results were interpreted relative to the field average. Statistical analysis of the data was performed using the IBM Spss statistical software. Descriptive characteristics such as mean, median, standard deviation, minimum and maximum values were calculated. For the analysis of significant differences, the ANOVA and Duncan test, as well as the Kruskal-Wallis and Mann-Whitney tests were applied. Pearson correlation coefficients and regression lines were determined to study the relationships between variables.

RESULTS AND DISCUSSIONS

The observation of the different hybrid traits was made in the filed and in the laboratory, post-harvest. Leaf colour, position and total number, anthers and stigma colour, cob position and plant height variation were the characteristics that were taken under observations

Leaf colour - The colour of the leaves is light green for 5 (33.3%) hybrids: Lv101, Lv102, Lv103, Lv104, Lv106, Lv107 and dark green for the remaining 10 hybrids (66.67%).

Leaf position - The insertion angle (leaf position) is erect for 8 (53.33%) of the hybrids and semi-erect for 7 (46.67%) of the hybrids (Lv101, Lv102, Lv103, Lv104, Lv106, Kerala, Replik).

Anthers colour (tassel) - The colour of the anthers is white-yellowish for 7 (46.67%) hybrids (Lv101, Lv102, Lv104, Lv107, HSLvOana, LG 31377, 3520R) rose for 7 (46.67%) hybrids (Lv103, Lv105, Lv106, Multipel, CERA 320, Kerala, Replik) respectively yellow-rose for 1 hybrid, namely P0217.

Stigma colour (silk) - The colour of the stigmas is yellow for 7 (46.67%) hybrids (Lv101, Lv102, HSLvOana, LG 31377, 3520R), rose for 4 (26.67%) hybrids (Lv103, Lv104, Lv105, Lv106) and yellow-rose for the remaining 4 (26.67%) hybrids.

Cob position (degrees) - The position of the cob was at angles of 25 (2.22%), 30 (6.67%), 35 (37.78%), 40 (2.22%) and 45 (51.11%) degrees, the minim angle being registered in the measuring of the hybrid Lv 107

Total number of leaves, Number of leaves up to the main cob - The total number of leaves varies between 9 and 15 leaves per cob, with a mean value of 13.4 leaves, respectively the number of leaves to the main cob varies between 6 and 9 leaves, with a mean value of 7.55 leaves.

Between the number of leaves to the main cob and the total number of leaves one can observe a significant direct correlation ($R=0.6$, $p=0.000$).

Plant height (m) - Regarding the total plant height, this varied between 1.60 m for the hybrid Lv102 and a maximum of 2.5 m for the hybrid Lv103, and the mean height was 1.986 m (Figure 1).

Applying ANOVA, one observes that there are significant differences between the studied hybrids regarding the total plant height ($F=68.092$, $p=0.000$). The hybrids Lv103, LG 31377, Multipel, Kerala and Po217 are those which presented significant differences in positive sense with respect to the mean of the field (Table 1).

The data regarding the insertion height of the cob show that this has varied between0.50m for the hybrids Lv101, Lv102, Lv104, lv106, Lv107 and 1.10 m for the hybrids HSLvOana and LG 31377 with a mean of the field of 0.7709 m (Figure 2).

The hybrids differ significantly from the point of view of the variable Main cob insertion height ($F=35.570$, $p=0.000$). One observes that the hybrids Lv103, HSLvOana, LG 31377 and 3520R, Multipel, are those which have presented significant differences in positive sense with respect to the mean of the field (Table 2).

Table 1. Numerical data associated to plant height

	Mean	Std. Deviation	95% Confidence Interval for Mean		Min	Max	The difference between the averages and the average of the field	P	Semnif.
			Lower Bound	Upper Bound					
Media campului	1.9860	0.16882	1.9706	2.0013	1.60	2.50	-	-	-
Lv101	1.8160	0.12770	1.7683	1.8637	1.62	2.10	-0.17000	0.000	000
Lv102	1.8000	0.08280	1.7691	1.8309	1.60	2.00	-0.18600	0.000	000
Lv103	2.1203	0.16420	2.0590	2.1816	1.80	2.50	0.13433	0.000	***
Lv104	1.8200	0.08741	1.7874	1.8526	1.68	2.00	-0.16600	0.000	000
Lv105	1.9817	0.10554	1.9423	2.0211	1.80	2.20	-0.00433	0.883	Ns
Lv106	1.9173	0.11588	1.8741	1.9606	1.70	2.10	-0.06867	0.020	0
Lv107	1.9470	0.10103	1.9093	1.9847	1.70	2.12	-0.03900	0.185	Ns
HSLvOana	1.9867	0.08563	1.9547	2.0186	1.83	2.19	0.00067	0.982	Ns
LG 31377	2.3217	0.06259	2.2983	2.3450	2.20	2.45	0.33567	0.000	***
3520R	1.9690	0.05671	1.9478	1.9902	1.85	2.09	-0.01700	0.563	Ns
Multipel	2.1353	0.07664	2.1067	2.1640	2.02	2.30	0.14933	0.000	***
CERA 320	1.8083	0.07456	1.7805	1.8362	1.69	1.98	-0.17767	0.000	000
Kerala	2.0697	0.06881	2.0440	2.0954	1.92	2.20	0.08367	0.005	**
Replik	1.9780	0.07014	1.9518	2.0042	1.84	2.12	-0.00800	0.785	Ns
Po217	2.1187	0.10699	2.0787	2.1586	1.96	2.34	0.13267	0.000	***

Ns= Not significant. * The mean difference positive and is significant at the 0.05 level. ** The mean difference positive and is significant at the 0.01 level. *** The mean difference positive and is significant at the 0.001 level. 0 The mean difference negative and is significant at the 0.05 level. 00 The mean difference negative and is significant at the 0.01 level. 000 The mean difference negative and is significant at the 0.001 level.

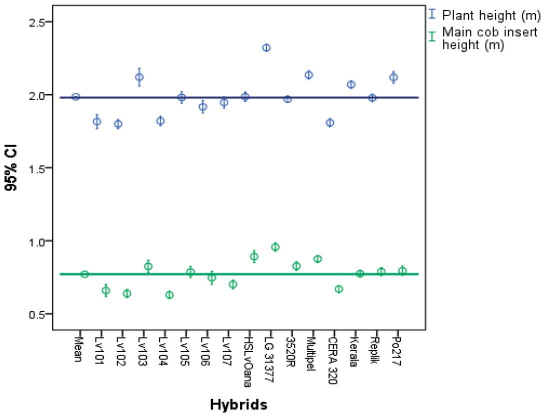

Figure 1. Means and confidence intervals for the means of the variables Total plant height, Main Cob insertion height vs mean of the field

Table 2. Numerical data associated to cob insertion height

	Mean	Std. Deviation	95% Confidence Interval for Mean		Min	Max	The difference between the averages and the average of the field	p	Semnif
			Lower Bound	Upper Bound					
The average of the field	0.7709	0.12575	0.7595	0.7824	0.5	1.1			
Lv101	0.6593	0.11117	0.6178	0.7008	0.5	0.92	-0.1116	0.000	000
Lv102	0.6383	0.07502	0.6103	0.6663	0.5	0.8	-0.1326	0.000	000
Lv103	0.8247	0.11082	0.7833	0.866	0.6	1.05	0.0538	0.045	*
Lv104	0.629	0.0664	0.6042	0.6538	0.5	0.79	-0.1419	0.000	000
Lv105	0.7863	0.1045	0.7473	0.8254	0.6	1.05	0.0154	0.564	Ns
Lv106	0.7467	0.12027	0.7018	0.7916	0.5	1	-0.0242	0.365	Ns
Lv107	0.7017	0.0797	0.6719	0.7314	0.5	0.9	-0.0692	0.010	0
HSLvOana	0.8917	0.11008	0.8506	0.9328	0.6	1.1	0.1208	0.000	***
LG 31377	0.957	0.07702	0.9282	0.9858	0.8	1.1	0.1861	0.000	***
3520R	0.8267	0.0804	0.7966	0.8567	0.6	0.94	0.0558	0.038	**
Multipel	0.8753	0.06163	0.8523	0.8983	0.75	1	0.1044	0.000	***
CERA 320	0.6693	0.06258	0.646	0.6927	0.55	0.8	-0.1016	0.000	0
Kerala	0.7747	0.06437	0.7506	0.7987	0.7	0.9	0.0038	0.888	Ns
Replik	0.7887	0.07328	0.7613	0.816	0.6	0.9	0.0178	0.507	Ns
Po217	0.7943	0.0878	0.7615	0.8271	0.6	1	0.0234	0.381	Ns

Ns= Not significant. * The mean difference positive and is significant at the 0.05 level. ** The mean difference positive and is significant at the 0.01 level. *** The mean difference positive and is significant at the 0.001 level. 0 The mean difference negative and is significant at the 0.05 level. 00 The mean difference negative and is significant at the 0.01 level. 000 The mean difference negative and is significant at the 0.001 level.

The leaf length of the main cob has registered values between 50 cm for the hybrid Lv107and 86 cm for the hybrid LG 31377, the maxim of the means of the lengths of the leaves being attained for LG 31377 (Figure 2).
The analysis of the length of the leaf of the main cob shows that there are significant differences regarding the analysed hybrids (F=23.530, p=0.000) and the mean of the field, the hybrids LG 31377, Multipel, Replik, Po217 being those which differ significantly in positive sense with respect to the mean of the field (Table 3).

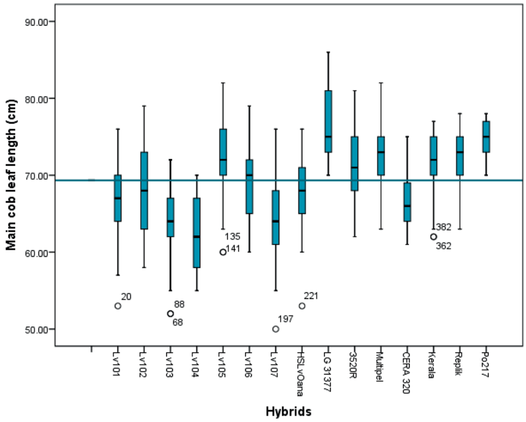

Figure 2. Box-plot diagram associated with the characteristic length of leaf of the main cob vs. mean of the field

Table 3. Numerical data associated to cob insertion height

	Mean	Std. Deviation	95% Confidence Interval for Mean		Min	Max	The difference between the averages and the average of the field	p	Semnif.
			Lower Bound	Upper Bound					
The average of the field	69.3333	6.08789	68.7791	69.8875	50.00	86.00			
Lv101	66.6667	5.68321	64.5445	68.7888	53.00	76.00	-2.6666	0.064	Ns
Lv102	68.5000	5.64923	66.3905	70.6095	58.00	79.00	-0.8333	0.561	Ns
Lv103	63.7667	4.93183	61.9251	65.6082	52.00	72.00	-5.5666	0.000	000
Lv104	61.9000	4.56637	60.1949	63.6051	55.00	70.00	-7.4333	0.000	000
Lv105	72.0000	5.90149	69.7963	74.2037	60.00	82.00	2.6667	0.063	Ns
Lv106	68.9333	5.17909	66.9994	70.8672	60.00	79.00	-0.3999	0.780	Ns
Lv107	64.0667	5.56425	61.9889	66.1444	50.00	76.00	-5.2666	0.000	000
HSLvOana	67.7333	4.54808	66.0351	69.4316	53.00	76.00	-1.5999	0.265	Ns
LG 31377	76.5667	4.24819	74.9804	78.1530	70.00	86.00	7.2333	0.000	***
3520R	71.6333	4.95833	69.7819	73.4848	62.00	81.00	2.300	0.109	Ns
Multipel	72.7000	3.87877	71.2516	74.1484	63.00	82.00	3.3667	0.019	**
CERA 320	66.6667	3.19842	65.4724	67.8610	61.00	75.00	-2.6666	0.064	Ns
Kerala	71.7333	4.44067	70.0752	73.3915	62.00	77.00	2.4000	0.095	Ns
Replik	72.2333	4.04017	70.7247	73.7420	63.00	78.00	2.9000	0.044	*
Po217	74.9000	2.46842	73.9783	75.8217	70.00	78.00	5.5667	0.000	***

Ns= Not significant. * The mean difference positive and is significant at the 0.05 level. ** The mean difference positive and is significant at the 0.01 level. *** The mean difference positive and is significant at the 0.001 level. 0 The mean difference negative and is significant at the 0.05 level. 00 The mean difference negative and is significant at the 0.01 level. 000 The mean difference negative and is significant at the 0.001 level.

The main width of the cob leaf varies between 5 cm and 11 cm and shows significant differences compared to both the field average and the hybrids considered in the study (F=34.323, p=0.000). Hybrids 3520R, Multipel, and Po217 differ significantly positively compared to the field average (Table 3, Figure 3).

Table 4. Numerical data associated to main cob leaf width

	Mean	Std. Deviation	95% Confidence Interval for Mean		Min	Max	The difference between the averages and the average of the field	p	seminif
			Lower Bound	Upper Bound					
The average of the field	7.4000	1.10327	7.2996	7.5004	5.00	11.00			
Lv101	6.6000	0.81368	6.2962	6.9038	6.00	8.00	-0.80000	0.001	000
Lv102	6.9000	0.99481	6.5285	7.2715	5.00	8.00	-0.50000	0.035	00
Lv103	6.4000	0.62146	6.1679	6.6321	5.00	7.00	-1.00000	0.000	000
Lv104	6.1333	0.50742	5.9439	6.3228	5.00	7.00	-1.26667	0.000	000
Lv105	7.3000	0.91539	6.9582	7.6418	6.00	8.00	-0.1000	0.673	Ns
Lv106	6.6000	0.62146	6.3679	6.8321	5.00	8.00	-.80000	0.001	000
Lv107	7.6000	0.81368	7.2962	7.9038	6.00	9.00	0.2000	0.399	Ns
HSLvOana	7.8333	0.94989	7.4786	8.1880	6.00	9.00	0.4333	0.068	Ns
LG 31377	7.6000	0.72397	7.3297	7.8703	6.00	8.00	0.2000	0.399	Ns
3520R	9.0667	0.98027	8.7006	9.4327	8.00	11.00	1.66667	0.000	***
Multipel	8.8333	0.46113	8.6611	9.0055	7.00	9.00	1.43333	0.000	***
CERA 320	7.0667	0.78492	6.7736	7.3598	6.00	8.00	-0.3333	0.160	Ns
Kerala	7.5333	0.77608	7.2435	7.8231	6.00	8.00	0.1333	0.574	Ns
Replik	7.3000	0.74971	7.0201	7.5799	5.00	8.00	-0.1000	0.673	Ns
Po217	8.2333	0.72793	7.9615	8.5051	6.00	9.00	0.83333	0.000	***

ns= Not significant. * The mean difference positive and is significant at the 0.05 level. ** The mean difference positive and is significant at the 0.01 level. *** The mean difference positive and is significant at the 0.001 level. 0 The mean difference negative and is significant at the 0.05 level. 00 The mean difference negative and is significant at the 0.01 level. 000 The mean difference negative and is significant at the 0.001 level.

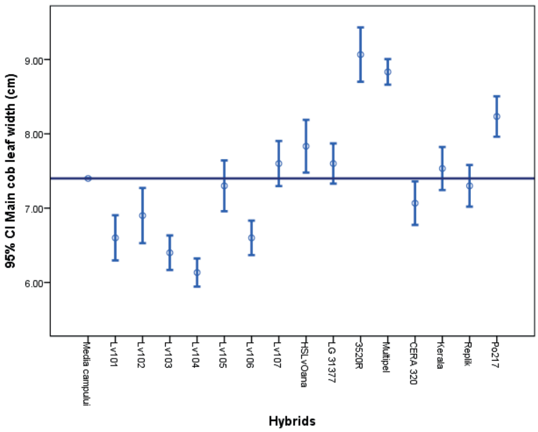

Figure 3. Means and confidence intervals of the mean for main cob leaf width (cm)

The average number of days to germination is 8.82 days with a standard deviation of 0.94 days. The hybrids Po217, Kerala, and Lv107 germinated after a minimum of 8 days, while the last hybrid to germinate was HSLvOana after a maximum of 12 days (Figure 4, Table 5). The median number of days to flowering is Me=69.67 days.

Table 5. Numerical data associated to main cob leaf width

	Mean	SD	Min	Max	Percentiles		Median
					25(Q_1)	75(Q_3)	
Days to plant emergence	8.82	0.94	7.67	11.33	8.33	9.33	8.33
Days to flowering	70.00	1.40	69.00	74.00	69.00	70.50	69.67
Days to silk	73.38	1.48	72.00	76.67	72.33	73.83	73.00

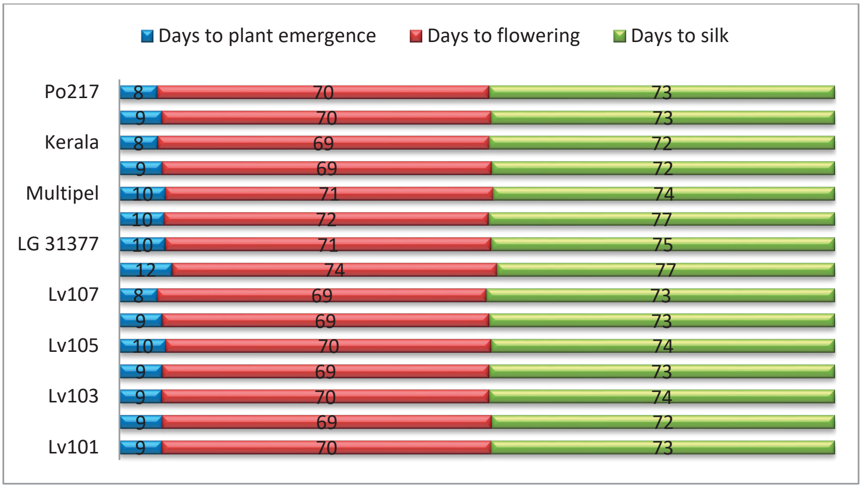

Figure 4. The number of days until emergence/flowering/silking for the hybrids considered in the study

The average of the accumulated growing degree days until flowering was 16238.20 (°C)/hour degrees, with a standard deviation of 464.40 (°C) (Table 6)

Table 6. Numerical descriptive characteristics associated with the variables of cumulative thermal degree sum until flowering (°C)/Degree hours, cumulative thermal degree sum until flowering (°C)/Degree days, cumulative thermal degree sum until tasselling (°C)/Degree hours, and cumulative thermal degree sum until tasselling (°C)/Degree days

	Mean	SD	Min	Max	Percentiles		Median
					25 (Q_1)	75 (Q_3)	
The sum of thermal degrees until flowering day(C) / Degrees hours	16238.20	464.40	15865.67	17487.00	15869.67	16471.50	16055.00
The sum of thermal degrees until flowering day(C) / Degrees days	674.78	19.35	659.00	726.67	659.33	684.50	667.33
The sum of thermal degrees to silky day (C) / Degrees hours	17608.29	396.56	17177.00	18490.00	17326.67	17707.00	17575.33
The sum of thermal degrees to silky day (C) / Degrees days	731.82	16.47	714.00	768.67	720.00	735.67	730.33

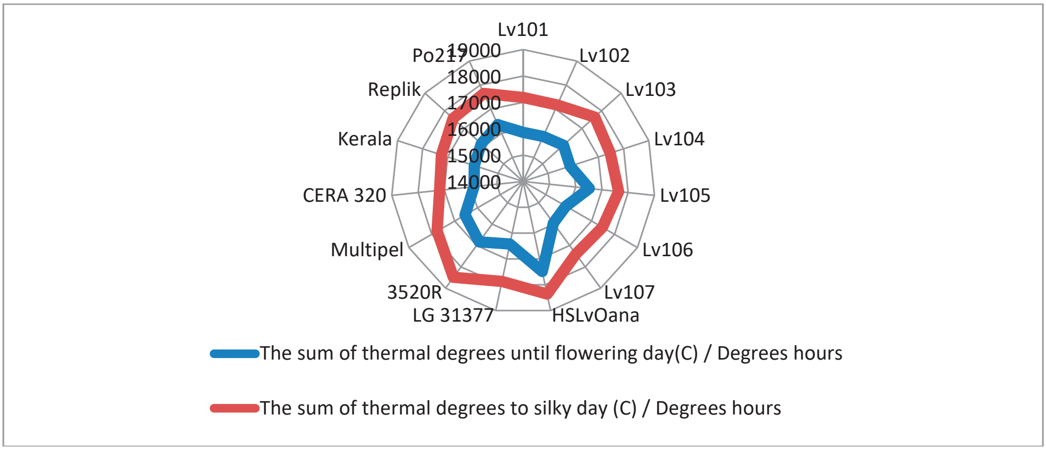

Figure 5. The cumulative thermal degree sum until flowering (°C) / degree hours and the cumulative thermal degree sum until tasseling (°C) / degree hours according to hybrids

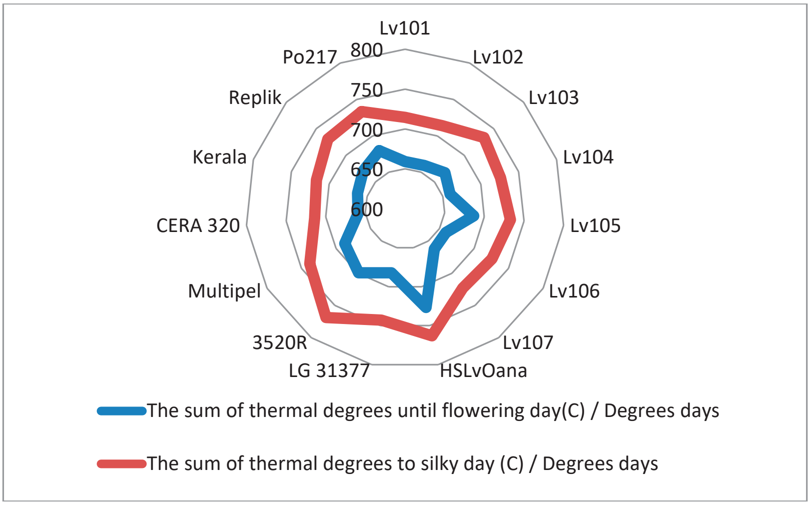

Figure 6. The cumulative thermal degree sum until flowering (°C)/degree days and the cumulative thermal degree sum until tasselling (°C)/degree days according to hybrids

Table 7 and Figures 7-8 present the production results for 15 hybrids evaluated in the experiment. Three hybrids (3520R, Multipel, and Po217) produced yields exceeding the field mean of 8064 kg/ha, with the hybrid P0217 producing the highest mean yield of 11807 kg/ha, followed by Multipel with 11322 kg/ha and 3520R with 10260 kg/ha. The differences in production among these hybrids were statistically significant. The hybrids Replik (9418 kg/ha) and LG31377 (9779 kg/ha) also produced yields above 9000 kg/ha, with differences from the field mean being statistically significant. The production of four hybrids, HSLvOana, CERA 320, Kerala, and Lv101, were close to the field mean, with no statistically significant differences. Three hybrids, Lv106, Lv104, and LV102, produced yields below the field mean by 2000-3000 kg/ha, with statistically significant differences in a negative sense. The hybrids Lv103, Lv105, and Lv107 produced yields lower than the field mean by 1300-1800 kg/ha.

Table 7. Kernel yield (kg/ha; u=14%)

	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum	Diferenta mediilor fata de media campului	semnif	Tukey HSD
			Lower Bound	Upper Bound					
Lv106	4954	318.11	4835.21	5072.79	4394	5391	-3110.14	000	A
Lv104	5080.67	262.84	4982.52	5178.81	4620	5612	-2983.47	000	A
Lv102	5962.33	440.56	5797.82	6126.84	5130	6620	-2101.81	000	B
Lv103	6188.47	546.95	5984.23	6392.7	5448	6989	-1875.67	00	B, C
Lv107	6582.93	496.96	6397.36	6768.5	6002	7399	-1481.21	0	C
Lv105	6755.9	284.73	6649.58	6862.22	6380	7228	-1308.24	0	C, D
Lv101	7228.67	714.13	6962.01	7495.33	6264	8308	-835.47	Ns	D
Media campului	8064.14	2219.85	7397.22	8731.05	4549.10	13575.10	-	-	-
Kerala	8424.53	972.23	8061.5	8787.57	7081	9754	360.39	Ns	E
CERA 320	8488.3	467.56	8313.71	8662.89	7846	9386	424.16	Ns	E
HSLvOana	8707.97	457.53	8537.12	8878.81	8171	9503	643.83	Ns	E
Replik	9418.5	846.18	9102.53	9734.47	8224	10601	1354.36	**	F
LG 31377	9779.77	433.57	9617.87	9941.66	9319	10503	1715.63	**	F,G
3520R	10260.1	561.97	10050.26	10469.94	9250	11027	2195.96	***	G
Multipel	11322.5	869.66	10997.76	11647.24	10088	12482	3258.36	***	H
Po217	11807.47	1338.44	11307.69	12307.25	10222	13760	3743.33	***	H

DL 5%=1167.329; DL 1% =1571.623; DL 0.1%=2089.803

ns= Not significant. * The mean difference positive and is significant at the 0.05 level. ** The mean difference positive and is significant at the 0.01 level. *** The mean difference positive and is significant at the 0.001 level. 0 The mean difference negative and is significant at the 0.05 level. 00 The mean difference negative and is significant at the 0.01 level. 000 The mean difference negative and is significant at the 0.001 level. Homogeneous subsets for alpha = 0.05 are displayed.

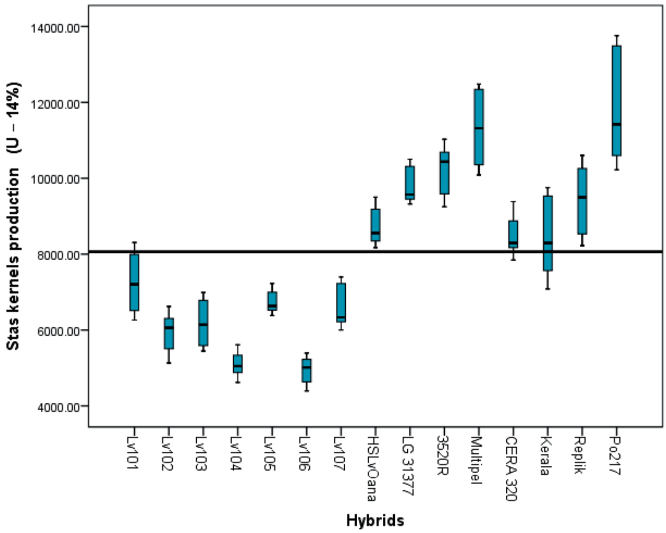

Figure 7. Box plot diagram associated to yield of each hybrid compared to the field mean

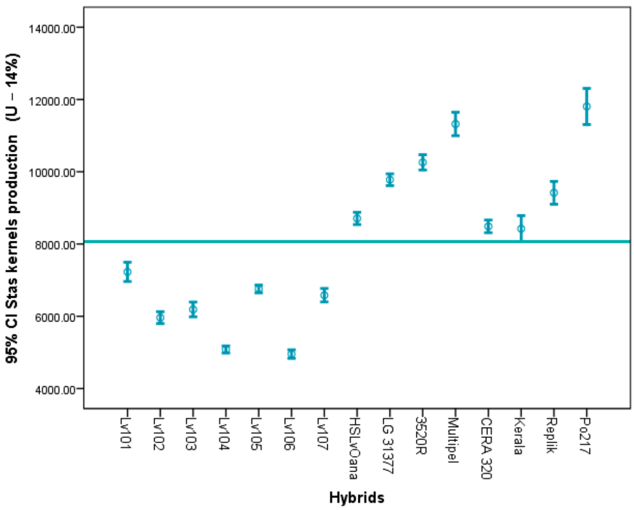

Figure 8. The means and confidence intervals for the means associated with the yields of the hybrids considered in the study compared to the field mean

CONCLUSIONS

The results regarding the behavior of 15 maize hybrids in the climatic conditions of the Agricultural Research and Development Station Lovrin area highlight different behaviors depending on their vegetation period, information that can be very useful for farmers in choosing the hybrid variety. In terms of production capacity, compared to the field average of 8064 kg/ha, the highest bean production was obtained for the Po217 hybrid - 11807 kg/ha, followed by the production of the Multipel hybrid - 11322 kg/ha and 3520R - 10260 kg/ha. Productions of over 9000 kg/ha were obtained for the Replik and LG31377 hybrids, 9418 kg/ha and 9779 kg/ha, respectively, indicating that these hybrids have a strong production capacity and resistance to water stress.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

DRY PERIODS INFLUENCE UPON MORPHOLOGICAL CHARACTERS OF TURDA 248 MAYZE HYBRID COBS

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Abstract

Tre current characteristics of maize plant are evolving by new breeding tolerance for dry season during vegetation period. The habit of the plant in general, but also the cobs prove true productive performances. However, the expression of the specific characteristics of the cobs is closely related to the natural provision of water. Being a moisture-loving plant, it is not uncommon to encounter periods of deficiency, especially during the deposition of dry matter in the grain. The intensity of these periods of drought causes some depressions in the morphology of the cobs and grains. The present study compares the cobs of the hybrid Turda 48 obtained in three different years, namely a relatively normal one and two years with obvious drought accents. From the data obtained, the cobs affected by the drought were 3 cm shorter and 0.2 cm thicker. The weight of the cobs decreased by 40-60 g, the number of grains on a cob decreased by 150, and the mass of the grains also decreased by 40-60 g. Grain percent of the cobs was reduced by 1-3 %. The grains formed were 0.5-1 mm shorter, the width remained at the same level, and the grain thickness was smaller by 0.6-0.8 mm. The mass of a thousand grains decreased by 20-40 g.

Key words: *cobs, grains, maize, morphological characters, variability.*

INTRODUCTION

Maize (*Zea mays* L.) is one of the most widespread cereals in the world (Tilman et al., 2002). The content of grains (Brown & Funk, 2008) is an important source of nutrients along with a variety of compounds (Lobell et al., 2011), such as carotenoids, phenolic components, phytosterols. Over time, the plant has undergone obvious evolutions through the desired characters (Doebley, 2000). Maize is considered today to be an important model (Haș et al., 2019), both for genetics and for perspective biology (Tollenaar & Lee, 2002). The origin of the plant is lost in time, so the starting point was a rustic species that produced small cobs, with a single bean 25 mm long. This cultivated plant between rows with *Zea mays mexicana* (*teosinte*), led in time to obtain several small cobs, a few centimeters, on a single plant. From that period there are currently three species of *Zea*, namely: *Z. mays* - ordinary corn, *Z. diploperennis* - *teosinte* perennial form and *Z. mays mexicana* - *teosinte* annual form. The name *Zea* comes from the Greek and means "that sustains life" and *mays*, a Taino word that means "gives life". Maize is expressed in the world both by *maize* - originally from *mahiz* (Spanish), which is the best description of the plant, and by the *corn*, which in some parts means cereal crop with expression and in a culinary context. Elsewhere, corn was developed from *Indian corn* = corn, referring to the multicolored flint horn, used for decorations (cobs with differently colored grains and woven and hanging sheets). The diploid plant contains 2n = 2x (2x10) = 20 chromosomes (Doebley, 2004), fixes the carbon on the C4 type, also having an increased efficiency of water recovery.

Being a unisexual monoecious species, maize has female flowers grouped in a spike-like inflorescence, with evident thickened axis (*spadix*). The maize spikelet has a long stigma with a role in capturing pollen grains, an ovary from which specific grains, awn and palea develop at the base. The mature cob has lengths of 3-50 cm and a diameter of 1.5-6 cm, being cylindrical, cylindrical-conical or fusiform. Their weight is between 50 and 500 g. 8-20 rows of grains are formed on a cob. The bean is a caryopsis with great variability in shape, size and color. The literature shows maize grains 2.5-22 mm long, 3-18 mm wide and 2.7-8 mm thick, and the mass of one thousand grains of 30-1200 g. The hybrid T. 248 studied has medium-sized cobs, with red spadix and normal yellow grains. The hybrid belongs to the indented form *(Zea mays* var. *indentata*, dent corn).

Research conducted to observe the variation of some characteristics of corn cobs (Lobell & Field, 2007), influenced by periods of drought (Sakurai et al., 2011) included: total length, diameter in the central portion, absolute weight, total number of grains, grain weight / cob, grain yield, grain length, width and thickness, and mass of one thousand grains (MTG).

MATERIALS AND METHODS

The variants have been cultivated in the last 3 years (2019-2021) with the hybrid Turda 248, from the semi-early group, FAO 380-390. The experience was set up according to the block method, with variants of 25 m^2 in 4 repetitions. The technology used was the one recommended by the resort. At full maturity, 25 cobs were randomly selected from each repetition (a total of 100), cut and brought to the laboratory. The 100 cobs were measured and determined: total length, thickness in the central area, weight, total number of grains, total grain weight, grain percent, grain length, grain width and thickness, mass of one thousand grains. The quality determined in the last year refers to the contents in protein, starch, oil and moisture at harvest. The morphological characters obtained were analyzed by the method of histograms (frequency polygons). In their expression, the class intervals established according to the specific sequence of values obtained were used. The study highlighted several aspects, namely: a) the mode values (with the highest frequencies), b) the limits of the intervals of variability of the studied characters and c) the specifics of each character of the maize ecotype in the analyzed area. Correlations were established between the analyzed characters, with the help of which their tendencies within the studied hybrid could be observed. Excel was used to express values. The significance of the correlation coefficients was obtained by comparing with the r_{max} values for the levels of 5%, 1% and 0.1% of the transgression probabilities. The quality indices were obtained with the help of the Perkin Elmer Inframatic 9500 analyzer. In the statistical calculation of all the values obtained, the analysis of variance (Anova test) was used on the variation strings. Statistical parameters were calculated using the formulas: $\bar{a} = \Sigma x/n$, where $\bar{a}$ = average of the determinations, and x = determined values, S^2 (variance) = $1/n\text{-}1[\Sigma x^2\text{-} (\Sigma x)^2/n]$, S (standard error) = $\sqrt{S^2}$ and CV% (coefficient of variation) = $S/\bar{a}.100$.

RESULTS AND DISCUSSIONS

Climatic characterization of maize vegetation. In general, the crop area has a favorable climatic regime (Sárvári & Pepo, 2014), from the beginning of vegetation to flowering (Table 1). The peak of rainfall is in May-June, after which the water deficit is installed until harvest. Lack of water (Dorland, 2000) thus affects the deposition of nutrients in the grain (Andersen et al., 2001; Rimski-Korsakov et al., 2009; Jolánkai et al., 2013), in close connection with the level of water supply in soil (Taylor et al., 2013). The normal thermal regime was exceeded in August the first year and in the period July-August in the other two years. For maize vegetation, the average temperatures exceeded the multiannual values by 0.66^0C, 0.78^0C and 1.08^0C, respectively.

The regime of rain falls in the maize vegetation (Huang et al., 2015) highlights the possibilities that the plant had in the formation of cobs and grains. As the volume of precipitation approached the necessary, consumption, the favorability of that year was more obvious. Of the three years analyzed, 2019 came closest to the necessary, being followed by the other two years with significant deficits. The hydroclimatic index (the ratio between rainfall and ETP) in 2019 had a positive balance, while in the other two years the balance was negative.

Variability of cobs and maize grains. The appearance and dimensions of the cob of this corn hybrid showed characteristic aspects. Thus, at the level of the three years, the length was generally between 13 and 24 cm. They dominated the lengths of 21 cm in the first year and 18 cm in the other two years (Figure 1). The graph shows the influence of the rainfall regime in the formation of cobs, of which the favorable year 2019 was highlighted. The thickness of the cobs in their central portion was generally between 3 and 4.8 cm (Figure 2). Dominant thicknesses were 4 cm in the second year (2020) year and 3.8 cm in the other two years.

Table 1. Climatic factors evolution from maize vegetation

Month	Temperatures, tn^{0}C				Rain, mm				Hydroclimatic index,HI*,%			
	Multi	2019	2020	2021	Multi	2019	2020	2021	Multi	2019	2020	2021
Apr.	11.0	11.3	11.8	9.0	56	50	24	47	86	77	37	72
May	16.3	16.4	15.8	16.0	81	68	**92**	**95**	105	88	**119**	**123**
June	19.5	21.8	19.9	19.9	94	**187**	**138**	80	99	**197**	**145**	84
July	21.7	21.8	**22.6**	**24.5**	81	48	10	50	53	32	7	35
Aug.	21.3	**23.9**	**23.6**	**23.7**	60	8	33	47	54	7	30	42
Mean, sum	17.96	19.04	18.74	18.62	372	361	297	319	79.4	80.2	67.6	71.2
±		**+1.08**	**+0.78**	**+0.66**	±	**-11**	**-75**	**-53**	±	**+0.8**	**-11.8**	**-8.2**

HI* % = P mm/ETP.100 (ETP, evapotranspiration potential)

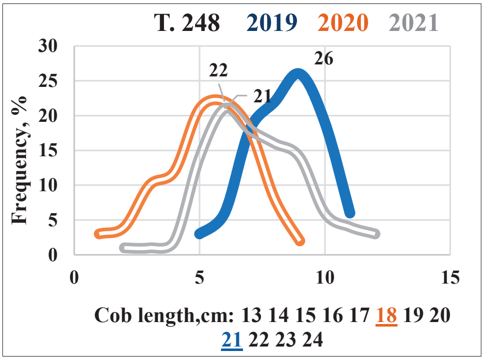

Figure 1. Frequencies of maize cob length

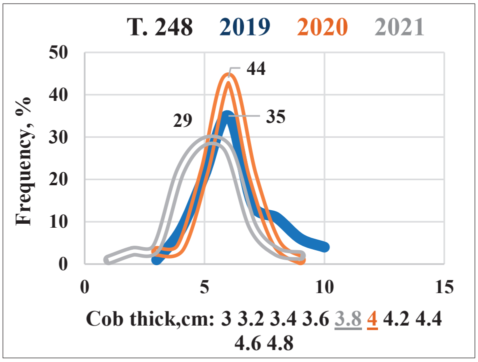

Figure 2. Frequencies of maize cob thick

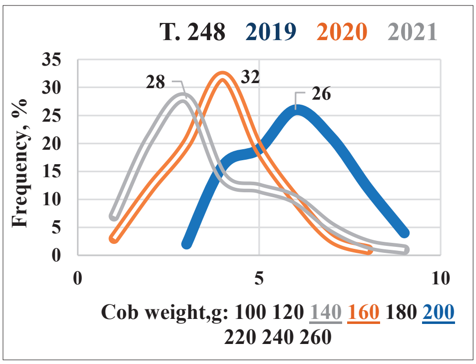

Figure 3. Frequencies of cob weight

Figure 4. The cobs aspect of Turda 248 hybrid

The weight of the cobs was generally between 100 and 260 g (Figure 3). The dominant cobs weighed 200 g in the favorable first year, 160 g in the second year and 140 g in the last year, respectively. The appearance, color and size of the cobs of this hybrid are shown in Figure 4.

The number of grains/cobs was between 400 and 850, at the level of the three years of cultivation (Figure 5). Of these, the cobs with 750 grains dominated in 2019 and the ones with 600 berries in the other two years. Regarding the weight of these grains on a cob, the values were between 80 and 220 g (Figure 6). Dominant were the grain biomasses of 180 g/cob in the favorable climate year 2019 and 140 g, respectively 120 g in the other two years, with the dry regime, 2020 and 2021 respectively. Grain percent of the cobs was within a wide range of values: 75% to 87%. Of these, 84% were dominant in the first year, 83% in the second year and 81% in the last year (Figure 7). Characteristics of the grains of this hybrid are shown in Figure 8.

The grain sizes were inscribed at values characteristic of the hybrid and the researched period. Thus, the length (or height) of the grains was generally between 8 and 13.5 mm (Figure 9). The dominant values were 11 mm in the first year, 10.5 mm in the second year and 10 mm in the last year. The width of these grains was 5.5 mm and 9 mm (Figure 10). The mod values of the three years were similar, namely at 7 mm. The third dimension, the grain thickness was between 3.6 mm and 5.4 mm (Figure 11). The grains with higher dominant thicknesses were in the first year, 4.8 mm. In the other two years, the grain thickness of 4.2 mm and 4 mm, respectively, are dominant.

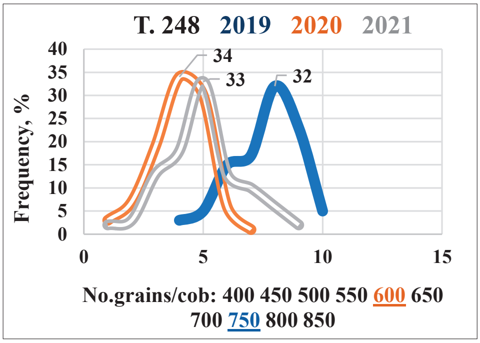

Figure 5. Frequencies of no. grains/cob

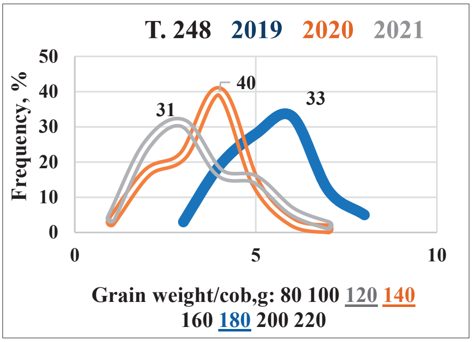

Figure 6. Frequencies of grains weight/cob

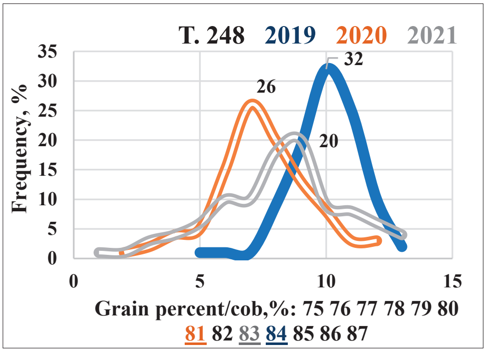

Figure 7. Frequencies of grain percent/cob

Figure 8. The grains aspect of Turda 248 hybrid

The absolute mass of the grains (thousand grains weight-TGW) was between 140 and 320 g (Figure 12). Dominant were grains with TGW of 240 g in the first year, 220 g in the second year and 200 g in the third year. Drought periods have obviously influenced the biomass of the grains of this hybrid.

From a qualitative point of view, the grains contained 7.0-7.4% protein, 4.1-4.5% oil, 72-74% starch, at the grain humidity of 16-18%.

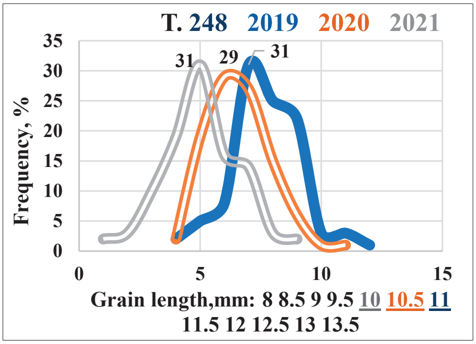

Figure 9. Frequencies of grain length

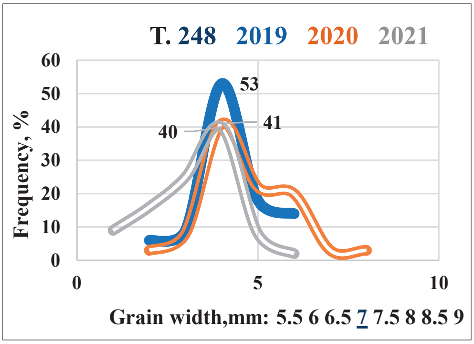

Figure 10. Frequencies of grain width

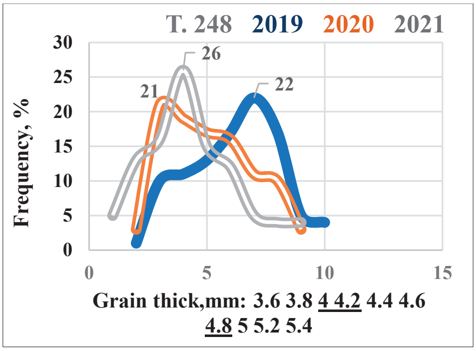

Figure 11. Frequencies of grain thick

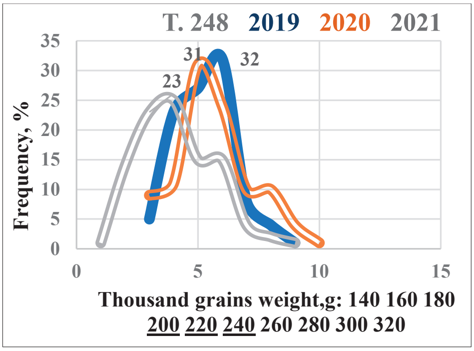

Figure 12. Frequencies of thousand grains weight

Correlations between main characters. If we analyze the whole set of correlations between all the analyzed characters, we find both positive and negative situations. Very obvious positive correlations were observed between the characteristics of the cob: length, thickness, weight, total number of grains, total weight of the grains. Negative correlations of different intensities of significance were observed in a higher proportion in the last year, 2021, a year with longer periods of drought (Table 2).

Statistical analysis of the variability of morphological characters in maize. The results obtained in the morphological analysis of the characters of the hybrid T. 248 in the three years showed specific aspects. Thus, the length of the cob was between 17.3 cm in 2020 and 20.4 cm in 2021. The variability showed small coefficients (7.2-10.9%). The thickness of the cob measured 3.80 cm to 4.01 cm, with a variation also reduced (5.2-10.4%), and its weight was between 145.1 g to 190.2 g (with medium to high variation). The grain percent/cob was between 81.4% in the second year and 84.0% in the first year. The average number of grains on a cob was 524.3 and 705.2 (average variability in all years) (Table 3). The weight of grains on the cob was 117.4 g to 159.6 g, with medium to high variability. The absolute weight of the grains (TGW) was on average between 203.3 g in 2021, 225.3 g in the first year and 234.8 g in the second year of cultivation. The variability of this character was reduced to average. The three grain sizes, namely length, width and thickness, had the lowest values in 2021 and the highest in the relatively more favorable year, 2019. From the average values obtained, differences emerged that explain the influence of drought in the last years of cultivation and more obviously in the last period of vegetation (Table 4).

Table 2. Correlations between the main characters of Turda 248 cobs and grains

Indices	Cob width cm	Cob weight g	No. grains	Grains weight g	% grains/ cob	Grain length mm	Grain width mm	Grain thick mm	TGW, g
	2019								
Cob length, cm	**.376**	**.827**	**.461**	**.805**	**-.238**	**.439**	**.358**	.075	**.699**
Cob width, cm	1	**.587**	**.387**	**.609**	.046	**.409**	**-.632**	-.119	**.489**
Cob weight, g		1	**.703**	**.989**	**-.245**	**.650**	.094	.059	**.702**
No. grains/cob			1	**.716**	-.033	**.449**	**-.292**	-.103	.020
Grains weight, g				1	-.107	**.648**	.061	.045	**.707**
% grains/cob					1	-.133	**-.254**	-.129	-.126
Grain length, mm						1	.086	.037	**.473**
Grain width, mm							1	.087	**.411**
Grain thick, mm								1	.188
	2020								
Cob length, cm	**.238**	**.797**	**.481**	**.799**	-.169	**.385**	**.261**	.140	**.596**
Cob width, cm	1	**.623**	**.489**	**.633**	-.069	**.480**	.108	.053	**.357**
Cob weight, g		1	**.582**	**.987**	**-.285**	**.569**	**.281**	.140	**.706**
No.grains/cob			1	**.606**	.006	**.364**	-.173	-.159	-.050
Grains weight, g				1	-.130	**.569**	**.286**	.132	**.697**
% grains/cob					1	-.136	.024	.075	.183
Grain length, mm						1	.166	.084	**.418**
Grain width, mm							1	**.222**	**.545**
Grain thick, mm								1	**.259**
	2021								
Cob length, cm	**.267**	**.687**	**.713**	**.708**	**-.236**	.116	.073	.031	**.217**
Cob width, cm	1	**.342**	**.232**	**.357**	-.081	-.009	-.030	-.030	**.223**
Cob weight, g		1	**.737**	**.991**	**-.246**	-.086	**-.205**	**-.237**	.062
No.grains/cob			1	**.756**	.080	**.258**	-.184	-.095	-.183
Grains weight, g				1	**-.422**	**-.222**	**-.262**	**-.303**	**.561**
% grains/cob					1	**.440**	**.497**	**.364**	**-.392**
Grain length, mm						1	**.726**	**.548**	-.244
Grain width, mm							1	**.735**	**-.225**
Grain thick, mm								1	**-.303**
	LSD 5 % = .190 LSD 1 % = .250 LSD 0.1 % = .320								

Table 3. Statistical indices of Turda 248 maize cobs

Indices	Cob length, cm	Cob width, cm	Cob weight, g	% grains/ cob	No. grains/ cob
	2019				
Mean, ā	**20.4**	**4.01**	**190.2**	**84.0**	**705.2**
Variance, s^2	2.15	0.06	805.2	2.87	4973
Std. error. s	1.46	0.24	28.35	1.69	70.52
Var. coef., s%	**7.2**	**6.0**	**15.0**	**2.01**	**10.0**
	2020				
Mean, ā	**17.3**	**3.95**	**150.0**	**81.4**	**524.3**
Variance, s^2	3.55	0.04	750.6	2.66	3767
Std. error, s	1.88	0.21	27.4	1.63	61.37
Var. coef., s%	**10.9**	**5.2**	**18.3**	**2.01**	**11.7**
	2021				
Mean, ā	**19.1**	**3.80**	**145.1**	**82.1**	**571.4**
Variance, s^2	4.30	0.11	1293	7.60	6847
Std. error. s	2.09	0.32	35.9	2.71	82.7
Var. coef., s%	**10.9**	**10.4**	**24.7**	**3.3**	**14.4**

S^2 variance, s standard deviation, VC variation coefficient

Table 4. Statistical indices of Turda 248 maize grains

Indices	Grains weight, g	TGW, g	Grain length, mm	Grain width, mm	Grain thick, mm
2019					
Mean, ā	**159.6**	**225.3**	**11.3**	**7.13**	**4.58**
Variance, s^2	543.5	482.1	3.04	0.14	0.20
Std. error s	23.31	21.95	1.74	0.37	0.44
Var. coef., s%	**14.6**	**9.7**	**15.4**	**5.18**	**9.6**
2020					
Mean, ā	**117.4**	**234.8**	**10.8**	**7.20**	**4.44**
Variance, s^2	1555	1125	0.39	2.83	0.16
Std. error s	39.4	33.5	0.63	1.68	0.39
Var. coef., s%	**33.6**	**14.3**	**5.8**	**23.4**	**8.9**
2021					
Mean, ā	**118.7**	**203.3**	**9.22**	**6.33**	**4.05**
Variance, s^2	746.8	960.7	3.91	1.04	0.44
Std. error s	27.3	30.9	1.93	1.02	0.58
Var. coef., s%	**23.1**	**15.2**	**21.6**	**16.2**	**15.8**

CONCLUSIONS

From a climatic point of view, the multiannual average of the hydroclimatic index (HI) is about 80% of what is needed for maize vegetation. In the first year this threshold was slightly exceeded, which means a relatively normal degree of favorability. In the other two years HI had deficiencies and characterize years with periods of drought.

The morphological characteristics of the cobs obtained in the three years, a relatively normal year - 2019 and the last two years with obvious periods of drought, were specific. Thus, in the dry years the average length of the cobs lost 3 cm, and their thickness decreased by 0.2 cm. The cobs weighed less by 40-60 g and formed with 150 grains less. The grains on the cobs weighed less by 40-60 g and had a mass of one thousand grains reduced by 20-40 g. The grain percent of the cobs was reduced by 3%. The grain of corn had lengths of 1.0 mm, the width remained constant, and the thickness decreased by 0.6-0.8 mm.

Simple correlations were established between all the studied characters, with some differentiations. The correlations between the size and biomass of cobs and grains were positive and very significant. Negative correlations were observed between the grain yield of the cobs with all the other characters, in all years. Of the three years, the most severe periods of drought in the last year have had the most unfavorable influence on the grains, in percentage, size and absolute weight.

The statistical indices studied showed average values affected by the drought periods of those years, and the variability of the analyzed characters ranged from low to medium and rarely very high.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

MICOFLORA ASSOCIATED WITH WHEAT SEEDS

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Abstract

The research followed the identification in laboratory conditions of the micoflora associated with wheat seeds. The biological material was represented by caryopsis from the Glosa and Boema varieties, used in the experiments monitored in our research. For both varieties, we worked with the PDA and malt agar culture medium variants and untreated seeds, seeds disinfected with distilled water and 70% ethanol solution. Each variant was represented in three repetitions. In the case of the Glosa variety, the most common micromycetes belonged to the genera Alternaria and Stemphylium, and in the case of the Boema variety, micromycetes from the genera Penicillium and Alternaria were detected. In both varieties, in the untreated(control) and disinfected with distilled water variants, Rhizopus spp. was established. The poorest micoflora was detected in the variant disinfecting the seeds with 70% ethanol solution. Seed germination was not affected.

Key words*: wheat, mycoflora, seeds, varieties.*

INTRODUCTION

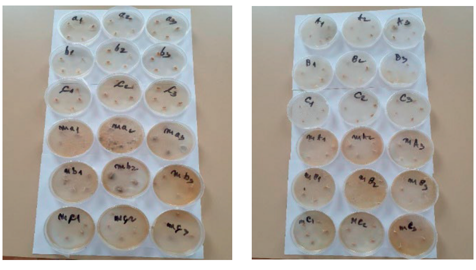
Wheat is considered the most important cultivated plant, providing the basic food of people (Muntean et al., 2003) with an area that includes temperate and subtropical zones of the world (Hashmi & Ghaffar, 2006). Seeds are a common means of spreading and transmitting plant pathogens. Contaminated or infected seeds can contribute to the spread of some dangerous pathogens for plants with the establishment of crops but also in the case of commercial exchanges (Agarwal & Sinclair, 1996). Seeds from diseased plants can cause reduction or inhibition of germination, plant growth, yield reduction and crop quality deterioration (Webwer et al., 2001; Dawson & Bateman, 2001; Bateman & Kwasna, 1999). The pathogens present internally or contaminating the seeds or associated in the seed mass are sources for the infections of cultivated plants.
The micoflora of wheat seeds transmitted through wheat seeds contributes to the quantitative and qualitative decrease of wheat production. The micoflora of wheat seeds is associated with pathogens such as: *Fusarium* spp., *Alternaria* spp., *Drechslkera sorokiniana*, *Cladosporium herbarum*, *Stemphylium botryosum* (Glazek, 1997; Nirenberg et al., 1994). Also, micromycetes from the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Trichoderma,* *Trichotecium*, *Tilletia* are associated with the micoflora of wheat seeds (Hashmi & Ghaffar, 2006; Raicu & Baciu, 1978; Hajihasani et al., 2012).
Micromycetes associated with wheat seeds can, under certain conditions, cause black point, a condition of the seeds that can negatively influence germination (Cristea et al. 2008; Gheorghieş et al., 2004). Also important is the fact that they are fungi that contaminate seeds and are sources of some mycotoxins (Chulze, 2010; Ittu M., 2006) dangerous for consumers in the food chain.

MATERIALS AND METHODS

The research aimed to identify the mycoflora of wheat seeds. The biological material was represented by seeds from the Glosa and Boema varieties, used in experiments set up during the research period at Moara Domneasca. The variants analyzed were not disinfected, disinfected in distilled water and disinfected in 70% ethanol solution. Three repetitions were made for each variant. Petri dishes with a diameter of 90 mm and the culture medium PDA Roth (potato extract glucose agar) intended for microbiology laboratories and malt agar were used. Samples of 15 seeds/variant were made. The disinfected seeds were placed in Petri dishes incubated at a thermostat at 21^0C±20^0C. The mycelial growth was monitored 3, 6, 9 days. After the incubation period each Petri dish was examined and the microscopic identification of the pathogens present. The incidence of seeds infected with the identified pathogen was calculated as the percentage of seed infection of each variant, with the formula: F (I) = (n/N) x 100, where, F(I) = frequency (incidence) of seed infection; n= number of seeds on which a fungal species was identified; N = number of seeds tested. The presence of the micromycete *Rhizopus* spp was measured as a percentage of the surface of the Petri plates. The microscopic identification was carried out according to the morphological characteristics of the fructifications of the fungi.

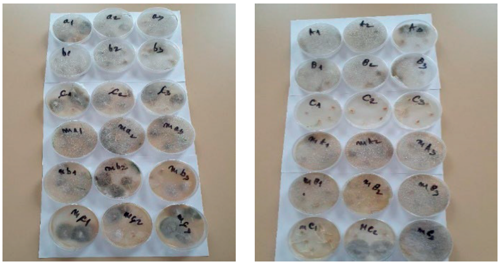
RESULTS AND DISCUSSIONS

For the examination of the micoflora, seeds from the varieties Glosa (A) and Boema (B) placed on PDA and MA culture media were used. The vegetative growth of the fungi was monitored after 3 days (Figure 1), after 6 days (Figure 2) and after 9 days (Figure 3) of incubation. After 9 days of incubation, the microscopic identification of the pathogens identified according to the characteristics of the specific fruits was carried out.



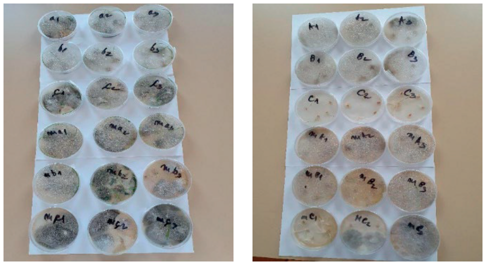
A (Glosa) B (Boema)

Figure 1. Growth of fungal colonies on PDA (A) and MA (B) culture medium 3 days after incubation



A (Glosa) B (Boema)

Figure 2. Growth of fungal colonies on PDA (A) and MA (B) culture medium 6 days after incubation



A (Glosa) B (Boema)

Figure 3. Growth of fungal colonies on PDA (A) and MA (B) culture medium 9 days after incubation

The data in Table 1 show the microflora associated with wheat seeds in the monitored varieties. In the case of the Glosa variety, on the PDA culture medium in the control variant, the micromycetes *Alternaria* spp. and *Stemphylium* spp. were detected in all the vessels, and in two of the repetitions the pathogen *Epiccocum* spp was also detected. In the distilled water variant, *Penicillium* spp. and *Alternaria* spp. and *Stemphylium* spp. were detected.

In the case of the 70% ethanol solution variant, fruiting belonging to the genera *Alternaria* and *Stemphylium* were identified, with poor fruiting. Regarding the development of the colonies and the identification of fungi on the malt agar medium, a richer fungal flora was found, possibly because the light disinfection with distilled water also stimulated the micoflora of the seeds in the case of untreated seeds (c) the presence of the fructifications of the micromycete *Cladosporium* spp. and in the case of the distilled water variant, the micromycete *Trichoderma* spp. was identified in one of the repetitive vessels. The presence of yeasts was also observed. The fructifications of the genera *Alternaria* and *Stemphylium* and yeasts were identified on the MA culture medium when disinfected with 70% ethanol. In the Boema variety, micromycetes from the genera *Alternaria* and *Stemphylium* and *Penicillium* were identified in the seeds from all plates in the PDA variety. With the disinfection variant with distilled water, *Aspergillus* genus fructifications were also identified. In the case of disinfection with 70% ethanol, micoflora was poor, consisting of young *Alternaria* and *Stemphylium* fruits, the latter predominating, and yeasts. On the malt agar culture medium, the micoflora consisted of fungi of the same genera and on the 70% ethanol disinfection malt agar variant it was composed of *Alternaria*-poor micoflora and small yeast colonies. The characteristic fructifications of the *Rhizopus* micromycete were identified in all the analyzed variants.

Table 1 Micoflora associated with wheat seeds

Variants			The pathogen (9 days)							
			Rhizopus spp.	*Alternaria* spp. + *Stemphylium* spp.	*Cladosporium* spp.	*Epicoccum* spp.	*Trichoderma* spp.	*Penicillium* spp.	*Aspergillus* spp.	Yeasts
Glosa	PDA	N(c)	+++	+++	-	++	-	-		-
		AD	+++	+++	-	-	-	+		-
		E 70%	-	+++	-	-	-	-		
	MA	N(c)	+++	+++	++	-	-	-		-
		AD	++	++	-		+	-		+
		E 70%	-	+++	-	-	-	-	-	+
Boema	PDA	N(c)	+++	+++	-	-	-	+++	-	-
		AD	+++	+++	-	-	-	+++	++	-
		E 70%	-	+	-	-	-	-	-	+
	MA	N	+++	++	-	-	-	+++	+	-
		AD	+++	++	-	-	-	+++	+	-
		E 70%	+	+	-	-	-	-	-	++

+ - detected in one of the three repetitions; ++- detected in two of the 3 repetitions; +++- detected in the three repetitions
N(C)- control variant; AD- distilled water variant; E- 70% ethanol solution

The incidence of fungi identified in the monitored variants was also calculated (Table 2) and it was found that in the case of the Glosa variety, the highest incidence values, over 80%, were calculated for micromycetes from the genera *Alternaria* and *Stemphylium* in both culture medium in the control variants and disinfection with distilled water. In the disinfection option with 70% ethanol, the incident values were 33.33% on the PDA medium and 46.66% on the seeds placed on the MA culture medium.
The frequency of the micromycete *Trichoderma* spp. was 33.33% and 13.33% values were recorded for micromycetes *Cladosporium* spp., *Epiccocum* spp. The incidence of yeasts with a value of 13.33% was recorded on the malt agar culture medium. A low frequency, 6.66%, was determined in the case of the micromycete *Penicillium* spp. on the PDA culture medium.
Regarding the Boema variety, it was found that the highest incident values were calculated in the case of the micromycete *Penicillium* spp. with values over 73%, followed by the micromycetes *Alternaria* spp. and *Stemphylium* spp with frequency values between 6.66% and 33.3% on both culture media, with higher values for seeds not disinfected or subjected to a light disinfection. Determinations were also made on the germination of the analyzed seeds and it was found that this was not affected.

Table 2. Incidence of fungi detected on wheat seeds (9 days)

Variants	The pathogens	PDA			MA		
		N (c)	AD	E 70%	N(c)	AD	E 70%
Glosa	*Alternaria* spp. +*Stemphylium* spp.	80	86.66	33.33	86.66	53.33	46.66
	Cladosporium spp.	-	-	-	-	13.33	-
	Epicoccum spp.	13.33	-	-		-	-
	Trichoderma spp.	-	-	-	-	33.33	-
	Penicillium spp.	-	6.66	-	-	-	-
	Drojdii	-	-	-	-	13.33	13.33
Boema	*Alternaria* spp. +*Stemphylium* spp.	26.66	13.33	6.66	33.33	26.66	20
	Cladosporium spp.	-	-	-	-	-	-
	Epicoccum spp.	-	-	-	-	-	-
	Trichoderma spp.	-	-	-	-	-	-
	Penicillium spp	73.33	73.33	-	73.33	60	-
	Aspergillus spp.	-	13.33	-	13.33	6.66	-
	Yeast			6.66			26.66

CONCLUSIONS

The micoflora of the wheat seeds included species of the genera *Alternari*a and *Stemphylium* with the highest incidence values in the case of the Glosa variety, followed by micromycetes from the genera *Trichoderma*, *Cladosporium*, *Epicoccum*. The micoflora of the seeds of the Boema variety included *Penicillium* spp. with the highest values of the incident, followed by *Alternaria* spp. and *Stemphylium* spp. and *Aspergillus* spp. Disinfecting the seeds with 70% ethanol ensured a decrease in the presence of contaminated micoflora in the caryopsis of the monitored varieties.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EFFECT OF SPOTLIGHT PLUS ON DESICCATION IN SOYBEAN

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Abstract

The present study aims to evaluate the effect of different doses of the selective herbicide Spotlight Plus on desiccation in soybean. The field experiment was set up in 2022 in the region of Knezha, Northern Bulgaria. Seven treatments, including untreated control, four experimental doses of the selective product Spotlight Plus, and two dose rates of the non-selective product Beloukha with a broad spectrum action, were analyzed. The products were applied with an experimental sprayer Pulvexper at BBCH stage 87. The results showed that using the selective product Spotlight Plus containing 60 g/l carfentrazone-ethyl as a desiccator does not negatively affect the crop's yield quantity or oil content. The application with both of the tested products increased the desiccation rate on pods, stems, and leaves in comparison to the untreated plots and enhanced the crop yield.

***Key words**: carfentrazone-ethyl, desiccation, herbicide, soybean, yield.*

INTRODUCTION

Legumes provide more than 69% of the protein as well as 30% of the oils needed for the human diet (Ge et al., 2016). Soybean is one of the most cultivated legume crop worldwide. The leading country in soybean production for 2020 was Brazil with 138 million tons followed by the USA with about 120.7 million tons. After that in the list are Argentina, China, and India with 46.5, 16.4, and 11.5 million tons. Although soybean is grown also in Europe, Bulgaria is not a typical soybean producer. Soybean in Bulgaria is not a fundamental crop. It is grown mainly for human consumption and for seed production for planting, or as a rotation crop. It has a favourable nitrogen balance and contributes to the long-term sustainability and higher profit (Iantcheva et al., 2021). The European Union is providing subsidies for protein crops, which could be a motivation for the farmers (Oilseeds and Products Annual Report 2021). According to Dima (2015), Romania, Bulgaria, and the Republic of Moldova could supply 5% of the European Union annual consumption of soybean (30% of non-GM soybeans annually used in the EU), equal to more than 2 million tons. Soybean has a high nutritional value. It is rich in essential amino acids as lysine, tryptophan and methionine, and for that reason soy proteins play an important role in human in animal nutrition (Iantcheva et al., 2021). Soybean meal is rich in minerals, mainly calcium, iron and potassium and contains vitamin B complex and beta-carotene. In the last years, soybean has been used as an important source of phytoestrogens (genistein) and isoflavones (Sakthivelu et al., 2008). Usually, before maturity, the soybean is treated with desiccant products. It is important for the products that are used for desiccation, to leave no residues above the accepted levels in the seed or to cause no negative effects on the yield quality or quantity, as they are used mainly for human or animal consumption. (McNaughton, 2015). Due to the fact that the crop is indeterminate and because of variations within the field, not all beans mature simultaneously (Soltani et al., 2015). To ensure uniform weed and crop desiccation and reduce these variations, as a pre-harvest aid, farmers often apply herbicides (Cole & Cerdeira 1982; Wilson & Smith, 2002). Albrecht et al. (2022) reported about plenty of benefits of pre-harvest desiccation of soybeans (*Glycine max* L.). According to the authors, this agricultural practice could control weeds, stabilize plants with green stem/leaf retention problems, accelerate and/or optimize harvest, and reduce damage from pests and fungi that may attack the crop at the end of the crop cycle (Griffin et al., 2010; Toledo et al., 2014; Bezerra et al., 2016; Bellaloui et al., 2020).

The active ingredient and the application time of the desiccation product are of great importance, as they could result in yield loss and leave herbicide residues in the grain (Azlin & McWhorter 1981; Cerkauskas et al., 1982; Smith, 1996; Wilson & Smith, 2002).

In recent years, products based on glyphosate, glufosinate, diquat, carfentrazone-ethyl and others have been widely used for the desiccation of leguminous crops. Those based on carfentrazone-ethyl gave the lowest results (Soltani et al., 2013). For this reason, desiccation was mainly carried out with diquat, glyphosate and glufosinate.

The use of glyphosate increased significantly after the cultivation of gene modified glyphosate resistant crops as rapeseed, corn, soybean, cotton and sugar beet (Cerdeira et al., 2007; Duke, 2018). As a result, glyphosate residues have been frequently detected in food (Zoller et al., 2018). According to the Food and Agriculture Organisation (FAO), both glyphosate and its primary degradation product, AMPA, lead to their accumulation in the food chain and increasing the potential risk for human health (Bai & Ogbourne, 2016).

In the last few years, however, the negative effects of the application of these active ingredients have been proven beyond doubt, and most of them have already been banned or are about to be banned for use in the next few years.

Carfentrazone-ethyl is classified as toxicity categories III and IV (USEPA, 1998) and is considered to be practically non-toxic to birds, but it is moderately toxic to aquatic animals (Han et al., 2007).

Therefore, the aim of the present study is to determine the effect of the application of carfentrazone-ethyl in soybeans, which has not been monitored in Bulgaria yet.

MATERIALS AND METHODS

Plant material

The field experiment was performed with soybean (*Glycine max* L.), cv. Srebrina - a modern Bulgarian cultivar, invented in the Experimental Station on Soybean in Pavlikeni, Bulgaria. The variety is mid-early, with growing season of 118-125 days. It has a compact habit and a resistant stem with a height of 80-98 cm, covered with short gray hairs. The mass of 1,000 seeds varies between 125-158 g. The variety is resistant to the diseases stem and bean canker, bean blight and resistant to ascochitosis, blight and bacteriosis.

Experimental design

The field experiment was set up in 2022 in the region of Knezha, Northern Bulgaria. Soybean cv. Srebrina was sown on 6 April 2022 with a sowing rate of 300,000 plants/ha. A randomized block design with four replicates was used. The young plants emerged on 20 April 2022. The treatment was performed on 19 August 2022. The air temperature during the application was 23.2°C and the relative humidity - 61%. As a test product a micro-emulsion (ME) selective herbicide Spotlight Plus (FMC Agro), containing 60 g/l carfentrazone-ethyl was chosen. As a reference product, the herbicide Beloukha (Belchim Crop Protection) was selected. It is a non-selective, broad spectrum, foliar-applied herbicide, containing 680 g/l pelargonic acid, made from sunflowers. The test variants included 1 - untreated control, 2 - Spotlight Plus (0.5 l/ha), 3 - Spotlight Plus (0.75 l/ha), 4 - Spotlight Plus (1 l/ha), 5 - Spotlight Plus (2 l/ha), 6 - Beloukha 16 l/ha, and 7 - Beloukha (32 l/ha). The products were applied with an experimental sprayer Pulvexper at BBCH stage 87 and the plant height during the application was 70 cm. The desiccation rate of leaves, stems, and beans was measured three times. The first measurement was done 3 days after the application, the second 1-9 days after the application, and the third 1-15 days after the application (on 2 August 2022, 28 August 2022, and 2 September 2022, respectively).

Statistical data analyses

The data are presented as mean of four replications. The experimental results were statistically processed with the SPSS program using a one-way ANOVA dispersion analysis, as well as Duncan's comparative method with a validity of differences determined at a 95% significance level.

RESULTS AND DISCUSSIONS

The rate of desiccation of leaves, stems, and pods is measured three times. The first observation was made 3 days after the treatment on 22 August 2022, the second 1-9 days after the treatment (28 August 2022), and the third 1-15 days after the treatment (2 September 2022 respectively). The data of the desiccation rate of leaves of soybean are presented in Figure 1.

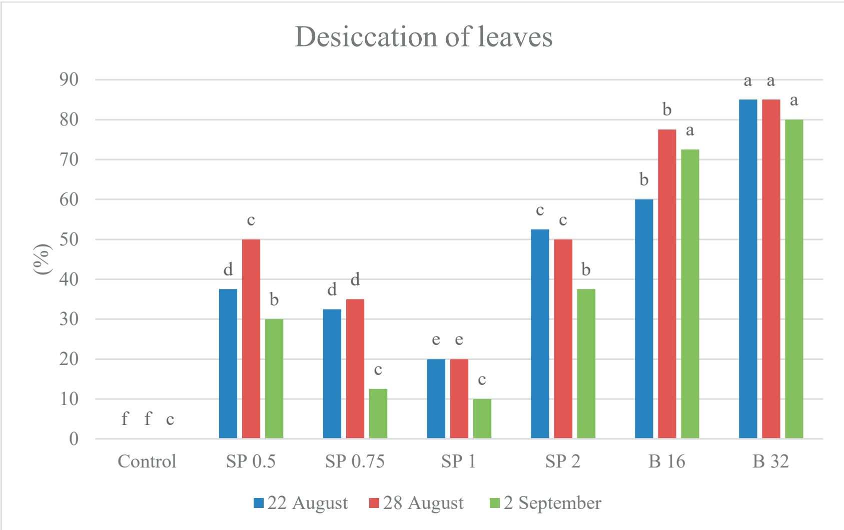

Figure 1. Desiccation rate of leaves of soybean (*Glycine max* L.) three, seven, and 14 days after herbicide application. SP - Spotlight Plus, B - Beloukha

The results show that the application of all the test products lead to an enhancement of the desiccation rate of leaves. The first observation was done three days after the herbicide treatment. A desiccation rate of about 37% higher than the control was observed when Spotlight Plus was applied in a dose of 0.5 l/ha. The increase in variant SP 0.75 and variant SP 1 was by 32% and 20%, respectively. The desiccation rate was about 52% higher when the selective product was applied in a dose of 2 l/ha. The desiccation was higher when the broad spectrum product Beloukha was applied. The percent of leaf desiccation in variants B16 and B32 was by 60% and by 85% higher than the control, respectively. The second estimation of the desiccation rate was performed three days after the first one (28 August 2022). On that date, the rate of the desiccation in variant SP 0.5 was 50% higher than the control. The dose of 0.75 l/ha lead to a 35% increase compared to the control. The rates of 1l/ha, and 2 l/ha increased the desiccation rate by 20% and 50% respectively. The desiccation on the leaves 15 days after the application enhanced by 30, 13, 10, and 38% for variants SP 0.5, SP 0.75, SP 1 and SP 2, respectively. The increase in variant B 16 and B 32 compared to the control was by 73% and 80%, respectively. The rate of desiccation of soybean stems is presented in Figure 2. At the first observation, it is seen that the application of carfentrazone-ethyl in a dose of 0.5 l/ha increased the desiccation rate by 11% compared to the control. The rate of 0.75 l/ha and 1 l/ha of the selective herbicide led to a 9% enhancement of the desiccation rate. After the application of 2 l/ha of the selective product the desiccation was 20% higher than the control.

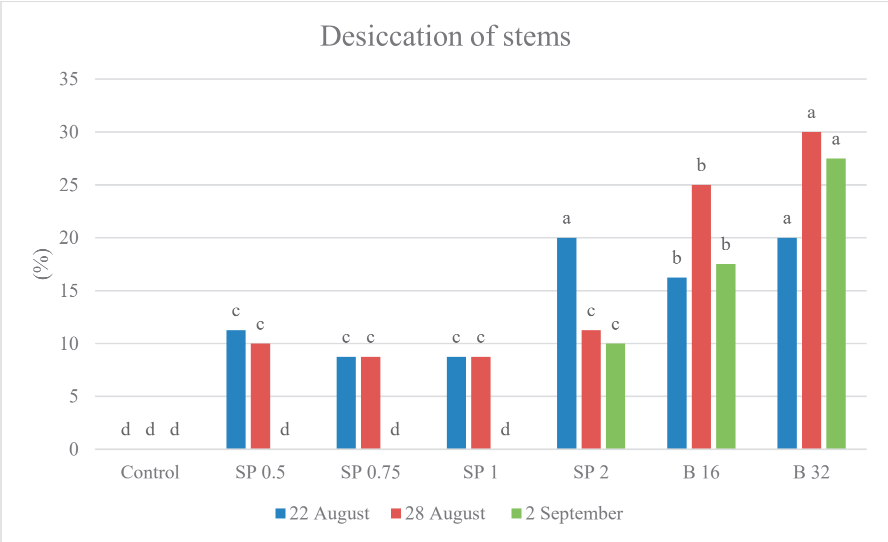

Figure 2. Desiccation rate of stems of soybean (*Glycine max* L.) three, nine, and 15 days after herbicide application. SP - Spotlight Plus, B - Beloukha

The treatment with the total herbicide increased the desiccation by 16% and by 20%, for dose rates of 16 and 32 l/ha, respectively. The second observation was performed tree days after the first one. It is seen that the rate of desiccation is the lowest in the untreated control. The enhancement in variants 2, 3, 4, and 5, where the selective product was used, was by about 10% compared to the control. A higher desiccation rate was observed when the non-selective product was applied. The increase in comparison to the untreated control was by 25 and 30%, respectively.

Fifteen days after the foliar treatment, there was no statistically proven differences between the desiccation rate of the stems of the control plants and the plants, treated with the selective product in all the rates that were lower than 1 l/ha. The spray with the highest dose (2 l/ha) increased the desiccation rate by 10% compared to the control. The application of the total herbicide increased the desiccation by 18 and 28% for the application doses of 16 and 32 l/ha, respectively.

The desiccation rate of pods is presented in Figure 3. The first observation was performed on 22 August, three days after the application of the products. The use of 0.5 and 0.75 l/ha of the selective product led to a 6% higher desiccation rate than the control. The rate of 1 l increased the desiccation by 5%, and the rate of 2 l/ha - by 16% compared to the untreated plots, respectively. The application of the non-selective herbicide in a dose of 16 l/ha increased the desiccation rate by 6%, and the dose of 32 l/ha - by 18% compared to the untreated plots, respectively.

The second observation was done six days after the first one, on 28 August 2022. The rate of desiccation was the lowest on the untreated plots. The rate increased on the plots, treated with the selective product by 14, 13, 11, and 20% for the dose rates of 0.5, 0.75, 1, and 2 l/ha, respectively. The rate of desiccation was higher when the total herbicide was used. Both of the rates applied led to a 30% higher desiccation rate in comparison to the untreated control.

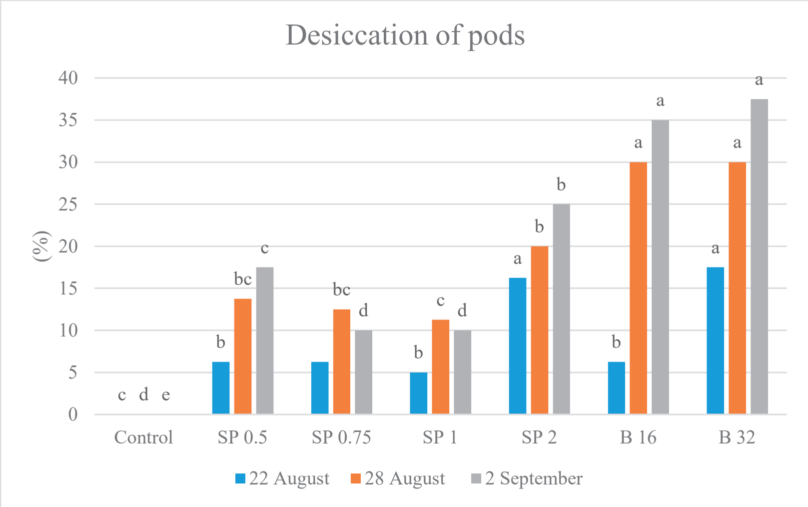

Figure 3. Desiccation rate of pods of soybean (*Glycine max* L.) three, nine, and 15 days after herbicide application. SP - Spotlight Plus, B - Beloukha

The last observation of pods desiccation was on 2 September (15 days after the application). The lowest rate of desiccation was measured on the untreated plots. In variant SP 0.5 the rate was 18%, and in variants SP 0.75 and SP 1, it was 10%, compared to the control. The highest dose of Spotlight Plus led to a 25% increase, and the application of Beloukha - 35% and 38% enhancement, compared to the untreated plots, respectively.

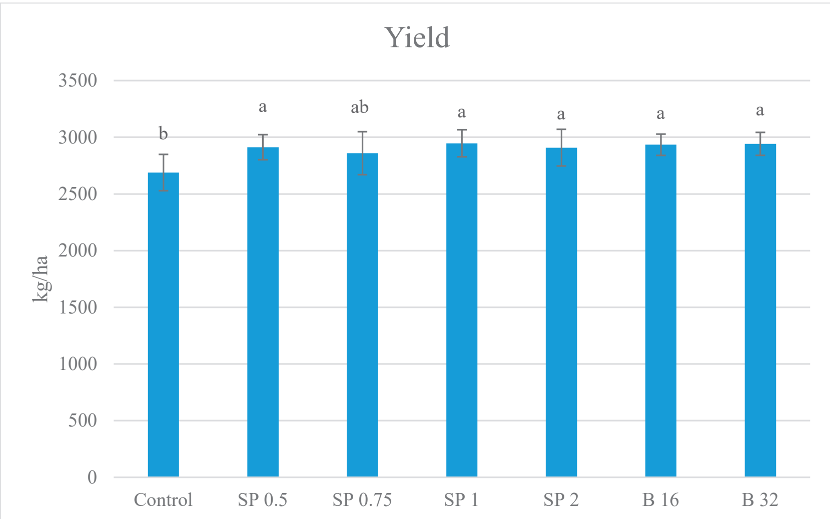

Figure 4. Yield of soybean (*Glycine max* L.) after the application of desiccation products. SP - Spotlight Plus, B - Beloukha

The yield is harvested and analyzed on 5 September 2022. The lowest yield was recorded on the untreated plots. The increase in variant SP 0.75 is by 8%. For all of the other variants the enhancement is by about 9%, compared to the untreated control.

Table 1. Parameters of the yield of soybean. SP - Spotlight Plus, B - Beloukha

Variants	TKW (g)	Moisture content (%)	Oil content (%)
Control	161.72	12.53a	36.45
SP 0.5	165.35	10.68c	36.33
SP 0.75	169.14	11.23bc	36.18
SP 1	156.77	11.93ab	37.2
SP 2	159.54	10.8bc	35.63
B 16	168.46	10.9bc	37.3
B 32	164.12	10.7c	36.73

The application of the different herbicides affected also the moisture content of the treated plants (Table 1). The moisture content was the highest in the untreated plots. There was a slight decrease in all of the other variants, varying between less than 5% in variant SP 1, and 15% in variant SP 0.5 respectively. The thousand kernel weight (TKW) as well as the oil content of soybean were not significantly affected by the treatments.
In the research of Havstad et al. (2022) a comparison between the action of Spotlight Plus, Beloukha and other herbicides was made. The effect of Beloukha was satisfactory, but as the price of that type of organic products is still high, this could be consider as a disadvantage. The treatment with Spotlight Plus had minimal or no effect in the experiment with clover, but in a research for its action in potato, the product was among the most perspective desiccants for that crop (Haesaert et al., 2004).

CONCLUSIONS

The experimental results showed that the selective herbicide Spotlight Plus, containing 60 g/l carfentrazone-ethyl, could be successfully used for pre-harvest desiccation on soybean in Bulgaria. The data demonstrated that the desiccation rate of leaves, stems and beans were higher on the sulfentrazone-ethyl-treated plots, compared to the untreated controls. According to the experimental doses, higher rates led to better desiccation, especially on pods, when applied at the rate of 2 l/ha. Two weeks after the application, some of the test plants demonstrated the tendency of recovering. The effect was satisfactory only when the highest doses were applied. Regarding the yield, the results show that both of the tested products were able to increase it and the differences were statistically proven. No statistically proven differences in oil content and thousand kernel weight were measured. After the analyses performed, as the tested product did not negatively affect the soybean yield quality or quantity, we could recommend the use of carfentrazone-ethyl in soybean in Bulgaria as a good alternative to the widely used active ingredients glufosinate and diquat.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

TESTING OF RETARDANTS ON DURUM WHEAT

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Abstract

In 2018-2021, the following retardants were tested at the Experimental and Experimental Base of the Agricultural University of Plovdiv, Bulgaria: Medax Top (1000 ml/ha); Baya (1500 ml/ha) and Bogotá (2500 ml/ha). The treatment of Durum wheat (Desf.) variety Saya was carried out in phenological phase BBCH 30-39. It was found that the highest grain yield was obtained when the durum wheat variety Saya was treated with the Medax Top retardant (1000 ml/ha), with an average of 4.632 t/ha or 9.0 % more than the water control over the three-year period. In second place is the Bogota retardant (2500 ml/ha) with a grain yield of 4.507 t/ha or 6.0%, followed by the Bayja 1500 ml/ha retardant with a yield of 4.410 t/ha or 3.7% more than the control.

Key words: *durum wheat, retardants, grain yield.*

INTRODUCTION

Durum wheat is one of the most significant socio-economic cereals. In terms of worldwide distribution, it holds second place after bread wheat. It comprises 10% of the wheat production and 8% of the sown land. The attention directed towards this culture is determined above all by the quality of its grain. It depends on the genetic traits of the variety, the technology of growing and harvesting, as well as on abiotic factors during the period of formation and filling of the grain, its storage and processing.

Durum wheat is an excellent raw material for the production of pasta, and the quantity of protein and gluten strength are the most important characteristics (Feillet & Dexter, 1996). On the international markets, durum wheat (*Triticum durum* Desf.) is highly valued also for its glossiness and hard grain with an amber-yellow colour due to the high content of yellow pigments.

In Bulgaria, as a country located between the 40th and 45th parallel, where Northern Spain, Southern France and Central Italy are positioned, and where the land occupied by durum wheat in these countries is significant, a tendency to increase the occupied areas and a restored interest in this traditional culture is expected in our country. The increased demand on the European markets and insufficient supply makes its cultivation profitable and economically rewarding.

It is of interest to study the influence of various growth regulators - stimulants, retardants and anti-stress products on the yield and chemical-technological properties of durum wheat grain and their inclusion in the cultivation technology (Delchev, 2004; Delchev, 2009; Delchev & Panayotova, 2010; Kolev et al., 2006; Jalal. A. Al-Tabbal et al., 2006).

The purpose of the conducted experiment is to establish which of the tested retardants - Medax Top, Baya or Bogota - is the most suitable for growing durum wheat.

MATERIALS AND METHODS

The experimental work was carried out in field conditions on the territory of the Department of Plant Growing at the Educational Experimental and Implementation Unit of the Agricultural University of Plovdiv - Bulgaria for three years, i.e. 2018-2021. The following retardants were tested: **Medax®Top** (active substance - prohexadione-calcium 50 g/l (calcium 3,5-dioxo-4-propionyl cyclohexane carboxylate) + mepiquat chloride 300 g/l (1,1-dimethylpiperidine chloride) in a dose of 1000 ml/ha. The treatment was carried out during phenophase BBCH 30-39 (elongation of the stem - the flag leaf). Recommended volume of the working solution: water 300 l/ha. Recommended acidity of the working solution

4.5-7 pH. Manufacturer: Company BASF SE, Germany.

Baya (active substance - 480 g/l ethephon) in a dose of 1500 ml/ha to limit the growth of the aerial parts of the plants. Application time: BBCH 31-39 (the first node is at least 1 cm above the tillering node - the flag leaf is fully opened). Quantity of working solution: water 300 l/ha. Maximum number of applications: 1. Authorization holder: ADAMA Agriculture B.V., Arnhemseweg 87, 3832 GK Leusden, The Netherlands.

Bogota® (active substances - 300 h/l chlormequat; 150 g/l ethephon) in a dose of 2500 ml/ha. Authorization holder: ADAMA Agriculture B.V., Arnhemseweg 87, 3832 GK Leusden, The Netherlands. Time of application BBCH 31-37 (1st node is at least 1 cm above the tillering node - flag leaf barely visible, still curled). Quantity of working solution: water 300 l/ha. The tested retardants were compared with a water control: 300 l/ha

The indicated retardants were tested on durum wheat variety Saya, which was recognized as an original one in 2016 (Executive agency for variety testing, approbation and seed control, 2016). The variety was created at the Institute of Field Crops in Chirpan by the method of intraspecific hybridization, by crossing the Bulgarian durum wheat variety Zagorka and the M-5359 line, and selection in subsequent generations. The Saya variety belongs to the medium-tall durum wheat group and has a very low lodging resistance compared to the Predel and Saturn 1 standard varieties.

Saya variety durum wheat is grown according to the adopted "Technology for the production of durum wheat" approved as a scientific product by the Standing Committee on Innovations and Technologies at the Agricultural Academy - Sofia with protocol RD-09-10 of 10.11.2017 (Bozhanova et al., 2018). Durum wheat was sown after the chickpea predecessor in the period of 20.10. until 5.11. with a sowing rate of 500 germinating seeds/m^2 and fertilization with 140 kg/ha P_2O_5 and 160 kg/ha N as an active substance, and the entire quantity of phosphorus fertilizer and 1/2 of the nitrogen fertilizer was applied before sowing, and the remaining ½ quantity of the nitrogen fertilizer was used early in the spring as nutrition.

The field experience was carried out on carbonate alluvial-meadow soil Molic Fluvisols (FAO - UNESCO, 1990), which has an average sandy-clay mechanical composition, a humus content of 1-2% and a slightly alkaline pH reaction (7.2-7.7), presence of carbonates (4.3-7.4%) and lack of salts. The content of nutrients in the soil layer from 0-20 cm is as follows: N - 15.1 mg/1000 g; P_2O_5 - 30 mg/100 g; K_2O - 45 mg/100 g. (Popova & Sevov, 2010). The soil has good physico-mechanical properties, loose structure, low plasticity and stickiness with good moisture ability and filtration ability (Tahsin & Popova, 2005).

The following structural elements of the yield were recorded: productive tillering (pcs.), wheat-ear length (cm), number of grains in the wheat-ear (pcs.), mass of the grains in the wheat-ear (g), and from the physical parameters of the grain - mass per 1000 grains (g), hectolitre mass (kg) and glossiness quality (%). Grain yield (t/ha) was reported by variants and repretitions. The harvest was carried out at full maturity, by direct harvesting with a small trial harvester Wintersteiger seedmaster universal.

The grain samples taken from the tested varieties of durum wheat were qualified according to the following indicators: mass per 1000 grains (TGW) according to BDS ISO 520:2003; hectolitre mass according to BDS ISO 7971-2:2000; glossiness quality of the grain according to BDS EN 15585:2008; protein calculated according to the formula N x 5.83 according to BDS 13490:1976; gluten BDS EN ISO 21415-1:2007 in Accredited laboratory complex for testing at the Agricultural University - Plovdiv.

BIOSTAT software was used for the statistical processing of the obtained data on the studied indicators (Penchev, 1998).

RESULTS AND DISCUSSIONS

Productivity and grain quality of durum wheat are affected by rainfall and its distribution and air temperatures during plant vegetation. Figures 1 and 2 show the quantity of precipitation and average monthly temperatures compared to the climate norm.

The quantity of precipitation during the vegetation of durum wheat in all three years of the field experiment (2018-2021) exceeded those for a multi-year time period. Their amount is as follows: in 2018/2019 - 466.1 mm, in 2019/2020 - 478.9 mm and in 2020/2021 - 467.4 mm compared to 419.6 mm for the climatic norm (Figure 1).

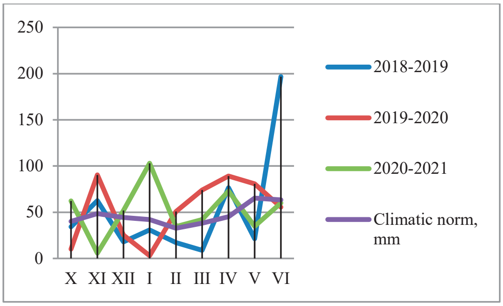

Figure 1. Precipitation by months, mm

Regarding the temperatures during the vegetation of durum wheat, higher average monthly temperatures were observed compared to the climatic norm, except for the months of November and April 2020-2021 and the month of April 2020 (Figure 2).

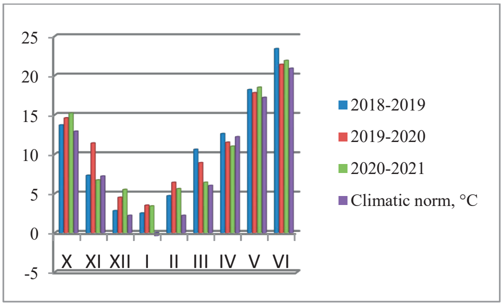

Figure 2. Monthly temperatures (average) (°C)

It is observed that the rainfall during the phenophases requiring normal moisture in the plant development determining the amount of the yield and the quality of the grain in the 2020-2021 harvest is more favourable for the growth of durum wheat. The spring of 2020 was characterized by more precipitation during the flowering period in the months of April and May by 44 mm and by 15.4 mm, respectively compared to a multi-year period, which negatively affected the pollination and fertilization of the flowers and this led to to formation of fewer grains and lower grain yield in 2020 (Figure 1 and Figure 2).

Biometric indicators

Plant height

Researchers such as (Berova et al., 2013) have proven the effect of phytohormones and synthetic regulators on the growth of durum wheat.

The tested retardants had varying degrees of effect in reducing plant height. The highest effect was obtained with the retardant Medax top in a dose of 1000 ml/ha, where the plants were lower by 2.7 cm compared to the water control. Followed by the retardants Bogota in a dose of 2500 ml/ha and Baya in a dose of 1500 ml/ha, in which the reduction of the stem height of durum wheat was respectively 1.4 cm and 0.9 cm. (Table 1[a]).

Table 1[a]. Biometric data (average 2018-2021)

Retardants	Plant height, cm	Productive tillering, number	Wheat-ear length, cm
Medax top 1000 ml/ha	85.2	2.48	8.23
Baya 1500 ml/ha	87.0	2.21	7.95
Bogota 2500 ml/ha	86.5	2.33	8.11
Water control	87.9	2.15	7.86
GD 5 %	1.57	0.16	0.21

Productive tillering varies depending on the applied retardant in the range of 2.21 to 2.48 tillers. A more significant increase in productive tillering was observed with the Medax top retardant by 15.3% (0.33 tillers) and with the Bogota retardant by 8.4% (0.18 tillers) (Table 1[a]).

The length of the wheat-ear

In their research, a number of authors 3emonstrate (Kolev & Terziev, 1996) that the length and density of the wheat-ear is primarily a characteristic of the variety, but the soil and climatic conditions, as well as the cultivation technology, significantly affect the values of this indicator. A favourable combination of these conditions has a positive effect on this indicator. The conditions in April and May of 2021 during the formation of the wheat-ear elements were favourable, which resulted in the reported good values of the wheat-ear length indicator. On average, for the three-year study period, the wheat-ears were the longest in the variants treated with the Medax top and Bogota retardants, respectively by 0.37 cm (4.7%) and by 0.25 cm (3.2%) more than the water control

(Table 1[a]). It can be seen that the application of all three retardants has a positive effect on wheat-ear length (Table 1[a]).

Number of spikelets in the wheat-ear

The number of spikelets and favourable conditions during flowering and fertilization ensure the formation of well-seeded wheat-ears. Weather conditions are good in this period in the spring of 2019 and 2021, which is a good prerequisite for the formation of more spikelets in the wheat-ear of durum wheat variety Saya. The largest number of spikelets was reported in the plants treated with Medax top - 29.1 pieces (8.2%) and in Bogota 28.3 pieces (5.2%) (Table 1[b]).

Table 1[b]. Biometric data (average 2018-2021)

Retardants	Number of spikelets in a wheat-ear	Number of grains per wheat-ear	Mass of grains in the wheat-ear
Medax top 1000 ml/ha	29.1	52.1	2.32
Baya 1500 ml/ha	27.5	48.7	2.26
Bogota 2500 ml/ha	28.3	50.4	2.19
Water control	26.9	47.2	2.11
GD 5%	1.32	3.12	0.14

Number of grains in the wheat-ear

This indicator is strongly related to yield. According to (Bergman et al., 1991), the increase in yield is due to the increased number of grains in the ear. The indicator is closely related to the conditions during the formation of spikelets and flowers. Another author (Rabie, 1996) reported an established relationship between the number of grains formed and the duration of flowering. The supply moisture to wheat during flowering and grain formation is of great importance, both for the number of grains in the ear and for their normal development. Researcher (Richards, 2004) highligths as one of the main focus for the development of modern selection, the increase in the number of grains in the wheat-ear. According to (Sekhon et al., 1994), the increased yield achieved in recent years is probably related to the increased number of grains in the wheat-ear.

The number of grains in the wheat-ear of the main tiller is of essential importance for the achievement of the productive abilities of the variety, as well as for the size of the yield. The number of grains formed depends a lot on the climatic conditions during flowering and fertilization. From the data presented in Table 1, it is clear that the most grains in the wheat-ear were formed in those treated with the Medax top retardant - 52.1 pcs (10.4%). The plants treated with the retardant Bogota follow next - 50.4 pcs (6.8%) and Baya - 48.7 pcs (3.2%) more than the water control (Table 1[b]).

Grain mass in the wheat-ear

Another very important indicator of the productive abilities of the variety and the size of the yield is the mass of grains in the wheat-ear. An important role in the time of formation of the grain is played by both weather conditions and various agrotechnical activities during the cultivation of durum wheat. Higher grain mass values in the wheat-ear compared to the water control were reported for all three retardants tested. For Medax top - 2.32 g (10.0%), Bogota - 2.26 g (7.1%) and Baya - 2.19 g (3.8%) (Table 1[b]).

Grain yield

Grain yield is the most important and accurate criteria for the influence of soil, climate, organizational and technological factors. Thus, the application of retardants together with the introduction of new, more productive varieties and durum wheat cultivation technologies are some of the most effective factors in increasing grain production and satisfying consumer needs. Obtaining more and high-quality grain is unthinkable without optimizing the varietal composition, sowing density, fertilization, disease, enemy and weed control, harvesting, storage and processing of durum wheat.

During the three-year period of the experiment, it was found that the highest grain yield was obtained when the durum wheat variety Saya was treated with the Medax Top retardant at a dose of 1000 ml/ha, and for the three-year period an average of 4,632 t/ha or 9.0% more was achieved than the water control.

The yield increase for plants treated with the Medax Top retardant by year is as follows: in 2019 by 0.439 t/ha, in 2020 by - 0.413 t/ha and in 2021 - by 0.290 t/ha. In second place in terms of productivity is the vairant treated with the Bogota retardant in a dose of 2500 ml/ha with a grain yield of 4.507 t/ha or by 6.0% more followed by the variant with the Baya retardant in a dose of 1500 ml/ha with a yield of 4.410 t/ha or 3.7% more than the water control (Table 2).

Table 2. Grain yield, t/ha

Retardants	2019 t/ha	2020 t/ha	2021 t/ha	Average	
				kg/ha	%
Medax top 1000 ml/ha	4.792	3.984	5.119	4.632	109.0
Baya 1500 ml/ha	4.592	3.657	4.981	4.410	103.7
Bogota 2500 ml/ha	4.659	3.825	5.038	4.507	106.0
Water control	4.353	3.571	4.829	4.251	100.0
GD 5%	0.296	0.245	0.193		

Physical and chemical-technological indicators

Thousand Grain Weight (TGW)

TGW is a physical indicator that characterizes the density of the grain; it depends on the characteristics of the variety and on the growing conditions. The low mass per 1000 grains means poorly fed grain and happens in the case of unfavourable weather conditions during the filling of the grain. From the data presented in Table 3, it is observed that the mass of 1000 grains of the tested retardants is within the limits of 42.1 g for the water control to 43.4 g for those treated with Medax top (Table 3).

Table 3. Physical and chemical-technological indicators

Retardants	TGW (g)	Test Weight (kg/hl)	Glossiness (%)	Protein (%)	Gluten (%)
Medax top 1000 ml/ha	43.4	76.2	97.2	14.89	31.9
Baya 1500 ml/ha	43.1	74.9	96.8	14.32	30.5
Bogota 2500 ml/ha	42.6	75.5	96.2	14.47	31.2
Water control	42.1	74.7	95.5	14.25	30.1
GD 5%	1.22	1.47	1.64	0.59	1.06

Test Weight

Test Weight is a species and variety indicator. It is a generally adopted trade indicator and is a measure of the health status of plants. According to BDS, 76-82 kg/hl is accepted as a base for the wheat. The highest values of this indicator were reported for Medax top 76.2 kg/hl, followed by Bogota with 75.5 kg/hl and Baya - 74.9 kg/hl, while in the water control the hectolitre mass was 74.7 kg/hl (Table 3).

Glossiness

Glassy quality is highly correlated with protein and starch content. It is influenced by many factors, but the genetic predisposition of the variety, the use of nitrogen fertilizers and the weather conditions during the formation and ripening of the grain are decisive.

In relation to the retardants studied, the glossiness quality varies from 96.2% for Bogota, 96.8% for Baya to 97.2% for Medax top. The increase of this indicator is respectively by 0.7%, 1.4 % and 1.8% compared to the water control (Table 3).

Protein

The significance of protein content for the technological qualities of wheat is direct and indirect. The yield of gluten closely correlates with it (Saldzhiev, 2008).

In the course of the field experiment, it was found (Table 3) that the protein content increased with all tested retardants. When treated with the Medax top retardant, the protein content was the highest at 14.89%. The variants treated with Bogota 14.47% and Baya 14.32% follow (Table 3).

Gluten

It has been established that the amount of protein and gluten depends up to 70% on the environmental conditions and on the cultivation technology and up to 30% on the genetic traits of the variety.

One of the important indicators of wheat quality is the quantity and quality of gluten. The amount of gluten depends more on the consistency than on the grain size. Only with the same consistency does the larger grain contain more gluten. The average gluten content for the experimental period was higher in the variant treated with the retardant Medax top - 31.9%, followed by Bogota - 31.2%, Baya - 30.5% and the control - 30.1% (Table 3).

CONCLUSIONS

The productivity of Saya durum wheat variety is the highest when treated with the Medax Top retardant (1000 ml/ha), with an average of 4.632 t/ha, or by 9.0% more grain than the water control over the three-year study period. Second comes the retardant Bogota (2500 ml/ha) with a grain yield of 4.507 t/ha or by 6.0% more that the control, followed by the

retardant Baya 1500 ml/ha with a yield of 4.410 t/ha or by 3.7% more than the control variant.
Plants treated with the Medax Top retardant are of the lowest height.
The structural elements of the yield, including productive tillering, wheat-ear length, number of grains in the wheat-ear, mass of grains in the wheat-ear have the highest values in the variants sprayed with the Medax Top retardant.
Plants treated with the Medix Top retardant form the most glassy grain with a high protein and gluten content.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Centre of research, technology transfer and protection of intellectual property rights - Project 14-17, Agricultural University, Plovdiv, Bulgaria.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF THE GENETIC VARIABILITY OF WINTER PEA VARIETIES (*Pisum sativum* L.) FROM THE COLLECTION OF IPGR - SADOVO

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Abstract

Subjects of the present research paper are 48 pea specimens - winter varieties taken from the collection of the Institute for Plant Genetic Resources - Sadovo. The study aims at establishing the rate of genetic similarity and genetic remoteness of the specimens kept in the national gen-bank. The specimens are mainly of French and Bulgarian origin. A mathematical approach was used for their group formation - a cluster, correlation and factor analysis using the following indicators - total nitrogen, crude fibres, crude ash, total sugars and tannins. There were established strong negative correlations between: total nitrogen and crude fibres (r = - 0.896); total nitrogen and crude ash (r = -0.853), and total nitrogen and total sugars (r = - 0.886). The group formation of winter pea varieties allows higher objectivity in evaluation, as well as more possibilities for use of pea collection.

Key words: *peas (Pisum sativum L.), total nitrogen, crude fibres, crude ash, total sugars and tannins, genetic similarity and remoteness, correlation, cluster analysis, factor analysis.*

INTRODUCTION

Climate changes call for the search of new alternative solutions in the structure of crops and their varietal composition. The complex evaluation and analysis of plant genetic resources /PGR/ and their main indicators as part of the national and some international collections, as well as the grouping of plant materials by significant qualitative features, is a necessary condition for their preservation, management and use (Angelova & Sabeva, 2018; Kalpakchieva, 2004; Mikic et al., 2021; Sabeva, 2019; Stanek et al., 2004).

Cereals and legumes, including peas, have indisputable contribution for solving the protein problem worldwide. Peas` high nutritive value is determined by the quality and quantity of plant protein, which contains the necessary amino-acids.

Peas is rich in starch - 45-50%; crude protein - 25-30%; fibres - 6-15%; ash - 2-5%; microelements and lysine from 1.4 to 1.8%. Taking into account the nutrients and the calories, mature pea seeds exceed meat almost 3 times; fish - 4 times; rye and wheat bread - 1.5 times; potatoes - 3.5 times; and cabbage - almost 6 times. Peas` chemical composition, as well as its ecological plasticity and adaptability, determine to a great extent the wide area of distribution and make it an irreplaceable source of protein (Sabeva et al., 2014; Sabeva, 2019).

The crude protein content is a varietal feature, which is influenced to a great extent by soil and climate - the conditions necessary for grain formation (Mikic et al., 2012; Vasileva & Kosev, 2021).

Variation of crude protein influenced by external factors is most highly expressed in the high-protein varieties, reaching up to 10%. Taking into account the protein content at deviation of 1,5%, the variety is considered stable regarding this indicator. The bigger the variation amplitude is, the more interesting the studied material is for the selection. (Ali Khan et al., 1995; Cervenski et al., 2017; Sabeva, 2019)

In a study of ours, ranging over 15 years, we have established that the content of *crude* protein in winter pea varieties is influenced by the year and the variety, as well as their interaction - *year x variety* (Sabeva, 2019; Sabeva & Angelova, 2022). It has been mathematically proven. The year has the strongest influence - 61.2%, followed by the variety - 32.4%, and the weakest influence has the interaction year x variety - 6.4%.

A number of authors, based on their studies, have reported that crude protein content varies depending on seed origin and soil-climatic conditions - from 20.35% to 31.35%. (Ali Khan et al., 1995*)* have proved that crude protein content varies from 21.1% to 28.3%, according to the region of cultivation in Canada. Within the frames of one and the same variety, variation is from 24.0% to 26.3%, and with relation to the year, it is from 25.8% to 27.4%.

With relation to the soil-climatic conditions, there have been established fluctuations in the following: crude fibres - from 3.0 to 6.0%, mineral salts - from 2.0 to 3.1%, crude fats - from 1.3% to 1.5%.

Seed size is a quality feature, which varies within wide limits depending on the soil-climatic conditions and the agro-machinery. Studying the dependence between seed size and crude protein content, it has been established the correlation coefficient - r= 0.42, which shows that it is difficult to combine high protein content and large grain (Gueguen & Barbot, 1988; Srivastava et al., 2009)

A number of studies have reported that pea genotypes possessing seeds with smooth and wrinkled surface and cultivated in the same environment have diversity between varieties with relation to the crude protein content (Gueguen & Barbot, 1988; Odoardi et al., 2004). Some researchers suggest that varieties with wrinkled seeds have higher contents of sugar than these with smooth seed surface. Some Canadian authors have proven in their studies that there is negative correlation dependence between the raw protein and starch content. They claim that specimens with high starch level have low content of raw protein and raw fibres (Gueguen & Barbot, 1988; Sabeva, 2019).

According to a study conducted by Angelova and Sabeva (2013), it has been reported the presence of a weak negative correlation only between crude protein and crude fibre content. Taking into account the pea varieties and their grouping by crude protein content, authors have reported that there is no proven correlation between the morphological features of seeds, the weight of 1000 seeds and their phenotype.

Vasileva and Kosev (2021) have reported for negative correlation dependence between seed yield and phosphorus content (r = - 0.35), crude protein (r = - 0.18) and crude fibres (r = - 0.03).

With relation to the source forms for selection, more and more attention is paid not only to the morphological and productive characteristics, but to the qualitative indicators also, which determine the nutritive value of forage. There is search of genotypes combining high nutritive quality and high yield. Taking into account the choice of qualitative indicators, it is important to clarify the variability in the contents of chemical compounds, which determine the qualitative composition of forages (Burstin and Duc, 2005; Mikich et al., 2012).

IPGR keeps up a collection of over 2500 pea specimens including old varieties, forms and populations, as well as newly-selected varieties and lines.

Systematization of the information related to the evaluation of pea specimens by qualitative features, along with their chemical characteristics, give possibilities for increase of effectivity of the selection programs (Sabeva, 2019).

The present study aims at establishing the rate of genetic similarity and remoteness of a cross section including 48 winter pea specimens by some bio-chemical indicators in order to be used effectively in the selection work with peas.

MATERIALS AND METHODS

Plant material

The studied cross section of winter pea varieties (48) consisted of specimens originated mainly from Bulgaria and France. The predominant varieties were early and middle-early forms for the use of green mass and grain. The specimens had leaves of a common type. 13 of them were white-blossom, and the rest 35 - purple-blossom. Seeds were round, sometimes with an irregular shape, and had a smooth surface. Coloration was diverse and varied from light-beige to light-brown, particolored, from grey-green and dark-green to dark-brown (Angelova & Sabeva, 2013; Sabeva et al., 2014; Sabeva, 2019).

Evaluation of the specimens regarding their content of: *total nitrogen (X1)*, *crude fibres*

(X2), crude ash (X3), total sugars (X4) and concentrated tannins (X5) was conducted in the bio-chemical laboratory of IPGR - Sadovo.

The content of *total nitrogen/ crude protein* in grain was determined by Kjeldhal`s Method; *crude fibres* - by Heneverg and Shtoman; *crude ash/total sugars* - by Schoorl`s Method (A.O. A. C, 1990); *concentrated tannins* in % - by Terrill et al. (1992) with butanol-salt acid (Ilieva and Dochkova, 1999).

Soil-climatic characteristics

The specimens were cultivated in the experimental field of IPGR, on cinnamon forest soil. The town of Sadovo is situated in the Upper Thracian Plain, with an altitude - 141 m and having continental climate (42º70´58´´ N; 024´55´58´´ E.). Winter in the region is softer compared to Northern Bulgaria, and summer is hot. The average annual temperature is 12.4°C with a minimum value in January and a maximum value in July. In particular years temperatures in January and February decrease under - 18°C for 2-3 days, and in some cases – under - 20°C, which is important for the winter varieties. Precipitation sum has the highest values in May – 47.5 mm, and the lowest values in September – 14.9 mm (Boyadjieva & Stankova, 1990).

Variability of the examined indicators was determined through the use of variation analysis. The interaction between the studied indicators was evaluated and expressed through the correlation coefficient r.

The correlation analysis showed the presence of statistically significant correlations between the studied indicators, followed by a factor analysis technique (Kline, 1994) in order to reduce the number of the seven initially included indicators. The factor analysis was performed by the principle component method (PCA). The number of principle components was determined by the number of eigenvalues of the correlation matrix that were greater than 1 (Kaiser's criterion). Eigenvalues show the contribution of the eigenfactor when explaining the total dispersion in the variables.

The factor model is usually determined by the factor weights, which represent the correlation coefficients between the respective observed indicators and the factors. Thus, a smaller number of generalized factors are determined, without their own meaning, but combining the properties of several indicators.

Adequacy assessment of the factor analysis was performed by using the Kaiser - Mayer - Olkin (CMO - test) and Bartlett tests.

The grouping of 48-th examined specimens was performed through a hierarchical cluster analysis. The method of intergroup connection was used (Dyuran & Odelly, 1977; Desheva et al., 2016; Ward, 1963).

The mathematical and statistical processing of empirical data was performed through the statistical program SPSS Statistics 19.

RESULTS AND DISCUSSIONS

Variation and correlation analysis

A qualitative indicator showing the forage value of the pea grain is its chemical composition (Angelova & Sabeva, 2013; Sabeva et al., 2014; Sabeva, 2019).

Table 1 shows the main descriptive features of grain bio-chemical composition of the cross section of winter pea varieties for three years in average.

The variation coefficient is an important strategic indicator, which is used for establishing the homogeneity of the examined feature. The values of the variation coefficient varied in our research study within the limits from 5.54% to 49.29% (Table 1).

Data analysis showed that relatively most variable was the indicator *content of concentrated tannins* (CV= 49.29%). Tannin content in peas is related to the dark colouring of the flowers and seeds (Ilieva and Dochkova, 1999; Sabeva, 2019). With relation to our study, the variation limits of tannin content was from 0.01% to 2.71%. Winter PGR of peas in the present research included the whole number of 48 specimens, 35 of them had purple blossoms (*P. arvense*), 13 - white blossoms (*P. sativum*) and diverse coloration of seed coat. The concentration of concentrated tannins in seeds of both pea types corresponded to the results received by other authors (Cervenski et al., 2017; Kotlarz et al., 2011; Stanek et al., 2004).

Table 1. Parameter of main descriptive characteristics for grain bio-chemical composition of winter pea varieties for three years averagely

INDICATORS	Number of specimens	Min.	Max.	Mean	SE	SD	CV, %
Total nitrogen, %	48	3.74	4.65	4.19	0.03	0.23	5.54
Crude fibres, %	48	2.78	6.64	4.78	0.12	0.85	17.85
Crude ash, %	48	1.94	3.38	2.76	0.05	0.31	11.36
Total sugars, %	48	3.67	7.15	4.89	0.10	0.67	13.66
Tannins, %	48	0.01	2.71	1.63	0.12	0.80	49.29

The total nitrogen contents in grain varied within close limits, which was confirmed by the variation coefficient (CV= 5.54%). There was established a low variation coefficient for the rest of the indicators: crude fibres (CV= 17.85%); crude ash (C V= 11.36%) and total sugars (C V= 13.66%). The content of the biochemical indicators examined in the present study is comparable to the results for other pea varieties reported by other authors (Cervenski et al., 2017; Kotlarz et al., 2011; Srivastava et al., 2009; Stanek et al., 2004).

In order to establish and evaluate the relations between the studied indicators, a correlation analysis was applied (Table 2).

There were established strong negative correlations between: *total nitrogen and crude fibres* (r = - 0.896); *total nitrogen and crude ash* (r = -0.853) and *total nitrogen and total sugars* (r = - 0.886). A strong positive correlation was observed between: *crude fibers and crude ash* (r = 0.937); *crude fibres and total sugars* (r = 0.968) and *crude ash and total sugars* (r = 0.963) (Table 2).

The received results reporting for relations between the studied indicators confirm the results of numerous authors. Ali-Khan and Youngs (1973) in their study with 506 introduced plants reported that there was an insignificant negative correlational dependence between *crude protein content*, *seed yield* and *seed size*.

Other authors (Srivastava et al., 2009; Stanek et al., 2004; Vasileva and Kosev, 2021) reported for the presence of correlational relations between *seed coarseness* and *crude protein content*, as well as between *crude protein* and *starch content*.

Hierarchical cluster analysis

In order to identify the similarity and closeness of winter genotypes, it was applied a hierarchical *cluster* analysis. Evaluation of the genetic closeness between specimens was performed through a comparison of the content of: *total nitrogen, crude fibres, crude ash, total sugars and concentrated tannins.*

Table 2. A correlation matrix between the examined chemical indicators

	Total nitrogen	Crude fibres	Crude ash	Total sugars	Tannins
	x_1	x_2	x_3	x_4	x_5
x_1	1	-0.896**	-0.853**	-0.886**	0.154
x_2		1	0.937**	0.968**	0.159
x_3			1	0.963**	0.204
x_4				1	0.083
x_5					1

Cluster analysis results are presented graphically with a dendrogram (Figure 1), which shows the sequence of object collating and cluster formation. The studied winter pea varieties were grouped into six clusters and fourteen independent groups. The first cluster consisted of two subgroups - **"a"** and "**b**" and two independent groups. Genotypes forming the cluster were mainly French lines with high plants and purple blossoms. Specimens in subgroup **"a"** were close with relation to the studied indicators, only their tannin content exceeded the average value of the indicator 1.63% ± 0.12 for the group.

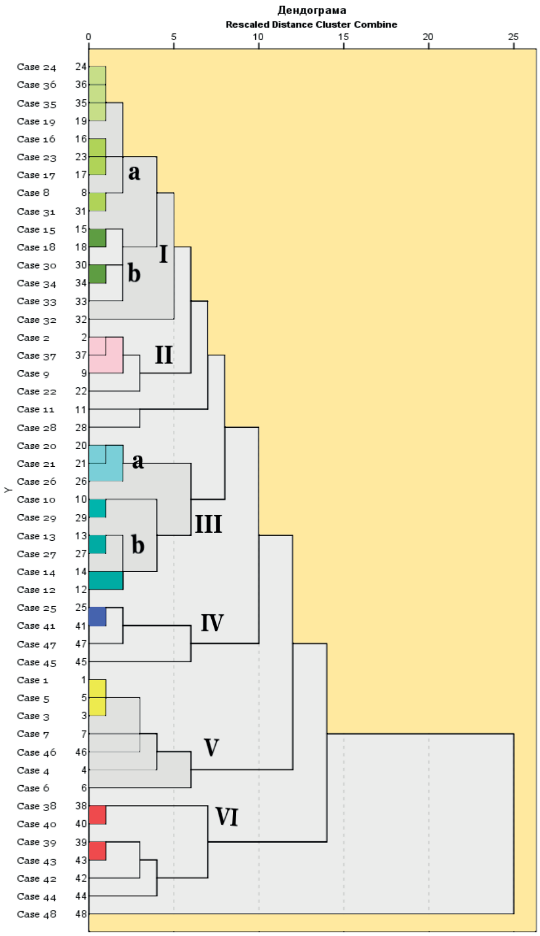

Figure 1. A dendrogram for the similarity of winter pea varieties

Subgroup "**b**"consisted of genotypes with higher content of total nitrogen and crude fibres than the average values of the group indicators as follows: 4.19% ± 0.03; 4.78% ± 0.12. The second cluster was formed by one subgroup and three independent groups. This cluster consisted of Mir variety and the populations 12 AB and П- 1 of Bulgarian origin, as well as three French lines. All, except the selection line 12 AB, were with purple blossoms. Specimens in this cluster had close values of the examined indicators, which were lower than the average reveal of the indicators. The third cluster also consisted of two subgroups - **"a"**and **"b",** and two independent groups. Varieties included in this cluster were characterized with close average values of the studied indicators, but with higher content of crude fibers and crude ash from the average reveal of the relevant indicators. They were different by phenotype, purple-blossom plants were predominant, only two of them – Pleven 10 and П-13 were white-blossom. The prevailing lines were those of French origin. Specimens in the fourth cluster were characterized with high content of total sugars and crude ash (with relation to the average value of the relevant indicator). Varieties in the fifth cluster were close by all studied indicators and did not contain tannins. Specimens in the sixth cluster had higher content of total nitrogen compared to the former group, and the rest of the studied indicators were under the average reveal of the indicators.

Dendrogram analysis showed that genotype formation into 6 groups was mainly due to the differences in the content of total nitrogen, crude fibres and tannins.

Most of the studied genotypes had good indicators and could be used as initial materials. The selection process could be directed towards the improvement of seed quality. Differences found in the present research study could be useful for the selection of potential parent couples for cross-breeding.

Analysis of the main components - PCA

Results from PCA application are presented in Table 3. Analysis showed that there were two own values, which determined the choice of *two* main components (Table 3).

The first component explained 35,09% of the total variation, and the second - 24,31%. The first component was related to the indicators - *crude fibres and total sugars*. *Total nitrogen, crude ash and tannins* formed the second component (Table 3).

Table 3. A factor matrix obtained by the method of the component principle

Factors	Components	
	PC 1	PC 2
Total nitrogen		0.660
Crude fibres	0.900	
Crude ash		0.415
Total sugars	0.846	
Tannins		0.762
% of the variance	35.09%	24.31%

Figure 2 shows the spatial visualization of the studied features and the relations between them, and Figure 3 presents the position of winter pea varieties in the component plane.

Taking into account the 48-th winter pea plants, there could be distinguished the following varieties as sources of total nitrogen variation: the French lines - 95202083, 302, 306 and 335; Pleven 10 Bulgarian variety and the populations - 35 [A], 37 [A], as well as Austria winter variety.

The rest of the specimens, which were closer to the centre of the component plane, could be unified into a core collection with relation to the studied features. This group of winter pea plants could be used for the creation of new varieties with appropriate qualitative features, as well as for their direct use in different production fields.

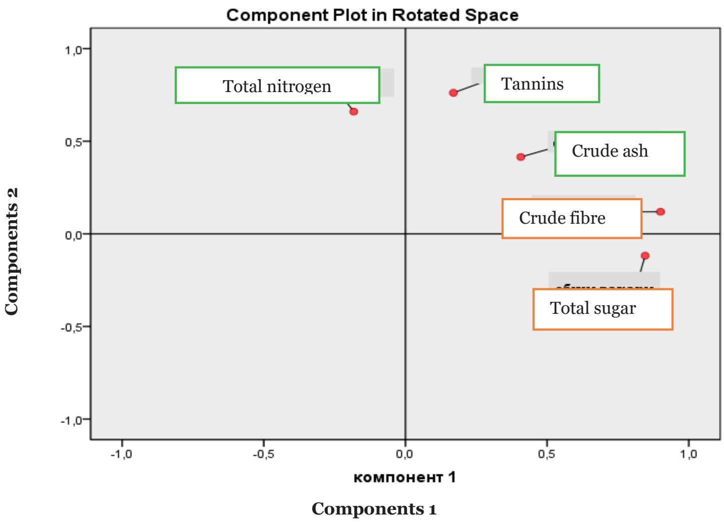

Figure 2. A projection of the examined features in the factor plane

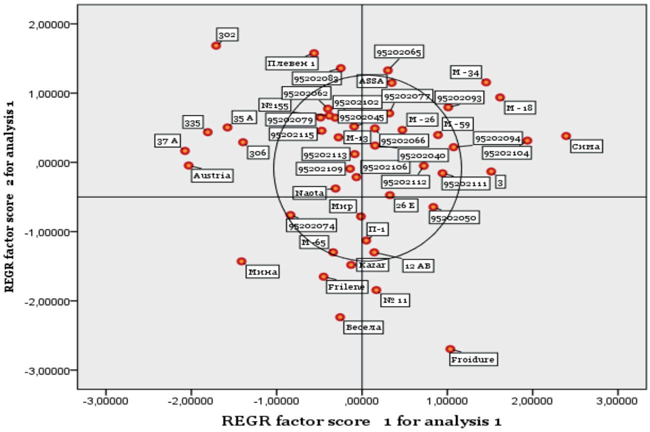

Figure 3. A projection of the examined specimens in the factor plane

CONCLUSIONS

In order to group genotypes by genetic similarity or remoteness, there could be successfully applied cluster and principle-component analyses. Their combined application allows the reception of a clearer concept for the influence of the studied indicators used for the differentiation of the examined genotypes.

There were established strong negative and positive correlations between: *total nitrogen and crude fibres* (r = - 0.896); *total nitrogen and crude ash (*r = -0.853), *and total nitrogen and total sugars* (r = - 0.886); *crude fibres and crude ash* (r = 0.937); *crude fibres and total sugars* (r = 0.968) and between *crude ash and total sugars* (r = 0.963).

Some winter pea plant genetic resources were determined as appropriate to be unified into core collections according to the examined indicators, as well as the possibilities to be included in different directions for application.

Database created according to the available connections between the studied indicators can be used in the selection and improvement work with peas for the search of relevant features.

The relationship " Yield - total ET " is best represented by Davidov's two-power formula. The same is expressed graphically through the S-curve, with a high correlation coefficient (R = 0.986) and value of powers n = 2.3 and m = 9.1.

The relationship Yield-evapotranspiration" by phases is the best presented by two-power formula with R = 0.921. The power's value for entire vegetation period is N = 1.3. The power values by phases are as follows: I period - m_1 = 0.05, II period - m_2 = 0.79, III period - m_3 = 0.49, IV period - m_4 = 0.28. this means that the sensitivity of the first period is the smallest, followed by the period including the time between the end of grain filling and ripening. The second and third periods show the highest sensitivity, with the advantage being on the side of the budding - flowering period.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

DETERMINATION OF VARIATION IN THE FATTY ACID COMPOSITION OF DIFFERENT FAR EASTERN WILD SOYBEAN ACCESSIONS AND IDENTIFICATION OF SUPERIOR GENOTYPES WITH OLEIC ACID CONTENT AS HIGH OLEIC POTENTIAL

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Abstract

Soybean [Glycine max (L.) Merr.] seeds contain 20% oil, 40% protein, and 12% soluble carbohydrates by dry weight. Because of its seed composition, it is one of the most economically important oil crops globally. Soybean is the largest oilseed crop worldwide with over half of the total oilseed production, followed by rapeseed, sunflower, and peanut. Direct progenitor of cultivated soybean [Glycine max (L.) Merr.], is wild soybean (Glycine soja Sieb. & Zucc.) and it is widely distributed in China, North Eastern Russia, Korea, Japan, and Taiwan. Wild soybean accessions originating from different geographical regions harbour genetic differences. Because of many genetic variations have been lost in the process of domestication of G. max, soybean breeders and geneticists are interested in identifying useful genes in G. Soja to improve different characteristics of cultivated soybeans. Therefore, it is necessary to collect and conserve the wild soybean from different regions. When soybean is consumed directly as food, polyunsaturated fatty acids such as linoleic and linolenic acid (ω-6, ω-3) are essential fatty acids. These fatty acids prevent diseases such as inflammation, cardiovascular, and Alzheimer's, as well as promote fetal development. The consumption rate between ω-6 and ω-3 concentration has an effect on human health. Healthy ratios of ω-6 to ω-3 fatty acids have been reported to range from 1:1 to 4:1. Generally, the ω-6/ω-3 ratio in the seed oils of wild (Glycine soja) and cultivated soybeans (G. max) is 3-4:1 and 6-7:1, respectively. Wild soybeans contain almost twice the α-linolenic acids ALA) in their seed oil. Twenty-four wild soybean accessions from three different countries were investigated under field conditions in 2021. The content of oil, oleic acid, linoleic acid, linolenic acid, palmitic acid and stearic acid ranged between 4.13-12.14%, 11.4-49.74%, 29.3-56.08%, 4.81-14.33%, 10.22-13.85% and 3.3-4.64%, respectively. Also ratios of ω-6 to ω-3 fatty acids varied between 3.368 and 6.091. To conclude, considerable variations in oil content, fatty acid composition, ω6/ω3 ratios were noticed among 24 wild soybean accessions in this study. The Nigata, Japan accession is one of the prominent wild soybean accession in the study with high oleic acid content (49.74%).

***Key words**: wild soybean, fatty acids, ω-6, ω-3, high oleic. molecular biology and biotechnology. CABI, Wallingford, UK. p. 165–168.*

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] seeds contain 20% oil, 40% protein, and 12% soluble carbohydrates by dry weight (Liu, 1997). Because of its seed composition, it is one of the most economically important oil crops globally. Soybean is the largest oilseed crop worldwide with over half of the total oilseed production, followed by rapeseed, sunflower, and peanut. Direct progenitor of cultivated soybean [*Glycine max* (L.) Merr.], is wild soybean (*Glycine soja* Sieb. & Zucc.) and it is widely distributed in China, North Eastern Russia, Korea, Japan, and Taiwan (Hymowitz and Singh 1987, Chung and Singh 2008). Wild soybean accessions originating from different geographical regions harbour genetic differences. Because of many genetic variations have been lost in the process of domestication of *G. max*, soybean breeders and geneticists are interested in identifying useful genes in *G. soja* to improve different characteristics of cultivated soybeans (Hyten et al., 2006). Therefore, it is necessary to collect and conserve the wild soybean from different regions.

Soybean is an important oil crop grown worldwide due to its diverse uses of oil and protein for humans and livestock. Soybean oil accounts for 60.85% of the world's oilseed production; therefore, it has become the most dominant vegetable oil (Abdelghany et al., 2020). Mainly, five major fatty acids are found in soybean oil: palmitic acid (11%), stearic acid (4%,), oleic acid (23%), linoleic acid (55%), and linolenic acid (8%). This profile of soybean oil has been modified depending on the end products of soybean oil to meet different demands of the markets (Fehr, 2007). Recent studies have revealed that soy food such as soy milk, tofu and soy souce has nutritious qualities that have positive effects on human health (Omoni and Aluko, 2005). This may have led to dramatic increases in soy food demand in the global market (Kulkarni et al., 2017). When soybean is consumed directly as food, polyunsaturated fatty acids such as linoleic and linolenic acid (ω-6, ω-3) are essential fatty acids. These fatty acids prevent diseases such as inflammation, cardiovascular, and Alzheimer's, as well as promote fetal development (Serhan et al., 2008; Swanson et al., 2012). Also, it is an important plant-based source of ω-3 polyunsaturated fatty acid for vegetarians and non-seafood eaters. Numerous studies have concluded that a higher intake of ω-3 fatty acid or fatty acids with relatively low ω-6/ω-3 ratios is appropriate from a human health perspective (Abel et al., 2004; Simopoulos, 2008). The consumption rate between ω-6 and ω-3 concentration has an effect on human health. Healthy ratios of ω-6 to ω-3 fatty acids have been reported to range from 1:1 to 4:1 (Mattson and Grundy, 1985; Renaud, 2002). A ratio of ω-6/ω-3 was found 5:1 ratio suppressed symptoms of asthma, for prevention of cardiovascular disease and improvement of blood circulation 4:1, 2:1 to 3:1 for suppression of rheumatoid arthritis; and 2.5:1 for inhibition of colon cancer cell proliferation (Simopoulos, 2008). Generally, the ω-6/ω-3 ratio in the seed oils of wild (*Glycine soja*) and cultivated soybeans (*G. max*) is 3-4: 1 and 6-7: 1, respectively (Asekova et al., 2014; Dhakal et al., 2013). Wild soybeans contain almost twice the α-linolenic acids ALA) in their seed oil (Pantalone et al., 1997). The accumulation of a high concentration of ALA in wild soybean was due to a different set of desaturate alleles controlling ALA in cultivated soybean (Pantalone et al., 1997). Therefore, it is necessary to develop new cultivated soybean lines with high ALA concentration using wild soybean as a genetic resource for increasing ALA lowering ω-6/ ω-3 ratios and concentration by classical breeding.

The functionality of soybean oil for both food and industrial uses is influenced by the fatty acid profile of the oil (Lee et al., 2008). Modification of saturates, oleic acid, and linolenic acid via breeding and biotechnology are being emphasized to develop desired fatty acid phenotypes. It is not practical to commercially develop all oil phenotypes (Asekova et al., 2014). To breed new varieties for desired traits, the gene pool of cultivars must be broadened by introducing wild species, landraces, and exotic germplasm into breeding programs.

Wild soybeans have been reported to contribute new and unique genes for high yield, high protein, resistance soybean cyst nematode, tolerance to salt, and high linolenic acid (Wang et al., 2004; Kabelka et al., 2005; Nichols et al., 2006; Lee et al. 2009; Pantalone et al., 1997; Lee et al., 2010). Therefore, the wild soybeans can be utilized as a genetic resource to develop new varieties with the desired fatty acid composition. The aim of this study was to evaluate the distribution and characterics of the major fatty acids of wild soybean accessions belong to some Far East countries.

MATERIALS AND METHODS

Plant Materials

Twenty-four wild soybean accessions belonging to South Korean, China and Japan wild soybean habitats were used as a plant material (from Chung's Wild Legume Germplasm Collection, Chonnam National University, Yeosu, South Korea) (Table 1).

Accessions were grown at the Experimental Farm of Cukurova University (36°59′N, 35°18′E, and 23 m), in Adana (Turkey) in 2021. In the Adana province, a typical Mediterranean climate prevails and the winters are warm and rainy whereas the summers are hot and dry. Before planting, 200 kg ha^{-1} of DAP (36 kg ha^{-1} N, 92 kg ha^{-1}P) fertilizers were applied. Ammonium nitrate (33%N) at the rate of 200 kg ha^{-1} was applied once before the first irrigation. Additional Ammonium nitrate (33% N) at the rate of 70 kg ha^{-1} was applied once before the first irrigation. (R1 stage). Soybean accessions were sown in the first week of May with distance of 2 m and row-to-row distance of 2 m according to a completely randomized design with three replications.

Table 1. Wild Soybean Material List

1	Jeonnam Shinan Abhaedo, Korea	**13**	Incheon Ganghwa Buleumdo, Korea
2	Jeonnam Shinan Bigeumdo, Korea	**14**	Yeongheungdo, Ansan, Gyeonggi-do, Korea
3	Gyeongnam Tongyeong Yokdo, Korea	**15**	Gyeongbuk Yecheon, Korea
4	Jeonnam Shinan Im Jado, Korea	**16**	Gyeongbuk Andong, Korea
5	Gangwon-do, Korea	**17**	Jilin, China
6	Gangwon Samcheok, Korea	**18**	Wonju, Gangwon, Korea
7	Liaoning, China	**19**	Gyeongbuk Uiseong, Korea
8	Liaoning, China	**20**	Jilin, China
9	Aomori, Japan	**21**	Liaoning, China
10	Akita, Japan	**22**	Ichinoseki, Japan
11	Niigata, Japan	**23**	Yamagata, Japan
12	Chuncheon, Gangwon, Korea	**24**	Japan Kumamoto

Oil extraction and GC analysis

The samples of 24 accessions grown in 2021 were subjected to oil extraction using a Soxhlet apparatus and a gravimetric method.

The experiments were performed three replicates and seeds were bulked (20 g from each replication) and 5 g clean and mature seed samples were taken for oil content and fatty acid analysis.

The oil content was determined by comparing the weights of 5 g seed samples before and after extraction using a Soxhlet apparatus (FOSS) with petroleum ether for 4 hours.

An oil sample of 500 mg was dissolved in 2 ml isooctane followed by 1.5 ml of 0.5 M methanolic NaOH. The tube was then vortexed and held in boiling water for 7 min and allowed to cool to room temperature. Two ml of BF_3 (Boron trifluoride) were added, vortexed, and held in boiling water for 5 min and allowed to come down to room temperature.

The tube was vortexed after adding 5 ml NaCl, centrifuge at 4,000 rpm for 10 min. The supernatant was used for GC analyses (AOAC 1984).

The fatty acid (FA) composition was analyzed using a GC Clarus 500 with auto sampler (Perkin Elmer, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m • 0.32 mm, ID • 0.25 lm, BP20 0.25 UM, USA).

The oven temperature was brought to 140°C for 5 min, then raised to 200°C at a rate of 4°C/min and to 220°C at a rate of 1°C/min, while the injector and the detector temperatures were set at 220°C and 280°C, respectively.

RESULTS AND DISSCUSSIONS

Oil Content and Fatty Acid Profile

The oil content and fatty acid compositions and their range of variation in 24 wild soybean accessions are shown in Table 2. The oil content of the 24 wild soybean accessions varied between 4.13-12.14%, and the average being 7.12%. The highest oil content was obtained from Yeongheungdo, Ansan, Gyeonggi-do, Korea accession, while the lowest value was obtained from Aomori, Japan**.** Different studies have shown that cultivated soybean genotypes have a higher oil content than wild soybean accessions (Kim and Park, 2005; Wee et al., 2017).

Fatty acid composition is one of the most important soybean seed quality traits and affected by genotype, environmental conditions, planting date, fertilization, and the interaction these factors (Kurt, 2018). A significant variation in oil content and fatty acids were found among the 24 wild soybean (*Glycine soja*) accessions as shown in Table 2. Average values for oil content and five major fatty acids components found in wild soybean 7.12, 12.34, 3.71, 18.48, 51.58, 11.58% oil content, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid, respectively. The palmitic and stearic acids fractions are saturated fatty acids and constitute 15% of the soybean oil. The remainder of the oil (about 85%) is made up of unsaturated fatty acids such as oleic, linoleic and linolenic acids (Lee et al., 2007). Since it is preferred that the amount of saturated fat of soybean oil used in food industry is low, the amount of saturated fatty acids of the characterized soybean genotypes was also determined. Palmitic acid is predominant saturated fatty acid in soybean oil and common soybean cultivars contain an average 1%1 palmitic acid. The lowest palmitic acid content was recorded for Nigata, Japan 2 (10.22%) accession, while the highest palmitic acid content was recorded for Gyeongnam Tongyeong Yokdo, Korea (13.85%) with 12.34 % average value. Stearic acid content varied between 3.3-4.64%. The highest value belonged to Gyeongbuk Yecheon, Korea accession, while the Yamagata, Japan accession had the lowest value. The observed stearic acid content in this study was low in comparison with previous studies (Qin et al., 2014; Abdelghany et al., 2020) but higher than reported earlier studies of *G. max* and *G. soja* (Fan et al., 2015; Wee et al., 2017; La et al., 2019). Also, palmitic acid content higher than previous studies of *G. max* and *G. soja* (Qin et al., 2014; Fan et al., 2015; Wee et al., 2017; Abdelghany et al., 2020).

Table 2. Fatty acids composition and ω6/ω3 ratios of wild soybean accessions

Origin	Oil %	Palmitic acid, %	Stearic acid, %	Oleic acid, %	Linoleic acid, %	Linolenic acid, %	ω6/ω3
Jeonnam Shinan Abhaedo, Korea	7.74	13.54	3.81	13.01	53.48	13	4.11
Jeonnam Shinan Bigeumdo, Korea	5.73	12.02	3.96	13.41	54.5	**14.33**	3.80
Gyeongnam Tongyeong Yokdo, Korea	5.41	**13.85**	3.43	**11.4**	54.62	13.7	3.99
Jeonnam Shinan Im Jado, Korea	4.71	13.38	4.01	14.91	52.18	13.78	3.79
Gangwon-do, Korea	5.55	11.46	3.65	13.85	56.94	12.06	4.72
Gangwon Samcheok, Korea	5.13	11.2	4	19.73	50ş79	12.51	4.06
Liaoning, China	5.41	12.42	3.51	13.3	55.8	13.18	4.23
Liaoning, China	7.32	12.05	3.58	12.33	55.26	13.67	4.04
Aomori, Japan	**4.13**	12.7	3.62	13.68	55.37	11.84	4.68
Akita, Japan	5.51	12.63	4.18	18.19	53.69	9.25	5.80
Nigata, Japan	8.23	**10.22**	3.49	**49.74**	**29.3**	**4.81**	**6.09**
Chuncheon, Gangwon, Korea	8.24	10.45	3.01	24.58	51.33	8.76	5.86
Incheon Ganghwa Buleumdo, Korea	11.52	12.97	3.58	19.02	52.3	9.95	5.26
Yeongheungdo, Ansan, Gyeonggi-do, Korea	**12.14**	13.21	3.36	14.24	54.59	11.76	4.64
Gyeongbuk Yecheon, Korea	8.13	13.56	**4.64**	22.91	47.44	9.63	4.93
Gyeongbuk Andong, Korea	7.42	12.75	3.54	16.14	51.92	13.43	3.87
Jilin, China	4.65	12.43	3.73	11.81	**56.08**	13.24	4.24
Wonju, Gangwon, Korea	6.96	13.27	3.68	13.79	51.3	15,23	**3.37**
Gyeongbuk Uiseong, Korea	7.67	10.85	4.18	23.11	52.21	7.886	6.62
Jilin, China	9.28	13.18	3.89	16.29	51.2	13.62	3.76
Liaoning, China	7.16	10.26	3.41	39.91	35.24	7.24	4.87
Ichinoseki, Japan	9.94	13.33	4.01	16.98	53.82	9.49	5.67
Yamagata, Japan	7.63	11.87	**3.3**	15.72	54.13	13.48	4.02
Japan Kumamoto	5.16	12.46	3.54	15.56	54.39	12.19	4.46
Ave.	7.12	12.34	3.71	18.48	51.58	11.58	4.62
Max.	12.14	13.85	4.64	49.74	56.08	14.33	6.09
Min.	4.13	10.22	3.3	11.4	29.3	4.81	3.37

Oleic and linoleic acids were found to be major unsaturated fatty acids in wild soybean accessions. The highest oleic (49.78%) and lowest linoleic acid (29.3%) percentage was recorded for Nigata, Japan 2 accession. Oleic acid content varied from 11.4-49.74% with an average value of 18.48%. Wee et al. (2017) reported that the oleic acid content of 319 wild soybean accessions they collected from different regions of Japan varied between 7.66 and 15.86%. La et al. (2018) reported a range of oleic acid varied between 10.7-16.2% in 80 wild soybean accessions from different origin. The higher concentration of oleic acid in soybean oil is preferred for wider use in food and industrial products, as it is more stable at high cooking temperatures (Lee et al., 2007; Warner and Gupta, 2005; La et al., 2014). Additionally, its high oleic acid content increases the oxidative stability of the oil and reduces the need for hydrogenation, which is negatively associated with heart health in humans (Ascherio and Willett, 1997; Wilson, 2004; La et al., 2014). Linoleic and linolenic acid contents varied between 29.3-56.08% and 4.81-14.33% in the wild soybean accessions. The present study showed a wider range of linoleic acid and linolenic acid content when compare d with previous studies (Wee et al., 2017; La et al., 2018). Abdelghany et al. (2020) reported that linolenic acid content of 633 soybean accessions varied between 3.43-12.76%. In another study performed with wild soybean accessions, Wee et al. (2017) reported that the oleic acid content varied between 12.1 and 25.4%. As the oils of soybean genotypes with high LNA content oxidize rapidly, it is not suitable for producing stable oil, as it induces off-flavor compounds in cooked foods. But, linoleic and linolenic acid is essential for human health and development (La et al., 2019). Furthermore, asthma, heart disease and other syndromes could affect human health due to lack of linoleic and linolenic acid in diets (Simopoulos, 2008; Wang et al., 2012; Abdelghany et al., 2020).

ω-6/ω-3 and PUFA/SFA ratios

Omega-6 (ω-6) and omega-3 (ω-3) fatty acids are essential and the human body, like other mammals, cannot synthesize them and must obtain them in their diets (Asekova et al. 2014). The ω-6/ω-3 ratio is as important as the amount of these fatty acids for human health. The ideal ω-6/ω-3 ratio should be between 3: 1 to 5: 1, as this ratio is associated with a reduction in the risk of cardiovascular and other chronic diseases, such as diabetes, and the improvement of the immune response and brain function (Simopoulos, 2002; Simopoulos, 2008).

The ω-6/ω-3 ratio ranged from 3.37, in "Wonju, Gangwon, Korea" to 6.09 in "Nigata, Japan" with mean a value of 4.62 (Table 2). Of the 24 wild soybean accessions, 18 of them have ω-6/ω-3 ratio of less than 5 and 6 of them have a ratio of less than 4. Lee et al. (2019) reported that the average ω-6/ω-3 ratio was 3.4/1 in their study with 80 wild soybean genotypes. The average value of ω-6/ω-3 ratio was found lower than cultivated soybean reported by Asekova et al. (2014) and Abdelghany et al. (2020). However, the average ω-6/ω-3 ratio in previous studies with wild soybean accessions ranges between 3 and 3.38 (Wee et al., 2001; La et al. 2019). The main reason for the low ω-6/ω-3 ratio of wild soybeans is the higher linolenic ratio than cultivated soybean. Therefore, in order to reduce the-6/ω-3 ratio, it is not only sufficient to lower the linoleic acid content, but also to increase the linolenic acid content. According to the results from the present study, the linoleic acid content of Nigata 2, Japan genotype is 29.3%, while the ratio of-6 / ω-3 is 6.09. However, although the linoleic acid ratio is 51.3% in the Wonju, Gongwon, Korea genotype, the ω-6/ω-3 ratio is 3.37. This is due to the linolenic acid content being 15.23%.

CONCLUSIONS

To conclude, considerable variations in oil content, fatty acid composition, ω6/ω3 and PUFA/SFA ratios were noticed among 24 wild soybean accessions in this study. The Nigata, Japan 2 accession is one of the prominent wild soybean accession in the study with high oleic acid content (49.74%).

Because, high oleic lines have been developed through different ways such as selection breeding, mutation breeding and genetic engineering. However, varieties developed by genetic engineering and mutation breeding have different disadvantages. For example, lines containing FAD2 mutant alleles have increased oleic acid content but decreased yields. In addition, most of the wild soybean accessions in the study had an ω6/ω3 ratio in the human health beneficial range (1-5), unlike those in cultivated soybeans. The identified diversity in oil content and fatty acid composition is useful for the identification of better parents with high oleic acid and linolenic acid contents for developing soybean varieties with traits which are beneficial to consumer health and industrial demands.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON THE PROTECTION OF RAPESEED CROP AGAINST DISEASES, WEEDS AND PESTS

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Abstract

Rapeseed (Brassica napus L.) is an important oilseed crop in Europe and in Romania because of their ability to germinate and grow at low temperatures in the temperate regions. Rapeseed is primarily used to produce edible vegetable oil and meal for animal feed. Rapeseed production is negatively influenced by several diseases, arthropod pests and weeds. Chemical control is still an indispensable method in effective rapeseed protection against these harmful organisms in Romania. The work falls into integrated management strategy for rapeseed harmful organisms based on risk assessment, monitoring and management of the rapeseed crop that can be used as a framework by growers to manage rapeseed bioaggressors with reduced plant protection products inputs is required. The research was carried out during the vegetation period of 2021 and 2022 in Poșta Câlnău commune, Buzău county.

***Key words**: rapeseed, protection, diseases, weeds, pests.*

INTRODUCTION

Rapeseed (*Brassica napus* L.) is a vegetable oil crop, widely used as a source of oil and protein for food and industrial applications (Raboanatahiry et al., 2021).

About 70 million tons of rapeseed are produced per year around the world, involving 66 countries: 34 countries in Europe, 15 countries in Asia, 9 countries in America, 6 countries in Africa, and 2 countries in Oceania (http://www.fao.org/faostat/en/#search/Rapeseed%20).

Rapeseed is the second most important oilseed crop of the world, and it is also a favourite plant for basic and breeding research (Friedt et al., 2018).

Winter-type rapeseed grows well in the climatic conditions of the Poșta Câlnău area.

Known for its production of high-quality vegetable oil, rapeseed competes with other crops in Romania, respectively soybean and sunflower.

In agricultural production, rapeseed is an indispensable component of crop rotations in our country.

If the introduction of high-yielding and hybrid cultivars and the opening of new markets in the food and feed sector have steadily increased rapeseed production since the 1980s, since the 1990s, however, the average growth rate of yields has declined in Europe, which has been associated with a less effective control of biotic stresses (Zheng et al., 2020).

Diseases, weeds and insect control largely relies on plant protection products, because crop rotation, soil management, resistant cultivars or biocontrol are often ineffective.

Chemical control is still an indispensable method in the effective protection of rapeseed in Romania against diseases, weeds and pests.

In heavily weed-infested soils, managing summer weeds plays an important role to avoid seeding delays, conserve nutrients and moisture for the rapeseed crop to use during the growing season.

Weed control in winter rapeseed has always been challenging. Yield loss from poor broad-leaved weed control can range from 3% up to 73% depending on the vigour of the crop and this does not take into account contamination of harvested crop with weed seed which can reduce marketability of the sample (https://ahdb.org.uk/new-approaches-to-weed-control-in-oilseed-rape).

In rapeseed growing regions revealed 16 diseases and 37 insect pests, as well as nematodes, slugs and snails. This biotic stresses predominantly affect leaves (10 diseases and 22 insect pests) and stems (7 diseases and 12 insect pests), while only 2 diseases and 11 insect pests affect pods and seeds of oilseed rape (Zheng et al., 2020).

Identification of rapeseed pathogens and pests in different areas of Romania is a permanent goal for phytopathological and entomological scientific activity in our country (Grozea et al., 2007; Paraschivu et al., 2011; Radu et al., 2011; Zală et al., 2012; 2023).

Several diseases negatively impact rapeseed production. Diseases caused by soil-borne pathogens, pose a risk of substantial yield loss since crop rotation schemes have become narrow as the time lapse between rapeseed crops in a field has been shortened (Wallenhammar et al., 2022).

Sclerotinia sclerotiorum, whose sclerotia can survive in the soil for more than 4 years, is able to infect more than 400 plant species (Mizubuti, 2019).

Pollen beetle (*Meligethes aeneus*), cabbage aphid (*Brevicoryne brassicae*), rape stem weevil (*Ceutorhynchus napi*), rape seed weevil (*Ceutorhynchus assimilis*), rape flea beetle (*Phyllotreta atra*), striped flea beetle (*Ph. nemorum*) are important insect pests that affect European. Eastern Europe has the highest number of pest species: 15, where damage by insect pests is a significant yield reducing factor in rapeseed production, with an average annual yield loss of 15% (Milovac et al., 2017).

Due to environmental concerns and concern for human health, the sustainable use of pesticides is required by promoting the integrated management of diseases, weeds and pests according to the European directive 2009/128/CE.

MATERIALS AND METHODS

The research was carried out in the rapeseed experimental fields from Poşta Câlnău-Buzău at 45°14′ latitude and 26°51′ longitude, in conditions of natural infection during the vegetation period of 2021 and 2022. The land belongs to the BioVitAgro farm.

Correct diagnosis of rapeseed pathogens, weeds and pest is the primary requirement in any integrated management practice.

Diseases, pests and weeds was detected under conditions of natural infection under the influence of climatic conditions during the vegetation period of the two years of studies.

The observations were made on the rape hybrid PT234. This hybrid with semi-early maturity, has a fast development in autumn and a very good tolerance to drought and low temperatures (https://www.corteva.ro/).

Visual observation is the fastest method to identify diseases based on symptoms shown by infected rape plants and identification pests and weeds based on their morphological characters.

The abundance of the species *Meligethes aeneus* was determined in the phenophase of maximum flowering (BBCH 65) with the entomological net both in the variants treated with the two insecticides, as well as in the control variant. The collected material (one thread/ total surface area variant, was placed in a 720 ml jar, which had pieces of paper soaked in acetone).

The attack value is represented by frequency (disease incidence)-F%, intensity (severity)-I% and attack degree (AD%). Frequency is the percentage of leaves or siliques attacked out of 100 examined leaves or siliques. The intensity, visually estimated, indicates the degree to which the leaf or siliques are attacked. The intensity was noted directly in percentage. The attack degree present severity of disease or pest in the crop and was calculated with the formula:

$$\text{AD}\ (\%) = \frac{\text{F}\ (\%)\text{xI}(\%)}{100}$$

The effectiveness of treatments with fungicides and insecticides applied was calculated according to the Abbott (1925) formula, based on the recorded attack degrees:

$$\text{E}\ (\%) = \frac{\text{AD control} - \text{AD treated}}{\text{AD control}}\text{x}\ 100$$

The harvestable surface of one variant was 20 m^2.

The experiments were arranged according to the method of randomized blocks, in 4 repetitions.

A standard technology was applied: plowing executed at 25 cm, sowing of a treated seed with Scenic Gold 375 FS (fluopicolid 200 g/l +

fluoxastrobin 150 g/l) - 1 l/100 kg of seed and Lumiposa (cyantraniliprol 625 g/l) - 1.14 l/ 100 kg of seed; NH_4NO_3-120 kg/ha (1/3 of the dose in autumn and 2/3 of the dose in early spring) and complex fertilizer NPK 18: 46: 0-100 kg/ha (applied to prepare the land for sowing). Regarding the preceding plant, this was autumn barley. Sowing in the two years of research was carried out in the first decade of September.

In the experiment with fungicides, 3 treatments with insecticides were also applied (one in autumn and 2 in spring). In the experiment with insecticides (V1-untreated, V2-Inazuma, V3-Decis Expert 100 EC) 2 treatments with fungicides were also applied (both in spring). In the herbicide experience (V1-untreated, V2-Salsa) 3 treatments with insecticides were applied (one in autumn and 2 in spring) and 2 treatments with fungicides were also applied (both in spring). The experiences with fungicides against powdery mildew included 3 experimental variants: V1 - untreated control, V2 - 1 treatment applied (Final: boscalid - 200 g/l + dimoxistrobin - 200 g/l - 0.5 l/ha; respectively Custodia 320 SC (azoxistrobin-120 g/l + tebuconazol – 200 g/l - 1.0 l/ha) in the stem elongation phenophase: BBCH 31; V3 - 2 treatments: one applied in the stem elongation phenophase (Final), and the second (Custodia) in the "yellow bud" phenophase: BBCH 59. Observations on the effectiveness of fungicides were carried beginning of ripening: BBCH 80 (Weber and Bleiholder, 1990). The insecticides applied were: Inazuma (acetamiprid-100 g/kg + lambda-cihalotrin - 30 g/kg) - 0.2 kg/ha and Decis Expert 100 EC (deltametrin) - 0.75 l/ha. A single treatment with each of the two insecticides was applied when pests (*Brevicoryne brassicae* and *Ceutorhynchus assimilis*) were detected on the plants, and efficacy scoring took place after 3 days. To combat the *Meligethes aeneus* species, insecticide treatment was applied in the "yellow bud" phenophase, in combination with the second fungicide treatment.

The notations regarding the attack of diseases and the presence of pests were performed on 100 leaves and siliques of each 4 plants/variant.

The herbicide applied were Salsa (etamet-sulfuron-metil 75%) - 25 g/ha + trend (adjuvant) - 250 ml/ha postemergence-BBCH 10-18. We used the numerical quantitative method for mapping the weeds, which consists in counting, by species, the weeds/m^2 using the metric frame (Chirilă, 2009); 2 determinations/variant with 3 days before herbicid application and efficacy scoring took place after 30 days.

For the observation of the climatic conditions necessary for the appearance and development of the disease, precipitation and temperatures were taken into account (Cotuna et al., 2022). The temperature recorded values slightly lower than the multi-year average only in April of 2021, otherwise the values were higher (Figure 1).

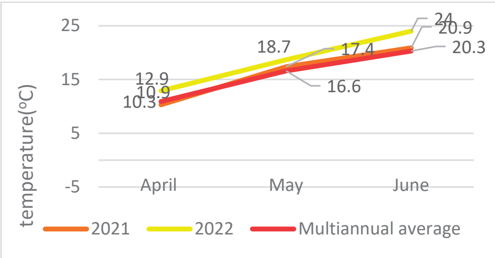

Figure 1. Average monthly temperatures (°C), Poşta-Câlnău, Buzău (source:https://www.meteoblue.com/en/weather/week/poșta-câlnău_romania)

As for the rainfall recorded in April, May and June, it was below the multi-year average values in each of the two years (Figure 2).

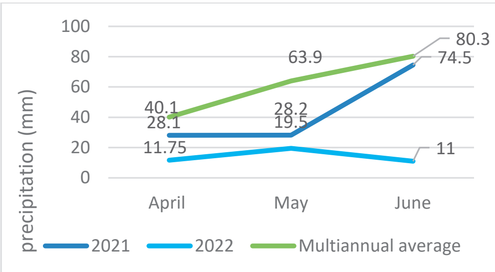

Figure 2. Average monthly precipitation (mm), Poşta-Câlnău, Buzău (source:https://www.meteoblue.com/en/weather/week/poșta-câlnău_romania)

RESULTS AND DISCUSSIONS

The attack of *Ceutorhynchus assimilis* larvae (Figure 3.) consists in consuming the newly formed seeds in siliques.

Figure 3. Observation of *C. assimilis* larvae
(foto: Lipianu S.)

The greatest damage caused by the pollen beetle is caused by the adults that cause reddening of the buds, which is why they will drop before they can produce flowers. Once the flowers have opened, damage by *Meligethes aeneus* adults (Figure 4) or larvae is negligible, and the pest will help pollinate the crop.

Figure 4. Observation of *Meligetes aeneus* adult
(foto: Istrate R.)

Continued feeding by cabbage aphid (Figure 5) causes yellowing, wilting and stunting of plants (Istrate and Roșca, 2009).

Figure 5. Observation of *Brevicoryne brassicae* colony
(foto: Zală C.R.)

The treatment with insecticides against *Brevicoryne brassicae* species (Table 1) was applied in the phenophase first flowers opening (BBCH 60). The efficacy of Inazuma insecticide was 96.3% in 2021 and 96.1% in 2022. The efficacy of Decis Expert 100 EC insecticide varied between 94.8% in 2022 and 94.9% in 2021, which resulted in an important decrease in the number of colonies/plant.

Table 1. Results regarding the efficacy of insecticide treatments against *Brevicoryne brassicae* species (colonies/plant)

Variants	**Years**	**Average density**		**E (%)**
		before	after	
		treatment		
Untreated	2021	21.7	-	
	2022	22.9	-	
Inazuma	2021	-	0.8	96.3
	2022	-	0.9	96.1
Decis Expert	2021	-	1.1	94.9
	2022	-	1.2	94.8

The treatment with insecticides against *Ceutorhynchus assimilis* species (Table 2) was applied at the end of the development of siliques stage. The effectiveness of the Inazuma insecticide was the highest, 97.5%, in 2021, when we recorded a decrease in the number of larvae/silique from 47.3 in the untreated variants to 1.2. The efficacy of Decis Expert 100 EC insecticide was average over the two years of 96.0%.

Table 2. Results regarding the efficacy of insecticide treatments against *Ceutorhynchus assimilis* species (larvae/silique)

Variants	**Years**	**Average density**		**E (%)**
		before	after	
		treatment		
Untreated	2021	47.3	-	
	2022	49.7	-	
Inazuma	2021	-	1.2	97.5
	2022	-	1.4	97.2
Decis Expert	2021	-	1.8	96.2
	2022	-	2.1	95.8

Regarding the effect of applying a single treatment with insecticides against the species Meligethes aeneus, we mention that in the control variant we captured (Figure 6) a total number of 483 adults (2021) during one week (one threading/day/total variant surface) and 514 adults in 2022.
Compared to the untreated control variant, the total number of specimens collected was 116 adults in the variant with the Inazuma insecticide (which represents a decrease of

76%) and in the variant with Decis Expert 126 adults were captured (a decrease of 74%).
In 2022, 139 specimens were captured (which represents a decrease of 73% compared to the untreated control variant) in the variant with Inazuma, and 152 specimens (the decrease being 70.5%) in the variant with Decis Expert.

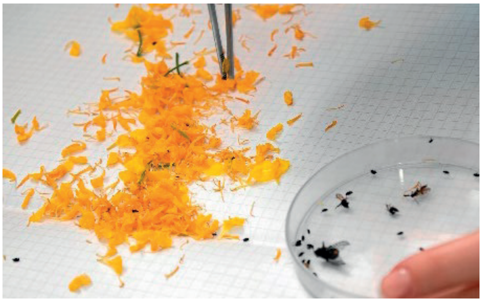

Figure 6. Counting pollen beetle captured following a threading (foto: Lipianu S.)

Alternaria appears on the siliques in the form of small light brown lesions that soon turn black (Figure 7, left) and these lesions on silique cause infection in the seed. Infected silique may ripen and shatter prematurely. while the crop is standing or in the swath.
Powdery mildew symptoms appeared as white colonies on both leaf surfaces and on siliques (Figure 7, central and right).

Figure 7. left): Alternaria on the siliques and Powdery mildew: central) on leafs, right) on siliques (foto: Zală C.R.)

Sclerotinia stem rot develops lesions greyish white, and infected tissues tend to shred easily. When the bleached stems of diseased plants are split sclerotia are visible (Figure 8).
From the data presented in Table 3, it can be seen that *Erysiphe cruciferarum* had more favorable conditions for its manifestation in 2022. The application of a single phytosanitary treatment against powdery mildew recorded the highest effectiveness in 2022, respectively 71.2% for Final fungicide and 69.6% for Custodia fungicide. The efficacy of the two treatments (the first with Final and the second with Custodia fungicide) was 98.5%.

Figure 8. Sclerotinia stem rot and some species of weeds (foto: Zală C.R.)

Table 3. Results regarding the efficacy of fungicide treatments against *Erysiphe cruciferarum*

Variants	Years	F (%)	I (%)	A.D. (%)	E (%)
Untreated	2021	42.2	52.5	22.2	-
	2022	43.6	59.6	26.0	-
Final	2021	22.1	29.8	6.6	70.3
	2022	23.8	31.5	7.5	71.2
Custodia	2021	23.4	30.4	7.1	68.0
	2022	24.5	32.1	7.9	69.6
T1-Final T2-Custodia	2021	6.3	5.5	0.34	98.5
	2022	6.5	6.2	0.4	98.5

From the data presented in Table 4, it can be seen that also alternaria had more favorable conditions for its manifestation in 2022. Since the alternaria manifested itself only at the level of siliques, only the effect of a single treatment in the “yellow bud” phenophase. Treatment effectiveness ranged around 92.0% in both years.

Table 4. Results regarding the efficacy of fungicide *treatments* against *Alternaria brassicae*

Variants	Years	F (%)	I (%)	A.D. (%)	E (%)
Untreated	2021	32.81	61.5	20.2	-
	2022	34.95	63.4	22.2	-
Final	2021	6.35	25.2	1.6	92.0
	2022	6.75	25.4	1.71	92.3
Custodia	2021	6.5	25.3	1.64	91.9
	2022	7.1	25.7	1.82	91.8

The nine species encountered in the non-herbicided rapeseed variants were (%-2021/2022): *Amaranthus retroflexus* (12/11), *Tripleurospermum inodorum* (18/17), *Chenopodium album* (11/10), *Echinochloa crus-galli* (8/7), *Convolvulus arvensis* (11/11), *Veronica hederifolia* (13/12), *Stellaria media* (12/11), *Sonchus arvensis* (8/11) and *Sinapis arvensis* (7/10). The average number of weeds was 265.2/m^2 in 2021 and 211.7/m^2 in 2022.

The average number of weeds in the variants herbicided with Salsa after 30 days from the application of the treatment was 6.63/m^2 in 2021, which represents an effectiveness of 97.5%; and at the level of 2022 the average number of weeds was 9.9/m^2, which represents an effectiveness of 95.3%.

CONCLUSIONS

The occurrence of diseases, pests and weeds reported in the rapeseed crop can be influenced by the geographical position of Romania.

The insects found in our experience are: pollen beetle (*Meligethes aeneus*), rape seed weevil (*Ceutorhynchus assimilis*) and the cabbage aphid (*Brevicoryne brassicae*).

Colonies of *Brevicoryne brassicae* are found on upper and lower leaf surfaces, in leaf folds, along the leafstalk, and near leaf axils.

Against the backdrop of higher temperatures and less precipitation in 2022 compared to 2021, the efficacy of insecticide treatment against *Brevicorine brassicae* was only 0.1-0.2% lower in 2022 compared to the previous year, a fact attributed to volatilization faster of the spray solution.

The diseases of the autumn rapeseed crop found in our experience are: powdery mildew (*Erysiphe cruciferarum*), alternaria (*Alternaria brassicae*) and sclerotinia stem rot (*Sclerotinia sclerotiorum*); powdery mildew and alternaria being the most common while the attack of sclerotinia stem rot was sporadic.

The severity of powdery mildew and alternaria it varied between the two years, based largely on the moisture and temperature situation.

The weeds control allows the rapeseed crop to be competitive, and controls early emerging weeds before they take up excessive nutrients and moisture. For this reason, the control and management of the weeding condition plays an important role in the technological links of rapeseed.

The average number of weeds and the effectiveness of the treatment were influenced by the climatic conditions at the level of the two years of research, water stress and higher temperatures decreased the number of weeds/m^2, but also caused a decrease in the effectiveness of the Salsa herbicide, at the level of the year 2022.

ACKNOWLEDGEMENTS

We thank the engineer A.V. Bălașu for the permission to carry out the experiments on his BIO VIT AGRO farm.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

WINTER WHEAT (*T. aestivum* L.) YIELD DEPENDING ON THE DURATION OF AUTUMN VEGETATION AND THE TERMS OF SPRING VEGETATION RECOVERY: 50-YEARS STUDY IN UKRAINE

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Abstract

The grain yield of the national soft winter wheat standards for 1967-2018 in Ukraine was analyzed and its connection with the duration of the autumn vegetation and the time of spring vegetation recovery was established. Significant effects of climate change have been identified on the cessation of autumn vegetation, the duration of winter dormancy and the duration of the growing season of soft winter wheat. Based on the analysis of yield and cessation of autumn vegetation for 50 years, it was found that soft winter wheat forms mostly higher yields (6.13 t/ha) under the late cessation of vegetation (from 19 November to 29 November). During the very early (until 28 October) and early cessation (from 28 October to 07 October) of autumn vegetation, the yield decreased slightly and amounted to 5.77 and 5.45 t/ha, respectively. The calendar dates for the cessation of autumn vegetation have a clear tendency to change to later dates. With a slight difference in the recovery time of spring vegetation over 10-year periods, its significant variability was observed during the research years from 90 days (2013) to 150 days (1990). The highest grain yield (7.26 t/ha) of winter wheat was obtained with early (until 03 March) recovery of spring vegetation. The lowest grain yield was in wheat (5.00 t/ha) with a late and very late (4.50 t/ha) recovery of spring vegetation. On the basis of the data analysis, it was established that duration the late stop of the autumn vegetation (45-55 days), winter wheat plants accumulate the optimal amount of plastic substances, which contributes to their better overwintering and the growth of grain yield.

Key words*: winter wheat, autumn vegetation, spring vegetation, yield, climate change.*

INTRODUCTION

Winter wheat is characterized by the longest growing season among annual field crops, which in turn has both advantages and disadvantages (Mostipan et al., 2021).

The growth, development, and productivity of soft winter wheat plants depend on their development during the autumn growing season, the degree of hardening, agrometeorological conditions during the winter dormancy, their condition after winter and the time of spring vegetation recovery (Alabushev and Popov, 2015).

Soft winter wheat actively grows at an average daily air temperature above +5°C - the effective temperature above the biological minimum (Korkhova, 2013; Boichuk and Bazalii, 2011). The calendar terms of the cessation of autumn and the recovery of spring vegetation depend on this temperature. As a result, winter wheat plants receive different amounts of heat in different years, which affects their physiological processes during the growing season and ultimately depends on the productivity of agrophytocenosis (Alabushev and Popov, 2015).

Wheat crops are highly sensitive to changes in the environment and climate (Porter and Semenov, 2005). During the last two decades, progress in wheat genetics has been partially offset by changes in Europe's climate (Brisson et al., 2010; Lobell et al., 2011). Climate change is characterized by an increase in the frequency of extreme events and is a major challenge for breeders (Semenov et al., 2014).

In recent years, a number of scientific institutions around the world, including Ukraine, have declared changes in climatic conditions during the winter wheat growing season. The anomaly is more observed in winter, which in turn affects the duration of the fall vegetation of winter crops, wintering conditions, growth and development of plants in spring and summer and their productivity (Goldvarg et al., 2019; Polovyi et al., 2017; Luo et al., 2018).

There is a lot of evidence that rising temperatures due to climate change negatively affect the yields of the main crops: corn, wheat, and barley (Gouache et al., 2012; Lobell et al., 2011; Lobell and Field, 2007), rice in Asia (Peng et al., 2004), corn and soybeans in the United States (Schlenker and Roberts, 2009), spring wheat in Mexico (Lobell et al., 2005). Regarding the winter wheat yield in Europe, Lobell et al. (2011) found that temperature trends during the wheat growing season significantly contribute to leveling or slowing the growth of its yield (Gouache et al., 2012).

One of the most obvious consequences of rising temperatures for wheat is the earlier onset of phenological stages. Optimal temperatures for grain formation range from 19.3°C to 22.1°C (Porter and Gawith, 1999). High temperatures above 34°C accelerate premature leaf death, significantly affecting grain yield (Wardlaw and Moncur, 1995).

Breeding measures may be more effective in adapting winter wheat to climate change. Existing genetic variations allow for the adaptation of phenology of wheat and its resistance to heat stress. It is the genetic tolerance of winter wheat to heat stress that was the most effective. This means that research and selection work must begin to take into account the resistance to heat stress in Europe. In the near future (2020-2049), there may be a slight increase in heat stress (Gouache et al., 2012). In the distant future (2070-2099), the frequency of thermal stress during grain filling should increase significantly. Therefore, winter wheat resistance to temperature changes is likely to be a key sign of increased yield potential and yield stability in Europe (Stratonovitch and Semenov, 2015).

There is strong evidence of historical and recent climate change in Ukraine, particularly with respect to increasing temperatures in winter. Furthermore, precipitation appears to decreasein the southern zone of Ukraine during 1961-2009 (Morgounov et al., 2013). Summer precipitation is likely to decline, and winter precipitation is expected to increase, while droughts may become more likely and intensify (Lioubimtseva and Henebry, 2012).

Numerous studies have proven the undeniable role of weather conditions in the early spring period in the formation of the winter wheat crop. The time of renewal of spring plant vegetation is of particular importance (Lykhochvor, 2018; Mostipan and Umrykhin, 2018). Autumn vegetation determines the wintering conditions of winter crops, so the contribution of agrometeorological conditions of the autumn-winter period in the formation of yields is 25 to 40%.

According to Holmer (2008), there are significant correlations between winter wheat yields and winter durations, suggesting that short winters tend to higher yields of this crop. The overall difference between climate and wheat yield is 26%, which is high for this climate variable. The positive effect of mild and short winters on winter wheat yields is confirmed by other studies in areas with winter temperatures below 5-6°C, while crop models show lower yields at higher temperatures (Holmer, 2008).

Studies have shown that the timing of the cessation of autumn vegetation affects the yield of winter wheat crops. The later the wheat plant vegetation stops, the greater the bushiness of the plants and, accordingly, the density of the stems. The prolongation of the autumn vegetation also affects the phytosanitary condition of the crops. Mostipan (2019) shows that the highest grain yield of winter wheat (5.67 t/ha) for black steam is formed in the years with the cessation of autumn vegetation in the third decade of November. Yerashova (2018) in the northern steppe of Ukraine found that the limiting factor in winter wheat plant growth in 2016 was the early cessation of vegetation processes. According to Netis (2011), early cessation of vegetation (until 20 November) leads to a decrease in yield, and late (after 5 December) - to its increase (Mostipan, 2019; Yerashova, 2018; Netis, 2011).

The time of cessation of autumn vegetation, wintering conditions, and the time of spring vegetation recovery are important factors for the formation of winter wheat. As a result of the analysis of the yield and time of cessation of autumn vegetation for the last 35 years (1979-2014), it is established that durum wheat forms a bigger crop at the late cessation of autumn vegetation after peas (4.70 t/ha) and black steam (5.55 t/ha) than with the early cessation of autumn vegetation - 4.37 and 4.65 t/ha, respectively.

Over the last 35 years (1980-2014) there has been an increase in temperature during the

dormant period of winter wheat; it increases annually at 0.93°C (Alabushev and Popov, 2015).
Studies by many scientists have shown that the growth, development and yield of winter wheat are significantly influenced by environmental factors as time of the spring vegetation recovery (TSVR) (Medenets, 1982; Brazhchenko et al., 2006; Khakhula, 2013). In the case of early TSVR with moderate solar radiation and cool weather, winter wheat produces higher yields than in the middle and late stages of its recovery (Khakhula, 2013).
The ecological effect of TSVR is not manifested annually, so it is not always possible to predict the type of plant development. But in such years to influence the processes of growth, development and survival of plants in spring and summer and the formation of their productivity can be through the application of such intensive technologies as differentiated crop care, optimization of mineral nutrition, application of plant growth regulators, trace elements, weeds, diseases, and pests protection (Ulich et al., 2014).
For Ukraine, higher, particularly during the second vegetative period in late spring and the ripening period, will compromise wheat yields while higher temperatures may have a slightly positive effect during the yield formation phase. In all growth phases, except during yield formation, lower temperatures were associated with higher crop yields. Higher temperatures in the yield formation phase, in contrast, correlate with higher yield in winter wheat (Fischer et al., 2014).
The later the winter wheat vegetation is recovered, the more total radiation that is absorbed the surface of the crops. Therefore, in the years with late spring, plants grow and develop in conditions of higher air temperature and greater solar energy. In the case of early spring, winter wheat vegetation occurs at lower temperatures and slow growth, which are more favorable for the regeneration of damaged organs, plant regrowth, and the course of all growth processes (Kulyk et al., 2020; Netis, 2011).
According to Alabusheva and Zbrailova (2001), no clear dependence of the average value of winter wheat yield on the time of spring vegetation recovery wa found.
Studies in the northern steppe of Ukraine have shown that the later the spring vegetation of winter wheat is recovered, the lower the yield. In the case of early vegetation recovery (III decade of February), the yield of crops with sowing in the period from late August to early October is almost the same and ranges from 6.44 to 6.96 t/ha. In the case of late resumption of vegetation (early April), the highest yields (3.86-3.91 t/ha) are formed by crops sowing from 10 to 25 September. The shorter the period from the temperature transition through 0ºC to +5ºC, the higher the winter wheat yield (Mostipan and Umrykhin, 2018).
According to Dorokhova and Vasyleva (2018), a significant deviation of hydrothermal conditions for 40 years (1979-2018) compared to long-term data (1929-1978) was revealed. Air temperatures increased 1.1-3.0°C, the amount of precipitation increased by 2.8-8.4 mm depending on the month of observations. Favorable conditions for winter wheat overwintering were observed in 77.5% of cases and unfavorable in 7.5%. There is a direct correlation between overwintering plants with average monthly temperatures in November ($r = +0.65$) and the first decade of December ($r = +0.34$), the inverse – with January temperatures ($r = -0.34$) and December precipitation ($r = -0.33$). With an increase in air temperature in the winter months, there is a sharp change in temperature during the cessation of fall vegetation (November-December) (Dorokhov and Vasylev, 2018).
Experiment observations at 120 agricultural meteorological stations spanning from 1981 to 2009 in China were found that the climate during the wheat growth period had changed significantly and the change had caused measurable impacts on wheat growth and yield. Changes in temperature, precipitation, and solar radiation in the past three decades jointly increased wheat yield in northern China by 0.9-12.9%, however, reduced wheat yield in southern China by 1.2-10.2%, with a large spatial difference (Tao et al., 2014).
Therefore, the question of the dependence of the soft winter wheat yield on the time of autumn cessation and the recovery of spring vegetation in the context of climate change is relevant.
The purpose of our research was to study the dependence of winter wheat grain yield on the duration of autumn vegetation and the terms of recovery of spring vegetation in Ukraine based on the analysis of data for 50 years.

MATERIALS AND METHODS

Soft winter wheat yield data for the period 1968-2018 were obtained at the Bila Tserkva Research and Breeding Station of the Institute of Bioenergy Crops and Sugar Beets of the National Academy of Sciences of Ukraine (49°43'23.6"N 30°05'53.4"E).

The soil of the experimental field is typical deep low-humus chernozem, coarse-grained medium, and light loam. According to the agrochemical survey in 2016, the humus content is 3.4-3.8%, alkaline hydrolyzed nitrogen - 118-134 mg/kg of soil, mobile phosphorus - 180-208, and metabolic potassium - 73-91 mg/kg of soil. The reaction of the soil solution is weakly acidic and close to neutral.

The climate is moderately continental; the average annual air temperature is 6.9°C with significant fluctuations over the months. The average annual rainfall is 538 mm, which is unevenly distributed during the growing season: in summer it is much more than in spring and autumn.

The technology to grow soft winter wheat was generally accepted for the Forest-Steppe zone in Ukraine. The predecessor of soft winter wheat in all years was peas. Data from the Bila Tserkva meteorological station were used to characterize agroclimatic indicators.

Yield indicators were obtained from soft winter wheat varieties, which in different years were national standards in the Forest-Steppe of Ukraine: Myronivska 808, Illichivka, Poliska 70, Kyanka, Myronivska 61, Donska napivkarlykova, Poliska 87, Bilotserkivska napivkarlykova, Perlyna Lisostepu, Podolanka, Lisova pisnia.

RESULTS AND DISCUSSIONS

The optimal sowing dates for soft winter wheat are factors that cannot be replaced or compensated by others. The timing of winter wheat sowing significantly affects the time of emergence and friendliness of seedlings, the subsequent growth and development of plants, and their productivity (Vorona et al., 2013; Zhirnyh, 2017).

Some scientists consider the best time for sowing soft winter wheat to be when seedlings did not reach the 23-24 phase of BBCH development until autumn vegetation stops. At the same time, with the cessation of the autumn vegetation, the plants must have a development in which the differentiation of the growth cone and the formation of the rudimentary ear quickly began during the spring recovery of the vegetation. Other scientists consider that the successful timing of winter wheat sowing must meet two criteria: the average daily air temperature at about 15°C, and the duration of autumn vegetation 40-50 days (Hanhur and Hanhur, 2010).

Analysis of the calendar dates for soft winter wheat sowing in our research shows that most of them took place in the optimal area for the Forest-Steppe zone of Ukraine. Somewhat later, sowing was carried out in 2000, 2007 (30.09), 2008, 2016 (27.09), 2013 (01.10), respectively, the cessation of autumn vegetation was late and very late: 24.11 (2000), 22.11 (2007), 21.11 (2008), 02.12 (2013), 12.12 (2016). The grain yield was close to 5.09 t/ha in 2014 and 5.27 t/ha in 2001 to the average for 50 years (5.39 t/ha), or much higher (6.36 t/ha in 2017, 7.03 t/ha in 2009 and 7.82 t/ha in 2008) (Table 1).

Table 1. The impact of the duration of the autumn growing season of soft winter wheat on grain yield

Years	Calendar sowing date	Sowing-emergency period, days	Calendar term of autumn vegetation cessation	Duration of autumn vegetation, days	Yield, t/ha
1967/68-1976/77	06.09* 03-08.09**	8 6-11	08.11 22.10-28.11	56 36-73	4.58 3.18-6.01
1977/78-1986/87	13.09* 07-20.09**	8 6-14	12.11 24.10-30.11	52 38-70	4.91 3.62-6.27
1987/88-1996/97	15.09* 09-22.09**	8 7-9	06.11 22.10-02.12	44 31-71	5.93 4.95-8.59
1997/98-2007/08	17.09* 07-30.09**	8 7-10	17.11 23.10-18.12	53 31-84	5.33 3.48-7.82
2008/09-2017/18	22.09* 14.09-01.10**	9 8-10	23.11 03.11-12.12	54 35-68	6.21 2.6-9.59

* - average for 10 years; ** - limits of variability

As a result of unfavorable conditions in the winter of 2002-2003, winter wheat plants almost completely died (95%), which, accordingly, led to the impossibility of harvesting.

Over ten-year periods, beginning in 1967, the calendar dates for sowing winter wheat were shifted to later ones. Therefore, for the average sowing date of 6 September 1967-1976, the average date in 2008-2017 was 22 September.

The phase of wheat germination, on average, was observed on the ninth day with fluctuations in years from 6 days (1971, 1987) to 14 days (1978) days.

Autumn vegetation of soft winter wheat should last 40-60 days. Thus, plants from sowing to steady transition through 5°C, towards smaller temperatures, should gain the sum of effective temperatures of 300-350°C Under such conditions, crops have time to accumulate a sufficient amount of plastic substances during winter, allowing them to better withstand harsh conditions of both winter and spring-summer growing seasons (Korkhova, 2013; Boichuk and Bazalii, 2011; Alabushev and Popov, 2015).

The analysis of calendar dates of the cessation of autumn vegetation in years of research, testifies to their shift to later terms. The exception is 1987-1996, when the average date of vegetation cessation was 6.11. Thus, if in 1967-1976 the average date of vegetation cessation was 8 November, then in 2008-2017 it was 23 November. For 50 years of research, the calendar terms of autumn vegetation cessation had a fairly wide range from 22 October to 18 December. It should be noted that for 1967-1991 the first term of the cessation of fall vegetationwas 22-24 October, while in 1992-2017 1-3 November (except for 1997 - 23 October). At the same time, the late cessation of vegetation in 1967-1991 was observed on 28.11-02.12, while in 1992-2017 - on 7-18 December. The data obtained in this way indicate significant climatic changes and their impact on the time of the cessation of the autumn vegetation and the development of wheat plants in the fall.

On average, 52-56 days passed during the ten-year periods from germination to the cessation of winter wheat fall vegetation. The exceptions were 1987-1996 with an average vegetation duration of 44 days. The highest variability (53 days) was determined in 1998-2008.

In our research, the average 50-year autumn vegetation cessation is November 13. Based on this, we classified the calendar dates of autumn vegetation cessation as follows: very early (until 28.10); early from 28.10 to 07.11; optimal from 08.11 to 18.11; late from 19.11 to 29.11 and very late after 29.11 (Table 2, Figure 1).

In 50 years of research, a very early cessation of autumn vegetation was observed seven times and early - 12 times. For 13 years, the autumn vegetation stopped at the optimal time. The late cessation of winter wheat vegetation was observed 11 times and very late - seven. The highest grain yield of winter wheat (6.13 t/ha) was obtained at the late cessation of autumn vegetation. In the years with very early and early cessation of plant vegetation, the yield decreased slightly and amounted to 5.77 and 5.45 t/ha, respectively. But a more significant decrease (4.79 t/ha) was observed at the optimal time of the cessation of the autumn vegetation. The too long period of autumn vegetation, which is characteristic in years with the cessation of plant vegetation after 29 November, also contributed to lower yields.

Table 2. Influence of calendar terms of the cessation of autumn vegetation on the grain yield of soft winter wheat

Autumn vegetation cessation	Calendar terms	Years	Yield, t/ha
Very early	till 28.10	1973, 1976, 1979, 1987, 1988, 1991, 1997	5.77* 3.81-6.73**
Early	from 28.10 to 07.11	1970,1974, 1975, 1980, 1981, 1986, 1992-1995, 1999, 2014	5.45 3.41-9.59
Optimal	from 08.11 to 18.11	1968, 1971, 1972, 1983-1985, 1989, 1998, 2001, 2003, 2004, 2011, 2012	4.79 2.6-6.82
Late	from 19.11 to 29.11	1967, 1969, 1977, 1982, 2000, 2005, 2007, 2008, 2010, 2015, 2017	6.13 3.18-9.17
Very late	after 29.11	1978, 1990, 1996, 2006, 2009, 2013, 2016	4.87 3.62-6.36

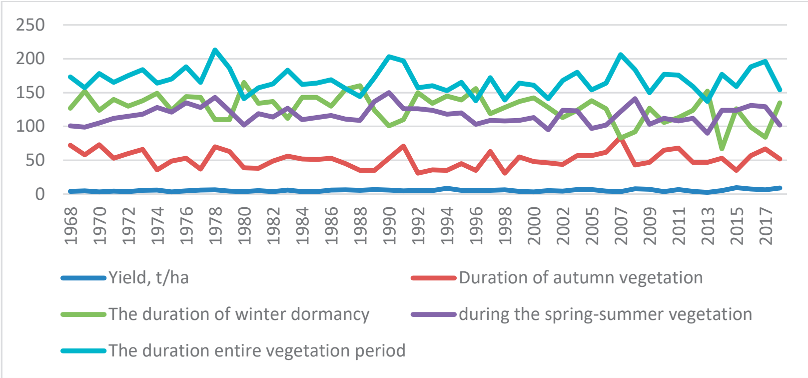

Figure 1. Yield, autumn vegetation cessation, duration of winter dormancy, duration spring-summer vegetation and duration vegetation period of winter wheat (for 1968-2008)

Netis (2007) concluded that with the early cessation of fall vegetation, the optimal sowing dates of wheat are shifted on 10-12 days toward the early ones. This is due to the fact that in early cooling of late sowing plants they do not have time to bush and form only 2-3 leaves.

Analysis of the grain yield of soft winter wheat shows that its highest formed in the late and in the very early and early calendar period of the cessation of autumn vegetation. But this pattern is not observed every year. Thus, in the 1967/68 and 1969/70 vegetation periods, with the late cessation of the autumn vegetation on 24 November and 28 November, the grain yield was 3.94 and 3.18 t/ha, respectively. It should be noted that in subsequent years, such deviations are not established. Therefore, it can be argued that the long autumn vegetation of soft winter wheat until the end of the third decade of November helps to increase the productivity of this crop.

We also noted deviations from the hypothesis during the early cessation of autumn vegetation. First of all, this applies to the vegetation period of 1979/80, when the grain yield was 3.81 t/ha. With a very early cessation of autumn vegetation (until 28 October), the period 1979/80 turned out to be atypical, with a grain yield of 3.81 t/ha. In the years with the optimal terms of the cessation of autumn vegetation, deviations were observed in 1972/73, 1985/86, 1989/90, 2003/04, 2004/05, when the grain yield reached 5.58-6.82 t/ha. Under a very late cessation of autumn vegetation, with an average yield of 4.87 t/ha, deviations from the average yield (6.36 t/ha) were established in 2016/17.

The prolonged stay of winter wheat plants at rest significantly affects the growth, development, and productivity of plants. It is proved that the longer the winter dormancy period of wheat, the weaker the plants come out of winter, and the crops are liquefied and sometimes die completely (Boichuk and Bazalii, 2011).

The obtained data on the duration of the winter dormancy period of soft winter wheat for 1967/68-2017/18 indicate its gradual reduction. At the same time, there is an increase in variation in the duration of this period. Thus, in 1967/68-1976/77 the duration of winter dormancy was 139 days with amplitude of 28 days. In 1977/78–2007/08, the period of winter dormancy was reduced with a variability of 59 days. In the last decade of research 2008/09-2017/18, the duration of this period averaged only 113 days with a variability from 67 days (2013/14) to 152 days (2012/13), which is three times higher than indicator 1967/68-1976/77 (Table 3).

Due to climate change observed in recent decades, the resumption of winter wheat spring vegetation of winter wheat often two to three weeks earlier than long-term (Goldvarg et al., 2019; Polovyi et al., 2017; Q. Luo et al., 2018).

Table 3. Duration of winter dormancy, spring-summer vegetation and the growing season of soft winter wheat

Years	Winter dormancy, days	Recovery time of spring vegetation	Duration of vegetation, days		Vegetation period, days
			from recovery to full ripeness of the grain	for the autumn period and from recovery in the spring to full ripeness of grain	
1967/68- 1976/77	139* 124-152**	23.03 18.03-13.04	114 99-135	170 156-188	309 294-332
1977/78- 1986/87	137 112-171	26.03 15.03-12.04	115 96-127	167 135-196	303 291-323
1987/88- 1996/97	135 101-160	16.03 22.02-07.04	121 103-150	165 138-203	299 291-307
1997/98- 2007/08	121 83-142	18.03 22.02-05.04	113 95-141	166 139-206	287 267-304
2008/09- 2017/18	113 67-152	17.03 29.02-15.04	114 90-131	168 137-196	281 244-289

* - average for 10 years; ** - limits of variability

The calendar term of spring vegetation recovery is generally caused by the receipt, circulation of warm or cold air masses. It is known that it is under their influence, and not under the influence of the sun, winter wheat can temporarily restore the growing season in winter. It is the atmospheric masses – warm or cold – that determine the time of the final onset of early or late restoration of vegetation (Brazhchenko et al., 2006).

The average ten-year duration of the period from TSVR to full ripeness of wheat grain ranged from 113 to 121 days. With slight differences over 10-year periods, there was significant variability of TSVR from 90 days (2013) to 150 days (1990). At the same time, there was an increase in the variation of this indicator from 36 days in 1968-1977 to 47 days in 1988-1997.

No significant changes in the duration of active soft winter wheat vegetation have been established in autumn and spring-summer for ten-year periods. Therefore, the average active vegetation in 1967/68-1976/78 was 170 days, and in 2008 09-217/18 - 168 days. At the same time, a significant increase in its variability was established from 32 days in 1967/68-1976/78 to 67 days (1997/98-2007/08).

The growing season of soft winter wheat, on average over 50 years of research, was 296 days with a variability of 224 to 332 days. These data indicate a reduction in this period in recent decades. Thus, in 1967/68-1976/78 the average duration of the growing season was 309 days, and in the following ten-year periods there was a gradual reduction of the winter wheat growing season and in 2008/09-2017/18 it was only 281 days. It should also be noted that in the last decade of research, the longest duration of the growing season was only 289 days.

The duration of TSVR for 1968-2018 in our studies was 52 days (from 22.02 to 15.04). For 50 years, the average date of TSVR is March 22. Therefore, the period of TSVR was divided as follows: very early - until 03.03; early - from 04.03 to 14.03; optimal - from 15.03 to 25.03; late - from 26.03 to 05.04; very late - after 05.04 (Table 4).

Table 4. Influence of the recovery time of spring vegetation on the grain yield of soft winter wheat

TSVR	Calendar terms	Years	Yield, t/ha
Very early	till 03.03	1990, 1995, 2008, 2016, 2018	7.26* 5.46-9.17**
Early	from 04.03 to 14.03	1989, 2002, 2007, 2014, 2015, 2017	5.95 3.62-9.59
Optimal	from 15.03 to 25.03	1971-1977, 1979, 1981-1983, 1986, 1991-1994, 2004, 2010-2012	5.26 3.41-8.59
Late	from 26.03 to 05.04	1968, 1970, 1978, 1984, 1985, 1988, 1997-2001, 2005, 2006, 2009	5.00 3.18-7.03
Very late	after 05.04	1969, 1980, 1987, 1996, 2013	4.50 2.60-6.23

In 50 years, the very early recovery of spring vegetation has been observed five times, the early - six. In the optimal time, TSVR was observed twenty times, late - fourteen times, and very late - five times.
The average grain yield of soft winter wheat according to TSVR indicates that the earlier it begins, the higher the productivity of this crop. Thus, with the early resumption of spring vegetation, the average grain yield was 7.26 t/ha, which is 1.87 t/ha higher than the long-term average. During the early recovery of spring vegetation, the yield was 5.95 t/ha with its variation - 5.97 t/ha.
In optimal terms of vegetation recovery, the grain yield was 5.26 t/ha with variability of 5.18 t/ha. The lowest wheat grain yield (5.00 t/ha) was under the late and very late (4.50 t/ha) TSVR. The yield variation was 3.85 t/ha for late and 3.63 t/ha for very late TSVR.
According to Mostipan et al. (2021) in the years of TSVR in the third decade of February, the productivity of winter wheat averages 6.42 t/ha, while in its late recovery, in the first decade of April, it decreases almost twice and is 3.29 t/ha (Mostipan et al., 2021).
We found that the pattern is not always confirmed; the earlier spring vegetation is restored, the higher the yield of wheat grain. Thus, during the early TSVR, its effect on wheat productivity was not observed in 2002, 2007, 2014 when the grain yield obtained (3.62-5.09 t/ha) was much lower than the long-term average (5.39 t/ha). This is usually due to unfavorable abiotic factors, which appear much later than TSVR.
Under the optimal terms of spring vegetation recovery in 1977, 1994, 2004 and 2011, the grain yield of winter wheat significantly exceeded the long-term average and amounted to 6.01-8.59 t/ha, which also does not coincide with the hypothesis of the impact of early harvest on crop productivity.
Atypical years also occurred during the late and very late recovery of spring vegetation. Thus, in 1978, 1987, 1998, 2005 and 2009 the grain yield of wheat was 6.23-7.03 t/ha, which is 0.84-1.64 t/ha higher than the long-term average.

CONCLUSIONS

The data obtained indicate a significant impact of climate change on the reduction of the winter dormancy period and the growing season of soft winter wheat in general. On average, in 1968-2018, the highest grain yield of soft winter wheat (6.13 t/ha) was obtained during the late cessation of autumn vegetation from 19.11 to 29.11. During the very early (until 28.10) and early cessation (from 28.10 to 07.10) vegetation of plants, the yield decreased slightly and amounted to 5.77 and 5.45 t/ha, respectively. It is established that the calendar dates for the cessation of the autumn vegetation have a clear tendency to shift to later dates.
With a slight difference in TSVR over ten-year periods, its significant variability was observed during the research years from 90 days (2013) to 150 days (1990). The highest grain yield of soft winter wheat (7.26 t/ha) was obtained with early TSVR, which is 1.87 t/ha higher than the long-term average. The lowest wheat grain yield was (5.00 t/ha) for TSVR late and very late (4.50 t/ha) TSVR. When creating varieties of winter wheat, breeders need to pay attention to meteorological changes and their significant impact on the ontogenesis of winter wheat and adapt the cultivation technology according to different climatic scenarios.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

MICROGRANULAR STARTER FERTILIZER EFFECTS ON GROWTH AND PRODUCTIVITY OF A HIGH-YIELD MAIZE HYBRID CULTIVATED UNDER CLIMATIC CONDITIONS OF ILFOV COUNTY

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Abstract

The researches presented in this paper were carried out during agricultural year 2019 in Grădiștea, Ilfov County and the main objective was investigation of the variability of yield for maize hybrid P0268 under application of a starter microgranular fertilizer with a 25 kg·ha^{-1} dose. The chosen hybrid is characterized by high yield, resistance to drought and high temperatures, being suitable for all types of soils, including those with low level of organic matter. The selected microgranular fertilizer for experiment ensures fast start of crops, accelerating the germination and influence positively the development of root system. The efficiency of microgranular fertilizer was evidenced at all maize vegetation stages and consisted in more vigorous and intense green colored plants, well developed radicular system and stems in comparison with unfertilized control variant. At harvest time, the grain yield in the case of fertilized variant was with 229 kg·ha^{-1}, higher in comparison with control. This experiment reveals the efficacy of microgranular fertilizer application on maize yield, but under climatic conditions of the agricultural year 2019, economic efficiency was low.

***Key words**: maize, microgranular fertilizer, yield.*

INTRODUCTION

To accomplish obtaining yields high enough to provide food for growing world population is essential to use inputs: mineral and organic fertilizers, different commercial formulations of fertilizers, phytosanitary products etc.

In the literature, there are many researches that emphasize the effects of fertilization on yield and quality parameters of yield for various crops.

Considering maize, it is known that high yields can be achieved by adequate supply and balance of essential nutritive elements and a limitative factor is water deficiency during any growth stage (Fageria & Baligar, 2011).

For instance, in the case of maize, the importance of fertilization is valued by many authors (Crista et al., 2014 (a, b); Barșon et al., 2021; Luță et al., 2022; Madjar et al., 2022).

Nowadays, beside mineral and organic fertilizers, application of microgranular fertilizers is currently increasing, due to their advantages: nutrients have immediate effects on plants, influence vegetative development leading to taller and more vigorous plants (Crista et al., 2014b), great spreading properties, higher yields (Balawejder et al., 2020), decrease of fertilizations costs (Jankowski et al., 2018) even reduce environmental impact (Thielicke et al., 2022). Furthermore, microgranular fertilizers favour intense roots development, assure a more efficient emergence and a better and homogenous density. In addition, in the case of late autumn or early spring sowing when plants could be affected by extreme climatic conditions, microgranular fertilizers ensure the reduction of negative effects resulted from these situations. Beside these, influence the efficient use of soil water and increase drought resistance. Also, provide resistance to weeds, pathogens and pests (https://agrointel.ro/48168/timac-agro-romania-10-motive-pentru-a-alege-ingrasamintele-microgranulate-pentru-fertilizarea-culturilor-agricole/).

A study (Jankowski et al., 2018) evidenced the efficacy of microgranular fertilizer application on winter oilseed rape: stimulated dry matter accumulation in rosette leaves, increased seeds and straw biomass and minimized the risk of soil salinization.

Application of microgranular fertilizer based on protein and calcined bones on maize crop increased the plant resistance to water stress during growing and consequently led to higher yields in comparison with control (Balawejder et al., 2020).

Other research (Haraga et al., 2022) investigated the effects of microgranular fertilizers over yield components and development of maize plants. The obtained results evidenced that applied fertilizer assured a better emergence of plants in the field combined with better development of root system, even increased seed yield in severe drought conditions.

Considering the importance of fertilization, it was developed an experiment with the purpose to evaluate the variability of yield for maize crop under application of 25 kg·ha^{-1} dose of microgranular starter fertilizer. The experiment site was Grădiştea, Ilfov County during agricultural year 2019.

MATERIALS AND METHODS

Experimental site

Experimental field was located at SC Picmar Prod Com SRL Society which is located in Grădiștea commune, Ilfov County (Figure 1). For researches were chosen two plots, sown with the same maize hybrid, one of them being fertilized with microgranular fertilizer.

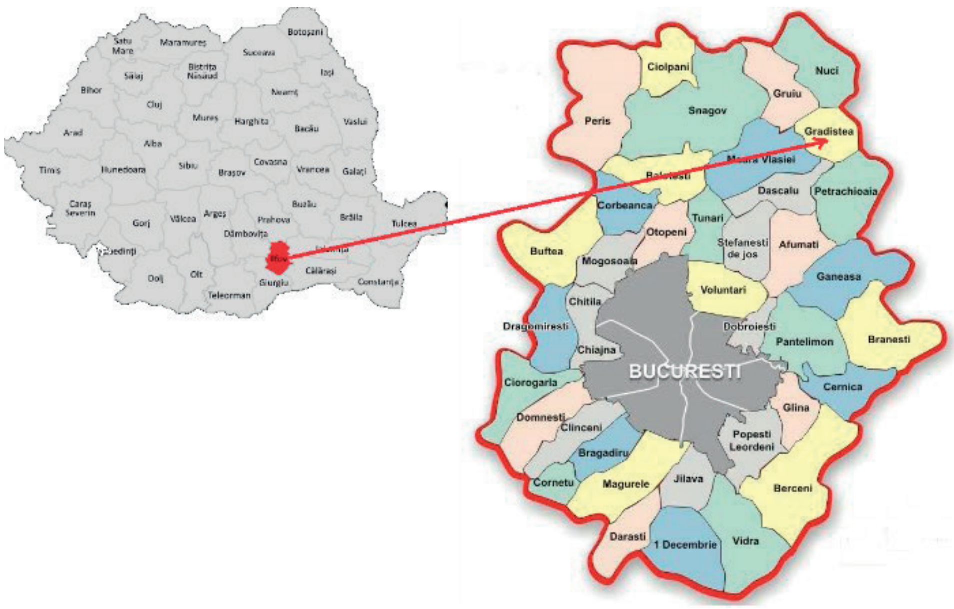

Figure 1. Position of Grădiștea commune on geographical map

Maize hybrid

For experiment was used P0268 a semi-late maize hybrid which is characterized by high yield, suitable for different types of soil, including those with low level of organic matter. Also, it is resistant to drought and high temperatures. Recommended densities are between 65000-70000 plants/ha for unirrigated plots and between 75000-84000 plants/ha for irrigated plots. In our experiment, P0268 maize hybrid was sown (April, Ist decade) with density of 70000 seeds/ha. Preceding crop was wheat.

Fertilization scheme

During experiment were applied several fertilizers: triple superphosphate (**TSP**), complex fertilizer NP 20:20 + 7SO_3 + Zn (**CF**), microgranular fertilizer (**MF**) and ammonium nitrate (33.5% N) (**AN**) as it is presented in fertilization scheme (Table 1).

Table 1. Fertilization scheme

Moment	Fertilizer	Dose ($kg \cdot ha^{-1}$)
October (II[nd] decade)	TSP	150
April (I[st] decade)	CF	250
	MF	25
May (II[nd] decade)	AN	140
June (I[st] decade)	AN	140

Used fertilizers

Characterizations of used fertilizers (**TSP, CF, MF, AN**) are depicted in Tables 2-5.

Table 2. Triple superphosphate (**TSP**) characterization

Characteristics	Details
Granule colour	grey
Granule size	1 to 5 mm (98% min.)
P_2O_5 content, %	46
P_2O_5 (soluble in citrate and water), %	45.5 (max.)
P_2O_5 (soluble in water), %	43 (max.)
Moisture, %	4 (max.)

Table 3. Complex fertilizer NP 20:20 + $7SO_3$ + Zn (**CF**) characterization

Characteristics	Details
Granule colour	dark grey
Granule size	3.3-4.1 mm
$N\text{-}NO_3^-$, %	7.5
$N\text{-}NH_4^+$, %	12.5
P_2O_5 (soluble in neutral ammonium citrate and water), %	20
P_2O_5 (soluble in water), %	16
SO_3 (total), %	7.5
SO_3 (soluble in water), %	6.5
Zn (total), %	0.01

Table 4. Microgranular fertilizer (**MF**) characterization

Characteristics	Details
Granule size	0.5-1.0mm
Formulation	NP 16:40 + 2MgO + $5SO_3$ + 2%Zn
$N\text{-}NH_4^+$, %	16
P_2O_5 (soluble in neutral ammonium citrate and water), %	40
SO_3 (soluble in water), %	5
MgO (total), %	2
Zn (total), %	2

Table 5. Granular ammonium nitrate (**AN**) fertilizer characterization

Characteristics	Details
Granule size	2-5 mm (90% min.) <1 mm (5% max.) <0.5 mm (3% max.)
Appearance	white slightly colored granules
N(total), %	33.5
$N\text{-}NO_3^-$, %	16.75
$N\text{-}NH_4^+$, %	16.75
pH	4.5 (min.)
Moisture, %	0.45 (max.)

Performed agrochemical analyses

Soil samples were collected and subjected to agrochemical analyses (Table 6).

Table 6. Soil agrochemical analyses

Analyses	Method
Moisture	gravimetry
pH_{H2O} (1:2.5)	potentiometry
K_{AL}	flame emission spectrometry
P_{AL}	spectrophotocolorimetry
Humus	Walkley-Black-Gogoașă method
$Zn_{AcNH4\text{-}EDTA}$ *	Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)
B_{H2O} #	Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)

P_{AL} = mobile form of phosphorus using ammonium acetate-lactate for extraction; **K_{AL}** = mobile form of potassium using ammonium acetate-lactate for extraction; **$Zn_{AcNH4\text{-}EDTA}$** = mobile form of zinc using ammonium acetate in the presence of EDTA for extraction; **EDTA** = ethylenediaminetetraacetic acid; **B_{H2O}** = soluble form of boron using boiling water for extraction; *NF X 31-120 (2003); #NF X 31-122 (1999).

Climatic conditions

Climatic conditions of year 2019 are presented in Table 7.

Table 7. Climatic conditions of year 2019

Month	Rainfall (mm)	Temperature (°C) (min./max.)
April	66.7	9-26
May	150.3	12-28
June	34.6	24-34
July	91.0	22-36
August	13.0	26-36
September	36.1	18-33

(Source:https://www.accuweather.com/ro/ro/gradistea/279663/september-weather/279663)

RESULTS AND DISCUSSIONS

Soil agrochemical characterization

The experiment was developed on reddish **preluvosol**, *parent material* - clay and loess deposits.

The agrochemical analyses performed for soil samples evidenced slightly acidic reaction, high level of mobile form of potassium (K_{AL}), middle level of humus and very low level of mobile form of phosphorus (P_{AL}) (Table 8).

Table 8. Results of soil agrochemical analyses

Analyses	Determined value	Interpretation
pH_{H2O}	6.35	slightly acidic
K_{AL}, mg·kg^{-1}	214	high level
P_{AL}, mg·kg^{-1}	6.74	very low level
Humus, %	2.48	middle level
Zn, mg·kg^{-1}	4.03	middle level
B, mg·kg^{-1}	0.44	middle level

Considering microelements (B, Zn), found concentrations are associated with middle levels.

In addition, solution used for extraction of mobile form of zinc (ammonium acetate in the presence of EDTA) has proven its efficacy and according to different authors (Vasile et al., 2006) offers the best results regarding mobility of metals in soil.

Visual evaluation of microgranular fertilizer application effects on maize plants

The radicular system of maize hybrid P0268 fertilized with MF is well developed, more vigorous, with increased root ramification as compared to control (without MF) (Figures 2 and 3).

According to Olbrycht and co-workers (2020), application of MF obtained from food industry by-products and fortified with proteins (30 kg·ha^{-1} dose) to maize was effective enough to have an impact on the intensive growth and development of the emerging root system.

At 10-12 leaves stage, the beneficial effects of MF application are more evident, since beside better formed radicular system, stem thickness is higher (Figure 3). Therefore, it could be considered that MF assured a better start in vegetation due to supply of direct accessible nutrients for plants.

Figure 2. Differences of radicular system at 4-6 leaves stage (left - without MF; right - with MF) (original images provided by SC Picmar Prod Com SRL)

Figure 3. Plant development at 10-12 leaves stage (left - without MF; right - with MF) (original images provided by SC Picmar Prod Com SRL)

Also, P_2O_5 high content of MF (40%) (Table 4) sustained proper the plants to overcome the pseudo (false) phosphorus deficiency which occurs mainly in cold, wet springs, as it has been spring of year 2019 (Table 7).

The efficiency of microgranular fertilizers with high content of P_2O_5 on maize crop is also sustained by researches reported by Haraga et al. (2022).

Visual comparative analysis of maize field evidenced the positive effects of MF application: dark green leaves, more vigorous stems, higher leaves surface (Figure 4).

Figure 4. Differences of maize field at 10-12 leaves stage (*left - without MF; right - with MF*) (original images provided by SC Picmar Prod Com SRL)

Results regarding maize yield

The analysis of the yield values evidenced the efficiency of MF application, more specific an increase for fertilized variant (V_2) with 229 kg·ha^{-1}, meaning 2.26% higher than control variant (V_1). Variance analysis indicates distinct significant differences for V_2 (Table 9). Some authors (Balawejder et al., 2020) reported an increase of maize yield by 6.6% in comparison with control after MF application of 30 kg·ha^{-1}.

Table 9. Variability of yield under influence of microgranular fertilizer (MF) application

Variants	Yield		Differences		
	kg·ha^{-1}	%	kg·ha^{-1}	%	sign
Control (**V_1**)	10125	100	Control	-	-
MF application (**V_2**)	10354	102.26	229	2.26	**
DL 5%=72 kg/ha; DL 1%=166 kg/ha; DL 0.1%= 529 kg/ha					

Beside proven beneficial effects on maize plants and yield, application of MF influenced also the moisture of seeds. Hence, in the case of V_2 the standard grain moisture of 14% has been achieved easily than in the case of V_1.

Variance analysis for crop yield at a standardized moisture content of 14% indicates distinct significant differences in the case of V_2 variant, the yield being with 257 kg·ha^{-1} higher, representing an increase of 2.50% over control (V_1) (Table 10).

Table 10. Variability of yield at standard moisture content of 14% under influence of microgranular fertilizer (MF) application

Variants	Yield		Differences		
	kg·ha^{-1}	%	kg·ha^{-1}	%	sign
Control (**V_1**)	10242	100	Control	-	-
MF application (**V_2**)	10499	102.50	257	2.50	**
DL 5%=79 kg/ha; DL 1%=183 kg/ha; DL 0.1%= 584 kg/ha					

Having in view the obtained results it could be concluded that application of MF (dose of 25 kg·ha^{-1}) has generated a yield higher with 229 kg·ha^{-1} (2.26% increase) in the case of fertilized variant (V_2).

Other perspectives

As gaining profit on the basis of obtained yield is an objective for each agronomist, in the context of our research was evaluated economic efficiency of MF application on maize crop.

Considering the costs with MF acquisition, applied doses, maize yield market value, it has been found that economic efficiency is very low corroborated with extreme climate events of year 2019 (low temperatures, intervals with high temperatures, heavy downpours, water shortage etc).

For example, for maize crop optimal rainfall levels are from 60-80 mm in May, 100-120 mm in June, 100-120 mm in July and 60-80 mm in August (Roman et al., 2011), conditions which for year 2019 were not fulfilled (Table 7). In addition, some authors (Butts-Wilmsmeyer et al., 2019) stated that both grain yield and compositional quality are related with water availability during flowering and grain fill. Moreover, good performances of yield components for maize crop under irrigation conditions were reported by Madjar et al. (2017).

However, the experiment has proven clearly the importance of choosing the proper maize hybrid suitable for agricultural area where it is cultivated, combined with proper fertilization system, including use of microgranular fertilizer. Still, despite all agronomic precautions, determinant factor it seems to be climatic conditions.

CONCLUSIONS

Influence of microgranular fertilizer on maize crop has evidenced positive effects on root development, plants were more vigorous and the grain yield in the case of fertilized variant was with 229 kg·ha^{-1}, higher in comparison with control.

Economic efficiency of microgranular fertilizer application it has been insignificant in the climatic conditions of year 2019. Therefore, it may be concluded that the influence of climatic conditions was higher than the influence of fertilizer application but the researches on this subject will be extended in the future.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

CONTROL OF RYE PESTS AND PATHOGENS ON SANDY SOILS IN SOUTH OF ROMANIA

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Abstract

*Due to climate change and human activity, biotic constrainers are anticipated to expand to regions where they were previously irrelevant. This will present new management challenges for crops, particularly in cropping systems dependent on minor cereals diversification. In Central and Eastern Europe, Secale cereale is a minor cereal that contributes to the diversity of crop species, particularly in marginal areas where wheat cultivation is unfavourable. During 2020-2021, using different chemical and biological pesticide formulations, a plant-pest-pathogen interaction profile was observed on Suceveana rye genotype in a randomized complete block design with three replications in dry area from South of Romania. The best protection against leaf rust (Puccinia recondita f. sp. secalis) was provided by Poliversum (Pythium oligandrum M1, 1×10.000.000 oospors/g product) for the 1st assessment - attack degree = 0.65% and for the 2nd assessment - attack degree = 1.42%, while against pests was provided by Bioinsekt and deltametrin (0.75 ml/ha). Negative and significant correlation of leaf rust attack with grain yield (r =-0.8561***) and pests attack with grain yield (r =-0.8631***) were found during 2020-2021 cropping season.*

Key words: *Secale cereale, attack degree, leaf rust, pesticides formulation, pests.*

INTRODUCTION

Climate change, the risk of epidemics and pandemics, the biodiversity created by invasive species, biotic and abiotic stresses, technological and genetic advancements have all had an impact on natural systems and agricultural and horticultural production around the world (Cotuna et al., 2021; Paraschivu & Cotuna, 2021; Răduţoiu, 2020; Răduţoiu & Băloniu, 2021; Răduţoiu & Stan, 2022. Răduţoiu & Ștefănescu, 2022; Velea et al., 2021; Zală, 2021). As a result, natural constrainers have grown up in importance posing a serious danger to global food security due to its numerous negative effects on the crops management, economy, society, technology, environment, especially in marginal areas characterized mainly by limited biophysical quality of the land (Durău et al., 2021; Matei et al., 2022a; Paraschivu et al., 2022a; Sărățeanu et al., 2023).

Worldwide, new cropping techniques, precise farming equipment, biotechnology, breeding for more resistant types, and high-tech solutions for controlling biotic and abiotic constrainers have profoundly transformed agriculture over the past three decades (Bălașu et al., 2015a; Matei et al., 2022b; Păunescu et al., 2022; Popescu et al., 2022; Zală et al., 2023). The conservation of genetic resources in agriculture and food security is a long-term challenge that transcends the borders of national interests (Bonciu, 2020; De Souza and Bonciu, 2022a; De Souza and Bonciu, 2022b). Maintaining access to safe, disease - and pest-free and affordable agricultural products and raw materials and ensuring sustainable agricultural production are challenges that must be faced in the context of increasing demand for agricultural products (Bonciu et al., 2021; Bonciu, 2022a; Bonciu 2022b; Paunescu et al. 2021).

By 2080, global temperature is anticipated to increase by 4,5-degree Celsius declining by 6% in productivity per each degree Celsius (Asseng et al., 2015).

Previous findings emphasised that climate change poses a serious danger to the health of wild and cultivated plants leading to food insecurity due to pathogens life cycles, increased incidence, pathogenicity, genetically recombination and aggressiveness traits (Bălașu et al., 2015b; Cotuna et al., 2022a; Cotuna et al., 2022b; Cotuna et al., 2022c; Cristea et al., 2015; Paraschivu et al., 2017; Paraschivu et al., 2019).

In this context, with 1.5 billion hectares of farmland and 3.4 billion hectares of pastures, agriculture occupies about 38% of the planet's surface (Alexandratos & Bruinsma, 2012). At the same time there are 2.7 billion ha of marginal land of which 1.5 billion ha are uncultivated, usable area for agricultural use, but an effective management could sort them out of this category (Tilman et al., 2011).

Development of land evaluation systems based on biophysical conditions have been started in the 20th century (Doran et al., 2018; Karlen et al., 1997).

The classification of land as prime, marginal, or unproductive often depends on the land's management or type of agriculture.

The marginal lands can be turned into productive land by appropriate land management or cultivation of suitable crops. In marginal areas from Central and Eastern Europe, where soil and climate are unfavourable for wheat production, rye (*Secale cereale*) is a minor cereal who plays a significant role in crop species diversity.

Ones of the most important biotic constrainers of rye in Central and Eastern Europe is Brown rust (BR), known also as Leaf rust (LR), caused by the obligate biotrophic basidiomycete *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) and pests (Meidaner et al., 2012).

Rye is susceptible to a number of pathogens and pests, despite being a significant source of resistance genes for wheat (Zhang et al., 2001; Saulescu et al., 2011).

Actually, genetic resistance is the disease's most cost-efficient and successful control strategy for Leaf rust (LR), but all-stage resistance is often not durable, because new pathogen`s virulent races evolve to overcome this type of resistance (race non-specific resistance) adapting to new environments.

Therefore, additional fungicides used is still remaining an important part of integrated disease management.

Little previous research is reported in controlling pathogens and pests in rye system in dry marginal areas. Therefore, the aim of the study was to evaluate the management of rye-pathogen-pests interactions in dry marginal environments from Southern Oltenia, Romania, using diverse formulations of conventional and biological pesticides.

MATERIALS AND METHODS

During 2020-2021 growing season, a plant-pest-pathogen interaction profile was observed on Suceveana rye genotype using different chemical and biological pesticide formulations in a randomized complete block design with three replications in dry area from Research and Development Station for Plant Culture on Sands Dăbuleni, located in Southern Oltenia, Romania (43°48′04″N 24°05′31″E), on sandy soil, poorly supplied with nitrogen (between 0.04-0.06%), well supplied with phosphorus (between 54 ppm and 77 ppm), reduced to a medium supplied with potassium (between 64 ppm to 83 ppm), low in organic carbon (between 0.12-0.48%) and weakly acidic pH to neutral (between 5.6 and 6.93).

Technological measures applied included broadcasting the fertilizers at sowing time with N80P80K80, one side nitrogen fertilization during vegetation with N_{70}, starter irrigation with 250 m^3 water/ha and supplemental irrigation with 300 m^3 water/ha at heading stage. Also, weeds control was done using Dicopur Top 464 SL (1 l/ha) applied in postemergence to control annual and perennial dicotyledons accordingly with the recommendations (cereals to the formation of the first internode and the weed species in the small phase of about 2-4 leaves and a maximum of 10-15 cm high for perennial weeds).

Plant–pathogen interaction was assessed in natural infection with *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) in a randomized complete block design (RCBD) with three replications.

Each plot had 5 m^2, a space of 1 m between blocks and 0.5 m between plots.

Disease observations were recorded since the first appearance (booting stage) of leaf rust infection on Suceveana rye genotype and at early dough stage (Zadoks scale) (Zadoks et al., 1974).

The Frequency (F%) and Intensity (I%) of leaf rust and insect's attack were determined for all assessed trail variants.

Leaf rust Intensity (%) was recorded for each trial variant assessing 10 plants randomly selected and pre-tagged plants of the central four rows of each plot and the mean of the ten plants was considered as the value for a plot.

Rust severity was determined by visual observation and expressed as percentage coverage of leaves with rust pustules (from 1% to up 75%) using the sale developed by Oladiran and Oso (1983) (Table 1):

Table 1. Leaf rust intensity expressed as percentage coverage of leaves with rust pustules (Oladiran and Oso, 1983)

Category	Percentage leaf rust infection relative to susceptible check
0	0 – no attack
1	1-10% of leaf area covered with rust pustules
2	11-25% of leaf area covered with rust pustules
3	26-50% of leaf area covered with rust pustules
4	51-75% of leaf area covered with rust pustules
5	> 76% of leaf area covered with rust pustules

For assessing the intensity of insect's attack was used the following scale (Table 2).

Table 2. Intensity of insect's attack expressed as percentage of damaged leaves

Category	Percentage of damaged leaves
0	0 - no attack
1	1-3% leaves damage
2	3-10% leaves damage
3	10-25% leaves damage
4	25-50% leaves damage
5	50-75% leaves damage
6	75-100% leaves damage

The attack frequency has been set with a metric frame (50 cm x 50 cm), taking in account the relative value of the attacked plants' number in report with the total number of the analysed plants or organs.

These parameters were used to calculate Attack Degree (AD%) using the formula: AD% = (F% x I%)/100 (Cociu and Oprea, 1989).

The treatment combinations are presented in Table 3.

Table 3. Treatments used in the experimental trial

Factor A fungicides	Factor B insecticides
a1-no treatment	b1-no treatment
a2- Poliversum -100 g/300 l water/ha	b2-Decis Expert 100 EC-75 ml/ha
a3-Mimox - 3 L/ha	b3-Bioinsekt – 0.5-1 L/ha
	b4-Neemex - 1-1.25 L/ha

In order to characterize the evolution of climatic parameters (air temperature, rainfall, humidity, wind speed) into the experimental field it was used an automatic weather station (AWS).

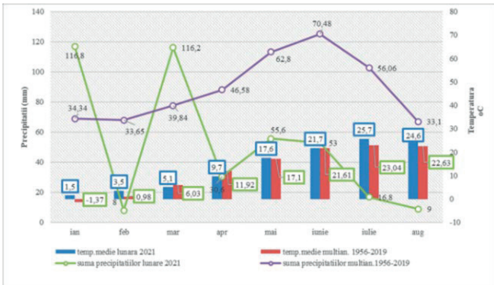
Means were compared with the no treated genotype Suceveana (control).

The experimental data were calculated and analysed, using MS Office 2019 facilities, while statistical analysis involved analysis of variance procedure (ANOVA) and significant differences were determined by the SD test at $P<0.05$ (Saulescu, 1967).

RESULTS AND DISCUSSIONS

Sandy soils in the dry south of Romania have insufficient natural resources for the growth of conventional crops, so it is necessary to grow crops that can cope with climate changes effects. Ones of the most suitable crops for these lands are sweet sorghum, sweet potato, rye, triticale, peanuts, cowpea, Jerusalem artichoke (Prioteasa et al., 2018; Prioteasa et al., 2019; Matei et al, 2020a; Matei et al., 2020b; Dima et al., 2021a; Dima et al. 2021b; Drăghici et al., 2021; Matei et al., 2021).

During 2019-2020 cropping season favourable climatic conditions led to the infection of rye with *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) and insects attack (*Schizaphis graminum, Eurygaster integriceps, Mayetiola destructor, Chlorops pumilionis*). Humidity was determined by the amount of rain of 406.00 mm, comparatively with multiannual average rainfall of 376.85 mm, while the monthly average temperature was 13.68°C comparatively with multiannual average temperature of 12.7°C (Figure 1).



* Automatic weather station DRSPCS Dabuleni, Romania

Figure 1. Climatic conditions during the study period (2021 year)

During January to August 2021 the monthly average temperature increased up to +0.93°C comparatively with multiannual average temperature for January to August between 1956-2019 for the same geographic area (+12.74°C), while rainfall amount for evaluated period was higher with 29.16 mm than multiannual amount for dry areas in Southern Romania. The rainfalls were higher in January and March, while for all other months the amount was lower than multiannual monthly average, impacting the leaf rust and pest's attack. The symptoms of Leaf rust disease exhibited for the first time at the end of April 2021. The fungus needs approximately six hours of moisture on leaves to start developing. With much moisture and suitable temperatures, lesions are formed within 7-10 days and spore production reduplicate another uredospore generation.

Identification of the fungus *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) and its characteristics were done in the Phytopathology Laboratory of Agriculture Faculty in University of Craiova, using MOTIC BIM-151B LED (40-1000x) microscope. The average size of uredospores release from uredinia is 20 mm in diameter and colour orange-brown having up to eight germ pores scattered in dense walls.

These findings suggest a modification of life cycle of the pathogen *P. recondita* f. sp. *secalis* by many generation numbers and higher resistance of uredospores to increased temperature.

Leaf rust pustules are small, with thousands of spores within, circular to oval shape, with orange to light brown dusty spores (uredospores) on upper surface of leaves surrounded by a light-coloured halo (Figure 2).

Figure 2. Pustules with uredospores of *Puccinia recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) (original photo Paraschivu Mirela, 2021)

Previous findings emphasized that Suceveana variety is very susceptible to *P. recondita* f. sp. *secalis* and it is necessary fungicide treatment as a part of integrated crop management (Paraschivu et al., 2021; Paraschivu et al., 2022b).

During the cropping season 2020-2021 the most affected variant by the attack of pests and pathogens was a1b1 (control - no treatment).

The results showed that after the 1st applying was applied the incidence of leaf rust severity was low for all fungicides applied comparatively with the control variant (no treatment) (AD =2.64%). There were not find significant differences between the two fungicides applied no matter insecticide combination (Table 4).

Table 4. The influence of the 1st treatment applied for controlling pathogens and insect's attack on rye during 2020-2021 cropping season

Fungi-cide	Insecti-cide	Attack degree the 1st det.- after the first treatment					
		Leaf rust			Insects		
		AD%	Dif. %	Signif	AD%	Dif. %	Signif
Untreated	Control	2.64	Mt		0.92	Mt	
	Decis Expert 100 EC	2.23	0.41	o	0.32	0.6	o
	Bioinsekt	2.37	0.27	o	0.46	0.46	o
	Neemex	2.04	0.6	o	0.60	0.32	
Poliversum -100 g/300 l water/ha	Control	0.98	Mt		0.85	Mt	
	Decis Expert 100 EC	0.65	0.33	o	0.41	0.44	o
	Bioinsekt	0.86	0.12	o	0.32	0.53	o
	Neemex	0.74	0.24	o	0.48	0.37	
Mimox 3 l/ha	Control	1.12	Mt		0.79	Mt	
	Decis Expert 100 EC	0.85	0.27	o	0.28	0.51	o
	Bioinsekt	0.96	0.16	o	0.35	0.44	o
	Neemex	1.06	0.06		0.72	0.07	
	LSD 5%		0.10			0.38	
	LSD 1%		0.45			0.87	
	LSD 0.1%		0.81			1.02	

*dif. < 5% significance level
no treatment variant = control

It was observed that Neemex insecticide has a slightly fungicide effect when it was applied alone (AD = 2.04%).

The best protection against leaf rust attack was noticed for Poliversum (AD = 0.65%) and Mimox (AD = 0.85%) mixed with Decis Expert 100 EC when the treatment has done at the beginning of booting stage.

After the 2nd determination it was observed that the evolution of leaf rust wasn't significant despite successive infections with uredospores suggesting that treatments applied were effective. Even for the 2nd treatment the lowest attack degrees for leaf rust were noticed for variants treated with fungicides (Table 5).

When insecticides were applied together with fungicides it was observed that insects attack degree was lower comparatively with the control (no treatment), than when they were applied alone.

Decis Expert 100 EC offered the best protection against insects' attack when it was applied alone for both treatments (AD = 0.32%, AD = 1.12%),

When insecticides were mixed with fungicides the best control of the insects was offered by Mimox+Decis Expert 100 EC (AD = 0.28%) for the 1st treatment and Poliversum -100 g/300 l water/ha + Bioinsekt (AD=0.54%) for the 2nd treatment.

Table 5. The influence of the 2nd treatment applied for controlling pathogens and insect's attack on rye during 2020-2021 cropping season

Fungi-cide	Insecti-cide	Attack degree the 2nd det.- after the second treatment					
		Leaf rust			Insects		
		AD%	Dif. %	Signif	AD%	Dif. %	Signif
Untreated	Control	4.76	Mt		2.76	Mt	
	Decis Expert 100 EC	4.12	0.64	oo	1.12	1.64	ooo
	Bioinsekt	3.86	0.9	oo	1.33	1.43	oo
	Neemex	3.62	1.14	oo	2.09	0.67	o
Poliversum -100 g/300 l water/ha	Control	2.03	Mt		2.14	Mt	
	Decis Expert 100 EC	1.57	0.46	o	0.87	1.27	oo
	Bioinsekt	1.42	0.61	o	0.54	1.60	ooo
	Neemex	1.88	0.15		1.25	0.89	o
Mimox 3 l/ha	Control	3.10	Mt		1.86	Mt	
	Decis Expert 100 EC	2.84	0.26		0.97	0.89	o
	Bioinsekt	2.61	0.49	o	0.62	1.24	oo
	Neemex	2.73	0.37	o	1.10	0.76	o
	LSD 5%		0.32			0.51	
	LSD 1%		0.61			1.21	
	LSD 0,1%		1.15			1.45	

*dif. < 5% significance level
no treatment variant = control

The highest yields were obtained for the variants with fungicides mixed with Bioinsekt (Poliversum + Bioinsekt = 3533.3 kg/ha and Mimox + Bioinsekt = 3266.7 kg/ha), while among variants treated only with insecticides the highest yield was recorded for Decis Expert 100 EC (2600 kg/ha) and Bioinsekt (2533.3 kg/ha).

Negative high correlations were observed between grain yield and pathogens and insects attack in 2020-2021 cropping season.

These results suggest that the influence of biotic constraints on plants, which reduced the amount of healthy plant tissue available for photosynthesis, decreased the output.

The response of rye to treatments applied along with grain yield (t/ha) suggested the presence of inverse relation between the disease and pests' severity and grain yield.

The highest significant loss percentages were found in no treated variant.

The value of determination coefficient (R^2 = 0.7329) indicated that up to 73% of variation in rye yield could be explained by leaf rust attack. It was noticed a highly significant correlation between leaf rust severity and grain yield (r = -0.8561***) (Figure 3).

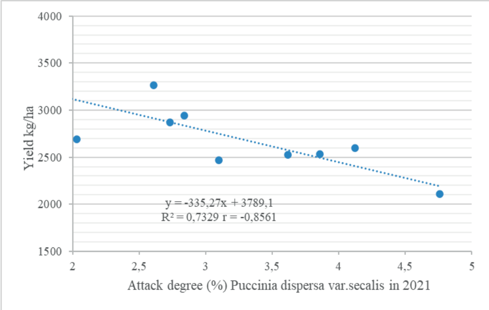


Figure 3. Relationship between Leaf rust severity and rye grain yield in 2020-2021 cropping season

Previous authors reported in Europe high yield losses due to Leaf rust in rye (Roux & Wehling, 2010; Meidaner et al., 2012).
Also, other authors showed that the application of fungicides led to 29% higher yields comparatively with untreated plots (Hartleb et al., 1995).
In experimental trials epoxiconazole, pyraclostrobin and fluxapyroxad showed high efficiency in controlling leaf rust in rye (Kupferund and Schröder, 2014)
The results showed that some pathogens and pests tend to become more aggressive even in cropping systems based on crops diversification by minor cereals.
The experiment's findings demonstrate that Leaf Rust is a major disease of rye in arid marginal areas of Romania, and climate variability can cause more outbreaks. The value of determination coefficient (R^2 = 0.745) indicated that up to 74% of variation in rye yield could be explained by insects' attack. It was noticed a highly significant correlation between insects' attack and grain yield (r = -0.8631***) (Figure 4).

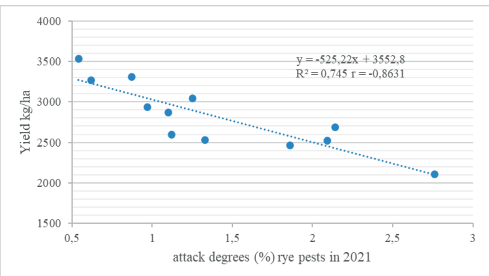


Figure 4. Relationship between insects' attack and rye grain yield in 2020-2021 cropping season

CONCLUSIONS

The present study was carried out to assess impact of different formulations of fungicides and insecticides on the attack of P. *recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) and insects (*Schizaphis graminum, Eurygaster integriceps, Mayetiola destructor, Chlorops pumilionis*) in natural conditions in dry area from Southern Romania during 2020-2021 cropping season. The increase of monthly temperature with +0.93°C lead to earlier incidence of the disease and insects attack starting even with the third week of April. The best protection against leaf rust was provided by Poliversum -100 g/300 l water/ha + Bioinsekt while the best control against pests was provided by Mimox+Decis Expert 100 EC (AD = 0.28%) for the 1st treatment and Poliversum -100 g/300 l water/ha + Bioinsekt (AD = 0.54%) for the 2nd treatment. For both pests and leaf rust control it was noticed the synergistic effect of insecticides and fungicides used in the experiment. Negative and significant correlations of pests and leaf rust attack degrees with grain yield (r = -0.8561***, respectively r = -0.8631***) were found during 2020-2021 cropping season.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Development Research Station for Plant Culture on Sands Dabuleni, Romania and was financed by the Ministry of Agriculture and Rural Development, Romania, through the ADER Project 1.4.2. (2019-2022).

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCHES CONCERNING THE INFLUENCE OF THE MAIZE HYBRID MATURITY AND THE IRRIGATION REGIME ON THE THOUSAND KERNEL WEIGHT AND HECTOLITER MASS

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Abstract

The scientific data presented in this paper were obtained in an experiment unfolded in 2017, 2018 and 2019 at ARDS Marculesti, Călărași County, Romania. The experiment was set up using the two-factor subdivided plot method, the A-factor was the corn hybrid and B factor-irrigation regime. The subdivisions of A factor have been: a1- P9175 (FAO 330), a2 - KWS BELLAVISTA (FAO 330), a3 - KWS SMARAGD (FAO 350), a4 - KWS KASHMIR (370 FAO) and a5 - KWS DURANGO (FAO 480). The subdivisions of B factor have been: b1 - rainfed; b2 - stressed at and after flowering: b3 - stressed before flowering and b4 - full irrigated throughout the vegetation period. The highest hectoliter mass values, as average, were obtained by the Bellavista hybrid and the lowest by the P9175 hybrid. As regard HLM, no significant values between irrigation treatments were recorded. The lowest TKW values were recorded in the Bellavista and Smaragd hybrids. The mass of one thousand grains registered very significantly positive differences between rainfed and irrigated, with very large amplitudes, close to 100 g.

Key words*: the thousand kernel weight (TKW), the hectoliter mass (HLM), corn hybrids, irrigation regimes.*

INTRODUCTION

Nowadays global warming has become an obvious, perceptible phenomenon. The global average soil surface temperature of 2022 year has recorded a 0.79^{0}C increase as compared to the last century. Moreover, the soil surface average temperature in 2022 was 0.24^{0}C higher than the last decade (1991-2020) (Figure 1).

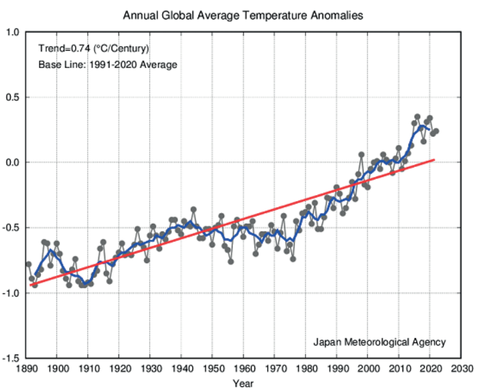

Figure 1. The soil global surface average temperatures since meteorological data have been taken (1890). Source: https://ds.data.jma.go.jp/tcc/tcc/products/gwp/temp/ann_wld.html

However, some researchers are of the opinion that the increase in the amount of carbon dioxide in the atmosphere is generated, to a greater extent, by natural phenomena, such as volcanic eruptions and the breathing of living things, and its fixation from the atmosphere can be efficiently produced by the absorption of ocean water and through more active photosynthesis. The effects of warming the Earth's atmosphere through the greenhouse effect are diverse and include increasing the number and intensity of extreme weather events such as storms and hurricanes, extreme drought, floods, etc. As the meteorological data show, in our country and in Europe, the warmest years were recorded in the last 20 years and the amplitude between very rainy and very dry years became much larger also in this time interval (Jaehyuk and Nazif, (2016). Other significant effects are the already felt water crisis, the decrease in ecological diversity, increased pest attack and the migration of human populations.

Modern agricultural technologies must take these changes into account. This can be done through two main directions: the creation of new corn hybrids, better adapted to the new climatic conditions and the improvement of technological measures related to soil tillage, plant density, weed, disease and pest control, fertilization, etc. (Campos et al., 2004; Duvick, 2005; Food and Agriculture Organization of the United Nations (FAO), FAO Statistical Databases, 2013; Löffler et al., 2005; Marin, 2004; Marin et al., 2008; Marin et al., 2015; Tardieu, 2012),

MATERIALS AND METHODS

The hectolitre mass (HLM) and the weight of a thousand grains (WTG) are defined as the ability of the grain to be able to form powder; this is also referred to as flour efficiency of the grain. These qualitative factors are the determinant of the economic value of grains as well as the packaging properties (Oniya et al., 2019).

Unfavorable climatic conditions, such as drought or heavy rainfall, the attack of diseases and pests can lead to a high percentage of chaff and a low content of flour. Therefore, the mass of one thousand grains and the hectoliter mass are very important economic and qualitative properties. The intrinsic features of maize hybrid are, also, important in this respect. Maize with low HLM often has a lower percentage of hard endosperm and consequently, produces a lower yield of prime, large grits when milled indicating that HLM can indeed be used as a quality indicator of maize (Engelbrecht, 2008).

Evaluation of maize quality is generally limited to visual inspection and laboratory milling tests (Watson, 1987, cited by Engelbrecht, 2008). Visual tests include inspection of the kernels for blemishes and insect damage. The hardness of maize kernels is also determined because of its importance during the maize milling process as it determines the milling performance (Engelbrecht, 2008). The way we have measured these units are presented in the current article.

The results regarding the mass of one thousand grains and the hectoliter mass presented in this article come from an experiment carried out at Agricultural Research and Development Station of Marculesti, Calarasi County, Romania, in 2017, 2018 and 2019 years. This experiment has had 2 factors, the factor A - maize hybrid and the factor B - irrigation regime. The setting up method was randomized blocks, with three replications. The investigated hybrids (factor A) were: a1- P9175 (FAO 330), a2 - KWS BELLAVISTA (FAO 330), a3 - KWS SMARAGD (FAO 350), a4 - KWS KASHMIR (370 FAO) and a5 - KWS DURANGO 480). The B factor (irrigation regime and period) had 4 graduations, namely: b1-rainfed; b2-stressed at and after flowering; b3-stressed before flowering and b4-full irrigated throughout the vegetation period. The irrigation method was drip to drip and there were automatic sensors to determine the actual soil moisture. Sowing and harvesting were done mechanically using the seed drilling machine and BAURAL SP2100 combine harvester (Figure 2). The production obtained on each experimental plot, the humidity of the grains at harvesting and the hectoliter mass were determined automatically with the equipment installed on the combine.

Figure 2. The Baural SP 2100 combine harvester for experiments

The mass of a thousand grains was determined using a photocell device for counting, along with a precision balance. Along with these determinations, measurements were made on the height of the cob's insertion, the height of the plant, the number of rows per cob, the number of kernels per row as well as the yield. The statistical interpretation was made by variance analysis.

RESULTS AND DISCUSSIONS

The climatic conditions of the experimental years, 2017, 2018 and 2019, as well as the 30 years average are presented in Figures 3, 4, 5 and 6.

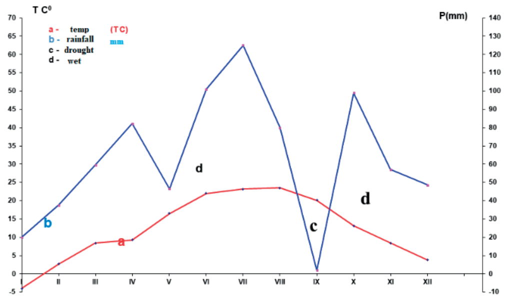

Figure 3. Climatic conditions of 2017 year at ARDS Marculesti by Walter Lieth climograme

From these data, it can be seen that 2017 was a year with average monthly temperatures close to the thirty years average but with very abundant precipitation in the summer months, June, July and August. In September, only 2 mm of precipitation was recorded, but this did not affect the maize production. Also, in May, 25 mm were recorded, compared to 45 mm, which is the thirty years average, something that determined the phenomenon of drought in the first growth phases of maize.

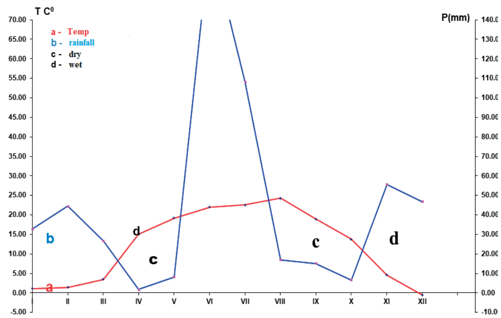

Figure 4. Climatic conditions of 2018 year at ARDS Marculesti by Walter - Lieth climograme

The year of 2018 recorded a very dry period in the spring months and May, with extremely low rainfall amounts of only 10 mm in two months, April and May. This greatly affected germination and growth in the early stages of maize growth. The situation changed dramatically in the summer months of June and July, when there were recorded rainfall of 180 mm, in June and 108 mm in July. The abundant rainfall from this activity favored the growth and development of maize plants in optimal conditions. The months of August and September were dry but this did not affect the maize production.

Temperatures recorded in 2018 were generally lower than the thirty years average in June and July due to the extremely heavy rainfall recorded. These climatic conditions led to very good pollen viability and fertility in maize.

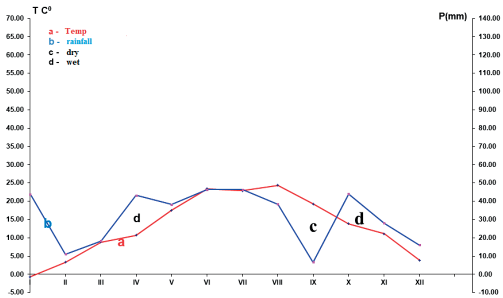

Figure 5. Climatic conditions of 2019 year at ARDS Marculesti by Walter - Lieth climograme

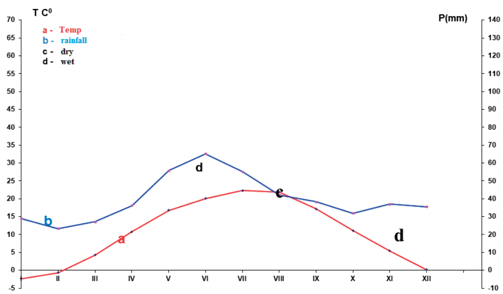

Figure 6. Climatic conditions of 30 years average from ARDS Marculesti, Walter - Lieth climograme

The year 2019 recorded average monthly values close to the thirty years average values, namely 10.7^{0}C in April, 17.8^{0}C in May, and 23^{0}C in June and July. Rainfall, however, was lower than the thirty years average and did not ensure optimal growth of corn plants. Thus, in May there were 30.2 mm compared to the 55.8 mm of thirty years average, 46.4 mm in June, compared to the 65.2 mm of thirty years average and 46.4 mm in July, compared to 55, 1 mm of thirty years average.

a. Hectoliter mass results

Hectoliter mass determination is believed to have been performed as earlyas the 17th or 18th centuries and to be of British origin (Greenaway et al., 1977, cited by Engelbrecht

Mandy, 2008). The first reported HLM determination was, however, performed in 1858 and the result was used as a grading factor for spring wheat in Milwaukee, Wisconsin (Phillip et al., 1936, cited by Engelbrecht, 2008). The Chicago Board of Trade adopted this measurement as a grading factor for spring wheat in 1859 (Phillip et al., 1936, cited by Engelbrecht, 2008). However, little is known about the design of early HLM devices or the procedure used to perform the tests (Engelbrecht Mandy, 2008). Hectoliter mass as a quality indicator for maize has not been proven very useful (Dorsey-Redding et al., 1991, cited by Engelbrecht, 2008). However, it has been shown that maize with low HLM has lower percentage of hard endosperm and therefore produces lower yield when milled (Rutledge, 1978, cited by Engelbrecht, 2008); thus HLM can be useful as an indicator of milling yield of maize (Engelbrecht, 2008).

The hectoliter mass data for 2017, 2018 and 2019 years are presented in Tables 1, 2 and 3.

From the hectolitre mass data, in 2017 year, it can be seen that the highest values of this character were recorded with the Bellavista hybrid and the lowest with the Kashmir hybrid (Table 1).

It is, also, observed that the rainfed and irrigated variants after flowering gave higher hectoliter masses than those irrigated before flowering and irrigated throughout the vegetation period, with very significantly positive differences (Table 1).

Other scientists have been had same results as we have been reported. This way, the nitrogen rate as well as the irrigation regime has influenced the mass of a thousand grains as well as the hectoliter mas in a way which means that the more nitrogen is applied and more water, the loose is the mass of a thousand grains. The hectoliter mass decreases with the increasing of the nitrogen rate and the amount of water applied by irrigation (Sapucay et al., 2020). Moreover the maize hybrid has been influencing the maize grain mass and bulk. This way, we have gained that several hybrids that have been researched have shown differential mass of a thousand grains as well as the hectoliter mass.

Table 1. The HLM values in function of corn hybrid and irrigation regime in 2017 at ARDS Marculesti

Hybrid	b1 - rainfed				b2 - stressed before flowering				b3- stressed after flowering			
	HLM. kg/hl	%	Dif. kg/hl	Sign.	HLM. kg/hl	%	Dif. kg/hl	Sign.	HLM. kg/hl	%	Dif. kg/hl	Sign.
a1	81.90	100	Ct		79.43	100	Ct		81.88	100	Ct	
a2	85.40	104	3.46	***	85.09	107	5.66	***	85.22	104	3.33	***
a3	80.00	97	-1.95	00	78.80	99	-0.63		80.00	97	-1.89	00
a4	79.43	96	-2.52	000	78.67	99	-0.75		78.74	96	-3.14	000
a5	82.3	100	0.38		82.32	103	2.89	***	82.64	100	0.75	
LSD 5% =1.19; LSD 1% = 1.61; LSD 0.1% =2.13												

Hybrid	b4 - full irrigated				Average of irrigation regimes				
	HLM. kg/hl	%	Dif. kg/hl	Sign.	Irrigation regimes	HLM. kg/hl	%	Dif. kg/ha	Sign.
a1	79.68	100	Ct		Rainfed	81.82	100	Ct	
a2	84.46	105	4.78	***	Stressed after flowering	80.86	99	-0.96	000
a3	78.80	98	-0.88		Stressed before flowering	81.69	100	0.13	
a4	78.42	98	-1.26	0	Full irrigated	80.63	99	-1.19	000
a5	81.76	102	2.08	**	LSD 5%=0.53; LSD 1%=0.72; LSD 0.1%=0.95				
LSD 5% =1.19; LSD 1% = 1.61; LSD 0.1% =2.13									

Legend: a1- P9175 (FAO 330), a2 - KWS BELLAVISTA (FAO 330), a3 - KWS SMARAGD (FAO 350), a4 - KWS KASHMIR (370 FAO) and a5 - KWS DURANGO 480).

The data obtained in 2018 year show the hybrids with the highest hectoliter mass values were Bellavista and Smaragd. However, all other hybrids compared to the a1 control gave highly significant positive differences (75.06 and 76.00 versus 73.14) (Table 2).

As regard the researched irrigation regimes, the highest values were also given by irrigation before flowering and irrigation throughout the maize vegetation period (Table 2).

The same trends are observed when we analyze the interaction of the two researched factors namely, the hybrid and the irrigation regime.

Table 2. The HLM values in function of corn hybrid and irrigation regime in 2018 at ARDS Marculesti

Hybrid	b1 - rainfed				b2 - stressed before flowering				b3- stressed after flowering			
	HLM. kg/hl	%	Dif.. kg/hl	Sign.	HLM. kg/hl	%	Dif.. kg/hl	Sign.	HLM. kg/hl	%	Dif.. kg/hl	Sign.
a1	72.63	100	Ct		73.55	100	Ct		72.21	100	Ct	
a2	79.44	109	6.81	***	80.32	109	6.76	***	79.93	110	7.73	***
a3	74.74	102	2.11	**	76.59	104	3.03	***	75.50	104	3.29	***
a4	74.66	102	2.03	**	75.32	102	1.76	**	74.77	103	2.56	**
a5	77.31	106	4.67	***	77.39	105	3.84	***	77.91	107	5.70	***

LSD 5% = 1.49 kg/hl; LSD 1% = 2.01 kg/hl; LSD 0.1% = 2.67 kg/hl

Hybrid	b4 - full irrigated				Average of irrigation regimes				
	HLM. kg/hl	%	Dif. kg/hl	Sign.	Irrigation regimes	HLM. kg/hl	%	Dif.. kg/ha	Sign.
a1	74.19	100	Ct		Rainfed	75.75	100	Ct	
a2	80.52	108	6.34	***	Stressed after flowering	76.63	101	0.88	*
a3	77.20	104	3.02	***	Stressed before flowering	76.06	100	0.31	
a4	75.55	101	1.37		full irrigated	77.10	102	1.35	***
a5	78.09	105	3.90	***	LSD 5%= 0.66 kg/hl; LSD 1%= 0.90 kg/hl; LSD 0.1%= 1.19 kg/hl				

LSD 5% = 1.49; LSD 1% = 2.01; LSD 0.1% = 2.67

Legend: a1- P9175 (FAO 330), a2 - KWS BELLAVISTA (FAO 330), a3 - KWS SMARAGD (FAO 350), a4 - KWS KASHMIR (370 FAO) and a5 - KWS DURANGO 480).

Unlike the previous two years, in 2019 the highest hectolitre mass values were obtained for the Smaragd hybrid, of 74.5 kg/hl and the lowest, for P9175, of 68.3 kg/hl. Almost all the hybrids compared with P9175 gave very significantly positive differences, except the Kashmir hybrid, in rainfed conditions, which gave a distinctly significant negative difference compared to the control P9175 (Table 3).

In 2019, the highest value of the hectoliter mass was obtained for rainfed treatment, of 73.33 kg/hl and the lowest, for irrigated throughout the vegetation period, of 71.67 kg/hl, the other irrigation regimes recording intermediate values. The differences between the irrigated and rainfed regimes, this year, were very significantly negative.

Table 3. The HLM values in function of corn hybrid and irrigation regime in 2019 at ARDS Marculesti

Hybrid	b1 - rainfed				b2 - stressed before flowering				b3- stressed after flowering			
	HLM. kg/hl	%	Dif. kg/hl	Sign.	HLM. kg/hl	%	Dif. kg/hl	Sign.	HLM. kg/hl	%	Dif. kg/hl	Sign.
a1	71.36	100	Ct		68.93	100	Ct		67.43	100	Ct	
a2	77.13	108	5.77	*	74.07	107	5.13	***	71.97	106	4.53	***
a3	75.20	105	3.83	***	73.60	106	4.67	***	73.46	108	6.03	***
a4	70.36	98	-1.00	00	71.96	104	3.03	***	73.23	108	5.80	***
a5	72.60	101	1.23	**	74.06	107	5.13	***	75.06	111	7.63	***

LSD 5% = 0.59 kg/hl; LSD 1% = 0.86 kg/hl; LSD 0.1% = 1.29 kg/hl

Hybrid	b4 - full irrigated				Average of irrigation regimes				
	HLM. kg/hl	%	Dif. kg/hl	Sign.	Irrigation regimes	HLM. kg/hl	%	Dif. kg/ha	Sign.
a1	65.5	100	Ct		Rainfed	73.33	100	Ct	
a2	69.53	106	4.03	***	Stressed after flowering	72.52	99	-0.81	000
a3	71.56	109	6.07	***	Stressed before flowering	72.23	99	-1.1	000
a4	75.50	115	10.00	***	full irrigated	71.67	98	-1.66	000
a5	76.26	116	10.77	***	LSD 5%= 0.37 kg/hl; LSD 1%= 0.50 kg/hl; LSD 0.1%= 0.66 kg/hl				

LSD 5% = 0.59; LSD 1% = 0.86; LSD 0.1% = 1.29

Legend: a1- P9175 (FAO 330), a2 - KWS BELLAVISTA (FAO 330), a3 - KWS SMARAGD (FAO 350), a4 - KWS KASHMIR (370 FAO) and a5 - KWS DURANGO 480).

On average over the three years of experimentation, the highest hectoliter mass values were obtained by the Bellavista hybrid and the lowest by the P9175 hybrid. The differences between these two hybrids were distinctly significantly positive at all irrigation regimes. The other hybrids, compared with P9175, did not show significant differences, except for the Smaragd hybrid, when irrigated, which gave a distinctly significant positive difference compared to the control P9175.The highest hectoliter mass values were recorded in 2017 and the lowest in 2019. These values are inversely proportional to the mass of a thousand grains (Table 4).

Table 4. The HLM values in function of corn hybrid and irrigation regime as average over three years of experimentation, 2017, 2018 and 2019 at ARDS Marculesti

Hybrid	b1 - rainfed				b2 - stressed before flowering				b3- stressed after flowering			
	HLM. kg/hl	%	Dif. kg/hl	Sign.	HLM. kg/hl	%	Dif. kg/hl	Sign.	HLM. kg/hl	%	Dif. kg/hl	Sign.
a1	75.31	100	Ct		73.97	100	Ct		73.84	100	Ct	
a2	80.66	107	5.34	**	79.82	107	5.85	**	79.04	107	5.20	**
a3	76.64	101	1.33		76.33	103	2.36		76.32	103	2.48	
a4	74.81	99	-0.50		75.32	101	1.35		75.58	102	1.74	
a5	77.41	102	2.10		77.92	105	3.96	*	78.53	106	4.69	**
LSD 5% = 3.10 kg/hl; LSD 1% = 4.51 kg/hl; LSD 0.1% = 6.77 kg/hl												

Hybrid	b4 - full irrigated				Average of irrigation regimes				
	HLM. kg/hl	%	Dif. kg/hl	Sign.	Irrigation regimes	HLM. kg/hl	%	Dif. kg/ha	Sign.
a1	73.12	100	Ct		Rainfed	76.97	100	Ct	
a2	78.17	106	5.05	**	Stressed after flowering	76.67	99.61	-0.30	
a3	75.85	103	2.73		Stressed before flowering	76.66	99.59	-0.31	
a4	76.49	104	3.37	*	full irrigated	76.47	99.35	-0.50	
a5	78.70	107	5.58	**	LSD 5%= 1.11 kg/hl; LSD 1%= 1.50 kg/hl; LSD 0.1%= 1.98 kg/hl				
LSD 5% = 3.10; LSD 1% = 4.51; LSD 0.1% = 6.77									

Legend: a1- P9175 (FAO 330), a2 - KWS BELLAVISTA (FAO 330), a3 - KWS SMARAGD (FAO 350), a4 - KWS KASHMIR (370 FAO) and a5 - KWS DURANGO 480).

b. Thousand kernel weight (TKW) results

Thousand kernel weight (TKW) is the average weight of a kernel, with a factor of 1,000 included to provide the necessary precision of the measurement (Hlynka & Bushuk, 1959, cited by Engelbrecht, 2008). Thousand kernel weight is a function of kernel size and density (Halverson & Zeleny, 1988, Dorsey-Redding et al., 1991, cited by Engelbrecht, 2008) and it gives the miller important information regarding the flour yield of wheat considering that large, dense kernels normally have higher ratio of endosperm to bran than smaller, less dense kernels (Halverson & Zeleny, 1988, cited by Engelbrecht, 2008) (Engelbrecht, 2008). The TKW data for 2017, 2018 and 2019 data are presented in the tables 5, 6 and 7. In 2017 year, the lowest TKW values were recorded by the Bellavista hybrid and the highest by the Kashmir hybrid. Irrigation at Management Allowable Depletion (MAD) point throughout the vegetation period or only in the two researched phenophases, were superior to the rainfed treatment in terms of the weight of a thousand grains; all these differences were very significantly positive (Table 5). In the same manner, within each hybrid, irrigation positively influenced the mass of a thousand grains. The interaction between hybrid and irrigation highlighted, through very significantly positive differences, the Kashmir hybrid and the irrigated treatment (Table 5).

Table 5. The TKW values in function of corn hybrid and irrigation regime in 2017 at ARDS Marculesti

Hybrid	b1 - rainfed				b2 - stressed before flowering				b3- stressed after flowering			
	TKW. g	%	Dif. g	Sign.	TKW. g	%	Dif. g	Sign.	TKW. g	%	Dif. g	Sign.
a1	269.3	100	Ct		284.67	100	Ct		316.67	100	Ct	
a2	245.6	91	-23.66	0	273.67	96	-11.00		295	93	-21.6	0
a3	280.6	104	11.34		296.33	104	11.66		309.67	97	-7.00	
a4	290.6	107	21.34	*	307.67	108	23.00	*	331	104	14.33	
a5	269.3	100	0.00		284.67	100	0.00		316.67	100	0.00	

LSD 5% =20.30; LSD 1% = 27.37; LSD 0.1% =36.32

Hybrid	b4 – full irrigated				Average of irrigation regimes				
	TKW. g	%	Dif. g	Sign.	Irrigation regimes	TKW. g	%	Dif. g	Sign.
a1	337.33	100	Ct		Rainfed	272.73	100	Ct	
a2	335	99.30	-2.33		Stressed after flowering	292.06	107	19.33	***
a3	338.33	100.29	1.00		Stressed before flowering	313.46	115	40.73	***
a4	379.33	112.45	42.00	***	full irrigated	348.79	128	76.06	***
a5	337.33	100.00	0.00		LSD 5%=9.08; LSD 1%=12.24; LSD 0.1%=16.24				

LSD 5% =20.30; LSD 1% = 27.37; LSD 0.1% =36.32

Legend: a1- P9175 (FAO 330), a2 - KWS BELLAVISTA (FAO 330), a3 - KWS SMARAGD (FAO 350), a4 - KWS KASHMIR (370 FAO) and a5 - KWS DURANGO 480).

During the 2018 year, the highest values of a thousand kernel weight were recorded by the Durango, Kashmir and P 9175 hybrids. The Bellavista and Smaragd hybrids showed very significantly negative differences compared to the control P 9175 and the Durango hybrid showed a very significantly positive difference compared to control (Table 6).

The irrigation before flowering and throughout the vegetation period gave de highest results on TKW (Table 6).

Table 6. The TKW values in function of corn hybrid and irrigation regime in 2018 at ARDS Marculesti

Hybrid	b1 - rainfed				b2 - stressed before flowering				b3- stressed after flowering			
	TKW. g	%	Dif. g	Sign.	TKW. g	%	Dif. g	Sign.	TKW. g	%	Dif. g	Sign.
a1	338.90	100	Ct		367.70	100	Ct		336.02	100	Ct	
a2	308.07	90	-30.83	000	321.79	87.51	-45.92	000	304.34	90.57	-31.68	000
a3	361.29	106	22.39	***	373.53	101.58	5.83		365.84	108.87	29.82	***
a4	343.17	101	4.27		353.37	96.10	-14.33	00	335.85	99.94	-0.17	
a5	289.85	85	-49.05	000	298.91	81.29	-68.79	000	291.22	86.66	-44.80	000

LSD 5% = 9.14 g; LSD 1% = 12.32 g; LSD 0.1% = 16.36 g

Hybrid	b4 - full irrigated				Average of irrigation regimes				
	TKW. g	%	Dif. g	Sign.	Irrigation regimes	TKW. g	%	Dif. g	Sign.
a1	376.96	100	Ct		Rainfed	327.40	100	Ct	
a2	324.23	86	-52.73	000	Stressed after flowering	345.92	105	18.52	***
a3	382.98	101	6.02		Stressed before flowering	326.68	100	-0.72	
a4	364.34	96	-12.62	00	full irrigated	352.56	107	25.16	***
a5	301.72	80	-75.24	000	LSD 5%= 4.08 g; LSD 1%= 5.51 g; LSD 0.1%= 7.31 g				

LSD 5% = 9.14 ; LSD 1% = 12.32; LSD 0.1% = 16.36

Legend: a1- P9175 (FAO 330), a2 - KWS BELLAVISTA (FAO 330), a3 - KWS SMARAGD (FAO 350), a4 - KWS KASHMIR (370 FAO) and a5 - KWS DURANGO 480).

The results recorded in 2019 highlighted the hybrid P9175, with a TKW of 318 g. The differences recorded compared to all other hybrids were significant and very significantly negative. The lowest value of TKW, this year was recorded for the hybrid Smaragd, of 258 g (Table 7).The researched irrigation regimes recorded increasing values, starting with the rainfed regime and ending with the irrigated one throughout the vegetation period.

The differences between all irrigated and rainfed regimes were very significantly positive (Table 7).

Table 7. The TKW values in function of corn hybrid and irrigation regime in 2019 at ARDS Marculesti

Hybrid	b1 - rainfed				b2 - stressed before flowering				b3- stressed after flowering			
	TKW. g	%	Dif. g	Sign.	TKW. g	%	Dif. g	Sign.	TKW. g	%	Dif. g	Sign.
a1	238.33	100	Ct		324	100	Ct		342.66	100	Ct	
a2	228.00	95	-10.3	000	262	80.86	-62.00	000	289.66	84	-53.00	000
a3	233.66	98	-4.67	0	278.33	85.90	-45.67	000	301.66	88	-41.00	000
a4	228.66	95	-9.67	000	279	86.11	-45.00	000	308.00	89	-34.67	000
a5	230.66	96	-7.67	00	255	78.70	-69.00	000	262.66	76	-80.00	000
LSD 5% = 4.39 g; LSD 1% = 6.39 g; LSD 0.1% = 9.59 g												

Hybrid	b4 - full irrigated				Average of irrigation regimes				
	TKW. g	%	Dif. g	Sign.	Irrigation regimes	TKW. g	%	Dif. g	Sign.
a1	368.66	100	Ct		Rainfed	231.86	100	Ct	
a2	310.00	84	-58.67	000	Stressed after flowering	279.66	121	47.8	***
a3	342.66	92	-26.00	000	Stressed before flowering	300.93	130	69.07	***
a4	336.33	91	-32.33	000	full irrigated	329.00	142	97.14	***
a5	287.33	77	-81.33	000	LSD 5%= 3.72 g; LSD 1%= 5.02 g; LSD 0.1%= 6.67 g				
LSD 5% = 4.39 g; LSD 1% = 6.39 g; LSD 0.1% = 9.59 g									

Legend: a1- P9175 (FAO 330), a2 - KWS BELLAVISTA (FAO 330), a3 - KWS SMARAGD (FAO 350), a4 - KWS KASHMIR (370 FAO) and a5 - KWS DURANGO 480).

On average over the three years of experimentation, the lowest TKW values were recorded in the Bellavista and Smaragd hybrids, with values of 291 and 288 g, respectively. The highest values were obtained by the P9175 hybrid, of 325 g. The Kashmir and Durango hybrids recorded TKW values close to those of the P9175 hybrid, the differences being insignificant at all irrigation regimes. As for the differences between the P9175 hybrid and the two hybrids with the lowest values, they were significantly and distinctly significantly negative, with the exception of the rainfed irrigation regime (Table 8).

Regarding the TKW values recorded by the 5 hybrids tested in the three years, it can be noted that the highest values were recorded in 2018, when the highest yields were, also, obtained for all hybrids. The highest value was obtained by the Durango hybrid, in 2018, of 371 g and the lowest, by the Smaragd hybrid, in 2019, of 259 g, with an amplitude of the recorded values of over 100 g.

The irrigation regimes recorded increases in TKW, from rainfed to irrigated throughout the vegetation period, from 277 g to 343 g, with an amplitude, as average, of 66 g.

However, throughout these three years of the experimentation period, taking in comparison each year to each other, the highest amplitude were recorded in 2018, in rainfed, compared to 2019, of almost 100 g. This shows the favorable influence of the massive rainfall recorded in 2018 on TKW and, finally, on production.

Table 8. The TKW values in function of corn hybrid and irrigation regime as average over three years of experimentation, 2017, 2018 and 2019 at ARDS Marculesti

Hybrid	b1 - rainfed				b2 - stressed before flowering				b3- stressed after flowering			
	TKW. g	%	Dif. g	Sign	TKW. g	%	Dif. g	Sign	TKW. g	%	Dif. g	Sign
a1	282.18	100	Ct		325.45	100	Ct		331.78	100	Ct	
a2	260.57	92	-21.61		285.81	87	-9.64	0	296.33	89	-35.45	0
a3	291.87	103	9.69		316.06	97	-9.39		325.72	98	-6.06	
a4	286.07	101	3.89		318.12	97	-7.33		325.00	97	-6.78	
a5	265.94	94	-16.24		283.97	87	-1.49	0	289.62	87	-42.16	0
LSD 5% = 34.82 g; LSD 1% = 50.66 g; LSD 0.1% = 75.99 g												

Hybrid	b4 - full irrigated				Average of irrigation regimes				
	TKW. g	%	Dif. g	Sign.	Irrigation regimes	TKW. g	%	Dif. g	Sign.
a1	360.98	100	Ct		Rainfed	277.33	100	Ct	
a2	323.07	89	-37.91	00	Stressed after flowering	305.88	121	28.55	***
a3	354.65	98	-6.33		Stressed before flowering	313.69	130	36.36	***
a4	364.20	100	3.22		full irrigated	343.45	142	66.12	***
a5	314.34	87	-46.64	00	LSD 5%= 15.53 g; LSD 1%= 20.94 g; LSD 0.1%= 27.80 g				
LSD 5% = 34.82 g; LSD 1% = 50.66 g; LSD 0.1% = 75.99 g									

Legend: a1- P9175 (FAO 330), a2 - KWS BELLAVISTA (FAO 330), a3 - KWS SMARAGD (FAO 350), a4 - KWS KASHMIR (370 FAO) and a5 - KWS DURANGO 480).

CONCLUSIONS

Maize is the most cultivated plant in Romania, with areas that exceed 3 million hectares every year. Romania's maize production and export are among the 15 largest corn producers in the world. In this context, the creation of new hybrids, more adapted to the constantly changing climatic conditions, is a perpetual task.

The hectoliter mass (HLM) and the thousand kernel weight (TKW) are very important indicators of grain yield, respectively the ratio of chaff and flour. Also, these qualitative indicators of maize kernels influence the packaging for transport as well as storage.

Regarding the hectoliter mass, in 2017, the highest values were obtained by the Bellavista hybrid (85.04 kg/hl) and the lowest by the Kashmir hybrid (78.82 kg/hl). The rainfed treatment and the one irrigated after flowering gave higher hectoliter mass values (81.82 kg/hl and, respectively, 81.69 kg/hl) than irrigation before flowering and irrigation throughout the growing season (80.86 kg/hl and, respectively, 80.63 kg/hl) at half of the AWC (Available Water Capacity).

In 2018, the results regarding the hectoliter mass of maize kernels were similar to those obtained in 2017, both in terms of the cultivated hybrid and the irrigation regime.

Due to unfavorable climatic conditions, much lower hectoliter mass values were obtained in 2019 than in the previous two years. So, the highest hectoliter mass values were obtained for the Smaragd hybrid, of 74.5 kg/hl and the lowest, for P9175, of 68.3 kg/hl.

About HLM, on average over the three years of experimentation there can be concluded the following:

- the highest hectoliter mass values were obtained by the Bellavista hybrid and the lowest by the P9175 hybrid;
- there were no significant differences between irrigation regimes;
- the highest hectoliter mass values were recorded in 2017 and the lowest in 2019.

Regarding TKW, in 2017, the lowest values were recorded for the Bellavista hybrid (287.33 g) and the highest, for the Kashmir hybrid (327.16 g), i.e. the opposite to the hectoliter mass values.

Irrigation at Management Allowable Depletion (MAD) point throughout the vegetation period or only in the two researched phenophases, were superior to the rainfed treatment in terms of the weight of a thousand grains; all these differences were very significantly positive.

Due to the very favorable vegetation conditions, in 2018, different results were obtained compared to the other two years, 2017 and 2019, in the sense that the P 9175 hybrid, taken as a control, had intermediate values compared to the other 4 researched hybrids.

The irrigated treatments gave higher values compared to the rainfed treatment.

In 2019, the P 9175 hybrid stood out, with a value of 318 g. The lowest TKW was recorded for the Smaragd hybrid, of only 258 g. The differences between the irrigated and rainfed Treatments were very significantly positive.

About TKW, on average over the three years of experimentation there can be concluded the following:

- the highest TKW values were obtained by the P9175 hybrid, of 325 g and the lowest, by the Bellavista and Smaragd hybrids, with values of 291 and 288 g, respectively;
- the irrigation treatments gave very significant positive differences over the rainfed treatment;
- the highest average TKW values were obtained in 2018 due to very favorable vegetative conditions in terms of rainfall.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

OPPORTUNITIES FOR CHEMICAL CONTROL OF SOME WEEDS IN WHEAT

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Abstract

In 2021 and 2022, a field experiment with the winter wheat variety 'Enola' was conducted. The trial was performed on the experimental field of the Agricultural University of Plovdiv, Bulgaria. The efficacy and selectivity of the following five herbicides was under evaluation - Pontos (240 g/l flufenacet + 100 g/l picolinafen) – applied in three rates, Pallas 75 WG (75 g/l piroxulam), Axial One (45 g/l pinoxaden + 5 g/l florasulam), Atlantis Flexx 20,25 WG (45 g/kg mesosulfuron + 67.5 g/kg propoxicarbazon-potassium + 90 g/kg mefenpir-dietil), and a tank mixture of Biathlon 4 D (714 g/kg tritosulfuron + 54 g/kg florasulam) and Axial 050 (50 g/l pinoxaden). The herbicidal treatments were compared with untreated control. The natural weed infestation was presented by the weeds corn chamomile, common poppy, wild mustard, wild radish, ivy-leaved speedwell, annual raygrass, and wild oat. Biological yield was reported for both years of the experiment. The highest average yield of the two experimental years was found for the variant treated with the tank mixture of Biathlon 4 D + Axial 050- 6.31 t/da.

***Key words**: wheat, weeds, herbicides, efficacy, selectivity.*

INTRODUCTION

Ensuring a sufficient amount of food to feed the population is a fundamental concern of mankind. A large part of the food products is procured through the cultivation of agricultural crops (Georgiev et al., 2019; Dimtrova et al., 2019; Nenova, 2019; Nenova et al., 2019; Marinov-Serafimov et al., 2017; Shopova & Cholakov, 2015; Shopova & Cholakov, 2014).
Winter wheat (*Triticum aestivum* L.) is main grain crop in Bulgaria. One of the factors limiting the development of cultivated plants is the annual ubiquitous appearance and development of weed vegetation (Yanev et al., 2014a; Yanev, 2015). The weeds are great competitors of wheat for nutrients, water, space, and light. The weeds can also cause indirect damages because many of them are hosts of harmful insects and diseases (Kalinova et al., 2012). Weeds have become an increasing problem in wheat and some weed species such as *Cirsium arvense*, *Consolida regalis*, *Polygonum aviculare*, *Consolida orientalis*, *Convolvulus arvensis*, *Centaurea cyanus*, *Erodium cicutarium*, and *Bifora radians* (Susuri et al., 2001; Mehmeti et al., 2009; Mehmeti and Demaj, 2010). The high weed infestation can decrease the yields by more than 70% (Atanasova and Zarkov, 2005). Weeds compete for wheat plants with high efficiency for the most critical growth requirements. As a result of this competition, the growth of the crop is weak, which is negatively reflected in the grain yield, as the competition of the weed with the wheat crop has a clear reflection in reducing the yield (Hammood and Safi, 2018; Hamad Al-Mafrajy, 2018; Garakishi, 2020). Yield losses due to weeds have been reported (Oerke and Dehne, 2004; Oerke, 2006; Dangwal et al., 2010).
One of the most frequently applied methods of weed control in cultivated plants is the chemical one. The choice of herbicide is one of the most important and responsible moments in the agrotechnics of the crops. The appropriate herbicide must meet a number of requirements – to be selective for the crop, highly effective against available weeds, its use not lead to the accumulation of residual amounts in the plant production and the soil, not to impair the quality of the production, to be harmless for soil microorganisms and the environment (Goranovska et al., 2022; Yanev, 2021; Yanev, 2020; Yanev & Kalinova, 2020; Mitkov et al., 2020; Neshev et al., 2020; Mitkov et al. 2018; Goranovska & Yanev, 2016; Kostadinova et al., 2016; Hristeva et al., 2015; Kalinova &

Yanev, 2015; Semerdjieva et al., 2015; Hristeva et al., 2014; Yanev et al., 2014b). Successful weed management in wheat depends not only on the proper herbicide choice, but also on the timing and dosage of its application (Abbas et al., 2009; Titiyanov et al., 2015; Petrova, 2017; Titiyanov et al., 2020; Yankova et al., 2020; Shaban et al., 2021; Yanev et al., 2021, Yanev, 2022).

The selection of herbicides suitable for wheat is determined by species diversity and weed density. Most herbicides control only a specific group of weeds, and to provide more broad-spectrum weed control, the use of herbicide combinations is recommended (Bostrom and Fogelfors, 2002; Walia et al., 2000). According to Chaudhry et al. (2008) when mixing anti-grain and anti-broadleaf herbicides, the density of wheat and broadleaf weeds decreased significantly (by 96.3% and 97.6%, respectively), and grain yield compared to their application alone increased by 15%. Several researchers have shown that tribenuron-methil and bifenox herbicides can control weeds in wheat (Brzozowska et al., 2008). Some investigators found positive effect for the interaction between cultivars and weed control treatments on weeds and yield of wheat crop (Abusteit et al., 1991; Singh and Singh, 1996). In some earlier studies of herbicide efficacy in wheat, Sinapis arvensis and Chenopodium album had also been found highly susceptible to fluroxypyr, 2.4-D, tribenuron-methyl and amidosulfuron (Elezović et al., 1994; Stanković-Kalezić et al., 1998; Radivojević et al., 2001). Galium aparine was the species that proved resistant to tested herbicides: Secator OD, Lintur 70 WG, Granstar 75WG, Mustang, Kambio, Starane-250, Arat and Kerto (Radivojević et al., 2006). The best weed control was accomplished by Safener 15 WP 2 (247 g/ha) as significantly lower weed counts per m (11.0) and higher percent weed control (73.4%). Based on better weed control and wheat yield, Safener 15WP (395.2 ml/ha) and Puma super 69EW (1250 ml/ha) were proved to be better in areas where wheat fields are predominantly infested by monocot weeds. Safener 15 WP and Puma super 69 EW were found effective against Avena fatua, less effective against Phalaris minor and ineffective against Poa annua (Mehmood et al., 2014). Therefore, the chemical method is one of the easiest and cheapest ways to control the weed, due to the tremendous economic returns resulting from the use of herbicides for their high effectiveness and speed of impact. These herbicides have been used to control wheat weeds in large areas of the world despite the emergence of environmental and health problems resulting from their use, which led to increased productivity, sometimes reaches more than 50% (Montazeri et al., 2005; Al-Aqidi, 2010; Gopal et al., 2017; Kaur et al., 2020).

The purpose of the study is to establish some possibilities for chemical control of individual weeds in common wheat variety "Enola".

MATERIALS AND METHODS

In 2021 and 2022, a field experiment with winter wheat variety “Enola” was carried out in the Educational and Experimental Field of the Department of Agriculture and Herbology at the Agricultural University - Plovdiv, Bulgaria. The experiment was carried out according to the block design in 4 replications with a size of the working plot of 20 m².

A preliminary inspection of the experimental field was performed. In the reporting field nine types of weeds, typical for the crop were identified. The average weed density in the two experimental years, per 1 m² was as follows: *Anthemis arvensis* L. - 5.5 specimens; *Papaver rhoeas* L. - 10 specimens; *Sinapis arvensis* L. - 7 specimens, *Raphanus raphanistrum* L. - 5 specimens, *Veronica hederifolia* L. - 9 specimens; *Lolium rigidum* L. - 34.5 specimens; *Avena fatua* L. - 36.5 specimens.

The study included the following treatments: 1. Untrated control; 2. Pontos (240 g/l flufenacet + 100 g/l picolinafen) - 0.5 l ha^{-1}; 3. Pontos - 0.75 l ha^{-1}; 4. Pontos - 1.00 l ha^{-1}; 5. Palas 75 WG (75 g/l pyroxulam) - 0.20 kg ha^{-1}; 6. Palas 75 WG - 0.25 kg ha^{-1}; 7. Axial One (45 g/l pinoxaden + 5 g/l florasulam) - 1.00 l ha^{-1}; 8. Atlantis Flex (45 g/kg mesosulfuron + 67.5 g/kg propoxicarbazon-natrii + 90 g/kg mefenpir-dietil) - 0.25 kg ha^{-1}; 9. Axial 050 (50 g/l pinoxaden) + Biathlon 4D (54 g/kg florasulam + 714 g/kg tritosulfuron) - 0.90 l ha^{-1} + 0.05 kg ha^{-1}. The herbicidal products were applied in the spring, in the tillering stage of the crop (BBCH 21-25).

The herbicide spraying was accomplished via electrical backpack sprayer SOLO model 417 (Solo, Germany) with a volume of the working solution 200 l ha^{-1}.

The predecessor of wheat was oilseed rape. The preparation of the area was carried out by plowing to a depth of 20 cm, as well as by two cultivations. Pre-sowing fertilization with NPK 15:15:15 at the rate of 300 kg ha^{-1} was accomplished. Sowing was done at the optimal time for wheat with a seed drill for crops with a fused surface at a row spacing of 12 cm, with a density of 450 pcs. germinating seeds per m^2. Spring dressing was carried out in March with ammonium nitrate 300 kg ha^{-1}). The herbicide efficacy evaluations were performed 14, 28, and 56 days after the herbicidal application. The 10-score scale of EWRS (European Weed Research Society) for visual rating was used. For herbicidal selectivity, the 9-score scale of EWRS was used.

The results of the conducted research with the software package of SPSS 17 program of one- and two-factorial analysis of variance were processed.

RESULTS AND DISCUSSIONS

Against *Anthemis arvensis* L., the results of the herbicide products used are presentend in Table 1. When counting the 14th day after the treatment with the herbicides, the efficacy varies widely, from the 10 to 75%. On the 28th day after the treatment, the efficacy increases progressively in all tested variants and reaches 30 - 90%.

Table 1. Efficasy of the studied herbicides against *A. arvensis* avarage fort the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Pontos - 0.5 l ha^{-1}	10	30	50
3. Pontos - 0.75 l ha^{-1}	25	45	65
4. Pontos - 1.00 l ha^{-1}	40	60	75
5. Palas 75 WG - 0.20 kg ha^{-1}	40	60	75
6. Palas 75 WG - 0.25 kg ha^{-1}	60	80	90
7. Axial Edno - 1.00 l ha^{-1}	70	85	95
8. Atlantis Flex - 0.25 kg ha^{-1}	60	80	90
9. Axial 050 + Biathlon 4 D – 0.90 l ha^{-1} + 0.05 kg ha^{-1}	75	90	100

Aproximately complete weed cotrol was reported on the 56th day after treatment (90 - 100%) only in the variants treated with the products Pallas 75 WG in rate of 25 kg ha^{-1}, Axial One, Atlantis Flex and from the combined application of Axial 050 + Biathlon 4 D. For the other treatments, the efficacy was unsatisfactory and varied from 50 to 75%.

Table 2 shows the dynamics of the herbicidal efficacy against *P. rhoeas* L. reported on the 14th, 28th and 56th days after treatment with the different products. It is noteworthy that no herbicidal effect (0%) was reported for Pallas 75 VG on all three reporting dates. In the remaining treatments of the experiment, a relatively low herbicidal efficiency of 20 to 70%, at the first reporting date was found. However, the efficacy reaches the maximum 100% at the last reporting date only for variant 9 (Axial 050 + Biathlon 4 D - 0.90 l ha^{-1} + 0.05 kg ha^{-1}). This proves that, regardless of the slower mechanism of action, the tank mixture of Axial 050 + Biathlon 4 D has excellent efficacy against weeds. The variant treated with the herbicide Axial One also had an approximately excellent effect on the 56th day after treatment (95%). Except for the highest tested rate of the herbicide Pontos, its variants with lower applied doses as well as Atlantis Flex had unsatisfactory efficacy at the last reporting date.

Table 2. Efficasy of the studied herbicides against *P. rhoeas* avarage fort the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Pontos - 0.5 l ha^{-1}	20	40	60
3. Pontos - 0.75 l ha^{-1}	35	55	75
4. Pontos - 1.00 l ha^{-1}	50	70	85
5. Palas 75 WG - 0.20 kg ha^{-1}	0	0	0
6. Palas 75 WG - 0.25 kg ha^{-1}	0	0	0
7. Axial Edno - 1.00 l ha^{-1}	55	75	95
8. Atlantis Flex - 0.25 kg ha^{-1}	35	55	75
9. Axial 050 + Biathlon 4 D – 0.90 l ha^{-1} + 0.05 kg ha^{-1}	70	85	100

From Table 3, it is clear that all applied herbicides in the experiment had excellent herbicidal efficacy against *S. arvensis* L. reported on the 56th after application of the products. Despite the lower efficacy rates at day 14 after treatment, the weed was relatively easy-to-contol by the different evaluated herbicides in the trial.

Table 3. Efficasy of the studied herbicides against *S. arvensis* L. avarage fort the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Pontos - 0.5 l ha^{-1}	75	85	95
3. Pontos - 0.75 l ha^{-1}	80	90	100
4. Pontos - 1.00 l ha^{-1}	85	95	100
5. Palas 75 WG - 0.20 kg ha^{-1}	80	90	95
6. Palas 75 WG - 0.25 kg ha^{-1}	85	95	100
7. Axial Edno - 1.00 l ha^{-1}	80	90	100
8. Atlantis Flex - 0.25 kg ha^{-1}	75	90	100
9. Axial 050 + Biathlon 4 D – 0.90 l ha^{-1} + 0.05 kg ha^{-1}	85	95	100

With the exception of the products Pontos applied in its lowest tested rate and Pallas 75 BG tested at the rate of 0.20 kg ha^{-1} against *R. raphanistrum* L. the efficacy of the other variants was 95 - 100%, reported on the 56th day after treatment (Table 4).

Table 4. Efficasy of the studied herbicides against *R. raphanistrum* L. avarage fort the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Pontos - 0.5 l ha^{-1}	70	80	90
3. Pontos - 0.75 l ha^{-1}	75	85	95
4. Pontos - 1.00 l ha^{-1}	80	90	100
5. Palas 75 WG - 0.20 kg ha^{-1}	70	80	90
6. Palas 75 WG - 0.25 kg ha^{-1}	80	90	100
7. Axial Edno - 1.00 l ha^{-1}	75	90	100
8. Atlantis Flex - 0.25 kg ha^{-1}	70	85	100
9. Axial 050 + Biathlon 4 D – 0.90 l ha^{-1} + 0.05 kg ha^{-1}	80	90	100

Already on the first date, the percentages ranged from 70 to 80% after the application of the diffarnt herbicides in the experiment. At the last reporting dtae for treatments 4, 6, 7, 8 and 9 an efficacy of 100 percent was recorded. The efficacuy for treatment 2 and 5 an efficacy of 90% was found. For treatment 3 the efficacy was 95%.

Table 5 shows the effect of the applied herbicides on *Veronica hederifolia* L. Except for the highest tested rate of Pontos and the tank mixture of the herbicides Axial 050 and Biathlon 4D, the weed was very poorly controlled by all other variants in the experiment. On the 56th day after the treatment, the recorded efficacy of Pontos was 80%, and 85% from the combined treatment with Axial 050 + Biathlon 4 D. In all other variants of the trial, herbicide control varied from 40 to 65% on the last reporting date.

Table 5. Efficasy of the studied herbicides against *V. hederifolia* L. avarage fort the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Pontos - 0.5 l ha^{-1}	10	30	45
3. Pontos - 0.75 l ha^{-1}	20	45	65
4. Pontos - 1.00 l ha^{-1}	40	60	80
5. Palas 75 WG - 0.20 kg ha^{-1}	5	25	40
6. Palas 75 WG - 0.25 kg ha^{-1}	10	30	50
7. Axial Edno - 1.00 l ha^{-1}	15	35	55
8. Atlantis Flex - 0.25 kg ha^{-1}	10	35	55
9. Axial 050 + Biathlon 4 D – 0.90 l ha^{-1} + 0.05 kg ha^{-1}	40	65	85

Against the annual, monocotyledonous weed *Lolium rigidum* L., an increase in the herbicidal effect with an increase in the rates of the herbicidal product Pontos (Table 6) was observed.

Table 6. Efficasy of the studied herbicides against *L. rigidum* L. avarage fort the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Pontos - 0.5 l ha^{-1}	10	35	55
3. Pontos - 0.75 l ha^{-1}	25	50	70
4. Pontos - 1.00 l ha^{-1}	40	60	80
5. Palas 75 WG - 0.20 kg ha^{-1}	30	50	70
6. Palas 75 WG - 0.25 kg ha^{-1}	40	65	85
7. Axial Edno - 1.00 l ha^{-1}	65	85	95
8. Atlantis Flex - 0.25 kg ha^{-1}	30	50	70
9. Axial 050 + Biathlon 4 D – 0.90 l ha^{-1} + 0.05 kg ha^{-1}	70	90	100

On the 14th day after treatment, the efficacy varied only from 10 to 40% for the individual herbicide rates. On the 56th day, the herbicide control was increased and reached from 55 (0.50 l ha^{-1}) to 80% (1.00 l ha^{-1}). A satisfactory herbicidal effect from the herbicide Pallas 75 VG and Atlantis Flex at the last reporting date was reported. The highest efficacy in the variants treated with Axial One (95%) and from the combined usage of Axial 050 + Biathlon 4 D (100%) on the same reporting date was found. In these two variants, the effect is entirely due to the active substance pinoxaden contained in the products Axial One and Axial 050, which have a higher herbicidal efficacy against weeds compared to other grass weed contoling herbicides.

From Table 7, can be conclude that the tested herbicide Pontos has a very low efficacy against *Avena fatua* L., recorded on the 14th day after its application, ranging from 5 to 20%. Gradually, at the following reporting dates, the effect increases, and on the 56th day after treatment, it reaches 35-60%, which is unsatisfactory. From Atlantis Flex a satisfactory efficacy of 85% at the same reporting date was recorded. At the two tested rates of Pallas 75 WG, the herbicidal efficay reached 90-95%. Excellent control was reported for the seventh and ninth variants, at the last reporting date, which is due to the Axial One and Axial 050 products.

Visible signs of phytotoxicity were not observed in any of the variants.

Table 7. Efficasy of the studied herbicides against *A. fatua* L. avarage fort the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Pontos - 0.5 l ha^{-1}	5	20	35
3. Pontos - 0.75 l ha^{-1}	10	30	50
4. Pontos - 1.00 l ha^{-1}	20	40	60
5. Palas 75 WG - 0.20 kg ha^{-1}	60	80	90
6. Palas 75 WG - 0.25 kg ha^{-1}	65	85	95
7. Axial Edno - 1.00 l ha^{-1}	70	90	100
8. Atlantis Flex - 0.25 kg ha^{-1}	45	65	85
9. Axial 050 + Biathlon 4 D – 0.90 l ha^{-1} + 0.05 kg ha^{-1}	80	90	100

Table 8 presents the results of yields obtained on average for the two years of the experiment. The differences in yields are determined by the herbicidal efficacy of the products and by their ability to control the weeds present in the experiment. The natural background of weeding with highly competitive weed species resulted in a lower mean yield than the untreated control (3.28 t ha^{-1}).

According to the degree of mathematical proof, six separate groups of herbicides are distinguished here (a, b, c, d, e, f,). It is also observed here that variant 9 (Axial One + Biathlon 4 E) is from group (f), the most distant from the group of the untreated control (a), that is, with the highest yield followed by variant 7 (Axial One). Due to the fact that the herbicides Pallas 75 ВГ (0.25 kg ha^{-1}), Pontos (1.00 l ha^{-1}) and Atlantis Flex control less available weeds in the experiment, their yield decreases compared to that of the above-mentioned product. At the lower tested rate of Pallas (0.20 kg ha^{-1}), the biological yield decreased more severely. Compared to all other treated variants, the lowest reported yields were from the herbicide Pontos applied in rates of 0.75 and 0.50 l ha^{-1}.

Table 8. Avearge wheat grain seed yield, t ha^{-1}

Treatments	Yelds
1. Untreated control	3.28 a
2. Pontos - 0.5 l ha^{-1}	3.87 *b
3. Pontos - 0.75 l ha^{-1}	4.00 *b
4. Pontos - 1.00 l ha^{-1}	5.20 *d
5. Palas 75 WG - 0.20 kg ha^{-1}	4.58 *c
6. Palas 75 WG - 0.25 kg ha^{-1}	5.19 *d
7. Axial Edno - 1.00 l ha^{-1}	5.76 *e
8. Atlantis Flex - 0.25 kg ha^{-1}	5.24 *d
9. Axial 050 + Biathlon 4 D – 0.90 l ha^{-1} + 0.05 kg ha^{-1}	6.31 *f

CONCLUSIONS

The number of dairy cows has continuously Herbicidal products Axial 050 and Biathlon 4 D applied in a tank mixture showed excellent efficacy against *Anthemis arvensis* L., *Papaver rhoeas* L., *Sinapis arvensis* L, *Raphanus raphanistrum* L., *Lolium rigidum* L. and *Avena fatua* L.

The product Axial One is superior in efficiency to the herbicides Pallas 75 VG and Atlantis Flex compared to *Papaver rhoeas* L. and *Lolium rigidum* L..

The herbicides Pallas 75 WG, Atlantis Flex, Axial One and Pontos applied in doses of 0.50 and 0.75 l ha^{-1} had a low herbicidal efficay against *Veronica hederifolia* L. (from 45 to 65%).

Against *Lolium rigidum* L., the efficacy of the herbicides Pontos applied in doses .50 and 0.75 l ha^{-1}, Pallas 75 WG (0.20 kg ha^{-1}) and Atlantis Flex was unsatisfactory (from 55 to 70%).

During the entire vegetation period of wheat, variety “Enola”, no visible signs of phytotoxicity were recorded in any of the variants, which indicates the high selectivity of the tested products.

The highest average yield was obtained from the variant with the combined use of Axial 050 + Biathlon 4 D (6.31 t ha^{-1}).
Of all variants treated with herbicides, the yield of Pontos tested at a dose of 0.50 l ha^{-1} was the lowest - 3.87 t ha^{-1}.

ACKNOWLEDGEMENTS

This experiment was carried out with the support of Project 17-12 at The Centre of Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University of Plovdiv, Bulgaria.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EFFECT OF CORIANDER (*Coriandrum sativum* L.) ESSENTIAL OIL CULTURE ON SOIL BIOGENICITY AND DETERMINATION OF ITS ANTIMICROBIAL ACTIVITY AGAINST *Escherichia coli*

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Abstract

Soil microbiological and agrochemical indicators were analyzed during biological cultivation of coriander, in greenhouse conditions, as main indicators of good plant development, studied for antimicrobial activity against E. coli, by testing different variants of plant extracts (decoction, tincture, medicinal wine, medicinal vinegar, medicinal oil). The results of the agrochemical analysis show that coriander does not have a major impact on the dynamics of macronutrients in the soil, while the biogenicity and activity of enzymes cellulase and catalase increased in soils with coriander culture compared to the no-vegetation control. Positive antimicrobial activity against the pathogenic microorganism E. coli was reported for all variants of coriander extracts, differing for individual parts of the plant and individual variant extracts. Root and whole plant extracts showed higher antimicrobial activity compared to leaf and stem extracts. The strongest antimicrobial activity of the plant extracts was found in the medicated oil and medicated vinegar variants and the weakest in the `decoction` variants. The choice of solvent and exposure time likely influence the diameter of the retention zone.

***Key words**: coriander, soil microorganisms, cellulase, catalase, antimicrobial activity.*

INTRODUCTION

The study of essential oil crops as biocides possessing antimicrobial activity is essential to determine their qualities as biological agents against pathogenic microorganisms. The term "biocide" is a uniting term for the antibacterial action of various substances (Russell, 2002). By their functional essence, these chemical substances attack and inactivate bacteria, exerting a toxic effect on their cells. Most often, the mechanisms include: disruption of bacterial cell homeostasis, lysis followed by the leakage of the internal contents of the cell, inhibition of the catalytic function of bacterial enzymes, disruption of electron transport and oxidation processes, negative interaction with macromolecules and biosynthetic processes of bacteria (McDonnell & Russell, 1999). Coriander (*Coriandrum sativum* L.) is a well-known herb of the Apiaceae family, widely used as a spice in the food industry, as well as for its medicinal properties in pharmacy. Coriander seed oil is the second most important essential oil in the world, exhibiting antimicrobial activity against Gram-positive and Gram-negative bacteria, some yeasts, dermatophytes and filamentous fungi (Silva & Domingues, 2017). Coriander essential oil showed the strongest antibacterial activity against *Bacillus subtilis*, followed by *Stenotropomonas maltophilia* and *Penicillium expansum* (Kačániová et al., 2020). Coriander seed extract has the highest rate of growth reduction of several pathogenic or alteration microorganisms (*Salmonella typhi*, *Staphylococcus aureus*, *Candida tropicals*, *Mucor* sp., *Emericella nidulans*), while *Aspergillus flavus* has the highest resistance against coriander extracts (Amin et al., 2021). The antimicrobial mechanisms found in these essential oils have been explained on the basis of their content in natural compounds such as carvacrol, thymol, p-cymene and c-terpinene, among others (Amin et al., 2021). According to a study by Saygi et al. (2021) in terms of antimicrobial activity, the Gram-negative bacterium *E. coli* was more sensitive to coriander oil than the gram-positive bacterium *S. aureus*.

Soil biogenicity depends on a number of factors - soil temperature and humidity, soil mechanical composition, soil pH, nutrient supply, type of vegetation, methods of soil treatment and fertilization, and other factors (Malcheva et al., 2018). A complex of factors (physico-chemical, chemical) affects the development of microorganisms and their activity, including the type of vegetation - differences are found in terms of the quantity, quality composition and activity of soil microorganisms when fertilizing with the same fertilizer products, but applied at different vegetation (Malcheva et al., 2018; 2019). Soil microorganisms and the enzymes produced by them are sensitive indicators of ongoing changes, including when growing essential oil crops (Malcheva et al., 2019). When studying the influence of fertilizers on the quantity and activity of soil microorganisms, Plamenov et al. (2016) found that, for equal treatments, soil biogenicity (mainly determined by bacteria, less actinomycetes and molds) was higher in the essential oil crop canola compared to wheat. Among different studied indexes the microbiological and enzyme activity are suggested by number of authors as universal indexes of the soil fertility and pollution (including contamination with pathogenic microorganisms) (Malcheva et al., 2021; Malcheva et al., 2022, Dilly et al. , 2003; Nannipieri et al., 2000; Li et al., 2008; Perucci, 1992; Pascual et al., 1999; García-Gil et al., 2000; Ros et al., 2003; Crecchio et al., 2004 ; Bastida et al., 2008; Marcote et al., 2001; Malcheva, 2014a; 2014b). The biochemical indices are most sensitive towards the changes in the soil properties and their measurement are used largely as indicators for the management effect over the condition of the soil and agricultural productivity (Bandick and Dick, 1999; Nayak et al., 2007).

Essential oils are used as a source of carbon and energy by quite ubiquitous soil microorganisms and provide evidence that they will not accumulate in soil if environmental conditions favor the growth of these microorganisms (Vokou and Liotiri, 1999). In a study of the herbicidal potential of essential oils, Jouini et al. (2020) found that soil microorganisms, after a transient shock period induced by the addition of essential oils, recovered their original function and biomass.

Plants produce secondary metabolites that can inhibit bacteria, fungi, viruses and pests. There is a general consensus that secondary metabolites in plant extracts can inhibit Gram-positive bacteria more than Gram-negative bacteria (Ait-Ouazzou et al., 2012; Chanda et al., 2011), i.e. Gram-positive bacteria are more susceptible to plant extracts (Rakholiya et al., 2013). This difference is simply a consequence of the difference in cell wall structure between these major classes of bacteria. The cell wall of Gram-negative bacteria is surrounded by an additional lipopolysaccharide membrane that provides a hydrophilic surface and functions as a permeability barrier for many plant extracts. However, this is not always true, as some plant extracts inhibit Gram-negative bacteria more than Gram-positive bacteria.

Over 1,340 plants have been identified with antimicrobial activity, and over 30,000 antimicrobial compounds have been isolated from plants (Tajkarimi et al., 2010; Vaou et al., 2021).. Medicinal plants and their natural products remain largely untapped as sources of antibacterial compounds. Eloff (2004) gives certain criteria for the efficacy of phytoproducts as follows: an extract or fraction is considered to have significant antibacterial activity if the minimum inhibitory concentration (MIC) against the given pathogenic microorganism is equal to or less than 100 μg /mL, and Kuete and Efferth (2010) defined compounds with significant antibacterial activity as those with an MIC equal to or less than 10 μg/mL. Gibbons (2004) in his study defined essential oils as having significant antibacterial activity if the MIC was equal to or less than 5 μL/mL. Since the density of essential oils is lower but close to 1 g/mL, the MIC value of essential oils = < 5 μg/mL is considered to be relevant and can serve as a reference limit. Following these criteria, 50 essential oils have been reported to possess high antibacterial activity against at least one bacterial species. Such observations confirm that plants and their natural products represent promising sources of antibacterial agents and that continued research is needed in this direction.

The purpose of the study is, on the one hand, to determine the influence of the coriander essential oil crop on the soil microflora, and on the other hand, to analyze the antimicrobial activity of the plant against the pathogenic microbial species *E. coli*.

MATERIALS AND METHODS

The experiment was carried out under controlled conditions in the greenhouse of the educational and experimental field of the Department of Plant Breeding at the Technical University - Varna.

Before planting the experiment, during the growing season and after harvesting the crop, soil samples were taken, for each of the options, to determine:

- The content of ammonium nitrogen (NH_4–N) and nitrate nitrogen (NO_3–N) was determined spectrophotometrically.
- The content of phosphorus and potassium was determined by the Egner-Riem double-lactate method.
- Soil pH (ISO 10390).

For the microbiological analysis, the method of dilution and triplicate inoculation of solid nutrient media was used with subsequent counting of colony-forming units (CFU) in 1 g abs. dry soil (Mishustin & Emtsev, 1989; Malcheva & Naskova, 2018; Nustorova & Malcheva, 2020). Systematic and physiological groups of aerobic microbes - bacilli and non-spore-forming bacteria (on nutrient agar), micromycetes (mold fungi) - on Chapek-Dox agar, actinomycetes and bacteria assimilating mineral nitrogen (on Actinomycetes isolation agar) were determined. The general microflora was determined. The mineralization coefficient was calculated according to the formula: bacteria assimilating mineral nitrogen / (non-spore-forming bacteria+bacilli) (Mishustin and Runov, 1957; Malcheva and Naskova, 2018).

To isolate *E. coli*, a solid culture was made on Endo agar. Typical *E. coli* colonies on Endo agar are dark red with a metallic sheen (Malcheva and Naskova, 2020). Certified reference material was used: *E. coli* WDCM 0090 VT000904.

Agar diffusion method is used to determine antimicrobial activity (Nustorova and Malcheva, 2020). The volume of inoculated extract in each well was 60 µl.

The catalase activity of soil microorganisms was determined by the manganometric method (Khaziev, 1976).

In the laboratory experiment to determine the cellulose-decomposing activity, soil with a thickness of about 7 mm was poured into a petri dish with a diameter of 10 cm, maintaining 60% PPV /maximum field moisture content/. In each Petri dish, 3 strips of sterile filter paper measuring 10/50 mm are placed on the soil and cultivated at 25°C. During 10 days, the area of the degraded cellulose is recorded with a standard mesh. Average values from the three bands are calculated.

The following variants of coriander extracts were prepared (Table 1).

Table 1. Extract variants

Variant	Method of preparation of the extract
Decoct	A decoction (potion) is the liquid obtained by boiling the chopped plant product with the necessary solvent, usually water. Recommended for roots, flowers, leaves, twigs, fruits. The extractive solution is filtered while hot.
Tincture	Therapeutic substances are extracted from the chopped herbs by soaking with ethyl alcohol at a concentration of 30%, usually for a time varying between 8-10 days. The operation is carried out in well-closed glass containers. Shaking, for good extraction, is necessary throughout the extraction period.
Medicinal wine	The extraction is carried out in a weak hydroalcoholic environment, at a slightly acidic pH. For preparation, pre-crushed herbs are soaked for 7-10 days in wine (of good quality and well stabilized), after which the preparation is filtered.
Medicinal vinegar	It is obtained by extracting the active substances from herbal drugs with wine vinegar. For preparation, previously crushed herbs are soaked for 7-10 days in vinegar (good quality), after which the preparation is filtered.
Medicinal oil	It is a form of soaking the herbs in olive oil. The duration of soaking is 4-6 weeks. Store in tightly closed glass bottles, in a dark and cool place.

Coriander extracts are available in the following variants: roots, stems, leaves, whole plant.

RESULTS AND DISCUSSIONS

An agrochemical analysis of soil samples was peformed before sowing coriander and at the end of the vegetation of the plant species (Table 2).

Table 2. Agrochemical analysis

Variant	pH	Macronutrients			
		NH_4 mg/kg	NO_3 mg/kg	P_2O_5 mg/100 g	K_2O mg/100 g
Control (no vegetation)	7.26	4.03	8.24	17.1	17.7
Coriandrum sativum L.	7.24	3.97	5.99	16.2	15.4

Although after sowing the coriander, the values of the available forms of N, P and K decrease comparing with the limit values for the stocking of the soil with available nitrogen compounds, mobile phosphates and available potassium, it can be concluded that the soil is poorly stocked with nitrogen. In revanche, it has a good degree of storage in terms of phosphorus and potassium, and the soil reaction is slightly alkaline, relatively favorable for the development of coriander.

The biogenicity of the investigated variants includes the determination of ammonifiers (non-sporing bacteria and bacilli), actinomycetes and micromycetes (Table 3).

Table 3. Quantity and qualitative composition of soil microorganisms (CFU x 10^3 / g abs. dry soil)

Variant	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actinomycetes	Micromycetes	Bacteria assimilating mineral nitrogen	Mineralization coefficient
Control (before placing the trial, no vegetation)	3259.2	2667.2	400	57.6	134.4	4920	1.60
Coriandrum sativum L. (at the end of the growing season)	4102	2953.6	506.8	292	349.6	5936.4	1.72

The results show that the biogenicity of the soils is higher in the variant with vegetation compared to the control (no vegetation). This trend applies to the individual studied groups of microorganisms and, accordingly, to the general microflora. The rate of decomposition of organic matter in soils correlates with the amount of microorganisms.

In both variants, non-spore-forming bacteria take the main share in the composition of the general microflora, followed by bacilli, and the least represented are micromycetes (mold fungi) and actinomycetes (Figures 1 and 2).

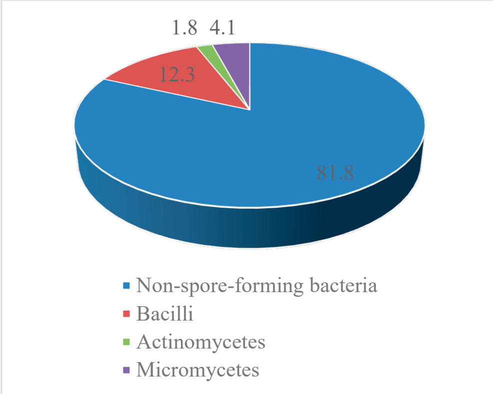

Figure 1. Percentage participation of microorganisms in the composition of the total microflora (control)

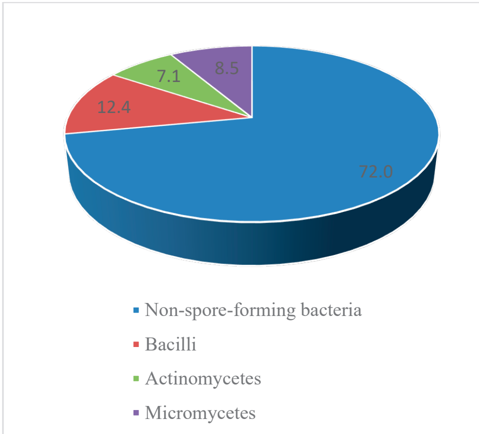

Figure 2. Percentage participation of microorganisms in the composition of the total microflora (*Coriandrum sativum* L.)

The percentage participation of non-spore-forming bacteria in the variant without vegetation is higher, while in the variant with coriander, the amount of this group of microorganisms decreases at the expense of an increase in the amount of actinomycetes and micromycetes. The amount of spore-forming bacteria - bacilli - remains relatively constant in both variants. Non-spore-forming bacteria and bacilli are mainly involved in the initial stages, and actinomycetes and micromycetes in the final stages of decomposition of organic matter.

Catalase is a respiratory enzyme that breaks down H_2O_2 (toxic), which is released when proteins are broken down. The catalase activity of the tested variants is presented in Figure 3.

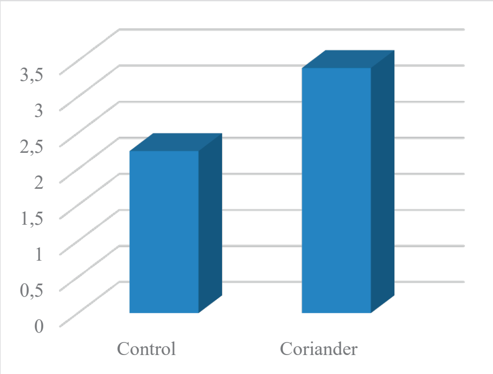

Figure 3. Catalase activity of soil microorganisms ($ml\ O_2$/30 min)

The results showed that in the variant with coriander, catalase activity increased 1.5 times, compared to the variant without vegetation. In addition to microbial origin, there is also catalase of plant origin. Catalase values correlate with the amount of soil microorganisms. A number of factors are important for enzyme activity: soil type, soil humidity and temperature, nutrient content, amount and composition of microflora, type of vegetation and others.

Cellulase catalyzes the hydrolysis of cellulose, in which cellulose is initially broken down to cellobiose, which under the action of ß-glucosidase is broken down to glucose. The cellulase activity of the tested variants is presented in Figure 4.

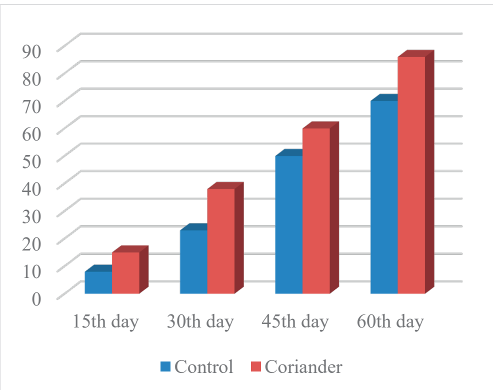

Figure 4. Cellulase activity of soil microorganisms (% degraded area)

Microbiological and enzymatic activity were approached by a number of authors as sensitive soil indicators, including contamination with pathogenic microorganisms (Malcheva et al., 2021; Malcheva et al., 2022, Dilly et al., 2003; Nannipieri et al., 2000; Li et al., 2008; Perucci, 1992; Pascual et al., 1999; García-Gil et al., 2000; Ros et al., 2003; Crecchio et al., 2004; Bastida et al., 2008; Marcote et al., 2001; Malcheva, 2014a, 2014b).

The results were negative for the presence of the tested pathogenic species *E. coli* in both variants, which allowed to test the prepared variant coriander plant extracts for antimicrobial activity (Table 4). Studies on the antioxidant and antimicrobial activity of *C. sativum* have mainly focused on the aerial parts (Kačániová & Ivanišová, 2019).

Table 4. Antimicrobial activity against *Escherichia coli* of the studied coriander extracts

Variant	Sterile area, cm			
	Roots	Leaves	Stems	Whole plant
Decoct	1.3	0.2	0.2	0.2
Tincture	0.9	0.6	0.5	0.9
Medicinal wine	1.4	0.8	1.1	15
Medicinal vinegar	0.7	0.5	0.5	0.8
Medicinal oil	1.5	0.9	1.3	1.5

The sterile zone for coriander root extracts decreases in the following order: medicinal oil > medicinal vinegar > decoction > tincture > medicinal wine. Compared to the root extracts, the sterile zone of the leaf extracts had lower values, indicating that coriander roots had a more effective effect against *E. coli* compared to their leaves. The sterile zone for coriander leaf extracts decreases in the following order: medicinal oil > medicinal vinegar > tincture > medicinal wine > decoction. Compared to root and leaf extracts, the sterile zone of stem extracts was intermediate between the results for root and leaf, indicating that coriander roots had a more effective effect and leaves had a weaker effect against *E.coli* compared to its stems. In medicinal wine, the same effect against the pathogen was found for leaf and stem extracts. The sterile zone for coriander stem extracts decreases in the following order: medicinal oil > medicinal vinegar > tincture = medicinal wine > decoction. Compared to other whole plant extracts, the sterile zone is comparable to root extracts – the largest retention zone compared to leaf and stem extracts. It is found that the sterile zone in the decoction variant is greater only in the root extracts, in the leaf, stem and whole plant extracts it is the lowest. The sterile zone for coriander whole plant extracts decreases in the following order: medicinal oil > medicinal vinegar > tincture > medicinal wine > decoction. In terms of antimicrobial activity, gram-negative bacteria (*E. coli* and others) are more sensitive to coriander oil than gram-positive bacteria (Saygi et al., 2021; Silva et al., 2011). Similar results against *E. coli* were obtained in the study of antibacterial activity of savory oil (Blažeković et al., 2010).

The nature of the solvent was found to be the main factor in the extraction of antioxidants and bioactive compounds from coriander (Kačániová et al., 2020). Additional conditions - lowering the pH of the medium when using medicinal vinegar and medicinal wine, the inclusion of additional plants - grapes (wine), vinegar (apples, grapes), olives (solvent olive oil for the medicinal oil) also affect the retention zone. In the variants with medicinal vinegar, a general sterile zone is formed around the wells, in the variants with medicinal wine - a bubble halo at a distance of 0.5 cm around each well - probably reactions from the created acidic environment. In the medicated oil variants, the sterile zone increases towards the interior, showing an enhanced effect of combining the root, leaf, stem, whole plant variants and combining the coriander extracts with olive extract (olive oil). As the time of action of the extracts increases (24 h, 48 h, 72 h), the diameter of the sterile zone increases by about 0.1 cm. Essential oil from the plant *Satureja hortensis* L. manifested varied antibacterial activity against *E.coli*, *Salmonella enteritidis*, and *B. subtilis*, depending on the concentration of essential oil used as well as the type of bacteria (Blažeković et al., 2010).

CONCLUSIONS

The obtained results of the agrochemical analyzes indicate that *Coriandrum sativum* does not have a great influence on the dynamics of macroelements in the soil. The macronutrient values obtained were close at the beginning and end of the experiment.

The variants with coriander increased the biogenicity of soil microorganisms, but in general the composition and percentage participation of the studied groups of microorganisms were preserved. In all variants, the main share in the composition of the general microflora is occupied by non-spore-forming bacteria, followed by bacilli, and the least represented are actinomycetes and micromycetes.

The values of the enzymes catalase and cellulase correlate with the amount of microorganisms and also their activity increases in the variant with coriander. A number of factors are important for enzyme activity: soil type, soil humidity and temperature, content of nutritional elements, amount and composition of microflora, type of vegetation and others.

Root and whole plant extracts showed higher antimicrobial activity against *E. coli* compared to leaf and stem extracts. The strongest antimicrobial activity of the plant extracts was found in the medicated oil and medicated vinegar variants and the weakest in the "decoction" variants (except for the root extracts).

The choice of solvent probably affects the diameter of the retention zone. As the exposure time increases, the sterile zone increases.
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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE INFLUENCE OF THE APPLIED MANAGEMENT ON THE PHYTODIVERSITY OF A *Festuca valesiaca* Schleich. EX GAUDIN PERMANENT MEADOW

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Abstract

The problem of diversity has reached the top of current concerns, because modern agriculture has recently been focused on the development of methods and procedures that allow the administration of a relatively small number of species, with the aim of immediate economic interest. The objective of this study was to determine the effect of the applied management on the phytodiversity of a Festuca valesiaca permanent meadow in the Moldovian forest-steppe. The experimental factor was: fertilization with seven graduations: V_1- unfertilized (control), V_2- $N_{50}P_{50}$ kg/ha annually, V_3- $N_{75}P_{75}$ kg/ha annually, V_4- $N_{100}P_{100}$ kg/ha annually, V_5-10 t/ha sheep manure annually, V_6-20 t/ha annually and V_7-30 t/ha annually sheep manure applied at two years. The applied fertilizers influenced the floristic composition, producing, quantitative and qualitative changes in the vegetal cover. The dominance and frequency of the species, as well as the variation of the Shannon diversity index, were largely influenced by the amount of mineral N, the amount of manure, but also by the number of years of the fertilization period.

Key words: *permanent grasslands, organic and mineral fertilization, plant diversity, species frequency.*

INTRODUCTION

Grassland areas described as green oceans are important for the ecosystem services they provide, with an essential role in the balance of the global ecosystem (Hopkins & Holz, 2005; Carlier et al., 2005; Lemaire et al., 2011; O'Mara, 2012). Currently, four ecosystem services are distinguished that grasslands provide, such as: support, supply, adjustment and cultural (Vîntu et al., 2004; Nábrádi, 2007; Boval & Dixon, 2012; Iacob et al., 2015; Duthie, 2015; Goliński et al., 2012; Avondo et al., 2013; Smit et al., 2015). In addition to the production of fodder for animals, the focus of current attention is the relationship of grasslands with climate and erosion control. Over time, the interaction between orographic, climatic, edaphic factors and applied management has determined a great diversity of grassland systems in our country. The *Festuca valesiaca* grasslands studied in the experiment are Natura 2000 habitats (Ponto-Pannonian *F. valesiaca* grasslands - R3415) and are specific to the steppe and forest-steppe areas, which have recently gained increasing importance in the face of climate change and biodiversity conservation, imposing their maintenance and sustainable use through the PAC. The development of these meadows depends to a large extent on the intensity of the applied management (Samuil et al., 2009; 2010a; 2010b; Samuil & Vîntu, 2012; Ciobanu et al., 2012, Vîntu et al., 2017). Fertilization, regardless of its nature (mineral or organic), determines together with the increase in production and the improvement of the main qualitative indices and the modification of the structure of the vegetal cover, something that has been demonstrated by numerous specialists, both nationally and internationally (Samuil et al., 2008; Rotar et al., 2004; Vîntu et al., 2006; Ciobanu, 2014; Molnár et al., 2020). Maintaining biodiversity through sustainable management of grassland ecosystems is a necessity and a major concern of researchers in this field (Jankowski et al., 2003).

Phytodiversity can be positively influenced at moderate intensification (Cŏp et al., 2010), and a minimum of intensity is needed to maintain the characteristic of floristic composition (Tonn & Briemle, 2010). In this context, to knowing to what extent the level of intensification influences the phytodiversity of a permanent meadow of *F. valesiaca* Schleich. ex Gaudin, during 2019-2020 observations were made regarding the changes in the floristic composition that occurred in the tested variants.

MATERIALS AND METHODS

This study presents the results of an experience organized in 2018 on a permanent meadow of *F. valesiaca* Schleich. ex Gaudin from the Moldavian forest-steppe, between the geographical coordinates 47°05'-47°10' North latitude and 27°28'-27°33' Eastern longitude, on a slightly sloped ground, with NE exposition. The climatic conditions in the area are characterized by an average temperature of 9.6°C and 517.8 mm of total annual precipitation. Between April and September, the average temperature is 17.3°C, and the precipitation amounts to 337.5 mm. As a result of the diversity of physical-geographical factors and, first of all, of the relief, the dynamics of the atmosphere is very active and complex and gives the researched area a climate with thermal and hydrous characteristics specific to temperate-continental regions.

The experiment was organized according to the method of randomized plots in three repetitions, and the experimental factor was fertilization with seven gradations: V_1 - unfertilized (control), V_2 - $N_{50}P_{50}$ kg/ha annually, V_3 - $N_{75}P_{75}$ kg/ha annually, V_4 - $N_{100}P_{100}$ kg/ha annually, V_5 - 10 t/ha sheep manure annually, V_6 - 20 t/ha annually and V_7 - 30 t/ha annually sheep manure applied at two years.

Two types of fertilizers were used within the experiment: organic, represented by a well fermented sheep manure (older than two years) and mineral, represented by a complex fertilizer with nitrogen and phosphorus ($N_{20}P_{20}$). The sheep manure had the following chemical composition: N - 0.692%, P_2O_5 - 0.320% and K_2O - 0.811%. Fertilizers were applied manually in early spring before the start of active vegetation growth. Each plot was 4 m × 2.5 m in size. Harvesting was done at earing-flowering stage of the dominant grasses, and the floristic composition was established using the Braun-Blanquet method (Cristea et al., 2004). Floristic data have been processed with Excel and PC-ORD. Within the PC-ORD multivariate analysis program (McCune & Grace, 2002), various methods were used to order the floristic relevés, namely: Summary (to determine the Shannon Index and the number of species), average frequency and dominance, and multidimensional NMDS Autopilot scaling (Păcurar & Rotar, 2014).

The statistical data was analyzed by SPSS using ANOVA and the Lowest Significant Difference test (LSD 5%).

RESULTS AND DISCUSSIONS

Biodiversity of meadows is the result of colonization and disappearance of species, influenced by different factors. In the management of meadows, the laws of ecology and evolution must be taken into account, in order to preserve biodiversity (Partel et al., 2005). The phytodiversity of grasslands is affected by several factors, including natural or anthropogenic actions (Duru et al., 2010; Lehman & Tilman., 2000; Mauz & Rémy, 2004; Pasho et al., 2011).

During the 2 experimental years (2019-2020), we followed how the type of fertilizer and the amount applied (mineral with $N_{50}P_{50}$, $N_{75}P_{75}$ and $N_{100}P_{100}$ kg/ha applied annually and organic with 10, 20 t/ha sheep manure applied annually and 30 t/ha sheep manure applied at two years) influences the floristic composition of the meadow under study.

Ordering the floristic composition with the help of NDMS Autopilot (multidimensional scaling), shows us the changes produced by the administration of mineral and organic fertilizers on the biodiversity of the vegetal cover in the period 2019-2020. In 2019, the analysis of the floristic composition of the *F. valesiaca* meadow revealed that the studied phytocoenoses do not overlap clearly, which shows that there is no great similarity, which shows a different floristic diversity from one fertilization option to another (Figure 1). The ordering chart of the floristic composition

(Figure 1) from 2019 confirms that the application of organic and mineral fertilizers produced important changes in the floristic composition of the vegetal cover. The fact that the phytocenosis of the control variant does not overlap in the graphic representation with the fertilized variants, and the distance between them is large, shows us that we have a low similarity in terms of floristic composition. As it can be observed within the Figure 1, a overlap between certain variants, such as the case of variant V_2 ($N_{50}P_{50}$) with V4 ($N_{100}P_{100}$) and respectively V_6 (20 t/ha of sheep manure applied annually), which proves that the similarity between them it could be a high one (Figure 1).

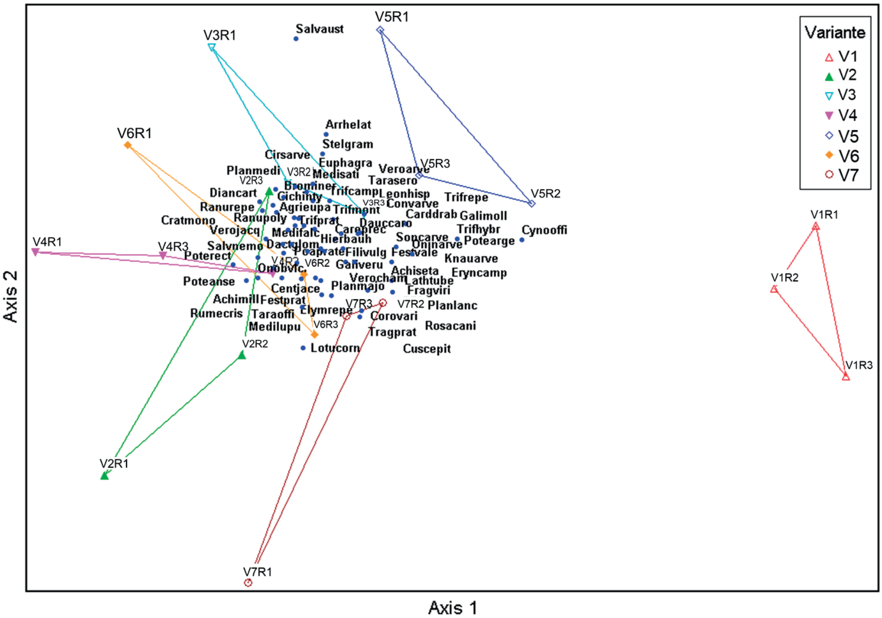

Figure 1. Ordination of floristic composition in 2019, influenced by fertilization (V_1 - unfertilized (control); V_2 - $N_{50}P_{50}$; V_3 - $N_{75}P_{75}$; V_4 - $N_{100}P_{100}$ kg/ha annually; V_5 - 10 t/ha ha of sheep manure applied annually, V_6 - 20 t/ha of sheep manure applied annually and V_7 - 30 t/ha of sheep manure applied at 2 years; R_1, R_2, R_3 - replicates)

(Legend: Species name: *Arrhenatherum elatius* (L.) J. Presl. et C. Presl = *Arrhelat*, *Bromus inermis* Leyss. = Brominer, *Dactylis glomerata* L. = Dactglom, *Elymus repens* (L.) Gould. = *Elymrepe*, *Festuca pratensis* Huds. = Festprat, *Festuca valesiaca* Schleich. ex Gaudin = Festvale, *Poa pratensis* L. ssp. *pratensis* = Poaprat, *Coronilla varia* L. =Corovari, *Lotus corniculatus* L. = Lotucorn, *Medicago falcata* L. = Medifalc, *Medicago lupulina* L. = Medilupu, *Medicago sativa* L. = Medisati, *Onobrychis viciifolia* Scop. = Onobvic, *Trifolium campestre* L. = Trifcamp, *Trifolium pratense* L. = Trifprat, *Trifolium repens* L. Trifrepe, *Trifolium montanum* L. = Trifmont, *Trifolium hybridum* L. = Trifhybr, *Ononis arvensis* L. = Ononarve, *Carex praecox* Schreb. = Careprae, *Agrimonia eupatoria* L. = Agrieupt, *Achillea millefolium* L. = Achimile, *Achillea setacea* Waldst. et Kit. = Achiseta, *Cardaria draba* (L.) Desv. = Carddrab, *Convolvulus arvensis* L. = Convarve, *Centaurea jacea* L. = Centjace, *Cichorium intybus* L. = Cichinty, *Cirsium arvense* (L.) Scop. = Cirsarve, *Cuscuta epithymum* (L.) L. ssp. *trifolii* (Bab.) Behrer = Cuscepit, *Crataegus monogyna* L. = Cratmono, *Cynoglossum officinale* L. = Cynooffi, *Daucus carota* L. ssp. *carota* = Dauccaro, *Dianthus carthusianorum* L. = Diancart, *Eryngium campestre* L. = Eryncamp, *Euphorbia agraria* M. Bieb. = Euphagra, *Fragaria viridis* Duchesne = Fragviri, *Filipendula vulgaris* Moench. = Filivulg, *Galium verum* L. = Galiveru, *Galium mollugo* L. = Galimoll, *Hieracium bauhinii* Besser = Hierbauh, *Knautia arvensis* L. = Knauarve, *Leontodon hispidus* L. = Leonhisp, *Plantago lanceolata* L. = Planlanc, *Plantago media* L. = Planmedi, *Plantago major* L. = Planmajo *Potentilla anserina* L. = Poteanse, *Potentilla argentea* L. = Potearge, *Potentilla recta* L. = Poterect, *Ranunculus repens* L. = Ranurepe, *Ranunculus polyanthemos* L. ssp. *polyanthemoides* (Borreau) Ahlfv. = Ranupoly, *Rosa canina* L. = Rosacani, *Rumex crispus* L. = Rumecris, *Salvia nemorosa* L. = Salvnemo, *Salvia austriaca* Jacq. = Salvaust, *Sonchus arvensis* L. = Soncarve, *Stellaria graminea* L. = Stelgram, *Taraxacum officinale* Weber. = Tragoffi, *Taraxacum serotinum* (Waldst. & Kit.) Fisch. = Tarasero, *Tragopogon pratensis* L. ssp. *pratensis* = Tragprat, *Veronica arvensis* L. = Veroarve, *Veronica chamaedrys* L. = Verocham, *Veronica jacquinii* Baumg. = Verojacq nomenclature after Sârbu et al., 2013; Ciocârlan, 2009)

There are, however, some species that entered the floristic composition of the phytocenosis following the application of certain doses of mineral and organic fertilizers, as follows: *Arrhenatherum elatius* (L.) J. Presl. et C. Presl, L., *Bromus inermis* Leyss., *Medicago sativa* L., *Trifolium campestre* L. when applying $N_{75}P_{75}$ (V_3), *Coronilla varia* L., *Lotus corniculatus* L. and the species *Tragopogon pratensis* L. ssp. *pratensis* when fertilizing with 30 t/ha of sheep manure applied at 2 years (V_7) (Figure 1).

The floristic diversity of the control variant (V_1) is highlighted in 2019 by a number of 36 species and a value of the Shannon - Wiener index (H') of 1.587, which shows us that the phytocoenosis of the control variant has a low diversity (Table 1). In 2019, it appears that in the variants fertilized with mineral fertilizers, the values of the Shannon – Wiener diversity index (H') varied between 2.234 and 2.510, indicating a low to medium diversity. The number of species in the case of the mineral fertilized variants varied between 45 and 50. In the case of the variants fertilized with sheep manure, the values of the Shannon Wiener diversity index (H') depending on the amount applied, were between 2.291 and 2.630, indicating low to medium diversity. The number of species in the case of organically fertilized variants varied between 43 and 50 (Table 1).

Regarding the frequency of species in the phytocenosis of the control variant (type *Festuca valesiaca* Schleich. ex Gaudin) in 2019, in addition to the dominant species, other species appear with a greater coverage and frequency, such as: *Achillea setacea* Baumg., *Centaurea jacea* L, *Fragaria viridis* Duchesne, *Galium verum* L., *Poa pratensis* L., *Elymus repens* (L.) Gould., *Lotus corniculatus* L., *Plantago lanceolata* L. etc., but also species with medium coverage and frequency: *Ranunculus polyanthemos* L. ssp. *polyanthemoides* (Borreau) Ahlfv., *Potentilla argentea* L., etc. (Figure 2).

Table 1. Relationships between experimental variants and some diversity indices in 2019 and 2010

Fertilization variants	Phytodiversity			
	2019		2020	
	H'	S	H'	S
V_1	1.587^{Mt}	36^{Mt}	1.875^{Mt}	33^{Mt}
V_2	2.234*	45*	2.352*	47*
V_3	2.510*	49*	2.638*	53*
V_4	2.493*	50*	2.612*	48*
V_5	2.291*	50*	2.160	40
V_6	2.630*	53*	2.594*	50*
V_7	2.296*	43*	2.386*	46*
LSD 5%	0.318	9	0.287	13

(H' = Shannon Index, S = number of species, Mt. = control,* = $p < 0.05$)

There are also species in the phytocenosis of the control variant, especially those with high fodder value, such as: *Arrhenatherum elatius* (L.) J. Presl. et C. Presl, L., *Bromus inermis* Leyss., *Dactylis glomerata* L., *Trifolium pratense* L., *Medicago lupulina* L., which had a very low coverage and frequency (Figure 2).

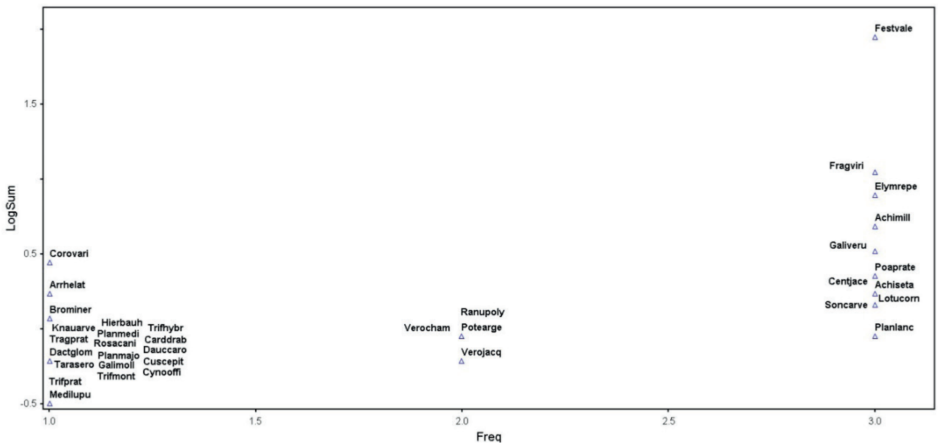

Figura 2. Dominant frequency diagram of control variant phytocoenosis *F. valesiaca* Schleich. ex Gaudin in 2019 (Legend: Freq= Species frequency, Logsum = Log base 10 of species dominance, for species name see the legend from Figure 1)

Comparing the floristic composition of the control variant with that of the variants on which mineral and organic fertilizers were applied, we find that certain species change their dominance and frequency (Figure 3).

Therefore, some species that in the control variant had a very low coverage and frequency, such as the case of the species: *Arrhenatherum elatius* (L.) J. Presl. et C. Presl, L., *Bromus inermis* Leyss., *Dactylis glomerata* L., *Trifolium pratense* L., *Medicago lupulina* L., *Plantago media* L. etc., are now found in a higher percentage with a medium to high frequency (Figure 3). Valuable species from a fodder point of view, such as *Medicago sativa* L. și *M. falcata* L., which were absent in the phytocenosis of the control variant, we observe that they increase their frequency-dominance as a result of mineral and organic fertilization (Figure 3).

Other species such as: *Salvia austriaca* Jacq., *Cynoglossum officinale* L., *Potentilla recta* L., *Leontodon hispidus* L., *Filipendula vulgaris* Moench. etc., presents a low frequency-dominance in most of the variants studied (Figure 3).

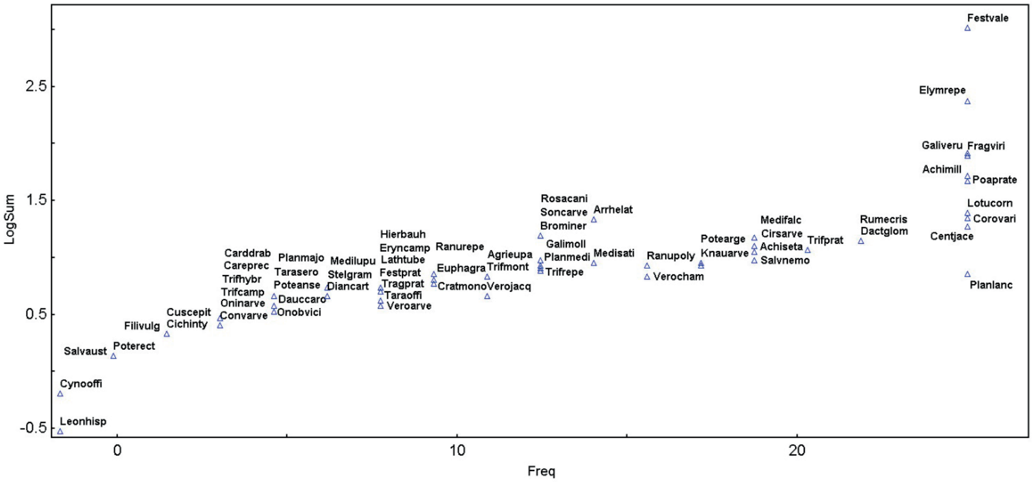

Figure 3. Dominant frequency diagram of organic and mineral fertilized variants phytocoenosis, without control variant, in 2019 (Freq= Species frequency, Logsum = Log base 10 of species dominance, for species name see the legend from Figure 1)

As in the previous year (2019), mineral and organic fertilization in 2020 determined major changes in the floristic composition, as can be seen from the graphic representation, the distance between the floristic composition of the control variant and that of the fertilized variants is very large, which indicates a different floristic diversity from one fertilization variant to another (Figure 4).

From Figure 4, it emerged that in the case of the unfertilized variant (control), there was a visible difference compared to that of the variants fertilized with mineral and organic fertilizers, and at small doses applied (V_2 - $N_{50}P_{50}$) or at (V_5 - 10 t/ha ha of sheep manure applied annually) the changes recorded in the structure of the vegetal cover were less.

In the variants fertilized with the maximum doses of fertilizers (V_4 - $N_{100}P_{100}$ and V_7 - 30 t/ha of sheep manure applied at 2 years) the ordering of the floristic composition was more distant from the homogeneous group of species. There was a homogeneous distribution of species within the variants fertilized with average doses of organic or mineral fertilizers, respectively the variants V_3 - $N_{75}P_{75}$ and V_6 - 20 t/ha of sheep manure applied (Figure 4).

The administration of fertilizers favored the installation of new species, at certain doses of fertilization, such as: *Lathyrus tuberosus* L., *Festuca pratensis* Huds. and *Prunus spinosa* L. in the variant V_3 ($N_{75}P_{75}$), *Trifolium montanum* L. și *Teucrium chamaedrys* L. in the variant V_6 (20 t/ha of sheep manure applied annually) the

Agrimonia eupatoria L. species in the V_4 variant ($N_{100}P_{100}$).

Analyzing the phytodiversity of the control variant (V_1), in 2020, we observe that the average value of the number of species is 33 and that of the Shannon - Wiener index of 1.875, which indicates a low biodiversity (Table 1).

As in the previous year, in 2020, the phytodiversity of the fertilized phytocenoses registered an increase depending on the type of fertilizer and the dose applied, compared to the phytocenose of the control variant. Therefore, in the case of the variants fertilized with mineral fertilizers, the values of the Shannon - Wiener diversity index (H') varied between 2.352 and 2.638, indicating a low to medium diversity. The number of species in the case of mineral fertilized variants varied between 47 and 53 (Table 1).

Organic fertilization in its doses determined the increase in the number of species, the values being between 40 and 50, depending on the amount of sheep manure applied. The values of the Shannon - Wiener diversity index (H') varied between 2.160 and 2.594, indicating a low to medium diversity (Table 1).

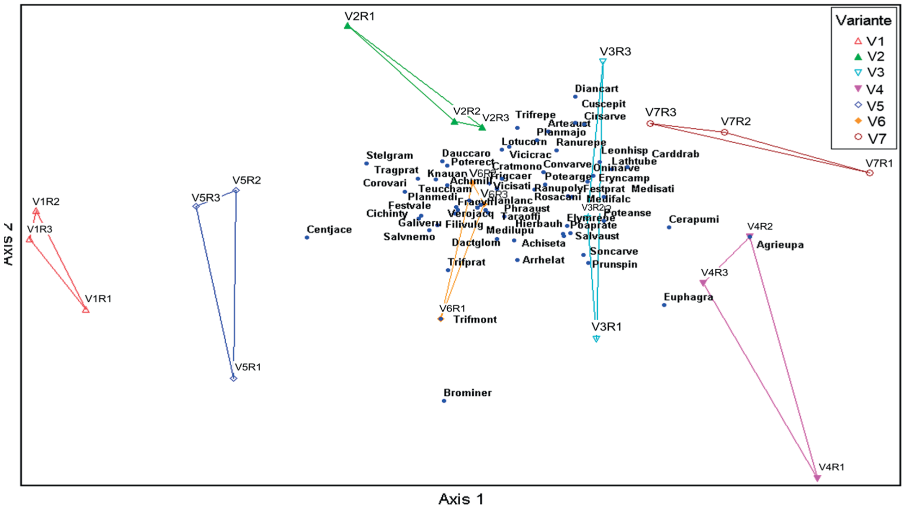

Figure 4. Ordination of floristic composition in 2020, influenced by fertilization (V_1 - unfertilized (control); V_2 - $N_{50}P_{50}$; V_3 - $N_{75}P_{75}$; V_4 - $N_{100}P_{100}$ kg/ha annually; V_5 - 10 t/ha ha of sheep manure applied annually, V_6 - 20 t/ha of sheep manure applied annually and V_7 - 30 t/ha of sheep manure applied at 2 years; R_1, R_2, R_3 – replicates)

(Legend: Species name: *Arrhenatherum elatius* (L.) J. Presl. et C. Presl = *Arrhelat*, *Bromus inermis* Leyss. = Brominer, *Dactylis glomerata* L. = Dactglom, *Elymus repens* (L.) Gould. = *Elymrepe*, *Festuca pratensis* Huds. = Festprat, *Festuca valesiaca* Schleich. ex Gaudin = Festvale, *Poa pratensis* L. ssp. *pratensis* = Poaprat, *Phragmites australis* (Cav.) Trin. ex Steud. = Phraaust, *Coronilla varia* L. = Corovari, *Lotus corniculatus* L. = Lotucorn, *Medicago falcata* L. = Medifalc, *M. lupulina* L. = Medilupu, *M. sativa* L. = Medisati, *Trifolium pratense* L. = Trifprat, *Trifolium repens* L. Trifrepe, *Trifolium montanum* L. = Trifmont, *Trigonella caerulea* (L.) Ser. = Trigcaer, *Vicia sativa* L. = Vicisati, *Ononis arvensis* L. = Ononarve, *Carex praecox* Schreb. = Careprae, *Agrimonia eupatoria* L. = Agrieupt, *Achillea millefolium* L. = Achimile, *Achillea setacea* Waldst. et Kit. = Achiseta, *Cardaria draba* (L.) Desv. = Carddrab, *Cerastium pumilum* Curtis. = Cerapumi, *Convolvulus arvensis* L. = Convarve, *Centaurea jacea* L. = Centjace, *Cichorium intybus* L. = Cichinty, *Cuscuta epithymum* (L.) L. ssp. *trifolii* (Bab.) Behrer = Cuscepit, *Crataegus monogyna* L. = Cratmono, *Daucus carota* L. ssp. *carota* = Dauccaro, *Dianthus carthusianorum* L. = Diancart, *Eryngium campestre* L. = Eryncamp, *Euphorbia agraria* M. Bieb. = Euphagra, *Fragaria viridis* Duchesne = Fragviri, *Filipendula vulgaris* Moench. = Filivulg, *Galium verum* L. = Galiveru, *Hieracium bauhinii* Besser = Hierbauh, *Knautia arvensis* L. = Knauarve, *Lathyrus tuberosus* L. = Lathtube, *Leontodon hispidus* L. = Leonhisp, *Plantago lanceolata* L. = Planlanc, *Plantago media* L. = Planmedi, *Plantago major* L. = Planmajo, *Potentilla anserina* L. = Poteanse, *Potentilla argentea* L. = Potearge, *Potentilla recta* L. = Poterect, Prunus spinosa L. = Prunspin, *Ranunculus polyanthemos* L. ssp. *polyanthemoides* (Borreau) Ahlfv. = Ranupoly, *Rosa canina* L. = Rosacani, *Rumex crispus* L. = Rumecris, *Salvia nemorosa* L. = Salvnemo, *Salvia austriaca* Jacq. = Salvaust, *Sonchus arvensis* L. = Soncarve, *Stellaria graminea* L. = Stelgram, *Taraxacum officinale* Weber. = Tragoffi, *Teucrium chamaedrys* L. = Teuccham, *Tragopogon pratensis* L. ssp. *pratensis* = Tragprat, *Veronica jacquinii* Baumg. = Verojacq nomenclature after Sârbu et al., 2013; Ciocârlan, 2009).

The increase of the number of species is due to the application of fertilizers, which changed the state of soil fertility.

This change in soil fertility allowed other mesotrophic and/or eutrophic species to establish in the fertilized variants.

This study demonstrated that fertilization with mineral and organic fertilizers in moderate doses contributed significantly to the increase in the number of species present in the vegetal cover.

Different other researchers, in similar experiments, have shown that fertilization regardless of its nature, applied in moderate quantities stimulates floristic diversity of meadows (Mrkvièka & Veselá, 2002; Rotar et al., 2003; Păcurar & Rotar, 2004; Chytrý et al., 2009; Ciobanu et al., 2013a; 2013b; Nettier et al., 2010; Samuil et al., 2013; Vîntu et al., 2017; Molnár et al., 2020). Fertilization applied in high doses causes the loss of species richness, results confirmed in some studies carried out by Dragomir et al., 2020; Boch et al., 2021; Ranta et al., 2021.

Analyzing the species frequency diagram in the control variant (type *F. valesiaca* Schleich. ex Gaudin), in the year 2020, we notice that in addition to the dominant species, other species are found with a high coverage and frequency, such as: *Elymus repens* (L.) Gould., *Poa pratensis* L., *Achillea millefolium* L., *Fragaria viridis* Duchesne, *Centaurea jacea* L, *Galium verum* L., *Trifolium pratense* L, *Plantago media* L., *P. lanceolata* L., *Coronilla varia* L., *Lotus corniculatus* L. etc., but also species with medium coverage and frequency: *Achillea setacea* Waldst. et Kit., *Filipendula vulgaris* Moench. and *Taraxacum officinale* Weber.

Species such as: *Dactylis glomerata* L., *Arrhenatherum elatius* (L.) J. Presl. et C. Presl., *Bromus inermis* Leyss., *Medicago lupulina* L., *Vicia sativa* L., *Daucus carota* L., *Cichorium intybus* L. etc., had a very low coverage in the control variant (Figure 5).

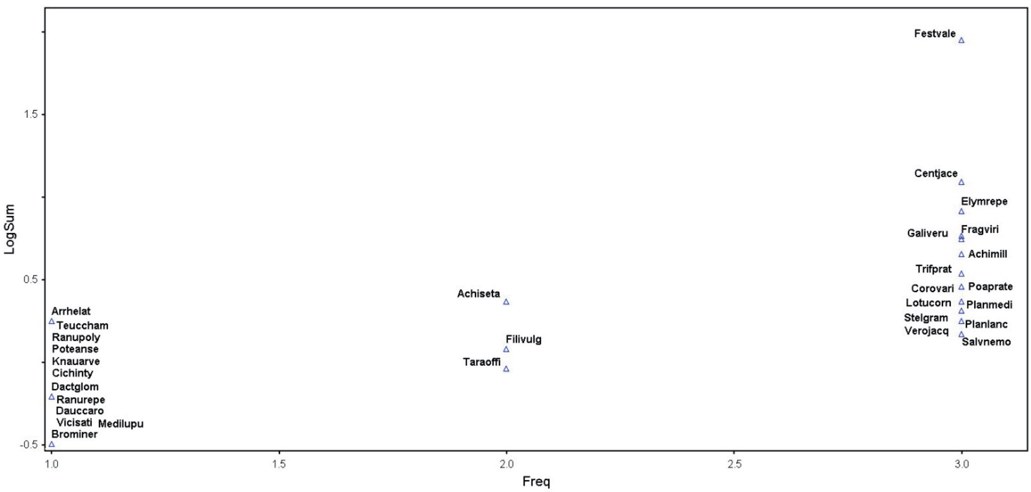

Figura 5. Dominant frequency diagram of control variant phytocoenosis *F. valesiaca* Schleich. ex Gaudin in 2020 (Legend: Freq= Species frequency, Logsum = Log base 10 of species dominance, for species name see the legend from Figure 4)

In 2020, comparing the floristic composition of the unfertilized variant with that of the variants where organic and mineral fertilizers were applied, we find, as in the previous year, that certain species change their dominance and frequency (Figure 6).

Therefore, some species that in the control had a large coverage such as the *Festuca valesiaca* Schleich species. ex Gaudin and *Elymus repens* (L.) Gould., are now found in a lower percentage (Figure 6). In the case of other species, such as *Arrhenatherum elatius* (L.) J. Presl. et C. Presl, and *Medicago falcata* L. we observed that their frequency has increased significantly and they are classified as dominant species, an aspect that is explained by their abundance in most of the studied variants. Other species that change their dominance and frequency are: *Dactylis glomerata* L., *Festuca pratensis* Huds., *Knautia arvensis* L., *Cichorium intybus* L., *Potentilla argentea* L., and so on. The species with the lowest abundance and frequency in most experimental variants are: *Agrimonia eupatoria* L., *Trifolium montanum* L., *Cerastium pumilum* Curtis., *Teucrium chamaedrys* L., *Euphorbia agraria* M. Bieb, etc. (Figure 6).

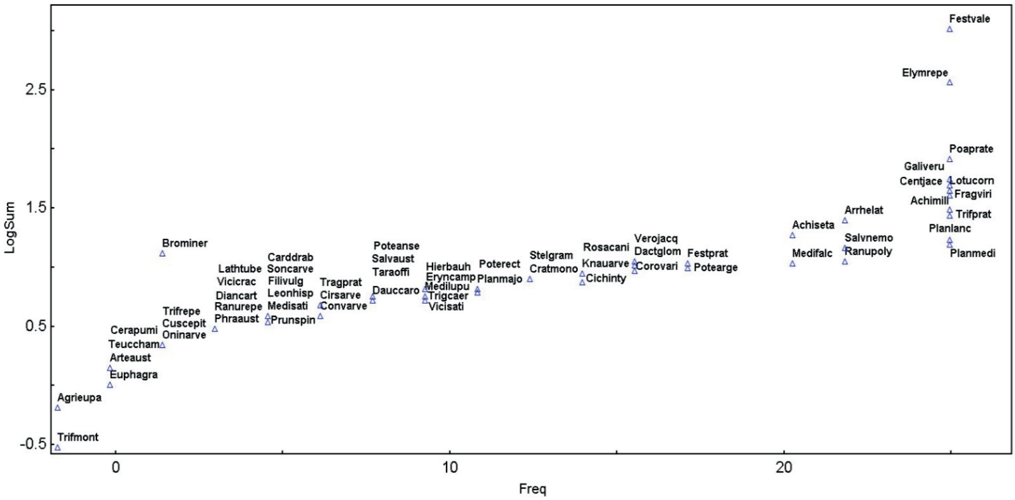

Figure 6. Dominant frequency diagram of organic and mineral fertilized variants phytocoenosis, without control variant, in 2020 (Freq= Species frequency, Logsum = Log base 10 of species dominance, for species name see the legend from Figure 4)

CONCLUSIONS

The administration of fertilizers on these permanent *F. valesiaca* meadows, in any form, causes obvious changes in the structure of phytocenoses compared to the unfertilized variant. The changes in the floristic composition of the vegetal cover, as well as the variation of the Shannon diversity index, were largely influenced by the amount of mineral N, the amount of manure, but also by the number of years of the fertilization period.

The increase of the number of species as well as their different way of dispersal in the studied variants could have been caused by the improvement of the soil nutrient content after fertilization.

As a result of our research it is found that the using a management based on fertilization with moderate amounts of organic and mineral fertilizers can help to preserve the biodiversity of *F. valesiaca* meadows, while high amounts of fertilizers can negatively influence it.

To maintain a high level of floristic diversity, we recommend the application of 20 t/ha of manure administered annually, and in the absence of manure, the application of a moderate dose of mineral fertilizer with $N_{75}P_{75}$.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

GROWTH, DEVELOPMENT, AND WEED SUPPRESSION CAPACITY OF *Camelina sativa* (L.) Crantz GROWN AS SOLE AND MIXED CROP WITH LEGUMES: PRELIMINARY RESULTS

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Abstract

Camelina sativa (L.) Crantz is a valuable crop with good drought resistance and has been proposed for cultivation in Organic farming system. The development stages of camelina, pea, and vetch grown as sole and mixed crops, and the existing weeds S. halepense and S. viridis in a field plot experiment were under evaluation. The trial was stated in the experimental field of the Agricultural University of Plovdiv, Bulgaria. On the last reporting date the camelina genotypes 'Luna' and 'Lenka' entered the beginning of the flowering stage, and the Bulgarian landrace showed better development and was in the full flowering stage. The pea and vetch were in the full flowering stage as well. The development stages of the mixed cropping systems were the same as the growing stages of their sole crops. The mixed cropping system of the well-developed Bulgarian camelina landrace grown in a mixed cropping system with pea and vetch suppressed the growth and development of the weeds reported. In the sole crops, the reported weeds developed faster and accumulated a greater amount of biomass.

Key words*: Camelina sativa, intercrops, growth, development, weeds.*

INTRODUCTION

One of the underestimated oil crops widespread in the wild environment of Bulgaria and neighboring countries is gaining large interest in the last decade. *Camelina sativa*, known also as false-flax or gold-of-pleasure, originated from southeastern Europe and southwestern Asia (Luo et al., 2019). It`s been used in culinary as valuable cold-pressed oil, a functional food supplement for the prevention of cardiovascular problems in pharmacology, but also the alfa-linolenic acid (aka omega-3) plays important role in the cell renewal of cosmetic products. The high oil content (up to 49%) and good protein (21-30%) justify the use of the meal for feed.

The increased industrial search for alternatives to fossil fuels for jet fuel and biodiesel can be answered with this easy-to-grow crop (Mondor and Hernández-Álvarez, 2022). The short vegetation and good tolerance to climate changes and soil limitations make the camelina low-input crop well-adapted to each environment (Bakhshi et al., 2021; Ahmet et al., 2017).

One of the oldest forms of agricultural production was based on mixed cropping (Plucknett and Smith, 1986). Cropping systems based on carefully designed plant mixtures reveal several advantages under various agricultural conditions (Willey, 1979; Malézieux, 2009). Some advantages of mixed cropping are nutrient use efficiency and yield stability (Lizarazo et al., 2020). As described by Silvertown (1982), in the mixed cropping system, two or more cultivated plants are grown together at the same time on the same land. For example, the forage of the grain legume-cereal mixed crop has higher crude protein content, and higher relative feed value (Strydhorst et al., 2008). According to Lauk and Lauk (2008), the highest yield for a combination of the pea-oat mixture was found. After growing pea-barley and pea-oilseed rape as mixed crops the yield was higher than those of the sole crops (Malhi, 2012).

According to Poggio (2005), mixing plant species may also reduce weed diversity and stand. Mixed cropping of peas together with camelina showed a significant suppressive effect on weed flora. Liebman and Dyck (1993) found that the composition of two or more crops decreases the weed biomass in the intercropping system when compared to the sole crops. According to Leclère et al. (2019) intercropping camelina with pea or barley showed a reduction in weed biomass. Weed suppression was probably due to the mutually enhanced competitiveness of both crops, indicating a mechanism based on the allocation of resources (Saucke and Ackermann, 2006).

There is still limited information about the growing *C. sativa* in mixed cropping systems, therefore this experiment aimed to evaluate some effects of camelina with pea or vetch as intercrops in organic farming conditions.

MATERIALS AND METHODS

The experiment was carried out in the certified biological experimental field of the Agro-ecology Center at the Agricultural University - of Plovdiv, Bulgaria.

Winter camelina (*Camelina sativa* (L.) Crantz) varieties Luna (K 1), Lenka (K 2), and a Bulgarian landrace (K 3) of false flax were used in sole crop and in an intercropping system with winter forage pea (*Pisum sativum* L.) variety Mir and vetch (*Vicia sativa* L.) variety Obrazetc 666.

The experiment was performed by using a randomized block design in three replications with an experimental plot size of 10 m^2. Recommended sowing rates were applied for the sole crop of camelina 800 germinated seeds/m^2, winter pea - 120 germinated seeds/m^2, and vetch - 200 germinated seeds/m^2. Intercropping was sown with a half rate of seeds.

Mineral nutrition was applied to ensure three kilograms of active substance nitrogen with certified organic farming fertilizer Bioazoto 12. The preceding crop was winter rye.

Deep ploughing and cultivating followed by tillage before and after the sowing of camelina were applied.

The development stages of the crops (camelina, pea, and vetch) and the existing weeds (*Sorghum halepense* (L.) Pers. and *Setaria viridis* L.) in the experimental plots were reported.

The observations started after the camelina genotypes entered the rosette stage (R) and ended after the Bulgarian camelina genotype (K 3) entered the full flowering stage.

The number and fresh weight of the weeds in the different cropping systems (sole and intercropped) were examined on 03.06.2022 (7 days after the full flowering stage of the K 3).

For statistical analyses of the collected data Duncan's multiple range test with the SPSS 19 program (Duncan, 1955). Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSIONS

On Table 1 the obtained data for the crops' development after the rosette stage till full flowering of the Bulgarian camelina genotype is presented.

The first observation was on the 22nd of April. On this date, all camelina genotypes were in the rosette stage. The sole crops pea and vetch as well as the intercropping system of pea with the three camelina genotypes were in the 4th-5th trifoliate stage. When the vetch was grown in a mixture with camelina, its growing stage was 2nd-3rd trifoliate.

On the second observation date (29.4.2022) the three camelina genotypes were still in the rosette stage. The sole crops pea and vetch as well as the intercropping system of pea and vetch with the tree camelina genotypes were in the 4th-5th trifoliate stage.

On the third reporting date (6.5.2022) the camelina varieties Luna (K 1) and Lenka (K 2) were still in the rosette stage. The Bulgarian camelina landrace (K 3) entered the pod setting stage. The sole crops of pea and vetch were in the 5th-6th trifoliate stage.

In the mixed cropping systems, the camelina varieties Luna (K 1) and Lenka (K 2) were still in the rosette stage as well. In the mixed cropping system of pea and vetch with the tree camelina genotypes, the legume plants were 4th-5th trifoliate stage.

On the fourth evaluation date (13.5.2022) the camelina varieties Luna (K 1) and Lenka (K 2) were still in the rosette stage. The Bulgarian camelina genotype (K 3) entered the beginning flowering stage. The sole crops pea and vetch were in the 6th-7th trifoliate stage. There were no observed differences in the development stages for the mixed cropping systems of the camelina varieties and the legume crops. The growing stages were the same as for the sole crops.

On the fifth observation date (20.5.2022) the sole crops of the camelina genotypes Luna (K 1) and Lenka (K 2) were in the pod setting stage. The Bulgarian camelina (K 3) entered the full flowering stage. The sole crops pea and vetch were at the beginning of flowering. The same growth stages were found for the intercropping of camelina with the legumes.

On the last reporting date (27.5.2022) the camelina genotypes Luna (K 1) and Lenka (K 2) entered the beginning of the flowering stage. The Bulgarian variety (K 3) was in the stage of full flowering. The pea and vetch were in the full flowering stage as well. The development stages of the mixed cropping system were the same as the growing stages of the sole crops grown in the study.

Table 1. Crop development after the rosette growth stage till full flowering of the Bulgarian camelina

Variants	22.4.2022	29.4.2022	6.5.2022	13.5.2022	20.5.2022	27.5.2022
Growth stage						
K 1 Luna	Rosette	Rosette	Rosette	Rosette	Pod setting	Beginning of flowering
K 2 Lenka	Rosette	Rosette	Rosette	Rosette	Pod setting	Beginning of flowering
K 3	Rosette	Rosette	Pod setting	Beginning of flowering	Full flowering	Full flowering
Pea	4-5 trifoliate	4-5 trifoliate	5-6 trifoliate	6-7 trifoliate	Beginning of flowering	Full flowering
Vetch	4-5 trifoliate	4-5 trifoliate	5-6 trifoliate	6-7 trifoliate	Beginning of flowering	Full flowering
K 1 / Pea	Rosette	Rosette	Rosette	Rosette	Butonization	Beginning of flowering
	4-5 trifoliate	4-5 trifoliate	5-6 trifoliate	6-7 trifoliate	Beginning of flowering	Full flowering
K 2 / Pea	Rosette	Rosette	Rosette	Rosette	Pod setting	Beginning of flowering
	4-5 trifoliate	4-5 trifoliate	5-6 trifoliate	6-7 trifoliate	Beginning of flowering	Full flowering
K 3 / Pea	Rosette	Rosette	Pod setting	Beginning of flowering	Full flowering	Full flowering
	4-5 trifoliate	4-5 trifoliate	5-6 trifoliate	6-7 trifoliate	Beginning of flowering	Full flowering
K 1 / Vetch	Rosette	Rosette	Rosette	Rosette	Butonization	Beginning of flowering
	2-3 trifoliate	4-5 trifoliate	5-6 trifoliate	6-7 trifoliate	Beginning of flowering	Full flowering
K 2 / Vetch	Rosette	Rosette	Rosette	Rosette	Butonization	Beginning of flowering
	2-3 trifoliate	4-5 trifoliate	5-6 trifoliate	6-7 trifoliate	Beginning of flowering	Full flowering
K 3/ Vetch	Rosette	Rosette	Pod setting	Beginning of flowering	Full flowering	Full flowering
	2-3 trifoliate	4-5 trifoliate	5-6 trifoliate	6-7 trifoliate	Beginning of flowering	Full flowering

On Table 2 are the results regarding the weeds' development after the rosette growth stage of camelina till full flowering of the Bulgarian camelina landrace. The evaluation dates were the same as those for reporting the growth stages of the crops.

The reported weeds were the grass species *Sorghum halepense* L. (Pers.) and *Setaria viridis* (L.). On the first date of observation (22.04.2022), both weeds were in the 2^{nd} – 3^{rd} leaf stage. There were no differences between the sole and the intercrops.

On the second evaluation date (29.04.2022) both weeds were in the 3^{rd}-4^{th} leaf stage. There were no differences between the sole and the intercrops.

Table 2. Weeds' development after the rosette growth stage till full flowering of the Bulgarian camelina

Dates	22.4.2022	29.4.2022	6.5.2022	13.5.2022	20.5.2022	27.5.2022
K 1 Luna						
S. halepense	2-3 leaf	3-4 leaf	5-6 leaf	6-7 leaf	Beginning of inflorescence	Inflorescence
S. viridis	2-3 leaf	3-4 leaf	5-6 leaf	6-7 leaf	Beginning of inflorescence	Inflorescence
K 2 Lenka						
S. halepense	2-3 leaf	3-4 leaf	5-6 leaf	6-7 leaf	Beginning of inflorescence	Inflorescence
S. viridis	2-3 leaf	3-4 leaf	5-6 leaf	6-7 leaf	Beginning of inflorescence	Inflorescence
K 3						
S. halepense	2-3 leaf	3-4 leaf	5-6 leaf	6-7 leaf	Beginning of inflorescence	Inflorescence
S. viridis	2-3 leaf	3-4 leaf	5-6 leaf	6-7 leaf	Beginning of inflorescence	Inflorescence
Pea						
S. halepense	2-3 leaf	3-4 leaf	5-6 leaf	6-7 leaf	Beginning of inflorescence	Inflorescence
S. viridis	2-3 leaf	3-4 leaf	5-6 leaf	6-7 leaf	Beginning of inflorescence	Inflorescence
Vetch						
S. halepense	2-3 leaf	3-4 leaf	5-6 leaf	6-7 leaf	Beginning of inflorescence	Inflorescence
S. viridis	2-3 leaf	3-4 leaf	5-6 leaf	6-7 leaf	Beginning of inflorescence	Inflorescence
K 1 / Pea						
S. halepense	2-3 leaf	3-4 leaf	3-4 leaf	4-5 leaf	6-7 leaf	Beginning of inflorescence
S. viridis	2-3 leaf	3-4 leaf	3-4 leaf	4-5 leaf	6-7 leaf	Beginning of inflorescence
K 2 / Pea						
S. halepense	2-3 leaf	3-4 leaf	3-4 leaf	4-5 leaf	6-7 leaf	Beginning of inflorescence
S. viridis	2-3 leaf	3-4 leaf	3-4 leaf	4-5 leaf	6-7 leaf	Beginning of inflorescence
K 3 / Pea						
S. halepense	2-3 leaf	3-4 leaf	3-4 leaf	4-5 leaf	5-6 leaf	6-7 leaf
S. viridis	2-3 leaf	3-4 leaf	3-4 leaf	4-5 leaf	5-6 leaf	6-7 leaf
K 1 / Vetch						
S. halepense	2-3 leaf	3-4 leaf	3-4 leaf	4-5 leaf	6-7 leaf	Beginning of inflorescence
S. viridis	2-3 leaf	3-4 leaf	3-4 leaf	4-5 leaf	6-7 leaf	Beginning of inflorescence
K 2 / Vetch						
S. halepense	2-3 leaf	3-4 leaf	3-4 leaf	4-5 leaf	6-7 leaf	Beginning of inflorescence
S. viridis	2-3 leaf	3-4 leaf	3-4 leaf	4-5 leaf	6-7 leaf	Beginning of inflorescence
K 3/ Vetch						
S. halepense	2-3 leaf	3-4 leaf	3-4 leaf	4-5 leaf	5-6 leaf	6-7 leaf
S. viridis	2-3 leaf	3-4 leaf	3-4 leaf	4-5 leaf	5-6 leaf	6-7 leaf

There were no differences in the weeds' growth stages on the third reporting date (6.5.2022). The weeds were in the growing stage 5^{th}-6^{th} leaf for the variants with sole crops (for the three camelina genotypes) and for the legumes (pea and vetch). On this evaluation date, when the crops camelina and legumes (independently of the variety and crop), the intercropping system leads to suppression of both weeds development. In the intercropping systems of the three camelina varieties together with pea and vetch as well, the growth stage of both weeds was suppressed and they were still in the 3rd - 4th leaf stage.

On the fourth evaluation date (13.5.2022) in the plots with sole crops (camelina or legumes) the weeds were in the growing stage 6th-7th leaf, and in the mixed cropping systems the weeds developed lower leaf number and were in 4th-5th leaf stage.

On the fifth reporting date (20.5.2022) the weeds in the sole-crop camelina, pea, and vetch reached the beginning of the inflorescence stage. In the plots of the variants with the camelina varieties Luna (K 1) and Lenka (K 2) mixed with pea and vetch as well, both weed species were in the 6th-7th leaf stage. The better-developed Bulgarian camelina genotype in intercropping system with pea and vetch depressed the weeds and they were in the 5th-6th leaf stage.

On the last reporting date (27.5.2022) the weeds in the sole-crop camelina, pea, and vetch reached the inflorescence stage. In the plots of the variants with the camelina varieties Luna (K 1) and Lenka (K 2) mixed with pea and vetch as well, both weed species were in the beginning of the inflorescence stage. The better-developed Bulgarian camelina landrace in the cropping system with pea and vetch depressed the weeds and they were in the 6th-7th leaf stage.

Many studies confirm that found that the weed biomass in the intercrop was significantly lower than in the sole crop (Liebman and Dyck, 1993; Szumigalski and Van Acker, 2005; Paulsen et al., 2006; Gu et al., 2022).

On Table 3 is presented the data concerning the weeds' number and weeds' fresh biomass weight.

The highest number of the weed *S. halepense* per m^2 (21.67) was found to be for the sole crop of the camelina variety Luna (K 1). For this variant, the highest weed fresh weight was recorded - 34.04 g. In the plots of pea and vetch as sole crops the number of the weed *S. halepense* was lower (8.67 and 8.00, respectively) but the weight of the weed was greater - 30.70 g for the pea and 27.23 g for the vetch.

Table 3. Number and fresh weight of the weeds in the different cropping systems (sole and mixed)

Treatments	Number / m^2	
	S. halepense	*S. viridis*
K 1	21.67 a	8.37 bc
K 2	12.67 b	5.35 bc
K 3	9.67 bc	8.00 bc
Pea	8.67 bc	14.67 a
Vetch	8.00 bc	9.30 b
K 1 / Pea	11.00 bc	6.36 bc
K 2 / Pea	9.33 bc	8.30 bc
K 3 / Pea	5.67 bc	4.33 bc
K 1 / Vetch	8.00 bc	5.64 bc
K 2 / Vetch	7.67 bc	8.13 bc
K 3 / Vetch	9.67 bc	3.67 c
Treatments	**Fresh weight, g**	
	S. halepense	*S. viridis*
K 1	36.04 a	5.50 cd
K 2	15.70 bc	4.67 cd
K 3	15.57 bc	5.33 cd
Pea	30.70 a	13.50 ab
Vetch	27.23 ab	15.93 a
K 1 / Pea	12.27 bc	6.90 cd
K 2 / Pea	8.40 c	7.07 cd
K 3 / Pea	5.17 c	3.40 d
K 1 / Vetch	6.57 c	4.63 cd
K 2 / Vetch	6.90 c	9.23 bc
K 3 / Vetch	7.40 c	3.12 d

Means with different letters are with proven differences according to Duncan's Multiple Range test ($p<0.05$).

When the camelina varieties were grown as an intercropping system with pea and vetch the weed *S. halepense* was suppressed and formed lower fresh biomass weight.

Regarding the weed species *S. viridis* the highest number was counted in the sole crops of pea and vetch - 14.67 and 9.30, respectively. The results are with proven differences in comparison to the other treatments. The lowest number of specimens was recorded in the mixed crop system of the Bulgarian camelina variety with vetch - 3.67. The highest weed biomass was recorded for the sole crop vetch - 15.64 g. The results are with proven differences in comparison to the other treatments. The lowest *S. viridis* fresh biomass 3.40 and 3.12 g for the intercropping system of the Bulgarian camelina landrace with pea and vetch respectively were recorded.

CONCLUSIONS

The Bulgarian camelina landrace developed faster when compared to the foreign genotypes (Luna and Lenka varieties). Differences in the growing stages of winter pea did not differ between the sole or intercrop with the three camelina genotypes. Differences in the growing stages of vetch as sole or intercrop with camelina differed only on the first reporting date where the vetch was behind in the development.

In the intercropping system with the Bulgarian camelina genotype (K3) grown together with pea and vetch the development of the weeds *S. halepense* and *S. viridis* was suppressed to a greater extent than the mixed cropping system of the foreign camelina varieties Luna (K1) and Lenka (K2) grown in an intercropping system with the legume crops (pea and vetch). In the sole crops, the reported weeds developed faster. The mixed cropping system of the well-developed Bulgarian camelina landrace (K 3) grown as an intercrop with pea and vetch suppressed the development of the weed *S. halepense*, while the suppression of the weed *S. viridis* was higher.

ACKNOWLEDGEMENTS

The research was financially supported by CORE Organic ERA-NET and the Bulgarian National Science Fund (BNSF) under project KP - 06-ДО 02/4 SCOOP: Developing intercropping systems with camelina to increase the yield and quality parameters of local underutilized crops.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

CLIMATE CHANGE AND ITS IMPACT ON WATER CONSUMPTION IN THE MAIN AGRICULTURAL CROPS OF THE ROMANIAN PLAIN AND DOBROGEA

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Abstract

Drought is the limiting factor that manifests itself on the largest agricultural area.The agricultural areas most vulnerable to soil water scarcity are those of Dobrogea, the south of the Romanian plain, the south-east and east of Moldova, as well as the west of the Tisei Plain. The purpose of the research was to calculate the moisture deficit and the need for water for irrigation based on climate scenarios made with different global climate models, as well as determining the water consumption of irrigated crops according to the expected climate change in the Romanian climate regime. In order to predict the effects of climate change, it was followed in the areas served by Constanta and Tecuci weather stations the modification of the elements of the irrigation regime in case of possible increases in average temperatures by 2^0C respectively 5^0C. The management of irrigation water in a given area was established according to the estimated moisture deficit as the difference between water consumption and useful rainfall during the growing season of crops.

***Key words**: limited water supplying, watering rate, irrigation norm, water consumption.*

INTRODUCTION

The global climate is undergoing dramatic and irreversible changes. Climate change will also lead to variability in agricultural production, increasing extreme weather events such as heat waves, droughts, and heavy rainfall. Limiting dangerous climate change requires huge reductions in emissions, as well as the use of alternatives to fossil fuels world-wide (Dragotă et al., 2019). It is estimated that human activities can cause a rise of global temperature by 1ºC above pre-industrial levels, with a likely range of 0.8ºC to 1.2ºC (Huppmann et al., 2018). Global warming will likely reach 1.5 ºC between 2023 and 2052 if it continues to rise at the same intensity. Analysing the long-term warming trend of the pre-industrial period, the observed global average temperature for the decade 2006-2015 was 0.87ºC (IPCC Press Release, 2018). The negative impact on the economy, health, nature, agriculture and population varies across Europe, depending on the region and territory (European Environment Agency EEA, 2014). The agricultural sector contributes approximately 10% to the total anthropogenic greenhouse gas emissions in the EU-27 (EEA, 2010). Crop production varies each year, being heavily influenced by fluctuations in climatic conditions and by extreme meteorological events (Wheeler et al., 2013).

Climate variability influences all sectors of the economy, but agriculture remains the most vulnerable, and its impact is more acute at present, because climate change and variability is becoming more and more pronounced (Chitu et al., 2015). In Romania, the effect of climate change is felt, and these will be manifested by increasing temperatures, changing the rainfall regime, melting ice and snow, and raising sea levels.

Extreme weather phenomena leading to negative environmental impacts (floods and droughts) will become more frequent and intense in many regions (Rummukainen, 2012). Effects on ecosystems, economic sectors, population health, and vulnerability vary from region to region (Daniel et al., 2019). Due to climate change, production will be significantly reduced unless action is taken accordingly. The important role belongs to breeders to create varieties and hybrids with a low consumption of evapotranspiration (IPCC Press Release, 2018). The climatic changes have increased the growth of weeds, insect pests and diseases in many geographic areas, and increased problems for sustainable agriculture (Bojariu et al., 2015). Agronomic approaches such as adjusting sowing dates, nutrient management, water management, use of plant hormones and osmoprotectors, should be applied significantly to mitigate the negative effect of climatic parameters (Mustafa et al., 2023). The impact of climate change on yields of main crops in the world is expected to be negative (Roudier et al., 2011) while the exact impact remains highly uncertain when elevated temperatures, higher atmospheric CO_2 and changed rainfall occur simultaneously (Roudier et al., 2011). If specific action is not taken and people continue to consume current levels of fossil fuels (e.g. oil and coal), the average temperature of the Earth will rise by 2ºC to 6.4ºC by the end of the 21st century (IPCC Press Release, 2018).The average global temperature has increased by 0.74ºC over the past 100 years (1906-2005) (Korean Meteorological Agency, 2008; Rasul et al., 2012; Abbas et al., 2017; Bokhari et al., 2017).

MATERIALS AND METHODS

The objective of the research paper was to calculate the moisture deficit, the need for water for irrigation, as well as the water consumption of maize based on climate scenarios. To achieve this objectives, three possible climatic scenarios were established namely:

1. Tn - current average temperatures;
2. Tn +2ºC - increasing of current average temperatures with 2ºC;
3. Tn +5ºC - increasing of current average temperatures with 5ºC.

The ETRO value was estimated by Thornthwaite method, based on correlation between water consumption of a crop and air average temperature. Depending on the values of the average normal temperatures, the values of the average monthly temperatures were obtained in the case of increase by 2ºC or 5ºC. After obtaining these values, the annual values of the thermal index were calculated for the three climatic scenarios (Tn; Tn+2ºC and Tn+5ºC) in the two pedoclimatic areas, Dobrogea and the Romanian Plain (Table 1).

Table 1. Monthly, annual average temperature and monthly and annual thermal index values (ºC) at Tecuci and Constanta meteorological stations during 1991-2020 period

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	SUM I
Tecuci													
t_m°C	-1.8	0.3	5.2	11.4	17.2	21.1	22.9	22.4	16.9	10.9	5.2	-0.3	-
i	0.00	0.01	1.06	3.48	6.49	8.82	10.01	9.68	6.32	3.26	1.06	0.00	50.1
t_m°C +2°C	0.2	2.3	7.2	13.4	19.2	23.1	24.9	24.4	18.9	12.9	7.2	-0.1	-
i	0.01	0.31	1.74	4.45	7.67	10.15	11.37	11.02	7.49	4.20	1.74	0.0	60.1
t_m°C+5°C	1.2	5.3	10.2	16.4	22.2	26.1	27.9	27.4	21.9	15.9	10.2	0.2	
i	0.12	1.09	2.94	6.04	9.55	12.21	13.50	13.14	9.36	5.76	2.94	0.0	76.6
Constanta													
t_m°C	0.0	1.8	5.8	10.9	16.7	21.3	23.4	23.0	18.0	12.5	7.1	1.7	-
i	0.00	0.21	1.25	3.26	6.21	8.97	10.35	10.08	6.95	4.10	1.70	0.20	53.2
t_m°C +2°C	2.0	3.8	7.8	12.9	18.7	23.3	25.4	25.0	20.0	14.5	9.1	3.7	-
i	0.25	0.66	1.96	4.20	7.37	10.28	11.71	11.44	8.16	5.01	2.48	0.63	64.1
t_m°C+5°C	5.0	6.8	10.8	15.9	21.7	26.3	28.4	25.0	20.0	17.5	12.1	6.7	
i	1.00	1.59	3.21	5.76	9.23	12.35	13.87	11.44	8.16	6.66	3.81	1.56	78.6

i = monthly thermal index
I= annual thermal index

With the obtained values it was possible to calculate the Etro for the maize crop according to climatic scenarios and pedoclimatic zones. In the calculations of the water balance in the soil the values of the initial reserves, of the minimum ceilings and of the watering norms specific to the two studied areas were used. Romania being crossed by the 45th parallel it is quite possible that the current value of annual precipitation will remain the same. In the paper the soil water balance was calculate using the current rainfall values. Thus, the rainfall values used in the soil water balance calculations where the average values over of at least 29 years (1991-2020) of the studied areas with the calculation assurance of 80% (Table 2).

Table 2.Average rainfall (mm) recorded at Constanta and Tecuci meteorological stations in 1991-2020 period

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	SUM I-XII	SUM V-IX
Constanta	26.5	21.6	29.0	32.1	45.2	57.5	64.1	36.4	49.7	38.7	33.8	35.0	469.6	285
Tecuci	24.0	23.8	31.0	41.3	54.9	73.4	58.5	50.6	50.3	51.2	35.1	36.0	530.1	329

After calculating the soil water balance for the maize crop in the two pedoclimatic areas and for the three scenarios, a 2 x 3 bifactorial model was obtained. The need to apply irrigation for a particular area is determined according to the moisture deficit calculated as the difference between water consumption and water supply sources of the soil and maize.

RESULTS AND DISCUSSIONS

The average daily evapotranspiration will increase in the two pedoclimatic areas studied by 2 m^3/ha/day in the case of 2ºC and 5 m^3/ha / day in the case of heating with 5ºC increase, which requires finding technical and agrophytotechnical methods to create genotypes with a reduced sweating coefficient and which react positively in the case of moisture stress (Table 3).

The total optimal real evapotranspiration will increase by values that will exceed 1,246 m^3/ha in the case of 5ºC heating of the average temperature, which means an important volume of irrigation water that must ensure the normal growth and development of plants (Table 4).

Table 3. Synthesis of data on the influence of the increase in average temperatures in the maize crop on the average daily evapotranspiration between 1991-2020 period

Variant		Daily ETRO		Diferences and Significance	
		m^3/ha	%	m^3/ha	Signif
Constanta	t_n	32	100	Control	
	t_n + 2ºC	34	106.25	2	***
	t_n + 5ºC	37	115.62	5	***
Tecuci	t_n	33	100	Control	
	t_n + 2ºC	35	106.06	2	***
	t_n + 5ºC	38	115.15	5	***

LSD 5%=0.75 LSD 1%= 1.03 LSD 0.1 %=1.35

Table 4. Synthesis of data on the influence of the increase of average temperatures in the maize crop on the total average evaporation between 1991-2020 period

Variant		Total ETRO		Differences and Significance	
		m^3/ha	%	m^3/ha	Signif
Constanta	t_n	5,225	100	Control	
	t_n + 2ºC	5,522	106.26	297	***
	t_n + 5ºC	5,963	114.12	738	***
Tecuci	t_n	5,370	100	Control	
	t_n + 2ºC	6,109	113.76	739	***
	t_n + 5ºC	6,616	123.20	1,246	***

LSD 5%=167.53 LSD 1%= 222.35 LSD 0.1 %=291.85

Table 5. Synthesis of data on the influence of the increase in average temperatures on the average water deficit in the maize crop between 1991-2020 period

Variant		Hydric deficit		Differences and Significance	
		m^3/ha	%	m^3/ha	Signif
Constanta	t_n	3,240	100	Control	
	t_n + 2ºC	3,526	108.64	286	***
	t_n + 5ºC	3,957	122.13	717	***
Tecuci	t_n	3,865	100	Control	
	t_n + 2ºC	4,341	112.32	476	***
	t_n + 5ºC	4,841	125.25	976	***

LSD 5%=222.43 LSD 1%= 310.28 LSD 0.1 %=421.65

With the increase of the average temperatures will increase and the values of the moisture deficit and the average water requirement for irrigation At Constanta in case of increase of average temperatures by 5ºC deficit will be 3,957 m^3/ha and at Tecuci with 4,841 m^3/ha.

The average irrigation water requirement will be between 1,239 m^3/ha and about 2,817m^3/ha at an increased temperature higher by 5ºC. This need for irrigation water will reduce the irrigated areas if the volume of irrigation water used today is maintained or the current irrigated areas are maintained but with the use of additional volumes of irrigation water. The increase in the need for irrigation water will increase the expenditure on irrigation water and the need for equipment and manpower (Table 5 and Table 6).

Table 6. Synthesis of data on the influence of the increase of average temperatures in the maize crop on the average water requirement between 1991-2020 period

Variant		Water need		Differences and Significance	
		m^3/ha	%	m^3/ha	Signif
Constanta	t_n	742	100	Control	
	$t_n + 2°C$	1164	156.87	422	**
	$t_n + 5°C$	1239	166.98	497	**
Tecuci	t_n	1300	100	Control	
	$t_n + 2°C$	1732	13.23	432	**
	$t_n + 5°C$	2817	216.69	1517	***

LSD 5%=233.72 LSD 1%= 419.48 LSD 0.1 %=654.94

Table 7. Synthesis of data on the influence of average maize temperature increase on the number of waterings between 1991-2020

Variant		Number of watering		Differences and Significance	
		m^3/ha	%	m^3/ha	Signif
Constanta	t_n	3	100	Control	
	$t_n + 2$ °C	5	166.67	2	***
	$t_n + 5$ °C	6	200.00	3	***
Tecuci	t_n	7	100	Control	
	$t_n + 2$ °C	8	114.30	1	***
	$t_n + 5$ °C	9	128.57	2	***

LSD 5%=0.25 LSD 1%= 0.36 LSD 0.1 %=0.49

Table 8. Synthesis of data on the influence of average maize temperature increase on average irrigation rate between 1991-2020

Variant		Average irrigation rate		Differences and Significance	
		m^3/ha	%	m^3/ha	Signif
Constanta	t_n	2835	100	Control	
	$t_n + 2°C$	4725	166.67	1890	***
	$t_n + 5°C$	5670	200.00	2835	***
Tecuci	t_n	3934	100	Control	
	$t_n + 2°C$	4496	114.30	562	***
	$t_n + 5°C$	5058	128.57	1124	***

LSD 5%=202.3 LSD 1%= 286.70 LSD 0.1 %=393.69

In the two studied locations will increase the number of waterings depending on the average temperature that will be achieved, namely by 2 in the case of heating with 2ºC of the normal average temperature and by 3 waterings in the case of heating with 5ºC of the normal average temperature.

The irrigation norm will exceed the current norm by about 1,800 m^3/ha in Constanta and by 562 m^3/ha in Tecuci in the case of heating with 2ºC of the normal average temperature and in the case of increasing the average temperature by up to 5ºC the irrigation norm depending on the pedoclimatic area will increase by 1,124-2,835 m^3/ha (Table 7 and Table 8).

CONCLUSIONS

Due to these climatic changes, the aridification process will intensify, the periods of droughts being more numerous and of longer duration. The association between atmospheric and pedological drought will have particularly damaging effects for agricultural crops, diminishing or totally compromising the crops. By 2029, the average temperatures in our country are expected to increase by 1.5ºC, and by 2099 by 2-5ºC, depending on the climate scenario (IPCC, 2013).

The average daily evapotranspiration will increase in both areas by about 6% if the temperature rises by 2ºC and by about 15 % at a temperature above 5ºC. The average water deficit is possible to increase by more than 15% at tn + 2ºC and by 19% at tn+5ºC.

As regards the average water requirement for irrigation, it will increase compared to the current values with values of approximately 61% at tn + 2ºC and 74% at tn + 5ºC. The studied area presents the most favourable pedological conditions for the growth and development of the maize crop, but in the case of increasing the average temperature it is necessary to find technical and agrophytotechnical methods for reducing the water consumption an important role in this regard will be played by the breeders who have to create genotypes with a reduced sweating coefficient, and which react positively in the case of.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON VEGETATION INDICATORS IN *Primula officinalis* Hill. SPECIES USING FIELDSCOUT CM 1000 NDVI METER

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Abstract

Studies were carried out during the growing season of Primula officinalis Hill., to obtain information on the health status of the plants and the influence of the measurement date on NDVI values. The FIELDSCOUT® CM1000 NDVI Meter measures the NDVI differentially in the presence of light at 660 nm and 840 nm wavelengths. Three determinations in dynamics were made. Three plants were analysed for each variant/repeat/row, with each plant having three readings on three different leaves, resulting in a total of 729 readings and 243 plants analysed in each determination. The study of the influence of the measurement date on the NDVI values in leaves of Primula officinalis Hill. in 2018 shows a slight increase in the values measured on 03.05.2018 by 0.08 units, which is distinctly significant compared to the first measurement, considered as a control. The results on the interaction of the two factors on NDVI values in 2019 showed that on 20.05.2019 all planting variants had distinctly significant and highly significant values relative to the control.

Key words*: measurements, NDVI, Primula officinalis Hill., vegetation index.*

INTRODUCTION

Medicinal and aromatic herbs are an essential part of our lives, from birth till oldness, they accompany us both in joy and sorrow. Almost each of us uses medicinal herbs in the form of teas when we relax or struggle with health issues. Just like the human beings, plants go through different stages of evolution; from emergence, maturity, to senescence.

Therefore, it’s highly recommended to use the applied technologies in the organic farming system, the use of free hormones inputs, GMOs, pesticides, antibiotics and other synthetic substances and all of these allow the preservation of the bio-availability of the active compounds during processing and the elimination of toxicological risks in final food products (Săvescu & Popescu 2022).

Calculation of the Normalized Difference Vegetation Index (NDVI) is often used around the world to monitor drought, forecast agricultural production, assist in forecasting fire zones, and desert offensive maps. Farming apps, like Crop Monitoring, integrate NDVI to facilitate crop scouting and irrigation, among other field treatment activities, at specific growth stages (https://eos.com/make-an-analysis/ndvi/).

NDVI is calculated in accordance with the formula:

$$NDVI=\frac{NIR-RED}{NIR+RED}$$

NIR - reflection in the near-infrared spectrum;
RED - reflection in the red range of the spectrum.

According to this formula, the density of vegetation (NDVI) at a certain point of the image is equal to the difference in the intensities of reflected light in the red and infrared range divided by the sum of these intensities (https://eos.com/make-an-analysis/ndvi/).

Reflectance is the ratio of energy that is reflected from an object to the energy incident on the object. The spectral reflectance of a crop differs considerably in the near-infrared region (λ = 700-1300 nm) and in the visible red range (λ = 550-700 nm) of the electromagnetic spectrum (Kumar & Silva, 1973).

Chlorophyll is a plant's health indicator, strongly absorbs visible light, and the cellular structure of the leaves strongly reflects near-infrared light. When the plant becomes dehydrated, sick, afflicted with disease, etc., the spongy layer deteriorates, and the plant absorbs more of the near-infrared light, rather than reflecting it. Thus, observing how NIR changes compared to red light provides an accurate indication of the presence of chlorophyll, which correlates with plant health (https://eos.com/make-an-analysis/ndvi/).

Researches' results concerning the dynamics of the normalized difference vegetation index (NDVI) showed vast opportunities to use these non-invasive measures to monitor the health status of different crops.

Piekkielek & Fox, 1992; Chapman & Barreto, 1997; Evans, 1989, discovered that the NDVI is successful in predicting photosynthetic activity, because this vegetation index includes both near-infrared and red light. Plant photo-synthetic activity is determined by chlorophyll content and activity. The relationship between leaf N and leaf chlorophyll has been demonstrated for maize.

Verhulst, & Govaerts, 2010 consider that the underlying factor for variability in a typical vegetation index cannot be blindly linked to a management input without some knowledge of the primary factor that limits growth. For example, in a field where N is the limiting factor to growth, the NDVI may show a strong correlation with the N availability in the soil; however, in another field, where water is the limiting factor, the NDVI may be just as strongly correlated with plant-available soil moisture (https://earthobservatory.nasa.gov/features).

Field experiments were conducted in many countries, such as those from Southern Greece to assess the Normalized Difference Vegetation Index (NDVI) in estimating Camelina's crop growth and yield parameters under different tillage systems (conventional and minimum tillage) and organic fertilization types (compost, vermicompost and untreated control) Angelopoulou et al., 2020.

Zhang, et al., 2014 showed that crop condition assessment in the early growing stage is essential for crop monitoring and crop yield prediction. A normalized difference vegetation index (NDVI)-based method is employed to evaluate crop condition by inter-annual comparisons of both spatial variabilities (using NDVI images) and seasonal dynamics (based on crop condition profiles).

The past two decades have seen an increasing demand for timely, transparent, and accurate information on global agricultural monitoring for enhancing food security at global, national, and sub-national scales. Information about the condition of crops in early crop-growing stages (before harvest) can help indicate potential food surpluses and shortages and support related decision-making (Meng, 2008).

The image classification method, which first made a supervised or unsupervised classification on the remote sensing data, then labels each category as a certain increase level with the observed data on the state of growth of the seedling that has spatial and temporal attributes (Liu, 1999).

MATERIALS AND METHODS

The research was carried out at The National Institute of Research and Development for Potato and Sugar Beet Brașov, Technology and Good Practices in Agriculture Department, Laboratory of Medicinal and Aromatic Plants.

The research was carried out for gathering information about the plant's health status through a non-invasive evaluation, in unirrigated field conditions, following at the same time the measurement date's influence, on the NDVI values.

The study took place during the years of 2018-2019. Analyses were made, using the FIELDSCOUT® CM1000 NDVI Chlorophyll Meter. Three determinations in dynamics were made, measurements that indicate the difference in vegetation index in the presence of light, for estimating a plant's health status. At each variant/repetition/row, three plants were examined, and at each plant three readings were on three different leaves, resulting in a total number of 729 readings and 243 analysed plants on each determination.

The biological material, on which the researches were made, was brought in the spring of 2016 from the spontaneous flora of Brașov County, this study focused on aspects of biology and technology regarding the introduction into the culture of the species *Primula officinalis* Hill.

The experiment was bifactorial, set according to the model of randomized blocks, in three repetitions, being established by seedling in the fall of 2016, with the aim of determining the optimal nutrition space for the *Primula officinalis* Hill. species.

Factor A - the distance between rows with the following graduations: 25 cm, 50 cm, 75 cm;

Factor B - the distance between plants per row, with the following graduations: 10 cm, 25 cm, 50 cm;

Interaction with 10/25 density is considered the variant control of this research (Nițu (Năstase) et al., 2021).

Normalized difference vegetation index measurements using the NDVI Meter.

The FIELDSCOUT® CM1000 NDVI (Normalized Difference Vegetation Index) Meter (Figure 1) senses light at wavelengths of 660 nm and 840 nm to estimate plant health. The ambient and reflected light at each wavelength is measured. Chlorophyll absorbs 660 nm light and, as a result, the reflection of that wavelength from the leaf is reduced compared to the reflected 840 nm light. Light having a wavelength of 840 nm is unaffected by leaf chlorophyll content and serves as an indication of how much light is reflected due to leaf physical characteristics such as the presence of a waxy or hairy leaf surface.

Figure 1. NDVI measuring device
(source: Original photo, Sorina Nițu)

RESULTS AND DISCUSSIONS

From the influence of measurement date upon NDVI values of the *Primula officinalis* Hill. leaves, there can be observed a slight increase in the values measured on 03.05.2018 by 0.08 units, this being distinctly significant compared to the first measurement, considered as a control; measurements made on 11.05.2018 indicate an average value of 0.75 units, insignificant compared to the control variant. Measurements taken, indicate a good health status of the *Primula officinalis* Hill. plants (Table 1).

Table 1. The influence of the measurement date on the NDVI values in *Primula officinalis* Hill. leaves 2018

No.	Date of measurement	Average NDVI	Relative value (%)	Difference	Sig.
1	25.04.2018	0.72	100.0	0.00	Mt
2	03.05.2018	0.79	110.5	0.07	**
3	11.05.2018	0.75	104.2	0.03	-
DL (p 5%)				0.04	
DL (p 1%)				0.06	
DL (p 0.1%)				0.12	

The vegetation status of *Primula officinalis* plants, indicated by the NDVI measurements, was different during 2019, with deviations from the first measurement date, considered control, being distinctly significant and very significant (Table 2).

Table 2. The influence of the measurement date on the NDVI values in *Primula* officinalis Hill. leaves 2019

No.	Date of measurement	Average NDVI	Relative Value (%)	Difference	Sig.
1	25.04.2019	0.77	100.0	0.00	Mt.
2	13.05.2019	0.80	104.5	0.03	**
3	20.05.2019	0.84	108.5	0.07	***
DL (p 5%)				0.02	
DL (p 1%)				0.03	
DL (p 0.1%)				0.06	

The influence of planting distance on NDVI values in *Primula officinalis* Hill. leaves increases at the variants with a 50 cm distance between rows, with more significant values than the control variants, and those planted at a 75 cm distance between rows had the same values as the control, planted at a 25 cm distance between rows (Table 3).

Table 3. The influence of planting distance on NDVI values in *Primula officinalis* Hill leaves 2018

No.	Distance between rows	Average NDVI	Relative value (%)	Difference	Sig.
1	25	0.72	100.0	0.00	Mt
2	50	0.81	112.3	0.09	***
3	75	0.72	100.0	0.00	Mt
DL (p 5%)				0,02	
DL (p 1%)				0,02	
DL (p 0.1%)				0,03	

NDVI values, registered in the year of 2019 were insignificant at the 50 cm distance between rows and distinctly negatively significant at the 75 distance between rows (Table 4).

Table 4. The influence of planting distance on NDVI values in *Primula officinalis* Hill leaves 2019

No.	Distance between rows	Average NDVI	Relative Value (%)	Difference	Sig.
1	25	0.82	100.0	0.00	Mt.
2	50	0.80	98.5	-0.01	-
3	75	0.79	96.6	-0.03	00
DL (p 5%)				0.02	
DL (p 1%)				0.02	
DL (p 0.1%)				0.03	

The interactions between planting distance and the date of NDVI measurements (Table 5) have shown that on 25.04.2018, planted variants at 50 cm between rows had the highest values, with significant differences.

The variants planted at 75 cm between rows, at this date, have very significant negative values compared to the control. On 03.05.2018, the NDVI values of the variants planted at 50 cm between rows increase very significantly, the other variants having values close to the control.

Table 5. Interaction between planting distance and NDVI measurement data on *Primula officinalis* Hill. species 2018

No	Planting distance (cm/cm)	Date of measure ment 2018	Average values NDVI	Relative value (%)	Diff.	Sig.
1	25	25.04.	0.73	100.0	0.00	Mt
2	50		0.77	105.5	0.04	*
3	75		0.66	90.4	-0.07	000
4	25	03.05.	0.77	100.0	0.00	Mt
5	50		0.84	109.6	0.07	***
6	75		0.78	101.7	0.01	-
7	25	11.05.	0.68	100.0	0.00	Mt
8	50		0.83	122.7	0.15	***
9	75		0.74	108.4	0.06	**
DL (p 5%)				0.03		
DL (p 1%)				0.04		
DL (p 0.1%)				0.06		

When performing the last analysis, the variants planted at 50 cm register very positive values, and those planted at 75 cm have distinctly significant values compared to the control.

In 2019, the NDVI values analyzed at the experience regarding the interaction between the planting distance and the measurement date were insignificant, close to the control variants (Table 6).

Table 6. Interaction between planting distance and NDVI measurement data on *Primula officinalis* Hill. species 2019

No.	Planting distance (cm/cm)	Date of measurement 2019	Average values NDVI	Relative value (%)	Diff.	Sig
1	25	25.04.	0.79	100.0	0.00	Mt
2	50		0.77	97.5	-0.02	-
3	75		0.75	94.9	-0.04	00
4	25	13.05.	0.81	100.0	0.00	Mt
5	50		0.81	100.4	0.00	-
6	75		0.79	97.5	-0.02	-
7	25	20.05.	0.85	100.0	0.00	Mt
8	50		0.83	97.6	-0.02	-
9	75		0.83	97.3	-0.02	-
DL (p 5%)					0.03	
DL (p 1%)					0.04	
DL (p 0.1%)					0.06	

The interaction between the NDVI measurement date and the planting distance shows distinctly significant differences in the case of variants planted at 75 cm between rows and significant in the case of those planted at 50 cm between rows, in both analyzed data. The variants with graduations of 25 cm between rows present insignificant values on 03.05.2018, with a slightly significant decrease on 11.05.2018 (Table 7).

Table 7. Comparisons of the interaction between the NDVI measurement date and the planting distance at *Primula officinalis* Hill. Species 2018

No	Date of measurement	Distance between rows	Average values NDVI	Relative value (%)	Diff	Sig
1	25.04.2018	25cm	0,73	100,0	0,00	Mt
2	03.05.2018		0,77	105,0	0,04	-
3	11.05.2018		0,68	93,1	-0,05	0
4	25.04.2018	50cm	0,77	100,0	0,00	Mt
5	03.05.2018		0,84	109,1	0,07	*
6	11.05.2018		0,83	108,3	0,06	*
7	25.04.2018	75cm	0,66	100,0	0,00	Mt
8	03.05.2018		0,78	118,3	0,12	**
9	11.05.2018		0,74	111,7	0,08	**
DL (p 5%)				0,05		
DL (p 1%)				0,07		
DL (p 0.1%)				0,12		

The normalized difference vegetation index in the presence of light estimates a very good state of plant health at a nutrition space of 50 cm between rows in very favorable years for the growing season, such as 2018. In less favorable years (2019), NDVI differences become insignificant between cultivation methods in the first phases of vegetation and with distinct and very significant values in full plant development (20.05).

The analysis of the results regarding the interaction of the two factors on the NDVI values in 2019 showed that on 20.05.2019, all three planting distances had distinctly significant and very significant values compared to the control (Table 8).

Table 8. Comparisons of the interaction between the NDVI measurement date and the planting distance at *Primula officinalis* Hill. Species 2019

No.	Date of measurement	Distance between rows	Average values NDVI	Relative value (%)	Diff.	Sig.
1	25.04.2019	25cm	0.79	100.0	0.00	Mt
2	13.05.2019		0.81	102.5	0.02	-
3	20.05.2019		0.85	107.6	0.06	**
4	25.04.2019	50cm	0.77	100.0	0.00	Mt
5	13.05.2019		0.81	105.6	0.04	*
6	20.05.2019		0.83	107.8	0.06	**
7	25.04.2019	75cm	0.75	100.0	0.00	Mt
8	13.05.2019		0.79	105.3	0.04	*
9	20.05.2019		0.83	110.2	0.08	***
DL (p 5%)			0.03			
DL (p 1%)			0.05			
DL (p 0.1%)			0.07			

CONCLUSIONS

The influence of the measurement date upon NDVI values on the leaves of *Primula officinalis* Hill., notices, in 2018, a slight increase of the values measured on 03.05.2018 by 0.08 units, this being distinctly significant compared to the first measurement considered control; the measurements made on 11.05.2018 indicate an average value of 0.75 units, this being insignificant compared to the control.

The health status of the *Primula officinalis* Hill. plants, indicated by the NDVI measurements, were different during 2019, the deviations from the first measurement date, considered as a control, being distinctly significant and very significant. The measured NDVI values indicate good plant's health.

The influence of the planting distance upon NDVI values at *Primula officinalis* Hill. values, increases at the variants with 50 cm between rows, with very significant values compared to the control variants, and the 75 cm between rows variants had values close to those of the control variants, planted at a distance of 25 cm between rows.

NDVI (Normalized Difference Vegetation Index) analysis at the *Primula officinalis* Hill. species, is the first of this kind, made in Romania with this device on medicinal plants, opening, in this way, new research boundaries in the field of medicinal plants, which can determine the plant's health status, from emergence till senescence.

The use of normalized difference vegetation index can also help to establish the harvest season, correlated with an optimal accumulation of active substances in plants.

ACKNOWLEDGEMENTS

The study was conducted as part of research PhD programme of the first author. The first author is thankful to NIRDPSB Brașov for support during PhD programme.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

NEW BREEDING METHODS IN SUNFLOWER HYBRID RESEARCH AND DEVELOPMENT

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Abstract

Sunflower cultivation is particularly important for vegetable oil production worldwide. Therefore, breeding programs have intensified research and development to increase yields by producing F1 hybrid seeds from crosses of inbred lines through the introduction of cytoplasmic androsterility. For the experiment, we used sterile sunflower inbred lines and restorer inbred lines to isolate the restorer gene in a segregation ratio of 1:2:1 in self-fertilization and 1:1 in backcrossing with PET1-type cytoplasm. The results of our research offer breeders the possibility of achieving greater dynamics in the development of inbred lines, the introgression of genetic resistances to sunflower diseases and the stimulation of a greater number of genotypes to increase the combined capacity of sunflower inbred lines. This was achieved by reducing the breeding program from three inbred lines to two inbred lines using sterility genes and heterozygous fertility genes. The use of segregating restoration genes on sterile cytoplasm enables the breeder to form valuable genotypes, adapt quickly to new herbicide technologies and efficiently introduce resistance genes to new disease nomenclatures.

Key words: *sunflower, breeding, hybrid, inbred lines, cytoplasmic androsterility, restoring genes.*

INTRODUCTION

Since it s introduction in the 17th century (Park & Burke 2020), the sunflower oil industry has produced up to 20 million tonnes of seeds per year worldwide (Havrysh et al., 2023), with breeders playing a key role in developing several strategies and technologies that increase production efficiency and seed oil quality (Lukomets, 2021; Rani, 2017; Ahmad et al., 2005). The first breakthrough in sunflower breeding came when the first high oleic genotypes were developed by V.S. Pustovoyt using mutagenesis techniques. The newly developed germplasm led to a significant increase in oil extraction from sunflower seed production and the development of several varieties (Gavrilova and Anisimova, 2017). The mutagenesis technique has also been used to increase oil quality (Radanović et al., 2018).

Varietal use then changed with the introduction of the first sunflower hybrids Romsun 52 and 53, which were released in 1971 and had a significant increase in seed and oil yield. The first hybrids in the world were produced in Romania at the Fundulea Research Institute (Bran, 2018). The first hybrids developed based on nuclear male sterility (Makarenko et al., 2019) were later replaced with cytoplasmic sterility to increase the efficiency of F1 seed production (Ahmad et al., 2005; Hladni et al., 2007). Breeding programs started to focus more on hybrid production since new sources of cytoplasmic sterility (CMS) were obtained from Helianthus petiolaris (PET1) by Leclercq P. 1969 (Goryunov et al., 2019). CMS genotypes are the result of mutagenesis developed in mitochondria (Makarenko et al., 2019), fertility that can be restored by Rf genes (Horn et al., 2003; Horn et al., 2019; Kusterer et al., 2005). Accordingly, inbred lines of the CMS-Rf system have started to be implemented in research enterprises due to the heterosis effect (Li et al., 2022; Saif et al., 2023) achieved by isolating the A+B (CMS-mentor) line development from the Rf line development program to increase the expression of the heterosis effect to its maximum biological potential of inbred line crosses (Farrokhi et al., 2011; Habib et al., 2011). For oil breeding programs, dominant Rf1 genes are used to restore CMS (PET1), which is present on linkage group 13 (Gholinezhad et al., 2014), and for

confectionary sunflower programs, Rf3 genes, both of which are capable of restoring PET1 cytoplasm fertility.
The presented selection method aims to transform the N-rf1rf1 B-line into S-Rf1rf1 with sterile cytoplasm due to Rf1 genes in heterozygous state, which is required for maintenance (self-pollination or sibling pollination). Sterile plants derived from the S-Rf1rf1 B-line (self-pollination or pollination by siblings) are used in hybrid development, in the development of new populations to increase combining ability or to integrate new technologies and new sources of disease resistance through recurrent selection. In this way, the breeding programme gains genetic flexibility, which makes it possible to maintain and preserve the inbred line efficiently, so that the breeder can choose, depending on the stage of the breeding programme, how to approach the development of high-performance hybrids in a cost-effective way.

MATERIALS AND METHODS

The experiment was carried out using the AG1001B (N-rf1rf1) line B, the AG1001A (S-rf1rf1 or CMS) line A+B and the AG121R (S-Rf1Rf1) line Rf, developed in the Timisoara research, as sources for the experiment (Figure 1). The resulting sterile genotypes (CMS) were used as non-recurrent parents and the Rf line as a recurrent parent to develop new B lines (S-Rf1rf1).
Conversion of the B line N-rf1rf1 to S-Rf1rf1 was carried out by successive backcrosses (BCn) using the same initial CMS as in the non-recurrent case and resulted in progeny until complete conversion, as shown in Figure 1. Given that the initial donor is an Rf line, we recommend that the BC process be carried out from F1BC1 (75%) to F1BC10 (99.9%), as incomplete conversion of the Rf line could degenerate the B-line background of the new population. On the other hand, if the donor is a B line S-Rf1rf1 or Rf1Rf1 for another conversion, the genetic dynamics of the conversion will leave valuable progeny along the BC process that can be used in the breeding program. The new B line on sterile cytoplasm with heterozygous Rf1 genes will change the structure of the sunflower breeding program in terms of inbred line maintenance, population development and combining ability testing. However, in the seed production process, several options can be used in which the reconversion of line B on sterile cytoplasm with heterozygous Rf1 to normal cytoplasm without Rf1 can be carried out using the method proposed by Carvalho and Toledo. (Carvalho and Toledo, 2008).

The percentage of sterile and fertile plants was recorded to track the segregation ratio of the Rf1 gene during backcrossing, self-pollination, and sibling pollination.

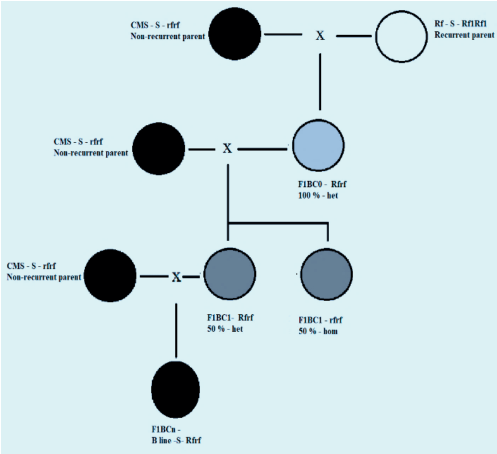

Figure 1. Conversion model of normal B-line with normal cytoplasm into B-line with sterile cytoplasm and heterozygous fertility restoring gene (Rf1)

Segregation ratio analysis was performed using the $\chi2$ test, in which the segregation ratio of fertile and sterile plants in the generations analyzed was compared to theoretical Rf1 gene segregation values of 3F:1S for self-pollinated progeny and 1F:1S for backcrossed (BC) genotypes (Sujatha & Shankar, 2011).

RESULTS AND DISCUSSIONS

According to our results in the F1 generation, depending on homozygous or heterozygous Rf1 sources, we can obtain by crossing CMS (PET1) a fully fertile offspring in the homozygous case and 50% fertile and 50%. sterile in the case where we use heterozygous Rf1 donor sources as the recurrent parent.
The F_2 ($\chi2$=0.27) results indicate a single dominant gene for Rf1 with segregation ratio

(1: 2: 1, Rf1Rf1-fertile plants, Rf1rf1-fertile plants, rf1rf1-sterile plants). Similar results for the Rf1 segregation ratio on CMS sources (PET1) have been observed in other research papers (Horn et al., 2003).

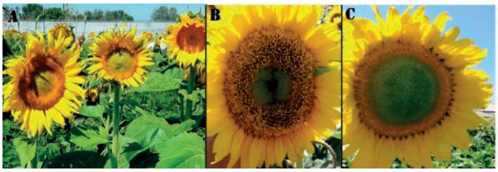

Figure 2. B-line with sterile cytoplasm and heterozygous fertility restoring gene (Rf1). (A)- Sterile and fertile plants on the new B-line developed F_1BC_n, (B)-Fertile plants, (C)-sterile plants

Our results indicate a 1: 1 ratio in terms of fertile and sterile plants in the BC. In the F_2BC_1 generation a ratio of fertile to sterile plants of 1: 1 was obtained (χ^2=2.50). The same ratio can be observed for the F_2BC_2 generation (χ^2=0.40).
The results are in agreement with those of other authors (Zhang and Stewart, 2001; Yue et al., 2010) who report the same segregation ratios for the Rf_1 gene in both BC and self-pollinated. Therefore, the concept of B-S -Rf1rf1 inbred line development formation is valid (Table 1).

Table 1. Segregation ratio of fertile and sterile plants in the B-S line Rf1rf1

Generation	Number o plants	B-line S-Rf1rf1		Theoretical value		χ^2
		Fertil	Steril	Fertil	Steril	
F2	80	62	18	60	20	0.27
F2BC1 (SIB1)	40	25	15	20	20	2.50
F2BC2 (SIB2)	40	18	22	20	20	0.40
F3BC2(SELF1)	40	26	14	30	10	2.13
Test.cross(F1)	80	80	0	80	0	0

Also, in the F_3BC_2 we observed a segregation ratio of 3: 1 (χ^2=2.13) meaning that the Rf1 genes keeps the segregation ratio in normal standards when using inbred lines of B-line S-Rf1rf type (Table 1). When converting the normal B line N-rf1rf1 to S-Rf1rf1, all progenies from BC_1 to BC_2 generation crosses gave 50% fertile and 50% sterile plants (Figure 2).
Successive backcrossing with the CMS version of line B was carried out using the fertile plants from the resulting progeny. It is very important to know that if we want to achieve a complete conversion of the B line, we need to ensure that there are enough CMS seeds that can
stand in for the non-recurrent parent until the new S-Rf1rf1 B line is fully developed. A complete transformation of the line will be carried out in parallel with a fertility testing procedure that can be controlled by crossing with CMS tests or using molecular markers.
Maintaining of the B-line S-Rf1rf1 is mandatory on the heterozygous allelic state of the Rf1 genes, otherwise the homozygous state of the Rf1 genes will not allow their maintenance and it will be necessary to reintroduce the CMS form again of the normal B line to resume the heterozygous allelic state. The method of maintaining new inbred lines (Figure 3) can alternate either through self-pollination and sib-pollination, it can be only sib-pollination, or it can be only through self-pollination. Thus, the breeder can use a method depending on the goal pursued.

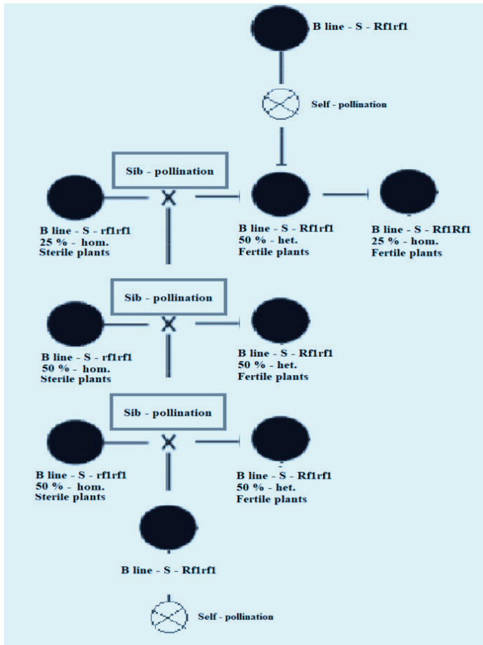

Figure 3. Maintenance procedures of the new B-line with sterile cytoplasm and heterozygous fertility restoring gene (Rf1rf1)

For testing the combining ability, sterile forms resulted plants during maintenance will always be used as non-recurrent forms together with an Rf-tester inbred line. The sib-pollination maintenance form will only continue if the test hybrid genotype is good enough to be maintain until the final evaluation of the combining ability of the new inbred line B-S-Rf1rf1. Inbred lines intended for registration can be propose for registration under B - S-Rf1rf1 form, or they will be re-converted into traditional inbred lines of type A+B. (CMS-maintainer B-N- rf1rf1) (Figure 4).

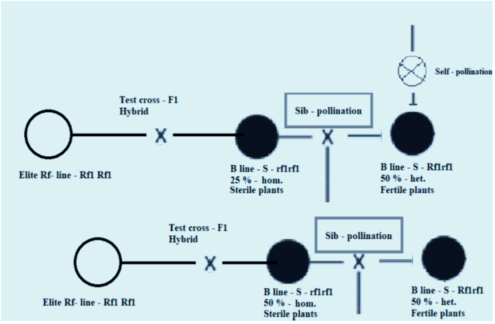

Figure 4. Testing the combining ability of the new B-line with sterile cytoplasm and heterozygous fertility restoring gene (Rf1rf1)

CONCLUSIONS

The Rf1 gene confirmed as single dominant gene being able to restore sterile cytoplasm PET1 according to our results in a segregation ratio for self-pollination of 1: Rf1Rf1 - fertile plants, 2: Rf1rf1 fertile plants and 1: rf1rf1-sterile plants in all our breeding procedures of conversion of the B line N-rf1rf1 into S-Rf1rf1. Also, sib-pollination register progenesis in rapport of 1: Rf1rf1 fertile plants (50%) to 1: rf1rf1- sterile plants (50%) when crossing sunflower genotypes with cytoplasm PET1 concluding the essential need of a single dominant gene for the conversion of B line N-rf1rf1 into S-Rf1rf1. Developing B-lines with S- Rf1rf1. can increase the dynamics of the sunflower breeding program reducing the workforce by reducing the breeding program from 3 inbred lines to 2 inbred lines, eliminating the emasculation process for developing new populations and conversions processes and testing in preliminary stages the combining ability of the new B-lines without CMS conversion. Other advantages consist is in avoiding unwanted effects that appear on CMS forms by protecting and maintaining a single Rf1 restorer gene to its exact segregation ratio and avoiding the formation of recessive restorer genes.

The method of maintaining new B-inbred lines S-Rf1rf1 can alternate either through self-pollination and sib-pollination, it can be only sib-pollination, or it can be only through self-pollination. Thus, the sunflower breeder can use these genetic dynamics of the method of maintenance depending on the goals of the research program.

ACKNOWLEDGEMENTS

This study was carried out with the support of the "Program for increasing performance and innovation in excellence doctoral and postdoctoral research" project - "PROINVENT" within the IRVA doctoral school in Timisoara.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

PRODUCTION OF STRAW CEREALS UNDER THE INFLUENCE OF SOIL TILLAGE AND CLIMATE CONDITIONS, FROM SOUTH-EAST ROMANIA

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Abstract

Supplying the necessary food to the global population is threatened by how pollution affects food quality. Pollution manifests itself through the defective management of natural resources and the instability of environmental factors. These variations in temperatures and rainfall become problematic with each passing year. The worrisome predictions of climate change and particularly its repercussions on agriculture and the survival of mankind challenge researchers to constantly look for solutions regarding crop technology and the type of cultivated plants so that the impact on the environment is minimum while obtaining rich and qualitative harvests. This paper aims to observe and analyze the adaptability of straw cereal species to the types of tillage that characterize the dry-farming work system under different conditions of abiotic stress recorded between 2019-2022. The triticale crop showed positive yield increases compared to the control tillage in all specific conservation agriculture tillages during the three-year study, while the rye crop showed instability regarding yields.

***Key words**: staw cereals, adaptability, conservative agriculture, yields.*

INTRODUCTION

Adapting soil tillage and crop plants to areas where classic, intensive agriculture caused over the years, the decrease of soil fertility and its degradation is of enormous agronomic and economic importance. Cereals represent the basis of the agri-food industry that supplies food to the global population. Therefore it is vital to obtain prosperous and valuable production, especially since these plants are subject to environmental pollution and its consequences.

Fluctuations in environmental factors caused by pollution, especially the lack of precipitation (Ghatak et al., 2017), influence the achievement of production to cover food needs globally (Lamaoiu et al., 2018). As a result, plants need to be tolerant to water deficit, strong winds, extreme temperature variations in a short time, salinity, and soil infertility (Halford et al., 2015). The effects of various conservation tillages on the utilization of limited soil nutrients under climate stress have become the main focus of researchers and primary concerns of farmers globally. Long-term studies on the productivity of crops established on land processed by the methods that are the basis of conservative agriculture take place at NARDI Fundulea. The principles underlying conservative agriculture rely on the importance of protecting the soil against erosion and subsidence (Chamen et al., 1992), conserving water inside the soil, and reducing costs with technology (Van den Putte et al., 2010; Bacenetti et al., 2015; Zentner et al., 2002). The response of plants to the instability of abiotic factors manifests itself through physical, morphological, and metabolic changes to adapt themselves and tolerate the damage caused by stress (Havrlentova et al., 2021; Basu et al., 2016). We can assist plants in the fight against harsh climatic conditions by choosing the tillage for the root system to develop optimally and have access to nutrients from great soil depths. Deep tillage loosens the soil and allows roots to reach nutrients and water stored inside the soil (Bengough et al., 2011). Doing so, the plants consume water and do not allow it to evaporate, which results in the absorption of more radiation and, as a result, a good development of the aerial organs, which contributes to obtaining rich harvests without compromising the quality of the seeds (Halford et al., 2015; Unkovich et al., 2023). Another considerable factor for increasing the tolerance to climate stress is the close connection between the root system and the microorganisms that live inside the soil (Hartman and Tringe, 2019), which also depends on the quality and method of soil processing.

MATERIALS AND METHODS

The experience placed in the Chiscani Experimental Field, within ARDS Braila, was conducted during 2019-2022. From a geomorphological point of view, the mentioned perimeter represents a relatively flat area with an absolute altitude between 14-15 m. The soil is chernozem type, vermic subtype, with moderate-carbonate variety, loamy texture, formed on loess deposits with a predominantly loamy texture up to 1 m depth, and sandy loam at greater depths. The calcium carbonate content varies between 4.5-5.0% in the upper horizons, and the humus supply is in the middle class (2.4-3.1%). Total nitrogen content is specific to the soil type, and mobile phosphorus is very good (74-225 ppm). The mobile potassium content is optimal in the 0 - 20 cm depth and good in the Am horizon. The soil reaction has pH values between 7.9-8.4 (dominantly alkaline).
The study was conducted on four straw crops: wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L., 1753), rye (*Secale cereale* L., 1753), triticale (*Triticosecale* Muntzing, 1936), five tillage types, on three repetitions each (Figure 1): the classic tillage A1 - Plowing (control), and the tillages of conservative agriculture: A2 - Paraplow, A3 - Scarification, A4 - Heavy-disk and A5-Minim-till. The yields obtained according to the soil works, under the influence of the variability of climatic factors, were analyzed

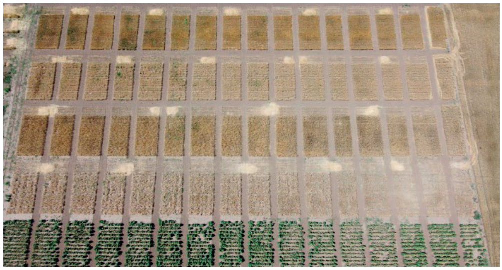

Figure 1. Field placement of cereal crops. From upward to downward: barley, wheat, rye, triticale. From left to right: A1- Plowing (control), A2 - Paraplowing, A3 - Scarification, A4 - Heavy disk, A5 - Minim-till

The study was conducted on four straw crops: wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L., 1753), rye (*Secale cereale* L., 1753), triticale (*Triticosecale* Muntzing, 1936), five tillage types, on three repetitions each (Figure 1): the classic tillage A1 - Plowing (control), and the tillages of conservative agriculture: A2 - Paraplow, A3 - Scarification, A4 - Heavy-disk and A5 - Minim-till. The yields obtained according to the soil works, under the influence of the variability of climatic factors, were analyzed.

RESULTS AND DISCUSSIONS

The analysis of average temperatures and accumulated precipitation in the study years (2019-2022) highlights the severity of the in-consistency of abiotic factors (Figures 2 and 3).

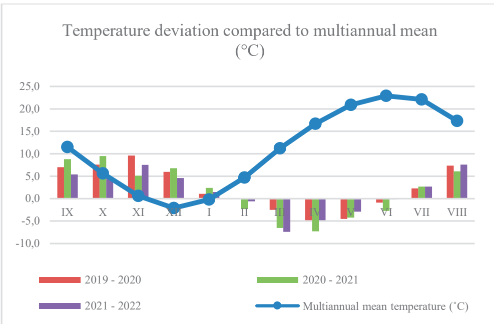

Figure 2. Mean temperatures recorded in the three years, in Braila - Romania

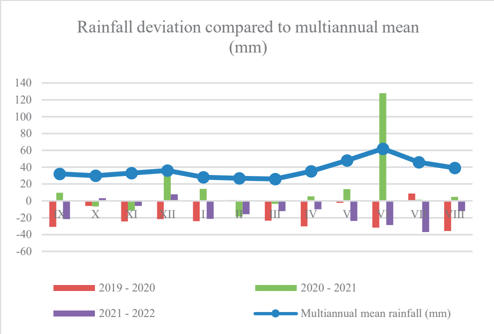

Figure 3. Rainfall recorded in the three years, in Braila - Romania

The 2019-2022 mean annual temperature was 1.8°C above the multi-annual mean of 10.9°C, with increases of 2.5°C in 2019, 1.5°C in 2020, and 1.5°C in 2021. Significant differences in temperature compared to the multi-annual mean, are observed between February and June. These negative temperatures prolong the dormancy period of straw crops, causing a delay in the onset of vegetation and an extension of the period until full maturity. One hypothesis of this extension of dormancy is that the plants become weaker, and the seeds produced suffer in terms of the quality of the constituent substances, directly affecting both food value and economic value.

Regarding the variation of temperatures over time, no significant differences are observed from year to year.

The effects of global warming can be seen through the amount of precipitation. Except for December and June of 2020-2021, rainfall was in deficit compared to the multi-annual monthly sum. The recorded precipitations over the three years were 77 mm below the multiannual sum of 442 mm. Although 2020 - 2021 recorded a deviation of +172 mm above the multi-annual sum, it was not enough to cover the deficit recorded in 2020 and 2022. Another worrying aspect is the variation of rainfall from year to year, namely 221 mm in 2020, 618 mm in 2021, and 264 mm in 2022. An anticipated hypothesis related to precipitation may be that it varies in opposite directions from one year to another, alternating a dry year with a rainy year. However, we must continue the studies to observe if this alternation persists, in which case we must choose crop plants that can tolerate better climatic stress.

The barley crop did not show enlarged differences in production compared to the control tillage-plowing in 2021, abundant in rainfall. In the dry year 2020, maximum increases were in A4 and A5 plots (663 kg/ha and 770 kg/ha). In 2022, the yield in A3 ranked first (4673 kg/ha). Although both agricultural years were poor in precipitation, the difference between yields is due to the increased temperatures of +2.5°C and +1.5°C. Considering the average yields over the three years of study, the type of tillages favorable for barley crops are a4 and A5, with yields of 4031 kg/ha and 4138 kg/ha compared to the control work (3368 kg/ha). In the case of sowing in stubble, the productions remained consistently superior to the control work, with minimal influences of environmental factors.

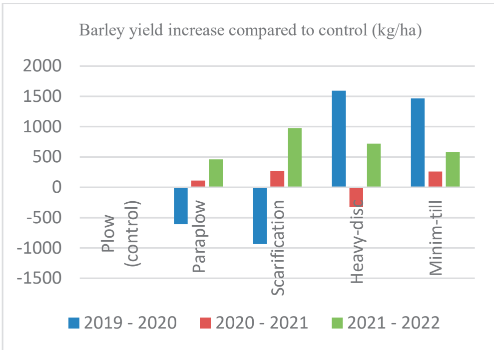

Figure 4. The difference in yield obtained in barley crop, compared to control tillage A1-Plowing

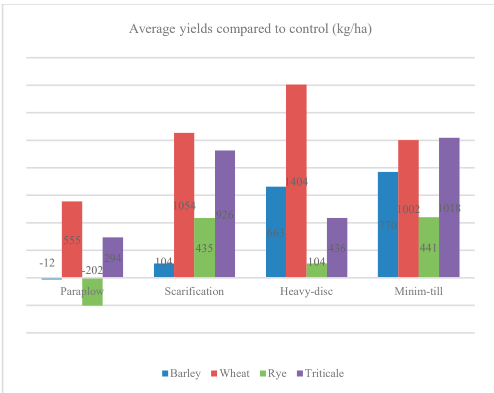

Figure 5. The difference in yield obtained in all crops, compared to control tillage A1-Plowing

Figure 6. Barley crop before harvesting

Figure 7. Wheat crop before harvesting

Tillage A2 (2846 kg/ha) and stubble sowing A5 (3293 kg/ha) contributed to productions below the control tillage A1 (3297 kg/ha), in the study years 2019-2022, for the wheat crop. In dry years, like the barley crop, tillage A4 helped to obtain the maximum yields compared to the other tillages of the dry-farming system, namely 3300 kg/ha in 2020 and 3500 kg/ha in 2022. Therefore we can presume that the root system of wheat does not have the power to penetrate the soil in the absence of humidity, developing at shallow depths. When rainfall is abundant and the soil stores water at great depths, the root system grows at greater depths, which is why in 2021, the yield obtained in the A3 plot was 4553 kg/ha.

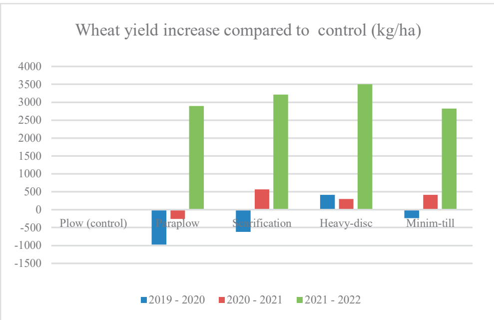

Figure 8. The difference in yield obtained in wheat crop, compared to control tillage A1-Plowing

The yields of the rye crop were superior to A1-control tillage in 2021, with abundant precipitation. Increases between 27 - 267 kg/ha were obtained, except for the heavy disc tillage from which we recorded a loss of 562 kg/ha. During the dry years, conservative tillages recorded unstable yields. In 2020, the maximum yields were obtained from the A4 and A5, while in 2022, they ranked below the control - A1.

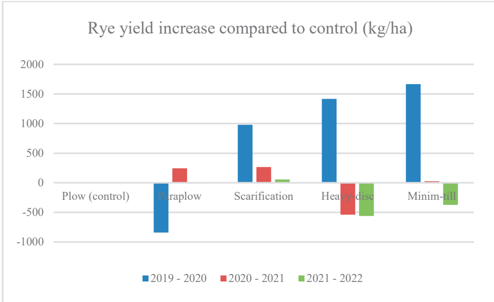

Figure 9. The difference in yield obtained in rye crop, compared to control tillage A1-Plowing

Figure 10. Triticale crop (left) and rye crop (right)

Triticale is a cereal with remarkable plasticity in conservative farming technologies. They produced superior harvests to the classical tillage A1-plowing, regardless of the total amount of precipitation. In A3 and A5 plots, triticale reached average productions of 4217 kg/ha and 4309 kg/ha in the studied period. Regarding the A5 (Minim-till), these results highlight the importance of the vegetal carpet in preventing the evaporation of water and its efficient use both by the root system of the plants and by the microorganisms that live in symbiosis with it.

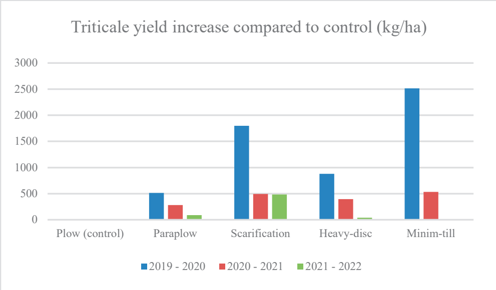

Figure 11. The difference in yield obtained in triticale crop, compared to control tillage A1-Plowing

The transition from classical to conservative agriculture is a long-term process that involves changing the quality and structure of the soil but also the accommodation of crops to these changes. Some species (triticale) adapt quickly, while others require several years to produce constant yields. Based on rye yields, it did not follow a pattern as triticale. Thus we can assume that rye responds more difficult to changes which is why further studies are necessary. In time, we must see whether the results will become conclusive and decisive for crops more sensitive to change and hold for cultures that adapt quickly. Another reason for the variation in yields obtained in 2020 and 2022 for the rye crop (both years were deficient in rainfall) may be the remaining soil water reserve from 2021, a year in which a redundancy of 176 mm was recorded.

CONCLUSIONS

The three studied years were hot, with temperatures above the multi-annual monthly average. Rainfall was in deficit in 2020 and 2022, which makes them very dry.

The triticale crop adapted to the specific tillages of the dry-farming system, registering positive increases in all three years of the study, regardless of the climatic conditions. Thus, the plasticity and adaptability of this plant to conservative farming techniques stand out.

The increases in yields between 460 and 977 kg/ha for barley and 2826-3500 kg/ha for wheat in 2022 are due to the accumulated rainfall in June 2021 (127.4 mm). The water was absorbed and stored by the soil. In autumn, at sowing time, the wheat and barley plants used it effectively, developing very well until the winter. At the beginning of spring, they resumed vegetation with high vigor and were able to tolerate the drought and heat of the next growing season.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Ministry of Agriculture and Rural Development which financed the Sectorial Project: ADER 1.2.1./27.09.2019: "Research on the identification of technical solutions and technological elements for the practice of the dry-farming work system in Southern Romania".

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

A STUDY ON THE GERMINATIVE CAPACITY AND HERBA YIELD OF *Hyssopus officinalis* L.

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Abstract

Hyssopus officinalis L. has a long and rich history as a medicinal plant due to the health benefits transmitted from generation to generation of usage as a carminative, antiseptic, and cough reliever. These benefits have drawn the attention of the researchers, whom, in the last years, have started to fundament scientifical elements of the hyssops crop technology. This study aims to follow and report the germinative capacity and both fresh and dry herba yield of three hyssops varieties (white, blue and pink). The assessing of herba yield, was done at SCDA Lovrin and the germinative capacity of the studied varieties was determined in the Laboratory for Seed Quality from the Faculty of Agriculture from Timisoara. The results are highlighting significant differences between the varieties regarding germinative capacity, the mass of 1000 grains and both dry and fresh herba yield.

***Key words**: hyssop, herba yield, medicinal plants, germinative capacity.*

INTRODUCTION

Hyssopus, originating from the Mediterranean and Central Asia, is mentioned in the Bible and in the works of Theophrastus, Dioscorides, Columella, and Pliny. It is cultivated as a medicinal plant with expectorant, antiseptic, and digestive functions stimulating properties (Dragland et al., 2003; Jung, 2004; Lugasi et al., 2006, Imbrea, 2016). Additionally, hyssopus is used as a honey plant, spice, and ornamental plant (Kapelev, 1986; Păun, 1986; Verzea, 2002). According to the Flora României, vol. VIII, the cultivated species is *Hyssopus officinalis* L., with subspecies *officinalis* Briq and *canescens* (DC) Briq. *Officinalis* subspecies includes 3 varieties (*vulgari*s Benth, *decussatus* Pers., and *angustifolius* (Bieb) Benth). Additionally, within the *vulgaris* Benth. variety, plants with blue-violet flowers (f. *cyaneus* Alef.), red-carmine flowers (f. *ruber* (Mill) Alef.), or white flowers (f. *albus* Alef.) can be found (Fleischer, 1988; Mechraz, 1989; Muntean, 2007; Roman, 2008).

MATERIALS AND METHODS

The study was conducted on three colour varieties, namely pink, blue, and white, obtained from the collection of the Phytotechnics discipline within the Faculty of Agriculture in Timisoara, from three years of cultivation. Germination capacity testing was carried out in the Laboratory for Testing Seed Quality and Plant Material within the Faculty of Agriculture. For the processing and interpretation of the experimental results, the following programs were used: for analysis of variance - statistics [ANOVA], MSTATC; for correlations and regressions - statistics - Regressions and Graphs; procedures with formulas on factor contribution and DLs - in EXCEL; Cluster Analysis.

RESULTS AND DISCUSSIONS

Thousand grain weight (TGW) is an indicator that shows the size of the seeds, provides information on their quality and cultivation method, expressing their potential as a multiplication material. Table 1 and Figure 1 present the results regarding the thousand seed weight depending on the colour variety. Compared to the experience average, which recorded a value of 0.894 g, only the white colour variety recorded a higher value (1.001 g), statistically significant. In the case of the pink colour variety, the smallest value of the thousand seed weight was obtained (0.739 g), respectively a difference of 0.155 g, statistically significant in a negative sense. For the blue colour variety, the values of the thousand seed weight were very close to the field average (0.943 g), the difference of 0.049 g was not statistically assured.

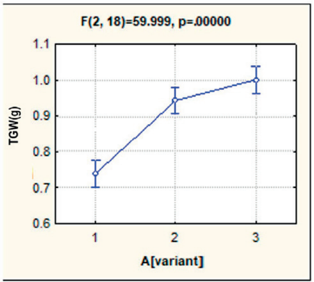

Figure 1. TGW variation in the 3 varieties

Table 1. TGW variation depending on colour variety

Factor A - colour variety	TGW [g]	Difference [g]	Significance
a1 - v1	0.739	-0.155	000
a2 - v2	0.943	0.049	ns
a3 - v3	1.001	0.106	***
Average	0.894	mt	
DL 5% = 0.053 g; DL 1% = 0.072; DL 0.1% = 0.098			

The variation of TGW in the three colour varieties under study shows an increasing trend. The values of TGW ranged from 0.74 g in the pink variety to approximately 1.0 g in the white variety. The differences between the varieties are highly significant (p<0.001). The results regarding the evolution of TGW according to the colour variety and year of culture are presented in Tables 1 and 2, and Figures 1 and 2.

Table 2. TGW variation depending on cultivation year

Factor B - cultivation year	TGW [g]	Difference [g]	Significance
b1	0.946	0.051	ns
b2	0.911	0.017	ns
b3	0.826	-0.068	0
Average	0.894	mt	
DL 5% = 0.053 g; DL 1% = 0.072; DL 0.1% = 0.098			

The obtained data show that compared to the field average, the seeds obtained from the crops in the first, second, and third year do not present significant differences for the first and second year, only for the third year of culture, the difference of 0.068 g of the mass of 1000 grains is statistically significant in a negative sense. It should also be noted that, of the three experimental years, in terms of precipitation deficit during the growing season, the year 2022 had the highest deficit and the highest values of monthly average temperatures.

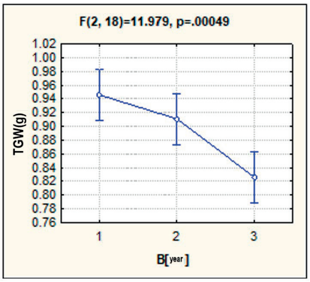

Figure 2. TGW variation depending on cultivation year

The values of the 1000 grain weight decrease with the year of cultivation, with the highest value obtained in the first year of vegetation of 0.95 g, followed by the second year of vegetation with 0.91 g, and the lowest value was obtained in the third year of vegetation.
The interaction between colour variety of hyssop and the crop year on the 1000 seed weight is presented in Table 3 and Figure 3.

Table 3. Results on interaction of colour variety and cultivation year on TGW

			Factor B [cultivation year]			
			B1	B2	B3	Average
Factor A [colour variety]	A1 - pink	TGW [g]	0.801	0.798	0.619	0.894
		Dif[g]	-0.094	-0.096	-0.275	
		Sig	0	0	000	
	A2 - blue	TGW [g]	1.014	0.937	0.878	
		Dif [g]	0.12	0.04	-0.01	
		Sig	*	ns	ns	
	A3 - white	TGW [g]	1.022	0.998	0.982	
		Dif [g]	0.128	0.103	0.088	
		Sig	**	*	ns	
DL 5% = 0.091 g DL 1% = 0.125; DL 0.1% = 0.170						

Compared to the control, the average interaction between year and colour variety was expressed as follows:
For the pink variety [v1], all differences obtained in the three years were statistically significant (highly significant in b3 - year 3, significant in b2 - year 2, and b1 - year 1), but in a negative sense.
For the blue variety [v2], only in the first year was an interaction recorded expressed by a statistically significant positive difference, while for the II and III years, the differences are

insignificant, and the values of the 1000 seed weight are close to the field average.
For the white variety [v3], the interaction between factors was expressed by statistically significant positive differences (distinctly significant in b1 [year 1], significant in b2 [year 2]) and insignificant in b3 [year 3].

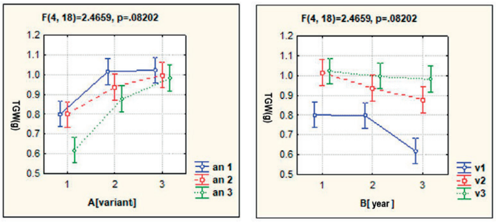

Figure 3. The variation of the 1000 grain weight depending on the interaction between cultivation year and color variety [A x B]

Over the three years of cultivation, the 1000 seed weight shows an upward trend with respect to color variety. The highest 1000 seed weight is obtained in a3 [blue variety], while the lowest is obtained in a1 [pink variety]. Comparing the years, it can be deduced that the highest value for 1000 seed weight was obtained in year 1 of cultivation, while the lowest was obtained in year 3 of cultivation. Figure 4 shows the contribution of the experimental factors (colour variety, crop year, and their interaction) to the realization of the TGW.

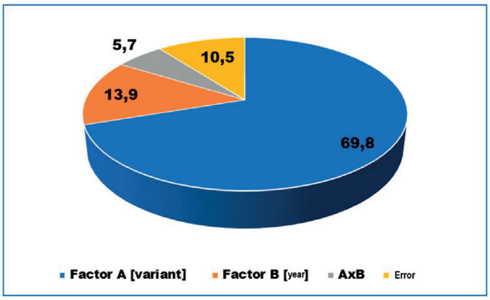

Figure 4. The contribution of the A [colour variety], B [crop year], and the A x B interaction factors

The A [colour variety] factor contributes 69.8% to the realization of the TGW, the B [crop year] factor contributes 13.9%, and the A x B interaction contributes 5.7%. Therefore, the colour variety factor has the highest contribution, followed by the B [crop year] factor and the A x B interaction. Cluster analysis is a multivariate analysis technique that includes a number of algorithms for classifying objects [elements or individuals] into homogeneous groups. A dendrogram is one of the methods of representing the clustering and can provide a summary of the classification.
Figure 5 shows the dendrogram of the TGW based on the nine combinations (three colour varieties and three crop years).

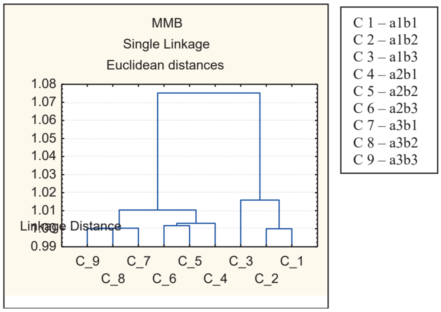

Figure 5. TGW dendrogram of the nine AXB combinations

The highest value of TGW 1.02 g is obtained from the combination a3b1 - class A, which differs significantly from all other combinations that do not contain the letter A. Other combinations belonging to class A include: a2b1, a3b2, and a3b3. Therefore, all four combinations yield approximately the same value of TGW at around 1 g. The four combinations [white in all three years of cultivation and blue in the first year of cultivation] are not significantly different from each other, but are significantly different from a2b3, a1b1, a1b2, and a1b3.
The lowest value of TGW 0.62 g - class D is obtained from a1b3, which differs significantly from all other combinations.
In conclusion, the expression of the weight of 1000 seeds is a character influenced by colour variety, and depending on the year of cultivation, with hyssop being a perennial plant, the highest value of the TGW is obtained in the first year of growth, followed by a reduction in subsequent years. Based on this finding, it is recommended that seeds harvested in the first year of growth be used for the production of hyssop seedlings.
For hyssop, which is a plant with small seeds and propagated by seedlings, seed germination

is a crucial process influenced by their quality. Table 4 and Figure 6 show seed germination according to colour variety.

Table 4. Seed germination depending on colour variety and cultivation year

Factor A [color variety]	Germination [%]	Difference [%]	Significance
a1	90.35	4.00	***
a2	93.66	7.31	***
a3	75.02	-11.32	000
Average	86.34	mt	
DL 5% = 0.048 %; DL 1% = 0.066; DL 0.1% = 0.091			

The results obtained show a very significant effect of the hyssop colour form, meaning that there are very significant differences in germination between the three colour variants studied in the experiment. Compared to the control - the experiment's average, very significant increases were obtained for all three-colour variants, noting that for variant 3 (white), the increase was negative, meaning that the germination rate for white hyssop was below the experiment's average. The germination percentage increases from variant 1 (pink) to variant 2 (blue), after which it decreases. Therefore, we have an upward trend from v1 to v2 and a downward trend from v2 to v3. The germination rates obtained for the three variants were 90% for v1 (pink), 94% for variant 2 (blue), and 75% for variant 3 (white).

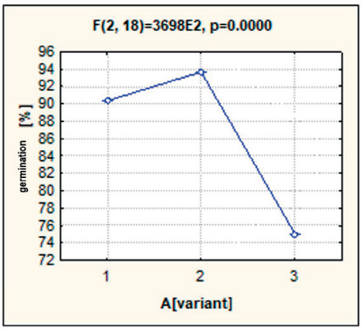

Figure 6. Germination variation of the three colour Hyssopus variety

Table 5. Percentage of germination by cultivation year

Factor B [year of cultivation]	Germination [%]	Difference [%]	Significance
b1 – an 1	91.37	5.03	***
b2 – an 2	88.31	1.97	***
b3 – an 3	79.35	-6.99	000
Average	86.34	mt	
DL 5% = 0.048 %; DL 1% = 0.066; DL 0.1% = 0.091			

The highest germination percentage was obtained from the seeds harvested in the first year (91.37%). Compared to the control - the experience mean (86.34%), statistically significant differences were obtained in all three years of production, noting that in the third year, the difference was negative, meaning that the germination rate was below the experience mean. The germination percentage decreased from year 1 to year 3, indicating a decreasing trend depending on the year of culture. The values of the germination percentage varied between 91% (year 1) and 79% (year 3). The differences between the years are highly significant (p<0.001).

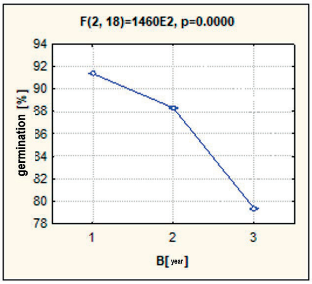

Figure 7. Germination variation during the three cultivation years

It should be noted that the germination percentage obtained in the first and second year of cultivation exceeds the value of 85%, which recommends their use as material for multiplication.

The germination percentage obtained for the interaction of color variety and year of cultivation [AXB] is presented in Table 6 and Figure 8.

Table 6. Percentage of germination obtained at the interaction of colour variety and cultivation year

			Factor B [cultivation year]			
			b1	b2	b3	Average
Factor A [colour variety]	a1	Germination	96.03	93.99	81.02	86.34
		Diff [%]	9.69	7.65	-5.32	
		Sig	***	***	000	
	a2	Germination	98.07	95.93	86.98	
		Diff [%]	11.72	9.59	0.63	
		Sig	***	***	***	
	a3	Germination	80.01	75.01	70.05	
		Diff [%]	-6.33	-11.34	-16.29	
		Sig	000	000	000	
DL 5% = 0.091 g DL 1% = 0.125; DL 0.1% = 0.170						

The highest germination percentage was obtained in the first year for blue hyssop

(98.07%), followed by pink hyssop (96.03%). Values over 90% germination were also obtained in the second year of cultivation for the same color varieties (95.93% for blue and 93.99% for pink). It is worth noting that for white hyssop, where the highest 1000 seed mass was obtained, the germination percentage ranged from 70.05% in the third year of cultivation to 80.01% in the first year of cultivation, values much lower than the experience average (86.34%).

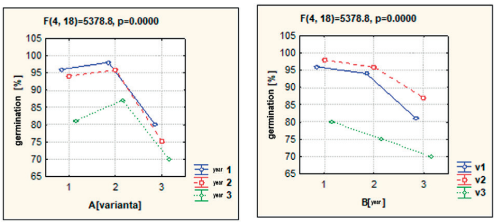

Figure 8. Germination variation [AxB interaction]

In the 3 years of cultivation, germination shows a decreasing trend regardless of the variant. The highest germination value is obtained for v2 [blue isop], while the lowest is for v3 [white isop]. Comparing the years, it can be deduced that the highest germination value was obtained in year 1 of cultivation, while the lowest was in year 3 of cultivation. The contribution of factors A [color variety], B [cultivation year], and the interaction of A x B, is presented in Figure 9.

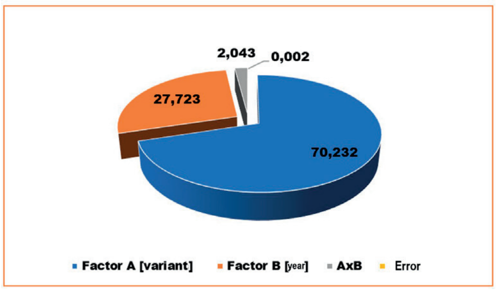

Figure 9. The contribution of factors A [color variety], B [cultivation year], and the interaction A x B

Factor A [color variety] contributes to the germination achievement in proportion of 70.23%, factor B [crop year] contributes with 27.7%, interaction A x B with 2.043%. The CLUSTER analysis and germination dendrogram is presented in figure 10.

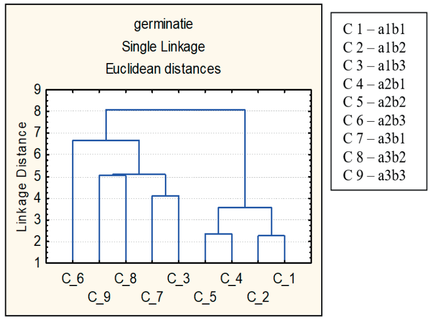

Figure 10. Dendrogram of the 9 AxB interactions

Each combination belongs to a different homogeneity class. Therefore, any combination differs from all other combinations. The highest germination value of 98.07% is obtained for combination a2b1 - class A, significantly different from all other combinations. The lowest germination value of 70.05% - class I is obtained for a3b3, significantly different from all other combinations.

CONCLUSIONS

The research conducted on the TGW and germination of three color varieties of hyssop (pink, blue, and white) provides information that contributes to improving the knowledge related to the technology of this species, which, although known since biblical times, is little known in our country and in Europe.

The TGW is a character influenced by the color variety, and depending on the year of cultivation, hyssop being a perennial plant, the highest value of the TGW is obtained in the first year of vegetation, with the value decreasing in the following years. Based on this observation, it is recommended to use seeds harvested in the first year of vegetation for the production of hyssop seedlings.

Analyzing the contribution of experimental factors to the realization of the TGW, the results show that the color variety contributes to the realization of TGW by 69.8%, the year of cultivation contributes by 13.9%, and the interaction of the two factors by 5.7%. Therefore, the color variety factor has the greatest contribution, followed by the year of cultivation factor and their interaction.

Regarding the germination percentage, the highest values were obtained in the first year of cultivation for blue hyssop (98.07%), followed by pink hyssop (96.03%). Values of over 90% germination were also obtained in the second year of cultivation, for the same color varieties (95.93% for blue and 93.99% for pink). It should be noted that for white hyssop, where the highest TGW was obtained, the germination percentage ranged from 70.05% in the third year of cultivation to 80.01% in the first year of cultivation, values much lower than the experience average (86.34%).

Factor A [color variety] contributes to germination by 70.23%, factor B [year of cultivation] contributes by 27.7%, and the interaction of A x B by 2.043%.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

MODELING DRIP-IRRIGATED RICE YIELD USING NORMALIZED DIFFERENCE VEGETATION INDEX: A PRELIMINARY STUDY

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Abstract

Rice is one of the major food crops with a growing demand on the global market. The need for water-saving and environmentally friendly technologies presses current agricultural science to look for alternative ways of rice irrigation. The most prospective one is drip irrigation. Yield prediction is also of great importance for sustainable agriculture. The goal of the study was to create a pilot model for drip-irrigated rice yield prediction in the conditions of the South of Ukraine using spatial normalized difference vegetation index. The index values were taken from OneSoil AI platform for the drip-irrigated rice cultivated in 2016-2017 within the framework of cultivation technology studies. The highest index value was recorded in the stage of "tillering-heading" and applied for the regression and neural network-based models. It was established that the performance of various regression models was quite similar in fitting quality and accuracy, while neural network-based one provided significantly higher precision. It is reasonable to simulate drip-irrigated rice yield with a good accuracy (MAPE<15%) using simple linear regression model. Further improvement of predictions is expected through the increase of the sample size.

***Key words**: artificial neural network, regression, remote sensing, statistics, yielding scale.*

INTRODUCTION

Yield prediction is a valuable branch of modern agricultural science with a growing demand in practice of precision agriculture. Timely prediction of a crop yield is essential for economic planning of agricultural production; besides, it is necessary to identify and correct possible weak places in the cultivation technology in advance to avoid yield losses, and for this purpose modern farmers can use the latest achievements of yield simulation in various decision support systems. Some of them are integrated with geoinformation systems and remote sensing data, such as vegetation indices, which are widely implemented to characterize growing conditions of plants cultivated in the field. Normalized difference vegetation index (NDVI) proposed by Rouse et al. (1974) is among the most frequently used in these purposes due to its wide availability on various online platforms, both paid and free (Pettorelli, 2013). Most crop yield prediction models are based on the vegetation index, which is applied as the only input or in combination with additional inputs to simulate crop yield. There are crop yield prediction models almost for every crop, cultivated in Ukraine, for example, sweet and grain corn, beans, grain sorghum, soybeans, wheat, barley, major oil crops, etc. (Becker-Reshef et al., 2010; Franch et al., 2019; Lykhovyd, 2020; Vozhehova et al., 2020; Lykhovyd, 2021; Lykhovyd, 2022; Lavrenko et al., 2022). On the global scale, NDVI-based models for yield prediction are developed to some extent almost for every major crop, with the greatest focus on such valuable ones as wheat, corn, and rice.

Rice is one of the major staple food crops in the world. It is cultivated in more than 100 countries with a gross yield exceeding 450 million tons per year. Although rice is one of the most cultivated crops in the world, there is still lack of provision with this crop because of extremely fast increase in the population. As it is estimated, the provision of rice should be at the level of 700 million tons, therefore, now there is a discrepancy of nearly 250 million tons between the proposal and demand on the global market (Bhandari et al., 2017). Therefore, an enhancement of rice productivity is of a great relevance for current agricultural science.

Rice is on the list of cereals cultivated in the South of Ukraine, mostly concentrated in Kherson and Odesa regions. The areas are about 30,800 ha, mostly - paddy rice, cultivated by high-input and extremely costly (especially, in the initial stage) technology with high freshwater uptake. In the modern conditions of the global freshwater scarcity in all the branches of economy, it is necessary to develop the technology for water saving during rice cultivation. In this regard, notwithstanding the fact of biological mismatching, drip irrigation could be a prospective technology for rice cultivation, therefore, drip-irrigated rice grows more and more popular among the leading crop producers and scientists in South Asia, India, China, and also Ukraine (Sharda et al., 2017; Parthasarathi et al., 2018; Osinnii et al., 2020; Xu et al., 2020). One of the tasks is not only to improve the crop cultivation technology, but also to provide novel reliable tool for the yield prediction in advance to harvesting. In this regard, remote sensing combined with geoinformation technologies could serve a good service.

The main goal of this study is to provide a preliminary result on the estimation of drip-irrigated rice yield, cultivated in the South of Ukraine, using the highest NDVI value as the only input for the crop productivity estimation. This prospective study is aimed to determine main regularities of yield prediction under novel cultivation technology, and provide an assessment of the maximum attainable crop productivity under such conditions.

MATERIALS AND METHODS

Models for drip-irrigated rice yield prediction using normalized difference vegetation index (NDVI) were developed by the yielding data collected in the field trials with the crop conducted within 2016-2017 at the fields of "Raiz-Pivden" farm located in the Oleshky district of Kherson region, Ukraine (geographical coordinates of the experimental field are 46°28'22.52" N 33°09'38.60" E; altitude 13 m). The drip-irrigated rice was cultivated using common cultivation technology, apart from the factors of tillage (skimming at 10-12 cm; chisel plowing at 30-32 cm), fertilization (N_0P_0; $N_{90}P_{30}$; $N_{120}P_{45}$; $N_{150}P_{60}$), and irrigation (120, 140, 160% of ETc adj). The experiment was performed in four replications, therefore, in general, we had 192 different rice plots with different yield levels for the two years of the study. The study was conducted with the variety Flahman (var. *italic* Alef). The field was monitored using NDVI from the OneSoil AI platform (combined Sentinel ½ images) with the resolution 250 m. Only cloud-free images were used for computation of NDVI. The highest values of the vegetation index were used for yield simulation. This falls to the phenological phase "tillering-heading", specifically, July 24th in 2016, and July 11th in 2017. The grid of experimental plots was superposed over the NDVI images and NDVI values were correspondingly extrapolated and assigned for each plot. Average yield and vegetation index values for every plot were further used in the crop modelling.

Crop modelling was performed using standard algorithms of various regression functions (Freund et al., 2006). The calculations were performed in BioStat v.7 software, add-in for Microsoft Excel 2019. Models' accuracy was estimated by the value of mean absolute percentage error (MAPE), while fitting quality – by the value of coefficient of determination R^2. Combined model of regression and neural network was created through the method of simple superposition and adjustment coefficient computation by the method proposed in the work of Lavrenko et al. (2022). Pure artificial neural network-based model was developed using Tiberius XL software, which is a back-propagation neural network tool with the generalized delta rule training algorithm, with the following parameters: 48 samples, 5 hidden neurons, 1000 epochs, learning rate 0.80 (Adamowski, 2008). Figure was created in Microsoft Excel 2019.

RESULTS AND DISCUSSIONS

The input data for drip-irrigated rice yield modelling are generalized in the Table 1. The results of regression modelling by seven different approaches are represented in the Table 2. It is evident that the models do not significantly differ from each other in terms of prediction accuracy with MAPE amplitude of 0.3951%. The same is true for the fitting quality, assessed by the values of R^2 coefficient. The best fitting is in cubic regression function, while the worst one is attributed to functional regression. However, we must admit that the difference between all the applied regression approaches is so subtle that it could be neglectable. This is explained by relatively small sample size, where complicated non-linear regression computations had no opportunity to show their superiority over simple linear regression analysis (Frost, 2020). As all the developed models fall under the criteria of good forecast by the magnitude of prediction error MAPE (Blasco et al., 2013), and are classified as moderate or strong correlation between the crop yield and NDVI by various interpretations of correlation coefficient used in modern science (Taylor, 1990; Akoglu, 2018), it is reasonable to create a scale for rough drip-irrigated rice yield estimation based on a single chosen model among the developed ones. Therefore, the basis for the development of rice yield scale in connection with NDVI values in our study is the linear model as the simplest one but not inferior in quality.

Table 1. Data on rice yields and corresponding NDVI values in the field trial

Sample number	Yield, t ha^{-1}	NDVI	Yield, t ha^{-1}	NDVI
	2016		2017	
1	3.14	0.55	3.12	0.50
2	3.79	0.60	4.32	0.56
3	4.58	0.65	5.11	0.59
4	4.06	0.62	4.62	0.57
5	3.73	0.59	3.76	0.53
6	4.56	0.65	5.29	0.60
7	5.61	0.72	6.33	0.65
8	5.17	0.70	5.92	0.63
9	3.44	0.57	3.44	0.52
10	4.20	0.63	4.88	0.58
11	5.00	0.68	5.57	0.62
12	4.59	0.65	5.12	0.59
13	3.93	0.61	4.70	0.57
14	5.02	0.68	6.24	0.65
15	5.61	0.73	6.90	0.68
16	5.14	0.70	6.31	0.65
17	4.76	0.67	5.58	0.62
18	6.03	0.76	7.54	0.71
19	6.90	0.82	8.57	0.76
20	6.51	0.79	8.15	0.74
21	4.56	0.65	5.24	0.60
22	5.59	0.72	7.00	0.69
23	6.25	0.77	7.65	0.72
24	5.84	0.74	7.11	0.69

Table 2. Results of regression analysis and models of the drip-irrigated rice yield correspondence to the NDVI values at the stage of "tillering-heading"*

Model type	Regression statistics and equations			
	R	R^2	MAPE (%)	Equation for rice yield derivation from NDVI
Linear	0.7525	0.5662	14.5368	13.2552x – 3.2919
Quadratic	0.7571	0.5732	14.4264	$-17.1049x^2 + 35.7071x - 10.5670$
Cubic	**0.7593**	**0.5765**	14.4479	$-124.9179x^3 + 229.3117x^2 - 124.7109x + 23.8913$
Power	0.7462	0.5568	14.4886	$10.7582x^{1.6767}$
Functional	0.7354	0.5408	14.7219	0.9797×12.9214^x
Logarithmic	0.7560	0.5716	**14.3268**	9.0982 + 8.6334 – ln(x)
Hyperbolic	0.7559	0.5713	14.5176	13.9147 – 5.5128/x

*The best performance by each statistical index is marked with bold font.

Visual assessment of the linear model for drip-irrigated rice productivity prediction is presented in the Figure 1. It is obvious that the best fitting quality is attributed to the samples with median productivity of the crop, while with the increase in the crop yield, as well as with its decrease, the discrepancy between the lines becomes more distinct.

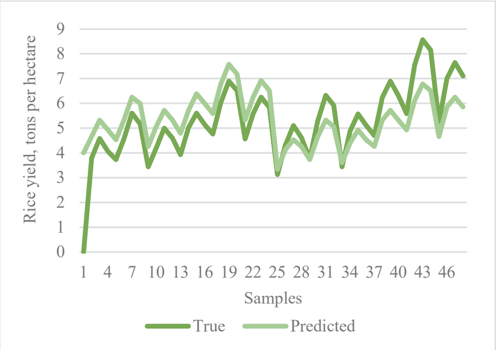

Figure 1. Visual Approximation of the Drip-Irrigated Rice Yield Prediction Model

The scale for drip-irrigated rice yields is represented in the Table 3. We used 0.05 step of NDVI values, starting the scale with the minimum of 0.50, and ending with the maximum of 0.85 (as the values of NDVI exceeding 0.85 are uncommon for OneSoil AI platform). The yield is given ± possible dispersion (taking into account the MAPE of the linear model).

Table 3. Drip-irrigated rice yield depending on the NDVI values at the stage of "tillering-heading"

NDVI	Yield, t ha^{-1}
0.50	3.34±0.49
0.55	3.99±0.58
0.60	4.66±0.68
0.65	5.32±0.77
0.70	5.99±0.87
0.75	6.65±0.97
0.80	7.31±1.06
0.85	7.98±1.16

For better understanding yield dynamics with better crops condition reflected by NDVI values we provide a graphical visualization of the developed scale in the Figure 2. As the prediction model testifies, the highest expected productivity of drip-irrigated rice is about 9.0-9.2 t ha^{-1}.

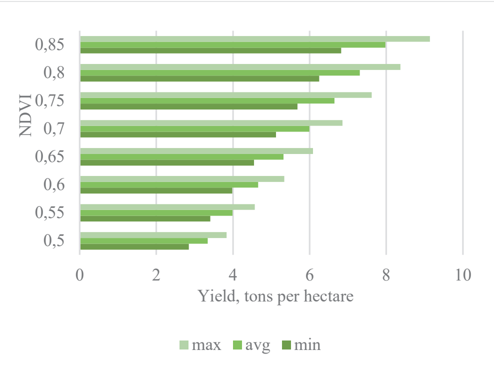

Figure 2. Drip-Irrigated Rice Yield Simulated by NDVI Values at the Stage of "Tillering-Heading"

The developed scale is the first one created to predict rice yields in the conditions of drip irrigation on the basis of remotely sensed NDVI. Most similar models are developed to predict paddy rice yields. Some of them are of comparable accuracy, and some (mostly complex, not pure NDVI-based ones) are superior to ours. For example, Huang et al. (2013) developed more accurate (overall relative error averaged to 5.82%) rice yield model based on NDVI, but it is used for paddy rice. Korean scientists developed integrated NDVI-Meteorological model for paddy rice yield prediction (Na et al., 2012). The model is more complicated than pure NDVI-based ones, but it provided somewhat higher accuracy comparing with our model: correlation coefficient 0.7840 vs. 0.7525. Another successful complex paddy rice yield models involved a combination of NDVI, enhanced vegetation index (EVI), leaf area index (LAI), and weather data. Performance of the models was very high - R^2 values were within 0.70-0.82 (Hong et al., 2012). Therefore, inclusion of additional inputs apart from NDVI is a useful strategy to improve predictive quality of mathematical models for drip-irrigated rice yield estimation. Though, some scientific groups achieved incredibly high accuracy of regression models (correlation coefficient up to 0.94) for rice prediction even at using NDVI as the only input (Faisal et al., 2019; 2020). Good prediction model for rice yield estimation in the conditions of Egypt with R^2 0.85 was proposed by Noureldin et al. (2013). At the same time, NDVI-based regression models for rice yield assessment developed by Harrell et al. (2011) are inferior to ours with R^2 0.36-0.42. We suppose that model quality strongly depends on the factors of the input's quality (from what platform and satellites NDVI imagery is derived and what GIS software is used to pre-process them), presence of additional inputs (such as meteorological data), total number of data pairs (sample size), and mathematical algorithms applied. The complex model with the best quality of inputs, large sample size will provide the best prognostic performance under the proper mathematical processing.

Apart from the complexity level and sample size, good results could be achieved through the usage of modern computation techniques. We have tried to enhance the model quality through the combination of artificial neural network (ANN) approach with linear regression analysis, as it had a success in case of the yield modelling for beans (Lavrenko et al., 2022). However, in this study the combined model (with the following equation 14.2136x - 3.5299) was inferior in prediction quality even to the simple linear one, notwithstanding the fact that the pure ANN-based model showed significantly better results (Table 4). Unfortunately, "black box" nature of the ANN-based model makes it impossible to derive the way of achieving the crop yields, so this one is unsuitable for use in practice. Therefore, the model for drip-irrigated rice yield prediction through NDVI data needs further improvement mainly through the enlargement of the sample size.

Table 4. Comparison of the simple, combined linear regression models and ANN-based one for drip-irrigated rice prediction using the NDVI values at the stage of "tillering-heading"

Statistics	Simple linear model	Combined linear model	ANN-based model
Correlation coefficient R	0.7525	0.7525	0.9999
Determination coefficient R^2	0.5662	0.5662	0,9998
Mean absolute percentage error MAPE (%)	14.5368	15.7500	5.0434

CONCLUSIONS

It is possible to predict drip-irrigated rice yield with a good accuracy (MAPE<15%) using simple linear regression model. Better performance in yield prediction is attributed to neural network-based model (MAPE<10%), but its use is limited in practice. A combination of regression and neural network did not result in significant performance improvement. Yield scale was developed based on the simple linear regression model. The scale is of use both in science and practice for drip-irrigated rice yield prediction using remote sensing data on normalized difference vegetation index in the period of "tillering-heading" of rice. Further improvements in the model are expected through the enlargement of the sample size.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

BIOSYNTHETIC AND BIOCONTROL POTENTIAL OF ENDOPHYTIC YEAST STRAINS YP6 AND YBS14 FOR IMPROVEMENT THE GROWTH AND DEVELOPMENT OF SOLANACEAE PLANTS

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Abstract

YP6 was isolated from Triticum aestivum L. seeds and YBS14 was isolated from the roots of Helichrysum italicum L. Partial sequence analysis of ITS5-5.8-ITS4 region of the nuclear ribosomal DNA with universal primers identified YP6 as Pichia fermentans and YBS14 as Saccharomyces cerevisiae. Both yeast strains produced indole-3-acetic acid when cultivated in a medium supplemented with 0.2 % L-tryptophan. The antimicrobial activity of yeast strains against plant pathogenic fungi was determined. YP6 and YBS14 were tested for endophytic colonization of Solanaceae plants by soil drenching and leaf spraying. To establish colonization in the various tissues of tested plants, samples were taken and explants were inoculated on yeast malts agar. The effect of the microbial endophytes on photosynthesis, stomatal conductivity, and transpiration intensity was analyzed by using the portable photosynthetic system for CO_2 analysis in plants. In all treated plants photosynthesis was intense and growth stimulation was observed. The final aim of the present study is to evaluate endophytic yeast and demonstrate their PGP activity.

Key words: *endophytic yeast, Solanaceae, biosynthetic potential, physiological parameters, antimicrobial activity.*

INTRODUCTION

Local varieties of Solanaceae plants and populations obtained as a result of long evolutionary development are of significant potential interest in this direction. They are well adapted to different agro-ecological conditions and are carriers of valuable economic, taste and technological qualities. This is confirmed by the enormous interest shown by foreign breeders, scientific institutes and private companies in genetic material from local Bulgarian varieties. Unprecedented advances in science and new biotechnologies, including microbiology, microbiomes, etc., enable the development of flexible management models involving local genetic resources, research and the development of innovative biopreparations for pathogen control and yield enhancement.

Yeasts inhabit all aerobic habitats, but most species are known for their biotechnological applications and/or medical relevance, rather than their potential application in agricultural production systems or their biological function in the environment (Buzzini et al., 2012; Cantrell et al., 2011; Sundh et al., 2011). Yeast endophytes are found in intercellular space or inside cells of host plants causing no apparent damage (Saikkonen et al., 1998). They benefit plants by promoting plant growth (Dai et al., 2008), improving resistance to multiple stress (Lewis, 2004; Malinowski et al., 2006, protecting from diseases and insects (Wilkinson et al., 2000; Tanaka et al., 2005; Vega et al., 2008). Relatively few yeast-based products for plant protection have reached advanced developmental stages and were suggested as commercial products for postharvest applications. *Saccharomyces cerevisiae* strain LAS02, is also being evaluated for its biological activities (Droby et al., 2016; EFSA, 2015; Liu et al., 2013). These examples clearly demonstrate the potential use of yeasts for plant production and protection, but also indicate their limited exploitation for commercial applications in agriculture. In addition to biocontrol, yeasts have been implicated in benefiting soil structure and plant nutrition, and biostimulation of growth, development, and stress resistance (Yakhin et al., 2016). The elucidation of the modes of action underlying all bio-activities is a prerequisite for the development of science-based, reliable products. Until recently, however, the mode of action underlying the beneficial activities of yeast and yeast-based products has been little understood.

Endophytes may contribute to their host plant defences against phytopathogenic organisms through plant physiology control (Giménez et al., 2007). An increase in plant growth will prevent a variety of abiotic and biotic stresses, reflecting plant vigour or persistence and being considered as potential protection against pathogen challenges (Kuldau and Bacon, 2008; Kim et al., 2006). Many studies demonstrate that plants infected with endophytes obtain growth promotion (Barka et al., 2002; Petkova et al., 2022), resistance to drought stress (Swarthout et al., 2009) and tolerance to unsuitable soil conditions (Belesky and Fedders, 1995; Malinowski et al., 2006). Previous research has examined the effect of single and double treatment with yeast *Z. bailii* YE1 and *S. cerevisiae* YD5 inoculum on the development and biochemical parameters of tobacco (Petkova et al., 2022). According to this publication the effect of leaf spraying versus root drench and the effect of single treatment versus duplicate treatment, *Z. bailii* YE1 strain was the most effective by foliar inoculation, while for *S. cerevisiae* YD5 application was better with single soil treatment.

In the current study, the focus of research is the biosynthetic and biocontrol potential of two endophytic yeast strains YP6 and YBS14 on four different Solanaceae plants. Endophytic inoculation with yeast strains was done by soil drenching and leaf spraying and the observation of the physiological and biometric parameters of treated plants was accomplished. Finally, current experiments must also assess whether it is worthy or useful to promote crop growth, development, photosynthesis, and disease protection.

MATERIALS AND METHODS

1. Isolation and molecular identification of yeast

YP6 was isolated from surface-sterilized *Triticum aestivum* L. seeds and YBS14 was isolated from surface-sterilized roots of *Helichrysum italicum* L., grown on the training-experimental field Institute of Plant Genetic Resources “K. Malkov”- Sadovo. The surface sterilization to remove the adhering microorganisms was done according to Petkova et al. (2022). Yeast colonies, isolated from each explant were subculture on separate yeast malts agar (YMA) (Himedia, Mumbai, India) plates at room temperature, morphologically analyzed by microscope observation and then identified. To demonstrate the success of surface sterilization, the wash water from the last wash was inoculated with YMA without antibiotics and the plates were incubated at 27°C for 5 days.

For molecular identification, DNA was isolated with a HiPurA fungal DNA purification kit (Himedia, Mumbai, India) (Petkova et al., 2022). Control of the purity and concentrations of genomic DNA is performed by agarose gel electrophoresis. The protocol for DNA extraction of filamentous fungi was conducted according to Rosa et al. (2009). The internal transcribed spacer (ITS) domains of the rRNA gene were amplified with the universal primers ITS1 (5’-TCCGTAGGTGAACCTGCGG-3’) and ITS4 (5’-TCCTCCGCTTATTGATATGC-3’), as described by White et al. (1990) and 0.5 μL Taq polymerase mix (5 U/μL, Canvax, Spain). Amplification of the ITS region was performed as follows: 95°C for 5 min; followed by 35 cycles consisting of 94°C for 1 min, 58.5°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 5 min. Amplified PCR products were sent to Macrogen (Seoul, South Korea) for DNA sequencing. The nucleotide sequences of endophytic yeast isolates were aligned using the BLAST software package (http://www. https://blast.ncbi.nlm.nih.gov) and submitted to the GenBank database.

2. The biosynthetic potential of the studied strains

2.2.1. *Production of indole-3-acetic acid, phosphate solubilization, ZnO solubilization, and proteolytic activity*

Yeast inoculum was prepared by cultivation in a 5 ml sterilized broth which contains in one litre of water 10 g/l yeast extract, 20 g/l peptone, and 20 g/l dextrose and the culture was incubated at 30°C for 48 h. Quantification of indole-3-acetic acid was accomplished by the addition of an equal volume of Salkowski reagent. Measurement was done spectrophotometrically according to Limtong and Koowadjanakul (2012). Statistical analysis data are calculated as mean standard deviation. Student t-tests were used for the expression of the differences between groups and variance was considered statistically significant at $p<0.05$ (Silva-Hughes et al., 2015).

The inoculum density was adjusted to an optical density at 600 nm (OD600) and 100 µl was spotted onto Pikovskaya's agar (Zaidi et al., 2006) and incubated at 30°C for 5 days. The formation of a clear zone around the colonies was considered a positive result for phosphate solubilization. PSE (Phosphate Solubilization Efficiency) = Z/C x 100, where, Z is the clearance zone including microbial growth and C is the colony diameter (Nagaraju et al., 2017).

To determine the zinc solubilization ability, research strains YP6 and YBS14 were plated into the 9 mm agar wells of ZnO agar according to Rokhbakhsh-Zamin et al., 2011. The plates were incubated at 30°C for 3 days and observed for showing a zone of enlightenment around the colonies. The halo area was measured to determine the zinc solubilization efficiency (ZnE) of the strains using the method of Dinesh et al., 2018: ZnE = (HZ/C) × 100, where HZ is the diameter of the solubilizing halo area, and C is the diameter of the colony.

The phosphate and zinc oxide solubilization efficiency was calculated as a ratio between the diameter of the halo zone and the diameter of the colonies.

Proteolytic activity was determined from a clear zone in skimmed milk agar (Himedia, India) (Petkova et al., 2022). The agar plates were prepared and then YP6 and YBS14 were plated into the 9 mm agar wells and inoculated at 30°C for 3 days. The halo zone around the colony was measured and accepted as positive for cell wall degrading enzyme production.

2.2.2. *Screening for siderophore production*

The production of siderophore from yeast was performed in 2% yeast malts agar with the addition of chrome azurol S (CAS) (Himedia, India) according to Schwyn and Neilands (1987). After inoculation of the medium, the yeast was incubated at 27°C for 5 days in the dark. The development of a yellow-orange halo around the colony was considered a positive result for siderophore production producing ability.

2.2.3. *Antifungal activity of YP6 and YBS14 against Fusarium, Rhizoctonia, and Alternaria*

The studied yeast strains were tested to determine their antifungal action against *Fusarium solani*, *Rhizoctonia solani*, and *Altenaria solani* as was performed by the agar diffusion method (Cardenas et al., 1999). Phytopathogenic fungi have been isolated from damaged tobacco plants. Pathogenic species were identified by morphology-based identification of spore and mycelium characteristics (Matute et al., 2019). The fungal plant pathogens were tested for their ability to cause host damage under the application of Koch's postulates.

3. Plant colonization by endophytic *P. fermentans* YP6 and *S. cerevisiae* YBS14

For the purposes of the experiment, seedlings of well-developed tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.) and tobacco (*Nicotoana tabacum* L.) plants were used. On the 7^{th}, 14^{th}, and 21^{st} day after treatment (DAT), some samples of the studied plants were taken to determine the presence of yeast in them by inoculating explants from leaves, stems and roots on yeast extract agar (Merck, Germany) as was described by Petkova et al., 2021. The isolation frequency (IF) of the colonization of tobacco by yeast strains is calculated by the following formula published by Petrini and Fisher (1986).

The soil application was done by the root application and the leaf spraying was made on all leaves with a suspension at a concentration of 1×10^4 yeast cells, separately for each strain as was well-defined by Petkova, 2021. Control plants were sprayed only with water without treatment with yeast suspension. The effect of yeast isolates on the roots of tobacco was studied after treatment, using a magnification of 4 x on light microscope Leika M320.

4. Biometrical and physiological analysis of tobacco plants after the first and the second treatment with *P. fermentans* YP6 and *S. cerevisiae* YBS14

Biometrical analysis by the important traits such as stem height, root and leaf number, and leaves biomass of the aboveground part of plants were taken weekly. Measurements were conducted in three biological replicates and the statistical data were calculated with MS Excel 2010.

Physiological measurements were performed on three fully developed leaves by the utilization of a portable photosynthetic system Q-box CO650 - Plant CO_2 Analysis Package (Qubit Systems Inc., Canada). The intensity of photosynthesis (A, μmol m-2 s-1), stomatal conductance (Gs, μmol m-2 s-1), and intensity of transpiration (E, μmol m-2 s-1) were monitored and the results are presented as arithmetic mean ± SD. Parameters of photosynthesis and transpiration measurements were done by using a portable photosynthetic system Q -box CO650 - Package for CO_2 analysis in plants (Qubit Systems Inc., Canada). Statistica 7.0 software was used for the statistical evaluation (Stat Soft Inc. 2004).

RESULTS AND DISCUSSIONS

1. Identification of yeast strains

To isolate endophytes from selected plants, healthy and well-developed plants were selected. YP6 was isolated from *Triticum aestivum L.* seeds. YBS14 was isolated from the roots of *Helichrysum italicum L.*, which is mainly used for its limited habitat and importance as a medicinal plant. DNA-based methods are used to classify and identify yeast and are extremely valuable in the study of microbial species isolated from natural habitats. Using molecular identification methods, the nucleotide sequences of the resulting 950 bp PCR fragments were sequenced. After processing the results of sequencing and performing BLAST analysis with the available data in GenBank, the identity of the strains is determined. Partial sequence analysis of ITS5-5.8-ITS4 region of the nuclear ribosomal DNA with universal primers identified YP6 as *Pichia fermentans* and YBS14 as *Saccharomyces cerevisiae* with accession number in the Gene bank MZ798453 and MZ798454, respectively.

2. Biosynthetic potential of the studied strains

The result in table 1 revealed that the isolated strains possess high (solubilization zone greater than 2 cm) phosphate-solubilizing activity, measured as the phosphate-solubilization index (PSI) on PVK medium. The highest dissolution index was observed in YBS14 (135.37%), followed by YP6 (134.50%). Acid phosphatases and phytases synthesized by rhizosphere microorganisms are involved in the organic solubilization of soil phosphorus (Thaller et al. 1995). The established ability of three of the investigated strains to improve the solubility of inorganic phosphates is an important characteristic of PGP-microorganisms. Several fungi and bacteria have been shown to be phosphate- and Zn - solubilizers (Young et al. 2013; Sharma 2011; Kumar et al. 2009) but few of them exhibited also antimicrobial activities. The ZnO dissolution index for *P. fermentans* YP6 was calculated to be 132.85%. When inoculation was performed with *S. cerevisiae* YBS14, no solubilization of zinc oxide was observed.

Phytohormone IAA is the most common naturally occurring and methodically examined plant growth regulator. The amino acid tryptophan is a prominent precursor of IAA. The current experiment showed that the concentration of IAA production was strain specific. In the current study, yeasts were able to produce IAA. The tested yeasts have a medium level of IAA synthesis ranging from 28.3 ± 0.0134 mg/L by *P. fermentans* YP6 strain to 15.0 ± 0.026 mg/L by *S. cerevisiae*

YBS14 strain when there is 0.1% L-tryptophan in the medium (Table 1). Plant growth hormones are well-known to increase crop growth and yield (Saharan and Nehra, 2011).

Yeast can produce plant growth hormones such as auxin, gibberellic acid, and ethylene, which promote plant growth and yield (Amprayn et al. 2012; Nassar et al. 2005).

Table 1. Phosphate-solubilization index and IAA production, and qualitative determination of proteolytic activity and synthesis of siderophores of tested yeast strain

Yeast strains	Clear zone of phosphate-solubilization activities in mm ± SD, N=3	Phosphate-solubilization index (PSI) in %	ZnO solubilization as a zone in mm ± SD, N=3	ZnO solubilization index in %	IAA production ron tryptophan	Proteolytic activity, measured as zone in mm ± SD, N=3
P. fermentans YP6	27.6 ± 0.095	134.50	23.5 ± 0.086	132.85	28.3 ± 0.0134	23.53 ± 0.238
S. cerevisiae YBS14	28.3 ± 0.134	135.37	ND	ND	15.0 ± 0.026	18.63 ± 0.087

*SD - standard deviation; N-number of replicates. ND - not detected. The results are presented as arithmetic mean of three replicates (N = 3).

Synthesis of siderophores by yeasts in the rhizosphere also leads to the difficulty of iron access to harmful microflora and is reported as a significant PGP feature (Deshwal and Kumar, 2013). In the present study, the ability of strains to produce siderophores was determined on a solid medium after the addition of Chrome Azurol S (CAS) according to Schwyn and Neilands (1987). The synthesis of siderophores by the yeast strains was detected by a change in the colour of the culture medium (from blue to red-purple or yellow). Only *P. fermentans* YP6 showed activity when cultivated on CAS solid medium (Figure 1). Synthesis of siderophores leads to the deterrence of iron access to harmful microflora and is reported as a significant PGP feature (Deshwal and Kumar, 2013).

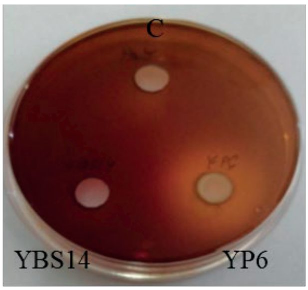

Figure 1. Synthesis of siderophores by yeasts YP6 and YBS14 strains

The main focus was to assess PGP effects on the nightshade family of plants. During seed bioassay, yeast inoculations improved root development as compared to the control. A study of the effect of YP6 and YBS14 culture filtrates on the tomato root showed a significant positive impact. S. cerevisiae YBS14 exhibited lower IAA production and as a result a smaller amount number of root hairs (Figure 2). In contrast, *P. fermentans* YP6 produced a 53% higher amount of IAA and in a co-cultivation experiment with *S. cerevisiae* YBS14, tobacco exhibited the development of a dense complex of root hairs. Hsu et al. published in 2010 that IAA stimulate plant cell enlargement, cambium cell, division, differentiation of phloem and xylem, root initiation and lateral root formation. This statement was confirmed by the results of the present study.

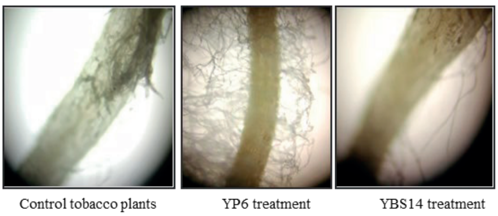

Figure 2. The interaction of yeast isolates with the roots of tobacco (*N. tabacum* L.*)* was studied after treatment, using a magnification of 4 $_x$ of a light microscope Leika M320

3. Antifungal activities of yeast strains against *Alternaria solani, Rhizoctonia solani,* and *Fusarium solani*

A large group of active yeast strains have been described as biocontrol agents as a result of their common ability to produce various antifungal metabolites (Silva-Hughes et al., 2015; Petkova et al., 2022). The antifungal activity of the tested yeast strains against

Fusarium solani, *Rizoctonia solani* and *Alteraria solani* and the demonstration of their inhibition effects are presented in Figure 3.

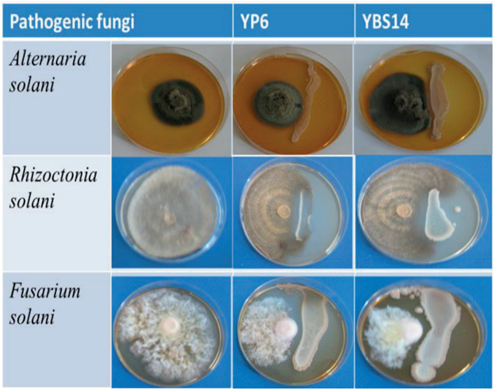

Figure 3. Antimicrobial activities of yeast strains against pathogenic fungi (*Alternaria solani, Rhizoctonia solani*, and *Fusarium solani*)

From the obtained results, it can be noted that two strains YP6 and YBS14 inhibited the growth of three of the test moulds *Fusarium solani*, *Rhizoctonia solani,* and *Alternaria solani.* The strongest effect of yeast is shown against *Fusarium* fungus. The weakest effect of YP6 and YBS14 is shown towards *Alternaria solani*. Their effect is probably due to the production of lytic enzymes and in the case of YP6 synthesis of siderophore. This result is similar to the previously published data by Petkova et al., 2022 with dought yeast strains YD5, YE1, and YSW1 which have been shown to inhibit the same phytopathogens and stimulate tobacco growth and development. Earlier findings reported the use of siderophores to control several pathogenic fungi such as *Pythium ultimum*, *Sclerotinia sclerotiorum*, and *Phytophthora parasitica* causing plant diseases (Mcloughlin et al., 1992). A large group of active yeast strains have been described as biocontrol agents due to their combined ability to produce various antifungal metabolites (Tian et al., 2009). Among these compounds, yeast produces low molecular weight antimicrobial molecules. Endophytes may contribute to their host plant defences against phytopathogenic organisms through plant physiology control.

4. Conducting pot and trials test cultures to track the effect of the tested strains separately

The highest colonization rates of leaves and stems of 85% to 100% were reported for YP6 and YBS14 of colonization root of tested plants (Figure 4). After data processing, the higher incidence rate in leaves and stems was impressive, even in the soil-inoculated experimental plants. Both downstream and upstream yeast migration was demonstrated up to the 21st DAT, where the percentage of colonization in different tissues of soil-treated and foliar-treated tobacco plants was quite similar to our previous results published by Petkova et al. (2022) (Figure 4). No colonization of plants was detected in control plants from the *Solanaceae* family.

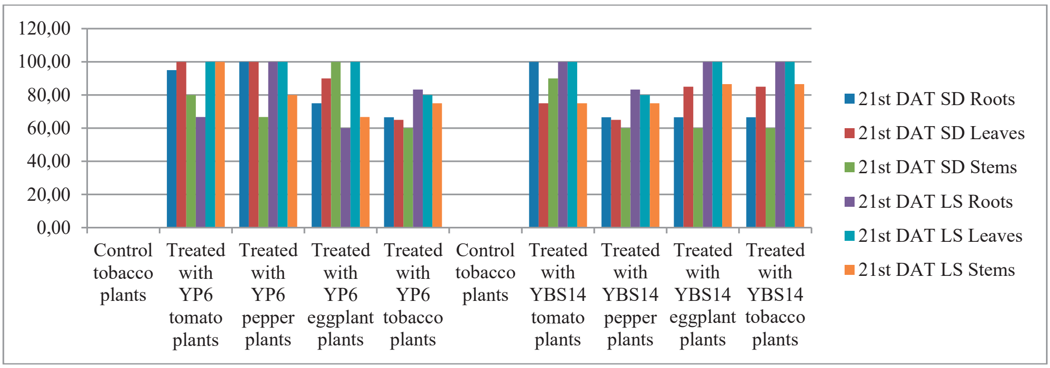

Figure 4. Percent colonization of tobacco plants at 21st days after treatment (DAT) with YP6 and YBS14 introduced by direct soil inoculation (SD) and leaf spray (LS)

From the results in Table 2, it can be seen that by the 21st day, the greatest increase in above-ground parts was recorded in tomato plants leaf-spraying treated with YP6, which reached a height of 29.45 cm, followed by YP6 treatment by soil application of the yeast 27.42 cm.

Soil-treated plants with YBS14 showed stem height close to that of control untreated plants. Both strains when applied by soil drench cause stress on root and lateral root growth.

Above-ground biomass (stem and leaves) was higher compared to control plants.

In the case of the experiments with pepper plants, there was variation in the height of the aboveground mass, but a threefold increase in root growth compared to the control (2.7 cm), after treatment with both yeast strains and in both treatments (12.3 to 16.6 cm) (Table 2). This leads to an increase in the biomass of the aerial part of the plants and the number of leaves.

Table 2. Biometry of tomato, pepper, eggplant, and tobacco to monitor the effect of the studied strains (YBS14 and YP6) separately on their growth and development

Plant	Days after treatment	Yeast Strain	Method of treatment	Stem height, cm ± SD*	Root length, cm ± SD*	Above-ground biomass (stem and leaves) in gram ± SD*	Number of leaves ± SD*
Tomato	21 DAT	Control	No treatment	21.83±1.14	5.96±0.15	6.55±1.79	8.00±1.29
		YBS14	LS	20.16±1.27	12.52±0.64	5.84±1.35	7.50±1.42
		YBS14	SD	27.83±2.34	24.86±1.56	6.66±1.12	7.33±1.25
		YP6	LS	29.45±2.56	17.71±1.42	2.96±0.07	6.50±1.67
		YP6	SD	27.42±2.16	11.6±1.06	7.5±0.43	8.00±1.36
Pepper	21 DAT	Control	No treatment	15.00±1.44	2.7±0.25	0.6±0.12	5.33±2.33
		YBS14	LS	14.03±1.92	12.13±0.84	1.86±0.35	7.00±2.16
		YBS14	SD	16.86±1.85	11.80±0.75	2.9±0.26	8.50±1.27
		YP6	LS	14.53±2.03	13.25±0.63	2.19±0.39	7.33±0.97
		YP6	SD	14.45±1.38	16.65±0.46	2.46±0.17	7.66±1.02
Eggplant	21 DAT	Control	No treatment	14.37±1.89	5.70±0.23	2.26±0.34	4.33±0.69
		YBS14	LS	15.55±1.72	8.25±0.42	2.05±0.29	5.30±1.49
		YBS14	SD	14.17±2.03	7.03±0.59	2.6±0.46	5.50±1.22
		YP6	LS	14.66±1.47	8.35±0.71	3.45±0.22	3.60±0.84
		YP6	SD	16.33±1.68	16.63±1.36	4.43±0.39	6.30±0.79
Tobacco	21 DAT	Control	No treatment	22.60±3.16	9.20±0.62	10.65±0.45	7.00±0.84
		YBS14	LS	18.00±2.67	17.63±0.53	13.2±0.51	8.33±1.27
		YBS14	SD	23.80±2.80	10.02±0.72	12.54±0.62	7.66±0.93
		YP6	LS	22.70±3.06	11.66±0.79	12.48±0.53	5.35±0.78
		YP6	SD	19.26±1.85	17.89±0.83	11.86±0.76	4.39±0.72

Legend: LS-leave spraying; SD-soil drenching. *SD - standard deviation; N-number of replicates was 10 plans for each treatment and 10 control plants.

Soil treatment with YP6 of eggplants showed a stimulating effect on plant biometric parameters at 21[st] DAT.

In contrast to the results with YP6, the soil treatment with the other tested strain YBS14 showed the weakest stimulation of stem, root, and leaf biomass growth in eggplant (Table 2).

The longest root length was recorded in soil treatment of tomato with YBS14, in pepper and eggplant in the soil treatment with strain YP6, in tobacco with strain foliar treatment with YP6 and soil treatment with YBS14.

Biometry data show that different yeast species have different effects on plants.

In the case of tobacco, the growth of the above-ground part of the plants in the initial stages had similar values in the soil and foliar treatments, but on the 21st day after treatment, it was seen that the plants with the foliar application of YP6 had almost twice higher growth (Table 2).

The greatest effect on stimulating stem length was shown by YP6 in leaf-spraying treatment of tomatoes and tobacco. Strain YBS14 showed better results in soil application of the yeast inoculum on tobacco growth. Biometric and quality indicators of plants also depend on applied agricultural techniques and growing conditions, which also contribute to changing the composition of microorganisms and the nature of interactions in general. This shows that it would be good to carry out additional studies in the future under more and different conditions in order to use the metabolic properties of the yeasts under investigation as effectively as possible.

Because the quality of Solanaceae plants depends on the specific qualities formed in the leaves and fruits depending on a number of factors. This requires additional research and in different conditions in order to use the metabolic qualities of the studied yeasts as effectively as possible. It is more specific to tested plants and should be investigated in the future because the biometric and quality indicators vary depending on the applied agricultural techniques and growing conditions.

The use of the biochemical properties of microorganisms in agriculture is a promising and rapidly developing field in modern agriculture, which has the potential to help solve important problems related to plant protection, fertilization and, respectively, environmental protection.

Periodical measurements on 7th, 14th, 21st, and 21st day after yeast inoculation on the intensity of photosynthesis, transpiration and stomatal conductivity of experimental plants clearly indicated that mutual plant-yeast coexistence causes no stress to the plant organism. More expressed significant positive impact on studied physiological traits exerted by the YP6 strain than YBS14 strain.

When regarding the effect of leaf spraying versus root drench the statistical evaluation of all studied biometric and physiological parameters highlighted that for YP6 strain the most effective is foliar, while for YBS14 is single soil and single leaf treatment ($p<0.05$) (Table 3). Photosynthesis in almost all experimental variants was recorded at a higher rate 21 days after treatment with the respective yeast inoculum. When measuring stomatal conductance and transpiration intensity, higher values were found in almost all investigated variants compared to the control plants.

Based on these findings it could be concluded that there is no inhibitory impact on plant health status and physiology caused by the endophytic yeast strains colonization. Furthermore, treatment with YP6 and YBS14 enhance the photosynthetic potential as well as the plant productivity which is well demonstrated also by data for photosynthesis rate monitoring (Table 2 and 3).

Table 3. The intensity of photosynthesis (A. µmol m-2 s-1) stomatal conductance (Gs. µmol m-2 s-1) and intensity of transpiration (E. µmol m-2 s-1) in the different experimental conditions variants on the 7th and 21st days after treatment of tomato, pepper and eggplant with the strains YBS14 and YP6

Tested *Solanaceae* plant	Days after treatment (DAT)	Yeast strain	Method of treating plants	Photosynthesi. mol m^{-2} s^{-1}	Stomatal conductance. mol m^{-2} s^{-1}	Transpiration intensity. mol m^{-2} s^{-1}
Tomato	7TH DAT	Control	No treatment	8.444±2.76	0.678±0.06	73.71±5.06
		YBS14	LS	12.039±3.39	0.149±0.05	16.94±2.48
		YBS14	SD	6.813±2.78	0.145±0.03	14.73±1.78
		YP6	LS	9.717±2.01	0.511±0.05	71.03±4.53
		YP6	SD	6.787±1.97	0.358±0.08	44.51±3.12
	21ST DAT	Control	No treatment	3.384±0.68	0.296±0.09	38.23±6.68
		YBS14	LS	4.901±0.98	0.133±0.08	16.74±3.07
		YBS14	SD	3.189±1.03	0.321±0.06	48.28±3.64
		YP6	LS	6.489±1.66	0.191±0.05	18.69±3.43
		YP6	SD	1.368±0.28	0.132±0.05	12.79±2.17
Pepper	7TH DAT	Control	No treatment	15.153±1.45	1.11±0.07	14.16±2.87
		YBS14	LS	8.922±1.32	0.183±0.04	19.06±3.54
		YBS14	SD	11.509±2.03	0.163±0.05	18.39±2.90
		YP6	LS	8.635±1.32	0.146±0.03	15.27±3.18
		YP6	SD	11.723±1.63	0.221±0.02	23.5±4.64
	21ST DAT	Control	No treatment	2.937±0.16	0.098±0.01	11.68±2.06
		YBS14	LS	8.671±1.56	0.26±0.02	36.88±3.78
		YBS14	SD	4.209±0.48	0.107±0.01	12.64±2.38
		YP6	LS	8.924±1.36	0.156±0.02	17.67±3.03
		YP6	SD	7.301±0.14	0.188±0.02	23.74±2.77

Eggplant	7TH DAT	Control	No treatment	9.096±0.98	0.417±0.03	43.01±3.54
		YBS14	LS	8.937±1.06	0.371±0.04	49.07±3.96
		YBS14	SD	11.681±2.03	0.596±0.04	96.01±4.25
		YP6	LS	16.31±2.56	0.848±0.06	146.97±6.78
		YP6	SD	18.831±2.67	0.622±0.08	83.61±5.74
	21ST DAT	Control	No treatment	3.03±0.676	0.11±0.02	12.31±2.58
		YBS14	LS	7.09±1.23	0.438±0.03	74.41±4.48
		YBS14	SD	4.502±0.690	0.293±0.05	40.97±3.28
		YP6	LS	11.578±1.93	0.227±0.03	27.65±2.49
		YP6	SD	13.474±2.36	0.158±0.02	17.82±3.63
Tobacco	7TH DAT	Control	No treatment	4.665±2.28	0.648±0.04	18.98±3.78
		YBS14	LS	6.265±1.36	0.581±0.09	17.35±2.99
		YBS14	SD	7.887±1.96	0.644±0.06	18.02±3.01
		YP6	LS	4.683±1.93	0.192±0.03	6.04±1.34
		YP6	SD	6.685±1.32	0.534±0.05	15.50±2.87
	21ST DAT	Control	No treatment	12.086±4.33	0.136±0.01	4.14±1.69
		YBS14	LS	13.402±2.76	0.139±0.01	3.32±1.10
		YBS14	SD	15.528±2.58	0.182±0.01	4.65±1.25
		YP6	LS	12.689±3.54	0.257±0.03	6.51±2.30
		YP6	SD	15.931±3.91	0.391±0.06	10.25±3.55

Legend: LS-leave spraying; SD-soil drenching

CONCLUSIONS

In conclusion, the newly isolated strains were characterized by physiological-biochemical and molecular-genetic studies. In both inoculation methods, colonization was observed in all parts of the tested plant. This testifies to the ability of the tested yeasts to move up and down in plants. *P. fermentans* YP6 and *S. cerevisiae* YBS14 are capable of endophytic colonization of Solanaceae plants without damaging their tissues. YP6 and YBS14 can produce and synthesize physiologically active substances (indole-3-acetic acid. siderophores) and stimulate plant growth and reduce pathogen infection as well as biotic and abiotic stress. They have a stimulating effect on photosynthesis, stomatal conductivity, and transpiration intensity. YP6 and YBS14 have a high potential to be used as a biocontrol agents in agriculture. The results are of interest for the development of multifunctional biological preparations with different biological activities. YP6 and YBS14 strains are promising for inclusion in commercial PGP products for sustainable agriculture.

ACKNOWLEDGEMENTS

We are grateful for the financial support by the Centre of Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University of Plovdiv by grant number 01-20.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

QUALITATIVE CHARACTERISTICS OF FODDER FROM LEGUME AND GRASS CROPS IN PURE AND MIXED GRASS STANDS

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Abstract

The data from the analysis indicate that T. pratense in a pure crop shows the highest resistance and adaptability in foot-hill conditions, and in mixed with L. perenne maintains the highest presence. Grass stands of T. repens had the highest CP content (210.9 g kg^{-1}). The excess was by 9.6% (compared to L. corniculatus), 16.6% (compared to T. pratense) and 32.1% (compared to the average value). It is the crop with the lowest content of CFr (a decrease of 27.2% compared to the average value). The highest protein content was found in the biomass of L. perenne with T. pratense (151.5 g kg^{-1}), followed by the mixture with L. corniculatus (144.8 g kg^{-1}) and T. repens (129.7 g kg^{-1}). Grass stands of L. corniculatus and its mixtures with L. perenne had the highest content of DM. T. repens (63.7 g kg^{-1}) and the mixture of L. perenne with T. pratense (63.4 g kg^{-1}) had the highest ash content.

***Key words**: legume and grass fodder crops, two-component mixtures, fodder quality.*

INTRODUCTION

The increased interest in ecologically clean agriculture leads to the search for crops with high adaptation and adjustability to the agro-ecological characteristics of the area. Cultivation of fodder species typical for mountain conditions is a suitable alternative for obtaining high yields and quality plant matter that satisfies the fodder needs of animals.

The competitive strength of the monoculture depends on the relative share of the species involved in the above-ground mass. Species interactions (synergistic or antagonistic) affect use regime, biodiversity, yield and weed infestation in grass stands (Finn et al., 2013; Black et al., 2017). The biological cultivation of garsses as monocultures is a prerequisite for vigorous weed infestation of crops and limited nitrogen supply of plants. The legume components increase the amount of nitrogen in the grass mass and a decrease in weed infestation degree (Arlauskienė et al., 2021). In a number of agroecosystems, nitrogen is an element that influences the provision of high yields in the formation of fodder mass (Thilakarathna, 2016), whereas mixed crops have a lower weed infestation degree (8%) compared to monocultures (43%) (Finn et al., 2012). Perennial ryegrass grown in mixed crops with legumes contributes to higher yields (Vasileva, 2015).

For the foot-hill conditions of Central Northern Bulgaria, Bulgarian selection populations of perennial ryegrass were studied in mixtures with red clover (Naydenova et al., 2010). For the experimental period, the legume component strongly dominated the grass stand. The perennial ryegrass in the mixture contributes to increase the quality characteristics of the fodder, as well as to maintain a sustainable balance between the cereal and leguminous components. Red clover (*T. pratense*) is the main species for maintaining fodder resources in the ecosystems of the foot-hill and mountain regions of Bulgaria (Naydenova & Bozhanska, 2014), white clover (*T. repens*) plays an important role in obtaining high-quality fodder (Mihovski & Sabeva, 2011), and the fodder mass of bird's-foot-trefoil (*L. corniculatus*) has a high nutritional value and digestibility ranging from 65% to 75% (Churkova et al., 2016; Churkova & Churkova, 2020).

The objective of the present experiment is to make a qualitative characterization of fodder resulted from legume and grass meadow species, grown as monocultures or in mixtures, under mountain conditions by following the botanical and chemical composition of the grass vegetation.

MATERIALS AND METHODS

The experiment was conducted in the period 2020-2022 at the Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Bulgaria), on a light gray pseudopodzolic soil under non-irrigated conditions. The experiment was based on the block method, in 4 replications with a plot size of 5 m^2. The preparation of the experimental areas included: plowing in autumn, and in spring - disking and milling. The seeds were spread by hand, and then the land was rolled. For sowing the monoculture and two-component mixed grass stands, the following cultivars were used, respectively: perennial ryegrass, red clover, white clover and bird's-foot-trefoil.

The experimental variants included:
Monoculture grass stands
1. Perennial ryegrass (*Lolium perenne* L.)
2. Red clover (*Trifolium pratense* L.)
3. White clover (*Trifolium repens* L.)
4. Bird's-foot-trefoil (*Lotus corniculatus* L.)

Mixed grass stands:
5. Perennial ryegrass (*Lolium perenne* L.) + Red clover (*Trifolium pratense* L.)
6. Perennial ryegrass (*Lolium perenne* L.) + White clover (*Trifolium repens* L.
7. Perennial ryegrass (*Lolium perenne* L.) + bird's-foot-trefoil (*Lotus corniculatus* L.)

The grass stands with monoculture were mowed manually in the initial phase of ear formation of grasses and bud-formation period until the beginning of blossoming of the legumes. Crops with mixed cultivation of grass and legume species were harvested in the phase of ear formation of the grasses and bud-formation period of the legume meadow grasses.

The following indicators were studied:
Botanical composition of the grass stand (%) - determined by weight, by analyzing grass samples by groups (grasses, legumes and weed vegetation), taken immediately before mowing.
The chemical composition of the dry mass according to the Weende method includes:

o Crude fiber (CFr, g/kg^{-1}) according to the *Weende* analysis - the sample is treated sequentially with solutions of 1,25% (w/v) H_2SO_4 and 1,25% (w/v) NaOH. The residue is dried, ashed and weighed.

o Crude protein (CP, g/kg^{-1}) according to Kjeldahl (according to BDS/ ISO-5983) - to decompose the organic matter, the sample is boiled with sulphuric acid in the presence of a catalyst. The acidic solution is alkalified with sodium hydroxide solution. The ammonia is distilled and collected in a certain amount of sulphuric acid, the excess of which is titrated with a standard solution of sodium hydroxide. Alternatively, the separated ammonia is distilled in surplus of boric acid solution and then titrated with hydrochloric or sulphuric acid solution.

o Crude fats (CF, g kg^{-1}) - were analyzed by extraction in a Soxhlet type extractor with a non-polar organic solvent. After the extraction, the sample is dried in a laboratory dryer at 95ºC to a constant weight.

o Ash (g/kg^{-1}) - decomposition of organic matter by gradual combustion of the sample in a muffle furnace at 550°C.

o Moisture content (g kg^{-1}) - drying the sample in a laboratory dryer at a temperature of 105°C to a constant weight.

o Dry matter (DM, g/kg^{-1}) - empirically calculated from % of moisture.

o Calcium (Ca, g kg^{-1}) - according to Stotz (complexometric) and Phosphorus (P, g kg^{-1}) – with a vanadate-molybdate reagent according to the method of Gerike and Kurmis (Sandev, 1979) - spectophotometer (*Agilent* 8453 UV - *visible Spectroscopy System*) measuring in the 425 ηm region.

o NFE (Nitrogen-free extractable substances) = 100 – (CP, % + CFr, % + CF, % + Ash, % + Moisture, %), converted to g kg^{-1}.

Statistical analysis of data included one-way analysis of variance (ANOVA) and multiple comparison of means by least statistically significant difference ($LSD_{0.05}$).

RESULTS AND DISCUSSIONS

Botanical composition of monoculture grass stands of grass and legume fodder crops

Weed vegetation was present at a high percentage in the grass stand with the monocultures of grass and legumes in the year of sowing (Figure 1). In the variant with perennial ryegrass as a pure crop, the ratio of grasses compared to weed vegetation was 56.5:43.5%, clearly indicating significant weed infestation of the grass stand. Of the legume crops, white clover had the lowest presence in the crop, and its ratio with weed vegetation was 57.5:42.5%. The ratio of components in the biomass of bird's-foot-trefoil was 61.2:38.8%, respectively legume: weed.

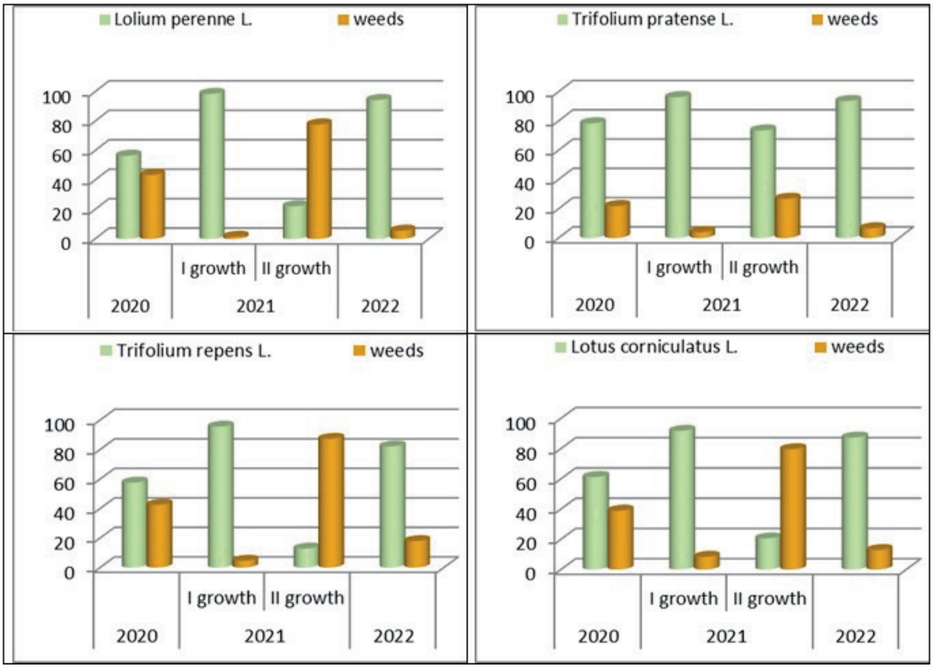

Figure 1. Botanical composition (%) of monoculture grass stands of grass and legume meadow species (by years)

Under favourable conditions and good seeding density, red clover significantly suppressed the development of weed vegetation (Naydenova, 2002). The results of the study indicate that in the first vegetation, the monoculture grass stands of red clover had a lower weed infestation degree, as the legume:weed ratio was 78.3:21.7%.

For the second experimental year, the weed infestation of the monoculture grass stands in the legume crops in the first cut ranged from 3.8% (*T. pratense*) to 8.3% (*L. corniculatus*). The results of the analysis indicate that the main crop had a high presence in the grass stand, namely: red clover with 96.2%, white clover with 95.5% and bird's-foot-trefoil with 91.7%.

L. perenne also showed a high share percentage (98.6%) in the formed biomass and a low percentage of weed infestation (1.4%).

In the second cut in 2021, the weed infestation rate was high in all tested variants of the legume and grass fodder crops. White clover had the largest share of weed vegetation in the grass stand (87.2%), whereas bird's-foot-trefoil and perennial ryegrass had close indicator values of 79.5% and 77.8%, respectively. Red clover had the highest share percentage in the plant biomass at a ratio of 73.3:26.7% between legumes: weeds, respectively.

In the third vegetation, the highest degree of weeding was registered in the monoculture grass stands with white clover (legume: weed ratio of 82.1:17.9%), followed by bird's-foot-trefoil (legume: weed ratio of 87.2:12.8%) and red clover (legume: weed ratio of 93.5:6.5%).

Perennial ryegrass had a high share (94.4%) in the above-ground mass compared to weed vegetation (5.6%).

Botanical composition of perennial two-component mixtures of grass and legume fodder crops

The proportional share and specific features of the ingredients in the composition of the grass stands are decisive for the sustainability and use of the mixture (Bozhanska, 2017).

In the year of sowing, perennial ryegrass occupied the largest share in mixtures with bird's-foot-trefoil. The ratio of grass and legume crops was 56.0:20.0%. *L.perenne* was also a dominant component (with 41.5%) in the mixture with white clover (23.3%), where was the highest percentage of weeds (35.2%) - Figure 2. Borawska-Jarmułowicz et al. (2012) tested meadow grasses in mixed crops indicating that perennial ryegrass (cv. Naki and Bajka) in the first experimental year appeared as an aggressive species compared to the other components in the mixture.

In the first experimental year, the mixed crop of perennial ryegrass with red clover had the most balanced ratio of both components (grasses: legumes - 43.1:36.8%). The grass stands also had the lowest weed infestation (20.1%) compared to the mixtures of perennial ryegrass with bird's-foot-trefoil (24.0%) and white clover (35.2%).

In the first cut of the second experimental year (2021), the weed infestation of the crops in the three tested variants was relatively low. The perennial ryegrass-red clover mixture had an absolute balance of both components (47.6:47.6%) and the highest share of weed vegetation (4.8%) in the above-ground biomass. Perennial ryegrass dominated the spring regrowths of the white clover and bird's-foot-trefoil mixtures, as the share of grass, legume and weed vegetation was 80.9:17.0:2.1% and 85.7:13.4:0.9%, respectively. The variants of perennial ryegrass with bird's-foot-trefoil had the highest share of the grass component and the lowest weed infestation.

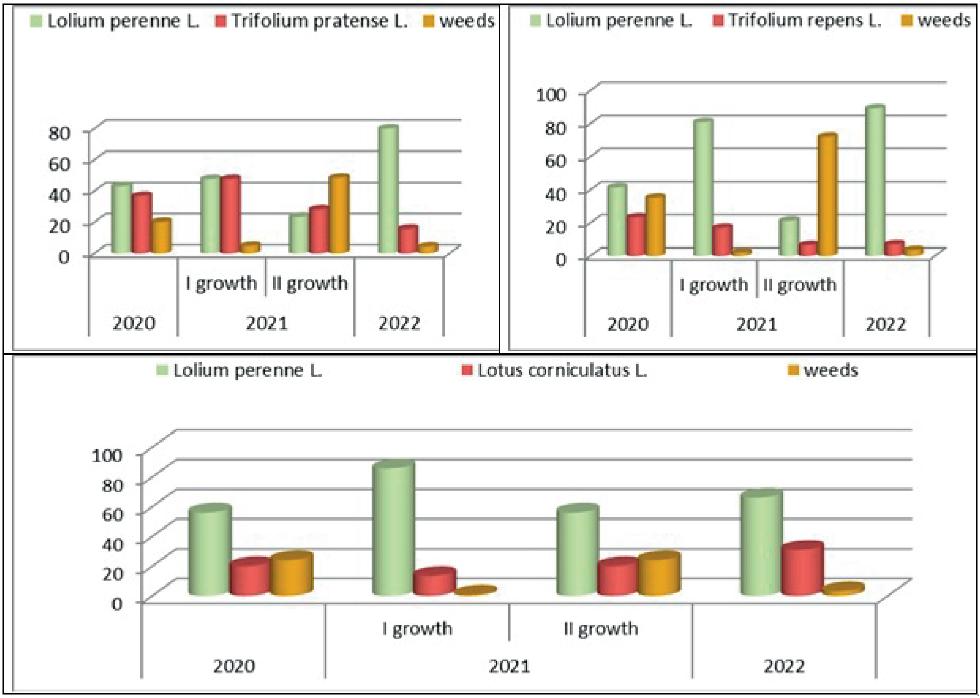

Figure 2. Botanical composition (%) of perennial two-component mixtures of grass and legume meadow species (by years)

In contrast, the second cut of the second vegetation marked significant weed infestation of the tested perennial grass-legume mixtures. The percentage of weeds varied from 24.0% (*L. perenne* + *L. corniculatus*) to 72.0% (*L. perenne* + *T. repens*). Perennial ryegrass had the highest share in the mixed crop with bird's-foot-trefoil, as the ratio of grass to legume component was 56.0:20.0%.

The mixture of perennial ryegrass with red clover also in this regrowth had the most balanced percentage ratio of the tested components with 23.4:28.1% compared to the other two variants. The lowest share of grass and legume crops in the grass stand was reported for the mizture of *L. perenne* + *T. repens*, respectively 21.3:6.7% at a high degree of weed infestation.

In the third experimental year, the weed infestation level in the mixed grass stands was relatively low. The values of the indicator vary from 3.1% (*L. perenne* . + *L. corniculatus*) to 4.3% (*L. perenne* + *T. pratense*). Perennial ryegrass dominated all three tested mixtures, as the highest share was found in the white clover grass stands, where the grass: legume: weed ratio was 89.2:7.2:3.6%, respectively.

Studies in mixed grass stands show that with the age of the grass stands, the share of grasses in the grassland increases and that of legumes and weeds decreases (Nešić et al., 2007). Compared with the second vegetation, where in the mixture with red clover the share of the components was almost equal, there was a significant decrease of the legume component in the third vegetation. The share of the perennial ryegrass was 80.0%, and the red clover was 15.7%.

In the third vegetation, the highest share of bird's-foot-trefoil was found in the grass stand (30.9%) with perennial ryegrass, compared to the first and second experimental years. The least weed infestation degree was registered in this mixture (3.1%), in comparison with other miztures, with a high share of perennial ryegrass (66.0%).

The botanical analysis of the monoculture grass stands with legume fodder crops indicated that *T. pratense* had the highest resistance and adaptability to the pre-mountain conditions of the Central Balkan Mountain. This variant had the lowest weed infestation degree and the highest share of the main crop in the formed biomass. This trend is also confirmed with the share of the legume component in the mixed grass stands. Compared to the other legume species during the experimental years, red clover retained relatively the highest share (over 32.0%) in mixtures with perennial ryegrass, followed by bird's-foot-trefoil (over 21.0%) and white clover (over 13.0%). *L. perenne* as a monoculture, registered a high share of the grass component (over 67.0%) in the above-ground matter volume, with a share of weed vegetation less than 33.0%. In the mixed grass stands, perennial ryegrass maintained the highest share in the two-component mixture with bird's-foot-trefoil (<65%), followed by the mixture with white (<58%) and red clover (<48%).

Main chemical composition of monoculture and mixed grass stands of legume and grass fodder crops

The chemical composition of the fodder mass is a determining factor for the quality of the grass stands (Bozhanska, 2017).

In the year of sowing, in the monoculture grass stands of legume meadow grasses, white clover recorded the highest crude protein (214.1 g kg^{-1} DM), ash (83.6 g kg^{-1} DM), calcium (26,0 g kg^{-1} DM) and phosphorus (2.2 g kg^{-1} DM) - Table 1. The dry matter of the bird's-foot-trefoil contained the highest fiber content (338.9 g kg^{-1} DM) and the lowest level of macronutrients, such as calcium (14.7 g kg^{-1} DM) and phosphorus (0.7 g kg^{-1} DM).

Red clover as a monoculture has the lowest amount of protein fraction (181.5 g kg^{-1} DM) and the highest content of nitrogen-free extractable substances (391.6 g kg^{-1} DM), but compared to the other two-component grass stands, its mixture with perennial ryegrass had the highest values regarding the concentration of crude protein (139.8 g kg^{-1} DM) and mineral substances (83.7 g kg^{-1} DM).

Perennial ryegrass is grass fodder crops with great competitive ability (Bozhanska, 2017) and rapid development (Katova, 2016). During the first vegetation, it prevails in the plant biomass compared to the legume representative, which is a prerequisite for the formation of biomass with a high carbohydrate content. In its mixture with red clover, the values of nitrogen-free extractable substances were the highest (382.1 g kg^{-1} DM). The mixed grass stands of *L. perenne* with *L. corniculatus* had the lowest crude protein (127.0 g kg^{-1} DM), ash (71.5 g kg^{-1} DM) and NFE (350.5 g kg^{-1} DM) and those with white clover had the highest concentration of crude fat (35.2 g kg^{-1} DM) and calcium (21.5 g kg^{-1} DM).

In the second vegetation, in monoculture grass stands of *Trifolium* species, white clover again had the highest crude protein content (217.3 g kg^{-1} DM), followed by bird's-foot-trefoil (194.4 g kg^{-1} DM) and red clover (168.2 g kg^{-1} DM). Bird's-foot-trefoil biomass has the highest values of dry matter (926.0 g kg^{-1} DM) and crude fiber (413.3 g kg^{-1} DM), whereas red clover had the lowest with 1.6% respectively (for DM) and 36.5% (for CFr). The amount of macronutrients in the dry matter in the monoculture cultivation of legumes varied from 15.3 g kg^{-1} DM (*L. corniculatus*) to 17.8 g kg^{-1} DM (*T. pratense*) - for calcium and from 2.5 g kg^{-1} DM (*T. pratense*) to 3.2 g kg^{-1} DM (*T. repens*) - for phosphorus.

Animals are more willing to eat grass stands including many species from grass families of cereals and legumes (Baikalova et al., 2019).

Table 1. Chemical composition (g kg^{-1} DM) of legume and grass fodder crops in monoculture grass stands and perennial two-component mixtures, over the years and average for the period 2020-2022

Variants	DM	CP	CFr	CF	Ash	NFE	Ca	P
				2020				
Lolium perenne L.	900.9	101.6	416.8	40.7	86.3	255.5	14.7	2.5
Trifolium pratense L.	894.1	181.5	220.6	24.9	75.5	391.6	17.0	1.0
Trifolium repens L.	890.7	214.1	197.8	29.7	83.6	365.5	26.0	2.2
Lotus corniculatus L.	899.4	196.2	338.9	30.5	60.8	273.0	14.7	0.7
L. perenne L.+*Tr. pratense* L.	894.0	139.8	257.1	31.3	83.7	382.1	12.5	2.0
L. perenne L.+*Tr. repens* L.	895.0	135.6	277.8	35.2	76.6	369.8	21.5	2.2
L. perenne L.+*L. corniculatus* L.	897.2	127.0	313.6	34.6	71.5	350.5	21.4	3.1
				2021				
Lolium perenne L.	916.8	102.1	413.9	25.1	50.8	325.0	17.7	1.9
Trifolium pratense L.	911.1	168.2	302.8	44.9	69.8	325.6	17.8	2.5
Trifolium repens L.	916.6	217.3	349.8	26.4	70.4	252.8	16.6	3.2
Lotus corniculatus L.	926.0	194.4	413.3	36.0	59.2	223.2	15.3	2.9
L. perenne L.+*Tr. pratense* L.	920.7	147.4	486.2	22.3	59.9	204.9	12.2	2.2
L. perenne L.+*Tr. repens* L.	916.7	131.8	403.2	31.7	55.8	294.4	12.2	1.9
L. perenne L.+*L. corniculatus* L.	921.3	151.0	365.3	26.5	53.6	325.0	13.2	2.2
				2022				
Lolium perenne L.	910.3	105.5	359.1	16.3	44.2	385.2	17.9	2.1
Trifolium pratense L.	907.1	192.9	381.4	19.3	39.0	274.5	21.2	3.4
Trifolium repens L.	911.1	201.4	259.8	26.5	37.2	386.2	21.1	2.6
Lotus corniculatus L.	911.9	186.9	278.4	23.6	45.2	377.8	10.1	3.3
L. perenne L.+*Tr. pratense* L.	913.3	167.3	388.3	11.3	46.7	299.7	14.5	1.9
L. perenne L.+*Tr. repens* L.	915.6	121.8	387.9	16.3	45.2	344.4	12.2	5.5
L. perenne L.+*L. corniculatus* L.	911.6	156.3	374.9	20.7	40.6	319.1	11.0	4.1
				2020-2022				
Lolium perenne L.	909.3	103.1	396.6	27.4	60.4	321.9	16.8	2.1
Trifolium pratense L.	904.1	180.9	301.6	29.7	61.4	330.6	18.7	2.3
Trifolium repens L.	906.1	210.9	269.1	20.9	63.7	334.8	21.2	2.7
Lotus corniculatus L.	912.4	192.5	343.5	30.0	55.1	291.3	13.4	2.3
L. perenne L.+*Tr. pratense* L.	909.3	151.5	377.2	21.6	63.4	295.6	13.1	2.0
L. perenne L.+*Tr. repens* L.	909.1	129.7	356.3	27.7	59.2	336.2	15.3	3.2
L. perenne L.+*L. corniculatus* L.	910.0	144.8	351.3	27.3	55.2	331.5	15.2	3.1
Average	***908.6***	***159.0***	***342.2***	***26.4***	***59.8***	***320.3***	***16.2***	***2.5***
MAX	***912.4***	***210.9***	***396.6***	***30.0***	***63.7***	***336.2***	***21.2***	***3.2***
MIN	***904.1***	***103.1***	***269.1***	***20.9***	***55.1***	***291.3***	***13.1***	***2.0***
Significance of the differences in the values of the indicator over the years	P > 0.05	P < 0.001	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Significance of differences in indicator values between variants	**P < 0.001**	P > 0.05	**P = 0.01**	**P < 0.01**	**P < 0.001**	P > 0.05	P > 0.05	P > 0.05

Multi-species cenoses are more productive and more stable than single-species ones provide a well-balanced nutritious food (Khramoj et al., 2019; Eichler-Löbermann et al., 2020). Plants from the Fabaceae family synthesize about 1.5-

3 times more protein than Poaceae (Stagnari et al., 2017; Kulkarni et al., 2018).
In the second vegetation, monoculture grass stands with perennial ryegrass were lower in ash (5.4 to 17.9%) and phosphorus (2.1 to 18.0%), but richer in nitrogen-free extractable substances (with from 0.4 to 58.6%) and calcium (with from 33.7 to 45.3%) compared to the mixed ones. Associated with legume fodder crops, the crude protein in the dry matter of its two-component mixtures increased from 29.1% (*L. perenne* + *T. repens*) to 48.0% (*L. perenne* + *L. corniculatus*) , and the crude fiber content varied from 365.3 g kg^{-1} DM (*L. perenne* + *L. corniculatus*) to 486.2 g kg^{-1} DM (*L. perenne* + *T. pratense*).
In the third vegetation, the maximum values of crude protein (201.4 g kg^{-1} DM), crude fat (26.5 g kg^{-1} DM) and nitrogen-free extractable substances (386.2 g kg^{-1} DM) were found in the monoculture grass stands with white clover. The biomass is distinguished by the lowest content of crude fiber (259.8 g kg^{-1} DM) and mineral substances (37.2 g kg^{-1} DM).
Grass stands with *T. pratense* had the highest fiber content (381.4 g kg^{-1} DM), calcium (21.2 g kg^{-1} DM) and phosphorus (3.4 g kg^{-1} DM) and low concentration of crude fat (19.3 g kg^{-1} DM) and nitrogen-free extractable substances (274.5 g kg^{-1} DM).
The grass mass of *L. corniculatus* had the lowest crude protein content (7.8% lower than the maximum indicator value) in dry matter and a relatively low crude fiber concentration (37.0% lower than the maximum value of the indicator) compared to that of the other legume grasses grown as monocultures.
In the third vegetation, the share of *L. perenne* in the white clover grass stand was < 89.0%, which affected the percentage of protein in the fodder mass. The mixture, compared to the others included in the experiment (*L. perenne* + *T. pratense*; *L. perenne* + *L. corniculatus*), had the highest content of dry matter (915.6 g kg^{-1} DM), NFE (344.4 g kg^{-1} DM) and phosphorus (5.5 g kg^{-1} DM), but poorest in crude protein (121.8 g kg^{-1} DM).
The values of these indicators are completely opposite in the mixture of perennial ryegrass with red clover, where the amount of crude protein (167.3 g kg^{-1} DM) and calcium (14.5 g kg^{-1} DM) was the highest, and that of nitrogen-free extractable substances (299.7 g kg^{-1} DM) and phosphorus (1.9 g kg^{-1} DM) – the lowest. In the *L. perenne* + *T. pratense* mixture, the following regularity was observed: the high protein content was combined with the highest crude fiber content (388.3 g kg^{-1} DM). This atypical manifestation is due to the botanical characteristics of the crops in the grass association, in this case the reaction of the legume crop to the grass crop and the regime (mowing) of grass stands use. In Norway, mixed grass stands dominated by perennial ryegrass include species (*Agrostis capillaris* L., *Trifolium pratense* L., *Trifolium repens* L.) that balance the composition of the grass stand, increase the productivity and quality of the fodder mass (Jørgensen et al., 2019).
The mixture *L. perenne* + *L. corniculatus* recorded the lowest content of fiber fraction (374.9 g kg^{-1} DM) and calcium (11.0 g kg^{-1} DM), and the highest of crude fat (20.7 g kg^{-1} DM).
The amount of mineral substances in the mixed grass stands varied from 40.6 g kg^{-1} DM (*L. perenne* + *L. corniculatus*) to 46.7 g kg^{-1} DM (*L. perenne* + *T. pratense*).
Averaged over the period, white clover in monoculture grass stands with legume fodder crops had the highest dry matter crude protein content (210.9 g kg^{-1}). The excess is respectively 9.6% (compared to bird's-foot-trefoil), 16.6% (compared to red clover) and 32.6% (compared to the average). The difference in the values of the indicator is reliable ($P < 0.001$) and highly dependent on the conditions of the year. In the two-component mixtures, the biomass of perennial ryegrass with red clover had the highest protein content was (151.5 g kg^{-1} DM) followed by its mixture with bird's-foot-trefoil (144.8 g kg^{-1} DM) and white clover (129, 7 g kg^{-1} DM).
The difference in the crude fiber content of the studied monoculture and mixed grass stands of perennial ryegrass and legume fodder crops was statistically significant ($P = 0.01$). The lowest fibre content (269.1 g kg^{-1} DM) was found in the dry mass of white clover from the group of legumes. The decrease was 27.2% compared to the average value of the indicator. In the mixed grasslands, the amount of crude fiber was 2.6% higher (*L. perenne* +

L. corniculatus) to 10.2% (*L. perenne* + *T. pratense*) compared to the average (342.2 g kg^{-1} DM).
It was established that the cultivation method and the composition of the grass stand are factors that have a significant impact (*P < 0.001*) on the concentration of dry matter and mineral substances in the investigated grass stands. On average for the period, the monoculture grass stands of *L. corniculatus* (912.4 g kg^{-1} DM) and its mixtures with *L. perenne* (910.0 g kg^{-1} DM) had the highest dry matter content. The excess in the average values of the indicators were by 0.2-0.4% respectively. White clover (63.7 g kg^{-1} DM) and the mixture of perennial ryegrass with red clover (63.4 g kg^{-1} DM) recorded the highest ash content with an excess of 6.6 to 7.2% over the average of the indicator.

CONCLUSIONS

In monoculture cultivation, the relative share of *L. perenne* in the grass stand was over 67.0%, and in mixed crops it maintained the highest share in the two-component mixture with bird's-foot-trefoil (<65%), followed by the mixture with white (<58%) and red clover (<48%).
Monoculture grass stands of *T. pratense* show the highest resistance and adaptability in foot-hill conditions, and in mixed grass stands with *L. perenne* it retained the highest share (over 32.0%).
Averaged over the period, white clover in monoculture grass stands had the highest crude protein content (210.9 g kg^{-1}) in the dry matter. In the two-component mixtures, the biomass of perennial ryegrass with red clover had the highest protein content (151.5 g kg^{-1} DM). The excess in the values of the indicator was by 16.8% (compared to the mixture with white clover) and 4.6% (compared to the mixture with bird's-foot-trefoil).
1. When growing *L. perenne* in two-component mixtures with L. corniculatus, *T. repens* and *T. pratense*, the proportion of unwanted vegetation in the obtained biomass decreases significantly compared to that of its monoculture cultivation.
2. The mixed cultivation of *L. perenne* with the indicated medium-long-lasting leguminous grasses adapted to the area positively affects the main indicators of forage quality of the obtained biomass. The biomass of the mixture of *L. perenne* and *T. pratense* has the highest protein content. The studied mixtures are also distinguished by a more balanced protein and fiber content, which causes a higher digestibility of the feed.
3. *T. pratense* can be defined as the most suitable legume component for creating hay mixtures with *L. perenne* for the foothill conditions of the Central Balkan Mountain.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

WHEAT YIELD RESULTS UNDER THE INFLUENCE OF N, P, K FERTILIZATION AND CLIMATIC CONDITION

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Abstract

The aim of the work was to carry out a study on the influence of climatic conditions and fertilization with chemical fertilizers on wheat production in the specific conditions of Didactical Farm (SDE) of the University of Life Sciences "King Mihai I" from Timisoara (ULST). A Fundamental contribution to the increase of the yield per unit area is made by the level of N, P. K fertilization and optimal soil and climatic conditions for exploiting the productive potential of the cultivated variety. The high ecological plasticity of wheat and its constant productions means that farmers are still very interested in this crop. Growers are also interested in the crop with the highest yield per unit area. The aim of this paper is to highlight the yield results of Ciprian wheat variety, obtained in the soil and climatic conditions of SDE during 2019-2020, under the influence of nitrogen, phosphorus and potassium fertilization in order to determine the growers to choose an optimal wheat fertilization option. Wheat yield obtained was determined by nitrogen fertilizer application from the control variant as follows: N_{30}- 485gk/ha, N_{60}-584 kg/ha, N_{90}-605 kg/ha, at all four rates the differences are statistically assured as highly significant.

Key words: *cultivars, fertilization, soil and climatic conditions, yield components.*

INTRODUCTION

For more than 35% of the world's population, wheat (*Triticum aestivum* L.) is the main source of nutrition, providing more than 45% of calories and more than 40% of protein for the world's population (Imbrea, 2011, Erekul, 2012). Nutrition management is one of the approaches to improve crop yields (Sowers, 1994), Wheat depletes nutrients from the soil, so if it is not properly fertilised, soil fertility starts to decline (Arregui, 2008). Therefore, fertiliser applications are essential to maintain a positive nutrient balance by replacing nutrients that are absorbed and lost during cultivation (Blandino, 2015). However, increasing nutrient use efficiency is essential to achieve the expected yield using as little fertiliser as possible (Şmuleac, 2020). Using the right fertilizer in the right amount is one of the most important management strategies to increase fertilizer efficiency and maximize crop productivity. (Školníková, 2022) The application of mineral fertilisers in wheat increases the nitrogen, phosphorus and potassium available in the soil (Schlesinger, 1992). Optimal fertiliser dosage improves wheat yield and fertiliser use efficiency as well as reducing pollution In addition, the right combination of primary nutrients is also important to increase wheat yield and nutrient use efficiency (Harrison, 2001). In cereal crops, overall N use efficiency has been found to be 33% (Mohammed, 2013). Nutrient use efficiency decreases with increasing N dose, while increasing crop yield reported lower N use efficiency (27.1%) from a N dose of 120 kg N ha compared to a N dose of 30 kg N ha with a N use efficiency of 39.27% (2021). One of the reasons for the lower N efficiency is N losses, limiting only 50% of the applied N fertilizer available for cereals (Raun, 1999; Chen, 2008; Cameron, 2013). Apart from the individual effects of nutrients, the interaction between nutrients is also crucial for nutrient yield and efficiency. Nitrogen helps plants to use potassium, phosphorus and other nutrients efficiently. The efficiency of N and P use, as well as the productivity and quality of agricultural products could benefit from increased K fertilisation (Kozlovský, 2018).

MATERIALS AND METHODS

The wheat variety used in the research was Ciprian, a variety developed at SCDA Lovrin, recognized for its superior quality characteristics and currently grown on large areas in the western part of the country.

In the experimental field located in the SDE Timisoara, were organized every year bifactorial experiments, with the grading of experimental factors:

Factor A - P and K fertilization level, with 5 graduations: a1 - P_0K_0; a2 - $P_{40}K_0$; a3 - $P_{80}K_0$; a4 - $P_{40}K_{40}$; a5 - $P_{80}K_{80}$

Factor B - N fertilisation level, with 5 graduations: b1 - N_0; b2 - N_{30}; b3 - N_{60}; b4 - N_{90}; b5 - N_{120}.

Elements of the applied technology: - The experiment located in the Experimental Station Timisoara in the first year of the experiment the sowing of wheat was carried out on 20.10.2018 and the harvesting of the crop was carried out on 07.07.2019; in the second year of the experiment the date of sowing the crop was 19.10.2019, with the harvesting of the wheat on 29.07.2020; and in the third year of the experiment the sowing was carried out on 10.11.2020 with the harvesting on 22.07.2021. The sowing density was 550 germinable grains/m^2.

In the first year in the wheat crop, an application of the systemic herbicide Pallas 250 g/ha + adjuvant 0.5 l/ha and a second herbicide application with Priaxor at a rate of 250 ml/ha was carried out during the vegetation, at the appearance of the first internode in wheat and in the rosette phase of weeds. Herbicides were applied when the air temperature was between 8-10°C.

In the 2020 and 2021 crops, one application of Gramitrel herbicide, 1 l/ha and one application of Trinity herbicide (2 l/ha) were made in vegetation, at weed rosette stage and at first internode emergence in both microzones studied.

Insecticides used for disease and pest control were: Catapult, at tillering stage (0.6 ml/ha); Krima (100 g/ha) and Twist Plus (1 l/ha), at bud stage and Cyperguard (0.6 ml/ha) at the emergence of spikelet.

For the processing and interpretation of the experimental results, the following programs were used: for analysis of variance - statistics [ANOVA], MSTATC; for correlations and regressions - statistics - Regressions and Graphs; procedures with formulas on factor contribution and DLs - in EXCEL; Cluster Analysis.

RESULTS AND DISCUSSIONS

Nitrogen fertilization has determined very significant increases, compared to the control variant, with the exception of the dose of N_{30} where a distinctly significant increase is obtained, as shown in Table 1.

Doses N_{30} - N_{120} exceed the control by 8% to 13%. The difference in production between the productions obtained at these doses compared to the N_0 control are between 378-688 kg/ha, differences statistically assured as distinct and very significant. The influence of fertilization with phosphorus and potassium on the wheat production obtained, the average of the years 2019-2021 is presented in Table 2. Compared to the unfertilized control P_0K_0 [a1], insignificant increases were obtained, except for the dose of P40K0 where a difference is obtained very significant, this difference is negative 397 kg/ha, i.e., the production obtained at the dose $P_{40}K_0$ compared to the control dose P_0K_0 is lower by 387 kg. Negative differences were obtained at all doses of P and K.

The production analysis carried out at the 5 doses of PK [factor A] which highlights the fact that it is between 4900 kg/ha obtained by applying the dose of $P_{40}K_0$ and approx. 5300 kg/ha obtained at the other 4 doses at P_0K_0, $P_{80}K_0$, $P_{40}K_{40}$ and $P_{80}K_{80}$. (Figure 1).

Table 1. Influence of nitrogen fertilization on wheat production, average years 2019-2021

Factor N	Yield		Diff	Signif.
	Kg/ha	%		
N_0	4811	100	mt	
N_{30}	5189	107.9	378	**
N_{60}	5498	114.3	688	***
N_{90}	5418	112.6	607	***
N_{120}	5264	109.4	453	***

DL 5% = 221 kg DL 1% = 293 DL 0.1% = 380

The production increases up to the dose of N_{60}, after which it decreases, as we can see from Figure 2. The lowest production is obtained with the unfertilized variant N_0 - 4800 kg/ha, and the highest with N_{60} -5500 kg/ha. So, there is a non-linear relationship between N and production.

Table 2. Influence of phosphorus and potassium fertilization on wheat production, average years 2019-2021

Factor P,K	Yield		Diff	Signif.
	Kg/ha	%		
P_0K_0	5316	100	mt	
$P_{40}K_0$	4929	92.7	-387	000
$P_{80}K_0$	5317	100.0	1	
$P_{40}K_{40}$	5315	100.0	-1	
$P_{80}K_{80}$	5304	99.8	-12	

DL 5% = 221 kg DL 1% = 293 DL 0.1% = 380

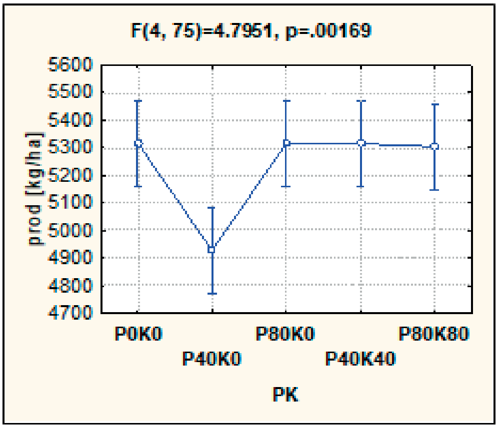

Figure 1. Variation of production under the influence of P and K fertilization (average years 2019-2021)

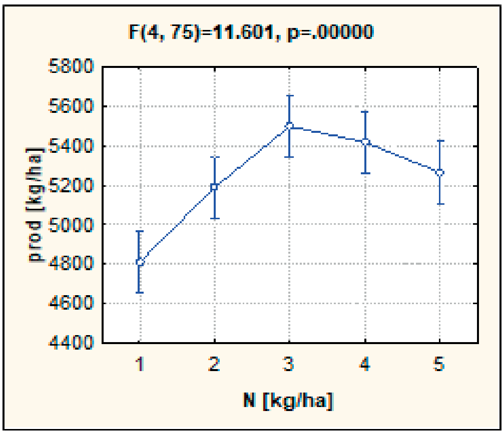

Figure 2. Variation of production under the influence of N fertilization (average years 2019-2021)

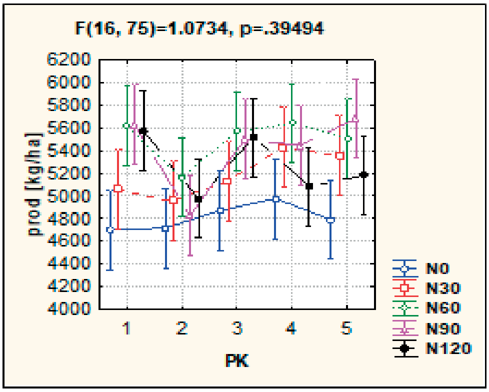

Figure 3. Variation of production by interaction of fertilization with P, K (average years 2019-2021)

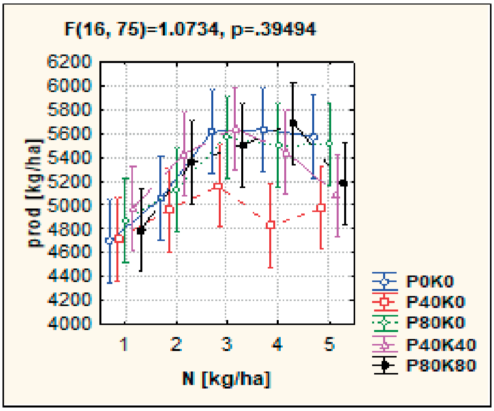

Figure 4. Variation of production by interaction of fertilization with N (average years 2019-2021)

Factor A[PK] contributes to the achievement of production in a proportion of 12.2%, factor B[N] with 29.4%, and the interaction AxB with 10.9%. (Figure 5).

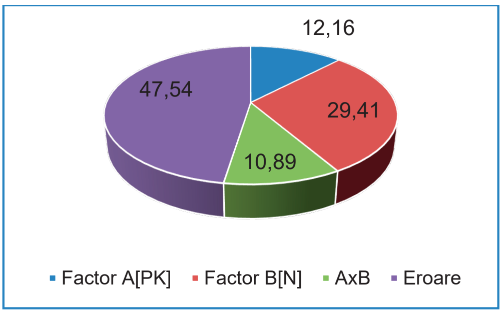

Figure 5. Contribution of P, K and N fertilisation and macroelement interaction (average years 2019-2021)

The influence of phosphorus and potassium fertilizers on protein content in the 2019-2021 cycle, in the experimental field at SDE Timişoara, presented in Table 3, shows us that, compared to the unfertilized control variant a1[P_0K_0], the following increases were obtained:

- insignificant, at the $P_{40}K_0$ dose level
- significant at the dose of $P_{80}K_0$
- distinctly significant on the agrofund of $P_{40}K_{40}$
- the highest value was statistically assured as highly significant at the dose of $P_{80}K_{80}$.

In conclusion, the increases varied between 0.2 and 0.7%. The results presented in Table 4, regarding the influence of nitrogen fertilizers on the average protein content (%) of the years 2019-2021, show us that, compared to the control b1[N_0], very significant increases were obtained regardless of the nitrogen dose applied. Spores have values between 2-5.3%.

Table 3. Influence of phosphorus and potassium fertilizers on protein content (%) average years, 2019-2021

Factor P, K	Protein	Diff	Signif.
P_0K_0	13.12	mt	
$P_{40}K_0$	13.32	0.19	ns
$P_{80}K_0$	13.58	0.46	*
$P_{40}K_{40}$	13.67	0.54	**
$P_{80}K_{80}$	13.80	0.67	***

DL 5% = 0.38 DL 1% = 0.51 DL 0.1% = 0.66

Table 4. Influence of nitrogen fertilizers on protein content (%), average years, 2019-2021

Factor N	Protein	Diff	Signif.
N_0	10.34	mt	
N_{30}	12.38	2.04	***
N_{60}	14.03	3.69	***
N_{90}	15.10	4.76	***
N_{120}	15.63	5.29	***

DL 5% = 0.38 DL 1% = 0.51 DL 0.1% = 0.66

The analysis of the protein content achieved at the 5 doses of PK [factor A] highlights the fact that it is between 13.1-13.8%. (Figure 6). Protein content increases with PK dose, increases are small from dose to dose.
The analysis of the protein content achieved at the 5 doses of N [factor A] (Figure 7), highlights the fact that it is between 10.3-15.6 percent. The protein content increases with the increase of the applied nitrogen dose. The highest percentage of protein [15.6] is obtained at N_{120}.
Protein content increases with nitrogen dose regardless of PK dose [b]. The highest values of the protein content are obtained at N_{90} and N_{120} regardless of PK [a], and the lowest at N0. (Figure 8).
Analysis of results regarding the influence of the interaction of AxB factors [PK x N], on protein content, 2019-2021 (Figure 9).
Factor A - fertilizers with P and K contribute to the protein content by 1.45%, factor B by N doses by 91.1%, and the AxB interaction by 1%.

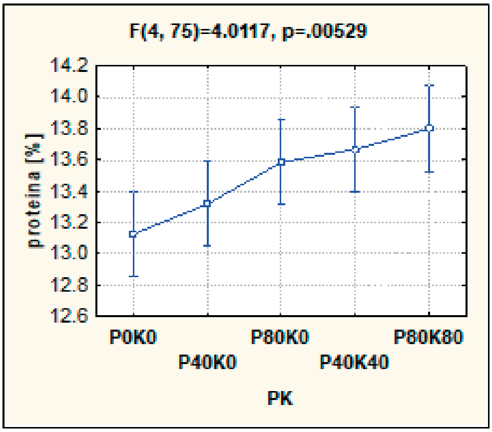

Figure 6. Variation in protein content [Factor A] 2019-2021

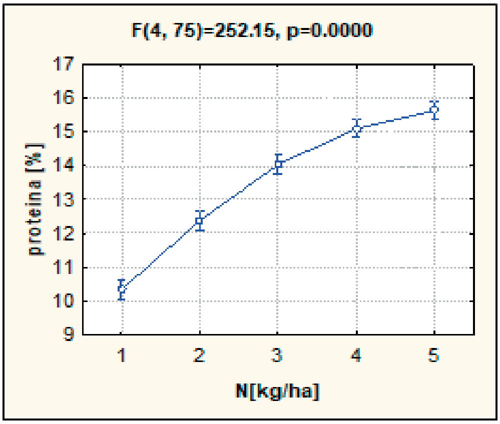

Figure 7. Variation in protein content [Factor B] 2019-2021

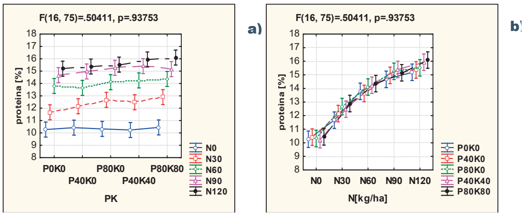

Figure 8. Variation in protein content [Factor AxB] 2019-2021

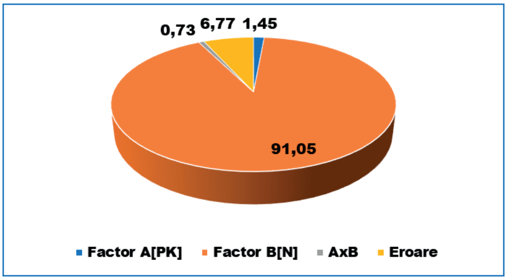

Figure 9. Contribution of factors A[PK], B[N] and interaction AxB 2019-2021

CONCLUSIONS

The research results demonstrated the favorability of the microzone (SDE Timişoara) for wheat cultivation and also the suitability of the Ciprian variety for cultivation in this area.

Chemical fertilizers have contributed to obtaining production increments, Thus, on cambic chernozem from SDE Timişoara, fertilizers with P and K contribute to the achievement of production in a proportion of 8.7% and those with N contribute with 13%.

The highest wheat production of 5680 kg is obtained on the $P_{80}K_{80}N_{90}$ farm, followed by the $P_{40}K_{40}N_{60}$ farm, with 5640 kg, $P_0K_0N_{90}$, with 5634 kg and $P_{80}K_0N_{60}$, with a production of 5570 kg/ha.

The production analysis carried out at the 5 doses of PK highlights the fact that it is between 4900 kg/ha obtained by applying the dose of $P_{40}K_0$ and approx. 5300 kg/ha obtained at the other 4 doses, i.e. at P_0K_0, $P_{80}K_0$, $P_{40}K_{40}$ and $P_{80}K_{80}$. The protein content obtained under the conditions of the Timişoara microzone, at the 5 P and K doses, was between 13.12% obtained at P_0K_0 and 13.80% obtained at $P_{80}K_0$ and $P_{80}K_{80}$. The analysis reflects the variation of the protein content, obtained at the 5 N doses, in the Timişoara microzone, between 10.34% (N_0) and 15.63% (N_{120}).

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON FOLIAR DISEASES OF BARLEY, MURIGHIOL LOCATION, TULCEA COUNTY

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Abstract

The research followed the evolution of the mycosis attack of autumn barley cultivated in the classical culture and during the conversion period to switch to the ecological culture, in the agricultural year 2021-2022, in Tulcea county. The frequent micromycetes were Pyrenophora teres, Pyrenophora graminea, Puccinia hordei, Rhynchosporium secalis and Blumeria graminis f.sp. hordei. In the barley cultivated in the two systems, the incidence of attack by Pyrenophora teres and Puccinia hordei was 100%. The frequency of the Pyrenophora graminea attack was 5% and 6% in the control variants. The powdery mildew attack was more sensitive to barley in the conventional culture and the Rhynchosporium secalis attack was subunit in both culture systems. The application of the treatment in the conventional system ensured an effectiveness of over 64%.

Key words*: barley, pathogens, diseases, degree of attack, conversion period.*

INTRODUCTION

The phytosanitary status of barley crops constitutes a condition for high and quality productions. Barley is attacked by specific pathogens such as *Pyrenophora graminea* which causes the leaf stripe disease, *Pyrenophora teres* which induces net blotch of barley, rust caused by the micromycete *Puccinia hordei, Rhynchosporium secalis*, responsible for the appearance of scald, *Blumeria graminis* f. sp. *hordei* causes powdery mildew. Barley pathogens induce changes in plants and severely reduce barley production (Neate & McMullen, 2005). When the attack of *Blumeria graminis* f. sp. *hordei* is not controlled it can become devastating (Haugaard et al., 2001), and the control by using fungicides is usually fast but the fungus has the potential to develop tolerance to the most used chemical products (De Waard et al., 1993). *Pyrenophora graminea*, a pathogen with a major impact on seeds as well (Zad et al., 2002; Manole & Cristea, 2015) requires adequate control (Cristea & Gheorghies, 1997), the losses produced by this pathogen being important for barley culture (Porta-Puglia et al., 1986; Damgaci & Aktuna, 1983). *Pyrenophora teres* has become one of the most common diseases of barley (Shipton et al., 1973; Popescu & Cristea, 2022) which produces yield losses of up to 40% in sensitive plants (Beckes et al., 2021) with an impact on seeds and even compromising production (Liu et al., 2011). The manifestation of the disease differs with the virulence of the pathogen, the genotype of the host, environmental conditions and losses being different for sensitive and resistant varieties (McLean et al., 2009). *Rhyncosporium secalis* causes leaf blotch or scald of barley and is found wherever barley is grown (Goodwin, 2002) causing production losses of over 35% (Shipton et al., 1973) being also present on other plant species (Caldwell, 1937). The attack of the micromycete *Puccinia hordei* differs with the genotype and causes production reductions of up to 62% in sensitive varieties (Park et al., 2015). The measures to prevent and control the pathogens of cultivated plants have in mind an integrated control, with an emphasis on the cultivated genotype (Pana et al., 2015) and where the application of treatments is required, it is necessary to calculate their effectiveness (Toth & Cristea, 2020; Toth &

Cristea, 2018; Jaloba et al., 2019). Biopesticides based on natural components, extracted from microorganisms constitute a means of biological control (Haugaard et al., 2001; Schonbeck & Dehne, 1986; Reglinski et al., 1990).

MATERIALS AND METHODS

The aim of the research was to monitor barley diseases in the conventional culture and the culture in the conversion period for the transition to the ecological culture in the year 2021-2022. The experiments were located in Sarinasuf, Murighol, Tulcea County. The Cardinal variety was cultivated in a conventional system and conversion for the transition to ecological culture. For the culture experience in the conventional system, the seed was treated with the fungicide Amiral (0.5 l/t), and the vegetation was treated with the fungicide falcon, in a dose of 0.8 l/ha, together with the foliar fertilizer First Power and the biostimulator Aminoplant, 1 l/ha. No treatments were applied to the control variant. In the case of the experience with barley during the conversion period for the transition to ecological culture, the seed was untreated, but passed through the selector. Foliar fertilization was carried out with the product Inofol 10 l/ha with the Delfan biostimulator, in a dose of 2 l/ha, these fertilizers being ecologically certified. The control variant was free of treatment. Based on the clinical picture, the characteristic symptoms were identified and the responsible pathogens were identified microscopically.

Observations were made regarding the incidence of the attack (F%), the intensity (I%), calculating the degree of attack for the detected diseases based on the data obtained. Calculation formulas were used: Frequency (F %) = n x 100/N, where N = number of plants observed (%), n = number of plants specific symptoms (%), Intensity (I%) = Σ (ixf)/n (%) where, i = percentage given, f = number of plants/organs with the respective percentage, n = total number of attacked plants/organs, GA = attack degree (%), F = frequency (%), I = intensity (%). The effectiveness of the tratment was calculated acoording to the formula E (%) = [(GA var c – GA var t)/GAvar c], where: GA var c = degree of attack in the control variant and si Ga var t = degree of attack in the traded variant. The Cardinal variety was cultivated in a conventional system and conversion for the transition to ecological culture. The experiments were located in Sarinasuf, Murighol, Tulcea County. For the culture experience in the conventional system, the seed was treated with the fungicide Amiral, (0.5l/t), and the vegetation was treated with the fungicide Falcon, in a dose of 0.8l/ha, with the foliar fertilizer First Power and the biostimulator Aminoplant, 1 l/ha. No treatments were applied to the control variant. In the case of the experience with barley during the conversion period for the transition to ecological culture, the seed was untreated, but passed through the selector. The foliar fertilization was carried out with the Inofol 10 l/ha product together with the Delfan biostimulator, in a dose of 2 l/ha, these fertilizers being ecologically certified. The control variant was free of treatment.

RESULTS AND DISCUSSIONS

In the conditions of the year 2021-2022 (Figure 1) in the experimental area, barley culture was monitored in the conventional system (Figure 2) and in the conversion period for the transition to ecological culture (Figure 3). The detected diseases were: net blotch, caused by the micromycete *Pyrenopfora teres*, the leaf stripe disease, caused by the pathogen *Pyrenophora graminea*, rust, produced by *Puccinia hordei* (Popescu & Cristea, 2021), the powdery mildew caused by the fungus *Blumeria graminis* f. sp. hordei, scald caused by *Rhynchosporium secalis* (Table 1). The data regarding the attack of diseases in the experience of barley grown in the conventional system show that, in the case of the reticular leaf spot attack, a frequency of 100% was found on barley leaves in both culture systems. Regarding the intensity of the attack of the pathogen *Pyrenophora teres* in the conventional system, the value was 30.5% in the control variant and in the treated variant the value of the intensity of the attack was lower, of 9.5%, which led to values of the degree of attack of 30.5% and respectively 9.5. In the case of the leaf tearing attack, the incidence on the leaves was noted, resulting in a frequency of 6% in the control variant and 2% in the treated variant. The net blotch and barley stripe leaf disease were present with different values of the attack in barley varieties cultivated in Romania (Pana et al., 2015). The attack of the micromycete *Puccinia hordei* was manifested with the maximum frequency (F = 100%) so the values of the intensity of the attack made the difference regarding the degree of attack. The intensity of the rust attack was 26.5% in the control variant and 9.5% in the treated variant. The powdery mildew attack was 2.6% in the control and was reduced below 1% in the control variant. The biggest difference in attack values was found in attack frequency, respectively 31% in the control variant and 14% in the treated variant. Regarding the intensity value, it was 6.5% for the treated variant and 8.5% for the control variant. The attack of the micromycete *Rhynchosporium secalis* on the leaves was also noted with a frequency of 8% and an intensity of 4.5% in the treated variant and F = 12% and I = 6% in the control variant. The values of the degree of attack when burning the leaves was below 1% in both cases (Table 1).

Research has shown that the application of treatments reduced the attack of barley pathogens (Cristea & Gheorghies, 1997). The data from the same table regarding the attack of pathogens detected in the experience with barley in the conversion period for the transition to ecological culture showed that the values of the attack of pathogens were higher. Thus, in the case of the *Pyrenophora teres* attack, it manifested itself with maximum frequency, but the intensity values were 27.5% in the control variant and 20.5% in the variant to which the certified products were applied. The attack of *Pyrenophora graminea* fungus recorded an incidence of 3% and 5%, respectively, and the frequent rust attack on all analyzed plants varied between 11.5% and 7.5% in the variants of this culture system. In the case of the *Blumeria graminis* f. sp. *hordei* attack, attack values of around 1% were recorded, and as regards the presence of the *Rhynchosporim secalis* fungus, it had a higher but still sub-unit attack level.

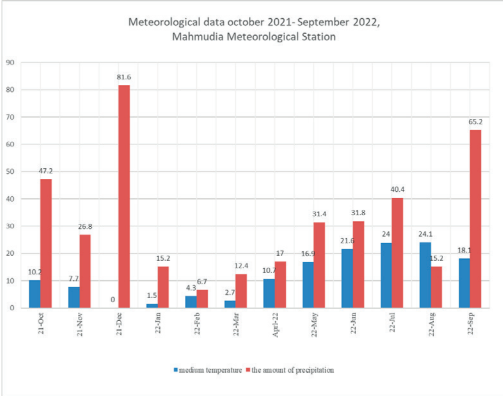

Figure 1. Meteorological data October 2021 - September 2022, Mahmudia Meteorological Station, Tulcea County

Figure 2. Barley crop aspect - in the conventional culture (original)

Figure 3. Barley crop aspect- in the conversion period (original)

Table 1. Data regarding the attack of foliar diseases in barley (2021-2022), Murighiol, Tulcea county

Variety/ culture system	Variant Trait/ Control	The pathogen/disease												
		Pyrenophora teres/ Barley net blotch			*Pyrenophora graminea*/ Barley leaf stripe	*Puccinia hordei*/ rust			*Blumeria graminis* f.sp. *hordei*/ Powdery mildew			*Rhynchosporium secalis* / Scald		
		F (%)	I (%)	GA (%)	F (%)	F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)
Cardinal/ conventional system	Trait	100	9.5	9.5	2	100	9.5	9.5	14	6.5	0.9	8	4,5	0.3
	Control	100	30.5	30.5	6	100	26.5	26.5	31	8.5	2.6	12	6	0.7
Cardinal/ conversion period	Trait	100	20.5	20.5	3	100	12.5	12.5	16.5	7	1.1	11	5.5	0.6
	Control	100	27.5	27.5	5	100	28.5	28.5	15.5	9.5	1.5	16	6.5	0.8

The application of the treatment to the seed and in vegetation to barley in the conventional culture, reduced the attack of the detected pathogens and ensured effectiveness of over 65% in the case of the net blotch attack, leaf strep disease, and powdery mildew. In the case of the attack of rust, the effectiveness was 64.1% and in the case of the attack of rhynchosporiosis, the effectiveness was 57%. In the barley crop in the conversion period, where the sorting of the seed and the application of certified products in the vegetation had an important role, these interventions ensuring a level of attack that does not worry in the second year of observation (Popescu & Cristea, 2022) (Table 2). The application of treatments ensures the effectiveness in the control of the attack of plant diseases with an impact on agricultural production (Buzatu et al., 2018). Management on net blotch requires the consideration preventive and curative control measures (Backes et al., 2021).

Table 2. The effectiveness of phytosanitary intervention on foliar diseases in barley

Variety/ culture system	Variant Treated/ Control	The pathogen/ disease									
		Pyrenophora teres/ net blotch		*Pyrenophora graminea*/ leaf stripe		*Puccinia hordei*/ Rust		*Blumeria graminis* f.sp.*hordei*/ powdery mildew		*Rhynchosporium secalis*/ scald	
		GA (%)	E (%)	GA (%)	E (%)	GA (%)	E (%)	GA (%)	E (%)	GA (%)	E (%)
Cardinal / conventional system	Treated	9.5	68.8	2	66.6	9.5	64.1	0.9	65,4	0.3	57.1
	Control	30.5	-	6	-	26.5	-	2.6	-	0.7	-
Cardinal / conversion period	Treated	20.5	25.4	3	40	12.5	56.1	1.1	21.4	0.6	20
	Control	27.5	-	5	-	28.5	-	1.4	-	0.8	-

CONCLUSIONS

The frequent diseases of autumn barley in the experimental area in the conditions of the year 2021-2022 cultivated in the conventional system and during the conversion period were caused by the micromycetes of *Pyrenophora teres*, *Pyrenophora graminea, Puccinia hordei, Blumeria graminis* f. sp. *hordei*, *Rhynchosporium secalis*. The incidence of the attack was maximum in the micromycetes *P. teres* and *P. hordei* in all experimental variants.

The application of treatments to barley grown in the conventional system reduced the attack of the monitored pathogens. The attack of the micromycete *P. graminea* was less in the barley in the conversion period compared to the untreated variant in the conventional system, which we attribute to the seed selection operation used at sowing.

ACKNOWLEDGEMENTS

We wish to thank “Dima Ancuta” PFA, Tulcea County for technical support.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

CONTRIBUTIONS TO THE DEVELOPMENT OF THE CULTIVATION TECHNOLOGY OF CASTOR HYBRIDS (*Ricinus communis* L.)

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Abstract

Castor bean is cultivated in Romania as an ornamental plant and very little for its seeds rich in oil used in the textile and chemical industry or in pharmacy and cosmetics. Castor oil is at the same time a good melliferous and energetic plant. In 1989, 26,300 ha of castor were cultivated in Romania, and currently insignificant areas are cultivated. Interest in this culture has grown a lot in recent years due to the development of castor hybrids with clearly superior productions compared to the old varieties. The research aimed at the development of an economically efficient cultivation technology, in the pedoclimatic conditions of the north of Moldova. A numar of 6 castor hybrids of French origin were tested, cultivated in rows at a distance of 70 cm, using precision seeders from corn in 3 variants of spacing per row: 70 cm, 100 cm and 130 cm. The highest seed productions were for the hybrid LS-CB-18-04 (4,871 kg/ha) and hybrid LS-CB-18-02 (4,774 kg/ha), at a sowing distance of 130 cm per row (10,989 plants/ha).

Key words*: castor bean, hybrid, cultivation technology.*

INTRODUCTION

Castor (*Ricinus communis* L.) is an endemic plant in sub-tropical and tropical regions and belongs to the Euphorbiaceae family (Scarpa et al., 1982). Its spread was possible due to its high resistance to drought, but also salinity, an abiotic factor that intensifies its action from one year to another (Lungoci et al., 2023; Lungoci et al., 2022). Domains such as encapsulation, incorporation into polymer matrixes of film or gel type (Lungoci et al., 2023), nanoparticles (Ojha et al., 2017) and other products, have taken a great extend, and plant extracts and non-drying castor oil finds its place.

The plant has been known since antiquity for its medicinal properties, being mentioned numerous times in Egyptian papyri, especially for the treatment of skin diseases (Severino et al., 2013). Some other uses of castor oil and extracts are shown in Figure 1 (Iqbal et al., 2011; Sandhyakumary et al., 2003; Shokeen et al., 2008; Rakesh et al., 2011; Taur et al., 2011; Upasani et al., 2011).

Castor oil has a growing international market, as evidenced by its more than 700 uses, from medicines and cosmetics to replacing petroleum products in the production of plastics, biodiesel and lubricants (Ramanjaneyulu et al., 2013), for the insecticide effect, antimicrobial (Soni et al., 2017).

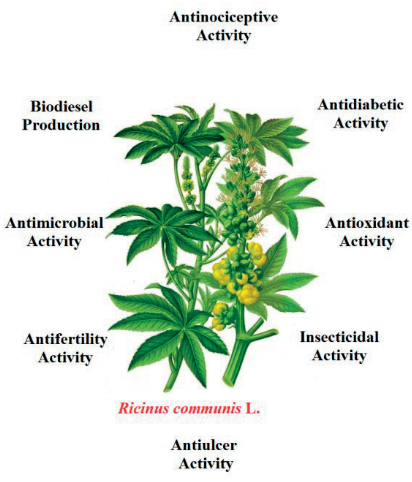

Figure 1. *Ricinus communis* L.

This species was also acclimatized on the European continent, until 1980 the areas were quite extensive. In the area of origin, it is a perennial specie, but in European countries it is known as an annual crop (Patanè et al., 2019). Castor is cultivated in Romania as an ornamental plant and in very small surfaces for its oil-rich seeds, used in the textile and chemical industry or in pharmacy and cosmetics. Castor is at the same time a good melliferous and energetic plant. From an agronomic point of view, it is a valuable plant, due to the penetrating power of the root, destroying the hardpan (Zoz et al., 2021). In 1989, in Romania, 26,300 hectares were cultivated with castor, and currently insignificant areas are cultivated. The interest for this crop has increased greatly in recent years due to the development of castor hybrids with significantly higher yields than the old varieties (Roman et al., 2012).

MATERIALS AND METHODS

The experimental field was carried out in a commercial farm in Ripiceni, Botosani, according to the following experimental protocol: monofactorial experiment, laid out according to the randomized block method, in three repetitions (155 square meters/repetition).
The tested factor was the distance between the castor plants in a row: A_1-70 cm, A_2 - 100 cm and A_3 - 130 cm.
The applied technology was the traditional one: after harvesting the wheat that was the predecessor plant, weeding was carried out, followed 14 days later by basic fertilization with 400 kg/ha of NPK 16/16/16 type complex fertilizers and plowing at a depth of 33 cm. The shredding and leveling of the land was done with a combine about 20 days after plowing. The soil was prepared in the spring, through a work with the combiner at a sowing depth of 7 cm, carried out on the day of sowing, an operation by which the amount of 100 kg/ha of urea (46% nitrogen active substance) was incorporated into the soil. The sowing was carried out at the end of April, using six hybrids from France that are in the testing and homologation phase: LS-CB-18-01, LS-CB-18-02, LS-CB-18-03, LS-CB-18 -04, LS-CB-18-11, LS-CB-18-12. The seed was treated with Maxim XL 035 FS (fludioxonil 25 g/l + metalaxyl-M (mefenoxam) 10 g/l) to prevent soil infections with *Fusarium* spp.

After sowing, weeding was carried out in the pre-emergence with the product Dual Gold (960 g/l s-metolachlor) in a dose of 1.4 l/ha, and during the growing season three manual weedings were carried out to keep the crop free of weeds. Currently, there is no approved herbicide for the castor crop in Romania. The plants sprouted 12-13 days after sowing and were protected from pests by the preventive application of the pyrethroid insecticide Karate Zeon (lambda-cyhalothrin 50 g/l) in a concentration of 0.1%. At the beginning of flowering, the second treatment was applied against polyphagous pests using the product Mospilan (acetamiprid 200 g/kg) in a concentration of 0.1%: the red spider (*Tetranycus urticea*), the fruit caterpillar (*Helicoverpa armigera*). The culture did not show specific disease symptoms during the entire vegetation period.
Analyzing the climatic conditions of the area, monitored by the nearby weather station, we can observe that, in the case of temperatures, in most months they recorded values much lower than the multi-year average. An exception is the month of January where the average is 0.7 higher than the multi-year average and February, where the difference is 1°C. The results are presented in Figure 2.

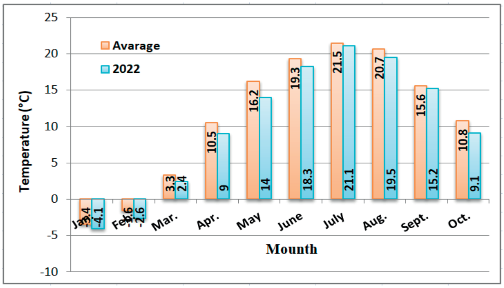

Figure 2. Monthly temperatures recorded in 2022

The rainfall regime recorded values between 25.5 mm in February and 65.5 mm in September. We encounter a positive deviation in September, the difference compared to the multi-year amount being 20 mm. In the other months, the values are close to the multiannual amount. The data are presented in Figure 3.

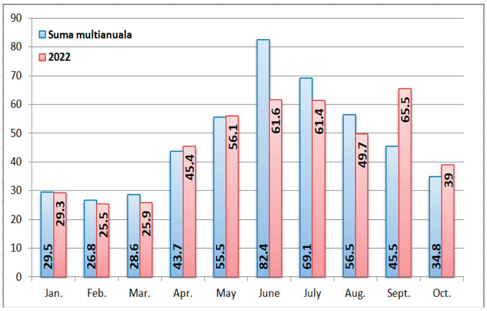

Figure 3. Monthly amount of precipitation in 2022

Soxhlet extraction

A quantity of 150 g shelled and crushed castor seeds was placed in a Soxhlet extractor fitted with a 1000 ml glass flask containing 350 ml of ethanol. The extraction was carried out at the boiling temperature of ethanol (78°C), for 3 hours. After extraction, the solvent was evaporated with the help of a rotoevaporator until a complete removal of the ethanol necessary to determine the weight of the extract was found (Danlami, 2015; Randall, 1978). After the extraction process, the castor seed residues was dried in an oven for 24 hours at 50°C to determine the weight of the dry material.

The hectoliter mass was determined according to the current standard ISO 7971-2:2019, and the TKW according to the SR ISO 520/2002 standard (Zaharia et al., 2017).

RESULTS AND DISCUSSIONS

Table 1. The influence of the hybrid on the production of the 130 cm between rows variant

Hybrid	Yield	Difference (%)	Difference (kg/ha)	Significance
LS-CB-18-04	4165.7	118.93	663.1	***
LS-CB-18-02	4092.3	116.84	589.7	***
Avarage	3502.6	100	0	Mt
LS-CB-18-03	3341	95.39	-161.6	ooo
LS-CB-18-11*	3243.7	92.61	-258.9	ooo
LS-CB-18-01	3153.7	90.04	-348.9	ooo
LS-CB-18-12*	3019	86.19	-483.6	ooo
			DL 5%	42.9 kg/ha
			DL 1%	61.0 kg/ha
			DL 0.1%	88.3 kg/ha

The distance of 130 cm between the rows has a negative influence compared to the other variants analyzed. The hybrids LS-CB-18-04 and LS-CB-18-02, have the highest yields and the differences from the control, which is the average, being 663.1 kg for the first hybrid and 589.7 for the second. These statistically ensured differences are very significant and positive. In the other 4 hybrids, the differences from the average are very significant, but negative.

Table 2. The influence of the hybrid on the production of the 100 cm between rows variant

Hybrid	Yield	Difference (%)	Difference (kg/ha)	Significance
LS-CB-18-04	4592	117.21	674.1	***
LS-CB-18-02	4491.7	114.65	573.8	***
Avarage	3917.9	100	0	Mt
LS-CB-18-03	3771	96.25	-146.9	ooo
LS-CB-18-11*	3636.3	92.81	-281.6	ooo
LS-CB-18-01	3588.7	91.6	-329.2	ooo
LS-CB-18-12*	3427.7	87.49	-490.2	ooo
			DL 5%	37.7 kg/ha
			DL 1%	53.6 kg/ha
			DL 0.1%	77.6 kg/ha

In the case of the distance of 100 cm between the rows, the productions were much higher than in the previous version. The highest productions were also recorded for hybrids LS-CB-18-04 and LS-CB-18-02, varying between 4871 kg/ha for the first and 4774 kg/ha for the second. The differences from the average being very significant and positive. Compared to the previous version, we can see that production has increased for all variants. For the hybrid LS-CB-18-03 the production is very significant, negative, similar being also in the last two hybrids.

Table 3. The influence of the hybrid on the production of the 70 cm between rows variant

Hybrid	Yield	Difference (%)	Difference (kg/ha)	Significance
LS-CB-18-04	4871	115.42	650.8	***
LS-CB-18-02	4774	113.12	553.8	***
Avarage	4220.2	100	0	Mt
LS-CB-18-03	4096	97.06	-124.2	
LS-CB-18-11*	3935	93.24	-285.2	oo
LS-CB-18-01	3903	92.48	-317.2	ooo
LS-CB-18-12*	3742	88.67	-478.2	ooo
			DL 5%	37.7 kg/ha
			DL 1%	53.6 kg/ha
			DL 0.1%	77.6 kg/ha

The variant of sowing at 70 cm between the rows of plants brought the highest yield increase, the hierarchy of the hybrids being the same. The highest production was obtained with the LS-CB-18-04 variant with a value of 4871 kg/ha and a difference compared to the control variant of 650.8 kg/ha, followed by the LS-CB-18-02 variant which obtained a production of 4774 kg/ha, with a positive difference of 553.8 kg compared to the control variant.

For the LS-CB-18-03 variant the production is not statistically assured, while for the LS-CB-18-01 variant it is distinctly significant, negative. In the last two variants, the production is very significant, negative.

Table 4. The influence of the distance between rows on the TKW at Ricinus communis L. hybrids

Hybrid	Yield	Difference (%)	Difference (g)	Significance
LS-CB-18-03	34.3	108.2	2.6	
LS-CB-18-11*	34	107.26	2.3	
LS-CB-18-01	33.4	105.36	1.7	
Avarage	31.7	100	0	Mt.
LS-CB-18-12*	29.9	94.32	-1.8	
LS-CB-18-04	29.5	93.06	-2.2	
LS-CB-18-02	29	91.48	-2.7	
			DL 5%	4.1 g
			DL 1%	5.9 g
			DL 0.1%	8.5 g

The interaction between hybrid and row distance resulted in variability of MMB values. They varied between 29 and 34.3 g. The highest MMB was obtained in the variant LS-CB-18-03 with a value of 34.3, with a value close to the average, and the lowest value was obtained in the variant LS-CB-18-02 and was 29 g. All analyzed variants were not statistically assured.

Table 5. The influence of row spacing on MH at *Ricinus communis* L. hybrids

Hybrid	Yield	Difference (%)	Difference (kg)	Significance
LS-CB-18-04	51.9	102.98	1.5	
LS-CB-18-03	51.4	101.98	1	
LS-CB-18-02	51.1	101.39	0.7	
LS-CB-18-01	50.6	100.4	0.2	
Avarage	50.4	100	0	Mt.
LS-CB-18-12*	49.4	98.02	-1	
LS-CB-18-11*	48.2	95.63	-2.2	
			DL 5%	2.9 kg
			DL 1%	4.2 kg
			DL 0.1%	6.1 kg

Similar to MMB also for MH the variants were not statistically assured. The values varied between 51.9 and 48.2 g. Positive differences were registered by hybrids LS-CB-18-04, LS-CB-18-03, LS-CB-18-02 and hybrid LS-CB-18-01.
Lower than average values wore obtained in the case of LS-CB-18-12* and LS-CB-18-11* hybrids.
The correlation analysis shows us that there is an interaction between TKW and production, the correlation coefficient being r^2 =0.85 and r^2=0.74 in the case of the MH-production interaction.

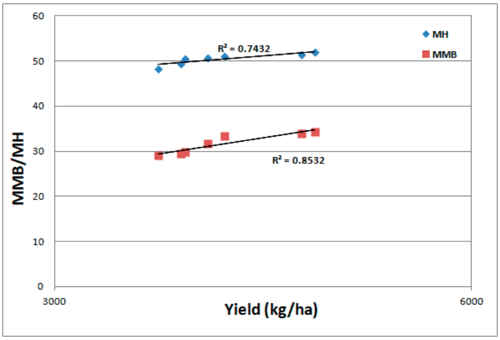

Figure 3. Correlation between MMB and MH for the studied hybrids

The oil content analyzed by the Soxhlet method shows us that there is no great difference between the oil content and the characteristics of the hybrid. The highest percentage is obtained in the LS-CB-18-01 hybrid, which is 45.4%.

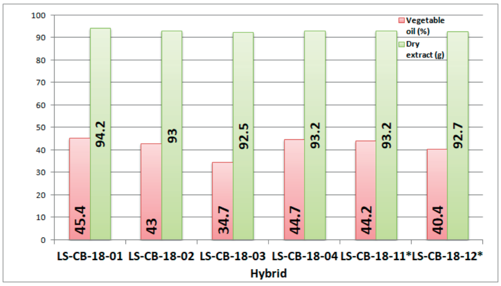

Figure 4. Oil and dry matter content of the 6 hybrids analyzed

CONCLUSIONS

Concluding what has been presented, we can state that at a distance of 70 cm between the rows, the highest production was obtained between it and the main analyzed indicators (MMB, MH) with a weak interaction. Also, there is no positive correlation between oil content and yield.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INFLUENCE OF SOWING TIME ON MORPHOLOGICAL CHARACTERISTICS OF SUNFLOWER PLANTS

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Abstract

Morphological characteristics of six sunflower hybrids with different precocity were assessed to notice the effect of sowing time (ST). ST was set taking into account the Celsius degrees at the soil depth of 7 cm at 7 a.m.: ST1 at 5°C, ST2 at 7°C and ST3 at 9°C. The research was performed in the field experiments in Tulcea County in 2021 under rainfed conditions. The number of leaves per plant increased upon the sowing delay from 27.7 at ST1 to 30.9 at ST3, while the number of days from sowing to flowering decreased from 62.7 at ST1 to 48.5 at ST3. Stem diameter had the highest value at ST2. The average plant height for the five hybrids increased from ST1 to ST3. ST2 provide the highest head diameter (19.8 cm) followed by ST1 (19.3 cm) and ST3 (18.5 cm).

***Key words**: sowing time, morphological characteristics, sunflower plants.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the main oil crop in Romania being cultivated on 1.11 million ha in 2021 (retrieved from: https://www.https://insse.ro/cms/sites/default/files/com_presa/com_pdf/prod_veg_r21.pdf).

In European Union, Romania it is the largest sunflower producer followed by Bulgaria and France (FAOSTAT database).

One of the most important factors that influence morphological characteristics and yield for many crops is sowing time.

For sunflower an early sowing can avoid the dry atmosphere and water deficiency during flowering and seed filling stages but on the other hand the emergence can be extended and different weeds (such as *Poygonum convolvulus* L., *Sinapis arvenis* L., *Chenopodium album*) can cause problems (Vranceanu, 1974).

When planting is delayed soil moisture gained during winter season can be inefficient valued due to evapotranspiration, the crop did not have enough time to fill achenes (Killi & Altunbay, 2005) and the yield decreased owing to high temperature during flowering (Ahmed et al., 2020).

It was found that stem diameter, plant height and number of days from sowing to flowering increased when the sowing was performed earlier (Ozturk et al., 2017; Demir, 2019) or later (Birck et al., 2016; Mijic et al., 2020). Balalic et al. (2016) turned out that head diameter at maturity is influenced mostly by sowing date and less by the year and hybrid. It is expected that the number of leaves will be higher at an early sowing because their development stage is completed in long-day conditions (Vranceanu, 1974). In the last years there were studies which confirm this fact (Aliloo, 2018; Morsy et al., 2022).

Morphological characteristics of sunflower can also be influenced by the plant density, hybrid (Kalenska et al., 2020), drought tolerance indices (Ghaffari et al., 2012), irrigation (Shahin et al., 2018) or fertilization (Labao et al., 2017; Coelho et al., 2022).

The importance of these studies comes from providing farmers with up-to-date information specific for their area.

In the past we could have considered a proper period for sowing by looking at the calendar but then the temperatures did not vary like nowadays. This study aims to find determine the influence of sowing time consider the temperature factor and not calendar day on the main morphological characteristics of six sunflower hybrids in the climatic conditions specific for Dobrogea area in South-East Romania.

MATERIALS AND METHODS

Plant material and field trials. The experiment was carried out in the field experiments in the South of Tulcea county (Beidaud - 44º42' N latitude and 28º34' E longitude) during 2021 on a chernozem argiloiluvial soil under rainfed conditions. Two hybrids included in the study were certified (P64LE99 and FD15E27) and four were in the process of certification (DS001, DS002, DS003 and HS7083). They were sown at three different sowing time (ST) taking into account the Celsius degrees at the soil depth of 7 cm at 7 a.m.: ST1 at 5ºC (1st April), ST2 at 7ºC (17th April) and ST3 at 9ºC (23rd April). Sowing density was 55,000 germinable seeds ha-[1]. The space between rows was 70 cm. The plot size was 210 m2 (4.2 m x 50 m). The previous crop was winter barley. The weeds were controlled with herbicide Pantera 40 EC (40 g/l quizalofop-P-tefuryl) 0.8 L/ha applied at 2-4 leaves stage and a hoeing before the emergence of inflorescence.

Determinations. The number of days from sowing to flowering was determined when 75% of the plot was flowered. The number of leaves per plant was determined at the flowering stage. Height of plants, stem diameter (at 1 m above the ground) and head diameter were assessed at maturity. All observations were performed for 10 plants in four replications.

Weather conditions. At Beidaud area during the sunflower growing period (April-August), the mean temperature has increased continuous from 9ºC (April) to 24.4ºC (June) and decreased slightly to 23.6ºC in August. The sum of rainfall for the same period was 400.8 mm sufficient for covering the sunflower water requirements for a good development which is over 400 mm (Pejic et al., 2009). Rainfall was irregular during the months of sunflower vegetative period, the rainiest month was June (147.7 mm) and the driest was August (32.2 mm) (Figure 1).

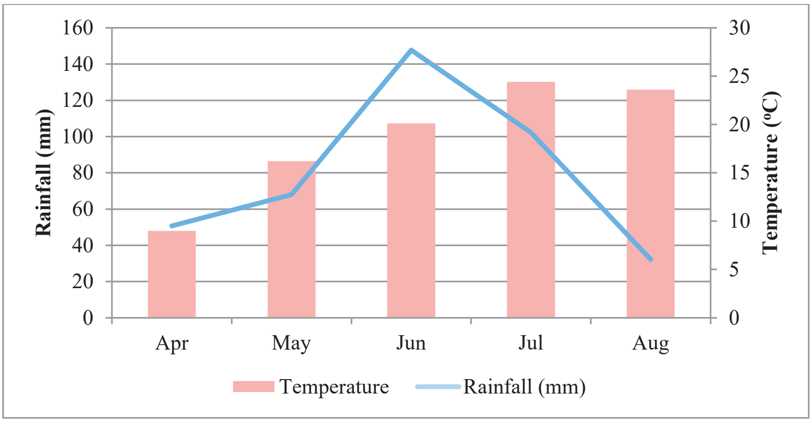

Figure 1. Average temperature (°C) and monthly distribution of rainfall (mm) during the sunflower growing season

Statistical analysis. Collected data were statistically analysed by ARM-9 software using Tukey's HSD (Honestly Significant Difference) test (P< 0.05).

RESULTS AND DISCUSSIONS

In the present study it is clearly presented that sunflower plants need more days to reach flowering stage when they are sowing at 5ºC compared to those sowed at 7ºC and 9ºC (Table 1). This fact it is attributed to lower temperature that extend the growth period for plants sowed at ST1. With all this, plants from ST1 flowered first. These results are in accordance with those obtained by Ahmed et al. (2020) and Morsy et al. (2022).

Table 1. Number of days from sowing to flowering stage of six sunflower hybrids sowed in three sowing times

Sowing time	Hybrid						Average
	P64LE99	DS001	DS002	DS003	FD15E27	HS7083	
ST1	61	63	64	66	61	61	62.7
ST2	50	51	53	55	51	50	51.7
ST3	49	49	49	51	46	47	48.5
Average	53.3	54.3	55.3	57.3	52.7	52.7	

The lowest value was for DS001 at ST1 (194.08 cm) and the highest value was for DS002 at ST3 (226.37 cm). Between hybrids at ST1 there were no significant differences. With one exception (P64LE99) all hybrids followed the same trendline: the height continued to raise upon sowing delay (Table 2). This is in contrast with observations made by Demir (2019) and Ozturk et al (2017). Mean rates of ST were higher than those reported by Birck et al. (2016) - 170.2 cm, Capone et al. (2011) - 98.9 cm or by Allam et al. (2003) - 140.8 cm. The plant height varies depending of the ability of hybrids to adapt to environmental conditions. Even if the plant height was great there were no lodging symptoms.

Table 2. Plant height (cm) of six sunflower hybrids sowed in three sowing times

Hybrid	ST1	ST2	ST3
P64LE99	211.58-	209.92bc	215.03bc
DS001	194.08-	203.94cd	211.98bc
DS002	212.03-	226.37a	226.78a
DS003	208.4-	216.7b	218.13ab
FD15E27	202.13-	205.67cd	207.58c
HS7083	197.55-	198.02d	204.85c
Tukey's HSD P< 0.05	21.21	7.76	10.27

Different letters in columns differ at significant difference according to Tukey's HSD test; P< 0.05; "-": no significant difference.

Number of leaves/plant was influenced by both hybrid and ST. All hybrids generate the most leaves when were sowed at 9ºC and generate less when were sowed at 5ºC. Hybrid FD15E27 had the most leaves/plant at all ST while DS002 had the less (Table 3).

Table 3. Number of leaves/plant of six sunflower hybrids sowed in three sowing times

Hybrid	ST1	ST2	ST3
P64LE99	28.63b	27.73c	30.40b
DS001	25.75c	29.85ab	32.15a
DS002	25.88c	26.45d	28.80c
DS003	26.60c	27.13cd	29.83bc
FD15E27	31.55a	30.78a	32.20a
HS7083	29.10b	29.05b	32.20a
Tukey's HSD P< 0.05	1.767	1.160	1.514

Different letters in columns differ at significant difference according to Tukey's HSD test; P< 0.05; "-": no significant difference.

The hybrid with the highest head diameter correlated with ST was DS003 at ST2 - 20.88 cm and the smallest head diameter was for DS002 at ST1 (Table 4). Mijic et al. (2020) tested 22 sunflower hybrids and concluded that head diameter ranged greatly from 19.2 cm to 30 cm. Head diameter as well as plant height and 100-seed weight at phenotypic and genotypic levels indicated that selection for any of them is effective for improving the other ones especially seed weight plant^{-1} (Ahmed et al., 2020).

Table 4. Head diameter (cm) of six sunflower hybrids sowed in three sowing times

Hybrid	ST1	ST2	ST3
P64LE99	16.8bc	19.07ab	18.01ab
DS001	18.67ab	19.29ab	19a
DS002	15.75c	17.58b	16.47b
DS003	18.25ab	20.88a	19.76a
FD15E27	18.ab	19.55ab	18.58ab
HS7083	19.31a	22.16a	18.45ab
Tukey's HSD P< 0.05	2.41	3.21	2.22

Different letters in columns differ at significant difference according to Tukey's HSD test; P< 0.05; "-": no significant difference.

For stem diameter there were no statistically differences. With the exception of FD15E27, all hybrids had the highest value at ST2. In this experiment stem diameter was influenced by the ST but not by hybrid. The results varied from 1.73 cm for DS001 at ST1 and 2.33 for HS7083 at ST2 (Table 5).

Table 5. Stem diameter (cm) of six sunflower hybrids sowed in three sowing times

Hybrid	ST1	ST2	ST3
P64LE99	1.91-	2.14-	2.09-
DS001	1.73-	2.1-	2.07-
DS002	1.80-	2.21-	1.98-
DS003	2.05-	2.18-	1.99-
FD15E27	2.2-	2.04-	2.17-
HS7083	1.98-	2.33-	2.26-
Tukey's HSD P< 0.05	0.7	0.29	0.34

Different letters in columns differ at significant difference according to Tukey's HSD test; P< 0.05; "-": no significant difference.

Number of leaves/plant increased continuous from ST1 to ST2 and to ST3 as with plant height which makes us believe that there is direct relation between these two morphological characters. The results are opposite with those obtained by Morsy et al. (2022) where the number of leaves were highest in the first sowing dates. Ahmed et al. (2015) obtained the highest number of leaves/plant at the middle sowing date.

At ST2, the average head diameter was 19.75 cm and was statistically higher than the first ST (17.91 cm) and the last ST (18.38 cm). It was demonstrated that head diameter is strongly influenced by irrigation level from 11.1 cm at $ET_{75\%}$ to 17.6 $ET_{100\%}$ (Shanin et al., 2018). Balalić et al. (2016) point out that head diameter affects the number of flowers and grains per head, which directly affects the grain yield per plant. In the study carry out by Demir et al. (2019) when five sowing dates were evaluated head diameter decreased continuous from the first sowing date to the last one.

Stem diameter had the highest value at ST2 fallowed by ST3 and ST1 as in the case of head dimeter (Table 6). This is in accordance with the results obtained by Allam et al. (2003). The ST average was 2.06 cm which is bigger than that reported by Birck et al. (2016) which was 2.39 cm.

Table 6. Differences between ST for number of leaves/plant, stem diameter (cm) and head diameter (cm)

Sowing time	Plant height (cm)	Number of leaves/plant	Head diameter (cm)	Stem diameter (cm)
ST1	204.29b	27.68b	17.91b	1.94b
ST2	210.1a	28.5ab	19.75a	2.17a
ST3	214.05a	30.92a	18.38b	2.09ab
Tukey's HSD P< 0.05	5.7	2.76	1.12	0.19

Different letters in columns differ at significant difference according to Tukey's HSD test; P< 0.05; "-": no significant difference.

CONCLUSIONS

Morphological characteristics are influenced by hybrid but mostly by sowing time.

Sunflower plants sowed early need more days to reach the flowering stage but even so they flowered first before those sowed later. Plant height and number of leaves/plant increased their value upon sowing delay while head and stem diameter had the highest value in the middle sowing time.

Similar researches have to be repeated at specific periods due climate change in all climatic regions.

ACKNOWLEDGEMENTS

The study was performed with financial support by The Minister of Agriculture and Rural Development, through 4262/18.09.2018 Project and with support of the Doctoral School of Engineering and Management of Plant and Animal Resources and the Faculty of Agriculture of the University of Agronomic Sciences and Veterinary Medicine in Bucharest.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

PRELIMINARY RESULTS ON MAIZE BIOMASS UNDER THE INFLUENCE OF TILLAGE IN THE CONTEXT OF CLIMATE CHANGE

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Abstract

Environmental pollution has reached such an advanced stage that no measure can effectively stop the adverse effects shortly. The effort of many future generations is necessary globe wide to be able to restore within normal limits the damage that has been reached at present. Agriculture must contribute to these remedies by recycling plant residues, improving cultivation technologies to make them as environmentally friendly as possible, using biological pesticides and alternative methods of fighting harmful organisms, as well as redirecting farmers to choose new crops that can complete the range and diversify it to practice sustainable agriculture. The purpose of this research was to observe the quantity of maize biomass depending on the type of tillage by comparing specific conservative agriculture tillage with the classic soil tillage - plowing in drought and heat conditions of 2021-2022. The maximum values of green biomass (37.04 Mg ha⁻¹) and dry biomass (10.57 Mg ha⁻¹) were recorded in the scarified plot - L3, and the minimum values of 22.63 Mg ha⁻¹ respectively 6.93 Mg ha⁻¹, in the control plot L1 - plowed.

***Key words**: biomass, corn, conservative agriculture, plant residues, drought.*

INTRODUCTION

The biomass of plants used as fertilizer is closely related to the activity of microorganisms in the soil, the structure of the soil, and its fertility. The soil stores microorganisms that compose the living organic matter in it. Microorganisms are essential to plant growth and development due to their activities as a rich source of C, N, P, and S. They transform nutrients and degrade pesticides, they form a protective structure that prevents soil degradation and act as an indicator of soil fertility (Salinas-Garcia et al., 2002). When tillage, plant residues, and microorganisms interact in a calculated way, the benefits are seen not only on the crop but the entire ecosystem (Shukla et al., 2004).

Maize biomass is used in the bioethanol and biomaterials industry. The lignin present in its vegetable mass can be extracted for the development of advanced composites, polymer blends, carbon fibers, plastics, and nanomaterials. Maize biomass has antioxidant and antibacterial properties, UV protection effectiveness, and thermal stability (Grabovskyi et al., 2021; Camargos et al., 2019). Another use of corn biomass is its transformation into fiberboards (Theng et al., 2015). Used as a cover layer, dry biomass provides the soil with minerals such as calcium, magnesium, phosphorus, and potassium (Batista et al., 2009), improving at the same time soil organic carbon (Wilhelm et al., 2007).The efficiency of agriculture from an agricultural, economic, and environmental protection point of view means the use of crops seen from the angle of the primary production for human food, the secondary production for improving soil fertility and biodiversity by mixing plant residues with the soil, but also using it as a source of biomass for the energy sector.

Agriculture can be a comfortable source of raw materials used as biomass in the energy sector.

MATERIALS AND METHODS

The experience was carried out in 2022 in the Chiscani Experimental Field within A.R.D.S. Braila. The experiment was established on a vermic, phreatic, wet, moderately carbonated loess, clay-sandy, arable soil, with a clay content between 22.5-24.2% in the upper horizons, 29.2-21.2% in the transition horizons and 17.6-17.9 % at the base (at depths greater than 130 cm). This type of soil is well supplied in mobile phosphorus (P2O5), with values of 174-225 ppm in the upper horizons (0-20 cm) and middle, below 32 ppm in the transition horizon. The total nitrogen content is standard in the upper horizons, the soil reaction is dominantly alkaline with pH = 7.9-8.4, and the average humus content in the upper horizons is 2.4-3.1%, which decreases to 1.6% in the transition horizon (A/C).
The design of the experiment was distributed in three repetitions on five soil tillages: L1 - plowed (control), L2 - Paraplow, L3 - Scarified, L4 - Disc-Heavy and L5 - sown in stubble, without tillage Minim-Till.

RESULTS AND DISCUSSIONS

The year 2022 is considered very dry due to the lack of precipitation. In recent years, the only certainty about climate factors is that they are constantly changing and worsening. The agricultural year's monthly mean temperature of 12.4°C exceeded the multi-year monthly mean by 1.5°C. This atmospheric heat was all the more difficult for the crop plants to bear as the drought was also present, the precipitation deficit recorded compared to the multi-annual monthly average of 442 mm, was 178 mm, which means that the total precipitation in the agricultural year was 264 mm.
The application of land processing methods specific to the dry-farming work system that characterizes conservative agriculture has as an objective the performance of minimal soil works that allow the optimal growth of crops without harming the soil and the environment. In the presence of abiotic stress factors, the choice of soil work and crops to obtain the maximum yields is the object of study at the global level.

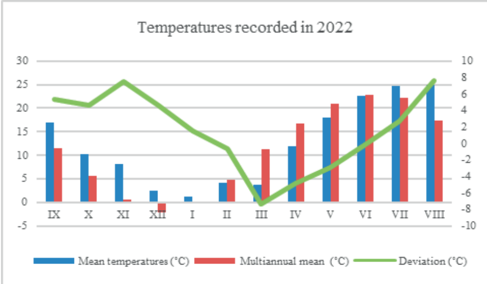

Figure 1. Mean temperatures recorded in 2021-2022 in Braila

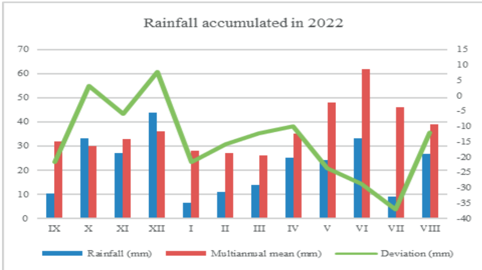

Figure 2. Rainfall accumulated in 2021-2022 in Braila

KyleW. Freeman et al. (2007) cited the authors Sadler et al. (2000) who conducted studies on the influences of soil type on productivity elements and biomass of crops under drought conditions. The corn crop, wheat, sunflower, and other crops often introduced in rotations in Romania are increasingly affected by drought and heat. Since the yields of corn crops obtained in 2022 in the study area were below 2 to/ha, the biomass was studied, and the possibility of its utilization as a source of renewable energy and fertilizer to reduce the damage caused by drought.

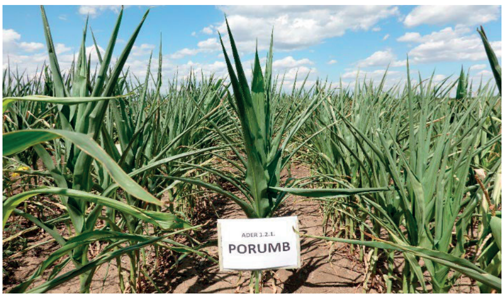

Figure 3. The effect of abiotic stress on plant development

Theng D. et al. (2015) state that the biomass of maize is composed of 60% stalks, 25% leaves, and 15% other parts of the plant, excluding cobs.

These proportions coincide with those obtained in our study in the Eastern area of Romania (Table 1).

The average green mass per plant and the average dry mass per plant were 532.4 g and 484.7 g, respectively 151.9 g and 142.5 g in the L3 - Scarified and L4 - Heavy-disc plots (Table 2). The control plot L1 - Plowing, recorded the minimum values, namely 325.3 g green mass and 99.7 g dry mass/plant.

The biomass increments obtained are superior to the L1 - control with values between 1.2 g and 207.2 g/plant (Table 2). Based on these results, the incapacity of the classic tillage can be deduced to contribute to the good development of the maize crop under climate stress conditions, both grain production and biomass production.

Maize biomass values according to tillage are statistically ensured and highlighted in Table 3.

Table 1. Corn plant's green mass divided into stems and leafs

Green Mass		
Tillage	**Stem (%)**	**Leafs (%)**
Plow (Control)	60.86	24.14
Paraplow	63.14	21.86
Scarification	60.06	24.94
Heavy-disc	59.01	25.99
Minim-Till	58.25	26.75

Table 2. The increase in green and dry biomass obtained, compared to the control

Tillage	The difference compared to Control	
	GM (g)	DM (g)
Plow (Control)	-	-
Paraplow	1.2	11.0
Scarification	207.2	52.2
Heavy-disc	159.5	42.8
Minim-Till	61.5	14.1

Table 3. Statistical assurance of the obtained biomass differences

Tillage	GM (g)	Difference	Symbol	DM (g)	Difference	Symbol
Plow (Control)	325.3	-	-	99.7	-	-
Paraplow	326.5	1.2	-	110.7	11.0	-
Scarification	532.4	207.2	***	151.9	52.2	**
Heavy-disc	484.7	159.5	***	142.5	42.8	**
Minim-Till	386.8	61.5	-	113.8	14.1	-
LSD5%=	*83.6*			*28.1*		
LSD 1%=	*121.7*			*40.9*		
LSD 0.1%=	*182.5*			*61.3*		

Maize plants lost between 21.5% and 25.12% moisture from stalks and 37.6% and 61.65% from leaves.

A loss of 61.65% was recorded in L2, and the minimum loss was in L3. Regarding the stems, the maximum and minimum losses were observed in L4 and L1, respectively.

A large amount of water stored in the leaves in the case of L2 and L1 may be a reaction to ensure the plant's survival.

To cope with stressors, plants redirect water into the leaves to carry out photosynthesis and thus survive. Regarding L3 and L4 tillage, the water was stored in the stem to develop the reproductive organs, namely the cobs.

The green mass per hectare obtained based on the studied soil tillage was between 22.63 Mg ha^{-1} and 37.04 Mg ha^{-1}, with L3 and L4 ranking first. It is due to the depth of tillage that allowed better penetration of the corn roots into the deep layers and efficient use of available water and nutrients.

The dry mass per hectare was in the range of 6.93-10.57 Mg ha^{-1}.

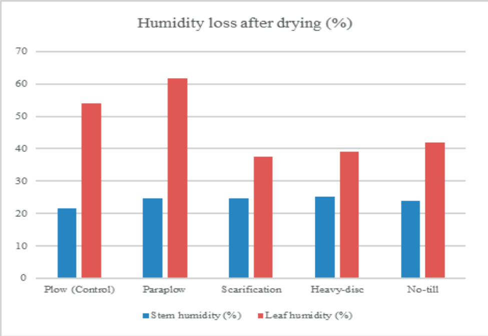

Figure 4. Humidity loss of corn plants after complete drying

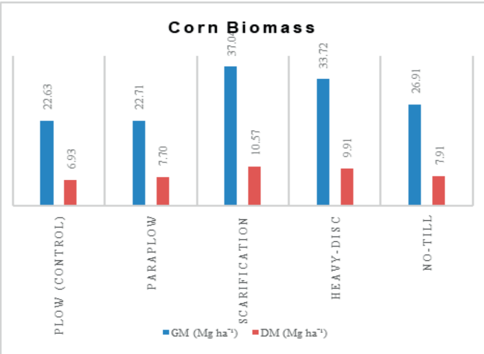

Figure 5. Green and dried biomass of corn plants based on soil tillage

Figure 6. The difference in plant height and drought tolerance of corn plants, based on soil tillage (From left to right: L1 - Plowed, L2 - Paraplowed, L3 - Scarified, L4 - Heavy-disk, L5 - Minim Till)

Based on these results, taking into account the poor yields obtained under such temperature and rainfall conditions, maize cultivation can be considered for the use of green mass as green manure and dry mass (plant residues) in restoring soil fertility, soil carbon and as a raw material in various industries (Wilhelm et al., 2007; Hooker et al., 2005; Igathinathane et al., 2006), including as a source of biomass for renewable energy, thus registering a major transformation of secondary production into primary production.

CONCLUSIONS

The year 2022 was both hot and dry, and the increase in temperatures associated with the lack of precipitation imposes conditions that are hard to tolerate for crop plants causing suffering even for drought-resistant ones.

The high yield losses of the corn crop in the current climatic conditions are a reason to consider cultivating this plant for the use of biomass in various industrial branches and agronomic purposes.

The soil tillages of conservation agriculture, L3 - scarification and L4 – heavy-disk, contributed to the valorization of water and nutrients from deep soil depths, thus allowing good development of the maize plants and helping them tolerate the stress factors of the experimental year.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Ministry of Agriculture and Rural Development which financed the Sectorial Project: ADER 1.2.1./27.09.2019: “Research on the identification of technical solutions and technological elements for the practice of the dry-farming work system in Southern Romania”.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

HERBICIDES APPLICATION FOR WEED CONTROL IN WINTER BARLEY (*Hordeum vulgare* L.)

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Abstract

An uncontrolled weed flora can decrease winter barley's grain yield and quality. Winter barley (Hordeum vulgare L.) variety 'Emon' field trial was conducted in the growing seasons 2020/2021 and 2021/2022. The trial was stated in the experimental field of the Agricultural University-Plovdiv, Bulgaria. The experiment aimed to study the efficacy of herbicides for broadleaf weed control in barley. The following herbicides efficacy was evaluated: Sekator OD - 0.15 l ha^{-1}; Axial One - 1.00 l ha^{-1}; Biathlon 4 D - 55 g ha^{-1}; Granstar 75 DF - 15 g ha^{-1}; Aminopielik 600 SL - 2.00 l ha^{-1}. The herbicides were applied in spring at the phenophase end of tillering - the beginning of spindling of the crop (BBCH 29-31). The herbicide efficacy was recorded by the 10-score visual scale of EWRS (European Weed Research Society). At the particular infestation with Sinapis arvensis L., Anthemis arvensis L., Galium aparine L., Consolida regalis Gray., and Viola arvensis L. the highest overall efficacy and highest yield were recorded when Biathlon 4 D - 55 g ha^{-1} was applied.

Key words: *barley, weeds, control, yield, herbicide.*

INTRODUCTION

Barley (*Hordeum vulgare*) is a member of the grass family and it is a major cereal grain plant grown in temperate climates around the globe. It was one of the first cultivated grain crops (from more than 10,000 years ago) (Zohary and Hopf, 2000). According to data from the Bulgarian Ministry of Agriculture and Forestry in 2021the areas sown with barley were126 957hectares and the average barley grain yields were5.411t ha^{-1} (www.mzh.government.bg).

Weed infestation is one of the main factors leading to yields and quality reduction (Tonev et al., 2007). The frequent violations and mistakes that are made in practice, in the technology of growing winter cereal crops, in particular in the case of barley, require the mass use of the chemical weed control method. However, this is possible only through a good and thorough knowledge of the continuously updated list of new herbicidal products and their competent, correct, and timely application according to the specific requirements of the crop, the agro-ecological and economic conditions of the area (Tonev et al., 2007).

Parameters to consider when optimizing herbicide doses are weed species and stage of development, crop competitiveness, climatic conditions, application technique, formulation/adjuvant and combination with other pesticides (Kudsk, 2008), and applied systems for tillage (Young and Thorne, 2004).

A large number of experiments related to the chemical control of weeds in barley have been carried out on a global and national scale. It was found that the winter barley varieties "Veslets", "Aheloy-2", "Emon", and "Perun" obtained the highest yield when treated with the herbicide Weedmaster (Atanasova, 2010). The influence of herbicides for broadleaf weeds control applied alone and in combination with herbicides for grass weeds control was investigated. It was found that the herbicides Derby 175 SK, Sekator WG, and Lintur 70 WG showed good efficacy against the weeds (Atanasova, 2008). Chhokar et al. (2008) reported that the active substance pinoxaden successfully controlled the grass weeds *Phalaris minor* Retz., *Avena ludoviciana* Dur. and *Polypogon monspeliensis* (L.) Desf. in barley.

Experiments were conducted with two-row winter barley to determine the efficacy of 4 herbicides and their tank mixtures on *Avena sterilis* L. and *Sinapis arvensis* L. After the combined application of pinoxaden + 2.4-D +

florasulamthe efficacy of the treatment against weeds was the highest (92%) (Pala, 2020).
The current study aims to establish the efficacy of some herbicides against broadleaf weeds and the influence of the treatments on some growth and reproductive parameters of barley.

MATERIALS AND METHODS

The experiment was carried out in the experimental field of the Department of Agriculture and Herbology at the Agricultural University - of Plovdiv, Bulgaria. The experiment was carried out according to the randomized block design in three replications. The size of the harvesting plot was 20 m².
The variants of the trail were as follows:
1. Untreated control;
2. Sekator OD (25 g/l *iodosulfuron* + 100 g/l *amidosulfuron*) - 0.15 l ha^{-1};
3. AxialOne (45 g/l *pinoxaden* + 5 g/l *florasulam*) - 1.00 l ha^{-1};
4. Biathlon 4 D (54 g/kg *florasulam* + 714 g/kg *tritosulfuron*) - 55 g ha^{-1};
5. Granstar 75 DF (750 g/kg *tribenuron-methyl*) - 15 g ha^{-1};
6. Aminopielik 600 SL (600 g/l *2.4 D amine salt*) - 2.00 l ha^{-1}.

Herbicides were applied in the phenophase end of tillering (BBCH 30).
The efficacy against the weeds *Sinapis arvensis* L., *Anthemis arvensis* L., *Galium aparine* L., *Consolida regalis* Gray., and *Viola arvensis* L. by using the 10-score visual scale of EWRS (European Weed Research Society) was under evaluation. The herbicidal efficacy was recorded on the 14th, 28th, and 42nd day after treatment. The selectivity of the herbicide was recorded by the 9-score scale of EWRS on the 14th, 28th, and 42nd day after treatment.
For the experiment, the Bulgarian barley variety "Emon" was grown. The sowing density was 500 germinated seeds/m². In both years of the experiment, the preceding crop of barley was winter oilseed rape (*Brassica napus* L., hybrid PT 228 CL), which was grown according to the Clearfield® technology.
The tillage carried out before barley sowing was deep plowing, followed by discing and harrowing. Before sowing, fertilization with 300 kg ha^{-1} with NPK 15:15:15 and spring dressing with 300 kg ha^{-1} NH_4NO_3 was done.

The following biometrical and productive parameters of barley were studied:
- Plant height at the end of the vegetation (m);
- Ear length at the end of the vegetation (cm);
- Absolute seed mass (g) (Tonev et al., 2018);
- Hectoliter seed mass (kg) (Tonev et al., 2018);
- Barley grain yields (t ha^{-1}) by harvesting the entire experimental plot of all three replicates of each variant. The harvest was carried out with a Wintersteiger®field trial harvester.

Duncan's method with the SPSS 19 program (Duncan, 1955) was used for the statistical processing of the obtained data. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSIONS

On Table 1 is presented the established herbicidal efficacy against the weeds. The application of carfentrazone-ethyl or metsulfuron-methyl in all studies effectively reduced the density and biomass of broadleaf weeds. (Bhullar et al., 2013). In our trial, on average for the period, on the 14th day after treatment, the efficacy against *S. arvensis* ranged from 47.5% for treatment 6 (Aminopielik 600 SL - 2.00 l ha^{-1}) to 72.5% in treatment4 (Biathlon 4 D - 55 g ha^{-1}) was found. On the next reporting date, the efficiency increased and reached 67.5-87.5% between variants on average over the experimental years. On the 42nd day after treatment, on average for the conditions of the experiment, the efficacy against the *S. arvensis* reached 100% in variants 3 (Askial One - 1.00 l ha^{-1}) and 4 (Biathlon 4 D - 55 g ha^{-1}). The efficiency of Sekator OD - 0.15 l ha^{-1} and Granstar DF - 15 g $ha^{-1}$was 90% and that of Aminopielik 600 SL - 2.00 l ha^{-1}-77.5% (the lowest among treatments).
On average for the period, the efficacy against *A. arvensis*on the 14th day ranged from 57.5% for treatment 6 (Aminopielik 600 SL - 2.00 l ha^{-1}) to 77.50% fortreatment 3 (Askial One - 1.00 l ha^{-1}) was recorded. At the next reporting date, the efficacy increasedto82.5-92.5% on average between treatments. On the 42ndday after treatment, on average for the conditions of the trial, the efficacy reached 100% in variants 3 (Askial One - 1.00 l ha^{-1}) and 4 (Biathlon 4 D - 55 g ha^{-1}).

Table 1. Efficacy of the studied herbicidal products against the weeds (%)

Treatments	*S. arvensis*								
	2021			2022			Average		
	14 day	28 day	42 day	14 day	28 day	42 day	14 day	28 day	42 day
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Sekator OD - 0.15 l ha^{-1}	60	80	90	65	85	90	62.5	82.5	90
3. Axial One - 1.00 l ha^{-1}	65	85	100	75	85	100	70	85	100
4. Biathlon 4 D - 55 g ha^{-1}	70	90	100	75	85	100	72.5	87.5	100
5. Granstar 75 DF - 15 g ha^{-1}	60	80	90	55	75	85	57.5	77.5	87.5
6. Aminopielik 600 SL - 2.00 l ha^{-1}	45	65	75	50	70	80	47.5	67.5	77.5
Treatments	*A. arvensis*								
	2021			2022			Average		
	14 day	28 day	42 day	14 day	28 day	42 day	14 day	28 day	42 day
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Sekator OD - 0.15 l ha^{-1}	70	90	100	75	80	95	72.5	85	97.5
3. Axial One - 1.00 l ha^{-1}	75	95	100	80	90	100	77.5	92.5	100
4. Biathlon 4 D - 55 g ha^{-1}	75	95	100	70	80	100	72.5	87.5	100
5. Granstar 75 DF - 15 g ha^{-1}	65	85	95	60	80	100	62.5	82.5	97.5
6. Aminopielik 600 SL - 2.00 l ha^{-1}	60	80	90	55	80	90	57.5	80	90
Treatments	*G. aparine*								
	2021			2022			Average		
	14 day	28 day	42 day	14 day	28 day	42 day	14 day	28 day	42 day
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Sekator OD - 0.15 l ha^{-1}	50	70	85	60	75	95	55	72.5	90
3. Axial One - 1.00 l ha^{-1}	55	75	85	60	80	85	57.5	77.5	85
4. Biathlon 4 D - 55 g ha^{-1}	60	80	85	70	75	90	65	77.5	87.5
5. Granstar 75 DF - 15 g ha^{-1}	50	65	70	55	60	65	52.5	62.5	67.5
6. Aminopielik 600 SL - 2.00 l ha^{-1}	40	50	55	45	50	65	42.5	50	60
Treatments	*C. regalis*								
	2021			2022			Average		
	14 day	28 day	42 day	14 day	28 day	42 day	14 day	28 day	42 day
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Sekator OD - 0.15 l ha^{-1}	50	70	85	55	75	90	52.5	72.5	87.5
3. Axial One - 1.00 l ha^{-1}	60	80	90	70	85	95	65	82.5	92.5
4. Biathlon 4 D - 55 g ha^{-1}	65	85	100	70	90	100	67.5	87.5	100
5. Granstar 75 DF - 15 g ha^{-1}	60	70	90	60	65	95	60	67.5	92.5
6. Aminopielik 600 SL - 2.00 l ha^{-1}	60	80	85	55	90	95	57.5	85	90
Treatments	*V. arvensis*								
	2021			2022			Average		
	14 day	28 day	42 day	14 day	28 day	42 day	14 day	28 day	42 day
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Sekator OD - 0.15 l ha^{-1}	50	60	65	45	70	75	47.5	65	70
3. Axial One - 1.00 l ha^{-1}	60	70	85	55	75	80	57.5	72.5	82.5
4. Biathlon 4 D - 55 g ha^{-1}	65	85	90	75	90	95	70	87.5	92.5
5. Granstar 75 DF - 15 g ha^{-1}	50	60	70	45	70	80	47.5	65	75
6. Aminopielik 600 SL - 2.00 l ha^{-1}	40	50	55	45	55	60	42.5	52.5	57.5

On the 14^{th} day after the herbicidal application, an efficiency of 65% against the weed *G. aparine* was reported for treatment4 (Biathlon 4 D - 55 g ha^{-1}). The efficiency of the other treatments on this datevaried from 42.5 to 57.5%. On the next reporting date, the efficacy increased and varied from 50 to 77.5%.

On the last evaluation date, the highest efficacy was recorded for treatment 2 (Sekator OD - 0.15 l ha^{-1}), and the lowest results for treatment 6 (Aminopielik 600 SL - 2.00 l ha^{-1}) - 60% only, which is insufficient to successfully control *G. aparine*.

On average for the period, on the 14th day after treatment, the efficacy against *C. regalis* was from 52.5% for treatment 2 (Sekator OD - 0.15 l ha^{-1}) to 67.5% in treatment 4 (Biathlon 4 D - 55 g ha^{-1}). On the next evaluation date, the efficacy increased and was from 72.5-85% among the variants on average for the research period. On the 42nd day after spraying, on average for the conditions of the study, the efficacy against *C. regalis* was 100% for variant 4 (Biathlon 4 D - 55 g ha^{-1}). An efficacy of 92.5% was reported for treatment 3 (Askial One - 1.00 l ha^{-1}) and 5 (Granstar 75 DF - 15 g ha^{-1}). The control of the weed by Aminopielik 600 SL - 2.00 l ha^{-1}(variant 6) was 90% on the third reporting date. On the last reporting date, the lowest efficiency was recorded for option 2 (Secator OD - 0.15 l ha^{-1}) - 87.5%.

In a trial conducted by Degenhardt et al. (2005), the application of the herbicidesfluroxypyr + 2.4-D provided efficient control of the weed *V. arvensis*. In our study, on average for the period, on the 14th day after application, the obtained efficacy was ranging from 42.5% in treatment 6 (Aminopielik 600 SL - 2.00 l ha^{-1}) to 70% in treatment 4 (Biathlon 4 D - 55 g ha^{-1}). On the 42nd day after spraying, on average for the study conditions, the efficacy against the weed *V. arvensis* reached the highest values with variant 4 (Biathlon 4 D - 55 g ha^{-1}) - 70%. Efficacy of 57.5% was reported for variants 3 (Askial One - 1.00 l ha^{-1}). In the other treatment, the control of the weed was not sufficient, and this species turned out to be one of the most difficult to fight under the conditions of the experiments conducted in 2021 and 2022.

Table 2 presents the established results for vegetative indicators of barley. Higher values for plant height were found in all variants where herbicide treatment was performed against weeds. The statement corresponds with other research findings (Hristova et al., 2021; Shabanet al., 2021; Yanev et al., 2021). The highest plants were measured in variant 3 (Biathlon 4 D - 55 g ha^{-1}) - 1.10 m average for the period. The plants from the control are the lowest - 0.87 m.

The plants from the untreated control have the shortest ears - 8.04 cm average for the period. All herbicide-treated variants had longer ears, with lengths from 13.13 to 13.50 cm. The longest ears were measured from the plants of variant 4 (Biathlon 4 D - 55 g ha^{-1}).

Table 2. Plant height (m) and ear length (cm)

Treatments	Plant height (m)		
	2021	2022	Average
1. Untreated control	0.83 c	0.90 c	0.87
2. Sekator OD - 0.15 l ha^{-1}	1.02 b	1.11 a	1.07
3. Axial One - 1.00 l ha^{-1}	1.12 a	1.01 b	1.07
4. Biathlon 4 D - 55 g ha^{-1}	1.06 b	1.14 a	1.10
5. Granstar 75 DF - 15 g ha^{-1}	1.09 a	1.00 b	1.05
6. Aminopielik 600 SL - 2.00 l ha^{-1}	0.98 b	1.07 c	1.03
Treatments	Ear length (cm)		
	2021	2022	Average
1. Untreated control	8.97c	7.11 c	8.04
2. Sekator OD - 0.15 l ha^{-1}	13.23 a	13.02 a	13.13
3. Axial One - 1.00 l ha^{-1}	13.57 a	12.99 a	13.28
4. Biathlon 4 D - 55 g ha^{-1}	13.59 a	13.41 a	13.50
5. Granstar 75 DF - 15 g ha^{-1}	13.64 a	13.17 a	13.41
6. Aminopielik 600 SL - 2.00 l ha^{-1}	12.87 b	11.78 b	12.33

Figures with different letters are with a proven difference by Duncan's multiple range test ($p < 0.05$).

According to Georgiev et al. (2014), the absolute seed mass is among the most important seed quality indicators. In our study, the parameter was influenced by the treatments and weed infestation. The highest absolute seeds mass was reported for variant 3 (Askial One - 1.00 l ha^{-1}) - 41.54 g. average for the period the lowest absolute seed mass was recordedfor the untreated control - 37.43 g (Table 3).

From the obtained data concerning the hectolitre seed mass of barley, it was observed that all herbicide-treated variants had higher values for hectoliter seed mass compared to the untreated control. In the treated variants, the hectoliter mass varies from 69.75 to 70.83 kg. The seeds of the untreated control had a hectoliter mass of 67.92 kg on average.

Weed control leads to yield increase (Balyan and Malik, 1994; Tonev et al., 2007; Atanasova, 2008; Talgre et al., 2008; Buttar et al., 2015; Hristova et al., 2021; Shaban et al., 2021; Yanev et al., 2021). A high average yield was reported in all variants where herbicide treatment was performed (Table 3). The highest grain yield in variant 4 (Biathlon 4 D - 5.5 g/da) was reported - 6.33 t ha^{-1}. For the other variants, yields varied from 6.02 to 6.22 t ha^{-1}. The lowest yields were recorded in the untreated control - 4.61 t ha^{-1} (Table 3).

Table 3. Absolute seed mass (g), hectoliter seed mass (kg), and barley grain yields (t ha^{-1})

Treatments	Absolute seed mass (g)		
	2021	2022	Average
1. Untreated control	38.14 c	36.71 d	37.43
2. Sekator OD - 0.15 l ha^{-1}	41.29 b	42.11 a	41.70
3. Axial One - 1.00 l ha^{-1}	41.54 a	42.05 a	41.80
4. Biathlon 4 D - 55 g ha^{-1}	41.33 b	42.12 a	41.73
5. Granstar 75 DF - 15 g ha^{-1}	41.30 b	41.69 b	41.50
6. Aminopielik 600 SL - 2.00 l ha^{-1}	41.22 b	40.74 c	40.98
Treatments	Hectoliter seed mass (kg)		
	2021	2022	Average
1. Untreated control	67.33 b	68.50 d	67.92
2. Sekator OD - 0.15 l ha^{-1}	70.00 a	71.50 a	70.75
3. Axial One - 1.00 l ha^{-1}	70.33 a	71.33 a	70.83
4. Biathlon 4 D - 55 g ha^{-1}	70.67 a	70.50 b	70.59
5. Granstar 75 DF - 15 g ha^{-1}	70.50 a	71.00 a	70.75
6. Aminopielik 600 SL - 2.00 l ha^{-1}	70.00 a	69.50 c	69.75
Treatments	Grain yields (t ha^{-1})		
	2021	2022	Average
1. Untreated control	4.47 d	4.75 c	4.61
2. Sekator OD - 0.15 l ha^{-1}	5.97 c	6.06 b	6.02
3. Axial One - 1.00 l ha^{-1}	6.19 b	6.25 a	6.22
4. Biathlon 4 D - 55 g ha^{-1}	6.37 a	6.29 a	6.33
5. Granstar 75 DF - 15 g ha^{-1}	6.26 a	6.11 b	6.18
6. Aminopielik 600 SL - 2.00 l ha^{-1}	6.10 b	6.17 b	6.13

Figures with different letters are with a proven difference by Duncan's multiple range test ($p < 0.05$).

CONCLUSIONS

The application of Askial One - 1.00 l ha^{-1} (Treatment 3) and Biathlon 4 D - 55 g ha^{-1} (Treatment 4) controlled the weeds *S. arvensis* and *A. arvensis* to100%.

The application of Biathlon 4 D controlled *C. regalis* to 100% as well.Secator OD - 1.5 ml/da (Treatment 2) showed the highest efficacy results against the weed *G. aparine,* andthe highest average efficacy against *V. arvensis* was recorded after the application of Askial One - 1.00 l ha^{-1} (Treatment 3).

The highest results for the plant height, ear length, absolute seed mass, and barley grain yields after the application of Biathlon 4 D - 55 g ha^{-1} (Variant 4), and the highest hectoliter mass of the seeds was found after the application of Axial One - 1.00 l ha^{-1}(Treatment 3).

ACKNOWLEDGEMENTS

The research was financially supported by Project 17-12 at The Center of Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University of Plovdiv, Bulgaria.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF THE EFFICIENCY OF A BIOSTIMULANT CONTAINING ORGANIC SUBSTANCES BY USING LABELED NITROGEN 15N

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Abstract

Fertilizers with foliar application or those containing substances that have a nutrient-stimulating effect have indicated that the use of biostimulants alone in crop treatment often does not lead to significant effects on yield and quality. The carried out research aimed to establish, using the labeled nitrogen 15N as a tracer, the contribution of complex foliar fertilizers containing natural organic substances, to increase the efficiency of using different forms of nitrogen from the soil applied fertilizer. The degree of recovery from soil to plant was evaluated using the sunflower test plant (Helianthus an-nuus). The procedure was performed under foliar application conditions of two fertilizers containing macronutrients, secondary elements and microelements with / without organic substances (protein hydrolyzate). Stable 15N isotopes have been used to examine nitrogen (15N) uptake from soil-applied chemical fertilizers. Depending on the nitrogen species applied, an increase of the 15N/N ratio was observed as follows: amide nitrogen (-NH_2) < ammoniacal nitrogen (-NH_4) < nitric nitrogen (-NO_3).

***Key words**: foliar fertilizers, labeled nitrogen 15N, protein hydrolysate, sunflower, biostimulants.*

INTRODUCTION

The use of fertilizer products from the class of biostimulators for agriculture is expanding rapidly (Chiaiese et al., 2018; Xu L. et al., 2018; du Jardin, 2015; Calvo et al., 2014). Applied to plants, seeds or soil, many formulations are available today thanks to intensive research and continuous experimentation that provides information on their effectiveness and mechanisms of action (Caradonia et al., 2019; Garcia-Gonzalez et al., 2016; Michalak et al., 2016; Sharma et al., 2014).

Many fertilizers contain organic substances from the category of protein hydrolysates (Gupta et al., 2021; Gimondo et al, 2019; Maurya et al., 2016; du Jardin, 2015; Shak, et al., 2014), humic substances and fulvic acids (Gupta et al., 2021; Olivares et al., 2017; Tudor et al., 2017; Narwal et al., 2006; Chung et al., 2000), plant or algae extracts (Consentino et al., 2021; Gupta et al., 2021; Hashem et al., 2019; Ronga et al., 2019; Battacharyya et al., 2015), which have the ability to stimulate the metabolism of nutrients and to facilitate absorption of ionic species or molecules (Gupta, S. & Van Staden, 2021; Colla G. et al., 2017; Colla, G. et al., 2014).

In general, products with a bioregulatory role are organic substances, which, applied in small concentrations, participate in the physiological processes of plant growth and development, with favorable effects, both quantitative and qualitative, on crops, contributing to reducing the polluting impact of chemical fertilization on the environment (Bartucca et al., 2022; Del Buono and D. Can, 2021; Wan et al., 2021; Ronga et al., 2019; Salvi et al., 2019; Colla et al., 2017, Tudor et al., 2017; Amirkhani et al., 2016; Colla et al., 2014).

One way to track the efficiency of nitrogen fertilizer uptake by plants is to use nitrogen labeled with a stable isotope such as 15N. Stable isotope-labeled (15N) tracers allow knowing the amounts of nutrients differentially absorbed by plants from soil and fertilizers, as well as the transformations that take place in the complex soil-plant-fertilizer system (Congreves et al., 2021; Langelier et al., 2021; Zhang et al., 2021; Anas et al., 2020).

The production of nitrogen fertilizers is energy intensive (Teske et al., 2022) and large amounts of N-fertilizer are currently intensively supplied by growers every season in the form of nitrate, ammonium or urea (Goñi et al., 2021).

The researches carried out have shown that vegetable protein hydrolyzate is a source whose use in fertilizers with foliar application can lead to a decrease in the amount of nitrogen coming from mineral fertilizers that are obtained with high energy consumption.

MATERIALS AND METHODS

The paper presents the results obtained from experiments carried out in a greenhouse for sunflower crop.

Using the labeled nitrogen 15N for the basic fertilization of the sunflower crop, the effect of the foliar application of two fertilizers on the absorption of different forms of nitrogen from the soil into the plant was investigated.

In the experiment carried out, the FERT fertilizer containing macronutrients, secondary elements and microelements and the HFERT product with the same matrix as FERT to which soy protein hydrolyzate was added were used (Table 1).

The vegetable protein hydrolyzate used for the introduction into the HFERT product was obtained from soybeans, applying mixed hydrolysis with a first chemical step and then an enzymatic one with Alcalase 2.4 L.

The compositional characteristics of the fertilizers used are presented in Table 1.

Table 1. Compositional characteristics of FERT and HIDROFERT fertilizers

Compositional characteristics	FERT	HFERT
	Content (%)	
Nitrogen, N total, including:	18.5	21.2
ammoniacal	3.6	3.9
nitric	5.4	6.1
amidic	9.5	9.5
organic	0	1.7
Phosphorus, P_2O_5	18.3	19.2
Potassium, K_2O	18.2	18.9
Boron, B	0.01	0.01
Copper, Cu	0.005	0.006
Iron, Fe	0.047	0.052
Magnesium, MgO	0.23	0.25
Manganese, Mn	0.023	0.025
Molybdenum, Mo	0.001	0.001
Sulfur, SO_3	0.45	0.51
Zinc, Zn	0.01	0.01
Organic substances, including:	0.60	10.4
protein hydrolysate	0	9.7
free amino acids	0	0.08
pH	6.58	6.72

The experiments were organized in the vegetation house of the National Research and Development Institute for Soil Science, Agro-chemistry and Environment Protection-RISSA Bucharest, having as test plant sunflower (*Helianthus annuus*), the variety NEOMA. The agrochemical experiments on the sunflower culture, were carried out on a chernozem soil with the following physico-chemical characteristics: 3.48% humus, 0.17% nitrogen total, 146 mg/kg mobile phosphorous (PAL), 224 mg/kg mobile potassium (KAL), 2.01% organic carbon, and mobile forms of cations in solution at the level: 13.1 mg/kg Zn, 2.74 mg Cu, 86 mg/kg Fe, 8.6 mg/kg Mn and pH 6.78.

The experiences that have taken place have involved the following activities:

- organizing and setting up the experience in pots vegetation containing 10 kg cambic chernozem soil;
- basic fertilization by incorporation into the soil, before sowing ($N_{180}P_{45}K_{45}$), this means 180 kg of nitrogen, 45 kg of phosphorus and 45 kg of potassium per hectare;
- the sowing itself, making sure that the seed material is uniform, calibrated (appearance, weight);
- fertilization using products containing ^{15}N isotopically labeled nitrogen in the amidic, ammoniacal and nitric groups after sprouting with a dose of 30 mg ^{15}N/pot and 10 mg ^{15}N/ plant;
- the maintenance of the plants, following daily watering conditions using 70% water of the field capacity;
- preparation of dilute fertilizer solutions and application to plants, in a dose of 10 ml solution with a concentration of 1% / plant;
- application of 3 foliar treatments at an interval of 7 days apart from the previous one.

The experimental scheme of agrochemical testing is presented in Table 2. Each variant was with three replicates.

Table 2. Experimental scheme of agrochemically testing

No. var.	Codes	Basic fertilization	Foliar fertilization	^{15}N nitrogen species applied
V1	WBF	Without basic fertilization	Without foliar application	-
V2	WBF+FERT	Without basic fertilization	Foliar application FERT	-
V3	WBF+HFERT	Without basic fertilization	Foliar application HFERT	-
V4	BF + ^{15}N-NH_2	Basic fertilization ($N_{180}P_{45}K_{45}$)	Without foliar application	^{15}N-NH_2
V5	BF + ^{15}N-NH_4	Basic fertilization ($N_{180}P_{45}K_{45}$)	Without foliar application	^{15}N-NH_4
V6	BF + ^{15}N-NO_3	Basic fertilization ($N_{180}P_{45}K_{45}$)	Without foliar application	^{15}N-NO_3
V7	BF + ^{15}N-NH_2 + FERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with FERT	^{15}N-NH_2
V8	BF + ^{15}N-NH_4 + FERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with FERT	^{15}N-NH_4
V9	BF + ^{15}N-NO_3 + FERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with FERT	^{15}N-NO_3
V10	BF + ^{15}N-NH_2 + HFERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with HFERT	^{15}N-NH_2
V11	BF + ^{15}N-NH_4 + HFERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with HFERT	^{15}N-NH_4
V12	BF + ^{15}N-NO_3 + HFERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with HFERT	^{15}N-NO_3

The following ^{15}N labeled fertilizers applied by incorporation into soil using a dose of 30 mg / pot were used in the experiments:

- 20% amide (N-NH_2) labeled ^{15}N nitrogen urea;
- 20% ammoniacal (N-NH_4) labeled ^{15}N nitrogen ammonium nitrate;
- 20% nitric (N-NO_3) labeled ^{15}N nitrogen ammonium nitrate;

After 45 days of sprouting, these plants were harvested as green mass, dried and ground in order to perform isotopic examination.

RESULTS AND DISCUSSIONS

The analysis of the achieved results by applying the nuclear technique including the use of the ^{15}N stable isotope concerned the nitrogen recovery rate and the evolution of nitrogen export depending on the applied fertilization and the labeled nitrogen species applied in soil, using the same nitrogen dose (source $^{15}NH_4$-ammonium nitrate, ammonium nitrate-$^{15}NO_3$, $^{15}NH_2$-urea).
The direct method on 15N add is the most appropriate to determine the recovery efficiency of N derived from fertilizers.
The isotopic determinations of the dried plant material samples were performed using a Thermo Delta V mass spectrometer (IRMS) with an interface for elemental analysis NC 2500.
The following parameters were evaluated to quantify the effect of soil and foliar fertilization on the sunflower crop:

- nitrogen (N, %);
- examining the isotopic ratio or the percentage of atoms, $^{15}N/N$ (%), in the samples of plant material depending on the ^{15}N species applied;
- examining the $\delta^{15}N$ parameter, which represents the accumulation of the ^{15}N isotope in the analyzed sample. This represents the corrected value of the ^{15}N isotope measured against a primary reference scale. The main reference scale for $\delta^{15}N$ used was atmospheric air. The value of $\delta^{15}N$ represents the $^{15}N/^{14}N$ ratio and expressed in units per million (‰);
- ^{15}N isotope export in sunflower plant according to the ^{15}N species applied and foliar fertilization applied; The uptake of 15N enriched fertilizer added to soil will result in a 15N/14N ratio greater than 0.3663% within the plant, the extent of which is a reflection of uptake of the labelled 15N fertiliser;
- the recovery rate for ^{15}N isotope applied depending on the species of ^{15}N marked nitrogen applied, due only to foliar fertilization.

Part of the obtained results were presented in a previously published article (Nicu et al., 2021).
In order to evaluate the effect of foliar fertilization on nitrogen uptake from chemical fertilizers applied to the soil, the following parameters in the plant material samples were evaluated:

- the isotopic ratio or the percentage of atoms, $^{15}N/N$ (%), in the samples of plant material depending on the ^{15}N species applied;
- determining the $\delta^{15}N$ parameter, representing the accumulation of the ^{15}N isotope in the analyzed sample (‰);
- the export and the recovery rate of ^{15}N isotope in the sunflower plant according to the ^{15}N species applied in soil.

The results obtained through the analysis of the plant material and their interpretation are presented in the following figures (Figures 1-3):

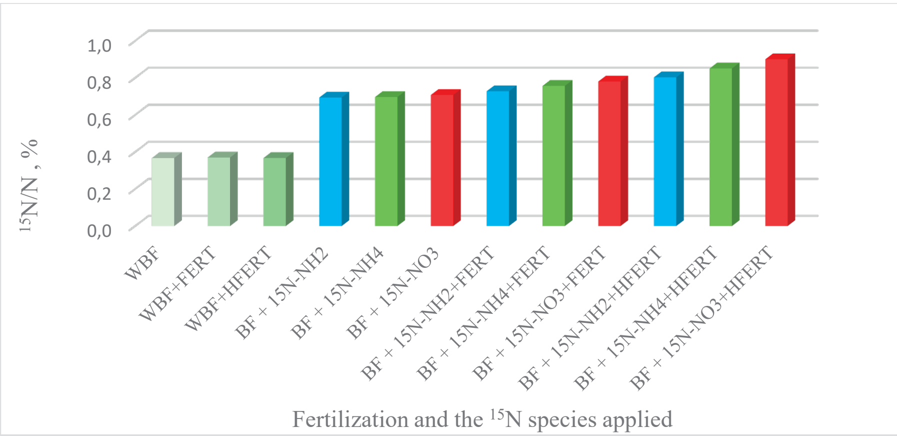

Figure 1. Evolution of the ratio 15N/N, % depending on the basic and foliar fertilization applied

Plants are able to absorb essential elements through their leaves. The absorption takes place through their stomata and also through their epidermis. Base fertilization is essential to ensure adequate nutrition and to make foliar application more efficient (Figure 1). Also, the proteins and amino acids in the HFERT product contributed to a superior uptake and metabolism of the nitrogen nutrient from the basic fertilization, a fact proven with the help of the ^{15}N tracer (Figure 2).

Products containing protein hydrolysates have been shown to be effective with benefits on growth, yield, product quality, resource efficiency and stress tolerance of a wide range of agronomic crops (Rouphael and Colla, 2020; Colla et al., 2017; Calvo et al., 2014).

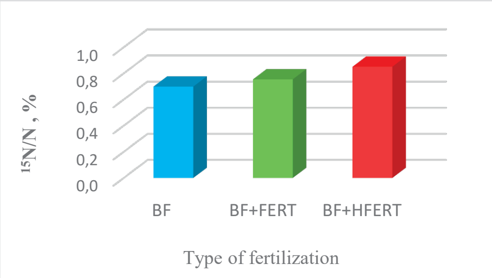

Figure 2. Evolution of the ratio 15N/N, % depending on foliar fertilization

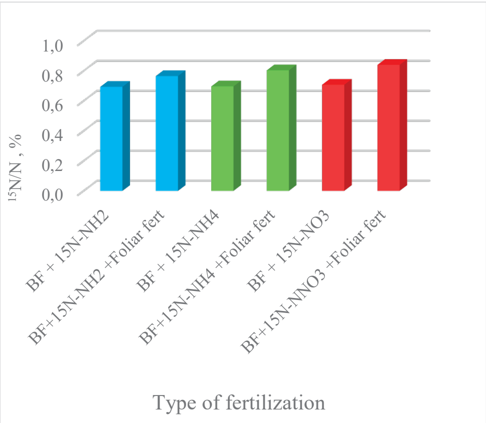

Figure 3. Evolution of the ratio 15N/N, % depending on 15N species applied and fertilization

The analysis of the experimental results revealed that the isotopic ratio $^{15}N/N$ in the plant material samples increased in the order of basic fertilization, basic fertilization including FERT fertilizer foliar application and basic fertilization including HFERT foliar application (Figure 2).

The increase of the $^{15}N/N$ ratio was 7.9% compared to the only basic fertilization variant for foliar application of FERT fertilizer and 21.7% for HFERT fertilizer. The foliar application of the HFERT fertilizer has led to an increase of the $^{15}N/N$ ratio with 12.8% compared to the FERT fertilizer variant (Figure 2). Between the two foliar applied fertilizers the difference was significant by applying HFERT variant.

Compared to the control with the basic fertilization (BF) the differences obtained with the foliar application were statistically significant for FERT and distinctly significant for HFERT (Figure 2).

Depending on the nitrogen species applied, an increase of the $^{15}N/N$ ratio was noted as follows: amide nitrogen ($-NH_2$) < ammoniacal nitrogen ($-NH_4$) < nitric nitrogen ($-NO_3$). The application of cumulative foliar fertilization (FERT + HFERT) compared to the control to which no foliar treatments were applied ensured an increase of the 15N/N ratio by 10.3% for $^{15}N-NH_2$, by 15.4% for $^{15}N-NH_4$ and, respectively, 18.7% in the case of $^{15}N-NO_3$ (Figure 3).

The evolution of the isotopic ratio $^{15}N/N$ in the plant material samples and of the parameter $\delta^{15}N$ depending on the used foliar fertilizer and the ^{15}N marked nitrogen species applied in soil, are shown in Figures 4 and 5.

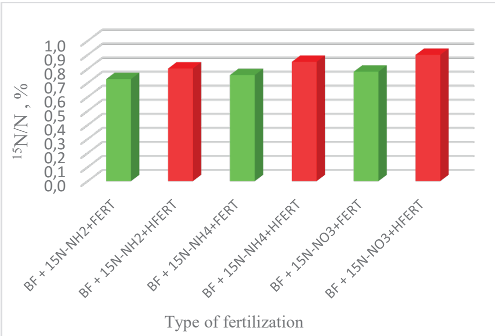

Figure 4. Evolution of the $^{15}N/N$ ratio, %, depending on the foliar fertilizer used and the labeled nitrogen ^{15}N species applied

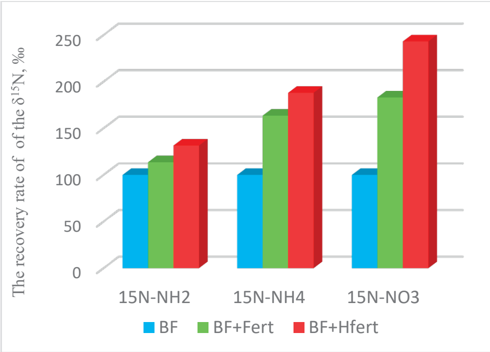

Figure 5. The recovery rate of the $\delta^{15}N$, ‰, depending on the foliar fertilizer used and the labeled nitrogen 15N species applied, compared to the non-foliar fertilized variant (100%)

The compositional difference between the applied foliar fertilizers consists in the fact that the HFERT product contains, in addition to FERT, hydrolyzed soy protein. If we compare the effect of this hydrolyzate on ^{15}N labeled fertilizers applied by incorporation into soil, we notice that the application of the HFERT product leads to increased accumulations in the plant of ^{15}N compared to the FERT product.

Depending on the nitrogen species applied, an increase of the $^{15}N/N$ (%), was noted as follows: amide nitrogen ($-NH_2$) < ammoniacal nitrogen ($-NH_4$) < nitric nitrogen ($-NO_3$). The increase due to the variant BF + $^{15}N-NO_3$ + HFERT is 15.4% compared to BF + $^{15}N-NO_3$ + FERT, 12.5% for the $^{15}N-NH_4$ and 10.3% for the $^{15}N-NH_2$ species (Figure 4). These data show us the effect that the protein hydrolyzate has on increasing the root activity of assimilating the nitrogen nutrient from the fertilizers incorporated in the soil, due to the increase in photosynthesis processes.

For the parameter $\delta^{15}N$ (‰), representing the accumulation of the ^{15}N isotope, it increased ascending from basic fertilization, basic fertilization including FERT fertilizer foliar application and basic fertilization including HFERT foliar application. The increase of $\delta^{15}N$ was 55.4% compared to only the basic fertilization variant for FERT foliar application and 86.4% for HFERT application.

The foliar application of the HFERT fertilizer led to an increase for the parameter δ15N (‰), compared to the FERT variant with 15.7% for the $^{15}N-NH_2$ species, 15.0% for the $15N-NH_4$ species and 32.8% for the $^{15}N-NO_3$ species.

Depending on the applied nitrogen species, an increase of the parameter $\delta^{15}N$ in the order of amidic nitrogen ($-NH_2$) < ammoniacal nitrogen ($-NH_4$) < nitric nitrogen ($-NO_3$) was noted. By reference to the basic fertilized variant but without foliar application considered 100%, these increases were between 13.6% ($^{15}N-NH_2$) and 83.1% ($^{15}N-NO_3$) for the FERT variant. The same trend is maintained for the variants where the HFERT product was applied, but the increases were between 31.5% ($^{15}N-NH_2$) and 143.2% ($^{15}N-NO_3$) (Figure 5).

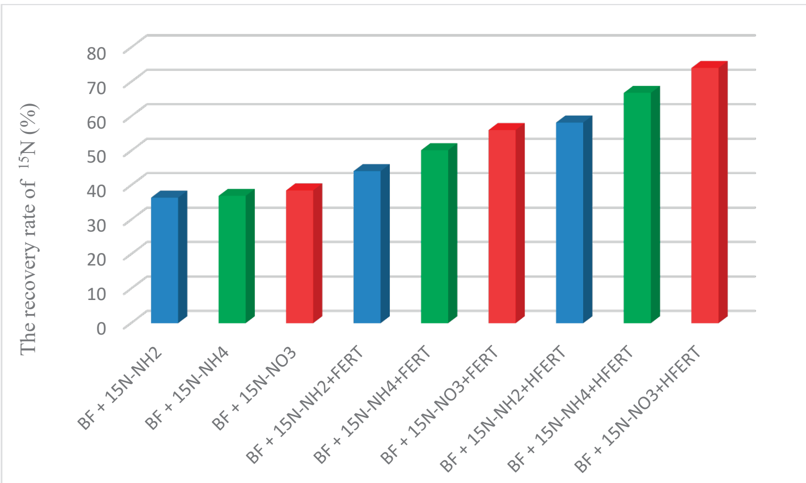

Figure 6. Evolution of the recovery rate for the labeled nitrogen (^{15}N, %) applied depending on the used fertilization and the nitrogen species marked ^{15}N applied

The average recovery rate for isotopically labeled nitrogen ^{15}N (%) depending on the used fertilization was 37.30% for the variant using basic fertilization only, 50.13% in case of additional application of FERT foliar treatment and 66.38% for the application of HFERT foliar treatment (Figure 6).

The data obtained in this study are slightly higher than those presented by other authors (Yan et al., 2020), in which fertilizer ^{15}N recovery for different crops was between 23% and 30%, but these are consistent with the non-foliar fertilized variants (BF + $^{15}N-NH_2$, BF + $^{15}N-NH_4$, BF + $^{15}N-NO_3$) which ranged from about 36% to 38%.

The isotopically labeled nitrogen recovery rate, depending on the applied species, as well as that due only to the foliar application of the two fertilizers, are shown in Figure 7. The highest rate of nitrogen recovery was noted for the nitric form (65.06%), followed by the ammoniacal and the last one for the amidic form (51.19%), regardless of the foliar applied fertilizer.

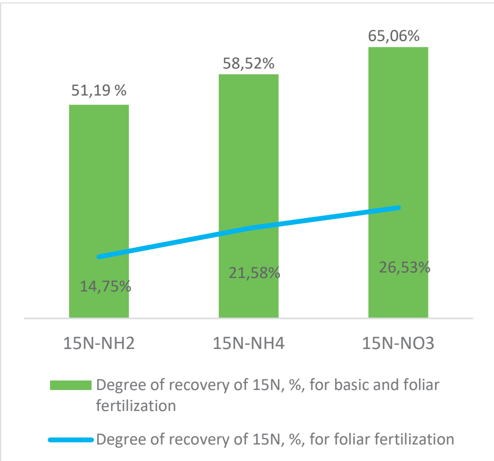

Figure 7. The degree of recovery of ^{15}N for basic and foliar fertilization and due only to foliar fertilization

The assessment of the recovery rate for nitrogen due to foliar fertilization alone ranged between 14.75% ($^{15}N-NH_2$) and 26.53% ($^{15}N-NO_3$) Figure 7. In the case of the corn crop, the application of nitrogen-15 enriched ammonium nitrate showed an efficiency of fertilizer use between 43 to 57% of applied N (Reddy and Reddy, 1993).

The nitrogen recovery rate due to the presence of protein hydrolysate in the fertilizer's matrix was not different for the ^{15}N labeled species and ranged between 24.19% ($^{15}N-NH_2$ species) and 24.89% ($^{15}N-NH_4$ species). These data show us that the positive effect due to the presence of hydrolyzed protein is not influenced by the form of nitrogen in the basic fertilization.

CONCLUSIONS

In order to evaluate the degree of translocation of different nitrogen forms from the soil into the plant, chemical fertilizers with the ^{15}N labeled isotope applied by incorporation into the soil, was used. The degree of translocation was evaluated using the sunflower test plant (*Helianthus annuus*). The procedure was performed under foliar application conditions of two fertilizers containing an NPK matrix including microelements, with / without organic substances (protein hydrolyzate).

The isotopic ratio $^{15}N/N$, in plant material samples increased as follows: basic fertilization, basic fertilization including FERT foliar application and basic fertilization including HFERT foliar application, ranged from 0.69% (BF), ranged from 0.75% (BF+FERT), and to 0.85% (BF+HFERT).

The presence of the protein hydrolyzate in the NPK matrix of the biostimulant ensured an increase in the $^{15}N/Nt$ ratio by 21.7% compared to the unfertilized foliar control and by 12.8% respectively compared to the FERT foliar fertilizer.

Depending on the applied nitrogen species, an evolution of the parameter $\delta^{15}N$ was noted as follows: amide nitrogen ($-NH_2$) < ammoniacal nitrogen ($-NH_4$) < nitric nitrogen ($-NO_3$).

The presence of the protein hydrolyzate in the NPK matrix of the biostimulant ensured an increase in the $\delta^{15}N$ parameter by 13.6% in the case of amide nitrogen ($-NH_2$), by 63.6% in the case of ammoniacal nitrogen ($-NH_4$) and by 88.1% in the case of nitric nitrogen ($-NO_3$), by applying the HFERT foliar fertilizer.

The assessment of the recovery rate for nitrogen due to foliar fertilization alone ranged between 14.75% ($^{15}N-NH_2$) and 26.53% ($^{15}N-NO_3$).

ACKNOWLEDGEMENTS

This research was conducted under the NUCLEU Program, Contract No. 19 34N/2019 - Sustainable soils for high-performance agriculture and a healthy environment – SAPS, Project PN 19 34 03 01 "Innovative products for sustainable agriculture and food security in the context of global change", NUCLEU Program, Contract No. 23 29N/2022 - “Innovative solutions for maintaining and restoring soil health under climate changes adaptation - SOL-SAN”, Project PN 23 29 02 01 "New organic fertilizer products for efficient use of natural resources in sustainable agriculture" and project number 44 PFE /2021, Program 1 - Development of national research-development system, Subprogramme 1.2 - Institutional performance - RDI Excellence Financing Projects.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

AGRONOMIC RESPONSE OF RAPESEED SEED AND OIL YIELD ON DIFFERENT MINERAL FERTILIZING SCHEME

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Abstract

Rapeseed is a crop that has a significant agronomic response in terms of seed yield and oil content and yield. This study aims to assess the influence of fertilizer type, quantity and the combined effect of them on both seed and oil yield in the pedoclimatic conditions of Western Plain in Romania. The research was carried out on a chernozem soil with a weak acidic reaction and for biological material, it consisted of one rapeseed hybrid developed by Limagrain company, Astronom. The experimental field was uniformly fertilized at the same time with sowing with 200 kg/ha of 27:13.5:0 complex fertilizer and in order to assess the influence of fertilizers, in the spring, three graduations of fertilizers were used, as follows: a1 - E34 (10:24:0 + 0.1Zn + 0.1Br +20 SO_3); a2 - DAP (18:46:0) and a3 - 20:20:0. The results highlight the increase of both seed and oil yield, depending on the type of the fertilizer, with as much as 20% compared to the field average.

Key words*: rapeseed, oil, yield, fertilizers.*

INTRODUCTION

Autumn rapeseed, due to its use both in nutrition and as biofuel, as well as the yield determined by the level of production compared to the investment level, has experienced significant development in the last decade (Imbrea, 2017; Fridrihsone, 2020). Yields in rapeseed are dependent on levels of mineral fertilization, due to the high consumption of elements such as K_2O, N, P_2O_5, S, Mg, Ca, and B (Wang et al., 2010; Men et al., 2020) and soil moisture (Wick, 2012; Zhang et al., 2013; Eyni et al., 2020; Smuleac et al., 2020; Bečka et al., 2021).

Another important element of rapeseed fertilization is the rate of nutrient absorption, considering that the most intense absorption of macroelements and sulphur occurs from the resumption of vegetation (February-March) until flowering (Grosz, 2011; Suvet, 2021), as well as the fact that each element in particular has an important contribution to the expression of the potential of rapeseed production (Louvieaux, 2020).

Regarding the absorption of micronutrients, boron, zinc, and molybdenum have a special role in fruiting, flower formation, and pollination, reaching a maximum absorption during the flowering and silique formation period (Haneklaus, 1999; Bătrâna et al., 2021).

MATERIALS AND METHODS

The effect of the type of mineral fertilizer and the crop establishment period on the level of production, content, and oil yield, as well as the interaction between the experimental factors, was studied in a bifactorial experiment set up at the Didactical Farm (SDE) of University of Life Sciences "King Mihai I" from Timisoara in the agronomical year 2020-2021 on chernozem, which is the most common soil in the region. The experience was arranged according to the method of randomized blocks, with the following grading of the experimental factors: Factor A - type of mineral fertilizer, with three gradations: a1 - E34 (10:24:0 + 0.1Zn + 0.1Br +20 SO_3); a2 - DAP (18:46:0) and a3 - 20:20:0. Factor B - crop establishment period, with three gradations: b1 - 10-20.08, b2 - 21-31.08; and b3 - 1-10.09. The fertilizers were applied in fractions, 200 kg/ha of 27:13:5:0 at seedbed preparation and 200 kg of each of the three fertilizers in the spring. The rapeseed hybrid used was Astronom. The preceding crop was winter wheat.

RESULTS AND DISCUSSIONS

Table 1 presents the results of seed yield according to fertilization. The average seed yield recorded values ranging from 2841 kg/ha on agrofund a3 to 3626 kg/ha on agrofund a1.

Table 1. Seed yield by fertilizer type

Factor A Fertilizer type	Seed production		Difference [kg/ha]	Significance
	kg	%		
a1 - E34	3626	112.2	393	***
a2 - DAP	3231	99.9	-2	ns
a3 - 20.20	2841	87.9	-391	000
media	3233	100.0	ns	
DL 5% = 13.69 kg; DL 1% = 18.75; DL 0.1% = 25.55				

From the presented data, it can be observed that compared to the control - the average of the experiment (3233 kg/ha), two levels of fertilization resulted in statistically significant yield increases, at a1 and a3: the increase obtained at a1 was 393 kg, meaning that the production obtained at a1 exceeded the experiment's average by 12.2%, and at a3, around 391 kg, but the production is below the experiment's average, meaning that the increase is negative. Seed production according to the sowing period is presented in Table 2.

Table 2. Seed yield according to the sowing period

Factor B Sowing period	Seed production		Difference [kg/ha]	Significance
	kg	%		
b1 - 10-20.08	3201	99.0	-31	000
b2 - 20-31.08	3227	99.8	-6	ns
b3 - 1-10.09	3270	101.1	37	***
media	3233	100.0	mt	
DL 5% = 13.69 kg; DL 1% = 18.75; DL 0.1% = 25.55				

Compared to the control - the experimental average (3233 kg/ha), only in the variant sown in the first decade of September, the yield exceeded the control by 37 kg, a difference statistically significant as highly significant. In the case of the other two experimental variants, the yields obtained were below the experimental average. The seed production obtained at the interaction of factors A x B and the significance the production differences compared to the control are presented in Table 3.

Table 3. The seed production obtained with the A x B interaction
and the significance of the production differences compared to the control - average

Factor B Sowing period	Factor A -Fertilizer type											
	a1 - E34				a2 - DAP				a3 - 20.20			
	Crop		Diff. [kg]	Signif.	Crop		Diff. [kg]	Signif.	Crop		Diff. [kg]	Signif.
	kg	%			kg	%			kg	%		
b1 - 10-20.08	3605	111.5	372	***	3197	98.9	-35	00	2802	86.7	-431	000
b2 - 20-31.08	3623	112.1	390	***	3216	99.5	-17	ns	2842	87.9	-391	000
b3 - 0-10.09	3650	112.9	417	***	3280	101.5	47	***	2880	89.1	-353	000
Average	3233											
DL 5% = 23.71 kg	DL 1% = 32.48	DL 0.1% = 44.26										

Compared to the control - the experience average (3233 kg/ha), the following yields were obtained:

- Statistically significant increases for fertilization with a1 and a3, regardless of the sowing time. For fertilization with a1, the increase ranged between 372 kg [b1] and 417 kg [b3], meaning that the yield obtained at a1 exceeded the experience average by 11.5% [b1] and 12.9% [b3]. It is worth noting that for a3, the increases were negative, regardless of the sowing time.
- A highly significant increase was obtained for fertilization with a2, in sowing time b3, a significant increase in b1, and a non-significant increase in b2.

The influence of the type of fertilizer on seed production is shown in Figure 1.

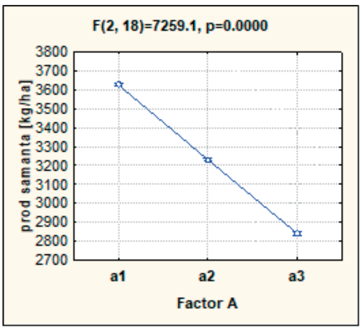

Figure 1. Seed yield variation according to the fertilization level [Factor A]

The seed production has a descending trend, varying between 3626 and 2840 kg. The type of fertilizer used has an influence on seed production, with the highest yield obtained in variant a1, fertilized with E34, and decreasing for variants a2 (DAP) and a3 (20:20:0). The differences in yields between fertilizer levels are statistically very significant [$p<0.001$].

The influence of sowing time on seed production is presented in Figure 2.

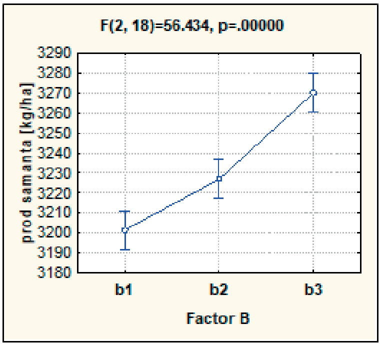

Figure 2. Seed yield variation according to the sowing period [Factor B]

The seed production increases with the sowing period, and the two variables are directly proportional. The evolution of seed production is ascending. The production varies between 3200[b1] kg and 3270[b3] kg. The increase in production from b1 to b2 is slower than the increase from b2 to b3.

The analysis of the results regarding the influence of the interaction between factors A x B [fertilizer type x sowing period] on seed production is presented in Figure 3

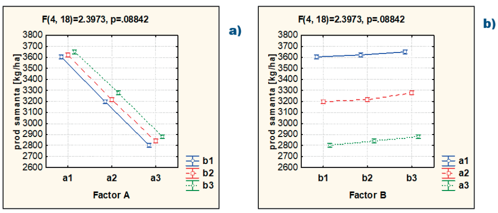

Figure 3. Seed yield variation [A x B]

Regardless of the sowing time, the seed yield has a decreasing trend as the level of fertilization increases.

The highest yield is obtained in a1, while the lowest is in a3.

The yields obtained in a1, at the three sowing times varied between 3605-3650 kg/ha, in variant a2 between 3200-3300 kg/ha, and those in variant a3 varied between 2802-2880 kg/ha.

Figure 4 presents the contribution of the experimental factors and their interaction in achieving the production.

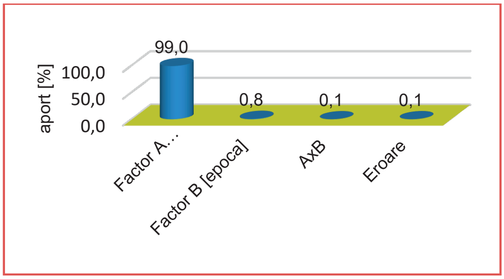

Figure 4. The contribution of factors A [fertilizer type], B [sowing period], and the interaction of A x B

Factor A [fertilizer type] contributes to the production in a proportion of 99.0%, factor B [seeding time] contributes with 0.8%, and the interaction A x B with 0.1%. Therefore, the highest contribution is made by the type of fertilization factor, followed by factor B [seeding time] and the interaction A x B.

The oil content depending on the type of fertilization, seeding time, and the interaction A x B is presented in Table 4.

Based on the type of fertilization, the oil content varied between 42.3% (20:20:0) and 44.1% (E34). The average experience recorded a value of 43.1%, and only in variant a1 (E34) was there a statistically significant increase of 1% compared to the average.

The sowing period determined oil content ranging from 42.9% (second decade of August) to 43.2% (last decade of August and first decade of September).

Table 4. Oil content depending on the type of fertilization, the sowing period and the A x B interaction

Experimental factor	Factor levels	Oil content [%]	Std.Err.	-95.00%	+95.00%	Diff. [%]	Signif.
Factor A [Fertilizer type]	a1 - E34	44.1	0.123779	43.79100	44.36187	1.0	***
	a2 - DAP	43.0	0.221703	42.47740	43.49990	-0.1	ns
	a3 - 20.20	42.3	0.115329	41.99917	42.53107	-0.8	000
	Average	43,1				mt	
	DL 5% = 0.36 %; DL 1% = 0.49; DL 0.1% = 0.67						
Factor B [sowing period]	b1 - 10-20.08	42.9	0.306068	42.17330	43.58489	-0.2	ns
	b2 - 20-31.08	43.2	0.297936	42.53226	43.90634	0.1	ns
	b3 - 0 - 10.09	43.2	0.305181	42.52806	43.93556	0.1	ns
	Average	43,1				mt	
	DL 5% = 0.36 %; DL 1% = 0.49; DL 0.1% = 0.67						
AxB Interaction							
Factor A	Factor B						
a1 - E34	b1 - 10-20.08	44.1	0.034264	43.91871	44.21356	1.0	**
	b2 - 20-31.08	44.0	0.207695	43.10638	44.89366	0.9	**
	b3 - 0 - 10.09	44.2	0.364435	42.59511	45.73118	1.1	**
a2 - DAP	b1 - 10-20.08	42.2	0.173968	41.43612	42.93317	-0.9	00
	b2 - 20-31.08	43.5	0.188647	42.73541	44.35877	0.4	ns
	b3 - 0 - 10.09	43.2	0.121145	42.71298	43.75547	0.1	ns
a3 - 20.20	b1 - 10-20.08	42.4	0.160351	41.69659	43.07646	-0.7	0
	b2 - 20-31.08	42.1	0.116490	41.60957	42.61199	-1.0	00
	b3 - 0 - 10.09	42.3	0.317024	40.93403	43.66211	-0.8	0
	Average	43,1					
	DL 5% = 0.62%; DL 1% = 0.85; DL 0.1% = 1.15						

Regarding the interaction between factors, compared to the control - the average experience, the following increases were obtained: a distinct significant increase in fertilization type a1 (E34) regardless of the sowing period, a distinct significant increase in type a2 (DAP) in the b1 period (second decade of August), while the increases in the b2 (last decade of August) and b3 (first decade of September) periods were not significant. In the case of fertilization type a3 (20:20:0), a distinct significant increase was obtained in b2 (last decade of August), while the increases in b1 (second decade of August) and b3 (first decade of September) periods were significant.

It is noteworthy that for the fertilization levels of a2 (DAP) and a3 (20:20:0), the increases are below the average experience, meaning negative increases.

The influence of the type of fertilization on the oil content is presented in Figure 5

The oil content decreases with the level of fertilization, the two variables being inversely proportional. The trend of the oil content is descending. The oil content varies between 44% and 42.3%. The differences between the levels of fertilization are highly significant [$p<0.001$].

The influence of the sowing period on the oil content is shown in Figure 6.

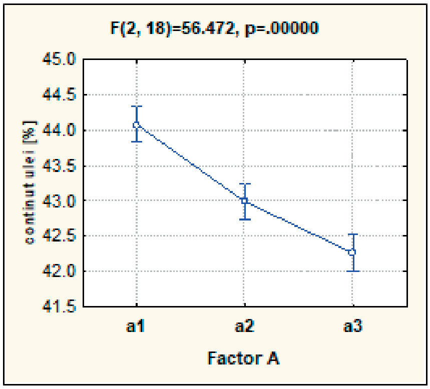

Figure 5. Oil content variation for the three types of fertilizers [factor A]

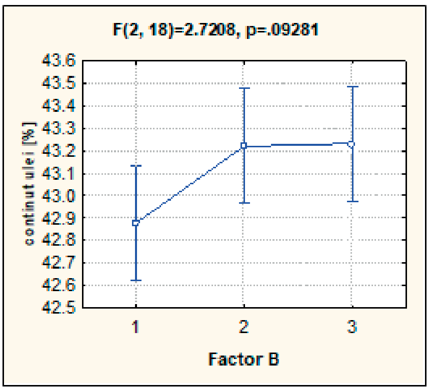

Figure 6. Oil content variation for the three sowing periods [factor B]

The oil content increases with the sowing period, thus from b1 (the second decade of August) to b2 (the last decade of August), there is an upward trend [increasing from 42.9% to 43.2%], while from b2 (the last decade of August) to b3 (the first decade of September), the oil content registers approximately the same value [from 43.22% to 43.23%]. The analysis of the results regarding the influence of the interaction between the factors AXB [fertilization x sowing period] on the oil content is shown in Figure 7.

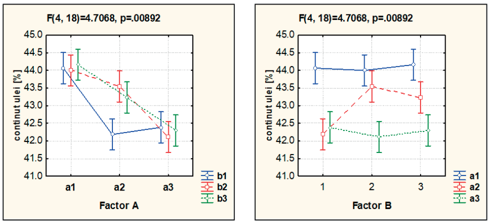

Figure 7. Oil content variation [A x B interaction]

Regardless of the sowing period, the oil content has a decreasing trend with respect to the fertilization level, with the exception of the b1 period (the second decade of August) which has a decreasing trend from a1 (E34) to a2 (DAP), and from a2 (DAP) to a3 (20:20:0), the trend is ascending. The highest oil content is obtained at a1 (E34), while the lowest is at a3 (20:20:0). The oil contents obtained at a1 (E34) for the three sowing periods ranged from 44.1% to 44.2%, with a decreasing trend from b1 (the second decade of August) to b2 (the last decade of August), and an ascending trend from b2 (the last decade of August) to b3 (the first decade of September), with values ranging from 44.1% [b1], 44.0% [b2] and 44.2% [b3]. The same trend applies to a3 (20:20:0). The oil content for a3 (20:20:0) ranged from 42.4% [b1], 42.1% [b2], and 42.3% [b3]. The contribution of the factors A [type of fertilization], B [sowing period], and the interaction of AxB in achieving the oil content is presented in Figure 8.

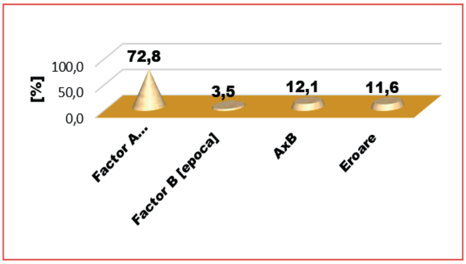

Figure 8. The contribution of factors A [type of fertilizer], B [sowing season], and the A x B interaction

Factor A [type of fertilizer] contributes to achieving the oil content by 72.8%, factor B [sowing season] contributes by 3.5%, and the A x B interaction by 12.1%. Therefore, the greatest contribution is made by the fertilizer factor, followed by the A x B interaction and the B [sowing season] factor.

The oil production according to the type of fertilizer, sowing season, and A x B interaction is presented in Table 5.

Table 5. Oil crop depending on the type of fertilization, the sowing period and the A x B interaction

,Experimental factor	Factor levels	Oil crops [%]	Std.Err.	-95.00%	+95.00%	Diff. [%]	Signif.
Factor A [Fertilizer type]	a1 - E34	1598	4.60179	1587.468	1608.692	202	***
	a2 - DAP	1389	10.70854	1364.392	1413.780	-7	ns
	a3 - 20.20	1201	5.16708	1188.938	1212.769	-195	000
	Average	1396					
	DL 5% = 9.29 kg; DL 1% = 12.73; DL 0.1% = 17.34						
Factor B [sowing period]	b1 - 10-20.08	1375	58.25851	1240.653	1509.342	-21	00
	b2 - 20-31.08	1397	57.39366	1264.709	1529.409	1	ns
	b3 - 0 - 10.09	1416	56.92218	1284.700	1547.225	20	***
	Average	1396					
	DL 5% = 9.29 kg; DL 1% = 12.73; DL 0.1% = 17.34						
AxB Interaction							
Factor A	Factor B						
a1 - E34	b1 - 10-20.08	1588	2.933613	1575.843	1601.088	192	***
	b2 - 20-31.08	1594	4.966161	1572.567	1615.302	198	***
	b3 - 0 - 10.09	1612	8.448273	1575.490	1648.190	216	***
a2 - DAP	b1 - 10-20.08	1349	5.579258	1324.789	1372.801	-47	000
	b2 - 20-31.08	1401	7.056626	1370.138	1430.863	4	ns
	b3 - 0 - 10.09	1418	1.191520	1412.835	1423.088	22	*
a3 - 20.20	b1 - 10-20.08	1188	5.223971	1165.256	1210.210	-208	000
	b2 - 20-31.08	1197	2.154135	1187.474	1206.011	-199	000
	b3 - 0 - 10.09	1218	6.740849	1189.083	1247.090	-178	000
	Average	1396					
	DL 5% = 16.09 kg DL 1% = 22.04 DL 0.1% = 30.04						

The highest oil yield (1598 kg/ha) was obtained in the a1 (E34) fertilization variant, where the highest seed yield and oil content were also achieved. The yield increase of 202 kg/ha is statistically significant. In the a2 and a3 fertilization variants, the oil yield was below the experimental average (1396 kg/ha).
Regarding the influence of the sowing season on oil yield, compared to the experimental average (1396 kg/ha), the highest yield was obtained in variant b3 (the first decade of September) with 1416 kg/ha, a difference of 20 kg/ha, which is statistically very significant.
The interaction between the type of fertilizer and the sowing season influenced the oil yield. The highest oil yields ranging from 1588 -1612 kg/ha were obtained in the a1 (E34) fertilization variant in all three sowing seasons. The yield differences ranging from 192 kg/ha to 216 kg/ha are statistically very significant. For the other fertilization levels in interaction with the sowing season, except for the a2 (DAP) variant and the b3 sowing season (the first decade of September), where a yield increase of 22 kg/ha was obtained compared to the experimental average, statistically significant, the oil yield did not exceed the experimental average.
The influence of fertilization on oil yield is presented in Figure 9.

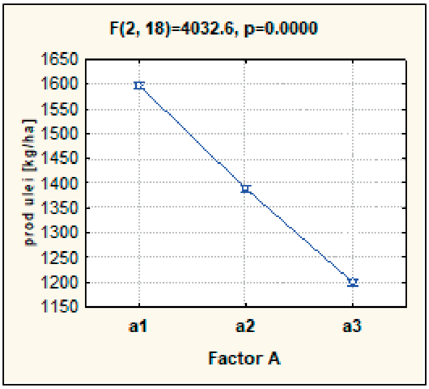

Figure 9. Oil yield variation depending on used fertilizer [Factor A]

The oil yield decreases with the level of fertilization, with the two variables being inversely proportional. The oil yield exhibits a decreasing trend. The yield varies between 1600 kg/ha (a1 - E34) and 1200 kg/ha (a3 -20:20:0). The differences in yield between fertilizer levels are highly significant [$p<0.001$]. The influence of sowing time on the yield of oil is shown in Figure 10.

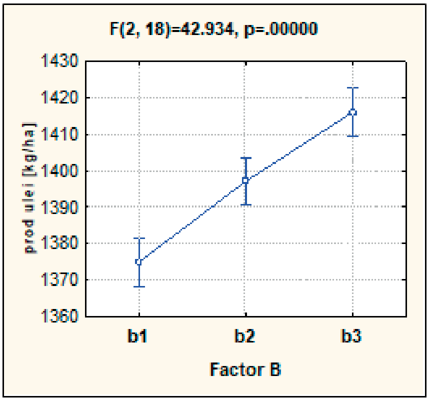

Figure 10. Variation of oil yield at the three sowing times [factor B]

The oil yield increases with the sowing period, the two variables being directly proportional. The trend of oil yield is ascending. The yield ranges from 1375 kg/ha (B1 - second decade of August) to 1416 kg/ha (B3 - first decade of September). The analysis of the results regarding the influence of the interaction between factors A x B [type of fertilization x sowing period] on the oil yield is presented in Figure 11.

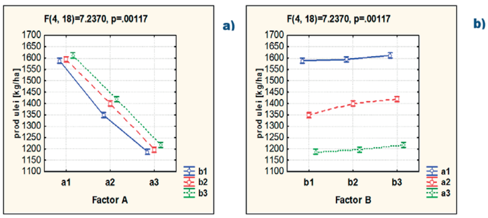

Figure 11. Variation of oil yield [A x B interaction]

Regardless of the sowing time, the oil yield shows a decreasing trend with increasing fertilizer level. The highest yield is obtained with a1 (E34) and the lowest with a3 (20:20:0). The yields obtained with a1 (E34) at all three sowing times varied between 1590 kg/ha (second decade of August) and 1610 kg/ha (first decade of September), while those obtained with a3 (20:20:0) varied between 1200 kg/ha (second decade of August) and 1220 kg/ha (first decade of September).

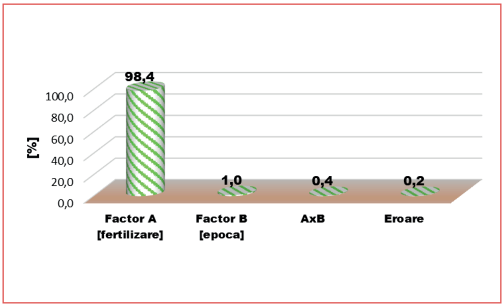

Figure 12. Contribution of factors A [fertilization], B [sowing time] and their interaction A x B

Factor A[fertilization] contributes to production achievement in proportion of 98.4%, factor B [sowing period] contributes with 1.0%, and interaction A x B with 0.4%. Therefore, the greatest contribution is made by factor A[fertilization], followed by factor B[season] and interaction A x B.

CONCLUSIONS

Analyzing the production results, oil content, and oil production depending on the type of fertilization and sowing time, important conclusions can be drawn to support rapeseed farmers in the western part of the country. Regarding fertilization, the highest production (3233 kg/ha) was obtained in the case of fertilization with E34 (10:24:0 + 0.1Zn + 0.1Br + 20 SO_3), a fertilizer that, along with nitrogen and phosphorus, contains zinc, boron, and sulfur. As for the highest oil content (44.1%) and the highest oil production (1598 kg/ha), they were also obtained in the variant fertilized with this type of fertilizer. In recent years, due to climate change and especially the lack of soil moisture during the germination period, farmers in the western part of the country have started to sow rapeseed crops in the second half of August. The results of the study show that the highest and most secure rapeseed yields for the climatic conditions in the researched area are obtained in the variant sowed in the first decade of September (3650 kg/ha). This period also produced the highest oil content and the highest oil production (1416 kg/ha). Regarding the interaction between the type of fertilizer and the sowing period, the best results were obtained in the variant fertilized with E34 (10:24:0 + 0.1Zn + 0.1Br + 20 SO_3) and sowed in the first decade of September, with a seed production value of 3650 kg/ha, oil content of 44.2%, and oil production of 1612 kg/ha.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY REGARDING INFLUENCE OF VARIETY, BIOLOGICAL MATERIAL AND NUTRIENT SPACE ON POTATO MINITUBERS PRODUCTION

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Abstract

The main purpose of this research was to follow the minituberization process for 3 Romanian potato varieties, created at NIRDPSB Brasov. The biological material called "minitubers" is part of the first links of the national system of potato production for seed. The trifactorial experience of this research (3x2x2), on 3 repetitions, included the following factors: experimental factor A: variety, with three gradations: a1 - Marvis; a2 - Castrum; a3 - Ervant (considered control); experimental factor B: biological material used for planting, with two gradations: b1 - plantlets (considered control); b2- microtubers; experimental factor C: the volume of the nutrition space, with two graduations: c1-1.5 l (considered control) and c2- 2 l. The determinations were made for number and weight of minitubers/plant, in function of experimental factors. The average number of minitubers obtained/nutrition space was between 5.75 (Marvis variety) and 10.25 (Ervant variety), and the weight of minitubers ranged from 28.22 g (Ervant variety) to 93.53 (Castrum variety). By increasing nutrition is noted very significant positive differences for both analysed parameters.

***Key words**: genotype, microtubers, minitubers, plantlets, potato, rapid multiplication, seed.*

INTRODUCTION

Potato is most important cultivated food crop and is believed to contribute significantly to sustain future global food security (Kumar et al., 2021).

The profitability of agricultural production is largely determined by the quality and yield properties of seeds and planting material. In potato seed production, a promising direction at the current stage of development of the industry is microclonal propagation, the advantage of which is the ability to get healthy planting material in large quantities in a short time (Filippova et al., 2020).

The production of healthy seed is very crucial to sustain the production and productivity of potato (Sadawarti et al., 2020). Nowadays potato can be rapidly multiplied using nodal cuttings produced in vitro and involving following minitubers production (Dimante and Gaile, 2014). Use of tissue culture technique in seed production has resulted in mass production of potato in a very short period of time. The system is characterized by very flexible and rapid multiplication giving a higher planting propagule (Beukema and Van de Zaag, 1990; Pruski, 2001; Muthuraj et al., 2016, quoted by Sadawarti et al., 2020).

Seed potato production involving minituber production systems has found its place all over the world. This system creates a bridge between the in vitro rapid multiplication and the field multiplication of seed tubers and is thus a classical way to multiply or acclimatize in vitro material before its use in the open field (Sharma and Pandey, 2013).

Soil planting of micropropagated plants has been reported to be a rapid and efficient method for producing potato mini-tubers (Ahloowalia, 1994, quoted by Sharma and Pandey, 2013).

High yields of potato (*Solanum tuberosum* L.) in the field can be ensured by use of high-quality seed potato (Calori et al., 2017, quoted by Sadawarti et al., 2020)

The screen house minitubers production is aimed to get a large number of diseases free mini- tubers in the short time and low cost of production to restore the farmer who used his/her own rotated seed potatoes for extended years in open field with stored pathogens that resulted in high yield and quality losses (Tolessa, 2021). Minitubers are usually defined as the progeny tubers produced on in vitro derived plantlets or microtubers (Rykaczewska, 2016). Minitubers are small seed potato tubers that can be produced year-round in glasshouses on in vitro propagated plantlets planted at high density (Lommen, 1995). They are considered to be the most suitable propagule to reduce the number of field multiplications in a seed programme (Lommen & Struik, 1992, quoted by Lommen, 1995). The production of minitubers consists of two phases: the multiplication of plantlets in vitro and the production of minitubers on these plantlets in the glasshouse.

MATERIALS AND METHODS

The research took place in the Research Laboratory for Vegetal Tissue Culture of National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania, in 2022. The activity of the laboratory has as its main objective: obtaining of a virus-free biological material, starting from meristem culture. After 6-8 months from meristem inoculation, plantlets are developed. The multiplication phase extends over several subcultures, with a duration of 3-4 weeks/subculture until the required number of plantlets is reached. An alternative for in vitro production of healthy material is obtaining microtubers, but in different conditions compared to plantlets. In spring, in period April-May, the biological material obtain *in vitro* (plantlets or microtubers) conditions is transferred in “insect-proof” space. All the stages of minitubers production are shown in Figure 1. The main purpose of this research was to follow the minituberization process for three Romanian potato varieties, created at NIRDPSB Brasov. Marvis, Castrum and Ervant. As biological material it was used plantlets and microtubers. The culture vessels in which they were planted had different volume (1.5 l and 2.5 l).

The trifactorial experience (3 x 2 x 2), on three repetitions, included the following factors:

- experimental factor A - variety, with three gradations: a1 - Marvis; a2 - Castrum; a3 - Ervant (considered control);
- experimental factor B: biological material used for planting, with two gradations: b1 – plantlets (considered control); b2- microtubers;
- experimental factor C: the volume of the nutrition space, with two graduations: c1-1.5 l (considered control) and c2- 2 l.

The results recorded after minitubers harvesting (which represent Prebase material in the production of seed potatoes) were processed by statistical program ANOVA POLIFACT.

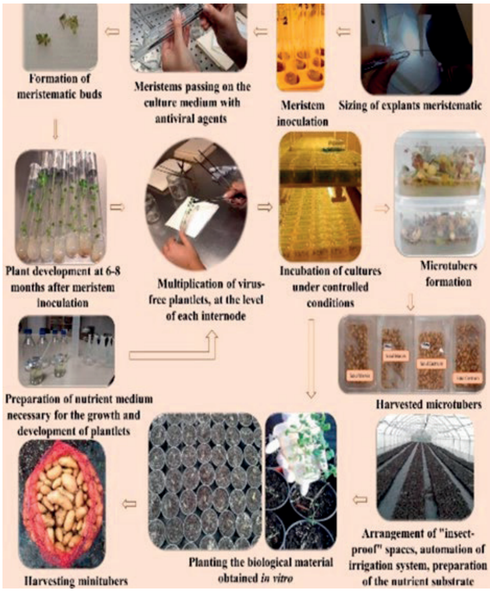


Figure 1. Schematic presentation of the technology for obtaining potato minitubers in "insect-proof" spaces

RESULTS AND DISCUSSIONS

Regarding the influence of variety (Table 1) on the average minitubers number/plant, distinctly significant negative differences are observed for Marvis (-4.50 minitubers) and Castrum varieties (-4.33 minitubers), compared to the control genotype. The Ervant variety stands out with a high value of minitubers number (10.25 g).

When studying the influence of variety on minitubers weight, distinctly significant positive differences are highlighted for Castrum (65.31 g) and Marvis varieties (55.69 g), compared to the control variety, which is at a lower level of the minitubers weight value (28.22 g). Thus, this variety presented a high production capacity of minitubers, but their weight was low.

Table 1. Influence of variety on the average number of minitubers obtained/plant and on the average weight (g) of minitubers/plant

Variety (a)	Minitubers number /pl.	Diff. (g)	Sign.	Minitubers weight/pl. (g)	Diff. (g)	Sign.
Marvis (a_1)	5.75	-4.50	oo	83.91	55.69	**
Castrum (a_2)	5.92	-4.33	oo	93.53	65.31	**
Ervant (a_3) (Ct)	10.25	-		28.22	-	

LSD 5% = 2.48; 1% = 4.10; 0.1% = 7.68. LSD 5% = 24.45g; 1%= 40.45g; 0.1% = 75.71g.

From the analysis of biological material influence on minitubers number/plant and on their weight, insignificant differences are observed, the two types of biological materials not influencing the parameters studied.

On a detailed analysis of the minituberization process, by using microtubers at planting, we find a higher value of minitubers number, compared to the biological material considered as control (plantlets).

Also, the positive effect of microtubers used for planting is reflected in obtaining a higher value of the weight of minitubers (82.23 g), compared to the control biological material (54.88 g), with a positive difference, close to the first threshold of limit differences (Table 2).

Table 2. The influence of biological material on the average number of minitubers obtained/plant and on the average weight of minitubers/plant (g)

Biological material (b)	Minitubers number/pl.	Diff. (g)	Sign.	Minitubers weight/pl. (g)	Diff. (g)	Sign.
Microtubers(b_1)	7.56	0.50	ns	82.23	27.35	ns
Plantlets (b_2) (Ct)	7.06	-		54.88	-	

LSD 5% = 1.52; 1% = 2.30; 0.1% = 3.70. LSD 5% = 29.31g; 1%= 44.38 g; 0.1% = 71.29 g.

By comparing the experimental differences with the limit differences calculated in studying the influence of nutrition space volume on the average number of minitubers/plant, the beneficial effect of the increased nutrition space is noted, leading to a very significant positive difference (3.28 minitubers). Examining the results regarding the minitubers weight /plant highlights the positive influence of the increased nutrition space (Table 3), expressed by a highly significant positive difference (39.73 g).

Table 3. The influence of nutrition space volume on the average number of minitubers obtained/plant and on the weight of minitubers/plant (g)

Nutrition space volume (l) (c)	Minitubers number /pl.	Diff.(g)	Sign.	Minitubers weight/pl. (g)	Diff.(g)	Sign.
1.5 (c_1) (Ct)	5.67	-		48.69	-	
2 (c_2)	8.94	3.28	***	88.42	39.73	***

LSD 5% = 0.80; 1% = 1.13; 0.1% = 1.59. LSD 5% = 11.83 g; 1%= 16.61 g; 0.1% = 23.44 g.

Ervant variety is distinguished by high values of minitubers number for both biological materials used for planting (Table 4). For the Castrum variety, microtubers using showed a positive effect in the formation of minitubers number, with a difference close to the first threshold of the limit differences (2.50 minitubers). When comparing the differences obtained between the Marvis variety and the control, negative differences are found: distinctly significant (-4.83 minitubers) when using microtubers and significant (-4.17 minitubers) when plantlets were used as biological material.

By comparing the differences obtained between the second variety and the control genotype, negative differences are observed: significant for microtubers (-3.00) and distinctly significant (-5.67 minitubers) for control biological material.

Table 4. The combined influence of variety and biological material on the average number of minitubers obtained/plant

Variety/Biological material	Marvis (a_1)		Castrum (a_2)		Ervant (a_3)		a_1-a_3/ Sign.	a_2-a_3/ Sign.
Microtubers (b_1)	5.33	-0.83 ns	7.17	2.50 ns	10.17	-0.17 ns	-4.83 oo	-3.00 o
Plantlets (b_2) (Ct)	6.17	-	4.67	-	10.33	-	-4.17 o	-5.67 oo

LSD 5% = 2.64; 1%= 3.99; 0.1% = 6.41. LSD 5% = 2.61; 1%= 4.18; 0.1% = 7.41

Ervant and Castrum varieties are distinguished by high values of minitubers number for the increased nutrition space, determining the registration of positive, very significant differences of 8.83 g and 3.832 g. Thus, the nutrition space increasing strongly influenced the minitubers formation for these varieties (Table 5). The increased nutrition space showed a less beneficial effect for Marvis variety, which obtained a lower value of minitubers number (4.33 minitubers) with a highly significant negative difference (-2.83 minitubers).

Table 5. The combined influence of variety and volume of nutrition space on the average number of minitubers obtained/plant

Variety (a)/ Nutrition space volume (l) (c)	Marvis (a_1)		Castrum (a_2)		Ervant (a_3)		a_1-a_3/Sign.	a_2-a_3/Sign.
	Minitub. no./pl.	Diff./ Sign.	Minitub. no./pl.	Diff./ Sign.	Minitub. no./pl.	Diff./ Sign.		
1.5 (c_1) (Ct)	7.17	-	4.00	-	5.83	-	1.33 ns	-1.83 ns
2 (c_2)	4.33	-2.83 ooo	7.83	3.832 ***	14.67	8.83 ***	-10.33 ooo	-6.83 ooo

LSD 5% = 1.39; 1%= 1.95; 0.1% = 2.76. LSD 5% = 2.06; 1%= 3.33; 0.1% = 6.00.

Studying of combined influence of biological material used at planting and nutrition space volume on the average number of minitubers obtained/plant reveals the beneficial effect of increased nutrition space, both for microtubers and plantlets, with positive differences: very significant (4.89 minitubers) and distinctly significant (1.67 minitubers). By planting microtubers in the increased nutrition space, a distinctly significant positive difference (2.11) is obtained, compared to the use of plantlets (Table 6).

Table 6. The combined influence of biological material used at planting and nutrition space volume on the average number of minitubers obtained/plant

Biological material (b)/ Nutrition space volume (l) (c)	Microtubers(b_1)		Plantlets (b_2)		b_1-b_2/ Sign.
	Minitub. no./pl.	Diff./ Sign.	Minitub. no./pl.	Diff./ Sign.	
1.5 (c_1) (Ct)	5.11	-	6.22	-	-1.11 ns
2 (c_2)	10.00	4.89 ***	7.89	1.67 **	2.11 **

LSD 5% = 1,14; 1% = 1,59; 0,1% = 2,25. LSD 5% = 1.36; 1% = 2.03; 0.1% =3.17.

When examining the combined influence of variety and the biological material used for planting on the weight of minitubers (g) obtained/plant, distinctly significant positive differences are observed for the varieties Marvis (79.23 g) and Castrum (85.63 g), compared to the control genotype, by using microtubers. Also, a significant positive difference is noted for the Castrum variety (44.99 g), compared to the control genotype when using plantlets (Table 7).

Table 7. The combined influence of cultivar and biological material on minitubers weight (g)/plant

Variety (a)/ Biological material (b)	Marvis (a_1)		Castrum (a_2)		Ervant (a_3)		a_1-a_3/Sign.	a_2-a_3/Sign.
	Minitubers weight (g)/pl.	Diff./ Sign.	Minitubers weight (g)/ pl.	Diff./ Sign.	Minitubers weight (g)/ pl.	Diff./ Sign.		
Microtubers (b_1)	106.50	45.18 ns	112.91	38.75 ns	27.28	-1.89 ns	79.23 **	85.63 **
Plantlets (b_2) (Ct)	61.32	-	74.16	-	29.17	-	32.15 ns	44.99 *

LSD 5% = 50.76 g; 1%= 76.85 g; 0.1% = 123.48 g. LSD 5% =40.38 g; 1%= 62.80 g; 0.1% = 106.02 g.

Examining the combined influence of biological material used for planting and nutrition space volume on minitubers weight (Table 8) reveals the beneficial effect of the increased nutrition space for both biological materials, determining the registration of positive differences: very significant (52,80 g) and distinctly significant (26.65 g).

Table 8. The combined influence of the biological material used at planting and the volume of the nutrition space on the weight of minitubers obtained (g)/plant

Biological material (b)/ Nutrition space volume (l) (c)	Microtubers (b_1)		Plantlets (b_2)		b_1-b_2/ Sign.
	Minitubers weight (g)/pl.	Diff./ Sign.	Minitubers weight (g)/pl.	Diff./ Sign.	
1,5 (c_1) (Ct)	55.83	-	41.56	-	14.27 ns
2 (c_2)	108.63	52.80 ***	68.21	26.65 **	40.42 **

LSD 5% = 16.73 g; 1%= 23.48 g; 0.1% = 33.15 g. LSD 5% = 24.16 g; 1%= 36.17 g; 0.1% = 57.08 g

From the analysis of varieties behavior regarding the weight of the minitubers obtained on the two nutrition spaces, Castrum (98.12 g) and Ervant (33.81 g) varieties stand out, which determine the achievement of very significant positive differences for the increased nutrition space.

The variety/nutrition space interaction highlights: very significant positive difference (78.97 g) and positive significant difference (33.16 g), by comparing Marvis and Castrum varieties, with the control variety, by using reduced nutrition space and significant differences (32.41 g) and very significant (97.47 g) positive by comparing the previously mentioned varieties with the control variety, but when increased nutrition space was used (Table 9).

Table 9. The combined influence of variety and nutrition space volume on weight of minitubers (g) obtained/plant

Variety (a)/ Nutrition space volume (l) (c)	Marvis (a_1)		Castrum (a_2)		Ervant (a_3)		a_1-a_3/Sign.	a_2-a_3/Sign.
	Minitubers weight (g)/pl.	Diff./ Sign.	Minitubers weight (g)/pl.	Diff./ Sign.	Minitubers weight (g)/pl.	Diff./ Sign.		
1.5 (c_1) (Ct)	90.28	-	44.48	-	11.32	-	78.97 ***	33.16 *
2 (c_2)	77.53	-12.75 ns	142.59	98.12 ***	45.13	33.81 ***	32.41 *	97.47 ***

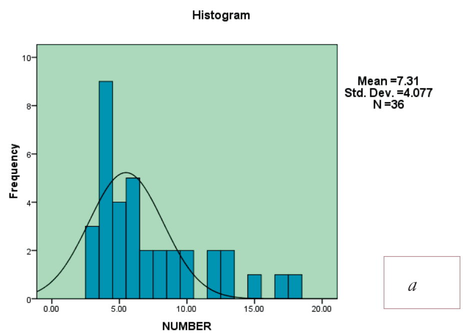
LSD 5% = 20.49 g; 1%= 28.76 g; 0.1% = 40.61 g. LSD 5% = 23.32 g; 1%= 36.77 g; 0.1% = 64.17g.

When comparing the differences between biological materials used, a distinctly significant positive difference is found by increasing the nutrition space (40.42 g). There was an extremely significant positive correlation between number and variety and volume of space nutrition. The weight was positively correlated with volume of space nutrition (Table 10).

Table 10. Correlation Matrix of Pearson Correlation Coefficients

Pearson Correlation	Number	Weight	Variety	Biological material	Volume of nutrition space
Number	1	.093	.457**	-.062	.408**
Weight	.093	1	-.441**	-.265	.385*
Variety	.457**	-.441**	1	.000	.000
Biological material	-.062	-.265	.000	1	.000
Volume of nutrition space	.408**	.385*	.000	.000	1

**. Correlation is significant at the 0.01 level (1-tailed).
*. Correlation is significant at the 0.05 level (1-tailed



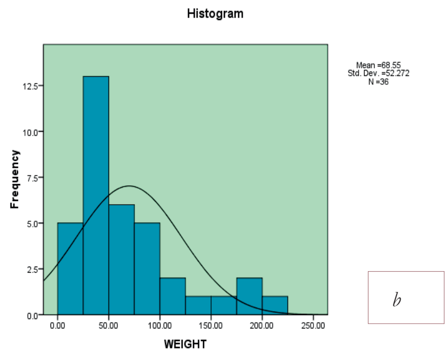


Figure 2. Number distribution (a) and weight (b) for studied genotype

CONCLUSIONS

The average number of minitubers obtained/nutrition space was between 5.75 (Marvis variety) and 10.25 (Ervant variety), and the weight of minitubers ranged from 28.22 g (Ervant variety) to 93.53 (Castrum variety). For the Ervant variety, it can be observed that although it obtained a high number of minitubers/nutrition space (10.25), their weight is low.

The microtubers using as planting material showed a beneficial effect on the average number of minitubers and on the weight, thus

obtaining higher values (7.56 minitubers and 82.23 g), compared to the biological material consisting of plantlets, for which -they recorded 7.06 minitubers and a weight of minitubers/plant of 54.88 g.
Planting the biological material in the increased culture space favored the minituberization process for the two studied parameters (8.94 minitubers and 88.42 g).
The analysis of varieties behaviour in minituberization draws our attention to Ervant (14.67 minitubers) and Castrum varieties (7.83 minitubers), by using the increased nutrition space.

ACKNOWLEDGEMENTS

This research work was carried out with the support of National Institute of Research and Development for Potato and Sugar Beet Brasov and also was financed from ADER 5.1.2.
Project "Research on the production of minitubers under specific isolation conditions".

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SOME BIOLOGICAL PECULIARITIES AND THE BIOMASS QUALITY OF COMMON BUCKWHEAT, *Fagopyrum esculentum*, GROWING UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

Common buckwheat - Fagopyrum esculentum Moench, Polygonaceae family, is a short-season crop, cultivated for its grain-like seeds and as a melliferous and cover crop, enabling more complete adaption to its environment than other traditional crops. The goal of this research was to evaluate some biological peculiarities and the biomass quality of common buckwheat Fagopyrum esculentum cultivated in the experimental sector of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chisinau, Republic of Moldova. The prospects of using harvested biomass as forage and substrates for biomethane production were assessed. We found that whole-plant dry matter, cut in the early flowering stage, contained 15.4% CP, 9.4% ash, 37.5%CF, 39.1% ADF, 57.7% NDF, 6.6% ADL, 32.5% Cel, 18.1% HC, 6.9% TSS, with nutritive and energy values: 584 g/kg DDM, RFV= 95, 11.74 MJ/kg DE, 9.51 MJ/kg ME and 5.54 MJ/kg NEl, but in seed formation stage - 10.8% CP, 8.0% ash, 36.2% CF, 38.3% ADF, 58.9% NDF, 6.9% ADL, 31.4% Cel, 21.6% HC, 10.1% TSS, with nutritive and energy values: 591 g/kg DDM, RFV= 93, 11.88 MJ/kg DE, 9.62 MJ/kg ME and 5.62 MJ/kg NEl. The quality of the silage prepared from whole plants cut in the seed formation stage was: pH = 3.76, 39.9 g/kg lactic acid, 0.2 g/kg butyric acid, 10.9 g/kg acetic acid, 226/kg DM with 9.6% CP, 7.7% ash, 35.5% CF, 36.2% ADF, 55.5% NDF, 5.5% ADL, 30.7% Cel, 24.5% HC, 8.4% TSS, with nutritive and energy values: 607 g/kg DDM, RFV = 102, 12.00 MJ/kg DE, 9.85 MJ/kg ME and 5.87 MJ/kg NEl. It has been estimated that the green mass substrates for anaerobic digestion, have C/N=20.5-29.5, optimal amount of lignin and hemicelluloses, the biochemical methane potential reaches 292-305 l/kg ODM, but in ensiled substrate - C/N=33.3 and biochemical methane potential 314 l/kg ODM.

Key words: *biochemical composition, biological peculiarities, biomass, biomethane, Fagopyrum esculentum, green mass, silage.*

INTRODUCTION

The continuous global population growth has resulted in the intensification of food and energy production, which has been necessary in order to cover the rising demands to maintain the standards of living.

The family *Polygonaceae*, as mentioned by "The Plant List", includes 59 plant genera, with 5,385 scientific plant names, 1,384 are accepted species names. The representatives of this family are present worldwide, but are most diverse in the North Temperate Zone. It consists mostly of herbs and some trees, shrubs and vines. Several species are cultivated for seeds and vegetables, forage, medicinal plant, energy biomass, also as ornamentals and cover crop. The genus *Fagopyrum* Mill. is composed of 26 diploid/tetraploid species of which common buckwheat, *Fagopyrum esculentum* Moench. and tartary buckwheat, *Fagopyrum tataricum* Gaertn. are the most cultivated species, their edible gluten-free seeds are eaten as groats or used sometimes in flour, particularly for buckwheat pancakes, and portions of the plant are frequently included in animal feed. Common buckwheat, *Fagopyrum esculentum* Moench., is one of the oldest domesticated food crops from Asia. It is native to south-central China and Tibet and has been introduced into suitable climates across Eurasia, Africa and the Americas. It is an annual herbaceous plant, stems – ascending or erect, ribbed, reddish, with internodes, branched above, glabrous or papillate on one side, reaching up to the height of 1.5 m. Leaves petiolate, petiole 0.5-2 cm long, grooved lower leaves with long petiole, upper ones subsessile; lamina triangular or sagittate, cordate, basal lobes rounded to acuminate, 1.5-10 x 1-8 cm, ochrea 2-3 (5) mm long, hyaline, pubescent near the base. The inflorescence is composed of numerous clusters of flowers (panicles in long axillary spikes) containing 5-6 flowers attached to the nodes of the stem, flowers white or cream, pink or red. The indeterminate growth habit whereby ripened seeds coexist with green seeds and few flowers in the same plant. The fruits are achenes - about 5-7 mm with 3 prominent sharp angles, flat surfaces, pale brown. *Fagopyrum esculentum* has deep rooted, branched, taproot system. It tolerates a wide range of soil types and fertility levels, but performs best when the climate is cool and moist. Common buckwheat is low fertiliser requirements, the seeding rate depends on the purpose of cultivating the crop, thus, 30-40 kg/ha are needed when used as a grain crop or about 50 kg/ha when used as a cover/fodder crop; a low seeding rate has the potential to increase weed competition because of the low density of the plant population and hence results in low yield; sowing depth depends on the type of soil and climatic conditions. The *Fagopyrum esculentum* has a wide range of agronomic and health benefits that make it a promising crop for sustainable agricultural production. The achenes are gluten free, have high nutritional quality of proteins and are rich in minerals; the levels of fibre and starch with reduced speed of digestion and rutin made buckwheat products favourable for healthy nutrition, patients with diabetes and coeliac disease. Young plantlets can be consumed in salads it is rich in amino acids, minerals and fibres, more valuable than seeds from a nutritional point of view, with higher concentration of lysine and vitamin C (Campbell, 1997; Ohnishi, 1998; Treadwell & Huang, 2008; Jacquemart et al., 2012; Mariotti et al., 2015; Farooq et al., 2016; Arduni et al., 2016; Arduni & Mariotti, 2018; Heuzé et al., 2019; Ohsako & Li, 2020).

Fagopyrum esculentum is of interest to beekeepers because flowering lasts generally until the first frosts, a period when there are few other melliferous plants. Buckwheat honey has dark colour and strong taste. Best common buckwheat cultivars produce from 150 to 300 kg/ha (Campbell, 1997; Cawoy et al., 2008; Alekseyeva & Bureyko, 2000; Ion et al., 2018; Liszewski & Chorbiński, 2021).

The goal of this research was to evaluate some biological peculiarities and the quality of fresh and ensiled biomass of common buckwheat *Fagopyrum esculentum,* as fodder for ruminant animals, as well as substrate for the production of biomethane by anaerobic digestion.

MATERIALS AND METHODS

The common buckwheat *Fagopyrum esculentum,* which was cultivated in the experimental plot of the National Botanical Garden (Institute) of Moldova, Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″, served as subject of research and the traditional crop alfalfa, *Medicago sativa* and corn, *Zea mays*, were used as control variants. The experimental design was a randomised complete block design with four replications, and the experimental plots measured 10 m^2. *Fagopyrum esculentum* was sown in the middle of May, at 25-cm row spacing and a rate of 5 g/m^2. The green mass was harvested manually at 5 cm cutting height, in the flowering and seed formation period. The leaf/stem ratio was determined by separating the leaves from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems). The common buckwheat silage was prepared from green mass harvested in the seed formation period, cut into small pieces and compressed in glass containers. The containers were stored for 45 days, and then, they were opened and the organoleptic assessment and the determination of the organic acid composition of the persevered forage were done in accordance with the Moldavian standard SM 108. The dry matter content was detected by drying samples to constant weight at 105°C. For biochemical analysis, the plant samples were dried in a forced air oven at 60°C, milled in a beater mill equipped with a sieve with mesh diameter of 1 mm and some of the main biochemical parameters, such as crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM), digestible organic matter (DOM) were determined by near infrared spectroscopy (NIRS) using PERTEN DA 7200 with standardized methods at the RDI for Grassland Brasov, in Romania. The concentration of hemicellulose (HC), cellulose (Cel), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl) and relative feed value (RFV) were calculated according to standard procedures.

The carbon content of substrates was obtained using an empirical equation according to Badger et al. (1979). The biochemical methane potential was calculated according to the equations of Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

As a result of the study on the biological peculiarities of studied taxa of common buckwheat *Fagopyrum esculentum*, the emergence of the seedlings was observed on the 4th-6th days after sowing, the development of the stem - in 7-8 days after seedling emergence, the formation of the flower buds occurred at the end of June and the flowering period during July-August, the seed formation period in middle July, the seed ripening August-September.

The structure of the common buckwheat harvested aerial plant biomass and its yield are shown in Table 1. At the flowering stage, the height of *Fagopyrum esculentum* plants was 116.1 cm, while in seed formation stage it reached 126.4 cm, *Medicago sativa* plants, at first cut, were 84.5 cm tall, but at the third cut - 53.8 cm. The green mass productivity of *Fagopyrum esculentum* plants cut in flowering stage reached 29.2 t/ha green mass or 3.8 t/ha dry matter with 44.5% leaves and flowers, while those cut in seed formation stage produced 12.8 t/ha green mass or 3.8 t/ha dry matter with 61.8% leaves, but the leguminous forage crop *Medicago sativa* at the first cut yielded 27.7 t/ha green mass, 7.2 t/ha dry matter with 52.9 % leaves and flowers, while at the third cut - 9.6 t/ha green mass, 2.2 t/ha dry matter with 62.5% leaves, respectively.

Table 1. Some agrobiological peculiarities and the structure of the green mass of the studied species

Plant species	Plant height cm	Stem, g		Leaf + flower, g		Yield, t/ha	
		fresh mass	dry matter	fresh mass	dry matter	fresh mass	dry matter
Fagopyrum esculentum, flowering stage	106.9	28.10	2.84	11.40	2.28	29.20	3.80
Fagopyrum esculentum, seed formation stage	126.4	20.75	4.92	16.30	3.67	30.60	7.11
Medicago sativa, first cut	84.5	6.21	1.63	6.92	1.81	27.70	7.20
Medicago sativa, third cut	53.8	5.93	0.92	5.81	1.53	9.60	2.01

Analysing the results of the common buckwheat fresh mass quality (Table 2), we concluded that the dry matter of the whole plants harvested in the flowering stage contained of 15.4% CP, 9.4% ash, 37.5% CF, 39.1% ADF, 57.7% NDF, 6.6% ADL, 32.5% Cel, 18.1% HC, 6.9% TSS, with nutritive and energy values: 584 g/kg DDM, RFV = 95, 11.74 MJ/kg DE, 9.51 MJ/kg ME and 5.54 MJ/kg NEl, but the harvested whole plants in seed formation stage - 10.8% CP, 8.0% ash, 36.2% CF, 38.3% ADF, 58.9% NDF, 6.9% ADL, 31.4 % Cel, 21.6% HC, 10.1% TSS, with nutritive and energy values: 591 g/kg DDM, RFV= 93, 11.88 MJ/kg DE, 9.62 MJ/kg ME and 5.62 MJ/kg NEl, respectively. The common buckwheat natural forage contained optimal amount of crude protein, structural carbohydrates, lignin and energy concentration, as compared with the *Medicago sativa* natural forage. As compared with green corn forage, the buckwheat forage stands out due to its higher concentration of crude protein, minerals, cell wall fractions and low level of total soluble sugars, digestibility, relative feed value and energy concentration.

Literature sources indicate considerable variation in the chemical composition and nutritional value of *Fagopyrum* whole plants. According to Larin (1952), buckwheat green fodder contained in dry matter - 10.7% CP, 2.8% EE, 28.2% CF, 48.7% NFE, hay - 12.2% CP, 2.4 % EE, 31.5%CF, 41.4% NFE, straw 6.0% CP, 1.9% EE, 40.0% CF, 44.5% NFE. Campbell (1997) showed that buckwheat green fodder is characterised by 36.6% DM, including 4.6% CP, 0.9% EE, 8.0% CF, 19.5% NFE, 3.6% minerals. Amelchanka et al. (2010) mentioned that the use of fresh buckwheat reduced ruminal ammonia concentrations and enhanced estimated microbial nitrogen growth efficiency, the contents of net energy for lactation in fresh mass was 4.3 MJ/kg, but in ensiled mass 4.9 MJ/kg. Kälber et al. (2011) compared the forage quality of green mass from flowering catch crops and remarked that buckwheat green forage contained 160 g/kg DM, 13.8% CP, 1.97 % EE, 50.2% NDF, 38.3% ADF, 8.26% ADL, 11.2 % ash; phacelia forage - 120 g/kg DM, 14.9% CP, 2.44% EE, 46.9% NDF, 34.9% ADF, 8.35% ADL, 15.0% ash; berseem clover forage –117 g/kg DM, 17.1% CP, 2.59% EE, 43.2% NDF, 33.9% ADF, 7.03% ADL, 12.8% ash; chicory forage - 104 g/kg DM, 19.1% CP, 3.46% EE, 40.1% NDF, 29.4% ADF, 7.46% ADL, 19.0% ash, but ryegrass forage -131 g/kg DM, 19.0% CP, 3.23% EE, 56.0% NDF, 33.1% ADF, 4.92% ADL, 12.9% ash. Kara (2014) reported that the mineral nutrient content in harvested buckwheat biomass was 1.42-3.29% N, 1.99-3.43% Ca, 0.204-0.294% P, 1.93-5.76% K, 0.314-0.643% Mg. Mariotti et al. (2015) remarked that herbage quality of buckwheat plant in the green achene stage was 12.0 % ash, 14.4 % CP, 2.2 % EE, 45.2 % NDF, 27.9 % ADF, 6.2 % ADL, 30.3 % NFC, 15.6 % HC, 23.5 % Cel, 53.3 % TDN and RFV=135.7, but in the brown achene stage: 9.6% ash, 10.3% CP, 1.7 % EE, 41.8% NDF, 26.7% ADF, 6.8% ADL, 34.9% NFC, 15.1% HC, 19.9% Cel, 58.5% TDN and RFV = 152.2, respectively. Görgen et al. (2016) found that *Fagopyrum esculentum* forage contained 95-142 g/kg DM with 14.3-23.8% CP, 41.2-57.6 % NDF, 31.3-33.4% ADF, 10.1-15.2% ash, but *Pennisetum glaucum* forage contained 127-172 g/kg DM with 20.2-24.2% CP, 52.1-55.1% NDF, 25.1-27.5% ADF, 10.6-12.2% ash. Leiber et al. (2018) reported that the chemical composition of the experimental dietary components from total aerial part of buckwheat plants was 917 g/kg OM, 11.0% CP, 1.5% EE, 46.0% NDF, 28.4% non-NDF carbohydrates, 4.8% total extractable phenols, but the ryegrass fodder contained 910 g/kg OM, 16.6% CP, 3.8% EE, 52.6% NDF, 17.2 % non-NDF carbohydrates, 0.82% total extractable phenols. Heuze et al. (2019) remarked that the average dry matter content and fodder value of common buckwheat fresh mass was: 159.8 g/kg DM, 14.6% CP, 0.9% EE, 28.4% CF, 46.3% NDF, 33.9% ADF, 7.7% lignin, 11.4% ash, 25.6 g/kg Ca, 2.4 g/kg P, 60.2% DOM, 17.6 MJ/kg GE, 10.6 MJ/kg DE and 8.5 MJ/kg ME. Bhardwaj & Hamama (2020) remarked that *Fagopyrum esculentum* forages contained 20.8 % CP, 5.6 % EE, 38.4% NDF and 31.4% ADF. Omokanye et al. (2021) mentioned that the content of dry matter and concentration nutrients in *Fagopyrum esculentum* biomass was 227 g/kg DM, 12.2% CP, 57.0% NDF, 38.8% ADF, 14.0 g/kg Ca, 2.5 g/kg P, with 57.0 % TDN, 1.40 Mcal/kg net energy of maintenance and RFV=96. Erol et al. (2022) reported that buckwheat forage harvested in the flowering stage consisted of 30.49-48.30% leaf rate, the dry matter contained 19.39-22.66% CP, 19.89-27.58% ADF, 30.86-40.14% NDF and RFV=158.92-221.40, but the forage harvested in the milk stage contained 20.00-44.54% leaves, 12.80-16.17% CP, 26.59-30.30% ADF, 36.60-45.04% NDF and RFV = 115.39-175.48. Zhou et al. (2022) compared the forage quality of eight Tartary buckwheat cultivars harvested in different growth stages mentioned that nutritional contents were 3.15-7.08% CP, 3.39-5.39% EE, 6.00-20.20% CF, 29.92-50.04% NDF, 24.72-42.02% ADF, 52.25-68.35% NFE, 7.38-17.49% ash, 0.20-0.34% Ca, 0.16-0.40% P, 50.83-64.07% TDN, RFV = 121.31-217.39 and RFQ = 124.90-224.54.

Ensiled fodder is a key element for productive and efficient ruminant livestock farms, which provides a uniform level of high-quality feed for ruminants, particularly in the autumn - middle spring period, but also throughout the year. The prepared silage from common buckwheat cut in seed formation stage was distinguished by light olive leaves and yellow stems with pleasant smell specific to pickled vegetables, but corn silage - by homogeneous yellow colour with pleasant smell like pickled fruits; the consistency was retained, in comparison with the initial plant green mass, without mould and mucus. As a result of the performed analysis (Table 3), it was determined that the silage fermentation profile of prepared silages was pH = 3.76-3.77, 5.60-17.00 g/kg free lactic acid, 5.10-5.50 g/kg free acetic acid, 21.10-39.00 g/kg fixed lactic acid, 5.20-5.40 g/kg fixed acetic acid, 0.20 g/kg fixed butyric acid. In buckwheat silage, the concentration of total organic acids is higher, and fixed lactic acid predominates, as compared with corn silage.

Table 2. The biochemical composition and the nutritive value of the harvested green mass of the studied species

Indices	*Fagopyrum esculentum*		*Medicago sativa*		*Zea mays*
	flowering stage	seed formation stage	first cut	third cut	
Crude protein, g/kg DM	154	108	170	141	84
Minerals, g/kg DM	94	80	90	90	52
Crude fibre, g/kg DM	375	362	341	383	248
Acid detergent fibre, g/kg DM ,	391	383	365	393	271
Neutral detergent fibre, g/kg DM	577	589	558	579	474
Acid detergent lignin, g/kg DM	66	69	63	66	48
Total soluble sugars, g/kg DM	69	101	63	69	336
Cellulose, g/kg DM	325	314	302	327	223
Hemicellulose, g/kg DM	181	216	193	186	203
Digestible dry matter, g/kg DM	584	591	605	583	678
Relative feed value	95	93	101	94	133
Digestible energy, MJ/ kg	11.74	11.88	11.96	11.57	13.28
Metabolizable energy, MJ/ kg	9.51	9.62	9.82	9.50	10.90
Net energy for lactation, MJ/ kg	5.54	5.62	5.83	5.51	6.91

Table 3. The fermentation profile, the nutrient composition of the silage prepared from the studied species

Indices	*Fagopyrum esculentum*	*Zea mays*
pH index	3.76	3.77
Content of organic acids, g/kg DM	51.00	48.60
Free acetic acid, g/kg DM	5.50	5.10
Free butyric acid, g/kg DM	0	0
Free lactic acid, g/kg DM	5.60	17.00
Fixed acetic acid, g/kg DM	5.40	5.20
Fixed butyric acid, g/kg DM	0.20	0.20
Fixed lactic acid, g/kg DM	39.90	21.10
Total acetic acid, g/kg DM	10.90	10.30
Total butyric acid, g/kg DM	0.20	0.20
Total lactic acid, g/kg DM	39.9	38.10
Acetic acid, % of organic acids	21.37	21.19
Butyric acid, % of organic acids	0.38	0.41
Lactic acid, % of organic acids	78.24	78.40
Crude protein, g/kg DM	96.00	80.00
Crude fibre, g/kg DM	355.00	245.00
Minerals, g/kg DM	77.00	59.00
Acid detergent fibre, g/kg DM	362.00	258.00
Neutral detergent fibre, g/kg DM	555.00	469.00
Acid detergent lignin, g/kg DM	55.00	37.00
Total soluble sugars, g/kg DM	84.00	326.00
Cellulose, g/kg DM	307.00	221.00
Hemicellulose, g/kg DM	245.00	211.00
Digestible dry matter, g/kg DM	607.00	688.00
Relative feed value	102	136
Digestible energy, MJ/ kg	12.00	13.45
Metabolizable energy, MJ/ kg	9.85	11.04
Net energy for lactation, MJ/ kg	5.87	7.06
Carbon, g/kg DM	513.00	522.78
Nitrogen, g/kg DM	7.10	12.80
Ratio carbon/nitrogen	72	41
Biomethane potential, L/kg VS	303	338

As compared with the initial fresh mass, the *Fagopyrum esculentum* silage (Table 3), had optimal concentration of crude protein, total soluble sugars and minerals, reduced content of neutral detergent fibre and acid detergent lignin, which had a positive impact on dry matter digestibility, relative feed value and net energy for lactation. It has been found that the concentration of crude protein, acid detergent lignin and minerals in buckwheat silage is higher, but the concentration of carbohydrates and energy is lower than in corn silage.

Different results are presented in the literature regarding the biochemical composition and feed value of buckwheat silage. Kälber et al. (2012) found that the ensilability was optimal for buckwheat sward, and the buckwheat silage contained 135 g/kg CP, 15.8 g/kg EE, 445 g/kg ADF and 75.7 g/kg ADL. Mariotti et al. (2015) remarked that the quality of ensiled forage of buckwheat in the green achene stage was pH = 3.8, 1.4% lactic acid, 12.1% ash, 14.2% CP, 1.3% EE, 49.1 % NDF, 35.2% ADF, 8.9% ADL, 26.0% NFC, 13.9% HC, 26.3% Cel, 50.1% TDN and RFV = 117.2, but in the brown achene stage, respectively, pH = 4.1, 1.4% lactic acid, 10.1% ash, 10.1% CP, 1.8% EE, 44.2% NDF, 32.0% ADF, 8.7% ADL, 35.7% NFC, 12.2% HC, 23.3% Cel, 55.5% TDN and RFV = 135.3. Herrmann et al. (2016) studied the biochemical composition of silages made of various crops in Germany and remarked that buckwheat silage contained 231 g/kg dry matter with 89.9 % organic matter, pH = 5.0, 4.5% lactic acid, 1.3% acetic acid, 0.6% butyric acid, 11.4% CP, 1.7% EE, 44.4% NFE, 52.2% NDF, 42.6% ADF and 13.4% ADL; the silage made from buckwheat/phacelia mixtures - 256 g/kg dry matter with 91.5% organic matter, pH = 4.0, 3.9% lactic acid, 2.1% acetic acid, 0.3% butyric acid, 9.4% CP, 2.7% EE, 52.3% NFE, 42.9% NDF, 37.8% ADF, 8.5% ADL, but maize silage - 302 g/kg dry matter with 95.8 % organic matter, pH= 3.7, 5.1 % lactic acid, 1.6 % acetic acid, 0 % butyric acid, 7.8 % CP, 2.6 % EE, 64.7 % NFE, 41.2 % NDF, 24.0 % ADF and 2.9 % ADL. Keles et al. (2016) reported that buckwheat silages contained 89.8% OM, 2.9% EE, 12.7% CP, 47.2% NDF, 38.1% ADF, 9.5% ADL, 71.9% DOM, 1.92 Mcal/kg ME, but maize silage - 94.1% OM, 2.6% EE, 7.5% CP, 50.9% NDF, 27.3% ADF, 3.9% ADL, 71.9% DOM and 2.18 Mcal/kg ME.

A developing society needs energy to sustain its growth. Renewable energy has become the core element of sustainable development nowadays. Versatile energy sources such as biomass, are used for various purposes, including biogas production. The product of this process is combustible methane, carbon dioxide, some hydrogen and digested effluent can be used as fertilizer. Plant biomass may be used for biogas production directly after harvest and as ensiled substrates. The results regarding the quality of the *Fagopyrum esculentum* substrates and the potential for obtaining biomethane are shown in Table 4. Methanogenic bacteria need a suitable ratio of carbon to nitrogen for their metabolic processes. The nitrogen content in the investigated buckwheat substrates ranged from 15.36 g/kg to 24.64 g/kg, the estimated content of carbon - from 503.33 g/kg to 512.78 g/kg, the C/N ratio varied from 20.42 to 33.38; the alfalfa substrates contained 22.56-27.20 g/kg nitrogen, 500.0 g/kg carbon and C/N = 18.38-22.16; the corn substrates contained 12.80-13.44 g/kg nitrogen, 511.11-527.00 g/kg carbon and C/N = 39.00-39.93. Essential differences were observed between the acid detergent lignin and hemicellulose contents. The buckwheat green mass substrates contained lower amounts of hemicellulose and higher amounts of lignin than buckwheat silage substrate, the biochemical methane potential varied from 292-305 l/kg VS in green mass substrates to 314 l/kg VS in silage substrate. The biochemical methane potential of buckwheat substrates, as compared with alfalfa substrates, does not differ essentially, but as compared with corn substrates, it is lower. According to Pabón Pereira (2009) *Fagopyrum esculentum* substrate contained 170 g/kg DM, 90% OM, 44 % total fibre, 5% lignin, 26% Cel, 12% HC, 4% starch, 14% protein, with biochemical methane potential 320 l/kg, but *Hordeum vulgare* substrate contained 380 g/kg DM, 95% OM, 65 % total fiber, 2% lignin, 23 % Cel, 40 % HC, 22 % starch, 9% protein, with biochemical methane potential 300 l/kg. Herrmann et al. (2016) mentioned that buckwheat silage substrates had C/N = 26.4, biochemical methane potential 210.4 l/kg VS; buckwheat/phacelia mixture silage - C/N=29 and biochemical methane potential 232.9 l/kg VS, but maize silage C/N = 37 and biochemical methane potential 328.2 l/kg VS. Elsayed et al. (2019) reported that buckwheat husk substrate had 97.60% volatile solids, 47.50% total carbon, 2.30% total nitrogen, C/N = 20.65 and cumulative methane yields achieved 200 mL/g VS, but wheat straw substrate contained 95.64% volatile solids, 47.62% total carbon, 0.30% total nitrogen, C/N = 158.73 and 230 mL/g VS methane yields.

Table 4. The biochemical biomethane production potential of the researched substrates

Indices	*Fagopyrum esculentum*			*Medicago sativa*		*Zea mays*	
	green mass, flowering stage	green mass, seed formation stage	silage	green mass, first cut	green mass, third cut	green mass	silage
Crude protein, g/kg DM	154.00	108.00	96.00	170.00	141.00	84.00	80.00
Minerals, g/kg DM	94.00	80.00	77.00	90.00	90.00	52.00	59.00
Nitrogen, g/kg DM	24.64	17.28	15.36	27.20	22.56	13.44	12.80
Carbon, g/kg DM	503.33	511.11	512.78	500.00	500.00	527.00	511.1
Ratio carbon/nitrogen	20.42	29.58	33.38	18.38	22.16	39.00	39.93
Hemicellulose, g/kg DM	181.00	216.00	245.00	193.00	186.00	203.00	221.0
Acid detergent lignin, g/kg DM	66.00	69.00	55.00	63.00	69.00	48.00	37.00
Biomethane potential, L/kg VS	305	292	314	314	298	321	338

CONCLUSIONS

The *Fagopyrum esculentum* whole plants cut in the early flowering stage contained 15.4% CP, 9.4% ash, 37.5% CF, 39.1% ADF, 57.7% NDF, 6.6% ADL, 32.5% Cel, 18.1% HC, 6.9% TSS, with nutritive and energy values: 584 g/kg DDM, RFV= 95, 11.74 MJ/kg DE, 9.51 MJ/kg ME and 5.54 MJ/kg NEl, but in the seed formation stage - 10.8% CP, 8.0% ash, 36.2% CF, 38.3% ADF, 58.9% NDF, 6.9% ADL, 31.4% Cel, 21.6% HC, 10.1% TSS, with nutritive and energy values: 591 g/kg DDM, RFV= 93, 11.88 MJ/kg DE, 9.62 MJ/kg ME and 5.62 MJ/kg NEl.

The quality of the silage prepared from *Fagopyrum esculentum* whole plants cut in the seed formation stage was: pH= 3.76, 39.9 g/kg lactic acid, 0.2 g/kg butyric acid, 10.9 g/kg acetic acid, 226 g/kg DM with 9.6 % CP, 7.7 % ash, 35.5 % CF, 36.2 % ADF, 55.5 % NDF, 5.5 % ADL, 30.7 % Cel, 24.5 % HC, 8.4 % TSS, with nutritive and energy values: 607 g/kg DDM, RFV= 102, 12.00 MJ/kg DE, 9.85 MJ/kg ME and 5.87 MJ/kg NEl.

The *Fagopyrum esculentum* green mass substrates for anaerobic digestion, have C/N = 20.42-29.58 and the biochemical methane potential reaches 292-305 l/kg ODM, but in ensiled substrate - C/N = 33.38 and biochemical methane potential was 314 l/kg ODM.

The *Fagopyrum esculentum* plants develop well under the climatic conditions of Moldova, and green mass and silage have optimal feeding value, and also may be used as feedstock for biomethane production.

ACKNOWLEDGEMENTS

The study has been carried out in the framework of the project: 20.80009.5107.02 *"Mobilization of plant genetic resources, plant breeding and use as forage, melliferous and energy crops in bioeconomy"*.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

PRODUCTIVE CAPACITY OF RAPESEED HYBRIDS GROWN IN THE CONDITIONS OF CENTRAL SOUTH BULGARIA

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Abstract

This study was conducted in the course of three years (2017-2020) in the EEIB (Educational and Experimental Implementation Base) of the Department of Plant-growing at the Agricultural University of Plovdiv. The experiment was implemented according to the block method in 4 repetitions with the size of the experimental plot 20 m^2, on a meadow lightly solonetz soil. Subject of the experiment are the hybrids PT234, PT225, PT271 and PT264. The purpose of the study is to determine their productivity when grown in the conditions of Central South Bulgaria. Regardless of the different weather conditions during the years of the experiment, the highest seed yield was reported for hybrid PT 234.

***Key words**: rapeseed, hybrids, yield.*

INTRODUCTION

Rapeseed is a plant that in recent years has taken an important place among crops cultivated in our country. The range of its applications is various, including from the food industry to the use in biofuels. Thanks to its deep root system, it improves the structure of soil and increases its water permeability. Therefore, intensive selection activity is being done in a number of countries in the world with the purpose of creating new high-yielding and high-quality hybrids (without erucic acid and low content of glucosinolates) (Zheng et al., 2022; Seyis et al., 2006; Nita et al., 2022; Bopp et al., 2021; Zhang et al., 2022). The lack of selection activity in our country necessitates testing and implementation in practice of foreign hybrids and varieties. For this reason, the goal of this work is to test several introduced rapeseed hybrids, establish their phenological development, structural elements, productivity and mass of 1000 seeds in the conditions of Central South Bulgaria with a recommendation for cultivating the most suitable of them.

MATERIALS AND METHODS

The study was conducted in the area of the Training, Experimental and Implementation Unit of the Department of Plant growing in the Agricultural University of Plovdiv during the period 2017-2020. The experiment was set up according to the block method in 4 repetitions with the size of the experimental plot 20 m^2. The hybrids included in the experiment are PT234, PT225, PT271 and PT264. Wheat was used as a precursor, and rapeseed was grown according to the adopted technology.

The following indicators are recorded: the phases of development during the vegetation of the crop, plant height (cm), number of branches per plant (pcs), number of fruits and seeds per plant (pcs), fruit length (cm), weight of seeds per plant (g), seed yield per hectare (t) mass per 1000 seeds (g) . Processing of the mathematical data was done by variance analysis with the Biostat software package, version 5.1 (Penchev, 1998).

RESULTS AND DISCUSSIONS

The quantity of precipitation during the years of the experiment varied from 600.5 mm in 2017-2018 to 474.4 mm in 2018-2019 (Figure. 2). The quantity of precipitation fallen in all three years is higher compared to that of the multi-year period (419 mm).

The most favourable for rapeseed development is 2018-2019, which is characterized by the best combination of temperature and moisture

during the critical periods of plant development. (Figures 1 and 2).
Negative temperatures during the years of the study were recorded in the months of March (-10.6°C for 2017-2018 and February -11.2°C in 2018-2019 and the month of January -10.5°C in 2019-2020) (Figure 3).

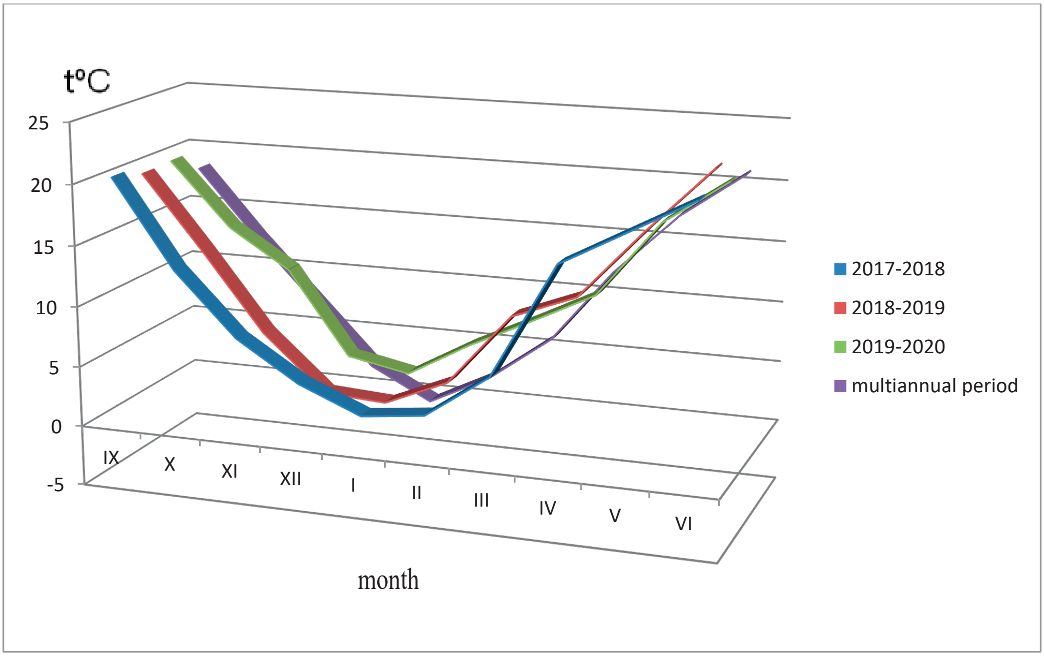

Figure 1. Month mean temperature for the experimental period, °C

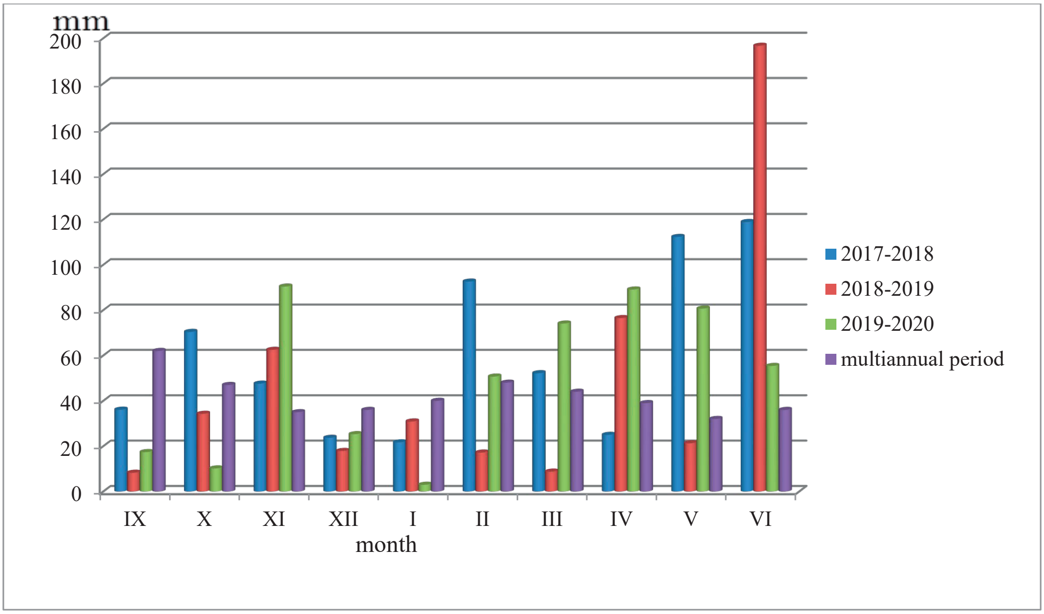

Figure 2. Quantity of rainfalls, mm

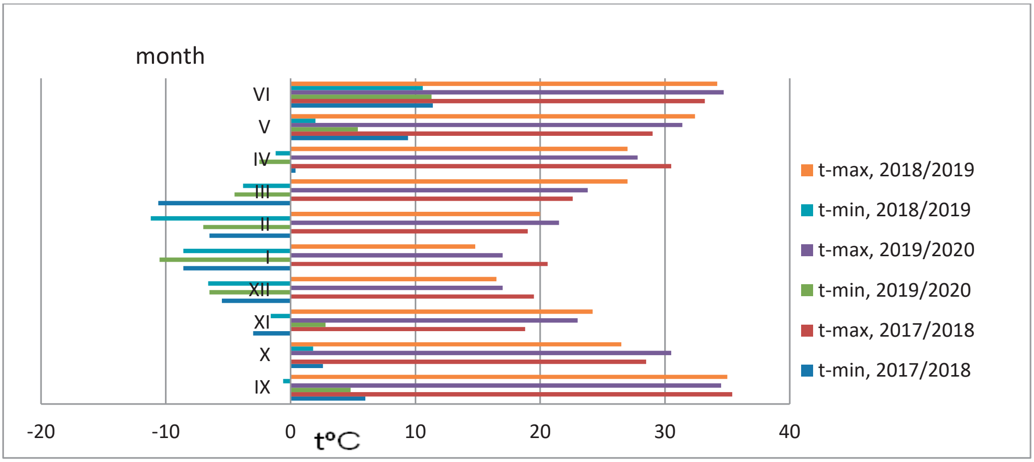

Figure 3. Absolute minimum and maximum air temperatures, °C

The phenological observations show that, due to the weather conditions and the specifics in the development of the tested hybrids, the development phases occur at different times (Table 1).

A major factor for the development of rapeseed, especially during germination and the first phase of the development of the crop is moisture content.

That is why, the precipitation that fell during the germination period (36.1mm - 2017; 8.3 mm - 2018; 17.4 mm - 2019), created conditions for the plants in 2018 to sprout the earliest (20.09), followed by 2019 (27.09) and the latest - in 2017 at 28.09.

About a month after sprouting, all tested hybrids reach the 6th-8th leaf stage.

Depending on the year of the experiment, the tested hybrids reach this phase from 26.10 to 30.10.

When temperatures drop in December (-5.5°C - 2017; - 6.6°C - 2018; -6.6°C - 2019), rapeseed suspends its vegetation.

This period in the first year of study occurred on 12.12, in the second - on 9.12, and the third - on 8.12.

The increase in temperatures in all three study years in the beginning of March led to the resumption of vegetation (the time from 7.03 to 10.03).

The next stage which is stem formation was reported 1 to 3 days earlier in 2020 compared to 2018. In 2019 this stage occurred up to 1 day earlier than in 2018 and 1 to 2 days later than in 2020.

During the three years of the experiment, the hybrids entered the stage of stem formation as follows: PT264 (26.03; 25.03; 23.03), PT271 (25.03; 25.03; 24.03), PT234 (26.03; 25.03; 24.03) and PT225 (27.03; 27.03; 25.03).

The plants of all tested specimen entered the budding stage, from 8.04 to 11.04.

Hybrid PT234 entered the earliest in this stage (10.04; 9.04; 8.04), and the latest - PT 225 (11.04; 11.04; 10.04).

Hybrid PT234 (10.04; 9.04; 8.04) entered this stage at the earliest, and hybrid PT 225 (11.04; 11.04; 10.04) at the latest.

During the period of the experiment, the large-scale flowering stage occurred in the third ten days of April. For individual hybrids, this stage was recorded on 26.04; 25.04; 23.04 for PT234 hybrid, on 26.04; 27.04; 25.04 for PT271 hybrid, on 26.04; 26.04; 24.04 for PT264 hybrid and on 27.04; 28.04; 26.04 for PT225 hybrid.

Full maturity stage occurred a few days later in 2018 compared to the other two years.

PT234 hybrid (29.06; 25.06; 24.06) entered this stage at the earliest, and PT 225 hybrid - at the latest (30.06; 29.06; 27.06).

The vegetation period of the tested hybrids varies from 272 to 283 days, the shortest being for PT234 hybrid - from 272 to 279 days, and the longest is for PT225 hybrid - from 275 to 283 days.

Table 1. Phenological observation

Phenological observation	Hybrids											
	PT234			PT 225			PT271			PT264		
	Years											
	2017 2018	2018 2019	2019 2020	2017 2018	2018 2019	2019 2020	2017 2018	2018 2019	2019 2020	2017 2018	2018 2019	2019 2020
Sowing	5.09	3.09	5.09	5.09	3.09	5.09	5.09	3.09	5.09	5.09	3.09	5.09
Prouing	28.09	20.09	27.09	28.09	20.09	27.09	28.09	20.09	27.09	28.09	20.09	27.09
6st-8th leaf	29.10	26.10	29.10	30.10	28.10	30.10	29.10	27.10	29.10	30.10	27.10	30.10
Finish vegetation	12.12	9.12	8.12	12.12	9.12	8.12	12.12	9.12	8.12	12.12	9.12	8.12
Beginning vegetation	10.03	7.03	9.03	10.03	7.03	9.03	10.03	7.03	9.03	10.03	7.03	9.03
Stem development	26.03	25.03	24.03	27.03	27.03	25.03	25.03	25.03	24.03	26.03	25.03	23.03
Bud stage	10.04	9.04	8.04	11.04	11.04	10.04	10.04	10.04	9.04	10.04	10.04	9.04
Beginning of flowering - 10%	18.04	17.04	15.04	19.04	20.04	17.04	17.04	18.04	16.04	19.04	17.04	16.04
Full flowering - 75%	26.04	25.04	23.04	27.04	28.04	26.04	26.04	27.04	25.04	26.04	26.04	24.04
Wax ripeness	14.06	13.06	11.06	16.06	16.06	14.06	15.06	15.06	13.06	14.06	13.06	11.06
Full maturity	29.06	25.06	24.06	30.06	29.06	27.06	30.06	28.06	26.06	30.06	26.06	25.06
Vegetation period, days	274	279	272	275	283	275	274	282	274	275	280	273

The indicators that have a direct impact on seed yield are presented in Tables 2 and 3.

They change over the cultivating years due to the weather conditions.

On average over the study period, the plant height of the tested hybrids ranged from 156.8 to 167.1 cm.

As a result of the more favourable combination of climatic factors in 2019, the height of the plants at the end of the growing season had higher values in all tested hybrids, except for PT 225 hybrid.

The reason for the formation of lower plants in 2019-2020 is the irregular distribution of precipitation in the critical periods of rapeseed development.

The greatest height during the term of the experiment was recorded for PT 234 hybrid (168.3 cm; 169.6 cm; 163.5 cm), and the lowest, except for the first year, for PT 264 hybrid (157.3 cm; 157.4 cm; 155.9 cm).

Over the years of the study, the number of branches in all tested hybrids was the highest in 2019, except for PT225 hybrid, where a higher number of branches was recorded in 2018. Of the hybrids tested, PT 234 had the highest values, from 8.2 to 8.4 pieces, and with the lowest, PT 264 hybrid - from 7.5 to 7.7 pieces.

The highest number of branches on average for the study period was recorded for PT234 hybrid (8.3 pieces), and the least for hybrids PT271 and PT264 (7.6 pieces).

The more favourable effect of climatic factors in 2019 is a precondition for the formation of a larger number of fruits (from 214.9 to 229.7 pieces) per plant compared to other years (from 203.0 to 221,9 pieces).

On average for the study period, the number of fruits was the highest for PT 234 hybrid (224.2 pieces) and the least in PT 264 hybrid (210.9 pieces).

The length of the fruits during the years of study and averagely over the period varied within small limits.

The highest values for this indicator were reported for PT234 hybrid (from 8.0 to 8.4 cm). For the rest of the hybrids, the length of the fruits on average during the experiment ranged from 7.8 cm to 7.9 cm.

The number of seeds in one fruit varies within a limited range. On average for the study period, the highest values were recorded for PT 234 hybrid (27.8 pieces), and for the other hybrids the number of seeds was between 26.3 and 27.2 pieces.

Fruit and seed weight are the structural elements that have the strongest influence on the seed yield of a plant. Higher values of these indicators in a combination with the climatic factors in the period of formation - filling and ripening of the seeds were reported in 2019.

The highest values of these indicators in 2019 were reported for hybrid PT 234 (26.9 g) and the lowest for hybrid PT 264 (25.8 g) regarding fruit weight, while regarding seed weight per plant - from 14.6 for hybrids PT 264, PT271 - up to 15.4 g for PT 234 hybrid.

In the other two years, the values of these indicators were from 25.5 g to 26.5 g with regards to fruit weight and from 14.4 g to 15.2 g with regards to seed weight.

On average for the study period, the weight of the pods had the highest values for PT 271 hybrid (11.4 g) and the lowest in PT264 hybrid (11.2 g).

Both in individual years and on average for the period, the weight of the pods in the studied hybrids was almost the same.

Structural elements that have the greatest influence on seed yield have the highest values in PT 234 hybrid.

The data in Table 4 show the seed yield obtained during the years of the experiment depending on the climatic conditions and the studied hybrids.

Higher yields in all tested hybrid variants were reported in 2019, except for PT225 hybrid.

A precondition for this is the better combination and distribution of meteorological factors this year.

The highest seed yield in the first year of study was obtained from PT234 hybrid (3.829 t/ha) followed by PT225 (3.813 t/ha), PT264 (3.712 t/ha) and PT 271 (3.549 t/ha).

In the second and third years of the study, the highest yield was obtained from hybrid PT234 (3.917 and 3.557 t/ha), and the lowest from PT264 (3.759; 3.354 t/ha).

For the hybrids subject of this study, on average during the test period, the highest seed yield was obtained from hybrid PT234 (3.788 t/ha), followed by PT225 (3.656 t/ha), PT264 (3.608 t/ha), and lowest - from PT 271 (3.574 t/ha).

Table 2. Structural analysis of yield elements

Hybrids	Height plants (cm)				Number of branches in a single plant (pcs)				Number of fruits in a single plant (pcs)				Fruit length (cm)			
	Years															
	2017 2018	2018 2019	2019 2020	Average	2017 2018	2018 2019	2019 2020	Average	2017 2018	2018 2019	2019 2020	Average	2017 2018	2018 2019	2019 2020	Average
PT234	168.3	169.6	163.5	167.1	8.2	8.4	8.2	8.3	221.9	229.7	220.9	224.2	8.1	8.4	8.0	8.2
PT225	159.8	158.0	156.7	158.2	8.0	7.9	7.4	7.8	217.8	215.3	205.3	212.8	8.0	7.9	7.8	7.9
PT271	156.9	157.6	156.0	156.8	7.6	7.7	7.6	7.6	211.2	214.9	207.7	211.3	7.9	7.9	7.9	7.9
PT264	157.3	157.4	155.9	156.9	7.6	7.7	7.5	7.6	213.4	216.4	203.0	210.9	7.9	7.8	7.6	7.8

Table 3. Structural analysis of yield elements

Hybrids	Number of seeds in one fruit (pcs)				Weight of the fruit in a single plant (g)				Weight of the seeds in a single plant (g)				Weight of pods in a single plant (g)			
	Years															
	2017 2018	2018 2019	2019 2020	Average	2017 2018	2018 2019	2019 2020	Average	2017 2018	2018 2019	2019 2020	Average	2017 2018	2018 2019	2019 2020	Average
PT234	27.8	28.0	27.5	27.8	26.5	26.9	26.0	26.5	15.2	15.4	15.0	15.2	11.3	11.5	11.0	11.3
PT225	27.2	26.8	26.5	26.8	26.1	26.1	25.7	26.0	14.8	14.7	14.5	14.7	11.3	11.4	11.1	11.3
PT271	26.6	26.7	26.6	26.6	25.9	26.0	25.9	25.9	14.5	14.6	14.5	14.5	11.4	11.4	11.4	11.4
PT264	26.7	26.7	26.3	26.6	25.8	25.8	25.5	25.7	14.6	14.6	14.4	14.5	11.2	11.2	11.1	11.2

Table 4. Yield of seeds t/ha

	2018	2019	2020	Average
PT234	3.829	3.917	3.557	3.788
PT225	3.813	3.772	3.383	3.656
PT 271	3.549	3.740	3.432	3.574
PT264	3.712	3.759	3.354	3.608
GD 5%	0.0624	0.020	0.0610	

From the mathematical processing of seed yield by year, provenance at CD 5% was found between hybrid PT234 and all other hybrids except hybrid PT 225 in the first year.

The mass of 1000 seeds gives an idea of the physical properties of the seeds and is directly related to obtaining high yields (Table 5).

Agro-climatic conditions, agricultural techniques and the difference between hybrids influence this indicator.

The highest mass per 1000 seeds during the years of study was recorded in 2019, and the lowest - in 2020.

On average for the growing period, the highest mass per 1000 seeds was reported for PT234 hybrid (5.17 g).

Over the years of study, on the basis of the mathematical processing done, evidence regarding this indicator at CD 5% has been established between hybrid PT234 and the other specimen.

Table 5. Mass of 1000 seeds

Hybrids	Mass of 1000 seeds (g)			
	2018	2019	2020	Average
PT234	5.17	5.20	5.15	5.17
PT225	5.12	5.11	5.07	5.10
PT 271	5.00	5.01	5.00	5.00
PT264	5.01	5.02	4.96	5.00
GD 5%	0.04	0.07	0.05	

CONCLUSIONS

During the years of the experiment, the vegetation period for the tested hybrids varied from 272 to 283 days.

The structural elements of yield for all hybrids have the highest values in 2018-2019. The only exception is hybrid PT225, where the highest values were recorded in the first year.

During the three years of study, the highest values of all structural elements were recorded for PT234 hybrid.

The obtained seed yield in 2018-2019 is higher compared to the other two years. Only for PT225 hybrid higher yield was reported in the first year.

In all three years of the study and averagely over the period, the highest seed yield was obtained from PT234 hybrid.

The mass of 1000 seeds in the three years and averagely over the period was highest for PT234 hybrid (5.17; 5.20; 5.15 g.).

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

COMPARATIVE TESTING OF OLD WINTER WHEAT VARIETIES UNDER CHANGING CLIMATIC CONDITIONS

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Abstract

The experiment was conducted in the experimental field of IRGR - Sadovo in the period 2018-2020. The sixteen old varieties of common winter wheat, created in IRGR, were tested in yield for two years. The yield, plant height and physical properties of the grain were obtained: 1000 g kernel weight and test weight (kg/hl). The obtained data are processed by statistical methods - dispersion, variation and analysis of the main components. The results show that the influence of the genotype, environment and their interaction has been proven in all monitored traits. All varieties have significantly higher yields than the standard. The highest grain yield was reported for the varieties Joanna, KM 135, Diamand and Guinness. There is no significant higher 1000 kernel weight and the test weight of only two varieties is significantly higher than the standard. The aim of the study is to test the effect of climate change on the grain yield, plant height and physical properties of old varieties of common winter wheat, as the main food crop, and to assess their resilience to climate change.

***Key words**: old varieties, winter wheat, resistance, climatic changes, yield.*

INTRODUCTION

Wheat is counted among the 'big three' cereal crops, with over 600 million tons being harvested annually. For example, in 2007, the total world harvest was about 607 mil. tons compared with 652 mil. tons of rice and 785 mil. tons of maize (http://faostat.fao.org/). However, wheat is unrivalled in its range of cultivation, from 67º N in Scandinavia and Russia to 45º S in Argentina, including elevated regions in the tropics and sub-tropics (Feldman, 1995). It is also unrivalled in its range of diversity and the extent to which it has become embedded in the culture and even the religion of diverse societies (Shewry, 2009).

Despite its relatively recent origins, bread wheat shows enough genetic diversity to allow the development of more than 25.000 species of Feldman et al. (1995), which are adapted to a wide range of moderate environments. In the presence of sufficient water and mineral nutrients and ensuring effective control of pests and pathogens, yields can exceed 10 tons ha^{-1}, showing high similarity to other moderate crops. However, the shortage of water and nutrients and the impact of pests and pathogens cause the average world yield to be low, about 2.8 tons ha^{-1}.

The perspectives for global wheat production in 2021 has been reduced this month, and the latest FAO forecast now stands at 778.8 million tons, although still 4 million tons (0.5%) higher than the 2020 production forecast year. The limitation of the global production forecast is mainly related to the European Union (EU), where less than expected previously planted area led to a decrease of 4 million tons compared to the previous forecast. However, EU production is still expected to increase by 6 percent on an annual basis to 133.3 million tons and, together with favorable prospects in the United Kingdom, the United States and Ukraine, supports the overall positive outlook for world production this year (http://www.fao.org/worldfoodsituation/csdb/en/). One of the challenges facing humanity as a result of climate change is to increase the number of people at risk of starvation compared to reference scenarios with climate change. In 2006, the global estimated number of malnourished people was 820 million, according to FAO data.

Wheat (*Triticum aestivum* L.) is a major food crop and feeds billions of people daily. Its productivity is expected to decrease significantly with increasing temperature. Due to global climate change, wheat yields are expected to decrease by 6% for each 1°C temperature increase. Therefore, wheat yields must increase by 60% by 2050 to meet the food needs of a growing world population (Asseng et al., 2015). The yield of bread wheat is most interesting for breeders. It depends largely on the genotype, the conditions and the interaction between them. The results of the genotype X environment interaction are manifested in the adaptation and stability of the genotypes. When such an interaction exists, the arrangement of the genotypes will be different under different conditions. Productivity stability plays a very important role. In most cases it is typical for the old varieties. The creation of varieties with high and stable yield and good quality is an important and main factor in the wheat production. The environment has a negative effect on yield, but it can be increased by improving the growing conditions with increasing agricultural techniques. The intermittent yield is a result of changes in climatic conditions in the study areas. According to Dotlacil et al. (2000) old varieties with low yields have greater stability in the individual years than these varieties with high yield potential. Genotype x environment interactions complicate the cultivation of superior genotypes (Hintsa & Fetien, 2013). Therefore, an assessment of each genotype without including its interaction with the environment is incomplete (Crossa, 1990).

The study of genetic diversity has both historical value and immediate practical impact on the breeding of cultivated plants. However, diversity itself is limited in use (Novoselovi´c et al., 2016). But it gives breeders an advantage to know the best sources and to include them in cross-breeding schemes. The old varieties are the basis of any selection program. Their use allows the inclusion of valuable qualities in modern varieties. Assessing correlations to determine the extent of relationships between different traits associated with yield is an important issue that reveals a complex chain of associations (Majumder et al., 2008; Mohammadi et al., 2012). Wheat grain yield is a dominant feature of the economic value of the crop (Shewry, 2007). Test weight and 1000 grains weight are important traits for breeding programs in wheat (Aydin et al., 2010). Grain size is one of the most important indirect traits and can be used as a criterion in the early generations of decay. Traits such as optimal plant height and 1000 grains weight are key elements of yield (Li & Gill, 2004; Gupta et al., 2006). The plants height, although not a direct element of productivity, is the focus of breeding programs for wheat. Its importance is due to its direct connection on the one hand with the lodging of plants and on the other with the size of the harvest index (Hedden, 2003).

There are three possibilities for increasing production - creating new higher yielding varieties, testing the suitability of old varieties to changing conditions, and increasing the area. It is also necessary for the varieties to be characterized by both high yields and good traits of physical properties. They are crucial for selling them.

The aim of this research is to study the yield, height and physical properties of old varieties of common winter wheat under changing climatic conditions.

MATERIALS AND METHODS

The experiment was conducted in the experimental field of IPGR - Sadovo in the period 2018-2020. Varietal experiments were performed in a block diagram in three replications, with a size of the experimental plot of 10 m^2. The experiment was done using the technology of cultivation after the bean predecessor, adopted in IPGR. Sowing was carried out in the optimal time. Sadovo 1 was used as a standard. The grain yield was calculated at a standard humidity of 13%.

The main factors influencing the growth and development of wheat are temperature, precipitation and their combination. Their interaction during the important stages of development is especially important. 16 varieties of winter common wheat were monitored on yield and traits: plant height (cm), 1000 grains weight (g) and test weight (kg/hl). Statistical methods were used for evaluation - variance (ANOVA), variation and principal component analysis.

The statistical program SPSS 19 was used. The degree of variation of the traits was determined by a coefficient of variation based on average values for the study period. It is accepted that the variation is considered weak if the coefficient of variation is up to 10%, medium - when it is greater than 10% and less than 20%, and strong - when it is over 20% Dimova & Marinkov (1999). From the analysis of the variant, the influence of the sources of variation - genotype, environment and their interaction on the yield, the 1000 grains weight and the test was calculated.

RESULTS AND DISCUSSIONS

For the first vegetation period it can be summarized that the average monthly temperatures are higher than the perennial ones, and the precipitation was not evenly distributed. In November, rainfall fell, more than the multi-year norm and favored the development of plants. There was a delay in the development of wheat before the rain in April.
After the large amount of precipitation in June, secondary weeding occurred (Figure 1 and Figure 2). The meteorological conditions during the vegetation year 2019-2020 differ from those in the previous year. Conditions during the period from sowing to the end of March are variable and are not the most favorable for the development of wheat due to the snowfall at the end of March and the beginning of April 1 (Figure 3 and Figure 4).
The average monthly temperatures in April alone were lower than the norm, with temperatures higher than 1.94°C in December and 6.47°C in January. During the important phases of spinning, hatching and pouring the grain, no lack of moisture was reported. The rainfall in April and May favored the formation of a higher stem compared to the typical varieties, large grain and the formation of high yields. The figure with the meteorological data shows that the precipitation during the vegetation year is more than the norm, but it is not evenly distributed. The second growing season of the study as a whole can be defined as very favorable for ordinary winter wheat. Confirmation of this are the high yields obtained.

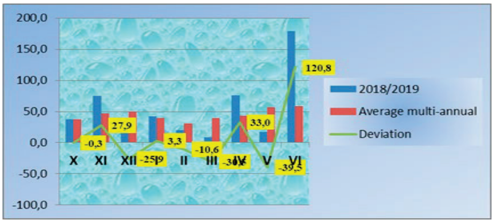

Figure 1. Sums of month rainfall (mm) during vegetation year 2018-2019

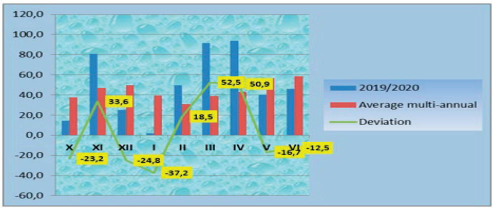

Figure 2. Sums of month rainfall (mm) during vegetation year 2019-2020

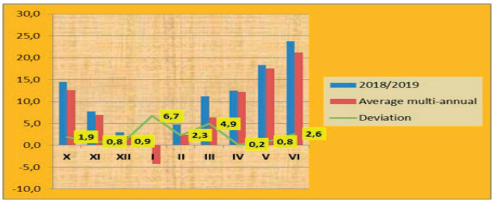

Figure 3. Average temperature sum (t°C) of months during vegetation year 2018-2019

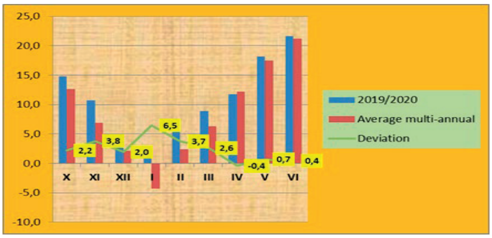

Figure 4. Average temperature sum (t°C) of months during vegetation year 2019-2020

On average for the two years of testing, the highest grain yield was reported for the varieties Joanna, KM 135, Diamond and Guinness (Table 1).

Yields from the old varieties in the present study ranged from 431.5 kg/da for cultivar Sadovo 1 to 651.5 kg/da for Joanna.

It is noteworthy that the average yield of all participants is proven to be higher than that of the standard variety. The variation in this indicator is a marginal 10.2% between low and medium variation. It is the highest in this indicator compared to the other two, ie the grain yield is the most variable in the present study.

On average for test period, the highest plant height was reported for the varieties Bononiya, Mustang and Sadovo 552. With the smallest height of the stem are the varieties Yoana, KM 135, Geya 1, Diamant, Pobeda, Sadovska beliya and Niky. The differences are statistically proven. The highest plant height in the present study ranged from 105.3 cm for cultivar Mustang to 82.5 cm for Lysil (Table 1).

The 1000 grains weight is one of the most important indirect traits, characterizing the grain size, its mill quality (relative content of endosperm, potential yield of flour with low ash content) and its quality as seed (Popov et al., 1965; Filipov, 2004; Stoeva et al., 2009; Yanchev & Ivanov, 2012; Delibaltova et al., 2014; Taneva et al., 2014; Ivanov, 2019). Water deficit during the grain setting period can cause grain yield decrease by reducing grain weight per spike and 1000 kernel weight (Genç et al., 1987; Koç et al., 1994).The trait 1000 grains weight does not show a proven higher 1000 grains weight compared to the standard. Six varieties have a proven lower weight per 1000 grains (Table 2). The maximum value is for the Mustang variety and the lowest for the Guinness variety. Our previous studies obtained similar results: the influence of genotype on the trait mass per 1000 grains is significant (Angelova et al., 2020). The variation is low.

Table 1. Study characteristics of common wheat varieties

Genotype	Yield, mean	Differ.	Sign.	PH, mean	Differ.	Sign.
Sadovo 1 st.	431.5			97.8		
Bononiya	527.6	96.0	+++	104.5	6.7	+++
Niky	531.0	99.5	+++	92.5	-5.3	--
Lysil	619.7	188.2	+++	82.5	-15.3	---
Sad. beliya 1	573.1	141.6	+++	98.8	1.0	n.s
Tsarevets	526.3	94.8	+++	98.5	0.7	n.s
Pobeda	515.2	83.6	+++	103.2	5.3	--
Mustang	607.2	175.6	+++	105.2	7.3	+++
Geya 1	604.7	173.1	+++	83.7	-14.2	---
Diamant	613.1	181.6	+++	103.2	5.3	--
Murgavets	594.9	163.3	+++	97.2	-0.7	n.s
Sadovo 552	532.4	100.8	+++	105.3	7.5	+++
Sadovo 772	527.4	95.9	+++	98.5	0.7	n.s
Guinness	612.0	180.5	+++	99.3	1.5	n.s
Yoana	651.5	219.9	+++	82.8	-15.0	--
KM 135	628.8	197.3	+++	89.0	-8.8	--
Mean	568.5			96.4		
Minimum	431.5			82.5		
Maximum	651.5			105.3		
Std.Dev.	57.7			8.0		
Coef.Var..%	10.2			8.3		
Stand. error	14.4			2.0		
GD 5%	11.0			3.6		
GD 1%	14.6			4.7		
GD 0.1%	18.9			6.2		

The test weight in only two varieties is significantly higher than the standard, and in 7 it is proven to be lower (Table 2). The variation is low. The highest value was reported for a variety in Bononia, and the lowest in Pobeda.

Table 2. Study characteristics of wheat varieties

Genotype	1000 GW, mean	Differ.	Sign.	TW, mean	Differ.	Sign.
Sadovo 1 st.	45.4			75.5		
Bononiya	45.2	-0.2	n.s	80.2	4.7	+++
Niky	43.4	-1.9	--	72.8	-2.8	--
Lysil	46.8	1.5	n.s	72.9	-2.6	--
Sad. beliya 1	44.1	-1.2	n.s	71.4	-4.1	---
Tsarevets	40.2	-5.1	---	74.6	-0.9	n.s
Pobeda	43.1	-2.2	--	68.3	-7.2	---
Mustang	47.4	2.1	+	71.7	-3.8	---
Geya 1	44.6	-0.8	n.s	72.8	-2.7	--
Diamant	41.7	-3.6	---	77.5	2.0	-
Murgavets	45.2	-0.2	n.s	75.7	0.2	n.s
Sadovo 552	46.1	0.7	n.s	75.9	0.4	n.s
Sadovo 772	44.1	-1.2	n.s	74.2	-1.3	n.s
Guinness	39.2	-6.2	---	77.6	2.1	+
Yoana	43.8	-1.6	n.s	76.1	0.5	n.s
KM 135	44.2	-1.2	n.s	75.3	-0.3	n.s
Mean	44.0			74.5		
Minimum	39.2			68.3		
Maximum	47.4			80.2		
Std.Dev.	2.2			2.9		
Coef.Var..%	5.0			3.8		
Stand. error	0.5			0.7		
GD 5%	11.0			1.7		
GD 1%	14.6			2.2		
GD 0.1%	18.9			2.9		

The results show that the influence of the genotype, environment and their interaction has been proven for all monitored traits.The strongest influence in the formation of the grain yield, the 1000 grains weight and the test weight, expressed by η, is the influence of the medium. In the three studied traits it is over 61% and the strongest grain yield reaches 82% (Table 3).

Table 3. ANOVA - Influence of the sources of variation on the studied traits

Trait	Sources of variation	SS	df	MS	F exp.	F tab.	η	Sign.
Yield	Genotype, Factor A	300003.5	15	20000.2	443.5	3.0	10.1	***
	Environment, Factor B	2426022.1	1	2426022.1	53793.1	11.9	82.0	***
	Interactio, A x B	230675.8	15	15378.4	341.0	3.0	7.8	***
	Error	2886.3	64	45.1			0.1	
	Total	2959587.7	95				100.0	
PH	Genotype, Factor A	5695.2	15	379.7	79.4	3.0	51.4	***
	Environment, Factor B	3901.5	1	3901.5	816.0	11.9	35.2	***
	Interactio, A x B	1177.8	15	78.5	16.4	3.0	10.6	***
	Error	306.0	64	4.8			2.8	
	Total	11080.5	95				100.0	
1000 GW	Genotype, Factor A	434.7	15	29.0	29.7	3.0	19.4	***
	Environment, Factor B	1578.7	1	1578.7	1619.0	11.9	70.6	***
	Interactio, A x B	160.7	15	10.7	11.0	3.0	7.2	***
	Error	62.4	64	1.0			2.8	
	Total	2236.4	95				100.0	
TW	Genotype, Factor A	737.3	15	49.2	47.8	3.0	17.5	***
	Environment, Factor B	2565.8	1	2565.8	2495.8	11.9	61.0	***
	Interactio, A x B	839.9	15	56.0	54.5	3.0	20.0	***
	Error	65.8	64	1.0			1.6	
	Total	4208.7	95				100.0	

SS - sum of squares; gf - degrees of freedom; MS - variance; F exp. - F experimental; F tab. - F tabular; η - force of influence of the factor (%); *** - proved at α = 0.001.

The strength of the genotype influence is also significant, but the values of η are much lower and range from 19.4 for the 1000 grains weight to 10.1 for the grain yield. Only in the case of plant height is the influence of the genotype higher than that of the environment. The strength of the influence of the genotype 51.4%, and the environment is 35.2. The strength of the impact of the interaction is also low (7.2 of 20.0).According to a number of authors (Fufa et al., 2005; Akcura et al., 2006; Kaya et al., 2006; Tsenov et al., 2006; Plamenov & Spetsov, 2008) the impact of the conditions of the year on the productivity and quality of the common winter wheat is more important than the influence of genotypes. In our previous studies in southern Bulgaria, it was established through the analysis of the variant that climatic conditions have the strongest influence on the yield of the general variation (Uhr & Chipilski, 2017; Ivanov et al., 2018). In the cultivation of foreign varieties under the conditions of Dobrudzha also the factor year was determining for the formation of the production potential of the studied foreign wheat cultivars (Chamurliyski et al., 2015).

Based on the data for the studied indicators of yield, height and physical properties of the grain, a factor analysis was performed by the method of the main components of 15 old varieties of common winter wheat and the Sadovo 1 standard. According to Rymuza et al. (2012) the applied principal component analysis (PCA) method allows a complex assessment of the relationships between the characteristics. The greatest distinctive force, which diversifies the studied plants, is manifested by the 1000 grains weight and grain yield. Our results from the application of PCA are presented in Tables 4, 5, 6 and Figures 5 and 6. The values of the two components for each of the studied traits were calculated empirically (Table 4). The analysis shows that the first component justifies 36.819% of the total variation, and the second - 30.842%. The two factors together justify 67.661% of the total variation in experience. This relatively small percentage illustrates the existence of complex relationships between the studied traits. For example, the signs of yield and plant height are related to the first component. The second component is correlated with the test weight and 1000 grains weight (Table 5).

Table 4. Component analysis of the variance in the studied traits

Comp.	Total	% of Variance	Cumulative,%
1	1.473	36.819	36.819
2	1.234	30.842	67.661
3	0.797	19.932	87.593
4	0.496	12.407	100.0

Table 5. Explained significant components by traits

№	Trait	Component	
		1	2
1	Yield	-0.809	0.340
2	Plant hight	0.870	0.015
3	1000 grain weight	-0.184	-0.732
4	Test weight	0.167	0.763

Table 6. Explained significant components by varieties

№	Variety	Component	
		1	2
1	Sadovo 1	-1.512	-0.484
2	Bononiya	-0.971	0.965
3	Niky	-0.118	-0.391
4	Lysil	1.514	-1.237
5	Sadovska beliya 1	-0.152	-0.651
6	Tsarevets	-0.582	0.994
7	Pobeda	-1.083	-1.139
8	Mustang	-0.263	-1.300
9	Geya 1	1.278	-0.650
10	Diamant	-0.006	1.530
11	Murgavets	0.221	0.022
12	Sadovo 552	-1.013	-0.197
13	Sadovo 772	-0.580	-0.175
14	Guinness	0.265	2.178
15	Yoana	1.845	0.394
16	KM 135	1.159	0.141

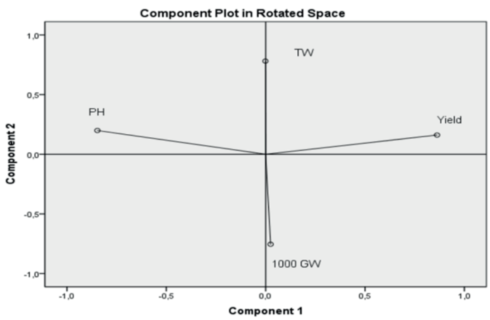

Figure 5. PC analysis of traits in common winter wheat

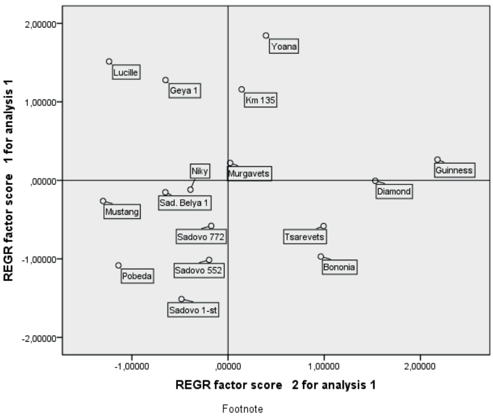

Figure 6. PC analysis of 15 wheat genotypes

The distribution of the varieties, according to their values for the first and second components, is presented in Table 6 and Figure 6. The first component includes the varieties Sadovo 1, Bononia, Lucil, Gaia 1, Murgavets, Sadovo 552, Sadovo 772, Joanna and KM 135. The second component includes Niki, Sadovska belia, Tsarevets, Pobeda, Mustang, Diamant and Guinness. By placing the figures next to each other, the connection of the varieties in groups can be determined by a certain feature. The closer the points of the varieties are to the point of a Figure 5 shows the PC analysis of the traits included in the study. Figure 5 shows that the test weight and the 1000 grain weight are in a very strong negative correlation. This means that varieties with larger grains have a lower test weight. Plant height and yield are also very strongly negatively correlated, ie. varieties with lower plant heights have higher yields. There are no reliable positive correlations between the individual traits. Figure 6 shows a PC analysis of the common wheat genotypes included in the study. When comparing the two figures (Figures 5 and 6), the separation of the genotypes into groups can be assessed according to certain traits. Compared to the examined traits from Figure 5, it can be seen which variety is characterized by which trait. The Diamond and Guinness varieties are characterized by yield. The varieties Joanna and KM 135 with the test weight. The varieties Mustang, Sadovska beliya and Niki are characterized by the plants height. These varieties, which are located on the periphery of the figure, are characterized for the most part by the rays of the features of Figure 5. On the other hand, those located in the middle of Figure 6 are more balanced with respect to all the traits.

CONCLUSIONS

The influence of genotype, environment and their interaction is significant for all studied traits.

The strength of the influence of the environment is the range from 61 to 82% in terms of yield, test weight and 1000 kernel weight. The genotype is determining for the plants height , but the difference with the influence of the environment is not large.

Based on the PC analysis, it was found that the Diamant and Guinness varieties are characterized by yield. The varieties Joana and KM 135 with the test weight. The varieties Mustang, Sadovska beliya and Niki are characterized by the plant height.

On average for the two years of testing, the highest grain yield was reported for the varieties Yoana, KM 135, Diamant and Guinness. They can be re-listed in the institute's variety list and offered to farmers as resistant to climate change.

This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018".

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON THE INFLUENCE OF DISTANCE BETWEEN ROWS AND FERTILIZATION ON SOME MORPHOPRODUCTIVE ELEMENTS IN *Bromus inermis* Leyss. SPECIES

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Abstract

The research conducted during the period 2021-2022, at the Research and Development Station for Meadows, Vaslui (46°40'-36°10' north latitude and 27°44'-20°40' east longitude) followed the influence of fertilization and the distance between rows on the plants height (cm), shoots number (shoots·m^{-2}), panicle length (cm), number of nodes per inflorescence, number of branches per inflorescence and seed production (kg·ha^{-1}) for smooth brome (Bromus inermis Leyss.*). The organized experience was bifactorial, 3 x 5 type, it was placed according to the method of subdivided plots, with the plot harvestable area of 20 m^2 (2 m x 10 m), in three replications, and the studied factors were: A - the distance between rows with three graduations and B - fertilization with five graduations. Following the study, it was found that by applying mineral fertilizers and by sowing at bigger distances between rows higher plants were obtained, with a higher number of shoots·m^2, also the panicle length, the number of nodes per inflorescence and number of branches per inflorescence and seed production were higher.*

***Key words**: plants height, shoots number, panicle length, number of nodes per inflorescence, number of branches per inflorescence, seed production, Bromus inermis L.*

INTRODUCTION

Smooth brome (*Bromus inermis* Leyss.) it is a species of perennial grasses with high fodder value and high productivity and is used for the establishment of temporary grasslands (Liu et al., 2014).

This species is characterized by high tolerance to drought and low temperatures and average resistance to soil salinity.

The species is also used for the restoration of degraded meadows and the establishment of pastures (Liu et al., 2008; Antonova et al., 2015). In addition, the smooth brome species is of great importance in the feeding of animals, due to its high production capacity and good digestibility of feed (Dumlu et al., 2013; Unal and Mutlu, 2015). The species can also be used to prevent soil erosion due to the well-developed root system and the presence of stolons (Cinar et al., 2016; Saritas et al., 2017).

Seed production is undoubtedly of particular importance for oversowing or resowing permanent grasslands and the establishment of temporary grasslands by providing the necessary seed material. The technology of growing the seed batches of fodder species is different from the technology of fodder production, with a tendency to improve the morphological peculiarities of seed production and seed quality indices (Ene and Mocanu, 2016; Samuil et al., 2012).

MATERIALS AND METHODS

The purpose and objectives of the research conducted during the period of March to October 2021, at the Research and Development Station for Meadows, Vaslui (46°40'-36°10' N latitude and 27°44'-20°40' E longitude), were represented by the analysis of the influence of row spacing and fertilization on plant height (cm), shoots number (shoots·m^{-2}), panicle length (cm), number of nodes per inflorescence, number of branches per inflorescence and seed production (kg·ha^{-1}), at the smooth brome (*Bromus inermis* Leyss.), the seed crop, in the second year of vegetation.

To achieve the proposed purpose, it was organized a bifactorial experience, 3 x 5 type, placed according to the method of subdivided plots, with the plot harvestable area of 13.5 m^2 (1.5 m x 9 m), in three replications. The factors studied were: A - the distance between rows with three graduations (a_1 - 25 cm, a_2 - 37.5 cm and a_3 - 50 cm) and B - fertilization with five graduations (b_1 - unfertilized, b_2 - $N_{50}P_{50}$, b_3 - $N_{50}P_{50}K_{50}$, b_4 - $N_{75}P_{75}K_{75}$ and b_5 - $N_{100}P_{100}K_{100}$).
The biological material used was represented by the Mihaela smooth brome variety, variety approved in 2010 at the Research and Development Station for Meadows, Vaslui (Silistru, 2010).
Fertilizer was applied early in the spring, at the start of plant vegetation.
The plants height (cm) by measuring, in 3 repetitions, the shoots on the rows located 1 m from the edge of the plot.
The shoots number ($shoots \cdot m^{-2}$) was determined by counting the shoots, in 3 repetitions, from 1 linear m of the rows located 1 m from the edge of the plot, then the obtained number was expressed to m^2.
Panicle length (cm) was determined by measuring the length of the panicle, from 1 linear m of the rows, then the obtained number was expressed to m^2.
Number of nodes per inflorescence was determined by counting the nodes on the inflorescence, from 1 linear m of the rows, then the obtained number was expressed to m^2.
Number of branches per inflorescence was determined by counting branches on inflorescence, from 1 linear m of the rows, then the obtained number was expressed to m^2.
Seed production ($kg \cdot ha^{-1}$) was determined by weighing the manually harvested seeds on each variant, then reporting to the area unit.
The agricultural year 2021-2022 was a very dry year. The amount of precipitation was 250.2 mm lower than the annual average (533.2 mm), affecting the growth and development of plants. In terms of temperatures it was a warm year, less favorable to the growth and development of smooth brome plants. The results were statistically interpreted by analyzing the variance and calculating the least significant differences and by analyzing the correlations between the study parameters.

RESULTS AND DISCUSSIONS

Seed quality is essential for the establishment of new crops. The size of the seeds, their weight, the content of reserve nutrients are the main elements of seed quality on which the development of future *Bromus inermis* Leyss. plants depends (Marco and De, 1990; Smart and Moser, 1999; Snider et al., 2016; Sousa et al., 2016).
Analyzing the influence of the interaction between row spacing and fertilization on the plants height at the smooth brome seed crop in the second year of vegetation (Table 1), it emerged that this indicator had values between 118 cm at the a_1b_2 variant (row spacing 25 cm, fertilized with $N_{50}P_{50}$) and 129 cm at a_2b_3 variant (row spacing 37.5 cm, fertilized with $N_{50}P_{50}K_{50}$). In general, by sowing at greater distances between rows, higher values of this indicator were obtained, plants with a higher height namely.
From the point of view of the effect of fertilization on smooth brome plants, the tendency was generally to increase the height of the plants, with the increase in the doses of mineral fertilizers.
Analyzing the influence of the interaction between row spacing and fertilization on the number of $shoots \cdot m^{-2}$ (generative shoots) (Table 1), it is found that they generated values between 415 $shoots \cdot m^{-2}$ at the a_3b_1 variant (row spacing of 37.5 cm, unfertilized) and 1272 $shoots \cdot m^{-2}$ at the a_1b_4 variant (row spacing of 25 cm, fertilized with $N_{75}P_{75}K_{75}$).
Regarding the generative number of $shoots \cdot m^{-2}$, for the variants where the sowing distance between rows was 25 cm, higher values were obtained than the sowing at greater distances.
By applying mineral fertilizers, the values for the number of $shoots \cdot m^{-2}$ had an increasing trend, and for the variants with maximum fertilization doses ($N_{100}P_{100}K_{100}$), the highest values were usually obtained.
Analyzing the influence of the interaction between row spacing and fertilization on the panicle length (Table 1.), It was found that they generated values between 17.3 cm at a_1b_5 variant (row spacing of 25 cm, fertilized with $N_{100}P_{100}K_{100}$) and 24.1 cm at a_3b_3 variant (row spacing of 50 cm, fertilized with $N_{50}P_{50}K_{50}$).

It was observed that the highest values at this indicator were obtained when sowing was carried out at a distance of 37.5 cm between rows or 50 cm between rows.
By applying mineral fertilizers the values had an increasing trend and stabilized when applying the $N_{50}P_{50}$ and $N_{50}P_{50}K_{50}$ doses, after which the values began to decrease.
In addition to correlations between study factors and analyzed parameters, correlations between study parameters such as the number of generative shoots and the length of the panicle were determined (Figure 1).

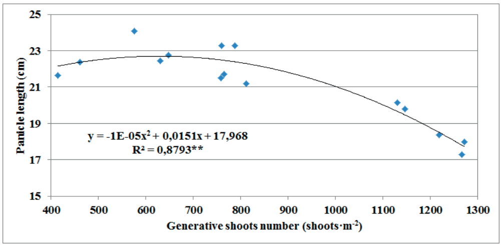

Figure 1. Correlation between generative shoots number and panicle length

The greater the distance between the rows, the smaller the number of shoots·m^{-2} and the longer the panicle was. When the shoots reached the range of 600-800 shoots·m^{-2}, the length of the panicle tended to stabilize, then decrease.
At a small number of shoots·m^{-2} the length of the panicle was higher, but they can be uneven and with few seeds per panicle, so the production can be less.
Analyzing the influence of the interaction between row spacing and fertilization on the number of nodes per inflorescence (Table 1), it was found that they generated values between 8.1 at the a_1b_5 variant (row spacing of 25 cm, fertilized with $N_{100}P_{100}K_{100}$) and 9.0 at the a_2b_3 variant (row spacing of 37.5 cm, fertilized with $N_{50}P_{50}K_{50}$) and a_2b_5 variant (row spacing of 37.5 cm, fertilized with $N_{100}P_{100}K_{100}$).
At this indicator, the highest values were obtained by sowing at a distance of 37.5 cm between rows and the administration of $N_{50}P_{50}$ mineral fertilizers.
Following the research, the positive correlation between the length of the panicle and the number of nodes per inflorescence was also revealed (Figure 2).

Analyzing the influence of the interaction between row spacing and fertilization on the number of branches per inflorescence (Table 1), it was found that they generated values between 25.8 at a_1b_5 variant (row spacing of 25 cm, fertilized with $N_{100}P_{100}K_{100}$) and 32.1 at a_1b_5 variant (row spacing of 50 cm, fertilized with $N_{50}P_{50}$).

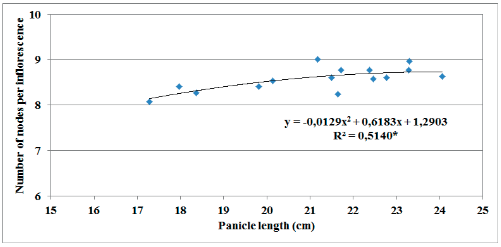

Figure 2. Correlation between panicle length and number of nodes per inflorescence

By applying $N_{50}P_{50}K_{50}$ mineral fertilizers doses, the number of branches per inflorescence had an increasing trend and stabilized, after which the values started to decrease. By sowing at 50 cm between rows the highest values were obtained.
The correlation between the length of the panicle and the number of branches per inflorescence was determined (Figure 3.). It was seen that the number of branches per inflorescence obtained almost constant values at all the factors studied, but by applying the established technologies at the longer sowing distance (37.5 cm between rows), the values were slightly higher.

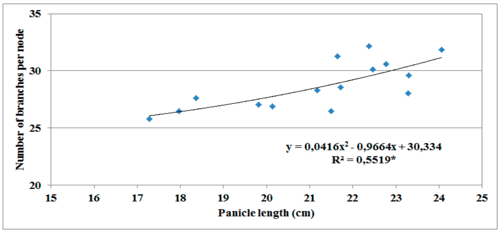

Figure 3. Correlation between panicle length and number of branches per node

The smooth brome plants that had the longer panicle generated a higher number of branches per inflorescence, and at the size of the panicle between 20-23 cm (the length of the panicle) the number of branches per inflorescence was higher and resulted in higher seed production.

Analyzing the influence of the interaction between row spacing and fertilization on seed production at the smooth brome seed crop in the second year of vegetation (Table 1), it was found that they generated values between 276 $kg \cdot ha^{-1}$ at the a_3b_2 variant (row spacing of 50 cm, fertilized with $N_{50}P_{50}$) and 437 $kg \cdot ha^{-1}$ at the a_1b_2 variant (row spacing of 25 cm, fertilized with $N_{50}P_{50}$).

By sowing at shorter distances between rows, 25 cm, higher seed yields were obtained than sowing at distances of 37.5 cm and 50 cm between rows.

By applying mineral fertilizers, the values had an increasing trend, the variants fertilized with $N_{75}P_{75}K_{75}$ achieving the highest values on average.

The correlation between the number of $shoots \cdot m^{-2}$ (generative shoots) and seed production (Figure 4), in the second year of vegetation, revealed that the higher number of generative shoots resulted in higher seed production.

In Figure 5 there are presented aspects of the experimental field (vegetation starting in the second year of exploitation of the smooth brome seeds production crop).

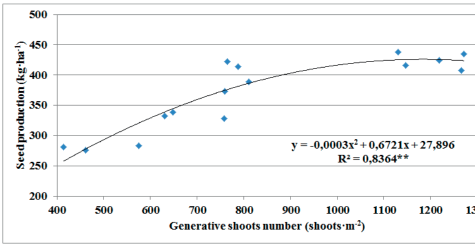

Figure 4. Correlation between generative shoots number and seed production

Figure 5. Aspects of the experimental field, in the second year of vegetation

Table1. Influence of the distance between rows and fertilization on some morphoproductive indicators of *Bromus inermis* Leyss. species

Variant		Plants height (cm)	Generative shoots number ($shoots \cdot m^{-2}$)	Panicle length (cm)	Number of nodes per inflorescence	Number of branches per inflorescence	Seed production ($kg \cdot ha^{-1}$)
a_1 - 25 cm (C)	b_1 - unfertilized (C)	116^C	1147^C	19.8^C	8.4^C	27.0^C	416^C
	b_2 - $N_{50}P_{50}$	118	1131	20.1	8.5	26.9	437
	b_3 - $N_{50}P_{50}K_{50}$	119	1219	18.4	8.3	27.6	424
	b_4 - $N_{75}P_{75}K_{75}$	122*	1272**	18.0	8.4	26.4	435
	b_5 - $N_{100}P_{100}K_{100}$	121*	1267**	17.3	8.1	25.8°	408
a_2 - 37,5 cm	b_1 - unfertilized	125***	$758^{\circ\circ\circ}$	21.5	8.6	26.4	$328^{\circ\circ\circ}$
	b_2 - $N_{50}P_{50}$	124**	$760^{\circ\circ\circ}$	23.3**	8.8	28.0	$373^{\circ\circ}$
	b_3 - $N_{50}P_{50}K_{50}$	129***	$788^{\circ\circ\circ}$	23.3**	9.0**	29.6***	413
	b_4 - $N_{75}P_{75}K_{75}$	127***	$765^{\circ\circ\circ}$	21.7	8.8	28.5*	422
	b_5 - $N_{100}P_{100}K_{100}$	127***	$812^{\circ\circ\circ}$	21.2	9.0**	28.3*	388
a_3 - 50 cm	b_1 - unfertilized	122**	$415^{\circ\circ\circ}$	21.6	8.2	31.3***	$281^{\circ\circ\circ}$
	b_2 - $N_{50}P_{50}$	122*	$461^{\circ\circ\circ}$	22.4*	8.8	32.1***	$276^{\circ\circ\circ}$
	b_3 - $N_{50}P_{50}K_{50}$	124**	$576^{\circ\circ\circ}$	24.1***	8.6	31.8***	$283^{\circ\circ\circ}$
	b_4 - $N_{75}P_{75}K_{75}$	125***	$631^{\circ\circ\circ}$	22.5*	8.6	30.1***	$332^{\circ\circ\circ}$
	b_5 - $N_{100}P_{100}K_{100}$	122**	$648^{\circ\circ\circ}$	22.8*	8.6	30.6***	$339^{\circ\circ\circ}$
	LSD 5%	*5*	*75*	*2.4*	*0.5*	*1.2*	*30*
	LSD 1%	*7*	*101*	*3.2*	*0.6*	*1.7*	*40*
	LSD 0.1%	*10*	*134*	*4.3*	*0.8*	*2.2*	*54*

C - Control variant.

CONCLUSIONS

From the analysis of the obtained results it was differentiated that each of the factors studied influenced the morpho-productive parameters analyzed, but in the climatic conditions specific to the agricultural year 2021-2022 the results obtained were influenced to a large extent by the small amount of precipitation.

By sowing at longer distances between rows and applying mineral fertilizers, shoots with a higher height were generated.

By sowing at a distance of 25 cm between rows and maximum fertilization with mineral fertilizers, $N_{100}P_{100}K_{100}$, the highest number of shoots/m^2 was obtained.

By applying mineral fertilizers the length of the panicle was higher, and the highest values at this indicator were obtained when the sowing had a distance of 37.5 cm between rows or 50 cm between rows.

By applying mineral fertilizers the number of nodes per inflorescence also had a general trend of growth, the highest values being recorded at the sowing distance at 37.5 cm between rows.

The application of mineral fertilizers and sowing at shorter row spacing had a positive effect on seed production, resulting in higher yields on these variants.

ACKNOWLEDGEMENTS

The research topic is part of the project No. 26/20.01.2021, Project domain (according to the RDI 2014-2020 Strategy of MADR): Grassland culture.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

YIELD COMPONENTS AND GRAIN YIELD OF TEN GENOTYPES OF WINTER WHEAT (*Triticum aestivum* L.) CULTIVATED UNDER CONDITIONS OF A.R.D.S. SECUIENI

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Abstract

Straw cereals, especially wheat (Triticum aestivum L.), are the most widely cultivated plant in the world, grown in over 100 countries, and are a prime commercial source. In this respect, the study was carried out in the growing season 2021-2022, in the agro technology conditions of Secuieni area, Neamț County, Romania, where 10 genotypes of winter wheat (Triticum aestivum L.) were studied, including 9 wheat varieties cultivars (Trublion, Centurion, Katarina, Glosa, Aspekt, Izvor, Avenue, Solehio, Alcantara) and 1 wheat hybrids variety (Hyxperia). We have used a sowing density of 500 germinable grains /sm, 360 germinable grains/sm and 250 germinable grains /sm. In this paper are presented data concerning grain yield related to the unit area (kg/ha) and yield components represented by the plant density after emergence (plants/ sm), plant density in spring (main shoots and tillers/ sm), head plant density at the harvest time (ears/ sm), the weight of the grains in the spikes (g/ ear), as well as main physical indices related to the quality, i.e. thousand kernels weight (TGW) and hectolitre mass (HLM).

Key words: *grains yields, HLM, sowing rate, TGW, wheat varieties.*

INTRODUCTION

World food production remains dominated by cereals, the demand for increasing global food security is one of the main objectives of breeding programs aimed at increasing yields associated with heterosis (hybrid vigor) in wheat (Whitford et al., 2013; Longin et al., 2012).
For high yield potential of wheat cultivars varieties, the number of harvestable ears should be between 600 and 700/sm, which can be accomplished by sowing 500-550 seeds/sm (Cvetkovic et al., 2016; Hristov et al., 2012; Cociu, 2014). Winter wheat (*Triticum aestivum*) is a rustic and drought-tolerance plant, with very good adaptability to different climatic and soil conditions, from south to north areas.
In Romania, the areas cultivated with wheat have undergone minor changes in recent decades. Thus, in 1938, 2.5 million ha of wheat were cultivated with wheat, and the areas were gradually reduced to 2.1 million ha during 1979-1981. In recent years, surface oscillations can be reported, around this value (2.1-2.2 million ha) (Roman et al., 2012).
The three main groups of grain classification according to protein content are: feed wheat (below 12.5%), baking wheat (12.5-14.5%) and durum wheat or premium wheat (>14.5%) (Tabără et al., 2008).
Wheat cultivation offers the following advantages:
- kernels has a high content of carbohydrates and proteins, corresponding to the requirements of the human body;
- kernels has a good shelf life over long periods of time;
- the grains are easily transported over long distances;
- wheat grains have different alternatives for capitalization;
- wheat grains are an important source of trade on the world market;
- wheat can be grown in different soil and climatic conditions, ensuring satisfactory yields wherever it is grown;
- cultivation technology is completely mechanized and well developed, without special problems;
- wheat is a very good precrop for most crops;

- according to the early wheat varieties, successive crops can be sown, especially if the varieties are irrigated (Ion, 2010).

The vegetation period of winter wheat lasts, in the conditions of our country, about 9 months (270-290 days). During this period, from germination to maturity, wheat plants go through certain phenological phases (stages), which are recognized by changes in the external appearance of plants and which are accompanied by internal changes in plant biology. Usually, it is difficult to strictly delimit these phases, because they partially overlap, or run in parallel (Roman et al., 2011).

It is generally accepted to divide the vegetation period of wheat plants into the following phenological phases: germination (emergence); rooting; twinning; straw formation (elongation); sprouting - flowering - fertilization; grain formation and ripening.

In turn, the presented phases are grouped in the vegetative stage (period), characterized by the development of the vegetative organs of plants (from germination to twinning) and the generative (reproductive) stage, characterized by the development of inflorescence, flowers and berry formation (from beginning of straw elongation and until full ripening) (Axinte et al., 2006). Practically, choosing the good agricultural practices, especially related to the soil management, is a key factor in granting food, clean water, feed, energy, safe climate, diverse ecosystem services and biodiversity for future generations (Mușat et al., 2021).

The yields obtained, by applying organic system, show that differentiated results can be obtained, the choice of the working variant in relation to the crop plant being decisive (Guș et al., 2004).

In last years, organic system of winter wheat has gained more and more ground. Organic wheat is one of the best listed and most sought-after organic products on the world market.

For organic system, wheat varieties grown in conventional agriculture are also used.

Beneficial effects of organic farming at farm level:

- Restoring the natural balance of water and nutrients and infesting weeds, diseases, insects and other pests.
- The restoration of natural balances is achieved, both by using classical technological measures (fertilization, soil work, etc.) and by using ecological measures (rotation, associated and intercropping crops, agroforestry curtains, hedges, grassy strips, etc.), soil improvement measures (green manure, mulching, conservation work, etc.) and plant protection (preventive, biological, biotechnical methods, etc.).
- Sustainable growth of soil has the healthiest methods and means of restoring and preserving soil fertility by stimulating the activity of soil microorganisms and the use of compost, green manure and long rotations with perennials and annuals with rich and / or deep root system.
- Decreasing soil erosion. The reduction of soil erosion is achieved as a result of soil improvement (increasing the organic matter content and improving the structure) and its better coverage (mulching, protection crops, etc.).
- Better water conservation in the soil. High soil organic matter content leads to better water retention and conservation in the soil, which has the effect of reducing irrigation needs (Roman et al., 2011).
- Respecting the intrinsic needs of animals regarding food, shelter, movement (http://madr.ro/docs/dezvoltare-rurala/rndr/buletine-tematice/PT4.pdf-page 11).

In the case of *organic system* soil fertility must be maintained as follows:

- cultivation or tillage practices maintain or increase soil organic matter, improve soil stability and biodiversity, and prevent soil compaction and erosion;
- practicing multiannual crop rotation, including legume crops and green manures;
- the application of fertilizers of animal origin or organic matter, both preferably composted, from organic production;
- use of biodynamic preparations.

The sources of organic agriculture are represented by the three currents that have emerged in Europe. The first is the one that appeared in Germany in 1924 under the impetus of Rudolf Steiner, with the name of biodynamic agriculture. The second current, published in Britain in 1940, was based on the theory developed by Sir Albert Howard and Lady Eve Balfour under the name of organic agriculture. Last but not least, the third current, called organ-biological agriculture, was developed in Switzerland by Hans Peter Rush and H. Müller.

In the 1940s, in Switzerland, Hans Peter Rush and Müller H. emphasized the autarky of producers and the interest of short market circuits. These ideas have resulted in a method that the authors have called organic farming that focuses on renewable resources to ensure food security for the population. Organic farming is defined as a productive system that avoids the use of synthetic fertilizers, pesticides, plant growth regulators, feed additives in animal husbandry. Technological elements are allowed and practiced various sowing processes, use of plant resources after harvest, manure, legumes, green manure, mechanical cultivation, use of rock dust - a mineral source for maintaining high fertility, biological and physical control pests, diseases and weeds. The fundamental aims of this model of organic farming are: - long-term maintenance of soil fertility, - avoidance of all forms of pollution that can be caused by agricultural techniques, - production of sufficient quantities of food of high nutritional quality, - minimization of use fossil energy - non-recoverable energy in agricultural practice, (Toncea et al., 2012).

It has begun to take on a clearer outlook since the last decade in our country as well. Agriculture has been "ecological" since its inception, but in recent years the application of systematic vision and modern technologies to agriculture has been sought. *Organic farming* promotes the cultivation of the land through those means that ensure a balance between agroecosystems and the environment (generating "specific agro climaxes") (Puia & Soran, 1981).

It is based on the use of those means and six methods offered by society, by the scientific and technical achievements that ensure the obtaining of large, constant and high-quality productions, in the conditions of environmental protection. *Organic farming* is in fact becoming synonymous with the agriculture of the coming years, which ensures the integrity of the biosphere, maximizing the production capacity of agroecosystems and obtaining good quality products (Ionescu et al., 1978).

It will require more conscientious and imaginative work and will ensure an abundance of food while reducing fossil energy consumption, maintaining or increasing the natural fertility of soils, improving man's living environment and protecting the environment as a whole. Organic farming, agriculture that is being born now for the future, is and must be thought of on an ever-widening, efficient and generous scale, ensuring the prosperity of society and nature on all the meridians of the globe. The structure of the new curricula and analytical programs in higher agronomic education must respond to the guidelines on the development of agriculture on ecological principles and in our country. For this reason, agricultural scientific research in our country must act on a systemic basis, both in the field of creating varieties (hybrids) of plants and animal breeds, and in improving the technologies of plant cultivation and animal husbandry, non-polluting, the protection of flora and fauna, the preservation of ecological balances and the protection of the environment (Toncea et al., 2012).

The characteristics to be followed in the choice of a wheat variety are its adaptability to the pedoclimatic conditions of the area, the increased tolerance to the specific pathogenic pressure and the efficient use of nitrogen, in order to maximize yields (Roman et al., 2009).

Taking in consideration these aspects, the objective of the present paper is to put into evidence the effects of different winter wheat sowed in different sowing rate in location A.R.D.S. Secuieni, from Romania.

MATERIALS AND METHODS

The experimental results were obtained in the pedo-climatic conditions from A.R.D.S. Secuieni, Neamț County, Romania in the 2021-2022 growing season.

The study focused on observations and comparative determinations on some genotypes of winter wheat, local and foreign origin (cultivars and hybrids), as follows: 9 wheat varieties cultivars (Trublion, Centurion, Katarina, Glosa, Aspekt, Izvor, Avenue, Solehio, Alcantara) and 1 wheat hybrids variety (Hyxperia). The experimental variants were placed according to the randomized block method, in three replications. The harvest surface of each plot was 10 m^2. Sowing was done at the optimum time, using a sowing density of 500 germinating seeds/sm, 360 germinating seeds/sm and 250 germinating grains/sm, at a distance between rows of 12.5 cm and the sowing depth of 4-5 cm. The agrotechnical management performed in the field was in organic farming condition used bio products. Results was based on variance analysis, according to the poly-factorial method of field setting (Săulescu & Săulescu 1967).

The main purpose of the research was to find out the influence of genotypes on grain yield related to the unit area (kg/ha) and yield components, such as the plant density after emergence (plants/sm), plant density in spring (main shoots and tillers/sm), plant density at the harvest time (ears/sm), the weight of the grains in the spikes (grams/ear), as well as the main physical index, i.e. thousand grains weight (TGW) and hectoliter mass (HML).

Researches were carried out in field experiments at the Agricultural and Development Research Station Secuieni (ADRS Secuieni) located in North Romania (Neamț County) in the years 2021-2022 under organic farming conditions (Figure 1).

The researches were performed on a soil of cambic chernozem type.

The soil has a medium nitrogen supply (20.7 ppm $N\text{-}NO_3$); well supplied with phosphorus (74.8 ppm, P_{AL}); poor potassium supply (142.6 ppm, K_2O); well supplied with calcium and magnesium (1.6 meq/100 g soil); humus - 2.44% and pH (in water) = 5.55.

Experimental design

The experiment was based on the method of subdivided plots into 3 replications, with the following factors:

- Factor A - variety, with 10 graduations:
  - a_1 = Trublion;
  - a_2 = Centurion;
  - a_3 = Katarina;
  - a_4 = Glosa;
  - a_5 = Aspekt;
  - a_6 = Izvor;
  - a_7 = Avenue;
  - a_8 = Solehio;
  - a_9 = Alcantara;
  - a_{10} = Hyxperia.
- Factor B - plant density, with 3 graduations:
  - b_1 = 250 germinable kernels/sm;
  - b_2 = 360 germinable kernels/sm;
  - b_3 = 500 germinable kernels/sm.
- Factor C - locations, with 1 graduation:
  - c_1 = Secuieni.

Crop management

The previous crop was peas.

Seed treatment was with Bordeaux mixture in a concentration of 5%, spring fertilizer with manure. All studied variants were sown on 28th of October.

For disease and insect control were apply two times the product Ortimag - 100 ml/100 l water, insecto-fungicide homologated for organic agriculture (fist application on 10 April and second application on 25 May) .

The productivity elements were evaluated at 10 plants chosen at random from each experimental variant.

The calculation and interpretation of the results was done based on the analysis of variance (Săulescu & Săulescu, 1967).

The percentage of protein contain in the wheat seeds was determined with the device Nir Noise Instruments Quick Analyzer, Agri Check Plus model.

RESULTS AND DISCUSSIONS

From a climatic point of view (Table 1), in the winter months, the evolution of the thermal factor was favorable for winter crops. Although temperatures below the freezing point were set early (in October).

Figure 1. General view of research field in A.R.D.S. Secuieni

Table 1. Climatic conditions at Secuieni, Neamț County during the 2021-2022 growing season

Month	Temperature (^{0}C)		Rainfall (mm)	
	Average for 2021-2022	*Average**	*Average for 2021-2022*	*Average**
October	8.1	7.2	10.8	46.0
November	5.6	3.5	39.0	56.6
December	0.0	-1.5	5.4	40.6
January	-0.1	-3.9	4.6	18.2
February	2.7	-2.1	0.8	15.7
March	2.5	2.6	38.4	64.5
April	9.5	8.4	20.8	29.3
May	16.9	15.6	40.1	85.0
June	20.8	18.9	56.6	85.0
Average (Oct-Jun)	7.3	5.4	24.1	49.0

*average for 15 years

The monthly average temperatures of the 2021-2022 growing season were higher than the normal average in the area, according to the climatological norm. High temperatures were registered starting with October (0.9ºC) and continuing with November (2.1ºC), January (3.8ºC) and February (4.8ºC). The coldest month of the year was January, when it was -0.1ºC, with 3.8ºC higher than the normal average. Thus, we can say that wheat crops benefited from a mild winter from a thermal point of view. Starting from March, the monthly average temperatures were higher or at the same level with the normal average, in March (+0.1ºC), April (+1.1 ºC), May (+1.3ºC) and June (+1.9ºC). May had an unfavorable influence on crop growth, given to the temperature and rainfall influence.

The rainfall amount during the vegetation period (1 October 2021 - 30 June 2022) was 179.4 mm, i.e. 176.7 mm, under the normal amount in the area (356.1 mm). Lower rainfall was recorded in the months of October (10.8 mm of rainfall, 35.2 mm less than the normal amount in the area), December (5.4 mm, 35.2 mm less than normal amount in the area), January (4.6 mm, 13.6 mm less than normal amount in the area), March (38.4 mm, 26.1 mm less compared to the normal amount in the area) and June (56.6.0 mm, 28.4 mm less compared to the normal amount in the area). Concerning the climatic conditions, in the 2021-2022 growing season, was not favorable for cereals crops due to the lack of water from (practically from late October to June we had drought conditions.

The sowing densities has complied the technological norms for each genotype (varieties and hybrid). Thus, the obtained cultivars densities after emergence were considered very well. It ranged from 128 plants/sm at Avenue variety, in sowing rate 250 germinable seeds to 355 plants/sm at Izvor variety, in sowing rate 500 germinable seeds/sm (Table 2).

Concerning the determination of the density, (number of plants includes the main shoots and tillers/mp), they were performed when the vegetation resumed. This, for the cultivars obtained by breeding, the average densities were between 232 main shoots and tillers/sm for Aspekt variety in sowing rate This, for the cultivars obtained by breeding, the average densities were between 232 main shoots and tillers/sm for Aspekt variety in sowing rate 250 seeds/sm) and 646 main shoots and tillers/sm for the Trublion variety (in sowing rate 250 seeds/sm).

The final element of the plant density expression of the 10 winter wheat varieties is the number of ears they formed per unit area (sm). The fertile tillers capacity is specific to each genotype studied, led to a mean number of harvestable ears between 408 ears/sm in Avenue variety (in sowing rate 360 seeds/sm) to 768 ears/sm in Hyxperia hybrid wheat (in sowing rate 360 seeds/sm) the rainfall for all the vegetation period

Table 2. Influence of the genotype on the evolution plant density from emergence to the harvest

No.	Wheat varieties	Sowing rate (density g.k./sm)	Sprouting density (plants/sm)	%	Main shoots and tillers (sm)	%	Harvestable ears (sm)	%
1	Trublion	250	175	107	646	155	464	89
		360	209	96	377	77	664	112
		500	303	103	303	103	632	103
2	Centurion	250	181	111	399	96	501	96
		360	237	109	498	101	472	80
		500	272	93	272	93	592	97
3	Katarina	250	159	97	460	113	440	84
		360	249	114	549	113	552	93
		500	315	107	315	107	608	100
4	Glosa	250	179	109	375	90	628	120
		360	207	95	331	67	708	120
		500	320	109	320	109	680	111
5	Aspekt	250	133	81	253	61	612	117
		360	176	81	352	72	608	103
		500	232	79	232	79	648	106
6	Izvor	250	183	112	292	70	544	104
		360	265	122	637	130	648	109
		500	355	121	355	121	636	104
7	Avenue	250	128	78	422	101	508	97
		360	203	93	507	103	408	69
		500	261	89	261	89	536	88
8	Solehio	250	155	95	340	82	548	105
		360	227	104	612	124	584	99
		500	304	104	304	104	544	89
9	Alcantara	250	181	111	598	144	528	101
		360	205	94	575	117	508	86
		500	303	103	303	103	608	100
10	Hyxperia	250	164	100	377	91	448	86
		360	200	92	480	98	768	130
		500	269	92	269	92	624	102
Averages		**Varieties average (250 g.k./sm)**	163.8	100	416.2	100	522.1	100
		Varieties average (360 g.k./sm)	217.8	100	491.8	100	592	100
		Varieties average (500 g.k./sm)	293.4	100	293.4	100	610.8	100

This one variety formed the largest number of fertile tillers per plant.

Tillering capacity is another favorable factor that influences the grain yield. The genotypes tested in the experiment, highlight the special value of the cultivars tested, but also the clear superiority of the data obtained in the winter wheat hybrids, in terms of tillering capacity (Table 3).

The final element of the plant density expression of the 10 winter wheat varieties is the number of ears they formed per unit area. The fertile tillers capacity is specific to each genotype studied, led to a mean number of harvestable ears, fertile tillers plants between 1.2 ears/sm for Glosa variety, in sowing rate 360 g.k./sm and Aspekt variety in sowing rate 500 g.k./sm to 3.2 fertile tiller/sm in Alcantara variety, in sowing rate 250 g.k./sm. This one variety formed the largest number of fertile tillers/plant.

Tillering capacity is another favorable factor that influences the grain yield. The genotypes tested in the experiment, highlight the special value of the cultivars tested, in terms of tillering capacity and in grains weight of ears/sm. The higher quantity in terms of grains ear it was for Centurion variety, 1.7 grams/ear in sowing rate 250 g.k./sm.

Table 3. Influence of the genotype on tillering capacity and the grain weight ear

No.	Wheat varieties	Sowing rate (density g.k./sm)	Spring density (tillers plants/sm)	%	Harvest density (fertile tillers plants)	%	Grains weight ears/sm (g)	%
1	Trublion	250	3.7	145.1	2.8	123.3	1.3	95.7
		360	1.8	80.0	1.5	78.9	1.5	108.7
		500	2.0	86.6	2.0	101.5	1.1	100.9
2	Centurion	250	2.2	86.3	2.0	88.1	1.4	104.3
		360	2.1	93.3	1.7	89.5	1.7	123.7
		500	1.8	77.9	1.4	71.1	1.4	130.9
3	Katarina	250	2.9	113.7	2.5	110.1	1.4	102.9
		360	2.2	97.8	2.0	105.3	1.3	96.7
		500	2.4	103.9	2.1	106.6	1.0	89.1
4	Glosa	250	2.1	82.4	1.7	74.9	1.0	69.6
		360	1.6	71.1	1.2	63.2	1.1	84.0
		500	2.1	90.9	2.0	101.5	1.3	117.3
5	Aspekt	250	1.9	74.5	1.8	79.3	1.5	109.4
		360	2.0	88.9	1.5	78.9	1.1	81.0
		500	1.4	60.6	1.2	60.9	1.0	88.2
6	Izvor	250	1.6	62.7	1.5	66.1	1.4	102.2
		360	2.4	106.7	2.2	115.8	1.3	96.7
		500	2.2	95.2	1.8	91.4	1.2	109.1
7	Avenue	250	3.3	129.4	2.9	127.8	1.5	105.1
		360	2.5	111.1	2.1	110.5	1.4	105.7
		500	2.5	108.2	1.9	96.4	1.1	101.8
8	Solehio	250	2.2	86.3	2.1	92.5	1.7	120.3
		360	2.7	120.0	2.2	115.8	1.3	93.7
		500	2.4	103.9	1.6	81.2	1.2	108.2
9	Alcantara	250	3.3	129.4	3.1	136.6	1.3	92.8
		360	2.8	124.4	2.5	131.6	1.3	99.0
		500	3.0	129.9	2.8	142.1	1.1	100.0
10	Hyxperia	250	2.3	90.2	2.3	101.3	1.4	97.8
		360	2.4	106.7	1.9	100.0	1.5	110.9
		500	3.3	142.9	2.9	147.2	1.0	89.1
Averages		**Varieties average (250 g.k./sm)**	2.6	100.0	2.3	100.0	1.4	100.0
		Varieties average (360 g.k./sm)	2.3	100.0	1.9	100.0	1.3	100.0
		Varieties average (500 g.k./sm)	2.3	100.0	2.0	100.0	1.1	100.0

The lower weight per ear were for Katraina variety, in higher density, 500 g.k./sm, Aspekt, 500 g.k./sm and Hyxperia, also in 500 g.k./sm. (Table 3).

From the point of view of the productive potential of the 10 winter wheat genotypes studied, a great variability is observed (Table 4). This, in the case of varieties average, the grain yield was 6,746 kg/ha with values between 5,338 kg/ha for the Aspekt variety (in sowing rate 250 seeds/sm) and 7,878 kg/ha for the Izvor variety in sowing rate 500 seeds/sm.

Following the determinations concerning the main physical indicators that show the quality of wheat grains, respectively the thousand kernels weight (TGW) and the hectoliter mass (HLM) we noticed that there were no significant differences between the 10 varieties of winter wheat tested in the experiment (Table 5).

This, the TGW values in the case of cultivars were between 32.9 g of Katarina (500 seeds/sm) and 43.5 g of Centurion (360 seeds/ sm).

Table 4. Results of the grain yields (kg/ha) obtained at A.R.D.S. Secuieni

No.	Wheat varieties	Grain (density g.k./sm)	Grain yield (kg/ha)	%	Difference from CT* (kg/ha)	Significance
1	Trublion	250	6223.0	98.9	-66.8	o
		360	6675.0	98.7	-86.1	o
		500	6853.0	95.3	-334.9	oo
2	Centurion	250	6580.0	104.6	290.2	
		360	6887.0	101.9	125.9	
		500	7501.0	104.4	313.1	
3	Katarina	250	6964.0	110.7	674.2	
		360	7213.0	106.7	451.9	
		500	6960.0	96.8	-227.9	o
4	Glosa	250	6655.0	105.8	365.2	
		360	6959.0	102.9	197.9	
		500	7453.0	103.7	265.1	
5	Aspekt	250	5338.0	84.9	-951.8	ooo
		360	5600.0	82.8	-1161.1	ooo
		500	6330.0	88.1	-857.9	ooo
6	Izvor	250	6392.0	101.6	102.2	
		360	7409.0	109.6	647.9	
		500	7868.0	109.5	680.1	
7	Avenue	250	6365.0	101.2	75.2	
		360	6292.0	93.1	-469.1	oo
		500	6647.0	92.5	-540.9	oo
8	Solehio	250	5371.0	85.4	-918.8	ooo
		360	6869.0	101.6	107.9	
		500	7302.0	101.6	114.1	
9	Alcantara	250	6828.0	108.6	538.2	
		360	6714.0	99.3	-47.1	o
		500	7468.0	103.9	280.1	
10	Hyxperia	250	6182.0	98.3	-107.8	oo
		360	6993.0	103.4	231.9	
		500	7497.0	104.3	309.1	
Averages		**Varieties average (250 g.k./sm)**	6289.8	100.0		
		LSD% 5=416.5	***LSD 1%=564.69***		***LDS 0.1%=756.82***	
		Varieties average (360 g.k./sm)	6761.1	100.0		
		LSD% 5=414.02	***LSD 1%=626.95***		***LDS 0.1%=1007.17***	
		Varieties average (500 g.k./sm)	7187.9	100.0		
		LSD% 5=309.71	***LSD 1%=424.74***		***LDS 0.1%=578.12***	

**CT - average of the field*

Figure 2. Centurion variety in sowing rate 250 seeds/sm

Figure 3. Centurion variety in sowing rate 500 seeds/sm

Table 5. Influence of the genotype on physical quality indices TGW and HLM

No.	Wheat varieties	Grain (density g.k./sm)	Thousand Kernel Weight (g)	%	HLM (kg/hL)	%
1	Trublion	250	35.7	92.8	77.4	95.8
		360	34.7	92.1	77.4	96.1
		500	35.7	94.5	77.8	96.6
2	Centurion	250	41.9	108.9	80.0	99.0
		360	43.5	115.4	80.2	99.6
		500	43.5	115.2	80.6	100.1
3	Katarina	250	38.1	99.0	83.1	102.8
		360	32.9	87.3	80.5	100.0
		500	32.9	87.1	80.4	99.9
4	Glosa	250	40.2	104.4	82.3	101.9
		360	39.4	104.6	82.2	102.1
		500	38.2	101.1	82.1	102.0
5	Aspekt	250	35.5	92.2	81.7	101.1
		360	34.3	91.0	81.5	101.2
		500	33	87.4	80.9	100.5
6	Izvor	250	38.2	99.2	83.5	103.3
		360	37.7	100.1	83.5	103.7
		500	38.2	101.1	83.6	103.9
7	Avenue	250	38.1	99.0	81.5	100.9
		360	38.4	101.9	81.4	101.1
		500	39.4	104.3	80.6	100.1
8	Solehio	250	38.5	100.0	80.3	99.4
		360	36.4	96.6	80.9	100.5
		500	39.2	103.8	81.5	101.2
9	Alcantara	250	38.3	99.5	80.2	99.3
		360	38.6	102.4	81.0	100.6
		500	38.7	102.5	79.8	99.1
10	Hyxperia	250	40.4	105.0	77.9	96.4
		360	40.9	108.5	78.9	98.0
		500	38.9	103.0	78.1	97.0
Averages	**Varieties average (250 g.k./sm)**		**38.49**	100.0	80.8	100.0
	Varieties average (360 g.k./sm)		**37.68**	100.0	80.5	100.0
	Varieties average (500 g.k./sm)		**37.77**	100.0	80.5	100.0

CONCLUSIONS

The correct choice of variety and cultivation technology according to the climatic conditions of the year are decisive factors in obtaining stable productions. Sowing density is an important technological sequence in increasing productivity and is determined according to the cultivation area and the phenotypic characteristics of the variety.

The morphological characters of wheat are not significantly influenced by the sowing density; they are influenced by the variety and to the greatest extent by the climatic conditions.

Productivity elements are influenced by seeding density, variety and experimental conditions.

The Katarina variety achieves high and stable yields (6964 kg/ha in lower sowing rate 250 seeds/sm), with a TGW of 38.1% and HLM 83.1 kg/hl.

The Hyxperia hybrid wheat obtained the highest yields (6,993 kg/ha in sowing rate 360 seeds/sm) regardless of the climatic conditions of the year, proving stability and ecological plasticity, with a protein content of 13.5%.

The Avenue variety is the earliest variety tested, with high production potential but lower protein content (11.3%).For the northern part of the country in the year 2021-2022, good results were obtained when 500 seeds/sm were used, at which density the reduction in productivity was compensated by the increase in the number of seeds/sm, the production obtained being 7868 kg/ha on average over the years of experimentation, with the variety Izvor.

The year 2021-2022 is relevant when, due to the climatic conditions, wheat had a very poor rainfall, in average 49 mm during all the vegetation period (October-June) with a major influence on wheat production.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

MODELS OF QUANTITATIVE ASSESSMENT OF THE INFLUENCE OF ELEMENTS OF TECHNOLOGY ON SEED YIELD OF PARENTAL COMPONENTS OF MAIZE HYBRIDS UNDER IRRIGATION CONDITIONS

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Abstract

The results of research on establishing the correlation-regression dependence of the assimilation surface area of plant leaves of inbred lines-parental components of corn hybrids of different FAO groups and seed yield depending on the density of plants and treatment with the drug Retengo® are highlighted. The research is based on a comparative assessment of three parent lines of different FAO groups: DK247 (FAO 290), DK205710 (FAO 380), DK445 (FAO 420). The effectiveness of the application of the fungicide with growth-stimulating action Retengo® at different plant densities was determined. The yield calculation showed that the realization of the seed yield potential for each inbred line depends on the genotype, in accordance with the established individual parameters of the optimum leaf surface and phytocenosis density in the crop. The use of the fungicide with growth-stimulating action Retengo® strengthens the correlation of the area of leaves of plants of lines and the yield of seeds.

***Key words**: maize, lines - parental components, plant density, fungicide, leaf area, seed yield.*

INTRODUCTION

In recent decades, the yield of corn hybrids has increased significantly due to the use of new genotypes and innovative agricultural technologies (Assefa et al., 2018; Kaminskiy & Asanishvili, 2020).

The creation and introduction into production of innovative corn hybrids of various FAO groups, characterized by a high effect of heterosis and high potential yield, depends on the genetic potential of the parent lines included in the pedigree of these hybrids. An important issue is increasing the seed production of parental components of promising corn hybrids for their accelerated introduction into production. However, the realization of the genetic potential of parental components is carried out to the full extent only under conditions of application of optimal measures of agricultural technology (Abelmasov & Bebeh, 2018).

The formation of the crop depends on the intensity of photosynthesis, since the processes of accumulation of organic matter are closely related to the activity of the leaves and their ability to accumulate solar light energy. According to the modern theoretical understanding of the functioning mechanisms and interrelationships of the donor-acceptor system in a plant, it is possible to ensure an intensive production process by modifying the morpho-physiological features of the culture, forming a powerful photosynthesizing surface, and extending the life of the photosynthetic apparatus. The intensity of biomass accumulation is determined by the optimal content and ratio of photosynthesis pigments (carotene, xanthophyll, pheophytin a, pheophytin b, chlorophyll a, chlorophyll b), which depend not only on the direction of photosynthesis, but also on the speed and nature of plant metabolism (anatomical and morphological changes) (Milas et al., 2018; Odabas et al., 2013).

The formation of the corn grain mainly occurs due to the photosynthesis of the upper leaves. Higher productivity is ensured by hybrids in which the leaves of the middle and lower tiers intensively use the weakened insolation, and the upper ones are better adapted to the intensive supply of PAR. The maximum size of the leaf area is reached on the 70th day after the emergence of seedlings, which coincides with the phase of "ejection - flowering of the panicle". After that, there is a gradual decrease in the area of the leaf surface and reaches zero on the 130th day of vegetation (Su et al., 2018). The increase in leaf surface occurs unevenly during the growing season and is largely determined by the amount of nutrients in the soil, including trace elements (copper, zinc, etc.) (Yunusov et al., 2020).

The loss of 25% of leaves by plants at all stages of development, except for the period of "dropping the panicle - milk ripeness", leads to a decrease in the yield of corn grain by 10% (Boedhram et al., 2001).

Inbred lines - parent components of corn hybrids, as a product of long-term forced self-pollination, are more demanding on growing conditions, differ in increased sensitivity to the influence of adverse factors, and have a smaller plant habit compared to hybrids. The genotypic features of the line affect the phenotypic manifestation of traits; therefore, it is necessary to take into account the biological features of the parental components and technological recommendations for growing hybridization plots. In this regard, scientific developments on the optimization of technological techniques for growing seeds of inbred lines of corn, parental components of promising hybrids, are becoming of urgent importance (Dziubetskyi et al., 2020).

Plant density plays an important role in the complex of agrotechnical measures of corn cultivation, which depend on the yield and its quality. A significant harvest can be obtained due to high individual productivity and the maximum permissible stem density in a specific growing area (Youngerman et al., 2018; Bahan, 2022).

The density of plants is one of the main factors that determines the efficiency of using fertility, temperature and water regimes of the soil, solar energy and other components of the vital activity of the agrocenosis. Optimizing the conditions for growing agricultural crops allows to increase the productivity of both a single plant and the crop as a whole (García-Martínez et al., 2020; Sarlangue et al., 2007; Liu Y. et al., 2021). Since the formed corn seed is a product of the photosynthetic activity of the plant, it is important to determine the optimal area of the assimilation surface of the leaves, which is the basis of photosynthesis. Leaf area is closely related to seeding density. Thus, it is possible to state that the density of plants in the field is the main condition that determines the area of plant leaves, according to existing growing conditions. The optimal density of the phytocenosis ensures the appropriate intensity of photosynthesis and, therefore, the final yield of one plant and the entire crop yield (Shapiro & Wortmann, 2006). On the other hand, in sowing, there is competition between plants for obtaining space, light, water, mineral nutrition and carbon dioxide, which affects the individual productivity of the plant and the sowing as a whole (Attia et al., 2021).

Considering the above, we can assert the importance of determining the optimal seeding density for each genotype. Plant density depends on growing conditions (duration of the vegetation period, sum of effective temperatures, availability of moisture, nutrients, etc.) and the genotype of the plant (Kharchenko et al, 2019; Ren et al., 2021; Sher et al., 2017).

The use of growth regulators is one of the promising directions in agricultural production. The use of regulators is especially important for crops of self-pollinated lines of corn, which, due to morphological and biological features, differ in low germination energy, weak initial growth, sensitivity to damage by pests, phytoinfections, etc. (Ivanyshyn, 2020; Vozhehova et al., 2022).

Fungicides have a positive effect on plants due to the reduction of fungal diseases of corn, such as *Ustilago zeae* (Beckm.), *Pseudomonas hoici Kendr.* and *Erwinia dissalvens* (Burkh.), *Helminthosporium turcicum* (Pass.). In addition, treatment with fungicidal drugs with a re-regulating effect increases the stress resistance of plants to adverse biotic and abiotic conditions (Vozhehova et al., 2021; Marchenko, 2019).

However, for new inbred lines-parental components of hybrids, there are no recommendations on the optimization of technology elements; therefore research is needed on the influence of plant density and re-regulating drugs on the area of the assimilation surface and the yield of parental lines under drip irrigation conditions.

MATERIALS AND METHODS

The purpose of the research was to determine the dynamics of the area of the assimilation surface of plant leaves and to record the yield of seeds of inbred lines-parental components of hybrids of corn of different FAO groups, depending on the density of plants and treatment with the drug Retengo®.

The response of corn hybrids to different cultivation conditions was studied at the Institute of Irrigated Agriculture, Kherson, Ukraine (46°44'33" N; 32°42'28" E; 50 m above sea level) (location A) during 2018-2020. A three-factor experiment (factor A - parent lines, B - treatment of plants with Retengo®, C - density of plants in sowing) was established by the method of randomized split blocks. The sown area of the plots was 50.0 m^2, the accounting area was 30.0 m^2. The research was carried out in quadruplicate.

The material for the research was the parental components of different maturity groups: DK445 (Mixed germplasm, FAO 420) - parent component of hybrids Arabat, Vira, Hileya; DK205710 (Iodent germplasm, FAO 380) - the parent component of the Kakhovsky hybrid; DK247 (Mixed germplasm, FAO 290) - parent component of hybrids Skadovs′kyy, Oleshkivs′kyy (the lines belong to the Institute of Grain Crops of the National Academy of Agrarian Sciences of Ukraine and were used in joint breeding programs with the Institute of Irrigated Agriculture of the National Academy of Agrarian Sciences of Ukraine). The plant density of the parent components was 60,000, 70,000, 80,000, 90,000, 100,000 plants ha^{-1}. The treatment of the parent components of corn was carried out with the drug Retengo® - a fungicide with a re-regulating effect from the AgCelence® group of drugs, which is included in the State Register of pesticides and agrochemicals approved for use in Ukraine. Plants were treated with Retengo® in the 7-8 leaf phase (BBCH 16-19).

The area of the leaf surface was determined by the linear method with subsequent calculation according to the formula: $S = k * l * n$, where S - leaf area, cm^2; k - average correction factor, is equal to 0,75; l - leaf length, cm; n - the width of the leaf at its widest point, cm.

Agricultural cultivation techniques and research methods are generally accepted for irrigation conditions in addition to the studied factors. Surface drip irrigation was used, the level of pre-irrigation soil moisture being 80% of the lowest moisture content in the 0–50 cm soil layer (Vozhehova et al., 2014).

Mathematical processing of research results was carried out by the method of dispersion analysis using the Agrostat computer program package (Ushkarenko et al., 2009)

RESULTS AND DISCUSSIONS

Currently, there are many models for predicting the yield of agricultural crops, in particular corn (Kharchenko et al., 2019).

Researchers believe that early-ripening hybrids of corn and low-growing plants better withstand the thickening of plants, and increase the yield on the sowing area. Later-ripening plants are better grown in sparse crops, where they simultaneously increase the biometric indicators of the plant and the yield of one plant. This response of hybrids to seeding density can be predicted using cluster analysis and cluster diagram (Palamarchuk et al., 2021).

For the optimal passage of the photosynthesis process, corn crops must have a certain area of the leaf surface, which acts as a means of accumulating plastic substances for the formation of the seed crop of parent components. In conditions of irrigation, the intensity of physiological processes of corn increases - the area and productivity of the leaf apparatus of plants increases (Marchenko et al., 2020).

During the determination of the area of the leaf apparatus of corn in the section of the development phases, a positive dynamic of its increase until the flowering phase and a decrease of the indicator during the phase of milk ripeness of the grain were established. In the phase of 15 leaves (BBCH 37-39), a difference in the indicator in the cross section of the line is noted. Thus, the DK 445 line was leafier and provided an indicator value within the range of 0.344-0.395 m^2/plant, while the DK 247 line had a leaf area value from 0.262 to 0.316 m^2/plant (Table1).

Table 1. The area of the assimilation surface of one plant of lines–parental components of corn hybrids depending on the factors of the experiment (average for 2018-2020), m^2/plant

The line is the parent component (Factor A)	Drug treatment (Factor B)	Density, plants ha^{-1} (Factor C)					Average by factor	
							A	B
		60,000	70,000	80,000	90,000	100,000		
in the phase of 15 leaves (BBCH 37-39)								
DK 247 (FAO 290)	no processing, control	0.291	0.282	0.273	0.268	0.262	0.285	0.274
	Retengo®	0.316	0.305	0.291	0,289	0.287		0.296
DK 205710 (FAO 380)	no processing, control	0.343	0.332	0.322	0.321	0.315	0.329	0.319
	Retengo®	0.362	0.351	0.342	0.337	0.329		0.340
DK 445 (FAO 420)	no processing, control	0.373	0.362	0.353	0.351	0.344	0.362	0.349
	Retengo®	0.395	0.384	0.372	0.374	0.365		0.374
Average by factor C		**0.347**	**0.336**	**0.326**	**0.323**	**0.317**		
LSD_{05}		0.034	0.034	0.034	0.035	0.034		
in the flowering phase (BBCH 63-67)								
DK 247 (FAO 290)	no processing, control	0.382	0.371	0.362	0.351	0.319	0.376	0.357
	Retengo®	0.423	0.412	0.401	0.382	0.359		0.395
DK 205710 (FAO 380)	no processing, control	0.434	0.423	0.411	0.401	0.378	0.426	0.409
	Retengo®	0.472	0.461	0.452	0.427	0.402		0.443
DK 445 (FAO 420)	no processing, control	0.484	0.473	0.454	0.435	0.413	0.462	0.452
	Retengo®	0.505	0.495	0.471	0.453	0.432		0.471
Average by factor C		**0.450**	**0.439**	**0.425**	**0.408**	**0.384**		
LSD_{05}		0.040	0.041	0.037	0.034	0.037		
in the phase of milky seed maturity (BBCH 83-85)								
DK 247 (FAO 290)	no processing, control	0.341	0.330	0.323	0.311	0.282	0.337	0.317
	Retengo®	0.383	0.372	0.361	0.342	0.324		0.356
DK 205710 (FAO 380)	no processing, control	0.394	0.383	0.371	0.351	0.331	0.385	0.366
	Retengo®	0.431	0.422	0.403	0.392	0.367		0.403
DK 445 (FAO 420)	no processing, control	0.444	0.433	0.414	0.398	0.381	0.422	0.414
	Retengo®	0.465	0.454	0.432	0.404	0.395		0.430
Average by factor C		**0.410**	**0.399**	**0.384**	**0.366**	**0.347**		
LSD_{05}		0.041	0.041	0.036	0.033	0.038		
LSD_{05}		0.042	0.042	0.039	0.036	0.033		

Until the phase of 15 leaves (BBCH 37-39) the density of plants did not significantly affect the size of the leaf surface area of one plant, and after this period (in the second half of the growing season), a clear pattern of a decrease in the leaf surface of a plant with an increase in plant density was noted. Thus, thickening from 60,000 plants ha^{-1} to 100,000 plants ha^{-1} contributed to the reduction of leaf area: in the 15-leaf phase by 7.6-9.9%, and in the flowering phase by 14.2-17.3%. In the flowering phase (BBCH 63-67) the area of the corn assimilation apparatus reached its maximum. The genotype of the FAO group of parental component lines had the greatest influence on the indicators of the area of the assimilation surface. In the flowering phase, the largest plant leaf area was in the mid-late line DK 445 (FAO 420), and the average factor A (parental line) was 0.462 m^2/plant. A smaller area of the plant assimilation surface was formed by the mid-ripe line DK

205710 (FAO 380) - 0.426 m^2/plant, and the mid-early line DK247 - 0.376 m^2/plant.

In the phase of milk ripeness (BBCH 83-85) the area of the leaves of the corn line decreased because the growth processes stopped, the generative period began, during the origin of which nutrients are redistributed, the drying of the lower leaves was noted. Thus, in the phase of milk ripeness, the values were lower than in the flowering phase by 0.039-0.041 m^2/plant.

The genotype of the line influenced the leaf area of the parental components of the corn hybrids. The maximum value of this indicator, on average according to factor A, was 0.422 m^2/plant in the mid-late line DK 445, the minimum value was in the mid-early line DK247 (0.337 m^2/plant). Plant density also has a significant effect on the "plant leaf area" feature. The maximum assimilation surface area, on average over the years of research (0.347-0.450 m^2/plant), was shown by plants at a density of 60,000 plants ha^{-1}, and all parent lines, regardless of genotype, showed maximum leaf area at this density. The minimum indicators of the area of the assimilation surface of the leaves were observed at a density of 100,000 plants ha^{-1} - 0.317-0.384 m^2/plant.

Treatment of plants with the drug Retengo® increased the area of the assimilation surface by 0.016–0.039 m^2/plant or by 3.8–12.3%, compared to the untreated control.

According to Li et al. (2015) yield of corn grain and dry matter when growing corn hybrids under drip irrigation depended on plant density. The optimal seeding density is set $\leq$ 4.7 plants/m^2. With an increase in density to 8.3 plants/m^2, the relationship with the productivity index was not established, but the yield of grain and dry matter decreased. As the density increased to 10.7 or more plants/m^2, the productivity index decreased.

Using the Bayesian computational methods, Lacasa et al. (2021) significant relationships were established between the yield of agricultural crops and the density of sowing. Using linear regression to determine the response to changes in corn planting density, Assefa et al. (2018) reported that the share of the effect of plant density on corn yield ranged from 8.5 to 17%.

Sun et al. (2016), using the APSIM model, which takes into account the variability of weather factors, according to data from an 11-year field experiment, confirmed that plant density is one of the most important factors affecting corn yield. At the same time, the density of plants correlates with the optimal time of sowing.

In our studies, the seed yield varied depending on the line and the density of the cenosis. Thus, the medium-early DK 247 line showed the maximum seed yield at a density of 90,000 plants ha^{-1} and treatment with Retengo®– 4.94 t ha^{-1}. The medium-ripe line DK 205710 showed the maximum seed yield at a density of 80,000 plants ha^{-1} and treatment with Retengo®– 5.61 t ha^{-1}.

The highest seed yield was shown by the mid-late line DK 445, which is the parent form of the Arabat, Hileya, Vira hybrid at a density of 70,000 plants ha^{-1} and treatment in the 7–8 leaf phase with the re-regulating fungicide Retengo® - 7.08 t ha^{-1} (Figure 1).

Effective seed production of corn lines and hybrids involves accelerated reproduction of parental components and obtaining seeds with high sowing qualities. In order to improve the methods of selection for the productivity of corn lines under irrigation conditions, an important issue is to find out the specifics of the relationship between seed yield and the area of the assimilation surface of the plant. These two key indicators may have been antagonistic.

The area of the assimilation surface increases on thinned crops and, at the same time, seed yield may decrease. Therefore, correlation-regression modeling of the variation of these features was carried out. Analyzing the obtained data, it is possible to conclude that the high leaf surface area of the parent components does not always indicate a high seed yield.

A high negative correlation between the area of the assimilation surface and yield was found in the mid-early line-parental component DK 247 ($r = -0.771$). The use of the drug Retengo® weakened the negative correlation and, at a certain interval, the increase in the area of the leaf surface (from 0.372 to 0.381 m^2/plant) and productivity went in parallel (Figure 2).

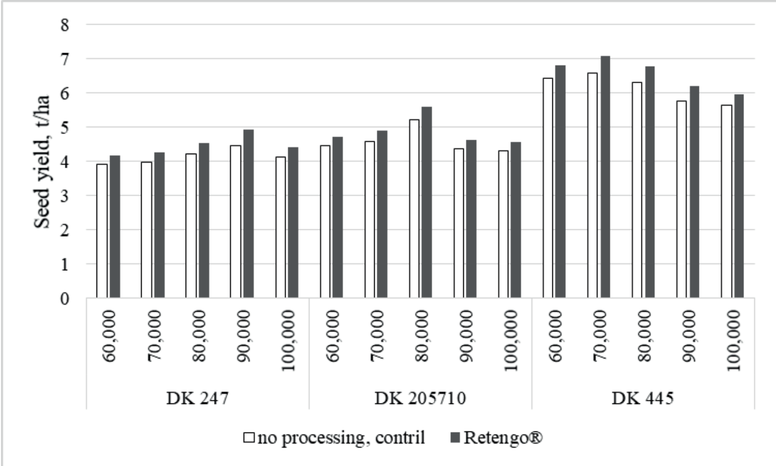

Figure 1. Seed yield of parent lines of corn hybrids depending on plant density and treatment with Retengo®, t ha^{-1} (average for 2018-2020)

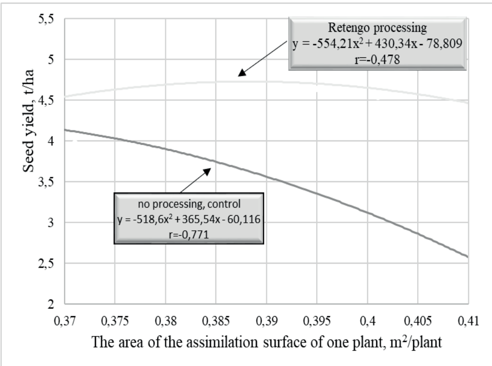

Figure 2. Correlation-regression model of the dependence of seed yield and the area of the assimilation surface of one plant of the mid-early line DK 247 (average for 2018-2020)

An increase in the assimilation surface over 0.38 m^2/plant was accompanied by a drop in productivity. It was found that without the use of Retengo®, an increase in the area of the assimilation surface of one plant leads to a significant drop in productivity.

In the mid-late line DK 445, the increase in the area of the assimilation surface was accompanied by an increase in yield (Figure 3). Such changes take place in parallel under the action of Retengo® and on the control version. However, the growth of the area of the assimilation surface is limited to an area of 0.473-0.495 m^2/plant, this level of plant leaf area corresponds to a density of 70,000 plants ha^{-1}.

Therefore, it is possible to conclude that the main factor for obtaining a high yield of quality seeds in the DK445 line is the density of 70,000 plants ha^{-1} and the use of Retengo®.

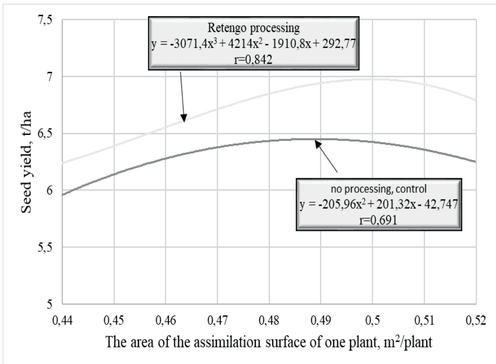

Figure 3. Correlation-regression model of the dependence of seed yield and the area of the assimilation surface of one plant of the mid-late line DK445 (average for 2018-2020)

The increase in productivity and the area of the assimilation surface in the lines of the parent components was due to a decrease in the incidence of fungal diseases of corn, such as *Ustilago zeae* (Beckm.), *Pseudomonas hoici* Kendr. and *Erwinia dissalvens* (Burkh.), *Helminthosporium turcicum* (Pass.). In addition, this drug has a re-regulating effect in the direction of increasing stress resistance to biotic and abiotic conditions.

CONCLUSIONS

It was established that in the flowering phase the area of the assimilation surface of the leaves of corn plants reached its maximum, its values ranged from 0.319 to 0.505 m^2/plant. Treatment of plants with the drug Retengo® increased the area of the assimilation surface by 0.016-0.039 m^2/plant or by 3.8-12.3%, compared to the untreated control.

An increase in plant density from 60,000 plants ha^{-1} to 100,000 plants ha^{-1} contributed to a decrease in leaf area: in the 15-leaf phase by 7.6-9.9%, and in the flowering phase by 14.2-17.3%.

The calculation of seed yield showed that the maximum values of the inbred mid-late line DK 445 (FAO 420) were formed on the variant at a density of 70,000 plants ha^{-1} and for treatment with the fungicide Retengo® – 7.08 t ha^{-1}. The mid-germination DK 247 line showed the maximum seed yield at a density of 90,000 plants ha^{-1} after treatment with the drug Retengo ® – 4.94 t ha^{-1}. The medium-ripe line DK 205710 showed the maximum seed yield at a density of 80,000plants ha^{-1} and application of Retengo® – 5.61 t ha^{-1}. The maximum values of seed productivity were formed by those variants in which the optimal area of the leaf apparatus of plants was observed.

Under the conditions of the optimal irrigation regime, parent lines of corn hybrids of different FAO groups form seeds with high sowing qualities at different plant densities – from 60,000 to 100,000 plants ha^{-1}. The density of the cenosis is of decisive importance for the formation of seed yield, and for each line-parental component there is an optimum density of plants. The correlation of seed yield and the area of the assimilation surface can be positive (r = 0.691 - 0.842) and negative (r = -0.478 - –0.771) depending on the norm of the reaction of the genotype, but this dependence is not linear and each line has its own optimum a combination of these indicators.

Treatment with the drug Retengo® changed the correlations of seed yield and the assimilation surface area of plants in the direction of increasing the positive dependence of these characteristics.

ACKNOWLEDGEMENTS

The research was performed in accordance with the State Research Program No 14 of the National Academy of Agrarian Sciences of Ukraine "Technologies for growing cereals. Maize and sorghum selection" under assignment 14.02.00.15.P "Improving the elements of technology for cultivating new intensive type hybrids and their parental forms under drip irrigation in the southern steppe of Ukraine" (State registration No 0119U000026).

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

APPLICATION OF HERBICIDES FOR WEED CONTROL BEFORE GERMINATION AND IN THE EARLY VEGETATION IN MAIZE

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Abstract

In 2020 and 2021, a field trial with the maize hybrid P 9241 in the experimental field of the Agricultural University - Plovdiv, Bulgaria was conducted. The herbicidal products Stomp Aqua - 4.00 l ha^{-1} and Gardoprim Plus Gold - 3.50 l ha^{-1} applied to soil, as well as Adengo - 0.44 l ha^{-1} and Camix - 2.50 l ha^{-1} applied in the 1st - 2nd leaf stage of maize were evaluated. The efficacy of the studied products by the 10-score visual scale of EWRS was reported. Selectivity was also assessed using the 9-score scale of EWRS. The highest herbicidal efficacy against Amaranthus retroflexus L., Xanthium strumarium L., Abutilon theophrasti Medik., Solanum nigrum L., Sorghum halepense (L.) Pers. developed from seeds was reported after the application of Adengo. Of the studied herbicides, the highest control against Chenopodium album L. was reported for Camix. The biological yields of maize, as well as the structural elements of the maize yield were the highest after the application of Adengo and Camix applied in 1st - 2nd leaf stage of the crop.

Key words: *maize, herbicides, weeds, efficacy, yield's structural elements.*

INTRODUCTION

The growth of the world's population is insufficiently providing it with a sufficient amount of food products. This is possible through the implementation of output technologies, methods, and means leading to the sustainable production of agricultural products (Georgiev et al., 2019; Shopova & Cholakov, 2015; Shopova & Cholakov, 2014; Calkins & Swanson, 1995).

Maize (*Zea mays* L.) is one of the most widely spread and important cereal crops in the world (Ram et al., 2017). Its production is used in three directions - for grain, for green fodder, and for animal silage (Iken & Amusa, 2004). *Zea mays* L. is characterized by very good adaptability and high productive potential (Aldrich et al., 1975).

Weeds are the main limiting factor in maize cultivation, leading to a decrease in the yield and quality of the produced product (Tonev et al., 2019; Saleem et al., 2015; Arnold et al., 2013). Weeds have been found to consume a significant proportion of soil-applied fertilizers (Mundra et al., 2002).

Depending on the species composition and weed density, as well as the duration of the competitive relationship between maize and weeds, the grain yield of *Zea mays* L. can be reduced from 18% to 96.7% (Gharde et al., 2018; Dimitrova et al., 2018; Ehsas et al., 2016; Jagadish & Prashant, 2016; Kakade et al., 2016; Yakadri et al., 2015; Dimitrova et al., 2014a; Dimitrova et al., 2014b; Mukherjee & Puspajit, 2013; Jat et al., 2012; Oerke & Dehne, 2004; Khan et al., 2003; Zhalnov & Raikov, 1996).

Crop yield reduction, apart from the development of weeds in the crop, can also be caused by nutrient deficiency (Ivanov et al., 2019; Manolov & Neshev, 2017; Neshev & Manolov, 2016; Manolov et al., 2015; Neshev & Manolov, 2014; Neshev et al., 2014; Goranovska et al., 2014).

Depending on the latitude and agroecological conditions, maize may be infested by different types of weeds. In Bulgaria, the most common weeds in *Zea mays* L. are *Amaranthus retroflexus* L., *Datura stramonium* L., *Xanthium strumarium* L., *Solanum nigrum* L., *Chenopodium album*, *Abutilon theophrasti* L., *Sinapis arvensis* L., *Echinochloa crus gali* L., *Setaria glauca* L., *Sorghum halepense* L., *Convolvulus arvensis* L, *Cinodon dactilon* L., and *Cirsium arvense* L. (Mitkov et al., 2019; Hristova et al., 2012; Kalinova et al., 2012; Mitkov et al., 2009).

The weed association of maize fields in Kahramanmaras, Turkey is composed of

Amaranthus retroflexus L., *Convolvulus arvensis* L., *Solanum nigrum* L., *Chenopodium album* L., *Sorghum halepense* (L.) Pers., *Xanthium strumarium* L., *Cyperus rotundus* L., *Datura stramonium* L., *Portulaca oleracea* L., *Tribulus terrestris* L., and *Anagallis arvensis* L. (Tursun et al., 2016).

In Greece, *Amaranthus* spp. is most commonly found in maize crops (Vizantinopoulos & Katranis, 1998).

In Fundulea, Romania, maize is infested mainly by *Setaria viridis*, *Echinochloa crus-galli*, *Sorghum halepense*, *Chenopodium album*, *Xanthium strumarium*, and *Cirsium arvense* (Şerban et al., 2022).

Demjanová et al. (2009) and Týr & Vereš (2012) found that in Slovakia maize mainly infested by *Chenopodium album*, *Amaranthus* spp., *Echinichloa crus galli*, *Datura stramonium*, *Fallopia convolvulus*, *Persicaria* spp., *Convolvulus arvensis*, *Cirsium arvense*, *Elytrigia repens*, *Avena fatua*, and *Abutilon theophrasti*. According to Smatana et al. (2015), the dominant weeds in the crop were *Atriplex* spp. and *Setaria viridis*.

The most common weeds in Poznań, Poland are *Echinochloa crus-galli*, *Chenopodium album*, *Polygonum convolvulus*, *Polygonum aviculare*, *Geranium pusillum*, and *Viola arvensis* (Skrzypczak et al., 2011).

Weed infestation in the maize fields of India is presented by *Polygonum* spp., *Stellaria media, Stellaria aquatica, Oldelandia diffusa, Oldenlandia umbellate, Physalis minima, Solanum nigrum*. In the Belgaum district of Karnataka, India, the most common weeds are *Cynodon dactylon, Dinebra retroflexa, Echinochloa colonum, Eleusine indica, Cyperus rotundus, Parthenium hysterophorus, Commelina benghalensis, Portulaca oleracea, Cynotis cuculata, Phyllanthus niruri*, and *Amaranthus viridis* (Soren et al., 2018; Mukherjee & Puspajit, 2013; Haji et al., 2012).

Weed control in agricultural crops (Mitkov, 2021; Mitkov, 2014; Tonev et al., 2010; Tityanov et al., 2010; Tityanov et al., 2009a; Tityanov et al., 2009b) and particularly in maize, is most often accomplished by herbicidal application (Mitkov, 2022; Mitkov, 2020; Goranovska & Kalinova, 2018; Goranovska et al., 2017; Janak & James, 2016; Sevov et al., 2015; Umesha & Sridhara, 2015; Goranovska & Kalinova, 2014; Dimitrova et al., 2013b; Skrzypczak et al., 2011; Pannacci & Covarelli, 2009, Tonev et al., 2009b).

According to Dimitrova et al. (2013a) efficient control of grass and broadleaf weeds was achieved with soil application of Gardoprim plus gold 500 SK at a rate of 4.00 l ha^{-1}, Lumax 538 SK at a rate of 4.00 l ha^{-1}, and Wing at a rate of 4.00 l ha^{-1}.

For the control of *Abuthilon theophrasti* L. and *Solanum nigrum* L. Mitkov et al. (2018) recommended Merlin Duo at rates of 1.00 l ha^{-1} to 2.00 l ha^{-1} after sowing before crop emergence.

Very good efficacy against *Amaranthus retroflexus* L., *Setaria viridis* (L.) Beauv., *Sinapis arvensis* L., and *Solanum nigrum* L. was found after treatment with foramsulfuron at a rate of 20.3 g ai ha^{-1}. At a higher dose of 20 to 50 g ai ha^{-1}, the herbicide also provides very good control against *Abutilon theophrasti* Medik., *Chenopodium album* L. and *Echinochloa crus-galli* (L.) Beauv. (Pannacci, 2016).

Kalinova et al. (2000) found that Stomp 33 EK + Mistral 4 SK in rates of 3.00 l ha^{-1}+1.30 l ha^{-1} controlled *Sorghum halepense* L., *Convolvulus arvensis* L., *Echinochloa crus gali* L., *Chenopodium album* L., *Amaranthus retroflexus* L., and *Abutilon theophrasti* L. in *Zea mays* L.

If there is mixed weed infestation, Kierzek et al. (2012), recommend the soil application of s-metolachlor + terbuthylazine + mesotrione in a tank mixture and nicosulfuron with adjuvant Atpolan Bio 80 SL.

Many scientists have studied the biological efficacy of atrazine in combination with other herbicides in maize (Acharya et al., 2022; Bottcher et al., 2022; Burhanuddin Wiqar et al., 2022; Choudhary et al., 2022; Jha et al., 2022; Khanna et al., 2022; Pinsupa et al., 2022; Wasnik et al., 2022). Soil application of atrazine followed by vegetational treatment with tembotrione vegetative was found to successfully control the weeds in maize (Arunkumar et al., 2019). Bada et al. (2022) also reported efficient weed control by the system involving soil application of atrazine followed by foliar treatment with tembotrione or topramezone.

Very good efficacy against *Xanthium strumarium*, *Amaranthus retroflexus*, *Datura stramonium*, and *Chenopodium album* in maize was observed after application of tembotrione at 100 g ai ha^{-1} and tembotrione at 100 g ai ha^{-1} in combination rimsulfuron at 10 g ai ha^{-1}, nicosulfuron at 40 g ai ha^{-1} and foramsulfuron at 60 g ai ha^{-1} (Damalas et al., 2018).
The present study aims to study the application of herbicides for weed control before germination and in the early vegetation in maize.

MATERIALS AND METHODS

In 2020 and 2021, a field trial with the maize hybrid P 9241 (370 FAO) in the experimental field of the Agricultural University - Plovdiv, Bulgaria was conducted.
The variants of the experiment were: 1. Untreated control; 2. Stomp Aqua (455 g/l pendimethalin) - 4.00 l ha^{-1} (BBCH 00); 3. Gardoprim Plus Gold (312.5 g/l S-metolachlor + 197.5 g/l terbutylazine) - 3.50 l ha^{-1} (BBCH 00); 4. Adengo (225g/l isoxaflutole + 90 g/l thiencarbazone-methyl + 150 g/l tsiprosulfamid - antidote) - 0.44 l ha^{-1} (BBCH 11-12); 5. Camix (60 g/l mesotrione + 500 g/l S-metolachlor) - 2.50 l ha^{-1} (BBCH 11-12).
The trial was performed by the randomized block design in 4 replications (Dimova and Marinkov, 1999) with a size of the experimental plot of 28 m².
The herbicidal products Stomp Aqua - 4.00 l ha^{-1} and Gardoprim Plus Gold - 3.50 l ha^{-1} were applied to the soil. Adengo - 0.44 l ha^{-1} and Camix - 2.50 l ha^{-1} were applied in the 1st - 2nd leaf stage of maize. The treatment was carried out via electrical backpack sprayer SOLO (model 417), with a size of the working solution 300 l ha^{-1}.
During the two experimental years, maize was grown as a monoculture under non-irrigated conditions. An experimental field was fertilized with 25 kg/da N:P:K (15:15:15) followed by deep plowing. Before sowing the maize, disking was carried out at 15 cm and two cultivations at 8 cm of depth. Sowing was carried out in the optimal period of the crop at a row spacing of 70 cm, with a density of 65000 plants per hectare. Spring dressing with 25 kg/da NH_4NO_3 was also carried out.
The experimental area was naturally infested with *Chenopodium album* L., *Amaranthus retroflexus* L., *Xanthium strumarium* L., *Abutilon theophrasti* Medik., *Solanum nigrum* L., and *Sorghum halepense* (L.) Pers. developed from seeds and rhizomes.
The biological efficacy was reported on the 14th, 28th, and 56th day after the herbicide application. The efficacy against the weeds was evaluated by the 10-score visual scale of EWRS. The efficacy results were compared with the untreated control.
The selectivity of the studied herbicides was evaluated on the 7th, 14th, 28th, and 56th day after the treatments by the 9-score visual scale of EWRS (at score 1 - there is no damage on the crop, and at score 9 there is complete death of the crop).
The following indicators of maize were evaluated and analyzed: ear length (cm); a number of grains per ear; ear diameter (cm); absolute seed mass of 1000 air-dry seeds (g), hectolitre seed mass (kg), and maize grain seed yield (t ha^{-1}).
Duncan's method with the SPSS 19 program (Duncan, 1955) was used for the statistical processing of the obtained data. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSIONS

In 2020 and 2021, weed species belonging to only two biological groups were reported in the experimental area with maize. The species of the late-spring weeds were *Chenopodium album* L., *Amaranthus retroflexus* L., *Xanthium strumarium* L., *Abutilon theophrasti* Medik., and *Solanum nigrum* L. Species from the perennial group of weeds was *Sorghum halepense* (L.) Pers. developed from seeds and rhizomes.
On the 14th day after applying the herbicides, the highest efficacy against *Ch. album* averaged over the two years was registered with Camix - 2.50 l ha^{-1} (BBCH 11-12) - 100%. Approximately excellent efficacy in the control of this weed was also registered with Adengo - 0.44 l ha^{-1} (BBCH 11-12) and Gardoprim Plus Gold - 3.50 l ha^{-1} (BBCH 00) - 97.5%. During the first reporting date, on average for the period, the lowest herbicide efficacy was registered with Stomp Aqua at a

dose of 4.00 l ha^{-1}, applied after sowing before emergence - 87.5%.

On day 28 after the treatment with Camix, the efficacy against *Ch. album* was again the highest compared to the other herbicides (Table 1). Gardoprim Plus Gold at a rate of 3.50 l ha^{-1}, applied after sowing before germination of the crop showed a higher efficacy against the weed compared to Adengo at a rate of 0.44 l ha^{-1}. The efficacy of the two products on the 28th day after treatment on average for the two experimental years was 87.5% and 77.5%, respectively.

Average for the two years, on the 56th day, the highest control of *Ch. album* - 82.5% was registered for Camix - 2.50 l ha^{-1} (BBCH 11-12). Gardoprim Plus Gold - 3.50 l ha^{-1} (BBCH 00) controlled the wed on average of 75%. Similar efficiency was reported for Adengo - 0.44 l ha^{-1} (BBCH 11-12) - 72.5%. During the third reporting date, the lowest herbicidal efficacy against *Ch. album* - 60%, for Stomp Aqua at a rate of 4.00 l ha^{-1} was found. Higher control of *Ch. album* - 99%. Şerban et al. (2022) found high control of the weed after the application of Diniro (40 g/kg prosulfuron + 400 g/kg dicamba + 100 g/kg nicosulfuron) at a rate of 500 g ha^{-1} + Trend (adjuvant) in dose 0.25 l ha^{-1}, applied in the 4th - 6th leaf stage of maize.

Table 1. Efficacy of the studied herbicides against *Chenopodium album* L. (%)

Variants	2020			2021			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Stomp Aqua - 4.00 l ha^{-1} (BBCH 00)	90	75	65	85	70	55	87.5	72.5	60
3. Gardoprim Plus Gold - 3.50 l ha^{-1} (BBCH 00)	100	90	80	95	85	70	97.5	87.5	75
4. Adengo - 0.44 l ha^{-1} (BBCH 11-12)	100	80	75	95	75	70	97.5	77.5	72.5
5. Camix - 2.50 l ha^{-1} (BBCH 11-12)	100	95	85	100	90	80	100	92.5	82.5

Regarding the weed *Amaranthus retroflexus* L., on the 14th day after application of the herbicides, 100% efficacy of Camix - 2.50 l ha^{-1} (BBCH 11-12) and Gardoprim Plus Gold - 3.50 l ha^{-1} was recorded (BBCH 00). High efficiency was also reported for Adengo - 0.44 l ha^{-1} (BBCH 11-12) - 97.5%. On the first reporting date, the lowest efficacy against *A. retroflexus* after treatment with Stomp Aqua at a dose of 4.00 l ha^{-1} applied to the crop was observed (Table 2).

Table 2. Efficacy of the studied herbicides against *Amaranthus retroflexus* L. (%)

Variants	2020			2021			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Stomp Aqua - 4.00 l ha^{-1} (BBCH 00)	90	80	70	85	75	65	87.5	77.5	67.5
3. Gardoprim Plus Gold - 3.50 l ha^{-1} (BBCH 00)	100	95	90	100	90	80	100	92.5	85
4. Adengo - 0.44 l ha^{-1} (BBCH 11-12)	100	100	100	95	100	100	97.5	100	100
5. Camix - 2.50 l ha^{-1} (BBCH 11-12)	100	100	100	100	100	100	100	100	100

On the 28th day after treatment, the efficacy of Adengo against *A. retroflexus* reached 100%. Camix at a dose of 2.50 l ha^{-1} on the 28th day maintains excellent control against *A. retroflexus* - 100%. In both experimental years, the foliar application of the herbicides was more effective than the application of soil herbicides applied after sowing before the germination of the crop (Table 2). On the second reporting date, the lowest control against the weed was observed for Stomp Aqua at a rate of 4.00 l ha^{-1} - 77.5%.

On the 56th day, the results for the *A. retroflexus* control showed that soil-applied herbicides were less effective than early-vegetation-applied herbicides. The highest efficacy against the weed was from Camix and Adengo - 100%. One hundred percent control against *A. retroflexus* can also be achieved after alone application of nicosuffuron (Dobbels & Kapusta, 1993).

Of the soil-applied herbicides, higher efficacy against *A. retroflexus* was obtained with Gardoprim Plus Gold - 85%. The control against this weed after the application of Stomp Aqua was significantly lower - 67.5% (Table 2).Results related to the control of *Xanthium strumarium* L. showed distinct differences between herbicides applied after sowing before crop emergence and in the early vegetation of maize. On average for the two experimental years, on the 14th day after the application of Adengo and Camix, 100% percent efficacy was recorded. With Stomp Aqua and Gardoprim Plus Gold, the efficacy against *X. strumarium* L. is unsatisfactory and is only 7.5% (Table 3).

On the 28th day after treatment, excellent control of *X. strumarium* L. was observed for the treatment with Adengo. The evaluation of the efficacy of Camix showed a decrease from 100% to 87.5% on the second reporting date. The Stomp Aqua and Gardoprim Plus Gold variants showed no efficacy against *X. strumarium.*

On the 56th day after treatment with Adengo – 0.44 l ha^{-1} (BBCH 11-12) the control against *X. strumarium* L. was excellent again. Satisfactory efficacy - 75% against the weed after application of Camix was also observed. Zero efficacy against weed was registered with the soil-applied herbicides Stomp Aqua and Gardoprim Plus Gold (Table 3).

Table 3. Efficacy of the studied herbicides against *Xanthium strumarium* L. (%)

Variants	2020			2021			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Stomp Aqua - 4.00 l ha^{-1} (BBCH 00)	10	0	0	5	0	0	7.5	0	0
3. Gardoprim Plus Gold - 3.50 l ha^{-1} (BBCH 00)	10	0	0	5	0	0	7.5	0	0
4. Adengo - 0.44 l ha^{-1} (BBCH 11-12)	100	100	100	100	100	100	100	100	100
5. Camix - 2.50 l ha^{-1} (BBCH 11-12)	100	90	80	100	85	70	100	87.5	75

In contrast to *X. strumarium*, the studied herbicides showed higher efficacy against *Abutilon theophrasti* Medik. During the first reporting date, 100% control against *A. theophrasti* in the variants with Adengo at a dose of 0.44 l ha^{-1} (BBCH 11-12) and Camix at a dose of 2.50 l ha^{-1} (BBCH 11-12) was observed. In the variants with Gardoprim Plus Gold and Stomp Aqua the control of the weed was 92.5% and 87.5% respectively.

On the second reporting date, 100% control of *A. theophrasti* was again observed for variants 4 and 5 on average for the two years. In the variants with soil herbicides (3 and 2), good control of weeds was reported, respectively 87.5% and 82.5% (Table 4).

On the 56th day after treatment, the herbicides Adengo and Camix applied early in the growing season maintained 100% percent control of *A. theophrasti*. The efficacy of soil-applied Gardoprim Plus Gold and Stomp Aqua was lower, 77.5% and 72.5% respectively (Table 4).

Table 4. Efficacy of the studied herbicides against *Abutilon theophrasti* Medik., (%)

Variants	2020			2021			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Stomp Aqua - 4.00 l ha^{-1} (BBCH 00)	90	85	75	85	80	70	87.5	82.5	72.5
3. Gardoprim Plus Gold - 3.50 l ha^{-1} (BBCH 00)	95	90	80	90	85	75	92.5	87.5	77.5
4. Adengo - 0.44 l ha^{-1} (BBCH 11-12)	100	100	100	100	100	100	100	100	100
5. Camix - 2.50 l ha^{-1} (BBCH 11-12)	100	100	100	100	100	100	100	100	100

The control results of *Solanum nigrum* L. in the trial area show the following. On the 14th day after treatment with Adengo - 0.44 l ha^{-1} (BBCH 11-12) and Camix - 2.50 l ha^{-1} (BBCH 11-12) the weed was controlled 100%. Very good efficiency after the treatment with Stomp Aqua - 4.00 l ha^{-1} (BBCH 00) and Gardoprim Plus Gold – 3.50 l ha^{-1} (BBCH 00) was also reported - 92.5% and 97.5%, respectively (Table 5).

It is noteworthy that during the second reporting date, the excellent efficacy against *S. nigrum* of Adengo and Camix was the same. Stomp Aqua and Gardoprim Plus Gold also provided good control of the weed - 82.5% and 92.5%, respectively.

On the 56th day after treatment, average for the period 100% control of *S. nigrum* L. was observed only after the usage of Adengo. In second place in terms of effectiveness against the weed was Camix - an average of 92.5%. Of all the studied products, the lowest control of *S. nigrum* for Stomp Aqua - 67.5% was recorded (Table 5).

Table 5. Efficacy of the studied herbicides against *Solanum nigrum* L. (%)

Variants	2020			2021			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Stomp Aqua - 4.00 l ha^{-1} (BBCH 00)	95	85	70	90	80	65	92.5	82.5	67.5
3. Gardoprim Plus Gold - 3.50 l ha^{-1} (BBCH 00)	100	95	90	95	90	85	97.5	92.5	87.5
4. Adengo - 0.44 l ha^{-1} (BBCH 11-12)	100	100	100	100	100	100	100	100	100
5. Camix - 2.50 l ha^{-1} (BBCH 11-12)	100	100	95	100	100	90	100	100	92.5

All treatments showed high efficacy against *Sorghum halepense* (L.) Pers. developed from seeds on the 14th day after treatment - from 97.5% to 100%.

On the 28th day, 100% control of *S. halepense* developed from seeds only at Camix - 2.50 l ha^{-1} (BBCH 11-12) was reported. For the other variants, the efficiency varied from 92.5% to 97.5%. On the 56th day for Adengo - 0.44 l ha^{-1} (BBCH 11-12) excellent control against *S. halepense* developed from seeds was found. Similar high efficiency (97.5%) was also observed for Camix - 2.50 l ha^{-1} (BBCH 11-12) and Gardoprim Plus Gold - 3.50 l ha^{-1} (BBCH 00) (Table 6).

Table 6. Efficacy of the studied herbicides against *Sorghum halepense* (L.) Pers. developed from seeds (%)

Variants	2020			2021			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Stomp Aqua - 4.00 l ha^{-1} (BBCH 00)	100	95	90	95	90	85	97.5	92.5	87.5
3. Gardoprim Plus Gold - 3.50 l ha^{-1} (BBCH 00)	100	100	100	95	95	95	100	97.5	97.5
4. Adengo - 0.44 l ha^{-1} (BBCH 11-12)	100	100	100	95	95	100	97.5	97.5	100
5. Camix - 2.50 l ha^{-1} (BBCH 11-12)	100	100	100	100	100	95	100	100	97.5

The most difficult-to-control weed species in the study was *Sorghum halepense* (L.) Pers developed from rhizomes. The results for the herbicidal control of the weed are presented in Table 7. The soil-applied herbicides Stomp Aqua - 4.00 l ha^{-1} (BBCH 00) and Gardoprim Plus Gold - 3.50 l ha^{-1} (BBCH 00) showed no efficacy on the weed (0%). In the case of early vegetation herbicides, the efficacy is slightly higher but still insufficient.

Table 7. Efficacy of the studied herbicides against *Sorghum halepense* (L.) Pers. developed rhizomes (%)

Variants	2020			2021			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Stomp Aqua - 4.00 l ha^{-1} (BBCH 00)	0	0	0	0	0	0	0	0	0
3. Gardoprim Plus Gold - 3.50 l ha^{-1} (BBCH 00)	0	0	0	0	0	0	0	0	0
4. Adengo - 0.44 l ha^{-1} (BBCH 11-12)	30	20	15	25	20	10	27.5	20	12.5
5. Camix - 2.50 l ha^{-1} (BBCH 11-12)	15	10	0	10	0	0	12.5	0	0

On the 56th day, 12.5% efficacy only with Adengo - 0.44 l ha^{-1} (BBCH 11-12) was observed, which is practically unsatisfactory. In the remaining variants, the control was 0% (Table 7).

Satisfactory efficacy against *S. halepense* from rhizomes in maize was observed after the application of nicosulfuron (Eleftherohorinos and Kotoula-Syka, 1995).

During the two experimental years, the selectivity of the applied products to maize hybrid P 9241 was also studied. Under the conditions of the experiment and during the four reporting dates of the two years, no visible manifestations of phytotoxicity were found in all variants with herbicides - score 1 on the EWRS scale.

In addition to the biological efficacy and selectivity of the tested herbicides, the productivity of the maize hybrid P 9241 was also monitored during the experiment.

The comparative analysis of the ear length of maize, hybrid P 9241 showed that there are proven differences in all variants. It was statistically proven that the plants of treatment 4 (Adengo - 0.44 l ha^{-1} (BBCH 11-12) had the longest ears, with 20.43 cm in 2020 and 19.57 cm in 2021. It is also worth noting the variant with Camix - 2.50 l ha^{-1} (BBCH 11-12), where this indicator in 2020 is 19.36 cm and in 2021 - 18.42 cm. It was mathematically proven that of all variants, the shortest ear length in the untreated control was recorded, where in 2020 it was 11.22 cm, and in 2021 - 10.18 cm (Table 8).

Table 8. Maize ear length (cm)

Variants	2020	2021	Average
1.	11.22 e	10.18 e	10.70
2.	14.74 d	14.15 d	14.45
3.	18.23 c	17.50 c	17.87
4.	20.43 a	19.57 a	19.99
5.	19.36 b	18.42 b	18.89

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

Table 9. Number of seeds per maize ear

Variants	2020	2021	Average
1.	186.00 d	238.00 d	212.00
2.	394.00 c	322.00 c	358.00
3.	410.00 c	378.00 b	394.00
4.	608.00 a	532.00 a	570.00
5.	594.00 b	522.00 a	558.00

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

The results for the parameter number of seeds in a cob are presented in Table 9. The lowest number of seeds in a maize ear, on average for the period was recorded in the untreated control - 212.00. On average for the two experimental years, the highest number of seeds per ear after the application of Adengo - 0.44 l ha^{-1} (BBCH 11-12) - 570.00 was recorded (Table 9).

Regarding the ear diameter in 2020 and 2021, significant differences were recorded between the untreated control and all variants with herbicides. In 2020, the highest ear diameter (4.30 cm) was registered for Adengo - 0.44 l ha^{-1} and Gardoprim Plus Gold - 3.50 l ha^{-1}. With Camix - 2.50 l ha^{-1} and Stomp Aqua - 4.00 l ha^{-1}, the ear diameter was 4.20 cm. It is fair to note that there is no mathematically proven difference between these four treatments. The ear diameter was the lowest in the untreated control - 3.20 cm.

In 2021 the highest ear diameter after the application of Adengo - 0.44 l ha^{-1} (BBCH 11-12) (4.10 cm) was reported. In the remaining variants with herbicides, the ear diameter varied from 3.90 cm to 4.00 cm. There was no mathematically proven difference between variants 2, 3, 4, and 5. The maize ear diameter was the lowest in the untreated control (2.80 cm) in the second year as well (Table 10).

Table 10. Maize ear length (cm)

Variants	2020	2021	Average
1.	3.20 b	2.80 b	3.00
2.	4.20 a	4.00 a	4.10
3.	4.30 a	3.90 a	4.10
4.	4.30 a	4.10 a	4.20
5.	4.20 a	3.90 a	4.05

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

Absolute seed mass depends on the size and nutritional status of the seeds. On average for the two years, the highest results for this indicator for Adengo - 0.44 l ha^{-1} (BBCH 11-12) and Camix - 2.50 l ha^{-1} (BBCH 11-12) 279.15 g and 275.57 g, respectively were found. Both in 2020 and in 2021, there is no statistically proven difference between variants 4 and 5. A slightly lower absolute seed mass was registered in the variants with Stomp Aqua - 257.03 g and Gardoprim Plus Gold - 262.28 g. From all the treatments, the lowest values of the studied indicator for the untreated control were obtained, where in 2020 it was 244.33 g, and in 2021 - 223.50 g (Table 11). The significantly lower values of absolute seed mass in the untreated control compared to the herbicide variants were due to the high weed infestation. Bastegan et al. (2022) reported that weed development in sweet corn (*Zea mays* L. var. *saccharata*) resulted in a reduction of 1000 grain weight. Fang et al. (2022) found that successful weed control by mechanical weeding combined with low doses of herbicide led to an increase in the 1000-grain weight.

Table 11. Absolute seed mass of maize (g)

Variants	2020	2021	Average
1.	244.33 c	223.50 c	233.92
2.	261.40 b	252.65 b	257.03
3.	267.12 b	257.43 b	262.28
4.	288.10 a	270.19 a	279.15
5.	283.44 a	267.69 a	275.57

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

The hectoliter mass is determined by the size and protection of the grain, by the presence of impurities, including weeds, etc. (Dimitrova et al., 2006). The highest values of hectoliter mass on average for the two years after the application of Adengo - 0.44 l ha^{-1} (BBCH 11-12) and Camix - 2.50 l ha^{-1} (BBCH 11-12) were registered - respectively 75.50 kg and 75.25 kg. For this indicator as well, the untreated control has the lowest values - 63.25 kg on average for the experimental period (Table 12).

Table 12. Hectolitre seed mass (kg)

Variants	2020	2021	Average
1.	65.50 d	61.00 d	63.25
2.	72.00 b	70.00 b	71.00
3.	74.00 a	72.50 ab	73.25
4.	76.00 a	75.00 a	75.50
5.	75.50 a	75.00 a	75.25

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

Table 13 shows the maize yield from the present study. The obtained results showed that there is a positive correlation between the effect of herbicides against the weeds and the structural elements of the maize yield and the biological yields of maize.

As a result of the weed infestation, the lowest maize yield was recorded in the untreated control, where in 2020 it was 4.11 t ha^{-1} and in 2021 it was 2.93 t ha^{-1}. Studies under different agrometeorological conditions show that maize grain yield can be reduced to varying degrees depending on the type and density of weeds (Choudhary et al., 2022; Wiqar et al., 2022; Mitkov, 2020; Dimitrova et al., 2018; Tursun et al., 2016; Skrzypczak et al., 2011; Walia et al., 2005).

The highest yield for Adengo - 0.44 l ha^{-1} (BBCH 11-12), and in 2020 it was 9.12 t ha^{-1}, and in 2021 it was 8.48 t ha^{-1} was reported. The other variant with early vegetation treatment is close to this yield. With Camix in a dose of 2.50 l ha^{-1}, applied in the 1st - 2nd leaf of the crop in 2020, the reported yield was 8.85 t ha^{-1}, and in 2021, 8.35 t ha^{-1}.

Compared to the variants with early vegetation application, lower yields were recorded when applying the herbicides to the soil. On average for the two experimental years, for Gardoprim Plus Gold at a rate of 3.50 l ha^{-1} (BBCH 00), a yield of 7.22 t ha^{-1} was reported, and with Stomp Aqua at a rate of 4.00 l ha^{-1} (BBCH 00), a yield of 6.17 t ha^{-1} (Table 13).

Table 13. Maize grain seed yield (t ha^{-1})

Variants	2020	2021	Average
1.	4.11 e	2.93 d	3.52
2.	6.23 d	6.10 c	6.17
3.	7.42 c	7.02 b	7.22
4.	9.12 a	8.48 a	8.80
5.	8.85 b	8.35 a	8.60

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

CONCLUSIONS

Adengo at a rate of 0.44 l ha^{-1} applied in the 1st - 2nd leaf stage of maize on the 56th day after treatment provides 100% control against *Amaranthus retroflexus* L., *Xanthium strumarium* L., *Abutilon theophrasti* Medik., *Solanum nigrum* L., and *Sorghum halepense* (L.) Pers. developed from seeds. However, none of the herbicides studied provided effective control against *Sorghum halepense* (L.) Pers. developed rhizomes. The highest control against *Chenopodium album* L. – 82.5% on average for the two years of the study for Camix at a dose of 2.50 l ha^{-1} was reported.

The herbicides applied early in the vegetation were more effective against existing weeds than herbicides applied after sowing before the germination of maize.

Under the conditions of the experiment, no visible signs of phytotoxicity were found on maize, hybrid P 9241 after the application of pendimethalin; S-metolachlor + terbutylazine; isoxaflutole + thiencarbazone-methyl; mesotrione + S-metolachlor.

The maize ear length, the number of seeds per ear, the ear diameter, absolute seed mass, hectolitre seed mass, and maize grain seed yield were the highest for the variants with Adengo - 0.44 l ha^{-1} (BBCH 11-12) and Camix - 2.50 l ha^{-1} (BBCH 11-12).

ACKNOWLEDGEMENTS

The research was financially supported by Project 17-12 at the Center of Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University of Plovdiv, Bulgaria.

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MISCELLANEOUS

Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SOME SEEDS CHARACTERISTIC AND BIOMASS QUALITY OF SOME *Brassicaceae* AND *Fabaceae* SPECIES IN MOLDOVA

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Abstract

The goal of our research was to fulfil the potential of traditional, underutilized and less known plant species of the families Brassicaceae and Fabaceae, which grow in the experimental plots of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chisinau, Republic of Moldova. The seeds and biomass of these plant species have high potential for use as food, forage and raw material for circular economy, including bioenergy production. The determination of physical and mechanical properties of seeds and agricultural products is important in the design of harvesting, handling and processing equipment. Our research revealed that the characteristic dimensions of seeds of the studied Brassicaceae species varied in the following ranges ℓ: b: δ ≈ (1.98-12.60): (1.67-3.67): (1.63-2.05) mm; the angle of repose α=24.6°-30.6° and the flow angle on steel is α_1=15.8°-31.7°, on wood α_1=18.1°-37.3° and on enamelled surface α_1=15.3°-30.5°; M_{1000} = 4.30-9.73 g and bulk seed density 88.3-766.9 kg/m^3. The seeds characteristic of the studied Fabaceae species was ℓ: b: δ ≈ (1.53-8.00): (1.20-5.47): (1.47-5.01) mm; the angle of repose α=21.5°-33.0°, flow angle on steel is α_1=14.3°-27.7°, on wood α_1=14.7°-29.8° and on enamelled surface α_1=14.2°-27.3°; M_{1000} = 1.35-170.04 g and bulk seed density 370.8-830.0 kg/m^3. The harvested green mass from studied Brassicaceae species is characterized by 16.1-23.5%CP, 63.2-69.9% DMD, RFV=117-162, 10.21-11.20 MJ/kg ME, 5.18-6.76 MJ/kg NEl and from Fabaceae species 14.2-23.4% CP, 58.4-69.5% DMD, RFV=91-168, 9.51-11.15 MJ/kg ME, 5.53-7.17 MJ/kg NEl. The biochemical biomethane potential from vegetal substrates is 305-379 L/kg of organic matter.

Key words: *biomass quality, Brassicaceae species, Fabaceae species, methane potential, nutritive value, seed characteristic.*

INTRODUCTION

World plant biodiversity, its conservation and prospects for practical use are becoming an increasingly pressing problem in the 21st century (ECPGR,2021).

Current climate change can cause reduction in the abundance of food and fodder, crop productivity and quality indices. The increase of food and fodder production in the context of climate change and increasing prices for energy resources will be based on more rational use of traditional crops and identification of new species that will expand the range of crops. The diversification of crop production has to be achieved by mobilization, acclimatization and implementation of new and non-traditional plant species from local flora and other floristic regions.

In the context of the acute shortage of protein in the food of humans and animals, it is necessary to expand the areas where protein crops are cultivated by mobilizing new species that would extend the assortment of both: crops for the production of food for humans and fodder necessary for the development of the animal husbandry sector and poultry farming, as well as raw material for various industries. The species of the family *Fabaceae* and *Brassicaceae* are of particular interest.

The family *Brassicaceae* Burnett (syn. *Cruciferae* Juss.) includes 372 genera and

4.060 accepted species. Most of them are herbaceous annuals, biennials and perennials, warm season shrubs and trees; some are used as agricultural crops and ornamental plants. Most of the species occur in the temperate zone of the Northern Hemisphere (especially in the Mediterranean and Irano-Turanian region) and much less in the Southern Hemisphere. In the spontaneous flora of Bessarabia, the *Brassicaceae* family is currently represented by 48 genera with 97 species. Many *Brassicaceae* species are known as important agricultural and horticultural crops, which provide edible roots, leaves, stems, buds, flowers and seeds, some species are used as human food in many different forms, while others are also used as fodder, energy and cover crops (Jabeen, 2020).

The family *Fabaceae* Lindl. (syn. *Leguminosae* Juss.) is the third largest family among the angiosperms, consists of about 730 genera and about 20,000 species of trees, shrubs, vines, and herbs and is worldwide in distribution. In the spontaneous flora of the Bessarabia the family *Fabaceae* is represented by 146 species of 35 genera. The *Fabaceae* species are of great agro-ecologic importance due to their symbiotic relationship with nitrogen fixing bacteria, thus, they improve the physical properties of soil, form a large amount of organic raw material for circular economy and, besides, they are an important source of proteins, beneficial to human and animal nutrition. The benefits of leguminous forage plants within farming systems have long been recognized and include a higher production of meat, milk and wool by ruminants, improvement of soil fertility, root disease management in cropping systems, increasing biodiversity and risk management in diversified systems, particularly under the conditions of climate change. Many *Fabaceae* species have valuable medicinal properties and multi-purpose use in various industries; besides, they are excellent honey plants and cover crops (Stoddard, 2013; Stinner, 2015; Petcu et al., 2022).

Underutilized and less known *Fabaceae* and *Brassicaceae* species constitute potential source of food, fodder and raw material for circular economy, including bioenergy production.

The physical properties, such as length, width, thickness, bulk and true density, 1000 seed weight, surface area, and sphericity, are basic characteristic parameters for optimization and design of internal run roller plate seed metering structure. Information on physical properties can be valuable for plant breeders, engineers, scientists, and seed industries experts, machine designers, processing structures and controls; and to determining machine performance efficiency. Variation in seed properties caused by the genetic pattern, soils and environmental conditions, or handling may influence the processing, storage and utilization of seed. Physical, mechanical and chemical characteristics of seed materials are important in designing the equipment for seeding, harvest, transport, storage, processing, cleaning, hulling and milling (Togo et al., 2018).

The goal of our research was to evaluate some seed characteristics and green biomass quality of some *Brassicaceae* and *Fabaceae* species, as fodder for livestock, as well as substrate for the production of biomethane by anaerobic digestion.

MATERIALS AND METHODS

The *Brassicaceae* species: *Crambe cordifolia, Isatis tinctoria, Raphanus sativus* var. *oleifera, Sinapsis alba* and *Fabaceae* species: *Astragalus galegiformis, Galega orientalis, Glycine max, Lablab purpureus, Lotus corniculatus, Medicago sativa, Onobrychis arenaria, Onobrychis viciaefolia, Pisum arvense, Vicia sativa, Vicia tenuifolia, Vigna radiate, Vigna unguiculata,* which grow in the experimental plot of the National Botanical Garden (Institute) of Moldova, Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″, served as subjects of research.

Samples of seeds of the studied species were collected, the evaluation of the seed characteristics of each species were carried out based on the measurement of the following parameters: length, width, thickness, friability (angle of repose α, angle of friction α_1), mass of 1000 seeds, bulk seed density. The angle α was determined by two methods: a) *general*, measuring the height h of the cone with a depth calliper and the diameter of the base D in two perpendicular planes with the metal ruler KLB 300 x 19 x 0.5 mm; the angle α was calculated according to the formula $tg\alpha = 2h/D$; b) *local*, measuring the angle α by applying the digital inclinometer on the inclined surface of the cone. The flow angle α_1 of the seeds was measured using a table with the upper surface rotating vertically. On this surface, it is possible to attach plates made of different materials. We used plates of steel 10, wood and enamelled steel. The angle α_1 was measured using a digital inclinometer. The weight of 1000 seeds were determined by using an electronic balance with accuracy of 0.01 g. The measurement was replicated 5 times for 1000 seeds selected at random. For bulk density measurement, an empty cylindrical container of 1000 ml volume was filled with the seeds, then weighing the content, the bulk weight was then recorded. This was done in 10 replications.

The dry matter content in harvested green mass was detected by drying samples up to constant weight at 105°C. For biochemical analysis, green mass samples were dried in a forced air oven at 60°C, milled in a beater mill equipped with a sieve with diameter of openings of 1 mm, and some assessments of the main biochemical parameters: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL), total soluble sugars (TSS) have been made by near infrared spectroscopy (NIRS) technique using the equipment PERTEN DA 7200. The concentration of hemicellulose (HC), cellulose (Cel), digestible dry matter (DDM), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl) and relative feed value (RFV) were calculated according to standard procedures.

The carbon content of the substrates was determined using an empirical equation according to Badger et al. (1979). The biochemical methane potential was calculated according to the equations of Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

The size of the seeds is an important characteristic of a plant species. The results of the evaluation of the physical characteristics of the seeds of the studied *Brassicaceae* and *Fabaceae* species species are shown in Table 1. The obtained results have demonstrated the following values of the characteristic dimensions (length - ℓ, width - b, thickness - δ) for the studied *Brasicaceae* species seeds ℓ: b: δ ≈ (1.98-12.60): (1.67-3.67): (1.63-2.05) mm, the highest values were recorded by the species *Isatis tinctoria* and *Crambe cordifolia.* The dimensions of *Fabaceae* species seeds have the following values ℓ: b: δ ≈ (1.53-8.00): (1.20-5.47): (1.47-5.01). We noticed that the lowest seed sizes were of species *Lotus corniculatus* and *Medicago sativa,* but highest seeds dimensions of species *Vigna unguiculata* and *Glycine max.*

The flow capacity (friability) of seed material, angle of repose α and flow angle α_1 is an important practice in the design of warehouses, transport and conditioning facilities (different types of transport means, elevators, cleaning and drying devices etc.) (Ene & Mocanu, 2016).

The obtained values of the angle of repose α and angle of friction α_1 confirm the results of the dimensional and morphological analyses: the friability of *Brassicaceae species* seeds have the following values: angle of repose α = 24.6°-30.6° and angle of friction α_1 = 15.8°-31.7° - on steel, α_1 = 18.1°-37.3° - on wood, α_1 = 15.3°-30.5° - on enameled surface; *Fabaceae* species seeds - angle of repose α = 21.5°-33.0° and angle of friction α_1 = 14.3°-27.7° - on steel, α_1 = 14.7°-29.8° - on wood, α_1 = 14.2°-27.3° - on enameled surface.

The enamelled and steel surfaces, seeds have a better flow capacity than on those made of wood. Our study shows that in the *Brassicaceae* family, the *Crambe cordifolia* have the highest friability, the next level of friability, a little lower, were found at *Raphanus sativus* var. *oleifera* and *Sinapsis alba* seeds. We would like to mention that in studied Fabaceae species the *Vicia sativa, Vicia tenuifolia, Vigna radiate, Vigna unguiculata* have the highest friability and lowest friability showed *Galega orientalis, Medicago sativa, Onobrychis arenaria, Onobrychis viciaefolia.*

The weight of 1000 seeds (M_{1000}) is an important physical indicator of quality when establishing the sowing norm, but the bulk seed density is of practical interest and are necessary for designing the storage spaces, the organization of the transport, the customization of the equipment and the appreciation of some technological norms. The analysis of the mass parameters, weight of 1000 seeds and bulk seed density, of studied *Brassicaceae* and *Fabaceae* seeds (Table 2) demonstrates that their values correlate with the structure and geometric parameters. The values of the mass of 1000 seeds depending plant species. Among the researched *Brassicaceae* species, higher mass seeds values are found at *Raphanus sativus* var. *oleifera* and from *Fabaceae* species - *Pisum arvense, Glycine max, Vigna unguiculata.* The *Astragalus galegiformis, Lablab purpureus, Medicago sativa, Vicia tenuifolia, Vigna unguiculata* is distinguished by a seed bulk density that exceeds 800 kg/m^3. *Crambe cordifolia* and *Isatis tinctoria* seed siliques, *Onobrychis arenaria* and *Onobrychis viciifolia* seed pods have lowest bulk density.

Table 1. The results of the evaluation of the physical characteristics of the seeds of the studied species

Species	Dimensional parameters, mm			Angle of repose **α**, Methods			Flow angle **α₁**, Surfaces		
	ℓ	**b**	**δ**	general	local	average	steel	wood	Enamel
Brasicaceae									
Crambe cordifolia	5.33 ±0.16	4.47 ±0.28	4.15 ±0.30	25.1 ±0.3	24.0 ±1.6	24.6	15.8 ±0.2	18.1 ±0.9	15.3 ±0.6
Isatis tinctoria	12.60 ±0.70	3.67 ±0.24	1.63 ±0.19	28.0 ± 1.1	32.1 ± 1.5	30.0	31.7 ± 0.4	37.3 ± 0.9	30.5 ± 0.4
Raphanus sativus var. oleifera	3.50 ±0.24	2.43 ±0.21	2.05 ± 0.04	27.4 ±0.4	28.9 ±1.6	28.2	21.3 ±0.3	26.0 ±0.8	22.7 ±0.2
Sinapsis alba	1.98 ±0.11	1.67 ±0.03	-	25.3 ±0.5	29.3 ±1.3	27.3	22.0 ±0.6	27.4 ±1.5	24.7 ±0.3
Fabaceae									
Astragalus galegiformis	3.73 ±0.07	2.25 ±0.17	1.69 ±0.13	26.0 ±1.1	26.9 ±1.3	26.5	22.8 ±0.4	26.3 ± 0.5	-
Galega orientalis	3.56 ±0.4	1.88 ±0.22	1.44 ±0.07	32.5 ±0.9	33.4 ±0.8	33.0	27.7 ±0.3	29.8 ±0.8	27.3 ±0.4
Glycine max	7.07 ±0.25	5.4 0 ±0.27	5.20 ±0.25	27.0 ±0.6	25.5 ±0.7	26.2	15.2 ±1.1	16. ±0.9	14.7 ±0.3
Lablab purpureus	6.50 ±0.40	4.85 ±0.20	4.67 ±0.27	23.2 ±0.6	27.3 ±1.0	25.3	18.0 ±0.5	18.0 ±0.7	17.0 ±0.4
Lotus corniculatus	1.53 ±0.08	1.34 ±0.11	1.07 ±0.08	24.9 ±0.7	25.3 ±0.7	25.1	19.0 ±0.6	22.7 ±0.4	23.0 ±0.6
Medicago sativa	2.17 ±0.10	1.20 ±0.06	1.07 ±0.06	30.2 ±0.3	31.5 ±0.4	30.9	27.3 ±0.4	33.6 ±0.9	26.7 ±0.2
Onobrychis arenaria	5.62 ±0.3	3.96 ±0.21	2.33 ±0.15	28.4 ±0.7	31.8 ±1.3	30.1	23.0 ±0.1	29.0 ±0.7	22.0 ±0.1
Onobrychis viciaefolia	6.07 ±0.07	3.88 ±0.20	2.75 ± 0.18	28.4 ±0.7	31.8 ±1.3	30.1	23.0 ±0.1	29.0 ±0.7	22.0 ±0.1
Pisum arvense	5.80 ±0.20	5.05 ±0.07	5.01 ±0.03	25.9 ±0.6	26.9 ±0.4	26.4	20.5 ±1.5	21.3 ±1.6	19.0 ±1.1
Vicia sativa	4.65 ±0.33	3.73 ±0.40	_	21.7 ± 0.3	21.3 ±0.5	21.5	14.5 ±0.3	14.7 ±0.8	14.5 ±0.3
Vicia tenuifolia	3.65 ±0.25	3.16 ±0.04	3.05 ± 0.05	21.8 ±0.5	23.5 ±1.2	22.7	14.3 ±0.3	15.7 ±0.5	14.2 ±0.1
Vigna radiata	4.83 ±0.03	4.28 ±0,06	_	23.2 ±0.7	26.0 ±1.8	24.6	15.6 ±0.3	17.4 ±0.4	16.0 ±0.1
Vigna unguiculata	8.00 ±0.25	5.47 ±0.27	4.23 ±0.03	24.9 ±0.8	28.3 ±1.3	26.6	18.0 ±0.9	18.3 ±1.2	18.3 ±0.3

The results obtained in this work are included in the results of other researches. Prina (2009) remarked that *Crambe cordifolia* fruit diameter was 4.0-6.5 mm. Spataro & Negri (2008) mentioned that *Isatis tinctoria* siliques length were 9.5-14.3 mm and siliques wing 2.5-3.7 mm. Bojñnanský & Fargašová (2007), reported that seeds dimensions of *Astragalus galegiformis* was 3.7-3.9 mm length and 2.5-2.7 mm width. The authors Kibar & Öztürk (2008) and Chhabra & Kaur (2017) present the following dimensional parameters soybean hybrids and cultivars ℓ: b: δ ≈ (6.1-8.19): (4.5-7.12): (4.4-6.23) mm. Ene & Mocanu (2016) and Togo et al. (2018) reported that alfalfa seeds dimensions are given ℓ: b: δ ≈ (2.00-2.80): (1.00-1.70): (0.50-1.35) mm. Kilonzi et al. (2017) remarked that *Lablab purpureus* seeds is characterized by ℓ: b: δ ≈ (9.7-10.7): (6.80-7.40): (5.20-5.60) mm.

Table 2. The mass parameters of the studied *Brassicaceae* and *Fabaceae* seeds

Species	Bulk seed density, kg/m^3	Weight of 1000 seeds **M_{1000}**, g
Brasicaceae		
Crambe cordifolia	273.75±0.73	4.30±0.12
Isatis tinctoria	88.3±0.13	5.40±0.19
Raphanus sativus var. *oleifera*	738.02±1.33	9.73±0.33
Sinapsis alba	766.93±1.99	4.82±0.13
Fabaceae		
Astragalus galegiformis	802.92±3.13	10.11±0.38
Galega orientalis	784.23±3.77	6.27±0.05
Glycine max	719.29±2.44	147.2±0.44
Lablab purpureus	817.32±4.03	74.53±1.23
Lotus corniculatus	795.08±0.99	1.35±0.05
Medicago sativa	818.7±2.59	1.78 ±0.03
Onobrychis arenaria	370.8±2.76	14.63 ±0.12
Onobrychis viciaefolia	392.16±3.13	17.45±0.28
Pisum arvense	713.92±5.28	170.04±1.78
Vicia sativa	778.09±3.09	59.25±0.59
Vicia tenuifolia	824.7±2.04	21.14±0.35
Vigna radiata	790.06±3.26	59.00±0.35
Vigna unguiculata	830.00±2.76	124.07±1.85

Ene & Mocanu (2016) found that seeds dimensions of studied *Lotus corniculatus* crops was ℓ: b: δ ≈ (1.10-1.80): (0.80-1.60): (0.70-1.40) mm and *Onobrychis viciaefolia* seeds was ℓ: b: δ ≈ (5.00-8.20): (3.00-7.50): (2.20-4.50) mm. Taser et al. (2005) reported that length of the common vetch seeds ranged from 4.27 to 6.29 mm, the width ranged from 3.60 to 5.49 mm, while the thickness ranged from 3.15 to 4.63 mm. Davies (2011) reported that *Vigna unguiculata* seeds characteristic were ℓ: b: δ ≈ (9.87-15.21): (7.00-10.88): (5.49-8.47), M_{1000} = 253.80-671.4 g, α = 24.3-29.7°. N'Danikou et al. (2022) found that weight of 1000 seeds studied genotype of legumes crops was: 90-410 g for *Glycine max,* 21-75 g for *Vigna radiate* and 83-150 g for *Vigna unguiculata.*

The biochemical composition, nutritive and energy value of the harvested green mass from the tested species are presented in Table 3. Analysing the results of the biochemical composition of green mass, we found that the dry matter of the studied species differs essentially in the concentration of nutrients and energy. The harvested green mass from studied *Brassicaceae* species is characterized by 16.1-23.5% CP, 63.2-69.9% DMD, RFV = 117-162, 10.21-11.20 MJ/kg ME, 5.18-6.76 MJ/kg NEl and from *Fabaceae* species 14.2-23.4% CP, 58.4-69.5% DMD, RFV = 91-168, 9.51-11.15 MJ/kg ME, 5.53-7.17 MJ/kg NEl. We found that *Isatis tinctoria, Raphanus sativus* var. *oleifera, Sinapsis alba, Astragalus galegiformis, Galega orientalis, Vicia tenuifolia* green mass was characterised by very high content of crude protein, optimal cell wall fractions (NDF, ADF, ADL, hemicellulose and cellulose), nutritional value and energy supply of the feed as compared with traditional fodder plants *Lotus corniculatus, Medicago sativa, Onobrychis viciaefolia, Pisum arvense, Vicia sativa.*

The results of the estimation of fodder quality of green mass from studied *Brassicaceae* and *Fabaceae* species are given in the specialized literature. Thus, the *Crambe cordifolia* fodder has a content of 19.6% CP, 2.6% EE, 29.3% CF, 36.4% NFE, 17.9% TSS, 10.5-12.1% ash, 75.0% DOM (Medvedev & Smetannikova, 1981; Vergun et al., 2018); *Isatis tinctoria* fodder 20.2-24.9% CP, 3.0-4.5% EE, 12.5-32.4% CF, 30.7-48.5% NFE, 10.8-12.5% ash (Kshnikatkina et al., 2005; Țîței, 2016); *Raphanus sativus* var. *oleifera* fodder 15.0-25.9% CP, 2.0-.5% EE, 19.0-24.0% CF, 35.4-57.0% NFE, 14.7-23.6% ash (Medvedev & Smetannikova, 1981); the *Sinapsis alba* fodder contained 12.0-22.2% CP, 1.6-3.3% EE, 19.5- 34.0% CF, 9.1-15.8% ash, 36.4-43.3% NFE, 50.42% NDF, 43.84% ADF, 15.61% ADL, 6.58% HC, 28.23%Cel, 64.56% IVTD, RFV 101 (Medvedev & Smetannikova, 1981; Kshnikatkina et al., 2005; Kiliç et al., 2021); *Astragalus galegiformis* fodder 17.2-20.9% CP, 1.8-4.5% EE, 23.2-34.8 % CF, 45.6-48.5% NFE, 4.6-8.8% ash, 11.9 MJ/kg ME (Kshnikatkina et al., 2005; Chibis et al., 2011; Teleuţă et al., 2015; Bondarchuk, 2019); *Galega orientalis* fodder 16.18-22.84% CP, 2.95-3.90% EE, 24.58-36.85% CF, 47.50-57.90% NDF, 28.90-43.90% ADF, 3.70-6.30% lignin, 10.2 % sugar, 7.51-12.48% ash, 64-82% DMD, RFV = 97-133, 10.2 MJ/kg ME (Medvedev & Smetannikova, 1981; Kshnikatkina et al., 2005; Teleuţă et al., 2015; Teleuţă & Ţîţei, 2016; Coşman et al., 2017; Meripõld et al., 2017; Żarczyński et al., 2021); *Glycine max* fodder 15.3-30.1% CP, 1.1-4.4% EE, 31.2% CF, 45.3-66.3% NDF, 31.2-42.5% ADF, 5.8-8.1% lignin, 9.3% ash, 64.0-88.1% IVDMD, 17.5-18.1 MJ/kg GE, 11.6 MJ/kg DE and 9.2 MJ/kg ME (Heuzé et al., 2016b; Peiretti et al., 2018); *Lablab purpureus* fodder 18.4% CP, 2.6% EE, 28.2 % CF, 44.6% NDF, 32.0% ADF, 7.2% lignin, 8.9% sugar, 11.1% ash, 67% DOM, 18.2 MJ/kg GE, 11.7 MJ/kg DE and 9.2 MJ/kg ME (Heuzé et al., 2016c); *Lotus corniculatus* 16.4-21.1% CP, 3.3-4.1% EE, 25.7-35.7% CF, 48.3% NDF, 28.20% ADF, 9.9% lignin, 33.8% NFE, 4.0-6.2% sugar, 7.3-10.2% ash, 68.8% DOM, 9.8 MJ/kg ME (Medvedev & Smetannikova, 1981; Kshnikatkina et al., 2005; Heuzé et al., 2015; Teleuţă & Ţîţei, 2016; Coşman et al., 2020); *Medicago sativa* fodder 14.50-20.26% CP, 2.49-2.90% EE, 26.70-33.31 % CF, 37.20-39.41% NFE, 39.90-65.50% NDF, 30.90-55.70% ADF, 7.6% lignin, 7.02-11.50% ash, 68.50% % DOM, 18.1 MJ/kg GE, 11.9 MJ/kg DE and 9.4 MJ/kg ME (Medvedev & Smetannikova, 1981; Gryazeva, 2005; Kshnikatkina et al., 2005; Stavarache et al., 2015; Heuzé et al., 2016a; Teleuţă & Ţîţei, 2016); natural sand sainfoin fodder 16.7-20.6% CP, 2.3-4.2% EE, 21.5-30.2% CF, 46.70% NDF, 31.7% ADF, 4.8% lignin, 35.6-46.0% NFE, 10.0% sugar, 26.9% Cel, 15.0% HC, 6.0-8.8% ash, 67.7% DMD, 12.5 MJ/kg DE, 10.28 MJ/kg ME, 6.56 MJ/kg NEl, RFV= 127 (Gryazeva, 2005; Dronova et al., 2016; Teleuţă& Ţîţei, 2016; Demydas et al., 2019; Heuzé et al., 2016; Ţîţei, 2021); *Onobrychis viciifolia* contained 15.3-20.8% CP, 1.9-3.6%EE, 6.4-8.0 % ash, 21.2-33.9%CF, 39.4-47.4% NFE, 30.78 % ADF, 39.80 % NDF, 10.2 MJ/kg ME (Medvedev & Smetannikova, 1981; Kshnikatkina et al., 2005; Okcu & Şengül, 2014; Dronova et al., 2016; Teleuţă & Ţîţei, 2016; Demydas et al., 2019); *Pisum arvense* 10.2-16.9% CP, 38.1-44.1% NDF, 27.6-34.9% ADF, 9.1-11.6% ash, 61.7-67.4% DMD, RFV=131-166 (Ates, 2012; Cacan et al., 2019); *Vicia sativa* forage contained 21.5% CP, 31% NDF, 22% ADF, 60% IVDMD, but *Vicia villosa* ssp. *dasycarpa* 16.5% CP, 40% NDF, 31% ADF, 46% IVDMD (Ates et al., 2013); *Vicia tenuifolia* harvested in small pod stage contained 20.97% CP, 1.48% EE, 31.19% CF, 39.50% NFE, 6.86% ash and 51.83 mg/kg carotene (Maevsky et al., 2013).

Biomass play an important role in terms of energy supply and positive environmental effects. The valorification of phytomass substrate through anaerobic digestion is carried out in biogas generators with a wide variety of microorganisms, resulting in methane gas as a fuel for the production of heat and electricity and carbon dioxide, and the digested residue is rich in macro- and micronutrient and it is widely used in production farms as a fertiliser in organic farming. Many microorganisms promote a number of chemical processes in converting the biomass to biogas. The quality of feedstock for biogas production depends on the nutrient composition and on how accessible it is to enzymes and microbes (Vintilă & Neo, 2011; Dandikas et al., 2015). The carbon to nitrogen ratio constitutes a basic factor governing the correct course of methane fermentation. It is a commonly known fact that methanogenic bacteria need a suitable ratio of carbon to nitrogen for their metabolic processes, ratios higher than 30:1 were found to be unsuitable for optimal digestion, and ratios lower than 10:1 were found to be inhibitory, because of low pH, poor buffering capacity and high concentrations of ammonia in the substrate.

The results regarding the biochemical biomethane production potential of investigated *Brassicaceae* and *Fabaceae* substrates are shown in Table 3. The carbon to nitrogen ratio falls within the established norms, ranged from 13 to 22. Petcu et al. (2022) found that in winter peas herbage C/N=9.24-12.63.

Table 3. The green biomass quality of the studied *Brassicaceae* and *Fabaceae* species

Variant	**CP %**	**Ash %**	**CF %**	**ADF %**	**ADL %**	**NDF %**	**TSS %**	**Cel %**	**HC %**	**DDM**	**RFV**	***DE***	***ME***	***NEl***	**C/N**	***BMP***
Crambe cordifolia	16.1	10.0	30.4	33.0	5.4	50.4	9.7	27.6	17.4	63.2	117	12.44	10.21	6.23	20	326
Isatis tinctoria	23.5	11.3	26.7	29.0	4.4	43.4	7.7	24.6	14.4	68.1	146	13.32	10.94	6.69	13	356
Raphanus sativus var. *oleifera*	22.8	12.0	22.0	24.4	2.9	40.1	16.4	21.5	15.7	69.9	162	13.64	11.20	7.22	13	379
Sinapsis alba	22.9	10.9	24.9	28.3	4.8	43.9	6.3	23.5	15.6	66.9	142	13.08	10.74	6.77	14	349
Astragalus galegiformis	23.4	12.6	23.3	24.8	3.1	40.1	18.3	21.7	15.3	69.5	161	13.58	11.15	7.17	13	377
Galega orientalis	23.4	10.5	26.6	28.8	3.6	47.7	10.0	25.2	18.9	66.0	128	12.95	10.63	6.76	13	371
Glycine max	17.8	9.4	28.6	31.0	4.9	48.4	14.2	26.1	17.4	64.8	124	12.73	10.45	6.46	18	337
Lablab purpureus	17.6	9.0	26.1	28.1	4.4	45.3	19.6	23.7	17.1	67.0	168	13.13	10.78	68.0	18	345
Lotus corniculatus	18.9	10.2	29.4	32.5	5.1	51.4	9.7	27.4	18.9	63.58	115.1	12.52	10.28	6.29	17	337
Medicago sativa	17.2	9.1	33.1	34.7	5.8	51.0	8.3	28.9	16.3	61.9	113	12.50	10.26	6.04	18	321
Onobrychis arenaria	15.6	9.3	27.5	30.0	4.5	47.2	18.4	25.5	17.2	65.5	129	12.86	10.56	6.58	20	339
Onobrychis viciaefolia	16.6	9.4	24.9	27.6	4.2	43.4	19.5	23.4	15.8	67.40	144	13.20	10.84	6.85	19	345
Pisum arvense	14.2	8.9	37.1	39.2	6.5	59.8	9.1	32.7	20.6	58.4	91	11.58	9.51	5.53	22	305
Vicia sativa	20.6	12.0	28.8	31.3	4.6	50.7	10.2	26.7	19.4	64.5	118	12.68	10.41	6.43	15	349
Vicia tenuifolia	23.0	12.0	27.2	28.4	4.2	46.5	14.4	24.2	18.1	66.8	140	13.09	10.75	6.76	13	350

Essential differences were observed between concentrations of hemicellulose and acid detergent lignin. The *Raphanus sativus, Astragalus galegiformis*, *Galega orientalis* substrates had low concentration of acid detergent lignin. The biochemical methane potential of the tested *Brassicaceae* substrates ranges from 32 l/kg (*Crambe cordifolia*) to 379 l/kg (*Raphanus sativus*) and in *Fabaceae* substrates ranges from 305 kg (*Pisum arvense)* to 377 l/kg (*Astragalus galegiformis*).

Data on biomethane production potential are presented in other publications. The *Isatis tinctoria* substrate has a potential of 153- 245 l/kg (Carchesio et al., 2014; Țîței, 2016), *Raphanus sativus* substrate 297-474 l/kg (Molinuevo-Salces et al., 2013; Ahlberg& Nilsson, 2015), *Sinapsis alba* substrate - 251-379 l/kg (Molinuevo-Salces et al., 2013), *Medicago sativa* substrat 120-270 l/kg (Wang & Schmidt, 2010; Teleuţă & Ţîţei, 2016; Hunady et al., 2021), *Glycine max* substrat - 266 l/kg (Morozova et al., 2020), *Onobrychis* sp. substrates 140-277 l/kg (Teleuţă & Ţîţei, 2016; Hunady et al., 2021). Pabón-Pereira et al (2020) reported that biochemical methane potential was 290 l/kg ODM in *Vicia sativa* substrates, 370 l/kg ODM in *Pisum sativum* substrates and 350 l/kg ODM in *Vicia faba* substrates 350 l/kg ODM. Wang& Schmidt (2010) reported that methane potential of vetch substrate achieved 320 L/kg VS.

CONCLUSIONS

The seeds characteristic of the studied *Brassicaceae* species was: ℓ: b: δ ≈ (1.98-12.60): (1.67-3.67): (1.63-2.05) mm, the angle of repose α=24.6°-30.6° and the flow angle on steel is α_1=15.8°-31.7°, on wood α_1=18.1°-37.3° and on enamelled surface α_1=15.3°-30.5°; M_{1000} = 4.30-9.73 g and bulk seed density 88.3-766.9 kg/m^3.

The seeds characteristic of the studied *Fabaceae* species was ℓ: b: δ ≈ (1.53-8.00): (1.20-5.47): (1.47-5.01) mm; the angle of repose α=21.5°-33.0°, flow angle on steel is α_1=14.3°-27.7°, on wood α_1=14.7°-29.8° and on enamelled surface α_1=14.2°-27.3°; M_{1000} = 1.35-170.04 g and bulk seed density 370.8-830.0 kg/m^3.

The harvested green mass from studied *Brassicaceae* species is characterized by 16.1-23.5%CP, 63.2-69.9% DMD, RFV=117-162, 10.21-11.20 MJ/kg ME, 5.18 -6.76 MJ/kg NEl and from *Fabaceae* species 14.2-23.4%CP, 58.4-69.5% DMD, RFV=91-168, 9.51-11.15MJ/kg ME, 5.53-7.17 MJ/kg NEl.

The biochemical biomethane potential from *Brassicaceae* species vegetal substrates varied from 349 to 379 L/kg of organic matter and from *Fabaceae* species from 305 to 377 L/kg of organic matter.

ACKNOWLEDGEMENTS

The study has been carried out in the framework of the project: 20.80009.5107.02 *"Mobilization of plant genetic resources, plant breeding and use as forage, melliferous and energy crops in bioeconomy"*.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE MAIN CHARACTERISTICS OF THE GENETIC SYSTEM IN SOME FOREST TREE SPECIES

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Abstract

The genetic system represents the genetic pathway of organisms for organizing and transmitting genetic material that determines the balance between coherence, gene recombination, and control over the amount and type of gene combinations. The objective of this paper is to present the main characteristics of the genetic system of several species of forest trees, namely: Picea abies, Abies alba, Larix decidua, Pinus sylvestris and Quercus robur. The main characteristics of the genetic system taken into account refer to: the mode of reproduction; population dynamics type; chromosomal cycle; recombination index; presence or absence of chromosomal genetic polymorphism, etc. The used methods included searching of the various databases with the latest publications in the field and identification of some relevant results. In the case of forest trees, the genetic systems present a special situation, compared to other higher plants; is about a particular configuration of the systems components. An optimal genetic system of long-lived species, such as forest trees, is characterized by a high recombination index and cross-pollination; however it is possible to change the rate of genetic recombination through selection.

Key words*: genetic system, forest tree, recombination, chromosomal cycle.*

INTRODUCTION

Forests provide, directly or indirectly, important health benefits for all people. Health-enhancing qualities of forests are a result of multiple and mutually reinforcing benefits.

The conservation of forests is relevant to all aspects of sustainable development and human wellbeing; also, the forestry maintains an ecological balance (Ma et al., 2022).

The genetic system refers to all the characteristics of a natural population that determine its hereditary behavior over time periods sufficient for evolutionary (Rajora and Zinck, 2021). In another formulation, the genetic system is essentially a set of instructions stored in nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

The genetic system includes the number of genes and chromosomes, the type of chromosomal structures, ploidy, the average rate of mutations, the frequency with which the crossing-over occurs, the peculiarities of the reproductive system, the type of sexual determinism, the mechanisms of the regulation of gene activity, etc.

Through the genetic system of a species it is ensured that valuable information is stored or transmitted, that other new information is created, suitable in addition to existing information or that replaces less valuable information. Genetic material, including genes and DNA, controls the development, maintenance and reproduction of organisms.

The genetic system and its components determine a population's ability to withstand evolutionary change. The evolution of the genetic system means the evolution of those mechanisms that achieve and affect genetic variability.

Being one of the most ancient groups of seed plants, the conifers are considered as a link between angiosperms and pteridosperms. The conifer genomes have a number of features that distinguish them from other plants; the most notable is their enormous genome size, which is not a result of recent polyploidization (Bondar et al., 2022).

Picea abies, popularly known as "spruce", is a particularly resistant and very easy-to-maintain specimen, suitable for a special garden. It adapts well to any type of soil and is equally resistant to frost and drought.

The spruce always has a pyramidal-conical crown, always green, with acicular leaves. The cones are 10-15 cm long, green or red when are young, brown when ripe, with persistent, thin, rhomboidal scales, with a truncated, ridged or even wrinkled tip.

Abies alba, the European silver fir or silver fir, is a fir native to the mountains of Europe. The eastern limit of the fir passes through Romania. Tree of size I/II, pyramidal crown, with acicular leaves, arranged radially, silver color with pendulous cones and parchment scales.

The European larch (*Larix decidua* Mill.) has large ecological amplitude. In the Alps and Tatra Mountains it grows in continental climates, with cold, dry and snowy winters. It grows on well-drained soils, not tolerating waterlogging, with a pH range from neutral to acid. The larch is very cold and wind tolerant during winter (dormant period), and it has a cold hardiness limit of around -30°C. Its deciduous habit confers a significant advantage by reducing desiccation damage on foliage during winter (Da Ronch et al., 2016).

Pinus sylvestris is a monoecious plant with pendulous blue-green or gray-green drooping cones. Leaf buds are ovoid, non-resinous. The male cones consist of an axis with scale-shaped stamens, with two pollen sacs on the upper face. The female cones are large, formed by carpel scales that present two anatropous ovules on the upper face. After fertilization the ovules become winged seeds.

Quercus robur (oak) is part of the *Fagaceae* family; it is widespread in the temperate climate areas of Europe, Asia and some regions of North Africa. The wood from oaks is hard and durable and valued for several purposes including for construction, furniture, veneer, fencing and firewood. It has a high tannin content, which makes it resistant to insect and fungal attacks and is particular useful for wine and spirit barrels.

Oak species also have an important ecological role, as they support insects and their acorns provide a valuable food source for many birds and mammals. The canopy of oaks allows a fair amount of light to pass through, permitting a diverse and enriched understory.

Pedunculate oak is very tolerant to soil conditions and the continental climate. It can be found in periodic wet areas by streams and rivers, but prefers fertile and well-watered soils. Pedunculate oak is a pioneer species in plains and hills, while it is a late successional species in valleys and floodplains.

Agriculture, horticulture, forestry, economy and environmental health are interconnected and essential for identifying the best practices available (Cotuna et al., 2022a; 2022b; 2022c).

The climate variability and climate changes impacting directly crops yield and indirectly the biotic constrainers might result in genetic diversity disruption (Bonciu, 2019; Velea et al., 2021), through invasion of weeds, pests and pathogens in areas where they have not been relevant before (Cotuna, 2021; Paraschivu, 2022; 2021). The conservation of the biodiversity of both agricultural and forestry ecosystems involves sustainable management measures, through the application of intensive treatments that promote the natural regeneration of species and by forest conservation (Hampe & Petit, 2005).

MATERIALS AND METHODS

These papers present some of the main characteristics of the genetic system of several species of forest trees, namely: *Picea abies, Abies alba, Larix decidua, Pinus sylvestris* and *Quercus robur*.

The main characteristics of the genetic system taken into account refer to: the mode of reproduction; population dynamics type; chromosomal cycle; recombination index; presence or absence of chromosomal genetic polymorphism, etc. The research method consisted in identifying, accessing and selecting of several scientific results in the field, published in some journals indexed in WOS, Clarivate Analytics, Scopus and Springer databases.

RESULTS AND DISCUSSIONS

The main characteristics of the genetic system to *Picea abies*

Due to the large and complex genome of conifers, this important group of plants was, until recently, lacking species with available reference genomes (De La Torre et al., 2014). In 2013 the first draft assembly of the *P. abies* genome was published (Nystedt et al., 2013).

Norway spruce (*Picea abies*, 2n = 24) belonging to the family *Pinacea*e, is one of the most important conifers in Europe. Unfortunately, recent forest decline caused by pollution concerns mainly this species and emphasis a necessity to study genetic background of the response to biotic and abiotic stresses.

Spruce presents a periodic flowering, the time between two abundant fruiting varying with the geographical position, determined especially by latitude and altitude. Male and female unisexual flower buds are formed in the summer before flowering, on annual spurs, and can be distinguished from September to October.

The anthesis process is influenced by air temperature; the opening of the pollen sacs takes place above 5^0C, variable from one tree to another and probably genetically conditioned. It should be noted that heavy rains during the flowering period can reduce pollen production by about 25% of the potential capacity. After fertilization, the zygote divides twice and four free nuclei are formed, which are placed at the base of the archegonia, in a single plane. All cells, except those in the archegonia area, divide rapidly and are filled with substances that are nutrients for the embryo.

Genome mapping of *P. abies* genome is complicated by the biology of the species and from the complexity of the nuclear genome. One way to simplify its analysis is fractionation into individual chromosomes. The urgent need for genetic improvement contrasts with a poor knowledge of the genome (Űberall et al., 2004).

The *Picea* maps were based on diploid F1 crosses with the densest composite map containing only 2,300-2,800 markers per framework map (Pavy et al., 2017).

When karyotyping the species *P. abies* var. *acuminata* it was found that 10 chromosomes have the median centromere and 2 chromosomes have the submedian centromere; also, 5 chromosomes with median centromeres showed secondary constrictions, with the possibility of forming nucleoli. Following the karyotype study of *P. abies*, it was found that their genome is very large (~20 Gbp) and contains a high fraction of repetitive DNA (Bernhardsson et al., 2019). The current *P. abies* genome assembly covers approximately 60% of the total genome size but is highly fragmented, consisting of >10 million scaffolds (Bernhardsson et al., 2019). These authors suggest that approximately 3.8% of the anchored scaffolds and 1.6% of the gene models covered by the consensus map have likely assembly errors as they contain genetic markers that map to different regions within or between linkage groups.

According to Westin et al. (1999), some *P. abies* clones showed no consistent difference in mitotic index, either in period or in general levels (Figure 1). The response of mitotic index to temperature differed in spring and fall. Also, the differences in cold hardiness between the clones were not directly coupled to differences in mitotic index (Westin et al., 1999).

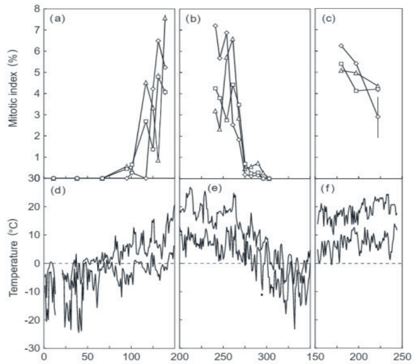

Figure 1. Mitotic indices for three spruce clones (a-c) and standard errors of the means (d-f)
Source: Westin et al. (1999)

The use of roots offers important advantages over other systems used to isolate mitotic chromosomes. Seedlings are easy to handle, root meristems are karyologically stable and can be synchronized to obtain a high frequency of metaphase cells. In Figure 2 is selected some aspects of the *P. abies* mitosis (Băra et al., 2004).

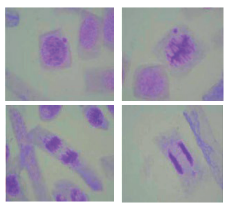

Figure 2. Aspects of the *Picea abies* mitosis
Source: Băra et al., 2004

The main characteristics of the genetic system to *Abies alba (Silver fir)*

Although the genus *Abies* includes over 40 species, many of them of considerable commercial importance, too few researches has been undertaken on their genetics and especially on cytogenetic relationships. With one exception (*A. firma*, in which a tetraploid with 48 chromosomes was identified), all the investigated species have n = 12, respectively 24 chromosomes in the sporophyte tissues (2n).

A. alba is a large conifer that can be found in central Europe and some parts of Southern and Eastern Europe. It is one of the tallest tree species of the genus *Abies* in Europe. This tree is considered an important ecological and functional balancer of European forests and a fundamental species for maintaining high biodiversity in forested ecosystems. Its future distribution is subject of a debate between palaeoecologists and modelers, with contrasting climate-response forecasts. Owing to its good cleavability and durability, especially under humid conditions, it is suitable for the production of shingles and for hydraulic engineering. Young trees of *A. alba* are very popular as Christmas trees.

A. alba has been showing a declining trend in its growth at its south-western limit that is related to increasing temperatures and in general in its distribution range (Dalmaris et al., 2022). Its main distribution is concentrated in Central Europe, on the Suisse plateau and in South and Eastern Germany as well as in the Czech Republic and Austria. There are conspicuous numbers in the Pyrenees, Southern Alps of Northern Italy and Ticino and the Eastern Alps, the Carpathians and Albania. It is also found more sporadically in Eastern France, on the Massif Central, and in the Apennines (Figure 3).

Figure 3. Distribution map of *Abies alba* (European Silver Fir)
Source: https://commons.wikimedia.org/wiki/File

A. alba occupies a broad range of environmental conditions and possesses a deep root system that allows to access deep water under summer drought. It is therefore considered to better cope with future drought events under climate change than *P. abies* that often occupies similar habitats (Vitasse et al., 2019).

The flowers are unisexual monoecious; the male ones are grouped in the form of thick and elongated, yellow catkins, while the female flowers are cylindrical, greenish and arranged mostly at the top of the tree's crown. The duration of the microsporogenesis phenomenon is 30-40 days. Flowering takes place from April to mid-June, depending on altitude, latitude and exposure. Unlike the spruce, the fruiting of the silver fir is more regular, more abundant and more constant. From a management perspective it is important not only to know the thresholds that determine the maximum or minimum recruitment, but also the maximum response of recruitment to a limiting factor over the gradients most frequently encountered in forest management (Trifković et al., 2023).

Silver fir is a wind-pollinated, generally outcrossing species. In dense stands with a sufficient number of mature individuals, its outcrossing rate is over 80% of all seeds produced, which is similar to many other conifer species. However, in occurrences with a reduced population size, and during years of low flower production, self-fertilization takes place (up to 95% of all seeds produced on some trees) (Wolf, 2003).

Silver fir has long been considered to be less variable than other conifers because of its low morphological variation. To preserve the population-specific genetic structures of silver fir, i.e. locally common alleles and the area-specific allele frequency distribution, many different populations from various distribution areas should be selected systematically for gene conservation purposes. The most effective way to conserve larger occurrences of silver fir and their genetic resources is through in situ conservation of stands and populations as well as their natural regeneration using long-term and small-scale regeneration methods (Wolf, 2003).

Figure 4 shows the banding and mapping of genes on chromosomes of root tip meristem cells of *A. alba* (Puizina et al., 2008). The karyotype is characterized by three heterobranchial chromosomes, with the shortest arms, and two other chromosomes with short arms, which may or may not be heterobranchial.

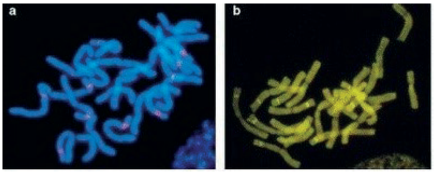

Figure 4. Banding and mapping of genes on chromosomes of root tip meristem cells of *Abies alba*
Source: Puizina et al., 2008

The nuclear reference genome sequence of *A. alba* with an estimated size of 18.16 Gb was generated from DNA representing the adult tree AA_WSL01 (Birmensdorf, Switzerland) in a community-based effort of the Alpine Forest Genomics Network and the chloroplast genome of this genotype was assembled (120,908 bp) (Mosca et al., 2019).

The main characteristics of the genetic system to *Larix decidua* (European larch)

European larch is one of the few deciduous conifers. It is one of the fastest-growing conifer species in Western and Central Europe (Figure 5), producing more than 10 m^3 of wood per ha annually under optimal conditions. Because of its fast juvenile growth and its pioneer character, larch has found numerous applications in forestry and agroforestry.


Figure 5. Distribution map of the native range of *Larix decidua* (European Larch)
Source: https://commons.wikimedia.org/wiki/File

European larch has a high level of genetic variability for most silvicultural traits both between and within populations. International provenance experiments have indicated the best larch populations in terms of silvicultural quality. The fastest growing populations were found among the Sudetes and Central Polish larch; Alpine populations were slowest growing. The populations from Central Europe showed also the highest stability across environments (low G x E), even across ecologically contrasting conditions, while south-western alpine larch populations performed well only at high elevation sites (Matras and Pâques, 2008).

L. decidua is monoecious and wind pollinated. Larch pollen is small and round without air bags. As a result it is transported by the wind over only relatively short distances of up to 300 m. Larch trees reach sexual maturity at the age of 15 years in open stands but at the age of 35-40 years in closed stands. Larch produces seeds every 3-4 years on average.

The first information on the number of chromosomes in L. decidua was provided by Strassburger as early as 1892 (n = 12). From the point of view of the karyotype, individual chromosomes can be classified into three groups, depending on the difficulties of individualization, as follows: easily identifiable chromosomes (I, II, III, IV and VII); chromosomes relatively easy to identify (V, VI and VIII); hard-to-identify chromosomes (IX, X, XI and XII), which practically cannot be distinguished from each other.

Of the haploid set of 12 chromosomes, six are isobrachial and six are heterobrachial. Chromosome I is the longest (144 units), with a median centromere. Of interest is the relatively long chromosome IV (118 units), which shows a satellite with a length equal to 32% of the length of the short arm and which can be very difficult to identify, because of the very narrow secondary constriction and a light strip that extends throughout chromosome width.

The satellites DNAs confer important functions with roles in cell division, chromatid separation, and chromosome stability (Jagannathan et al., 2018). Due to their fast evolution and defined chromosomal localization, may represent valuable targets to trace repeat evolution and divergence over long, evolutionary timeframes in conifers (Heitkam et al., 2021).

For *L. decidua*, idiograms have been published based on Chromomycin and DAPI banding and/or on chromosome length, arm length ratio and the visibility of secondary constrictions. But these criteria were not sufficient for an unambiguous individualization of all of the chromosomes due to similarity in size and arm length ratio, varying degrees of condensation of chromosomes between and within single metaphases and varying degrees of extension, and visibility of individual secondary constriction. Thus, Lubaretz et al. (1996) reported karyotyping chromosomes of *Picea abies, Pinus sylvestris* and *Larix decidua* (Figure 6), based on chromosomal localization of 18/25S and 5SrRNA genes by fluorescent in situ hybridization and on computer-aided chromosome analysis. By means of these techniques, the chromosomes of the Norway spruce and some of the Scots pine and European larch karyotype can be distinguished individually (Lubaretz et al., 1996).

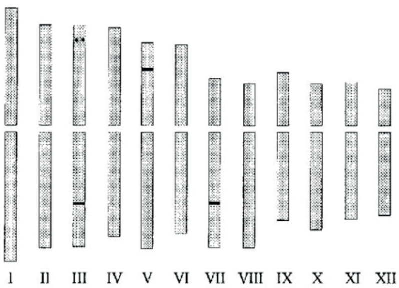

Figure 6. Chromosomes of *Larix decidua* arranged according to their relative length, arm length ratio and position of rDNA loci
Source: Lubaretz et al., 1996

Larch seed can be stored for at least 30 years in gene banks. Pollen can also be stored *ex situ*. Cryopreservation of somatic embryogenic lines is another possibility for conservation of larch genetic resources conservation since most technical problems have been solved recently.
European larch requires special management if it is to survive and flourish, especially in mixed forests. The drawing up of general rules of silviculture is necessary to ensure the establishment of progenies from the natural populations of larch and maintenance of larch stands. Assistance to natural regeneration can be provided through weed control, opening of stand canopy, complementary planting and other management efforts (Matras and Pâques, 2008).

The main characteristics of the genetic system to *Pinus sylvestris*

Pinus sylvestris (Scots pine) is a long-living, coniferous tree characterized by its orange trunk. Globally, it is the most widely distributed pine and is found throughout all of Eurasia. The genetic variety is immense and several different subspecies exist across its distribution.
Scots pine is, especially in the north of Europe, an economically important species. The wood is strong and easy to work with, making it excellent for general constructions, furniture-making and the pulp and paper industry. It is also used for stabilizing sandy soils.
This species has 2n = 24 chromosomes and, as with other conifers, the fact that it has long chromosomes can complicate the karyotype study for the purpose of establishing geographic variation or characterizing different taxon’s. Chromosomes of medium length and those without satellites are difficult to distinguish from each other. However, the number of satellite chromosomes does not correspond to the number of nucleoli.
The karyotype of the *P. sylvestris* species highlights the 12 pairs of chromosomes of different lengths (Figure 7); 9 pairs show metacentric centromere (I-IX) and 3 pairs show submetacentric centromere (X-XII).

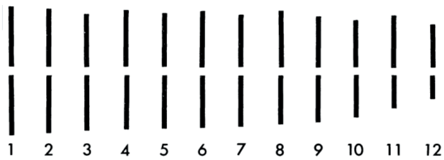

Figure 7. *Pinus sylvestris* idiogram

The formation of female and male flower primordia takes place between April and July, depending on the climate and stationary conditions. Scots pine is a normal cross-pollinated species, but self-fertilization can occur easily, and even complete self-compatibility exists. In practice, however, absolute autogamy is never achieved. Self-pollination is possible because the phenological gap between the maturation of female and male flowers is very small (usually half a day to a

day). The consequence of self-fertilization is given by the high percentage of sterile seeds and especially by embryonic mortality.
Regarding the precocity of Scots pine flowering and the formation of cone clusters, we can speak of a genetic control.
Under natural conditions Scots pine is not readily interfertile with other pine species. Spontaneous hybrids with *P. nigra, P. densiflora* and *P. mugo* have been reported. Towards other taxa, the species shows a robust hybrid incompatibility (Mátyás et al., 2004).
When selecting gene conservation units along a continuous cline, ecological information should be preferred to neutral markers. In the absence of drift, in a contiguous distribution range adaptively different populations may be expected at distances where annual mean temperature differs by a minimum of 1.0-1.5°C (equal to ca. 200 km in a flat landscape) (Mátyás et al., 2004). The size of gene conservation units of Scots pine should be sufficiently large to compensate for and buffer against outside gene flow: 100 ha should be considered the minimum. Nearby occurrences of genetically degraded or otherwise unsuitable stands should be either avoided or removed. A conservation unit should consist of numerous adjoining stands of various ages, provided their origin is the same. In areas of scattered occurrence, initial size may be 10 ha as a minimum, which can be increased during successive regenerations (Mátyás et al., 2004).

The main characteristics of the genetic system to *Quercus robur* (oak)

The genus *Quercus* comprises approximately 400 to 500 species and has been divided into two subgenera: subgenus *Cerris* characterized by rugulae visible in mature pollen grains or weakly masked and subgenus *Quercus* characterized by receptor-independent sporopollenin masking rugulae in mature pollen grains.
The genus *Quercus* spreads all over the northern hemisphere in Asia, North America, Europe and Africa, down to the Equator. Over the whole genus, there are about as many species in America (200 to 245) than Eurasia (196). However, Europe comprises only 22 species and Asia +100. Diversity is highest between 15° and 30° Northern latitude in central America (particularly Mexico) and South Asia (Figure 8) (https://quercusportal.pierroton.inrae.fr/).

Figure 8. Distribution map of genus *Quercus*
Source: https://charlois.com/en/quercus/

Oaks are considered by many societies as sacred trees, symbols of strength and endurance, with high cultural and historical value. They provide important environmental services like carbon sequestration, reservoir of biodiversity, soil and water protection, etc. They also provide several economic and cultural services like carpentry, furniture, cabinet making, veneer, cask industry, fuel wood, hunting and fungus gathering (https://quercusportal.pierroton.inrae.fr/).
Oak has 24 chromosomes in its diploid phase. Extra chromosome 2n = 24+1, 2 or 3 extra chromosomes have been reported as consequences of irregular segregation in mitoses (Zoldos et al., 1998).

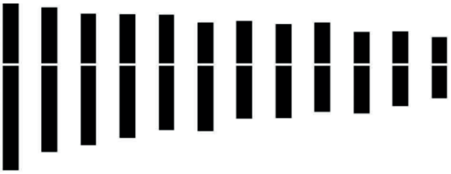

Figure 9. *Quercus robur* idiogram

Pedunculate oak (*Q. robur* L., 2n = 2× = 24) is an outcrossing, highly heterozygous diploid species. Flow cytometry analysis has shown that this species has a genome of 740 Mb per C[33], where the C-value is the amount, in picograms, of DNA contained within a haploid nucleus (Plomion et al., 2018).
Works on the genome of *Quercus robur* tree native from Europe began in January 2012, at INRA Bordeaux. Few years later, this genome has been sequenced, assembled and annotated.

In its last version, are characterized around 26,000 genes and estimated that 50% of the 750M bp of haploid genome was made of repetitive elements (Plomion et al., 2018). This first oak genome assembled so far, provides a foundation to study the biology and evolution of these species. In particular, it will allow identifying genes essential for the adaptation of these long-lived organisms to their environment, genes involved in the symbiotic relationships between the mycelia of truffles and its root, genes responsible for the biosynthesis of wood extractives such as tannins and lactone that gives their flavor and taste to alcohol and wine (https://www.oakgenome.fr/?page_id=244).

Although vegetative characters are important and frequently preferred in the identification of oaks, these are considered as quite risky because of hybridization between oak taxa. Scientific interest has recently moved from classic description to biological understanding of oak evolution by means of molecular markers (Denk and Grimm 2010; Simeone et al. 2013). However, oak taxonomy is still problematic and under debate due to often insufficient diagnostic morphological characters (Yılmaz, 2018).

Being a plant with typical anemophilic pollination, the oak produces a large amount of pollen which the upward currents can climb up to about 3000 m. Self-pollination and self-fertilization are not excluded, but there is a selectivity mechanism during fertilization that ensures preferential fertilization with pollen foreigner.

CONCLUSIONS

Picea abies, Abies alba, Larix decidua, Pinus sylvestris and *Quercus robur* are five species of *Pinaceae* that are of considerable economic and ecological importance in Central Europe. Similar to nearly all of the about 200 species of *Pinaceae* and many other gymnosperms, they have more or less symmetric karyotype consisting of 2n = 24 morphologically rather similar chromosomes.

The satellites DNAs confer important functions with roles in cell division, chromatid separation, and chromosome stability. Due to their fast evolution and defined chromosomal localization, may represent valuable targets to trace repeat evolution and divergence over long, evolutionary timeframes in conifers.

The genetic systems present a special situation, compared to other higher plants; is about a particular configuration of the systems components.

The conifer genomes have a number of features that distinguish them from other plants; the most notable is their enormous genome size, and this can create some difficulties in their research due to limit of computational resources. There are several factors responsible for a large genome size in conifers, but the main is polyploidy and amplification of transposable elements. Also, these elements present a source of genetic variation, because contribute to mutations increase and affecting the gene expression.

An optimal genetic system of long-lived species, such as forest trees, is characterized by a high recombination index and cross-pollination; however it is possible to change the rate of genetic recombination through selection. The priorities for specific gene conservation to conifers measures differ regionally. Preservation of genetic resources depends of the forest management practices, the extent of protected or unmanaged areas and the fragmentation of the species. Of course, the urgency to set up gene conservation units is much higher in an area with fragmented populations than in a region which sustainable forestry.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

BIOCHAR - A PRODUCT WITH VALUABLE APPLICATIONS

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Abstract

Biochar, a product manufactured through a technology with negative emissions obtained through the valorisation and superior use of agro-wastes, proves to have multiple uses (agriculture, environment, industry). The use in agriculture as an amendment or in the composition of some fertilizers that improve their nutrient supply properties contributes to the decrease of farmers` dependence on chemical fertilizers and the reduction of their application doses. This technology of using biochar supports farmers by combating soil degradation, increasing carbon content, simultaneously decreasing leached nutrients (especially nitrogen) and greenhouse gases, and decreasing the content of contaminants in agricultural products through the retention effect in the biochar structure of pesticides and heavy metals, which affect the production and quality of crops. At the same time, biochar represented a means of combating climate change, improving the physico-chemical properties of the soil and offering benefits to agricultural crops

***Key words**: biochar, agriculture, soil, carbon, fertilizer.*

INTRODUCTION

In addition to the development of new technologies such as precision agriculture, genetically modified crops, robotics, etc., inspiration can also be found in the application of traditional methods of improving soil fertility in modern agriculture. History shows a number of useful examples where people have tried to improve soil properties by applying charred wood (Sheil et el., 2012; Downie et al., 2011; Ogawa & Okimori, 2010; Blackmore et al., 1990).

The best-known example in the scientific literature is the creation of anthropogenic chernozems, also called Terra Preta de Índio, more than 8000 years ago (Glaser, 2007).

These soils resulted from a substantial accumulation of organic waste, such as household waste, excrement, and residual biomass, along with unburned woody debris. These were further broken down and resulted in soils of surprisingly high fertility (Glaser & Birk, 2012).

The phenomenon of Terra Preta de Índio has become the main inspiration of many scientists in the last two decades or so, with the main focus of their research on the role of biochar in improving soil properties and the question of whether the effect observed in Amazonia can be reproduced in other areas of the world (Horák et al., 2020).

An economic way to manage agricultural waste is to transform it into a valuable product called biochar.

Biochar production is becoming an alternative strategy for using agricultural waste in agricultural input. Residue production in large quantities in agro-forestry could be used as an abundant source of biochar preparation and become a solution for waste management (Gwenzi et al., 2015; Meng & Chen, 2013).

Biochar, a product manufactured through a technology with negative emissions obtained through the valorization and superior use of agro-wastes, proves to have multiple uses (agriculture, environment, industry).

The use of biochar covers several directions, making this material a valuable and multipurpose product due to its properties, namely:

- waste management (recycling of waste and promotion of green energy) (Das et al., 2022);
- climate mitigation (decrease green house gases) (Šimanský et al., 2016; Cayuela et al., 2013; Ennis et al., 2012; Kameyama et al., 2012);
- soil enchancement (improve soil quantity and fertility) (Burachevskaya et al., 2020;

Horák et al., 2020; Yazhini et al., 2020; Gondek et al., 2019; Shareef & Zhao, 2016);
- environmental remediation (removal of organic and inorganic polluants) (Toková et al., 2020; Mohamed et al., 2017; Gwenzi et al., 2015; Meng & Chen, 2013; Teixidó, et al., 2011; Cao et al., 2009);
- energy storage (Liu et al., 2019);
- carbon dioxide capture (sequesters carbon dioxide in good amounts, descreasing climate effects) (Fawzy et al., 2022; Horák et al., 2020; Van Zwieten et al., 2010);
- hydrogel - composite (supports growts of plants, increase plant available water) (Ambika et al., 2022; Das et al., 2022);
- nano-technology (supports nano-structuresuch as nano fertilizers) (Tan et al., 2016).

More and more studies are dedicated to analysing the properties of biochar to better understand the potential benefits and risks of large-scale application in agriculture and environmental remediation. Therefore, the development, obtaining and application of biochar is very important and can predicts its benefits and behavior in the future (Lian & Xing, 2017; Mierzwa-Hersztek et al., 2017).

Biochar is a stable "black carbon" that can be obtained from the pyrolysis of plant material, biomass, under anaerobic conditions. Biochar has gained great attention worldwide due to its specific properties and versatile activities in agriculture and the environment. Several definitions of biochar have been supported by several researchers. Of these, the most standardized and accepted definition is: “A solid material obtained from thermochemical conversion of biomass in an oxygen limited environment” (European Biochar Foundation-EBC 2012; International Biochar Initiative-IBI 2012).

The sustainable production of biomass and its non-energetic use in materials or in the form of pyrolysis products (biochar and pyro-oils) is the simplest, safest and fastest method that can be implemented globally.

Growing interest and research in soil biochar applications has been steadily increasing in recent years (Lehmann & Joseph, 2015; Verheijen et al., 2014).

Biochar has physico-chemical properties that allow it to be used for a long time, safely accumulate carbon in the environment and improve soil health (Wang et al., 2021; Meena et al., 2020; Joseph et al., 2020).

Numerous studies carried out in different soil and climate conditions have indicated a positive effect of biochar on soil chemistry (Horák et al., 2020; Gondek et al., 2019), of its ability to supply crops with nutrients (Beusch et al., 2019; El-Naggar et al., 2019; Mia et al., 2017; Yavari et al., 2015), improving the biological and physical properties of the soil (Ajayi & Horn, 2016; Biederman & Harpole, 2013; Lehmann et al., 2011).

Incorporating biochar into soil can help reduce soil erosion by improving soil structure and increasing water infiltration. This can be particularly beneficial in areas prone to erosion or landslides.

It should be noted that many studies have focused on problem soils (acidic, saline, with low soil organic carbon content) where biochar addition has led to substantial improvements in physical, chemical and biological characteristics (El-Naggar et al., 2019; Nguyen et al., 2018; Hseu et al., 2014; Jien & Wang, 2013).

However, it has been observed that biochar application can have the greatest effect on more fertile agricultural soils, where there is great economic and practical potential. Although there are many studies focusing on the short-term effects of biochar application on soil properties, there have been only a limited number of published studies looking at the medium-term effects of biochar (>5 years).

These studies can be divided into several groups. Some studies focus on the effect of repeated application of biochar over a period of several years (El-Naggar et al., 2019; Nguyen et al., 2018).

The other group of studies includes works with a single application of biochar at the beginning of the establishment of the experiment, followed by a monitoring period reaching up to 3-4 years (Aydin et al., 2020).

There is a huge body of literature devoted to the results of biochar testing on almost all soil types. There are numerous studies on agricultural use that show that biochar can serve as an excellent soil amendment

(Burachevskaya et al., 2020; Shareef & Zhao, 2016).
From the total number of articles studied, they were chosen for this paper only those that refer to some of the properties of biochar and its use in agriculture (143). We used the Web of Science and Google search engines Academic following the keyword: biochar.

BIOCHAR IN AGRICULTURE

The management of crop residues is a difficult problem for farmers to ensure a balance between costs and that of sustainable agriculture. Although burning is the easiest way to destroy plant waste, it not only causes drastic environmental changes through the greenhouse gases generated, but also large amounts of nutrients are removed. Therefore, a technique is needed that protects the environment and, at the same time, improves the quality of the soil. Therefore, pyrolysis of biomass residues can become an alternative technique, viable compared to previous approaches. Biochar can be produced through the pyrolysis process and can be used as a soil amendment. The application of biochar improves the physico-chemical properties of the soil and improves production in the context of an ecological, sustainable agriculture (Yazhini et al., 2020).
In recent years, the application of biochar as a soil amendment or attempts to formulate fertilizers with biochar content represents a new trend, mainly to reduce the leaching of nutrients and to improve the efficiency of their use, simultaneously with the improvement of the physical properties soil chemistry and the reduction of greenhouse gas emissions, in the context of carbon sequestration policy (Shy et al., 2020; Olad et al., 2018; Lehmann & Joseph, 2015; Zheng et al., 2013).
Dependence only on inorganic, classical fertilizers for future agricultural growth would mean further loss of soil quality, possibilities of groundwater contamination and environmental pollution. In addition, the use of conventional fertilizers is expensive due to low nutrient use efficiency.
Therefore, a rational and "personalized" fertilization of crops, effective, taking into account the pedo-climatic conditions, must be achieved by using technologies that do not disturb the environment, preserve, improve soil fertility, simultaneously with the improvement of the efficiency of agricultural systems and the implementation of sound and ecological agronomic practices (Francis et al., 2020; da Costa et al., 2019; Wu, 2011).
One of the characteristics of biochar that makes it attractive as a soil amendment is its highly porous structure, potentially responsible for improved water retention and increased contact surface area. The addition of biochar to soil has also been associated with increased nutrient use efficiency, either through the nutrients contained in the biochar or through physico-chemical processes that allow better use of soil-own or fertilizer-derived nutrients (Kumawat et al., 2021; Trenkel, 2021; Francis et al., 2020; Shi et al., 2020; da Costa et al., 2019; Wu, 2011).
A number of studies have shown that biochar as a soil amendment has the potential to mitigate the effects of climate change by increasing soil organic carbon content and improving soil quality (Zhang et al., 2012; Laird et al., 2010).
Importantly, it is the apparent biological and chemical stability that allows biochar to act as both a carbon sink and provide long-term soil benefits (Fawzy et al. 2022; Das et al., 2020).
A preliminary analysis shows that European countries are already taking steps towards greater efficiency in the use of resources, mainly due to economic concerns related to their dependence, the energy crisis and the increase in the prices of raw materials worldwide.
Crop quality and productivity can be improved by implementing responsible nutrient fertilization, combining the application of mineral, organo-mineral fertilizer products with slow/controlled nutrient release fertilizers and biofertilizers, respectively (Kumawat et al., 2021; Trenkel, 2021; Shi et al., 2020; Bhatt et al., 2019; Hermida & Agustian, 2019; Iftime et al., 2019; Cole et al., 2016; Bhattacharjee & Dey, 2014; Xiaoyu et al., 2013).
Kuwagaki & Tamura, 1990 proposed seven properties to be measured for an agronomic biochar quality assessment: pH, volatile compounds content, ash content, water holding

capacity, bulk density, pore volume and specific surface area.

The potential benefits of biochar as a soil amendment are well identified in the literature. These include carbon sequestration, improved crop yields and increased water retention (Yu et al., 2013). According to several studies, an improvement in soil quality could be long lasting after the addition of biochar (Bruun et al., 2017), but also improving the state of soil fertility (Mašek et al., 2019; Atkinson et al., 2010). Despite the fact that biochar is of increasing interest through the possibility of being used both as an amendment and in fertilizer products with slow/controlled nutrient release properties, their release mechanism and formulation face limitations that still require multiple and complex interdisciplinary research (Pogorzelski et al., 2020; Chen et al., 2018; El Sharkawiet al., 2018; Wen et al., 2017). Biochar application improves soil physical qualities, i.e., improves aeration, water holding capacity, increases porosity and reduces evapotranspiration and soil bulk density (Schulz et al., 2014; Mukherjee & Lal, 2013; Herath et al., 2013). However, the actual effect of this amendment depends on the type of biochar, production conditions, soil condition and the amount of biochar applied (Wang et al., 2018).

Aslam et al. (2014) attributed the improvement in soil physical properties after biochar introduction to the type of input material, pyrolysis conditions, biochar application rate, and the type of soil in which the biochar was applied and incorporated (Šimanský et al., 2019). Different mechanisms have been proposed that increase nutrient availability to plants in different ecosystems provided by biochar application. Among these mechanisms are: the incorporation of soluble nutrient sources in biochar for a better availability for the needs of crops (Biederman & Harpole, 2013; Sohi et al., 2010), reduction of nutrient leaching due to the physico-chemical properties of biochar (Liang et al., 2006) and minimizing nitrogen losses through NH_3 volatilization and denitrification (Šimanský et al., 2016; Cayuela et al., 2013; Ennis et al., 2012; Kameyama et al., 2012). Biochar has a large surface area and different functional groups like carbonyl (R-C=O), carboxyl (-O-C=O), hydroxyl (-O-H), ethers (C-O-C), aromatic or alkyl groups (Prasannamedha et al., 2021; Kameyama et al., 2012), with a role for the transport of nutrients (El-Naggar et al., 2018; Xu et al., 2016; Prommer et al., 2014; Biederman & Harpole, 2013) and the elimination of pollutants (Qian et al., 2019; Tan et al., 2015; Cao et al., 2009).

These attributes increase the nutrient retention capacity of biochar, even nitrate and phosphate anions (Prommer et al., 2014; Biederman & Harpole, 2013; Kameyama et al., 2012; Major et al., 2010). Thus, biochar could stabilize soil organic matter and increase respiration and reduce the bioavailability and phytotoxicity of heavy metals (Ennis et al., 2012; Park et al., 2011). Biochar has also been shown to increase crop yields (El-Naggar et al., 2018; Xu et al., 2016; Biederman & Harpole, 2013), reduces greenhouse gas (GHG) emissions and increases soil carbon storage (Horák et al., 2020; Rizhiya et al., 2019; Zhao et al., 2019; Horák et al., 2017; Kameyama et al., 2012; Zhang et al., 2012; Galinato et al., 2011; Van Zwieten et al., 2010; Lehmann et al., 2006). A nutrient-deficient or degraded soil can be treated with biochar, which is produced from a certain raw material and certain conditions of the pyrolysis process, ensuring the modification of the specific properties of the soil and the improvement of production (Robb et al., 2020; Ippolito et al., 2012). Various studies have reported a positive effect of biochar on soil hydrophysical and hydraulic properties (Salinas et al., 2018; Lehmann & Stephen, 2015; Arthur et al., 2015; Hardie et al., 2014). Biochar application positively affected bulk density (Zhang et al., 2012), soil porosity (Walters et al., 2018; Mukherjee et al., 2013; Jones et al., 2010), soil water capacity (Karhu et al., 2011) and soil hydraulic conductivity (Lei & Zhang, 2013; Makó et al., 2020). Due to the highly porous nature of biochar, its incorporation into soil can improve soil physical properties by creating new pores (Jones et al., 2010) attributed the partial filling of large cavities between coarse sand particles to the application of biochar.

Castellini et al. (2015) consider that biochar has a potential impact on the physical properties of the soil and therefore may affect the water balance of the ecosystem. The study of Sun & Lu (2014) showed a positive effect of biochar application on soil porosity and the ability to retain water and make it available to plants, resulting in an increase in crop yields.

Biochar can serve as a habitat for beneficial soil microbes, including bacteria and fungi, that promote nutrient cycling and plant growth. This can lead to healthier, more productive agricultural systems.

The appearance of some changes in the function and structure of the soil microbiome through the application of biochar is due to the modification of the C and N cycle, soil respiration and the flow of nitrogen oxides. Biochar coating tends to reduce nitrous oxide emissions (Zhang et al., 2016; Jia et al., 2010; Van Zwieten et al., 2010).

The application of biochar improves the quality of the soil by improving its physico-chemical properties, its fertility and the efficiency of using nitrogen from fertilizers (Chan et al., 2007).

Applying biochar to the soil will become the affordable solution for soil modification with low nutrient content, acidity, increased salinity (Yazhini et al., 2020; Tian et al., 2018; Dai et al., 2017; Lin et al., 2015; Carter et al., 2013; Asai et al., 2009). The results of the experiments carried out so far clearly confirm the positive effect of biochar application on the organic content of the soil shortly after application (Igaz et al., 2018; Juriga et al., 2018) which was noticed up to 3 years after the experiment (Horák et al., 2020; Šimanský et al., 2018).

However, negative or no effects on soil characteristics and crop yields were also recorded (Jeffery et al., 2017), emphasizing the need for further studies to analyze the effects of biochar in a diversity of soil types and cropping systems. It was found that, despite the variability introduced by soil and climate, the addition of biochar to soil generally increased crop yield, soil microbial biomass, plant and soil nutrient concentration, and total soil carbon. Despite the ability of the biochar to favourably modify the physico-chemical properties of the soil it also has some disadvantages:

-the selection of raw material needs more attention;

-sometimes the results may not show the significant effect of increasing crop yields by increasing nutrient availability (Spokas et al., 2012);

-hazardous by-products such as polycyclic aromatic hydrocarbons have the potential to result during the pyrolysis process (Weidemann et al., 2018; Gasco et al., 2016; Laghari et al., 2016).

In addition, due to the very stable nature of biochar it cannot be easily removed from the soil ecosystem (Jones et al., 2012).

PROPERTIES OF BIOCHAR

Biochar is obtained from thermal conversion of biomass. biochar is the main pyrolysis product, obtained by the thermochemical conversion of biomass in the absence of free oxygen at temperatures varying between 450 and 850 ˚C for a period between 6 and 8 h (Tibor & Grande, 2022). The biochar is a solid carbon-rich, porous material produced by the thermochemical conversion of a diverse range of biomass feedstocks under an inert atmosphere (i.e., in the absence of oxygen) (Ghodake et al., 2021). Feedstocks are a primary factor governing the chemical and physical properties of biochar. Carbonization of agricultural waste can be advantageous compared to disposal as waste by other means (Demirbas, 2006). The properties of biochar vary greatly depending on the type of raw material used and the pyrolysis conditions. If the content of lignocellulosic material in the feedstock is high, it gives a higher biochar yield because the lignin is converted to carbon during the pyrolysis process (Ippolito et al., 2020; Antunes et al., 2017; Schimmelpfennig & Glaser, 2012; Antal & Grønli, 2003). Biochar has heterogeneous properties due to the different raw materials that can be subjected to pyrolysis, such as stalks, cereal husks, seeds, wood waste, manure and sludge, vegetable and household waste, and others (Haddad et al., 2021; Khiari et al., 2020; Pariyar et al., 2020; Qian et al., 2019; Ahmed et al., 2018; Dunnigan et al., 2018; Shaaban et al., 2014; Angın, 2013; Kim et al., 2013; Tang et al., 2013; Ahmad et al., 2012; Zhang et al., 2012; Galinato et al., 2011; Atkinson et al., 2010). The increasingly advanced study of biochar, as well as pyrolysis technologies, deliberately controls the production of biochar, which allows obtaining a product with specific properties defined and suitable for the way of use (Rajapaksha et al., 2016). Thus, increasing the pyrolysis temperature results in a larger surface area, a higher fixed carbon and a lower oxygen content (Ippolito et al., 2020; Manyà, 2012). Higher pyrolysis temperatures result in lower ash content, higher pH, higher buffering capacity and finally an increase in the aromatic structure of the biochar which provides a high heating rate, heating time and post-pyrolysis of the biochar (Ippolito et al., 2012; Manyà, 2012). The technology for obtaining biochar is one of the few that is carried out with negative emissions, an important aspect for limiting global warming (Mašek et al., 2019). Biochar has a large, negatively charged internal surface that is resistant to degradation. Due to its properties, biochar acts as a porous carbonaceous sorbent generally produced from materials of biological origin (Tan et al., 2015). Thus, the control of these factors can lead to obtaining a biochar with specific characteristics such as surface area, volume and pore size, adsorption capacity, pH, carbon percentage, chemical composition, physical characteristics and cation exchange capacity (Ippolito et al., 2020; Somerville & Jahanshahi, 2015; Cimò et al., 2014; Ronsse et al., 2013). For example, the pore volume for the biochar obtained from wheat straw at 400°C increased from 0.016 (cm^3g^{-1}) to 0.034 (cm^3g^{-1}) in the case of pyrolysis at 600°C of the same raw material (Manna & Singh, 2015). In addition, the heating temperature of the raw material influences the surface of the biochar. For example, rice husk pyrolysis at 350°C and 650°C having the surface area of 32,7 (m^2g^{-1}) and 261,72 (m^2g^{-1}) (Schmidt et al., 2015; Claoston et al., 2014). Another study mentions that the surface area of biochar obtained from wood increased from 1 m^2g^{-1} to 317 m^2g^{-1} by increasing the temperature of the pyrolysis process from 450°C to 700°C (Brewer et al., 2014).

CONCLUSIONS

Biochar is a material that has received attention in the last 20 years, a fact proven by the large number of articles. An increase in the importance of the use of this material can be seen against the backdrop of environmental policies and the current political context. Based resistance to microbial decomposition and, therefore, it will improve carbon sequestration (Ippolito et al., 2012; Spokas et al., 2012; Singh et al., 2010). The properties of biochar can be influenced by the nature of the raw material, the pyrolysis process and the various process variables such as: pressure, heating temperature,

on our findings, biochar application leads to sustainable soil management in terms of carbon sequestration and conservation of soil structure. Although there are numerous studies from which the positive effects on the soil, agricultural yields and the environment result, there remain knowledge gaps regarding the biochar-soil-plant-environment relationship and interactions in the field in the long term. Important aspects that require research are related to the yield and long-term effects of repeated applications and the influence on the mobilization / retention of heavy metals for planning doses and frequency of applications.

ACKNOWLEDGEMENTS

This research was conducted under the NUCLEU Program, Contract No. 23 29N/2022 - “Innovative solutions for maintaining and restoring soil health under climate changes adaptation - SOL-SAN”, Project PN 23 29 02 01 “New organic fertilizer products for efficient use of natural resources in sustainable agriculture” and project number 44 PFE /2021, Program 1 - Development of national research-development system, Subprogramme 1.2 - Institutional performance - RDI Excellence Financing Projects.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE EVALUATION OF THE BIOMASS QUALITY OF *Spartina pectinata* AND PROSPECTS OF ITS USE IN MOLDOVA

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Abstract

Prairie cordgrass - Spartina pectinata Bosc ex Link, Poaceae family, is a warm-season, C_4 perennial rhizome grass native to North America. The main objective of this research was to evaluate the quality indices of green and ensiled mass substrate, solid dry biomass of the introduced taxa of Spartina pectinata, grown in monoculture in the collections of the National Botanical Garden (Institute), Chişinău. It was established that Spartina pectinata fresh mass substrate used for anaerobic digestion contained 43 g/kg CP, 66 g/kg ash, 437 g/kg Cel, 301g/kg HC, 59 g/kg ADL, 55 g/kg TSS, with C/N = 74 and biochemical methane potential 299 l/kg, but the ensiled substrate - 44 g/kg CP, 76 g/kg ash, 473 g/kg Cel, 323 g/kg HC, 57 g/kg ADL, with C/N = 72 and biochemical methane potential 303 l/kg, respectively. It has been determined that prairie cordgrass solid dry biomass harvested in winter period contained 453 g/kg Cel, 294 g/kg HC, 82 g/kg ADL, the estimated theoretical ethanol yield averaged 543 L/t. The prepared solid fuel, briquettes and pellets, had significantly higher net calorific value than perennial sorghum and corn stem fuel. The investigated introduced taxa of prairie cordgrass, may be used as multi-purpose feedstock for renewable energy production in Moldova.

***Key words**: biochemical composition, biomass, biomethane, cell wall components, solid fuel, Spartina pectinata, theoretical ethanol potential.*

INTRODUCTION

Energy availability is a crucial developmental factor for all areas of the economy. In recent decades there has been a considerable global increase in urban population, industrial productivity, energy demand, waste generation, and the emission of greenhouse gases from energy conversion.

Replacing fossil fuels with renewable energy alternatives has become a major global issue of the XXI century and a key to sustainable development. Lignocellulose is the most abundant and accessible renewable biomass in the world, it has great potential for being a source of energy and value-added products in circular economy.

In order to maintain biomass production on marginal land, developing energy crops with abiotic stress tolerances, it is necessary and will result in a reduction in competition for land with food crops, as well as improve sustainable cellulosic biomass feedstock and bioenergy production. *Poaceae* species with C_4 photosynthesis type are of great interest among non-traditional plant species, due to their high resilience to climate change effects: high temperature, water insufficiency and salinity stress. Therefore, it is necessary to study and develop perennial grass species having high biomass yields and stress tolerance that have been underutilized or unexploited.

Prairie cordgrass - *Spartina pectinata* Bosc ex Link, *Poaceae* family (syn. *Spartina michauxiana* Hitchc.; *Spartina pectinata* Bosc ex Link var. *suttiei* (Farw.) Fernald; *Sporobolus michauxianus* (Hitchc.) P.M. Peterson and Saarela) is a warm-season, C_4 perennial rhizome grass native to North America. The central culm is light green, terete, hollow, hard and sturdy that may reach 3 m in heights and 3-11 mm thick, during the growing season, the stems become woody and coarse. The glossy dark green leaves have sharp, serrated edges, are 5-15 mm wide and 20-96 cm long, with prominent linear veining and taper to a fine tip. The panicle may be up to 50 cm long and may have many branches, with 10 to 20 spikelets up to 2.5 cm in length. The seeds are flat, paper-like with barbed awns that attach firmly to fur or fabric. The root system is fibrous and strongly rhizomatous, and penetrates almost vertically downward to depths of 2.4-3.3 m. Clonal colonies of plants are often produced from the rhizomes. It is well adapted to both wet and dry soils and has a potential biomass yield of up to 21 t/ha in northern environments. The period of use of a plantation is 15-20 years. In Europe, it is grown primarily as an ornamental plant and, at the same time, it is used for strengthening sandy embankments, dams and dykes. Irrespective of the compactness of the soil, its strong and sharp-pointed roots permit its penetration. It should be noted that prairie cordgrass has a considerable anti-erosion function and, by growing on the banks of streams, it prevents flooding during periods of intensive rainfalls (Walkup, 1991; Fraser & Kindscher, 2005; Boe & Lee, 2007; Boe et al., 2009; Friesen et al., 2015; Kim et al., 2020).

Due to the intensive growth of biomass during the growing season *Spartina pectinate* has the prospect of being used for renewable energy production and feedstock for particleboard manufacture (Kowalczyk-Juśko, 2013; Maj & Piekarski, 2013; Helios et al., 2014; Kim et al., 2015; Guo, 2017; Janiszewska et al., 2022; Steinhoff-Wrzesniewska et al., 2022).

The main objective of this research was to evaluate the quality indices of green mass, ensiled mass, and solid dry biomass of the introduced taxa of *Spartina pectinate* in the third year of vegetation, and the prospects of its use as feedstock for renewable energy production.

MATERIALS AND METHODS

The introduced taxa of prairie cordgrass, *Spartina pectinata*, which was grown in monoculture in the experimental plot of the National Botanical Garden (Institute) Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″ longitude, served as subject of the research, and the cv. *Argentina* of perennial sorghum, *Sorghum almum*, and the hybrid *Porumbeni* 458 of corn, *Zea mays*, were used as control variants.

The green mass was harvested manually, *Spartina pectinate* and *Sorghum almum* in the flowering stage, but *Zea mays* - in the milk-wax kernel stage. The harvested green mass was chopped with a stationary forage chopping unit. The dry matter content was detected by drying samples up to constant weight at 105°C. For ensiling, the chopped green mass was shredded and compressed in well-sealed glass containers. After 45 days, the containers were opened, and the sensorial and fermentation indices of the conserved forage were determined in accordance with standard laboratory procedures – the Moldavian standard SM 108 for forage quality analysis. For chemical analyses, the green mass and silage samples were dried in a forced air oven at 60°C, milled in a beater mill equipped with a sieve with diameter of holes of 1 mm and some assessments of the main parameters, such as crude protein (CP), crude fibre (CF), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL) and total soluble sugars (TSS), have been made by near infrared spectroscopy (NIRS) technique using PERTEN DA 7200 of the Research-Development Institute for Grassland Brasov, Romania. The concentrations of hemicellulose (HC) and cellulose (Cel) were calculated according to standard procedures. The carbon content of the substrates was obtained using an empirical equation according to Badger et al. (1979). The biochemical methane potential was calculated according to the equations of Dandikas et al. (2015). The solid dry biomass was collected in winter. The total carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) amounts were determined by dry combustion in a Vario Macro CHNS analyser, according to standard protocols. The some physical and mechanical properties of dry biomass, briquettes and pellets were determined according to the standards: SM EN ISO 18134, SM EN ISO 18122; SM EN ISO 18125. The acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL) in solid biomass have been determined using PERTEN DA 7200, the concentrations of hemicellulose (HC) and cellulose (Cel) were calculated according to standard procedures. The Theoretical Ethanol Potential (TEP) was calculated according to the equations of Goff et al. (2010) based on conversion of cellulose and hemicellulose into hexose (H) and pentose (P) sugars:

H = [%Cel+(%HC x 0.07)]x 172.82

P = [%HC x 0.93] x 176.87

TEP = [H + P] x 4.17

RESULTS AND DISCUSSIONS

Under the conditions of the Republic of Moldova, in the 3rd year of life, *Spartina pectinata* plants come out of dormancy in mid-April, while *Sorghum almum* plants - in the first days of May. The most intense growth was recorded during June and July. *Sorghum almum* plants bloom at the end of June and *Spartina pectinata* plants - in the first days of August. At the harvest time, the *Spartina pectinata* plants were 150-155 cm tall and the weight of a shoot was on average 23 g green mass or 9.1 g dry matter, consisting of 56.0% leaves and panicle.

The biogas production as renewable energy has recently become of major interest in Europe. Biomethane is a sustainable and renewable alternative to fossil fuels, can be used as vehicle fuel, as fuel in combined heat and power systems, or to produce electricity and heat, and the digested effluent (digestate) can be utilized as fertilizer. Plant biomass - phytomass substrates may be used in biogas generators as fresh mass and as ensiled mass. Analysing the results of the assessment of the biochemical composition of the studied *Poaceae* species, it can be noted that the harvested whole plants of prairie cordgrass were characterized by lower content of crude protein and total soluble sugars, optimal amount of minerals and higher cell wall fractions (NDF, ADF, ADL). The green mass substrate of perennial sorghum contained higher content of crude protein and minerals, optimal total soluble sugars and a much lower amount of cell wall fractions than prairie cordgrass substrate. The very high level of total soluble sugars, optimal levels of crude protein and reduced levels of minerals and cell wall fractions were found in corn green mass substrate. It is a commonly known fact that methanogenic bacteria need a suitable ratio of carbon to nitrogen for their metabolic processes, ratios higher than 30: 1 were found to be unsuitable for optimal digestion, and ratios lower than 10: 1 were found to be inhibitory, because of low pH, poor buffering capacity and high concentrations of ammonia in the substrate. The nitrogen content in the studied green mass substrates ranged from 6.90 to 17.00 g/kg, the estimated content of carbon - from 502.00 to 527.00 g/kg, the C/N ratio varied from 30 to 74. The C/N ratio in the prairie cordgrass mass substrate was very high and had a negative influence on the activity of bacteria and methanogenesis processes. The estimated biochemical methane potential of the studied green mass substrates varied from 299 l/kg VS to 332 l/kg VS (Table 1). The *Spartina pectinata* green mass substrate contained high level of acid detergent lignin (59 g/kg) and reduced biochemical methane potential - 299 l/kg VS. According to Nicholson & Langille (1965), unfertilized prairie cordgrass harvested in 10% head stage contained 388 g/kg DM, 1.32% N and 4.2% ash, but nitrogen-fertilized prairie cordgrass - 264 g/kg DM, 1.78 % N and 4.9% ash. Pabón-Pereira (2009) remarked that *Spartina anglica* substrate contained 320 g/kg DM, 89% OM, 77% total fibre, 5% lignin, 26% Cel, 46% HC, 0% starch, 12% protein, with biochemical methane potential 290 l/kg, but *Hordeum vulgare* substrate contained 380 g/kg DM, 95% OM, 65% total fibre, 2% lignin, 23% Cel, 40% HC, 22% starch, 9% protein with biochemical methane potential 300 l/kg. Kupryś-Caruk & Kołodziejski (2016) revealed that the yield and chemical composition of fresh biomass of *Spartina pectinata* was 32.5 t/ha, 329 g/kg DM, 96.2% OM, 39.8% CF and C/N = 35.5, but - of *Festuca arundinacea* - 25.0 t/ha, 296 g/kg DM, 89.3% OM, 29.7%CF and C/N = 22.9. Kupryś-Caruk et al. (2023) reported that the chemical composition of the cordgrass fresh mass was 307 g/g DM. 95.1% ODM, 7.1% CP, 36.7% Cel. 22.5% HC, 6.3% LIG, 2.5% WSC, 55.4% DDM, but *Miscanthus* fresh mass - 172 g/g DM. 92.4% ODM, 9.3%CP, 37.1% Cel. 23.7% HC, 6.3% LIG, 3.5% WSC and 55.4% DDM. The quality of silage depends on the plant species, the harvesting period, on the bio-morphological peculiarities of the harvested herbage, on techniques and technology of preparation, on conditions of its storage and on many other factors. As a result of the performed sensorial analysis, the investigated *Spartina pectinata* silage was distinguished by homogeneous olive colour, pleasant smell specific of pickled vegetables, and the consistency was maintained in comparison with the initial green mass, without mould and mucus. The biochemical composition and the biomethane potential of silage substrate from the studied Poaceae species are shown in Table 2. It was determined that the pH index of the ensiled prairie cordgrass was 4.57, but in the control variants pH = 3.77-3.87. The concentration of total organic acids in *Spartina pectinata* silage was lower (36.90 g/kg), predominantly in fixed form; a higher content of butyric acid was detected (15.75% of organic acids). We would like to mention that the dry matter of *Spartina pectinata* silage had lower concentration of crude protein, optimal mineral content and higher cell wall fractions content, with a minor decrease in the acid detergent lignin content, but total soluble sugars were detected as compared with the initial green mass. The corn and perennial sorghum silages were characterized by high content of total soluble sugars, optimal level of crude protein and minerals, low level of hemicellulose and acid detergent lignin as compared with prairie cordgrass silage. The nitrogen concentration in the tested ensiled substrates ranged from 7.10 g/kg to 15.40 g/kg, the estimated content of carbon - from 503 g/kg to 523 g/kg, the C/N ratio varied from 33 to 72. The biochemical methane potential varied from 303 l/kg VS in prairie cordgrass silage substrate to 338 l/kg VS in corn silage substrate.

Several studies have evaluated the biochemical composition and biogas potential of *Spartina* ensiled substrates. According to Kupryś-Caruk & Kołodziejski (2016), the *Spartina pectinata* silage substrate was characterized by pH 5.2, 0.5 g/kg lactic acid, 17 g/kg acetic acid, 267 g/kg DM, 4.7% ash, 8.8% CP, 1.9% EE, 5.8% monosaccharides, 33.2% Cel, 6.6% HC, 3.8% lignin, 60.6% DDM and the maximum biogas yield measured after 21 days of incubation was 722.7 m^3/t, but the silage substrate from *Festuca arundinacea* had pH 5.2, 89.7 g/kg lactic acid, 2.3 g/kg acetic acid, 214 g/kg DM, 11.7% ash, 10.0% CP, 2.2% EE, 5.5% monosaccharides, 30.4% Cel, 5.7% HC, 3.0% lignin, 62.9% DDM and biogas yield 734.1 m^3/t. Piątek et. al. (2016) revealed that the biogas yield from *Spartina pectinata* reached 404.5 m^3/t ODM, from *Miscanthus × giganteus* 487.9 m^3/t ODM and from *Andropogon gerardii* 546.6 m^3/t ODM. Kupryś-Caruk & Podlaski (2019) found that the chemical composition of silage from perennial grass *Spartina pectinata* was the following: pH 5.2, 2.5 g/kg lactic acid, 2.5 g/kg acetic acid, 357 g/kg DM, 10.1% CP, 29.4% CF, 2.7% monosaccharides, 6.2% ash, 404.5 m^3/t ODM biogas yield with 55% content of methane in biogas, but maize silage substrate had pH 3.9, 11.6 g/kg lactic acid, 1.9 g/kg acetic acid, 284 g/kg DM, 9.9% CP, 23.8% CF, 6.7% monosaccharides, 5.5% ash, 737.8 m^3/t ODM with 55% methane content. Kupryś-Caruk et al. (2021) remarked that the silage prepared from *Spartina pectinata* without silage additive had pH 4.7, 12 g/kg lactic acid, 3.5 g/kg acetic acid, 3.5 g/kg butyric acid, 346 g/kg DM, 4.5% CP, 37.9% Cel, 34.6% HC, 0.65% WSC, 1.21% EE, 54.2% DDM with observed biogas production 592.7 l/kg, but the ensiled mass with silage additives: pH 4.4-4.6, 8.5-24.7 g/kg lactic acid, 3.4-10.7 g/kg acetic acid, 1.2 g/kg butyric acid, 341-348 g/kg DM, 4.5-4.6% CP, 37.8-38.0% Cel, 32.0-32.5% HC, 0.65-0.77% WSC, 1.19-1.20% EE, 54.3-54.8% DDM with observed biogas production 533.8-653.0 l/kg. Kupryś-Caruk et al. (2023) reported that the chemical composition and methane yield of the cordgrass silage substrate was: 306 g/g DM, pH 5.2, 26.8 g/kg lactic acid, 42.5 g/kg acetic acid, 0 g/kg butyric acid, 94.5% ODM, 6.6% CP, 36.3% Cel. 22.6% HC, 6.6% LIG, 0% WSC, 56.3 % DDM and 267.7 l/kg VS, but the cordgrass silage prepared with the inoculant: 299 g/g DM, pH 5.1, 69.5 g/kg lactic acid, 109.3 g/kg acetic acid, 0 g/kg butyric acid, 94.6% ODM, 6.7% CP, 36.9% Cel, 21.9% HC, 6.0% LIG, 0% WSC, 55.0 % DDM and 329.3 l/kg VS.

The possibility of converting lignocellulosic biomass in bioethanol fuel is currently an area of great interest around the world. The bioethanol yields are influenced by tissue composition, ratios of cellulose, hemicellulose and lignin. Analysing the cell wall composition of straw substrates (Table 3), we would like to mention that the concentration of neutral detergent fibre in *Spartina pectinata* straw substrate was 829 g/kg, including 453 g/kg cellulose, 294 g/kg hemicellulose and 82 g/kg lignin, but *Sorghum almum* substrate contained

490 g/kg cellulose, 291 g/kg hemicellulose and 62 g/kg lignin; in *Zea mays* substrate, there were 423 g/kg cellulose, 244 g/kg hemicellulose and 83 g/kg lignin, respectively. The estimated theoretical ethanol yield averaged 543 L/t in *Spartina pectinata*, compared to 485 L/t in *Zea mays* substrate and 567 L/t in *Sorghum almum* substrate.

Table 1. The biochemical composition and the biomethane potential of the studied green mass substrate

Indices	*Spartina pectinata*	*Sorghum almum*	*Zea mays*
Crude protein, g/kg DM	43.00	106.00	84.00
Crude fibre, g/kg DM	487.00	392.00	248.00
Minerals, g/kg DM	66.00	96.00	52.00
Acid detergent fibre, g/kg DM	496.00	421.00	271.00
Neutral detergent fibre, g/kg DM	797.00	670.00	474.00
Acid detergent lignin, g/kg DM	59.00	45.00	48.00
Total soluble sugars, g/kg DM	55.00	110.00	336.00
Cellulose, g/kg DM	437.00	376.00	223.00
Hemicellulose, g/kg DM	301.00	249.00	203.00
Carbon, g/kg DM	508.00	502.00	527.00
Nitrogen, g/kg DM	6.90	17.00	13.44
Ratio carbon/nitrogen	74	30	39
Biomethane potential, l/kg VS	299	332	321

Table 2. The biochemical composition and the biomethane potential of studied silage substrate

Indices	*Spartina pectinata*	*Sorghum almum*	*Zea mays*
pH index	4.57	3.87	3.77
Content of organic acids, g/kg DM	36.90	48.00	48.60
Free acetic acid, g/kg DM	2.00	3.30	5.10
Free butyric acid, g/kg DM	1.80	0	0
Free lactic acid, g/kg DM	7.50	12.70	17.00
Fixed acetic acid, g/kg DM	1.90	3.80	5.20
Fixed butyric acid, g/kg DM	3.90	0	0.20
Fixed lactic acid, g/kg DM	19.80	28.20	21.10
Total acetic acid, g/kg DM	3.90	6.10	10.30
Total butyric acid, g/kg DM	5.70	0	0.20
Total lactic acid, g/kg DM	27.30	40.90	38.10
Acetic acid, % of organic acids	10.57	12.71	21.19
Butyric acid, % of organic acids	15.45	0	0.41
Lactic acid, % of organic acids	73.98	77.29	78.40
Crude protein, g/kg DM	44.00	93.00	80.00
Crude fibre, g/kg DM	523.00	405.00	245.00
Minerals, g/kg DM	76.00	94.00	59.00
Acid detergent fibre, g/kg DM	530.00	405.00	258.00
Neutral detergent fibre, g/kg DM	853.00	630.00	469.00
Acid detergent lignin, g/kg DM	57.00	40.00	37.00
Total soluble sugars, g/kg DM	0	52.00	326.00
Cellulose, g/kg DM	473.00	365.00	221.00
Hemicellulose, g/kg DM	323.00	225.00	211.00
Carbon, g/kg DM	513.00	503.00	522.78
Nitrogen, g/kg DM	7.10	15.40	12.80
Ratio carbon/nitrogen	72	33	41
Biomethane potential, l/kg VS	303	338	338

Table 3. The composition of cell walls and the theoretical ethanol potential of the studied dry biomass substrates

Indices	*Spartina pectinata*	*Sorghum almum*	*Zea mays*
Acid detergent fibre, g/kg DM	535	552	506
Neutral detergent fibre, g/kg DM	829	843	750
Acid detergent lignin, g/kg DM	82	62	83
Cellulose, g/kg DM	453	490	423
Hemicellulose, g/kg DM	294	291	244
Hexose sugars, g/kg	81.84	88.20	76.05
Pentose sugars, g/kg	48.44	47.87	40.14
Theoretical ethanol potential, L/t	543	567	485

Several literature sources describe the composition of cell walls in *Spartina pectinata* straw and ethanol yield. Kim et al. (2015) reported that in *Spartina pectinata* substrate glucan concentrations ranged from 301.8 to 373.5 g/kg biomass, xylan concentrations were in the range of 181.5-214.6 g/kg biomass, total lignin ranged from 155.3 to 188.7 g/kg biomass, the ethanol yields produced by *Saccharomyces cerevisiae* SR8 varied from 205.0 to 275.6 g/kg biomass, these ethanol yields are comparable with those of switchgrass, corn stover and bagasse. Guo (2017) mentioned that the feedstock quality of prairie cordgrass was characterized by 292.9-316.3 g/kg HC, 399.8-421.4 g/kg Cel, 63.3-73.8 g/kg lignin. Thapa et al. (2022) found that *Spartina pectinata* contained 30.07-31.75% HC, 37.20-38.66% Cel, 4.99-5.67% lignin, 4.97-5.47 % ash, *Andropogon gerardii* 30.61% HC, 39.91% Cel, 5.47% lignin, 4.80% ash; *Miscanthus* × *giganteus* 29.11 % HC, 40.00% Cel, 6.31% lignin, 3.63 % ash; *Panicum virgatum* 31.04 % HC, 38.88% Cel, 5.09% lignin, 4.51 % ash.

From the perspective of the direct combustion of solid biomass, knowing its properties is essential. The moisture content, ash content, elemental composition of biomass are significant assets that define dry biomass and are key factors that affect the calorific value, technologies for the production of solid biofuels that are clean and efficient in energy conversion systems The higher carbon and hydrogen contents, the higher the calorific value. In contrast, high oxygen and nitrogen values decrease the calorific value, decreasing the energy potential of the sold biofuel. Chlorine, nitrogen and sulphur have an effect on ash formation as well as significant impact on the formation of harmful emissions, mainly mono-nitrogen oxides and sulphur oxide and highly corrosive hydrochloric acid. The gas produced by combustion of sulphide and chloride is the main substance of acid rain, which have huge environmental impact. The elemental composition, ash content and calorific value of biomass and solid fuel from studied *Poaceae* species are illustrated in Table 4. We would like to mention that the dry matter of the studied biomass contained 44.35-45.87% C, 5.31-5.75% H, 0.30-0.83% N, 0.06-0.12% S, 0.04-0.05% Cl, 3.56-4.48% ash, 18.64-19.51 MJ/kg GCV. and 17.69-18.46 MJ/kg NCV. The higher content of carbon and the lower content of nitrogen and ash in *Spartina pectinata* biomass have positive impact on the calorific value as compared with *Zea mays* and *Sorghum almum.* The nett calorific value of pellets varied from 17.59 MJ/kg to 18.50 MJ/kg and - of briquettes from 17.24 MJ/kg to 18.20 MJ/kg. The *Spartina pectinata* densified solid fuel, namely, pellets and briquettes, is characterized by excellent nett calorific value (18.20-18.50 MJ/kg).

Table 4. The elemental composition, ash content and calorific value of studied dry biomass

Indices	*Spartina pectinate*	*Sorghum almum*	*Zea mays*
Carbon	45.87	44.35	45.17
Hydrogen	5.31	5.60	5.75
Nitrogen	0.30	0.41	0.83
Sulphur	0.08	0.06	0.12
Chlorine	0.04	0.05	0.04
Ash content of biomass, %	3.56	4.42	4.48
Gross calorific value, MJ/kg	19.51	18.64	18.78
Nett calorific value of pellets, MJ/kg	18.50	17.59	17.77
Nett calorific value of briquettes, MJ/kg	18.20	17.24	17.54

Some authors mentioned various findings about the elemental composition, calorific value of biomass and quality of solid fuel from *Spartina* species. Kowalczyk-Juśko (2010) found that the elemental composition and energy parameters of *Spartina pectinata* biomass were 4.3-5.4% ash, 41.92-42.73% C, 0.31-0.72% N, 0.08-0.14% S, 14.90-15.38 MJ/kg calorific value, as compared with hard coal: 4.3-5.4% ash, 66.57 % C, 1.16% N, 0.46% S, 26.1 MJ/kg calorific value. Stolarski et al. (2014) mentioned that the energy biomass harvested in April from *Spartina pectinata* contained 49.17% C, 5.79% H, 0.054% S, 2.58% ash, 19.09 MJ/kg HHV and 14.48 MJ/kg LHV, but *Miscanthus x giganteus* biomass contained

50.43% C, 5.84% H, 0.028% S, 1.90% ash, 19.18 MJ/kg HHV and 14.16 MJ/kg LHV. Uchman et al. (2016) remarked that *Spartina pectinata* contained 8.0% moisture, 3.24% ash, 77.5% volatile materials, 45.8 % C, 7.28% H, 0.26% N, 1.45% S, 19.29 MJ/kg LHV, but *Sida hermaphrodita* 9.0% moisture, 2.6% ash, 78.8% volatile materials, 44.8 % C, 7.4% H, 0.37% N, 1.40% S, 19.0 MJ/kg LHV, *Miscanthus x giganteus* 7.6% moisture, 1.36% ash, 75.4% volatile materials, 46.6 % C, 7.16% H, 0.16% N, 1.35% S, 19.45 MJ/kg LHV, *Panicum virgatum* 8.5% moisture, 3.23% ash, 78.1% volatile materials, 46.0 % C, 6.9 % H, 0.55% N, 1.43% S, 18.35 MJ/kg LHV. Maj& Piekarski (2013) reported that the pellets made from prairie cordgrass contained 7.55% moisture, 4.86 % ash, the heat of combustion reached the value of 18.31 MJ/kg and the calorific value was 16.93 MJ/ kg. Guo (2017) remarked that the prairie cordgrass feedstock contained 43.7-50.7 g/kg ash, 6.1-7.5 g/kg N, 0.9-1.0 g/kg S. Urbanovičová et al. (2017) remarked that the briquettes from *Spartina pectinata* reached a density of 800 kg/m^3 and 95% durability with calorific value 14-17 MJ/kg, but the briquettes from *Sida hermaprodita* - 820 kg/m^3 and 95% durability with calorific value 15 MJ/kg, respectively.

CONCLUSIONS

The introduced ecotype of prairie cordgrass, *Spartina pectinata*, under the climatic conditions of the Republic of Moldova, was characterized by optimal growth rates and productivity.

The *Spartina pectinata* fresh mass substrate used for anaerobic digestion contained 43 g/kg CP, 66 g/kg ash, 437 g/kg Cel, 301g/kg HC, 59g/kg ADL, 55 g/kg TSS, with C/N = 74 and biochemical methane potential 299 l/kg, but the ensiled substrate - 44 g/kg CP, 76 g/kg ash, 473 g/kg Cel, 323 g/kg HC, 57 g/kg ADL, with C/N = 72 and biochemical methane potential 303 l/kg.

The prairie cordgrass solid dry biomass harvested in winter period contained 453 g/kg Cel, 294 g/kg HC, 82 g/kg ADL, the estimated theoretical ethanol yield averaged 543 L/t.

The prepared solid fuel, briquettes and pellets, had significantly higher net calorific value than perennial sorghum and corn stem fuel. The investigated introduced taxa of prairie cordgrass, may be used as multi-purpose feedstock for renewable energy production in Moldova.

ACKNOWLEDGEMENTS

The study has been carried out in the framework of the projects: 20.80009.5107.02 *"Mobilization of plant genetic resources, plant breeding and use as forage, melliferous and energy crops in bioeconomy"*.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE EVOLUTION OF AGRICULTURAL YIELDS. A CASE STUDY ON TIMIS COUNTY

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Abstract

Over the last period of time, we are witnessing a perturbation of the climate, this having a direct influence over the agricultural yields. Luckily, on one hand Timis County has a soil with a higher quality, suitable for a large span of crops due to the close levels of groundwater, thus granting the crops to overcome more easily long periods of drought. On the hand modern agricultural equipment grants the farmers the usage of specifical crop technologies, in order to keep the water into the soil and to prevent erosion and the scarce effects of drought. This study aims to follow the evolution of yield performance over the course of three years - 2018, 2019 and 2020. The performance of agricultural establishments can be appreciated through the results obtained over the course of production process. Although over the course of the three years deviations of both rainfall and temperatures were registered, superior yields were reported for all Timis County.

Key words*: yield, climate change, agricultural production, evolution.*

INTRODUCTION

Although the Timiș County has an agricultural potential above the average of most agricultural regions in Romania, the context of economic and social changes recorded in Romanian society after 1989 has determined a sinuous stage of redefinition, determined by a series of socio-economic processes such as: re-privatization, economic restructuring, rural-urban migration, external migration, intense development of localities located near major cities, or depopulation of isolated localities. (Toma et al., 2009; Otiman, 2011; MADR, 2014; Vasile et al 2006).

All these phenomena have left their mark on the level of productivity and profitability of this sector with very good climatic favorability conditions, land with natural fertility superior to other regions, and a tradition in cultivating plants. (Da Rocha, 2006; Afonso, 2007; Antohi, 2011; Eftimie & Matei, 2014; Panait & Alecu, 2016).

With a total area of 869,665 ha (3.65% of the total area of the country), respectively 702,066 ha of agricultural land (80.7% of the total area), of which: 531,037 ha arable land (of which: irrigated arable land = 7,011 ha, non-irrigated arable land = 526,113), 121,347 ha natural pastures, 28,619 ha natural meadows, 3,871 ha vineyards, 9,171 ha orchards (of which: irrigated = 240 ha), 82 ha grapevine plantations. Timiș county is the most important agricultural county in the country, both in terms of cultivated area and volume of production.

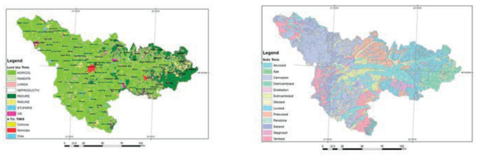
Funds attracted through financing programs for agriculture, as well as private and foreign investments in this sector, have led to the consolidation of agricultural land and the establishment of commercial agricultural farms, which support the level of production achieved.

MATERIALS AND METHODS

Through this study, an analysis was conducted on the agricultural potential of the county, expressed through the natural fertility and suitability of agricultural land for cereal crops, legumes and technical plants.

Most of the county is occupied by zonal soils, namely in the north-western part there are leached chernozems and chernozems of hayfields, and then in the hilly area, different types of ilvastre soils succeed from west to east.

The soil of Timiș county offers favorable conditions for the cultivation of agricultural crops, especially for cereal crops, but also for technical and forage plants, fruit trees and grapevines (Figures 1 and 2).



(a) (b)

Figure 1. (a) Land use in Timis County;
(b) Soil types in Timis County

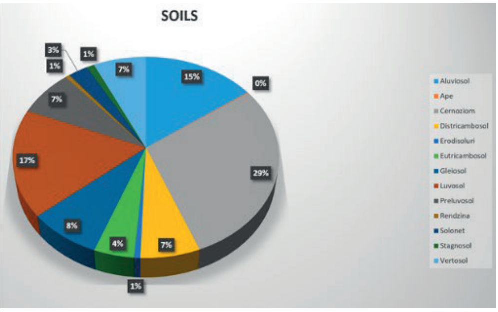


Figure 2. The distribution of soil types in Timis County

From the perspective of the legal status of those working on agricultural land in Timiș County (Figure 3), it can be observed that 55% belongs to legal entities and 45% to individuals, of which 33% are men and only 13% are women.

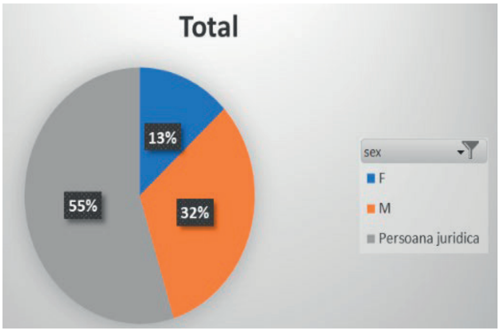


Figure 3. Distribution by legal status

RESULTS AND DISCUSSIONS

Compared to the reference year 2018, in which the largest cultivated area was recorded (507,536 ha), in 2019 the cultivated area decreased by 7,206 ha. However, this reduction was due to owners who hold small land areas abandoning their cultivation due to various reasons, especially aging and lack of financial resources. In 2020, the year when the Covid-19 pandemic started, the cultivated area decreased significantly by 165,013 ha compared to the area cultivated in 2018 (Table 1). The main reason for this situation was the restrictions imposed at the beginning of the spring campaign and the delays in the supply chain for inputs, which made it impossible for large agricultural farms in the county to establish spring crops. As a result, this reduction in the private sector's cultivated area is also observed.

Table 1. Evolution of the cultivated areas in Timiș County

Cultivated area	2018		2019		2020	
	Total (ha)	Privat (ha)	Total (ha)	Privat (ha)	Total (ha)	Privat (ha)
	507.5	502.9	500.3	496.1	342.5	337.4
%	100	100	99	99	67	67
Diff			-7.20	-6.821	-165.0	-165.4

Table 2 presents the evolution of wheat cultivated areas, average production, and total production at the level of Timiș County during the analyzed period.

Table 2. The evolution of cultivated areas, average and total wheat yield at the level of Timiș County

	Total To	Avg. kg/ha	Total To	Avg. kg/ha	Total To	Avg. kg/ha
Year	2018		2019		2020	
Yield	702.36	5.12	735.01	5.50	502.58	4.64
Diff			32.65	378	-199.77	-474
Area	137100		133615		108106	
Diff			-3484		-28994	

The largest area cultivated with wheat was achieved in 2018, with 137,100 hectares, followed by the area in 2019 with 133,615 hectares, a decrease of 3,484 hectares, mainly due to the areas cultivated by individuals, which also contributed to the reduction of the total cultivated area in the county. However, the weather conditions in the autumn of 2019, especially the severe drought from June to September, followed by a period of almost continuous rainfall in October, led to a reduction in the wheat sown area by 28,994 ha. Regarding the average yields achieved, these ranged from 4,649 kg/ha in 2020 to 5,501 kg/ha in 2019. Data regarding the total wheat production in Timiș County show yields ranging from 502,583 tons in 2020, a year with the smallest cultivated area and the lowest average production per hectare, to 735,018 tons in 2019, a year that recorded a smaller cultivated area of approximately 3,500 hectares, but whose average production favored obtaining the highest total production. In fact, of the three years analyzed, 2019 was the most favourable for wheat cultivation.

Table 3 shows the evolution of the areas, average and total yields of corn in Timiș County during the analyzed period.

Table 3. The evolution of the surface area, average and total production of corn at the level of Timiș County

	Total	Avg.	Total	Avg.	Total	Avg
	To	kg/ha	To	kg/ha	To	kg/ha
Year	2018		2019		2020	
Yield	1.505.7	9.42	1.726.0	10.42	741.71	6.639
Diff			220.35	995	-764.01	-2.786
Area	159759		165652		111721	
Diff			5892		-48038	

The highest maize yield was achieved in 2019 (1,726,089 tons), the year when the largest area of 220,359 ha was cultivated and the highest average yield of the analyzed period was achieved (10,420 kg/ha). It is worth mentioning that 2019 was an exceptional year from a climatic point of view, especially in terms of the distribution of precipitation during the maize growing season. In 2020, the restrictions imposed by the pandemic and the problems in securing inputs (customs blockades and the inability to secure hybrid seeds and fertilizers) were the causes that led to a drastic reduction of the cultivated area by 48,038 ha, and due to the mentioned reasons, the average yield was only 6,639 kg/ha.

Table 4 presents the evolution of the areas, average yields, and total yields of rye in Timiș County during the analyzed period.

Table 4. Evolution of the areas, average and total yields of rye at the level of Timiș County

	Total	Avg.	Total	Avg.	Total	Avg
	To	kg/ha	To	kg/ha	To	kg/ha
Year	2018		2019		2020	
Yield	3	2.000	59	1.123	92	5111
Diff			56	-877	89	3111
Area	1,5		53		18	
Diff			51		17	

Rye, a cereal crop with many agricultural uses and advantages, is very rarely cultivated in Timiș County. However, based on the analyzed data starting from 2019, this crop is on an upward trend both in terms of cultivated areas and average yields per hectare.

Table 5 shows the evolution of the cultivated areas, average yields, and total yields of barley in Timiș County during the analyzed period.

Table 5. Evolution of the areas, average and total yields of barley at the level of Timiș County

	Total	Avg.	Total	Avg.	Total	Avg
	To	kg/ha	To	kg/ha	To	kg/ha
Year	2018		2019		2020	
Yield	33.19	5.867	70.14	5.15	49.13	4.73
Diff			36.95	-709	15.94	-1.13
Area	5657		13600		10373	
Diff			7943		4716	

It should be noted that barley is also on an upward trend in terms of both cultivated areas and average yields per hectare, with the largest areas being cultivated in 2019 (16,600 ha) and 2020 (10,373 ha). However, the Timiș County provides very favourable conditions for wheat cultivation, which is why farmers have significantly reduced the areas cultivated with other cereal crops.

In table 6, the evolution of the cultivated areas, average yields, and total productions of triticale in Timiș county is presented for the analyzed period. By analyzing the data related to the cultivated area and yields of triticale, it can be observed that this crop is grown on approximately 3,500-4,000 ha each year, with an average yield ranging between 4,700-5,400 kg/ha.

Table 6. Evolution of the areas, average and total yields of triticale at the level of Timiș county

	Total	Avg.	Total	Avg.	Total	Avg
	To	kg/ha	To	kg/ha	To	kg/ha
Year	2018		2019		2020	
Yield	20.642	5.339	18.093	4.968	19.758	4.799
Diff			-2.549	-371	-884	-540
Area	3866		3642		4117	
Diff			-224		251	

Table 7 shows the evolution of the areas, average yields, and total production of oats in Timiș County during the analyzed period. The areas cultivated with oats in Timiș County during the analyzed period ranged from 3055 ha in 2020 to 3294 ha in 2019, while average yields ranged from 2300 kg/ha to approximately 3000 kg/ha. It should be mentioned that the areas cultivated with oats are of the spring type, and farmers only cultivate them on those areas where they were not able to sow wheat in the fall

Table 7. Evolution of the areas, average and total yields of triticale at the level of Timiș County

	Total To	Avg. kg/ha	Total To	Avg. kg/ha	Total To	Avg kg/ha
Year	2018		2019		2020	
Yield	8.981	2.797	9.581	2.909	7.292	2.387
Diff			600	112	-1.68	-410
Area	3211		3294		3055	
Diff			83		-156	

Table 8 presents the evolution of sunflower acreage, average and total production in Timiș county during the analyzed period. The data highlights the importance of this crop in the structure of crops at the county level, except for the issues mentioned in 2020. In 2018, the cultivated area of sunflowers reached 80,383 ha, with an average production of 2548 kg/ha and a total production of 204,816 tons. In the 2019 agricultural year, the cultivated area reached 78,239 ha, with an average production of 3529 kg/ha and a total production of 276,105 tons. We consider that the good distribution of precipitation during the sunflower vegetation period in 2019 was the main cause for obtaining an average production of over 3500 kg/ha.

Table 8. Evolution of the areas, average and total yields of sunflower at the level of Timiș County

	Total To	Avg. kg/ha	Total To	Avg. kg/ha	Total To	Avg kg/ha
Year	2018		2019		2020	
Yield	204.81	2.548	276.10	3.529	61.716	2.640
Diff			71.289	981	-143.1	92
Area	80383		78239		23377	
Diff			-2144		-57006	

Table 9 presents the evolution of the areas, average and total yields of rapeseed at the level of Timiș county, during the analyzed period. Rapeseed, the second oilseed crop both nationally and in Timiș county, covers areas of around 24000-27000 ha, not due to lack of interest from farmers but due to restrictions on crop rotation with sunflower and soybeans (annually, the cultivated areas with these three oilseed crops represent 30% of the arable land in the county). Average yields, depending on weather conditions, range between 2600 kg/ha and 3100 kg/ha.

Table 9. Evolution of the areas, average and total yields of rapeseed at the level of Timiș County

	Total To	Avg. kg/ha	Total To	Avg. kg/ha	Total To	Avg kg/ha
Year	2018		2019		2020	
Yield	80.251	2.935	43.551	2.670	76.933	3.104
Diff			-36.70	-265	-3.318	169
Area	27343		16311		24785	
Diff			-11032		-2558	

In table 10, the evolution of soybean surfaces, average and total production in Timiș County is presented for the analyzed period. Soybean is also an important crop and present in the crop structure, especially in commercial farms. The largest area cultivated in the analyzed period was in 2018, with 31,070 ha. The highest average yield per hectare was obtained in 2019 (2800 kg/ha), a very favorable year in terms of accumulated precipitation during the vegetation period.

Table 10. Evolution of the areas, average and total yields of rapeseed at the level of Timiș County

	Total To	Avg. kg/ha	Total To	Avg. kg/ha	Total To	Avg kg/ha
Year	2018		2019		2020	
Yield	55.336	1.781	59.352	2.800	33.307	2.141
Diff			4.016	1.019	-22.02	360
Area	31070		21197		15557	
Diff			-9873		-15513	

Table 11 presents the evolution of the surfaces, average and total production of sugar beet at the level of Timis County, during the analyzed period. Sugar beet, with all the advantages it offers both economically and agronomically, being a very good crop for rotation, has a very small share in the crop structure of Timis County. In practice, farmers were forced to gradually reduce the cultivated areas and abandon this crop starting from 2020, due to the closure of processing factories, despite the fact that there were very favorable conditions in terms of soil fertility, temperature, and humidity.

Table 11. Evolution of the areas, average and total yields of sugar beet at the level of Timiș County

	Total To	Avg. kg/ha	Total To	Avg. kg/ha	Total To	Avg kg/ha
Year	2018		2019		2020	
Yield	53.08	60.45	10.29	17.09		
Diff			-42.79	-43.36		
Area	878		602			
Diff			-276			

Table 12 presents the evolution of the areas, average and total yields of fodder beet at the level of Timiș County, during the analyzed period. As far as the cultivated areas and average yields of fodder beet in Timiș County are concerned, around 150 ha are cultivated with an average yield of 20 tons/ha.

Table 12. Evolution of the areas, average and total yields of sugar beet at the level of Timiș County

	Total	Avg.	Total	Avg.	Total	Avg
	To	kg/ha	To	kg/ha	To	kg/ha
Year	2018		2019		2020	
Yield	2.960	20.600	2.664	18.600	2.664	19.800
Diff			-296	-2.000	-296	-800
Area	144		143		135	
Diff			-1		-9	

CONCLUSIONS

The geographic conditions and suitability of arable land in Timiș County are accurately reflected by the crop structure. The most significant areas, around 70% of arable land, are cultivated with cereals, with wheat and corn representing 90% of these crops. In terms of territorial favorability, it can be said that the restrictive factor is caused by the lack of moisture during the vegetation period. In terms of thermal and natural fertility, there are very favorable conditions for a large number of species. Oilseed crops such as sunflower and rapeseed occupy about 20% of the county's arable land, and if soybean areas are added, the percentage increases to 25%, which is beneficial in terms of ensuring a minimum three-year rotation. The conditions generated by the Covid-19 pandemic have had an impact on both cultivated areas and the average yields achieved in 2020. Another beneficial aspect for obtaining high and consistent yields is represented by the method of cultivation of these areas. At the county level, 55% of the areas are cultivated within commercial farms, following appropriate technologies, which are reflected in the level of yields obtained in the end.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVOLUTION THE CURRENT SITUATION OF GREEN STINK BUGS POPULATIONS IN AGRICULTURAL AREAS OF TIMIS COUNTY AND EFFECTIVE CONTROL STRATEGIES

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Abstract

Green stink bugs (as polyphagous pest) are still active in agroecosystems in Romania producing negative effects in various types of agricultural and horticultural areas. In this paper, we focused on the population capacity of the pest in 8 localities belonging to Timis County, each representing a type of area, but also on the monthly dynamics during 5 months (June-October) from 2019-2022. We also focused on testing both chemical and non-polluting control products currently used by farmers and gardeners in the western part of the country. From the ones analysed, we found that in all the monitored localities the pest was present at varying levels from low to high. Regarding the monthly dynamics, the most specimens were observed in August and September, with a maximum recorded in 2020 followed by 2021. The most effective control strategy involves a chemical product applied individually, i.e Karate Zeon (lambda - cihalotrin) but also the Laser 240 SC (spinosad) product, 1-2 applications. Their combination is essential in keeping harmful populations under control.

***Key words**: control, agroecosystem, green stink bug, populations.*

INTRODUCTION

The origin of the green stink bug (*Nezara viridula* L.) (Hemiptera: Pentatomidae) is debatable, some mention that it comes from Asia area (Yukawa and Kiritani, 1965) and others from the African area (Hokkanen, 1986). One thing is certain, however, that it is expanding more and more in the temperate areas, not necessarily warm like the ones of origin. Later, the species spread everywhere, in Africa (Poutouli, 1995;), Asia (Kiritani and Hokyo, 1962; Kaul et al., 2007; Dehghani-Zahedani et al., 2022), Australia (Clarke, 1992), New Zeeland (Rea, 1999), South America (Panizzi, 2008), North America (Jones, 1988; Sosa-Gomez and Moscardi, 1995; Capinera, 2001) and Europe (Colazza et al., 1985; Rédei and Torma, 2003). In Romania, green stink bug has been present and identified as pest since 2010 (Grozea et al., 2012) and then it has spread to the east (Kurzeluk et al., 2015).

There is no complete list of plant species that it damages, but it certainly falls into the category of polyphagous with a wide spectrum of cultivated or wild plants (Solanaceae, Cruciferae, Malvaceae, Poaceae) (Panizzi and Slansky, 1991; Panizzi, 1997).

The great ability to spread in different places has made the pest species adapt to new and new plant species, both in the category of agricultural plants and horticultural plants.

Thus, *Nezara virdula* in all its damaging forms (nymphs and adults) was found on the aerial organs of vegetable (Uddin et al., 2013; Girish et al., 2014; Esquivel, 2016; Looney, 2019), agricultural (Costea and Grozea, 2022; Edde, 2022) or ornamental plants (Ciceoi et al., 2017).

In Africa, Asia and the U.S., the pest is considered of great economic importance, the production losses being high (Edde, 2022).

The control strategies adopted until now are diverse and applicable depending on the economic level or the geographical location. High costs with chemical treatments are associated with Africa (Taylor et al., 2018). But there are other approaches, such as biological ones (natural enemies, biopesticides) that are applied, but especially in the US, Europe and Australia (Knight and Gurr, Marcu and Grozea, 2018; 2007; Ademokoya et al., 2022).

Although it has been present in Romania for some time, there have been no comprehensive monitoring investigations (as far as we know) or in finding solutions to stop it except at the level of closed space as solar system (Marcu and Grozea, 2020). Continuous adaptation to new host plants could be a future problem for agro-horticultural crops.

In this context, we proposed that through this work we update the situation of the harmful species *Nezara viridula* and try to find combined solutions to keep it below the economic threshold of damage.

MATERIALS AND METHODS

The experimental design

In order to establish the population level of the pest *Nezara viridula* for the period 2019-2022, we made observations in 8 localities in Timis county (Figure 1). Their choice was made based on the availability and variety of cultures. Being known that the pest has a wide range of host plants, we focused on plants already established as host plants.

Figure 1. The positioning of the places where the observations were made

In order to facilitate the interpretation of population level results, each place was coded according to the data in Table 1. The study places, more precisely, localities were coded as follows: TI-1-GS, PI-2-F, PE-3-GA, GR-4-F, JI-5-GS, NI-6-F, DU-7-FO and IO-8-GA being associated with a certain category of crops or plants. Among these, we chose to focus on corn and sunflower (as agricultural field plants), tomato and pepper (as vegetables from garden) or lilac purple, magnolia and elderberry (as ornamental plants from green spaces or forest).

Depending on the category of the host plant, we delimited the surfaces to be analysed (Table 1). So, for the large field crops, 300m^2, for those in the gardens, we chose smaller areas (available) of 100m^2 and for the ornamental plants in green spaces (shrubs) we went for 5 plants/approximately 50m^2.

Months of observations

For the quantitative and evolutionary establishment of green sting bugs from the above-mentioned localities, we chose to make monthly observations, between the 10th and 20th day of each month (June-October) covering the period of intense vegetation for plants/crops, the activity of nymphs and adults of the pest but also the movement to all the distant localities.

Testing of chemical products and bioinsecticides

In order to find the best control strategy, we tested in 2022 a chemical product and a bioinsecticide currently used by farmers and gardeners in the western part of the country.

Thus, in laboratory (Phytosanitary Diagnosis and Expertise Laboratory from ULST) we tested 1 chemical product (Karate Zeon, a.s lambda - cihalotrin) of 0.02%) and 1 bioinsecticide (Laser 240 SC, a.s spinosad in dose of 0.05%) in 3 repetitions (R1, R2, R3).

Since testing in the field would have been difficult to do because in an open field green stink bugs can migrate immediately after treatments, we resorted to testing in a closed space, using parts of corn plants 30 insects each collected from the field (GR-4-F) and the tomato garden (PE-3-GA).

Statistical interpretation of data

For the assessment of the population level of the pest, descriptive statistics were made regarding the mean, minimum, maximum value, standard deviation, quartiles, asymmetry coefficient and variance, and for the monthly evolution, comparative graphs.

In order to establish the effectiveness of the control products, we resorted to the Significance Test (Duncan) by which the active pests remaining in the terrariums were evaluated.

Table 1. Identification and characterization of study sites

Place (Code)	Identification of GPS coordinates	Category	Plants or crops	Areas or plants analyzed
Timisoara (TI-1)	45°46'55.2"N 21°13'48.1"E	Green space (GS)	Lilac purple	5 plants/50 m^2
Pischia (PI-2)	45°87'61.5"N 21°42'01.1"E	Field (F)	Corn	300 m^2
Pesac (PE-3)	45°99'45.8"N 20°83'02.4"E	Garden (GA)	Tomato	100 m^2
Grabat (GR-4))	45°52'26.9"N 20°45'13.7"E	Field (F)	Corn	300 m^2
Jimbolia (JI-5)	45°79'75.3"N 20°73'67.5"E	Green space (GS)	Magnolia	5 plants/50 m^2
Nitchidorf (NI-6))	45°58'62.1"N 21°51'49.7"E	Field (F)	Sunflower	300 m^2
Dumbravita (DU-7)	45°78'30.7" N, 21°24'19.8"E	Forest (FO)	Elderberry	5 plants/50 m^2
Iohamisfield (IO-8)	45°5685.5"N 52°75'53.7"E	Garden (GA)	Pepper	100 m^2

RESULTS AND DISCUSSIONS

Evaluation of the population level and monthly dynamics

In all the places analysed, the active stages were present, both larvae and nymphs. They were present on all the relevant aerial organs of plants, on leaves, inflorescences, corn cobs, stems and fruits (in the case of vegetables) (Figure 2).

Figure 2. Green stink bugs in different forms on various plants: 1. adult on the lilac plant; 2. nymph in advanced stage on corn; 3. nymph in early stage on tomato

The population level was varied, and from what we found, a high level was recorded in populated cities and less so in smaller villages or open countryside (Figure 3). Which also explains the results of other researchers who integrate green stink bugs into the category of cosmopolitan pests (Aldrich et al., 1987).

The size of the populations over the entire period is varied from 8 to 464 specimens (Figure 3).

We also found that the type of plants or space is important, in gardens and green spaces, forests the pest was more present than in the field.

The most adults and nymphs were found in 2020 (550 specimens), followed by 2021 (256 specimens), then 2019 (239 specimens) and 2022 (152 specimens) (Figure 3). A sudden increase is observed in the period 2019-2020, then a gradual decrease (2021-2022).

From the descriptive analysis of the raw data (Table 2) it was extracted that the most pests were present in places with green spaces, namely x = 116.5 /TI-1-GS followed by forest with an average value of x = 49.7/DU-7-FO and then of gardens with x = 24.2/PE-3-GA. Regarding the open fields with corn and sunflower, the values were lower (x = 2.0/PI-2-F and x = 0.80/NI-6-F0.800). Intermediate values from all categories of spaces were placed at the average value of 48.2/ IO-8-GA, 20.1/GR-4-F) but also x = 38.1/(JI-5-GS).

The monthly evolution shows a gradual increase starting with June and reaching the peak in August (287 specimens) then a sudden increase until September (Figure 4). The fewest green stink bugs were recorded in June (4 specimens) but also in September (7 specimens).

Practically, it is a natural evolution that follows the course of the development of the stages of hemimetabolous insects from hibernating adults, nymphs and then the new adults to which the availability of food and climatic conditions have contributed. August is known as the hottest month of the year and green sting bugs are thermophilic species.

However, it was not possible to quantify the possibility that some of individuals temporarily migrated to other plants, especially in large spaces, such as those of corn and sunflower, therefore the values can be considered estimates. There is still no clear and efficient method for quantifying of green stink bugs in general.

Table 2. Descriptive statistics for number of green stink bugs observed in the study sites in Timis County

Place (code)	Descriptive elements[1] No. stink bugs/2019-2022							
	Mean	Min.	Max.	Low.Q.	Up. Q	R	Var.	SD
Timisoara (TI-1-GS)	116.500	0.00	366.000	0.00	180.000	366.000	299.10	176.5423
Pischia (PI-2-F)	2.0000	0.00	26.000	0.00	12.500	26.000	19.00	11.160
Pesac (PE-3-GA)	24.2000	0.00	142.000	0.00	71.000	142.000	4056.30	69.0811
Grabat (GR-4-F)	20.1000	0.00	102.000	0.00	55.000	103.000	2999.20	57.1600
Jimbolia (JI-5-GS)	38.100	0.00	166.000	0.00	20.000	166.000	3911.10	79.125
Nitchidorf (NI-6-F)	0.800	0.00	1.9000	0.00	0.9000	1.9000	8.07	10.005
Dumbravita (DU-7-FO)	49.700	0.00	222.000	0.00	45.000	222.000	890.40	94.1570
Iohamisfield (IO-8-GA)	48.200	0.00	102.000	0.00	59.000	102.000	44.20	12.160

[1]mean, minimum, maximum, quartiles (lower and upper), range, variance standard deviation

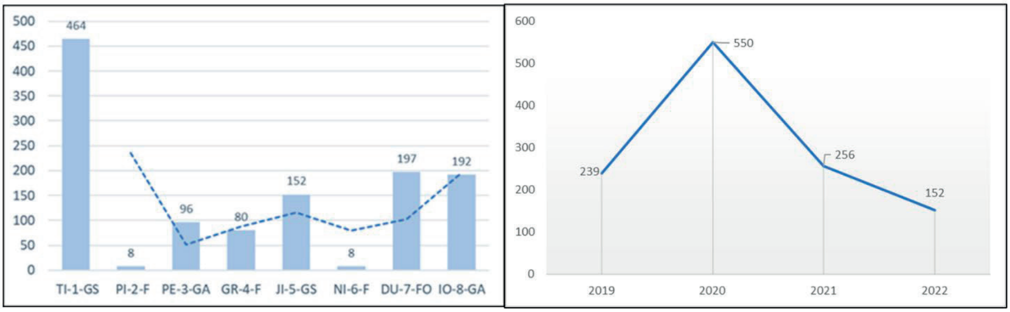

Figure 3. Total number of green stink bugs recorded in the period 2019-2022
in the study sites and annually where GS- Green space, F-Field, GA- Garden, FO-Forest

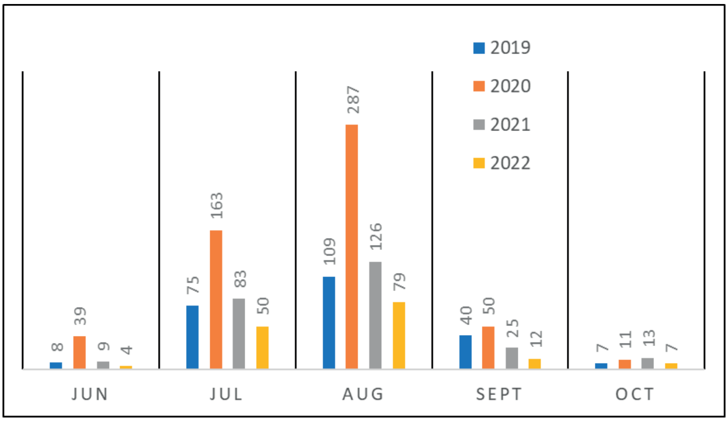

Figure 4. The monthly evolution (June-October) of the pest in the period 2019-2022

Assessing the effectiveness of treatments

From the analysis of the descriptive statistical data between the variants representing untreated, treated with insecticides and with bioinsecticides at different intervals (Table 3), the values were varied. Thus, in the Control variant, the most active stink bugs were recorded both at 3, 7 and 10 days (x = 25,000), while in the variants with insecticide Karate Zeon, lambda - cihalotrin the values were much lower, more chosen 7 and 10 days after their application (x = 6.50 and x = 1.50/Karate Zeon). In the versions with bioinsecticide (Laser 240 SC) it was found that the number of

green stink bugs remained at medium and high values. On the 3rd day after the application of the bioinsecticide there were on average 23.00 individuals, then after 7 days there were 15.70 and on the 10th day, 12.35 individuals remained.
The statistical analysis of the results by expressing the significance test ($p<0.05$, $p>0.05$) from Table 4, shows that between all the Control variants (a, b, c) and the variants with the insecticide Karate Zeon on 7th day and the 10th day after the treatment, there is significant differences, i.e. p=0.039 (a-e), p=0.048 (b-e), p=0.047 (c-e) and p=0.034 (a-f), p=0.035 (b-f) and p=0.0345 (c-f). And between the Control variants and those in which the Laser 240 SC bioinsecticide was applied at 10 days, there were significant differences (p=0.042 (a-i), p=0.041(b-i) and p=0.039(c-i).

Table 3. Statistical interpretation of values through descriptive elements expressing number of live green stink bugs left after treatments

Variable	Descriptive elements[1] Number of live green stink bugs left after treatments (control, insecticide and bio-insecticide)							
	Mean	Min.	Max.	Low.Q.	Up. Q	R	Var.	SD
Control (3rd day)	25.000	10.000	30.000	10.000	30.000	30.000	100.00	9.469
Control (7th day)	25.000	10.000	30.000	10.000	30.000	30.000	100.00	9.469
Ins (3rd day)[2]	20.350	7.000	24.000	7.500	24.000	6.000	87.326	8.824
Ins (7th day)[2]	6.550	2.000	14.000	2.500	12.500	12.000	42.351	5.569
Ins (10th day)[2]	1.500	1.000	3.000	1.500	3.500	6.000	13.000	3.155
BioIns (3rd day)[2]	23.000	8.000	27.000	9.500	27.500	22.000	93.153	8.588
BioIns (7th day)[2]	15.700	7.000	22.000	7.500	20.500	17.000	42.693	7.234
BioIns (10th day)[2]	12.350	5.000	17.000	5.500	17.000	7.000	14.336	5.119

[1]mean, minimum, maximum, quartiles (lower and upper), range, variance standard deviation
[2]Ins- Karate Zeon, BioIns- bioinsecticide Laser 240 SC

Table 4. Approximate probabilities by Duncan's Test for evaluating the effectiveness of treatments according to the number of live green bugs at different day intervals

Variable 1 (Var.2)	The Duncan test for evaluating the effectiveness of treatments by the number of green stink bugs alive. at different day intervals Approximate Probabilities for Post Hoc Test								
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
Co(3rd d)(a)		1.00	1.00	0.55	0.039	0.034	0.89	0.13	0.042
Co(7th d)(b)	1.00		1.00	0.58	0.048	0.035	0.88	0.140	0.041
Co(10th d(c)	1.00	1.00		0.62	0.047	0.0345	0.785	0.11	0.039
Ins1(3rd d)(d)	0.55	0.58	0.62		0.35	0.56	0.099	0.25	0.41
Ins1(7th d)(e)	0.039	0.048	0.047	0.35		0.76	0.048	0.046	0.035
Ins1(10th d)(f)	0.034	0.035	0.0345	0.56	0.76		0.01	0.022	0.25
BioIns(3rd d)(g)	0.89	0.88	0.785	0.099	0.048	0.01		0.44	0.21
BioIns(7th d)(h)	0.13	0.130	0.11	0.25	0.046	0.022	0.44		0.09
BioIns(10th d)(i)	0.042	0.041	0.039	0.41	0.035	0.25	0.21	0.09	

(a)-Control variant after 3 days; (b)-Control variant after 7 days; (c)-Control variant after 10 days; (d)-variant with Karate Zeon insecticide after 3 days; (e)-variant with Karate Zeon insecticide after 7 days; (f)-variant with Karate Zeon insecticide after 10 days; (g)-variant with Bio-insecticide Karate Zeon after 3 days; (h)-variant with Bio-insecticide Karate Zeon after 7 days; (i)-variant with Bio-insecticide Karate Zeon after 10 days

Between the variants with bioinsecticide at 3 and 7 days and those with Karate Zeon insecticide at 7 and 10 days there were significant differences, where $p<0.05$, namely p=0.048 (g-e), p=0.01 (g-f) and p=0.046 (h-e), p=0.022 (h-f). And between the bioinsecticide applied at 10 days and Karate Zeon insecticide applied at 7 days, with p=0.035 (i-e) there are differences.

CONCLUSIONS

From the results in this paper, it appears that green stink bugs are still present in agricultural and horticultural crops as well as forests, and the population level is one to be taken into account, even if the trend is of a slight decrease.
It is obvious that they prefer places such as cities or gardens near people's houses, and which we suspect are protected from the wind and retain warm air.
Regarding the control, from our tests we found that insecticide Karate Zeon (lambda-cihalotrin) are effective after 7-10 days after application. Likewise, the bioinsecticide Laser 240 SC (spinosad) can be considered effective to a lesser extent.

With all this in mind, we consider that application with tested insecticide (Karate Zeon) alternating with the tested bioinsecticide could be the ideal combination for keeping the pest under control and avoiding over pollution of the environment.

ACKNOWLEDGEMENTS

This research work was carried out with the support of owners of crops and gardens from Timis county. The testing of control products was carried out in the Phytosanitary Diagnosis and Expertise Laboratory of the University of Life Sciences "King Mihai I" from Timisoara.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

BIOLOGICAL FEATURES AND BIOMASS QUALITY OF SOME *Helianthus* SPECIES UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

The genus Helianthus Asteraceae family comprising 71 species of annual and perennial plants, most of them native to North America and Central America. The species Helianthus annuus, Helianthus mollis, Helianthus strumosus Helianthus tuberosus, which grow in the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chisinau, Republic of Moldova served as research subjects. It was found that the researched species had different growth and development rates, which affected the accumulation of biomass and nutrients. The harvested green mass of the studied Helianthus species contained 78-121 g/kg CP, 67-103 g/kg ash, 251-382 g/kg CF, 288-404 g/kg ADF, 456-604 g/kg NDF, 41-71 g/kg ADL, 40- 186 g/kg TSS, 239-332g/kg Cel, 164-209 g/kg HC with nutritive and energy value 415-681 g/kg DDM, 376-679 g/kg DOM, RFV = 89-135, 11.43-13.03 MJ/kg DE, 9.38-10.66 MJ/kg ME and 5.40-6.72 MJ/kg NEl. The biochemical methane potential of green mass substrates varied from 282 to 333 l/kg VS. The dehydrated stems of studied Helianthus species contained 474-511 g/kg cellulose, 237-263 g/kg hemicellulose and 102-121 g/kg acid detergent lignin with estimated theoretical ethanol potential 523-559 L/tone. The energy biomass from Helianthus species is characterized by 46.30-47.04% C, 5.19-5.58% H, 0.25-0.48% N, 0.05-0.06% S, 0.03-0.04% Cl, 1.56-3.18% ash with calorific value 18.05-18.65 MJ/kg GCV and 16.93-17.45 MJ/kg NCV.

***Key words**: biochemical composition, biochemical methane potential, biological features, calorific, value Helianthus species, nutritive value, theoretical ethanol potential.*

INTRODUCTION

Within the past decade, increased attention has been paid to the need for development and diversification of the uses of traditional crops and to the domestication of new plant species to meet new demands for food, forage, fibres, fuel, pharmaceuticals, chemicals and other important raw materials, which could stimulate economic growth.

Asteraceae is one of the two largest families of flowering plants, thus "The Plant List" mentions approximately 27.773 species, belonging to 1.765 plant genera. Some of these species are of particular interest due to their biological peculiarities, productivity and tolerance to biotic and abiotic factors. Among them, there is the genus *Helianthus* with 71 species of annual and perennial plants, native to North and South America. The popular annual species *Helianthus annuus* and the perennial *Helianthus tuberosus* are cultivated in temperate regions and some tropical regions as food crops for humans, forage for cattle and poultry, energy biomass and as ornamental plants (Johansson et al., 2015; Cabral et al., 2018; Wróbel et al., 2018; Wang et al., 2020; Liava et al., 2021; Ţîţei& Roşca, 2021).

Sunflower, *Helianthus annuus* is one of the most important cultivated oilseed and melliferous crops in our region. Sunflower is one of the important energy plants the above-ground biomass of which could be used as substrate for producing biogas. The sunflower dry biomass varied from 8.05 to 11.69 t/ha (Ion et al., 2015; Băşa et al., 2018). Sunflowers can grow in certain environments where corn, another silage crop, grows poorly, and sunflowers have a comparable nutrient content. Sunflowers have been used as silage, a type of livestock feed, for decades (Ray, 1919).

During the past decades, the narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from wild relatives, which have provided a continuous source of agronomic traits for crop breeding. Researchers have identified genes that provide resistance to several pathogens, such as *Puccinia helianthi*, *Plasmopara halstedii, Orobanche cumana*, *Sclerotinia* head rot, *Sclerotinia* stalk rot, and resistance to insects such as *Homoeosoma electellum* from the wild *Helianthus* species and successfully transferred them into cultivated sunflower. Wild *Helianthus* species are an important reservoir of useful genes for sunflower, *Helianthus annuus*, breeding programs aimed at providing resistance to various abiotic factors. *Helianthus paradoxus* might be a suitable genetic resource for improving the salt tolerance of sunflower, and *Helianthus pauciflorus* for influencing its cytoplasmic male sterility (Balogh, 2008; Seiler et al., 2017; Anton et al., 2018

Among them, the cultivated sunflower - *Helianthus annuus* and Jerusalem artichoke - *Helianthus tuberosus* and several ornamental plant species *Helianthus argophyllus, Helianthus debilis*, *Helianthus decapetalus*, *Helianthus maximiliani*, *Helianthus mollis Helianthus petiolaris*, *Helianthus salicifolius* etc. are of practical importance, having also industrial uses (Seiler, 2007; Adams et al., 2019; Rost, 2021; Blinkov et al., 2022; Peni et al., 2022). Some of the *Helianthus* species are invasive or dangerous weeds (Balogh, 2008); in the Republic of Moldova, *Helianthus decapetalus*, *Helianthus tuberosus* and *Helianthus tuberosus* var. *subcanescens* have been mentioned (Mârza, 2010).

Ashy sunflower, *Helianthus mollis* Lam., is a perennial herbaceous plant, with erect, hirsute to villous stems, up to 1.5 m tall, spreading by means of underground rhizomes. Leaves are sessile. The plant is greyish-white, the stem and the involucral bracts are densely covered in whitish down. Flower heads develop singly from the upper stems, with each flower having 15-30 showy petal-like yellow ray florets surrounding a central disk of tiny darker yellow disk florets. The achenes are wedge-shaped to egg-shaped, dark brown or brown-mottled, tipped by 2 scales with pointed tips, enclosing small seed. The root system is fibrous and rhizomatous. This plant tends to form dense colonies. *Helianthus mollis* grows on prairies, roadsides, dry open woods, rocky glades, fields, and thickets. Ashy sunflower has been used for various purposes, such as restoration of degraded lands, feeding the livestock and wildlife and as ornamental plant. Dairy cattle, beef cattle and sheep could consume *Helianthus mollis* when grazing in a new pasture with native plants or farmers could intentionally grow it and store the plant matter in silos (Ray, 1919; Taylor, 2021; Rost, 2021).

Woodland sunflower, *Helianthus strumosus* L. is native to eastern North America, is a rhizomatous perennial plant, stems erect, hairless or has sparse, long hairs downwards from the inflorescences, often glaucous, growing up to two meters tall. Leaves are up to 10 cm long and cuneate to subcordate in shape. The composite flower heads can be up to 9 cm at the peduncle. The ray florets are dark yellow, and disc florets - orange-brown in the centre. Achenes have an elongated rounded base and a truncated top with two relatively long, weakly attached awns, and are 4 to 5.5 mm long. Rhizomes are normally well developed, thin or a little thick, may sometimes be tuberous. *Helianthus strumosus* is frequently found in dry forests, but also in completely open habitats, on watersides, roadsides and prairies (Balogh, 2008).

The mobilization and study of some *Helianthus* plant species as forage and energy crops may be necessary.

The main goal of this research was to evaluate some biological features, the quality of green mass and dry stem biomass of the species: *Heliathus annuus, Helianthus mollis, Helianthus strumosus* and *Helianthus tuberosus* grown under the climatic conditions of the Republic Moldova.

MATERIALS AND METHODS

The Asteraceae species: *Heliathus annuus, Helianthus mollis, Helianthus strumosus* and *Helianthus tuberosus* cultivar 'Maria', which grow in the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chisinau, Republic of Moldova served as research subjects. The green mass was mowed in early flowering stage. The leaf/stem ratio was determined by separating the leaves, buds and flowers from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems). The harvested green mass was chopped with a stationary forage chopping unit. The dry matter content was detected by drying samples up to constant weight at 105°C. For biochemical analyses, the plant samples were dried in a forced air oven at 60°C, milled in a beater mill equipped with a sieve with diameter of openings of 1 mm and some assessments of the main biochemical parameters: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM), digestible organic matter (DOM) have been determined by the method of near infrared spectroscopy (NIRS) using PERTEN DA 7200. The concentration of hemicellulose (HC), cellulose (Cel), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl) and relative feed value (RFV) were calculated according to standard procedures. The carbon content of the substrates was obtained using an empirical equation reported by Badger et al. (1979). The biochemical methane potential was calculated according to the equations of Dandikas et al. (2015).

The *Helianthus annuus* stems, after seed removal, were collected at the end of September, but *Helianthus mollis, Helianthus strumosus* and *Helianthus tuberosus* stems were collected in March. The harvested stems were chopped and disintegrated in a knife mill with a sieve with the mesh size of 1 mm. To perform the analyses, the biomass samples were dried in an oven at 85°C. After that, the total carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) amounts were determined by dry combustion in a Vario Macro CHNS analyser. The content of ash was determined at 550°C in a muffle furnace HT40AL according to SM EN ISO 18122; the automatic calorimeter LAGET MS10A with accessories was used to determine the calorific value, according to SM EN ISO 18125. The content of cell walls was evaluated using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200. Theoretical Ethanol Potential (TEP) was calculated according to the equations of Goff et al. (2010) based on the conversion of hexose and pentose sugars.

RESULTS AND DISCUSSIONS

While researching the characteristics of growth and development, several differences between the studied *Heliathus* species were identified. It has been found that the seedlings of *Helianthus annuus* emerged at the soil surface in 12 days after sowing, the stems developed intensively by the middle of May, the flower heads started developing at the beginning of July, the full flowering stage occurred at the middle of July and seed ripening – in August. *Helianthus mollis* plants, in the first year of life, went through all the ontogenetic stages, but the flowering stage occurred very late and the plants did not produce viable seeds. In the second year, *H. mollis* plants came out of dormancy at the beginning of April, the intensive stem development was recorded at the middle of May, the flower heads developed in the middle of June and bloomed in August, and the seed ripening stage lasted from the end of August until the middle of September.

Helianthus strumosus, as well as some other perennial Asteraceae species, in the first year of growth, formed a rosette and the stalk with erect growth reached 50-55 cm in height. In the second year and the in further years of vegetation, in the middle of April, when the air temperature exceeded 10°C, the regrowth of *Helianthus strumosus* plants started from generative buds. From the end of May, the growth and development of the plants intensified, and by the middle of July the shoots were 135-155 cm tall. In the middle of September, shoots developed and passed into the generative phase, and in the first days of October, the plants bloomed, but produced few seeds and most of them did not germinate. *Helianthus strumosus* has the ability to propagate easily from small rhizome fragments, going through all the ontogenetic phases in the year when it is planted. It was found that the number of the stems per plant varied from 5 to 23.

In our previous articles, we mentioned the agro-biological features of the species *Helianthus tuberosus*. According to the results of our research on plants of *Helianthus tuberosus* ‘Maria’, the tubers were planted in the first days of April, and after 25 days the seedlings emerged at the soil surface. In the middle of May, the initiation of stem development was observed, and in mid-June - the formation of stolons. In the following period - July-September, growth and development intensified and the plants exceeded 250 cm in height. At the end of September, the development of flower heads was observed, and in October, the full flowering stage occurred, but the plants did not produce viable seeds.

Plant height, stem thickness and leaf/stem ratio have significant impact on the yield, but also affect the quality of the phytomass. Results regarding some biological peculiarities and the structure of the harvested phytomass of the studied *Helianthus* species are presented in Table 1. At the time of harvesting, plant height varied from 162 cm (*Helianthus strumosus*) to 215 cm (*Helianthus tuberosus*), the leaf share of the fodder from 34.0% (*Helianthus strumosus*) to 53.9% (*Helianthus mollis*).

Anton et al. (2018) reported that in the collection of wild *Helianthus* species from NARDI Fundulea, Romania, in the flowering stage, *Helianthus mollis* plants reached 165 cm in height, *Helianthus strumosus* - 250 cm and *Helianthus tuberosus* - 140-290 cm.

The biochemical composition, nutritive and energy value of the green mass from the studied *Helianthus* species are presented in Table 2. Analysing the results of the biochemical composition of green mass, we found that the dry matter of the studied species contained 78-121 g/kg CP, 67-103 g/kg ash, 251-382 g/kg CF, 288-404 g/kg ADF, 456-604 g/kg NDF, 41-71 g/kg ADL, 40-186 g/kg TSS, 239-332g/kg Cel and 164-209 g/kg HC. The nutritive and energy value of the harvested natural fodder was: 415-681 g/kg DDM, 376-679 g/kg DOM, RFV = 89-135, 11.43-13.03 MJ/kg DE, 9.38-10.66 MJ/kg ME and 5.40-6.72 MJ/kg NEl. *Helianthus mollis* fodder is characterized by higher concentration of protein and lower – of lignin. *Helianthus tuberosus* and *Helianthus mollis* do not differ essentially in the content of ash, neutral and acid detergent fibres, cellulose and energy concentration. *Helianthus strumosus* had lower concentration of protein, total soluble sugars and higher concentration of structural carbohydrates, which had a negative effect on digestibility, relative feed value and net energy for lactation.

Table 1. Some biological peculiarities and the structure of the harvested mass from the studied *Helianthus* species

Plant species	Plant height, cm	Stem, g		Leaf + flower head, g		Total weight of a shoot, g		The leaf share of the fodder, %
		green mass	dry matter	green mass	dry matter	green mass	dry matter	
Helianthus annuus	170	511.2	66.7	230.0	36.0	741.2	102.7	35.1
Helianthus mollis	154	38.1	13.6	60.6	15.9	98.7	29.5	53.9
Helianthus strumosus	162	51.5	17.3	29.2	8.9	80.7	26.2	34.0
Helianthus tuberosus	215	386.4	82.3	232.6	52.6	619.0	134.9	39.0

Table 2. The biochemical composition and the nutritive value of the green mass from the studied *Helianthus* species

	Helianthus annuus	*Helianthus strumosus*	*Helianthus mollis*	*Helianthus tuberosus*
Crude protein, g/kg DM	90	78	121	115
Crude fibre, g/kg DM	358	382	255	251
Minerals, g/kg DM	67	84	100	103
Acid detergent fibre, g/kg DM	367	404	288	292
Neutral detergent fibre, g/kg DM	576	604	460	456
Acid detergent lignin, g/kg DM	49	71	44	53
Total soluble sugars, g/kg DM	145	40	163	186
Cellulose, g/kg DM	318	332	244	239
Hemicellulose, g/kg DM	209	201	172	164
Digestible dry matter, g/kg DM	531	415	622	681
Digestible organic matter, g/kg DM	510	376	579	679
Relative feed value	97	89	134	135
Digestible energy, MJ/ kg	11.92	11.43	13.03	12.98
Metabolizable energy, MJ/ kg	9.79	9.38	10.70	10.66
Net energy for lactation, MJ/ kg	5.81	5.40	6.72	6.67

Literature sources indicate considerable variation in the chemical composition and nutritional value of *Helianthus* plant species. According to Medvedev & Smetannikova (1981), the chemical composition of green mass from early-blooming sunflower cultivars was 7% CP, 32.4% CF, and 8.6% ash, but from late-blooming sunflower cultivars- 12.4% CP, 26.2% CF and 11.5% ash. Seiler et al. (1991) remarked that *Helianthus annuus* contained 13.1-20.4% CP, 3.3-2.0-2.1% EE and 17.9-19.5% ash; *Helianthus decapetalus* - 4.7% CP, 1.5 % EE and 15.5% ash; *Helianthus mollis* - 5.3% CP, 1.5 % EE and 18.6% ash; *Helianthus strumosus* - 7.1% CP, 2.0 % EE and 10.4% ash; *Helianthus tuberosus* - 3.8% CP, 1.0 % EE and 8.5% ash. Seiler (1993) mentioned that *in vitro* digestibility of dry matter of the Jerusalem artichoke cultivars varied from 542 to 715 g/kg in whole plants in the flowering stage. According to Cosgrove et al. (2000), the Jerusalem artichoke tops contained 270 g/kg dry matter, 5% crude protein and 67% total digestible nutrients. Mello et al. (2006) mentioned that the dry matter content and the nutritional quality of sunflower whole plant forage, as influenced by sowing dates and peculiarities of different hybrids, was 328-347 g/kg DM, 90.8-93.0 % OM, 8.7-14.8 % CP, 39.5-52.5 % NDF, 36.3-48.8 % ADF, 9.0-9.7 % ADL. For comparison, Seiler (2007) remarked that *Helianthus grosseserratus* had a protein concentration of 201 g/kg, *Helianthus arizonensis* of 184 g/kg, *Helianthus simulans* of 181 g/kg, *Helianthus petiolaris* subsp. *fallax* of 173 g/ kg, and *Helianthus neglectus* of 162 g/kg. Kerckhoffs et al. (2011) revealed that the dry matter content and the biomass composition of *Helianthus annuus* plants was: 360 g/kg DM, 11.8% CP, 8.1% EE, 3.5% sugars, 0.4% starch, 20.5% cellulose, 10.2% hemicellulose, 24.9% CF, 36.9% NDF, 26.7% ADF, 6.2% lignin, 12.2% ash, but *Helianthus tuberosus* plants contained 282 g/kg DM, 4.7% CP, 0.7% EE, 5.0% sugars, 0.3% starch, 27.7% cellulose, 12.6% hemicellulose, 32.3% CF, 48.0% NDF, 35.5%ADF, 7.8% lignin, 8.8% ash. Teleuţă&Ţîţei (2013) found that green mass of the cultivar "Solar" of *Helianthus tuberosus* harvested during the formation of flower buds, contained 255 g/kg of dry matter and its chemical composition was: 9.32% of raw protein, 1.93% of raw fat, 21.29% of raw cellulose, 8.75% of minerals, 58.71% of nitrogen free extract. Heuze et al. (2015a; 2015b) remarked that the average feed value of Jerusalem artichoke fresh mass was: 32.3% DM, 15.3% CP, 2.2% EE, 15.1% CF, 40.6% NDF, 34.5% ADF, 11.5% lignin, 14.4% ash, 63% DOM, 16.8 MJ/kg GE, 10.1 MJ/kg DE and 8.2 MJ/kg ME, but sunflower fresh mass – 15.8% DM, 13.0% CP, 2.2% EE, 25.0% CF, 39.6% NDF, 35.9% ADF, 9.7% lignin, 13.1% ash, 64.4% DOM, 17.7 MJ/kg GE, 11.0 MJ/kg DE and 8.9 MJ/kg ME. Silva et al. (2016) revealed that the chemical composition of sunflower plants was 12.5% CP, 16.13% EE, 11.78% ash, 40.16% NDF, 31.27% ADF; corn plants contained 8.31% CP, 2.09% EE, 8.45% ash, 58.24% NDF, 32.50% ADF; sorghum plants - 5.57% CP, 1.74% EE, 4.63% ash, 63.73% NDF, 38.14% ADF. Ersahince & Kara (2017) have found that the chemical composition of Jerusalem artichoke green mass harvested in early flowering stage was 7.37% protein, 1.70% fats, 40.15% NFC, 39.03% aNDFom, 31.7% ADFom, 6.78% ADL. Adams et al. (2019) revealed that the crude protein content varied in sunflower species, between the following values: 14.6-20.1% CP in *Helianthus grosseserratus*, 15.9% CP in *Helianthus angustifolius*, 10.8-15.3% CP in *Helianthus maximiliani*, 12.9% CP in *Helianthus strumosus*, 12.1% CP in *Helianthus tuberosus*, 9.3% CP in *Helianthus tuberosus x annuus*, 8.6-8.7% CP in *Helianthus annuus*, 6.6-8.9% CP in *Helianthus mollis* and 2.6% CP in *Helianthus divaricatus.* Farzinmehr et al. (2020) evaluated the effect of the maturity stage and harvesting frequency of Jerusalem artichoke forage, and depending on the stage of maturity, it contained 146-247 g/kg DM, 9.25-14.5% CP, 1.43-1.87% fats, 34.0-46.7% NDF, 25.5-34.7% ADF, 5.96-11.0% ADL, 5.65-12.1% WSC, 29.9-35.5% NFC, 11.8-16.4% ash, 58.1-69.1% OMD, 7.69-9.37 MJ/kg ME. Pınar et al. (2021) revealed that Jerusalem artichoke green mass could provide a good source of nutrients for ruminants: 5.82-13.36% CP, 0.65-2.42% EE, 0.95-1.67% condensed tannins, 31.67-45.71% ADF, 38.77-53.27% NDF, 9.89-16.85% ash, 1.6-4.5% Ca, 0.5-2.9%P, 2.0-3.3% K, 0.3-0.7% Mg, 43.30-60.20% OMD, 5.82-8.52 MJ/kg ME, 2.65-4.93

MJ/kg NEL. Manokhina et al. (2022) found that the chemical composition of Jerusalem artichoke green mass from early leaf wilting cultivars was 2.8% CP, 3.3% fats, 4.2% sugars, 10.9% cellulose 7.6% others nutrients, but in cultivars with late leaf wilting respectively - 3.1% CP, 3.5% fats, 4.0% sugars, 13.1% cellulose 6.1% others nutrients.

Biogas is produced by microorganisms, such as methanogens and sulphate-reducing bacteria, performing anaerobic respiration inside a bioreactor, primarily consisting of methane, carbon dioxide and hydrogen sulphide, is an environmentally-friendly, renewable energy source. The biodegradation of different types of lignocellulosic biomass feedstock depends on the chemical structure, primarily on the content of cellulose, hemicellulose, lignin and the C/N ratio. The results regarding the quality of the investigated substrates and the biochemical potential biomethane are illustrated in Table 3. We found that the carbon content in the studied substrates ranged from 491.7 g/kg DM to 518.3 g/kg DM, and the nitrogen content from 12.5 g/kg DM to 19.4 g/kg DM, the C/N ratio varied from 25 to 41, respectively. The optimal C/N = 25-27 was found in *Helianthus mollis* and *Helianthus tuberosus* substrates. Essential differences were observed between the hemicellulose and lignin contents, which played an important role in biomethane yield. A high yield of biomethane can be provided by the *Helianthus mollis* substrate (333 l/kg VS), but the *Helianthus strumosus* substrate has low potential because of the very high content of lignin (282 l/kg VS). The biochemical methane potential of green mass substrates from *Helianthus annuus* and *Helianthus tuberosus* reached 317-320 l/kg VS.

Table 3. Biochemical composition and biomethane production potential of substrates from the studied *Heliathus* species

Indices	*Heliathus annuus*	*Helianthus strumosus*	*Helianthus mollis*	*Helianthus tuberosus*
Minerals, g/kg DM	67	84	100	103
Nitrogen, g/kg DM	14.4	12.5	19.4	18.4
Carbon, g/kg DM	518.3	508.9	500.0	491.7
Ratio carbon/nitrogen	36	41	25	27
Hemicellulose, g/kg DM	209	201	172	164
Acid detergent lignin, g/kg DM	49	71	44	53
Biomethane potential, L/kg VS	320	282	333	317

Several literature sources describe the biochemical composition and biomethane potential of substrates from *Helianthus* species. In Finland, Lehtomäki (2006) determined that the methane potential of *Helianthus tuberosus* tops was 300-430 l/kg VS. Amon et al. (2007) reported that the specific methane yield of substrates from two sunflower cultivars in the course of the growing season significantly decreased from 454 to 190 L/kg VS. Mursec et al. (2009) reported that the highest biomethane production was achieved by the sunflower substrate 283 L/kg VS, followed by the sorghum 188 L/kg VS and maize substrate 187 L/kg VS, amaranth substrate 225 L/kg VS and the Jerusalem artichoke substrate 115 L/kg VS. Heiermann et al. (2009) found that the fresh mass of Jerusalem artichoke contained 234 g/kg DM, 86.9% OM, 16.8% CP, 0.6% EE, 24.9% CF, 26.9% sugar, C/H=16.8, methane yield 220 L/kg. Alaru et al. (2011) reported that the sunflower substrates contained 5.18-7.29% HC, 27.99-34.06% Cel, 7.72-8.28% lignin, with methane yield 280-290 L/kg VS, but Jerusalem artichoke substrates contained 5.48% HC, 20.95% Cel, 5.05% lignin, with methane yield 325 L/kg VS. Kerckhoffs et al. (2011) revealed that the specific yield in biomass feedstock from maize was 304-310 L/kg VS, from sorghum 293-303 L/kg VS, but from sunflower 255 L/kg VS. Seppälä (2013) found that the specific methane yield of Jerusalem artichoke was 340 L/kg VS, but in sunflower substrate 380 L/kg VS. Kikas et al. (206 remarked that the methane yield of Jerusalem artichoke substrate was 325 L/kg TS. Sotnar et al. (2015) reported that the average specific biogas production from the aerial parts of Jerusalem artichoke was 484 L/kg; the methane content was 53.26% on average, corresponding to 249 L/kg. Opurum et al. (2021) remarked that the feedstock from fresh leaves and stalks of sunflower contained 909 g/kg total solids, 761 g/kg volatile solids, 21.5% CP, 2.1% fats, 28.6% CF, 3.4% nitrogen, 39.4% organic carbon, C/N = 11.5, but the livestock manure feedstock contained 905 g/kg total solids, 598 g/kg volatile solids, 9.1% CP, 2.9% fats, 30.3% CF, 1.5% nitrogen, 54.3% organic carbon, C/N= 37, the biogas yield of sunflower only substrate was 130 L/kg VS and sunflower with 50% livestock manure - 460 L/kg VS. Oleszek & Matyka (2018) reported that the theoretical methane yield of sunflower was 484 L/kg VS and the experimental methane yield 160 L/kg VS. Spyridonidis et al. (2019) remarked that the methane yields of stalk and head residues were 112.2 and 183.0 L/kg TS, but after alkaline pre-treatment the methane yields were 150.7 and 196.4 L/kg TS. A study conducted in Poland by Peni et al. (2022) showed that the biogas yield from *Helianthus salicifolius* averaged between 269.29 L/kg VS for raw biomass and 286.6 L/kg VS for silage. Zhurka et al. (2020) reported that the highest methane production, 268.35 L/kg VS, was achieved from the pre-treated sunflower head residues.

During the first decades of the 21th century, there has been an enormous interest in the production and usage of liquid biofuels (biodiesel or bioethanol) as promising substitutes for fossil fuels. Bioethanol is an attractive alternative fuel because it is a renewable resource and it is oxygenated, providing thus potential for reducing emissions in engines. Second generation bioethanol produced from lignocellulosic plant biomass is attracting attention as an alternative energy source and it is currently a topic of great interest for researchers around the world. The hydrolysis of lignocellulosic biomass to monomeric sugars is necessary before microorganisms can metabolize them. Analysing the cell wall composition of dehydrated stems of studied *Helianthus* species, Table 4, we could mention that the concentrations of structural carbohydrates in *Helianthus annuus* and *Helianthus tuberosus* substrates are much higher in comparison with *Helianthus strumosus* substrate. The *Helianthus annuus* substrate had high content of cellulose, hemicellulose and optimal content of acid detergent lignin as compared with *Helianthus tuberosus* substrate. The estimated theoretical ethanol yield from cell wall carbohydrates averaged 559 L/t in *Helianthus annuus* substrate, 543 L/t in *Helianthus tuberosus* substrate as compared with 523 L/t in *Helianthus strumosus* substrate.

Some authors mentioned various findings about the quality of lignocellulosic substrates from *Helianthus* species. Wróblewska et al. (2009) reported that the *Helianthus tuberosus* stalks have an average content of 40.95% Seifert cellulose, 22.65% pentosans, 20.48% Klason lignin and 35.46% soluble substances. Gunnarsson et al. (2014) determined the chemical components of the Jerusalem artichoke aerial mass: 15.7-24.8% cellulose, 11.2-12.4% hemicellulose, 16.6- 19.0% lignin, 1.6-1.8% protein, 3.2-3.8% lipids, 10.9-12.2% extractives, 12.3-14.5% uronic acid and 5.8-12.2% ash. Kikas et al. (2016) reported that the quality of sunflower plant was characterized by the following indices 5.18% HC, 34.06% Cel, 7.72% Lig, 9.78% ash, 49 g/m^2 potential ethanol yield, 33.08% hydrolysis efficiency and 66.36% fermentation efficiency, but - of Jerusalem artichoke harvested in September and October - 4.50-5.48% HC, 20.95-25.99% Cel, 5.05-5.70% Lig, 4.56-5.15% ash, 38-40 g/m^2 potential ethanol yield, 74.70-77.88% hydrolysis efficiency and 38.14-44.19% fermentation efficiency, respectively. Liu et al. (2015) found that, in Jerusalem artichoke stems, the concentration of cellulose was 284-481g/kg, hemicellulose 55-1751 g/kg, lignin 47-1201 g/kg, the ethanol potential yield from cellulose and hemicellulose in aboveground biomass varied from 1821 to 5.930 L/ha. Mathias et al. (2015) remarked that sunflower stem bark contained 48% cellulose and 14% lignin, but sunflower stem pith 31.5% cellulose and 2.5% lignin. Barbash et al. (2016) noted that chemical composition of sunflower stalks was: 67.32% holocellulose, 41.83% cellulose, 24.36% pentosans, 20.12% Klason lignin and 3.07% ash. Fiserova et al. (2006) reported that the Jerusalem artichoke stalks contained 28.5% alpha-cellulose, 23.1% hemicelluloses, 14.8% lignin, 33.9% extractives, 3.1% ash. Prusov et al. (2019) remarked that Jerusalem artichoke stem cortex contained 51.1% alpha-cellulose, 16.3% hemicelluloses, 12.5% lignin, 1.8% ash, but

Jerusalem artichoke stem pith 67.7% alpha-cellulose, 4.6% hemicelluloses, 7.6% lignin, 1.3% ash. Gholami-Yangije et al. (2019) reported that sunflower stalks contained 2.26% CP, 85.09 % NDF, 72.72 % ADF and 15.25 % ADL. Rossini et al. (2019) noted that Jerusalem artichoke ethanol yield from tubers ranges from 1500 to 11000 L/ha and from aerial biomass - 2835 to 11230 L/ha. Ţîţei et al. (2021) revealed that dry stems of *Helianthus tuberosus* 'Solar' contained 276 g/kg cellulose, 176 g/kg hemicellulose, 98.04 g/kg hexose sugars and 45.4 g/kg pentose sugars, the theoretical bioethanol yield from stems 598 l/kg.

Table 4. The cell wall composition and theoretical ethanol potential of substrates from the studied *Heliathus* species

Indices	*Helianthus annuus*	*Helianthus strumosus*	*Helianthus tuberosus*
Acid detergent fibre, g/kg	614	576	632
Neutral detergent fibre, g/kg	877	823	869
Acid detergent lignin, g/kg	107	102	121
Cellulose, g/kg	507	474	511
Hemicellulose, g/kg	263	247	237
Hexose sugars, g/kg	90.80	84.90	91.18
Pentose sugars, g/kg	43.21	40.63	38.98
Theoretical ethanol potential, L/tone	559	523	543

Table 5. The elemental composition, ash content and calorific value of stem biomass from studied the *Heliathus* species

Indices	*Helianthus annuus*	*Helianthus strumosus*	*Helianthus tuberosus*
Carbon	46.30	46.44	47.04
Hydrogen	5.21	5.19	5.58
Nitrogen	0.48	0.25	0.29
Sulphur	0.06	0.05	0.05
Chlorine	0.04	0.04	0.03
Ash content of biomass, %	3.18	2.68	1.56
Gross calorific value, MJ/kg	18.05	18.35	18.65
Net calorific value, MJ/kg	16.93	17.21	17.45

The chemical composition of dry biomass is a key factor that affects the calorific value and the technologies to be implemented for the production of solid biofuels. The elemental composition of biomass is a significant asset that defines the amount of energy and evaluates the clean and efficient use of biomass materials, provides significant parameters used in the design of almost all energy conversion systems and projects, for the assessment of the complete process of any thermochemical conversion techniques (Lawal et al., 2021). The main constituents of dry biomass are carbon (C), oxygen (O) and hydrogen (H). As carbon and hydrogen are oxidised in the combustion process, they release energy. Carbon is obviously representing foremost contributions to overall heating value. Furthermore, higher hydrogen content determines and leads to higher net calorific value. Nitrogen (N), sulphur (S) and chlorine (Cl) contents are some of the main causes of air pollution from biomass combustion. A higher percentage of these elements generally results in a higher level of air contaminants being released. The elemental composition, ash content and calorific value of stem biomass from the studied *Helianthus* species is presented in Table 5. We found that the studied stem biomass was characterized by 46.30-47.04% C, 5.19-5.58% H, 0.25-0.48% N, 0.05-0.06% S, 0.03-0.04% Cl, 1.56-3.18% ash, 18.05-18.65 MJ/kg GCV and 16.93-17.45 MJ/kg NCV. The higher content of carbon and hydrogen, and the lower content of nitrogen, sulphur and chlorine in *Helianthus tuberosus* stems had positive impact on calorific value as compared with *Helianthus annuus* biomass. According to Unal & Alibas (2006) the sunflower stalks contained 10.5 % moisture, 68.0% volatile matters, 7.0% ash, 48.2% carbon, 24.4 % fixed carbon, 5. 7% hydrogen, 1.1% nitrogen, 0.1% sulphur, 18.65 MJ/kg HHV and 17.19 MJ/kg LHV, but wheat straw 12.8 % moisture, 73.8% volatile matters, 6.2% ash, 44.8% carbon, 20.0 % fixed carbon, 5.4% hydrogen, 0.6% nitrogen, 0.2% sulphur, 17.88. MJ/kg HHV and 16.23 MJ/kg LHV, respectively. Wróblewska et al. (2009)

found that *Helianthus tuberosus* biomass contained 45.9% C, 6.1% H, 0.3% N, 2.5% ash and had 16.653 MJ/kg calorific value, in dry and ash-free state. Hăbăşescu & Cerempei (2012) mentioned that sunflower stem biomass had 3.78 % ash, 0.08%S, 14.76 MJ/kg LHV, maize stems 5.14 % ash, 0.09%S and 14.2 MJ/kg LHV, and wheat straw 6.25 % ash, 0.15 % S and 14.36 MJ/kg LHV, respectively. Kowalczyk-Juśko et al. (2012) reported that Jerusalem artichoke biomass contained 5.4-5.6% ash and had 16.10-16.30 MJ/kg calorific value. Teleuţă & Ţîţei (2013) determined that Jerusalem artichoke stems collected at the end of December had 15% humidity, the bulk density of the crumbled stems was 288 kg/m^3 and the specific density of the briquettes reached 720 kg/m^3 with gross calorific value 18.7 MJ/kg. Huang (2014) mentioned that the briquettes from sunflower stems were characterized by a calorific value of 4300 kcal/kg (18.0 MJ/kg) and 4.3% ash, wheat straw briquettes 4100 kcal/kg (17.1 MJ/kg) and 8.00 % ash, as compared with wood chips – 4785 kcal/kg (20.0 MJ/kg) and 1.2 % ash content. Stolarski et al. (2014) found that *Helianthus tuberosus* harvested in April contained 17.934% moisture, 3.87% ash, 46.60% carbon, 5.68% hydrogen, 0.032% sulphur, 18.75 MJ/kg HHV and 13.35 MJ/kg LHV. Maj (2015) mentioned that the heat of combustion of tested Jerusalem artichoke plant biomass was 14.22-14.85 MJ/kg, but Virginia mallow biomass was 16.92-17.55 MJ/kg. Mathias et al. (2015) reported that the heat capacity value of sunflower stem bark was 14 MJ/kg and sunflower stem pith – 13 MJ/kg. Szostek et al. (2018) found that the aboveground *Helianthus tuberosus* biomass contained 5.35-6.18% ash, 15.41-16.02 MJ/kg HHV and 14.19-14.72 MJ/kg LHV. Pavlenco (2018) found that sunflower whole plant contained 55.0% DM, 42.50% C, 5.10% H, 1.11% N, 0.11% S, 11.80% ash, 16.9 MJ/kg GCV and 15.8 MJ/kg NCV. Zapalowska & Bashutska (2017) noted that Jerusalem artichoke pellets were characterized by 6.81% moisture, 2.04% ash, 18.85 MJ/kg HHV. Țîței et al. (2019) reported that the Jerusalem artichoke milled chaffs contained 2.12% ash and 19.1 MJ/kg HHV, but pellets 2.10% ash and 17.7 MJ/kg LHV.

CONCLUSIONS

The harvested green mass of the studied *Helianthus* species contains 78-121 g/kg CP, 67-103 g/kg ash, 251-382 g/kg CF, 288-404 g/kg ADF, 456-604 g/kg NDF, 41-71 g/kg ADL, 40-186 g/kg TSS, 239-332 g/kg Cel, 164-209 g/kg HC with nutritive and energy value 415-681 g/kg DDM, 376-679 g/kg DOM, RFV = 89-135, 11.43-13.03 MJ/kg DE, 9.38-10.66 MJ/kg ME and 5.40-6.72 MJ/kg NEl.

The biochemical methane potential of green mass substrates varies from 282 to 333 l/kg VS.

The dehydrated stems of the studied *Helianthus* species contain 474-511 g/kg cellulose, 237-263 g/kg hemicellulose and 102-121 g/kg acid detergent lignin with estimated theoretical ethanol yield 523-559 L/ton.

The dehydrated stem mass of the studied *Helianthus* species is characterized by the following indices: 46.30-47.04% C, 5.19-5.58% H, 0.25-0.48% N, 0.05-0.06% S, 0.03-0.04% Cl, 1.56-3.18% ash, 18.05-18.65 MJ/kg GCV and 16.93-17.45 MJ/kg NCV.

Helianthus mollis has high potential as fodder for livestock and as substrate for biogas stations, and *Helianthus strumosus* - for the production of solid biofuels.

ACKNOWLEDGEMENTS

The study has been carried out in the framework of the project: 20.80009.5107.02 *"Mobilization of plant genetic resources, plant breeding and use as forage, melliferous and energy crops in bioeconomy"*.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

AN INVESTIGATION OF THE EFFECTS OF INTRODUCING AN ALTERNATIVE CATTLE FEEDING METHOD ON A DAIRY FARM

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Abstract

With the cost of farm labour in the UK remaining stable through 2020 and the cost of milk rising Reeve (2021) (AHDB, 2022), there is an increasing incentive to capitalise on these unique circumstances. Dairy farms have recognised for years, the vitality of providing the correct sustenance to cattle. It is recognised that correct levels of feed supplied to the cattle has an impact on milk yield. Another factor to consider, when it comes to feed, is the quality of the mix that is produced from a diet feeder. With diesel prices continuing rise, a more fuel efficient alternative to traditional feeding is being sought, without sacrificing quality. This report investigates the financial effects recorded when implementing a self-propelled diet feeder into a 146 cow dairy farm in Lancashire. Using primary research, gathered from a series of tests, carried out between a self-propelled diet feeder and the conventional trailed machine. The results showed the benefits of the self-propelled machine outweighed the negative aspects. Due to the study only being carried out on a single farm the data cannot represent other farms. However, on this farm, the increased value of milk yield exceeded the additional cost of diesel fuel used and time taken by the self-propelled unit. As a conclusion an increased daily income of £61.10, when using the self-propelled, it would take a projected nine years to pay off the machine with current labour, diesel and milk prices.

Key words: *farm labour, milk production, self-propelled, diet feeder Lancashire.*

INTRODUCTION

Design briefs in the agricultural engineering industry feature two main words: productivity and efficiency (Careers, 2022). Productivity is measured by the level of input vs the level of output from a certain operation (Krugman, 1990). These inputs could be quantified as labour, economic input or materials for example (Milano, 2019). An increase in productivity is notable when a company or manufacturer increases its outputs while maintaining the same level of inputs (Bjorkman, 1992). Meanwhile, efficiency is defined as producing as little to no waste when aiming to achieve an end goal or product (Dincer, 2018). In other words, productivity relates to the quantity of goods produced, while efficiency is the amount of materials used to produce that work (Hemphill, 2018). Companies which can strike a balance between the two fundamentals are able to offer a product that will bring the end consumer profitability in both working time and the materials used in the production of the end product (Fried, 1993).

The aspect of efficiency vs productivity can be applied to more or less any industry (Coelli, 2005). The agriculture and agricultural engineering industry has many different areas that could be analysed in relation to efficiency and productivity. Over the past 100 years, the world's population has quadrupled (Roser, 2019). In order to maintain the necessary outputs required from the agricultural sector, the industry has had to adapt and evolve to meet increased demand (Almond, 2021). These adaptations have had to allow organisations and businesses to increase output with the same input while also maintaining economic viability by limiting waste (Ghebremichael, 2013). One way of analysing whether or not an agricultural business is viable is by completing studies into the efficiency and productivity of a certain area (Oum, 1999). With the agricultural sector looking to increase outputs, maintain inputs and reduce waste, both efficiency and productivity are vital to reaching this goal (Latruffe, 2012).

This study will focus on the cattle production sector for dairy and beef. Within this area, there are many different inputs and outputs that can be analysed. However, the feeding of cattle is the highest input on the farm (in terms of capital invested) (Halladay, 2018). Therefore, if there is a change in either productivity or efficiency in this area, there will be a direct effect on the bottom line, whether that be positive or negative. It will also analyse the effects that altering feeding practices on a case study farm will have on a range of inputs and outputs, plus examine any changes in efficiency and productivity. In addition it will analyse the effects that such an alteration has on every aspect of the dairy-production process, from feeding through to milk yield. The alteration on the farm in question is the implementation of a self-propelled diet feeder when compared to the traditional trailed diet feeder used previously.

The farm in question has a 146 average head of dairy cattle along with 90 head of beef cattle, 53 head of heifers and 25 dry cows. The data collected will investigate diesel use between the two methods of feeding, the difference in milk yield in the dairy cows and the overall time taken to feed up.

MATERIALS AND METHODS

There are three main areas of concern that must be focused upon during data collection. These areas are reliability, validity and generalisability. The best way to improve reliability is through explanation of the methods used during a research project (Swetnam, 2009). The main question of reliability is, would the same procedures carried out again yield the same results? One way of increasing reliability is by ensuring that procedures and tactics are both followed and well documented. By following rigorous standards, this also ensures that validity is sufficient. Validity relates to the accuracy of the means of measurements (Bell, 2014); accurate means of measuring ensure that the results gained are valid to the highest level of reliance. The means of measuring also need to be able to bear the weight of the results or the claims made, otherwise the project would be open to valid criticism. Generalisability relates to the relevance of the research in question to the wider industry (Briesch, 2014). All of the points below address how this study will approach each factor stated above.

The use of primary data has many advantages over using secondary data, one of which is the authenticity of the results gained (Formplus, 2021). While secondary data is vital in research projects in which the option to gather primary data is not available, the gathering and analysing of primary data is often seen as more reliable. For example, when using secondary data from manufacturer studies, the data could be biased towards their product. This would be beneficial to a manufacturer, as inflated savings and data could make a product more appealing to the consumer. Ana Dolores Franco Valdez (2018) states that exaggerated or inflated product claims directly affect consumer evaluations of a company. Along with Ms Valdez, the Elaboration Likelihood Model (ELM) developed by Richard E. Petty and John T. Cacioppo (Petty, 1986) states that even strong claims from a manufacturer or company will have a profound effect on a consumer's ideologies of a company. Therefore the use of primary data (in research projects) both validates claims by companies and manufacturers while also providing useful information to prospective consumers from an unbiased point of view.

Due to the nature of this study, however, results will be obtained entirely from primary data that has been collected down at the dairy and beef units of Myerscough Farms (more specifically Lodge Farm). The first method of data collection used was a series of tests carried out between two separate machines. The two machines in question are a self-propelled Keenan MechFibre 345SP (known in this study as SP) and a Keenan MechFibre 365 (Known is this study as TM). The key difference between the two machines is that the MechFibre 345SP is a 2017 self-propelled unit fitted with a milling head which feeds certain raw materials into the tub, while the MechFibre 365 is a trailed unit which requires a telescopic handler or a loader tractor in order to put certain materials into the tub, as well as a power unit on the diet mixer itself. The associated tractor that powered the MechFibre 365 was a 2017 McCormick X6.430 and the associated telescopic handler unit used, a 2021 Kramer KT 357.

Figure 1. Trailed feeder wagon with the associated tractor and telescopic handler unit

Figure 2. Self-propelled feeder wagon used in this study

One of the first and more obvious advantages of using a self-propelled machine as opposed to the conventional trailed method is that the operation of loading and mixing is executed by a single machine. From a machinery point of view, in theory, this should limit the maintenance and diesel requirements. However, to verify these claims. Diesel consumption tests were carried out on both the self-propelled feed mixer and trailed feed mixer. These tests were carried out between 14/02/2022 and 04/03/2022. This time frame allowed 8 days of tests for each diet feeder while also allowing for a contingency, of a few days, in case of any situations in which testing would not be possible. The feeding structure at Lodge consists of five feed mixes spread across a circuit of two days. For example, day one would consist of a beef feed mix and then a high milking mix and a low milking mix, and day two would consist of the high and low milking mixes as well as a heifer and a dry cow mix. This structure means that the beef, heifer and dry cows are fed once every other day while the milking cows (known in this study as PD Plus (low milking) and open cow (high milking)) are fed once every day. Due to the eight-day testing period, this would therefore result in eight PD plus and open cow mix diesel consumption results for both the self-propelled and the trailed feeder wagon, as well as four sets of results for beef, heifer and dry cows for both feed mixing methods. As the most frequent source of income on the farm comes from the sale of milk, the feeding and therefore the yield of milk was under the closest scrutiny. The higher number of milking mix results that were gathered then facilitated a higher level of analysis, which could then be directly compared to any differences in milk yield.

The diesel consumption tests were carried out firstly on the self-propelled machine then on the trailed machine in blocks of eight days. These tests were carried out using one-litre measuring cylinders that were accurate to +/- 10 cm^3 or 10 millilitres. Two five litre and one two litre jugs were used throughout the tests to streamline the process and therefore prevent excess disruption to the feeding schedule. The five and two-litre jugs were filled between feeds using the one-litre measuring cylinder. This tactic was adopted after a series of four trial tests, as it was seen to greatly reduce the time taken to refill the machine after each test and would therefore create the least amount of disruption while maintaining the maximum level of accuracy. Prior to each day's feeding schedule the machine was filled to the specified level, which, on all machines in these tests, was just as the filler neck on the diesel tank widened out to the main tank. Once filled, the machine would then complete its usual gathering, mixing and dispensing of feed to the specified group of cattle. Once the whole feeding process was complete, the machine would then return to the yard and the engine would be switched off. At this point, the tank would be filled back to the specified fill point and the process would then repeat between three and four times a day. The level of diesel left in either the two-litre jug, five-litre jug or one-litre measuring cylinder was then measured, and the total combined diesel for the self-propelled was recorded. During the trailed diesel tests the same principles were adopted, however the diesel use for the telescopic handler and the tractor were measured individually.

Another area for investigation, on a dairy farm, is the time taken to complete tasks (Jackson, 2009). To accurately assess this area, which the self-propelled mixer claims to improve, the time was noted when the engine was started at the beginning of every feed mix and when the engine was stopped at the end of every feed mix. This would give a better understanding of the length of time taken to complete each feed cycle individually recorded, with a tolerance of +/- 59 seconds. As per the diesel consumption tests, the time was recorded from engine start to engine cut off for both the telescopic handler and the tractor separately. This allowed for comparison between time taken and diesel consumption on a litres-per-hour basis.

One area for consideration when carrying out the diesel consumption and time tests was the mix percent that was being made up. The mix percent relates to how much of the TMR is being made. For example, if the cattle only consumed 90% of the previous mix, the mix percent would be lowered to 90%. This changing of percentage mix would ensure that there was minimal waste between mixes and that 100% of what was being mixed would be consumed by the cattle. This changing of mix percent would directly affect the diesel and time taken tests as the higher the mix percentage then the higher the load on the machine and theoretically the higher the rate of diesel consumption.

The final method of data collection in this project is through a fully-structured interview. This interview was carried out with a member of staff who currently works on the farm. The participant is work based but also carries out managerial duties. Due to the comparative nature of this thesis, a structured interview was selected, as this means the results are more easily compared to one another (Horton, 2004). In the case of this research project, a structured interview was also beneficial as questions could be pre-planned while the test was taking place. This was more common where there were operation-based questions. The interviewee was selected due to them occupying the most job roles on the farm, and this may cause there to be differences in answers given based on what benefits and drawbacks directly affect the individuals as either operators or managers. The initial method would to have been to interview several of the farm staff, however due to time constraints and working commitments, only one member of staff was available to be interviewed. Finally, through working with farm management, access was granted to milk yield data and feed ingredient data, which will also be used in this study.

RESULTS AND DISCUSSIONS

The results of the tests from this research project have allowed a multitude of different angles to be assessed. These findings have allowed operators to be compared in terms of diesel use and time taken. However, due to the logistics of the farm, the same frequency of operators was not available for the second set of tests on the trailed feeder wagon. Therefore, new operators as well as operators from the first set of tests featured in the second set of tests as is seen from the data below. As well as operator comparison, the findings have also allowed analysis of which mixes tend to use the most and least amounts of diesel. As a whole, the results have allowed large-scale comparison between the two types of feeder wagons as shown on the following pages. These results show clear trends between the two diet feeders, however further analysis is needed in order to define which diet feeder is preferable in terms of pricing. The farm worker referred to in the main text as Patrick appears as Pat in tables and interviews.

Table 1. Data sheet for the diesel tests for the self-propelled feeder wagon (14-21/02/2022)

	Test Number	Date and time	Diesel used (litres)	Operator	Type of feed	Time Taken
SP feeder wagon	1	14/02/20	10.31	Pat	Dry	49
	2	14/02/20	4.9	Pat	PD Plus	31
	3	14/02/20	6.46	Pat	Open Co	45
	4	15/02/20	8.96	Pat	Heifer M	42
	5	15/02/20	4.82	Pat	Beef Mix	20
	6	15/02/20	2.13	Pat	PD Plus	27
	7	15/02/20	9.42	Pat	Open Co	45
	8	16/02/20	4.77	Ben	Open Co	29
	9	16/02/20	7.67	Ben	PD Plus	40
	10	16/02/20	7.94	Ben	Dry	56
	11	17/02/20	11.75	Pat	PD Plus	37
	12	17/02/20	5	Pat	Open Co	31
	13	17/02/20	3.845	Pat	Beef Mix	19
	14	17/02/20	6.6	Pat	Heifer M	29
	15	18/02/20	7.46	Ben	Dry	40
	16	18/02/20	6.45	Ben	PD Plus	39
	17	18/02/20	6.605	Ben	Open Co	38
	18	19/02/20	7.85	Ben	PD Plus	27
	19	19/02/20	4.015	Ben	Heifer M	23
	20	19/02/20	2.925	Ben	Beef Mix	13
	21	19/02/20	8.18	Ben	Open Co	43
	22	20/02/20	6.91	Pat	PD Plus	38
	23	20/02/20	5	Ben	Dry	28
	24	20/02/20	5	Ben	Open Co	34
	25	21/02/20	11.76	Pat	Heifer M	44
	26	21/02/20	3.955	Pat	Beef Mix	16
	27	21/02/20	4.6	Pat	PD Plus	38
	28	21/02/20	6.86	Pat	Open Co	56

Table 2. Data sheet for the trailed diet feeder wagon

	Date and time	Tractor diesel use	Kramer diesel used	Type of feed	Total diesel used	Time taken
Trailed feeder wa	24/02/2022 1:24pm	5.29	2.3	Heifer	7.585	51
	24/02/2022 2:16pm	2.16	1.14	Dry Cow	3.3	44
	24/02/2022 3:02pm	1.91	1.31	PD Plus	3.22	24
	24/02/2022 3:31pm	2.53	1.48	Open Cow	4.005	43
	25/02/2022 2:05pm	3.75	0.77	Beef Mix	4.52	20
	25/02/2022 2:32pm	1.85	1.04	PD Plus	2.89	44
	25/02/2022 3:18pm	3.4	1.27	Open Cow	4.67	52
	26/02/2022 5:29am	7.34	2.96	Dry Cow	10.3	48
	26/02/2022 6:21am	1.39	1.51	Heifer	2.9	21
	26/02/2022 6:43am	4.32	1.89	Open Cow	6.21	29
	26/02/2022 7:17am	1.67	1.5	PD Plus	3.165	26
	27/02/2022 2:10pm	3.23	0.91	Beef Mix	4.135	21
	27/02/2022 2:34pm	1.61	1.01	PD Plus	2.615	24
	27/02/2022 3:03pm	1.72	1.34	Open Cow	3.06	50
	28/02/2022 2:18pm	6.86	1.68	Heifer	8.54	35
	28/02/2022 2:55pm	1.62	1.08	PD Plus	2.7	27
	28/02/2022 3:22pm	2.34	1.65	Open Cow	3.99	40
	28/02/2022 4:04pm	2.97	1.47	Dry Cow	4.435	41
	01/03/2022 3:10pm	8	1.51	PD Plus	9.51	33
	01/03/2022 3:47pm	2.58	1.73	Open Cow	4.31	40
	01/03/2022 4:29pm	3	0.87	Beef Mix	3.87	16
	03/03/2022 1:09pm	3.72	1.2	Beef Mix	4.92	33
	03/03/2022 1:42pm	3	1.13	PD Plus	4.13	29
	03/03/2022 2:23pm	2.16	2	Open Cow	4.16	41
	04/03/2022 11:23am	5.55	1.44	Open Cow	6.99	35
	04/03/2022 12:03pm	2.2	1.15	Dry Cow	3.35	24
	04/03/2022 12:32pm	1.58	1.01	Heifer	2.59	19
	04/03/2022 3:33pm	2.13	2.18	PD Plus	4.31	35

This table shows the data collected during the trailed feeder wagon and telescopic handler tests. All the data and tables and figures are the result of data gathered during diesel tests. Finally, milk yield data collected from the farm will be used as a reference point to assess change between feed wagons.

Average Diesel Used Trailed	4.65642857
Average Diesel Used Self-Propelled	6.62177419

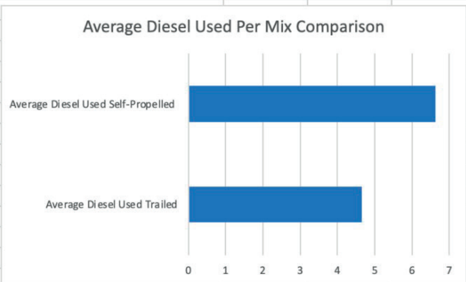

Figure 3. The average diesel used per mix for both the self-propelled diet feeder and the trailed diet feeder

The data shows that the trailed feeder used 1.96 litres less per mix, on average, than the self-propelled diet feeder.

Average Time Taken Trailed	34.25
Average Time Taken Self-Propelled	34.6129032

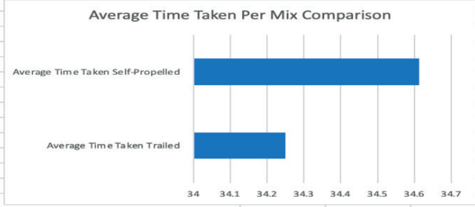

Figure 4. The average time taken per mix for both the self-propelled diet feeder and the trailed diet feeder

From the data and the graph it is clear that the self-propelled took an average of 22 seconds longer.

Self Propelled		
	Trailed	Self Propelled
Day 1 Test	4801	4679
Day 2 Test	4763	4636
Day 3 Test	4486	4794
Day 4 Test	4731	4713
Day 5 Test	4498	4887
Day 6 Test	4517	4839
Day 7 Test	4489	4993
Day 8 Test	4627	4899

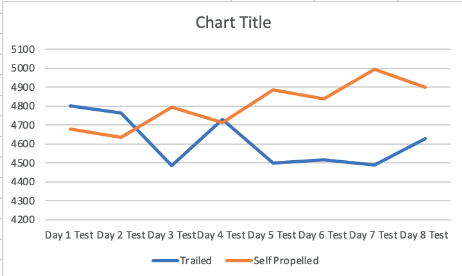

Figure 5. The changes in milk yield during the times of testing

The results appear to be sporadic and don't show a clear trend. However, it must be taken into account that the first 2 days with the trailed feeder shows the time where the cattle were likely still benefiting from the quality of feed mix provided by the self-propelled unit.

In the primary stages of this project, the initial hypothesis was that due to the self-propelled occupying the role of two machines, the diesel use would be lower. In addition to this, due to the self-propelled constantly mixing, this means that static mixing is not required once all materials are loaded into the diet feeder. It was expected that the self-propelled mixer would complete feeding in a more timely fashion than the trailed alternative. From the data it is clear that the

original hypothesis was incorrect however, as predicted there was an increase in milk yield.
When it comes to justifying the purchase of a self-propelled diet feeder on any farm, there are a number of different factors that need to be assessed and analysed before a decision can be made. The most pertinent of these factors are as follows.

Purchase Price
With a base model Keenan self-propelled on the market for £169,000 (Mowbray, 2017) and a trailed unit costing roughly £50,000 (French, 2022), there is a minimum capital sum of £119,000 to be put forward in order to equip a farm with a self-propelled diet feeder as opposed to a trailed diet feeder. The bulk of this transaction will come from the power train that the self-propelled unit is fitted with. However, with a trailed diet feeder the required elements - tractor and telescopic handler or loader tractor - are already commonplace on farms up and down the country. In the interview with Patrick, he shared the same view as Mowbray, saying that the upfront cost of switching to a self-propelled diet feeder would be a stumbling block for smaller dairy units with less throughput.

Diesel use
When any consumer in any industry purchases a piece of machinery with a fuel intake, one key factor that will be taken into consideration will be the efficiency of that fuel intake. Therefore, one point that manufacturer, Keenan, was claiming is a fuel consumption reduction of up to 25% (Keenan, 2021). However, the data in this study has shown that the average fuel consumption for the self-propelled diet feeder at Myerscough Farms was 1.97 litres more per mix (Figure 6) than the conventional trailed method. This is an increase of 42.2% per mix average. However, one of the main discussion points, with regards to the trailed unit, is the change in practices since the implementation of the self-propelled diet feeder. As stated by Patrick in his interview, the milling of the straw by the self-propelled feeder is very high quality, whereas with the trailed unit the straw isn't processed nearly as well. Prior to the self-propelled mixer, the farm used to rely on the trailed unit and pre-chopped the straw through a straw chopper before entering it into the diet feeder. The claimed fuel consumption from the trailed mixer, therefore, does not incorporate the fuel that used operating the pre-chopper. The pre-chopping of the straw would add an increase to the diesel fuel consumption using the trailed unit.

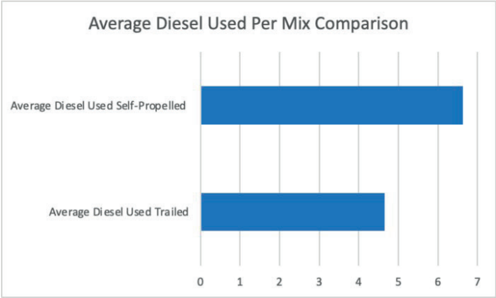

Figure 6. Comparison of the average diesel used per mix

This shows that the self-propelled feeder wagon used more fuel, when the average was calculated, compared to the trailed feeder wagon and telescopic handler (Figure 7).

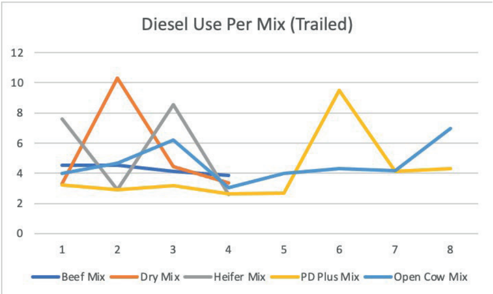

Figure 7. Diesel use per mix for the trailed machine

There are clearly four main anomalies in the data, these occur on day 1 with the heifer mix, day 2 with the dry mix, day 3 with the heifer mix and day 6 with the PD Plus mix.

Table 3. Type of feed

	Date and time	Tractor diesel use	Kramer diesel used	Type of feed	Total diesel used	Time taken
Trailed feeder wa	24/02/2022 1:24pm	5.29	2.3	Heifer	7.585	51
	24/02/2022 2:16pm	2.16	1.14	Dry Cow	3.3	44
	24/02/2022 3:02pm	1.91	1.31	PD Plus	3.22	24
	24/02/2022 3:31pm	2.53	1.48	Open Cow	4.005	43
	25/02/2022 2:05pm	3.75	0.77	Beef Mix	4.52	20
	25/02/2022 2:32pm	1.85	1.04	PD Plus	2.89	44
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	26/02/2022 5:29am	7.34	2.96	Dry Cow	10.3	48
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	28/02/2022 4:04pm	2.97	1.47	Dry Cow	4.435	41
	01/03/2022 3:10pm	8	1.51	PD Plus	9.51	33
	01/03/2022 3:47pm	2.58	1.73	Open Cow	4.31	40
	01/03/2022 4:29pm	3	0.87	Beef Mix	3.87	16
	03/03/2022 1:09pm	3.72	1.2	Beef Mix	4.92	33
	03/03/2022 1:42pm	3	1.13	PD Plus	4.13	29
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	04/03/2022 12:03pm	2.2	1.15	Dry Cow	3.35	24
	04/03/2022 12:32pm	1.58	1.01	Heifer	2.59	19
	04/03/2022 3:33pm	2.13	2.18	PD Plus	4.31	35

One common factor regarding the dates and the anomalies is that the spikes occur on the first test of the day on the 24/02, 26/02, 28/02 and 01/03. Overnight the tractor doesn't move and isn't run, however the fuel cools down once the tractors have been topped up while still warm. One possible explanation for this apparent increase in fuel consumption is the expansion of fuel during the period of mixing feed. Then, overnight, the fuel cools/contracts and this leads to a lower fuel level in the morning compared to the day before. To tackle this problem, the machine should have been filled up prior to testing at the start of each day rather than at the end of each day to prevent incorrect data. However, a problem that would then arise would be, for example, if the machine took somewhere between four and six extra litres, where that fuel data would be accredited to. One possible solution would be to fill up the tractor once it was warm and complete all tests while the machine was warm. However, amending these results would only decrease the trailed diet feeders overall average diesel consumption. With the trailed mixer already showing that it burns less diesel, for the purpose of this investigation, the results will remain.

Milk Yield Change

One method to access the quality of the mix is to measure waste from the feed, i.e. what the cows have sorted through, and left, in the troughs and compare with the associated milk yields. In Patrick's interview he claimed that due to the self-propelled's ability to process straw to a much higher standard, "*the cows are eating the same ration all the time whereas with the trailed there are spikes where they'll pick things out of the ration that they shouldn't be able to*". This statement highlights two factors of the self-propelled where the trailed is inferior. The first of these factors is that the cows do not sort through the feed as much and are therefore eating the same volume and mix contents every day. The second factor is the consistency of the feed that is put out; with the feed out of the self-propelled being far more consistent, K.A. Beauchemin (2018) and Mohammadreza Ebrahimi (2018) claim that it is better for the cows to ingest a more even mix with the correct levels of micronutrients being spread across the whole mix rather than having concentrated patches of mix containing more vitamins. Patrick also claims that this is due to the way the diet feeder is loaded. 'With the trailed feeder you've got to fill the mixer in two halves because it doesn't transfer mix from the front to the back. Whereas with the self-propelled because it's throwing it in an arc over the tub it's dropping out all the way along so you get a much more consistent mix.' Ali Hassanpour (2011) corroborates this statement with both Patrick and Ali explaining the benefits from the conveyor from the milling head 'throwing' the material into the tub, the material is effectively placed along the full length of the tub rather than in sections like in the trailed feeder. During the tests it was clear to see that care had to be taken when loading the trailed feeder and materials such as silage and straw had to be dropped in evenly in the front and the back. When asked whether the level of refusal and wasted feed had gone up or down, Patrick claimed that, "*because the feed is a lot more consistent we throw less away*". He put the reduction in wasted feed down to the more consistent feed, meaning the cows were unable to sort through the feed and leave undesirables in the trough.

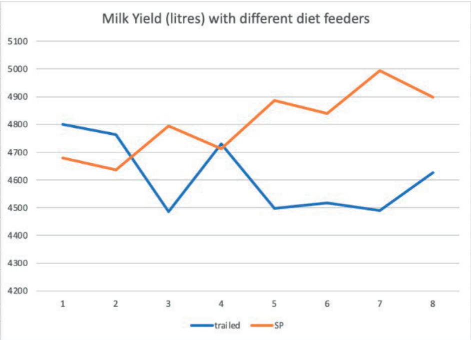

Figure 8. Milk yield comparison between the trailed diet feeders.

The milk yield changed over the course of the testing period while using both the self-propelled and the trailed feeders. It is clear, from the graph in Figure 8, that the milk yield during the trailed period is erratic. This inconsistency would corroborate Patrick's claim that the trailed mixer supplied the cows with irregular feed and therefore would bring the milk yield down or make it unstable. The plateaued, high section at the start of the trailed mixing period could be explained by the cattle still ruminating the feed from the self-propelled mixer and then, once dependant on the trailed mixer, the graph shows a general downward to resting trend around 4,500 litres with a single anomaly on day four of testing. As with the self-propelled, from the start there is a general upward trend with regular peaks.

Table 4. Typical rates of pay and costs

	Self-Propelled	Trailed
Average Hours Per Day	2.04	2
Rate of Pay	£17.96	£17.96
Average Pay Per Day	£36.64	£35.92

The average salary for a herd manager in the United Kingdom in 2022 is £17.96/hour (Talent, 2022).

Table 5. The UK cost of agricultural diesel (2022)

	Self-Propelled	Trailed
Average Diesel Burnt Per Day	22.768125	16.2975
Cost Per Litre	£1.04	£1.04
Total	£23.68	£16.95

Table 6. The UK price of milk (2022)

	Self-Propelled	Trailed
Average Litres Per Day	4805	4614
Price Per Litre	0.3589	0.3589
Total sale value per day	£1,724.51	£1,655.96

The price of milk in the UK was 35.89 pence per litre in February 2022 (AHDB, 2022).

Table 7. Comparison chart showing costs/income for the two machines

	Self-propelled	Trailed
Labour Outgoings	£36.64	£35.92
Fuel Outgoings	£23.68	£16.95
Milk Yield Income	£1,724.51	£1,655.96
Total income	£1,664.19	£1,603.09

From the table above and the previous Tables 4-7, it is clear to see that although labour and fuel costs are higher for the self-propelled, due to the increase in milk yield the self-propelled still boosts the farm's profits by £61.10 per day. Over the course of a year this equates to £22,301.50 profit from the self-propelled compared to the trailed. However, there is still the factor of the £180,000 initial purchase price to consider.

Break Even Analysis:

In order to fully understand how beneficial this machine would be, a break-even analysis must be carried out to see how long the machine would need to be implemented in order to pay itself off.

Table 8. Year-by-year income

Financial status	Year	Annual savings @ £61.10/day
	1	£22,301.50
	2	£44,603
	3	£66,904.50
	4	£89,206
	5	£111,507.50
	6	£133,809
	7	£156,110.50
	8: Base Model Pay Off Point	£178,412
	9: Current Method Pay Off Point	£200,713.50

From the Table 8 it shows that it would take nine years of operating at the same level as it did during the tests in order to pay itself off. More specifically, it would take exactly 420.86 weeks in order to pay itself off or 2,946 days.

From the data shown, it is clear why a small farm would not be able to overcome the initial purchase cost of an item of machinery such as a self-propelled diet feeder.

CONCLUSIONS

- The break-even point when purchasing a self-propelled diet feeder, in this instance, is 9 years.
- When supplied with feed from the self-propelled diet feeder, cow milk yield rose by 4.06% when compared to the trailed diet feeder.
- The refusal rate from the cattle was significantly reduced when using the self-propelled diet feeder compared to the trailed diet feeder therefore reducing waste.
- Fuel consumption for the trailed diet feeder was 33.13% lower on average than that of the self-propelled.
- The self-propelled diet feeder took on average 0.4 hours longer per day when compared to the trailed alternative.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE IMPACT OF VARIOUS PEST CONTROL OPTIONS ON ARTHROPOD STRUCTURE AND DIVERSITY IN SWEET POTATO AGRO-ECOSYSTEM IN THE CONDITIONS OF SANDY SOILS FROM SOUTHERN ROMANIA

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Abstract

The paper aimed to evaluate the influence of three different pest control options (chemical, biological and a combination of them) on epigeic fauna diversity in the conditions of sandy soils from southern Romania in two succesive years (2021-2022), through the analysis of biological material resulting from the collection of samples in pitfall traps and direct observations at the foliage level. The arthropod fauna was represented by 69 species belonging to a number of 26 families and 13 taxonomic orders: Acarina, Araneae, Collembolla and the insect orders Coleoptera, Dermaptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Mantodea, Neuroptera, Orthoptera and Thysanoptera. The composition of the fauna spectrum were dominated by Collembolla species in 2021 and Coleoptera species in 2022. The highest richness of species and values of Shannon-Wiener and Simpson diversity index were found in treatments with biological control either alone or in combination.

Key words: *arthropod structure, arthropod diversity, sweet potato, Southern Romania.*

INTRODUCTION

According to FAOSTAT (FAO, 2020) sweet potato (*Ipomoea batatas* L.) was ranked the fifth most important food crop worldwide after maize, rice, wheat and cassava. Sweet potatoes are more tolerant than other major crops to climate shocks and stresses, are fairly drought-tolerant, and do not need lots of organic matter or soils with high fertility levels. The sweet potato adaptability to all these factors, have aroused the interest for the introduction to culture on the sandy soil of the Dabuleni area from southern Romania (Diaconu et al., 2017), the researchers from Research and Development Station for Plant Culture on Sands Dabuleni (R.D.S.P.C.S.D. Dabuleni), Romania recently finalizing the culture technology, available now for local producers (Diaconu et al., 2018).

Crop monitoring for maintenance of crop health is essential to ensure high yield and quality of the sweet potato crops. Arthropod surveillance is mandatory in order to identify the spectrum of the species that can cause damage during vegetation period (Iamandei et al., 2014). On the other hand, soil arthropods might be affected by various management practices and play a relevant role as bioindicators of agroecosystem soil quality and health (Menta & Remelli, 2020). The study aim was to establish the arthropods fauna structure in sweet potato crop and to evaluate the influence of three different pest control interventions on epigeic fauna diversity in the conditions of sandy soils from southern Romania.

MATERIALS AND METHODS

The research was conducted in the experimental area of R.D.S.P.C.S.D. Dabuleni (N 43.80700, E 024.05516) in 2021 and 2022 sweet potato growing seasons. The experimental area was located on a typicallty sandy soil, with low fertility, protected with PE mulch, and set-up according to the method of subdivided plots. The crops preceding the sweet potato trial plots were: Sorghum

(*Sorghum bicolor* (L.) Moench) in 2021 and cowpea (*Vignia unguiculata* (L.) Walp) in 2022.

The surface of one treatment plot was 36 m.p. The sweet potato shoots, KSP1 variety, also obtained at S.C.D.C.P.N. Dabuleni greenhouse, were planted in field on last decade of May each year. The experimental treatments are described in Table 1.

Table 1. Experimental treatments used on sweet potato crop at R.D.S.P.C.S.D. Dabuleni in 2021 and 2022

Treatment number	Treatment description		
1	chemical treatment	chemical products, applied in vegetation	Cabrio top + Calypso 480 SC
2	biological treatment	biological insecticide and fungicide, applied at the soil preparation right before crop set-up in the field	Bio-insecticide based on *Metarhizium anisopliae* applied to the soil in a dose of 2.5 g/plant and incorporated immediately and granular product based on *Bacillus subtilis* - strain Dj3
3	complex treatment	mixed biological insect-fungicide + chemical in vegetation	Combination of treatment 2 + 1

The evaluation of the arthropod fauna present in the experimental plots organized at S.C.D.C.P.N Dăbuleni was carried out through the analysis of biological material resulting from the collection of samples in pitfall traps and direct observations at the foliage level. The soil fauna was monitored from May to August each year, using pitfall traps with formalin 4% as preservative solution. One pitfall trap per treatment plot was installed on the space between rows, left in the field for 7 days per months and afterwards the samples were transferred to Research and Development Institute for Plant Protection, Bucharest (R.D.I.P.P., Bucharest) entomology laboratory, where the collected material was washed and all the specimens where preserved in alcohol 70% for further analysis. Also, field observations of the arthropod populations were conducted at 4 week intervals when adults and larvae were counted on 100 sweet potato leaves per treatment. The specimens easily identified were left in the field and the rest were transferred to the R.D.I.P.P. entomology laboratory. The collected material was preserved in alcohol or dry and further processing of the samples consisted of sorting and identification of the arthropod fauna at the level of taxonomic group, family, genus or species. Data were tabulated and identified genera/species were listed. Total abundance and relative frequency were calculated for each sampling method in order to analyse the effects of the variants on arthropod faunal communities, for every year and overall experiment. Shannon-Wiener and Simpson diversity indices were calculated to interpret the results obtained in the edaphic entomofauna collection. Graphical representations and determination of diversity indices were performed using the Past 4.03 application (Hammer, 2018).

RESULTS AND DISCUSSIONS

The fauna of arthropods collected or identified by both methods in the experimental variants organized at S.C.D.C.P.N. Dăbuleni in 2021-2022 at the sweet potato crop was represented by 69 species belonging to a number of 26 families and 13 taxonomic orders: *Acarina, Araneae, Collembolla* and the insect orders *Coleoptera, Dermaptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Mantodea, Neuroptera, Orthoptera and Thysanoptera* (Table 2). Sweet potato crops can be affected by over 270 species of pest insect's worldwide (Amalin and Vasquez, 1993). Previous data obtained in the Dabuleni area noticed the presence of 14 pest species out of fifty one identified in sweet potato crop (Iamandei et al., 2014).

The results of the arthropods monitoring in the experimental variants organized at S.C.D.C.P.N. Dăbuleni in 2021 shows a total of 48 species, 26 collected in pitfall traps and 26 observed directly on plant. Predators species were generally prevalent in visual observation (Table 2).

Table 2. The general structure of the arthropod species collected in the experimental treatments organized at S.C.D.C.P.N. Dabuleni in the years 2021-2022 to the sweet potato crops

	Taxonomic group/Order: Family	Species	2021		2022	
			Pitfal trap	**Visual obser-vation**	**Pitfal trap**	**Visual obser-vation**
1	*Acarina/ Trombidiformes Tetranychidae*	*Tetranychus urticae* Koch, 1836	+	+	-	+
2	*Araneae Lycosidae*	*Pardosa amentata* (Clerck, 1757)	+	+	-	+
3		*Alopecosa* sp.	-	+	-	+
4	*Collembola Entomobryidae*	*Entomobryia arborea* (Tulberg, 1871)	+	-	+	-
5		*Lepidocyrtus* sp.	-	-	+	-
6	*Collembolla Bourletiellidae*	*Bourletiella arvalis* (A.Fitch, 1863)	+	-	+	-
7	*Collembolla Sminthuridae*	*Sminthurus viridis* (Linnaeus, 1758)	+	-	+	-
8	*Hemiptera Aphididae*	*Aphis gossypi* (Glover, 1877)	-	+	-	+
9		*Aphis* sp.	-	-	-	+
10		*Aulacorthum solani* (Kaltenbach, 1843)	-	-	-	+
11		*Macrosiphum euphorbiae* (Thomas, 1878)	-	-	-	+
12	*Hemiptera Pyrrhocoridae*	*Pyrrhocoris* sp.	-	-	+	-
13	*Hemiptera Aleyrodidae*	*Trialeurodes vaporariorum* (Westwood, 1856)	-	+	-	+
14	*Hemiptera Anthocoridae*	*Anthocoris nemorum* (Linnaeus, 1761)	-	+	-	+
15		*Orius* sp.	-	-	-	+
16	*Hemiptera Pentatomidae*	*Nezara viridula* (Linnaeus, 1758)	-	+	-	+
17	*Thisanoptera Thripidae*	*Frankliniella occidentalis* (Pergande, 1895)	+	+	+	+
18	*Ortoptera Acrididae*	*Acrida hungarica (Herbst, 1786)*	-	+	-	+
19		*Calliptamus italicus* (Linnaeus, 1758)	-	+	-	+
20	*Dermaptera Forficulidae*	*Forficula auricularia* (Linnaeus, 1758)	+	-	+	-
21	*Mantodea Mantidae*	*Mantis religiosa* (Linnaeus, 1758)	-	+	-	+
22	*Coleoptera Chrysomelidae*	*Chaetocnema* sp.	+	+	-	+
23		*Phyllotreta atra* (Fabricius, 1775)	+	-	+	-
24		*Phyllotreta nemorum* (Linnaeus, 1758)	+	-	+	-
25	*Coleoptera Coccinellidae*	*Adalia bipunctata* (Linnaeus, 1758)	-	+	-	+
26		*Hippodamia variegata* (Goeze, 1777)	-	+	-	+
27		*Coccinella septempunctata* (Linnaeus, 1758)	-	+	+	+
28		*Coccinella quinquepunctata* (Linnaeus, 1758)	-	-	+	-
29		*Harmonia axyridis* (Pallas, 1773)	-	+	-	+
30		*Scymnus* sp.	-	-	-	+
31	*Coleoptera Anthicidae*	*Notoxus monoceros* (Linnaeus, 1760)	+	-	+	+
32		*Notoxus brachycerus* (Faldermann, 1837)	-	-	+	-
33	*Coleoptera Carabidae*	*Bembidion quadrimaculatum* (Linnaeus, 1760)	-	-	+	-
34		*Harpalus azureus* (Fabricius, 1775)	-	-	+	-
35		*Harpalus calceatus* (Duftschmid, 1812)	+	-	+	+

	Taxonomic group/Order: Family	Species	2021		2022	
			Pitfal trap	Visual obser-vation	Pitfal trap	Visual obser-vation
36		*Harpalus pubescens* (O.F.Müller, 1776)	-	-	+	-
37		*Harpalus tardus* (Panzer, 1796)	-	-	+	-
38		*Scarites terricola* (Bonelli, 1813)	-	-	+	-
39	*Coleoptera Curculionidae*	*Tychius* sp.	-	-	+	-
40	*Coleoptera Cryptophagidae*	*Cryptophagus* sp	-	-	+	-
41	*Coleoptera Elateridae*	*Agriotes obscurus* (Linnaeus, 1758)	-	+	-	+
42		*Drasterius bimaculatus* (Rossi, 1790)	+	-	+	-
43	*Coleoptera Latridiidae*	*Corticarina* sp.	+	-	+	-
44		*Latridius* sp.	-	-	-	+
45	*Coleoptera Scarabaeidae*	*Scarabaeus typhon* (Olivier, 1789)	+	-	-	+
46		*Scarabaeus* sp.	-	-	+	-
47		*Anomala vitis* (Fabricius, 1775)	-	-	+	+
48		*Pentodon idiota* (Herbst, 1789)	-	-	-	+
49		*Anoxia villosa* (Fabricius, 1781)	-	+	-	-
50	*Coleoptera Tenebrionidae*	*Blaps lethifera* (Marsham, 1802)	-	-	+	-
51		*Pedinus femoralis* (Linnaeus, 1767)	+	-	-	+
52	*Coleoptera Staphylinidae*	*Atheta myrmecobia* (Kraatz, 1856)	+	-	+	+
53	*Neuroptera Chrysopidae*	*Chrysoperla carnea* (Stephens, 1836)	-	+	-	+
54	*Hymenoptera Ichneumonidae*	*Ichneumon formosus* (Gravenhorst, 1829)	+	-	+	-
55	*Hymenoptera Formicidae*	*Formica fusca* (Linnaeus, 1758)	+	-	+	-
56		*Formica rufa* (Linnaeus, 1761)	+	-	+	-
57		*Myrmica rubra* (Linnaeus, 1758)	+	-	+	-
58	*Lepidoptera Sphingidae*	*Agrius convolvuli* (Linnaeus, 1758) adult	-	+	-	+
		Agrius convolvuli (Linnaeus, 1758) larvae	-	+	-	+
59	*Lepidoptera Noctuidae*	*Helicoverpa armigera* (Hübner, 1808) adult	-	+	-	+
		Helicoverpa armigera (Hübner, 1808) larvae	-	+	-	+
60		*Spodoptera exigua* (Hübner, 1808) adult	-	+	-	+
		Spodoptera exigua (Hübner, 1808) larva	-	-	-	+
61		*Hadena syriaca* (Osthelder, 1933) adult	-	+	-	+
62		*Autographa gamma* (Linnaeus, 1758) adult	-	-	-	+
63	*Lepidoptera Pyralidae*	*Loxostege sticticalis* (Linnaeus, 1761) adult	-	-	-	+
64	*Diptera Syrphidae*	*Episyrphus balteatus* (De Geer, 1776)	-	+	-	+
65	*Diptera Chloropidae*	*Elachiptera cornuta* (Fallén, 1820)	+	-	-	-
66		*Chlorops pumilionis* (Bjerkander, 1778)	+	-	-	-
67	*Diptera – Muscidae*	*Stomoxys calcitrans* (Linnaeus, 1758)	+	-	+	-
68		*Musca domestica* (De Geer, 1776)	+	-	+	-
69	*Diptera Sciaridae*	*Sciara analis* (Schiner, 1863)	+	-	-	-

Legend: "+" - present in samples; "-" absent in samples

Four species of aphids (*Aphis gossypii, Aulacorthum solani, Macrosiphum euphorbae* and *Aphis* sp.), one species of thrips (*Frankliniella occidentalis*) as well as whitefly, known vectors of sweetpotato blight, were observed on the crop foliage. During the course of the observations made at the foliage level, on leaves where there were colonies in the early stages of these vectors, the presence of a large number of beneficial species was noted.

The most frequent species were *Coccinella septempunctata, Adonia variegata, Adalia bipunctata, Harmonia axyridis* and the larvae of *Episyrphus balteatus* followed by spider mite species of the family *Lycosidae*. Also very abundant in the untreated version were the predatory bugs *Orius* sp. and *Anthocoris nemorum* (*Hemiptera: Anthocoridae*).

The use of the pitfall trap method allowed the capture of a large number of individuals of *Hymenoptera Formicidae* species and the identification of the predators *Atheta myrmecobia and Harpalus calceatus*. These species are between the main antagonists of aphids in the study area, contributing to the protection of sweet potato crops against viral diseases (Iamandei et al., 2014). A number of foliage pests such as *Chaetocnema* sp*., Notoxus monocerus and Tetranychus urticae* were also identified using Barber traps. Butterflies of the species *Hadena syriaca, Spodoptera exigua and Helicoverpa armigera* (*Noctuidae*) and the specific pest *Agrius convolvuli*, whose larvae were present on the foliage, were also captured and subsequently identified, with sporadic presence noted during direct observations.

The results of the arthropods monitoring in the experimental variants organized at S.C.D.C.P.N. Dăbuleni in 2022 shows a total of 65 species, 33 collected in pitfall traps and 41 observed directly on plant. The same four species of aphids (*Aphis gossypii, Aulacorthum solani, Macrosiphum euphorbiae* and *Aphis* sp.), *Frankliniella occidentalis* as well as whitefly, known vectors of sweetpotato blight, were noticed in 2022. During the observations made at foliage level, a large number of specimens of useful fauna species were observed on leaves where colonies of these species were present. The most frequent species were *Coccinella septempunctata, Adonia variegata, Adalia bipunctata, Harmonia axyridis*, and the larvae of *Episyrphus balteatus* followed by spider species of the family *Lycosidae*. The species *C. quincvepunctata* and *Scymnus* sp. were noticed for the first time in 2022 monitoring. Also very abundant in the biological treatment variant were the predatory bugs *Orius* sp. and *Anthocoris nemorum* (*Hemiptera:Anthocoridae*) but other predators such as *Mantis religiosa, Chrysoperla carnea* and the staphylinid *Atheta myrmecobia* were also recorded.

The noctuid species *Hadena syriaca, Spodoptera exigua, Autographa gamma and Helicoverpa armigera* were captured and during the first decade of August an intense flight of the species *Loxostege sticticalis* was recorded. As for the specific pest *Agrius convolvuli*, their larvae were present on the foliage, with sporadic presence noted during direct observations. The use of the pitfall trap method allowed the capture of a large number of individuals of *Hymenoptera Formicidae* species and the identification of predators of *Atheta myrmecobia* species and a number of 6 carabid species *which,* together with coccinellids and neuroptera, are the main aphid antagonists in the study area, indirectly contributing to the protection of sweet potato crops against viral diseases. A number of foliage pests such as *Chaetocnema* sp*., Notoxus monocerus, N. brachycerus and Tetranychus urticae* were also identified using pitfall traps.

Regarding the prevalence of epigean fauna identified from pitfall trap material, in 2021, the highest numerical abundance was recorded by *Collembolla*, followed by *Acarina* and *Coleoptera* (Figure 1 and Table 3). The abundance of species was higher in both variants with biological treatments. Also, the highest value of the Shannon-Wiener diversity index was recorded in treatment 3 (1.817), followed by treatment 2 (1.795), while the lowest in treatment 1 (1.55). The same trend was also recorded for the Simpson index, the highest value in treatment 3 (0.7996), followed by treatment 2 (0.7637), while the lowest in treatment 1 (0.7313). In the year 2022, the highest numerical abundance was recorded by *Coleoptera*, followed by *Hemiptera* and *Collembolla* (Figure 2 and Table 3).

Table 3. The abundance and diversity of the taxonomic groups collected using soil traps in the three treatment options organized at S.C.D.C.P.N. Dabuleni in the years 2021-2022 to the sweet potato crops

	V1		V2		V3		Total 2021		V1		V2		V3		Total 2022	
	A	F %	A	F %	A	F %	A	F %	A	F%	A	F%	A	F%	A	F%
Acarina	30	38.9	6	6.7	26	30.2	62	24.5	0	0	0	0	0	0	0	0
Araneae	2	2.60	7	7.8	5	5.8	14	5.53	3	9	9	18	3	6.38	15	11.5
Hemiptera	1	1.30	5	5.6	8	9.3	14	5.53	2	6	7	14	12	34.0	25	19.2
Dermaptera	1	1.30	5	5.6	2	2.3	8	3.16	1	3	3	6	0	0	4	3.1
Coleoptera	21	27.3	12	13.3	18	20.9	51	20.2	18	54	15	30	22	46.8	55	42.3
Hymenoptera	0	0.00	7	7.8	1	1.2	8	3.16	0		3	0	1	2.13	4	3.08
Orthoptera	2	2.60	1	1.1	1	1.2	4	1.58	1	3	1	6	0	0	3	2.31
Diptera	4	5.19	8	8.9	5	5.8	17	6.72	0	0	2	2	1	2.13	2	1.54
Thysanoptera	1	1.30	0	0.00	1	1.2	2	0.79	0	0	0	0	1	2.13	1	0.77
Collembola	15	19.5	39	43.33	19	22.1	73	28.8	8	24	10	4	3	6.38	21	16.15
Total	77	100	90	100	86	100	253	100	33	100	50	20	47	100	130	100
Shannon–Wiener index	1.553		1.795		1.817		1.85		0.342		0.544		0.4234		0.7707	
Simpson index	0.7313		0.7637		0.7996		0.8031		2.854		5.724		3.0280		3.963	
Species richness	20		24		22		**26**		21		30		27		**33**	

A-Abundance; F- relative frequency

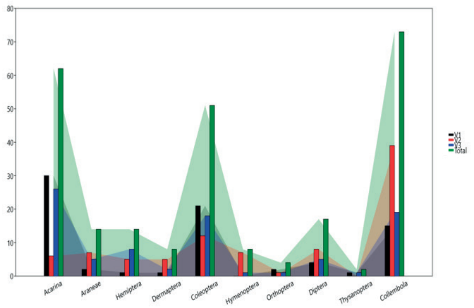

Figure 1. Abundance of epigean arthropod groups collected using soil traps in the three pest control options for sweet potato in 2021

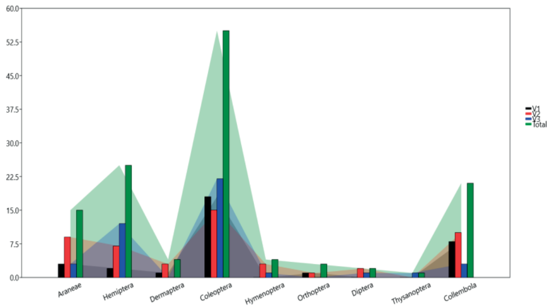

Figure 2. Abundance of epigean arthropod groups collected using soil traps in the three pest control options for sweet potato in 2022

In 2022, the abundance of epigeic species was higher in both variants with biological treatments. Also, the highest value of the Shannon-Wiener diversity index was recorded in variant 2 (0.5439), followed by variant 3 (0.42338), while the lowest in variant 1 (0.3414).

The same trend was also recorded for the Simpson index, with the highest value recorded in variant 2 (5.724), followed by variant 3 (3.028) and the lowest in variant 1 (2.854).

The species richness's was higher in treatments with biological control of the pest in both years of research.

Of the epigean invertebrates, *Coleoptera, Collembolla, Hemiptera* and *Aranea* were eudominant in all treatments, both years.

CONCLUSIONS

In the experimental conditions of 2021 and 2022, the diversity of arthropod species identified in the sweet potato crop in the experimental fields of S.C.D.C.P.N. Dăbuleni was represented by 69 species belonging to a number of 26 families and 13 taxonomic order.

In term of abundance, the composition of the sweet potato pest spectrum was dominated by coleopteran, with the genus *Notoxus* and *Drasterius bimaculatus* having the highest catches.

Damage caused by these pests can be significant in some years, and their aggregate presence can create a dangerous situation with undesirable effects in terms of yield and quality. Overall, data analysis showed that the incidence of crop-specific pest attack was sporadic, dangerous pests such as white grubs, wireworms, codling moth larvae and *A. convolvuli* had low numerical abundances.

The useful fauna has favorable activity conditions, successfully keeping the sweetpotato's virus-transmitting species under control. The presence of predator species from particular groups such as *Araneae, Carabidae, Coccinelidae, Chrysopidae, Syrphidae*, *Mantidae* and *Araneae* indicated the ecological balance, quality and sustainability of the variants consisting of biological treatments.

The three types of pest control interventions influenced structural biodiversity indices such as soil fauna abundance, specific arthropod richness and diversity in the batat crop during the 2021 and 2022 growing seasons and sandy soil conditions in Dabuleni.

ACKNOWLEDGEMENTS

This study was performed within the project ADER 7.3.4, financially supported by the Ministry of Agriculture and Rural Development, Sectoral Plan for 2019-2022.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

BIOWASTE COMPOST – AN ALTERNATIVE SOURCE OF NUTRIENTS FOR AGRICULTURAL CROPS

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Abstract

Important amounts of biowaste are produced daily both in the rural and urban areas of Romania. Composting is the most sustainable way for their treatment, and compost can be used as fertilizer, including in organic agriculture if it meets specific quality standards. The agronomic value of the compost is given by the macronutrient and organic matter content, and its quality by the absence of phytotoxic compounds (heavy metals and other pollutants), and the absence of pathogens for humans, animals and plants. This paper presents part of the results of a study made to characterize the agronomic value of three types of compost (a. poultry manure + cereal straw; b. poultry manure + vegetable food waste; c. biodegradable household waste). Mixtures of compost and soil were made where the compost was integrated in proportions of 25, 50 and 75% g/g. A test plant, oats (Avena sativa L.), was cultivated in the greenhouse, in pots, on the mixtures made. The intake of macronutrients (N, P, K, Mg, Ca) was analyzed in biomass samples, harvested at the grain filling stage.

Key words: *biowaste, composting, macronutrients, sustainable agriculture, agronomic value.*

INTRODUCTION

Nowadays, humanity is facing a demographic growth that tends to reach 8.5 billion inhabitants in 2030 (United Nations, 2015), wich brings a multitude of social challenges such as poverty, hunger, lack of jobs, lack of hygiene, lack of water, diseases and also economic and financial impact by diminishing natural resources which has a massive impact on the climate, therfore, sustainable development remains the only way to meet the needs of present generations and protect the environment at the same time (United Nations, 2015). In meeting many of the strategic objectives (poverty reduction, food supply, renewable energy production, responsible production, etc.) of the United Nations (UN) 2030 Agenda, agriculture has a key role to play (FAO, 2016), as it is one of the economic areas that can fundamentally integrate the concept of Circular Economy (EC), which has the role of increasing the efficiency of natural resource use, maximizing the added value of products and minimizing environmental and climate impact (Nordin et al., 2022; Velasco-Munoz et al., 2022; Rodríguez-Espinosa et al., 2023). As agriculture needs large quantities of fertilising materials, it may increase the use of organic fertilisers and composts instead of synthetic fertilisers that could contribute to reducing energy consumption, making better use of residual biomass resources, improving the physical, chemical and biological soil properties, increasing the resilience of agroecosystems and reducing climate impact (Chojnacka et al, 2022; De Corato, 2020; Wainaina et al, 2020).

Biowaste such as biodegradable garden and park waste, food and kitchen waste from households, offices, restaurants, wholesale warehouses, canteens, caterers or retail premises and comparable waste from food processing plants (Directive 2081/851/CE, PE) may be recycled through composting which is one of the most sustainable ways to dispose them. Using compost as fertilizing material offers many benefits to agriculture and the environment, directly and indirectly by increasing the natural fertility of the soil, increasing ecosystem services, improving the health of crop plants, improving water quality by reducing pollution caused by the use of synthetic fertilizers, increasing the quality of agricultural products, etc. (Rashid et Shahzad, 2021; Martinez-Blanco et. al, 2013).

In Romania, increasing the recycling of bio-waste would help to fulfill the objectives of the EU circular economy action plan as well as to reduce the amount of waste. In 2021, 77,148,372 poultry were registered in Romania, which resulted in a large amount of poultry manure, which mostly ended up in landfills.

In 2020, 131 kg/capita/year of food waste was produced in Europe, of which 14 kg from primary food production (agriculture, fisheries and aquaculture), 26 kg from food and beverage manufacturing, 9 kg from retail and distribution, 12 kg from restaurants and catering and 70 kg from households representing 53.43%, the highest share (Eurostat, 2023). Of these, vegetables, fruits and cereals are the food groups that become the largest share of food waste and are generated in the consumption phase, respectively 46%, which means that a large part of household food ends up in the trash (Sanchez et al., 2020). Given the increased consumption of animal products, globally, large quantities of poultry manure are produced every day on farms but also in rural households. The data published in specialized journals on the amount of litter produced by poultry per 1000 heads / day, respectively: chicken for meat – 65kg, geese – 200 kg, ducks – 190kg (Augustyńska-Prejsnar et. al, 2018). Composting of biowaste has many benefits because it helps to reduce the amount of waste in landfills and to produce composts that can replace chemical fertilizers, can improve soil quality and reduce pollution, and, at the same time, contribute to increase of food production which is very important due to the global population that is constantly raising.

The use of poultry manure as fertilizing material has been made since ancient times due to its high nitrogen content (Munch et al., 2022; Rizzo et al., 2022; Rech et al., 2020). However, applying poultry manure directly to the soil is undesirable because fresh poultry manure is phytotoxic and cannot be used as fertilizer (Bargougui et al., 2020). So, composting it with other types of waste, such as food waste or garden waste, is suitable. Zubair et al. (2020) highlighted the importance of using manure of all types, especially for nutrient recovery from these types of waste but concluded that aerobic composting and anaerobic digestion processes can effectively remove and recover nutrients from solid manure and represent a surplus of nutrients used as fertiliser. Numerous studies have revealed the favorable effects of using compost from animal manure on plant production. So, the use of poultry manure compost significantly increased potato tuber production (Minin et al., 2020); composted cattle manure used in cauliflower cultivation increased the total biomass weight (Simarmata, 2016); poultry manure and dry straw compost applied to cereals increased the production of triticale and wheat (Rusakova et al., 2015). Compost has a valuable content of organic matter essential to agriculture. The application of organic matter to the soil has the potential to alter the nitrogen dynamics of an ecosystem (Wang et al, 2022; Rusakova et Eskov, 2015; Hoang et al, 2022). Therefore, it is important to supplement synthetic fertilizers with organic amendments that provide the plants with the nutrients necessary for growth but at the same time help the environment. This paper presents the results of a study regarding the agronomic value, especially macronutrient availability (N, P, K, Mg, Ca) three types of compost obtained from food waste and manure from chickens raised on the ground within individual households in rural areas. The study was conducted in greenhouse with oat plants (*Avena sativa L.*).

MATERIALS AND METHODS

In this study, were used 3 types of compost obtained domestically in a rural locality in Ilfov County, Romania, using composting containers with a capacity of 320L, 65x65x75, namely:

C1_PMWS - poultry manure + dry wheat straw – 3:1 ratio (12 luni);

C2_PMFW – poultry manure + food scraps – 3:1 ratio (12 luni);

C3_HHC – compost from household waste (garden and kitchen scraps, 12 months).

The compost samples were taken to the National Institute of Research and Development for Pedology, Agrochemistry and Environmental Protection, to determine chemical analyses, in November 2021. The soil used for this experiment came from the Didactic Station for Agronomic Research and Development Moara Domnească, in Găneasa commune, Ilfov county.

Table 1. Physico-chemical characteristics of soil and compost used in experiments

Analyzed parameters	Soil	C1_PMWS	C2_PMFW	C3_HHC
pH	5.89	7.14	7.10	8.36
Humidity (%)	19.3	47.21	42.69	44.64
Dry matter (%)	80.7	52.59	56.31	55.36
C_org. (%)	2.02	14.92	13.62	9.71
C:N	9.35	11.39	10.72	9.23
Nt (%)	0.22	1.31	1.27	1.04
N-NO3̅ (mg kg−1d.m.)	28.67	229	136	910
N-NH4 + (mg kg−1d.m.)	9.12	59.33	53.67	86
P (%)	0.16	1.47	1.62	0.46
K (%)	-	1.23	1.48	1.48

pH is a very important parameter of composting, which depends on the characteristics of the materials that make up the substrate. According to existing studies, a pH range between 6.5 and 9 allows good activity during microbial activity. Both C2_PMFW and C1_PMWS had a pH around 7, resulting in a neutral pH. Similar results have been obtained by other researchers (Tampio et al., 2016; Montovani et Spadon ,2017; Jalili et al., 2019). C3_HHC had a pH of 8.36.
The total nitrogen content was 1.3% in C1_PMWS, compost obtained from poultry manure and wheat straw, and 1.26 for C2_PMFW compost, obtained from poultry manure and plant residues and 1.04 C3_HHC. The results obtained are similar to other experiments where the total nitrogen content of the compost was between 0.9 and 2% (Wilden et al., 2001; Peng et Pivato, 2019; Coelho et al., 2018). The phosphorus (P) content was 1.61% in C2_PMFW, 1,46% in C1_PMWS (Tampio et al., 2016; Peng and Pivato, 2019) and slightly lower in C3_HHC, respectively 0.46%.
The organic carbon content of the composts obtained was 13.62% in C2_PMFW, 14.92% in C1_PMWS and 9.71% in C3_HHC.
The values of nitrates (N-No3) and ammonium (N-NH4) were 136 mg./kg DM N-No3 for C2_PMFW and 229 N-No3 mg/kg DM in C1_PMWS and 54 mg/kg DM N-NH4 in C2_PMFW and 59 mg/kg DM N-NH4 in C1_PMWS. C3_HHC resulted in 910 mg/kg N-No3. Total N normally ranges from less than 1 % to 2,5 % (dry subst.) in finished composts (Wilden et al. ,2001; Peng et Pivato, 2019; Coelho et al., 2018; Jalili et al., 2019).

Table 2 -Content of heavy metals in soil and composts

Treatment	Cd (mg kg $^{-1}$d.m.)	Cr (mg kg $^{-1}$d.m.)	Cu (mg kg $^{-1}$d.m.)	Pb (mg kg $^{-1}$d.m.)	Ni (mg kg $^{-1}$d.m.)	Zn (mg kg $^{-1}$d.m.)	Mn (mg kg $^{-1}$d.m.)
Control - Soil	nd	32.5	26.8	12.3	19.6	661	-
C1_PMWS	nd	183.33	44.1	9.93	19.5	285.67	332
C2_PMFW	nd	126	47.9	19.2	26.83	257.33	439.33
C3_HHC	Nd	34.7	34.5	10.5	19.9	413	-

In Table 2 results on heavy metals content in soil and composts C1_PMWS, C2_PMFW and C3_HHC are presented. No cadmium (Cd) was detected in the three types of compost. In C2_PMFW compost contains 126 mg/kg DM. Cr and C1_PMWS 183.33 mg/kg DM. Cr, noticing a significant difference, a smaller amount having C3_HHC, namely 34.7 mg/kg. In terms of copper (Cu) and zinc (Zn), the results were similar between C1_PMWS and C2_PMFW, with C3_HHC having 351 mg/kg. Higher levels of lead (Pb) and nickel (Ni) have been detected in C2_PMFW, compost produced from poultry manure and plant debris.
Experiment establishment. The experiment was organized in the greenhouse of the University of Agronomic Sciences and Veterinary Medicine Bucharest, in March 2022

(Figure 1). 3 soil-compost mixtures were used, in concentrations of 25%, 50% and 75%. A control variant with a content of 100% soil was used.

V1: 100% soil
V2: 25% soil + 75% C1_PMWS
V3: 50% soil + 50% C1_PMWS
V4: 75% soil + 25% C1_PMWS
V5: 25% soil + 75% C2_PMFW
V6: 50% soil + 50% C2_PMFW
V7: 75% soil + 25% C2_PMFW
V8: 25% soil + 75% C3_HHC
V9: 50% soil + 50% C3_HHC
V10: 75% soil + 25% C3_HHC

After harvesting the plants, they were finely chopped and put in different containers, in order to weigh the production and perform chemical analyzes, carried out at the National Institute for Research and Development for Pedology, Agrochemistry and Environmental Protection Bucharest.

Figure 1. Pre-harvest experiment

RESULTS AND DISCUSSIONS

Compost maturity – Germination index.
The seed germination index (GI) is a widely used indicator of compost maturity and is a mandatory index in many international standards. The newest organic fertilizer standard (NY525–2021) introduced GI as a compost maturity assessment index and added the requirements for the first time, namely GI ≥ 70% (Wang et al., 2022). Tiquia et al (1996) discovered concluded that the GI value greater than 80%, usually means the final product, compost, does not exhibit phytotoxicity.

The determination of germination index (GI) in seeds of Lepidium sativum L. was made according to the method used by Cesaro (2015), namely:

$$GI(\%) = \frac{G1 \times L1}{G2 \times L2} \times 100$$

Where: G1 = no. germinated seeds in contact with the resulting compost solution;
L1= average length of germinated roots;
G2 = No. seeds germinated in contact with distilled water;
L2= average length of germinated roots.

To perform phytotoxicity tests, the compost sample (100 g) was mixed with water to a moisture content of 85%, kept for 2 hours and centrifuged at 6000 rpm for 15 min. Petri dishes were lined with filter paper and moistened with 3 ml of test solution or deionised water, used as a blank. Each plate was seeded with 10 seeds of L. sativum and incubated in the dark at 25 ± 1◦C for 72 hours. Subsequently, the number of germinated seeds was counted and the total length of seedlings was assessed, including both root and shoot. According to UNICHIM (2003), the seed was considered to germinate when the root was over 0.5 mm long.

C2_PMFW had a germination index of 81.44%, while C1_PMWS had an germination index of 91.96%, values according to which composts do not have phytotoxicity, so they can be used for growing green plants.

The content of macronutrients in oat plants.
The N content of oat plants harvested from all composted variants is higher than the control variant containing 100 % soil (Figure 2).

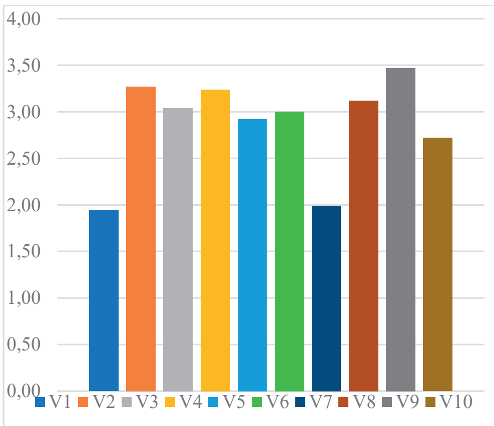

Figure 2. N content of oat plants (%)

Thus, in variants where C1_PMWS was used, the N content was higher by 68.55% in V2 (75% C1_PMWS), 56.7% V3 (50% C1_PMWS) and 67% V4 (25% C1_PMWS). In variants where C2_PMFW was used, the increase was 50.52% V5 (70% C2_PMFW), 54% V6 (50% C2_PMFW) and 2.58% V7 (25% C2_PMFW) and in variants where C3_HHC was used, the value was higher by 60.82% V8 (75% C3_HHC), 78.87% in V9 (50% C3_HHC), also the highest concentration of N, 3.47%) and 40.2% V10 (25% C3_HHC), respectively.
The highest P content was obtained from V3_50%PMWS (P = 0.641%), 6.31% more than the control variant, followed by V4-C1_PMWS and V6-C2_PMFW with a P content 5.32% lower than the control variant. Compared to the control variant, the lowest concentrations of P had plants harvested from the variants where C3_HHC was used, which had lower values by 32.39% in V6 (75% C3_HHC), 40.36% in V7 (50% C3_HHC), respectively 47.34% in V8 (25% C3_HHC), the latter being also the variant with the lowest P content (0.317%), as can be seen in Figure 3.

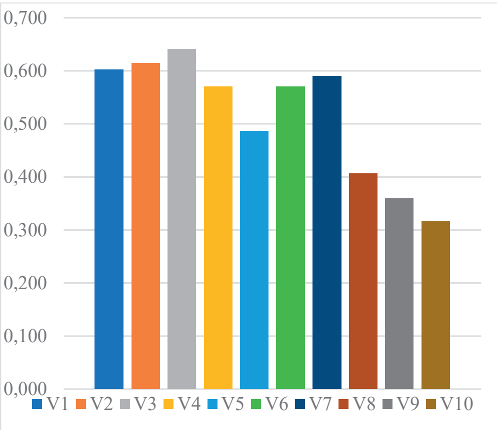

Figure 3. P content of oat plants (%)

The K content had the lowest value in the control, namely 5.69%, all variants that included compost, with concentrations higher than K, with 13.88% (V2 - 75% C1_PMSW), 22.67% (V3 - 50% C1_PMSW), 47.28% (V4 - 25% C1_PMSW). V5 - 75% C2_PMFW had a K content of 5.89%, 3.52% more than the control variant but V6_50% C2_PMFW and V7 - 25%C2_PMFW had a K content higher by more than 20%. The variants where C3_HHC was used had a K content higher by over 37.08% V10 (25% C3_HHC), 42.88% V9 (50% C3_HHC) and over 47.28% V8 (75% C3_HHC). Thus, plants grown in V4 and V8 had a K content about 50% higher than the control variant. See Figure 4.

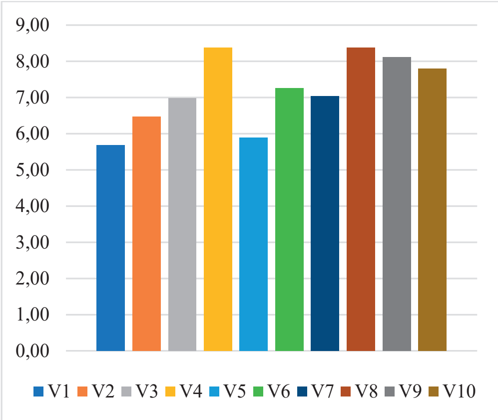

Figure 4. K content of oat plants (%)

The Ca content of oat plants was lower in most compost variants, only plants grown in V6 (50% C2_PMFW) and V10 (25% C3_HHC) had a higher content by 18.64% and 21.19%, respectively, than the control variant and the lowest Ca concentration had V4 (25% C1_PMWS), with 24.86%. However, the smallest amounts of Ca among the variants resulted from V8 (75% C3_HHC) and V9 (50% C3_HHC), as shown in Figure 5.

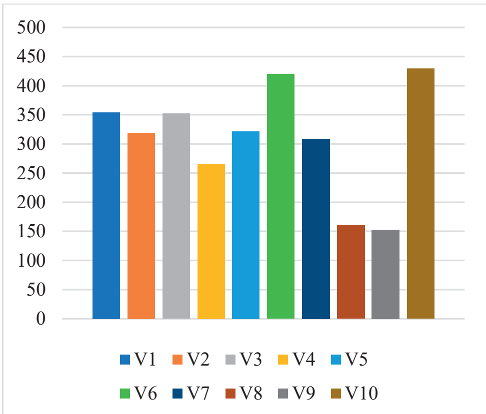

Figure 5. Ca content of oat plants (Mg/kg)

The Mg content of oat plants grown in compost mixtures exceeded in most control variants by 7.8% V3 (50% PMWS), 21.49% V4 (25% PMWS), 10.12% V6 (50% C2_PMFW) and 16.36% V7 (25% C2_PMFW), 11.12% V8 (75% C3_HHC), 27.6% V9 (50% C3_HHC), except V2 (75% C1_PMWS), V5 (75% C2_PMFW) and V10 (25% C3_HHC), having lower Mg concentrations compared to Mt, by 22.67%, 10.12% and 16.36%, respectively (Figure 6).

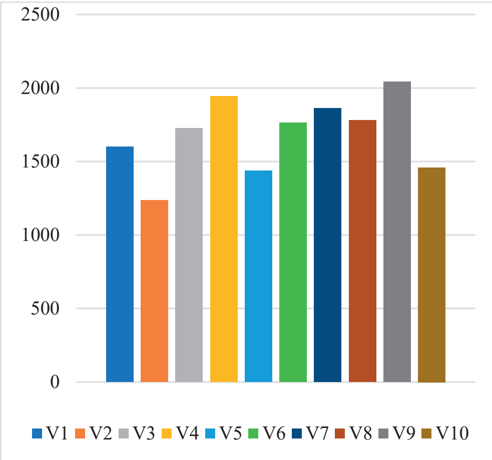

Figure 6. Content of Mg in oat plants (mg/kg)

CONCLUSIONS

Composting requires compliance with a set of rules regarding the types of waste from which to compost. Future studies in this area could lead to the evolution of compost used as fertilising material.

This study was carried out to verify the hypothesis that compost from bio-waste may be an alternative source of nutrients for plants, which is confirmed by the N and K content of oat plants grown from all variants containing compost, regardless of compost type, all of which have higher concentrations of N and K than the control variant where 100% was used.

These results are a first step towards positioning the use of compost as a fertilising material among the healthiest organic waste recycling practices.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY ON AN ECOLOGICALLY FRIENDLY METHOD TO FIGHT WITH *Pteridium acuilinum* IN MOUNTAIN CONDITIONS

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Abstract

The aim of the experiment is to determine the staged annual mowing of a population of Pteridium acuilinum (harmful species), in a natural grass stand of Chrysopogon gryllus type. The results of the conducted research show a progressive reduction of the harmful species from the first to the last experimental years, ranging from 72.0-78.0 pcs. of plants/m^2 (2014) up to 14.2-15.9 pcs. of plants/m^2 (2019). A positive trend was established regarding the recovery of grass cover and an increase in the number of useful fodder species such as: Festuca arundinaceae Scherb, Agrostis alba L., Dactylis glomerata L., Trifolium hybridum L. and Chrysopogon gryllus L.

***Key words**: mechanical control, grass stand, Pteridium acuilinum.*

INTRODUCTION

The problem with the invasive species *Pteridium aquilinum* (L.) Kuhn has been relevant for decades, covering various aspects, such as business, ecological, economic and actually it has transformed into a worldwide problem. Bracken weed infestation leads to a loss of agricultural land and disrupts biodiversity in certain ecosystems. The content of highly toxic substances in *Pteridium aquilinum* has a serious negative impact on the health of farm animals and people, moreover when it is consumed, causes poisoning and carcinomas (Marrero et al., 2001; Vetter, 2009). Bracken disrupts forest ecosystems and limits tree growth (Senyanzobe et al., 2020). *Pteridium aquilinum* extracts and spores can contaminate water sources and are genotoxic (Fernández & Sierra, 2022). The spread of the invasive species in pastures creates conditions for severe degradation, leading to disturbance of biodiversity (Sarateanu et al., 2021), and the application of various agrotechnical measures for weed control, including the use of herbicides or sowing with suitable competitive species, such as *Festuca rubra* and *Vicia cassubica* (Petrov & Marrs, 2000; Stewart et al., 2007; Ghorbani et al., 2006; Milligan et al., 2016; Akpınar et al., 2023). Controlling *Pteridium aquilinum* is very difficult in natural grass areas and requires different approaches. This includes some agrotechnical interventions (ploughing, harrowing, varying frequency of mowing, rolling, controlled burning of the aboveground mass), as the results have been characterized by variable success so far (Stewart et al., 2007; Argenti et al., 2012; Berget et al., 2015, Cervacio et al., 2016). A number of studies indicate that ecological approaches such as grazing control (Alday et al., 2013) and, in particular, staged mowing applied over a long period of time, greatly suppress the development of bracken (Cox et al., 2007). The destruction of the invasive species is very effective when an approach of several mowings per year is applied (Stewart et al., 2008) combined with a long time period of not less than 10 years (Akpınar et al., 2023), as the authors point out that this method is also the most expensive.

The aim of the present study is to destroy an invasive population of bracken that has invaded a completely natural grass area through staged mowing over a 6^{-year} period and to restore the share in the grass cover of beneficial plants from the groups of legumes and grasses.

MATERIALS AND METHODS

For six years (2014-2019), mechanical control was conducted (by mowing - up to two mowings per year, according to the formed regrowth) on an invasive population of bracken, established in a natural meadow (*Chrysopogon gryllus* type at the foot of the Central Balkan Mountain with geographical location: (latitude_N - 42° 51'35.45", longitude_E - 24.41° 36' 34" and 448 m above sea level (Krushaka locality), Bulgaria. The experimental area bordered on the west with a catchment area (The Osam River), and from the east with a coniferous forest massif. The experiment was carried out using the method of long plots (Tsade's method) in four replications, with 200 m^2 experimental plot size. The experimental variants were of the same type (by mowing), as for each individual variant were provided two periods (early and late). The following variants for mechanical control by mowing the harmful grass stand up to twice a year were studied as mowing variants/dates:

1. Mechanical mowing/dates of mowing: 01 June 1st mowing and 20 July - 2nd mowing (variant 1)
2. Mechanical mowing/dates of mowing: 10 June 1st mowing and 28 July - 2nd mowing (variant 2)
3. Mechanical mowing/dates of mowing: 20 June 1st mowing and 10 August - 2nd mowing (variant 3)
4. Mechanical mowing/dates of mowing - 30 June 1st mowing and 25 August - 2nd mowing (variant 4)

The removal of the bracken above-ground biomass was carried out with hand-held professional motorized brush cutters - STIHL FS 261 with a large cutting diameter (520 mm), suitable for use in hard-to-reach areas.

Study indicators

The following indicators were studied:

Phenological observations of the invasive population of bracken.

The density of bracken (number of plants/m^2) - was calculated in each variant (using measuring tapes of 5 pcs.).

Botanical composition of the grass stand (%) - determined by weight, by analyzing grass samples taken immediately before mowing, establishing the percentage share of the invasive species and the main botanical groups (grasses and legumes, and motley grasses). Changes in the botanical composition of the grass stand were studied from the 2nd (2015) to the 5th (2019) experimental years of each first regrowth.

For the second mowing, botanical analysis was not performed because of the lack of new regrowth from the three main biological groups (grasses, legumes and motley grasses).

The statistical processing of the data was done by two-factor analysis of variance, using the software product *Analysis Toolpak for Microsoft Excel* 2010.

RESULTS AND DISCUSSIONS

According to the results of the two-factor analysis of variance, mowing variants significantly (P <0.001) affected the density of the harmful species, as the interaction between mowing variant and year conditions had the strongest impact (42.7%). The force of their combined action is greater than the independent impact of each one of them (Table 1).

If they are ordered by strength of impact, the factor of mowing variants takes the second place with 11.9%, being almost equal in terms of impact to the factor of conditions of the year, which registered 11.0%.

Variant 3 shows the best results, in terms of reduction of the harmful species, after the first mowing on average over a 6-year period, when mowing was conducted on 20 June.

Table 1. Results of two-factor analysis of variance and Strength of impact of factors in (%) after 1st mowing on population density of *Pteridium aquilinum* (L)

Source of Variation	SS	df	MS	F	P-value	F crit	Degree of factorial impact, %
Conditions in the experimental year	4221.4	5.0	844.3	6.1	0.0001	2.3	11.0
Variants/mowings	4584.1	3.0	1528.0	11.1	0.0000	2.7	11.9
Interaction	16418.4	15.0	1094.6	8.0	0.0000	1.8	42.7
Within	13194.8	96.0	137.4				
Total	38418.7	119.0					

In the second mowing of the invasive *Pteridium aquilinum* population, factors differed significantly in their effect on the density of the harmful species.
Here, the factor of conditions of the experimental year has the strongest impact with 83.6% (Table 2).
The factor of mowing variants has a very small impact here with only 4.1%.
The interaction of both factors, such as variants of mowing by conditions of the year (4.6%) is significantly less than the conditions of the experimental year as an independent factor (83.6%).
A proven lower number of bracken plants after the second mowing was reported for 4 and 3 variants, with respective mowing dates of 25 August and 10 August.

Table 2. Results of two-factor analysis of variance and Strength of impact of factors in (%) after 2nd mowing on population density of *Pteridium aquilinum* (L)

Source of Variation	SS	df	MS	F	P-value	F crit	Degree of factorial impact, %
Conditions of the experimental year	48814.2	5.0	9762.8	208.1	0.0000	2.3	83.6
Variants/mowings	2373.5	3.0	791.2	16.9	0.0000	2.7	4.1
Interaction	2685.5	15.0	179.0	3.8	0.0000	1.8	4.6
Within	4504.4	96.0	46.9				
Total	58377.6	119.0					

The analysis of the dynamics in the density of the bracken by years shows a significant tendency to limit the harmful species by mowing. The application of mechanical control in the first year (2014) led to a slight decrease in the bracken density. After the mowing of the harmful mass (first mowing) in June (01 June and 20 June), the population of bracken in the experimental/mowing variants (variants 1 and 3) varied within the limits of 17.1 plants/m^2 up to 17.2 plants/m^2. A significantly higher density per unit area of the invasive bracken population was recorded in the next experimental variants (2nd and 4th), from 71.2 plants/m^2 up to 72.0 plants/m^2 (Figure 1). The second mowing, carried out in stages on the later dates (20 July, 28 July, 10 August, 25 August), had no effect on the density of the invasive bracken population. The values for all experimental variants are within the range of 72.0 plants/m^2 up to 76.0 plants/m^2 (Figure 2), which confirms the resistance of the invasive species due to its reproduction mechanisms (very strong and branched root system and strong spore formation on the aerial part of the plant).
In the second experimental year (2015), a significant share of the noxious plants per unit area were destroyed. The bracken density after the first mowing (on 01 June, 20 June, 30 June), and after the second mowing (on 20 July, 10 Augus, 25 Augus), in variants 1, 3 and 4 (Figure 1), was reduced from 43.2; 44.8 and 39.2 plants/m^2 up to 30.8, 31.2 and 12.8 plants/m^2 (Figure 2). An exception is the first experimental variant (variant 1), where the course of mowing (two mowings carried out on 01 June and 20 July) has a smaller effect on the reduction of harmful plants from 58.3 to 49.8 plants/m^2.
In the third experimental year (2016), both readings (mowings) showed a statistically significant reduction in weed density. This is strongly expressed in the 3rd and 4th variants, as in the specified variants and terms the density of the harmful species was reduced from 23.2 plants/m^2, 24.0 plants/m^2 after the 1st mowing (Figure 1), up to 12.8 and 14.4 plants/m^2 during the second mowing (Figure 2) of the grass stand (variants 3 and 4).
In the 4th experimental year (2017), after the first mowing of the grass stand on 01 June and 10 June in the first two variants (variants 1 and 2), the density of the bracken moved within the limits of 57.6 plants/m^2 at variant 1 to 40.8 plants/m^2.
For variants 3 and 4 mowed on 20 June and 30 June, similar results were reported, as the density of the specified species was within the limits of 40.0 plants/m^2 up to 44.8 plants/m^2. After the second mowing at the later dates for the different variants, the course of reduction is positive and shows significantly fewer harmful plants per unit area.

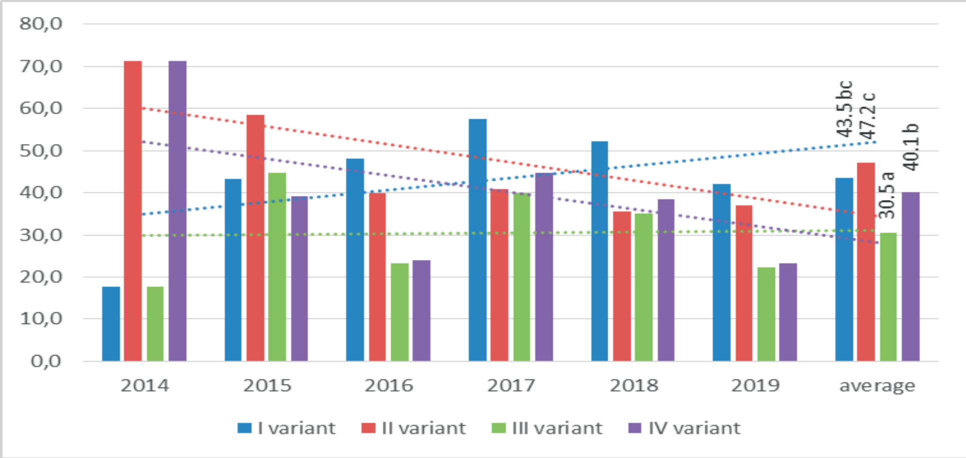

Figure 1. Density of *Pteridium aquilinum* L. (number of plants/m^2) by years and variants (second regrowth)

During the year, both readings (mowing dates) showed a significant reduction in weed density in all experimental options.

In the experimental plots, the destruction of the bracken population by mowing is most visibly expressed in the 3rd variant (mowing dates 20 June and 10 Augus and the 4th variant (mowing dates 30 June and 25 Augus). The course of reduction after the first and second mowing in the indicated variants reduced *Pteridium aquilinum* share in the grass cover (variant 3) from 40.0 to 17.6 plants/m^2, and from 44.8 to 18.4 plants/m^2 (variant 4).

The effect of mowing on the reduction (suppression) of the invasive population of *Pteridium aquilinum* was most significant in the last two experimental years (2018 and 2019).

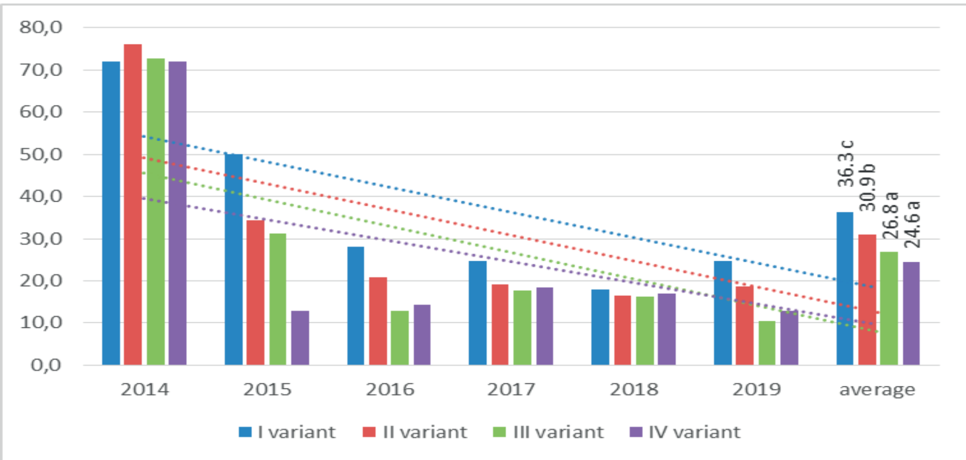

Figure 2. Density of *Pteridium aquilinum* L. (number of plants/m^2) by years and variants (second regrowth)

In the fifth experimental year (2018), after the first mowing of the grass stand (on 01 June and 10 June), in the first two variants, the bracken density moved within the limits of 52.2 plants/m^2 up to 35.6 plants/m^2 (Figure 1). For the 3rd and 4th variants mowed on 20 June and 30 June similar results were reported, as the density of the specified species was weaker and within the limits of 35.2 plants/m^2 up to 38.4 plants/m^2. In the second stage mowing of the grass stand (Figure 2) and the stipulated terms, the reduction of the invasive species was statistically significant, as a significantly lower number of plants per unit area was recorded.

During the year, both readings (mowings) showed a reduction in weed density, most pronounced in variants of the 2nd mowing (on 10 June and 28 Augus), and the 3rd mowing on 20 June –10 Augus. As a result of the applied mowing in the specified variants, the population of bracken was reduced from 35.6 plants/m^2 after the first mowing up to 16.4 plants/m^2 counted after the second mowing (variant 2), and from 35.2 plants/m^2 up to 16.2 number of plants per unit area (variant 3). Under the influence of the planned mowings, the dynamics in the density of *Pteridium aquilinum* established in the 1st and 4th variants marked a decrease, respectively, after the first dates of mowing (01 June and 30 June) from 52.2 plants/m^2 and 38.4 plants/m^2 (Figure 1), up to 18.0 plants/m^2 and 17.0 plants/m^2 established during the second dates (20 July-25 Augus) of mowing (Figure 2).

In the sixth last experimental year (2019), the destruction of harmful plants was most pronounced. After the first mowing periods, the bracken share was reduced from 46.2 plants/m^2 (variant 1) up to 33.5 plants / m^2 (variant 4). Similar values were reported for mowing variants (variants 2 and 3), as the density of harmful plants was even lower and within the limits of 31.4 plants/m^2 up to 31.6 plants/m^2 (Figure 1). The dynamics in the reduction of the population during the second mowing (Figure 2) confirms to an even greater extent the course of reduction in harmful plants, as in the individual variants (variants 1, 2, 3 and 4) and the corresponding planned dates for mowing (20 July, 28 July, 10 Augus, 25 Augus), their density was reduced to: 24.8, 18.6, 10.4, 12.8 plants/m^2. On average for the 6-year study period, in all variants, the most harmful *Pteridium aquilinum* plants were destroyed during the second mowing periods (Figure 2). The reduction in the density of the noxious grass stand (plants/m^2) for the individual mowing variants is in the interval: 36.3 (variant 1), 30.9 (variant 2), 26.8 (variant. 3), 24.6 (variant 4).

The values show that staged mowing has an effect on the destruction of harmful plants, but requires a long period of time to achieve the maximum effect.

Botanical composition of the grass stand after the mechanical control of the bracken

After the conducted phenological observations and botanical analysis, it was established that the partial formation of useful grass vegetation began in the second experimental year (2015).

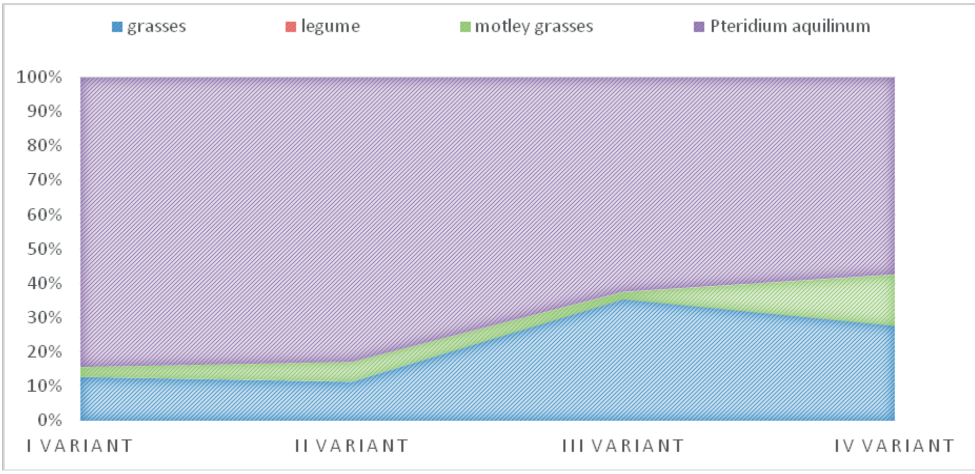

Figure 3. Changes in the botanical composition (%) of *Pteridium aquilinum* (L.) grass stand after the 1st mowing in 2015

In the same year, useful plants were represented only by the group of grasses. They occupied the largest share of 35.4% in the 3rd variant and the smallest in the 2nd variant with 11.3%. After the staged mowing (1st mowing dates) in June, the predominant species in all variants were *Agrostis alba*, *Poa pratensis* and *Dactylis glomerata*. Involvement of species from the group of legumes meadow grasses was not established. The presence of species with low economic value (the group of weeds) also increased, from the 1st to the 4th variants in the interval from 2.8% to 15.0%. The lowest share of *Pteridium aquilinum* was found in the 3rd and 4th experimental variants/mowing, as its aquilinum involvement ranged from 62.5% to 57.4%. In the remaining two variants (variants 1 and 2), *Pteridium aquilinum* dominated with a participation share in the grass cover over 80% (Figure 3).

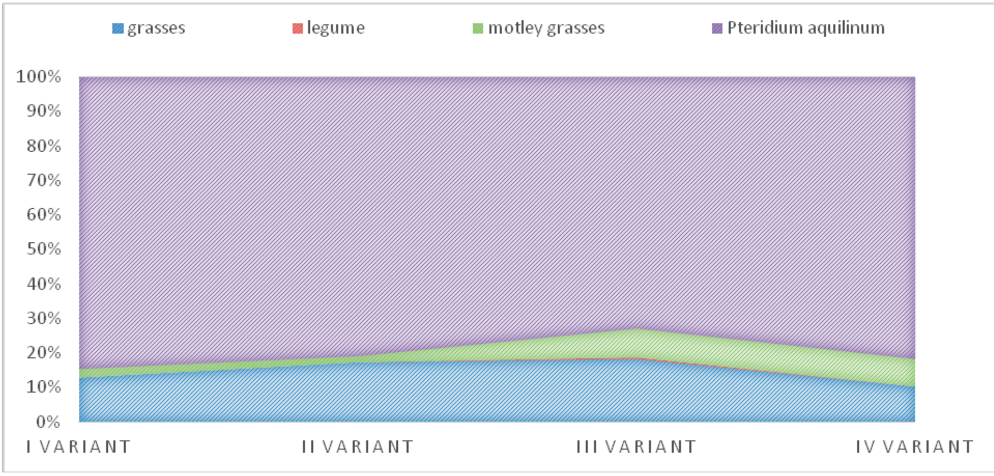

Figure 4. Changes in the botanical composition (%) in grass stand with *Pteridium aquilinum* (L.) after the 1st mowing in 2016

Studying the botanical changes in the experimental year (2016) shows that the recovery of useful plants in the grass stand is less pronounced due to the increased presence of bracken. After mowing the noxious vegetation, grasses dominated as economically important species and plants of low economic value (motley grasses). As a general assessment, the share of beneficial grasses in the grass stand was low. The group of grasses occupied the largest share in the first three variants (variants 1, 2 and 3), the course of increase is in the range of 12.8%, 17.3% and 18.3% (Figure 4). The grass meadow species were observed: *Dactylis glomerata, Agrostis alba, Festuca fallax*.

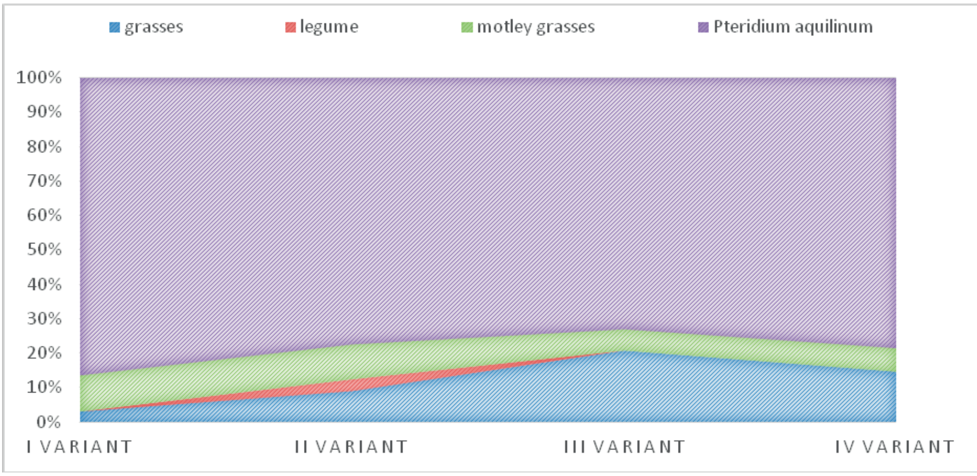

Figure 5. Changes in the botanical composition (%) in a grass stand with *Pteridium aquilinum* (L.) after the 1st mowing in 2017

After the first mowing in June (2017), in the individual variants, the highest share of harmful plants (bracken) was registered in the first mowing variant of 86.3% and lowest in the 3rd variant up to 72.9%.
The partial recovery of useful plants in the same experimental year (2017) was more pronounced despite the dominant position of the invasive species.
In the same year, under the influence of mowing, the grass composition changed followed the trend of the previous years with mainly grasses (Figure 5). The group of grasses has the largest share in the 3rd experimental variant (20.8%) and 4th (14.7%), opposite to the them, the group of legumes registered a significantly lower share ans took 3.4% in the grass stand. In the studied coenosis, *Bothriochloa ischaemum, Agrostis alba* had the greatest presence from the group of grasses.
The staged mowing of the harmful grass stand also stimulated the development of plants from the *Fabaceae family*, but to a much lesser extent, only one representative (*Trifolium hybridum*) was found. The presence of weeds (motley grasses) also increased, as their group occupied a share in the different variants from 6.3% (for variant 3) to 10.5% for the first experimental variant (variant 1).
The relative shares of the three groups (grasses, legumes and motley grasses) in the studied grass stand versus invasive species for the experimental year before the last are shown in Figure 6.

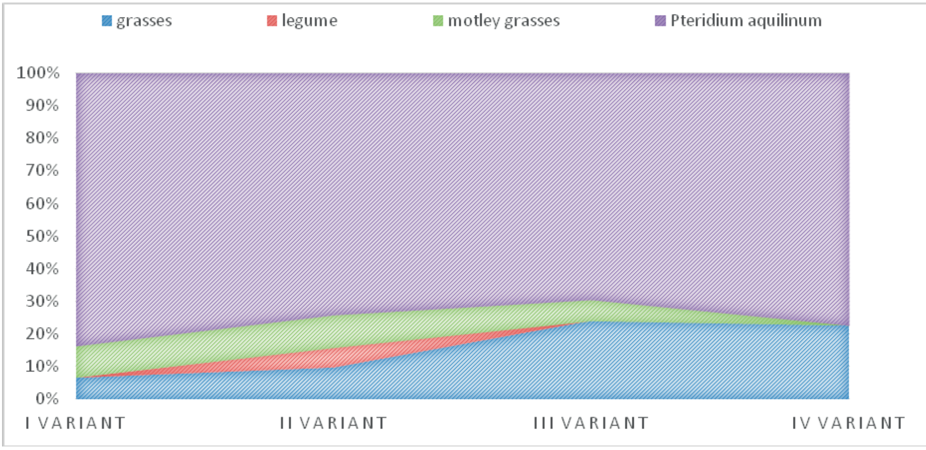

Figure 6. Changes in the botanical composition (%) of the *Pteridium aquilinum* (L.) grass stand after the 1st mowing (2018)

It can be seen that after the first mowing date (30 June 2018 year) the trend from the previous years has continued (Figures 3, 4 and 5) showing a constant high participation of the invasive species. During the year, *Pteridium aquilinum* recorded the largest shares in two of the mowing variants (variants 1 and 4) from 83.7% to 77.2% (Figure 6). There are also changes in the quantitative share of grass groups. The largest share of useful plants continues to be occupied by grasses. Tracking the curve shown shows that their share increased in the interval from the 1st (6.6%), to the 3rd (25.6%), and 4th (22.6%) mowing variants. Under the influence of mowing, the share of useful grass species is stimulated: *Agrostis alba, Festuca arundinaceae, Dactylis glomerata, Triodia decumbens* and *Agrostis capillaris*. Staged mowing of the noxious grass stand increased the share of legumes to 6.0%, as *Trifolium hybridum* and *Trifolium pratense* were found among the representatives of this group. The presence of weeds also increased. They occupied a share in the interval from 7.0% (variant 4) to 10.1% (variant 2).
The changes taking place in the grass cover during the final experimental year (2019) show that after mowing the grass stand, the bracken still retained a higher share compared to the useful vegetation (the grasses of economic importance) - Figure 7.

The largest share of the invasive species (*Pteridium aquilinum*) was in the first two variants from 68.5% to 70.8% and the least in the 3rd variant - up to 57.4%.

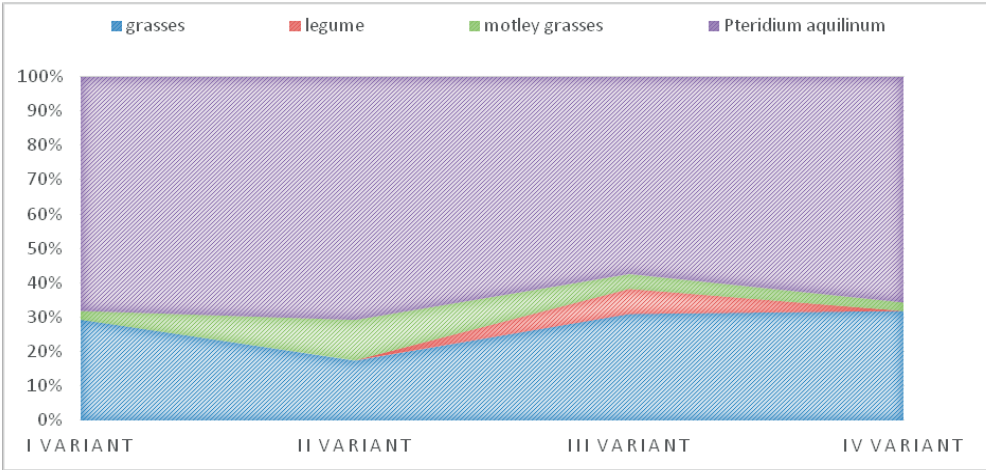

Figure 7. Changes in the botanical composition (%) of the *Pteridium aquilinum* (L.) grass stand after the 1st mowing (2019)

The presence of the useful meadow vegetation is dominated by the group of grasses. It occupied the following shares in the different variants: 29.4%, (variant 1), 17.3% (variant 2), 30.9% (variant 3) and a maximum value of (31.8%) in variant 4.

The tracking of meadow legume crops shows that this group registered the smallest share (7.4%), a trend continuing from previous years. For the conditions of the present experiment, mowing stimulated the emergence mainly of grasses and motley grasses, whereas the group of legumes had the smallest share.

The observed grass (dominant) species during the year were: *Agrostis alba*, *Dactylis glomerata* L. and *Chrysopogon gryllus*. As a result of the mowing, other types of grasses appeared, which had been in a suppressed state, such as *Chrysopogon gryllus* and *Agrostis capillaris*. The group of meadow legume crops had an unsatisfactory share in the grass cover, occupying a share of 7.4%.

Only one representative *Trifolium pratense* was observed in the third experimental variant, as the presence of legumes in the other variants was not established. After surveying the grass stand, the group of grasses from other botanical families (motley grasses) recorded the largest share of 11.9% (variant 2) and the smallest of 2.5% in the fourth experimental variant (variant 4).

CONCLUSIONS

The filed experiment in the conditions of the Central Balkan Mountain in the format of mechanical control shows the positive impact of staged mowing in a natural grass area heavily overgrown with *Pteridium aquilinum*. The mechanical control significantly reduced the population of bracken, as the number of harmful plants progressively decreased during individual years from 72.0-78.0 plants/m^2 (2014) to 14.2-15.9 plants/m^2 (2019), in all experimental variants.

On average for a six-year mowing period (2014-2019), the highest effect of the applied mowings was achieved in var. 3 and 4, as mowings were conducted on 20 June and 10 August; 30 June and 25 August, where the number of harmful plants per unit area was reduced from 72.0-72.8 plants/m^2 up to 26.6-24.6 plants/m^2. Under the influence of mowing, a process of partial restoration of the grass cover and stimulation of the growth and development of the useful grass vegetation was unlocked. It has been established that the course of restoration processes in the grass stand is most pronounced in the group of grasses and motley grasses, and to a much lesser extent in the meadow legume crops. The share of useful forage species such as, *Festuca arundinaceae*, *Agrostis alba*, *Dactylis* *glomerata, Agrostis capillaris*, *Trifolium pratense*, *Trifolium hybridum* and *Chrysopogon gryllus* was observed.

To achieve the maximum effect of the applied mechanical control against the harmful grass stand from bracken, it is necessary to extend the time frame from 6 to 10-12 years.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

MONITORING GRAPES' INFECTION WITH *Botrytis cinerea* BASED ON LACCASE ACTIVITY

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Abstract

Botrytis cinerea causes grey mould and is one of the most damaging disease occurring in vineyards worldwide, resulting in loss of grape production and wine quality. Laccase enzymes produced following fungal infection are involved in the oxidation of phenolic substances during the development of grey mould and has been used as an indicator of the degree of infection. Between the laccase activity of resulting juice of Botrytis cinerea infected bunches and the severity of infection observed in the vineyard a moderate to good correlation has been highlighted over the measurements carried out over veraison (BBCH81 – 88), and a strong correlation has been highlighted for the measurements carried out over the full ripening growth stage (BBCH 89). As a consequence of the degradation of polyphenolic compounds and the intensification of laccase activity in the infected musts, the change of chromatic characteristics, in the sense of increasing the values of the „Hue" parameter and the yellow color of musts was highlighted, in direct correlation with the oxidative processes.

Key words*: Botrytis cinerea, laccase, Vitis vinifera, polyphenolic compounds.*

INTRODUCTION

Gray mold caused by *Botrytis cinerea*, a necrotrophic fungus, is one of the most damaging grapevine diseases, along with downy mildew and powdery mildew. High values of relative humidity and a moderate temperature during the vegetative cycle of grapevine favour the development of the disease (Wan et al., 2015).

The infection with *Botrytis cinerea* on grapes determined the modification of their physico-chemical composition with important consequences on the quality of musts and wines (Bar Nun et al., 1988; Mayer, 2006).

Throughout the infection, numerous extracellular enzymes intervene, such as polygalacturonase (Johnston & Williamson, 1992; Have et al., 1998), xylanase (Brito et al., 2006), laccase (Grassin & Dubourdieu, 1984; 1989), as well as non-specific phytotoxic metabolites (microbial terpenes, botcinic acid) also known as virulence factors. As a results of their action, enzymatic degradation of cells and colonization of the host's substrate occurs (Breia et al., 2020). Among these enzymes, laccase (p-diphenol oxidoreductase) is considered to be the most important due to the negative effects on the quantity and quality of grape production.

This enzyme determines the protein oxidation of white wines and the color reduction of red wines as a result of the oxidation of phenolic compounds (Iland, 2000), the destruction of the aromatic and colouring substances in the grape berries (Kontek et al., 1997) affecting negatively the quality of wines (Grassin & Dubourdieu, 1989; Dewey et al., 2008).

Wines produced from grapes infected with *Botrytis* lack freshness and fruitiness and may present a predominant aromatic character of mold. Since correlations have been found between laccase activity and infection with *B. cinerea* (Roud et al., 1992; Dewey et al., 2008), laccase is considered an indicator of grape's infection with this fungus, activities higher than 3 U/mL is indicating the susceptibility for oxidative enzymatic

degradation of wine (Grassin & Dubourdieu, 1989).
The purpose of this study was to assess the risk of infection with *B. cinerea* in grapevine plantations based on the laccase activity and to monitor and evaluate from quantitative and qualitative point of view the grape harvest in order to apply an integrated management to combat gray mold.

MATERIALS AND METHODS

The biological material was represented by five wine grapevine cultivars: three varieties highly susceptible to gray mold - Chardonnay, Sauvignon blanc, Fetească albă, and two varieties with medium resistance - Fetească neagră and Cabernet Sauvignon.
Monitoring of the risk infection with *Botrytis cinerea* was performed during the growing season, special attention being paid to the stages of development represented by veraison, beginning of ripening (BBCH 81-88) and at harvest (BBCH 89) (Lorenz et al., 1995).
During beginning of ripening and full ripening phenophases grape bunches affected by *Botrytis* were hand harvested, the berries have been detached at the pedicel level and sorted according to the degree of infection into five categories of severity: 0 = no visible signs of infection/healthy grapes, 1 = up to 25% mycelium with visible spores, 2 = mycelium with spores affecting up to 50% of the berries, 3 = mycelium with spores affecting between 50% and 75% of the berries. Each sample unit was manually crushed into sterile plastic bag. The resulted must was analysed for soluble sugar content, total acidity, and laccase activity (Grassin & Dubourdieu, 1984; 1989).
The sugar content was measured by refractometry and expressed in g/L by conversion according to the OIV database (OIV-MA-AS313-01).
Total acidity was measured by titration using NaOH 0.1 N% phenolphthalein and the results were expressed in g/L^{-1} H_2SO_4 (OIV-MA-AS313-01 method).
Laccase activity was determined according to Grassin and Dubourdieu method (1984; 1989), slightly modified. The method is based on the use of syringaldazine as a substrate and the monitoring of the enzymatic reaction at 530 nm, where the extinction coefficient of oxidized syringaldazine is 65000 $nmol/ml^{-1}$.
In order to not inhibit the reaction, the phenolic compounds are fixed by using PVPP. A quantity of 1 mL of must containing laccase, as a result of the fungal attack with *Botrytis cinerea*, was filtered using a syringe containing 0.15 g of PVPP.
The results are expressed in units of laccase/mL, one unit of enzymatic activity being defined as the amount of enzyme that catalyzes the oxidation of a nanomole of syringaldazine per minute.
The production of laccase by *Botrytis cinerea* facilitates the infection process, the amount of laccase in the must and wine being considered an indicator of the degree of infection.
According to this method, musts with less than 0.2 UL/mL are considered to come from healthy grapes. The critical threshold is considered to have values higher than 3 UL/mL which will undergo to oxidative processes which lead to oxidative brownness of the wines.
In addition to the white varieties, the varieties for red wines were also analyzed in terms of chromatic parameters (color intensity, hue, % yellow, % red, % blue color), and polyphenolic potential, respectively, total polyphenol index, anthocyanin content and anthocyanin potential.
Chromatic parameters were determined according to the UV/Vis spectrophotometry method proposed by Glories (1984).
The phenolic potential was assessed by the standard ITV method (Cayla et al., 2002), based on the following analytical parameters: anthocyanins, total anthocyanins potential and total polyphenol index. The phenolic compounds were extracted from the grapes with an acidic aqueous solution. A quantity of 200 grape berries were randomly collected from each sample, weighed, then crushed and macerated for one hour at room temperature.
Fifty grams of the resulting must were treated with a solution composed of 85 mL of 0.1% HCl (37%) and 15 mL of ethanol (95%) and shacked for one minute every fifteen minutes. For obtaining clear solutions the coloured extracts were filtered through glass wool.
The sample were diluted in distilled water to 1/100, then the absorbance was measured at an optical density of 280 nm against a blank of

distilled water, and total polyphenolic index (TPI) was determined as follow:

Total polyphenol index (TPI) = OD 280 x 100 x [(weight of grape juice + 100)/ weight of grape juice].

Other samples were diluted 1/20 in 1% hydrochloric acid solution and absorbance was measured at OD 520nm against a blank of distilled water. The concentration of anthocyanin and the total anthocyanin potential were determined by using the following formulas:

Anthocyanins (mg/L) = OD 520 x 22.75 x 20

Total anthocyanins potential (mg/kg) = anthocyanins (mg/L) x [(weight of grape juice + 100)/ weight of grape juice].

Data was statistically analysed by applying the Dunnett test, in order to compare multiple groups with a single control group (Dunnett, 1964).

RESULTS AND DISCUSSIONS

Climatic conditions favorable for the *Botrytis cinerea* attack

Taking into account the average temperature during the period of leaf wetness and duration of leaf wetness, the quantity of precipitation and the relative humidity value, favorable conditions for the gray mold development were in June (127.7 hours), July (166.1 hours), and August (127.5 hours) of 2020, on the optimal temperature background of 22.7°C, 24.2°C, respectively 23.8°C, and in the months of June (95.4 hours) and August (68.8 hours) of 2021, based on the optimal temperature background of 19.4°C, respectively 21.4°C (Table 1).

Table 1. Climatic parameters favorable for the *Botrytis cinerea* development

Month	Medium temperature during the leaf wetness (°C)		Duration of leaf wetness (hours)		Conditions for *Botrytis cinerea* development (hours)	
	2020	2021	2020	2021	2020	2021
April	17.9	9.5	2.2	276.5	2.2	276.5
May	19.6	18.1	1.3	43.8	1.3	43.8
June	22.7	19.4	127.7	95.4	127.7	95.4
July	24.2	24.2	97.9	41.0	166.1	41.0
August	23.8	21.4	85.5	68.8	127.5	68.8
September	20.8	16.9	155.9	11.7	203.4	11.7

Monitoring of *Botrytis cinerea* infection risk through the attack degree evaluation

In accordance with the OIV methodology (2009) regarding the determination of grape resistance degree against the *Botrytis cinerea* fungus attack, in the climatic conditions of 2020 and 2021 and with the phytosanitary treatments applied, the grapes varieties studied presented a good and very good resistance, the attack degree on the grape production being very low. Thus, only a few isolated attacks of low intensity were reported, both on leaves and on grapes, these being mainly influenced by the sensitivity of the variety and the microclimate of the plantations (Table 2).

Table 2. Analysis of the resistance degree of *Vinifera* cultivars against the *Botrytis cinerea* attack

Variety	Veraison (BBCH 81-88)			Ripening, harvest (BBCH 89)		
	Frequency of attack (%)	Intensity of attack (%)	Degree of attack (%)	Frequency of attack (%)	Intensity of attack (%)	Degree of attack (%)
2020						
Chardonnay	3	0.16	0.01	19	6.3	1.2
Sauvignon	6	0.32	0.02	15	7.3	1.1
Fetească albă	15	5.7	0.86	16	5.9	0.95
Fetească neagră	8	5.63	0.45	20	2.8	0.56
Cabernet Sauvignon	10	0.86	0.09	15	4.7	0.71
2021						
Chardonnay	21	9.52	2	15	6.3	0.95
Sauvignon blanc	18	4.6	0.83	17	7.3	1.24
Fetească albă	12	5.45	0.65	18	5.9	1.06
Fetească neagră	8	0.45	0.04	10	2.55	0.25
Cabernet Sauvignon	10	0.86	0.09	11	4.7	0.52

Monitoring of *Botrytis cinerea* infection risk through the laccase analysis

The determinations made during the veraison phenophase hightlighed a moderate to good correlation (R ranged between 0.64 and 0.70) between the laccase activity of resulting juice from *Botrytis cinerea* infected bunches and the severity of infection observed in the vineyard. The laccase activity registered values ranging between 1.02 U/mL in case of 10% degree of the attack and 5.42 U/mL for 25% degree of attack (Figure 1a).

A very good correlation (R ranged between 0.6485 and 0.91) has been highlighted for the measurements carried out over the full ripening growth stage, at harvest. The laccase activity registered values ranging between 6.20 U/mL in case of 10% degree of attack and 16.24 U/mL for 75% degree of attack (Figure 1b).

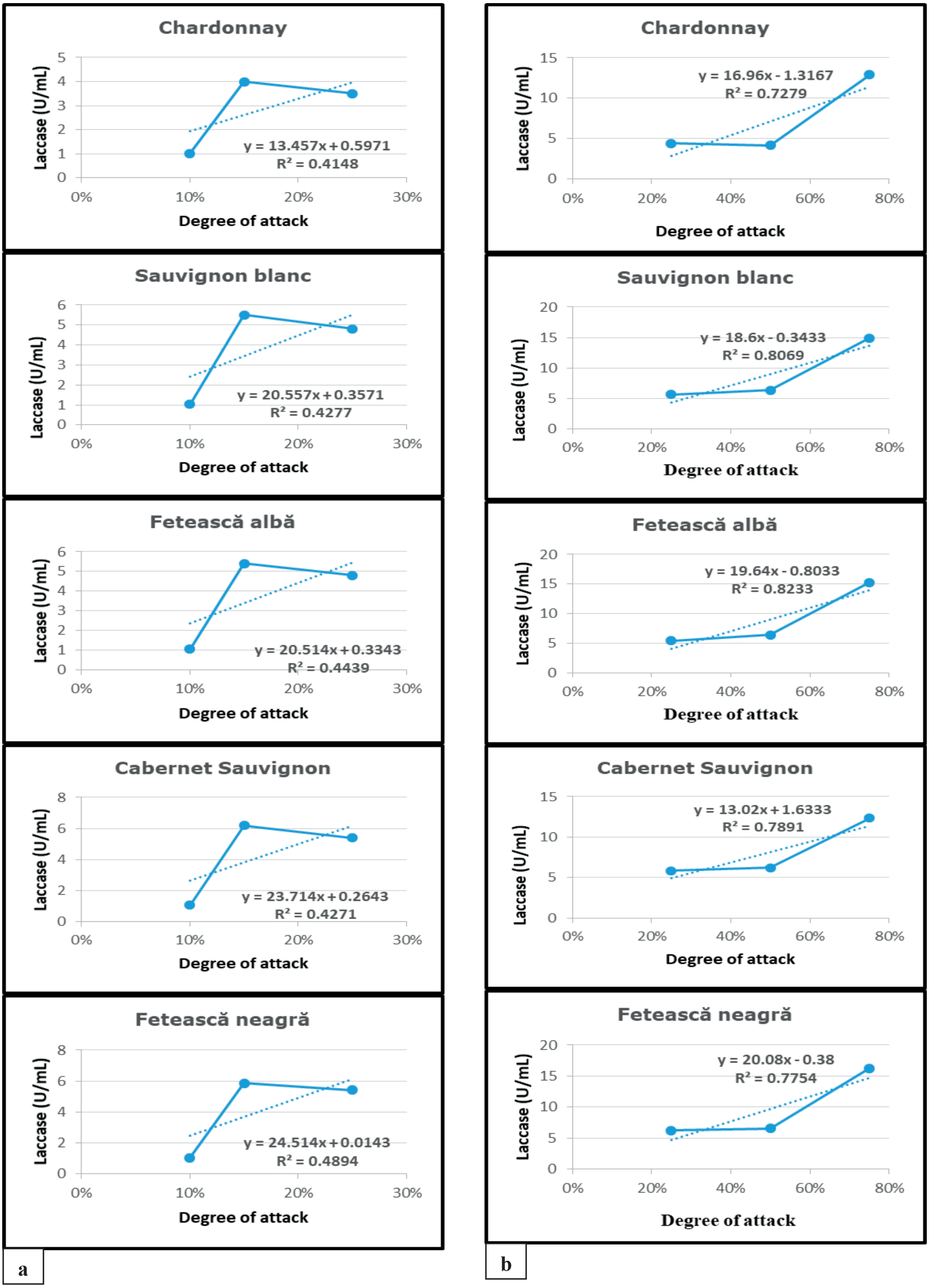

Figure 1. Level of laccase activity function of the attack degree with *Botrytis* at veraison (a) and at harvest (b)

Characterization of grapes and musts affected by the gray mold

In the veraison phenophase, in isolate cases, grapes affected by the *Botrytis cinerea* attack were identified, but with a degree of infection rate less than 25%. At harvesting, the attack frequency had values close to those recorded in the veraison phenophase but, the infection level increased up to 75%, as an effect of the change in the intensity of the attack. The soluble sugar content and total acidity of the must in the veraison phenophase registered values with max differences of +/- 5% compared with the control but even these small differences were statisticaly significant in terms of total acididy. At harvesting the sugar content from the must increase significative depending on the infection level of the grapes (Table 3).

Table 3. General physicochemical composition of grapes

Variety-Variant	Veraison				Harvesting			
	Sugar (g/L)	% +/-	Total acidity (g/L acid tartaric)	% +/-	Sugar (g/L)	% +/-	Total acidity (g/L acid tartaric)	% +/-
Chardonnay								
Control IA* 0%	114		14.32		209		7.79	
IA < 25%	114	0	14.35**	0.21	243**	16.27	7.95**	2.05
IA 25 – 50%	116	1.75	14.72**	2.79	247**	18.18	7.98**	2.44
IA 51 – 75%					252**	20.57	8.05**	-0.9
Sauvignon blanc								
Control IA* 0%	90		17.42		219		7.42	
IA < 25%	89	-1.11	18.2**	4.48	241**	10.05	7.95	7.14
IA 25 – 50%	90	0	18.53**	4.59	247**	12.79	7.08	-4.58
IA 51 – 75%					249**	13.7	6.98	-5.93
Fetească albă								
Control IA* 0%	115		14.24		200		6.44	
IA < 25%	116	0.87	14.56	2.23	237**	18.5	6.75**	4.81
IA 25 – 50%					241**	20.5	7.13**	10.71
IA 51 – 75%					249**	24.5	7.29**	13.2
Fetească neagră								
Control IA* 0%	162		8.22		219		7.16	
IA < 25%	164	1.23	8.12	-1.22	232**	5.94	7.2	0.56
IA 25 – 50%					268**	22.37	7.53**	5.17
IA 51 – 75%					272**	24.2	7.68**	7.26
Cabernet Sauvignon								
Control IA* 0%	154		9.18		213		7.2	
IA < 25%	153	-0.65	8.73	-4.9	232**	8.92	7.48**	3.89
IA 25 – 50%					238**	11.74	8.23**	14.31
IA 51 – 75%					242**	13.62	8.25**	14.58

**The mean difference is significant at the level P< 0.001 according to Dunnett t-tests.

Diffrences between -0.90% and 14.58% were observed in the total acidity values registred in the healthy must compared with the grapes affected by *Botrytis* attack.

The phenolic potential of grapes at harvesting

The phenolic potential of grapes changes significantly depending on the degree of harvest damage. The total polyphenol index (IPT) of the must resulted from the infected grapes is almost twice lower compared with the one registered in the healthy must. It was observed that amount of anthocyanins and the anthocyanin potential are reducing significatively depending on the attack degree with approximately 32-39%, at an attack intensity of 25%, reaching a reduction of 47-50% at an attack intensity of 51-75% (Table 4).

Table 4. Phenolic potential of grapes at harvest

Variety/ Attack intensity (AI)	Polyphenolic index		Anthocyanins (mg/L)		Anthocyanin potential (mg/kg)	
	Value	% reduction	Value	% reduction	Value	% reduction
Fetească neagră						
Control – AI 0%	55		258		774	
AI < 25%	38**	30,91	175**	32.17	525**	32.82
AI 25 – 50%	29**	47,27	131**	49.22	394**	49.10
AI 51 – 75%	26**	52,73	127**	50.78	382**	50.65
Cabernet Sauvignon						
Control – AI 0%	66		317		952	
AI < 25%	41**	37,88	193**	39.12	580**	39.08
AI 25 – 50%	39**	40,91	166**	47.63	498**	47.69
AI 51 – 75%	33**	50,0	166**	47.63	497**	47.79

**The mean difference is significant at the level P< 0.001 according to Dunnett t-tests.

The degradation of poliphenolic compunds in infected must can be explained by the increasing of laccase activity in the infected must with Botrytis from 5.86 U/mL 6.2 U/mL (Cabernet Sauvignon) and 6.2 U/mL (Fetească neagră), at an attack intensity of 25%, at values between 12.37 U/mL (Cabernet Sauvignon) and 16.24 U/mL (Fetească neagră) at an attack intensity of 51-75%. As a cosequence of the degradation of poliphenolic compunds and the intensification of laccase activity in the infected musts, the change of chromatic characteristics, in the sense of increasing the values of the „Hue” parameter and the yellow color of musts was highlighted, in direct correlation with the oxidative processes. The color intensity was also significatively affected by the *Botrytis cinerea* attack. The percentage of the yellow pigment in the must increases with the degree of fungal attack while the percentage share of the red pigment decreased (Table 5).

Table 5. Chromatic properties of grape juice

Variety/ Attack intensity (AI)	Color intensity	Color hue	% yellow pigments	% red pigments	% blue pigments
Fetească neagră					
Control - AI 0%	7.33	7.227	36.9	51.16	11.87
AI < 25%	9.99**	14.841**	51.25	34.53	14.21
AI 25 - 50%	9.20**	15.686**	53.17	33.90	12.93
AI 51 - 75%	8.83**	14.219**	51.53	36.24	12.33
Cabernet Sauvignon					
Control - IA 0%	8.27	6.082	33.98	55.86	10.16
AI < 25%	7.41**	12.345**	48.31	39.14	12.55
AI 25 - 50%	8.45**	14.487**	53.49	36.92	9.59
AI 51 - 75%	8.85**	14.308**	52.54	36.72	10.73

**The mean difference is significant at the level P< 0.001 according to Dunnett t-tests.

CONCLUSIONS

Botrytis cinerea causes gray mold disease on grapevine, determining the reduction of grape production and modification of wine quality. The infection with *Botrytis cinerea* on grapes determined the modification of their physico-chemical composition with important consequences on the quality of musts and wines As a consequence of degradation of the polyphenolic compounds and the intensification of laccase activity in the infected musts, the change of chromatic characteristics, in the sense of increasing the values of the „Hue" parameter and the yellow color of musts was highlighted, in direct correlation with the oxidative processes.

Laccase activity may be used as an indicator of grape's fungal infection with *Botrytis cinerea,* strong correlations being found between laccase activity and the infection produced by *B. cinerea*. Evidence of the presence of laccase in grapes and musts can be used to detect *Botrytis* infection and to monitor its evolution, as well as for the timely application of corrective measures.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

BIOLOGICAL PECULIARITIES AND QUALITY OF PHYTOMASS FROM SOME *Salix* L. AND *Populus* L. SPECIES

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Abstract

The rapid development of global bioenergy makes it necessary to find and develop fundamentally new approaches to forest management. Promising plants are species and varieties of the genera Salix L. and Populus L., which are characterized by fast rates of biomass growth. Research has established that the annual growth of species and varieties of the genus Salix L. is 80-145 cm, and that of the genus Populus L. is 120-165 cm. The Salix viminalis phytomass is characterized by 3.53% ash, 44.9% cellulose, 24.7% hemicellulose, 9.1% acid detergent lignin, 50.22% C, 6.00% H, 0.89% N, 0.07% S, 0.05% Cl, 19.77 MJ/kg HHV and 18.46 MJ/kg LHV. The Populus alba phytomass contained 2.82% ash, 50.6 % cellulose, 19.1% hemicellulose, 7.3% acid detergent lignin, 47.83% C, 5.98% H, 0.80% N, 0.16% S, 0.04% Cl, 19.22 MJ/kg HHV and 17.91 MJ/kg LHV. The estimated theoretical ethanol yield from cell wall carbohydrates averaged 510.4 L/t in Salix viminalis substrate and 505.4 L/t in Populus alba substrate, as compared with 476.3 L/t in pruning residues substrate. The creation of short-rotation plantations of Salix L. and Populus L. will make it possible to reduce resource pressure on other categories of forests, increase the forest cover of territories and the productivity of plantations, shorten the time of growing wood with the possibility of its further use for energy purposes.

Key words*: biological peculiarities, chemical properties, energy properties, phytomass, Populus, Salix, theoretical ethanol potential.*

INTRODUCTION

Biomass is a versatile raw material that can be used for production of heat, power, transport fuels, and bioproducts. The use of plant biomass as an energy carrier is vital due to the zero balance of the carbon cycle (C) in nature. It plays a critical role in reducing carbon dioxide (CO_2) emissions and creates an alternative to fossil fuels as a renewable energy source. The use of biofuels must benefit the environment regarding CO_2 emissions and other components of exhaust gases (Kraszkiewicz et al., 2022).
The problem of developing energy-saving technologies and finding alternative sources of renewable fuels is more acute than ever before for the global community. Despite this, the development and use of solid biofuels production in Ukraine and Moldova is constrained and lags behind both the country's domestic needs and the global dynamics of its development. Every year Ukraine consumes about 200 million tons of fuel and energy resources, covering its needs by only 53%. The UN program stipulates that the share of renewable energy sources in the global fuel and energy balance in 2050 may reach 50%, and according to the World Energy Council, it will reach 80-90% by the end of this century (Energy Strategy, 2011; 2013; Geletukha et al., 2015).
Among all fuels, biomass ranks 4th in the world and accounts for 14% of total primary energy consumption in the world. The largest share of solid biofuels in total energy consumption is in Sweden (Geletukha et al., 2015; 2016). In Ukraine, the share of electricity generated from renewable energy sources amounted to 14.6% or 13.9 GW of capacity in 6 months of 2021, including 230 MW of bioenergy (Kuz'min, 2021).

Today, there are several dozen fast-growing plants that can be grown for plant biomass. The most promising among them are varieties of the genera *Eucalyptus* L'Hér., *Populus* L., *Salix* L., *Robinia* L., *Miscanthus* Anderss., *Zea* L., *Saccharum* L., etc. The collected biomass is used to produce heat and electricity, and can also serve as a raw material for the production of solid biofuels, such as fuel granules, briquettes, and pellets.

Of all the energy crops in the world, it is the varieties of the genus *Salix* and *Populus* L. that are currently used as the main energy crop for solid fuel production. The first energy variety of the *Salix* genus was registered in the Netherlands in 1968, and today more than 230 *Salix* varieties are registered worldwide (Roik et al., 2015). Over the past 10 years, the United States and Poland, as well as Argentina and Romania, have been leading the way in registering new varieties (Lawrence et al., 2015; Roik et al., 2015). In Sweden, the Netherlands, and Germany, most of the energy crop varieties of *Salix* were registered in the 1990s. In Europe, where about 10 thousand crossed plants are produced annually, the breeding of energy crops of the *Salix* genus began in 1987. The period for developing a new variety is 10 years. Currently, there are 24 varieties authorized by EU standards. Every year 1-2 new varieties appear in the world (Holland Plant Ukraine, URL: http://hopu.com.ua/shvedska-energetychna-verba/)

Countries such as Sweden, England, Ireland, Poland, and Denmark have experience in growing *Salix* varieties. In Italy, Germany, Argentina, and Poland, the creation of special plantations of fast-growing *Salix* and *Populus* varieties is widely practiced. In Northern India, plantations of fast-growing species of *Populus* and *Eucalyptus* occupy 50-60 thousand hectares, where about 3.7 million tons of wood are harvested annually. In Europe, large areas are occupied by *Salix* species in Sweden - 18-20 thousand hectares, in Poland - more than 6 thousand (Roik et al., 2015). In Ukraine, the Swedish Energy Willow program has also been operating since 2006. Plantations of *Salix* energy varieties have been planted in Rivne, Volyn, Lviv, Ivano-Frankivsk, Ternopil, Vinnytsia, and Kyiv regions (CMU's Resolution No. 902-p of 01.10.2104 "On the National Renewable Energy Action Plan until 2020" http://zakon4.rada.gov.ua/laws/show/902-2014-%D1%80).

The creation of energy plantations from varieties of the genus *Populus* is being developed in detail in the conditions of Kyiv Polissya and Kharkiv region (H.M. Vysotsky UkrNDILGA) (Vysots'ka, 2014; Fuchylo et al., 2011; Khivrych & Mel'nychuk 2016; Kunts'o & Humentyk, 2013; Ishchuk, 2014; 2015). The phytomass of willows in Kyiv Polissia was estimated by Holiaka et al. (2018).

There are about 5 million degraded lands in Ukraine where energy crops can be successfully grown. However, today the area under energy crops is only about 7,000 hectares. According to experts, energy crops grown on 4 million hectares could replace 20 billion cubic meters of natural gas annually (Kuz'min, 2021). However, despite the large amount of unused non-agricultural land in Ukraine, there are still few industrial energy plantations. In our opinion, reserves of energy crops should be sought among species of local flora that grow rapidly in the soil and climatic conditions of Ukraine and Moldova, given the increasing continentality and aridization of the climate. Therefore, the issue of establishing short-rotation plantations of *Salix* and *Populus* L. on degraded lands in Ukraine and Moldova is topical.

MATERIALS AND METHODS

The objective of the paper is to analyze the taxonomic composition, chorology, energy properties, and resource base of representatives of the *Salicaceae* Mirbel. family in Ukraine and Moldova and, based on the study of annual shoot growth and energy value of phytomass, to identify promising species and varieties for energy plantations.

The material for the work was based on the comprehensive results of the analysis of the chorology and resource base of autochthonous species and hybrid varieties of *Salix* and *Populus* in Ukraine and Moldova. In situ studies of shoot growth of *Salix* and *Populus* species were conducted at the biostation of the Bila Tserkva National Agrarian University by linear measurements of shoot length of willows and poplars every 10 days during June - August (Molchanov & Smyrnov, 1967). Phytomass was analyzed in the laboratory of energy crops of the National Botanical Garden (Institute) named after Alexander Chobotar.

The regulatory framework for energy crops of *Salix* and *Populus* species and cultivars in Ukraine is provided by the Laws of Ukraine "On Alternative Energy Sources" (2008), "On Alternative Fuels" (2009), and "On Seeds and Planting Material" (2010).

As energy biomass the *Salix viminalis* and *Populus alba* phytomass were harvested manually in February, apple tree pruning residues was used as control variant. The phytomass were chopped into chaff using a stationary chopping unit, milled in a beater mill equipped with a sieve with diameter of holes of 6 mm. To perform the analyses of the elemental chemical and content of cell walls, the biomass samples were dried in an oven at 85 °C and then milled (<1 mm) and homogenized. After that, the total carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) amounts were determined by dry combustion in a Vario Macro CHNS analyzer, according to standard protocols. The some physical and mechanical properties of dry biomass were determined according to the standards: the moisture content of the plant material was determined by SM EN ISO 18134 in an automatic hot air oven MEMMERT100-800; the content of ash was determined at 550ºC in a muffle furnace HT40AL according to SM EN ISO 18122; the automatic calorimeter LAGET MS-10A with accessories was used for the determination of the calorific value, according to SM EN ISO 18125.

To determine the cell wall components in the dry mass of tested species, the amounts of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were assessed using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200 at the Research-Development Institute for Grassland Brasov, Romania. The amount of cellulose was calculated as ADF minus ADL and hemicelluloses - NDF minus ADF. The Theoretical Ethanol Potential (TEP) was calculated according to the equations of Goff et al., 2010 based on conversion of hexose (H) and pentose (P):

$$H = [\% \text{ Cel}+(\% \text{ HC} \times 0.07)] \times 172.82$$

$$P = [\% \text{ HC} \times 0.93] \times 176.87$$

$$TEP = [H + P] \times 4.17$$

RESULTS AND DISCUSSIONS

Among the species diversity, the willows of the plain part of Ukraine, which include 16 taxa, are promising for energy plantations. *S. alba* L., *S. pentandra* L., *S. fragilis* L., *S. viminalis* L., *S. acutifolia Willd.*, *S. cinerea* L., *S. purpurea* L., *S. triandra* L., *S. caprea* L., *S. rosmarinifolia* L., *S. aurita* L. However, tall shrub species with an average annual growth of up to 0.8-1.45 m or more are the most promising for creating energy plantations: *S. viminalis* L., *S. acutifolia*, *S. triandra*, *S. purpurea*, as well as *S. alba*, *S. pentandra* and *S. fragilis*, which have a tree life form (Figure 1). Among the native poplar species, *P. nigra* L., *P. alba* L., *P. tremula* L. with an average annual growth of 1.1-1.55 m in height are promising in the temperate climate zone of Ukraine for the creation of energy plantations.

In addition to the autochthonous species, Polish and Swedish energy varieties of the *Salix* genus have been intensively imported and tested in Ukraine over the past ten years, and native *Salix* and *Populus* varieties have been created and tested. The most popular source material for breeding is *S. triandra* and *S. viminalis*. According to M. Roik et al. (Roik et al., 2015), shrub species of the *Salix* genus produce 14 times more biomass than conventional forest, which allows obtaining 24-30 t/ha of dry biomass. The selection of *Salix* species is focused on increasing the yield of clones, increasing the potential for genetic improvement, increasing genetic diversity (variability) and shortening the production cycle (cultivation).

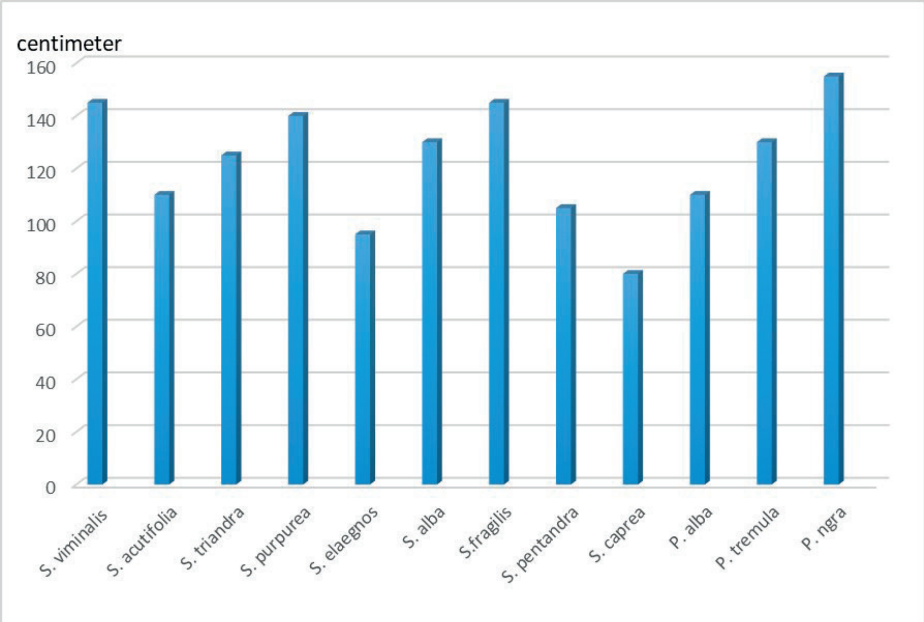

Figure 1. Average annual growth of three-year-old seedlings of *Salix* and *Populus* species in the forest-steppe of Ukraine

Since 2013, Ukraine has also started registering energy crop varieties of the *Salix* genus. As of February 2, 2023, 9 varieties of *S. viminalis* ('Panfyl's'ka 2', 'Marstyiana', 'Zbruch', 'Wilhelm', 'Linnea', 'Yevangelina', 'K2', 'M2', 'M3'), two varieties of *S. triandra* ('Yroslava', 'Panfyl's'ka'), two varieties of *S. fragilis* ('A3', 'Kozak', 'Adam 2') and one variety of *S. alba* ('N 1') (State register, 2023). The patent holders of these varieties are domestic scientific and educational institutions (Panfilska Research Station of the National Research Center "Institute of Agriculture of NAAS", Institute of Bioenergy Crops and Sugar Beet of NAAS, NUBiP of Ukraine), domestic and foreign companies (Salix Energy LLC (Ukraine), Jurelien Willov Briiding AB (Sweden), Lantmannen SW Seed AB. SE (Sweden) and private individuals.

A number of energy crop varieties of the *Populus* and *Salix* genera are being tested at the energy crops research and testing ground of the Leonid Pogorelyi Ukrainian Research Institute for Forecasting and Testing of Equipment and Technologies for Agricultural Production in Doslidnytske village, Obukhiv district. The experimental plots include *S. viminalis* and *S. triandra*, Swedish 'Inger', 'Tordis', 'Tora' and Polish '1047', '082', '1057' energy vatieties of *Salix* (Figure 2).

The creation of energy plantations from varieties of the genus *Populus* is being developed in detail in the conditions of Kyiv Polissia and Kharkiv region (Ukrainian Order "Sign of Honor" Research Institute of Forestry and Forest Melioration named after G. M. Vysotsky). In total, the collection of URIFFM includes 34 clones of *Populus* varieties and six clones of *Salix* varieties of N. Starova's breeding 'Lisova Pisnia', 'Lukash', 'Mavka', 'Olimpijs'kyj vohon', 'Pechal'na', 'Pryberezhna' (Figure 3).

We also studied the growth of three-year-old seedlings of the *Salix* and *Populus* varieties at the biostationary facility of the Bila Tserkva NAU. The most promising in the conditions of the northern forest-steppe were *S. hybrida* 'Inger', *S. hybrida* 'Tardis', *S.* cv. 'Ternopilska', 'Tardif de Champange', *P.* cv. 'Weresina', *P.* cv. 'Witschtejna' (Figure 4). In the spring of 2018, we also laid a 0.5-hectare experimental plantation of energy willow of the 'Linnea' variety in Bila Tserkva NAU.

Figure 2. Test plantation of energy crops *S. viminalis* 'Tora' in Doslidnytske village, Obukhiv district

Figure 3. Variety test plantation in the Southern Forestry of the State Enterprise "Kharkiv Forest Research Station"

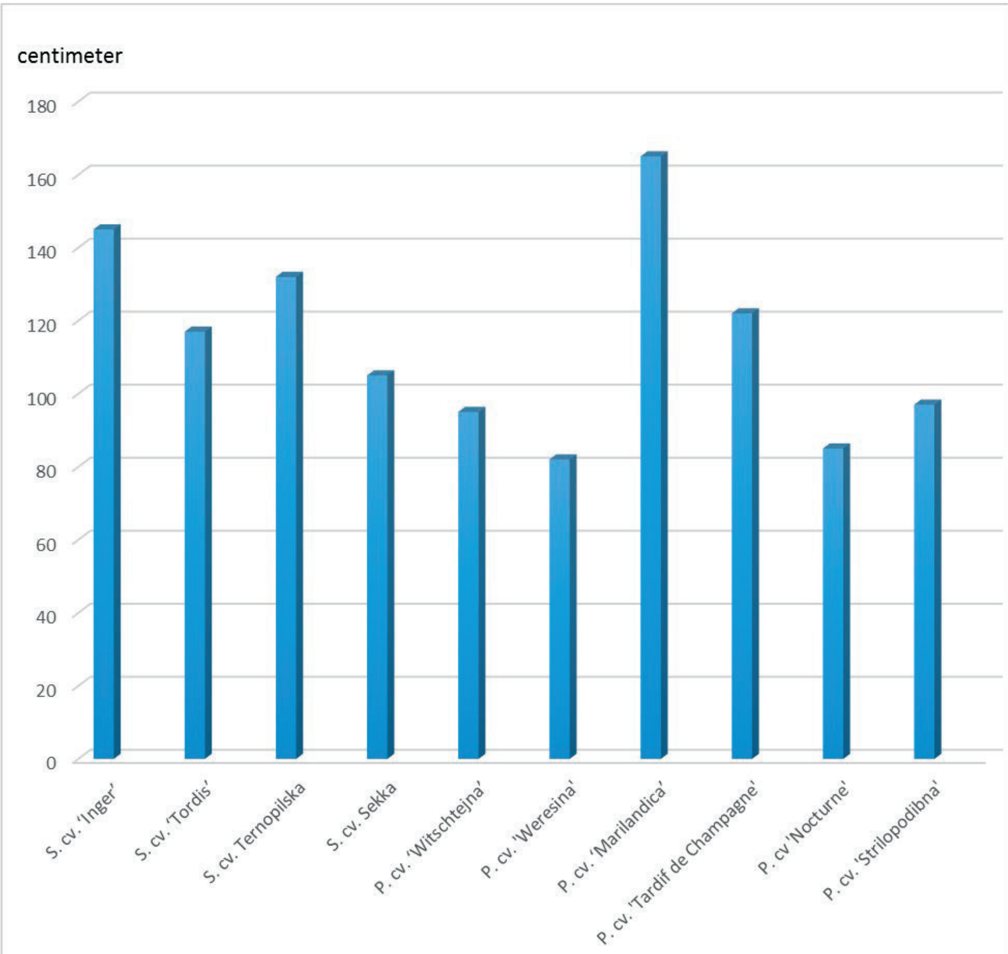

Figure 4. Average annual growth of three-year-old seedlings of *Salix* and *Populus* in the forest-steppe of Ukraine

In fact, the range of energy varieties of the *Salix* genus in Ukraine is much larger, but the long multi-year registration procedure significantly slows down the emergence of new varieties in the Ukrainian phytoenergy market. Another problem that hinders the development of bioenergy is the legal ban on planting *Salix* energy varieties on agricultural land, as *Salix* is classified as a forest crop in Ukraine. In neighbouring Poland, however, *Salix* is classified as an agricultural crop (Roik et al., 2015; Bohatov, 2021).

The development of *Salix* bioenergy is slowed down by the underdeveloped market for planting material for this crop. The market usually offers seedlings of different categories - licensed, unlicensed, Polish and Swedish varieties, and even seedlings cut right in the swamp. In Ukraine, only three companies work with licensed material, and two of them produce it only for their own needs.

Species and cultivars of the genus *Salix* and *Populus* in terms of calorimetric indicators produce from 7 to 20 tons of dry weight per hectare. For example, the yield of freshly cut shoots of *S. viminalis* is 40.7 - 47.3 m^3/ha, which is equivalent to 415 GJ × ha^{-1} of energy (Fuchylo et al., 2011). The estimated cost of production of one ton of fuel from *Salix* energy varieties is 1800-2000 euros (Ishchuk, 2014; Holland Plant Ukraine, 2022).

The definition of evaluation criteria to measure biomass quality is gaining increasing importance, especially in fast-growing forests.

Plant cell walls represent a vast reservoir of reduced carbon in the form of biopolymers, mostly cellulose, hemicellulose and lignin bound together in a complex network. Cellulose is the most abundant biopolymer on Earth and provides structural rigidity to plant cell walls. It is also a significant source of carbohydrates available for enzymatic hydrolysis and fermentation into liquid fuels, and represents the majority of substrates intended for second-generation biofuel production (Somerville et al., 2010). Hemicellulose is a heterogeneous biopolymer that adds strength to cells walls by linking cellulose microfibrils. Its composition differs greatly by plant species, but is mainly comprised of five-carbon sugar monomers and can therefore be a source of fermentable substrate using specialized or engineered microorganisms following chemical hydrolysis. Lignin has many important physiological roles in plants, including providing a hydrophobic surface in vascular tissues for water transport, and structural stability and resistance to disease and pest attack. However, it also presents a significant impediment to enzymatic cell wall depolymerization in liquid fuel production. Lignin has a higher energy density compared with cellulose and therefore is viewed as a desirable component for feedstocks used for thermal conversion. Lignocellulosic ethanol is an attractive biofuel because it is renewable, has reduced environmental impacts, and avoids competition with the food industry. The results regarding the quality of the investigated lignocellulosic substrates and its theoretical ethanol potential are illustrated in Table 1. We could mention that the concentrations of cellulose in *Populus alba* substrate are much higher in comparison with *Salix viminalis* substrate and pruning residues. The *Salix viminalis* substrate is richer in hemicellulose. The level of acid detergent lignin in *Populus alba* and *Salix viminalis* substrates were low as compared with the control, which had effect on the effective digestibility and decomposition of lignocellulose biomass. The estimated theoretical ethanol yield from cell wall carbohydrates averaged 510.4 L/t in *Salix viminalis* substrate and 505.4 L/t in *Populus alba* substrate, as compared with 476.3 L/t in pruning residues substrate. Several literature sources describe the composition of cell walls in willow and poplar energy biomass. According to Prosiński (1984) the lignocelulossic composition of *Salix alba* was 43.6% Seifert cellulose, 21.5% pentosans, 25.0% Klason lignin, but of *Populus alba* - 52.4% Seifert cellulose, 21.8% pentosans, 20.4% Klason lignin. Leple et al. (2007) remarked that *Populus trichocarpa* biomass contained 48.22-57.07% cellulose, 23.19-30.72% hemicellulose, 16.64-20.65% lignin. Karp&Shield (2008) reported that willow dry mass contained 55.9% cellulose, 14% hemicellulose, 19% lignin; poplar respectively 40% cellulose, 14% hemicellulose, 20% lignin bau in corn stover 35% cellulose, 28% hemicellulose, 10.4% lignin. Wróblewska et al. (2009) determined that young shoots of *Salix* *viminalis* content 39.29% Seifert cellulose, 17.05% pentosans, 26.04% Klason lignin. Mitsui et al. (2010) releved that dry matter of *Salix* spp. clones contained 78.9-81.2% hollocellulose, 27.2-32.3% lignin and 2.1-4.0% extractives with ethanol-benzen. Kim et al. (2014) reported that *Salix viminalis* var. *gigantea* stem was composed from 44.9% cellulose, 35.1% hemicellulose, 19.4% lignin and 0.6% of other extractives, the ethanol production 79.4 kg/t. Bajcar et al. (2018) remarked that the contents of cellulose, hemicellulose, and lignin in willow biomass was 44.6%, 32.1% and 14.5%, in rapeseed straw 41.4%, 29.8% and 18.6%; in wheat straw 38.1%, 30.6% and 21.3%, respectively. Majlingova et al. (2019) mentioned that willow mass contained 38.69% cellulose, 32.53% hemicellulose, 21.65% lignin and 11.25% extractives, but poplar- 36.60% cellulose, 32.55% hemicellulose, 24.69% lignin and 6.67% extractives.

Table 1. The cell walls composition and theoretical ethanol potential of studied biomass substrates

Indices	*Populus alba*	*Salix viminalis*	Apple tree pruning residues
Acid detergent fibre, g/kg	579	540	547
Neutral detergent fibre, g/kg	770	787	766
Acid detergent lignin, g/kg	73	91	110
Cellulose, g/kg	506	449	437
Hemicellulose, g/kg	191	247	219
Hexoses sugars, g/kg	89.8	80.60	78.17
Pentoses sugars, g/kg	31.40	41.80	36.02
Theoretical ethanol potential, L/tone	505.4	510.4	476.3

The chemical composition of dry biomass is a key factor that affects the caloric value and technologies for production of solid biofuels. The elemental composition of biomass is a significant asset that defines the amount of energy and evaluates the clean and efficient use of biomass materials, provides significant parameters used in the design of almost all energy conversion systems and projects, for the assessment of the complete process of any thermochemical conversion techniques (Lawal et al., 2021). The energy released during the combustion process is positively correlated with the carbon and hydrogen contents as a function of the energy value of these elements. In contrast, high oxygen and nitrogen values decrease the calorific value, decreasing the energy potential of the sold biofuel. Nitrogen, sulphur and chlorine contents are some of the main causes of air pollution from biomass combustion. A higher percentage of these elements generally results in a higher level of air contaminants being released. Ash content is one of the main factors of biomass quality, since higher amounts of ash diminishes the quality of solid fuels. The calorific value of a fuel can be expressed in two forms: the gross calorific value (GCV), or higher heating value (HHV) and the nett calorific value (NCV), or lower heating value (LHV). The HHV is the total energy content released when the fuel is burnt in air, including the latent heat contained in the water vapour and therefore represents the maximum amount of energy potentially recoverable from a given biomass source. In practical terms, the latent heat contained in the water vapour cannot be used effectively and therefore, the LHV is the appropriate value to use for the energy available for subsequent use. (MCKENDRY, 2002).

The elemental composition, ash content and calorific value of studied biomass is listed in Table 2. We found that the dry matter of the studied biomass contained 46.04-50.22% carbon, 5.84-6.00% hydrogen, 0.80-1.29% nitrogen, 0.04-0.18% sulphur, 0.04-0.07% chlor, 2.82-3.59% ash, 18.90-18.77 MJ/kg GCV and 17.69-18.46 MJ/kg NCV. The higher content of carbon and hydrogen, and the lower content of nitrogen, sulphur, chlorine and ash in *Populus alba* and *Salix viminalis* biomass which have positive impact on calorific value as compared with apple tree pruning residues. *Salix viminalis* biomass is characterized by higher level of carbon and optimal sulphur content, as compared with *Populus alba* biomass.

Table 2. The elemental composition, ash content and calorific value of studied biomass

Indices	*Populus alba*	*Salix viminalis*	Apple tree pruning residues
Carbon	47.83	50.22	46.04
Hydrogen	5.98	6.00	5.84
Nitrogen	0.80	0.89	1.29
Sulphur	0.16	0.07	0.18
Chlor	0.04	0.05	0.07
Ash content of biomass, %	2.82	3.53	5.59
Gross calorific value, MJ/kg	19.22	19.77	18.90
Nett calorific value, MJ/kg	17.91	18.46	17.69

Some authors mentioned various findings about the elemental composition and calorific value of biomass from *Salix* and *Populus* species. Xiong et al. (2008) reported that characteristics of willow biomass was 3.37% ash, 49.35% carbon, 6.31% hydrogen, 0.46% nitrogen and 19.09 MJ/kg HHV. Mitsui et al. (2010) found that calorific values of dried stem segments select willow (*Salix* spp.) clones were ranging from 18.7 to 19.1 kJ /g. Bilanzdija et al. (2012), raported that apple tree pruned biomass contained 73.50% volatile matters, 1.52% ash, 47.36% carbon, 6.42% hydrogen, 0.74% nitrogen, 45.30% oxygen, 0.18% sulphur and 17.06 MJ/kg LHV. Szyszlak-Barglowicz et al. (2012) remarked that willow coppice had 18 915 kJ/ kg heat of combustion and 17 688 kJ /kg heat value. Kang et al. (2014) noted that dry mass from poplar 79.7% volatile matters, 2% ash, 51.6 % carbon, 6.1% hydrogen, 0.6% nitrogen, 41.7% oxygen, 0.02% sulphur, but willow mass 74.2% volatile matters, 1.4% ash, 49.8 % carbon, 6.1% hydrogen, 0.6% nitrogen, 43.4% oxygen, 0.06% sulphur. Stolarski et al. (2014) found that *Salix viminalis* biomass contained 48.23-50.99% carbon, 5.67-5.88% hydrogen and 0.032-0.037% sulphur, 1.49-1.60% ash, 19.40-19.59 MJ/kg HHV, but *Salix dasyclados* 47.04-49.91% carbon, 5.49-5.94% hydrogen and 0.038-0.046% sulphur, 1.59-1.71% ash, 19.40-19.53 MJ/kg HHV. Williams et al. (2016) mentioned that the shrub willow contained 84.0% volatile matters, 1.5% ash, 50.3% carbon, 13.6 % fixed carbon, 6. 0% hydrogen, 0.36% nitrogen, 42.6% oxygen, 0.04% sulphur; hybrid poplar 84.0% volatile matters, 1.3% ash, 50.0% carbon, 14.6% fixed carbon, 6.0% hydrogen, 0.35% nitrogen, 42.8% oxygen, 0.03% sulphur, but herbaceous biomass respectively 79.1% volatile matters, 5.5% ash, 47.4% carbon, 15.4% fixed carbon, 5.8% hydrogen, 0.75% nitrogen, 41.0% oxygen, 0.10% sulphur. Monedero et al. (2017) reported that willow stem contained 83.59% volatile matters, 1.88% ash, 48.84% carbon, 6.18% hydrogen, 0.46% nitrogen, 0.03% sulphur, 0.0% clor, 17.98 MJ/kg LHV dry basis; poplar stem contained 82.37% volatile matters, 3.00% ash, 51.84% carbon, 6.39% hydrogen, 0.16% nitrogen, 0.04% sulphur, 0.01 % clor, 17.93 MJ/kg LHV dry basis. Bajcar et al. (2018) noted that raw willow mass is characterized by 10.3% moisture, 25.5% volatile matters, 3.15% ash, 48.1% carbon, 5.55% hydrogen, 0.55% nitrogen, 53.1% oxygen, 17.5 MJ/kg LHV, but torrefied mass 7.96-9.12% moisture, 23.1-44.9% volatile matters, 3.17-3.72% ash, 48.2-55.5% carbon, 3.64-5.87% hydrogen, 1.15-1.48% nitrogen, 42.2-44.9% oxygen, 19.2-21.5MJ/kg LHV. Dyjakon (2018) releved that pruning biomass had 0.8% ash, 19.31 MJ/kg HHV and 18.05 MJ/kg LHV. Gudima (2018) mentioned that studied *Salix* varieties contained 1.54-1.75% ash, 19.12-19.75MJ/kg GCV and 17.88-18.51 MJ/kg NCV. Pavlenco (2018) found that collected pruned biomass had 24.31-40.49% moisture and dry mass was characterized by 44.31-47.13% C, 5.37-6.11% H, 0.28-0.32% N, 0.02-0.04% S, 0.76-1.67% ash, 20.11-21.40 MJ/kg GCV and 18.78-20.07 MJ/kg NCV. Majlingova et al. (2019) revealed mentioned that willow mass had 1.28% ash, 19.63 MJ/kg HHV and 16.33 MJ/kg LHV, but poplar mass - 2.59% ash, 19.4 MJ/kg HHV and 16.18 MJ/kg LHV. Ţîţei & Roșca (2021) mentioned that calorific value of biomass from *Salix* and *Populus* species varied from 19.00 MJ/kg HHV to 19.5 MJ/kg HHV. According to Stachowicz & Stolarski (2023), the harvested willow mass contained 49.75% moisture, 78.38% volatile matters, 1.25% ash, 53.25% carbon, 20.37% fixed carbon, 5.97% hydrogen, 0.38% nitrogen, 0.016% sulphur, 0.019 % clor, 19.63 MJ/kg

50% carbon, 6.2% hydrogen, 0.6% nitrogen, 43.10% oxygen, 0.9% sulphur, 19.63 MJ/kg high heating values; poplar mass - 56.52 % moisture, 77.83% volatile matters, 1.67% ash, 53.46% carbon, 20.50% fixed carbon, 5.90% hydrogen, 0.43% nitrogen, 0.025% sulphur, 0.027% clor, 19.84 MJ/kg; black locust mass 38.89% moisture, 77.86% volatile matters, 1.40% ash, 52.60% carbon, 20.74% fixed carbon, 5.94% hydrogen, 0.91% nitrogen, 0.033% sulphur, 0.032 % clor, 19.46MJ/kg high heating values.

CONCLUSIONS

Therefore, promising plants are species and varieties of the genera *Salix* L. and *Populus* L., which are characterized by rapid biomass growth rates. Studies have shown that the annual growth of species and varieties of the genus *Salix* L. is 80-145 cm and of the genus *Populus* L. - 120-165 cm.

The *Salix viminalis* and *Populus alba* phytomass contained 2.82-3.53% ash, 44.9-50.6 % cellulose, 19.1-24.7% hemicellulose, 7.3-9.1% acid detergent lignin, 47.83-50.22% C, 5.98-6.00% H, 0.80-0.89% N, 0.07-0.16% S, 0.03-0.05 % Cl, 19.22-19.77 MJ/kg HHV and 17.91 18.46 MJ/kg LHV, but in apple tree pruning residues 5.59% ash, 43.7% cellulose, 21.9% hemicellulose, 11.0% acid detergent lignin, 46.04 % C, 5.84% H, 1.29% N, 0.18% S, 0.07% Cl, 18.90 MJ/kg HHV and 17.69 MJ/kg LHV. The estimated theoretical ethanol yield from cell wall carbohydrates averaged 510.4 L/t in *Salix viminalis* substrate and 505.4 L/t in *Populus alba* substrate, as compared with 476.3 L/t in pruning residues substrate.

The rapid development of global bioenergy causes the need to search for and develop fundamentally new approaches to plantation forestry. The introduction of plantation forestry in Ukraine will help reduce resource pressure on other categories of forests, increase the country's forest coverage and productivity of plantations, and reduce the time required to grow wood for further use for energy purposes. At the same time, a number of economic, environmental and social problems in rural areas may be resolved. Utilizing Ukraine's resource potential to the fullest extent, i.e. reforestation of vacant agricultural land, makes it possible to obtain an environmentally friendly renewable energy source in the form of biomass. The development of the latest biomass conversion technologies and their application in the energy sector of Ukraine and Moldova opens up the possibility of establishing energy independence for both countries. Accordingly, the economic situation in the countries will improve and the welfare of the population will increase.

ACKNOWLEDGEMENTS

The study has been carried out in the framework of the NARD projects: 20.80009.5107.02 and 20.80009.7007.19, Contract NBGI-BTNAU, Ukrainian Order "Sign of Honour" Research Institute of Forestry and Forest Melioration named after G. M. Vysotsky.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON THE IMPACT OF THE MULCHING SYSTEM ON STRAWBERRY YIELD

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Abstract

The research followed the impact of the mulching system on strawberry yield in the Giurgiu County area in 2022. Mulching provides a physical barrier between the soil and the plant, with positive effects on decreasing the degree of weediness, water losses, and attack by harmful organisms. The biological material taken in the study was of the varieties Magic and Alba. Two types of mulch were used, black polyethylene mulch and wheat straw-based organic mulch. In comparison to the control, where the yield was 25 t/ha, the Alba variety produced the highest yields, 40 t/ha in the black polyethylene mulching system variant and 30 t/ha in the wheat straw mulching system variant. In the Magic variety, the yield was higher in the wheat straw mulching system variant 35 t/ha compared to the black polyethylene mulching system variant 25 t/ha and the control 20 t/ha. The research showed that in Alba, the black polyethylene mulching system provided the highest yields, while in Magic, the wheat straw mulching system was more suitable. The results of the experiment demonstrate that the use of mulching systems is effective in increasing yield, with the best system differing by variety.

Key words: *strawberry, variety, mulching system, yield.*

INTRODUCTION

One of the most valuable fruits in the world is the cultivated strawberry (*Fragaria x ananassa* Duch). Romania's strawberry production has increased annually since 2014 by 0.6%. With 22,620 tons produced, the country produced the 29th-most strawberries in the world in 2019. Israel, which was ranked 28th with 24,622 tons, surpasses Romania, and Serbia comes in second place with 19,608 tons. With 3,221,557 tons in 2019, up 5% from 2018, China tops the list. In this ranking, the United States came in second, followed by Mexico and Turkey, and finally, the United States. The 5-year average growth rate for Moldova was +23.3% per year, while Kenya had the worst performance at -33.7% per year (FAO, 2019).

The strawberry crop is known to grown in numerous Romanian regions, but Hotarele, Giurgiu, one of the country's major strawberry producers, is particularly significant economically. In this region, over 200 hectares of strawberries were grown just in 2017 (Zmaranda, 2017). Strawberry fruits offer a significant financial advantage to the grower when compared to other crops and consumers like them because of their flavorful organoleptic characteristics and nutritional value. Due to the presence of vitamin C, strawberry fruits are highly regarded for their flavor, aromatic balance, and nutritional value. They are also valued for their high levels of polyphenols and ellagic acid, the latter of which is regarded as an anticancer agent (Temocico et al., 2019). There are many factors that influence strawberry yield and quality. Studies on this subject have demonstrated that, in addition to genetic characteristics, which are a significant factor, inadequate cultivation technology can also have a negative impact on plant development and fruit quality (Sayğı, 2022). The main factors that are important in production are the varieties selected for each region, the use of irrigation, the control of diseases and pests and the observation of culture technology, all with the objective of increasing financial income.

The application of the mulching system is a method that growers have been using for a long time to protect the plant and the soil surface and to produce higher quality fruit. The use of wheat straw or polyethylene film mulch is one of the most crucial cultural measures to prevent strawberry fruit infections with *Botrytis cinerea* because it helps to prevent direct contact with the soil and the same time of the pathogen with the fruit (Țane, 2022). The most popular mulching method is polyethylene film mulch (Santin et al., 2020). Researchers only recommend using black polyethylene mulch in agricultural systems because it is much more effective at blocking sunlight than transparent or colored mulch (Chang et al., 2016). Mulching can improve soil conservation by reducing water evaporation losses, regulating soil temperature, reducing weed growth, and enhancing microbial activities (Iqbal et. al., 2020). Numerous studies show that the use of a mulching system leads to appreciable increases in production for a variety of crops (Daugaard, 2008; Gao et al., 2019; Lamont and William, 2017; Singh et al., 2006). Organic material like wheat straw is another option for mulching soil. The benefits of wheat straw as a mulching system for the soil, including improved fertility, moisture retention and weed control, are cited by researchers as reasons to use it (Jabran and Chauhan, 2018; Ramakrishna et al., 2006). The objective of this paper was to evaluate the impact of several mulching techniques on the production of strawberries in Hotarele, Giurgiu.

MATERIALS AND METHODS

The present work was carried out in Hotarele, Giurgiu, located in the latitude 44,10° N, longitude 26,22° E and altitude of 29 meters. The climatic parameters for the area under study are: the average annual temperature 11.1°C; the absolute maximum daily temperature 40.5°C; the absolute minimum daily temperature -30.2°C; the average temperature of the summer months 20.4-23.2°C and the average temperature of the winter months 3 … -3.2°C. The average annual precipitation values are 556.1 mm, the relative air humidity has values between 72-74% and the average value of potential evapotranspiration is 729 mm. Global radiation recorded values between 122.5 and 127.5 kcal/cm^2, and the multiannual mean amplitude is 25.5°. The experimental field is located on a cambic chernozem where the water table is at approximately 2 m and the morphological profile is of the type An –Bv –Cca with accumulations of good quality humus and a large amount of organic substances. The pH of the soil is generally neutral or weakly alkaline with values of 7-7.5. Alba and Magic, were the strawberry cultivars used in this experiment. The plants were imported from a Dutch nursery for the Alba variety and from a Romanian nursery for the Magic variety. Magic is a very productive variety with medium-vigorous, dense, globular bush that is resistant to low temperatures. It produces a moderate amount of stolons. The fruit is glossy, uniformly red and conical in shape, weighing 10 to 15 g. The pulp has a consistent red color, is firm and is transport-resistant. It ripens at the end of May and the harvest lasts until the middle of June. It is one of the most popular and widely used strawberry varieties by growers because of its high production, high frost resistance (up to -25°C) and low soil requirements. Alba is a variety of strawberry adapted to the climatic conditions of our country. The plant is vigorous and productive, with large leaves. The fruit has a distinctive commercial appearance, is large (on average over 20 g), conical-elongated and has high firmness. It has a good flavor and a sweet-sour aroma. It is a very early variety; typically, the first harvest occurs between May 5 and 7.

The crop was established with refrigerated stolons in the first decade of July (July 10). In order to have a good production from the first year, the inflorescences that formed during this period were removed. The planting was done manually with spoon-shaped planters, on the irrigated area one or two days before. It is very important that when planting, the central bud (heart) is not buried because it leads to the death of the plant. After planting, the plants were watered. Planting distances: 1 m between rows and 0.30 m between plants per row. In the Alba variety, the cultivation is carried out on matted row system in equidistant rows, and in the Magic variety, the cultivation is carried out in the classic system in simple rows.

To control weeds, the herbicide Sultan 50 SC (metazachlor 500 g/l) was applied, pre-

emergent for annual monocotyledonous and dicotyledonous weeds from seed (0.5 l/ha in 300 liters of water). Additionally, during the vegetative period, a total of six soil-working methods were applied, three mechanical and three manual. The recommended irrigation rates during the vegetative period were 50-60 m^3/ha every two days.
The majority of the fertilization was done through fertigation (Table 1), which was done weekly based on the electrical conductivity of the soil and by analyzing the nutrient elements in the petiole of the leaves.

Table 1. Fertilization scheme

Phenological stage	Fertilizer	Dose (g/plant)	Application number
In the early stages of development BBCH 40 - 50	Phosphorus	1	1/week
Before blooming BBCH 55 - 60	Magnesium sulphate	0.2-0.5	
Full flowering, fruit formation BBCH 65 - 70	Calcium nitrate	0.1-0.12	
Fruit development BBCH 70 - 80	Urea	0.6	

In the experiment two mulching types were used: polyethylene film mulch and wheat straw mulch. Different stages of the mulching system's application were used. The following steps were taken in relation to the polyethylene film mulching system (Figure 1) the soil was modeled in the hill system, the drip irrigation system was installed, the black mulching film was applied, then the planting was carried out.

Figure 1. Black polyethylene mulching system

For the wheat straw mulching system (Figure 2), the soil was traditionally prepared in equal-distance rows, the drip irrigation system was installed, the planting operation was completed, while the wheat straw mulch was applied at the start of the flowering phenophase.

Figure 2. Wheat straw mulching system

The plants were planted on September 15. The fruit harvest began in May 7 and finished in June 15. The fruits were harvested at commercial maturity and shortly after, fruits were counted and weighed with a digital balance. Data on average fruit weight and total yield per plant were collected during vegetative growth and fruiting time.
Results were statistically analyzed by ANOVA and Tukey using the GraphPad Prism version 7.00 for Windows, GraphPad Software (La Jolla California USA).

RESULTS AND DISCUSSIONS

The results showed that Alba variety with polyethylene mulching film presented a bigger average weight by plant, as well as a bigger mean weight for fruit and production, when compared with Magic variety on the same mulching type. One of the most significant observations was that the polyethylene film mulching system significantly reduced the production of the Magic variety per plant because this variety is more sensitive to high temperatures than other varieties and the presence of the black polyethylene film which captures even more light, lead to an increase in temperature at the plant level, resulting in poor plant development and by default to poor fruit development. On the other hand, the wheat straw mulching system significantly improved the production for Magic variety. When compared, the production of the Alba variety, the polyethylene film mulching system recorded slightly increased values than the wheat straw mulching system (Figure 3).

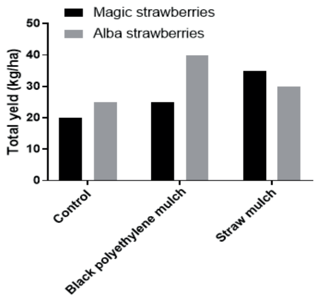

Figure 3. Total yield per hectare

As seen in Figure 4, in terms of the average weight fruit, significant variations were registered. At the Magic variety, the highest average fruit weight was registered in wheat straw mulching system, the difference from the control being significantly different (p<0.0001) and two times higher (14.87 g) than this. No difference was observed in polyethylene film mulching system comparing to control (p = 0.95). This could be explained due the increase sensitivity of this variety to high temperatures than others in the presence of the black polyethylene film.

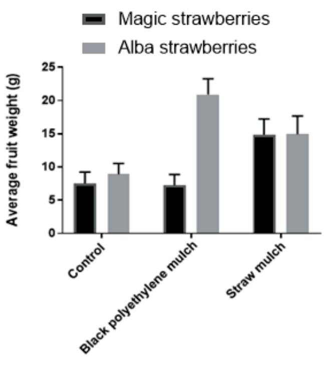

Figure 4. Effect of mulching types on average fruit weight

Alba variety registered the maximum average fruit weight of plant (20.92 g per fruit) from the treatment in the black polyethylene film mulching system, the difference from the control being significantly different (p<0.0001).

Strawberry grown under black polythene mulch produced higher yield per plant due to larger fruits produced by improved plant growth due to a favorable hydrothermal regime of the soil and a completely weed-free environment. Comparing to other topic research, results confirmed a similar observation of increased yield with larger fruits after mulching with black polythene (Bakshi et al., 2014). Adnan et al. (2017) obtained the highest fresh weight of fruits (23.01 g $fruit^{-1}$) in black polythene mulch. Pandey et al. (2015) found that among the various mulches tested, black polythene outperformed yield parameters (fruit fresh weight 36.74 g and fruit yield/plant 536.55 g). Angrej et al. (2007) also presented as results how polyethylene film improves the number of fruits per plant, average weight and size (length and width), and fruit yield. The increase of yield using black plastic mulch is reported also by Laugale et al. (2010). As I mentioned above, researchers have recommended polyethylene film mulch in numerous studies, but according to data collected on the Magic variety, which is a fairly cultivated variety in Romania, it only has good results in the presence of wheat straw mulch.

As seen in Figure 5 significant variation between varieties in terms of the total yield per plant were registered. At the Magic variety, the highest total yield per plant was registered in wheat straw mulching system, the difference from the control being significantly different (p<0.0001) and two times higher (1.2 kg per plant) than this. No difference was observed in polyethylene film mulching system comparing to control (p = 0.66).

Alba variety registered the maximum total yield per plant (1.75 kg per plant) from the treatment in the black polyethylene film mulching system, the difference from the control and wheat straw mulching system being significantly different (p<0.0001).

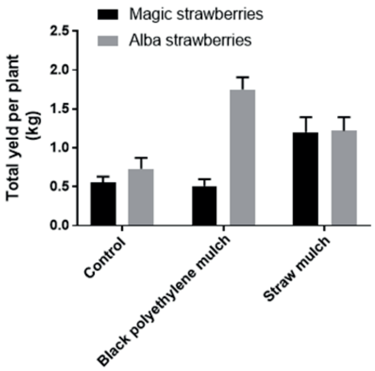

Figure 5. Effect of mulching types on total yield per plant

Regarding the control, the production was significantly reduced compared to the two types of mulching, because it did not control the weeds and direct contact of the fruit with the soil led to gray rot infections.

CONCLUSIONS

When mulching systems were used, both the Alba variety and the Magic variety experienced considerable production gains. The Alba variety works best with the method of mulching with polyethylene film, while the Magic variety works best with the system of mulching with wheat straw.

Mulching systems are crucial in strawberry cultivation because, in addition to highly increasing yields, they also help prevent the growth of weeds that can serve as hosts for different pathogens and, by avoiding direct soil contact with the fruit, they also ensure protection against numerous soil pathogens.

It is important to keep in mind that a high level of production is not mainly attributable to the mulching system applied, but rather to its harmonic integration with appropriate technology while taking into account the preferences of the selected variety.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

CHARACTERIZATION OF AN AGROFORESTRY SYSTEM FROM WEST OF ROMANIA THROUGH SUSTAINABILITY INDICATORS OF SOIL

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Abstract

The agroforestry systems in Romania are rare and low studied, although the advantages provided by these systems to agriculture are multiple. The aim of this research was to use several soil indicators widely used as indicators of soil sustainability to characterize an agroforestry system from western Romania, Timiș County (45.45418°N, 20.90334°E). The studied agroforestry system was a silvoarable ecosystem and consisted of two components: a woody perennial plant represented by Euro-American hybrid poplar trees (Populus deltoides x Populus nigra) and an agricultural crop - rapeseed (Brassica napus L.) - hybrid LG Architect. The soil parameters analyzed for both components were pH, humus, total nitrogen, plant-available phosphorus, and plant-available potassium. There have been found higher values of the soil parameters pH, humus, plant-available phosphorus and plant-available potassium in the soil cultivated with rapeseed than those of the soil planted with hybrid poplar, and statistically significant (p<0.01) correlations between several soil factors of the two components of the silvoarable system: between pH and plant-available potassium in the Populus spp. plantation and between humus and plant-available phosphorus in the rapeseed crop. The findings of this study show benefits expressed as nutrient increase of soil for the rapeseed crop in the silvoarable system poplar-based and emphasize the favourable association of these two types of plants: trees and crops.

***Key words**: poplar, Populus, rapeseed, canola, silvoarable, nutrients.*

INTRODUCTION

Agroforestry is an agricultural practice which combines trees with crops and/or livestock, and which is practised on 15 million hectares equivalent to 9% of the cultivated agricultural land or to 3.6% of the territorial land (or on 52 million hectares if the reindeer is included) in the European Union (Augère-Granier, 2020), and respectively on 1 billion hectares and by over 1.2 billion people worldwide (United Nation Food and Agriculture Organization - FAO, 2017).

The agroforestry systems in Romania are low studied despite their multiple advantages (Kay et al., 2019) for crop protection and production, soil protection and environmental buffer in climate changes mitigation. When these types of systems are studied in Romania, the investigations are predominantly focused on silvopastoral systems (Hartel et al., 2018; Smith et al., 2022) or on forest shelterbelts (Malschi et al., 2010; Mihaila et al., 2022a; 2022b). The Romanian potential in agroforestry absolute area is large as compared to other countries in Europe (den Harder et al., 2017). According to Augère-Granier (2020) citing the Proceedings of the 5th World Congress on Agroforestry 2022, Romania is the fourth funded country of the European Union through financial supporting programmes in agroforestry, after Spain, France, and Italy, which reveals the increasing interest on this type of agriculture due to its benefits and recognition as a main tool in mitigate several environmental challenges, such as climate change, protection of water, soil, and biodiversity resources, alongside with the reduction of the pesticide dependence and animal welfare threatening as agriculture consequences. Because of the rather recent interest in agroforestry, in Romania this activity is often not clearly recognized with this name, although this exists and this is performed and sometimes even funded and supported through financial national instruments as part of the common agricultural policy (CAP) of the European Union.

The hybrid poplars (*Populus* spp.) present several characteristics which recommend them to be used as part of the silvoarable agroforestry systems, such as: they are perennial plants with fast growth and weight gain, necessitate long period of crop rotation which make them very comfortable for farmers (between 2 and 40 years, depending on the local climate and on the purpose of cultivation) (Manevski et al., 2019), they have deep resistant root (Babi et al., 2023), able to explore the deep layers of the soil profile, and thus accessing different pools of water and nutrients, are excellent drainers of the water-excess soils (Manevski et al., 2019), increase the nutrient concentrations and availability in the vicinity crops (Pardon et al., 2017).

The aim of the paper is to describe a silvoarable agroforestry system from west side of Romania which combine hybrid poplar trees with rapeseed crop through several soil parameters widely used as sustainability indicators in ecosystem assessments: pH, humus, total nitrogen, plant-available phosphorus, and plant-available potassium, with the goal to emphasize the favourable association of these two types of plants: trees and crops.

MATERIALS AND METHODS

The study has been conducted in an agroforestry system (Figure 1) located in Timiș County (45.45418°N, 20.90334°E), Romania and aimed to characterize it through several soil parameters widely used as indicators of soil sustainability. The studied agroforestry system is a silvoarable ecosystem according to FAO three-types classification of agroforestry (2017) and consisted of two components: a woody perennial plant represented by Euro-American hybrid poplar trees (*Populus deltoides* × *Populus nigra*) and an agricultural crop - rapeseed (*Brassica napus* L.) - hybrid LG Architect bordered by the poplar plantation. The soil parameters analyzed for both components were pH, humus, total nitrogen (total N), plant-available phosphorus (plant-available P), and plant-available potassium (plant-available K), which are main physical and chemical indicators used in describing the sustainability of agroecosystems (Augusto et al., 2017). The soil of the analyzed agroforestry system is vertisol (World Reference Base for Soil Resources, 2022). The soil samples have been collected at different depths: in the poplar plantation at three depth: 0-10 cm, 10-20 cm, and 20-30 cm, and in the rapeseed crop at 10 cm. These intervals of sampling have been chosen taking into consideration the tillage management in the two plant types of the agroforestry system, respectively: no soil workings in the poplar plantation for eight years which determined non disturbed soil profile, and conventional soil workings in the rapeseed crop which meant the disturbing and mixing of the soil layers on a depth by 30 cm, a sufficient reason to sample this soil only for the depth of 0-10 cm. The soil analyses have been performed by OSPA (Office for Pedological and Agrochemical Studies) Timiș using the following methodology: determination of pH - SR 7184-13:2001-PS-03; determination of humus - STAS 7184/21-82-PS-01; determination of the total N - STAS 7184/2-85-PS-08; determination of the plant-available P - STAS 7184/19-82-PS-02; determination of the plant-available K - STAS 7184/18-80-PS-06. The statistical analysis has been performed using the software IBM SPSS 28.0.0.0.

Figure 1. The agroforestry site: rapeseed crop (*Brassica napus* L.) bordered by poplar (*Populus* spp.) plantation

RESULTS AND DISCUSSIONS

The values of the aimed soil parameters are listed in Table 1 both for rapeseed crop and for poplar plantation by depth intervals. Also, for the soil factors of the poplar plantation there has been calculated the mean of the three depths in order to compare them with the values recorded in the soil of rapeseed crop.

Table 1. The soil parameters (mean values) of the two components (rapeseed crop and *Populus* spp. plantation) of the studied agroforestry ecosystem, by soil depths

Soil depth (cm)	Soil parameters in *Populus* spp. (hybrid poplar) plantation (mean values)				
	pH	Humus (%)	Total N (%)	Plant available P (ppm)	Plant available K (ppm)
0-10 cm	6.55	2.33	0.14	4.38	126
10-20 cm	6.26	1.76	0.12	4.14	91
10-30 cm	6.27	1.06	0.13	5.65	97
Means of soil depths	6.36	1.71	0.13	4.72	104.66
Standard Deviation	0.16462	0.63611	0.1000	0.81144	18.71719
Soil depth (cm)	Soil parameters in *Brassica napus* (rapeseed) crop (mean values)				
	pH	Humus (%)	Total N (%)	Plant available P (ppm)	Plant available K (ppm)
0-10 cm	6.42	2.07	0.12	18.78	124
Standard Deviation	0.24434	0.65010	0.00577	15.61453	15.87451

According to ICPA Romania and considering the cultivation technology (normal/intensive), the values of the chemical indices of soil for both plant types were classified as presented in Table 2. Thus, there was identified a low supply with nitrogen of both plants and low-moderate with humus.

Table 2. The supply degree of soils with nutrients (rapeseed crop and *Populus* spp. plantation)

Soil depth (0-30 cm)	pH	Humus (%)	Total N (%)	Plant available P (ppm)	Plant available K (ppm)
Populus spp. (hybrid poplar) plantation (mean values)	6.36 low acid	1.71 low	0.130 low	4.72 very low	104.66 moderate
Brassica napus (rapeseed) crop (mean values)	6.42 low acid	2.07 moderate	0.126 low	18.78 moderate	124.00 moderate

There has been found lower values of the soil analyzed parameters (pH, humus, P and K) in the soil planted with hybrid poplar versus those of the soil cultivated with rapeseed (Figure 2), but the differences between means were not statistically significant ($p>0.01$, $p>0.05$) (paired-samples t-test) (Table 3).

There was compared the results achieved for the hybrid poplar plantations to other findings obtained in 2019 for the same area of study which aimed at that time to characterize the soil of an agricultural land for establishing its suitability for the cultivation of energy hybrid poplar (Cândea-Crăciun et al., 2019). The available data used for comparison have been pH, humus, and total nitrogen (Table 4). The results showed differences like a slightly increase of the pH value, a slightly decrease of the humus content, and a considerable decrease of the total nitrogen content.

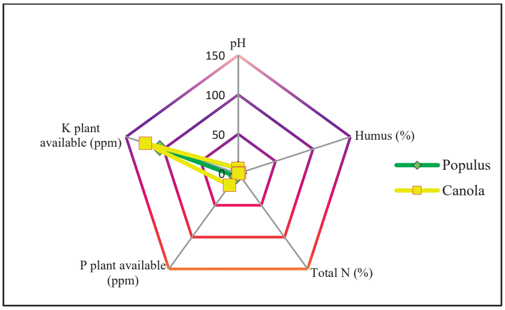

Figure 2. The soil parameters (mean values of soil layers depths) of the two components (rapeseed crop and *Populus* spp. plantation) of the studied agroforestry system

Table 3. Paired-samples t-test showing non-significant ($p>0.01$, $p>0.05$) differences between the soil parameters of the two components of the agroforestry ecosystem

Variables (means)	Mean	t	df	Significance (1-tailed)	Significance (2-tailed)
pH Hybrid poplar - pH Rapeseed	-0.0600	-0.933	2	0.225	0.449
Humus Hybrid poplar - Humus Rapeseed	-0.3566	-0.746	2	0.267	0.534
Total N Hybrid poplar - Total N Rapeseed	0.0020	0.480	2	0.339	0.678
P plant available Hybrid poplar - P plant available Rapeseed	-14.0566	-1.487	2	0.138	0.275
K plant available Hybrid poplar - K plant available Rapeseed	-19.6700	-2.409	2	0.069	0.138

Table 4. The supply degree of soils with nutrients for the study site, *Populus* spp. plantation, 2022 versus 2019

Soil depth (0-30 cm)	pH	Humus (%)	Total N (%)
2019 (Cândea-Crăciun et al., 2019)	5.90	1.76	0.17
2022	6.36	1.71	0.13
Difference	+1.07%	-2.85%	-23.53%

Although different from other studies, the pH values recorded in our study are situated within the recommended and appropriate range of pH values for the hybrid poplar growth, meaning between 5 and 8 (Hjelm et al., 2018). The values of the soil pH both in poplar plantation and in rapeseed crop remained in the appropriate range to avoid soil degradation and nutrient unavailability through acidity.

However, the attention should be paid to the humus, nitrogen, phosphorus and potassium content, because these nutrients are very important for the poplars (Rennenberg et al., 2010). Low content of humus in energy hybrid poplar plantations had been reported previously (Demo et al., 2013). The low supply with humus in the hybrid poplar plantation (Table 2) probably shows the poplar contribution to the nitrogen cycle through humus mineralization (Savin et al., 2019) because the chemical supplementary fertilization lacks in this plantation. A low non statistically significant decrease of the total nitrogen has been shown in the soil of rapeseed crop versus the soil of hybrid poplar, although the rapeseed crop has been nitrogen fertilized (RhizoStart 8-30-0 fertilizer - 2 weeks before seedling, 210 kg/ha; Nitrocalcar fertilizer NAC 27 N (27% nitrogen) - 2 months after seedling, 100 kg/ha; granular urea 46.2-00-00 (46% nitrogen) - six months after seedling, 200 kg/ha), but the soil sampling has been done at seven months distance from the last fertilization and therefore considered with no residual effect at the sampling time. Probably there are other reasons to explain the low content of total nitrogen in both cultures of the silvoarable system, such as those revealed by several studies (Fortier et al., 2017) which showed that the leaf litter chemistry and decay rate may influence the N mineralization and nutrient release during decomposition, depending on the poplar genotype. The hybrid poplars require fertile soils to grow at their whole capacity (Boysen & Strobl, 1991; Rytter et al., 2011), but these trees can supply their demand through alternative mechanisms. For example, the nitrogen and potassium uptake can be supplied from the foliar leaching through internal cycling determined by seasonal leaf senescence (Fortier et al., 2020). This is a different situation as compared with other tree species, because previous studies (Rennenberg et al., 2010) showed that many forest ecosystems have been grew in soils with nitrogen limited availability, and they relied more on the internal cycling of the nitrogen than on external input. But, there was shown by other researches that poplars are very efficient in finding, due to their root expansive system, alternative nitrogen sources, like that resulted from the limitation of nitrogen leaching (Hermansen et al., 2017; Pugesgaard et al., 2015). However, several studies indicate as possible necessary the supplementary fertilization of soils planted with hybrid poplar for energy biomass, at least for that with short rotation cycle, both at the start and at the end of a cycle and even during the entire vegetation cycle (Savin et al., 2019).

The higher values of P and K in the rapeseed crop as compared to the poplar plantation have been revealed also by other studies on silvoarable poplar-based systems (Sirohi & Bangarwa, 2017), due to the poplar ability to protect the adjacent crops against phosphate leaching (Dimitriou & Mola-Yudego, 2017; Savin et al., 2019) or potassium loss (Sirohi & Bangarwa, 2017).

In order to find out possible relationships between the analyzed soil parameters, statistical tests have been processed, but generally, there were not found statistically significant (Paired-samples t-test, $p<0.01$, $p<0.02$) differences between the mean values of the soil factors across layers, excepting the pH of 0-10 cm versus pH of 10-20 cm, the humus of 0-10 cm and 10-20 cm versus the humus of 20-30 cm, and the total N of 0-10 cm versus the total N of 20-30 cm (Table 5).

The significant differences of the pH values within the soil layers 0-10 cm and 10-20 cm as compared with the other layers could be explained by the lithology of the substrate (Savin et al., 2019) or through the soil biome contribution in the topsoil due to the litter presence and no-till management in the poplar plantation. The significant differences of humus content across soil layers is associated with its decrease with the depth in the soil profile, because humus is formed through decomposition and humification of organic residues available at soil surface (Chatterjee et al., 2018). The significant differences regarding the contents of total nitrogen between soil depths are because the total nitrogen is heterogeneously distributed in soil and this depends on many factors, such as soil type, parent material, land use (Li et al., 2022).

The statistical processing of data (Kendall's and Spearman's Correlations) indicated statistically significant ($p<0.01$) correlations between several soil factors of the two components of the agroforestry ecosystem: between pH and K plant-available in the *Populus* spp. plantation and between humus and P plant-available in the rapeseed crop (Table 6).

Table 5. Differences between soil parameters (mean values) by depth of soil layer (Paired-samples t-test, $p<0.01$, $p<0.02$) in hybrid poplar plantation

Paired Samples Test						
Soil factors by depth of soil layer	Paired Differences		t	df	Significance	
	Mean	Standard Deviation			(p<0.01)	(p<0.05)
pH 0-10 cm - pH 10-20 cm	0.29333	0.15373	3.305	2	**0.040****	0.081
pH 0-10 cm - pH 20-30 cm	0.27667	0.39311	1.219	2	0.174	0.347
pH 10-20 cm - pH 20-30 cm	-0.01667	0.23965	-0.120	2	0.458	0.915
Humus 0-10 cm - Humus 10-20 cm	0.57333	0.45654	2.175	2	0.081	0.162
Humus 0-10 cm - Humus 20-30 cm	1.27000	0.41509	5.299	2	**0.017****	**0.034***
Humus 10-20 cm - Humus 20-30 cm	0.69667	0.23861	5.057	2	**0.018****	**0.037***
Total N 0-10 cm - Total N 20-30 cm	0.01333	0.00577	4.000	2	**0.029****	0.057
Total N 0-10 cm - Total N 10-20 cm	0.01667	0.01528	1.890	2	0.100	0.199
Total N 10-20 cm - Total N 20-30 cm	-0.00333	0.01155	-0.500	2	0.333	0.667
P plant available 0-10 cm - P plant available 10-20 cm	0.23667	0.98855	0.415	2	0.359	0.719
P plant available 0-10 cm - P plant available 20-30 cm	-1.26667	5.38778	-0.407	2	0.362	0.723
P plant available 10-20 cm - P plant available 20-30 cm	-1.50333	5.30119	-0.491	2	0.336	0.672
K plant available 0-10 cm - K plant available 10-20 cm	35.0000	35.15679	1.724	2	0.113	0.227
K plant available 0-10 cm - K plant available 20-30 cm	29.0000	26.00000	1.932	2	0.097	0.193
K plant available 10-20 cm - K plant available 20-30 cm	-6.00000	10.00000	-1.039	2	0.204	0.408

Table 6. Correlations ($p<0.01$, $p<0.05$) of the agroforestry ecosystem

Correlation factor 1	Correlation factor 2	Statistic correlation	Significance (p<0.01)
pH Hybrid poplar	K plant available Hybrid poplar	Kendall's tau_b Correlation Coefficient	1.000**
		Sig. (2-tailed)	0
		Spearman's rho Correlation Coefficient	1.000**
		Sig. (2-tailed)	0
Humus Rapeseed	P plant available Rapeseed	Kendall's tau_b Correlation Coefficient	1.000**
		Sig. (2-tailed)	0
		Spearman's rho Correlation Coefficient	1.000**
		Sig. (2-tailed)	0
**Correlation is significant at the 0.01 level (2-tailed).			

The positive correlation found between plant-available P and humus in the rapeseed soil could be explained through the findings of Spohn (2020) regarding the P contribution, both as organic and inorganic fractions, in the stabilization of the organic carbon in soil. The positive correlation between pH and plant-available K found in the poplar soil within this study is important because at low pH the K availability is low and could be explained as determined by the soil properties such as soil aggregates properties, adsorption capacity (Bronick & Lal, 2005; Linquist et al., 2022) or as microbiologically mediated (Zheng et al., 2022).

The results found within this study regarding the aimed soil factors of sustainability showed that the soil cultivated with rapeseed bordered by the poplar in the analyzed silvoarable ecosystem has greater values for pH, humus, plant-available P and plant-available K than the soil planted with hybrid poplar clones, but further investigations are required and completed with another indicators of sustainability previously analyzed in various ecosystems of the same studied zone (Timiș County), such as direct or indirect biological indicators of soil (Iordache, 2012; Iordache & Borza, 2012; Iordache, 2018).

CONCLUSIONS

The findings of this study show increases in several contents of soil nutrients (humus, plant-available P, plant-available K) for the rapeseed crop versus the soil planted with hybrid poplar within the silvoarable system and statistically significant ($p<0.01$) correlations between several soil factors of the two components of the silvoarable system: between pH and plant-available K in the *Populus* spp. plantation and between humus and plant-available P in the rapeseed crop.

This study emphasizes the favourable association for the rapeseed crop with hybrid poplars within a silvoarable system in terms of nutrient increase of the soil.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

GRASSLANDS AS FODDER FOR ANIMALS AND RENEWABLE SOURCE OF ENERGY BIOMASS

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Abstract

Grasslands have a wide range of economic and ecological functions. We studied the productivity and quality of the biomass obtained from an area of grassland with Elymus repens, Poa pratensis and Festuca valesiaca, which is found within the boundaries of Orhei National Park, Republic of Moldova. The biomass was harvested at the beginning of the flowering stage and was dried under natural conditions. It was established that the productivity of grassland with Elymus repens were 4.72-9.31 t/ha dry matter, the grassland with Poa pratensis 1.38-3.63 t/ha dry matter and the grassland with Festuca valesiaca 1.59-3.77 t/ha dry matter. The biochemical composition of hay dry matter was: 6.62-13.90% crude protein, 1.31-3.43% crude fat, 26.65-40.78% crude cellulose, 43.27-53.02% nitrogen free extract, 2.41-6.82% sugars, 1.40-2.83% starch, 6.79-11.29% ash, 0.27-0.73% Ca, 0.15-0.30% P, 4.40-41.12 mg/kg carotene with energy concentration 17.78-18.44 MJ/kg GE, 7.93-9.73 MJ/kg ME and 4.28-5.50 MJ/kg NEl. The gas forming potential of the fermentable organic matter of the hay collected from the studied grassland varied from 400 to 598 l/kg VS, and specific methane yield varied from 210 to 314 l/kg VS.

***Key words**: biochemical composition, fermentable organic matter, grasslands, hay, productivity, specific methane yields.*

INTRODUCTION

Grasslands play an important role in global agriculture; they are a basic source of nutrients for herbivores and ruminants and play an important role in the prevention of erosion, the immobilisation of leaching minerals and in carbon storage, help regulating water regimes and the purification of soils from pesticides and fertilizers (Marușca et al., 2014; Marușca, 2016; Sărățeanu et al., 2023).

The natural grasslands in the Republic of Moldova are in a poor condition, being heavily affected by excessive, unregulated grazing throughout the year and totally lacking a pastoral management system, a fact that affects their productive potential. In recent years, our region has been facing severe droughts. This phenomenon manifests itself more strongly when the soil drought is accompanied by atmospheric drought, and also by soil salinization. This leads to a decrease in the production of plant biomass, the appearance of imbalances in food supply for animals and, implicitly, to a decrease in zootechnical production (Lazu, 2014; Leah, 2016).

Considering this aspect, it is necessary to undertake activities to evaluate the grass cover of meadows, to determine the content of nutrients in aerial biomass, to establish the technological elements of re-cultivation that will contribute to the manifestation of the productive potential, to reduce the negative effects of drought and salinization on providing farm animals with high-quality feed, but also obtaining biomass for energy production.

The rapid increase in prices and the insecurity of the supply of the necessary amount of oil, gas and coal, as well as the global warming caused by greenhouse gas emissions have made humanity turn to new stable, non-polluting and renewable energy sources. The complex issues of renewable energy production are now addressed, in terms of policies, at global and regional scale. The European Commission has approved the Energy Policy which foresees the following objectives for the year 2030: a 33.5% increase in energy efficiency, a 40% reduction in greenhouse gas emissions and achieving a 32% share of renewable energy sources (EU Directive 2018 /2001*). From a historical point of view, biomass was the first form of energy source that was used by people since the discovery of fire. Biomass is considered a "carbon neutral" energy source because the carbon emitted during combustion was previously absorbed through photosynthesis during plant growth. Nowadays, bioenergy represents 60-70% of renewable energy production and is seen as a key solution for encouraging the sustainable development of rural areas. The problem of using renewable energy sources is still relevant for the Republic of Moldova, which is totally dependent on the import of fossil energy resources. The Law on Renewable Energy (2016) *** and the Energy Strategy until 2030 (2013) were approved; the "Energy and Biomass in Moldova"** Project was promoted in 2011-2017, being financed by the European Union.

The Republic of Moldova has no nuclear power plants, oil and natural gas deposits, the forest areas are limited, and most of the traditional energy resources are imported at high prices.

Being aware of the quantity and quality of forage provided by grasslands is very important in the context of establishing and applying sustainable grassland management measures. The evaluation of the current productivity and quality of grasslands is necessary for establishing and observing the grazing capacity, as well as for the sustainable development of the animal husbandry sector and the increase of the living standards in the rural areas.

The botanical composition, soil fertility and climatic conditions, as well as the time and frequency of harvesting of grasslands are the major factors that determine the productivity and nutritive value of pastures and the quality of prepared hay.

At national level, there are few studies on the amount of fodder on permanent grasslands and their biochemical composition, a fact that prompted the realization of this study.

The main objective of this research was to evaluate the productivity and quality of the biomass (hay) obtained from an area of grassland with *Elymus repens, Poa pratensis* and *Festuca valesiaca,* and prospect it use as forage for livestock and also as substrate for biogas production.

MATERIALS AND METHODS

The research was carried out during the years 2020-2022, in 3 types of grasslands, in the territory of Orhei National Park - grasslands with *Elymus repens, Poa pratensis* and *Festuca valesiaca* - as part of a comprehensive study which included the entire area of pastures in this park. Also, in the study it was included a sector of meadow with sedge (*Poa pratensis*) on the territory of the "Alexandru Ciubotaru" National Botanical Garden (Institute) (NBGI) with a balanced phytosociological composition, rich in plant species from the Fabaceae family considered as a standard.

Hay production was calculated by the gravimetric method based on the grass harvested at the first cut. Grass samples were taken from the studied grassland sectors at the beginning of the full flowering stage from sample areas of 1 m^2, in 3 repetitions, weighing the harvested green mass. The grass was mowed by hand at a height of 5-7 cm. The samples were dried under natural conditions in open air and then weighed again to calculate the amount of dry matter (hay).

The evaluation of hay quality: crude protein (CP), crude cellulose (CF), crude fat (EE), nitrogen-free extract (NFE), soluble sugars (SS), starch, ash, calcium (Ca), phosphorus (P), carotene, were carried out in the Laboratory of Nutrition and Forage Technology of the Scientific-Practical Institute of Biotechnology in Animal Husbandry and Veterinary Medicine, in accordance with the methodological indications. The gross energy (GE), metabolizable energy (ME), net energy for lactation (NEl) were calculated according to standard procedures:

GE=23.9xCP+39.8xEE+20.1xCF+17.5xNFE;

ME=14.07+0.0206xEE-0.0147xCF-0.0114xCP+4.5%;

NEl=9.10+0.0098xEE-0.0109xCF-0.0073xCP.

The carbon content of the substrates was determined from data on volatile solids (organic dry matter), using an empirical equation according to Badger et al. (1979). The biogas production potential and specific methane yields were evaluated by the parameter "content of fermentable organic matter", according to Weissbach (2008). The data were statistically processed using the MS Excel program.

RESULTS AND DISCUSSIONS

The economic importance of grasslands is determined by the diversity of plant species that make up the grass cover, which, correlated with the seasonal conditions and the management of the meadows, ensures both a high productivity and quality of fodder for animal husbandry.

The studied grasslands differ according to the phytosociological composition, the conditions of the site on which they are located and the way of exploitation, as well as the lack or presence of maintenance and care works. The main characteristics of the studied grasslands are presented in Table 1. By analysing the data, it is found that the *Festuca valesiaca* grasslands are located on slopes with predominantly northern exposure, with 4-15° inclination. They are used in mixed mode. *Poa pratensis* and *Elymus repens* grasslands are floodplain meadows, used as hayfield or pasture.

Table 1. The general characteristic of the studied grasslands

Year	Name of the local public authority	Name of the grassland area	Plot, subplot	Plot area ha	Relief type	Slope exposure	Inclination angle	Grassland type	Way of usage
2020	Ivancea	"Lângă Ivancea"	3B	3.62	slope	N	9 °	grassland with *Festuca valesiaca*	mixed (hayfield - pasture)
	Ivancea	"La Iaz"	6A	10.87	slope	N	6 °	grassland with *Festuca valesiaca*	mixed (hayfield - pasture)
	Puțintei	"Opt Martie"	8A	7.49	plain	-	-	grassland with *Elymus repens*	mixed (hayfield - pasture)
	Puțintei	"Hîrtoape Lagăr"	20A	29.71	slope	NW	10 °	grassland with *Festuca valesiaca*	mixed (hayfield - pasture)
2021	Ghetlova	"Fîntîna Popii"	1A	13.83	plain	-	-	grassland with *Elymus repens*	mixed (hayfield - pasture)
	Ghetlova	"Șes la Ghetlova"	13A	5.61	plain	-	-	grassland with *Elymus repens*	mixed (hayfield - pasture)
	Ghetlova	"Budăi Hulboaca"	17B	12.62	slope	NE	15 °	grassland with *Festuca valesiaca*	mixed (hayfield - pasture)
	Morozeni	"Breanova"	1E	18.20	slope	NE	8 °	grassland with *valesiaca*	mixed (hayfield - pasture)
	Morozeni	"Breanova"	1F	12.17	slope	N	4 °	grassland with *Festuca valesiaca*	mixed (hayfield - pasture)
2022	Ivancea	"Dereneuca"	2 A,B	17.24	plain	-	-	grassland with *Poa pratensis*	mixed (hayfield - pasture)
	Trebujeni	"La Potîrcă-1"	4A	11.22	plain	-	-	grassland with *Poa pratensis*	pasture
	Trebujeni	"La Peșteri"	13B	22.16	plain	-	-	grassland with *Poa pratensis*	hayfield
	NGBI	"Luncă cu firuță"	NGBI	1.00	plain	-	-	grassland with *Poa pratensis*	hayfield
	Codreanca	"Valea Rohului"	3C	18.30	slope	SE	8 °	grassland with *Festuca valesiaca*	mixed (hayfield - pasture)

Based on the harvests from the sample areas, after the drying of the cut grass and the subsequent weighing of obtained dry mass, the production of hay per hectare was calculated. The average production per hectare for each type of grassland was also calculated and the productivity category was established. The obtained results are presented in Table 2.

The highest production of hay was obtained on *Elymus repens* grasslands, on average about 6 t/ha, which places them in the category of grasslands with very high productivity.

Poa pratensis grasslands have productivity of 1.38-3.63 t/ha dry matter. The average production obtained from these grasslands is about 2.5 t/ha, which corresponds to the high productivity category. According to the obtained data, the highest productions are obtained from the grasslands used as hayfields (the grassland 13B - "La Peșteri" belonging to the town hall of Trebujeni; the grassland "Luncă cu firuță" of NBGI).

The *Festuca pratensis* grasslands, although located on slopes, but used rationally in a mixed regime, provide good hay production, between 1.59 and 3.77 t/ha hay. The average production for this type of grasslands, obtained during the study period, is 2.7 t/ha, which corresponds to the medium productivity category.

Table 2. Hay production under the conditions of the years 2020-2022, by type of grasslands (t/ha)

Year	Name of the local public authority	Name of the grassland area	Plot, subplot	*Elymus repens*	*Poa pratensis*	*Festuca valesiaca*
2020	Ivancea	"Lângă Ivancea"	3B			2.03
	Ivancea	"La Iaz"	6A			2.69
	Puțintei	"Opt Martie	8A	4.72		
	Puțintei	"Hîrtoape Lagăr"	20A			1.68
2021	Ghetlova	"Fîntîna Popii"	1A	9.31		
	Ghetlova	"es la Ghetlova"	13A	5.03		
	Ghetlova	"Budăi Hulboaca"	17B			3.75
	Morozeni	"Breanova"	1E			3.77
	Morozeni	"Breanova"	1F			3.64
2022	Ivancea	"Dereneuca"	2 A,B		2.24	
	Trebujeni	"La Potîrcă-1"	4A		1.38	
	Trebujeni	"La Peșteri"	13B		3.63	
	NBGI	"Luncă cu firuță"	NBGI		2.88	
	Codreanca	"Valea Rohului"	3C			1.59
Average production, t/ha hay				6.35	2.53	2.74
Productivity category				Very high	medium	medium

Several literature sources have described the productivity of permanent grasslands. According to Medvedev & Smetannikova (1981), the hay yield of grassland with *Elymus repens* varied from 3.2-12.5 t/ha, but grassland with *Poa pratensis* 2.5-3.0 t/ha. Samuil & Vintu (2012) mentioned that the dry matter production of permanent grassland with *Festuca valesiaca* was 3.3 t/ha, but in the variants with fertilization treatments with different rates and combinations of organic and mineral fertilizers – it was 4.2-5.1 t/ha. Vintu et al. (2017) revealed that the productivity of non-fertilized *Festuca valesiaca* grasslands was 2.30 t/ha, but manure applied at 10-40 t/ha contributed to an increase in productivity between 54 and 67% or 3.55-3.83 t/ha DM.

Forages are a major source of nutrients for herbivores. Sometimes the balance of nutrients or the presence of some constituents in the forage will have positive or negative effects on animal health and productivity. The biochemical composition and energy concentration of prepared hays from the studied grasslands are presented in Table 3. The hay collected from grasslands with *Elymus repens* contained 6.62-11.79% CP, 1.31-2.47% EE, 30.78-37.17% CF, 40.64-53.01% NFE, 4.31-6.82% sugars, 1.40-1.79% starch, 7.12-7.69% ash, 0.27-0.49% Ca, 0.16-0.21% P, 4.40-9.0 mg/kg carotene with energy concentration 17.94-18.22 MJ/kg GE, 8.05-9.73 MJ/kg ME and 4.39-5.50 MJ/kg NEl. The forage quality of hay from grasslands with *Festuca valesiaca* was 7.85-12.20% CP, 1.99-3.15% EE, 29.92-40.78% CF, 40.42-49.03% NFE, 2.41-5.37% sugars, 1.78-2.93% starch, 6.79-8.44% ash, 0.31-0.48% Ca, 0.13-0.20% P, 3.45-29.00 mg/kg carotene, 18.17-18.41 MJ/kg GE, 7.93-9.48 MJ/kg ME and 4.28-9.48 MJ/kg NEl. The hay from grasslands with *Poa pratensis* contained 10.19-13.43% CP, 2.53-3.43% EE, 26.25-32.02% CF, 43.27-47.77% NFE, 5.24-8.23% sugars, 1.79-2.83% starch, 7.74-11.29% ash, 0.34-0.73% Ca, 0.27-0.30% P, 29.50-41.12 mg/kg carotene, 17.78-18.44 MJ/kg GE, 9.11-9.55 MJ/kg ME and 5.16-5.4 MJ/kg NEl.

Some authors mentioned various findings about the forage quality. According to Nissinen & Hakkola (1995), the quality of the hay from *Poa pratensis* was characterized by 10.0-11.8% CP, 32.7-33.9% CF and 70.8% OMD, but from *Festuca pratensis* - 8.7-11.4% CP, 34.2-36.2% CF and 63.9% OMD. Yagi et al. (2001) revealed that the hay prepared from *Elytrigia repens* contained 241 g/kg CP, 528 g/kg NDF, 295 g/kg ADF, 2.4 g/kg Ca, 3.9 g/kg P, and *Poa pratensis* hay - 210 g/kg CP, 579 g/kg NDF 330 g/kg ADF, 3.1 g/kg Ca, 3.5g/kg P; *Dactylis glomerata* hay - 194 g/kg CP, 525 g/kg NDF, 293 g/kg ADF, 2.9 g/kg Ca, 3.2g/kg P; *Lolium perenne* hay -224 g/kg CP, 511 g/kg

NDF, 283 g/kg ADF, 5.3 g/kg Ca, 3.4 g/kg P and *Phalaris arundinacea* hay contained 212 g/kg CP, 549 g/kg NDF, 309 g/kg ADF, 2.9 g/kg Ca, 3.2g/kg P, respectively. Dürr et al. (2005) found that the chemical composition of fodder from Kentucky bluegrass cultivars was 24.8-40.6 g/kg N, 524-565 g/kg NDF, 268-290 g/kg ADF, 3.8-5.1 g/kg Ca, 3.1-3.7 g/kg P. Maheri-Sis et al. (2008) mentioned that the chemical composition and nutritive value of hay from quackgrass, *Agropyron repens* harvested at late maturity was 950 g/kg DM, 887 g/kg OM, 8.9% CP, 1.44% EE, 34.30% CF, 66.0% NFE, 8.96% NFC, 69.60% NDF, 38.30% ADF, 5.70% ADL, 11.2% ash, 43.54% ODM and 6.58 MJ/kg ME. Tambe & Rawat (2009) reported the *Festuca valesiaca* fodder is characterised by 11 % CP, 81 % NDF, 41% ADF, 57% DDM, RFV=65. Samuil & Vintu (2012) mentioned that the dry matter collected from unfertilized permanent grassland with *Festuca valesiaca* contained 90.1 g/kg CP and 242.2 g/kg CF, but in the variants with fertilization treatments, 90.6-103.4 g/kg CP and 237.8-241.2 g/kg CF were obtained, respectively. Jankowska-Huflejt (2014) remarked that the hay from organic grasslands contained 10.75% CP, 2.98% EE, 30.59% CF, 7.31% ash, 0.64% Ca, 0.25% P. Vintu et al. (2017) found that the chemical composition and the nutritive value of forage from non-fertilized *Festuca valesiaca* grasslands was 7.8% CP, 75.2% NDF, 46.8% ADF, RFV=65, but in manure applied variants respectively 9.2-10.4% CP, 56.8-60.8% NDF, 41.9-42.4% ADF, RFV=87-93. Boob et al. (2019) remarked that the nutritive value of lowland hay meadows were 6.80-13.50% CP, 4.46-5.81 MJ/kg NEl. Nazare et al. (2019) reported the quality of the forage obtained from a *Festuca valesiaca* grassland, harvested at the ear formation stage, was 8.17% CP, 56.77% NDF, 34.84% ADF, RFQ=109.20, but in the variants with the application of fertilizers the respective values were obtained 8.86-12.36 % CP, 48.92-57.77% NDF, 32.29-35.29% ADF, RFQ=107.67-126.38. Çaçan (2022) revealed that quality characteristics of *Poa pratensis* were 11.07-13.50% CP, 5.77% ash, 1.40% EE, 51.5-59.66% NDF 26.7-32.3% ADF, 0.60-4.47% ADL, 63.5-65.65% DDM, RFV=102.2-123

Table 3. Nutrients and energy concentration in prepared hays from studied grasslands

Plot, subplot	Nutrients										Energy concentration		
	CP, %	EE, %	CF, %	NFE, %	sugars %	starch %	ash, %	Ca, %	P, %	carotene, mg/kg	GE, MJ/kg	ME, MJ/kg	NEl, MJ/kg
Elymus repens grasslands													
8A	6.62	2.47	30.78	53.01	-	-	7.12	-	-	4.40	18.01	9.73	5.50
1A	8.36	1.31	36.87	45.77	6.82	1.79	7.69	0.27	0.16	9.00	17.94	8.33	4.61
13A	11.79	2.07	37.17	40.64	4.31	1.40	7.60	0.49	0.21	6.50	18.22	8.05	4.39
Festuca valesiaca grasslands													
3B	10.35	3.15	30.42	47.64	-	-	8.44	-	-	13.00	18.17	9.48	5.33
6A	9.58	2.60	31.27	49.03	-	-	7.52	-	-	10.83	18.19	9.32	5.24
20A	12.20	2.98	29.92	47.38	-	-	7.52	-	-	24.64	18.41	9.29	5.24
17B	9.31	2.29	36.35	45.05	3.80	1.92	7.00	0.45	0.20	3.45	18.38	8.09	4.67
1E	9.16	2.17	40.03	40.42	2.41	1.78	8.22	0.48	0.13	28.8	18.18	7.94	4.28
1F	7.85	1.99	40.78	41.76	2.54	2.83	7.62	0.47	0.15	19.3	18.18	7.93	4.28
3C	7.94	2.34	35.70	47.23	5.37	2.17	6.79	0.31	0.17	29.00	18.27	8.77	4.86
Poa pratensis grasslands													
2AB	11.00	3.43	31.52	46.31	8.23	2.83	7.74	0.34	0.27	-	18.44	9.30	5.20
4A	13.90	2.79	26.65	47.77	6.32	1.94	8.89	0.46	0.30	41.12	18.15	9.55	5.46
13B	10.19	2.53	32.02	46.30	6.65	1.97	8.96	0.48	0.27	29.50	17.99	9.11	5.16
NBGI	13.43	2.86	29.15	43.27	5.24	1.79	11.29	0.73	0.30	34.12	17.78	9.24	5.21

Increasing biomass usage leads to the reduction of greenhouse gases emissions compared to the use of fossil fuels. After harvesting often such biomass is stored for long periods for composting due to the lack of alternative use strategy, excess biomass must be used for energy renewable production. In recent years considerations on grassland use for bioenergy have increased considerably, can be used as biomass feedstock for production solid fuel, lignocellulosic bioethanol, synthetic natural gas or synthetic biofuels, and in particular for biogas production. Grass is being considered as a potential feedstock for biogas production, due to its low water consumption compared to other crops, and the fact that it can be cultivated in non-arable lands, avoiding the direct competition with food crops. The grasses need to be collected and processed to the required condition, e.g. grass silage, hay, hay pellets, for use for energy production purposes (Prochnow et al. 2009; Rösch et al. 2009; Meyer et al. 2014; Dubrovskis et al. 2018).

Organic dry matter or volatile solid yield is an important factor influencing biogas and methane yield. Differences in gas formation potentials of crops are mainly due to specific chemical compositions of the plant material. Crude protein is the main nitrogen-containing nutrition component for microbes converting biomass. It is a commonly known fact that methanogenic bacteria need a suitable ratio of carbon to nitrogen for their metabolic processes, ratios higher than 30:1 were found to be unsuitable for optimal digestion, and ratios lower than 10:1 were found to be inhibitory, because of low pH, poor buffering capacity and high concentrations of ammonia in the substrate. The nitrogen content in the studied hay substrates ranged from 10.59 to 22.24 g/kg, the estimated content of carbon - from 492.9 to 517.8 g/kg, the C/N ratio varied from 23 to 29. It is well known that fat is a good source of energy. Carbohydrates supply most of the energy for maintaining vitality. The two carbohydrate fractions commonly used in evaluating the carbohydrate content of substrates are crude cellulose and nitrogen-free extract. The capability of biomass methanization is tightly associated with nutrient digestibility and plant species. When crude cellulose content increases, digestibility usually decreases. Nitrogen-free extract contains the most digestible portion of the carbohydrates. Fermentable organic matter represents the proportion of organic matter which can be biologically degraded under anaerobic conditions and, thus, can be potentially utilized in biogas facilities. The fermentable organic matter concentration in the tested hay substrates ranged from 500 to 747 g/kg, the gas forming potential of the fermentable organic matter varied from 400 to 598 l/kg VS, and specific methane yield - from 210 to 314 l/kg VS (Table 4). The best results were achieved in hay substrates collected from grassland with *Poa pratensis* with specific methane yield of 282-314 l/kg VS, likely low content of crude fiber. The methane yield per ha for grassland with *Elymus repens* ranged from 1217 to 2273 m^3/ha, and from grassland with *Poa pratensis* - 433 to 998 m^3/ha, while from grassland with *Festuca valesiaca* - 409 to 941 m^3/ha, respectively.

According to Mähnert et al. (2002) methane yields from grasses with intensive growth were 310-360 l/kg VS. Kaparaju (2003) found that the specific methane yield of grass hay substrates varied from 270 to 350 l/kg VS. Mähnert et al. (2005) compared the quality of biomass from seven types of grass, the results for biogas yield were: perennial ryegrass 904-929 l/kg VS, cocksfoot 718-800 l/kg VS, tall fescue 818-836 l/kg VS, red fescue 767-845 l/kg VS, meadow fescue 846-909l/kg VS, meadow foxtail 804 l/kg VS and timothy grass 591- 828 l/kg VS. Amon et al. (2007) mentioned that the specific methane yields of grassland from the mountain and from the valley region showed significant differences. Irrespectively of the number of cuts, only a low specific methane yield (128-221 l/kg VS) was measured from the biomass coming from the hill site, but the grass grown at the valley site produced 190-392 l/kg VS methane; the highest methane yield with one cut was reached with the late first cut in the three-cuts system namely 1872 m^3/ha; the average methane yield of the hill site was 910 m^3/ha. Seppälä et al. (2009) remarked that specific methane yields of grass substrates varied from 253 to 394 l/kg VS, and the methane and energy yields from different harvest years ranged from 1200 to 3600 m^3/ha/year.

Meyer et al. (2014) revealed that methane yield of roadside grass was 220-390 l/kg VS. Țîței (2016) reported that the methane yield of native perennial grasses (*Dactylis glomerata*, *Festuca arundinacea)* ranged from 2077 to 2243 m^3/ha, and from introduced *Agropyron* species - 1386-1605 m^3/ha, *Miscanthus* species 3348-4128 m^3/ha. Mattioli et al. (2017) reported that the biogas production from grass collected from public parks was 600-650 m^3/t. Dubrovskis et al. (2018) found that the grass hay pellet substrate achieved 666 l/kg VS biogas or 355 l/kg VS methane. Boob et al. (2019) mentioned that the methane yield of the substrate from lowland hay meadows was 259-320 l/kg VS.

Table 4. The methane production potential of hay substrates from studied grasslands

Plot, subplot	Nitrogen, g/kg	Carbon, g/kg	C/N	Fermentable organic matter, g/kg	Biogas potential, l/kg	Specific methane yield, l/kg	Methane production potential, m^3/ha
Elymus repens grasslands							
8A	10.59	516.0	49	672	538	282	1331
1A	13.38	512.8	38	580	464	244	2272
13A	18.86	513.3	27	575	460	242	1217
Festuca valesiaca grasslands							
3B	16.56	508.7	31	687	549	288	585
6A	15.33	513.8	34	682	546	286	769
20A	19.52	513.8	26	703	562	295	496
17B	14.90	516.7	35	598	478	251	941
1E	14.66	509.9	35	511	409	215	811
1F	12.56	513.2	41	500	400	210	765
3C	12.70	517.8	41	612	490	257	409
Poa pratensis grasslands							
2AB	17.60	512.6	29	676	540	284	636
4A	22.24	506.2	23	747	598	314	433
13B	16.30	505.8	31	654	524	275	998
GBNI	21.49	492.8	23	678	543	282	812

CONCLUSIONS

The productivity of grassland with *Elymus repens* were 4.72-9.31 t/ha dry matter, the grassland with *Poa pratensis* 1.38-3.63 t/ha dry matter and the grassland with *Festuca valesiaca* 1.59-3.77 t/ha dry matter.

The biochemical composition of hay dry matter was: 6.62-13.90% crude protein, 1.31-3.43% crude fat, 26.65-40.78% crude cellulose, 43.27-53.02% nitrogen free extract, 2.41-6.82% sugars, 1.40-2.83% starch, 6.79-11.29% ash, 0.27-0.73% Ca, 0.15-0.30% P, 4.40-41.12 mg/kg carotene with energy concentration 17.78-18.44 MJ/kg GE, 7.93-9.73 MJ/kg ME and 4.28-5.50 MJ/kg NEl. The gas forming potential of the fermentable organic matter of the hay collected from the studied grassland varied from 400 to 598 l/kg VS, and specific methane yield varied from 210 to 314 l/kg VS.

The hay collected from the studied grasslands can be used as fodder for livestock, also as energy biomass for biomethane production.

ACKNOWLEDGEMENTS

The study has been carried out in the framework of the projects: 20.80009.7007.01 and 20.80009.5107.02

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

AN OVERVIEW OF 50 YEARS OF STUDIES ON THE WET ZONES CORMOFLORA IN THE ROMANIAN BANAT

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Abstract

The paper contains our own data and observations issued from the scientifical papers, published between 1970 and 2020. Although, at a first approach, the flora of wet zones in the Romanian Banat region does not seem spectacular, our analysis shows the presence of several hundreds of species. In the past 50 years, many authors have studied these wet zones habitats bringing important contributions (floristic inventories, the study of rare or invasive species, general observations on the flora). Compared to the first part of the reference period, many species, considered common in the past, have become less frequent, some of them with uncertain spontaneous presence or extinct from the Romanian Banat: Hippuris vulgaris, Ludwigia palustris, Stratiotes aloides, Hottonia palustris, Lysimachia punctata, Potamogeton lucens, Zannichellia palustris, Marsilea quadrifolia. Significant changes are also noticed in cormoflora structure in terms of life forms and geo-elements. Reducing and degrading habitats and direct human pressures, the presence of invasive species (Amorpha fruticosa, Echinocystis lobata, Reynoutria japonica, Helianthus tuberosus, Asclepias syriaca, Lindernia dubia) are serious threats to wet zones cormoflora, including that of the protected areas.

Key words: *Romanian Banat, wet zones, cormoflora, historical changes, invasive species.*

INTRODUCTION

Having varied geographical conditions, the historical province of Romanian Banat (its limits presented by Gaudenyi & Milošević, 2023) harbors a relatively high plant diversity (our preliminary results show that approx. 40-45% of plant species from Romania are present in the Banat, given that the area of the Romanian Banat represents less than 1/10 of Romania's area), being studied by many botanists. Among the botanists who undertook studies and collected valuable data for the flora of this region, before 1900, we can mention (according to Coste et al., 1995): Rochel - 1823, Heuffel - 1858, Borbás - 1884. In the 20th century, Banat was the subject of numerous botanical studies which had as an object of study plants from aquatic and marshy habitats (according to Coste et al., 2002): Tökes - 1905, ZSÀK, Z. - 1916, Buia - 1942, Boşcaiu - 1942, 1944, 1965, 1966, 1971 etc., Soran - 1954, 1956, Pop - 1956, 1962, 1968, 1977, Bujorean - 1957, Bujorean et al. - 1959, 1961, 1962 etc., Gergely - 1964, Nyarady - 1966, Turenschi - 1966, Csürös et al. - 1968, Vicol - 1974, Grigore & Coste, 1976, Arvat - 1977, Oprea et al., 1974, Schrött & Sinitean, 1999, and many others. From these researches resulted valuable works such as doctoral theses, monographic books and scientific articles.

Even if the cormoflora of humid zones is not very rich compared to the flora of other types of habitats, it preserves important species. We only recall the research of Karácsonyi & Negrean (2010), in a humid microdepression in western Romania, where they reported a series of remarkable species such as: *Polygonum bistorta, Iris sibirica, Veratrum album, Crocus vernus, Narcissus poeticus* subsp. *radiiflorus, Lindernia procumbens*.

Historically, it can be noted the change of wet zones cormoflora, especially since the global extent of wetlands is estimated to have declined with 70% during the 20th century (Davidson, 2016). Species losses are also reported in the cormoflora of wetlands in our country (Neacşu, 2008). Some of the wet zones resources are

being conserved in the 20 designated Romanian Ramsar sites (https://www.ramsar.org/wetland/romania), two of which are partially located on the territory of the Romanian Banat, Iron Gates Natural Park and Mures Floodplain Natural Park.
Based on CLC (Corine Land Cover) data, Ianăș & Ișfănescu-Ivan (2022) shows that in the hill and plain areas of Banat, the dynamics of land use and land cover occur under drivers such as agriculture, urbanization, industrialization.

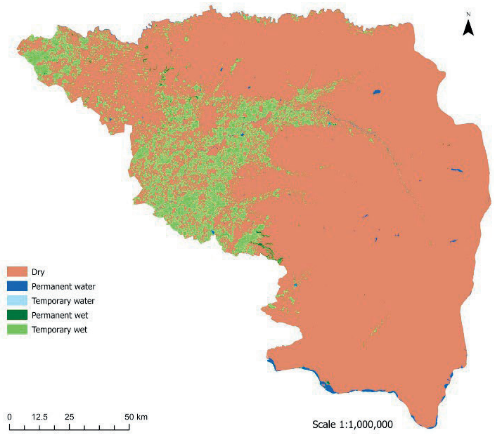

Figure 1. The wet zones distribution

The reduction of the wet zones areas in Banat (Figure 1) is expressed by the reduction of the habitats of the populations of paludicolous and aquatic species. The most recent (2022) *Romania's national communication under the United Nations Framework Convention on Climate Change* (p. 32) shows that much of the lowlands of Banat are at risk of drought. At the same time, Banat is the province where the hydro-ameliorative works (drainage) started the earliest (18th century) and continued intensively until the last years of the communist period; one of the main effects of drainages is the lowering of the underground water level in the lowland region (Nemeș & Constantinescu, 2012). The ecological effects of these works are treated among others by Buhociu (2001), Coste (2002), Coste & Oncia (2003), Coste et al. (1997), Ionescu (2001).

MATERIALS AND METHODS

In creating the general picture of the results of botanical research that had as its object the cormophyte flora of wetlands, we brought together our own results (or the results of research projects in which we participated: Arsene et al., 2002; 2005; 2015; Biro et al., 2021; Cucu et al., 2019; Neacșu, 2008; Neacșu et al., 2008; Neacșu & Arsene, 2017a; 2017b; Neacșu et al., 2018; Turcuș & Neacșu, 2013; Otveș et al., 2014; Stănescu et al., 2005) with those contained in the main bibliographic sources. The selection of these latter bibliographic references is made giving priority to monographic research (doctoral theses, treatises, syntheses published in book form) and scientific articles. In some cases, for the same author, if he has published as articles parts of his doctoral thesis, we have chosen to cite only this.
First of all, the classic theses of flora and vegetation should be mentioned here: Arvat, 1977; Coste, 1974; Grigore, 1971; Hoborka, 1974; Lovasz, 1995; Oprea, 1976; Peia, 1978; Pop, 1977; Schrött, 1972; Vicol, 1974 etc., or the excellent syntheses published by Drăgulescu (1995, 2013). We have considered here primarily species of conservation importance, as well as invasive ones, even if not all of them are aquatic or paludicolous species, but which are found within an area considered as a whole as a wetland or (more or less) permanent humid zones.

RESULTS AND DISCUSSIONS

From our own data and from the literature consulted, we estimate that the flora of the wet zones of Banat number about 400 species, almost half of the species being aquatic and paludicolous species. Most are species characteristic for these habitats, others arrived accidentally or were introduced as a result of human activities.
It should be noted that recently, compared to references from the 1970's or even earlier, the wet zones have significantly reduced their surfaces, due to the drainage works carried out, with the aim of introducing certain areas into the agricultural circuit.
That is why, using historical Austro-Hungarian maps, general maps and satellite images, in 2018, we carried out a comparative analysis of some wet zones in Timiș County (Figure 2) and we found that the natural wet surfaces have

regressed considerably, being replaced by arable land. We noticed the deep changes and alteration in the flora and also the decresed of aquatic macrophytes populations (Neacșu et al., 2018). Artificial wet zones are more numerous now (lakes, marshes, ponds), while only few natural areas have survived and their status is not adequate (Satchinez Swamps).

Figure 2. Lake Sânandrei (Timiș) after drying up (2015)

Although the surfaces and the type of habitat are different, we can have an image of some specific biodiversity losses, numerically considered. For example, the aquatic and paludicolous species identified in the Timiș-Bega interfluve (approx. 280 - Grigore, 1971), in the Lugoj piedmont (approx 150 - Vicol, 1974), in the perimeter of the Timiș, Pogoniș and Bârzava rivers (approx. 160 - Arvat, 1977), in the main reservoirs in Timiș (approx. 100 - Neacșu, 2008), or in the Timiș river basin (approx. 80 - Drăgulescu, 2013).

Comparing the list of aquatic and paludicolous flora near Timișoara, with the species present in the perimeter of the Satchinez Swamps (Figure 3), Arsene et al., 2005 found that a third of them are missing, compared to the initial list made by Tökes (1905).

Grigore (1971) mentions in the Timiș-Bega interfluve, a group of well-represented hydrophilic and hygrophilic species: *Marsilea quadrifolia, Salvinia natans, Polygonum amphibium, Ranunculus aquatilis, R. sceleratus, Rorippa amphibia, Trapa natans, Myriophyllum spicatum, M. verticillatum, Oenanthe aquatica, Sagittaria sagittifolia, Butomus umbellatus, Hydrocharis morsus-ranae, Alisma plantago-aquatica, A. lanceolatum, Juncus inflexus, J. conglomeratus, Potamogeton natans, P. crispus, Typha angustifolia, T. latifolia, Lemna minor, L. trisulca, Glyceria maxima, G. fluitans, Schoenoplectus lacustris, Heleocharis palustris, Carex* sp.

Vicol (1974) signals the remarkable presence of hydatohelophytes in the Lugoj piedmont and justifies it in close correlation with local conditions for the development and maintenance of wet zones. Among these species are: *Alisma plantago-aquatica, Lemna minor, L. trisulca, Potamogeton crispus, P. natans, Hydrocharis morsus-ranae, Trapa natans, Salvinia natans, Marsilea quadrifolia, Polygonum amphibium, Ranunculus aquatilis, Nuphar luteum*, *Typha latifolia, T. angustifolia, Heleocharis palustris, Oenanthe aquatica, O. banatica, Glyceria maxima, Elatine hexandra* (today this species is critically endangered and is included by Dihoru & Gavril, 2009 in *Red book of vascular plants of Romania*).

Arvat (1977) notes the luxuriant presence of the following species, on the edges of rivers, canals, marshes and ponds (in the space between the Timiș, Pogniș and Bârzava rivers): *Alisma plantago-aquatica, Ranunculus aquatilis, R. sceleratus, Butomus umbellatus, Ceratophyllum submersum, Glyceria fluitans, Heleocharis palustris, Hydrocharis morsus-ranae, Lemna minor, L. trisulca, Potamogeton natans, P. crispus, Oenanthe aquatica, Myriophyllum spicatum, M. verticillatum, Schoenoplectus lacustris, Typha angustifolia, T. latifolia, Phragmites australis, Polygonum amphibium, Juncus inflexus, J. conglomeratus, Carex sp*. Neacșu (2008) notes the common species such as: *Salix alba, Salix cinerea, Ranunculus repens, Polygonum amphibium, Lythrum salicaria, Typha angustifolia, Carex riparia, Bidens tripartita, Mentha aquatica* and less common species such as: *Lindernia procumbens, Peplis portula, Eleocharis acicularis, Leersia oryzoides, Najas minor, Oenanthe banatica*.

As can be seen, these lists are quite similar and most of these species are still found in wet zones flora today, but their populations are less represented. We only mention the current status of the species *Marsilea quadrifolia* - Near Threatened (IUCN), which in the past grew abundantly in this area (Grigore, 1971, Vicol, 1974). Among the species of aquatic/humid habitats listed in the annexes of the Habitats Directive, which were listed in Banat, we mention: *Marsilea quadrifolia, Cirsium brachycephalum, Eleocharis carniolica, Aldrovanda vesiculosa, Gladiolus palustris, Angelica palustris* (Annex IIb of the Habitat Directive), *Lindernia procumbens* (Annex IVb of the Habitat Directive).

From his study, Drăgulescu (2013) notes that among the 285 identified species in the Timiş River drainage basin, more than twenty are either rare or protected: *Thelypteris palustris, Marsilea quadrifolia, Nuphar lutea, Myosurus minimus, Ranunculus lateriflorus, Ranunculus lingua, Ranunculus ophioglossifolius, Montia minor, Rumex x gayeri, Ludwigia palustris, Apium nodiflorum, Peucedanum rochelianum, Elatine hexandra, Tozzia alpina* ssp. *carpatica, Cirsium brachycephalum, Taraxacum bessarabicum, Stratiotes aloides, Fritillaria meleagris, Gladiolus imbricatus, Narcissus poëticus* ssp. *radiiflorus, Wolffia arrhiza.* Most of these were taken from the bibliography, because they were no longer found in the field.

Figure 3. A population of *Ranunculus aquatilis* in a flooded area at Satchinez

Figure 4. The shore of the Lake Liebling (2006)

The life forms and geoelements structure has changed in recent decades due to the changes occurring within habitats, which has led to the expansion of some categories like therophytes, hemicryptophytes, mesophytes etc., in the disadvantage of hydrophytes and hygrophytes. For example, analysing the floristic structure of the reed communities (Figure 4), we noticed that compared to those described by Grigore (1971), in which helohydatophytes predominated, in those studied by us, therophytes and hemicryptophytes have a greater distribution and weight (Neacșu et al., 2008).

Figure 5. Meadow being invaded by *Amorpha fruticosa* (dark green) on the Mureș river, near Lipova

It should also be mentioned the species encountered sporadically nowadays or even with uncertain presence such as: *Hippuris vulgaris, Ludwigia palustris, Stratiotes aloides, Hottonia palustris, Lysimachia punctata, Potamogeton lucens, Zannichellia palustris* etc.

Otves et al. (2014) presents a list of 82 adventive species in Banat, some of them raising problems and disrupting the stability of wetland habitats: *Amorpha fruticosa* (Figure 5), *Echinocystis lobata, Reynoutria japonica, Helianthus tuberosus, Asclepias syriaca, Lindernia dubia* (Neacșu et al., 2021).

CONCLUSIONS

The decrease of wetland areas, anthropic pressure, climate changes, are factors that negatively influence the diversity of the cormoflora of the Banat areas.

We believe that against the background of these changes, ecological restoration actions of the wetlands in Banat are required.

Most of the species of aquatic and paludicolous plants in the Romanian Banat are also found in the Serbian Banat (Ljevnaić-Mašić, 2013; Ljevnaić & Mašić, 2016; Anđelković, 2020, etc.)

We have not found recent reports of species of the genus *Elatine* in the Romanian Banat, although their presence is certain in the Serbian Banat (e.g. Perić et al., 2016; Jenačković Gocić et al., 2020).

Wetland management must include viable strategies and concrete conservation measures to reduce biodiversity loss.

ACKNOWLEDGEMENTS

This paper is published from the own research funds of the University of Life Sciences "King Mihai I" from Timisoara.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE ALLUVIAL FOREST VEGETATION DISTURBED BY THE INVASIVE ALIEN PLANTS, IN THE DANUBE VALLEY, BETWEEN CETATE AND CALAFAT

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Abstract

Invasive species have a negative impact on forest, grassland, and agricultural ecosystems around the world, sometimes associating and forming segetal or ruderal plant communities. Exotic species compete with native species and threaten ecosystem stability. The paper presents the results of the investigations carried out in the alluvial forest vegetation in the Danube Valley from Oltenia, between Cetate and Calafat. In this region, the intensive abiotic activity, but not only that, has brought about the invasion of allochtone (invasive alien) species plants in the natural and semi-natural degraded ecosystems, especially in alluvial forest habitats. The species has been found in the following types of natural habitats: 91E0 - Alluvial forests with Alnus glutinosa and Fraxinus excelsior (Alno-Padion, Alnion incanae, Salicion albae); 92A0 - Salix alba and Populus alba galleries; 91F0 - Riparian mixed forests of Quercus robur, Ulmus laevis and Ulmus minor, Fraxinus excelsior or Fraxinus angustifolia along the great rivers (Ulmenion minoris); 91I0* - Euro-Siberian steppic woods with Quercus spp.*

***Key words**: invasive species, habitats, Oltenia, biodiversity, alluvial vegetation.*

INTRODUCTION

Invasive species have a negative impact on forest, grassland, and agricultural ecosystems around the world, sometimes associating and forming segetal or ruderal plant communities. Exotic species compete with native species and threaten ecosystem stability.

The studies on alien species are of particular interest today to protect natural habitats and reduce or eliminate ecological and economic damage. Invasive species are one of the most serious threats to biodiversity (Niculescu et al., 2017). The territory under study, located in the Danube Valley between the towns of Cetate and Calafat, is characterized by the existence of a high and permanent anthropogenic impact due to intense river transport, agricultural activities in the area, household activities and not least those carried out for tourist and recreational purposes. Invasive plant species and more have taken over more and more extensive territories in this territory. Also, the floods of recent years have influenced their spread in all types of habitat.

The most affected habitats in the Danube Valley are forest and aquatic habitats, swamps and tall grasses. In order to stop the spread of invasive plant species, it is first necessary to carry out inventory and monitoring actions in order to be able to find and then apply the most correct and sustainable measures to combat and at the same time restore the favorable conservation status of the affected habitats.

MATERIALS AND METHODS

Study area

The paper presents the results of the investigations carried out in the alluvial forest vegetation in the Danube Valley from Oltenia, between Cetate and Calafat (Figure 1).

Most of this territory is located within the four protected natural areas in southern Romania: ROSAC0045 Coridorul Jiului and ROSAC0039 Ciuperceni-Desa și ROSPA 0013 Calafat-Ciuperceni-Dunăre, ROSPA0023 Jiu-Danube Confluence.

Methods

The conspectus of the invasive alien plants in the study area from Danube Valley has been elaborated on the basis of personal researches undertaken since april-september in the period 2019-2022, as well as the little bibliographical information regarding this field.

In order to identify the species and the inter-taxa, we looked into: Romanian Flora, vols. I-XII (1952-1976) and Flora Europaea, vols. I-V (Tutin et al., 1964-1980; 1993).
Also to identify the invasive alien plants in the area study protected area we used the: Plante adventive in flora României (Sîrbu & Oprea, 2011).

Figure 1. Map of study area
(Source: https://earth.google.com/)

For the classification of the vegetal associations, we have used synthesis papers by Rodwell et al. (2002).

RESULTS AND DISCUSSIONS

Regarding the the invasive alien plants we found in the study area from the Danube Valley a number of 49 species.
These species are found in the floristic composition of the following types of natural habitats:
- 91E0* - Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-padion, Alnion incanae, Salicion albae*); CLAS. PAL.: 44.3, 44.2 and 44.13 (Gafta & Mountford - coord., 2008);
- 92A0 - *Salix alba* and *Populus alba* galleries, CLAS. PAL.: 44.141, 44.162 şi 44.6 (Gafta & Mountford - coord., 2008);
- 91F0 - Riparian mixed forests of *Quercus robur, Ulmus laevis* and *Ulmus minor, Fraxinus excelsior* or *Fraxinus angustifolia* along the great rivers (*Ulmenion minoris*), CLAS. PAL.: 44.4 (Gafta & Mountford - coord., 2008).

At the level of these habitats this invasive alien plants represents pressure and threats for the entire biodiversity.
Most of invasive species are highly aggressive and damaging ecosystems and is characterized by great dominance-abundance within some phytocoenotic (Niculescu et al., 2010; 2011).
The invasive alien plants identified in this area, after their introduction way, belong to two distinct categories: hemerophytes (deliberately introduced plants) and xenophytes (accidentally introduced plants) (Sîrbu, 2004; Sârbu & Oprea, 2011; Niculescu et al., 2012; 2016; Niculescu & Cismaru, 2013).
In Table 1 are presented the invasive alien plants identified in the alluvial forest vegetation, in the Danube Valley, between Cetate and Calafat.

Aspects regarding the vegetation edified by invasive alien plants in the in the studied territory

In phyto-sociological terms, the invasive alien plants can be one of the representative species, characteristic, dominant or accompanying to more plant communities:
1. *Stellario nemori-Alnetum glutinosae* (Lohmeyer,1957);
2. *Salicetum fragilis* (Passarge, 1957);
3. *Salicetum albae* (Issler, 1924);
4. *Quercetum roboris-pedunculiflorae* (Simon, 1960) (Syn.: *Fraxino angustifoliae-Quercetu pedunculiflorae* (Chifu et al., 1998; 2006);
5. *Fraxino pallisae-Quercetum pedunculiflorae* (Popescu et al., 1979; Oprea, 1997);
6. *Salici-Populetum* (Meijer-Drees, 1936; Rodwell et al., 2002).

The most aggressive species from an invasive point of view, which are represented by populations with many individuals, with a high spreading power, very prolific, with individuals that have a high reproductive power, very vigorous and with increased viability are: *Amorpha fruticosa, Ailanthus altissima, Ambrosia artemisiifolia, Phytolacca americana, Conyza canadensis, Aster lanceolatus, Reynoutria x bohemica, Echinocystis lobata, Helianthus* tuberosus

(Figure 2), *Erigeron annuus, Acer negundo, Sicyos angulatus* (Figure 3), *Lycium barbarum*, *Oenothera glazioviana.*
Invasive plant species were introduced intentionally (hemerophyte) or accidentally (xenophyte).


Figure 2. *Helianthus tuberosus* in the Danube Valley (foto: Mariana Niculescu)


Figure 3. *Sicyos angulatus* in the Danube Valley (foto: Mariana Niculescu)

Amorpha fruticosa (Figure 4) most often forms continuous strips of considerable width and very difficult to cross in meadow forest habitats, both at the edge and inside them.
Ambrosia artemisiifolia (Figure 5) is also a species with an extremely high invasiveness.
It is a phytosanitary quarantine species, very aggressive. The species is distributed both in grassy habitats and in forest and scrub habitats, changing the floristic composition of the plant communities in which it is found.
This species has in recent years conquered vast territories in the Danube meadow from the disputed territory and beyond, being a species that is very difficult to combat, continuously expanding and with negative effects not only in horticulture and agriculture, but also in terms of the condition of population health, since the number of people with allergies produced by this species is constantly increasing at the national and European level.

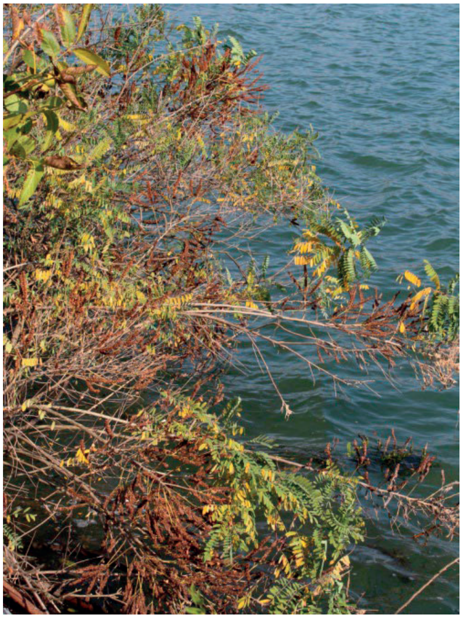
Figure 4. *Amorpha fruticosa* in the Danube Valley (foto: Mariana Niculescu)


Figure 5. *Ambrosia artemisiifolia* in the Danube Valley (foto: Mariana Niculescu)

Ailanthus altissima (Figure 6) has spread a lot in recent years in all habitats in the southwestern part of the country, being very difficult to combat due to the high content of seeds produced by a single plant and their very high germination powers as well as their very high adaptability to the climate changes of recent years.

Figure 6. *Ailanthus altissima* in the Danube Valley (foto: Mariana Niculescu)

Ailanthus altissima has spread a lot in recent years in all habitats in the southwestern part of the country, being very difficult to combat due to the high content of seeds produced by a single plant and their very high germination powers as well as their very high adaptability to the climate changes of recent years.

Table 1. Invasive plant species identified in forest habitats in the studied territory

Taxon	**Family**	**Introduction type**	**Natura 2000 Habitats**
Acer negundo L.	Aceraceae	hemerophyte	91E0*, 91F0
Alcea rosea L.	Malvaceae	hemerophyte	91E0*
Ailanthus altissima (Miller) Swingle	Simaroubaceae	hemerophyte	91E0*, 91F0, 92A0
Ambrosia artemisiifolia L.	Asteraceae	xenophyte	91E0*, 91F0, 92A0
Amorpha fruticosa L.	Fabaceae	hemerophyte	91E0*, 91F0, 92A0
Armoracia rusticana P. Gaertner, B. Meyer et Scherb	Brassicaceae	hemerophyte	92A0
Artemisia annua L.	Asteracea	xenophyte	Woodland light, forest edges, 91E0*, 91F0
Aster lanceolatus Willd.	Asteraceae	xenophyte	Woodland light, forest edges, 91E0*, 91F0
Bidens frondosa L.	Asteraceae	xenophyte	Woodland light, forest edges, 91E0*, 91F0, 92A0
Chenopodium ambosioides L	Chenopodiaceae	xenophyte	Woodland light, forest edges, 91E0*
Conyza canadensis (L.) Cronq. (L.) Cronquist	Asteraceae	xenophyte	91E0*, 91F0, 92A0
C. sumatremsis Retz.	Asteraceae	xenophyte	Woodland light, forest edges, 91E0*, 91F0, 92A0
Cosmos bipinnatus Cav.	Asteraceae	hemerophyte	Woodland light, forest edges, 92A0
Cyperus diformis L.	Cyperaceae	xenophyte	Woodland light, forest edges, canals and ponds in forests, 91E0*, 92A0
Datura wrightii Regel	Solanaceae	hemerophyte	Forest edges, 92A0
Echinocloa colona (L.) Link.	Poaceae	xenophyte	Forest edges, 92A0
Echinocystis lobata (Michx.) Torr. & A.Gray	Cucurbitaceae	hemerophyte	91E0*, 91F0, 92A0
Eclipta prostrata (L.) L. (*E. alba* L.) Hassk.)	Asteraceae	xenophyte	Forest edges, 92A0
Eigeron annuus (L.) Pers.	Asteraceae	xenophyte	91E0*, 91F0, 92A0
Fallopia dumetorum	Polygonaceae	hemerophyte	91E0*, 91F0, 92A0
Fraxinus pensylvanica Marshall (*F. pensylvanica* var. *lanceolata* (Borkh.) Sarg.; *F. lanceolata* Borkh.; *F. pubescens* Lam.)	Oleaceae	hemerophyte	91E0*, 91F0, 92A0
Galinsoga parviflora Cav.	Asteraceae	xenophyte	Woodland light, forest edges, canals and ponds in forests, 91E0*, 92A0
Galinsoga quadriradiata Ruiz et Pav.	Asteraceae	xenophyte	Woodland light, forest edges, canals and ponds in forests, 91E0*, 92A0
Helianthus tuberosus L.	Asteraceae	hemerophyte	Woodland light, forest edges, canals and ponds in forests, 91E0*, 92A0*
Ipomea purpurea Roth.	Convolvulaceae	hemerophyte	Forest edges, 92A0
Iva xanthifolia Rott.	Asteraceae	xenophyte	Forest edges, 92A0
Juncus tenuis Willd	Juncaceae	xenophyte	Woodland light, forest edges, canals and ponds in forests, 91E0*, 92A0*
Kochia scoparia (L.) Shrad. (*Bassia scoparia* (L.) A. J. Scott., *Chenopodium scoparia* (L.)	Chenopodiaceae	xenophyte	Forest edges, 92A0
Lycium barbarum L.	Solanaceae	hemerophyte	91E0*, 91F0, 92A0
Mentha spicata L. (*Mentha viridis* L.)	Lamiaceae	xenophyte	91E0*, 91F0, 92A0

Taxon	Family	Introduction type	Natura 2000 Habitats
Morus alba L.	Moraceae	hemerophyte	91E0*, 91F0, 92A0
Oenothera glazioviana Micheli	Onagraceae	xenophyte	91E0*, 92A0
Oxalix corniculata L.	Oxalidaceae	xenophyte	91E0*, 92A0
Oxalis stricta L. (*O. europaea* Jord.)	Oxalidaceae	xenophyte	91E0*, 92A0
Panicum capillare L.	Poaceae	hemerophyte	Forest edges, 92A0
Panicum miliaceum L.	Poaceae	hemerophyte	Forest edges, 92A0
Physalis alkekengi L.	Solanaceae	xenophyte	91E0*
Phytolacca americana L.	Phytolaccacea	hemerophyte	9130, 91E0*
Parthenocissus quinquefolia (L.) Planchon	Vitaceae	hemerophyte	91E0*
Polygonum orientale L.	Polygonaceae	hemerophyte	91E0*
Reynoutria x bohemica Houtt.	Polygonaceae	hemerophyte	91E0*, 92A0
Robinia pseudaccacia L.	Fabaceae	hemerophyte	91E0*, 92A0*
Solidago canadensis L.	Asteraceae	hemerophyte	Forest edges, 92A0*
Sicyos angulatus	Cucurbitaceae	hemerophyte	91E0*, 91F0, 92A0
Symphyotrichum lanceolatum Willd. (*Aster lanceolatum* (Willd.) G. L. Nesom)	Asteraceae	hemerophyte	91E0*, 92A0
Tanacetum parthenium (L.) Schultz Bip.	Asteraceae	xenophyte	Forest edges, 92A0*
Trigonella caerulea (Lam.) Ser. in DC	Fabaceae	xenophyte	Forest edges, 92A0*
Xanthium strumarium L.	Asteraceae	xenophyte	Woodland light, 91E0*
Xanthium orientale L. ssp. *italicum* (Moretti) Greuter	Asteraceae	xenophyte	Woodland light, 91E0*

CONCLUSIONS

Invasive species have a negative impact on forest, grassland, and agricultural ecosystems around the world, sometimes associating and forming segetal or ruderal plant communities. Exotic species compete with native species and threaten ecosystem stability.

The studies on alien species are of particular interest today to protect natural habitats and reduce or eliminate ecological and economic damage.

Numerous species identified in the researched territory develop explosively and have a very high tendency to destabilize the habitats from the point of view of physiognomy and floristic composition as well as from the syndynamic and conservative point of view.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

PHYSICAL-GEOGRAPHICAL AND SOCIO-ECONOMIC CONDITIONS DEFINING THE QUALITY OF THE ECO-PEDOLOGICAL RESOURCES IN THE TIMIŞOARA METROPOLITAN AREA

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Abstract

The ecopedological resources together with the physical-geographical factors and the regime of climatic factors constitute a subsystem called biotope or resort which is in close connection with the associations of plants and animals, together making up terrestrial ecosystems, they having the ability to transform the energy they store in biomass, between these and socio-economic interventions, relations of a varied and complex reciprocity can be established.The Timişoara metropolitan area, from this paper, is a project that includes the municipality of Timişoara, the city of Recaş and 25 other neighboring territorial administrative units in order to create an integrated administrative unit between the municipality of Timişoara and the neighboring localities, which would include 468,162 of inhabitants, on an area of 223952 ha, establishing relations of economic, social and cultural cooperation, territorial development, technical-building equipment and environmental protection, each locality maintaining its autonomy. In Romania, metropolitan areas were regulated by law 351 of July 6, 2001 an area constituted by association, based on voluntary partnership, between large urban centers and urban and rural localities located at distances of up to 30 km.

***Key words**: quality, eco-pedological, metropolitan, resources, sustainable.*

INTRODUCTION

The intervention of the human, begun with the elevation of the first mounds and waves of land, then continued with the hydro-meliorative works (initiated about 250 years ago) and with the intensification of social-economic activities, especially in the second half of the 20th century, also had, besides beneficial effects generated by economic increases, negative effects affecting, increasingly, the edaphic cover. Although the idea that soils are one of the most important components of the environment is unanimously accepted, the information provided by soil science is often underestimated or even ignored. The environment represented by water, air, soil, vegetation, and fauna is a set of spatial-temporal formations that function as cyber systems, with permanent exchanges of substances, energy and information, both between the phyto-coenotic and zoo-coenotic elements and with the environment, having the ability to transform cosmic energy into potential energy that they store in vegetable and animal biomass. In this context, the major directions of the Romanian Soil Science School (Florea et al., 1995; 2012; Ianoş et al., 1994; Niţă et al., 2018; 2019; Rogobete et al., 1997; Țărău et al., 2006; 2007; 2019; Uruioc et al., 1993) regarding the assurance of a unitary land research framework corresponding to the requirements of sustainable agriculture and environmental protection will have to solve, in order to connect to the European system, in full agreement and harmony, the following functions specific to the respective land: ecological, economic, technical, social, and legal. In view of these considerations, the authors of this paper try to present some aspects regarding the use of pedological information from pedological studies and stored in the O.S.P.A. Timişoara for the most part on classic support, but also on the basis of the Speed 1 computer system (used at O.S.P.A. Timişoara in 1988) and the BDUST-implemented system by I.C.P.A. Bucharest, since 2002, but also in the databases of research programs carried out over time by the authors (within the O.S.P.A., U.S.A.M.V.B. and U.P.T. in Timişoara, for the qualitative evaluation of the eco-pedological resources in the Timişoara Metropolitan Area and of possible pressures on them, but also of measures to promote some environmental social practice.

MATERIALS AND METHODS

The issue addressed refers to an area of 223952 ha in the area of Timişoara Municipality, the town of Recaş and 25 other neighbouring territorial administrative units (TAU) (Table 1).The starting data of the project of the Timişoara Metropolitan Area have existed since 2000 when, within a large private public partnership, the ***Strategic concept of economic and social development of the Timisoara Area***, which includes objectives and measures for both Timisoara and six communes on the first ring of development of the city: Dumbrăviţa, Ghiroda, Giroc, Moșnița Nouă, Săcălaz, and Sânmihaiu Român. This strategic concept was approved by the Timisoara Local Council and by all important political forces, and the Timiş County Council participated in the elaboration ensuring even the methodological consultant.

Table 1. Structure of the land fund for the main categories of use*

Nr.	UAT	nr. inhabitans**	Arable	Grasslands	Haymaking fields	Vineyards	Orchards	Agricultural	Forests	Waters	Other	Total
1	Timişoara[1]	331927	4088	155	84	8	6	4341	730	317	7539	12927
2	Becicherecu Mic	3158	3545	418	251	0	3	4217	0	0	448	4665
3	Biled	4062	4467	463	12	3	1	4946	0	173	392	5511
4	Bucovăţ	1804	2358	404	63	1	0	2826	208	44	174	3252
5	Chevereşu Mare	2272	4371	1040	571	1	5	5988	1092	241	796	8117
6	Dudeștii Noi	3341	3515	1013	248	0	0	4776	45	296	276	5393
7	Dumbrăviţa[2]	8935	543	50	1	1	1	596	1	46	1255	1898
8	Ghiroda[3]	6802	2241	338	218	3	12	2812	5	125	471	3413
9	Giarmata	7261	5977	54	148	60	292	6531	16	86	517	7150
10	Giroc[4]	11753	3580	291	32	0	0	3903	419	128	827	5277
11	Jebel	3614	5315	753	155	0	2	6225	17	148	353	6743
12	Liebling	4160	6671	658	443	0	2	7774	21	99	332	8226
13	Maşloc	2489	5175	1120	507	0	163	6965	983	27	316	8291
14	Mosnita Noua[5]	7890	4097	548	20	0	0	4665	174	0	2049	6888
15	Orţişoara	4600	11610	1558	524	2	74	13768	57	112	626	14563
16	Parţa	2372	4845	672	42	1	0	5560	35	181	361	6137
17	Pădureni	1749	2753	881	103	0	3	3740	1243	171	177	5331
18	Peciu Nou	5570	9113	1959	882	24	210	12188	54	206	526	12974
19	Pişchia	3148	7203	1206	513	285	489	9696	1963	188	514	12361
20	Recaş	9584	12121	4736	982	1589	230	19658	1810	403	1117	22988
21	Remetea Mare	2603	4815	903	163	61	14	5956	586	199	548	7289
22	Sacoşu Turcesc	3205	9202	1509	238	4	115	11068	381	452	552	12453
23	Satchinez	5026	8027	575	314	2	7	8925	10	482	571	9988
24	Săcălaz[6]	8807	9438	1174	0	4	6	10622	0	358	969	11949
25	Sânandrei	6902	6986	1002	233	1	5	8227	23	171	819	9240
26	Sânmihaiu Român[7]	7272	5335	1137	362	3	4	6841	10	225	450	7526
27	Şag	3437	2467	216	28	30	10	2751	42	75	534	3402
	Total	468162	149858	24833	7137	2083	1654	185565	9925	4953	23509	223952
	%		80.76	13.38	3.84	1.12	0.90	82.86	4.42	2.22	10.5	100

*Institutul Național de Statistică București, 2014

**Institutul Național de Statistică București, 2016 (Populaţia României după domiciliu)

The **municipality of Timişoara**, located at the intersection of the parallel of 45°47' North latitude, with the meridian of 21°17" Eastern longitude, being in the northern hemisphere, at almost equal distances from the North Pole and the Equator and in the Eastern Hemisphere, in the time zone of Central Europe, through its geographical position, the transport infrastructure, and the utility network is the engine of development in the intra-regional development plan. The naturalistic characterization of the studied area, the interpretation and processing of the entire volume of information (land, laboratory, office) were made in accordance with the methodology of elaborating pedological studies (M.E.S.P.) elaborated by I.C.P.A. Bucharest, in 1987, supplemented with elements (related to soil taxonomy) in the Romanian soil taxonomy system (SRTS-2003, respectively SRTS-2012). The analyses were performed in the U.S.A.M.V.B. Timişoara and O.S.P.A. Timişoara, according to national norms and standards, approved by the Standardization Association in Romania (A.S.ro.), and, since 2009, in the Laboratory of Physical-Chemical Analysis - O.S.P.A. U.S.A.M.V.B.T., Faculty of Agriculture, Banat University of Agricultural Sciences and Veterinary Medicine "King Mihai I of Romania" from Timișoara, Calea Aradului, no. 119, accredited RENAR according to STAS SR EN ISO/CEI 17025, by the accreditation certificate no. LI 1001/2013.

RESULTS AND DISCUSSIONS

From a geomorphological point of view, the researched perimeter is part of the great physical-geographical unit called the Banato-Crişană Plain, the subunit of the Timiş-Bega interfluve plain to the north of the Vinga Plain and the Tormac-Gătaia Plain. The Vinga Plain, the oldest and most complex in geographical aspect (Posea, 1997), is located south of Mureș, west of Lipovei Plateau, north of the subsident Bega-Timiş area, west of the Giucoşin-Aranca subsident area formed by the divergence of the glacis intensely modelled by a secondary network of current waters and valleys at an altitude of 95-200 m compared to the reference level. Part of the Southern Mureș Plain (Mihăilă et al., 1990,) has the appearance of a Piedmont plateau in steps, clearly detached under the altimetric, geomorphological, and structural report from the northern Mureș and the Lipovei Hills in the east. The Vinga Plain has four altitudinal steps arranged in a fan-matrix made by Mureș River, and of local tectonic influence, respectively by the hidden laccolite Luda-Bara, which produced a vault, in the east, a radial circular, asymmetric hydrography and a similar fragmentation of the plain, thus forming, towards the Lipovei Plateau, two interfluvial elongated fields towards south-west and other fields almost radially circular to south-west (Posea, 1997). Bizerea (1973) considers these steps more tectonic, distinguishing five levels: Seceani (180 m), Alioş (160 m), Vinga (150 m), Calacea (130 m), and Satchinez (100 m). Developing south of Mureș, between Lipova and Secusigiu (Satu Mare), the northern limit is given by Lunca Mureșului, also imposed by a subsident area. The eastern limit to Lipovei Plateau is marked by a level of 40-60 m, between Lipova and Maşloc, the limit altitude oscillating between 180-190 m (under the patches of terrace 5, which remain in the plateau at 200-220 m – Posea, 1997), and then by Beregsău to Sânandrei. On the left of Beregsău, the plain penetrates through meadows and low terraces on the valleys that descend from the Lipovei Plateau, in the south-east corner crossing the terrace-glacis on the right of the Bega. Between the terrace plain of Bega (Lucareţului Plain) and the Vinga Plain, the conventional limit can be drawn on the Ghertiamoş Valley, between Ianova and Remetea Mare (where a north-south fault seems to pass – Posea, 1997). In the south, towards the Timiş Plain, the limit follows the 100 m curve, sometimes even 95 m. Although the altimetric difference makes the limit less noticeable, in the field there is, generally, a level of 15-20 m and locally of only 3-5 m, unevenness made by the movement of the wandering rivers of the semi-circular hydrographic collectors of the Vinga Plain after their penetration into the subsident plain, i.e., Beregsău. The Tormac-Gătaia Plain, located between Timiș - Pogăniș - Bârzava, has an average altitude of 155 m in the east that descends slowly to west-north-west up to 135-110 m, connecting the Ramna Hills and the low Timișului Plain through a field with lots of small depressions and strongly influenced by the tributaries of the Birda stream. The transition to the subsidence Plain of Timiş is marked by a decrease in the depth at which the pedo-phreatic waters are found. The relief falls in steps from east to west, thus constituting a transitional area between the structural erosive Piedmont relief from the interfluves to the fluvial accumulative relief and division of the low fields, ending quite suddenly on the line of the localities Folea - Liebling - Sacoșu Turcesc - Buziaş. Located between the Vinga Plain and the Tormac-Gătaia Plain, this plain is mostly a recent plain, which, at first sight, appears as a rather flat surface; carefully researched, it is found to have numerous levels represented especially by abandoned meanders, micro-depressions, and long tops of bank ridges (made up of coarse materials). This is largely due to the uneven deposits of alluvial materials during the big floods caused by Timiş and Bega, before regularization, sewerage and damming, as well as subsequent compaction. Due to the varied micro-relief, the surface of the plain has numerous spring puddles after snow melting or in periods with heavy rainfall, puddles that disappear only by evaporation. Depending on the variation of the morpho-hydrographic and lithological nature of the generating agents, several subunits can be identified within it (Țărău et al., 2016): the Cenei - Beregsău - Săcălaz Plain, the Peciu Nou - Parţa - Şag Plain, the Timișoara Plain, the Bega - Timiș and Timișană bay Plain. The Cenei – Beregsău - Săcălaz Plain is located on the western periphery of the vast dejection cone generated by Timiş, Bega and Beregsău.

The Peciu Nou - Parţa - Şag Plain is part of its alluvial islands and, partially, in the Bega Plain, it is present as an alluvial flow of subsidence fluvio-lacustrine plain, with waterbeds suspended above the field (elevated by the alluvia transported by the rivers that once crossed the area) and, between them, low, marshy lands with the groundwater near the surface. The cover of loessoid materials is interrupted by significant areas with soluble mineral soils, as a result of the "plugs" of harmful salts arising in the path of water circulation. The contact between the alluvial area of the Bega river and the lower terrace of Timiş covered with loessoid materials is done at the level of the sandy tops of bank ridge areas (the former fruit plantation from Diniaş, respectively the one from the Sânmartinul Sârbesc) and of the vast depressive areas in which salsodisol predominates. The salty lands (from an area of 4 ha), with their specific flora, located near the Diniaş, are protected as a pedological reserve (protected natural area, Law no. 5 of March 6, 2000), a measure initiated and promoted in the 1960s by C. V. Oprea. Among the plant species present in the reserve are *Plantago maritima* (sea plantain), *Artemisia santonica* (artemisia), *Atriplex tatarica*, *Atriplex littoralis*, *Myosurus minimus* (mousetail), *Trifolium fragiferum* (strawberry clover), *Lotus augustissimus* (lotus), *Aster tripolium* (sea aster).In the area of the reserve (in the areas with stagnant water that are formed in the spring - called, by the locals, bald-spots), vegetates an alga (cyanobacteria) known as the star jelly (Nostoc commune). The Timișoara Plain is, most of it, a subsidy and division plain made up of a succession of river tops of bank ridge and river depressing areas characteristic of a continental delta, bordered in north-east by a higher step with altitudes of over 100 m, a portion that connects, on the Giarmata Vii - Dumbrăviţa line, with the high Vinga Plain. Studied in detail, it presents a number of local features represented especially by abandoned meanders, micro-depressions and tops of bank ridges. The *Bega – Timiș and Timișană bay Plain*, an important part of the Timiş Plain within the studied area, is characterized by a wide development of the main river meadows, with numerous meanders, diffuses, separations and deserted courses and is made up of two divisions: the low interfluve plain of Bega (Canal) - Timiş and the low plain located north of the Bega Canal, but having a great unevenness, both relief and parental materials, thus favouring swamping, for which since the 18th century they started doing the first works of regularization, sewerage, damming, and drainage. The Timișană Plain has large interfluves with slopes; it forms an intermediate step between the low Timiș Plain and the hilly plains (the Tormac - Gătaia Plain) within the studied area, i.e., the Buziaș Hills, where they make up bays. The transition from the hilly and pre-hill sector to the Timiș Plain is made by a succession of terraces with different widths. The geological past of the studied area is linked to that of the Banato - Crișană Plain, of which it is part, representing one of the eastern portions of the great sedimentation called the Pannonia Depression that sank along the alignments of ancient north-south faults, more to the west and fewer towards the Carpathians, starting with the Badenian, with a maximum during the Pannonian, after which it became slower (Ianoş et al., 1999), their presence being marked south from Gătaia by volcanic hillock Şumig or by the formation of the Lada-Bara laccolite along the Vinga - Seceani - Ianova alignment, as well as the mineralized waters from Calacea, Buziaş or Ivanda. From a lithological point of view, the cadastral territory is characterized by a succession of layers of different age, thickness, and granulometric composition depending on the forms of mezo- and micro-relief, the tops of bank ridges being made up of coarse texture deposits. Mihăilă et al. (1988), analysing drilling data, indicated four lithological complexes deposited under different sedimentation conditions. The first is made up of small gravel and sands with clays and very thick sandy clays (160-180 m) that were deposited directly on the Pannonian. The age of the complex is estimated from the upper Pliocene to the Medium Pleistocene. The fourth complex brings together superficial deposits throughout the plain, different in origin and age: loess and loessoid deposits, fine sands and clays, red clay (Upper Pleistocene). Florea et al. (1966) established a number of five red strips - fossil soils in the deposits of this field. The lithology of surface materials is generally represented by alluvial-calcic and wind formations, but especially Pleistocene ones. Quaternary sedimentary deposits have the highest development in the area, covering all the other geological formations with frequent thicknesses of 100-200 m. The important thickness of these deposits attests the extent that they had during the Quaternary the erosion, transport, and accumulation processes, at the same time with the phenomena of subsidence that affected the entire Pannonia basin, including the studied area. Over the basic gravel, follows an alternation of sands of different granulations, gravels, clays, sandy clays, marls, sandy marls, sediments that represent the deposits of several generations of dejection cones of Mureș and Timiş rivers, which have successively changed their courses as a result of the continuous subsidence in the Pleistocene (Mihăilă, 1989; Uruioc, 2002).

Hydrologically, the studied area is part of the group of south-western hydrological systems, the Timiş - Bega hydrographic basin, the complex hydro-meliorative system Șag - Topolovăţ, with a rich hydrographic network consisting of rivers, lakes, and canals. The course of the Bega river, the most southern tributary of the Tisa, is characterized by a water regime with very large variations of water levels and flows. Under these conditions, both to ensure the need for water to the waterway (started in 1728, on a route of the old course, completed in the first phase in 1756), as well as to protect the municipality of Timișoara (after the great flood of 1859), Bega was linked to Timiş through a system consisting of two canals. Thus, the hydrotechnical node from Coştei was conceived and realized, with the main function to ensure the transfer of water quantities from Timiş to Bega, depending on the needs and the amount of rainfall taken from the two rivers upstream. Beregsău springs from the Lipovei Hills (Ujvari, 1972), after which it descends east from Fibiş and Pişchia, respectively south from Cernăteaz and gathers most of the waters from the Vinga Plain, among which Măgheruș (both seem established on the old courses of the Mureș). The current course of Beregsău being regularized and turned into a canal, the flooding of the meadow takes place very rarely, only during the big floods. In the eastern part of the locality of Seceani all the valleys converge towards Măgheruș, so that Luda-Bara, an important tributary of Măgheruşului, gathers its water from the Seceani platform; after crossing from the north to the south of the village of Murani, it flows from north-west to south-west almost in parallel with Măgheruș. In rainy autumns and springs, the whole meadow is flooded for a long time. There, on an area of 200 ha located in the eastern part of Murani village, near the county road (DJ 693) that connects the town of Pișchia to Seceani, near the A1 highway, Bucharest - Nădlac (part of the IV Pan-European corridor) was included in a natural area. The Murani Marshes make up an area of special avi-faunistic importance due to the presence of several (migratory) bird species protected at European level through the EC Directive 147/EC of November 30, 2009 (on the conservation of wild birds. In the western part of Calacea, the area is drained by Apa Mare, around Carani by the Surduc Valley, and around Corneşti by Valea Lacului. Then, there are a series of valleys that change their direction several times from the springs to Beregsău. Apa Mare, which originates under the peak Luda Bara on the territory of Fiscut (commune of Şagu), has a well-drained valley from its entrance to Vinga to the place where the Arad - Timișoara road crosses. From the road to the centre of Vinga, it has low-drained partially soaked portions, and from there to the communal road leading to Secusigiu, it becomes a large marshy area with reeds. In the flooding meadows, because of the digressing watercourses, there are a number of ponds and marshes with developed grassy vegetation, especially in the perimeter of the Apa Mare meadow, declared a natural ornithological reserve, the area protecting a specific habitat for the aquatic fauna, especially (*Isobrycus minutus*), great crested grebe (*Podiceps cristatus*), spoonbill (*Platalea leucordia*), great egret (*Egretta alba*), little egret (*Egretta garzetta*), western march harrier (*Cyrcus aeruginosus*), European bee-eater (*Merops apiaster*), European pond turtle (*Emys orbicularis*), a reptile, as well as many other species of protected birds. Satchinez Marshes form an ornithological nature reserve that covers 242 ha in the border of Satchinez. It was established in 1942, at the proposal of the ornithologist Dionisie Linția, comprising marshy lands downstream (Journ. Cons. Min. No. 1166/1942). Currently, the reserve has an area of 1,194 ha, being nicknamed the "Banat Delta", with 40% of the bird species found on the territory of Romania living there. This natural habitat is a reminiscence of the old marshes that covered these lands until the middle of the 8th century. From the Lipovei Hills, the Gherteamoş stream is poured into Bega, crossing the studied area north-east to the west close to Ianova, then goes to southwest, collecting the waters of the erosion valleys and the torrential elements in the Piedmont area Stanciova – Herneacova – Ianova, in a fanlike pattern. Within this course, east of Innova, an accumulation lake was made, with a water surface of 10-15 ha, which represents an attractive area for recreational fishing enthusiasts. After leaving the territory of our country, Bega flows into the Tisa at Titel, in Serbia, but before that it also receives the waters of Bega Veche (Beregsău). Timişul (called in the Roman period Tibisis or Tibiscus, a name that could come from the Dacian "thibh-isjo" meaning marsh), the most important hydrographic artery in Banat, collects its waters from a river basin of 5248.0 km^2, 241.2 km in length. The lower course of Timiş starts from Coştei, from where it forms a wide valley, with numerous meanders, digressions and ponds, phenomena favoured by the very small slope and the depth of water-clayey deposits. Great hydrotechnical works started in the 18th century created the Bega-Timiş drainage and navigation system through canalling, regularization and damming of the courses of the two rivers, removing their lower basins from floods. Of the tributaries received by Timiş in its lower basin, the most important is Pogăniş, which, after it springs from the northern part of the Semenic Mountains, collects the waters from the hills of the same name, constituting a collection basin of 700 km^2 and a length of almost 100 km, the place of flow into the Timiș being on the territory of the commune of Sacoșu Turcesc, Timiș County, at the north-western limit of the village of Uliuc. The drainage regime, with strong floods that used to cause numerous floods, led to the regularization and damming of the riverbed. Part of the Pogănișului Meadow, especially the area of Tormac, Blajova and Berini, with an area of 75.5 ha, was declared a nature reserve, its objective being to protect the spotted tulip (*Fratillaria ileagris*), a Mediterranean species from the Liliaceae family (Nica, 2004; Banaterra.eu). The current hydrographic aspect completely different from the one in the not-too-distant past is the result of important hydro-meliorative works started over 250 years ago.

The climatic peculiarities of the studied area are determined by its geographical position so that it is characterized by a temperate moderate continental climate with shorter and milder winters, being frequently under the influence of cyclones and air masses that cross the Mediterranean and the Adriatic Sea. From a phytogeographic point of view, the studied area belongs to the central European geobotanical province, strongly influenced by the vicinity of the South-European geobotanical province. Thus, the natural floristic elements have different geographical sources: European, Euro-Asian, Boreal, Balkan, Mediterranean, Illyric, to which a series of endemic plants can be added. In this sense, floristic research in the studied area (and of course, in a larger area) were published by Fr. Griselini (1779) who, living between 1774 and 1777 in Timișoara Banat, wanted to inform in his 21 letters, the (science) world about the many aspects that drew his attention in this ancient Romanian province. In his 12th letter, Griselini makes a thorough description of the natural frame, relief, springs, and rivers that cross it, soil nature, and vegetation, etc. The landscape is diversified by the existence of small, solitary patches located near the former courses of the waters, or along former access roads, made up of *Salix fragilis* L., *Acer tataricum* L., *Robinia pseudacia* L. and, rarer, *Quercus cerris* L., *Quercus pedunculiflora* Koch. Bistra Forest with an area of 19.90 ha, located in the meadow on the left bank of the Bega river, 12 km from Timisoara, was declared an area protected by Law no. 5 of March 6, 2000 and represents an area foreshadowed protection for pedunculate oak (*Quercus robur*), Hungarian oak (*Quercus frainetto*) or Austrian/Turkey oak (*Quercus cerris*). Also, in the Bega meadow, there is the Bazoș dendrological reserve that extends over 60 ha. It includes Parcul Mare, Parcul American and several nurseries intended for the cultivation of exotic species. From 1994, the protected area was declared for the protection of biodiversity, gene fund, eco-fund and for maintaining the ecological balance in Timiș County. It is part of the International Association of Botanical Gardens. From the data presented, it can be observed that the diversity of the pedo-climatic conditions and the local particularities of the studied area had a strong influence on the structure of the land fund and of the land use, in general, and of agricultural lands (implicitly on their current and future productivity). To our regret, we do not have sufficient statistical data to be able to follow the evolution of land uses, but we could appreciate their dynamics guiding us after the natural vegetation areas and reconstructing the map of the old supposed uses (Coste et al., 1997; Coste, 2003).

It may be that, until the beginning of the 19th century, the areas remained almost unchanged, except for a slight decrease in forest areas. The bigger changes began there, as in the other regions of the country, after 1829, after the Treaty of Adrianople, when the trade with grain and wood was liberalized. However, the most radical changes in the use of the land in the last 250 years have taken place in the plains, where large areas of marshes have been transformed into arable land and pastures, with them also occurring areas of poor salty soils.

At the same time, the plain areas were densely populated, and the works of regularization, sewerage, drainage, and damming led to the improvement of the land and to an increase of the economic significance of these areas compared to the hilly ones. Drainage works reduced the areas of lakes and ponds, in general, of hydrophilic and hygrophile ecosystems, from 10,926 km^2 to areas of several tens of square kilometres; currently, there are aridization tendencies by decreasing the level of pedophreatic waters (Man, 2015)

According to the Romanian soil taxonomy system (SRTS-2012) with 12 types (Table 2) and soil associations identified, which mirror, by their geo-bio-chemical and morphological properties the main defining and determining characteristics for the growth and fructification of the main cultivated plants, expressed by grade.

Table 2. Main types of soil (ha/%)

	UAT	Agricultural ha	AS	CZ	FZ	EC	EL	LV	VS	PE	SG-GS	SN	AT*-TT	Asoc.
1	Timişoara	4341	343	864	0	1190	0	0	703	561	0 50	0	630	0
2	Becicherecu Mic	4217	70	2846	0	640	104	0	96	64	0 25	130	18	224
3	Biled	4946	0	4543	0	0	0	0	10	0	0 194	49	0	150
4	Bucovăţ	2826	285	0	0	1967	0	0	254	79	0 0	241	0	0
5	Chevereşu Mare	5988	132	0	0	379	4106	0	146	180	0 828	0	217	0
6	Dudeştii Noi	4776	0	3310	296	250	94	0	113	145	0 37	125	35	371
7	Dumbrăviţa	596	0	303	0	32	255	0	0	0	0 0	0	6	0
8	Ghiroda	2812	194	0	0	132	1530	0	315	36	67 200	20	0	318
9	Giarmata	6531	99	0	69	949	3688	0	478	858	98 249	0	43	0
10	Giroc	3903	458	0	0	2378	0	0	53	0	0 493	0	0	521
11	Jebel	6225	320	626	2275	2198	0	0	214	0	0 280	312	0	0
12	Liebling	7774	26	1978	0	11	3742	0	515	0	330, 985	126	3	58
13	Maşloc	6965	0	0	77	100	5658	0	380	185	280 86	0	199	0
14	Moşniţa Nouă	4665	1302	0	0	1640	0	0	1018	0	0 451	13	0	241
15	Orţişoara	13768	0	2193	0	0	8444	317	0	0	200 407	0	1007	1200
16	Parţa	5560	1024	407	638	2973	0	0	14	112	0 0	8	8	376
17	Pădureni	3740	189	368	1338	1357	0	0	126	0	0 179	183	0	0
18	Peciu Nou	12188	115	3983	1938	1885	0	0	864	1203	0 73	1103	11	1013
19	Pişchia	9696	27	0	0	999	6893	98	411	115	247 259	0	647	0
20	Recaş	19658	1579	0	29	4618	6521	1460	956	504	410 490	0	3032	59
21	Remetea Mare	5956	155	0	2007	1114	360	352	300	1144	346 131	0	47	0
22	Sacoşu Turcesc	11068	273	1445	3023	1814	3092	0	768	0	0 153	121	0	379
23	Satchinez	8925	428	6694	0	0	696	0	54	0	0 643	62	348	0
24	Sânandrei	8227	0	1277	0	552	4301	14	600	0	0 197	29	471	786
25	Săcălaz	10622	985	3719	617	2951	0	0	478	1320	0 249	303	0	0
26	Sânmihaiu Rom	6841	1446	774	0	177	0	0	2023	0	0 132	65	0	2224
27	Şag	2751	513	203	319	1461	0	0	7	55	0 0	2	4	187
	Suprafaţa	185565	9963	35533	12626	31767	49484	2241	10896	6561	1978,6791	2892	6726	8107
	%	100	5.36	19.14	7.00	17.10	26.63	1.21	5.87	3.51	1.07 3.65	1.56	3.63	4.36

The basic principle of the assessment method elaborated in our country is the one after which, for each homogeneous ecological unit (HEU) within a territorial administrative unit (TAU) defined according to the current methodologies for elaborating pedological studies, using the 23 indicators of value - *climatic indicators* (indicator 3C - average annual temperature - corrected values, indicator 4C - average annual rainfall – corrected values), indicators of some morphological, chemical, physical, hydro-physical characteristics and the volume of the soil cover (the indicator 14 - gleysation, indicator 15 - stagno-gleysation, indicator 16 or 17 - salinization or alkalization, indicator 61 - $CaCO_3$ content total on 0-50 cm, indicator 63 - soil reaction in the water or in the first 20 cm, indicator 144 - humus supply in layer 0-50 cm, indicator 23A - texture in water or first 20 cm, indicator 44 - total porosity in restrictive horizon, indicator 133 - useful edaphic volume), *indicators of relief characteristics* (indicator 33 - slope, indicator 38 - glides), indicators related to hydrography, hydrology and drainage of the territory (indicator 40 - flooding, indicator 181 - excess stagnant humidity, indicator 39 - depth of the pedo-phreatic water), *indicators of some anthropic interventions* (indicator 29 - pollution, indicator 271 - land improvements) as well as the interactions between the values of natural an anthropic features are turned into well-established value grades from 1 to 100, which support the quality classes from I to V (Table 3) depending on their suitability for arable use (Order M.A.D.R. no. 278/201), the situation being specific for each territory taken into account.

Table 3. Quality classes for "ARABLE"

Territorial Administrative Unit (TAU)	Arable	Class I (81-100 pts.) ha	Class II (61-80 pts.) ha	Class III (41-60 pts.) ha	Class IV (21-40 pts.) ha	Class V (1-20 pts.) ha	Levelled average grade
1. Timişoara	4088	146	1889	789	960	304	58
2.Becicherecu Mic	3545	1298	1303	507	220	217	69
3. Biled	4467	2375	1780	260	47	5	76
4. Bucovăţ	2358	81	827	1092	343	15	56
5. Chevereşu Mare	4371	0	1435	2572	350	14	57
6. Dudeștii Noi	3515	1160	1402	500	230	223	68
7 .Dumbrăviţa	543	245	287	7	4	0	69
8. Ghiroda	2241	0	1035	957	229	20	59
9. Giarmata	5977	128	924	3860	949	116	50
10. Giroc	3580	15	715	1990	653	207	47
11. Jebel	5315	305	2758	1674	454	124	59
12. Liebling	6671	722	1216	2298	1799	636	55
13. Maşloc	5175	78	762	2812	1260	263	48
14.Mosnita Noua	4097	146	1790	1855	253	53	58
15.Orțișoara	11610	1499	5273	3677	594	567	63
16. Parţa	4845	90	2200	1832	649	74	57
17. Pădureni	2753	160	1420	881	230	62	59
18. Peciu Nou	9113	425	4998	2663	440	587	60
19. Pişchia	7203	157	2977	2327	1389	353	54
20. Recaș	12121	0	1959	4984	3865	1313	42
21.Remetea Mare	4815	49	962	2473	967	364	53
22. Sacoşu Turcesc	9202	174	3748	3529	1674	77	57
23. Satchinez	8027	4127	2256	664	442	538	70
24. Sânandrei	6986	317	4026	1918	708	17	64
25.Săcălaz	9438	298	3967	2992	1747	434	57
26.Sânmihaiu Român	5335	192	1373	1150	2414	206	46
27.Şag	2467	46	1108	960	320	33	57
Total ha	149858	14233	54390	51223	23190	6822	
%		9.50	36.29	34.18	15.47	4.56	

The operation of classifying agricultural lands into quality classes based on the notes of value has highlighted a series of limiting factors that act on the production capacity of agricultural lands within the studied area, including: granulometric composition (soil texture), humus supply, soil reaction, degree of tingling or compactness, excess humidity; some of them are exemplified by the affected areas (Tables 4-6), and they refer to the limitations caused by the excess stagnant and groundwater (Table 4), poor and/or acidification of the soil (Table 6), respectively the degree of compaction and the deficit of humidity, for which, on a case-by-case basis, pedo-hydromeliorative measures (drainage, deep aeration, etc.) of achieving a balance airo-hydric regime and measures meant to favour the development of the processes of concentration of nutrients and organic matter in the soil (meliorative fertilization, long-term crop rotations with meliorative plants and perennial grasses, etc.) are required.

Table 4. Situation of land with excess surface and groundwater humidity

Nr. crt.	Commune Town Municipality	Total ha (agricultural)	Of which lands with:					
			Excess surface excess			*exces de umiditate freatică*		
			low	*moderate*	*high; excessive*	*low*	*high*	*very high; excessive*
1	Timişoara	4341	1270	111	0	2001	111	561
2	Becicherecu Mic	4217	923	1135	756	1190	390	290
3	Biled	4946	0	0	0	852	367	23
4	Bucovăţ	2826	767	560	230	950	265	241
5	Chevereşu Mare	5988	1566	2574	79	969	60	879
6	Dudeştii Noi	4776	990	1250	826	1280	560	320
7	Dumbrăviţa	596	135	95	-	65	7	0
8	Ghiroda	2812	201	80	-	261	230	235
9	Giarmata	6531	1059	950	596	825	495	250
10	Giroc	3903	1084	323	617	352	2495	519
11	Jebel	6225	160	77	0	1671	509	280
12	Liebling	7774	1980	1320	360	1757	1240	1104
13	Maşloc	6965	1106	1740	1040	340	99	124
14	Moşniţa Nouă	4665	1267	1120	890	980	680	455
15	Orţişoara	13768	1360	820	360	1120	350	415
16	Parţa	5560	25	990	360	1100	400	0
17	Pădureni	3740	950	120	0	1560	1020	179
18	Peciu Nou	12188	1690	1120	920	3119	2605	418
19	Pişchia	9696	1410	1076	506	950	390	260
20	Recaş	19658	3463	2454	750	1165	1845	650
21	Remetea Mare	5956	1550	1150	510	2560	620	255
22	Sacoşu Turcesc	11068	2960	1870	250	2960	2470	217
23	Satchinez	8925	1420	1387	219	1120	828	750
24	Sânandrei	8227	1690	960	512	1155	720	207
25	Săcălaz	10622	1280	2524	930	2223	981	266
26	Sânmihaiu Român	6841	1100	2250	870	644	668	132
27	Şag	2751	33	1070	395	1138	341	0
	Surface ha	185565	31539	26078	11976	34307	18251	9030
	%		17.00	14.05	6.45	18.48	9.84	4.87
	Surface ha/%		69,593 ha, 37.50%;			131,181 ha, 70.69%		
	Surface ha/%		61,588 ha, 33.19%;					

Thus, within the lands located in the Banato-Crișană Plain in relation to the form of micro-relief, permeability, and hydroclimatic balance, agricultural land can be grouped (according to indicator 181 of M.E.S.P., 1987 - excess surface humidity) depending on the period of stagnation (Table 5) as follows: low (2), with stagnation between 6-15 days, on 31,539 ha, 17.00%, moderate (3), with stagnation between 16-30 days, on 26,078 ha, 14.05%, high (4), very high (5) and extremely high (6), for periods that can exceed 60 days, with an area of 11,976 ha, 6.45%, representing together limiting factors on an area of 69,593 ha, 37.50%. Referring to the water regime of the soil, predominantly groundwater, the state of gleysation, or the excess of pedophreatic humidity, within the area investigated in relation to the intensity of the gleysation and the depth at which it appears were defined the grades of gleysation (indicator 14) resulting in the following land groups (Table 5): moderate (3), on 34,307 ha, 14.48% (of the studied area), high (4), on 18,251 ha, 9.84%, very high (5) and excessive (6), on 9,030 ha, 4.87%, representing limiting factors on an area of 61,588 ha, 33.19%. The two forms of excess humidity within the studied area represent limiting factors on an area of 131,181 ha, 70.69% (Table 5). One of the soil features with an important role in the way of excess and deficit of humidity occurs is compactness. This represents the property of the soil to oppose the forces that tend to mechanically undo the particles that make it. It is closely linked to granulometric composition, water content, humus content and its quality, as well as to the nature of adsorbed cations, the main ecological indicator that impacts the general way of working the soil with agricultural equipment and the plant root penetrating the soil. It is expressed in percentages, establishing the classes of soil compactness: very loose, loose, low compactness, moderate compactness, high compactness, features closely related to a complex indicator – the degree of compactness (indicator 44), which shows that, within the studied area, there is the following situation (Table 5): high (+25) on 59,596 ha, 32.12%, moderate (+15) on 71,975 ha, 38.79% and low (+5) on 26,683 ha, 14.38%, representing limiting factors on 158,254 ha, 85.29%.

The general compaction of the soil profile (Table 5) impacts the methods of retention and movement of water from the soil, the water retention force, which influences its accessibility for plants being constantly changing, depending on the degree of humidity of the soil (generated either by the water from precipitation, or by the pedophreatic intake and the degree of compaction, or by the relief form), for which, within the agricultural lands with an area of 185,565 ha, within the studied area, during one agricultural year, we encounter two extreme situations, respectively: excess moisture in the cold season on 158,254 ha, 85.29% (Table 5) and humidity deficit during hot years on 97,871 ha, 52.74% (Table 6).

Table 5. Situation of land affected by compaction and humidity deficit

Nr. Crt.	Commune Town Municipality	Total ha (agricol)	Of which lands with:					
			compactation			*Humidity deficit*		
			low	*moderate*	*high*	*low*	*moderate*	*high; excessive*
1	Timişoara	4341	104	1019	1515	1250	169	0
2	Becicherecu Mic	4217	848	3065	574	249	2251	0
3	Biled	4946	3354	435	65	1149	1268	0
4	Bucovăţ	2826	526	480	1270	1280	-	-
5	Chevereşu Mare	5988	2351	2055	1094	1904	2295	-
6	Dudeştii Noi	4776	948	3285	595	270	2550	0
7	Dumbrăviţa	596	265	150	180	356	-	-
8	Ghiroda	2812	201	2052	151	528	1278	-
9	Giarmata	6531	390	5550	340	1990	2710	490
10	Giroc	3903	1125	952	1236	991	1685	0
11	Jebel	6225	1513	1552	1570	496	1511	0
12	Liebling	7774	128	5453	1768	3222	2651	-
13	Maşloc	6965	525	226	6058	2640	3189	-
14	Moşniţa Nouă	4665	870	1140	2430	560	3150	-
15	Orţişoara	13768	180	7260	4160	1860	3960	2168
16	Parţa	5560	1020	2860	960	1260	0	0
17	Pădureni	3740	620	820	1517	360	1220	0
18	Peciu Nou	12188	1725	3812	3080	2960	1743	0
19	Pişchia	9696	2885	1760	4210	1960	2890	-
20	Recaş	19658	450	5060	9960	1860	6170	-
21	Remetea Mare	5956	939	870	3406	2690	-	-
22	Sacoşu Turcesc	11068	458	8379	1246	1790	5280	-
23	Satchinez	8925	1508	1470	1730	1884	2276	977
24	Sânandrei	8227	320	1010	6085	1980	2970	210
25	Săcălaz	10622	1217	6143	2073	1666	30	0
26	Sânmihaiu Român	6841	1112	3643	1915	1121	797	0
27	Şag	2751	1101	1474	408	1747	0	0
		185565	26683	71975	59596	41983	52043	3845
			14.38	38.79	32.12	22.62	28.05	2.07
			158,254 ha, 85.29%;			97,871 ha, 52.74%		

Referring to the state of soil reaction (indicator 63), as a result of the complex of physico-chemical properties in its natural or anthropic state, which expresses the ways in which the main biochemical processes of the soil determining the possibilities of growing and developing cultivated plants or those in natural biocoenoses, it presents altitudinal zoning from west to east (values decreasing with altitude), from the salsodisols of the subsidence plain to the strong acidic soils in the Piedmont areas. Depending on these values, the following groups of land can be identified (Table 6): *strong acidic* (4.7 with values between 4.4-5.0) on 3,924 ha, 2.11%, *moderately acidic* with values between 5.1-5.4 (5.2%) and values between 5.5-5.8 (5.6%) on 13,738 ha, 7.40%, *low acidic* with values between 5.9-6.8 (6.1-6.6) on 23,261 ha, 12.54%, representing limiting factors on 40,923 ha, 22.05%.

Table 6. Situation of land affected by salting and acidification

Nr. Crt.	Commune Town Municipality	Total ha (agricultural)	Of which lands with:					
			Salting			*acidifiere*		
			low	*moderată*	*Ptr. exce*	*low*	*moderate*	*High;*
1	Timişoara	4341	1805	405	50	1121	174	0
2	Becicherecu Mic	4217	1527	1384	133	601	102	0
3	Biled	4946	1705	1070	69	280	0	0
4	Bucovăţ	2826	40	-	-	1100	960	-
5	Chevereşu Mare	5988	460	230	-	3810	980	-
6	Dudeştii Noi	4776	1690	1760	130	730	104	0
7	Dumbrăviţa	596	-	-	-	340	20	-
8	Ghiroda	2812	40	377	20	1470	330	-
9	Giarmata	6531	940	-	-	1529	1670	70
10	Giroc	3903	701	21	0	1344	317	15
11	Jebel	6225	650	580	655	1555	348	0
12	Liebling	7774	935	55	136	4850	460	-
13	Maşloc	6965	-	-	-	1930	2726	-
14	Moşniţa Nouă	4665	125	498	13	1480	2060	-
15	Orţişoara	13768	80	60	-	4550	5650	13
16	Parţa	5560	317	126	8	1620	260	0
17	Pădureni	3740	630	735	183	960	360	0
18	Peciu Nou	12188	2223	986	1103	1663	422	0
19	Pişchia	9696	191	-	-	3195	4370	145
20	Recaş	19658	-	-	-	4280	11720	195
21	Remetea Mare	5956	10	-	-	2590	2395	16
22	Sacoşu Turcesc	11068	360	250	125	5958	2930	-
23	Satchinez	8925	620	506	68	4810	980	-
24	Sânandrei	8227	1050	670	29	2160	3450	135
25	Săcălaz	10622	4667	2679	837	1383	48	0
26	Sânmihaiu Român	6841	2375	1246	363	1680	326	0
27	Şag	2751	120	100	2	1320	396	0
		185565	23261	13738	3924	58309	43558	589
			12.54	7.40	2.11	3.42	23.47	0.32
				40923	22.05		102456	55.21

The soils included in the category of acid soils can be separated into two major groups regarding the type of acidification and soilification, with implications on the aerohydric regime. In a first group are the soil types that have horizon B clay-illuvial (Bt) and, in the second, soils with horizon B cambic (Bv) or spodic (Bs). The low fertility of acid soils is mainly caused by acidity, the presence of free aluminium in the soil solution, the deficiency of nutrients, and the defective aerohydric regime. Referring to the lands affected by salting (Table 7), the following groups of land resulted: *low alkaline* with values between 7.3-7.8 (7.5%), values between 7.9-8.4 (8.1%) on 58,309 ha, 31.42%, *moderate alkaline* with values between 8.5-9.0 (8.7%) on 43,558 ha, 23.47%, *high and excessively alkaline* with values between 9.1-9.4 (9.2%) on 589 ha, 0.32%, representing limiting factors on an area of 102,456 ha, 55.21%: solonetz, vertosols, pelosols, eutricambosols, chernozem (salsodic sub-types, etc.).

CONCLUSIONS

Overall, the relief of the studied area is characterized by a great complexity of morphological forms, with geological structures and specific pedo-geographic evolutions, related to the genesis in time and space of the Banato-Crișană Plain, of which it is part. The other feature is generated by the withdrawal of the Pannonia Lake, which left behind a vast and unhealthy area maintained until the end of the 18th century, during which 877,600 ha of marshes persisted, periodically fed from the many arms that were detached from the rivers transiting the area. The great structural and economico-social diversity determined mostly by the relief forms is also mirrored in the distribution of agricultural lands, which gives the Banat area a specific feature, in which the intensity of land fund use is close to maximum parameters, 82,86 % from the total area being used in different activities specific to agriculture (cereals, technical plants, etc., with 80,76 % being arable land). This is due, to a large extent, to the first works of drainage of the marshes and to the regularisation of the main rivers such as Beregsău, Bega, Timiş, Bârzava, etc., started in 1711-1728, and continued with other pedo-hydro-meliorative works until recently. eferring to the natural and anthropic conditions in the physico-geographical area located in the medium and inferior basins of the main courses - Bega, Timiş, Bârzava - these are, generally, favourable for the development of the agri-food sector, under all aspects, with an old tradition of cultivation of cereals and their capitalisation. However, we need to emphasise that, being located in the subsidence, divagation, and accumulation of Mureș, Timiș, and Bârzava rivers, the geomorphological evolution of the area studied is related to the evolution in time of the marine (Thetys) or lake (Pannonia) domains which generated the formation of soils (vertosols, pelosols, stagnosols, salsodisols, vertic-salsodic chernozes, etc.) which, during one agricultural year, have two extreme situations, respectively: excess of humidity in the cold season and deficit of humidity during the hot period of the year, both situations generating a series of stress forms with negative effects on the productivity and quality of the agroecosystems, which imposed the achievement in the area of agro-pedo-hydro-meliorative arrangements, the area being subjected, from its beginnings of local formation organisation, to more intense anthropic interventions than in other territories of the Timiş County.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

BENEFITS OF ORGANIC FERTILIZERS RESULTED FROM DIFFERENT ORGANIC WASTE CO-COMPOSTING: A REVIEW

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Abstract

Considering the increasing demography and the diversity of human activities, the quantity of waste is also increasing around the world, and the quantity of organic waste too. Therefore, the nature cannot deal with all waste by itself. Thus, people are looking for the most efficient ways to improve waste management. One of those ways, which is to reintegrate most of the residual materials as resources or as new products and to reduce their environmental impact is the circular economy (CE). Composting is nature's way of dealing with organic waste to turn it into simple components which will be able to integrate the natural cycles. So, composting is a part of CE that can transform organic waste into new products such as compost. This paper will explore the methods of composting, from traditional composting to the involvement of artificial intelligence (AI) in this process and will analyse the benefits of the organic fertilizers resulted from composting and co-composting of different materials. From this perspective, the most well-known composting systems will be addressed (home composting, on-farm composting, and industrial composting).

Key words*: co-composting, organic waste, circular economy, benefits, new products.*

INTRODUCTION

There are many different types of waste generated around the world and the organic waste is a big part of it. Organic waste generally refers to any material which is biodegradable, compostable, mainly produced by living organisms, such as animals and plants, classified as: garden and park waste, food and kitchen waste from households, restaurants, caterers and retail premises and comparable waste from food processing plants; animal by-products used in a biogas or composting plant (Directive 2008/98/EU).

It cannot be traced back in time to the moment when the idea of the CE appeared, however, the concept becomes more popular as the need to reduce the impact of human activity on the environment increases. European Union (EU) aims to transit from a traditional linear economy to a circular economy, and to achieve this target, the European Commission (EC) adopted its first circular economy action plan in 2015 with 54 concrete actions which covered the whole life cycle of the products (EC, 2015). Only 40% of the waste produced by EU households is recycled, with a wide variation between Member States (MS) and regions (above 80% recycling rate in some areas and below 5% in other) (EC, 2015). Forward, in 2018 it was revised the legislative framework (Directive 2008/98/EU) on waste which provides a hierarchy of waste and defines the types of waste and by-products, giving directions to MS on how to address the issue of waste management and sets the target for waste-recycling to 60% by 2030 and 65% by 2035 (Directive 2018/851/EU). Statistical reports showed that the total waste generated in EU by all economic sectors decreased with 0.99% in 2020 compared with 2004. The waste produced only by agriculture sector during 2004-2020 decreased with 0.33% in EU (Table 1).

In 2020 the waste recovery rate was 54.08% in EU (Table 2), from which 39.9% is being recycled, the rest of it goes to landfilling, incineration or it's disposed in other ways, and a smaller percentage is used for energy recovery (Eurostat, 2023). Even if the treatment of the waste overall has a descending trend, the recycling of the waste produced by agriculture sector has increased (Table 3).

Wanting to become the first climate-neutral continent by 2050, in 2019 EC adopted European Green Deal (EGD) which has a focus on reducing the greenhouse gas emissions (GHG) with 55% by 2030, compared to 1990 levels (EC, 2019).

According to the Fifth Biennial Report from the European Union under the UNFCCC (2022), in 2020, the total GHG emissions in the EU was 3700 million tonnes CO_2 equivalents, which means 34.3% below 1990 levels. Agriculture is the second largest sector that produces GHG emissions in EU (11% of the total GHG). The main sources of GHG emissions from agriculture comes from application of fertilizers and manure management, and enteric fermentation (including methane emissions).

The reports submitted by the EU and MS show a decrease of 31% in 2020 compared with 1990 in the use of inorganic fertilizers and a decrease of 4% in the use of nitrogen from organic fertilizers (EEA, 2022).

In accordance with the European Green Deal, EC published Farm to Fork strategy, a 10-year plan which aims to accelerate the transition to a sustainable food system and to reduce the impact on the environment of the food supply chain. Besides that, in 2022 through the proposal of a new Regulation on the Sustainable Use of Plant Protection Products (EC, 2022), EC decided to reduce with 50% the use of chemical pesticides and 50% the use of more hazardous pesticides by 2030, in line with the EU's Farm to Fork and Biodiversity strategies.

The reports from 2020 presents a decrease of 14% in the use of chemical pesticides, and 26% in the use of more hazardous pesticides, compared to 2015-2017 (Eurostat, 2022).

One of the cleaner technologies for waste management and GHG reduction is composting, a process of biological decomposition and stabilization of organic matter with a resulting product (compost) that is stable, free of pathogens and plant seeds, that can be beneficial for soils and plants (Haug, 1993; Martínez-Blanco et al., 2010; Scotti et al., 2016).

The compost can be applied to the soil, with amending and fertilizing purpose (Naeini & Cook, 2000; Scotti et al., 2016) and to recover degraded soils (Scotti et al., 2016); to exert plant disease suppressiveness (Pane et al., 2013; De Corato et al., 2019; Pane et al., 2020); to sequester carbon into the soil thus reducing global warming (Favoino & Hogg, 2008); to reduce production costs and negative impacts of agricultural activities by limiting inputs of fertilizers and pesticides (Martínez-Blanco et al., 2009b; Sánchez et al., 2017; Vázquez & Soto, 2017).

This paper addresses the most used composting methods currently used, the typology of composting materials and the benefits of the composts obtained from them. It also addresses the co-composting of waste from farms with medicinal plants to increase the compost suppressiveness against plant pathogens.

The purpose of this study is to support a series of experimental research on the co-composting of aromatic and medicinal plant residues with different residues from farm to obtain a product (compost) with significant suppressive properties to be used in ecological agriculture.

Table 1. Waste generation excluding major mineral wastes during 2004-2020 period (million tons)

Waste generation excluding major mineral wastes			
Period	2004	2020	2020/2004
Total UE	780.52	775.21	-0.99
Agriculture UE	62.34	20.73	-0.33

Source: Eurostat (env_wasgen)

Table 2. Waste treatment by category in EU during 2004-2020 period (%)

	Recovery (recycling + backfilling + energy recovery)		Disposal (landfilling + incineration + others)	
Time	2004	2020	2004	2020
Total UE	38.70%	54.08%	45.66%	37.45%

Source: Eurostat (env_wastrt)

Table 3. Treatment of waste excluding major mineral wastes in EU during 2010-2020 period (million tons)

Treatment of waste excluding major mineral wastes			
Period	2010	2020	2020/2010(%)
Waste treated UE	638.77	633.80	-0.99
Waste recycled UE	325.37	357.37	1.10
Waste recycled from agriculture UE	53.73	69.36	1.29

Source: Eurostat (env_wasoper)

MATERIALS AND METHODS

This study will review the relevant and current literature regarding composting methods, the material used for composting, and the impact that compost has on plant health, all of this in the context of the current legislation. For this, we pursue two objectives:

1. understanding the targets set by the EU to combat climate change and the actions taken to reach the targets set for the agriculture sector;
2. providing an overview on the benefits of compost, a solution for many difficulties faced by farmers.

To achieve our study objectives, we used the following methodology:

- European directives, regulations and communications related to waste management, circular economy and plant protection were searched and studied, as well as reports submitted by MS;
- a number of 22 keywords were established (“compost”, “co-composting”, “composting”, “bio waste”, “organic waste”, “green waste”, “waste”, “home composting”, “industrial composting”, on farm composting”, “aromatic plants”, “herbs”, “manure”, “lavender”, “*Lavandula*”, “pesticides”, “circular economy”, “greenhouse gas emissions”, “suppressiveness”, “plant pathogens”, “organic fertilizer”, “climate change”), and were used to search for scientific publications (original articles and reviews, as well as books) in international databases (Web of Sciences, Scopus, Google Scholar);
- a series of statistical data were collected from Eurostat databases and public reports;
- other free access papers.

Once the searching process was completed, the documents were screened, and the most relevant ones were selected to be further analyzed and studied, and following this, the information and data that satisfied the proposed objectives were retained.

RESULTS AND DISCUSSIONS

Composting methods

There are many methods of composting which can be applied either at home, in a specialized plant or at farm location. The material and location chosen for the composting dictates the method needed. Ayilara et al. (2020) enumerated some of the most common methods such as: in-vessel composting, window composting, vermicomposting, static composting, Berkley rapid composting, Indian Indore composting.

Home composting is considered the self-composting of the biowaste as well as the use of the compost in a garden belonging to a private household (EC, 2009) and it presents some potential benefits such as: avoiding the collection and transportation of the organic fraction of the municipal solid waste, which is translated in the reduction of economic, material, and energetic investments (McGovern, 1997; Ligon, & Garland, 1998; Boldrin et al., 2009; Storino et al., 2016; Vázquez & Soto, 2017), it allows a direct control of the process and the organic materials input (Martínez-Blanco et al., 2009a; 2010; Barrena et al., 2014) and it helps with the reduction of household waste and resource recovery (Cheng et al., 2022), reduction which is important especially in the countries where the collected organic waste is higher than the treatment capacity (Sulewski et al., 2021).

Considering the environmental aspects, home composting presents some issues: compost obtained is not always homogeneous and its quality cannot be known, odors and other pollutants such as methane, ammonia or nitrous oxide are emitted directly to the atmosphere due to the absence of gas treatment systems. (Amlinger et al., 2008; Martínez-Blanco et al., 2010; Barrena et al., 2014).

Industrial composting is one of the technologies environmentally friendly used mostly for the disposal of the organic fraction of the municipal solid waste (Haug, 1993; Martínez-Blanco et al., 2010; Andersen et al., 2012). It is also the most suitable way to compost polylactic acid (PLA) and polyhydroxy butyrate (PHB), compostable bio-thermoplastic blends which need elevated temperatures maintained for a long timeframe to be properly decomposed (Mistry et al., 2023; Fogašová et al., 2022). In the industrial composting plants the composting parameters (temperature, humidity, and aeration), the quality of the final product and the GHG emissions can be controlled (Peng et al., 2022; Haug, 1993). However, it requires separate collection of the urban waste, it implies energy consumption for transportation and processing (Haug, 1993) and the compost resulted is not always a high-quality product, due to heavy metals content (Barrena et al., 2014; Siles-Castellano et al., 2021).

On-farm composting is an efficient, environmentally safe, and cost-effective technology for recycling and valorization of the agricultural wasted (Scotti et al., 2016). Benefits of these composts could include: their ability to mediate soil-borne plant pathogen suppression (Pane et al., 2013; Scotti et al., 2016), increase the soil fertility (Scotti et al., 2015), and reduce the CO_2 emission (Altieri & Esposito, 2010; Pane et al., 2020).

Authors described on-farm composting as a strategic tool for agriculture solving the issue of disposal of crop residues and livestock waste and serving as an organic amendment which can improve the soil quality and plant health (Scotti et al., 2016; Pergola et al., 2018; De Corato, 2020b).

Preparation procedures and evaluation techniques of the compost

A high-quality compost is critical. Therefore, some authors directed their attention on the evaluation of the compost, others focused on the preparation procedures.

Preparation procedures of compost can affect the properties of compost and its suppressiveness. Research carried out on composts resulted from escarole (*Chicorium endivia* L.) processing mixed with chopped parts of cardoon (*Cynara cardunculus* L.), that were used as a bulking agent, show that the composts which were passively aerated and forced ventilated presented higher suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor* than the compost which was turned manually (Pane et al., 2019).

Bedolla-Rivera et al. (2022) directed their attention on the evaluation of the quality of the compost by developing new calculation formulas for the evaluation indexes providing an easy-to-interpret tool to measure the quality of the composts.

Other author studied how using machine learning (ML), a subset of AI can optimize the process, predict missing data, detect non-conformities, and can manage complex variables (Temel et al., 2023). ML can work with complex multivariate data, predict non-linear connections, and process missing data (Manley et al., 2022). Modeling composting process parameters is very significant in generating solutions and decision-making processes. Therefore ML can be used for higher accuracy of optimizing the processing parameters for improving compost quality and maturity (Xue et al., 2019; Dümenci et al., 2021; Ding et al., 2022; Yılmaz et al., 2022; Wan et al., 2022), monitor moisture content in industrial-scale composting systems (Moncks et al., 2022), estimate the enzymatic activity of compost (Chakraborty et al., 2014) and predict CO_2 output from the composting of feedstock manure (Li et al., 2022).

As any new technology, ML and AI algorithms have their challenges which can be studied more in the future.

Suppressive properties of aromatic and medicinal plants

There are very few studies on composts that were made from materials that included aromatic and medicinal plant residues. That is why, in this paper, we have included a chapter on the suppressive properties of the oils extracted from such species.

Considering that the soil-borne pathogens cause plant disease which have a negative impact on the agricultural productivity overall (De Corato, 2020a), some scientists have been focusing on understanding what activates the natural suppressiveness of the soil (Wei et al., 2020; De Corato, 2023; Yang et al., 2023) and how this can be improved throughout organic fertilizers such as composts (Sommermann et al., 2022; De Corato, 2020a; Ayilara et al., 2020), others have explored the possibility of controlling the pathogens using essential oils (EO) from aromatic and medicinal plants (Table 4).

Types of waste and by-products

Many authors have studied various recipes of composts trying to reintegrate in the economy different types of waste and by-products, while analyzing the benefits of the composts on the environment, soil, and plant health (Table 5).

Most of the waste produced buy agriculture is compostable waste, with animal or plant origin, such as: livestock manure, slaughtered animals, residues from plant processing, pruning biomasses, residues from distilled plants, or waste from biogas.

Considering the variety of residues available nowadays, the scientists are trying to learn more about the agricultural potential of each type of waste while using different composting methodologies.

Table 4. Properties of aromatic and medicinal plants extracts and essential oils against soil-borne organisms

Target Organism	Plant	Effect	Reference
Fusarium oxysporum f. sp. *ciceris* Padwick	*Thymus pallescens*, *Artemisia herba-alba*, *Laurus nobilis*, *Cymbopogon citratus* (de Candolle ex Nees) Stapf.	Inhibition of mycelial growth, sporulation, and spore germination	Moutassem et al., 2019
Verticillium dahlia Kleb.	*Mentha piperita* L., *Thymus vulgaris* L., *Lavandula angustifolia* Mill.	Inhibition of mycelial growth	Erdogan et al., 2016
Meloidogyne incognita, *Meloidogyne javanica*	*Orginum vulgare*, *Thymus citriodorus*	Nematostatic/nematicidal activity (paralysis of second-stage juveniles)	Ntalli et al., 2020
Penicillium citrinum	*Satureja thymbra*, *Rosmarinus officinalis*	Suppression of spore germination	Vokou et al., 1984
Mucor hiemalis	*Satureja thymbra*	Complete inhibition of mycelial growth	Vokou et al., 1984
Penicillium expansum	*Mentha spp.*	Inhibition of mycelial growth	Benomari et al., 2018
Alternaria alternata, *Aspergillus* spp., *Penicillium* spp., *Trichoderma viride*, *Cladosporium cladosporioides*, *Phomopsis helianthi*	*Origanum onites* L., *Salvia thymbra* L.	Strong antifungal activity against the microorganisms tested	Sokovic et al., 2002
Verticillium dahliae, *Fusarium oxysporum* f. sp. *lycopersici*, *Sclerotinia sclerotiorum*, *Pythium* spp.	*Origanum* spp.	Inhibition of mycelial growth	Wogiatzi et al., 2009
Phytophthora spp.	*Rosmarinus officinalis*, *Lavandula angustifolia*, *Lavandula spicata*, *Salvia officinalis*	Control of zoospore germination	Widmer et al., 2006
Fusarium oxysporum f. sp. *Cyclaminis*	*Salvia officinalis* L.	Suppression of fungal pathogen, reduction of disease indices in cyclamen roots and shoots	Ahmad et al., 2020

Table 5. The benefits of different composts

Composted materials	Purpose	Benefits	Reference
Steam distilled pruning residues from *Ocimum basilicum* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and old commercial urban-waste compost	Improving sustainability	Improve plant production	Zaccardelli et al., 2021
Gentamicin mycelial residue (GMR) and rice chaff	Recycling the residue of GMR	Improve plant growth	Bu et al., 2022
Biosolids resulted from urban wastewater treatment, bovine manure, and rice husks	Disposal of the biosolids resulted from urban wastewater treatment	Restoration of degraded soils	Bedolla-Rivera et al., 2022

Composted materials	Purpose	Benefits	Reference
Fresh sheep manure and cornstalks	Reduce gas emissions	Reducing the gas emissions	Li et al., 2022
Olive mill waste, wool waste, and wheat straw	Recycling the olive mill waste	Improve plant production	Altieri & Esposito, 2010
Swine manure and rice straw	Disposal of livestock manure	Improve plant growth	Qian et al., 2014
Dairy manure and rice straw	Disposal of livestock manure	Improve plant growth	Qian et al., 2014
Chinese medicinal herbal residues, food waste, and sawdust	Suppression of plant pathogens	Suppressiveness against *Alternaria solani* and *Fusarium oxysporum*	Zhou et al., 2016
Agricultural residues and pruning biomasses from leafy vegetables, fennel, and woodchips	Suppression of plant pathogens	High suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor*	Pane et al., 2020
Agricultural residues and pruning biomasses from leafy vegetables, basil, tomato, watermelon, and woodchips	Suppression of plant pathogens	High suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor*	Pane et al., 2020
Agricultural residues and pruning biomasses from leafy vegetables, basil, watermelon, and woodchips	Suppression of plant pathogens	High suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor*	Pane et al., 2020
Agricultural residues and pruning biomasses from leafy vegetables, basil, watermelon, and woodchips	Suppression of plant pathogens	Suppressiveness against *Rhizoctonia solani*	Pane et al., 2020
Agricultural residues and pruning biomasses from leafy vegetables, basil, and woodchips	Suppression of plant pathogens	High suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor*	Pane et al., 2020
Agricultural residues and pruning biomasses from leafy vegetables, basil, and woodchips	Suppression of plant pathogens	Suppressiveness against *Rhizoctonia solani*	Pane et al., 2020
Agricultural residues and pruning biomasses from leafy vegetables, artichoke, and woodchips	Suppression of plant pathogens	High suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor*	Pane et al., 2020
Agricultural residues and pruning biomasses from leafy vegetables, cabbage, walnut husk, and woodchips	Suppression of plant pathogens	High suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor*	Pane et al., 2020
Agricultural residues and pruning biomasses from leafy vegetables, basil, sorghum, tomato, pumpkin, and woodchips	Suppression of plant pathogens	High suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor*	Pane et al., 2020
Processing leftovers and unmarketable biomaterials of escarole (*Chicorium endivia* L.), chipped aboveground parts energy cardoon (*Cynara cardunculus* L.), and mature compost	Suppression of plant pathogens	High suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor*	Pane et al., 2019
C1 = composted 17.5% tomato residues, 15.5% escarole residues, 65% woodchips and 2% mature compost starter	Suppression of plant pathogens	High suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor*	Pane et al., 2013
C2 = composted 50.0% tomato residues, 48% woodchips and 2% mature compost starter	Suppression of plant pathogens	High suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor*	Pane et al., 2013
C3 = 78.0% artichoke residues, 20% woodchips and 2% mature compost starter	Suppression of plant pathogens	High suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor*	Pane et al., 2013
C4 = composted 43.5% artichoke residues, 23.5% fennel residues, 11.0% escarole residues, 20% woodchips and 2% mature compost starter	Suppression of plant pathogens	High suppressiveness against *Rhizoctonia solani* and *Sclerotinia minor*	Pane et al., 2013
Peat and compost (a mixture of lignocellulosic tree and grass cuttings and the	Suppression of plant pathogens	Suppressiveness against *Pythium ultimum* and	Loffredo & Senesi, 2009

Composted materials	Purpose	Benefits	Reference
organic fraction of municipal solid wastes)		*Fusarium oxysporum* f. sp. *callistephi*	
Coconut fiber and compost (a mixture of lignocellulosic tree and grass cuttings and the organic fraction of municipal solid wastes)	Suppression of plant pathogens	Suppressiveness against *Pythium ultimum* and *Fusarium oxysporum* f. sp. *callistephi*	Loffredo & Senesi, 2009
Composted defatted olive marc and fennel green waste	Suppression of plant pathogens	Suppressiveness against *Verticillium dahliae*	De Corato et al., 2019
Composted un-defatted olive marc and artichoke green-waste	Suppression of plant pathogens	Suppressiveness against *Verticillium dahliae*	De Corato et al., 2019
Composted spent coffee ground with green wastes of celery and carrot	Suppression of plant pathogens	Suppressiveness against *Verticillium dahliae*	De Corato et al., 2019
Composted spent tea bags with green wastes of tomato and lettuce	Suppression of plant pathogens	Suppressiveness against *Verticillium dahliae*	De Corato et al., 2019
Composted wood chips with green wastes of tomato and escarole	Suppression of plant pathogens	Suppressiveness against *Verticillium dahliae*	De Corato et al., 2019
Composted aspen chips with green wastes of artichoke and fennel	Suppression of plant pathogens	Suppressiveness against *Verticillium dahliae*	De Corato et al., 2019
Composted vineyard pruning wastes, vinery residues and wheat straw with green wastes of potato and pepper	Suppression of plant pathogens	Suppressiveness against *Verticillium dahliae*	De Corato et al., 2019
Composted tomato-waste	Suppression of plant pathogens	Suppressiveness against *Verticillium dahliae*	De Corato et al., 2019
Vermicompost produced from wastes of menthol mint (*Mentha arvensis*), chamomile (*Matricaria recutita*), geranium (*Pelargonium graveolens*), qinghao (*Artemisia annua*) followed by pyrethrum (*Chrysanthemum cinerariaefolium*), isabgol (*Plantago ovata*), African marigold (*Tagetes minuta*), Boerhavia (*Boerhavia diffusa*), mustard (*Brassica compestris*), lemongrass (*Cymbopogon flexuosus*) and garden mint (*Mentha viridis*)	Suppression of plant parasites	Inhibition in hatching of eggs of *Meloidogyne incognita* (root-knot nematode) and root-knot disease development	Pandey & Kalra, 2010

Co-composting of lavender with manure

A large quantity of bio waste consists in residual biomass resulted from the agricultural and industrial processes. The sector of aromatic and medicinal plants generates various kinds of residues like residual biomasses from distillation of aromatic herbs and non-utilized parts of medicinal plants (Santana-Meridas et al., 2012; Zhang et al., 2017; Saha & Basak, 2020; Wang et al., 2021; Zaccardelli et al., 2021; Li et al., 2022). Many of these residues are considered environmental liabilities if they are not ecologically recycled (Liang et al., 2011; Saha & Basak, 2020), but left on the field or incinerated (Lesage-Meessen et al., 2018). These residues still contain great nutrients and natural bioactive compounds such as cellulose, proteins, flavonoids, polysaccharides, organic acids (Su et al., 2018; Ni et al., 2020; Li et al., 2022), since the efficiency of the extraction of the EO is around 50% (Zhou et al., 2016; Greff et al., 2021b). Therefore, Lesage-Meessen et al. (2018) have studied the distilled straws of lavender and lavandin which proved to contain volatile molecules, usually found in the EO, and non-volatile phenolic compounds and flavonoids, as well as fungal enzymes involved in the degradation of lignocellulosic biomass.

Studies regarding the antifungal activity of lavender extracts and EO, proved that it can inhibit the mycelial growth of the fungus *Verticillium dahliae* Kleb. (Erdogan et al., 2016), or it can control of zoospore germination of *Phytophthora* spp. (Widmer et al., 2006).

Considering the previews scientific acquisitions, distilled straws of lavender might be a valuable component for co-composting, since plenty of fungal plant pathogens can survive during composting process by prolifically producing spores (Greff et al., 2021b; Chen et al., 2022; Liu, 2023), even though during composting most of the pathogens can be eliminated due to higher temperature through the decomposition (Wichuk et al., 2011; Liu, 2023).

Greff et al. (2021a) investigated the composability of post-extraction lavender waste by co-composting lavender with cattle manure, and González-Moreno et al. (2022) used a compost made of mature horse manure and lavender waste to create good quality vermicompost. Up to now, little information can be found about the benefits of co-composting lavender with sheep manure.

In the last 10 years, in Romania, the interest of small farmers for the cultivation of lavender in the "bio" system as well as for lavender oil production increased. Also, considering that sheep manure is a valuable resource of nutrients, and it is rich in organic matter, nitrogen, and phosphorus and it can be transformed into high-quality organic fertilizer (Ravindran et al., 2019; Li et al., 2022). In addition, since there is no other option for the elimination of lavender residues resulting from its processing to obtain the oil, our future research will focus on co-composting lavender residues with sheep manure. Besides the composting process, the quality of the compost, its agronomic value, etc., its antipathogenic effect will represent an objective to be studied.

CONCLUSIONS

The composting process is a complex process which can be the solution for many environmental issues we are facing nowadays. Our study showed that disposal of organic waste through composting it's a good strategy because of its low investment and operation costs, high benefits socially and environmentally and generation of a final product which can be used as fertilizer.

Even though there are studies which demonstrate the suppressive character of the compost, the use of compost as a suppressive agent has not been extended due to its complexity which was not entirely understood, therefore more studies should be conducted in this direction.

There are various materials that can be composted (co-composted), however less authors directed their attention on lavender waste and the benefits which this might bring.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

PEA CROP DISEASES - AN OVERVIEW

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Abstract

The pea (Pisum sativum L.) represents one of the most important leguminous crops worldwide, being in the top 10 vegetable crops, belonging to the Fabaceae family. Peas are grown both for human consumption, fresh or canned, and for animal feed in the dry state. The pea crop is affected by an important number of pathogens, which in favorable conditions can significantly decrease both the yield and the quality of the grains, even leading to total losses. Fungi, bacteria and viruses can cause a number of foliar diseases in peas. The most important pathogens that cause significant economic damage are: Didymella pinodes, Neocosmospora pisi, Pea enation mosaic virus¹, Peronospora pisi, Uromyces pisi, Pseudomonas pisi. Pea enation mosaic virus¹ can cause severe loss of pea harvest by up to 50% and Neocosmospora pisi leads to a decrease in yield by 15-60%. The Ascochyta blight disease complex can decrease the yield with values ranging from 10-60%. This bibliographic review provides an overview of recent studies on the main pathogens of pea crops.

Key words: *control, pathogens, Pisum sativum.*

INTRODUCTION

The pea (*Pisum sativum* L.) is native to the Middle East and is the oldest cultivated plant in the world (Zeven & Jukovski, 1975). Peas leave the soil enriched in nitrogen, having the ability to fix molecular nitrogen from the atmosphere (Popescu&Roman,2008), through nitrogen-fixing nodules following the symbiosis with bacteria of the genus Rhizobium, thus contributing to the improvement of soil fertility (Matsumiya et al., 2013). Production of dried peas has expanded in developed countries such as France, Canada and Australia, where it is used for protein supplements. Varieties of peas grown for processing shall be harvested when they are green and juicy and dried peas shall be harvested when the seeds reach a moisture content of 12% or less. Fresh peas are often grown in rotation with other vegetables. Pea is usually the first crop sown in the spring, and is therefore often planted on cool, wet soils (Grunwald et al., 2004). In Romania, the areas cultivated with peas are somewhat reduced, compared to other countries of the world. According to the latest FAO data, the area of peas cultivated in Romania increased in 2020, registering 94,360 thousand/ha compared to 2015 when 31,056 thousand/ha were cultivated. The seed is an important means of disease transmission to plants (Berca et Cristea, 2015; Dudoiu et al., 2016; Zaharia et al., 2022). Disease transmission through pathogen-bearing seeds also involves proper management of pathogen control (Couture et al., 2002). Fungi and bacteria can cause major foliar diseases of pea crops. Pathogenic fungi cause significant losses on the pea crop. Among the most important diseases caused by fungal pathogens we list: Downy mildew (*Peronospora pisi*), *Ascochyta* blight (*Didymella pinodes*) and rust (*Uromyces pisi*) (Grunwald et al., 2004).

PEA PATHOGENS

1. Ascochyta blight complex, is one of the main diseases affecting field pea production and can be caused by several pathogens of the genus *Ascochyta* (Tivoli & Banniza, 2007). The main pathogen that causes ascocytosis was initially named *Mycosphaerella pinodes* (Berk.

Et Blox) Nissel, the anamorph or conidial form *Ascochyta pinodes* (Br. Et Bl.) but Peever et al. (2007) proposed that the name *Didymella* be used because Internally Transcribed Spatial Region (ITS) DNA sequences are clustered with *Didymella* species and not with *Mycosphaerella* species. ***Didymella pinodes*** is the most widespread causative pathogen and the most damaging. In Australia, other *Phoma* species have also been shown to be pathogens of peas, including *Phoma koolunga* (Davidson et al., 2009), *Phoma herbarum* (Li et al., 2011) and *Phoma glomerata* (Tran et al., 2014), associated with the *Ascochyta* disease complex. All these pathogens can occur together in a pea crop, even on a single plant (Hare & Walker, 1944). Disease-causing fungi can be spread via seeds, but this is a minor source of infection compared to spores released from plant residues of the previous crop (Bretag et al., 2006). Spores can be carried by wind several kilometers, and once the crop is infected, the current plant lesions act as a secondary source of spores for further spread.

Symptoms. The disease manifests itself on all aerial organs of the plant: leaves, stems and pods. On plants that have just emerged, the disease makes its presence felt on the leaves, circular spots appear, dark brown in color, being basically isolated. On the stem and petiole, the spots are deep in the tissues and arranged longitudinally, showing a dark brown color with a dark and slightly raised edge. The characteristic form of manifestation of the disease appears on the pods, showing circular or irregular spots, confluent or isolated, light brown, outlined with a reddish border. If the infection occurs later, after the formation of the grains, the mycelium of the fungus also reaches the seeds, the disease manifesting itself in the form of dark or light yellow spots with a diffuse border. Next to the attacked tissues, small light brown or blackish dots appear during the vegetation period, which represent the pycnidia of the fungus. On the remains of diseased plants, the perithecia of the fungus are formed on the leaves over the winter, in the form of small black dots. *Didymella pinodes* can infect newly emerged seedlings and all above-ground parts of adult pea plants, causing seedling root rot, necrotic spots on leaves, lesions and blackening of stem base, and dark brown discoloration of seeds (Ahmed et al., 2015).

Life cycle. Transmission from one year to another occurs via infected seeds and plant debris. Dissemination of the fungus during the vegetation period is done by pycnospores and ascospores, carried by air currents. The primary inoculum (asexual conidia or ascospores) of all causative pathogens is spread by wind and rain on newly emerged crops (Carter & Moller, 1961). Only *D. pinodes* is known to produce ascospores (Punithalingham & Holliday, 1972), which develop from pseudothecia on infested stubble or senescent plant material, whereas all causative pathogens produce conidia. Regardless of the type of inoculum, the initial infection of the leaves results in small purple or black spots, which spread in wet conditions, leading to the death of the leaves (Roger & Tivoli, 1996b). Pycnidia develops in the resulting lesions and conidia is spread to neighboring plants (Schoeny et al., 2008). The conidial mode of spread increases disease severity more at the base of the plants than in the mid or upper parts of the plants (Tivoli et al., 1996). In Australia for example, conidia are considered of minor importance (Bretag et al., 2006) and ascospores are the main factor in the secondary spread of *D. pinodes*. Ascospores are produced in pseudothecia when infected leaves and stems become senescent and are forced into the air when moisture conditions are met. Ascospores are rapidly spread throughout the crop and subsequent precipitation promotes rapid infection, increasing disease severity (Roger & Tivoli, 1996). Stem infection may begin at the soil line and spread upward, with lesions often coalescing on the stem. When fungi develop on the petiole of an infected leaf, a stem lesion may begin at the base of the dead leaf, advancing above and below that point. Subsequent lesions eventually coalesce, surrounding the stem (Hare & Walker, 1944; Tivoli et al., 1996). Infection of the flowers causes them to dry up and drop, while infection of the pods causes them to become distorted and may drop. This effect may be transient and is often unobserved (Hare & Walker, 1944).

Primary air-borne inoculum. Moisture is an important factor in the release of ascospores. When the pseudothecia becomes moist, the asci enlarges and ruptures at the tip, releasing all the ascospores simultaneously into the air (Carter 1963; Hare & Walker, 1944). The timing of ascospore counts was analyzed and showed that dew is effective in causing ascospore release, with the highest numbers occurring during periods of rainfall (Carter & Moller, 1961; Carter, 1963). A diurnal spore release experiment was noted, with a peak in the late afternoon and a trough in the middle of the night (Bretag & Lindberck, 2006; Carter, 1963).

Soil-borne inoculum. Pathogens, with the exception of *A. pisi,* survive in the soil for many years, and grain yield was strongly correlated with the amount of fungus (*Ascochyta blight*) present in the soil (Bretag & Ward, 2001). This source of inoculum is particularly important in field pea growing areas, where inoculum accumulates in the soil if the pea has been grown in succession (Wallen & Jeun, 1968). Pathogens survive as chlamydospores, mycelium or sclerotia. *D. pinodes* is also a moderate saprophyte (Dickinson & Sheridan, 1968; Sheridan,1973), have shown an increase in detectable levels in soil in the 6-12 months after harvest, after an initial decrease in the first 6 months. Burying infested stubble decreased survival time below 12 months (Davidson et al., 1999; Sheriden, 1973; Zhang et al., 2005) possibly by preventing saprophytic growth by depleting oxygen and microbial activity.

Seed-borne inoculum. All pathogens can infect seeds (Kraft et al., 1998; Maude, 1996). Surface sterilization of seeds resulted in a decrease in *D. pinodes* infection from 60% to 18%, leading to the conclusion that most of the time the pathogen is carried on the seed coat (Bathgate et al., 1989). High seed infection is influenced by several factors, namely spring rainfall that disperses the inoculum, early sown crops that are exposed to large numbers of airborne ascospores, but also later harvested crops that are more likely to be exposed to the inoculum secondary (Bretag et al., 1995). Seed lots grown in areas with low rainfall, less than 350 mm per year, can be free from pathogens, making these areas suitable for seed production (Bathgate et al., 1989). Under controlled conditions transmission of pathogens from seed to the basal parts of the plant is frequent, for example 40% for *A. pisi* and 100% for *D. pinodes*, leading to death of young plants (Maude, 1966; Xue and Warkentin, 2001). However, the disease does not spread to the upper parts of the plant, suggesting that seed infection is not an important source of inoculum for an *Ascochyta blight* epidemic (Bretag et al., 1995; Moussart et al., 1998).

Secondary inoculum. During the growing season, both pycnidia and pseudothecia have been observed on the same plant organs, pycnidia are produced on both green and senescent plant organs, while pseudothecia appear only on senescent parts, appearing just before flowering. Discharge of both types of spores is initiated by precipitation or dew, so that epidemics are more severe in humid conditions (Roger & Tivoli, 1996). When ascospores are produced, the disease spreads rapidly to the top of the plant crown. The greatest damage caused by *Ascochyta blight* is produced by this secondary spread of ascospores (Bretag, 1991; Hare & Walker, 1944). In *D. pinodes* pseudothecia can appear 18 days after the appearance of lesions. Because they mostly form on senescent stems, the greatest number of pseudothecia are found on organs with senescent tissues. The number of ascospores remains relatively low until the end of the season, when pseudothecia develop on senescent material, and then the number of ascospores can triple (Roger & Tivoli, 1996). Pycnidia can form within 11 days from the onset of symptoms and increase in number to the end of the vegetative cycle of the crop. Roger and Tivoli (1996) found that pycnidia formed when lesions covered approximately 25% of the leaf surface.

Infection process. Conidia of *D.pinodes* germinate with one or more germ tubes, which frequently branch and form appressor-like structures on the leaf and cotyledon surface 6 h after inoculation (Clulow et al., 1991; Roger et al., 1999). Penetration occurs through epidermal walls 8 h after inoculation (Nasir et al., 1992; Roger et al., 1999), not through stomata, and then an infection vesicle is formed, located partly in the epidermal wall and partly in the cell lumen. From this the penetrating hypha arises and then initiates intra and intercellular hyphae (Nasir et al., 1992). Penetration occurs within 24 hours of inoculation with cell wall degrading enzymes (Heath & Wood, 1969; Roger et al., 1999) and rapid colonization by *D. pinodes* is soon followed by tissue collapse (Heath & Wood, 1969) in both resistant and susceptible genotypes (Nasir et al., 1992). In resistant types, the formation of infection vesicles and penetration hyphae is reduced, and the development and spread of lesions is delayed (Nasir et al., 1992). Symptoms may appear within 24 hours of inoculation and consist of brown spots, 2 mm in diameter, which continue to grow and coalesce, leading to death of the entire leaf, and pycnidia may form within 3 days (Heath & Wood, 1969; Roger et al., 1999). Symptoms on the cotyledon are visible only after 4 days after inoculation (Clulow et al., 1991). Increasing inoculum concentration increases disease severity by increasing the rate of lesion expansion (Heath & Wood, 1969; Roger et al., 1999) but inexplicably does not affect lesion number (Heath & Wood, 1969). The specialized literature indicates that there has been no clarification of this aspect since the study was carried out.

Detection and Quantification. Over time, several studies have been reported on the *D. pinodes* infection process on resistant and susceptible pea lines (Heath & Wood, 1969; Clulow et al., 1991; Nasir et al., 1992). Nasir et al. (1992) showed that the infection process of D.pinodes on pea leaves started with the germination of conidia and the formation of one or more germ tubes which later ramified and formed appressor-like structures on the surface the leaf. Subsequently, vesicle-like infection structures were formed, which formed penetrating hyphae and developed a network of hyphae with intra- and intercellular growth in the pea tissues. However, Clulow et al. (1992) showed that 32 h after inoculation, infection by *D. pinodes* on the leaf could occur without apressor formation by direct penetration of the cuticle through the germ tube tips. *D. pinodes* infection on the epicotyl, for a period of 30h after inoculation on susceptible pea lines, most germ tubes produced one appressorium that penetrated the cuticle (Clulow et al., 1992). Colonies on the nutrient substrate are generally light gray to almost dark gray in color, the pseudothecia and pycnidia are distributed along mycelial rays growing from the central point. After 20-30 mm growth, the pseudothecia and pycnidia become arranged in concentric rings in response to a 12 h photoperiod (Onfroy et al., 1999). Pycnidia production is high in light and decreased at low temperatures (Hare & Walker, 1944). On the stems, the pycnidia have a diameter of 100-200 μm. Conidia are hyaline, 1 or occasionally 2 septate, slightly constricted at sep and 8-16 x 3-4.5 μm (Punithalingham & Holliday, 1972). *D. pinodes* produces dark brown, globular pseudothecia with papillate ostioles 90 x 180 μm in diameter. As a general rule, pseudothecia will develop on poor or minimal media, while pycnidia are more likely to develop on highly nutritious media, although significant variability occurs among tupins (Hare & Walker, 1944; Roger & Tivoli, 1996). *D. pinodes* can produce pseudothecia on malt agar, Mathur agar medium, oatmeal agar medium. The favorable temperature for pseudothecia development and maturation was at 16°C, decreasing at 20°C, rare at 24°C and 28°C, and nonexistent at 30°C. From 12°C to 4°C, the same number of pseudothecia, but time to maturity increased from 35 to 100 days at lower temperature. At 16°C pseudothecia developed in 25-30 days (Hare & Walker, 1944).

Management. Cultivation of resistant varieties, use of healthy seed, crop rotation (3-4 years), collection and destruction of infected plant residues, deep plowing, weed control, seed treatment with specific recommended products, specific control methods. Unfortunately, sources of resistance to *Didymella pinodes* fungi are very limited, and pea varieties highly resistant to this disease have not yet been developed. Delayed seeding by 3-4 weeks reduces *D. pinodes* severity by more than 50%, however, such measures are not suitable at higher altitudes due to the shorter growing season. Crop management is the preliminary option to control the progress of the disease by minimizing the repainting of the inoculum, as well as the survival of the inoculum on plant residues and in the soil by avoiding reinfection. Burying infected residues also decreases the survival of pathogens. Integrating two or more control methods increases the chances of success (Jeger, 2004; Mc Donald & Peck, 2009). Jha et al. (2019) conducted research by applying foliar fungicides such as Hexaconazole @ 0.1% which was applied 2 times at 15 day intervals, this being the most effective in reducing the severity of *Ascochyta blight* up to 10.65% compared to the control (42.08%). The percent reduction in the incidence of the disease compared to the control after the application of the fungicide was 74.69%. In case of treatment with carbendazim 0.1%, the disease incidence was reduced to 12.74% compared to the control. In research conducted by Liu et al. (2016), fungicides based on tebuconazole, boscalid, iprodione, carbendazim and fludioxonil were found to be more than 80% effective in controlling the disease. Also, 3 biocontrol strains of *Bacillus* sp. and one of *Pantoea agglomerans* significantly reduced disease severity under greenhouse and field conditions (Liu et al., 2016). Other fungicides have been used to effectively control ascochyta blight but also to increase yield such as: mancozeb, chlorothalonil, thiabendazole (Xu and Warkentin, 1996; Bretang et al., 2006). However, the application of fungicides may increase production costs, and may also pose a risk to the environment due to diversion to other non-target areas. In addition, intensive application of fungicides can lead to strains that are resistant to commercial chemicals (Liu et al., 2016). Limitations on fungicide application have prompted the exploration of safer and more environmentally friendly biological control measures against *Ascochyta blight*. The bacterial antagonists *Pseudomonas fluorescens*, *Bacillus* spp., and *Serratia* spp. significantly reduced the severity of *Ascochyta blight* under greenhouse conditions (Wang et al., 2003). The mycoparasite *Clonostachys althaea* strain ACM941 represented an effective bioagent in the control of root rot complex caused by *A. pinodes* (Xue, 2003). The role of individual pathogens in the *Ascochyta* complex must also be understood in order to develop successful management practices that target all pathogens involved in this disease.

2. Downy mildew caused by the pathogen *Peronospora viciae* f. sp. *pisi* (Sydow) Boerema & Verhoeven (family Peronosporaceae, ord. Peronosporales). The pathogen exhibits long, thin, hyaline sporangiophores, ramified dichotomously in the upper third. The stegrims are short, divergent, unequal, slightly pointed at the tip. The sporangia are ovoid, yellowish, 22-27 x 1519 µm. Downy mildew leads to high yield and pod quality losses in pea crops. Peas are affected by a number of diseases, but downy mildew can be devastating in cool, wet conditions.

Symptoms. Downy mildew symptoms in peas can be local or systemic (Bathula and Singh, 2022). Systemic infection is the most severe form of the disease and usually results in stunting and stunting of plants leading to their death before flowering. Downy mildew usually appears on peas in the early stages of plant growth or when the weather is cool and wet. Diseased plants may show symptoms characterized by lack of vigor, small size, wilting and eventually death of the plants. In already developed plants, leaves appear, from the base of the plant, with chlorotic spots at first and later brown, usually located at the edges and covered on the underside with gray or purple powders (Melgarejo et al., 2010). Infected leaves turn yellow and die if the weather is cold and wet.

Life cycle. The fungus survives in the soil, on plant debris and in seeds. The disease is influenced by climatic conditions, namely continuous drizzle or days in which fog persists for more than 12 hours with temperatures between 15-20°C.

In these conditions, abundant conidia appear that infect the plants. From 20°C the disease stops evolving. The pathogen produces abundant inoculum in the form of sporangia on the surface of infected plants. Foliar infections are usually local and start on the underside of the leaf. The pathogen can infect and sporulate also on inflorescences and tendrils. Pod infection can occur in relatively high humidity conditions even in the absence of foliar infection. Infected pods become deformed and blister the surface. The optimal conditions for the development of downy mildew are cold and wet weather. Secondary spread of the disease occurs only through sporangia (Bathula and Singh, 2022). Systemic infection is the result of direct infection of the upper meristem. This type of infection is most common in cultivars with reduced stipule size (Matthews & Dow, 1983; Taylor et al., 1990). Local infections on leaves develop from conidia present on the plant surface (Mence & Pegg, 1971). Severe infections can lead to general plant deformity, which can lead to early plant death. *Peronospora pisi* can often go undetected by remaining asymptomatic until a 12-hour period occurs with at least 90% relative humidity, favoring an environment conducive to disease expression. This can lead to sporadic outbreaks, the severity and expression of the disease depending on environmental conditions and agricultural practices specific to each area (Marr et., 2021).

Soil-borne infection. Oospores in the soil are the primary inoculum early in the season. Oospores can survive for 10-15 years in soil (Olofsson, 1966).

Air-borne inoculum. The rate of disease progression is also greatly determined by relative humidity (RH). Exposure of leaves to moisture for a period of at least 3-4 hours is necessary to initiate infection (Olofsson, 1966; Pegg & Mence, 1970). The temperature can vary between 1 and 24°C, with an optimum between 12 and 20°C (Pegg & Mence, 1970). Initiation and production of conidia require greater than 90% RH for at least 12 hours (Olofsson, 1966) and reach a maximum at 100% RH. Most conidia lose viability within 3 days of removal (Pegg & Mence, 1970). Wind-distributed conidia from neighboring fields or from more distant cultivated areas are also important sources of primary inoculum. Conidia distributed by wind or dispersed by water droplets play an important role in the spread of the disease in pea crops. Young plants are more sensitive than mature plants. Results presented by Stegmark (1988) support the hypothesis that pea downy mildew mainly infects young tissue. Mence and Pegg (1970) demonstrated that terminal embryonic leaves, not yet developed at the time of inoculation, were found to be more susceptible to the disease than more mature leaves. In addition, increased resistance was found in older seedlings. This was found when seedlings of different ages, i.e. 2-6 nodes developed, were inoculated in the same experiment (Stegmark, 1991).

Detection and quantification. Singht et al. (2020) studied downy mildew intensity and meteorological parameters in different pea cultivars noting a negative correlation with minimum, maximum temperature, relative humidity and sunshine hours and a positive correlation with wind speed both in protected space as well as in the field.

Host resistance. Resistance variation in pea cultivars has been reported by Olofsson (1966), Allard (1970), Ryan (1971) and Stegmark (1988). Some pea cultivars are fully resistant to some isolates but are fully susceptible to others. However, there are also pea genotypes that have partially stable, never complete, resistance to different isolates (Stegmark, 1990). Race-specific complete resistance has been found in several cultivars, but there is no pea genotype with complete resistance to all known pathogen races (Ester and Gerlagh, 1979; Matthews & Dow, 1983). The *Pisum* gene bank, Weibullsholm Collection, kindly provided by Stig Blixt, was tested for resistance to oospore infection of germinated seeds at Nordreco (Stegmark, 1994). One line (L1382) showed complete resistance in replicate studies when pre-germinated seeds were soaked in a conidia suspension according to a method described by Ryan (1971). This line has red flowers and brown seeds. When the seed coat was removed before sowing, the seedlings were severely infested with downy mildew. This shows that the seed coat contributes most to the resistance of this line.

Partial resistance. The cultivar "Dark Skin Perfection" (DSP) was more resistant to downy mildew than other cultivars used in canning and freezing pea production (Olofsson 1966; Stegmark, 1988). However, DSP is also affected by downy mildew under conditions favorable to the pathogen. Stegmark (1988) described a pea breeding line with a high level of partial resistance. This line showed low susceptibility to all isolates of the fungus, but never complete resistance to any isolate (Stegmark, 1990).

Management. There are a variety of approaches to managing downy mildew, including growing resistant varieties, crop rotation, seed treatment and foliar fungicide applications. Implementing a good crop rotation is not only an economic strategy for reducing downy mildew impacts, but is also favorable from a general pest management perspective. Cultivating resistant cultivars and using fungal seed treatments are also economical options in downy mildew control. Plant infection is often caused by oospores present in the soil. Seed treatment with Aliette Super® (fosetyl-aluminium 528 g/kg + thiram 172 g/kg + thiabendazole 129 g/kg) and Wakil XL (50 g/kg fudioxonil + 175 g/kg metalaxylM + 100 g/kg cymoxanil) was also proven effective in controlling downy mildew (Pung et al., 2005). An advantage of seed treatment over foliar fungicide application is the lower cost of application when seed treatments are used. However, if downy mildew occurs in the crop, foliar fungicides can provide good control. Mixtures of phosphoric acid (2000 g a.i./ha) and chlorothalonil (1296 g a.i./ha) considerably reduced disease severity in field experiments (Pung et al., 2005). Regardless of the specific control measures used, pea crops should be routinely surveyed to prevent downy mildew occurrence and severity as part of a proactive pest management strategy. Further research by Falloon et al. (2000) showed that matalaxyl treatments applied to pea seeds were ineffective in controlling downy mildew in pea. Seed treatment with cymoxanil or fosetyl-Al provided better protection against the pathogen (Falloon et al., 2000).

3. Pea rust. Pea rust can be caused by different pathogens depending on the climatic conditions. In temperate regions of the world, pea rust is caused by *Uromyces pisi* (Pers.) Wint. (Emeram et al., 2005), while in tropical and subtropical regions, the fungus that causes the rust is *Uromyces fabae* (Pers.) de Bary (Rai et al., 2011). Based on the morphology of the telia and infection structures, these two species can be differentiated using internal transcription spatial (ITS) markers (Emeran et al., 2005; Barilli et al., 2006). *Uromyces pisi* can cause yield losses of over 30% (EPPO 2012) compared to *U. fabae* which can lead to losses of up to 50% (Kushawaha et al., 2006). Pea rust has become an important pathogen since the mid-1980s and is mainly distributed in Europe, North and South America, India, China, Australia and New Zealand, especially in regions with warm and humid weather (EPPO, 2012).

Symptoms. The attack is manifested by the appearance of discoloration spots on the leaves and stems, in the center of which small, dusty, light-brown dots appear. *Uromyces pisi* usually appears in mid-spring when the crop is in the flowering stage (Barilli et al., 2014). Later, spots appear on which groups of black spores open, more numerous on the underside of the leaves. Infected stems develop faster in the season than uninfected stems, as a result of increased concentrations of growth hormones (Pilet, 1953). Heavily attacked plants dry out prematurely and show scaly grains in the pods.

The host. *Uromyces pisi* is a heterotic macrocyclic fungus that completes its life cycle on *Euphorbia cyparissias*. The host range of *U. pisi* is wide, being able to affect plant species belonging to other genera (*Euphorbia*, *Medicago, Pisum*, etc.) (Barilli et al., 2012). *Uromyces viciae-fabae* commonly called bean rust, is reported to be a fungus that infects peas in addition to beans (Cummins, 1978).
Uromyces viciae fabae is the main causal agent of pea rust in tropical and subtropical regions such as India and China, where warm and humid weather favors the development of the fungus (Kushwaha et al., 2006). Asciospores are the infective structures of *U. fabae* (Kushawaha et al., 2006) while in *U. pisi* uredospores are the infective spores (Barilli et al., 2009). In Romania, the main pathogen that causes pea rust is represented by *Uromyces pisi*.

Life cycle. *Uromyces pisi* (fam. Pucciniaceae, order Uredinales) is a heteroeous pathogen of rust, which carries out its life cycle on two host plant species. The sexual stages are completed on *Euphorbia cyparissias*, while the asexual life cycle stages are completed on leguminous crops such as *Lathyrus, Orobus, Pisum* and

Vicia spp. *Euphorbia cyparissias* is an erect, branched perennial that usually grows up to 30 cm height. It occurs on poor and mainly dry soils, along roadsides and forests. The incubation time of the fungus on *Euphorbia* lasts a full year. Under European conditions, the fungus remains dormant during the winter in the roots of *Euphorbia cyparissias* and develops with the host as spring occurs. Infected host plants develop earlier in the season and are inhibited from flowering. The host plant is induced by the fungus to form pseudo-flowers; yellow leaves that grow in a rosette at the top of the stems and resemble true flowers in color and shape (Pfunder & Roy, 2000). The fungus produces a sweet smelling nectar on the surface of the yellow leaves, giving the appearance of a real flower. Nectar contains fungal gametes (spermatia) that are transferred by nectar-feeding insects (bees and ants) from one type of fungal mating to another.

Primary air-borne inoculum. The fungus survives on plant residues and the dispersion of the inoculum can occur from several sources, namely: infested residues, dust and soil. The number of days with precipitation during the growing season plays an important role in the spread of the disease than other meteorological parameters (Martins et al., 2022).

Ascospores produced by *U. pisi* on *E. cyparissias* are dispersed by wind to infect pea crops. Jørstad (1948) observed rust on field pea 25 km from the nearest source (*Euphorbia cyparissias*) in Norway, suggesting that long-distance wind dispersal is possible. The asexual stage begins with the release of ascospores, which are dispersed by wind and infect pea crops. Infection with ascospores results in the production of uredinia and subsequently uredospores. The primary source of uredospores may be from pea plants, infected earlier in the growing season, or from spores carried long distances by wind. As the host plant matures, telia is produced, resulting in the formation of teleutospores.

Detection and Quantification. When infected *Euphorbia cyparissias* cannot flower, but instead is induced by the fungus to form pseudoflowers. Pfunder and Roy (2000) hypothesized that fungi depend on insect visitation to achieve gamete mating. Pseudoflowers induced by *Uromyces pisi* interact with uninfected true host flowers via insects during their co-"flowering" period in early spring. Field experiments were conducted to test whether the two species (*Pisum sativum* and *Euphorbia cyparissias*) share their insects and whether they were mutually influenced by insect visitation. Following the results, real flowers received more visits from insects (Pfunder & Roy, 2000). In his work, Barilli et al. (2012) studied the response of pea to seven species of rusts that can infect related legumes, and found that indeed pea can be infected mainly by 2 pathogens, namely *Uromyces pisi* followed by *Uromyces viciae fabae.* Other pathogens that can cause rusts, such as *Uromyces striatus*, *Uromyces ciceris-arietini*, *Uromyces anthyllidis* and *Uromyces vignae,* can also infect and reproduce on peas, although to a lesser extent. Knowledge about the host range of a biotrophic fungus like *Uromyces* is of great agronomic and epidemiological importance. In fact, one of the constraints shown by the species belonging to this genus is that several rust species can infect the same host plants e.g. *U. viciae-fabae* and *U. pisi* on peas (Barilli et al., 2009). Furthermore, it is possible for a rust-causing fungus to infect a plant species that was thought to be resistant e.g. *Medicago* spp. which was recently added to the host range of *Uromyces ciceris-arietini* (Stuteville et al., 2010). These characteristics prevent a clear characterization of pathogens and, consequentl, their control.

Management. Rusts can cause significant damage to the pea crop. Sidenko (1960) reported that early tillering influences high-level occurrence of the pathogen in the Ukrainian steppe, especially if the crop is in close proximity to alternative hosts. An outbreak of pea rust can lead to a reduction in production area for a short period of time as a result of increased production costs, which would make field peas less competitive compared to other crops. According to Plant Health Australia (2009) germplasm with improved resistance to this disease has been identified in the Australian field pea breeding program but has not yet been published. The decision to apply one or more fungicides depends on the risk of rust epidemic in a given year. The rust epidemic is determined by the interaction of three important factors namely the host, the pathogen and the most important is the favorable environment for a certain period of time. Therefore, it is necessary to know the correlation between different meteorological parameters and the severity of rust. To prevent the spread of the disease, it is recommended to collect the remains of pea plants left in the field, as well as the *Euphorbia* plants around leguminous crops, to interrupt the biological cycle of the fungus. In case of strong attack, the pea plants will be treated with the following products Polyram combi 0.30%; Plantvax 75 wp 0.20% (Pârvu, 2010).

CONCLUSIONS

The pea crop is affected by numerous biotic and abiotic stressors. Fungal diseases such as rusts, downy mildew, ascochyta blight complex fall under the most widespread biotic stress. Rusts and downy mildews cause major crop damage in both tropical and temperate regions. Using fungicides to control plant diseases is a good approach, but excessive use of fungicides can cause environmental pollution and also lead to pathogen resistance. Therefore, to eliminate these constraints, we need to grow disease-resistant pea varieties. Their cultivation represents a safe and effective alternative method in controlling plant diseases.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EFFECTS OF BIOPESTICIDE CARBECOL, FUNECOL AND BIOFERTILIZER ECOLIT ON PHOTOSYNTHETIC PIGMENTS AND HYDROGEN PEROXIDE CONTENTS IN TOMATO (*Solanum lycopersicum* L.) PLANTS

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Abstract

The photosynthetic pigments and hydrogen peroxide content is a physiological marker to evaluate plant physiology and determine the response to application of different kinds of plant protection products. The aim of this study was to determine the response of leaf chlorophylls and hydrogen peroxide contents in tomato plants to application of biofungicide Carbecol alone or in combination with biofungicide Funecol and biofertilizer Ecolit. A greenhouse experiment was conducted out with tomato (Solanum lycopersicum L., cv Manusa) plants. Experimental results showed that plant treatments with Carbecol increased the contents of photosynthetic pigments in tomato plants, in particular chlorophyll a. The highest concentration of photosynthetic pigments was registered in treatment with integrated application of Carbecol, Funecol and Ecolit. The treatments with Carbecol alone increased the concentration of hydrogen peroxide in leaves. The highest level of this metabolite in leaves was observed in treatment with application of biofertilizer Ecolit. The integrate use of biofungicides and biofertilizer Ecolit increased tomato yield by 14%.

Key words: *Carbecol, Funecol, Ecolit, photosynthetic pigments, hydrogen peroxide, tomato.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the major vegetables in many countries, as well as in the Republic of Moldova. This crop plays an important role in human nutrition and food security. It is well known that tomato is sensitive to infection by many phytopaphogens, especially by *Phytophtora infestans*. To control the late blight producers use a large range of fungicides (Egel et al., 2019). Some studies demonstrated that application of protection products has negative consequences on physiological state of crops as well as on yield quality. Therefore, it is necessary to identify the innovative ecological plant protection products with minimum impact on plants on one hand, and on the other hand they must be effective for the protection of crops against phytopaphogens (Bahramisharif & Rose, 2019). Nowadays, ecological plant protection products have become a relevant particularity of durable agriculture development for increasing productivity with good quality of vegetables. The application of chemical pesticides diminishes the photosynthetic pigments, increases reactive oxygen species (ROS) in plants, and provokes imbalance of physiological constituents and metabolic processes (Cerný et al., 2018). Abd-El-Kareem et al. (2012) demonstrated that treatments of potato with potassium bicarbonates, essential plant oils and humic substances have a higher beneficial potential to reduce late blight disease accompanied with reducing yield losses. There is a body literature data which confirmed that biopesticides have an agronomic and economic importance with a large range of their functions to improve the plant resistance to some diseases, in particular to late blight of tomato and potato plants (Ngegba et al., 2022). The level of photosynthetic pigments concentrations determines the plant growth and productivity, the plant resilience to stress factors. The use of plant protection products has not only the capacity to control the diseases but they can alter a range of metabolites in leaves of plants. Likewise, the application of humic substances has ability to protect crops against phytopathogen attack and associated with accumulation of chlorophylls in leaves (Jindo et al., 2020). Hydrogen peroxide (H_2O_2) has been shown to play a key role in a range of physiological processes including photosynthesis, senescence, stomatal movement, plant growth and development (Noctor & Foyer, 1998). However, the hydrogen peroxide at higher concentration has a toxic effect responsible for reducing plant tolerance to unfavorable conditions (Nazir et al., 2020). At the same time, the hydrogen peroxide is considered a strong antiseptic due to its toxic effects on some infections of crops. Analysis of the literature data revealed that most of the studies were carry out to assess only the capacity of ecological protection products of diseases control of crops (Ngegba et al., 2022). Unfortunately, there are a few investigations focused to determine their influence on changes of photosynthetic pigments and hydrogen peroxide (Mourad et al., 2017). To address this knowledge gap, this study was aimed to investigate the effects of biopesticide Carbecol applied alone or in combination with biopesticide Funecol and biofertilizer Ecolit on the contents of chlorophyll *a, b*, carotenoids and hydrogen peroxide in leaves of tomato plants. Also, we determined the influence of treatments on tomato fruit yield.

MATERIALS AND METHODS

A greenhouse experiment was conducted to examine the potential effects of Carbecol alone or in combination with Ecolit and Funecol on photosynthetic pigments, hydrogen peroxide contents in leaves and productivity of tomato (cv Manusa). The experiment was performed out at the Institute of Genetics, Physiology and Plant Protection of the Republic of Moldova. The soil used for experiment was carbonated chernozem. The experiment included 6 treatments. The biorational products Carbecol and Funecol have the potential to control late blight of tomato. Individual and combined applications of the Carbecol, Ecolit and Funecol have been done at the following stages of the plant development: 1-st at 7 days after transplanting; 2-nd at the intensive growing stage; the 3-rd at flowering stage and the 4-th at the fruits development stage. Carbecol was applied taking in consideration the dose of 6 kg/ha. Biofertilizer Ecolit was applied at dose 3 L/ha and fungicide Funecol at concentration 0.4%. After the last treatment the leaves were collected from each variant in three replicates. Photosynthetic leaf pigments chlorophyll *a* and *b* and carotenoids were quantified by the method of Lichtenthaler & Wellburn (1987) through a spectrophotometer at 663 nm, at 644 nm and at 470 nm. Hydrogen peroxide concentration in leaves was determined according to Velikova et al. (2000). Absorbance was measured using a UV-Vis spectrophotometer at 390 nm. Hydrogen peroxide contents are expressed as µmol of H_2O_2 g^{-1} fresh weight. At physiological maturity, tomato fruits from each variant were harvested and weighed separately to determine fruit yield. The factorial treatments were distributed in three replicates according to the Complete Randomly Block Design. The data were analyzed by using STATISTIC 7 program. All the results were the means ± SE of three replicates.

RESULTS AND DISCUSSIONS

Evaluation of chlorophyll ***a*** and ***b***, and carotenoids contents is a biochemical marker to determine the plant responses to treatments with agrochemicals used in integrated crops protection. Likewise, the chlorophyll content is a tolerance parameter to unfavorable factors of plants (Hosseinzadeh et al., 2016). In general, we visually observed that tomato plants grown under treatments of ecological substances were greener compared to untreated plants (control). Mean data for photosynthetic pigments concentrations shows variable responses. Experimental results of this study revealed that concentrations of pigments in tomato leaves were related to treatments. The effects of treatments with Carbecol alone or in combination with biofertilizer Ecolit and

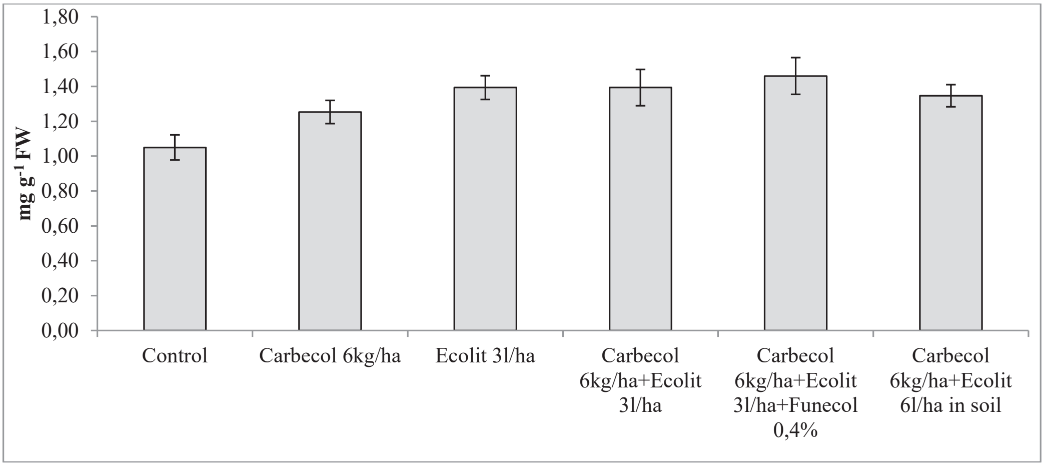

Figure 1. Effects of treatments of Carbecol alone or in combination with Ecolit and Funecol on the changes of the chlorophyll ***a*** content of *Solanum lycopersicum* L. plants. All the data are the means of three replicates (n = 3); vertical bars show standard errors (± SE)

Funecol on chlorophyll ***a*** and ***b*** contents are shown in Figures 1 and 2, respectively. It is necessary to note that in the variants with application of biopesticide tomato plants were less infected by *Phytophtora infestans* pathogen compared to control one (data are not shown). Experimental results shown that mean chlorophyll ***a*** contents ranged from 1.05 to 1.46 mg g/FW in depending on treatments. As we expected, the lowest concentration of chlorophyll ***a*** was observed in control plants. Our present findings are in line with the findings of other researchers (Egel et al., 2019). However, the most increase of chlorophyll ***a*** concentration (by 39%) was registered in treatment with integrated application of Carbecol, Ecolit and Funecol (variant 6). The chlorophyll ***b*** contents varied among different treatments, the differences between the treatments and control variant were significant (Figure 2).

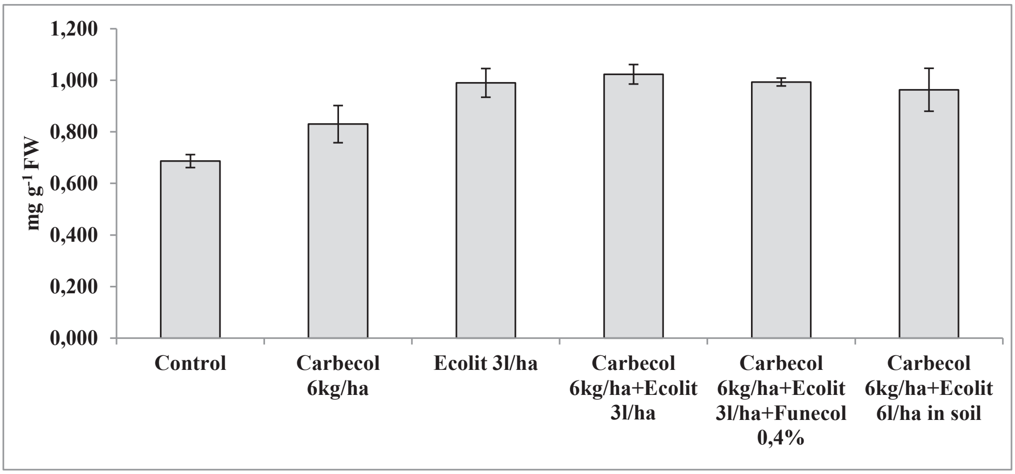

Figure 2. Effects of treatments of Carbecol alone or in combination with Ecolit and Funecol on the changes of the chlorophyll ***b*** content of *Solanum lycopersicum* L. plants. All the data are the means of three replicates (n = 3); vertical bars show standard errors (± SE)

Mean chlorophyll ***b*** contents ranged from 0.687 to 1.023 mg g/FW in depending on treatments. Hence, this implies that the application of ecological products was effective on the chlorophyll contents of the plants. Experimental data confirmed that chlorophyll ***b*** content increased with various treatments; moreover, the significant increase was recorded for Carbecol + Ecolit treatment. The lowest level of chlorophyll ***b*** was recorded in the control treatment. We suppose that integrated use of ecological products with biofertilizer stimulates the uptake of nutrients, in particular magnesium. This nutrient participates in the chlorophyll synthesis. At the same time combined treatments of biopesticide and biofertilizer Ecolit significantly reduced the attack by *Phytophtora infestans*. Hence, in this research the chlorophyll ***b*** content exhibited pronounced responses to application of biorational plant protection products. We may conclude that application of ecological protection products taken in this study could affect beneficially the photosynthetic pigments status of tomato plants. Chlorophylls are sensitive to oxidation and carotenoids play a significant role in the protection of chlorophylls against damage by different adverse factors. In addition, carotenoids are more stable pigments than chlorophylls (Loggini et al., 1999).

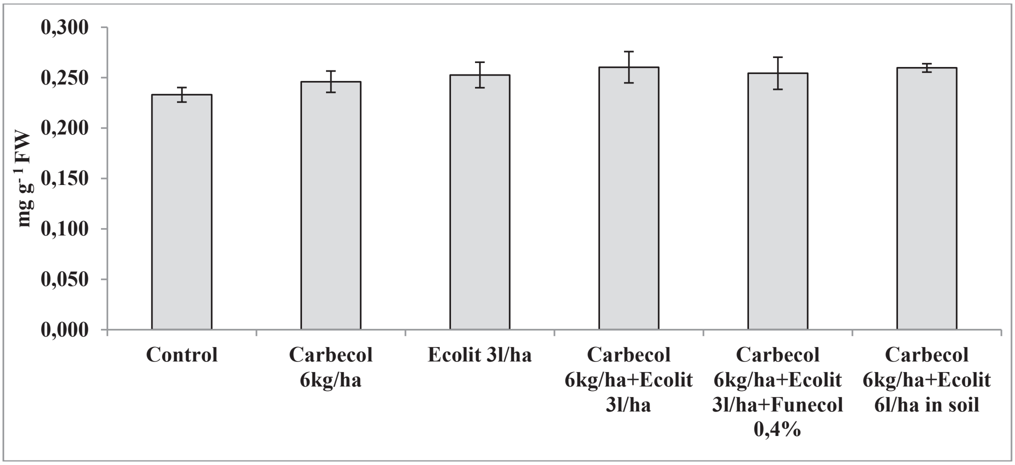

Figure 3. Effects of treatments of Carbecol alone or in combination with Ecolit and Funecol on the changes of carotenoids content of *Solanum lycopersicum* L. plants. All the data are the means of three replicates (n = 3); vertical bars show standard errors (± SE)

The analysis of carotenoids content in the leaves showed less responses to treatments than chlorophylls one. However, it was observed a moderate increase of carotenoids under different treatments in comparison with control plants (Figure 3). The highest (0.260 mg g/FW) and lowest (0.233 mg g/FW) concentrations of carotenoids were observed in the treatments with integrated use of all products and control variant, respectively. Therefore, the contents of carotenoids were light increased in leaves of tomato plants in response to application of plant protection ecological products. As we mentioned in the INTRODUCTION the hydrogen peroxide has a significant role in the physiological activity of the plants. As a rule this metabolite is produced predominantly in the processes of photosynthesis and photorespiration (Noctor & Foyer, 1998). To our knowledge, this research for the first time has examined the effects of treatments of plant protection products on changes of hydrogen peroxide content in tomato plants. The data of its concentration in leaves of tomato plants is illustrated in Figure 4. The experimental results revealed that the hydrogen peroxide contents in leaves were higher under treatments than their respective untreated plants. The accumulation of hydrogen peroxide in leaves changed under treatments with Carbecol alone or under combined application with biofertilizer Ecolit and biofungicide Funecol.

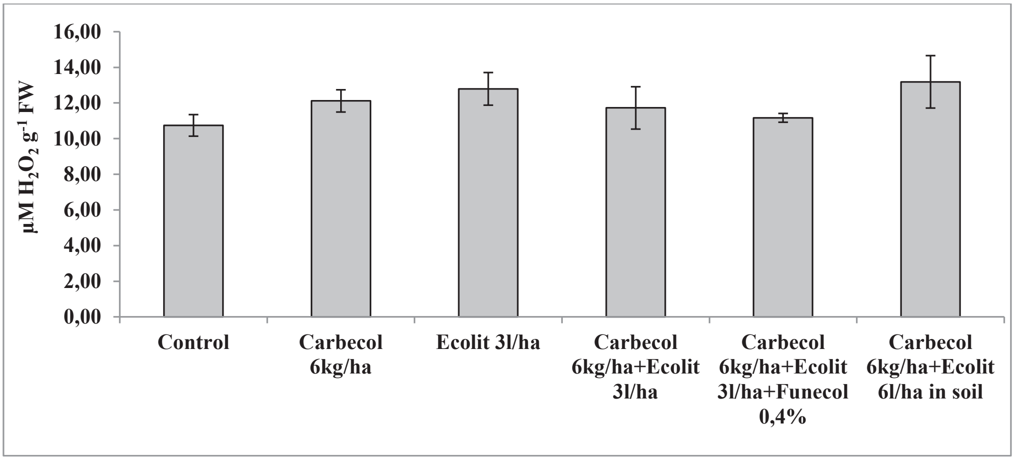

Figure 4. Hydrogen peroxide (H_2O_2, μmol g^{-1} FW) content in leaves of tomato exposed to different treatments. Bars represent the means of three replicates ± SE

Experimental results find out that the separate use of these products increased the concentration of hydrogen peroxide by 12.8% and 19.1%, respectively. As a whole, mean value of hydrogen peroxide contents were higher in treatment with integrated foliage application of the Carbecol and Ecolit applied in the soil (by 23.2%) over the control plants. However, it was observed that integrated application of Carbecol, Ecolit and Funecol insignificantly reduced its content in leaves (variant 5), but the value was higher than in control variant (10.74 micromoli H_2O_2/g FW). Our results are in concordance with the studies of Sidiqqui and coworkers (2009) in treatment of plants with extracts of compost tea. While treatments improved the biochemical parameters of plants in this study we also determined their influence on fruit yield of tomato. The data of tomato fruit yield is presented in Figure 5.

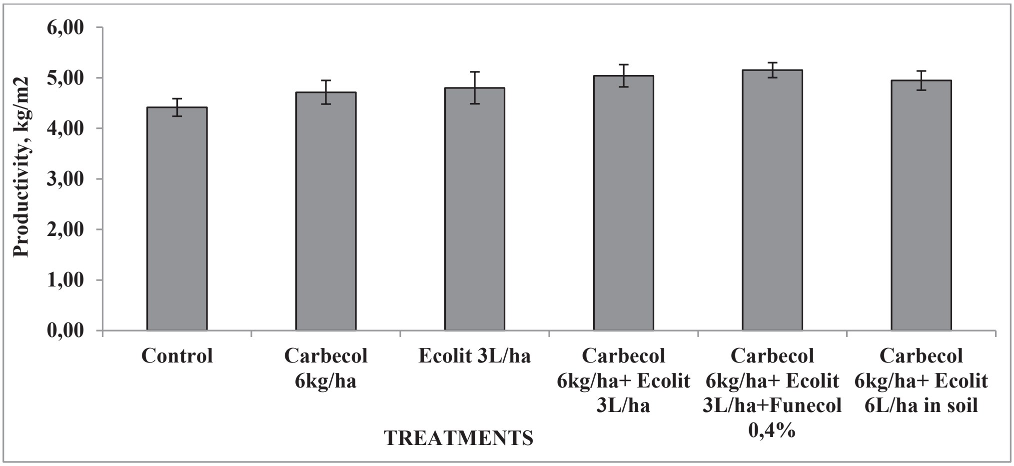

Figure 5. Effect of Carbecol alone or in combination with Ecolit and Funecol on the fruits productivity of tomato plants. Error bars represent LSD at $p \leq 0.05$ of a mean pooled from three replications

As we supposed, the application of Carbecol alone or in combination with biofertilizer Ecolit and fungicide Funecol had positive influence on plant productivity (Figure 5). The highest value of the yield was obtained in treatment with integrated application of Carbecol, Ecolit and Funecol. In this treatment the yield increased by 14% compare to untreated plants (control). As we expected the lowest yield of tomato fruits were registered in control variant. Probably, the negative effect of higher infection of untreated plants by pathogen *Phytophtora infestans* could be attributed the adverse influence of late blight disease on plants physiology on the one hand and lesser supply of nutrients on the other hand because the biofertilizer was not applied in control variant, and consequently decreased yield of tomato.

CONCLUSIONS

Experimental results demonstrated that under treatments with Carbecol applied alone or in combination with biofertilizer Ecolit and biofungicide Funecol increased photosynthetic pigments in tomato plants.
The application of biofertilizer Ecolit alone increases the carotenoids contents in plants.
The highest concentration of hydrogen peroxide was obtained under the application of ecological fungicide Carbecol and biofertilizer Ecolit.
The integrated treatments with Carbecol, Ecolit and Funecol improved photosynthetic pigments status of plants and increased fruits yield of tomato. A field study in this regard should be conducted to further verify these results.

ACKNOWLEDGMENTS

This research was carried out with the support of the National Agency for Research and Development of the Republic of Moldova, project 20.80009.5107.19.

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Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY OF THE INTERNAL STRUCTURE OF *Amaranthus retroflexus* LEAF

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Abstract

The Amaranthus retroflexus species studied has a stem up to 100 cm high, branched, light green to reddish in color, with few leaves at the base, and the upper part with leaves covered with hairy hairs. For the microscopic observation of the internal structure of the Amaranthus retroflexus leaf, the seedlings were first fixed in AFE solution (70% ethyl alcohol, glacial acetic acid, 40% formalin) for 48 hours, after which they were washed with distilled water and preserved in alcohol 70%. The internal structure of an Amaranthus retroflexus leaf was observed in cross-section. It consists of two epidermises, one upper and one lower, as well as mesophyll. Stomata open in the morning under the influence of light (photoactive reaction), when the stomatal cells have a higher degree of turgor than the accessory cells. It was also observed that the opening degree of the stomata reaches a maximum in sunny conditions, when the penetration of CO_2, necessary for photosynthesis, is more intense, which determines the transpiration of the leaves at a high intensity.

Key words: *Amaranthus retroflexus, internal structure, stomatal, leaf.*

INTRODUCTION

The study was conducted to examine the internal structure of the leaf of *Amaranthus retroflexus*, a common weed species, using various microscopic techniques. By sectioning the plant organ and creating temporary microscopic preparations, researchers can observe the cellular structures and arrangements within the leaf. This study can provide valuable insights into the anatomy and morphology of the leaf, which can be used to better understand the physiology and ecology of this species. Additionally, this research can contribute to the development of more effective methods for controlling the spread of this weed species in agricultural and natural ecosystems.

The leaf is a lateral vegetative organ of the stem or branches, with a flat shape, which fulfills the fundamental function in the process of photosynthesis, serving also for respiration and transpiration (Athanassova, 1996; Benvenuti & Macchia, 1999; Brainard & Bellinder, 2002). Being the most plastic vegetative organ of the plant, the leaf can metamorphose, adapting to perform other functions: protection, absorption, storage of reserve substances and water, vegetative reproduction (Fischer & Evert, 1982a; Fischer & Evert 1982b; Fischer & Evert 1982c).

The species *Amaranthus retroflexus* has a stem up to 100 cm high, simple or branched, light green to reddish in color, with few leaves at the base, and dense leaves at the top, covered with thin hairs (Dominguez et al. 1994; Ghorbani et al. 1999).

The leaves are ovoid, long-petiolate, bluish-green, alternate, with a pointed tip, usually reddish on the underside (Franssen et al. 2001a; Franssen et al., 2001b). The cotyledons have an elongated shape, a length of approx. 10-12 mm, with the underside usually reddish (Frost, 1971; Hügin, 1986; Hunt et al., 1985).

The flowers are small, clustered in greenish bunches at the extremity of the main stem or branches, with the perianth (the floral sheath differentiated between the calyx (sepals) and the corolla (petals) pointed like a thorn, the inflorescence prickly (Coetzer et al., 2002; Costea, 1998). The flowering period is June - September, and the number of seeds per plant is between 1000-5000 (Gallagher & Cardina, 1998a; Gallagher & Cardina. 1998b).

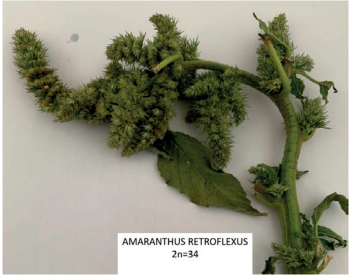

Figure 1. General aspect of the *Amaranthus retroflexus* species

MATERIALS AND METHODS

For provisional microscopic preparations, the fastest and most used technique for sectioning the plant organs is the manual one, with the anatomical razor/scalpel or blade (Gebauer et al., 1987; Ghorbani et al., 2000). The following types of sections are obtained: transverse when the scalpel passes perpendicular to the axis of the plant organ; longitudinal-radial, when the scalpel passes through the center of the organ, in the direction of its radius; longitudinal-tangential, when the scalpel passes perpendicular to the radius, but does not touch the center of the organ (Lonchamp, 1981; Schonbeck et al., 1980). Manual sectioning is performed as follows: the razor blade is applied to the surface of the object to be sectioned and a horizontal movement is imposed on the razor (Mapes et al., 1997; Marín et al., 2000; Schimpf, 1977). The movement must be uniform, without stops and restarts, so that the sections are thin (microtones) (Costea et al., 2001; Doyon et al., 1986). The thinnest sections are transferred to the port object slide, in a drop of water and covered with the slide (Warwick et al., 1980; Weaver, 1984; Weaver et al., 1986).
For the microscopic observation of the internal structure of the *Amaranthus retroflexus* leaf, the seedlings were first fixed in AFE solution (70% ethyl alcohol, glacial acetic acid, 40% formalin) for 48 hours, after which they were washed with distilled water and preserved in 70% alcohol. For the purpose of constructing the temporary microscopic preparation, a small fragment of the lower epidermis was detached with the help of sharp-tipped forceps, after which it was placed on the microscope slide (degreased beforehand) in a drop of water. The epidermal fragment was carefully stretched to remove creases, then covered with a glass coverslip and gently pressed to remove air bubbles. The obtained preparation was examined under a microscope, successively using 10x, 20x, 40x and 60x objectives.
In order to visualize the shape and arrangement of cell debris under the optical microscope, a temporary microscopic preparation was made by transversally sectioning the lower epidermis of the leaf and placing the respective fragment on the glass slide, in a drop of acetic carmine. The slide was then coverslipped and the slide was studied using progressive 10x, 20x, 40x, and 60x objectives.

RESULTS AND DISCUSSIONS

In the image below you can see the internal structure of an *Amaranthus retroflexus* leaf, in cross-section. It consists of two epidermises, one upper and one lower, as well as mesophyll.

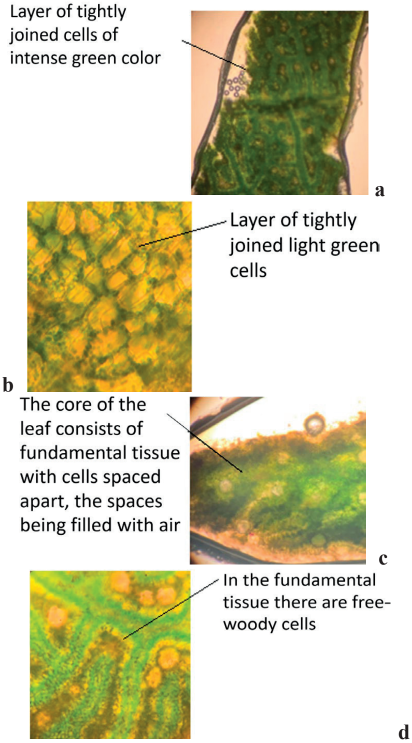

Figure 2. Transverse section through the leaf of *Amaranthus retroflexus*, viewed under the optical microscope, with different objectives (10x, 20x, 40x and 60x): a- upper epidermis; b- lower epidermis; c- mesophyll or core of the leaf; d- fundamental tissue

The upper epidermis, in turn, is made up of a layer of densely green cells closely united to each other. The lower epidermis consists of a layer of tightly joined light green cells. On both epidermises, but especially on the lower one, there are modified cells called stomata. The mesophyll or core of the leaf consists of ground tissue with cells spaced apart, the spaces being filled with air. In the fundamental tissue are found libero-wooden bundles.
On the lower epidermis, pluricellular hairs appear, in the shape of a shield, with a polygonal basal cell, a uniseriate pluricellular pedicel (several oblong-rectangular cells, superimposed on each other), and the gland is unicellular, approximately spherical-pyriform in shape, rich in cytoplasm.

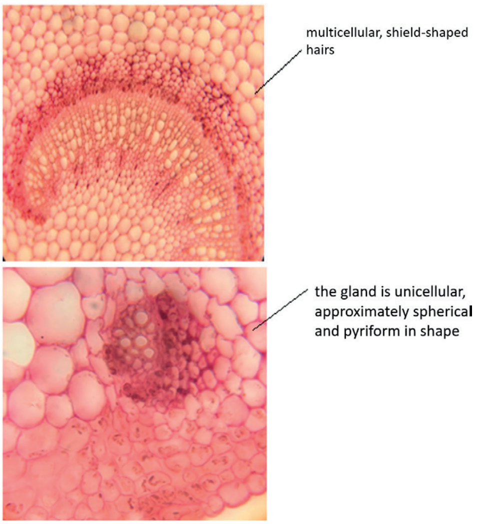

Figure 3. Multicellular hairs in *Amaranthus retroflexus* species

Stomata are specialized structures, in the form of small openings or pores, located at the level of the epidermis, having a role in gas exchange between the plant and the external environment, as well as in the release of water vapor (transpiration). Through the stomata, the carbon dioxide necessary for assimilation and the oxygen necessary for respiration enters the plant body, as well as the elimination from the plant body of various gaseous substances (oxygen and carbon dioxide) formed during the processes of respiration, respectively transpiration. Stomata help reduces water loss by closing when conditions dictate. Most of the stomata are located on the underside of the leaves, which reduces their exposure to heat and drafts.

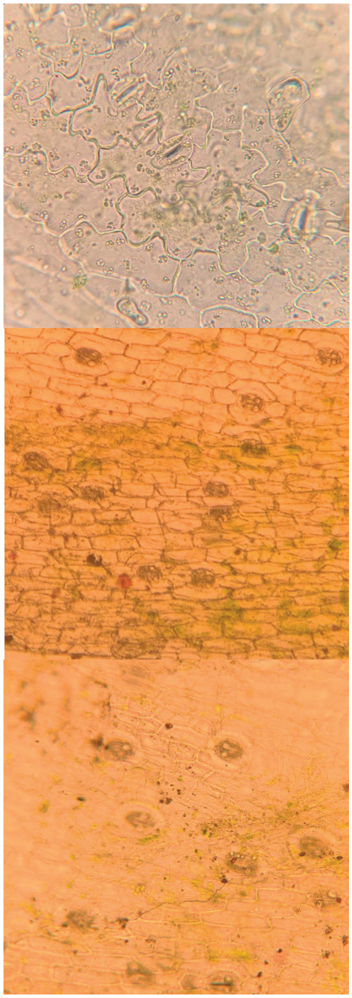

Figure 4. Appearance of stomata in the species *Amaranthus retroflexus* (40x)

In the process of transpiration, water is eliminated into the atmosphere mostly through the stomata and less through the cuticle of the epidermis. Cuticular transpiration is reduced in mature leaves and even more intense in young leaves, whose epidermal cells have a thin cuticle.
Transpiration through the stomata is about 10 times more intense than through the cuticle. The stomata perform opening and closing movements, which are the main means of regulating the intensity of transpiration. The amount of water removed in this way is

proportional to the degree of opening of the stomata, and the speed of water vapor diffusion is directly proportional to the surface and the diameter of the ostiole.

Stomata open in the morning under the influence of light (photoactive reaction), when the stomatal cells have a higher degree of turgor than the accessory cells. The opening degree reaches a maximum at midday, in sunny conditions, when the penetration of CO_2 necessary for photosynthesis is more intense. These phenomena cause the leaves to transpire at a high intensity.

CONCLUSIONS

In conclusion, the study of the internal structure of *Amaranthus retroflexus* leaf revealed that it consists of two epidermises, one upper and one lower, as well as mesophyll. Stomata in this species open in response to light in the morning due to a photoactive reaction, and their degree of opening is maximum in sunny conditions when the penetration of CO_2 is intense. This mechanism facilitates the process of photosynthesis, but it also leads to the transpiration of the leaves at a high intensity. The fixation and preservation techniques used in this study enabled the observation of the internal structure of the leaf in detail. This knowledge contributes to a better understanding of the anatomy and physiology of this species, which can be useful in various fields such as agriculture, botany, and ecology.

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ISSN 2285 – 5785
ISSN-L 2285 – 5785