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Economic and Energy Efficiency of Technologies for Growing Maize Hybrids Depending on the Type and Methods of Applying Fertilisers

Abstract. The urgency of solving the problem of the growth of maize grain production is unconditional, considering the needs of humanity, the economic, and energy efficiency of cultivation technologies, which substantially depends on the types of fertilisers and the frequency of their application. The purpose of this study was to prove the efficiency of maize grain production by perfecting the fertilisation system. Field research was conducted at the “Bohatyryvske” farm in the Romen district of the Sumy Oblast during 2019–2021 on dark grey podsolised soil. Mathematical and statistical processing was performed using the software package SAS 9.4 and “Agrostat 6”. The economic and energy evaluation of technologies was performed using the calculation method. Hybrids are flexible to growing conditions, respond positively to nutrition optimisation – yield varies from 6.07–6.16 in the control to 8.26–8.50 t/ha with carbomidoammonium nitrate 32 on the background of $N_{22}P_{57}K_{57}$. With the background application of $N_{22}P_{57}K_{57}$, the yield increased by 16.4%. Nitrogen fertilisers over the background provided an added increase in yield by 7.95–18.6%. With the introduction of carbomidoammonium nitrate 32, the yield increase was 2.19 t/ha and 2.44 t/ha or 36.1% and 38.0%, respectively, of the Concord and Asteroid hybrids. The reaction of hybrids to ammonia water and urea is lower compared to carbomidoammonium nitrate. Production costs for growing maize amounted to UAH 20.3–32.3 thous./ha. The introduction of carbomidoammonium nitrate against the background of $N_{22}P_{57}K_{57}$ provided a high conditionally net profit – UAH 19.2–19.9 thous./ha. The profitability of maize grain production is quite high – 44–80%. With the addition of Humilin Stimul, the yield increases by 0.23–1.71 t/ha, depending on the combination of fertilisers. With all types of fertilisers, the highest efficiency was obtained with one-time feeding on BBCH 15–17. The coefficient of energy efficiency for the application of fertilisers decreases. The results of this study are important for production and further areas of research, namely the biodiversity of soil microflora

Keywords: grain market, yield, production costs, conditional net profit, profitability

INTRODUCTION

The global political crisis sets back the global grain market [1]. Soaring prices for energy resources lead to higher prices for fuel, electricity, fertilisers, plant protection products, and other material and technical resources. This increases the cost of production of agricultural products and may reduce the volume of its production in 2022. According to the forecast of the Food and Agriculture Organisation of the United Nations (FAO) [2] the world

grain production in the 2021/2022 marketing year will amount to almost 2791.3 million tonnes, which will exceed the estimated data for the previous marketing year by 19.3 million tonnes (0.7%) [1; 3].

World grain production in 2020/21 was 2,772 million tonnes, production forecast for 2021/22 is 2,791.3 million tonnes, and consumption is 2,809.6 million tonnes. That is, consumption exceeds forecasted production. According to preliminary FAO forecasts [2; 4], it was assumed that

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in Ukraine in 2021/22 marketing year, grain production was to increase by 26.6% to 81.8 million tonnes due to a fairly significant increase in the production of maize, wheat, and barley [1; 4].

Maize, in terms of production and consumption, ranked first in the world among grain crops. It is predicted that in 2021/2022, the world maize production will be about 1.2 billion tonnes [5]. In 2020/21, the largest producer of maize in the world was the United States with almost 384 million tonnes, or a third of nominal world maize production. The largest maize producers in the world in 2019/20 were as follows: USA (31.1%); China (23.4%); Brazil (9.07%); EU (6%); Argentina (4.5%); Ukraine (3.2%); India (2.6%); Mexico (2.25%); South Africa (1.46%) and 16.5% – other countries [6; 7].

In 2020/2021, the United States exported over 61.5 million tonnes of maize, a third of total global maize exports. The largest consumers of maize in the world in 2020/21 were the following countries: USA – 12.0 billion bushels; China – 11.3; EU-27 – 3.13; Brazil – 2.77; Mexico – 1.73; India – 1.11; Egypt – 0.67; Japan – 0.63; Vietnam – 0.61; Argentina – 0.59 billion bushels. The total consumption in the world was 45.4 billion bushels [8].

The 2014-2020 global average maize grain price ranged from \$193 per tonne in 2014 to \$155 per tonne in 2017. However, in 2021, the price rose to \$250 per tonne and the tendency for the price to rise or stay at this level persists [9].

The effectiveness of agricultural crop cultivation technology is dictated both by the absolute productivity and the economic efficiency of the cultivation technology, and by the implementation of certain technological innovations [10; 11].

Maize is an intensive crop, which, to form a high yield, requires considerable production costs, by which it often exceeds other grain crops [12]. Under changing climatic conditions and growing technologies, the adaptability of maize hybrids plays a critical role [13-15]. Maize effectively responds to the optimisation of plant nutrition due to increased yield [16; 17].

The economic basis of modern crop production is the balanced production with the best ratio of material and energy costs and the assessment of the gross output of products. The main indicators of economic efficiency include gross production costs, evaluation of the received

gross products, profit, production prime cost, production profitability, general structure of costs [18]. Furthermore, modern cultivation technologies must be competitive in the technology market [19].

At the current stage, a feature of the agriculture development is that a 2-3-fold increase in yield is accompanied by a several-fold increase in the cost of non-renewable energy per unit of production. This gives reason to consider the production of plant products as an energy problem [20; 21].

Each technology involves different energy costs. To assess the practicality of a technological process or its individual methods from an energy perspective, it is necessary to assess their bioenergy efficiency quantitatively [22]. The final indicators of the bioenergetic assessment of maize cultivation technology include the gross energy costs, the energy intensity of the crop, and the energy efficiency coefficient.

The purpose of this study was to prove the economic and energy efficiency of maize cultivation technologies using diverse types of fertilisers and the frequency of fertiliser application.

MATERIALS AND METHODS

The study was performed by setting up and conducting field and laboratory experiments, followed by calculations of the economic and energy efficiency of cultivation technologies. The latter differed in terms of types, doses, and methods of applying fertilisers. Field research was conducted in 2019-2021 at the “Bohatyryvske” farm of Romen district of Sumy Oblast, in the northern part of the Left Bank Forest Steppe. The study was designed per the requirements of the methodology of the research case [23; 24] according to the method of split sections on dark grey podzolised soil. The degree of providing the soil with mobile forms of phosphorus, potassium, and nitrogen was as follows: phosphorus – 50-100 mg/kg; potassium – 40-80 mg/kg according to Chirikov, and nitrogen – 40-50 mg/kg according to Tiurin-Kononova [23].

To develop the elements of maize cultivation technology, namely the optimisation of maize fertilisation, in Experiment 1, the effectiveness of diverse types of fertilisers was investigated at the same rate of nitrogen application according to the active substance (N_{120}) on the background of $N_{22}P_{57}K_{57}$ per the developed scheme (Table 1).

Table 1. Scheme of the Experiment 1

Factor A Hybrid		Factor B Fertilisers		
Variant designation	Hybrid	Variant designation	Norm, kg/ha a.s.	Fertiliser
A1	ES Concord	B1	Control (without fertilisers)	
A2	ES Asteroid	B2	$N_{22}P_{57}K_{57}$ –background (B)	DAP
		B3	$B+N_{120}$	Ammonium water
		B4	$B+N_{120}$	UAM
		B5	$B+N_{120}$	Urea

The Experiment 2 was set up to establish the efficiency of maize cultivation with the combined use of nitrogen fertiliser types (ammonia water, UAM 32 fertiliser, and urea) and, against their background, of the

Humilin Stimul preparation in foliar feeding (Table 2). Humilin Stimul was fed once and twice in microstages BBCH 15-17 and BBCH 17-19. The preparation consumption rate was 3 l/ha.

Table 2. Scheme of the Experiment 2

Factor A Type of background fertiliser ¹		Factor B Humilin Stimul nutrition	
Variant designation	Fertiliser	Variant designation	Microstage, BBCH scale
A1	N ₂₂ P ₅₇ K ₅₇ –background (B)	B1	–
A2	B+Ammonium water	B2	15-17
		B3	17-19
		B4	15-17 and 17-19
A3	B+UAM	B5	15-17
		B6	17-19
		B7	15-17 and 17-19
A4	B+Urea	B8	15-17
		B9	17-19
		B10	15-17 and 17-19

The water-soluble complex fertiliser Humilin Stimul contains potassium humate (in terms of humic acids) – at least 40 g/l, succinic acid – 3 g/l, trace elements, other biologically active elements (gibberellins, auxins, cytokinins). The study was conducted with two hybrids (factor C) – ES Concord and ES Asteroid. The area of the accounting plot in the field experiment was 50 m², repetition was fourfold. Field studies were conducted with mid-ripening hybrids ES Concord (FAO 250) and ES Asteroid (FAO 290). Maize was sown when the soil was warmed up to 8°C: in 2019 – on April 15; in 2020 – on April 19; in 2021 – on April 23. Harvesting was performed in sections by continuous threshing. Before harvesting, plants were sampled to determine the biological productivity and structure of the crop.

The SAS 9.4 software package and the “Agrostat 6” computer program were used for mathematical and statistical processing of data from field experiments, namely on the yield. Indicators of economic efficiency

were calculated at the prices of 2021 [6; 7]. The main criterion for the energy evaluation of maize cultivation was the energy efficiency coefficient (C_{ee}). This is the ratio of the gross energy obtained with the crop to the gross energy of all technological operations and raw materials spent.

RESULTS AND DISCUSSION

According to the results of field research, it was found that the maize hybrids under study are quite flexible to the growing conditions, respond positively to the optimisation of the provision of nutrients and form yield, which differed little between hybrids, and the tendency of the reaction of hybrids to types of fertilisers was similar. The range of yield changes on average for 2019-2021 for the ES Concord hybrid was from 6.07 t/ha in the control version to 8.26 t/ha with the use of UAM 32 fertiliser at N₁₂₀ kg/ha a.s. against the N₂₂P₅₇K₅₇ background – DAP; ES Asteroid – from 6.16 t/ha to 8.50 t/ha, respectively (Table 3).

Table 3. Economic efficiency of growing ES Concord and ES Asteroid maize hybrids depending on the types of fertilisers, average for 2019-2021 (Experiment 1)

Variant ¹	Y ² t/ha ³	Yield increase				Cost of gross products	Production costs	Conditionally net profit	Profitability
		to control		to background					
		t/ha	%	t/ha	%				
ES Concord (A1)									
B1	6.07 ^a	–	–	–	–	36.4	20.3	16.1	80
B2	7.06 ^b	0.99	16.3	–	–	42.4	25.1	17.3	69
B3	7.67 ^c	1.60	26.4	0.61	8.64	46.0	31.8	14.3	45
B4	8.26 ^d	2.19	36.1	1.20	17.0	49.6	30.4	19.2	63
B5	7.74 ^c	1.67	27.5	0.68	9.63	46.4	29.2	17.3	59

Table 2, Continued

Variant ¹	Y ²	Yield increase				Cost of gross products	Production costs	Conditionally net profit	Profitability
	t/ha ³	to control		to background					
		t/ha	%	t/ha	%	UAH thous./ha			%
ES Asteroid (A2)									
B1	6.16 ^a	–	–	–	–	36.9	20.8	16.1	78
B2	7.17 ^b	1.01	16.4	–	–	43.0	25.7	17.4	68
B3	7.74 ^c	1.58	25.7	0.57	7.95	46.4	32.3	14.2	44
B4	8.50 ^d	2.44	38.0	1.33	18.6	51.0	31.1	19.9	64
B5	7.96 ^c	1.80	29.2	0.79	11.0	47.8	29.9	17.8	59
LSD	0.44								

Note: ¹ the content of the variant in Table 1; ²Y – yield; ³ substantial differences between values if indicated by different letters, SAS 9.4

The calculations of the economic efficiency of technologies show that the gross technological costs for the cultivation of maize hybrids differed substantially and ranged from 20.3 thousand UAH/ha in the control version to 32.3 thousand UAH/ha upon the introduction of UAM 32.

Calculations of the economic and energy efficiency of carrying out added fertilisation of crops with Humilin Stimul fertiliser allowed finding effective combinations of fertilisers and Humilin Stimul feeding. The scheme

of the experiment made provision for the establishment of the effectiveness of the joint application of a nitrogen fertiliser and Humilin Stimul feeding against the general background (N₂₂P₅₇K₅₇). Upon feeding with Humilin Stimul, the yield increased by 0.23-1.71 t/ha, depending on the combination of fertilisers (Table 4). It was proved that with all fertilisers under study, the highest effect was obtained with a one-time feeding in the BBCH 15-17 microstage (A2B2; A3B6; A4B9).

Table 4. Economic efficiency of growing maize hybrids using different nitrogen fertilisers and Humilin Stimul feeding, average for 2019-2021 (Experiment 2)

Variant designation ¹	Yield ²	Yield increase		Cost of gross products	Production costs	Conditionally net profit	Profitability
	t/ha	t/ha	%				
ES Asteroid							
A1B1	7.16 ^a	–	–	42.9	25.3	17.6	70
A2B2	7.99 ^c	0.83	11.6	47.9	29.9	18.0	60
A2B3	7.46 ^b	0.30	4.19	44.8	29.1	15.7	54
A2B4	7.81 ^c	0.65	9.08	46.9	29.9	16.9	56
A3B5	8.87 ^e	1.71	23.9	53.2	28.9	24.2	84
A3B6	7.99 ^c	0.83	11.6	47.9	27.6	20.3	73
A3B7	8.32 ^d	1.16	16.2	49.9	28.5	21.5	75
A4B8	8.37 ^d	1.21	16.9	50.2	27.9	22.4	80
A4B9	7.94 ^c	0.78	10.9	47.6	27.2	20.4	75
A4B10	8.14 ^{cd}	0.98	13.7	48.8	27.8	21.0	75
ES Concord							
A1B1	7.06 ^a	–	–	42.4	25.1	17.3	69
A2B2	7.77 ^c	0.71	10.1	46.6	29.2	17.4	60
A2B3	7.29 ^a	0.23	3.26	43.7	28.5	15.3	54
A2B4	7.58 ^b	0.52	7.37	45.5	29.2	16.3	56
A3B5	8.56 ^{de}	1.50	21.3	51.4	28.1	23.6	83
A3B6	8.03 ^c	0.97	13.7	48.2	27.3	20.9	77
A3B7	8.26 ^d	1.20	17.0	49.6	27.9	21.6	77
A4B8	8.33 ^d	1.27	18.0	50.0	27.4	22.6	82
A4B9	7.72 ^c	0.66	9.35	46.3	26.5	19.8	75
A4B10	7.97 ^c	0.91	12.9	47.8	27.2	20.6	76
LSD _{0.95}	0.41						

Note: ¹Designation of variants in Table 2. ²The difference is substantial for values indicated with different letters, SAS 9.4

When evaluating the effectiveness of technologies for growing agricultural crops in modern conditions, it is advisable to consider not the absolute yield, but a balanced ratio of the cost of the technology and the cost of the obtained products. Record harvests are rarely economically justified [10; 20]. Considering the strong positive response of maize to fertilisers, it is necessary to economically justify the effectiveness of fertilisers and establish the feasibility threshold [19]. This means not only the determination of the break-even point, but the economic expediency itself [18].

The use of new-generation fertilisers with macro- and microelement composition, nanofertilisers, and long-acting fertilisers ensures targeted use of nutrients by plants [25-27]. The increase in maize yield is closely correlated with the rate of nitrogen fertilisers, and their effectiveness increases with the joint use of nitrogen fertilisers and urease inhibitors, biological preparations that reduce the direct dependence on nitrogen fertiliser rates [28]. Fertilisers of biological origin and those obtained via secondary processing are widely used in production and require scientific justification of their effectiveness [29]. Fertiliser with the proposed mechanism of action can be successfully used to feed maize plants grown in areas with cyclic moisture deficit. Optimisation of plant nutrition is closely related to the preservation of soil fertility, microbiological biodiversity, and environmental safety [30; 31].

The potential fertility of the soils on which the study was conducted is quite high. The yield in the control variants in Experiment 1 – without fertilisers, in Experiment 2 on the background of $N_{22}P_{57}K_{57}$ – without fertilising crops, where fertilisers were not applied, was formed at a relatively high level – 6.07 t/ha and 6.16 t/ha, respectively, for the ES Concord and ES Asteroid hybrids. Application of $N_{22}P_{57}K_{57}$ in the form of DAP, based on the active substance, ensured an increase in yield by 0.99-1.01 t/ha or 16.4%. Added application of nitrogen fertilisers against the background of application of DAP provided an added increase in yield from 0.57% to 1.33%, or by 7.95-18.6%. With the introduction of UAM 32, the yield increase of the ES Concord hybrid was 2.19 t/ha or 36.1%; ES Asteroid hybrid – 2.44 t/ha or 38.0%, compared to the control variant. The reaction of hybrids to the introduction of ammonia water and urea, which was determined by the increase in productivity, was lower compared to the introduction of UAM 32 (Table 3).

The economic efficiency of cultivation technologies is dictated by the ratio of gross technological costs to the cost of gross products. The calculation of the economic efficiency confirmed that the fertilisation system is one of the most expensive items in the growing technology – costs ranged from 20.3 to 32.3 thous. UAH/ha depending on

the types of fertilisers. The introduction of UAM 32 on the background of $N_{22}P_{57}K_{57}$ (A1B4; A2B4) contributed to a high yield of hybrids, which ensured a high conditional net profit – 19.9 and 19.2 thous. UAH/ha, respectively, for the ES Asteroid and ES Concord hybrids. Added costs associated with the application of nitrogen fertilisers were paid off by a surplus harvest. The yield of maize grain production under study was extremely high – 44-80%. The economic efficiency of maize cultivation technologies, depending on the intensity of cultivation technologies in the conditions of the northern part of the Forest Steppe of Ukraine, was assessed by V.F. Kaminskyi and N.M. Asanishvili [19] and it was proved that the fertilisation system is costly, but the increase in yield covers all added costs.

Studies on the effectiveness of added fertilisation of crops with Humilin Stimul fertiliser allowed establishing that this technological solution is effective with various forms of fertilisers and Humilin Stimul feeding against the general background ($N_{22}P_{57}K_{57}$). Yu.O. Lavrynenko, O.A. Hozh, R.A. Vozhegova also indicate the high efficiency of microfertilisers with growth stimulants for growing maize under irrigation [13]. With all the fertilisers under study, the highest effect was obtained with a one-time feeding in the BBCH 15-17 microstage (A2B2; A3B6; A4B9) (Table 4). The yield increased by 0.23-1.71 t/ha, depending on the combination of fertilisers. Fertilisation of maize in the BBCH 17-19 microstage (A2B3; A3B6; A4B9) had a lower effect with earlier fertilisation. Carrying out two feedings, both in terms of productivity and economic efficiency, was inferior to the technology with one-time feeding. Production costs differed more depending on the types of fertilisers, with which Humilin Stimul fertilisation was carried out, and varied within 27.2-29.9 thous./ha against 25.3 thousand UAH/ha in the control background variant. In case of cultivation of the ES Asteroid hybrid, high efficiency was obtained upon one-time feeding with Humilin Stimul (BBCH 15-17) on the background of UAM 32: (A3B5) production costs amounted to UAH 28.9 thous./ha; conditionally net profit – UAH 24.2 thous./ha; profitability – 84%. A similar pattern was established for the ES Concord hybrid.

An important indicator for the assessment of technological measures in the cultivation of agricultural crops is energy costs, namely the comparison of the energy accumulated in the crop to the total energy spent on cultivation and harvesting [10; 18; 22].

The calculation shows that the lowest energy consumption for growing maize was in the control variant and varied from 16,919 in the ES Concord hybrid to 17,169 MJ/ha in the ES Asteroid hybrid (Fig. 1). Energy costs increased, both due to the added fertilisers applied and due to the costs of harvesting a surplus yield (Fig. 2).

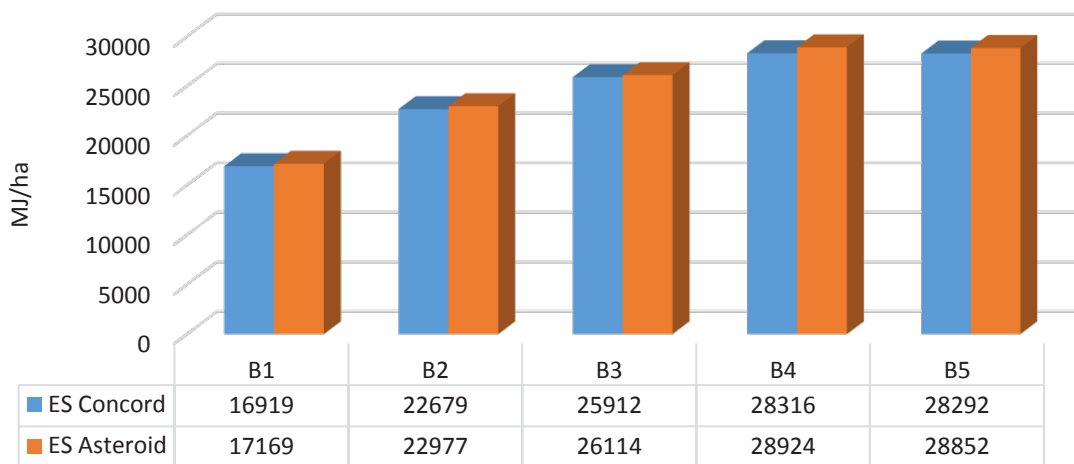


Figure 1. Energy costs for growing maize depending on the fertilisers, MJ/ha, average for 2019-2021, (Experiment 1)

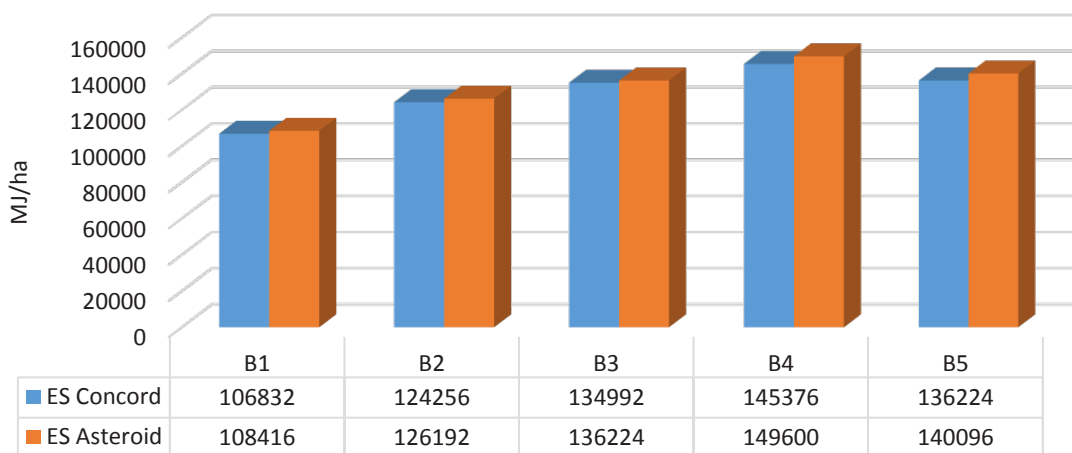


Figure 2. Gross energy yield of maize hybrids depending on the nitrogen fertilisers, MJ/ha, average for 2019-2021, (Experiment 1)

The energy efficiency coefficient has an inverse correlation with gross energy costs – it decreases with the growth of energy investments. Even an increase in productivity cannot balance energy costs and gross energy output. The energy efficiency coefficient in the control

version is 6.31, with the lowest energy costs for cultivation (Fig. 3). The coefficient of energy efficiency for the application of fertilisers decreases and is 4.81-5.49, depending on fertilisers applied for the cultivation of ES Concord and ES Asteroid hybrids.

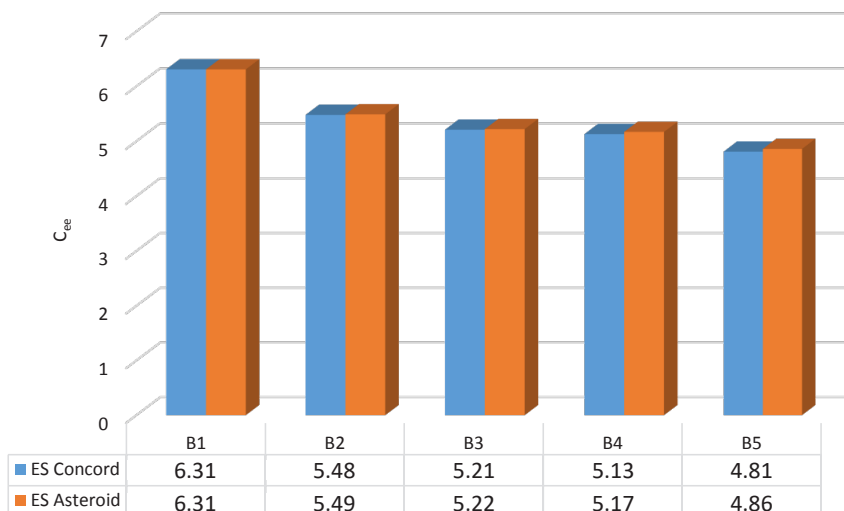


Figure 3. The coefficient of energy efficiency of maize cultivation depending on nitrogen fertilisers, average for 2019-2021 (Experiment 1)

Calculations of the economic and energy efficiency of added feeding of crops with Humilin Stimul fertiliser allowed proving the effective combinations of fertiliser and Humilin Stimul feeding. In case of the ES Asteroid hybrid cultivation, prominent efficiency was obtained from a one-time feeding with Humilin Stimul (BBCH 15-17) against the background of UAM 32: production costs amounted to UAH 28,979/ha; conditionally net profit – UAH 24,241/ha;

profitability – 84% (Table 4). A similar pattern was found for the ES Concord hybrid.

When feeding maize crops with Humilin Stimul, gross energy consumption ranged from 30,752 MJ/ha to 33,361 MJ/ha (Fig. 4). The lowest technological energy costs per hectare were in the control background version – 22,000 MJ/ha and 22,900 MJ/ha, respectively, for the ES Concord and ES Asteroid hybrids.

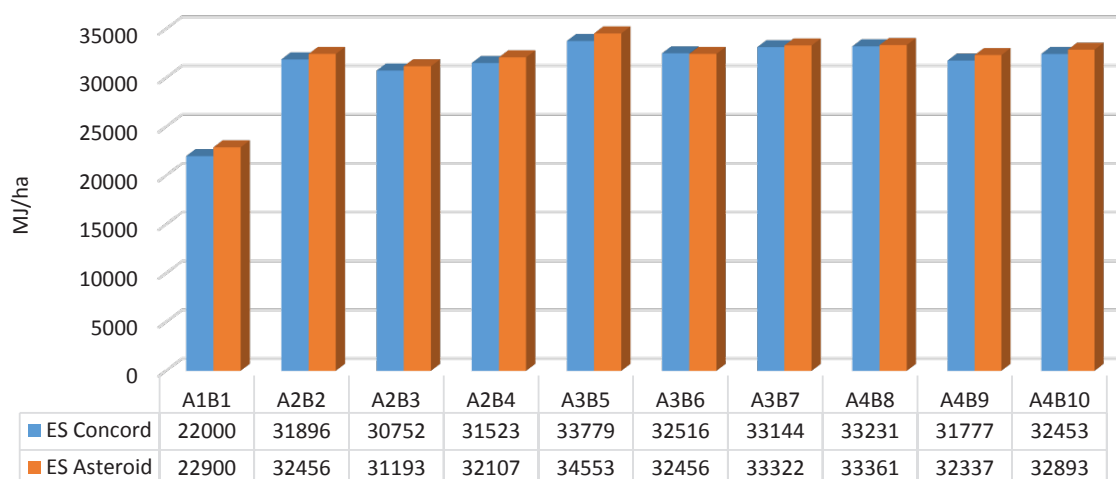


Figure 4. Energy costs for growing maize with the combined use of nitrogen fertilisers and the Humilin Stimul preparation, average for 2019-2021, thous. MJ/ha

Higher total technological energy costs were upon one-time feeding of crops with Humilin Stimul fertiliser in the phenological phase of 5-7 leaves on the background of nitrogen fertiliser UAM 32 (N120 kg/ha a.s.) – 34,553 MJ/ha and 33,779 MJ/ha, respectively, for the hybrid ES Asteroid

and ES Concord, which is associated with higher costs for harvesting an added yield.

Technologies for growing maize are characterised by elevated energy costs with a yield of 14,256-156,112 MJ/ha (Fig. 5).

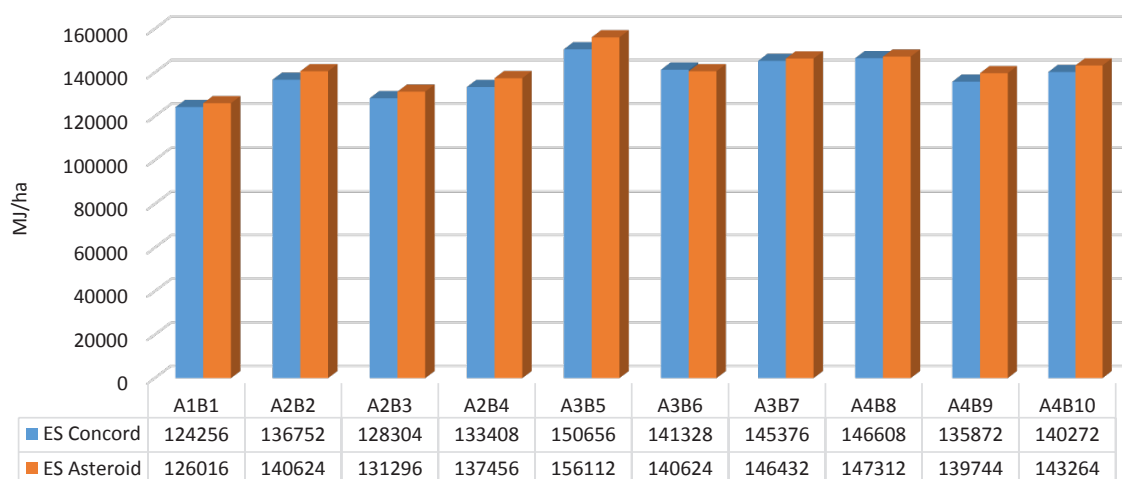


Figure 5. Gross yield energy of maize hybrids, depending on fertilisers and feeding with the Humilin Stimul preparation (Experiment 2)

Note: ¹Designation of variants in Table 2

Upon the joint application of several nitrogen fertilisers and feeding with the Humilin Stimul preparation, against the background of types of fertilisers, the energy

efficiency coefficient varied from 4.17 to 4.52, in the control version it was 5.48 (Fig. 6).

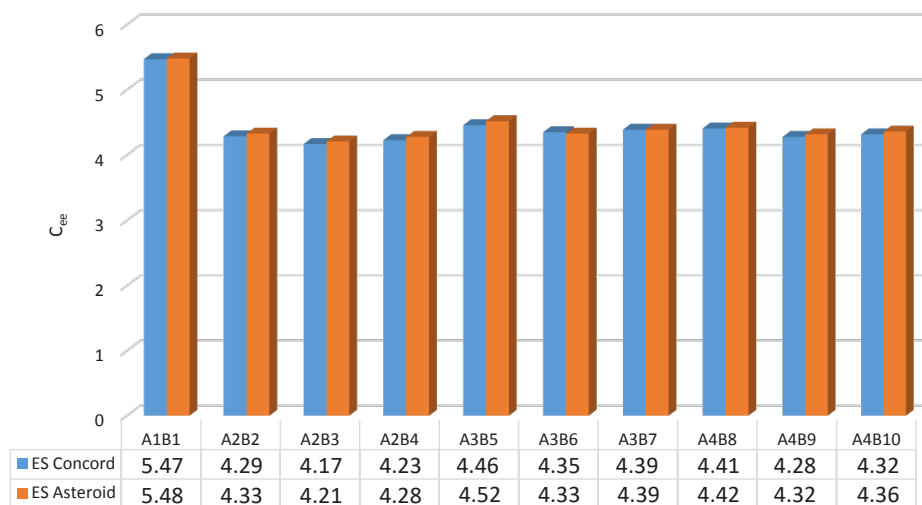


Figure 6. The energy efficiency coefficient of maize cultivation depending on the nitrogen fertilisers and feeding with the Humilin Stimul preparation, average for 2019-2021, (Experiment 2)

The decrease in the energy efficiency factor, even with increasing yield upon applying fertilisers and foliar feeding, is conditioned upon the rather elevated energy intensity of industrial chemical fertilisers.

The results of this study are original for the northern part of the Left Bank Forest Steppe of Ukraine since the effectiveness of various nitrogen-containing fertilisers for growing maize was investigated for the first time. A substantial difference in the type of fertilisers, productivity, and economic efficiency was proved.

CONCLUSIONS

Optimising the nutrition of maize hybrids using diverse types of fertilisers, application methods, and evaluating the economic and energy efficiency of growing technologies allowed proving their high economic and practical feasibility.

Maize hybrids are quite flexible to the growing conditions, respond positively to the optimisation of nutrition and form a yield that varies within 6.07 to 8.50 t/ha, and conditional net profit – UAH 15.7-24.2 thous./ha (ES Asteroid) and UAH 15.3-23.6 thous./ha (ES Concord). Application of $N_{22}P_{57}K_{57}$ in the form of DAP provides a yield increase by 0.99-1.01 t/ha, or 16.4%. Added application of nitrogen fertilisers against the background of DAP provides a yield increase from 0.57 t/ha to 1.33 t/ha, or by

7.95-18.6%. With the introduction of UAM 32, the yield increase of ES Concord amounted to 2.19 t/ha, or 36.1%; ES Asteroid – 2.44 t/ha, or 38.0%. Calculations of the economic and energy efficiency of added fertilisation with Humilin Stimul allowed proving effective combinations of fertilisers and feeding with Humilin Stimul. With the addition of Humilin Stimul, the yield increased by 0.23-1.71 t/ha, depending on the fertiliser. High efficiency was obtained with a one-time feeding of Humilin Stimul (BBCH 15-17) on the background of UAM 32 for the ES Asteroid hybrid: production costs were UAH 28,979/ha; conditionally net profit – UAH 24,241/ha; profitability – 84%. Technologies for growing maize are characterised by a high output of energy with a yield of 14,256-156,112 MJ/ha. The energy efficiency coefficient for the joint use of nitrogen fertilisers and maize fertilisation with Humilin Stimul on the background of the types of fertilisers changes from 4.17 to 4.52, in the control variant – 5.48.

The obtained results are important for the optimisation of maize nutrition and the introduction of the proposed forms of fertilisers and their combinations into production. At the same time, issues regarding the influence of different forms of fertilisers on soil biodiversity, preservation, and distribution of individual microbiological groups remain understudied. These areas of research are extremely relevant and require further investigation.

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Економічна та енергетична ефективність технологій вирощування гібридів кукурудзи залежно від виду та способів внесення добрив

Анотація. Актуальність вирішення проблеми зростання виробництва зерна кукурудзи є безумовною з врахуванням потреб людства, економічної та енергетичної ефективності технологій вирощування, яка суттєво залежить від виду добрив та кратності їх внесення. Метою дослідження є встановлення ефективності виробництва зерна кукурудзи за оптимізації системи удобрення. Польові дослідження проводили в фермерському господарстві «Богатирівське» Роменського району Сумської області впродовж 2019–2021 рр. на темно-сірому опідзоленому ґрунті. Математичну та статистичну обробку проведено з використанням програмного пакету SAS 9.4 та «Агрозат 6». Економічну та енергетичну оцінку технологій проводили з використанням розрахункового методу. Гібриди є пластичними до умов вирощування, позитивно реагують на оптимізацію живлення – урожайність варіює від 6,07–6,16 в контролі до 8,26–8,50 т/га за застосування карбонідоаміачної селітри 32 на фоні $N_{22}P_{57}K_{57}$. За фонового внесення $N_{22}P_{57}K_{57}$ урожайність зростала на 16,4 %. Азотні добрива по фоні забезпечували додаткове зростання урожайності на 7,95–18,6 %. За внесення карбонідоаміачної селітри 32 приріст врожайності склав 2,19 та 2,44 т/га або 36,1 % і 38,0 % відповідно гібриду Конкорд і Астероїд. Реакція гібридів на аміачну воду та карбамід нижча порівняно з карбонідоаміачною селітрою. Виробничі витрати на вирощування кукурудзи склали 20,3–32,3 тис. грн/га. Внесення карбонідоаміачної селітри на фоні $N_{22}P_{57}K_{57}$ забезпечувало отримання високого умовно чистого прибутку – 19,2–19,9 тис. грн/га. Рентабельність виробництва зерна кукурудзи є досить високою – 44–80 %. За підживлення Гумілін Стимул урожайність зростає на 0,23–1,71 т/га залежно від комбінації добрив. В комбінації з усіма видами добрив найвищу ефективність отримано за одноразового підживлення на ВВСН 15-17. Коефіцієнт енергетичної ефективності за внесення добрив знижується. Результати дослідження мають важливе значення для виробництва і подальших напрямів дослідження, зокрема біорізноманіття ґрунтової мікрофлори

Ключові слова: ринок зерна, урожайність, виробничі витрати, умовно чистий прибуток, рентабельність

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Adaptive Potential of Fenugreek Species at Different Sowing Times

Abstract. Among the unexplored technological elements of growing fenugreek in the conditions of Ukraine is the creation of conveyor cultivation of the culture. Therefore, the problem of the influence of sowing dates on the growth and development of plants, the parameters of the harvest and the qualitative composition of marketable products is acute. The purpose of this study was to substantiate the optimal sowing dates of fenugreek species to increase the yield of dried products in the conditions of Polissia on sod-podzolic soils. The leading methods employed to investigate this issue were the field method – to find the interaction of the object of research with biotic and abiotic factors, statistical method – to evaluate the research results to find the parameters of fenugreek's adaptive capacity. During 2012-2014, for different sowing periods, the average yield of dry mass in blue fenugreek varied from 0.5 to 1.3 t/ha, and in hay fenugreek – from 0.6 to 1.5 t/ha. The early spring period and the late spring period of the 1st sowing period were characterized by the highest overall adaptive capacity. The highest value of the specific adaptive capacity was established during the late spring 2nd sowing period. The relative stability index ranged from 2.6 to 11.4. Blue fenugreek during the late spring 1st and 2nd sowing periods responds well to improved growing conditions ($b_i > 1$), and fenugreek hay during the late spring 2nd and summer sowing periods. Early spring and late spring 1st sowing period samples are characterized by high breeding value. An increase in the sum of effective temperatures by 1°C contributed to the fluctuation of green mass yield from 4.82 to 128 kg/ha, and dry mass yield from 0.401 to 6.63 kg/ha. At the same time, an increase in precipitation by 1 mm affected the fluctuation of the yield of green mass from 15.0 to 146 kg/ha, and of dry mass from 0.145 to 25.9 kg/ha. Commercial production of fenugreek species was formed within the range of the sum of air temperatures ($>10^\circ\text{C}$) 383.9-487.1°C, the amount of precipitation 70.7-144.3 mm, and the growing season ranged from 35 to 55 days. The highest productivity of fenugreek species was found during sowing from the second decade of April to the first decade of May. The materials of this paper are of practical value for expanding the species diversity of vegetables and increasing the supply of the population with valuable food products and during the application of new and improved elements of technologies for growing valuable rare crops to achieve the maximum effect of fulfilling the genetic potential of plants

Keywords: blue fenugreek, hay fenugreek, sowing time, selection value of the genotype

INTRODUCTION

Since ancient times, fenugreek has been used as a spice, food, and medicinal crop, and more recently as a functional food (food that provides health and wellness benefits in addition to nutrients) [1]. Fenugreek seeds contain a considerable amount of fibre, phospholipids, glycolipids, oleic acid, linolenic acid, linoleic acid, choline, vitamin A, B1, B2, C, nicotinic acid, niacin, and many other functional elements [2; 3]. It is necessary to focus on the development of new cultivated varieties, to completely fulfil their potential as edible plants along with medicinal value [4].

Fenugreek is grown on different continents, in different soil and climatic conditions [5]. However, the development of plants is influenced not only by soil and climatic conditions, but also by technological interventions [6]. Sowing time and row width are important agronomic factors directly affect the yield level [7; 8]. The optimal timing of sowing opens the way to more efficient use of light, temperature, precipitation, and other environmental factors [9]. In general, early sowing times are better because of the expected beneficial effect on seed

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germination, plant growth and development, duration of the growing season, and yield [10].

Numerous studies have shown that climate change has adverse effects on food production and food security, and, according to forecasts, adverse effects will increase in the future [11-13]. Climate variability, such as being too wet or too dry, also affects yield [14; 15]. High average air temperatures affect crops through several mechanisms, including acceleration of plant growth and total evapotranspiration, and thus yields will decrease in response to increased temperatures [16]. Crops are especially sensitive to strong heat during the reproductive period, when it can substantially reduce the amount of grain and the final yield, and in the most negative cases lead to a complete loss of the harvest [17].

The changes in temperature and precipitation predicted by global climate models for the 2050s will lead to a global decrease in crop yields if planting and harvesting dates stay unchanged. However, adaptation of sowing dates and variety selection increases yield in temperate regions and allows avoiding 7-18% losses [18].

Technologies aimed at maximizing the protective reactions of the plant organism to adverse environmental conditions are of particular importance in vegetable production. Such technologies have a prominent degree of flexibility, considering environmental conditions in connection with a change in the set of technological operations, which are most suitable in particular conditions of the growing season [19]. The defining element in such technologies is the variety due to its biological, morphological, and genetic features, the constructed strategy for the selection of the predecessor, tillage, fertilizers, sowing, crop care, and harvesting.

A variety, as a biological system, should be considered in terms of the fulfilment of genetic potential in a particular ecological region [20]. The value of varieties is determined by their plasticity and stability, or the ability to form a certain level of yield in different environmental conditions [21]. The genotype \times environment interaction must be considered when developing selection programs focused on nutrition quality parameters, since selection in particular soil conditions can provide further optimization of nutrition quality [22; 23].

The purpose of this study was to find the best date of sowing to obtain the highest yield of dry mass of fenugreek species on sod-podzolic soils of Polissia.

MATERIALS AND METHODS

The research was conducted during 2012-2014 at the National University of Life and Environmental Sciences of Ukraine at the collection site of the Fruit Orchard, in the Kyiv Oblast. Two local samples of fenugreek were investigated: blue fenugreek (*Trigonella coerulea* (Desr.) Ser.) and hay fenugreek (*Trigonella foenum graecum* L.). Species were sown simultaneously in four terms: early spring – II-III decade of April (April 10 – 2012, April 24 – 2013, April 10 – 2014); late spring of the 1st term – 3rd decade of April – 1st decade of May (04/25 – 2012, 05/08 – 2013,

04/29 – 2014); late spring of the 2nd term – the 2nd decade of May (May 15, 2012, May 17, 2013, May 14, 2014); summer – 1st decade of June (06/10 – 2012, 06/04. – 2013, 06/05 – 2014). Moreover, the seeds for the early spring and the first late spring terms in 2013 had to be sown later due to the prolonged cold spring. Control – early spring sowing period (II-III decade of April). Repetition – three times with randomization. The registered area of the site was 5 m². Calculations were carried out on 30 plants – 10 from each repetition. The seeding scheme for fenugreek was 45 \times 15 cm. The depth of wrapping the seeds of blue fenugreek was 1.0-1.5 cm, hay fenugreek – 2-3 cm.

The field research method was used to determine the quantitative assessment of the effect of the researched factors on growth, development, plant productivity, and crop quality. The reliability of the obtained results was determined using a statistical method.

Analysis of variance (ANOVA) and correlation analysis were performed using the XLSTAT add-on in MS Excel. It was considered that the differences are significant when $\alpha=0.95$ [24].

The indicator of general adaptive capacity (GAC) was defined as the average value of the trait in various environmental conditions, and specific adaptive capacity (SAC) as a deviation from the general adaptive capacity in a certain environment [25]. The method of evaluating the GAC and SAC is based on testing a population of n genotypes in m environments. The number of repetitions is equal to c . Then (1):

$$x_{ikr} = U + V_i + d_k + (Vd)_{ik} + e_{ikr} \quad (1)$$

where x_{ikr} is the phenotypic value of (i) genotype grown in (k) environment in (r) replication; U is the overall average of the entire population of phenotypes; V_i is the effect of (i) genotype; d_k is the effect (k) of environment; $(Vd)_{ik}$ is the effect of interaction of (i) genotype with (k) environment; e_{ikr} is an effect due to random causes and attributed to the (ikr) phenotype.

The following restrictions were imposed on the elements of the model (2):

$$\sum_i v_i = \sum_k d_k = \sum_k (vd)_{ik} = \sum_k (vd)_{ik} = \sum e_{ik} = 0 \quad (2)$$

GAC effects (3):

$$GAC_i = V_i = X_i - u \quad (3)$$

The parameters of the adaptive capacity of fenugreek species were as follows (4; 5):

$$\sigma^2 SAC_i = \frac{1}{m-1} * \sum_R (d_R + Vd_{iR})^2 - \frac{m-1}{m} * \sigma^2 \quad (4)$$

$$\sigma SAC_i = \sqrt{\sigma^2 SAC_i} \quad (5)$$

To compare the variability of various features upon investigating fenugreek species and habitats, the relative stability of the species according to the following S_{gi} feature was used (6):

$$S_{gi} = \frac{\sigma_{SAC_i}}{U + GAC} * 100 \% \quad (6)$$

The reaction of fenugreek species to the improvement of environmental conditions was determined by the value of the regression coefficient b_i (7):

$$b_i = \frac{\sum X_{iR} * d_R}{\sum R d_R^2} \quad (7)$$

To select fenugreek species that combine productivity and stability, the selection value of the genotype was used (8):

$$SVG_i = U + GAC_i - p \sigma_{SAC_i} \quad (8)$$

To figure out when a certain stage of plant development will occur ("sowing-seedlings", "seedlings-beginning of budding", "beginning of budding-beginning of flowering", "beginning of flowering-beginning of ripening of beans", "seedlings-beginning of ripening of beans") the Growing Degree Days method was used, which is based on the real temperature [26]. The sum of effective air temperatures for the interphase period was calculated using the formula (9):

$$\sum t_{ef} = (t_{avg} - B) * n \quad (9)$$

where $\sum t_{ef}$ is the sum of the effective air temperatures for the period; °C, t_{avg} is the average active air temperature for the period, °C; B is the biological minimum, which in this study was taken as 10°C; n is the number of days in the period.

RESULTS AND DISCUSSION

The duration of the phenological phases of development of blue fenugreek from the 2nd decade of April to the 1st decade of June was established as follows: "sowing-seedling" ranged from 6 to 12 days and took place at total air temperatures (>10°C) of 21.3-70.5°C and total precipitation of 10.3-45.3 mm; "seedling-beginning of budding" – from 28 to 39 days, for total air temperatures (>10°C) of 310.0-316.2°C and total precipitation of 47.7-123.3 mm; "the beginning of budding-the beginning of flowering" – from 4 to 7 days, for the total air temperatures (>10°C) of 48.2-78.9°C and the total precipitation of 2.3-24.1 mm; "the beginning of flowering-the beginning of ripening of beans" – from 3 to 6 days, for the total air temperatures (>10°C) of 35.4-61.7°C and the total precipitation of 3.6-23.7 mm; "seedlings-the beginning of ripening of beans" – from 36 to 52 days, for the total air temperatures (>10°C) of 398.9-450.6°C and the total precipitation of 70.7-133.3 mm (Fig. 1).

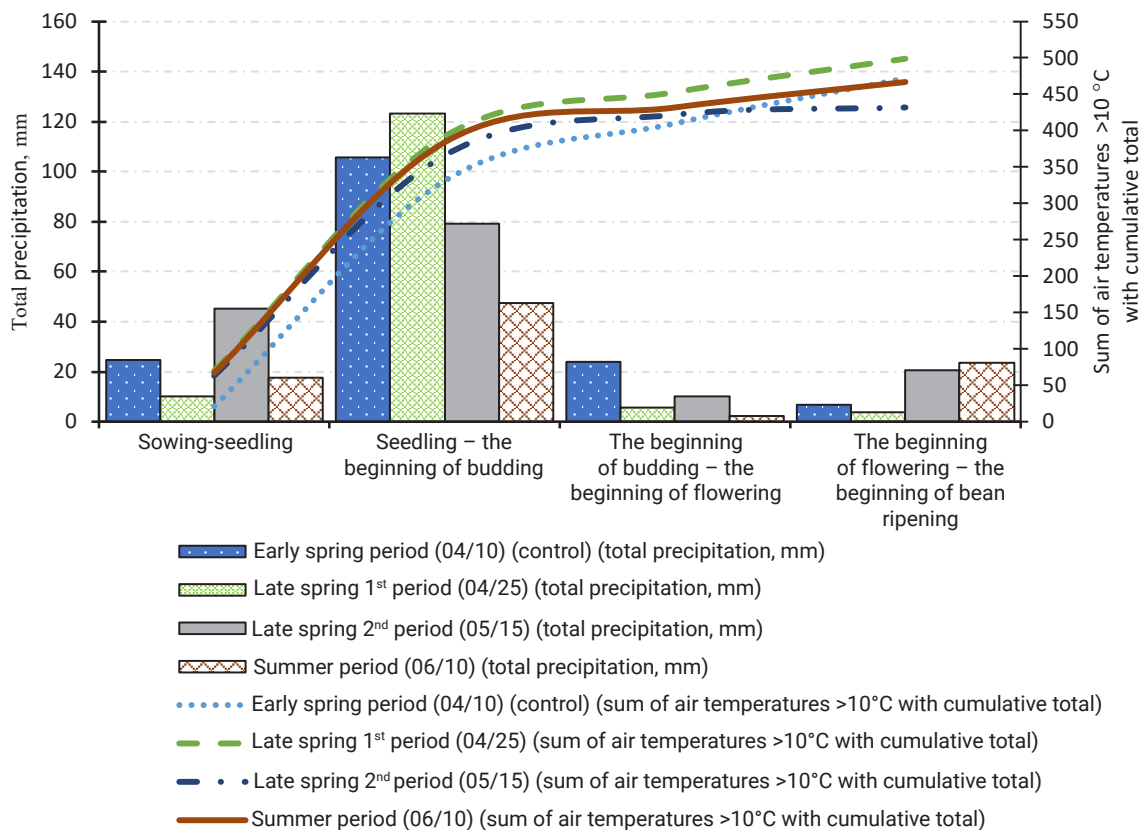


Figure 1. The sum of air temperatures above 10°C with a cumulative total and the total precipitation necessary to complete the phenological phases of the development of blue fenugreek at different sowing times (average for 2012-2014)

Depending on the timing of hay fenugreek sowing, the ranges of phenological phases of development were established as follows: “sowing–seedling” ranged from 7 to 12 days and took place during the total air temperatures (>10°C) of 24.6–82.1°C and the total precipitation of 12.0–20.7 mm; “seedling–beginning of budding” – from 26 to 37 days, for total air temperatures (>10°C) of 287.7–325.1°C and total precipitation of 40.7–122.0 mm; “the beginning of budding–the beginning of flowering” – from 5 to 6 days,

for the total air temperatures (>10°C) 48.0–63.0°C and the total precipitation 3.0–28.7 mm, “the beginning of flowering – the beginning of ripening beans” – from 3 to 12 days, for total air temperatures (>10°C) of 30.3–135.6°C and total precipitation of 21.3–35.7 mm; “seedlings–the beginning of ripening of beans” – from 35 to 55 days, for the total air temperatures (>10°C) 383.9–487.1°C and the total precipitation 71.3–144.3 mm (Fig. 2).

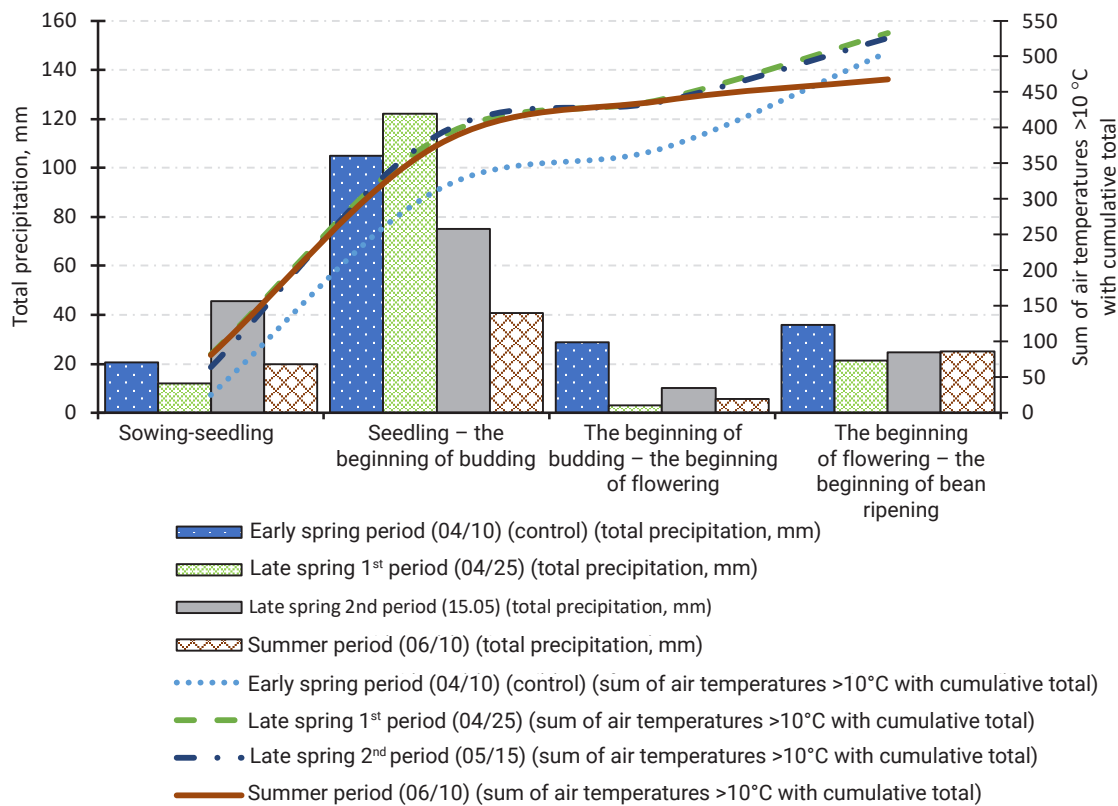


Figure 2. The sum of air temperatures above 10°C with a cumulative total and the sum of precipitation necessary to complete the phenological phases of hay fenugreek development during different sowing periods (average for 2012-2014)

C. Karma *et al.* (2017) found that different sowing dates in West Bengal differed significantly in number of days to germination, sowing on 30 December showed the maximum number of days (14.13 days) and the minimum number of days was achieved by fenugreek plants sown on 30 October (9.38 days) [27]. R. Sheoran *et al.* (2000) found that during a 14-day delay in sowing, the analysed fenugreek varieties formed inflorescences earlier and ripened faster. Furthermore, late sowing reduced plant requirements for warm temperatures, which is critical when plants requiring higher temperatures must be adapted to grow in more severe climates [28]. Ionescu and Roman (2013) note that fenugreek ripened in the third decade of July, after 95 days of vegetative growth, and the number of accumulated heat units was 922.2 (>10°C) [29].

In general, culture needs a cool climate during vegetative growth, and a dry climate during seed maturation [30]. Plants sown at the optimal time have more chances to achieve the correct phenological development. A delay in sowing, on the contrary, shortens the duration of the vegetation period of plants and successive phases in growth and development [31].

Based on the conducted analysis of variance, it was established that the yield of fenugreek species considerably depended on the genotype, environment, repetitions, and interaction of factors with the share of their influence being 18%, 36%, 24%, and 18%, respectively, with the predominance of the influence of the environment (Fig. 3)

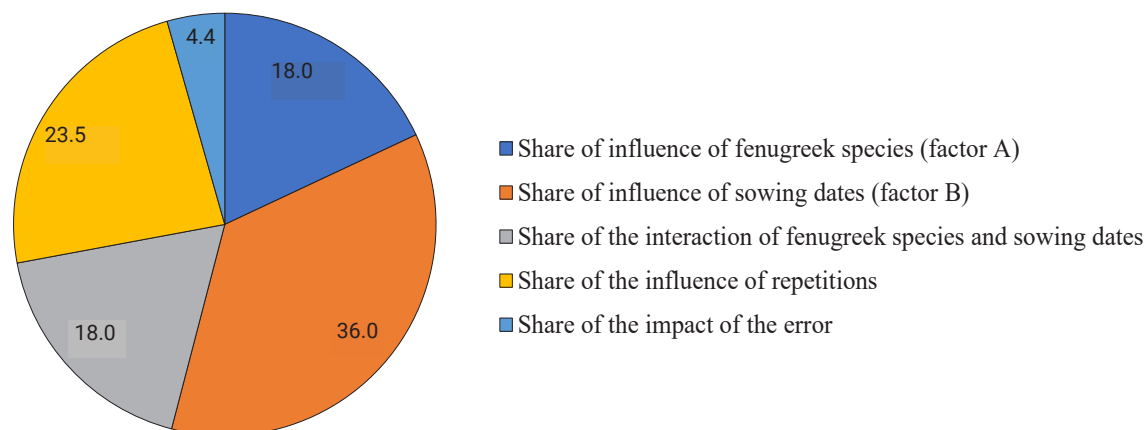


Figure 3. The share of influence of individual elements of the experiment on fenugreek productivity, % (average for 2012-2014)

Among the features of fenugreek species under study, yield is of particular importance as a holistic indicator of genetic potential. The range of yield variation shows the difference between the minimum and maximum value and characterizes the stability of a certain genotype. Varieties with a smaller range of variation have better adaptability. During the research period, the best value in terms of variation stability can be identified for fenugreek in the early spring period (0.2 t/ha) and late spring of the 1st sowing period (0.3 t/ha), and for blue fenugreek in the early spring period (0.3 t/ha), which were characterized by a high yield of dry mass within 1.1-1.4 t/ha (Table 1). The widest range of yield fluctuations (0.5 t/ha) was noted during the late spring 2nd period of fenugreek sowing. The time of sowing is crucial for the

growth and development of plants and obtaining the highest possible yield. Sowing too early or too late can hinder growth, reduce yield, and worsen the quality of the crop [32]. Radojka and Jevdjovic (2007) found that sowing in the first two weeks of April resulted in considerably higher yields compared to sowing at the end of April and during May [33]. In Kansas, A. Obour *et al.* (2015) reported no effect of sowing between April 1 and May 22 on dry matter yield [34]. In Poland, T. Bienkowski *et al.* (2016) found that sowing seeds as early as possible or with a delay of 10 days did not cause a significant decrease in seed yield, although delaying the sowing date by 20 days could reduce seed yield by 3-13%. Early sowing of fenugreek in West Bengal (October 30) is recommended for fast growth and high yield [35].

Table 1. Parameters of adaptive capacity by dry mass yield (average for 2012-2014)

Type (factor A)		Blue fenugreek				Hay fenugreek			
Sowing terms (factor B)		Early spring term (04/10) (control) 2012-2014	Late spring 1 st term (04/25) 2012-2014	Late spring 2 nd term (05/15) 2012-2014	Summer term (06/10) 2012-2014	Early spring term (04/10) (control) 2012-2014	Late spring 1 st term (04/25) 2012-2014	Late spring 2 nd term (05/15) 2012-2014	Summer term (06/10) 2012-2014
Dry mass yield, t/ha	Min	1.0	0.9	0.8	0.5	1.3	1.1	0.9	0.6
	Max	1.3	1.3	1.2	0.7	1.5	1.4	1.4	1.0
	Average	1.2	1.1	1.0	0.6	1.4	1.3	1.1	0.8
	Range of variation	0.3	0.4	0.4	0.2	0.2	0.3	0.5	0.4
Coefficient of variation (V), %		13.1	18.2	21.5	16.7	7.1	12.1	27.1	27.2
GAC _i		125	58	-75	-442	358	225	25	-275
SAC _i		61.4	82.4	86.0	36.6	36.6	61.4	121.1	86.0
Relative stability (S _{gi})		5.3	7.5	8.9	6.1	2.6	4.8	11.4	11.2
Plasticity (b _i)		0.84	1.16	1.21	0.58	0.58	0.84	1.57	1.21
Selection value (SVG _i)		742	529	371	347	1147	842	228	171

On average, the coefficient of yield variation for blue fenugreek was 17%, depending on individual sowing dates – from 13% to 22%; for hay fenugreek – 18%, depending on individual sowing dates – from 7% to 27%. In the case of blue fenugreek, a slight variation of the trait was found during the early spring sowing period, medium – late spring 1st season and summer, significant – late spring 2nd period. For hay fenugreek, a slight variation was found for the early spring sowing period, medium – for the late spring 1st sowing period, significant – for the late spring 2nd sowing period and summer.

Plasticity and stability allow predicting the behaviour of varieties in production conditions. Varieties where the regression coefficient is significantly lower than 1 belong to the neutral type. They do not respond well to changes in the environment. Under conditions of intensive farming, they do not reach high indicators, but under poor conditions, their indicators decrease to a lesser extent compared to varieties of the intensive type [36].

Varieties with a regression coefficient above 1 belong to the intensive type. In years with unfavourable weather conditions, including when there is a low agricultural background, they sharply reduce productivity. A close to zero or zero value of the regression coefficient reflects a variety that does not respond to environmental changes. With a smaller square deviation of the real indicators compared to the theoretically expected ones, the variety is more stable [37].

The coefficient of regression of dry weight yield of fenugreek species was different. During the late spring 1st and 2nd sowing periods, blue fenugreek responds well to improved growing conditions ($b_i > 1$): with an increase in average yield by 1 kg/ha, the increase in dry weight was 1.16 and 1.21 kg/ha, respectively. The lowest sensitivity to changes in environmental conditions for blue fenugreek was established during the early spring and summer sowing period: with an increase in average yield by 1 kg/ha, the increase in dry weight was 0.84 and 0.58 kg/ha, respectively.

Hay fenugreek is characterized by high sensitivity ($b_i > 1$) to the improvement of growing conditions during the late spring 2nd and summer sowing periods: with an increase in the average level of yield by 1 kg/ha,

the increase in dry weight was 1.57 and 1.21 kg/ha, respectively. Hay fenugreek reacted the least to the improvement of growing conditions during the early spring and late spring 1st sowing period: with an increase in the average level of yield by 1 kg/ha, the increase in dry mass was 0.58 and 0.84 kg/ha, respectively.

GAC characterizes the ability of a cultivated plant to obtain a consistently prominent yield under any conditions, and SAC indicates the plasticity of a cultivated plant, i.e., the ability to adapt to specific conditions (low temperature, drought, pests, etc.) [38].

At different sowing dates, the samples of fenugreek species under study ranged widely – from -442 to 358 according to the GAC parameter. The maximum GAC value was noted in hay fenugreek during the early spring and late spring 1st sowing period, with indicators of 358 and 225, respectively. For blue fenugreek, the maximum value was also established for the early spring and late spring 1st sowing period with indicators of 125 and 58, respectively. Summer sowing is characterized by the lowest GAC value.

The highest SAC value (86.0) for blue fenugreek was established in the late spring 2nd sowing period, and the lowest (36.6) – in the summer. For hay fenugreek, the highest SAC value (121.1) was established for the late spring 2nd sowing period, and the lowest (36.6) – for the late spring 1st sowing period.

The best variety for the test zone is the one that had the maximum GAC value, forms the highest yield under favourable growing conditions (weather and climatic conditions of the area, appropriate agricultural technology) and ensures prominent yield stability [39].

A variety with a high but unstable yield cannot guarantee maximum productivity in production conditions with insufficient care and adverse weather conditions. An integrated indicator that characterizes the combination of high productivity and stable yield in a variety is the selection value of the genotype (SVG_i) [40]. Early spring and late spring 1st sowing period samples are characterized by high breeding value.

Using correlation dependence calculations, the relationship between the parameters that evaluate the adaptive properties of genotypes was established (Table 2).

Table 2. Matrix of correlations between parameters of the adaptive capacity of fenugreek species (average for 2012-2014)

Indicator	Dry mass yield, t/ha	Range of variation, t/ha	Coefficient of variation (V), %	GAC _i	SAC _i	Relative stability (S _{gr})	Plasticity (b _i)	Selection value (SVG _i)
Dry mass yield, t/ha	1							
Range of variation, t/ha	-0.08	1						
Coefficient of variation (V), %	-0.60	0.82	1					

Indicator	Dry mass yield, t/ha	Range of variation, t/ha	Coefficient of variation (V), %	GAC _i	SAC _i	Relative stability (S _{gi})	Plasticity (b _i)	Selection value (SVG _i)
GAC _i	0.99	-0.08	-0.60	1				
SAC _i	-0.07	0.99	0.82	-0.07	1			
Relative stability (S _{gi})	-0.54	0.86	0.99	-0.54	0.86	1		
Plasticity (b _i)	-0.11	0.99	0.85	-0.11	0.99	0.88	1	
Selection value (SVG _i)	0.81	-0.64	-0.94	0.81	-0.64	-0.92	-0.66	1

The calculation of the correlation coefficient showed that the average yield has a direct strong relationship with the GAC index ($r=0.99$) and selection value ($r=0.81$) and an average inverse relationship with the coefficient of variation ($r=0.60$) and relative stability ($r=0.54$).

GAC has a strong direct relationship with the index of average yield ($r=0.99$) and selection value ($r=0.81$) and an inverse average relationship with the coefficient of variation ($r= -0.60$).

The SAC index has a strong direct relationship with the range of variation ($r=0.99$), plasticity ($r=0.99$), relative stability ($r=0.86$), and coefficient of variation ($r=0.82$).

A direct strong relationship was established between relative stability and the coefficient of variation ($r=0.99$), plasticity ($r=0.88$), range of variation ($r=0.86$), SAC ($r=0.86$) and inverse average relationship with GAC ($r= -0.54$).

The plasticity indicator has a direct strong relationship with the SAC index ($r=0.99$), the range of variation ($r=0.99$), relative stability ($r=0.88$) and the coefficient of variation ($r=0.85$) and an inverse average relationship with selection value ($r= -0.66$).

Selection value has a direct strong relationship with average yield ($r=0.81$), and an inverse average relationship with GAC ($r=0.81$). An inverse relationship was found between the selection value and the coefficient of variation ($r= -0.94$), relative stability ($r= -0.66$), SAC ($r= -0.64$), and range of variation ($r= -0.64$).

A direct strong relationship was found between dry mass yield at different sowing dates and the total effective temperatures (r =from 0.66 to 0.99). An inverse medium and strong relationship (r =from -0.48 to -0.99) was found between the yield of dry mass at different sowing times and the amount of precipitation.

According to the results of the regression equations, it was found that an increase in the total effective temperatures by 1°C contributed to the fluctuation of the yield of the blue fenugreek: in the early spring period (control) – by 128 kg/ha (green mass) or by 6.06 kg/ha (dry mass); in the late spring 1st period – by 53.0 kg/ha (green mass) or by 3.98 kg/ha (dry mass); in the late spring 2nd period – by -11.1 kg/ha (green mass) or by -1.81 kg/ha (dry mass); in the summer period – by -4.82 kg/ha (green mass) or by -0.401 kg/ha (dry mass). An increase in the

amount of precipitation by 1 mm affected the yield: in the early spring period (control) – by 146 kg/ha (green mass) or by 5.38 kg/ha (dry mass); in the late spring 1st period – by 28.3 kg/ha (green mass) or by 0.145 kg/ha (dry mass); in the late spring 2nd period – by -96.7 kg/ha (green mass) or by -22.7 kg/ha (dry mass); in the summer period – by -16.1 kg/ha (green mass) or by -3.42 kg/ha (dry mass).

An increase in the total effective temperatures by 1°C contributed to the fluctuation of hay fenugreek yield: in the early spring period (control) – by 14.1 kg/ha (green mass) or by 1.58 kg/ha (dry mass); in the late spring 1st period – by 33.9 kg/ha (green mass) or by 2.21 kg/ha (dry mass); in the late spring 2nd period – by 21.2 kg/ha (green mass) or by 6.63 kg/ha (dry mass); in the summer period – by 10.25 kg/ha (green mass) or by 4.33 kg/ha (dry mass). An increase in the amount of precipitation by 1 mm affected the yield: in the early spring period (control) – by 15.0 kg/ha (green mass) or by 1.07 kg/ha (dry mass); late spring 1st period – by 22.7 kg/ha (green mass) or by -0.693 kg/ha (dry mass); in the late spring 2nd period – by -77.3 kg/ha (green mass) or by 25.9 kg/ha (dry mass); in the summer period – by 18.4 kg/ha (green mass) or by 6.80 kg/ha (dry mass).

CONCLUSIONS

The obtained results suggest that the air temperature at different times of sowing had a great influence on the growth and development of plants, namely, the “sowing-seedling” period of fenugreek species varied from 6 to 12 days and passed for the total air temperatures (>10°C) 21.3-82.1°C and the total precipitation of 10.3-45.3 mm, “seedling–start of budding” – 26-39 days, 287.7-325.1°C, 40.7-123.3 mm; “beginning of budding–beginning of flowering” – 4-7 days, 48.0-78.9°C, 2.3-28.7 mm; “the beginning of flowering, the beginning of ripening of beans” – 3-12 days, 30.3-135.6°C, 3.6-35.7 mm; “seedling – the beginning of ripening of beans” – 35-55 days, 383.9-487.1°C, 70.7-144.3 mm.

Blue and hay fenugreek have the highest breeding value of the genotype (SVG_i =742 and 1,147, respectively) in terms of yield, high ecological stability (S_{gi} =5.3 and 2.6, respectively) and plasticity (b_i =0.84 and 0.58%, respectively) received during the early spring (II-III decade of

April) sowing period. To obtain a high yield of dry mass of plants, fenugreek should be grown during the early spring (II-III decade of April) and 1st late spring (III decade of April-I decade of May) sowing period, during which a more developed vegetative mass was formed and a higher yield of dry mass was established 1.3-1.4 t/ha – hay fenugreek and 1.1-1.2 t/ha blue fenugreek. The practical significance of the obtained results lies in the scientific substantiation of the best sowing dates of

fenugreek for correcting the cultivation technology on the sod-podzolic soils of Polissia.

Elucidating the use and potential of particular fenugreek species is a promising area for research, as the complex relationships between nutritional value, water availability, and soil quality remain understudied, as well as their consumer appeal and opportunities for inclusion in value-added chains.

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Адаптивний потенціал видів пажитника за різних строків сівби

Анотація. Серед невивчених технологічних елементів вирощування пажитника в умовах України залишається створення конвеєрного вирощування культури, тому гостро стоїть проблема впливу термінів сівби на ріст і розвиток рослин, параметри врожаю та якісний склад товарної продукції. Мета досліджень полягала в обґрунтуванні оптимальних строків сівби видів пажитника для підвищення врожайності сушеної продукції в умовах Полісся на дерново-підзолистих ґрунтах. Провідними методами дослідження цієї проблеми є польовий – для визначення взаємодії об'єкта досліджень із біотичними та абіотичними факторами, статистичний – проведення оцінювання результатів досліджень для визначення параметрів адаптивної здатності пажитника. Упродовж 2012–2014 рр. за різного строку сівби середня врожайність сухої маси в пажитника голубого змінювалася в межах від 0,5 до 1,3 т/га, а в пажитника сінного від 0,6 до 1,5 т/га. Найвищою загальною адаптивною здатністю характеризувався ранньовесняний строк та пізньовесняний 1-го строку сівби. Найбільше значення специфічної адаптивної здатності встановлено за пізньовесняного 2-го строку сівби. Показник відносної стабільності коливався від 2,6 до 11,4. Пажитник голубий за пізньовесняного 1-го та 2-го строку сівби добре реагує на покращення умов вирощування ($b_i > 1$), а пажитник сінний за пізньовесняного 2-го та літнього строку сівби. Високою селекційною цінністю характеризуються зразки за ранньовесняного й пізньовесняного 1-го строку сівби. Збільшення суми ефективних температур на 1°C сприяло коливанию врожайності зеленої маси від 4,82 до 128 кг/га, а сухої маси від 0,401 до 6,63 кг/га. Водночас збільшення суми опадів на 1 мм вплинуло на коливання урожайності зеленої маси від 15,0 до 146 кг/га, а сухої маси від 0,145 до 25,9 кг/га. Товарна продукція видів пажитника формувалася в межах суми температур повітря ($>10^\circ\text{C}$) 383,9–487,1 $^\circ\text{C}$, кількості опадів 70,7–144,3 мм, а вегетаційний період коливався від 35 до 55 днів. Найбільшу продуктивність видів пажитника виявлено під час сівби від II декади квітня по I декаду травня. Матеріали статті становлять практичну цінність для розширення видового різноманіття овочів і підвищення забезпечення населення цінними продуктами харчування та під час застосування нових та удосконалених елементів технологій вирощування цінних малопоширених культур для досягнення максимального ефекту реалізації генетичного потенціалу рослин

Ключові слова: пажитник голубий, пажитник сінний, термін сівби, селекційна цінність генотипу

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Effectiveness of Soybean Inoculation with Nodule Bacteria and Mycorrhizal Fungi in the Conditions of the Right Bank Forest Steppe of Ukraine

Abstract. The relevance of this study is determined by a complex of factors that shape soybean grain productivity – the effectiveness of symbiotic systems formed with nodule bacteria and mycorrhizal fungi, the activity of growth processes and the formation of vegetative organs by soybean plants, as well as their adaptive plasticity under the influence of various external factors. The purpose of this study was to investigate the morphobiometric characteristics of soybean plants, the productivity of the crop after pre-sowing treatment with biological preparations Rhizoline, Mycofriend, and Rhizosave. This paper presents the results of research on the use of soybean seed treatment with biological preparations to improve the growth and development of soybean plants and increase crop productivity. Field, laboratory, mathematical-statistical, and calculation methods were employed for this study. It was found that seed inoculation positively affects the leaf surface area in the budding phase, it increased from 5,313 cm² in the control to 6,426 cm² with the Rhizoline + Rhizosave + Mycofriend option, and in the phase of pod formation, respectively, from 8,704 cm² to 1,113 cm². The joint use of Rhizoline + Mycofriend + Rhizosave preparations most contributed to the increase in the mass of the root with nodules (+0.95 g), the number of nodules on the plant (+32 pcs), and the mass of nodules from the plant (+0.3 g). It was found that a comparable situation occurred during the phase of bean formation. The regularity of yield growth during the years under study showed that the average increase in yield varied from 0.41 t/ha with Rhizoline + Rhizosave inoculation to 0.6 t/ha with the combined use of Rhizoline + Rhizosave and the mycorrhizal preparation Mycofriend. It was substantiated that under appropriate conditions, the intensity of symbiotic nitrogen fixation increases thanks to nodule bacteria, as well as the absorption of water and nutrients by the root system of plants due to the activity of mycorrhizal fungi, the entry of amino acids, phytohormones, and vitamins into the plant, which positively affects the growth and development of soybean plants. The materials of this paper are of practical value for farmers in the development of new plant nutrition strategies involving biological preparations in the cultivation technology

Keywords: soybean seeds, biometric indicators, symbiotic productivity, number of nodules, biological preparation, productivity

INTRODUCTION

Increasing the production of high-quality products and increasing the level of soil fertility is one of the priority tasks of modern agricultural production [1]. Soybean is a unique protein and oil crop that is in great demand on the world and Ukrainian markets. Ukraine has great opportunities to increase the production of soybean seeds and obtain greater profits from its sale. Modern technologies

do not fully provide an opportunity to fulfil their productive potential in connection with the parity of prices for fuel, protection products, fertilizers – this increases technological costs and encourages the search for new technologies for growing soybeans [2]. The main measure aimed at the implementation of these tasks is the use of microbial preparations to restore microbiological

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processes in the soil [3-5]. Microorganisms are one of the important components of the soil formation and a link that ensures the ecological balance of the soil ecosystem [6; 7]. The productivity of this culture depends on the performance of many technological operations, namely inoculation. Nodule bacteria fix molecular nitrogen for plants and convert it into a form available for plants. Nodule bacteria synthesize amino acids and vitamins of group B and other biologically active substances. Therefore, optimization of the elements of soybean cultivation technology will help increase the yield of the crop and increase the efficiency of its cultivation.

Pre-sowing treatment of seeds with inoculants is one of the main measures for fulfilling the genetic potential of new soybean varieties. Thanks to the biological fixation of atmospheric molecular nitrogen, plants are supplied with nitrogen. A considerable role is played by nodule bacteria, which contribute to the formation of nitrogen-fixing nodules on the roots of leguminous plants, which also plays a significant role in restoring soil fertility [8; 9]. The use of biological preparations in the fertilization system for growing agricultural crops allows optimizing the level of mineral nitrogen from fertilizers to nitrogen biologically fixed by microorganisms from the air [10-12]. Using inoculation with nodule bacteria and treatment with fungicides, seed germination increases by 20%, plant resistance to diseases increases, and the formation of the symbiotic apparatus is stimulated by 28%, the structure of the crop (number of beans, weight of 1000 seeds) improves by up to 10%. [13; 14]. As a result of the activity of soil microorganisms, not only humus and nitrogen accumulate in the soil and the biological activity of the soil improves, but also mobile forms of nutrients, such as phosphorus and potassium [15-17].

A vital role in this process is played by mycorrhizal fungi, together with rhizospheric bacteria, which improve the supply of phosphorus and potassium to plants, improve water consumption, produce antibiotic substances and phytohormones, which positively affect their growth and development [18-20]. Research proved that under the action of biological preparations, the growth and development of soybean plants is activated, the area of its leaf surface increases, which contributes to an increase in the yield of the crop by 0.21 t/ha [21]. The use of biological preparations also positively increases biological activity, reducing the content of nitrates, heavy metal ions and radionuclides, intensifying the development of nitrogen-fixing and phosphate-mobilizing bacteria, therefore the ability to fix molecular nitrogen by plants is considered a valuable genetic resource of soybeans in the technology of its cultivation [22-24].

The use of biological preparations contributes to the supply of biological nitrogen, stimulates the growth and protection of plants from diseases, which allows obtaining high yields of soybean seeds without synthetic fertilizers and negative anthropogenic impact on the environment [25-27]. Some researchers experimentally

prove and theoretically substantiate that the maximum fulfilment of the potential of plant-microbial interactions is possible only with the correct selection of complementary pairs of plant varieties – strains of microorganisms [28-30]. However, many aspects of the complex application of biological preparations of different functionality remain understudied. Therefore, research aimed at improving the elements of crop cultivation technologies stays relevant.

The purpose of this study was to find the effect of the joint action of nodule bacteria and mycorrhizal fungi on the growth and development of plants, as well as the symbiotic and grain productivity of soybeans.

MATERIALS AND METHODS

Field work was carried out in 2017-2019 at the Khmelnytskyi State Agricultural Research Station Institute of Fodder and Agriculture of the Podillia National Academy of Sciences (north-western part of the Right Bank Forest Steppe of Ukraine).

In the experiment, soybeans of the Siverka variety were sown at 900,000 pieces/ha, selected by the NSC "Institute of Agriculture of the National Academy of Sciences". This is a precocious variety that ripens in 92-97 days. The predecessor was oats. The area of the accounting plot was 30 m², the sowing area was 42 m², the repetition was four times. Agricultural machinery generally accepted for the zone: the main cultivation is ploughing, mineral fertilizers are applied at kg/ha in a.s. – N₂₀P₄₀K₄₀ (ammonium nitrate, superphosphate, potassium chloride). Protection of crops from weeds was carried out – herbicides Basagran (2 l/ha), Harmony (8 g/ha) + surfactant, Trend 90 (0.2 l/ha), Cannoneer Duo insecticide (0.10 l/ha), Acanto Plus fungicide (0.7 l/ha).

Variants of treatment of soybean seeds with the following biological preparations were investigated:

1. Without inoculation (control).
2. Rhizoline 2 l/t + Rhizosave 2 l/t.
3. Mycofriend 1.5 l/t.
4. Rhizoline 2 l/t + Rhizosave 2 l/t + Mycofriend 1.5 l/t.

Biopreparations manufacturer "BTU-Tsentr".

Seeds were processed on the day of sowing. The proposed preparations differed in composition and properties. The Rhizoline biopreparation is a bioinoculant intended for pre-sowing inoculation of soybean, pea, and other legume seeds. Designed for rapid colonization of the legume root system with nodule bacteria and the formation of nodules on the roots, which enables plants to absorb molecular nitrogen from the atmosphere. The preparation is a concentrate of viable cells of nodule bacteria: *Bradyrhizobium japonicum* – symbiotic to soybeans, *Rhizobium leguminosarum* – symbiotic to peas and other strains of nodule bacteria, symbiotic to certain legumes. The bacterial titre is from 2.0×10⁹ CFU/cm³. The preparation also includes macro- and microelements, biologically active products of bacterial life (vitamins, heteroauxins, gibberellins, etc.).

The Mycofriend biopreparation is a mycorrhizal preparation intended for seed treatment, seedlings, in-row application, and fertigation. Created for rapid colonization of the root system of plants with mycorrhizal fungi and microorganisms of the rhizosphere of plants. Increases absorption of water and nutrients by the root system of plants, protects roots from diseases, provides plants with vitamins, phytohormones, and amino acids. The composition of the preparation includes microorganisms that support the formation of mycorrhizae and the rhizosphere of plants: *Streptomyces sp.*, *Pseudomonas fluorescens*; as well as phosphate-mobilizing bacteria: *Bacillus megaterium var. phosphaticum*, *Bacillus subtilis*, *Bacillus muciloginosus*, *Enterobacter sp.*, mycorrhizal fungi: *Glomus VS*, *Trichoderma harzianum*; The total number of viable cells is $(1.0-1.5) \times 10^8$ CFU/cm³.

The preparation includes biologically active substances: amino acids, phytohormones, vitamins.

The soil of the experimental site is medium-loamy chernozem with slightly podzol. Agrochemical characteristics of the soil: humus – 3.05-3.5%, pH – 5.75-6.2; hydrolytic acidity 1.9-2.35 mg/eq. per 100 g; hydrolysed nitrogen 170-136 mg/kg, mobile forms of phosphorus and potassium (according to Chirikov) (DSTU 4115:2002) [31] – 208-243 and 80-136 mg/kg, respectively.

According to the data of the Ukrainian Agrometeorological Centre [32], the weather conditions during the years of research differed from the average multi-year data. During the growing season of soybeans, the air temperature exceeded the average long-term indicators. On average, over the years of research, the increase was 2.29°C in 2017, 4.03°C in 2018, and 4.19°C in 2019 (Fig. 1; 2).

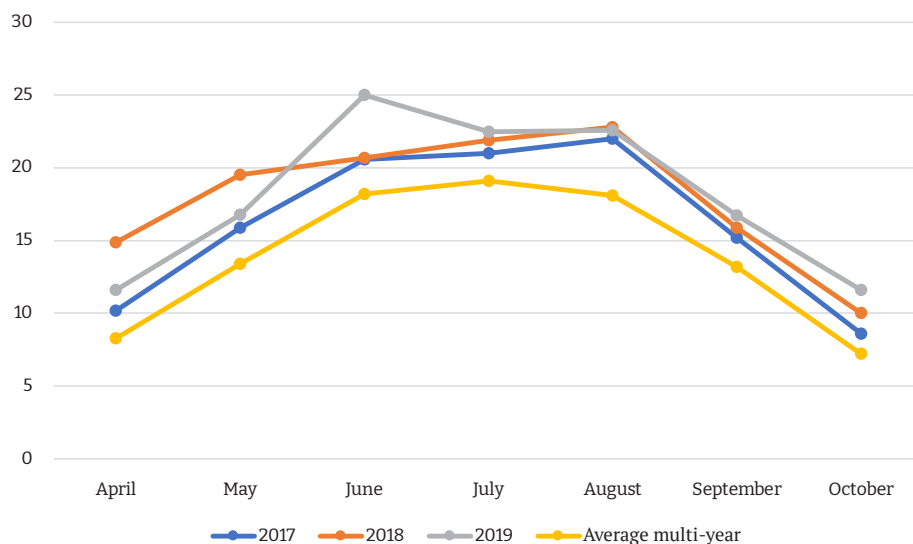


Figure 1. Dynamics of the average daily air temperature during the growing season of soybeans for 2017-2019 compared to the average multi-year data

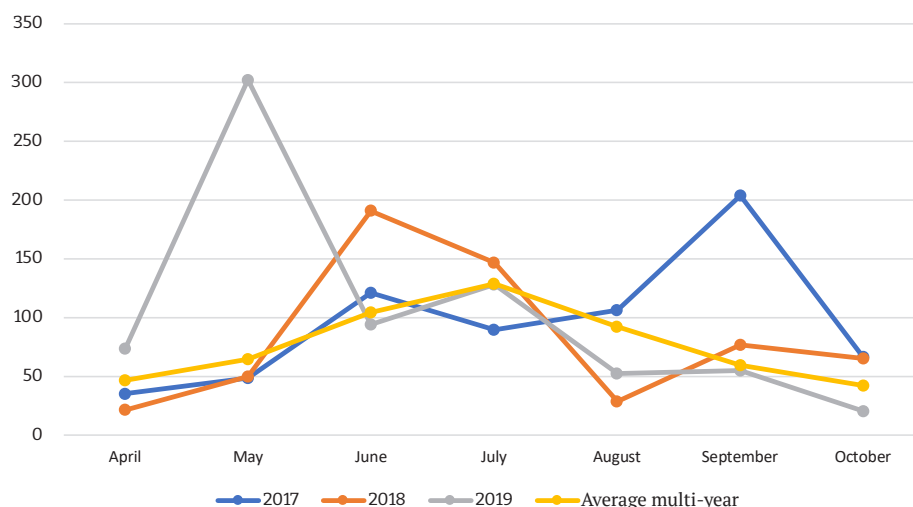


Figure 2. Precipitation dynamics for 2017-2019 compared to average long-term data

During the reporting period, precipitation exceeded the average multi-year data, the excess amounted to 131.8 mm in 2017, 40.9 mm in 2018, and 187.3 mm in 2019, while the unevenness of their precipitation was observed. Thus, a lack of precipitation was observed in April, May 2017-2018, July 2017, and August 2018-2019.

RESULTS AND DISCUSSION

The results of studies on the growth dynamics of soybean plants showed that seed treatment with nodule bacteria and mycorrhizal fungi in the budding phase promotes plant growth, the height increased from 64.95 cm in the control to 70.8 cm in the Rhizoline + Rhizosave + Mycofriend seed treatment. An analogous situation was observed in the phase of bean formation. In the control group, the weight of the plant and the weight of the leaves in the budding phase increased proportionately to the height of the plants and were 20.85 g and 3.9 g, respectively. Upon using the Rhizoline inoculant with the Rhizosave bioprotector, these indicators increased to 24.1 g

and 4.15 g, respectively. Some authors note that seed inoculation is a profitable technique in soybean cultivation, which improves the conditions of plant growth and development, the formation of a larger area of leaves, plant height, the number of branches, nodes, beans, seeds, and the growth of productivity up to 2.04 t/ha [33; 34].

Treatment of seeds with the Mycofriend mycorrhizal preparation did not substantially affect the weight of the plant and leaves, and the combined use of nodule bacteria (Rhizoline) and mycorrhizal fungi (Mycofriend) contributed to an increase in these indicators, respectively, to 29.7 g and 5.2 g, respectively, by 8.85 g and 1.3 g more than in the control. The corresponding regularity was also observed during the pod formation phase. Seed inoculation had a positive effect on the leaf surface area in the budding phase, it increased from 5,313 cm² in the control to 6,426 cm² in the Rhizoline + Rhizosave + Mycofriend variant, and in the phase of pod formation, respectively, from 8,704 cm² to 1,113 cm², which is 17% and 13%, respectively (Table 1).

Table 1. Biometric indicators of plants after treatment of soybean seeds with nodule bacteria and mycorrhizal fungi

Research variants	Plant height, cm	Plant weight, g	Mass of leaves, g	Leaf surface area, cm ²	Plant height, cm	Plant mass, g	Mass of leaves, g	Leaf surface area, cm ²
	Budding phase				Bean formation phase			
1. Without inoculation (control)	65.0	20.9	3.9	5,313	80.7	48.0	9.0	8,704
2. Rhizoline + Rhizosave (2 l/t+2 l/t)	65.0	24.1	4.2	5,398	83.9	51.2	9.75	9,889
3. Mycofriend (1.5 l/t)	67.1	24.3	4.7	6,402	85.0	53.9	11.1	1,044
4. Rhizoline + Rhizosave (2 l/t+2 l/t)+Mycofriend (1.5 l/t)	70.8	29.7	5.2	6,426	84.0	59.2	12.1	1,113
Average ± S_x	67.2±1.3	24.7±1.8	4.5± 0.3	5,884±306	83.4±0.9	53.0±2.4	10.5±9.8	10,044±514
V, %	3.8	14.8	12.9	10.4	2.2	8.91	13.2	10.3

It was established that modern biological preparations contribute to the formation of a developed root system and an active nitrogen-fixing symbiosis, activate the nitrogenase complex of bacteria and increase the resistance of plants to adverse conditions, under the action of biological preparations, plants formed a larger number of beans, seeds, nodules on the root system, and the area of the leaf surface increased [35]. In the experiment, the use of nodule bacteria and mycorrhizal fungi influenced the growth and development of roots and the formation of nodules. The root length of the control variant in the budding phase was 7.75 cm, the mass of the root with nodules was 2.1 g, the number of nodules on the plant was 45.8 pcs,

and the mass of nodules per plant was 0.54 g. The use of Rhizoline + Rhizosave increased the root length by 3.65 cm, root mass with nodules by 0.45 g, number of nodules per plant 12.7 pcs, and mass of nodules per plant 0.205 g. Treatment of seeds with mycorrhizal fungi (Mycofriend) compared to the use of Rhizoline + Rhizosave decreased root mass with nodules and mass of nodules, but a tendency to increase the number of nodules by inoculation of soybeans with aboriginal strains of nodule bacteria by 14% in the budding phase and 6% in the phase of bean formation was noted. The joint use of the preparations Rhizoline + Mycofriend + Rhizosave most contributed to the increase in the mass of the root with nodules (+0.95 g), the

number of nodules on the plant (+32 pcs) and the mass of nodules from the plant (+0.3 g). Thus, in the phase of bean formation, the number of nodules on the plant with the simultaneous application of preparations (Rhizoline + Rhizosave + Mycofriend) increased by 1.8 times compared to the control (without inoculation) – 56.1 pcs (Table 2).

Table 2. Symbiotic performance of soybean under seed treatment with nodule bacteria and mycorrhizal fungi

Research variants	Root length, cm	Root mass with nodules, g	Number of nodules on the plant, pcs	Mass of nodules on the plant, g	Root length, cm	Root mass with nodules, g	Number of nodules on the plant, pcs	Mass of nodules on the plant, g
	Budding phase				Bean formation phase			
1. Without inoculation (control)	7.75	2.10	45.8	0.54	10.4	5.00	56.1	1.01
2. Rhizoline + Rhizosave (2 l/t+2 l/t)	11.4	2.55	58.5	0.745	13.1	5.15	84.9	1.76
3. Mycofriend (1.5 l/t)	10.8	2.35	67.8	0.66	12.75	5.9	90	1.58
4. Rhizoline + Rhizosave (2 l/t+2 l/t) + Mycofriend (1.5 l/t)	11.8	3.05	77.8	0.84	13.1	6.45	102.3	2.19
Average $\pm S_x$	10.44 \pm 0.92	2.51 \pm 0.2	62.48 \pm 6.82	0.7 \pm 0.07	12.34 \pm 0.65	5.63 \pm 0.34	83.33 \pm 9.78	1.64 \pm 0.25
V, %	17.61	16.03	21.81	18.31	10.55	12.02	23.48	29.91

Seed inoculation with highly effective and active strains of nodule bacteria provides a prominent level of nitrogen-fixing capacity of symbiotic systems, which causes the intensification of soybean growth and development and, as a result, increases its yield up to 12% in growing technologies up to 38% [36-38]. According to the research carried out in 2017, soybean productivity increased from 2.18 t/ha in the control and up to 2.63 t/ha when using the Rhizoline biological preparation with the Rhizosave protector. The use of the mycorrhizal Mycofriend preparation increased the yield of the crop to 2.73 t/ha. By combining the Rhizoline biological preparation and the Rhizosave

protector with the mycorrhiza-forming Mycofriend preparation, the yield level increased to 2.76 t/ha, respectively, the increase compared to the control (without inoculation) was 0.58 t/ha. The analysis of research results in 2018 showed a comparable trend, the yield level fluctuated, respectively, from 2.13 t/ha in control to 2.72 t/ha with the joint use of biological preparations. The regularity of yield growth due to the introduction of nodule bacteria and mycorrhizal fungi, which was observed in 2017-2018, was confirmed in 2019 as well. Productivity increased from 2.26 t/ha in the control to 2.88 t/ha for the combination of biological preparations (Table 3).

Table 3. Soybean yield depending on seed inoculation with nodule bacteria and mycorrhizal fungi

Variant	Productivity, t/ha			Average for 2017-2018	b_i	V
	2017	2018	2019			
1. Without inoculation (control)	2.18	2.13	2.26	2.19	1.36	3.19
2. Rhizoline + Rhizosave (2 l/t+2 l/t)	2.63	2.58	2.58	2.6	0.12	1.15
3. Mycofriend (1.5 l/t)	2.73	2.64	2.73	2.7	1.07	1.85
4. Rhizoline + Rhizosave (2 l/t+2 l/t)+Mycofriend (1.5 l/t)	2.76	2.72	2.88	2.79	1.62	2.87
LSD ₀₅ , t/ha	0.04	0.10	0.04			

On average, over three years, the yield increase varied from 0.41 t/ha with Rhizoline + Rhizosave inoculation to 0.6 t/ha with the joint use of Rhizoline + Rhizosave with the Mycofriend mycorrhizal preparation with an excess of up to 22%. The analysis of the regression coefficient (b_i), which characterizes the average reaction of the research variant to the change in environmental conditions and allows predicting the change in the characteristic under study (yield), within the conditions available in the experiment, showed how biological preparations affect the reaction rate of the variety. Elevated productivity stability ($b_i=0.12$) of the crop was observed upon using the Rhizoline inoculant with the Rhizosave bioprotector, i.e., regardless of the growing conditions, the yield was at the level of previous years and consistently exceeded the control. The medium ecological plasticity ($b_i=1.07$) was characterized by the variant when treating seeds with the Mycofriend mycorrhizal preparation (1.5 l/t), while this variant consistently exceeded the control in terms of productivity. Joint use of Rhizoline inoculant with Mycofriend mycorrhizal preparation and Rhizosave bioprotector increases the plasticity of the variety ($b_i=1.62$). That is, when the conditions change towards improvement, the intensity of symbiotic nitrogen fixation due to nodule bacteria increases, as well as the absorption of water and nutrients by the root system of plants due to the activity of mycorrhizal fungi and the entry of vitamins, phytohormones, and amino acids produced by microorganisms into the plant. This leads to an increase in crop productivity by 0.6 t/ha compared to the control level of 2.19 t/ha.

CONCLUSIONS

The use of nodule bacteria and mycorrhizal fungi for the processing of soybean seeds contributes to the growth of symbiotic productivity, increasing the absorption of water and nutrients by the root system of plants, which ensures an increase in the leaf surface area and photosynthetic activity, which is reflected in the improvement of economic and valuable indicators and an increase in the yield of the crop. The best option for seed treatment turned out to be the variant of the combined use of Rhizoline (2 l/t) + Rhizosave (2 l/t) + Mycofriend (1.5 l/t), which is characterized by high productivity (2.79 t/ha) and ecological plasticity ($b_i=1.62$). It was proved that with the joint use of biological preparations, the leaf surface area increased by 13%, and the number of nodules per plant increased by 1.8 times comparing to the control level, the yield increased by 22% (2.19 t/ha in the control).

The obtained results are valuable from both a practical and a theoretical standpoint for improving ways of increasing the grain productivity of soybean plants by increasing the activity of symbiotic nitrogen fixation, the absorption area of the plant root system, and improving the availability of plant nutrients through the use of nitrogen-fixing bacteria and mycorrhizal fungi for the pre-sowing treatment of seeds of this strategic bean crop for Ukraine. The introduction of biological preparations into soybean cultivation technologies opens wide opportunities for economical use of resources and reducing the load of agrochemicals in fertilization systems.

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Ефективність інокуляції сої бульбочковими бактеріями та мікоризними грибами в умовах Правобережного Лісостепу України

Анотація. Актуальність досліджень зумовлена комплексом факторів, що формують зернову продуктивність сої – ефективністю утворених із бульбочковими бактеріями і мікоризними грибами симбіотичних систем, активністю ростових процесів та формуванням вегетативних органів рослинами сої, а також їхньою адаптаційною пластичністю за дії різних зовнішніх чинників. Мета наукової роботи – дослідити морфобіометричну характеристику рослин сої, продуктивність культури за передпосівної обробки біопрепаратами Різолайн, Мікофренд і Різосейв. У статті представлені результати досліджень використання обробки насіння сої біопрепаратами для покращення росту і розвитку рослин сої та підвищення продуктивності культури. Методи польові, лабораторні, математично-статистичні і розрахункові. Визначено, що інокуляція насіння позитивно вплинула на площу листової поверхні у фазу бутонізації, вона зростала від 5313 см² на контролі до 6426 см², варіант Різолайн + Різосейв + Мікофренд, а у фазу утворення стручків відповідно від 8704 см² до 1113 см². Сумісне використання препаратів Різолайн + Мікофренд + Різосейв найбільше сприяло підвищенню маси кореня з бульбочками (+0,95 г), кількості бульбочок на рослині (+32 шт.), та маси бульбочок з рослини (+0,3 г). З'ясовано, що аналогічна ситуація склалась і у фазу утворення бобів. Закономірність зростання урожайності протягом досліджуваних років, показала, що середньому приріст урожайності варіював від 0,41 т/га за інокуляції Різолайн + Різосейв до 0,6 т/га за сумісного використання Різолайн + Різосейв з мікоризним препаратом Мікофренд. Обґрунтовано, що за відповідних умов підвищується інтенсивність симбіотичної азотфіксації завдяки бульбочковим бактеріям, а також поглинання кореневою системою рослин води та елементів живлення за рахунок діяльності мікоризних грибів, надходження в рослину амінокислот, фітогормонів, вітамінів, що позитивно впливає на ріст та розвиток рослин сої. Матеріали статті становлять практичну цінність для аграріїв у розробці нових стратегій живлення рослин із залучення у технології вирощування біологічних препаратів

Ключові слова: насіння сої, біометричні показники, симбіотична продуктивність, кількість бульбочок, біопрепарат, урожайність

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Creation of Highly Productive Forms of Winter Wheat with Complex Resistance Against Diseases and Pests on Artificial Infectious Backgrounds of Their Causative Agents

Abstract. Diseases of field crops substantially reduce the yield and quality of the obtained agricultural products. Underselection of gross grain harvest due to diseases and pests can reach about 25-30% annually. This necessitates the creation of resistant varieties for winter wheat selection. The purpose of this study was to create a new highly productive selection material with complex resistance against the main most harmful diseases for further use in the selection of winter wheat. In the phase of emergence of plants in the tube, winter wheat plants were infected with spores of the causative agent of brown rust according to E.E. Heschel's method. To create an artificial infectious background of brown rust, a synthetic population of the pathogen (Institute of Plant Protection of the National Academy of Agrarian Sciences) was used as a reservoir of infection – a susceptible variety of Myronivska 10. To create an artificial infectious background of powdery mildew, a local population and an infectious reservoir of the American variety Keprock were used. In the F₃ hybrid nursery against the provoking background of powdery mildew, combinations highly resistant to the pathogen (up to 5%) were selected, created with sources of resistance: Zdar, Fakon, Pi170911, Bongo – Svitanok Myronivskiyi/Zdar, Kolos Myronivshchyny/Fakon, Berehynia Myronivska/Pi170911, Dostatok/Bongo. Resistance against the pathogen (up to 10.0%) was observed in two crossing combinations (Gorlytsia Myronivska/Gloria and Remeslivna/Wervok). Crossing combinations created with sources of resistance were selected on the artificial infectious background of hybrids of the fourth generation for resistance to brown rust: Flex, V 1275, Tobarzo, 203-238. It is worth noting the hybrids: Oberih Myronivskiyi/Flex, Smuhlianka/V 1275, Monotyp/Tobarzo, Kolos Myronivshchyna/203-238. The largest number of grains in an ear was obtained from the combination Svitanok Myronivskiyi/Zdar (61.5 pieces), and the largest weight of grain from an ear was obtained from the combinations Horlytsia Myronivska/Gloria and Berehynia Myronivska/Pi170911 (2.38 g and 2.37 g, respectively). The Oberih Myronivskiyi/Flex combination stood out for its resistance to brown rust and performance elements. The samples selected based on the results of the study are used as valuable raw material to create new winter wheat varieties resistant to diseases in the Forest Steppe of Ukraine

Keywords: *Triticum aestivum* L., source material, sources of resistance, pathogens, powdery mildew, brown rust, variety

INTRODUCTION

One of the main food crops in the world is soft winter wheat. Pathogenic microorganisms accompany it from the moment of sowing until harvesting. Even minimal

damage to seedlings by diseases and damage by pests lead to large total losses of the crop [1-3]. One of the key factors that determine the phytosanitary status of grain

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crops is the variety, as its contribution to the future harvest, according to scientists' calculations, can reach up to 70%. Presently, the most economically justified and most effective measure of ecological protection in the context of environmental protection is the creation of varieties highly resistant to a complex of diseases and pests, i.e., selection for resistance. Furthermore, the breeding of new, pest-resistant varieties with high ecological plasticity is one of the primary areas of increasing the yield of agricultural crops. In the system of integrated protection of sowing field crops, emphasis on the cultivation of highly resistant varieties with high productivity has always occupied a special place. It is such varieties that can fulfil the potential possibilities of productivity and quality and provide the greatest return from the agrotechnical techniques used [4; 5].

All plants have natural mechanisms of resistance against harmful organisms, which differ from plant to plant. Breeding work on the creation of varieties of field crops in general and winter wheat, which are resistant to pathogens of major diseases and pests, involves the use of both samples from the world collection and those tested in the conditions of a certain region [6]. The most dangerous and widespread diseases of winter wheat are brown rust, powdery mildew, septoriosi, fusariosi, hard soot, and root rot [2]. Since 2015, an increase in fusarium head blight has been noted in Ukraine, which considerably decreases the quality of flour and bread and leads to a substantial harvest shortage. As showed by H. Buerstmayr *et al.* (2009), H. Kovalyshyna *et al.* (2020) "...grain affected by some species of fusarium, due to its toxicity, becomes unsuitable for human consumption and animal feeding" [7; 8]. In wheat plants affected by root rot, their premature death, a significant manifestation of the spikelet, the invasion of saprophytic fungi on the spikelets, and a decrease in the sowing qualities of the seeds are noted en masse [9]. Young, actively growing wheat plants are more susceptible to damage by powdery mildew, the harmfulness of which is manifested in slowing down the growth and development of plants due to a decrease in the assimilation surface of the leaves, a delay in earing and a slowing down of grain filling, and as a result – a decrease in the content of raw gluten, protein, and starch. As indicated by K.P. Voss-Fels *et al.* (2019) "...harvest failure due to powdery mildew in some cases can reach 10-15%" [6]. Brown rust, causing the greatest damage in the phase of milk ripeness [10], reduces the assimilation surface by 30-40% in winter wheat plants, violates their water balance, causes premature death of leaves and grain brittleness due to increased transpiration [11-13]. Rusty fungi can reduce the content of protein and gluten in grains by up to 20% with severe damage to plants [8; 14]. Harvest losses due to damage by rust up to 40% amount to 30-40 kg/ha, and over 40% can reach 100 kg/ha and more [15-17].

Breeding work aimed at creating ecologically plastic varieties resistant to diseases and pests is one of

the main levers of influence on increasing the yield of field crops. Upon studying the immunological properties of the world collection, resistance of individual samples against 2-3 diseases with simultaneous susceptibility to others was revealed [3; 18]. Cultivation of winter wheat varieties with complex resistance against harmful pathogens can guarantee a yield increase of 1.2-1.5 t/ha without protection means. Therefore, the creation of such varieties is the main task of selection [6; 19]. As a result of cultivating varieties of field crops resistant to harmful pathogens and pests, not only does the pesticide load decrease, but also the danger of contaminating the crop and the environment is reduced. [20; 21]. The work on the selection of highly productive forms of winter wheat with complex resistance to diseases and pests on artificial infectious backgrounds and the creation of resistant lines based on them is a complex, multifaceted, creative, and long-term process that involves many years of research with an enormous amount of material. To create a new initial disease-resistant breeding material, it is necessary to replenish and use new sources of resistance.

The tasks of this study were as follows: to investigate the F₂ hybrid material and selection of disease-resistant genotypes of winter wheat on artificial infectious backgrounds of their pathogens (2018); to select highly productive forms of winter wheat with comprehensive resistance to diseases and pests in the F₃ hybrid nursery on artificial infectious backgrounds of their pathogens (2019); to investigate disease-resistant raw material of winter wheat obtained (2020).

The purpose of this study was to find a new genetically diverse selection material resistant to powdery mildew and brown rust.

MATERIALS AND METHODS

The research was conducted in 2017-2020 under artificial inoculation with pathogens in field infectious nurseries of the plant protection department of the V.M. Remeslo Myronivka Institute of Wheat of the National Academy of Agrarian Sciences (Tsentralne vilage, Obukhiv district, Kyiv Oblast) in the northern part of the Left Bank Forest Steppe.

To fulfil the set purpose, about 200 F₁ hybrids were sown for the 2017 harvest based on resistance against six pathogens and their complex and pests. In 2018, records were made of damage caused by pathogens of the main diseases of leaves, ears of corn, root rots, and pest infestation of F₂ hybrids. Forms resistant to certain diseases were selected, which were inhabited to a lesser extent by pests. For the 2019 harvest, 200 F₃ hybrids were sown on separate infectious backgrounds to carry out selections resistant to both individual diseases and their complex and pest infestation. In the F₃ winter wheat hybrid nursery, 108 hybrid populations were investigated for resistance to leaf diseases and 96 – for resistance to ear and root rot diseases.

In the tube emergence phase, winter wheat plants were infected with spores of the causative agent of brown

rust according to the method of E.E. Geschele [4]. To create an artificial infectious background, a synthetic population of the pathogen obtained from the Institute of Plant Protection of the National Academy of Agrarian Sciences was used, and the susceptible variety Myronivska 10 was used as an infection reservoir. To create an artificial infectious background of powdery mildew, a provocative background of the pathogen was created using a local population according to the generally accepted method [22]. The American variety Kepronck was used as an infection accumulator.

To investigate the dynamics of disease growth, the assessment during the period of maximum development of the disease was considered as the basis. For powdery mildew, brown rust – the phase of milk ripeness [23; 24]. Using the method of mowing with an entomological net, small mobile insects – leafhoppers, thrips, aphids, adults of cereal flies and sawflies were detected on winter wheat crops. In one field, depending on its size and the number of detected insects, 50-100 sweeps of the net were made in 5 or 10 places of the field. To calculate the number of pests per unit area, two sweeps were conventionally equated to an area of 1 m² [24; 25]. The pests that feed on the surface of plants (bugs, bread bugs, leeches, etc.)

were counted both directly on the plants and after shaking them off the plants into an entomological net [24-26].

To figure out the influence of weather conditions during the years of research, namely temperature and precipitation, on the development and spread of diseases, the calculation of the hydrothermal coefficient (HTC) was used [27]. The autumn of 2015-2019 was characterized by generally unfavourable weather conditions for the development of diseases and pests on winter wheat, as the lack of rain and high temperatures led to a delay in germination. In turn, the absence of pathogens and minimal infestation by pests was noted on the late seedlings of winter wheat. In 2016, during the period of recovery of spring vegetation – full maturity of winter wheat (HTC 1.5) as a result of optimal moisture, the development, and growth of pathogens and moderate pest infestation were noted (Fig. 1). In 2017, in the same period (HTC 0.8), drier weather conditions were noted, which limited the development of the main pathogens, moderate plant infestation by pests, and a complete absence of damage to plants by septorioses of leaves and brown rust. In the same year, a considerable development of the hard soot of the wheat ear was noted, which in some varieties reached 50.0%.



Figure 1. Indicators of HTC during the years of research

In 2018, the weather conditions were also not very conducive to the development of leaf diseases due to the abnormally dry conditions of the winter wheat growing season, as the temperature was unusually high almost every month. Precipitation in the 3rd decade of June resumed the growth of leaf septorioses and brown rust. In 2020, starting from the recovery of spring vegetation and until the full maturity of winter wheat varieties, the weather conditions contributed to the partial development of foliar diseases. As a result of the cool and dry weather in the first decade of June, the development of powdery mildew stopped and damage to plants did not exceed 0-15.0%. The development of hard soot, root rot, and fusarium head blight was 50.0, 31.3 and 7.5%, respectively.

RESULTS AND DISCUSSION

In the F₃ hybrid nursery, on separate artificial infectious backgrounds, records were made of plant damage by

powdery mildew, leaf septoria, fusarium wilt and hard ear smut, brown rust, and infestation by pests. Disease-resistant forms, less prone to colonisation by pests, were selected. Against the provocative background of powdery mildew (Fig. 2), 246 selections were selected and analysed. Highly resistant against the pathogen (up to 5%) were the combinations created with the following resistance sources: Zdar, Fakon, Pi170911, Bongo – Svitanok Myronivskiy/Zdar, Kolos Myronivshchyny/Fakon, Berehynia Myronivska/Pi170911, Dostatok/Bongo. Their population with thrips larvae varied from 5.2 to 14.5 spec/ear (Economic damage threshold or EDT 20.0-30.0 spec/m²), and leeches – 5.0-18.0 spec/m² (EDT 150.0-200.0 spec/m²). Resistance against the pathogen (up to 10.0%) was observed in two crossing combinations (Horlytsia Myronivska/Gloria and Remeslivna/Wervok).

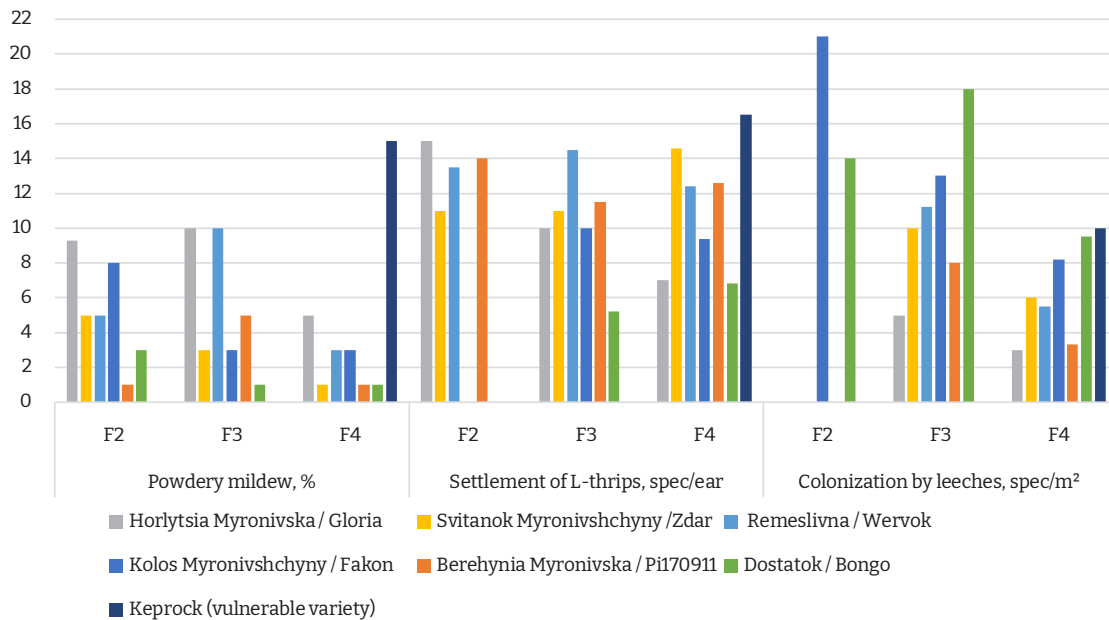


Figure 2. Complex resistance of the best F_2 - F_4 hybrids of soft winter wheat against powdery mildew pathogens and pests (2018-2020)

On the artificial infectious background of hybrids of the third generation, 280 selections were selected and analysed for resistance to brown rust (Fig. 3). The largest number of resistant offspring was obtained in crossing combinations created with the following resistance sources: Flex, V 1275, Tobarzo, 203-238. It is worth noting the hybrids: Oberih Myronivskyi/Flex, Smuhlianka/V 1275,

Monotyp/Tobarzo, Kolos Myronivshchyna/203-238. Damage to winter wheat plants by the causative agent of brown rust was observed within 3.0-10.0%. These combinations were also distinguished by a small population of pests (thrips larvae and leeches), and it was below the harmfulness threshold.

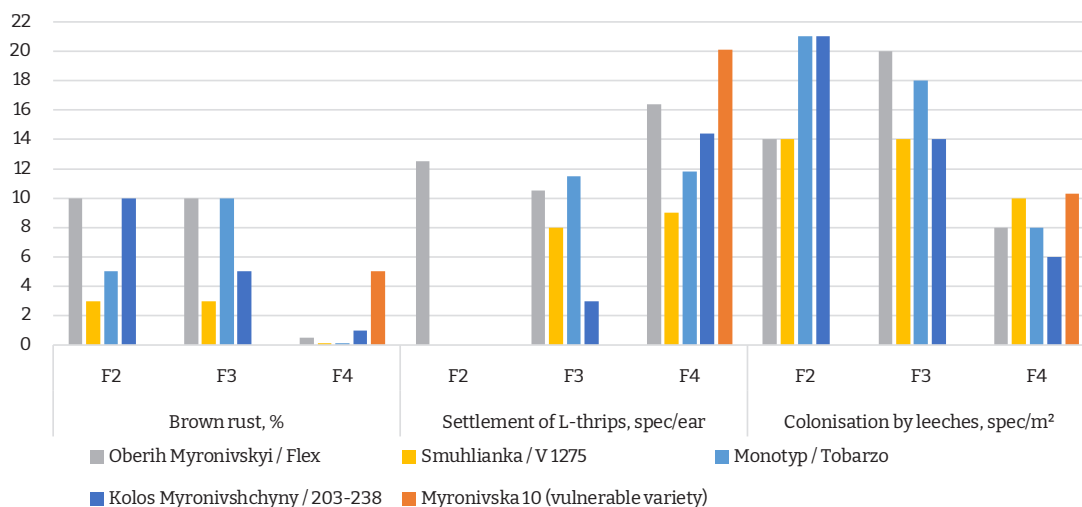


Figure 3. Complex resistance of the best F_2 - F_4 hybrids of soft winter wheat against brown rust pathogens and pests (2018-2020)

In the hybrid nursery of the fourth generation, 96 hybrid populations were investigated for resistance to leaf diseases and 126 – for resistance to ear and root rot diseases. On separate artificial infectious backgrounds, records were made of damage caused by pathogens of the main diseases and the colonization of plants by pests.

Disease-resistant forms that were less colonized by pests were selected. Against the provocative background of powdery mildew, 200 selections were selected and analysed. Highly resistant (up to 5%) against the pathogen of powdery mildew were the combinations created with the following resistance sources: Zdar, Fakon, Pi170911,

Bongo – Svitanok Myronivskiy/Zdar, Kolos Myronivshchyny/Fakon, Berehynia Myronivska/Pi170911, Dostatok/Bongo (Fig. 2). Their population with thrips larvae varied from 6.8 to 14.6 spec/ear (EDT 20.0-30.0 spec/ear), and leeches – 3.0-9.5 copies/m² (EDT 150.0-200.0 spec/m²).

On the artificial infectious background of hybrids of the fourth generation, 215 selections were selected and analysed for resistance to brown rust. The largest number of resistant offspring (see Fig. 3) was obtained in crossing combinations created with the participation of sources of resistance: Flex, V 1275, Tobarzo, 203-238. It is worth noting the hybrids: Oberig Myronivskiy/Flex, Smuglyanka/V 1275, Monotype/Tobarzo, Kolos Myronivshchyna/203-238. Damage to winter wheat plants by the causative agent of brown rust was not high. Only the first manifestation of the disease was observed in up to 1.0% (single pustules). These combinations were also distinguished by the small population of pests (thrips larvae (9.0-16.4 spec/ear) and leeches (6.0-10.0 spec/m²) and it was below the EDT – 20.0-30.0 spec/ear of thrips larvae and 150.0-200.0 spec/m² of leech on winter wheat.

Plant height of F₃ hybrids did not differ substantially from the parental forms, and spike length, number of spikelets in a spike, and weight of grains from a spike

had a noticeable positive difference with the maternal and parental forms. Exceeding ear parameters in hybrid combinations of the third generation of winter and spring wheat were observed in the studies of Ukrainian and foreign scientists. According to research results [28; 29] the inheritance of the soft winter wheat trait “the number of grains from an ear” in seven out of twelve hybrid combinations was according to the type of positive overdominance, in one – according to the type of positive dominance, in two – according to the type of intermediate inheritance; one has negative overdominance.

The selection value of the hybrid combinations created with the Berehynia Myronivska variety, which contains the Lr 34(+) allele, involved in crossbreeding to create the disease-resistant F₄ hybrid material. Valuable forms were selected based on the combination of elements of productivity (length of ear, number of grains in an ear, and mass of grain from an ear) and resistance to powdery mildew in comparison with parental forms (Table 1). The largest number of grains in an ear was obtained in the combination Svitanok Myronivskiy/Zdar (61.5 pcs), and the largest weight of grain from an ear – in the combinations Horlytsia Myronivska/Gloria and Berehynia Myronivska/Pi170911 (2.38 g and 2.37 g, respectively).

Table 1. Elements of productivity of hybrids and parental forms of soft winter wheat resistant to powdery mildew (2019-2020)

Parent forms, hybrid combinations	Plant height, cm		Spike length, cm		Amount of grain in an ear, pcs.		Mass of grains from an ear of corn, g	
	F ₃	F ₄	F ₃	F ₄	F ₃	F ₄	F ₃	F ₄
Ke Brock St (vulnerable variety)	95.0	105.5	6.9	7.6	36.7	48.7	1.6	1.72
♀ <i>Horlytsia Myronivska</i>	90	90.0	7.7	7.7	41.2	41.2	1.82	1.82
Horlytsia Myronivska/Gloria	90	91.2	7.9	8.1	53.1	53.3	2.34	2.38
♂ <i>Gloria</i>	108	106.1	7.9	7.9	30.4	31.4	1.09	1.12
♀ <i>Svitanok Myronivskiy</i>	80	74.3	8.5	9.8	43.4	50.5	1.46	1.97
Svitanok Myronivskiy/Zdar	84	73.5	9.8	11.2	49.5	61.5	1.79	2.08
♂ <i>Zdar</i>	62	62.0	6.0	7.1	32.5	36.5	0.98	0.98
♀ <i>Remeslivna</i>	85	81.1	8.0	9.5	40.7	40.7	1.36	1.48
Remeslivna/Werwok	70	82.5	10.0	11.6	48.4	59.2	2.06	2.23
♂ <i>Werwok</i>	90	86.0	8.7	8.9	40.1	41.4	1.62	1.68
♀ <i>Kolos Myronivshchyny</i>	105	103	7.8	8.8	42.8	49.7	1.66	1.96
Kolos Myronivshchyny/Fakon	90	89.5	9.7	9.6	55.7	55.7	2.28	2.28
♂ <i>Fakon</i>	80	81.0	6.9	6.7	34.1	36.4	1.23	1.43
♀ <i>Berehynia Myronivska</i>	95	96.0	7.3	8.3	30.5	32.5	1.27	1.36
Berehynia Myronivska/Pi170911	100	96.5	10.0	9.9	46.0	54.0	2.29	2.37
♂ <i>Pi170911</i>	85	84.0	7.0	7.8	34.2	33.4	1.41	1.53
♀ <i>Dostatok</i>	100	99.8	8.1	8.6	45.7	46.4	1.80	1.88
Dostatok/Bongo	108	104.2	8.8	9.8	51.1	55.1	1.94	2.06
♂ <i>Bongo</i>	105	105.2	6.7	7.7	29.7	29.9	1.31	1.39
LSD₀₅	2.2	5.9	0.5	0.5	5.1	5.9	0.22	0.26

Among the F₄ populations, 1,405 selections were selected and analysed on the artificial infectious backgrounds of brown rust, hard soot, fusarium head blight, root rot, and the provoking background of powdery mildew. A structural analysis was carried out on the elements of productivity of the selected selections, which were distinguished by their resistance against the main

pathogens and pests. Valuable forms were selected based on the combination of productivity elements (ear length, number of grains in an ear, and weight of grain from an ear) and resistance to brown rust in comparison with parental forms, presented in Table 2. The combination Oberih Myronivskyi/Flex stood out in terms of resistance to brown rust and productivity elements.

Table 2. Performance elements of hybrids and parental forms of soft winter wheat resistant to brown rust (2019-2020)

Parent forms, hybrid combinations	Plant height, cm		Spike length, cm		Amount of grain in an ear, pcs.		Mass of grains from an ear of corn, g	
	F ₃	F ₄	F ₃	F ₄	F ₃	F ₄	F ₃	F ₄
Myronivska 10 St (vulnerable variety)	115	98.4	7.5	8.6	33.8	43.3	1.42	1.66
♀ Oberih Myronivskyi	95	95.9	6.6	7.8	26.2	39.2	1.19	1.46
Oberih Myronivskyi/Flex	95	80.0	8.4	10.1	41.5	57.2	2.02	2.36
♂ Flex	120	98.2	6.0	6.8	30.3	42.3	1.01	1.19
♀ Kolos Myronivshchyny	105	70.5	7.8	7.8	42.8	43.6	1.66	1.68
Kolo Myronivshchyny/203-238	105	68.1	6.8	7.5	34.8	55.4	1.69	2.05
♂ 203-238	100	67.5	6.4	6.9	39.5	40.5	1.64	1.69
♀ Monotyp	92	92.6	8.0	8.1	48.8	48.3	1.69	1.78
Monotyp/Tobarzo	100	86.5	8.0	8.3	50.7	49.2	2.12	2.09
♂ Tobarzo	100	86.1	6.1	7.1	38.7	38.4	1.54	1.64
♀ Smuhlianka	85	85.3	7.6	7.8	30.5	31.7	1.26	1.36
Smuhlianka/ V1275	85	84.5	9.1	10.2	38.8	56.1	1.67	2.16
♂ V1275	85	84.2	6.4	7.2	30.5	31.5	1.04	1.00
LSD₀₅	2.3	5.7	0.6	0.6	5.4	6.0	0.25	0.28

According to the indicator, the height of the F₄ hybrid plants, which stood out for their resistance to diseases of the ear and root rot, did not differ substantially from the parental forms, and the length of the ear, the number of spikes in the ear, and the mass of grains from the ear had a noticeable positive difference between the maternal and parental forms. The combination of signs of complex resistance against foliar and root diseases with an increase in the height and productivity of the ear in hybrid combinations of the fourth generation of winter and spring wheat was noted in the studies of Ukrainian and foreign scientists. It is worth noting the results of research by Srinivasachary, N. Gosman *et al.* [30], who, using the example of winter wheat, conducted a study of the relationship between the genes of semi-dwarfism and susceptibility of the variety to fusarium head blight. The data obtained by them indicate that the highest value for selection for complex resistance to diseases has the forms that were selected based on a set of traits in years with a strong development of the disease. Furthermore, they

proved the possibility of combining disease resistance with high productivity and grain quality in one variety.

The complete dominance and transmission of disease-resistant maternal traits in the majority (78-84%) of the first-generation hybrids was noted in the studies of S.V. Retman [31] upon crossbreeding short-growing Western European wheats resistant to fungal diseases with unstable varieties of domestic selection in different years. In their studies of the development and distribution in the United States and Canada in 1991-1996 of the epiphytota of Fusarium head blight of wheat and barley, M. McMullen *et al.* [32] established that during splitting in the F₂-F₄ generation, plants with different degrees of damage were found – from 1-5% to 50-60%. For further selection work, scientists selected only resistant and medium-resistant promising forms of wheat and barley, which in the F₄-F₆ generations were involved in crossbreeding with highly winter-resistant productive varieties and lines of local selection to transfer genes for resistance to fusarium wilt to them.

According to the results of the study of constant lines of winter wheat, 11 disease-resistant lines were transferred from the selection nursery to the National Centre of Plant Genetic Resources of Ukraine (V.Ya. Yuryev Institute of Crop Production, Kharkiv) in 2018, and 9 in

2019. The obtained initial selection material, which stood out for its resistance to diseases and pests (Table 3), was transferred to the winter wheat selection laboratory for further use in the selection.

Table 3. Immunological characteristics of winter wheat lines in terms of resistance against pathogens of foliar diseases submitted to the National Agricultural Research Service of Ukraine (2020)

Line	Pedigree	Vulnerability, %		
		With powdery mildew	With leaf septoria	With brown rust
Powdery mildew				
Keprock	Vulnerable variety	15.0	25.0	1.0
Lutescens E.g.484/18	Pi. 170911/ Yuvilari Myronivskiy	5.0	10.0	0.5
Lutescens E.g.485/18	Snihurka/Selidon	3.0	10.0	0.1
Brown rust				
Myronivska 10	Vulnerable variety	10.0	20.0	5.0
Erythrospermum Pr.141/18	(TAM-200 x Myrlena) x Myrlena	5.0	8.0	0.1
Erythrospermum Pr.142/18	(Matyo x Kalynova) x Kalynova	1.0	10.0	0
Erythrospermum Pr.144/18	TAM-200 x Myrlena	5.0	10.0	0
Lutescens Pr.145/18	Kolos Myrnoivshchyny x Warwick	5.0	10.0	0.1
Erythrospermum Pr.146/18	Ekonomka x Tsarivna	8.0	10.0	0
Lutescens Pr.147/18	Beres x Myronivska 65	3.0	3.0	0.1

In 2020, certificates were received for two samples under the winter wheat breeding program for resistance to brown rust – Erythrospermum 141-18 and Erythrospermum 146-18 lines, in which TAM 200 and Ekonomka resistance sources were used as mother forms. They were assigned National Catalogue numbers UA0123473 and UA0123470, respectively, for a combination of high group resistance to powdery mildew, brown leaf rust, snow mould (score 9), and leaf septoria.

CONCLUSIONS

The results of the conducted study indicate the possibility of using resistant varieties of winter wheat as a means of restraining the growth of infection by pathogens without chemical protection agents. Highly resistant (up to 5%) F₄ hybrids against powdery mildew were selected as follows: Svitanok Myronivskiy/Zdar, Kolos Myronivshchyny/Fakon, Berehynia Myronivska/Pi170911, Dostatok/Bongo, which were created with resistance sources Zdar, Fakon, Pi170911, Bongo, and are characterized by complex resistance to their settlement with thrips larvae, the number of which varied within 6.8-14.6 spec/ear (EDT 20.0-30.0 spec/ear) and leeches – 3.0-9.5 spec/m² (EDT 150.0-200.0 spec/m²). Based on the combination of elements of

productivity (length of ear, number of grains in an ear, and weight of grain from an ear) and resistance to powdery mildew, the combination Svitanok Myronivskiy/Zdar with the largest number of grains in an ear (61.5 pcs.) was selected.

Selected combinations of crossing F₄, which are characterized by resistance to brown rust and a small population of pests (thrips larvae 9.0-16.4 spec/ear with EDT 20.0-30.0 spec/ear and leech 6.0-10.0 spec/m² with EDT 150.0-200.0 spec/m²): Oberih Myronivskiy/Flex, Smuhlianka/V 1275, Monotyp/Tobarzo, Kolos Myronivshchyna/203-238. The Oberih Myronivskiy/Flex combination is highlighted for its resistance to brown rust and performance elements.

Selected sources of resistance are used to create selection material with comprehensive resistance to foliar diseases and valuable economic traits. 220 genotypes were transferred to the winter wheat selection laboratory of the V.M. Remeslo Myronivka Institute of Wheat of NAAS of Ukraine from the selection nursery. Apart from group resistance to diseases, these genotypes are characterized by high productivity and quality of the grown products, have a wide further use in the selection.

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Створення високопродуктивних форм пшениці озимої з комплексною стійкістю проти хвороб та шкідників на штучних інфекційних фонах їх збудників

Анотація. Хвороби польових культур суттєво знижують врожайність та якість отриманої сільськогосподарської продукції. Недобір валового збору зерна за рахунок хвороб та шкідників щорічно може досягати близько 25-30 % тому створення стійких сортів є актуальним завданням в селекції пшениці озимої. Мета досліджень полягала у створенні нового високопродуктивного селекційного матеріалу з комплексною стійкістю проти основних найбільш шкідливих хвороб для подальшого використання в селекційному процесі пшениці озимої. У фазі виходу рослин в трубку проводили інфікування рослин пшениці озимої спорами збудника бурої іржі за методикою Е.Е. Гешеле. З метою створення штучного інфекційного фону бурої іржі використовували синтетичну популяцію збудника (Інститут захисту рослин НААН), як накопичувач інфекції – сприйнятливий сорт Миронівська 10. Для створення штучного інфекційного фону борошністої роси використовували місцеву популяцію та накопичувач інфекції американський сорт Кепок. У гібридному розсаднику F₃ на провокуючому фоні борошністої роси відібрано високостійкі проти збудника (до 5 %) комбінації, створені за участю джерел стійкості: Zdar, Fakon, Pi170911, Bongo – Світанок Миронівський/Zdar, Колос Миронівщини/Fakon, Берегиня Миронівська/Pi170911, Достаток/Bongo. Стійкість проти патогена (до 10,0 %) спостерігали у двох комбінацій схрещування (Горлиця миронівська/Gloria та Ремеслівна/Wervok). На штучному інфекційному фоні гібридів четвертого покоління за стійкістю проти бурої іржі відібрано комбінації схрещування, створені за участі джерел стійкості: Flex, V 1275, Tobarzo, 203-238. Варто зазначити гібриди: Оберіг Миронівський/Flex, Смуглянка/V 1275, Монотип/Tobarzo, Колос Миронівщини/203-238. Найбільшу кількість зерен у колосі отримано у комбінації Світанок Миронівський/Zdar (61,5 шт.), а найбільша маса зерна з колоса у комбінації Горлиця Миронівська/Gloria та Берегиня миронівська/Pi170911 (2,38 г та 2,37 г відповідно). За стійкістю проти бурої іржі та елементами продуктивності виокремилась комбінація Оберіг Миронівський/Flex. Виділені за результатами проведених досліджень зразки використовуються в якості цінного вихідного матеріалу для створення нових сортів пшениці озимої стійких до хвороб у Ліссестепу України

Ключові слова: *Triticum aestivum* L., вихідний матеріал, джерела стійкості, збудники хвороб, борошніста роса, бура іржа, сорт

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Identification of Mycobiota and Diagnosis of Soybean Seed Diseases

Abstract. Micromycetes that colonise soybean seeds reduce their sowing quality, causing loss of germination, rotting and death of seedlings, and the appearance of diseases on young plants. Infection-free seed is one of the factors that ensure crop growth. Therefore, the study of mycobiota of seeds is relevant for the implementation of preventive disease control measures. The purpose of this study was to identify phytopathogenic fungi that colonise soybean seeds and to establish their species composition. The seed samples were selected in the conditions of the Ukrainian Scientific Plant Quarantine Research Station (Chernivtsi Oblast) of the Institute of Plant Protection of the National Academy of Agrarian Sciences during 2019-2021. Mycological studies of soybean seeds were performed in the scientific laboratory of the Department of Phytopathology of the National University of Life and Environmental Sciences of Ukraine using the biological method from subsequent morphological and microscopic analysis of pathogens. It was established that the species composition of mycobiota of soybean seeds is diverse and is represented by 17 species: *Reronospora manshurica* (Naum.) Syd. (downy mildew pathogen), *Sclerotinia sclerotiorum* de Bary (white mold pathogen), *Botryotinia fuckeliana* Whetzel. (grey mold pathogen), *Diaporthe* sp. (Phomopsis pathogen); pathogens of fusariosis are *Fusarium okhusporum* (Schl.) Snyd. et Hans., *Fusarium graminearum* Schwabe, *Fusarium gibbosum* App. et Wr., *Fusarium solani* (Mart.) Sacc.; *Alternaria alternata* (Fries) Keissler (*Alternaria* pathogen), *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (cladosporiosis pathogen), *Colletotrichum* sp. (anthracnose pathogen); pathogens of various moulds – *Aspergillus niger* van Tiegh., *Aspergillus flavus* Link, *Trichothecium roseum* Link ex Fries, *Mucor mucedo* Fres. amend. Bref., *Penicillium expansum* Link and *Rhizopus nigricans* Ehrenb. The dominant species were *C. cladosporioides* and *A. alternata*, their frequency of occurrence was in the range of 28.5-47.8%. The obtained data on the symptoms of mycosis of soybean seeds, the species composition of pathogens and their frequency of occurrence are of practical importance for the diagnosis of diseases, the identification of pathogens and the implementation of preventive therapeutic control measures

Keywords: *Glycine max*, micromycetes, endophytic infection, mould, seed germination, types of pathogens

INTRODUCTION

Healthy seeds are one of the factors that ensure the growth of crop yields. At the same time, seed quality is strongly influenced by microbial communities, which consist of endophytes and epiphytes and take part in germination, growth, and development of plants and seed storage [1]. Seed-associated microbial populations consist of 9,000 different synergistic, commensal, and potential pathogenic microbial species [1; 2]. In general, over 80% of plant diseases are caused by fungi or fungi-like pathogens that threaten food safety [3].

Legume seeds are a favourable substrate for damage by various pathogens [4]. Soybean (*Glycine max* L.) is

infected with more than 200 types of phytopathogenic microorganisms, among which fungi are the most dangerous [5]. At the same time, about 30 types of pathogens can cause considerable economic losses [6]. Phytopathogenic fungi that colonise soybean seeds reduce its seeding quality, causing loss of germination, rotting and death of seedlings, disease manifestations on young plants [7; 8]. Infected seeds contribute to the spread of pathogens locally and over long distances [9]. Some fungal pathogens that colonise soybean seeds can produce mycotoxins [10; 11], dangerous for humans and animals.

In general, pathogens affecting soybean seeds, in addition to reducing germination and yield, also cause

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biochemical changes in the seed. Thus, the fungi *Phomopsis longicolla* and *Cercospora kikuchii* affect the quality of soybean seeds, reducing the content of isoflavone, protein, oil, and fatty acids, with malonlignin showing the greatest reduction [12]. Therefore, the study of the phytopathological state of the seeds, the identification of pathogens is relevant for the implementation of preventive disease control measures. The latter require information about the quality of the seed, its pathological condition since the seed can be a carrier of plant diseases [13; 14].

In various countries of the world, research is being conducted on the species composition of fungi that affect soybean seeds and reduce their quality. Phytopathogenic species of fungi of the *Diaporthe/Phomopsis* complex, which cause seed rot, pod, and stem rot on soybeans, are harmful in European countries and are represented by the species *D. longicolla*, *D. caulivora*, *D. eres*, and *D. novem* [15]. The analysis of the phytopathological state of soybean seeds in the conditions of Serbia confirmed its infection with the fungi *Peronospora manshurica*, *Macrophomina phaseolina*, *Botrytis cinerea*, *Cercospora kikuchii*, as well as species from the genera *Alternaria*, *Diaporthe*, and *Fusarium*. The dominant species of fungi were *Alternaria* and *Peronospora manshurica* [16]. The identification of fungi affecting soybean seeds grown in the Mediterranean region of Turkey confirmed its infection with four species, namely: *Aspergillus spp.*, *Penicillium spp.*, *Cladosporium spp.*, and *Fusarium spp.* [17].

The fungus *Diaporthe spp.* causes seed decay and deterioration of its quality. In wet production areas of the United States of America, up to 10 species have been extracted from soybean seed. Based on analysis of their morphology, associated species included *D. aspalathi*, *D. caulivora*, *D. kongii*, *D. longicolla*, *D. sojae*, *D. ueckeriae*, *D. unshiuensis*, and three new fungi *D. bacilloides*, *D. flavescens*, and *D. insulistroma* [18]. Seven species and six genera were identified from 55 fungal isolates obtained from soybean seeds (USA – southern Virginia, eastern Virginia, and north-eastern North Carolina). Fungi of the genera *Alternaria*, *Diaporthe*, and *Fusarium* were the most common [19]. Soybean anthracnose (pathogen – fungus *Colletotrichum truncatum*) is a dangerous disease in Brazil [20-22]. Potential yield losses from the disease can be up to 50% [22].

Fungi belonging to 13 genera were extracted from soybean seeds grown in Southwest China, among which *Fusarium* (*F. fujikuroi*, *F. proliferatum*, *F. verticillioides*, *F. asiaticum*, and *F. incarnatum*) accounted for 55.0%, *Colletotrichum spp.* – 23.0% [23]. In the conditions of Indonesia, the fungi *Aspergillus flavus* Link, *Aspergillus niger* van Tieghem, and *Cladosporium oxysporum* Berk & M.A. Curtis, *Colletotrichum dematium* (Pers. et Fr.) Grove f. sp. *truncata* (Schw.) Arx, *Curvularia pallescens* Boedijn, *Fusarium solani* (Mart.) Sacc., *Melanospora zamiae* Corda and *Nigrospora sp.* were extracted from the seeds of various soybean varieties [24].

Scientific literature has reports and experimental confirmations about the possibility of spreading the fungus *Corynespora cassicola* (Berk. & Curt.) with soybean seeds [25]. It was also established that with an increase in the duration of soybean seed storage (from 0 to 8 months), a change in fungal populations occurs, namely an increase in the incidence of *Aspergillus spp.* from 1.3% to 7.5% and *Penicillium spp.* from 0% to 17.5%, while the infestation of *Phomopsis spp.* decreases from 7.5% to 0-0.3% [26].

In the conditions of Ukraine, studies were previously conducted that confirm the infection of soybean seeds by various microorganisms of fungal and bacterial aetiology [27; 28]. At the same time, this information is insufficient for a comprehensive understanding of the entire spectrum of micromycetes that can be found in infected seeds.

The purpose of this study was to detect and identify micromycetes that infect soybean seeds, to establish the frequency of their occurrence and to investigate the symptoms of pathologies.

MATERIALS AND METHODS

Field research was performed in the conditions of the Ukrainian Research Station of Plant Quarantine, the Institute of Plant Protection of the National Academy of Agrarian Sciences during 2019-2021. For analysis, soybean seeds of the Xenia variety were selected, and an average sample was formed. For further research, the seed material was stored in a paper bag in laboratory conditions at 18-20°C.

Phytopathological analysis of soybean seeds was performed in the scientific laboratory of the Department of Phytopathology of the National University of Life and Natural Sciences of Ukraine according to generally accepted methods [29]. At the bottom of a Petri dish with a diameter of 90 mm, three layers of filter paper were placed, which were moistened with distilled water (excess water was drained). Petri dishes, filter paper, and water were sterile. Ten seeds were spread evenly (at 2 cm) on the surface of the filter paper. The total number of seeds under examination was 400 pieces. Next, the prepared seeds were incubated at 25±2°C for 7 days with a 12-hour alternation of light and darkness. After the end of the incubation period, the seeds were examined using a stereomicroscope (SIGETA MS-217 LED 20x-40x Bino Stereo) to identify the morphological structures of the fungi. Further work lied in making temporary microscopic preparations and using a monochrome microscope (Sigeta MB-103 40x-1600x LED Mono) to analyse mycelial morphology and sporulation. Soybean seeds affected by downy mildew were detected by macroscopic examination.

To detect internal infection, surface sterilisation of soybean seeds [30] and its incubation under the conditions given above were performed. To investigate the symptoms of diseases, in the pre-harvest period, soybeans with signs of pathologies were selected and diagnosed.

Micromycetes were also extracted from pure cultures and cultivated on potato-glucose agar [30; 31] in Petri dishes for 7 days at 22±2°C, which allowed evaluating the cultural and morphological characteristics of mushrooms. To identify pathogens, special literature was used [32; 33], where the species composition of soybean diseases in different regions of the world is covered, their symptoms on the vegetative and generative organs of plants are described, and attention is paid to the biological and morphological features of pathogens. Frequency of occurrence [30; 31] of micromycetes species were calculated according to formula (1):

$$Foc = \frac{n \times 100}{N} \quad (1)$$

where *Foc* is the frequency of occurrence, %; *n* is the number of seeds in which this species was found, pcs. *N* is the total number of examined seeds, pcs.;

RESULTS AND DISCUSSION

Three-year studies of the structure of micromycetes that colonise soybean seeds proved that their species composition is diverse and is represented by 17 species (Fig. 1): *Peronospora manshurica* (Naum.) Syd. (downy mildew pathogen), *Sclerotinia sclerotiorum* de Bary (white mold pathogen), *Botryotinia fuckeliana* Whetzl. (grey mold pathogen), *Diaporthe sp.* (Phomopsis pathogen); pathogens of fusariosis are *Fusarium okhusporum* (Schl.), Snyd. et Hans., *Fusarium graminearum* Schwabe, *Fusarium gibbosum* App. et Wr., *Fusarium solani* (Mart.) Sacc.; *Alternaria alternata* (Fries: Fries) Keissler (Alternaria pathogen), *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (cladosporiosis pathogen), *Colletotrichum sp.* (anthracnose pathogen); pathogens of various molds – *Aspergillus niger* van Tiegh., *Aspergillus flavus* Link, *Trichothecium roseum* Link ex Friet Fries, *Mucor mucedo* Fres. amend. Bref., *Penicillium expansum* Link and *Rhizopus nigricans* Ehrenb.

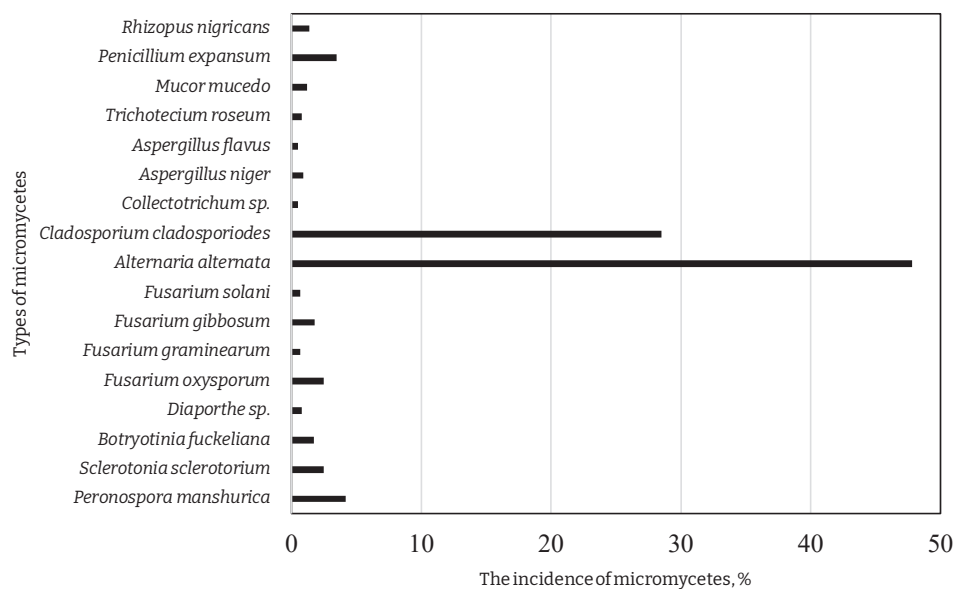


Figure 1. Frequency of occurrence of micromycetes affecting soybean seeds (epiphytic and endophytic mycoflora)

The frequency of the identified representatives of the mycobiota of soybean seeds was as follows: *P. manshurica* (4.2%), *S. sclerotiorum* (2.5%), *B. fuckeliana* (1.7%), *Diaporthe sp.* (0.8%), *F. oxysporum* (2.5%), *F. graminearum* (0.7%), *F. gibbosum* (1.8%), *F. solani* (0.7%), *A. alternata* (47.8%), *C. cladosporioides* (28.5%), *Colletotrichum sp.* (0.5%), *A. niger* (0.9%), *A. flavus* (0.5%), *T. roseum* (0.8%), *M. mucedo* (1.2%), *P. expansum* (3.5%), *R. nigricans* (1.4%).

Analysis of the distribution of micromycetes shows that the species *C. cladosporioides* and *A. alternata* were dominant. The frequency of their occurrence was within 28.5–47.8%. Pathogens of fusarium wilt were found in 0.7–2.5% of seeds. At the same time, the most common species was *F. oxysporum*. Among other phytopathogenic fungi (*Colletotrichum sp.*, *Diaporthe sp.*, *B. fuckeliana*, *S. sclerotiorum*, *P. manshurica*), which colonise seeds

during plant vegetation, their frequency of occurrence was within 0.5–4.2%. Representatives of saprotrophic mycoflora, which cause mould of seeds, had a prevalence ranging from 0.5% to 3.5%. The micromycete *P. expansum* was detected most often.

According to the results of mycological examination, the endophytic mycoflora of *S. sclerotiorum* and *B. fuckeliana* fungi was 95.3% and 92.5%, respectively (Fig. 2). Seeds infected with these fungi lost germination or formed sprouts that rotted (Fig. 3a, b). At the same time, the fungi *A. alternata* and *C. cladosporioides*, which prevailed in the general mycoflora of soybean seeds, were detected only in 4.5 and 2.7% during the analysis of internal infection (Fig. 2). This indicates their localisation in the seed coat. Such seeds created healthy seedlings (Fig. 3c). The internal infection of soybean seeds by the micromycete *P. expansum* was 0.5%.

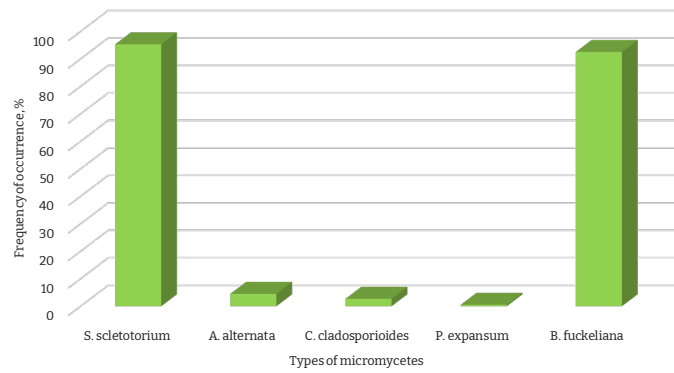


Figure 2. Frequency of occurrence of certain types of pathogens of soybean seeds (endophytic infection)

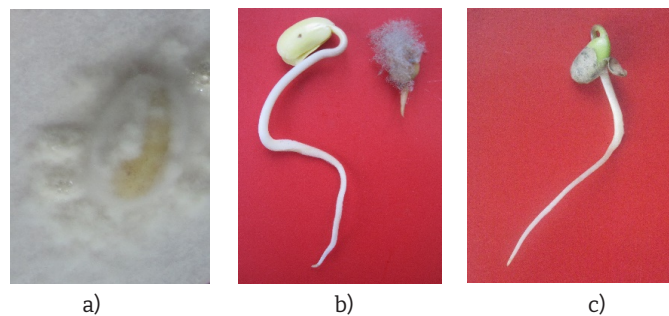


Figure 3. Soybean seed infected with micromycetes during germination: a – *S. sclerotiorum*, b – *B. fuckeliana* (healthy – left and affected – right), c – *A. Alternata*

As a result of visual diagnosis of affected soybeans with typical disease symptoms, macro- and microscopic analysis of the morphological structures of pathogens, clear pathological changes in the affected seed can be observed. For instance, seeds affected by the fungus *P. manshurica* are covered with a creamy shell of oospores of the fungus, become matte, whitish, and lose their lustre (Fig. 4a). In case of infection of soybeans with *S. sclerotiorum*, the seeds in them become discoloured, lose their lustre, and become thin. In the interseed space, sclerotia can form, which adhere to the seeds (Fig. 4b). Mushrooms *B. fuckeliana* and *Diaporthe sp.* cause the wrinkling of the seeds, the destruction of the skin,

putrefaction, and fragility (Fig. 4c, d). Grain affected by *Fusarium spp.* is thin, wrinkled. During germination, it is covered with mycelium (Fig. 4e) and becomes mouldy. The micromycetes *A. alternata* and *C. cladosporioides* cause the darkening of the seed coat, and sometimes fragility. During germination, heavily infected seeds become mouldy (4f, g). Seeds infected with *Colletotrichum sp.* are covered with brown spots, thin and underdeveloped. These symptoms of fungal seed diseases were observed in the case of a strong degree of disease progression on soybeans. Infection of soybean seeds with fungi *A. niger*, *A. flavus*, *T. roseum*, *M. mucedo* Fres., *P. expansum* (Fig. 4h), and *R. nigricans* caused its mould and loss of germination.

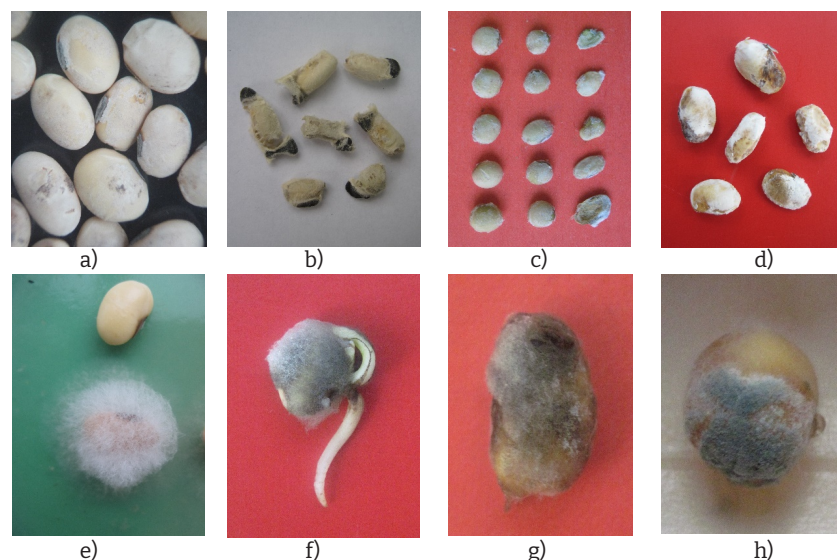


Figure 4. General appearance of soybean seeds severely affected by micromycetes: a – *Peronospora manshurica*, b – *Sclerotinia sclerotiorum*, c – *Botryotinia fuckeliana*, d – *Diaporthe sp.*, e – *Fusarium sp.*, f – *Alternaria alternata*, g – *Cladosporium cladosporioides*, h – *Penicillium expansum*

However, in all cases, for correct diagnosis of seed pathologies and identification of pathogens, it is necessary to use a biological method and microscopic analysis of pathogens, since pathogens were in the seeds in a latent state. The use of molecular biology methods is also important for the reliable identification of many types of pathogens [15; 34; 35].

Analysis of studies on the structure of populations of micromycetes affecting soybean seeds in different regions of the world show a wide variation in the species composition of pathogens and their frequency of occurrence. Thus, in the Poltava Oblast (Ukraine), damage to seeds belonging to 8 genera was detected: *Alternaria*, *Aspegillus*, *Botrytis*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, and *Peronospora*. The most widespread were *Alternaria* sp. – 26-35% and *Mucor* sp. – 22-47% [28]. In Serbia, the following pathogens of mycoses dominated: *Alternaria* sp. – 28.9%, *P. manshurica* – 44%, and *Fusarium* sp. – 10.3% [16]. In southwestern China, the population of fungi that spread with soybean seeds is represented by *Fusarium* sp., *Colletotrichum* sp., *Alternaria* sp., *Corynespora* sp., *Diaporthe/Phomopsis* sp., *Stagonosporopsis* sp., *Chaetomium* sp., *Podospora* sp., *Botryosphaeria* sp., *Thielavia* sp., *Macrophomina* sp., *Harzianum* sp., and *Didymella* sp. Isolates of the genus *Fusarium* dominated, the frequency of which was 55%, and *Colletotrichum* sp. – 22.3% [23]. In Brazilian conditions, among the identified pathogens of soybean seeds, 6 types of fungi were common: *Fusarium* sp., *Phomopsis* sp., *Cercospora* sp., *Colletotrichum* sp., *Rhizoctonia* sp., and *Penicillium* sp. [37]. In Ethiopia, damage to soybean seeds by fungi *Fusarium* sp., *A. niger*, *A. flavus*, *Penicillium* sp., and *Rhizopus* sp. was detected. The species *A. flavus* (29.18%) and *A. niger* (23.81%) predominated [38]. A study of seed-associated mycoflora in soybean under Indian conditions identified six fungal species, including *A. alternata*, *A. flavus*, *A. niger*, *F. verticillioides*, *Macrophomina phaseolina* and *Rhizopus stolonifer*. The most frequent species were *F. verticillioides* – 19.25%, *M. phaseolina* – 15.00% and *A. alternata* – 13.50% [39].

Research conducted in the conditions of Ukraine (Chernivtsi Oblast) allowed detecting and identifying 17 types of fungi that attack soybean seeds. Therewith,

the dominant position was occupied by *C. cladosporioides* and *A. alternata*. The last species also prevailed or was among the main representatives of the mycoflora of seeds in the studies of scientists from different countries [16; 28; 39]. The significant distribution of *A. alternata* is confirmed by its cosmopolitanism [40]. Seed damage by fungi *S. sclerotiorum*, *Colletotrichum* sp., and *Diaporthe* sp. was also identified, which are distributed in soybean cultivation regions [23; 37], but there is no information about them in Ukraine [28; 36].

CONCLUSIONS

Establishing the species composition of soybean seed pathogens is important for understanding the epidemiology of plant pathologies and developing effective measures to control them. As a result of the research, 17 types of micromycetes that colonise soybean seeds were identified: *Peronospora manshurica* (Naum.) Syd., *Sclerotinia sclerotiorum* de Bary, *Botryotinia fuckeliana* Whetzl., *Diaporthe* sp., *Fusarium oxysporum* (Schl.) Snyd. et Hans., *Fusarium graminearum* Schwabe, *Fusarium gibbosum* App. et Wr., *Fusarium solani* (Mart.) Sacc., *Alternaria alternata* (Fries: Fries) Keissler, *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *Colletotrichum* sp., *Aspergillus niger* van Tiegh., *Aspergillus flavus* Link, *Trichothecium roseum* Link ex Fries, *Mucor mucedo* Fres. amend. Bref., *Penicillium expansum* Link, and *Rhizopus nigricans* Ehrenb. The dominant species were *C. cladosporioides* and *A. alternata*. At the same time, they were localised in the seed coat. Endophytic micromycetes *S. sclerotiorum*, and *B. fuckeliana* caused loss of seed germination or seedling death.

Phytopathogenic fungi *S. sclerotiorum*, *Colletotrichum* sp., and *Diaporthe* sp. identified among the mycobiota complex of soybean seeds indicate the need for a more complex study of this issue in different soil and ecological conditions of Ukraine because these pathogens during the last decade have not been covered in the studies of Ukrainian scientists. Further research must establish the role of soybean varieties, growing region and meteorological conditions on the structure of the phytopathogenic complex of seed micromycetes.

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Ідентифікація мікобіоти та діагностика хвороб насіння сої

Анотація. Мікроміцети, які колонізують насіння сої, знижують його посівні якості, викликаючи втрату схожості, загнивання і загибель проростків, проявлення хвороб на молодих рослинах. Вільне від інфекцій насіння є одним із чинників, які забезпечують зростання врожаю. Тому вивчення мікобіоти насіння є актуальним для проведення превентивних заходів контролю хвороб. Мета роботи – виявити фітопатогенні гриби, які колонізують насіння сої і встановити їхній видовий склад. Вибір зразків насіння проводили в умовах Української науково-дослідної станції карантину рослин (Чернівецька область) Інституту захисту рослин Національної академії аграрних наук протягом 2019–2021 рр. Мікологічні дослідження насіння сої здійснювали у науковій лабораторії кафедри фітопатології Національного університету біоресурсів і природокористування України використовуючи біологічний метод із наступним морфологічним і мікроскопічним аналізом патогенів. Встановлено, що видовий склад мікобіоти насіння сої є різноманітним і представлений 17 видами: *Peronospora manshurica* (Naum.) Syd. (збудник пероноспорозу), *Sclerotinia sclerotiorum* de Bary (зб. білої гнилі), *Botryotinia fuckeliana* Whetzel. (зб. сірої гнилі), *Diaporthe* sp. (зб. фомопсису); збудниками фузаріозу – *Fusarium oxysporum* (Schl.) Snyd. et Hans., *Fusarium graminearum* Schwabe, *Fusarium gibbosum* App. et Wr., *Fusarium solani* (Mart.) Sacc.; *Alternaria alternata* (Fries: Fries) Keissler (зб. альтернاریозу), *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (зб. кладоспоріозу), *Colletotrichum* sp. (зб. антракнозу); збудниками різних пліснявінь – *Aspergillus niger* van Tiegh., *Aspergillus flavus* Link, *Trichothecium roseum* Link ex Friet Fries, *Mucor mucedo* Fres. emend. Bref., *Penicillium expansum* Link і *Rhizopus nigricans* Ehrenb. Домінуюче становище мали види *C. cladosporioides* та *A. alternata*, частота їх трапляння була в діапазоні 28,5–47,8 %. Одержані дані про симптоматику мікозів насіння сої, видовий склад патогенів і частоту їх трапляння, мають практичне значення для діагностики хвороб, ідентифікації збудників і проведення профілактичних терапевтичних заходів контролю

Ключові слова: *Glycine max*, мікроміцети, ендofітна інфекція, пліснявіння, проростання насіння, види патогенів

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A Hybrid Technique for Measuring the Content of Xenobiotics in Wild and Cultivated Blueberries

Abstract. Laboratory control requires testing of berry products for the content of residual amounts of mixtures of various xenobiotics: pesticides, polycyclic aromatic hydrocarbons. In this regard, the purpose of this study was to investigate the physicochemical conditions of extraction of xenobiotics from wild and cultivated blueberries and further chromatographic analysis of the obtained extracts. The leading approach to the development of a hybrid method of laboratory control of residual amounts of xenobiotics in berries is the construction of a theoretical model of the method and empirical studies of the proposed conditions of the method. The first stage of the study was sample preparation and extraction of xenobiotics. It was found that pesticides should be extracted from homogenized plant material, and for the extraction of polycyclic aromatic hydrocarbons, berries with an intact skin should be used. It was found that 1% acetonitrile solution of formic acid is a unified extractant of xenobiotics. The need for sequential chromatographic analysis of the obtained extracts by using high-performance liquid chromatography with a fluorescent detector, methods of high-performance liquid chromatography and gas chromatography with mass-selective detectors was substantiated. Approbation measurements showed that the limit of quantitative determination of residual quantities of pesticides is 0.01 ± 0.001 mg/kg, and the limit of quantitative determination of polycyclic aromatic hydrocarbons is much lower and is 0.5 ± 0.05 μ g/kg. The hybrid technique allows detecting and measuring the content of xenobiotics in extracts of blueberry berries within 90 minutes. The working ranges for controlling pesticide content are from 1.0 mg/kg to 0.01 mg/kg, for polycyclic aromatic hydrocarbons – from 20 μ g/kg to 0.5 μ g/kg. The magnitude of the error of the quantitative analysis was estimated using the root-mean-square deviation from the mean (σ , %). The completeness of extraction of xenobiotics was established as a percentage (r , %) using a series of studies of model samples artificially enriched with xenobiotics. It was found that the measurement error of xenobiotic content does not exceed 20%, the value of analyte extraction is within 90-108%, the method is suitable for laboratory control. The developed hybrid method is of practical value for performing laboratory control of chemical compounds dangerous to human health

Keywords: pesticides, polycyclic aromatic hydrocarbons, extraction, chromatography, safety of plant products

INTRODUCTION

During the last five years, many studies have been published, which report on modern technologies of growing, harvesting, post-harvest processing, storage, and processing of plant products, namely products of fruit and berry crops [1]. Therewith, the issues of laboratory control and determining the safety indicators of crop production per the requirements of regulatory documents of the countries of the European Union (EU) are still open. Ukraine cooperates with the EU countries in the

development of the market for Ukrainian berries, implements advanced approaches to successful export of a wide range of plant products, including wild berries [2] and needs the development of laboratory control methodology.

Some varieties of strawberries and blueberries, due to their taste properties and the ability to store and transport, are among the most represented products of the berry group in supermarkets of EU countries [3]. Notably, healthcare experts, namely nutritionists, single

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out blueberries as a valuable source of biologically active substances (BAS), recommend their consumption and use in the recipes of a wide list of food products useful for human health and pharmaceutical purpose [4]. Therewith, the consumption of fresh berries, or the consumption of food products made on their basis, must be safe for health and requires constant laboratory monitoring. Presently, laboratory control should provide not only the analysis of the content of useful BASs, but also the residual amounts of dangerous xenobiotics [4].

Food products must be safe and follow current sanitary and hygienic standards [5], namely regarding the content of xenobiotics, which are persistent pollutants, capable of being stored for a long time in the composition of food products, and when entering the human body, immediately realise their dangerous biological properties. Furthermore, resistant xenobiotics can migrate in the environment, contaminating water, harming human health not only when they enter the digestive tract, but also when in contact with the skin [6; 7]. When planning and carrying out laboratory control, scientists pay special attention to measuring the content of xenobiotics from the group of polycyclic aromatic compounds (PAC) because the compounds of the PAC group have carcinogenic, mutagenic, and teratogenic properties [8]. The most dangerous representative of compounds of the group of surfactants is the xenobiotic benzo(a)pyrene (BaP). Trace amounts of B(a)P contaminate food products, environmental objects, accumulate in the human body and cause the development of malignant tumours [8].

To obtain safe plant products, it is necessary to investigate and monitor chemical compounds of anthropogenic origin in various objects of the ecosystem where growing and harvesting take place. Some modern studies highlight the regularities of the influence of natural and anthropogenic factors of different geographical areas on the chemical composition of berries and confirm the need for the development of laboratory control, namely the expansion of the list of controlled compounds [9; 10]. Based on the results of previous research studies [11] of optimal laboratory control conditions and considering the results of the analysis of xenobiotics of agricultural products per standardized methods, namely: the European standard EN 15662:2018, the State Standards of Ukraine DSTU ISO 28540:2015 and DSTU 4689:2006 [12-14], it can be argued that the most promising laboratory methods are those that allow for a comprehensive study of products, promptly and with adequate quality to measure indicators [15].

Among xenobiotics, as a rule, the content of residual quantities of active ingredients of plant protection agents used in the technology of controlled culture cultivation is analysed [16]. The study of the safety of wild products growing in an environment not controlled by the product manufacturer should consider the possible anthropogenic load on the corresponding ecosystem and

the level of accumulation of anthropogenic compounds in products, specifically in berries. Therewith, literature sources do not cover the issue of laboratory control of carcinogenic compounds of the surfactant group in berries.

The purpose of this study was to develop a hybrid technique for laboratory control of residual amounts of xenobiotics (pesticides, polycyclic aromatic hydrocarbons) in berries of wild and cultivated blueberries.

MATERIALS AND METHODS

For the development of the hybrid method covered in this paper, the best conditions of modifications of the methods [12-14] used for the analysis of vegetables and fruits, including methods for monitoring environmental objects capable of accumulating anthropogenic compounds [17] were applied. The indicated modifications of the methods were tested and validated in 2017-2021 at the Ukrainian Laboratory of Quality and Safety of Products of the Agro-Industrial Complex at the National University of Life and Environmental Sciences of Ukraine (Kyiv-Sviatoshynskiy district, urban-type settlement of Chabany).

Approbation studies were carried out using a selection of samples totalling 125 pieces, which was formed from an equal number (25 pieces each) of wild blueberry samples, cultivated blueberries, model systems and samples of blueberries that were artificially enriched with xenobiotics. The list of xenobiotics at each stage of research contained anthropogenic chemical compounds from the group of pesticides: Acetamiprid, Alpha-Cypermethrin, Benomyl, Desmedipham, Imidacloprid, Chlorpyrifos-methyl, Chlorpyrifos; and groups of polycyclic aromatic hydrocarbons: Benzo(a)pyrene, Benzo(a)anthracene, Benzo(b)fluoranthene, Chrysene.

Solvents and reagents qualified for "chromatography" and "pure for analysis" were used in the study: acetonitrile, glycerine, deionized water, diethyl ether, isopropanol, calcium chloride, magnesium sulphate, methanol, formic acid, sodium hydroxide, sodium chloride, sodium citrate, acetic acid, sulphuric acid, trifluoroacetic acid, hydrochloric acid, chloroform. Organic solvents were used both individually and in a mixture with aqueous solutions of acids in various ratios of organic and inorganic phases. The acid content varied depending on the analysis conditions and ranged from 0.1% to 3.5%. Co-extractants, which are not target analytes, were extracted using solid-phase extraction on sorbents: Al_2O_3 and $nSiO_2$, activated carbon, graphitized activated carbon produced by Supelco™, solid-phase extraction columns (SPE – ChromSpher Pi, Varian™), mixtures of primary and secondary amines produced by Supelco™.

Sampling was performed following regulatory documentation harmonized with international standards: "Sampling methods for determining the maximum permissible levels of certain pollutants in food products for the purposes of state control" [18]. In the laboratory, each sample was divided into three parallel samples within the framework of one study.

The next stage of the study was the homogenization of samples. To detect pesticides, homogenization was performed by grinding the sample in a glass of an LZM-1 laboratory mill-homogenizer, at different temperature conditions within 4-25°C. To analyse the content of polycyclic aromatic hydrocarbons, homogenization of berry samples was not used, since polycyclic aromatic hydrocarbons (PAHs) contaminate environmental objects as a result of the combustion of fuel materials and are lipophilic compounds that contaminate the object in contact and adsorption on its surface [19]. In the samples under study, surfactants were concentrated in the skins of the berries and the plant leaves.

The extraction of xenobiotics begins with buffering the homogenized (or non-homogenized) sample by mixing it with "pure for analysis" chemical compounds, namely: magnesium sulphate, sodium chloride, sodium citrate, potassium citrate, calcium chloride, formic, and acetic acid. These salts were mixed before the study. For one sample, the weight of the salt mixture was 5.0 grams. Magnesium sulphate was included in the mixture in the amount of 1.5 g, sodium chloride – 1.5 g, sodium citrate – 1.0 g, potassium citrate – 0.5 g, calcium chloride – 0.5 g.

Extraction was performed according to the method of maceration with intensive stirring (200 rpm). Since matrix compounds affect the distribution of xenobiotics in the sample extraction system, the homogenized sample was cooled to +4°C. Additions of water, organic and mineral acids specified in the list of reagents were used to regulate the completeness of extraction of target xenobiotics by organic solvents. Additions of acids allowed shifting the balance of the process of dissociation of ionogenic analytes towards the formation of molecules and created conditions for the extraction of xenobiotic molecules from the homogenized raw material with the proper extractant.

To extract compounds of the PAH group from the surface of the berries, isopropanol without impurities of other chemicals was used. Phase separation of the extraction system was performed using a Thermo Scientific centrifuge for 10 minutes at constant rotation at a speed of 4,000 to 7,000 rpm, at temperatures in the centrifuge chamber from 4°C to 20°C. The obtained plant extract was isolated after centrifugation of the plant material and purified from co-extractive substances by dispersion solid phase extraction methods using organic solvents and sorbents (mixtures of primary and secondary amines, activated carbon, activated graphitized carbon). The purified extract of xenobiotics was concentrated and re-extracted using an IKA rotary evaporator, the resulting concentrate was dissolved in acetonitrile in a ratio of 1:20.

The obtained isopropanol PAH extract was concentrated, and the resulting thick residue was dissolved in acetonitrile. Acetonitrile is suitable for sample introduction in both high-performance liquid and gas chromatography.

Extracts were tested for the content of xenobiotics, pesticides, and PAHs by sequential chromatographic analysis using high-performance liquid chromatography with a fluorescence detector (HPLC/FLD), high-performance liquid chromatography and gas chromatography with mass-selective detectors (HPLC/MSD, GC/MSD). Analytical signals were processed using the unique characteristics of xenobiotics: retention time on a chromatographic column, characteristic parameters of the mass spectrum, mass spectral characteristics of xenobiotics recorded in the NIST 0.5 library.

Quantitative analysis of the content of xenobiotics in blueberry samples, model systems and samples of blueberry artificially enriched with xenobiotics was performed using calibration dependences, statistical analysis of the results (calculation of the measured average concentration (X_{avg}) and the root-mean-square deviation from the average (σ , %)) was performed using the software package Microsoft Office Excel and calculation formulas (1; 2):

$$X_{avg} = \frac{\sum_i^n X}{n} \quad (1)$$

where X_{avg} is the average concentration of xenobiotics in the sample; X is the xenobiotic concentration determined in each of the samples under study; n is the number of samples under study.

$$\sigma = \sqrt{\frac{\sum_i^n (X - X_{cp})^2}{n}} \cdot 100\% \quad (2)$$

where σ is the root-mean-square deviation from the mean value of xenobiotic content in the sample; X is the xenobiotic concentration determined in each of the samples under study; X_{avg} is the average concentration of the xenobiotic in the sample; n is the number of samples under study.

The study used algorithms for automatic integration of chromatographic peaks, which are components of the Dionex Chromeleon 7 Chromatography Data System software. Apart from quantitative analysis, after separation of xenobiotics by gas chromatography, the method of mass spectrometry allows working in the mode of screening search for analytes and estimating the presence of about a thousand xenobiotics without their quantitative calculation.

The proposed hybrid method includes the following stages: setting up the measuring equipment (120 min), sample preparation (extraction 150 min, including the time of action of the extractant on the homogenized sample from 5 to 25 minutes), synchronous chromatographic studies of three aliquots of the extract according to the methods of high-efficiency liquid and gas chromatography (90 min), results processing, report drafting (60 min).

RESULTS AND DISCUSSION

The results of the chromatographic study of acetonitrile solutions of xenobiotics, carried out by the HPLC/MSD method, are presented in Figure 1.

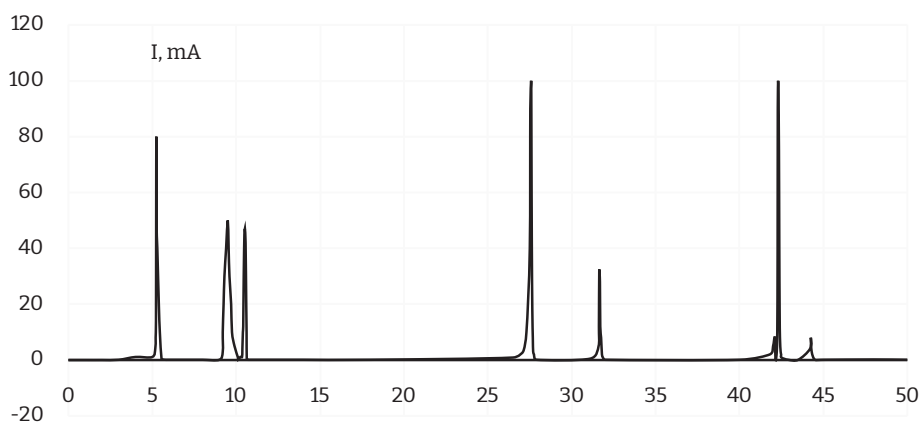


Figure 1. Chromatogram of an extract of a homogenized sample of wild blueberries enriched with xenobiotics. Device: Dionex Summit MSD-3200Q Trap. Column ChromSpher C18 3 μm , 4.6x5 mm. Mobile phase: 0.5% formic acid/methanol

The use of artificially enriched xenobiotics samples of blueberry crop production are the most convenient objects of research, which allow searching for the best physicochemical conditions of the method. The result of such chromatographic analysis, as a rule, is predictable, comprising chromatographic peaks of individual substances. This approach was used in the development of a hybrid technique, as it proved itself well during earlier studies of the content of residual amounts of pesticides and PAHs in the composition of samples of sunflower seeds, vegetable oil, and surface water [11; 17]. This approach allows detecting the analytical signal of a chemical substance on the chromatogram among the signals of matrix compounds and noise and simplifies the further determination of the control value of the minimum concentration of xenobiotics. Furthermore, it allows confidently comparing the values of fixed parameters of sanitary and hygienic norms with determined microquantities of xenobiotics and draw conclusions on the feasibility of using the developed method for investigating the safety control of crop production products. Similar stages of development of methods of laboratory control of xenobiotics are used by other researchers and have been covered in scientific publications for more than fifteen years [19-21].

As Figure 1 shows, individual peaks of chemicals with an analytical signal of up to 10 conventional units have an asymmetric shape, and peaks of greater intensity are symmetrical. In the methods of mass spectrometric detection, which is performed after the separation of xenobiotics by high-performance liquid or gas chromatography, the limit of quantitative determination of xenobiotics is 0.01 mg/kg, while the intensity of the analytical signal exceeded the noise by more than 6 times. The obtained results indicate an increase in the efficiency of the chromatographic system in comparison with earlier studies, where the target components were analysed at a noise:signal ratio of 1:3 [11; 15]. The increase in efficiency is associated with the improvement of the

extraction process of co-extractive substances at the stage of sample preparation.

According to the results of the chromatographic analysis, the list of target pesticides extracted by extractants based on acetonitrile and methanol had the same composition and contained Acetamiprid, Alpha-Cypermethrin, Benomyl, Desmedipham, Imidacloprid, Chlorpyrifos-methyl, Chlorpyrifos. The content of each of these pesticides was 0.05 mg/kg. Obtaining a satisfactory result regarding the expected content of pesticides, which was the amount artificially added to the samples (0.05 mg/kg) and characterized by the absence of xenobiotic losses at the stage of sample preparation, allowed choosing the most unified and environmentally friendly extractant – acetonitrile. Extractive removal of residual amounts of xenobiotics using acetonitrile was accompanied by acidification of the extraction system and aimed at the accumulation of organic acids in the composition of the extract of blueberries, the most complete transition to the extraction of the mixture of xenobiotics. Acidification of the extract with formic acid provides a shift in the balance of dissociation of organic acids towards the formation of molecules, the appearance of the extraction system changes. Although the researcher also controls the sample preparation visually, the decision regarding the effectiveness of the application of the acidified extractant is made based on the results of the chromatographic analysis. The total amount of extracted chemical compounds increases under the conditions of using extractants with formic acid content from 0.1% to 3.5%. Chromatographic analysis of the obtained extracts records the appearance of added peaks on the chromatograms, which are not the target components, while the increase in the percentage of formic acid creates conditions for the extraction of ballast substances of the matrix. Chemical compounds of the blueberry matrix in the extracts obtained by the extractants containing more than 1.0% formic acid complicate the chromatogram with non-target peaks, overload the chromatographic

system, and do not affect the amount of extracted target xenobiotics. The optimal content of added formic acid in the composition of the extractant is 1.0%.

Investigating the influence of the amount of analyte in the extract of the model system on the value of

the analytical signal on the chromatogram, it was established that the linear nature of the dependence for pesticides is within 1.0-0.01 mg/kg, for PAH the linear measurement range is within 20-0.5 $\mu\text{g}/\text{kg}$ (Fig. 2).

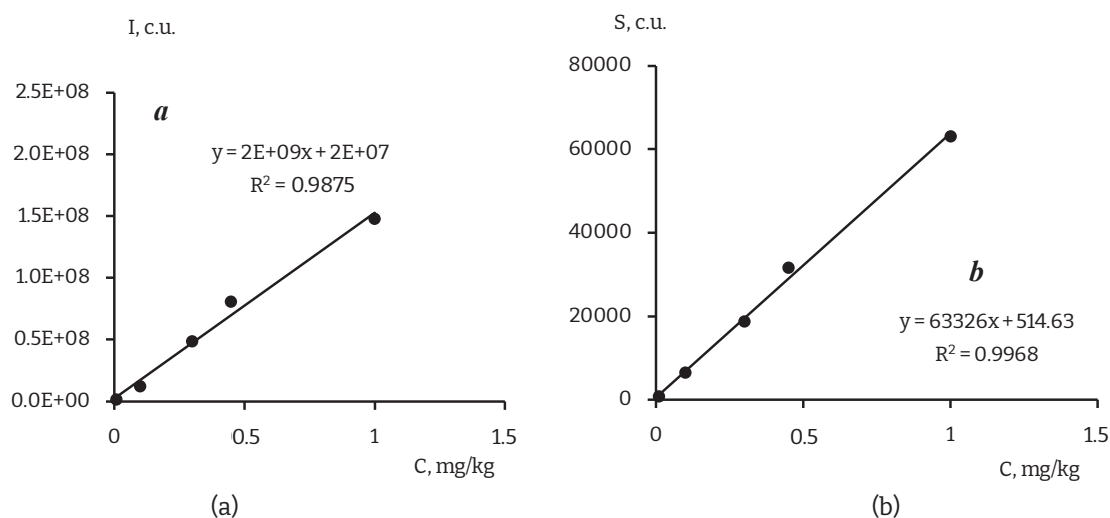


Figure 2. Grading dependences of the intensity of the analytical signal (a) and the peak area (b) of the imidacloprid xenobiotic on its amount in the calibration solution.

Figure 2 shows that the value of the lower point of the calibration dependence is 0.01 mg/kg (or 10 $\mu\text{g}/\text{kg}$) and is the minimum concentration of measuring the quantitative content (MQQ) of pesticides. This concentration can be measured chromatographically in case of the presence of xenobiotics of the pesticide group in the composition of product samples. In addition, the analysis of chromatograms showed that the noise:signal ratio of the analyte is 1:6, which allows detecting the qualitative characteristic of the method, namely the minimum concentration of xenobiotic detection (CXD) at 2 $\mu\text{g}/\text{kg}$. The specified quantitative and qualitative parameters are sufficient for conducting tests, obtaining results and deciding on the compliance of products with sanitary and hygienic requirements [5; 16].

Comparing the obtained quantitative characteristics of the method with the characteristics given in the literature, the developers of the methods achieve the simultaneous measurement of several pesticides, while the MQQ values can increase by two to nine times (0.02-0.09 mg/kg) [19; 20], or coincide with the level established using the hybrid method (0.01 mg/kg) [21]. Therewith, the techniques are aimed at measuring individual pesticides using chromatographic methods, which allows detecting and measuring much smaller amounts of xenobiotics. The authors of the study [22] achieved the MQQ of acetamiprid in the composition of sweet cherry fruits at 1.5 $\mu\text{g}/\text{kg}$, which is a lower level compared to the one achieved using the hybrid method. The minimum detection concentration of Acetamiprid is 0.5 $\mu\text{g}/\text{kg}$ [22], which is four times lower than the similar value of the proposed

hybrid method. Obtaining the ability to control lower levels of xenobiotics content is interesting for the organization of laboratory support for production and marketing of organically produced products. To ensure the control of the compliance of the safety indicators of crop production with sanitary and hygienic standards, a level of MQQ of 0.01 mg/kg (10 $\mu\text{g}/\text{kg}$) is sufficient [5; 16; 23].

A special attention of the researchers during the detection of the above-mentioned parameters of MQQ, CXD and creation of conditions for quantitative analysis is aimed at evaluating the results of the regression analysis of linear dependencies (Figure 2), namely the value of the correlation coefficient (R^2). The optimal conditions of the technique for measuring the content of xenobiotics in the fruits of tomatoes, cucumbers, and lettuce ensured the performance of a quantitative analysis using linear dependencies, which are described by a correlation coefficient of 0.999 [12; 23].

Examining similar parameters of the hybrid technique, it was found that the dependence of the intensity of the analytical signal (I, c.u.) of xenobiotics on their amount in the solution is linear, the calculated correlation coefficients (R^2) are within 0.957-0.991, indicating a sufficient consistency between the xenobiotic content in the sample and the intensity of its analytical signal. The next step in the statistical analysis was the verification and visualization of the dependence of the peak area of the analytical signal (S, c.u.) of the xenobiotic on its content in the analysed solution. The obtained results of the chromatographic analysis were used in the regression analysis of the values. An inherent feature of

the dependence of the peak area on the xenobiotic content is the range of correlation coefficients (R^2) within 0.987-1.00. Since the correlation coefficient testifies to the consistency and linear nature of the dependence of the analytical signal on the content of the analyte, it was the peak areas on the chromatogram that were used to construct the calibration dependences for calculating the content of xenobiotics in laboratory samples. Convergence of the results of quantitative analysis of xenobiotics in model samples increases if the calibration

dependence is constructed as a dependence of the plane of the analytical signal on the concentration of the analyte in the calibration solution. At the stage of GC/MSD research, two samples with residual acetamiprid content were found among ten wild blueberry samples. Furthermore, in all analysed samples, diethyltoluamide was identified according to the library of mass spectra (Table 1). In addition, one of the berry samples contained trace amounts of benzo(a)pyrene ($S_{BAP} \leq 0.05 \mu\text{g/kg}$) and benzo(b)fluoranthene in the amount of $0.75 \mu\text{g/kg}$.

Table 1. Test results of wild blueberry berry extracts in GC/MSD screening mode

Names of indicators, units of measurement	Test results	Limit of quantification	Norms	
			DSanPiN 8.8.1.2.3.4-000-2001	Regulation (EC) No 396/2005
Acetamiprid, mg/kg	0.011±0.002	0.01	Not standardised	2.0
Diethyltoluamide	qualitative analysis, identified	-	Not standardised	

As Table 1 shows, apart from the xenobiotic acetamiprid, which is included in the group of pesticides studied within the framework of the development of the hybrid research methodology, diethyltoluamide was detected on the chromatogram based on the results of a qualitative analysis (screening of xenobiotics according to the library). Qualitative identification of diethyltoluamide on chromatograms obtained by gas mass spectrometry became possible thanks to the presence of characteristic ions of diethyltoluamide in the mass spectrometer library. Since diethyltoluamide is the

active substance of several repellents used to protect humans and farm animals from tick bites, mosquitoes, midges [24; 25], the content of this xenobiotic in wild blueberries may be related to the human use of repellents during the collection of berries, or during the storage and sale of blueberries on the market.

Together with the results of the experimental approbation of the technique, the calibration studies allowed forming a database that provides quantitative characteristics of the suitability of the hybrid technique for performing practical tasks. Table 2 lists the obtained parameters.

Table 2. Results of chromatographic analysis of samples of blueberries artificially enriched with xenobiotics.

Compound, units of measurement	Measurement range	Measured average concentration, mg/kg (\bar{x}_{AVG})	Mean square deviation from the average (σ , %)
Acetamiprid, mg/kg	0.01	0.01	5.2
	0.05	0.04	7.1
	1.0	0.97	3.2
Alpha-Cypermethrin, mg/kg	0.01	0.01	5.5
	0.05	0.05	2.9
	1.0	0.96	4.5
Benomyl, mg/kg	0.01	0.01	5.8
	0.05	0.04	7.9
	1.0	0.95	4.1
Desmedipham, mg/kg	0.01	0.01	5.5
	0.05	0.05	3.9
	1.0	0.95	8.2
Imidacloprid, mg/kg	0.01	0.01	5.5
	0.05	0.045	4.9
	1.0	0.96	5.5
Chlorpyrifos-methyl, mg/kg	0.01	0.01	5.5
	0.05	0.05	3.9
	1.0	0.95	6.5

Table 2, Continued

Compound, units of measurement	Measurement range	Measured average concentration, mg/kg (X_{AVG})	Mean square deviation from the average (σ , %)
Chlorpyrifos, mg/kg	0.01	0.01	5.3
	0.05	0.05	3.9
	1.0	0.97	4.7
Benzo(a)pyrene, μ g/kg	0.5	0.55	4.3
	2.5	2.65	1.9
	10.2	10.5	1.7
Benzo(a)anthracene, μ g/kg	0.5	0.5	5.4
	3.0	2.9	2.3
	14.5	14.8	2.7
Benzo(b)fluoranthene, μ g/kg	0.5	0.6	5.3
	5.0	5.2	2.6
	20.0	19.8	1.0
Chrysene, μ g/kg	0.5	0.5	3.9
	5.0	5.1	2.5
	15.0	15.2	1.8

Table 2 shows that the proposed method can be used to figure out the residues of pesticides of various chemical groups and PAHs. The established average values of expected values (X_{AVG}) correspond to the levels of xenobiotic content introduced into the samples, namely: 0.01 mg/kg, 0.05 mg/kg, and 0.1 mg/kg. The measurement error does not exceed the set maximum level of 20% [22; 23]. To confirm the possibility of the method to

determine xenobiotics in fresh and dried berries, as well as to determine the influence of chemical compounds of the matrix, the corresponding plant extracts were analysed. Plant extracts were obtained from samples artificially enriched with pesticide markers in amounts of 0.01 mg/kg, 0.05 mg/kg, and 0.1 mg/kg; a marker of polycyclic aromatic hydrocarbons (benzo(a)pyrene) – in the amount of 1 μ g/kg (Table 3).

Table 3. Content of xenobiotics in blueberry extracts (n=10, P=0.95)

Marker name	Extractant	Introduced, μ g/kg	Found, μ g/kg	Extraction, %
Acetamiprid	1% acetonitrile solution of formic acid	0.50 \pm 0.01	0.47 \pm 0.02	95.1
Imidacloprid		0.50 \pm 0.01	0.48 \pm 0.01	96.5
Cypermethrin		0.50 \pm 0.01	0.45 \pm 0.02	90.0
Benzo(a)pyrene		1.0 \pm 0.1	1.08 \pm 0.2	108.0

Table 3 shows that the extraction process of xenobiotics is characterized by complete extraction from 90% to 108% of the introduced amount. Comparing the percentage of extraction of pesticides (90-96.5%) and PAH (108%), the extraction process from homogenized material leads to insignificant losses of analytes of pesticide groups. In its turn, PAH maceration is a process of washing chemicals from the surface of berry skins, no loss of analytes during extraction is observed.

CONCLUSIONS

Thus, this study investigated the specific features of extraction of xenobiotics of various groups from blueberries. It was proved that the use of a 1% acetonitrile solution of formic acid as an extractant allows extracting xenobiotics of various groups of pesticides and PAHs from plant material. The best conditions for the extraction

of xenobiotics are the temperature regime from 4°C to 25°C and the time of action of the extractant on the homogenized sample for 5-25 minutes. The method of gas chromatography with a mass-selective detector allows performing quantitative analysis of xenobiotics in plant extracts within 0.01-1.0 mg/kg. The measurement error does not exceed the maximum level (20%) set by the regulatory requirements for the quality of measurements. The percentage of extraction of xenobiotics ranges from 90% to 108%. Apart from the analytes involved in the approbation quantitative studies, residual amounts of the xenobiotic diethyltoluamide were detected using the screening mode of the gas chromatography mass spectrometer. Qualitative identification of diethyltoluamide on chromatograms obtained by gas mass spectrometry became possible due to the presence of characteristic ions of diethyltoluamide in the NIST 0.5 library.

The proposed sequence of procedures and tests of the hybrid method for the study of xenobiotics in the composition of blueberries is based on standardized methods and validated methods of chromatographic control of residual amounts of xenobiotics. The proposed method is

promising, it requires an expansion of the list of indicators of quantitative analysis, an expansion of approbation studies, it can be used in a comprehensive analysis of the safety indicators of berry products obtained in controlled and uncontrolled ecosystems.

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Гібридна методика вимірювання вмісту ксенобіотиків дикорослих та культивованих ягід чорниці

Анотація. Лабораторний контроль потребує здійснення випробувань продукції ягідництва на вміст залишкових кількостей сумішей різних ксенобіотиків: пестицидів, поліциклічних ароматичних вуглеводнів. У зв'язку з цим дана стаття спрямована на вивчення фізико-хімічних умов екстракційного вилучення ксенобіотиків із дикорослих та культивованих ягід чорниці та подальший хроматографічний аналіз отриманих витяжок. Провідним підходом для розробки гібридної методики лабораторного контролю залишкових кількостей ксенобіотиків у ягодах є побудова теоретичної моделі методики та емпіричні дослідження запропонованих умов методики. Перший етап дослідження – підготовка проби та екстракція ксенобіотиків. Встановлено, що вилучення пестицидів потрібно проводити з гомогенізованого рослинного матеріалу, а для екстракції поліциклічних ароматичних вуглеводнів застосовувати ягоди з неушкодженою шкуринкою. З'ясовано, що 1 % ацетонітрильний розчин мурашиної кислоти є уніфікованим екстрагентом ксенобіотиків. Обґрунтовано необхідність послідовного хроматографічного аналізу отриманих витяжок шляхом застосування вискоелективної рідинної хроматографії з флуоресцентним детектором, методів вискоелективної рідинної хроматографії та газової хроматографії з мас-селективними детекторами. Апробаційні вимірювання показали, що межа кількісного визначення залишкових кількостей пестицидів становить $0,01 \pm 0,001$ мг/кг, а межа кількісного визначення поліциклічних ароматичних вуглеводнів значно нижча та складає $0,5 \pm 0,05$ мкг/кг. Гібридна методика дозволяє протягом 90 хвилин виявити та виміряти вміст ксенобіотиків у витяжках ягід чорниці. Робочі діапазони для контролю вмісту пестицидів становлять від 1,0 мг/кг до 0,01 мг/кг, для поліциклічних ароматичних вуглеводнів – від 20 мкг/кг до 0,5 мкг/кг. Величину похибки кількісного аналізу оцінено за допомогою середнього квадратичного відхилення від середньої (σ , %). Повноту вилучення ксенобіотиків встановлено у відсотках (r , %) за допомогою серії досліджень модельних зразків, штучно збагачених ксенобіотиками. З'ясовано, що похибка вимірювання вмісту ксенобіотиків не перевищує 20 %, величина вилучення аналітів знаходиться в діапазоні 90–108 %, методика є придатною для виконання лабораторного контролю. Розроблений гібридний метод становить практичну цінність для виконання лабораторного контролю небезпечних для здоров'я людини хімічних сполук

Ключові слова: пестициди, поліциклічні ароматичні вуглеводні, екстракція, хроматографія, безпечність продуктів рослинного походження

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The Influence of Biological Preparations on the Microbiological Activity of Sod-Podzolic Soil for the Cultivation of Food Potatoes

Abstract. In the present-day world, the demand for organic products is growing, but there is an insufficient number of studies on the impact of biological preparations on soil fertility parameters. Indicators of microbiological activity are not the same for different soil types, which is why it is necessary to investigate them in detail in specific soil and climatic conditions, especially on zonal soils, which are sod-podzolic soils in Polissia. Research on the impact of various biological preparations on soil fertility indicators for the cultivation of one of the most popular crops in Ukraine – food potatoes – is relevant. The purpose of this study was to establish the effect of treatment of potato tuber seeds with biological preparations and introduction of a biodestructor for better decomposition of siderate on the focus of microbiological processes in the soil. The study was carried out in an experiment at the Volyn State Agricultural Research Station of the Institute of Potato Growing of the National Academy of Agrarian Sciences of Ukraine. The soil of the experimental site was sod-medium-podzolic sandy soil on water-glacial deposits. The stationary experiment included variants with the addition of siderate to the soil without a destructor (Background 1) and with the addition of the Ecostern destructor to the siderate (Background 2). The effectiveness of the following biological preparations was also investigated: Mycohelp, Agat, Regoplant, Fitohelp, Stimpo. Soil analysis for microbiological indicators was carried out in samples taken from the 0-20 cm layer. The focus of microbiological processes in the soil was found by the corresponding coefficients. It was found that the oligotrophicity coefficient was less than one on the variants using the Ecostern biodestructor during the growing season of potatoes, which indicates a good supply of easily digestible organic substances to the soil microbiota, while on the variants without Ecostern during the ripening phase, the oligotrophicity coefficients exceeded one. The coefficient of pedotrophicity changed more during the growing season than according to the variants of the experiment. In the seedling and flowering phase of potatoes, mineralisation-immobilisation coefficients were low in all variants of the experiment, which indicates the predominance of synthesis of organic matter. The materials of this paper are of practical value for farmers and producers of organic crop production in the issue of plant residue management for the use of biodestructors in the technology of growing food potatoes

Keywords: biodestructor, siderate, oligotrophic factor, pedotrophic factor, humification

INTRODUCTION

Ukraine is one of the five countries with the largest planting areas of this crop, but in terms of yield it is considerably inferior to other countries [1]. This is because 90% of the potato harvest comes from private households. The low yield of potatoes is caused by low-quality seed material, its damage by many pathogens [1] and insufficient nutrients [2-4]. O.Sh. Iskakova [3] notes that potato cultivation technologies should ensure both high yield and the achievement of a prominent level of profitability.

That is why it is important to form a favourable culture nutrition regime, as well as protection from stressful phenomena.

In Ukraine, the market of biological preparations is intensively developing. This is due to the growing demand for environmentally friendly products [5].

However, literary sources hold conflicting information regarding the positive or absent effect of biological preparations on indicators of biological activity

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of soils, which is why this issue is relevant and requires detailed investigation.

Research conducted in the Ternopil region proved the feasibility of treating potato tubers with Albit (100 ml per 1 tonne of seeds) followed by spraying twice during the growing season at 50 ml per 1 ha [6]. The yield of potatoes and their need for nutrients were more influenced by weather conditions, variety, and the content of nutrients in the soil [7; 8].

In poor sod-podzolic soils, nitrogen is in the first minimum among nutrients. Nitrogen affects the growth of plants, photosynthesis, metabolism, and as a result, the productivity and indicators of the crop quality [9]. When developing technologies for growing potatoes, it is important to find new alternatives to chemical fertilisers [10].

In Polissia, one of the most critical tasks in the area where sod-podzolic crops are spread is to ensure an increase in the content of organic matter in the soil, including humus. In the conditions of a shortage of conventional fertilisers, an effective alternative to humus is the use of crop residues [11].

Biologisation, the use of biological preparations, is a new trend in modern agar production [12]. According to studies [13], the effectiveness of biostimulants was manifested in the improvement of soil properties: the structural composition of the soil, the nutrient regime, the increase in biological activity in the deeper layers of the soil, the better development of the rhizosphere and the yield of winter wheat compared to the control.

According to researchers, biostimulants can be used as bioactive products in agriculture to increase plant productivity and resistance to stressful conditions [14].

The special role of microorganisms in the functioning of soils as elements of ecobiosystems: they take part in the small biological cycle of substances and energy in the soil, as well as in their influence on soil fertility indicators, namely on the transformation of nutrients in the soil, primarily nitrogen and carbon [15-17].

In the studies of R.A. Vozhehova *et al.* [18], the yield of summer-planted potatoes did not depend on the variants for treatment of tubers with chemical and biological preparations, and the average yield for three years was 20.86 t/ha, excluding the variant with additional treatment of plants with a biological preparation of fungicidal action Haupsin, where the yield increase was 0.33 t/ha.

The purpose of this study was to investigate the focus of microbiological processes in sod-medium-podzolic soil using a biodestructor for better decomposition of siderate and treatment of potato tuber seeds with biological preparations. The task of this study was to prove changes in the coefficients of oligotrophy, pedotrophy and mineralisation-immobilisation with the use of various biological preparations.

MATERIALS AND METHODS

The research was conducted under experimental conditions based on the Volyn State Agricultural Research

Station of the Institute of Potato Breeding of the National Academy of Sciences of Ukraine in 2019-2021 on sod-medium-podzolic sandy soil typical for the Polissia zone of Ukraine.

Potatoes of the Partner variety were grown in the experiment: a medium-early Ukrainian food potato variety. The vegetation period was approximately 107 days.

A two-factor stationary experiment included the following variants:

Factor A.

1. Control (without siderate biodestructor).
2. Biodestructor Ecostern in a dose of 1.2 l/ha.

The siderate was white mustard (200 c/ha), which was introduced into the soil.

Factor B. Treatment of potato tubers and 3-time spraying during the growing season of potato plants with biological preparations.

1. Control (without biological preparation).
2. Background – (introduction of Mycohelp 2.0 l/ha into the soil) + triple treatment during the growing season, Agat 25 – 100 ml/ha
3. Background + triple treatment during the growing season, Regoplant – 50 ml/ha.
4. Background + triple treatment during the growing season, Phytohelp – 1.0 l/ha.
5. Background + triple treatment during the growing season, Stimpo – 15 ml/ha.
6. Treatment of tubers with Mycohelp – 2.0 l/t + triple treatment with Mycohelp – 1.0 l/ha during the growing season.
7. Treatment of tubers with Regoplant 50 ml/t + triple treatment with Regoplant 50 ml/ha during the growing season.
8. Treatment of tubers with Fitohelp - 1.0 l/t + triple treatment with Fitohelp – 1.0 l/ha during the growing season.
9. Treatment of Stimpo tubers – 15 ml/t + triple treatment of Stimpo 15 ml/ha during the growing season.

Soil analysis for microbiological indicators was performed in samples taken from the 0-20 cm layer. In the studies, the number of microorganisms of the main ecological-trophic and taxonomic groups was found according to the method of microbiological sowing of soil suspension on different solid agarised nutrient media. Organotrophic bacteria were determined by sowing on meat-peptone agar, the number of microorganisms that assimilate nitrogen from mineral compounds and actinomycetes – on starch-ammonium agar, oligotrophic microorganisms – were grown on starvation agar, fungi – on Richter's nutrient medium. The number of microorganisms is given in colony-forming units (CFU) per one gram of dry soil, having previously determined the relative humidity of the soil sample using the thermostat-weight method. Subsequently, the obtained number of colonies was counted, considering the humidity coefficient, as well as the dilution of the soil suspension. Experimental studies were carried out in triplicate.

Microbiological soil monitoring was evaluated according to the methodical recommendations of V.M. Patyka and O.V. Sherstoboeva [19]. The focus of microbiological processes in the soil was determined according to the following coefficients.

The coefficient of oligotrophy (C_{ol}) was calculated as the ratio of the number of microorganisms capable of assimilating nutrients from very dilute solutions to the total number of eutrophic microorganisms according to the formula (1):

$$C_{ol} = N_{StA} / (N_{SAA} + N_{MPA}) \quad (1)$$

Next, the coefficient of pedotrophicity (C_{ped}) was determined according to the ratio of the number of microorganisms on soil agar (N_{SA}) to the number of those microorganisms that grew on meat-peptone agar (N_{MPA}) (2):

$$C_{ped} = N_{SA} / N_{MPA} \quad (2)$$

The coefficient of mineralisation-immobilisation (C_{m-i}) was determined as the ratio of the number of microorganisms that immobilise mineral forms of nitrogen to the number of organotrophs according to the formula (3):

$$C_{m-i} = N_{SAA} / N_{MPA} \quad (3)$$

where N_{SAA} , N_{MPA} is the number of microorganisms grown on starch-ammonium and meat peptone agar. Where N_{SA} is the number of microorganisms grown on starvation agar.

RESULTS AND DISCUSSION

Based on the ratio of ecological and trophic groups of microorganisms, namely nitrogen-fixing, oligotrophic, and microorganisms involved in the mineralisation of humic substances, it is possible to judge on the focus of the mobilisation processes in the soil. In this regard, the coefficients of oligotrophicity (StA/Zv), mineralisation (SAA/Zv) and pedotrophicity (SA/Zv) were calculated. These microbiological coefficients allow generalising the processes occurring in the soil, as well as to establish their focus. At elevated values of nitrogen mineralisation-immobilisation coefficients, changes in transformation of organic compounds occur, namely the dominance of organic matter decomposition over synthesis. On the other hand, a decrease in the pedotrophic factor can be interpreted as a decrease in the intensity of decomposition of soil organic matter. The results of the study of changes in the coefficients of the intensity of soil-biological processes in agrophytogeocenoses of potatoes under different systems of biological preparations are presented below.

The coefficient of oligotrophy (C_{ol}) for its growth indicates the spread of microorganisms in the soil that can exist in the absence or low concentrations of nutrients compared to other eutrophs. C_{ol} in the seedling and flowering phase of potatoes in all soil samples under study was low (≤ 1) (Fig. 1), which indicates a good supply of soil microorganisms with labile organic substances, as well as the presence of sufficient sources of carbon and nitrogen for the functioning of the soil microbial complex.

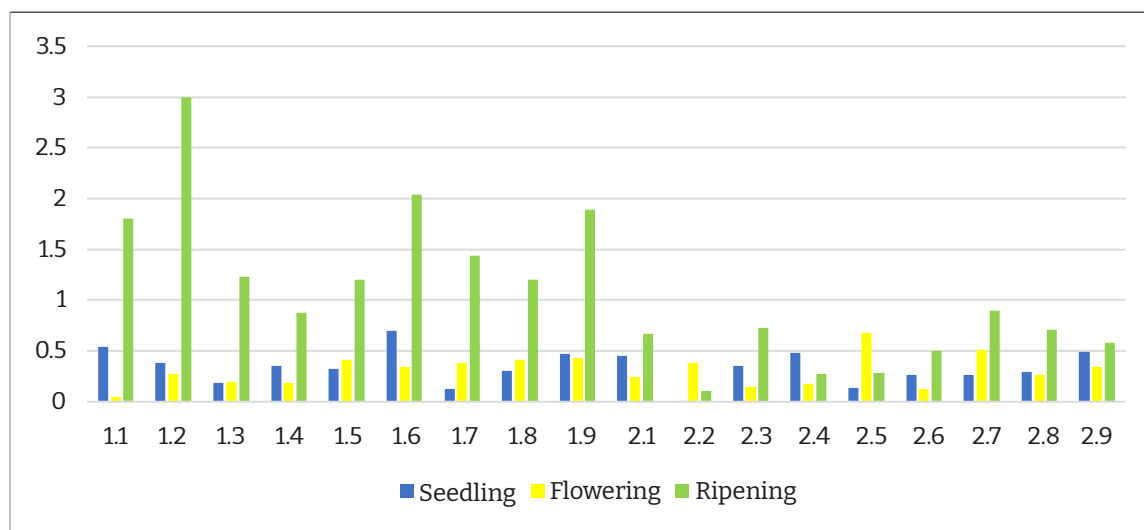


Figure 1. Microbiological coefficient of oligotrophy in sod-podzolic soil for growing potatoes

In the potato ripening phase, the situation changed slightly, C_{ol} increased (≥ 1) on background 1, where Ecoster biodestructor was not applied, which indicates a 10% decrease in the content of nutrients in the soil. The highest values of this indicator ranged from 1.2 on variants 3 and 8 (Treatment of tubers with Fitohelp – 1.0 l/t + triple treatment with Fitohelp – 1.0 l/ha during the growing season and Treatment of tubers with Fitohelp – 1.0 l/t + triple

treatment with Fitohelp – 1.0 l/ha during the growing season) to 2.9 in variant 2 (introduction of Mycohelp 2.0 l/ha to the soil + triple treatment during the growing season with Agat 25 – 100 ml/ha). On the variants of the background using Ecoster biodestructor at 1.2 l/ha, the C_{ol} on all variants of feeding potato plants was lower than 1 (C_{ol} ranged from 0.11 to 0.895), which indicates an elevated supply of soil nutrients, especially nitrogen.

The positive effect of biological preparations on improving the nutrient status of the soil, which is evidenced by the higher C_{oi} values compared to the control: on background 1 without the introduction of a biodestructor in the seedling phase, this indicator was 0.545, and in the phase of maturation of potato tubers – 1.8. Comparing different biopreparations in terms of their influence on the C_{oi} , it was established that the best feeding conditions for food potatoes were with Regoplant preparation in variant 7 ($C_{oi}=0.13$), slightly higher values were established in variant 3 with the introduction of Mycoplant and treatment with Regoplant during vegetation ($C_{oi}=0.185$). On variants 4, 5, and 8, the C_{oi} values in the seedling phase did not substantially differ from each other.

On the background 2 with the application of Ecosterne, the best conditions of the nutritional regime were on the variant 2 with the application of Mycohelp and the treatment with Agat vegetation, where the C_{oi} values were the lowest – 0.01. The highest values were set on variant 9 using Stimpo: on the background without the destructor, they were 0.43-1.89, and with the destructor – 0.345-0.58. The opposite dynamics were noted by O.Yu.Kolodiazhnyi and M.V. Patyka [20]: during the growing season of winter wheat, the coefficient of oligotrophy on the research variants decreased (0.03-0.67).

The intensity of decomposition of soil organic matter can be judged according to the coefficient of pedotrophicity. Table 1 shows changes in this coefficient due to the use of biological preparations on food potatoes.

Table 1. Microbiological coefficient of pedotrophicity (C_{ped}) in sod-podzolic soil during the main phases of potato plant development

Factor A	Factor B	Development phase		
		Seedling	Flowering	Ripening
Background 1. Without biodestructor	1. Control (without biological preparation)	1.795	0.055	0.305
	2. Application of Mycohelp 2.0 l/ha + triple treatment with Agat 25 – 100 ml/ha	0.835	0.035	0.12
	3. Application of Mycohelp 2.0 l/ha + triple treatment with Regoplant – 50 ml/ha	1.09	0.03	0.36
	4. Application of Mycohelp 2.0 l/ha + triple treatment with Fitohelp – 1.0 l/ha	0.695	0.3	0.36
	5. Application of Mycohelp 2.0 l/ha + triple treatment with Stimpo – 15 ml/ha	1.14	0.005	0.365
	6. Treatment of tubers with Mycohelp – 2.0 l/t + triple treatment with Mycohelp – 1.0 l/ha during the growing season	2.17	0.415	0.465
	7. Treatment of tubers with Regoplant – 50 ml/t + triple treatment with Regoplant – 50 ml/ha during the growing season	0.595	0.005	0.41
	8. Treatment of tubers with Fitohelp – 1.0 l/t + triple treatment with Fitohelp – 1.0 l/ha during the growing season	1.155	0.395	0.425
	9. Treatment of tubers with Stimpo – 15 ml/t + triple treatment with Stimpo – 15 ml/ha during the growing season	1.665	0.18	0.73
Background 2. Ecosterne 1.2 l/ha	1. Control (without biological preparation)	1.72	0.42	0.155
	2. Application of Mycohelp 2.0 l/ha (background) + triple treatment with Agat 25 – 100 ml/ha	0.355	0.095	0.025
	3. Application of Mycohelp 2.0 l/ha + triple treatment with Regoplant – 50 ml/ha	0.91	0.525	0.15
	4. Application of Mycohelp 2.0 l/ha + triple treatment with Fitohelp – 1.0 l/ha	1.63	0.405	0.065
Background 2. Ecosterne 1.2 l/ha	5. Application of Mycohelp 2.0 l/ha + triple treatment with Stimpo – 15 ml/ha	0.95	0.935	0.125
	6. Treatment of tubers with Mycohelp – 2.0 l/t + triple treatment with Mycohelp – 1.0 l/ha during the growing season	1.34	0.29	0.25
	7. Treatment of tubers with Regoplant – 50 ml/t + triple treatment with Regoplant – 50 ml/ha during the growing season	1.34	0.35	0.35
	8. Treatment of tubers with Fitohelp – 1.0 l/t + triple treatment with Fitohelp – 1.0 l/ha during the growing season	3.45	0.21	0.195
	9. Treatment of tubers with Stimpo – 15 ml/t + triple treatment with Stimpo – 15 ml/ha during the growing season	1.18	0.07	0.4

High values of C_{ped} (≥ 1) in the seedling phase of potatoes on background 1 without a biodestructor were observed on variants 1, 3, 5, 6, 8, 9, which indicates an increase in the intensity of decomposition of soil organic matter on these variants.

C_{ped} indicators proved the active decomposition by microorganisms of the remains of sideral culture. This indicator depended more on the period of soil sampling than on variants with biological preparations. In the seedling phase, variants 2, 4, 7 without a destructor were characterised by low C_{ped} values – 0.835; 0.695; and 0.595, respectively, which indicates a decrease in the amount of organic matter in the soil. In the phase of flowering and ripening of potatoes, C_{ped} decreased in all variants of the experiment and was within 0.03-0.75, which indicates a decrease in the amount of organic matter (residues of siderate) in the soil.

On background 2, with Ecoster 1.2 l/ha in the seedling phase of the culture, an elevated intensity of organic matter decomposition was observed in variants 1, 4, 6, 7, 8, 9. In variants 2, 3, 5, the intensity of organic matter

decomposition was insubstantial. During the flowering and maturing phase of potatoes, the C_{ped} did not exceed 1, which indicates insufficient supply of “fresh” organic matter to the soil and low intensity of its decomposition. The lowest C_{ped} values were found on variant 9 with the use of Stimpo – 0.07 and on variant 2 with Mycohelp and Agat treatment – 0.095. On variants 8, 6, 7, 4 and 1, the C_{ped} value was 0.21; 0.29; 0.35; 0.405; and 0.42, respectively. Upon applying Mycohelp to the soil and Stimpo vegetation treatment, the value was 0.935.

The C_{ped} value was also influenced by various agrotechnical measures. The studies of O.B. Panchenko, I.D. Prymak and I.A. Panchenko [21] established the influence of different tillage systems and found the lowest C_{ped} values for tillage, and the highest values for ploughing with different depths. The C_{m-i} shows the predominance of the decomposition processes of organic substances over their synthesis. During the seedling phase of potatoes, the C_{m-i} were low in all variants of the experiment, which indicates the predominance of organic matter synthesis processes (Fig. 2).

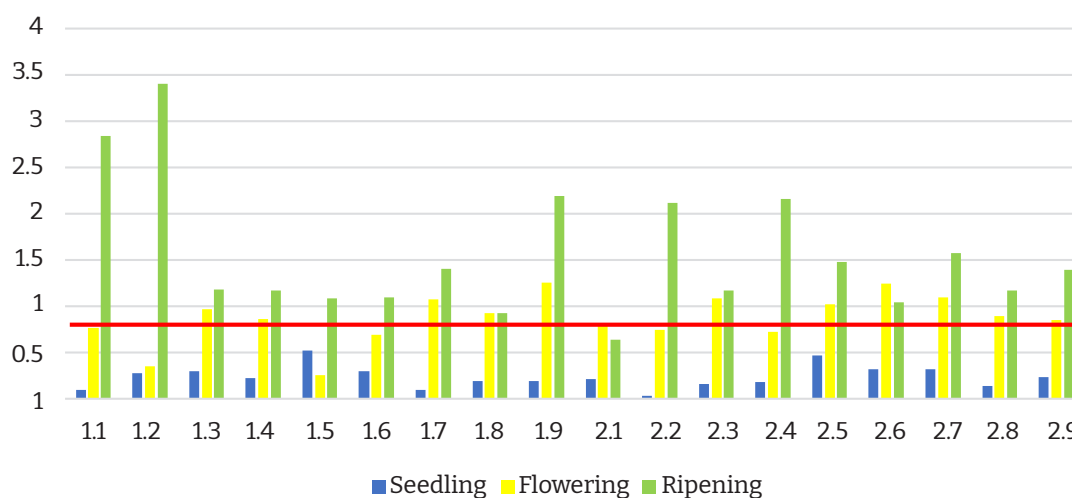


Figure 2. Microbiological coefficient of mineralisation-immobilisation (C_{m-i}) of nitrogen in sod-podzolic soil for growing food potatoes

A similar trend was observed in the flowering phase. The C_{m-i} coefficients were within 0.25-1.25 on background 1 without a destructor, and the highest values (1.25) were found on the Stimpo tuber treatment and vegetation treatment variants, and the smallest (0.25), which ensure flow in the soil of synthesis of organic matter, – on the variants with Mycohelp applied to the soil and vegetation treatment with Stimpo, as well as on the variant with the Mycohelp applied to the soil and vegetation treatment with the Agat PE preparation. In the seedling phase, the application of Ecoster stubble biodestructor did not cause any substantial differences in the values of this coefficient: C_{m-i} was 0.1-0.515 on background 1 without biodestructor, 0.025-0.205 on background 2 with biodestructor. Ecoster showed a more pronounced effect in the flowering phase, where the values were 0.25-1.25 and 0.715-1.24, respectively, on background 1 and 2. Thus, the

positive effect of the biodestructor on decomposition of white mustard and the supply of potato plants with nutrients during the flowering period of potatoes was established, which is also confirmed by the C_{ol} values.

During the ripening phase of potatoes, microbiological processes shifted even more towards the mineralisation of organic matter in all variants of the experiment. The C_{m-i} in the flowering and ripening phase approached the optimal indicators ($C_{m-i} = \text{approx. } 1.0$) when using Ecoster biodestructor 1.2 l/ha for all biological preparations, which indicated a balanced carbon-nitrogen cycle. On background 1 without a biodestructor, C_{m-i} was the highest on the variant with Mycohelp and Agat treatment (3.4), as well as on the variant with Stimpo (1.25); on background 2 – on variant 4 with Mycohelp + Phytohelp (2.16) and Mycohelp + Agat (2.12).

S.O. Hudz [22] established that the lowest C_{m-i}

values were for the biological fertilisation system. He also found an increase in C_{ped} values under the industrial fertilisation system, which indicates an increase in the intensity of decomposition of soil organic matter. As for C_{oi} , the optimal values were for ecological and biological fertilisation systems – 0.69-0.96 and 0.81-0.93, respectively.

CONCLUSIONS

1. The use of Ecosterne biodestructor at 1.2 l/ha on white mustard as a siderate on sod-medium-podzolic soil contributed to increasing the availability of nutrients during the growing season of food potatoes, as evidenced by the C_{oi} values of less than 1 (0.01-0.895). On the background without a stubble destructor, the lowest C_{oi} values were established when using Mycohelp and Regoplant biological preparations, and the highest values were set with the variant with Stimplo.

2. The C_{ped} depended more on the period of soil sampling than on variants with biological preparations and was 0.355-1.795 in the seedling phase, 0.005-0.935 in the flowering period, and 0.12-0.465 in the ripening period.

3. According to the C_{m-i} , the advantage was proved in the variant with Mycohelp soil treatment 2.0 l/ha + triple vegetation treatment with Agat 25 – 100 ml/ha. The positive effect of the biodestructor on the decomposition of organic matter of siderate was found. During the ripening period, the C_{m-i} in the flowering and ripening phase approached the optimal values (C_{m-i} = approx. 1.0) when using Ecosterne biodestructor 1.2 l/ha.

Studies on the impact of the destructor and biological preparations on improving the humus condition of sod-podzolic soil are promising.

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Вплив біопрепаратів на мікробіологічну активність дерново-підзолистого ґрунту за вирощування картоплі столової

Анотація. В умовах сьогодення у світі зростає попит на органічну продукцію, проте недостатня кількість досліджень із вивчення впливу біопрепаратів на параметри родючості ґрунтів. Показники мікробіологічної активності неоднакові для різних ґрунтових відмін, саме тому необхідно детально вивчати їх в конкретних ґрунтово-кліматичних умовах, особливо на зональних ґрунтах, якими в Поліссі є дерново-підзолисті. Актуальними є дослідження щодо впливу різних біопрепаратів на показники родючості ґрунту за вирощування однієї із найбільш популярних в Україні культур – картоплі столової. Мета роботи – встановити вплив обробки насіння бульб картоплі біопрепаратами та внесення біодеструктора для кращого розкладу сидерату на спрямованість мікробіологічних процесів у ґрунті. Дослідження проводили у досліді на Волинській державній сільськогосподарській дослідній станції Інституту картоплярства НААН України. Ґрунт дослідної ділянки – дерново-середньопідзолистий супіщаний ґрунт на водно-льодовикових відкладах. Стаціонарний дослід включав варіанти із заробкою у ґрунт сидерату без деструктора (Фон 1) та із внесенням десруктора Екостерн на сидерат (Фон 2). Також вивчали ефективність біопрепаратів: Мікохелп, Агат, Регоплант, Фітохелп, Стимпо. Аналіз ґрунту на мікробіологічні показники проводили у зразках, які відібрані з шару 0-20 см. Спрямованість мікробіологічних процесів у ґрунті визначали за відповідними коефіцієнтами. Встановлено, що на варіантах із застосуванням біодеструктора Екостерн протягом вегетаційного періоду картоплі коефіцієнт оліготрофності складав менше одиниці, що свідчить про добру забезпеченість ґрунтової мікробіоти легкозасвоюваними органічними речовинами, тоді як на варіантах без застосування Екостерна у фазу досягання коефіцієнти оліготрофності перевищували одиницю. Коефіцієнт педотрофності більше змінювався протягом вегетаційного періоду, ніж за варіантами досліду. У фази сходів і цвітіння картоплі коефіцієнти мінералізації-імобілізації були низькими на всіх варіантах досліду, що свідчить про переважання процесів синтезу органічної речовини. Матеріали статті становлять практичну цінність для фермерів та виробників органічної продукції рослинництва у питанні управління рослинними рештками за застосування біодеструктурів у технології вирощування картоплі столової

Ключові слова: біодеструктор, сидерат, коефіцієнт оліготрофності, коефіцієнт педотрофності, гуміфікація

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Efficiency of Use of Macronutrients by Spring Rapeseed Plants

Abstract. The relevance of this study is determined by the need to optimize the production process of agrocenoses of spring rapeseed and establish the regularities of the dynamics of macroelements in the soil under the influence of different rates of mineral fertilisers. In this regard, the purpose of this paper was to cover the regularities of changes in the content of macroelements in typical low-humus chernozems at different stages of spring rapeseed culture development in the conditions of the Right Bank Forest Steppe of Ukraine. Field, laboratory, analytical, and statistical research methods were used in the study. It was established that the highest content of macroelements in the soil at all levels of mineral fertiliser is in the phase of leaf rosette formation by spring rapeseed plants (BBCH 15-18). As plants grow through the phases, it decreases and reaches minimum values in the ripening phase (BBCH 85-88). The highest content of nitrogen mineral compounds was noted in BBCH 15-18: 52.0-60.2 mg/kg of soil depending on the fertiliser variant, the content in the variant without fertiliser application – 49.8 mg/kg of soil. It was found that the maximum content of mobile phosphorus compounds in the soil was recorded in the first half of the growing season. It was found that the content of mobile potassium in the soil is dictated by the amount of applied mineral fertilisers. The highest content was noted at the beginning of the vegetation of spring rapeseed, with a gradual decrease until the seed ripening phase (BBCH 85-88). A close correlation was established between the accumulation of dry matter in the BBCH 55-58 period and the yield level ($r=0.91$). The materials of this paper are of practical value for the improvement of processes related to the development of the productivity of spring rapeseed crops in the conditions of the Forest Steppe of Ukraine, as well as the rational use of mineral fertilisers on typical low-humus chernozems

Keywords: *Brassica napus* L., mineral nutrition, application rates of mineral fertilisers, dry matter, productivity

INTRODUCTION

The high competitiveness of spring rapeseed in the international market makes this crop promising due to the growing demand for oils characterised by a high content of oleic acid. Thanks to this argument, agrarians will have the opportunity to receive added profits, as well as for the cultivation of high-oleic sunflower, provided that certain technological elements of crop cultivation are optimised to use the soil and climatic conditions of Ukraine more effectively [1].

In the coming years, the promising high-oleic segment will grow in Ukraine according to world trends and ensuring demand in Europe [2]. In France, the area under winter rapeseed makes up over 50% of the area under winter rapeseed, while in Ukraine, this indicator is at 10-15%. The increase in the area sown under rapeseed allows stabilising the energy independence of Ukraine due to the increase in the gross collection of seeds for processing into biodiesel [3; 4]. Furthermore, the situation on the

world oil market can substantially affect the economic attractiveness of spring rapeseed cultivation [5].

Regulation of crop productivity formation processes encompasses the influence of many factors, both regulated and unregulated. Particular attention is paid to certain abiotic factors and certain elements of cultivation technology. Among abiotic factors, the main attention is paid to water and temperature regimes. Of the technological processes, the selection of the variety (hybrid), plant density, and feeding conditions are of immense importance [6; 7]. The emergence of many promising hybrids with high-yield potential on the market causes a problem related to their adaptation to growing conditions. Just as the rapid growth of the genetic potential of modern varieties and hybrids cannot be ensured at the proper level by abiotic factors [8]. A wide range of changes in biotic and abiotic environmental factors and the ability to adapt to them determines the degree of adaptability of modern hybrids [9].

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Rapeseed belongs to crops that react extremely sensitively to changes in mineral nutrition conditions. That is why due attention is paid to the regulation of the mineral nutrition regime for the cultivation of rapeseed [10].

Rapeseed cultivation technology, despite its prospects and wide distribution, still raises many questions aimed at individual technological processes, namely mineral nutrition intended to increase productivity and identify its impact on oil quality indicators.

Over the past decades, the issue of reproduction of fertility and preservation of soil cover has become relevant. One of the key factors in the fertility of soils is their provision of mobile forms of the main nutrients, which is carried out through the introduction of organic and mineral forms of fertilisers [11].

The increase in rapeseed yield, depending on the dose of mineral fertilisers, is 46-60% [12]. On chernozem soils of Ukraine, the greatest yield increase is obtained from the application of nitrogen fertilisers (about 30-40%) [1]. Phosphorous and potash fertilisers are less effective. Thus, in the conditions of the north-eastern part of Germany, full potassium nutrition increases the yield of rapeseed by 0.2-0.3 t/ha [13]. Moreover, the effectiveness of nitrogen fertilisers increases after stubble precursors, especially in wet years. Retail application of nitrogen fertilisers in conditions of insufficient soil moisture is ineffective, especially on soils with a heavy granulometric composition [1].

The analysis of literary sources indicates an increase in the yield of rapeseed under increased rates of nitrogen fertilisation, with possible lodging of plants [11; 13]. The use of nitrogen in the amount of 120-180 kg/ha causes a decrease in added production per 1 kg of nitrogen. Therewith, an increased accumulation of nitrates is noted in the obtained crop.

On chernozem soils, with insufficient application of nitrogen forms of fertilisers, the deficiency of the element is partially eliminated due to the natural reserves of the element in the soil. The analysis of earlier studies shows a direct dependence between the content of available forms of nitrogen in the soil and the amount of fertilisers applied [14].

Nitrogen, unlike phosphorus and potassium, is the most mobile, and therefore it is vital to monitor the nitrogen nutrition of crops, adjust the nitrogen dose in a timely manner and give plants the opportunity to form the highest and full-quality crops [15].

Based on this, it is of great interest to investigate the content and dynamics of typical ammonium and nitrate nitrogen in chernozems, i.e., mobile forms of nitrogen that are most available to plants.

Phosphorus is one of the main elements of plant nutrition. To obtain high and stable crops of superior quality, balanced mineral nutrition of spring rapeseed plants is necessary, where nitrogen and phosphorus play a significant role [1]. The supply and use of nitrogen by plants is related to the level of their phosphorus supply since

phosphorus metabolism in plants is closely related to carbohydrate-protein and energy metabolism [13].

An essential indicator of soil fertility is its availability of phosphorus, which is an organic and mineral soil compound. In plant nutrition, the main role belongs to mineral compounds of phosphorus, which are represented by the remains of apatite, phosphorite, salts of phosphoric acids. The content of phosphorus available to plants depends on the characteristics of the soil, the methods, and terms of applying fertilisers and their forms [16].

The assimilation of phosphorus compounds by the plant is characterised by two periods. The first period is the time of seed germination. There is a close connection between the growth processes of plants and the presence of phosphorus compounds in the soil. This is showed by the high concentration of the element in tissues where growth occurs. The second period corresponds to the seed ripening phase. In the seeds of plants, phosphorus compounds are in a storage form, which is phytin, the content of which can be 1-3%. In the vegetative organs of plants, phytin is absent or present in insignificant amounts [11].

Potassium reserves in the soil are a determining factor in the development of crop productivity under intensive cultivation technologies. Together with phosphorus, it helps reduce the adverse effect of excessive consumption of nitrogen by plants. An insufficient amount of potassium causes a decrease in photosynthetic activity, exposure to harmful organisms and disruption of the main processes of the plant organism [1].

Potassium fertilisers, upon interacting with the soil, are subjected to various transformations, which cause the formation of compounds of different mobility and availability for the plant organism. Potassium is one of the indispensable elements of the plant organism, which can enhance growth processes, respiration, and nitrogen exchange. Its role in the water regime of plant tissues is also important. Potassium deficiency manifests itself as early as at the initial stages of plant development, restraining their growth [13].

The need of spring rapeseed plants for potassium is observed most in the initial periods of its vegetation, contributing to the growth of plant resistance to adverse environmental factors and harmful organisms. With the optimal supply of plants with all nutrients, excluding potassium, the synthesis, and dissimilation of carbohydrates are inhibited, proteins are consumed for respiration, and the activity of enzymes ceases [11]. The supply of spring rapeseed plants with potassium at the proper level helps increase nectar production and improve seed quality indicators [17]. Under the conditions of optimal provision of the element during the growing season, friendly flowering of the crop, increased resistance to lodging, improvement of water exchange processes and, as a final result, increased productivity are observed.

Mobile potassium is the most available form for plants, which ensures the processes of their nutrition.

Potassium, in other forms, can be used under the condition of a number of chemical and physical transformations in the soil, such as mineralisation, destruction of minerals and microorganisms, which lead to the transition of insoluble compounds in the solution [16].

The purpose of this study was to identify the influence of different nutritional conditions created by fertiliser options on the development of the productivity of spring rapeseed agrocenoses and the dynamics of the main elements of mineral nutrition in the soil and plants in the conditions of the Right Bank Forest Steppe of Ukraine.

MATERIALS AND METHODS

Field experiments were carried out in the production unit of the NULES of Ukraine "Agronomic Research Station" in the stationary crop rotation of the Department of Plant Breeding during 2010-2013 and 2015-2017 on typical low-humus chernozems (humus content – 4.38-4.53%, $\text{pH}_{\text{saline}}$ – 6.9-7.3). Laboratory analyses were carried out in the laboratory of analytical research of the crop production department.

The territory of the experimental station belongs to the moderately warm, moderately humid agro-climatic sub-district of the Kyiv Oblast. The total amount of precipitation during the calendar year corresponds to an average of 543 mm, while for the period with temperature indicators above $+5^{\circ}\text{C}$ – 372 mm. Precipitation is unevenly distributed during the growing season [18].

The conditions of 2010, 2011 and 2016 can be characterised as conditions close to the average long-term indicators and favourable for the development of spring rapeseed.

The calculation of the coefficients of the substantiality of deviations (Cs) of the amount of precipitation and average temperatures indicates that the vast majority of months of crop vegetation belong to years with conditions close to normal (the first group of gradation).

The vegetation period of 2016 was characterised by the sum of effective temperatures exceeding the long-term indicators. The sum of the effective temperatures in April corresponded to 275°C , exceeding the long-term indicators by 115.4°C . This was reflected in the formation of shoots and the development of culture in the initial stages, speeding up their progress. May indicators were typical for this region. The summer months were characterised by exceeding the long-term indicators by $43-62.8^{\circ}\text{C}$.

The weather conditions in 2012 were close to the average long-term indicators. However, their distribution by months and decades at the initial stages of crop development did not meet the requirements of oil crops of the Brassicaceae family of the spring type of development, which was reflected in the formed productivity of the crop. The year 2012 was characterised by a lack of moisture during May and elevated temperature indicators in April-June. The weather conditions of 2013 were unfavourable for the development of rapeseed, which caused the lowest crop yield in the experiment.

The indicators of the sum of effective temperatures in 2015 exceeded the long-term average by 429.7°C and amounted to $3,302.7^{\circ}\text{C}$. In April and May, the sum of effective temperatures exceeded 37.9°C and 40.5°C , respectively. During the summer months, the sum of effective temperatures was within $633-685.1^{\circ}\text{C}$, with less precipitation compared to multiyear values. At the initial stages of rapeseed development, its soil reserves compensated the moisture deficit. June was characterised by drought and elevated temperatures. Weather conditions in 2017 were even more difficult and were characterised by a moisture deficit during the rapeseed growing season and stressful conditions caused by frost in the third decade of April. April's total effective temperatures were 49.9°C higher than the annual average. May turned out to be typical for this region, which ensured the early onset of the growing season. Excess of temperature indicators in June and July corresponded to 58°C and 28°C , respectively.

The subject under study was the Yura spring rapeseed hybrid. The technology used for the research was generally accepted for the Forest Steppe zone. Individual technological processes differed according to the scheme of the experiment. The predecessor is spring barley.

Scheme of the experiment – fertilisation options:

1. Without fertilisers (control).

2. $\text{N}_{30}\text{P}_{20}\text{K}_{35}$

3. $\text{N}_{60}\text{P}_{40}\text{K}_{70}$

4. $\text{N}_{90}\text{P}_{60}\text{K}_{105}$

5. $\text{N}_{120}\text{P}_{80}\text{K}_{140}$

Fertiliser rates were determined according to the balance method for the planned yield, considering the content of the main nutrients in the soil. The area of the accounting plot was 25 m^2 . Repetition – four times. Placement of variants was sequential. Field, laboratory, analytical, and statistical methods of scientific research were used for this study [19-21].

The content of easily hydrolysed nitrogen compounds was found according to the Tyurin-Kononova method, ammonium nitrogen – colorimetrically, using the Nessler reagent; mobile phosphorus and potassium – according to the Chirikov method [22]. The content of the main nutrients in dry plant samples was determined by first ashing them according to the method of K. Ginzburg *et al.* [22]: nitrogen – according to the photocolometric method using Nessler's reagent; phosphorus – photocolometrically according to the Denizhe method in the A. Levytskyi's modification [22]; potassium – using a flame photometer [19; 22].

RESULTS AND DISCUSSION

The obtained results indicate that the application of mineral fertilisers increases the content of nitrogen, phosphorus, and potassium in the soil: their content increases with an increase in the rates of fertilisation. The conducted studies established that the increase in nitrogen fertilisation rates leads to an increase in the concentration of mineral compounds in the soil.

The highest content of nitrogen compounds in the soil is noted in the leaf rosette phase of spring rapeseed (BBCH 15-18) and, depending on the fertiliser option, it varies from 52.0 to 60.2 mg/kg of soil, while the content in the control option without fertilisation is 49.8 mg/kg of soil (Table 1). As the spring rapeseed plants grow and develop, the nitrogen content in the soil decreases: during the ripening period of the seeds (BBCH 85-88), it changes from 26.0 to 30.1 mg/kg of soil, compared to 20.1 mg/kg in the control version.

Analysis of the data in Table 1 shows that the highest content of mobile phosphorus compounds was noted in the first half of the growing season of the crop, which is associated with insignificant assimilation by plants in this period. During the period of formation of the leaf rosette (BBCH 15-18), the highest content of mobile phosphorus compounds in the arable layer of the soil was noted for $N_{120}P_{80}K_{140}$ and was 92.9 mg/kg of soil against 61.1 mg/kg of soil in the control variant without fertiliser.

Table 1. Dynamics of the macronutrient content in the soil during the cultivation of spring rapeseed, mg/kg of soil (average for 2010-2013)

Stages of growth and development of spring rapeseed plants	Nutrition element	Unit of measurement	Fertiliser options				
			Without fertiliser (control)	$N_{30}P_{20}K_{35}$	$N_{60}P_{40}K_{70}$	$N_{90}P_{60}K_{105}$	$N_{120}P_{80}K_{140}$
BBCH 15-18	N	Content, mg/kg of soil	49.8	52.0	53.7	56.4	60.2
	P_2O_5		61.1	62.9	76	84.7	92.9
	K_2O		94.5	107.5	108.5	119.5	137.5
BBCH 55-58	N		38.2	41.9	43.8	45.3	48.5
	P_2O_5		49.9	52.1	68.2	72.1	82.6
	K_2O		82.4	95.4	98.4	102.4	113.4
BBCH 65-68	N		26.6	32.1	35.2	38.1	40.7
	P_2O_5		29.1	48.2	51.9	56.4	60.0
	K_2O		58.8	67.1	76.3	79.6	88.7
BBCH 85-88	N		20.1	26.0	27.2	28.8	30.0
	P_2O_5		21.1	36.0	42.4	51.1	54.1
	K_2O		45.5	49.6	56.5	57.5	59.7
LSD ₀₅	N		0.35				
	P_2O_5		0.63				
	K_2O		0.96				

The rate of application of mineral fertilisers directly affects the content of mobile potassium in the soil. Its highest content was noted at the beginning of the vegetation of spring rapeseed. Until the phase of full maturity (BBCH 85-88), it gradually decreased due to intensive use by plants. The maximum content of mobile potassium was noted in the rosette phase (BBCH 15-18) with the application of $N_{120}P_{80}K_{140}$ and was 137.5 mg/kg of soil, and in microstages BBCH 85-88, its content was 59.7 mg/kg of soil in this variant.

Thus, the application of mineral fertilisers positively affects the content of macronutrients in the soil. It was established that their number depends on both the application rate and the stage of development of spring rapeseed. In all variants of the experiment, the

highest content was noted in microstage BBCH 15-18 (phase of leaf rosette formation). During plant growth and development, the content decreases and the minimum value is observed in BBCH 85-88 (ripening phase of spring rapeseed).

The assimilation of mineral nutrients is closely related to the accumulation of dry matter. Deficiency in nutrition leads to a decrease in yield, including due to a decrease in stress resistance of the culture, which is also confirmed by other researchers [23]. Under conditions of intensive farming, the increase in the yield of agricultural crops is accompanied by an increase in the removal of mineral nutrients from the soil [24; 25].

It is known that 13 nutrients, primarily macronutrients, play a vital role in optimising plant nutrition [26].

Research results indicate that with an increase in the amount of applied mineral fertilisers, the content of nutrients in plants increases at all stages of plant growth and development. This trend can be observed in all variants of fertiliser application. In the stemming phase (BBCH 35-38), the highest content of potassium was found in the plant material, the content of which varied depending

on the rates of fertiliser application from 5.24% to 5.99%, while the content in the variant without fertiliser application was 4.93% (Table 2). Notably, as the phases of growth and development of rapeseed plants changed and, accordingly, the growth of vegetative mass and dry matter increased, the content of the main nutrients in plants gradually decreased.

Table 2. Dynamics of the content of mineral nutrients in spring rapeseed plants by growth phase, % of dry matter (average for 2015-2017)

Fertiliser options	Stages of growth and development of spring rapeseed plants											
	BBCH 35-38			BBCH 5-58			BBCH 65-68			BBCH 80-83		
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O
Without fertiliser (control)	2.76	1.02	4.93	1.71	0.71	2.99	1.1	0.48	2.51	1.02	0.31	1.79
N ₃₀ P ₂₀ K ₃₅	2.98	1.08	5.24	1.9	0.79	3.54	1.23	0.6	2.69	1.28	0.39	1.91
N ₆₀ P ₄₀ K ₇₀	3.26	1.13	5.55	2.0	0.85	3.96	1.51	0.65	2.77	1.36	0.5	1.97
N ₉₀ P ₆₀ K ₁₀₅	3.35	1.22	5.87	2.13	0.88	4.18	1.67	0.71	2.89	1.48	0.53	2.06
N ₁₂₀ P ₈₀ K ₁₄₀	3.59	1.26	5.99	2.27	0.92	4.59	1.81	0.73	3.1	1.54	0.6	2.14
LSD ₀₅	0.06	0.04	0.15	0.04	0.04	0.9	0.06	0.03	0.09	0.05	0.03	0.12

The content of nitrogen in plants is lower: it varied from 2.98% to 3.59%, compared to 2.76% in the control version. Phosphorus content in spring rapeseed plants with fertilisers varied from 1.08% to 1.26%, while it was 1.02% in the case without fertilisers.

In the later stages of growth and development of spring rapeseed, a decrease in the content of all nutrients was observed, as evidenced by the research results.

The positive effect of increased rates of mineral fertiliser application is confirmed by the results of research conducted in the north-eastern part of the Forest Steppe of Ukraine [27].

The conducted correlation-regression analysis of research results indicates a close correlation between the accumulation of dry matter in the BBCH 55-58 period and the level of productivity ($r=0.91$) (Fig. 1).

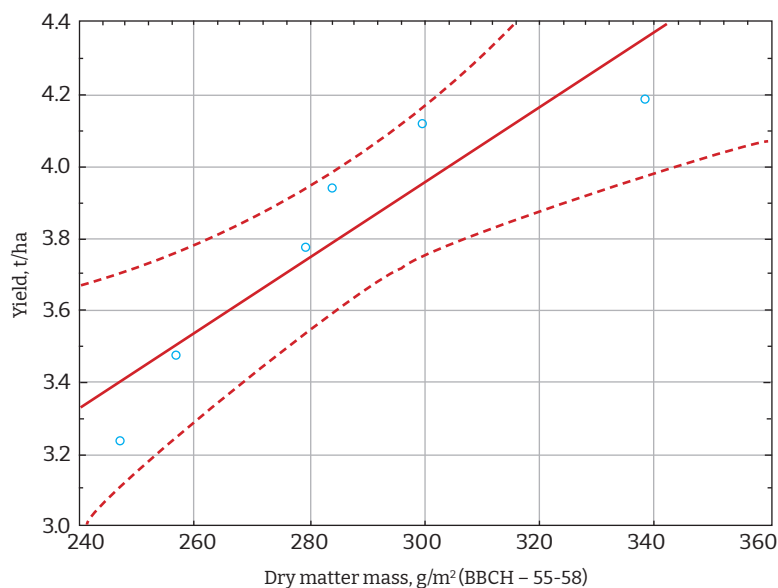


Figure 1. Correlation between yield (y) and dry matter mass (x) of spring rapeseed in BBCH microstages BBCH 55-58 (average for 2010-2013)

The resulting dependencies are described by the equations: Yield, t/ha=0.80369+0.01050 x mass of dry matter, g/m² (BBCH 55-58). Some scientists have obtained similar research results indicating an increase in accumulated dry matter by 7-32% under the influence of fertiliser application rates [1; 10; 17]. Use of mineral fertilisers in experiments by L. Harbar [1] positively affected the accumulation of dry matter during the entire vegetation period of rapeseed, which improved its productivity.

CONCLUSIONS

Based on the analysis of the current state of research in agronomic science on the cultivation of spring rapeseed, it was established that the application of mineral fertilisers in the maximum rates provides the best conditions for the formation of its crop.

Research established that the highest content of mineral nutrition elements in the soil is noted in the phase of spring rapeseed leaf rosette formation (BBCH 15-18) and gradually decreases towards the phase of full seed maturity (BBCH 85-88) due to their intensive use by plants.

The maximum content of nitrogen compounds in the soil in BBCH 15-18 and depending on the fertiliser variant varies from 52.0 to 60.2 mg/kg of soil, mobile phosphorus compounds – 92.9 mg/kg of soil (after N₁₂₀P₈₀K₁₄₀), mobile potassium – 137.5 mg/kg of soil (for N₁₂₀P₈₀K₁₄₀). It can be argued that the content of macroelements in the soil is dictated by the rate of application of mineral fertilisers: increased application rates positively affect the accumulation of basic nutrients in the soil. Crop productivity and dry matter accumulation largely depend on the fertilisation system. A close correlation was established between the accumulation of dry matter in the BBCH 55-58 period and the yield level (r=0.91).

The issue of the content of mineral nutrition elements in the post-harvest residues of spring rapeseed requires further study, including in the root system and investigating the effect of applied mineral fertilisers on the yield of subsequent crops in crop rotation. The question of obtaining ecologically safe products of crop production at high rates of application of mineral fertilisers is still unresolved.

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Ефективність використання макроелементів рослинами ріпаку ярого

Анотація. Актуальність досліджень зумовлена необхідністю оптимізації продукційного процесу агроценозів ріпаку ярого та встановлення закономірностей динаміки макроелементів у ґрунті за впливу різних норм внесення мінеральних добрив. В зв'язку з цим дана стаття спрямована на розкриття закономірностей зміни вмісту макроелементів в чорноземах типових малогумусних за різними стадіями розвитку культури ріпаку ярого в умовах Правобережного Лісостепу України. У процесі наукових пошуків за цією проблемою застосовували польовий, лабораторний, аналітичний та статистичний методи досліджень. Встановлено, що найвищий вміст макроелементів у ґрунті за всіх рівнів мінерального удобрення – у фазі формування рослинами ріпаку ярого розетки листків (ВВСН 15–18). У міру проходження фаз росту рослин він зменшується й у фазі дозрівання (ВВСН 85–88) досягає мінімальних значень. Найвищий вміст мінеральних сполук азоту відмічено у ВВСН 15–18: 52,0–60,2 мг/кг ґрунту залежно від варіанту удобрення, вміст у варіанті без внесення добрив – 49,8 мг/кг ґрунту. З'ясовано, що максимальний вміст рухомих сполук фосфору в ґрунті зафіксований у першій половині вегетаційного періоду. Встановлено, що вміст рухомого калію в ґрунті визначається кількістю внесених мінеральних добрив. Найвищий вміст відмічений на початку вегетації ріпаку ярого з поступовим зниженням до фази дозрівання насіння (ВВСН 85–88). Встановлено тісний кореляційний зв'язок між накопиченням сухої речовини у період ВВСН 55–58 та рівнем урожайності ($r=0,91$). Матеріали статті становлять практичну цінність для вдосконалення процесів, пов'язаних з формуванням продуктивності посівів ріпаку ярого в умовах Лісостепу України, а також раціональному використанню мінеральних добрив на чорноземах типових малогумусних

Ключові слова: *Brassica napus* L., мінеральне живлення, норми внесення мінеральних добрив, суха речовина, врожайність

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