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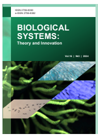

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The influence of microbiological inoculates on plant development in covering mixture

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Abstract. The study investigated seed germination, and plant development of a covering mixture consisting of 40% of spring vetch seeds and other crops under the influence of microbiological inoculants, namely Mycofriend-T and Bioinoculant-BTU-T. It was found that Mycofriend-T on bioinoculant-treated seeds of phacelia, long-stem flax, Sudan grass, and Alexandrian clover stimulated the germination of all crops in the mixture. The germination of the treated seeds increased by 2.5-5% compared to the control. The most significant increase in germination was found in spring vetch when treated with a complex of biological preparations – 7%. When treated with the microbial Mycofriend-T, the germination of seeds of this crop increased by 5%. The linear dimensions of the aboveground part of most plants of the mixture also increased by 10-40% compared to the control, depending on the crop. In the underground part of the plants, the linear dimensions also increased by 6-53% compared to the control, depending on the plant species. The total fresh weight of the aboveground part of plants when using Mycofriend-T

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and the bioinoculant complex increased by 60 and 59%, respectively, demonstrating the stimulating effect of the inoculant agents on the development of plants' leaf surface and stem. However, the most significant increase in fresh weight was found when analysing the data of the underground part of plants: when using Mycofriend-T + Bioinoculant-BTU-T, this indicator increased by 104%, while when using only the microbial Mycofriend-T, the increase was 43% compared to the control. The positive influence of bioinoculants on the functioning of the photosynthetic apparatus of Sudan grass plants was established. These research findings are important for farmers because they demonstrate how to improve the growth and physiological properties of cover crops, which perform many important functions for the soil and main crops

Keywords: *Trichoderma harzianum*; arbuscular fungi; *Rhizophagus irregularis* (*Glomus*); nodule bacteria; cover crops

INTRODUCTION

Harmonisation of the links between agricultural production, ecosystems, and climate is a task that can be solved by regenerative agriculture. In this approach, the use of cover crops is relevant forimproving the physicochemical properties of the soil and its biological properties, and reducing production costs. It is essential to form a layer of cover crops with good development of both the vegetative part of the plants and their well-developed root system. Growing cover crops is of interest to both large and small agricultural enterprises, which increases the demand for their seeds and simultaneously poses the problem of ensuring stable germination of crops in different environmental conditions. Cover crops, which can include several plant species from other families, require attention to the compatibility of each crop. Therefore, it is important to use environmentally acceptable methods to mitigate stress and eliminate restrictions on plant development during the growing season.

Studies show that plant growth under various stress conditions is enhanced by the influence of arbuscular mycorrhizal fungi, which stimulate plant nutrient uptake and increase plant resistance to stress factors. A. Wahab *et al*. (2023) analysed the current state of research on the effect of AMF on plants and showed that the symbiotic relationship between mycorrhizal fungi and plants facilitates the acquisition of nutrients and water by plants. The use of AMF is effective for plants to overcome drought stress, as shown in the study by S. Azizi *et al*. (2021). The researchers showed that myrtle plants tolerated drought better due to improved water supply and stimulation of antioxidant defence. N.O. Igiehon *et al*. (2020) showed that inoculation and mycorrhization of AMF alleviate drought stress and increase soybean yield, plant size, and seed oil content. An increase in the relative humidity of the above-ground shoots also accompanies the positive effect. Increased phosphorus uptake with AMF mycorrhization was shown in experiments with tomatoes by C.T.K. Tran *et al*. (2020), who, however, noted that mycorrhization increases the mobility of some forms of phosphorus, thereby increasing the potential for their leaching from the soil.

In addition to arbuscular fungi, *Trichoderma harzianum*, a well-known antagonist of soil-borne pathogenic fungi, also positively protects plants from phytopathogens. B. Glogoza *et al*. (2023) found that appropriate nodular bacterial inoculants can significantly accelerate plant development, especially in the *Fabaceae* family, which plays a crucial role in cover crop mixtures. According to their studies, rhizobacteria, which promote plant growth, and rhizobia, which can fix nitrogen, contribute to plant health and productivity in several ways. These microorganisms function as biofertilisers, improving plant nutrition, acting as biostimulants, regulating growth, and serving as biopesticides, increasing resistance to abiotic and biotic stress.

J. Poveda & D. Eugui (2022) demonstrated that the synergism between Trichoderma and bacteria brings more benefits than their separate use. However, the researchers noted that further investigation is needed to determine the specific mechanisms of this synergistic effect in increasing plant resistance to abiotic stresses. Microbial inoculants involve using live beneficial microorganisms together with seeds at sowing or by adding them to the growing medium. M. O'Callaghan *et al*. (2022) reviewed modern combinations such as co-inoculation and diverse microbial consortia that can fill different functional niches and increase the resilience of these microbial communities in the environment. The researchers also suggest that such combinations of microorganisms can act additively or synergistically. However, a review of research by A.S.M. Elnahal *et al*. (2022) raised the issue that the effectiveness of microbial inoculants is often controversial, especially in the field. Therefore, there is a critical need for their evaluation, particularly under field conditions. This problem highlights the importance of using research on various cultures in diverse environments.

Thus, several groups of microorganisms have been identified that positively impact the condition of plants and ecosystems. Therefore, obtaining new data on specific applications in combining such microorganisms, mainly when growing cover crops, is relevant. The purposeof the study was to investigate the effect of inoculants from different groups of microorganisms, namely, a combination of microbial preparations containing *Trichoderma harzianum*, arbuscular fungi *Rhizophagus irregularis (Glomus)*, bacterial complex and a culture of symbiotic nodule bacteria on the development of plants in a cover mixture.

MATERIALS AND METHODS

The studies were conducted in the educational and scientific laboratory of the Department of Ecobiotechnology and Biodiversity, Faculty of Plant Protection, Biotechnology, and Ecology at the National University of Life and Environmental Sciences of Ukraine in 2024. The research used plants from a cover crop mixture provided by Smart Crops LLC (Ukraine). The mixture consisted of seeds of spring vetch (*Vicia sativa* L.), phacelia (*Phacelia tanacetifolia* Benth.), common flax (*Linum usitatissimum*, L.), Sudan grass (*Sorghum bicolor subsp. drummondii* (Nees ex Steud.) Millsp. & Chase) and Alexandrian clover (*Trifolium alexandrinum*, L.). In the cover crop mixture, spring vetch seeds accounted for 40%, corresponding to the technological parameters for using cover crops containing representatives of the *Fabaceae* family. Thus, several groups of microorganisms have been identified that positively impact the condition of plants and ecosystems. Therefore, obtaining new data on specific applications in combining such microorganisms, mainly when growing cover crops, is relevant.

Conditions of the experiment. The study was conducted with preparations of microbiological origin Mycofriend-T and Bioinoculant-BTU-T produced by the biotechnology company BTU (Ukraine). The composition of the biologics Mycofriend-T included arbuscular *fungi Rhizophagus irregularis (Glomus)* and the *Trichoderma harzianum*. The microbial contained bacteria *Bacillus velezensis (Bacillus subtilis), Priestia megaterium (Bacillus megaterium var. phosphaticum), Paenibacillus mucilaginosus (Bacillus mucilaginosus), Agrobacterium salinitolerans (Enterobacter), Pseudomonas plecoglossicida (Pseudomonas fluorescens)*. The total number of viable cells (1.0-1.5)×10^8 CFU/ml. The biological preparation also included biologically active substances – products of the vital activity of microorganisms: phytohormones, vitamins, and amino acids.

The composition of the microbial Bioinoculant-BTU-T included symbiotic nodular bacteria *Rhizobium leguminosarum bv.viciae*, and biologically active products of bacterial life and nutrient medium components (macro-, microelements, etc.). The titre of the biologics ranged from 2.5×10^9 CFU/ml. Both biologics were based on a filler – peat. The research scheme included the following options: Control – no treatment; Option 1 – seed treatment with the biologics Mycofriend-T at the rate of 100 g of the biologics per 1 ha of crops; Option 2 – seed treatment with a complex of biologics Mycofriend-T + Bioinoculant-BTU-T at the rate of 100 g of each biologics per 1 ha of crops.

The seeds of the plants in the covering mixture were moistened for better adhesion and treated with biopreparations according to the research scheme. The seeds were sown in pots with a diameter of 16 cm^2 for cultivation. The plants were grown in a phytoclimatic chamber, "Silverbox evolution" (France), at a temperature of 20 ± 2°C and a lighting regime of light/darkness 14/10 hours. The air humidity regime in the chamber was maintained at 70-80%, and the soil humidity in the pots was 50-80% of the lowest moisture capacity. Peat substrate "Universal with biohumus" (Ukraine) was used for cultivation. For research, a sample of 4 plants of each species was used (20 plants in pot) in 3-fold replication for each variant.

The germination of crop seeds was assessed according to DSTU 4138-2002 (2004). In addition, to simulate the germination process in natural conditions, authors modified the germination device developed by them (Fig. 1). The containers were filled with a moistened peat mixture, into which seeds treated according to the scheme were placed. Germination was carried out in a thermostat at 25°C for 4 days.

Figure 1. Study the effect of biological preparations on the germination of spring vetch seeds (*Vicia sativa* L.)

Note: 1 – control; 2 – treatment with a complex of microbial preparations; 3 – treatment with the microbial preparation Mycofriend-T

Source: developed by the authors

The containers were covered with transparent lids, which allowed them to be placed vertically for the natural process of seedling root development and to observe the process. The method of Z. Hrytsayenko *et al*. (2003) was used to determine biometric indicators (lengths of underground and aboveground part) and plant mass. The functional state of Sudan grass leaves was assessed using the method of chlorophyll fluorescence induction (Brion *et al*., 2000). Statistical processing of the research results was carried out using the Microsoft Excel 2010 software suite ANOVA by methods of variational statistics using the t-test and one-way analysis of variance. Differences in the values of indicators with $p \leqslant 0.05$ were considered significant (Prylutsky *et al*., 2017). Microsoft Excel 2010 was used to construct graphs. The table values were $M \pm Sd$, where M – arithmetic mean, and Sd – standard deviation. The experimental studies of plants (both cultivated and wild), including the collection of plant material, were in accordance with institutional, national or international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

Seed germination is a critical moment in crop development that requires attention from producers. Pre-sowing treatment with biostimulants allows plants to be supported at the initial stage, which is important for plant growth in general. In cover mixtures where several different crops are grown, attention must be paid to their germination. Studies have shown that treatment with bioinoculants stimulated the germination of all crops in the mixture, and the increase in the number of germinated seeds was 2.5-5% compared to the control when using the biologics Mycofriend-T for seeds of phacelia, flax, Sudan grass, and Alexandrian clover (Table 1). The highest increase according to the indicator "seed germination" was found for spring vetch when treated with a complex of biological preparations – 7%. When treated with the biologics Mycofriend-T, the germination of seeds of this crop increased by 5%. The results of a preliminary assessment of the effects of microbial using a modified device (Fig. 1) also demonstrated a positive effect on the germination of spring vetch precisely with the combined action of two bioinoculants.

Table 1. Germination rates of crop seeds in the covering mixture when treated with bioinoculants, %

Crop	Experiment option		
	Control	Mycofriend-T	Mycofriend-T + Bioinoculant-BTU-T
Spring vetch	92.5 ± 0.2	97.5 ± 0.2	99.7 ± 0.2
Phacelia	72.5 ± 0.3	75.0 ± 0.1	-
Flax	90.0 ± 0.2	95.0 ± 0.2	-
Sudan grass	52.5 ± 0.2	57.5 ± 0.3	-
Alexandrian clover	87.5 ± 0.2	90.0 ± 0.3	-

Note: “-” no data available
Source: developed by the authors

In general, it is known that plant colonisation by arbuscular fungi can occur more or less efficiently, depending on the genomes of the fungi and plants entering into symbiosis, and this variability can be observed even at the level of plant varieties of the same species (Cervantes-Gámez *et al.*, 2015; Guigard *et al.*, 2023).

However, even the presence of fungi in the cortical root cells of a plant can stimulate an increase in shoot and root mass, believed to be due to the formation and development of lateral roots (Fiorilli *et al.*, 2018). During the exchanges, the fungus receives the products of plant photosynthesis; in return, the branched hyphal network contributes to the mineralisation and restoration of essential soil nutrients, thus increasing their bioavailability to the plant (Montero *et al.*, 2018; Jiang *et al.*, 2021). Arbuscular fungi play a particular role in increasing the bioavailability of phosphorus, as reported, through the mechanism of expression of genes whose products respond to inorganic phosphate and suppress the phosphorus starvation response of plants, which is typical for soils with low phosphorus content (Campo & San Segundo, 2020).

Thus, with a positive bioinoculation result, it is possible to observe an increase in plant growth and development. The studies have demonstrated a stimulating effect on plant development of the coating mixture of a separate preparation with arbuscular fungi and the complex use of two microbial preparations, which, in particular, shows the importance of choosing the target crop when using such microbial preparations (Fig. 2).

The use of biological products revealed an increase in morphological indicators of both the underground and aboveground parts of the plants of the covering mixture (Table 2). This was observed for all crops of the mixture, except phacelia, for which no positive effect of treatment with microbial products was found since the length of the aboveground part and the root system were slightly lower than in control. This may be due to the lower physiological performance of the seeds of this crop, as its germination was generally low.

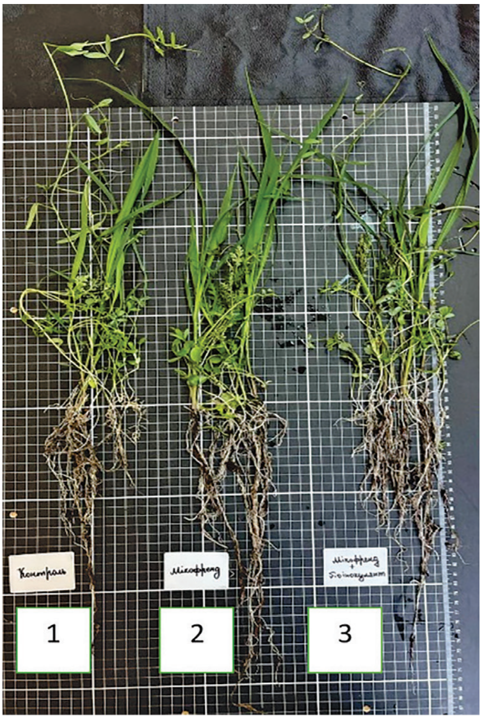

Figure 2. Development of plants in the cover mixture after seed treatment with bioinoculants 21 days after germination
Note: 1 – control; 2 – Mycofriend-T; 3 – Mycofriend-T + Bioinoculant-BTU-T
Source: developed by the authors

Table 2. Effect of bioinoculant preparations on biometric parameters of seedlings of cover crops

Crop	Experiment option	Indicators	
		Underground part, mm	**Aboveground part, mm**
Phacelia	Control	22.2 ± 1.5	31.4 ± 0.8
	Mycofriend-T	20.3 ± 1.2	30.2 ± 1.3
Flax	Control	47.4 ± 0.8	23.7 ± 1.1
	Mycofriend-T	50.3 ± 1.1	32.3 ± 1.5
Sudan grass	Control	11.1 ± 0.9	6.3 ± 1.4
	Mycofriend-T	24.3 ± 1.2	20.7 ± 1.2
Alexandrian clover	Control	19.4 ± 1.1	32.7 ± 1.3
	Mycofriend-T	21.2 ± 1.5	36.1 ± 1.4

Source: developed by the authors

When seeds were treated with the bioinoculant Mycofriend-T, the length of the root system of flax and clover seedlings increased by 6.0% and 9.0%, respectively, compared to the control. In contrast, the root system of Sudan grass seedlings exceeded the control value by 2 times. The length of the aboveground part of flax and clover seedlings increased by 35% and 10%, respectively, compared to the control, and the aboveground part of Sudan grass plants exceeded the control value by 3 times.

The stimulating effect of the treatment on the development of the aboveground and underground parts of the plants was also established in spring vetch, which was the main crop of the cover mixture under study. Combined treatment with microbial preparations increased the length of the root system by 53% compared to the control and when treated with Mycofriend-T – by 34%. The aboveground part of the plants also developed better under combined treatment, and its length increased by 40% compared to the control, while Mycofriend-T treatment increased this indicator by 24% (Fig. 3).

In addition, the authors used the preparation Bioinoculant-BTU-T, which contains nodule bacteria with a high tendency to colonise vetch plants, which also contributes to the enhancement of the development of the root system. With the combined treatment of Mycofriend-T and Bioinoculant-BTU-T, an increase in additional roots and root hairs in the root system of plants was also found, which affected the increase in their fresh weight (Fig. 4).

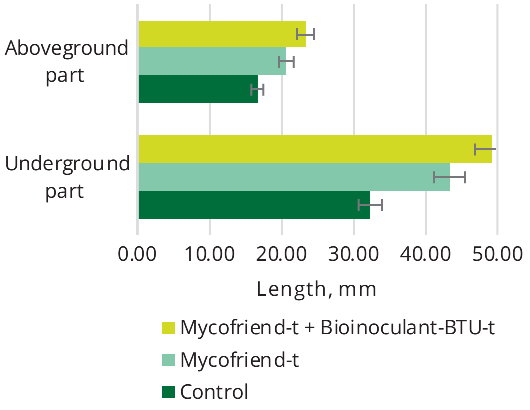

Figure 3. Assessment of the effect of bioinoculants on the growth of common spring vetch
Source: developed by the author

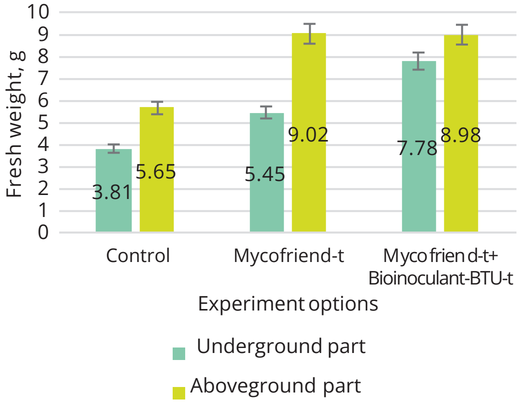

Figure 4. Accumulation of fresh weight by plants of the cover mixture when treated with bioinoculants
Source: developed by the authors

The total fresh weight of the aboveground part of plants when using Mycofriend-T and the complex of microbial preparations increased by 60% and 59%, respectively, demonstrating the stimulating effect of the inoculant agents on the development of plants' leaf surface and stem. However, the most significant increase in fresh weight was found when analysing the data of the underground part of plants: when using Mycofriend-T+ Bioinoculant-BTU-T, this indicator increased by 104%, while when using only the microbial preparation Mycofriend-T, the increase was 43% compared to the control. Thus, the effect of bioinoculants had a positive effect on the development of the root system in the initial period of plant vegetation.

The induction of chlorophyll fluorescence in Sudan grass leaves was determined to assess the condition of plants treated with microbiological inoculants since its leaf blade most closely matched the conditions for conducting diagnostics using the IF method. This method allows assessing the photosystem II of plants during life, which is important for determining long-term effects on plants and analysing the plant's general condition (Grusha *et al.*, 2014; Kovalyshyn *et al.*, 2016). An increase in the maximum fluorescence value (Fmax) was noted in plants in variants treated with inoculants compared to the control, which indicates that the photosynthetic process in plants is quite effective (Fig. 5).

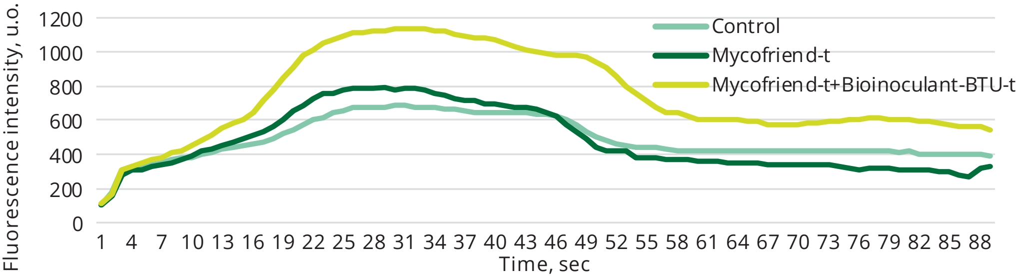

Figure 5. Induction curves of chlorophyll fluorescence in Sudan grass leaves upon treatment with microbial inoculants

Source: developed by the authors

To assess the induction of fluorescence in chlorophyll-bearing tissues, the calculated parameter Fv is used – the chlorophyll fluorescence variable, which is expressed as the difference between the highest fluorescence level and background fluorescence (Fmax – F_0) and provides information about the magnitude of the amplitude of changes in the Kautsky curve. It was found that this indicator increased when Sudan grass seeds were treated with bioinoculants: under the action of Mycofriend-T, it increased by 18%, and under the action of the complex of biological preparations Mycofriend-T and Bioinoculant-BTU-T – by 77% compared to the control. This also indicates a positive effect of bioinoculants on the functioning of the photosynthetic apparatus of Sudan grass plants (Table 3).

Table 3. Assessment of the influence of bioinoculants on the indices of chlorophyll fluorescence induction in Sudan grass leaves

Experiment option	IF indicators, conventional units					
	F_0	Fmax	Fst	Fv	(Fmax-Fst)/Fst	Fv/Fmax
Control	103	680	392	577	0.73	0.85
Mycofriend-T	104	784	328	680	1.39	0.87
Mycofriend-T+ Bioinoculant-BTU-T	112	1136	544	1024	1.09	0.9

Note: F_0 – background fluorescence level, Fmax – fluorescence maximum, Fst – stationary fluorescence level, Fv – variable fluorescent, (Fmax-Fst)/Fst – efficiency of photochemical conversion of energy in FS II, Fv/Fmax – the maximum quantum yield of the photochemistry of PSII

Source: developed by the author based on research

The maximum quantum yield of photochemistry of PSII is the expression Fv/Fmax. In healthy plants not exposed to stressors, the ratio Fv/Fm usually varies within 0.70-0.90. For the plants under study, this indicator was within the specified limits. The efficiency of photochemical energy conversion in PSII characterises the rate of linear electron transport and is an integrated indicator of the photosynthesis process. This indicator in the plants under study varied from 0.73 to 1.39 conventional units. It was higher in plants treated with inoculants, which indicates a positive effect of the treatment on the state and functioning of the photosynthetic system in Sudan grass plants.

The results presented demonstrate the improvement of vetch plant growth with AMF inoculation. The effectiveness of arbuscular fungal inoculants was shown in field studies conducted by K. Burak *et al*. (2024) on different vetch cultivars grown on depleted soils with high calcium content in southeastern Turkey. These studies showed that inoculation with arbuscular mycorrhizal fungi reduces the amount of lime in the soil and identified the most effective combinations of fungal isolates that positively affect the biochemical cycles of carbon, nitrogen, and phosphorus. T. Ding *et al*. (2020) also demonstrated the positive effect of the arbuscular mycorrhizal fungus *Sieverdingia tortuosa* on atrachnosis resistance and enhanced root growth of spring vetch.

Cover crops are an essential part of sustainable agriculture. Their role was recognised in soil recovery and reduction of weed infestation, including broader environmental impacts (Marcillo & Miguez, 2017; Daryanto *et al*., 2018), which could lead to an increase in crop productivity and quality over time. However, for cover crops to develop better in the face of climate change, it is important to support their growth and development. The use of bioinoculants improves plant growth and nutrient uptake under resource-limited conditions. In particular, V. Dragičević *et al*. (2020) highlighted the importance of using such inoculants in mixed cropping systems of legumes and cereals to enhance the productivity and resilience of agroecosystems. In this paper, the studies conducted by the authors confirmed the feasibility of pre-sowing treatment of cover crop mixtures with microbial biopreparations since they stimulate biometric and physiological indicators of plants. Treatment of cover crop mixtures seed contributed to improving seed germination, increased root system and shoots, increased green mass of plants, and optimised photosynthetic processes. Improving the above parameters will make the plant more resistant to abiotic stress factors. Other researchers who investigated the effect of biopreparations on seed treatment of various plants came to such conclusions.

In the study by M. Ebrahimi (2023), inoculation of clover seeds with microbes is recommended to improve their growth and development characteristics under drought conditions because of the positive effects of plant growth-promoting rhizobacteria. The studies by S. Yang *et al*. (2023) confirmed these findings, which indicate that mycorrhizal-based biopreparations improve plant growth parameters and physiological indicators. Namely, in this study, the role of mycorrhizal inoculation in nutrient absorption and its important contribution to plant greater biomass were discussed.

The paper by M. Baidalin *et al*. (2024) showed that double grass mixtures treated with nodule bacteria are more productive than untreated with biological products under the conditions of northern Kazakhstan. The use of inoculants increased the yield of green mass; this study demonstrated that inoculation with nitrogen-fixing bacteria significantly enhances the yield and nutritional quality of legume-cereal grass mixtures in northern Kazakhstan. In the study by K. Abdelaal *et al*. (2024), it was demonstrated that inoculation with mycorrhizal-based biopreparations positively affects the most studied traits, such as chlorophyll concentration and maximum quantum efficiency of PSII; the same trend was observed in the studies of the authors of the present paper under the conditions of using the microbiological Mycofriend-T + Bioinoculant-BTU-T (Table 3).

The stimulating effect of bacterial inoculants was confirmed by B. Glogoza *et al.* (2024). The researchers showed that treating corn seeds with bioinoculants stimulated the growth and development of the root system compared to the control; the mass of roots increased by 12-30%, and the height of plants treated with biological preparations exceeded the control plants by 15-23 cm. In this paper, the authors also demonstrated the stimulating effect of the application on the development of the root system and the mass of cover crops (Table 2, Fig. 3-4).

The combined application of bioinoculants and cover crops represents an effective and environmentally sound strategy for contemporary agriculture. This approach enhances plant resistance to various stress factors, while simultaneously enriching the soil microbiome with beneficial microorganisms. Additionally, it contributes to improving soil structure and fertility, leading to increased productivity and stability of major crop yields. By integrating these practices into existing farming systems, it is possible to promote sustainable agricultural development without compromising environmental integrity or long-term soil health.

CONCLUSIONS

The effect of microbiological inoculants on the development of plants in a cover mixture consisting of 40% spring vetch seeds, and the rest of phacelia, flax, Sudan grass, and Alexandrian clover was studied in laboratory conditions. The germination of the treated seeds increased by 2.5-5% compared to the control. The greatest increase in germination was found in spring vetch when treated with a complex of biological preparations – 7%.

In the aboveground part of the plants, the linear dimensions also increased by 10-40% compared to the control, depending on the plant species. For Sudan grass, the growth of the aboveground part was three times as high as compared to the control. In the underground part of the plants, the linear dimensions also increased by 6-53% compared to the control, depending on the plant species. For Sudan grass, the growth of the aboveground part was twice as high as compared to the control. The total fresh weight of the aboveground part of plants when using Mycofriend-T and the complex of microbials increased by 60% and 59%, respectively, demonstrating the stimulating effect of the inoculant agents on the development of plants' leaf surface and stem. However, the most significant increase in fresh weight was found when analysing the data of the underground part of plants: when using Mycofriend-T + Bioinoculant-BTU-T, this indicator increased by 104%, while when using only the Mycofriend-T, the increase was 43% compared to the control. The positive influence of bioinoculants on the functioning of the photosynthetic apparatus of Sudan grass plants has been established. In the future, further research is planned to assess the impact of bioinoculants on cover crops, study the mechanisms underlying them, and evaluate their performance under field conditions.

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CONFLICT OF INTEREST

None.

REFERENCES

[1] Abdelaal, K., Alaskar, A., & Hafez, Y. (2024). Effect of arbuscular mycorrhizal fungi on physiological, bio-chemical and yield characters of wheat plants (*Triticum aestivum* L.) under drought stress conditions. *BMC Plant Biology*, 24(1). doi: 10.1186/s12870-024-05824-9.

[2] Azizi, S., Kouchaksaraei, M.T., Hadian, J., Abad, A.R.F., Sanavi, S.A.M., Ammer, Ch., & Bader, M.K.-F. (2021). Dual inoculations of arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria boost drought resistance and essential oil yield of common myrtle. *Forest Ecology and Management*, 497(1), article number 119478. doi: 10.1016/j.foreco.2021.119478.

[3] Baidalin, M., Akhet, A., Baidalina, S., Ualiyeva, G., & Vasiljević, S. (2024). The effect of rhizobium inoculation on the nutritional value of crops in the legume-cereal intercropping system in Northern Kazakhstan. *Agronomy*, 14(11), article number 2574. doi: 10.3390/agronomy14112574.

[4] Brion, O.V., Korneev, D.Yu., Snegur, O.O., & Kitaev, O.I. (2000). *Instrumental study of the photosynthetic apparatus using induction of chlorophyll fluorescence: Methodological guidelines for students of the biology faculty*. Kyiv: Publishing and Printing Center "Kyiv University".

[5] Burak, K., Halil Yanardağ, I., Gómez-López, M.D., Faz, A., Yalçin, H., Sakin, E., Ramazanoğlu, E., Bars Orak, E., & Yanardağ, A. (2024). The effect of arbuscular mycorrhizal fungi on biological activity and biochemical properties of soil under vetch growing conditions in calcareous soils, *Heliyon*, 10(3), article number e24820, doi: 10.1016/j.heliyon.2024.e24820.

[6] Campo, S., & San Segundo, B. (2020). Systemic induction of phosphatidylinositol-based signaling in leaves of arbuscular mycorrhizal rice plants. *Scientific Reports*, 10, article number 15896. doi: 10.1038/s41598-020-72985-6.

[7] Cervantes-Gámez, R.G., Bueno-Ibarra, M.A., Cruz-Mendívil, A., Calderón-Vázquez, C.L., Ramírez-Douriet, C.M., Maldonado-Mendoza, I.E., Villalobos-López, M.Á., Valdez-Ortíz, Á., & López-Meyer, M. (2016). Arbuscular mycorrhizal symbiosis-induced expression changes in Solanum lycopersicum leaves revealed by RNA-seq analysis. *Plant Molecular Biology Reporter*, 34, 89-102. doi: 10.1007/s11105-015-0903-9.

[8] Convention on Biological Diversity. (1992, June). Retrieved from https://zakon.rada.gov.ua/laws/show/995_030#Text.

[9] Daryanto, S., Fu, B., Wang, L., Jacinthe, P.A., & Zhao, W. (2018). Quantitative synthesis on the ecosystem services of cover crops. *Earth-Science Reviews*, 185, 357-373. doi: 10.1016/j.earscirev.2018.06.013.

[10] Ding, T., Zhang, W., Li, Y., & Duan, T. (2020). Effect of the AM fungus sieverdingia tortuosa on common vetch responses to an anthracnose pathogen. *Frontiers in Microbiology,* 11, article number 542623. doi: 10.3389/fmicb.2020.542623.

[11] Dragičević, V., Simić, M., Dolijanović, Ž., Đorđević, S., Stoiljković, M., Dimkić, I., & Brankov, M. (2024). Combined effect of cover crops and bio-fertilizer on sustainable popcorn maize production. *Frontiers in Plant Science*, 14, article number 1250903. doi: 10.3389/fpls.2023.1250903.

[12] DSTU 4138-2002 "Seeds of Agricultural Plants. Methods for seed testing". (2004, January). Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=91465.

[13] Ebrahimi, M. (2023). Plant growth-promoting bacteria inoculation mitigates drought stress effects in *Trifolium pratense* L. seedlings. *PREPRINT*, 1-28. doi: 10.21203/rs.3.rs-3288040/v1.

[14] Elnahal, A.S.M., El-Saadony, M.T., Saad, A.M., Desoky, E.S.M., El-Tahan, A.M, Rady, M.M., AbuQamar, S.F., & El-Tarabily, K.A. (2022). The use of microbial inoculants for biological control, plant growth promotion, and sustainable agriculture: A review. *European Journal of Plant Pathology*, 162, 759-792. doi: 10.1007/s10658-021-02393-7.

[15] Fiorilli, V., *et al*. (2023). Omics approaches revealed how arbuscular mycorrhizal symbiosis enhances yield and resistance to leaf pathogen in wheat. *Scientific Reports,* 8, article number 9625. doi: 10.1038/s41598-018-27622-8.

[16] Glogoza B., Aldrich-Wolfe, L., Prasifka, J.R., & Prischmann-Voldseth, D.A. (2023). Impact of multiple soil microbial inoculants on biomass and biomass allocation of the legume crop field pea (Fabaceae: *Pisum sativum* L.). *Journal of Sustainable Agriculture and Environment*, 2(3), 314-327. doi: 10.1002/sae2.12060.

[17] Grusha, V.V., Gordiyenko, T.I., & Patyka, M.V. (2014). Assessment of the physiological state of crops by the method of photoinduction of chlorophyll fluorescence. In *Collection of scientific papers of the National Scientific Center "Institute of Agriculture of the NAAS"* (pp. 55-60). Kyiv: ECMO.

[18] Guigard, L., Jobert, L., Busset, N., Moulin, L. & Czernic, P. (2023). Symbiotic compatibility between rice cultivars and arbuscular mycorrhizal fungi genotypes affects rice growth and mycorrhiza-induced resistance. *Frontiers in Plant Science*, 14, article number 1278990. doi: 10.3389/fpls.2023.1278990.

[19] Hrytsayenko, Z.M., Hrytsayenko, A.O., & Karpenko, V.P. (2003). *Methods of biological and agrochemical research of plants and soils*. Kyiv: "Nichlava".

[20] Igiehon, N.O., Babalola, O.O., Cheseto, X., & Torto, B. (2021). Effects of rhizobia and arbuscular mycorrhizal fungi on yield, size distribution and fatty acid of soybean seeds grown under drought stress. *Microbiol Research*, 242, article number 126640. doi: 10.1016/j.micres.2020.126640.

[21] Jiang, F., Zhang, L., Zhou, J., George, T.S., & Feng, G. (2021). Arbuscular mycorrhizal fungi enhance mineralisation of organic phosphorus by carrying bacteria along their extraradical hyphae. *New Phytologist*, 230(1), 304-315. doi: 10.1111/nph.17081.

[22] Kovalyshyn, I.B., Pinchuk, A., Taran, M.V., & Shvets, R.L. (2016). Chlorophyll fluorescence induction of the genus clematis l. representatives leaves in Kyiv conditions. *Ukrainian Journal of Forest and Wood Science*, 238, 176-184.

[23] Marcillo, G.S., & Miguez, F.E. (2017). Corn yield response to winter cover crops: An updated meta-analysis. *Journal of Soil and Water Conservation*, 72 (3), 226-239. doi: 10.2489/jswc.72.3.226.

[24] Montero, H., Choi, J., & Paszkowski, U. (2018). Arbuscular mycorrhizal phenotyping: The dos and don'ts. *New Phytologist*, 221(3), 1182-1186. doi: 10.1111/nph.15489.

[25] O'Callaghan, M., Ballard, R.A., & Wright, D. (2022). Soil microbial inoculants for sustainable agriculture: Limitations and opportunities. *Soil Use and Management*, 38(3), 1340-1369. doi: 10.1111/sum.12811.

[26] Poveda, J., & Eugui, D. (2022). Combined use of Trichoderma and beneficial bacteria (mainly Bacillus and Pseudomonas): Development of microbial synergistic bio-inoculants in sustainable agriculture. *Biological Control*, 176, article number 105100. doi: 10.1016/j.biocontrol.2022.105100.

[27] Prylutsky, Yu.I., Ilchenko, O.V., Tsymbalyuk, O.V., Kosterin, S.O. (2017). *Statistical methods in biology*. Kyiv: Naukova Dumka.

[28] Tran, C.T.K., Watts-Williams, S.J., Smernik, R.J., & Cavagnaro, T.R. (2020). Effects of plant roots and arbuscular mycorrhizas on soil phosphorus leaching, *Science of The Total Environment*, 722(20), article number 137847. doi: 10.1016/j.scitotenv.2020.137847.

[29] Wahab, A., Muhammad, M., Munir, A., Abdi, G., Zaman, W., Ayaz, A., Khizar, C., & Reddy, S.P.P. (2023). Role of arbuscular mycorrhizal fungi in regulating growth, enhancing productivity, and potentially influencing ecosystems under abiotic and biotic stresses. *Plants*, 12(17), article number 3102. doi: 10.3390/plants12173102.

[30] Yang, S., Imran, & Ortas, I. (2023). Impact of mycorrhiza on plant nutrition and food security. *Journal of Plant Nutrition*, 46(13), 3247-3272. doi: 10.1080/01904167.2023.2192780.

Вплив мікробіологічних інокулянтів на розвиток рослин у покривній суміші

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Анотація. У дослідженні було вивчено проростання насіння та розвиток рослин покривної суміші, що складалася з 40 % насіння вики ярої та інших культур, під впливом мікробіологічних інокулянтів, а саме Мікофренд-Т та Біоінокулянт-БТУ-Т. Встановлено, що Мікофренд-Т на обробленому біоінокулянтами насінні фацелії, льону-довгунця, суданської трави та конюшини олександрійської стимулював проростання всіх культур у суміші. Схожість обробленого насіння зросла на 2,5-5 % порівняно з контролем. Найсуттєвіший приріст схожості виявлено у вики ярої при обробці комплексом біопрепаратів – 7 %. При обробці мікробним препаратом Мікофренд-Т схожість насіння цієї культури зросла на 5 %. Лінійні розміри надземної частини більшості рослин суміші також збільшилися на 10-40 % порівняно з контролем, залежно від культури. У підземній частині рослин лінійні розміри також збільшилися на 6-53 % порівняно з контролем, залежно від виду рослин. Загальна сира маса надземної частини рослин при застосуванні Мікофренд-Т та біоінокулянтного комплексу зростала на 60 та 59 % відповідно, що свідчить про стимулюючий вплив інокулянтів на розвиток листкової поверхні та стебла рослин. Однак найбільш суттєвий приріст сирої маси виявлено при аналізі даних підземної частини рослин: при застосуванні Мікофренд-Т + Біоінокулянт-БТУ-Т цей показник збільшився на 104 %, тоді як при використанні лише мікробного препарату Мікофренд-Т приріст становив 43 % порівняно з контролем. Встановлено позитивний вплив біоінокулянтів на функціонування фотосинтетичного апарату рослин суданської трави. Результати досліджень є важливими для аграріїв, оскільки демонструють шляхи покращення росту та фізіологічних властивостей покривних культур, які виконують багато важливих функцій для ґрунту та основних сільськогосподарських культур

Ключові слова: *Trichoderma harzianum*; арбускулярні гриби; *Rhizophagus irregularis* (*Glomus*); бульбочкові бактерії; покривні культури

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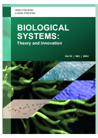

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Evaluation of powdery mildew (*Podosphaera pannosa* (Wallr.) de Bary) in roses

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Abstract. Roses are one of the main floricultural and decorative crops and are widely used in industrial floriculture and landscape architecture. During cultivation, rose plants are susceptible to various infectious diseases that impair their decorative properties. A common and dangerous pathology is powdery mildew, which is caused by the fungus *Podosphaera pannosa* (Wallr.: Fr.) de Bary. The study aimed to assess the susceptibility of rose varieties to powdery mildew in Kyiv. The study was conducted at the rose garden of the Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine and in the problematic research laboratory of Mycology and Phytopathology of the National University of Life and Environmental Sciences of Ukraine. The spread and intensity of powdery mildew development were assessed against a natural infection background using a scale that included a gradation from 0 to 4 points. A total of 79 rose varieties from 6 garden groups were examined. During the research, powdery mildew developed during May-October on all aboveground parts of plants (except lignified shoots), but most intensively on young shoots. During the vegetation period (2022-2023) in the open field, among the 17 varieties of the floribunda group examined, the disease did not develop in 12 varieties. The evaluation of powdery mildew on 16 varieties of hybrid tea roses showed its absence on 13. Among the 8 varieties of climbing large-flowered roses, the disease developed in 2 varieties. On roses of the shrub group, the pathology was not widespread in 23 varieties out of 33 studied. All 3 varieties of

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musk roses were not affected by *P. pannosa*, while in 2 varieties of ramblers, its presence was noted. In total, the disease was widespread in 22 rose varieties. The study of powdery mildew damage to roses is relevant for the development of control measures and the search for sources of resistance that can be used in the breeding process

Keywords: *Rosa* sp.; phytopathogen; resistance; cultivars and garden groups; disease development

INTRODUCTION

Roses are essential flowers and ornamental crops represented by many varieties. Cultivated roses are widely used in industrial floriculture, landscape architecture, home gardens, pharmaceutical production, food industry and other industries and have a variety of cultural and economic value. During cultivation, rose plants are susceptible to many diseases that significantly affect their decorative properties. One of the most dangerous pathogens on roses is the fungus *Podosphaera pannosa* (Wallr. : Fr.) de Bary (*Sphaerotheca pannosa var rosae* (Wallr. : Ex Fr.) Lev.), which causes powdery mildew.

Y. Bao *et al.* (2022) noted that rose powdery mildew is widespread in different regions of the world. It is dangerous when growing plants for cutting and is also the main disease of garden and indoor roses. The disease is widespread in Ukraine (Roses in the plantings of the city of Kyiv, 2020). It appears both in open and protected ground on all above-ground plant organs: leaf blades, leaf petioles, shoots, buds and flowers. M. Linde & N. Shishkoff (2003) described powdery mildew as one of the most serious diseases of the leaf apparatus. The authors emphasised that almost 40% of fungicides used on roses are designed to control powdery mildew.

In a review of scientific publications analysing a sustainable pest management strategy to reduce pesticide use, C.E. Góngora *et al.* (2024) highlighted the negative impact on the environment, human health and biodiversity of using only synthetic pesticides. There is also an issue related to the development of pest resistance to pesticides, which reduces the effectiveness of their use. At the same time, to ensure better control and environmental sustainability, it is necessary to reduce the use of chemicals by adopting original alternative strategies to maintain pest/pathogen populations below the level of economic harm and to achieve The European Green Deal (2019). Therefore, according to C.E. Góngora *et al.* (2024), one of the control strategies is the identification and cultivation of resistant varieties. A. Khan & S. Korban (2022) noted that the use of plant resistance is considered the most sustainable approach to protecting plants from various pathogens. The same applies to powdery mildew pathogens (Martins *et al.*, 2022).

The literature presents various data on the powdery mildew susceptibility of rose varieties of different garden groups, as well as wild rose species and forms. Y. Bao *et al.* (2022) indicated that powdery mildew caused by the fungus *P. pannosa* is the most common disease in different cultivation technologies of *Rosa multiflora* Thunb. J. Li *et al.* (2021) underlined the harmfulness of the disease, significant yield losses and deterioration of its quality in the areas of cultivation of *R. roxburghii* Tratt. X. Li *et al.* (2023) and K. Wang *et al.* (2024) emphasised the danger of disease development in *R. chinensis* Jacq. Plants, as there is a decrease in their decorative and economic qualities. L. Tan *et al.* (2022), for the first time, highlighted powdery mildew on *R. cymosa* Tratt plants caused by *P. pannosa*. The authors conclude that the occurrence of the disease in *R. cymosa* may pose a potential threat to other crops of the genus *Rosa* or *Prunus spp*.

X.Q. Qiu *et al.* (2015), in an analysis of the resistance of wild rose species to powdery mildew, proved that the genotypes of *R. laevigata* Michx., *R. longicuspis* Bertol., *R. luciae* Franch. & Rochebr. and *R. banksiae* R.Br. were immune to *P. pannosa*. High resistance to this pathogen was found in 12 samples of the *Pimpinellifoliae* section, *R. rubus* H. Lev. & Vaniot, *R. rugosa* Thunb. and others. The results of the experiments also showed that three sections *Pimpinellifoliae*, *Laevigatae* and *Banksianae* were more resistant to *P. pannosa* than others.

R.K. Mesta *et al.* (2021), among nine rose genotypes, Bugati, Carlet, Cherishma, Folklar, Gold Strike, Nobless and Tineke determined a moderately sensitive reaction to rose powdery mildew. Two genotypes Tajmahal and Papaya Red were susceptible to the disease. In general, none of the genotypes was resistant. N.K. Chandran *et al.* (2020) described the presence of resistance to powdery mildew at the genetic level in the IIHRR13-4 genotype. The comparison was made with the susceptible commercial variety Konfetti.

Despite active scientific research on the resistance of roses to powdery mildew in different countries of the world, this issue remains understudied in the conditions of Kyiv urban plant communities. Given that new varieties of roses are emerging that are resistant to diseases, there is a possibility of the emergence of new races of pathogens that can overcome plant resistance. Therefore, there is a need to study these processes. The study aimed to assess the intensity of powdery mildew damage to rose varieties of different garden groups.

MATERIALS AND METHODS

The study was conducted at the rose garden of the M.M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine. Powdery mildew was diagnosed visually based on typical and atypical symptoms (Pikovskyi & Solomiichuk, 2022). Samples of plant material with atypical symptoms were analysed in the problematic research laboratory of Mycology and Phytopathology of the National University of Life and Environmental Sciences of Ukraine. For this purpose, temporary solutions were produced, and the morphological structures of the pathogen were analysed and identified. Pathogen identification was carried out by microscopy of mycelium and conidial sporulation of *P. pannosa* and was performed using a Sigeta MB-103 40x-1600x LED mono monochrome microscope.

The assessment of the spread and intensity of powdery mildew development was conducted on a natural infectious environment (with preventive measures) during the period of its maximum development (second-third decade of September 2022-2023), using the following scale: 0 points – no disease symptoms; 1 point – up to 10% of the leaf surface (or bush surface) is affected; 2 points – 11 to 25%; 3 points – 26 to 50%; 4 points – more than 50% of the leaf surface (or bush surface). A total of 79 rose varieties from 6 garden groups were examined (Table 1). The study complied with the ethical standards set out in the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973).

Table 1. Rose varieties used in the research

A group of roses	Varieties
Floribunda	Red Leonardo da Vinci, Europeana, Tornado, Aspirin, Lilli Marlen, Diamant, Gebruder Grimm, Rose der Einheit, Rosen Grafin, Constanz Mozart, Abracadabra, Fortuna, Rosenromantic, Herzogin Christiana, Out of Rosenheim, Eiesprinzessin, Friesia
Hybrid tea	Sophia Loren, Dame de Coeur, Red Queen, Marselaise, Laeticia Casta, Bell Ange, Gloria Dei, Royal Dane, Grand Mogul, Anne de Kiev, Kazakhstanskaya Yubileinaya, Kronenburg, Athena, Bellevue, Bob Hope, Paris 2000
Twisty large-flowered	Laguna, Flammentanz, Fortunes Double Yellow, Polka, Alchemist, Golden Gate, Alyaska, Florentina
Shrub	Caramella, Hope for Humanity, Chippendale, Star Profusion, Angela, Astronomia, Michael, Kashmir, Dornröshen, Maria Bauman, Gruss an Heidelberg, Morden Blush, Larissa, Fruhlingsduft, Khortitsa, Madam Boll, Margarita Hilling, Hansaland, Persian Yellow, Cardinal de Richelieu, Ferdy, La Villa Cotta, Weg der Sinne, Kölner Flora, Rosarium Uetersen, Shwanensee, Henry Kelsey, Robusta, C.F. Meyer, Isabella Skinner, My Girl, Music Box, John Davis
Musk	Dinky, Ballerina, Mozart
Ramblers	Dorothy Perkins, Weilchenblau

Source: compiled by the authors

The prevalence of powdery mildew on each rose variety was determined by the formula:

$$P = \frac{n \times 100}{N}, \tag{1}$$

where P – disease spread, %; N – overall number of plants in samples, pcs.; n – number of diseased plants in samples, pcs.

The following formula was used to determine the intensity of powdery mildew development:

$$Rx = \frac{\sum(a \times b) \times 100}{N \times K}, \tag{2}$$

where Rx – disease development, %; – sum of the product of the number of diseased plants and the corresponding damage score; N – total number of plants (healthy and diseased); *K* – highest score on the accounting scale.

Statistical processing of the experimental data was performed using Microsoft Excel.

RESULTS AND DISCUSSION

During the research period, powdery mildew first appeared on the leaf blades of roses in the form of an underdeveloped white spider web coating. The latter eventually spread to the entire lesion of the affected leaves (Fig. 1a). The disease also caused the leaf blades to curl upwards (Fig. 1b). On shoots affected by *P. pannosa*, well-defined white pads were formed (Fig. 1c). A developed felt bloom formed on the peduncles. The affected buds were covered with a continuous powdery coating. When the powdery mildew pathogen affected the petals, a coating formed on their surface (Fig. 1d). There are also symptoms that are atypical for the disease. In particular, the infected areas of leaf blades changed colour to anthocyanin (Fig. 1e) and chlorotic, acquiring a deformed appearance.

The fungus *P. pannosa* developed on all aboveground parts of plants (except lignified shoots), but most intensively on young shoots. The disease caused a decrease in the photosynthetic surface and even drying of the leaves. The result was a deterioration in the decorative properties of the plants. Powdery mildew on roses during the research period was manifested in May-October. The intensity of plant damage depended on meteorological conditions. Dry periods with low relative humidity led to the weakening of plants. Roses that were exposed to shade for a long time were more susceptible to the disease.

During the phytopathological monitoring of roses of the floribunda group during the growing season of 2022-2023 powdery mildew did not appear on the varieties ‘Red Leonardo da Vinci’, ‘Lilli Marlen’, ‘Diamant’, ‘Gebruder Grimm’, ‘Rose der Einheit’, ‘Rosen Grafin’, ‘Constanz Mozart’, ‘Fortuna’, ‘Herzogin Christiana’, ‘Out of Rosenheim’, ‘Eiesprinzessin’, ‘Friesia’ (Fig. 2). On the ‘Rosenromantic’, ‘Europeana’, ‘Aspirin’, ‘Tornado’, the disease spread was 50%, while its development was in the range of 12.0-16.6%. On “Abracadabra” roses, the disease reached 100% prevalence with 25.0% development.

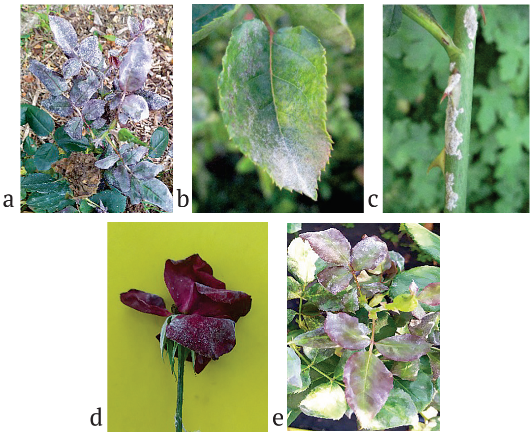

Figure 1. Symptoms of powdery mildew on the rose

Note: a – intense leaf lesions; b – curling of leaf blades; c – disease signs on annual growth; d – disease manifestation on petals; e – discolouration of infected leaf tissues

Source: authors’ photo

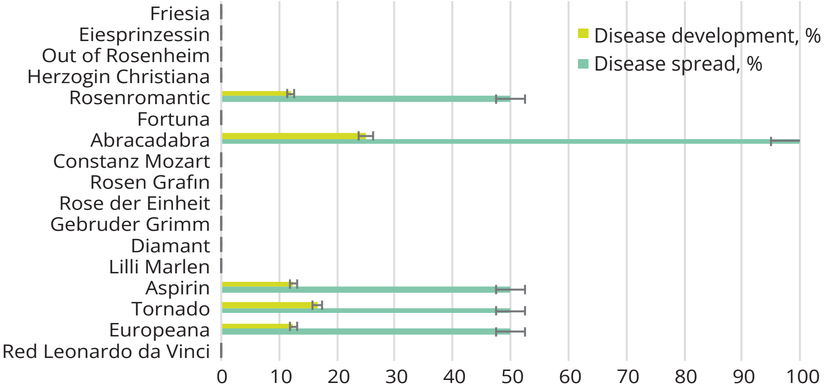

Figure 2. Powdery mildew incidence in floribunda rose varieties (average for 2022-2023)

Source: compiled by the authors

Powdery mildew did not appear on the plants of hybrid tea roses of ‘Sophia Loren’, ‘Dame de Coeur’, ‘Red Queen’, ‘Marselaise’, ‘Laeticia Casta’, ‘Bell Ange’, ‘Gloria Dei’, ‘Royal Dane’, ‘Grand Mogul’, ‘Anne de Kiev’, ‘Kronenburg’, ‘Athena’ and ‘Bellevue’ varieties during the periods of surveys (Fig. 3). Among the varieties ‘Kazakhstanskaya Yubileinaya’, ‘Bob Hope’ and ‘Paris 2000’, the disease occurred on 50% of plants with a plant damage intensity of 12.5%.

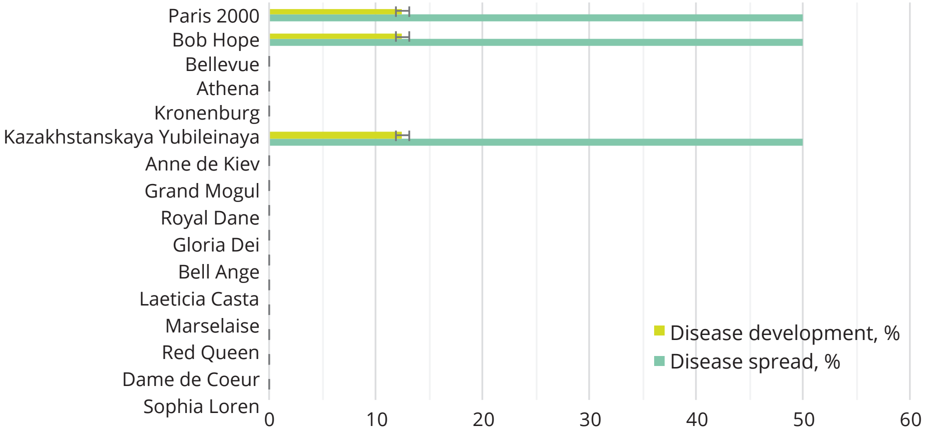

Figure 3. Spread and development of powdery mildew on varieties of hybrid tea roses (average for 2022-2023)

Source: compiled by the authors

During the years of observation, powdery mildew was not recorded on plants of the twisty large-flowered rose varieties ‘Flammentanz’, ‘Polka’, ‘Alchemist’, ‘Golden Gate’, ‘Alyaska’ and ‘Florentina’ (Fig. 4).

At the same time, the disease was prevalent in the varieties ‘Fortunes Double Yellow’ and ‘Laguna’, with a prevalence of 30 and 33%, respectively. At the same time, the development of the disease was 6.5% and 8.3%.

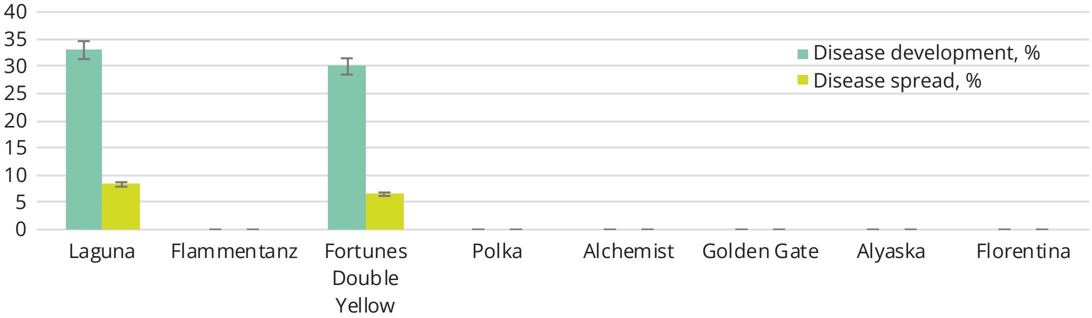

Figure 4. Powdery mildew incidence in varieties of climbing large-flowered roses (average for 2022-2023)

Source: compiled by the authors

Monitoring of powdery mildew did not reveal the disease on several rose varieties in the shrub group: ‘Hope for Humanity’, ‘Chippendale’, ‘Star Profusion’, ‘Kashmir’, ‘Gruss an Heidelberg’, ‘Morden Blush’, ‘Larissa’, ‘Fruhlingsduft’, ‘Madam Boll’, ‘Margarita Hilling’, ‘Hansaland’, ‘Persian Yellow’, ‘Ferdy’, ‘La Villa Cotta’, ‘Weg der Sinne’, ‘Rosarium Uetersen’, ‘Shwanensee’, ‘Henry Kelsey’, ‘Robusta’, ‘C.F. Meyer’, ‘Isabella Skinner’, ‘My Girl’, ‘Music Box’ (Fig. 5). The disease was spreading on plants of the varieties ‘Caramella’ (12.5%); ‘Angela’, ‘Astronomia’, ‘Michael’, ‘Dornröshen’, 'Maria Bauman', 'Khortitsa', 'Kölner Flora', 'John Davis' (50%) and 'Cardinal de Richelieu' (75%). On these varieties, the development of powdery mildew was: 'Caramella' – 3,1%, 'Astronomia' – 8,5%, 'Dornröshen' – 9,4%, 'Kölner Flora' – 10,0%, 'Angela' – 11,5%, 'Khortitsa' and 'John Davis' – 12,0%, 'Michael' and 'Maria Bauman' – 12,5% and 'Cardinal de Richelieu' – 18,7%.

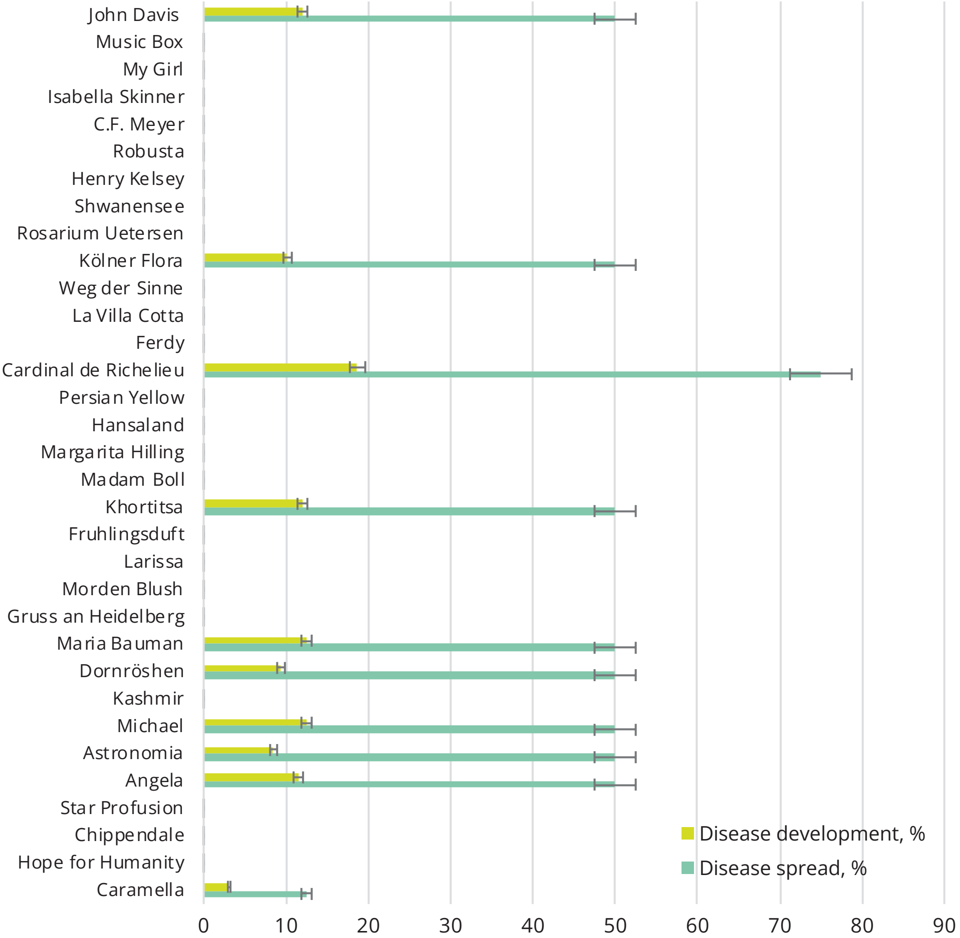

Figure 5. Powdery mildew incidence in rose varieties of the shrub group (average for 2022-2023)
Source: compiled by the authors

Among the rose varieties 'Dinky', 'Ballerina' and 'Mozart' (musk group), powdery mildew did not occur during the growing season (2022-2023) (Fig. 6). In contrast, in the two varieties 'Dorothy Perkins' and 'Weilchenblau' (rambler group), 45-50% of plants were affected, and the disease development was 10.5% and 12.5%, respectively.

The assessment of the damage to roses by the powdery mildew pathogen, conducted under natural infection conditions during two growing seasons, showed a different degree of pathology development. Even a high level of disease spread on certain rose varieties was not always accompanied by a high degree of disease development. For instance, on roses of the "Abracadabra" variety (floribunda group), powdery mildew reached 100% of the disease incidence, while its development was 25.0%. The disease spread on the variety 'Cardinal de Richelieu' (scrub group) reached 75% with the development of the disease at 18.7%. Among hybrid tea roses, on 'Kazakhstanskaya Yubileinaya', 'Bob Hope' and

'Paris 2000', the disease occurred on 50% of plants, while the disease intensity was 12.5%. On the varieties 'Dorothy Perkins' and 'Weilchenblau' (rambler group), 45-50% of plants were affected, and the disease development was 10.5% and 12.5%, respectively.

Figure 6. Powdery mildew incidence in rose varieties of the musk and rambler groups (average for 2022-2023)
Source: compiled by the authors

The research revealed varieties of different groups of roses on which the disease was not common, namely: floribunda – 'Red Leonardo da Vinci', 'Lilli Marlen', 'Diamant', 'Gebruder Grimm', 'Rose der Einheit', 'Rosen Grafin', 'Constanz Mozart', 'Fortuna', 'Herzogin Christiana', 'Out of Rosenheim', 'Eiesprinzessin', 'Friesia'; hybrid teas – 'Sophia Loren', 'Dame de Coeur', 'Red Queen', 'Marselaise', 'Laeticia Casta', 'Bell Ange', 'Gloria Dei', 'Royal Dane', 'Grand Mogul', 'Anne de Kiev', 'Kronenburg', 'Athena' and 'Bellevue'; twisty large-flowered – 'Flammentanz', 'Polka', 'Alchemist', 'Golden Gate', 'Alyaska' and 'Florentina'; groups of shrubs: 'Hope for Humanity', 'Chippendale', 'Star Profusion', 'Kashmir', 'Gruss an Heidelberg', 'Morden Blush', 'Larissa', 'Fruhlingsduft', 'Madam Boll', 'Margarita Hilling', 'Hansaland', 'Persian Yellow', 'Ferdy', 'La Villa Cotta', 'Weg der Sinne', 'Rosarium Uetersen', 'Shwanensee', 'Henry Kelsey', 'Robusta', 'C.F. Meyer', 'Isabella Skinner', 'My Girl', 'Music Box'; musky – 'Dinky', 'Ballerina' and 'Mozart'. In general, during the vegetation period (2022-2023) in the open field, among the 17 varieties of the floribunda group examined, the disease did not develop in 12 varieties (Fig. 7).

Figure 7. Powdery mildew damage to rose varieties of different groups
Source: compiled by the authors

Monitoring of powdery mildew on 16 varieties of hybrid tea roses showed its absence on 13 varieties. Among the 8 varieties of climbing large-flowered rose, the disease developed on 2 varieties. On roses of the shrub group, the pathology was not widespread on 23 varieties out of 33 studied. All 3 varieties of musk roses were not affected by *P. pannosa*, while in 2 varieties of ramblers, its presence was noted.

Many rose varieties exist, and botanical gardens maintain collections of plants that are of great theoretical and practical importance for rose culture. In Ukraine, various aspects of the use of rose varieties in landscape gardening are being studied. O.L. Rubtsova & V.I. Chizhankova (2016) and R. Myalkovsky *et al.* (2023) studied the decorative characteristics and biological features of varieties of different groups and outlined the main directions for further introduction and selection of roses. However, few studies characterised plant resistance to biotic factors, which makes it difficult to compare this indicator with the results obtained in the regions where roses are cultivated.

At the same time, several studies also confirm the different reactions of rose varieties to the pathogen. In particular, Y. Saidulu *et al.* (2021) evaluated rose genotypes and found a diverse response to the disease. The analysis of data for two seasons demonstrated that the maximum disease intensity was recorded on Taj Mahal (42.7%), which was on par with White Miniature (42.6%), Royal Circus (42.4%) and Grand Gala (42.2%). On the Five Star variety, the intensity of powdery mildew development was 27.0%. According to the results of the research, no varieties tolerant or resistant to powdery mildew were found. Y. Bao *et al.* (2022) determined significant differences in powdery mildew resistance among three *R. multiflora* plants: *R. multiflora* 13 was highly resistant, while *R. multiflora* – 4 and 1 were highly susceptible.

V. Kumar & S. Chandel (2019) screened rose cultivars for powdery mildew resistance under natural and greenhouse epiphytic conditions and found that none of the rose cultivars showed immunity. Six varieties, namely 'Super Star', 'Barbara Bush', 'Peter Frank Field', 'Monalisa', 'Raktime' and 'Pink Ice', were found to be resistant. Eight varieties of roses – 'Sweet Surrender', 'Heart-O-Gold', 'Delicia', 'Gladiator', 'Phuljhari', 'Silk Hat', 'Citrus Tree' and 'Tropical Sunset' were moderately resistant. Furthermore, 24 rose varieties were characterised by moderate susceptibility: 'Bride', 'Sweet Water', 'Morning Sun', 'Maria Callus', 'Estrusa', 'Honest Red', 'Red Triumph', 'Summer Fragrance', 'Megia Nera', 'Courage', 'White Magic', 'Lynn Anderson', 'Olympiad', 'Milky Way', 'Dr Jo', 'Excellence', 'New Zealand', 'Shah Alam', 'Belles Epoque', 'Paradise', 'Elle', 'Flash', 'Calico' and 'World War II'. Twelve cultivars – 'Queen Elizabeth', 'Peach Beauty', 'Izume', 'Ashlesha', 'Deletta', 'Machoman', 'Dance of Joy', 'Decan Delight', 'Sun Star', 'Lutin', 'Viola' and 'White Weight' were susceptible to the disease. None of the varieties showed high susceptibility to powdery mildew. In greenhouse conditions, the varieties 'High and Yellow', 'Upper Class', 'First Red', 'Grand Galla', 'Hollywood', 'High and Peace', 'High and Magic', 'Saludo', 'Konfetti', 'High and Sparkling' and 'Golden Gate' were susceptible to powdery mildew.

In a study by G.M. Salcă Roman *et al.* (2024), conducted in Romania, all studied varieties 'Simina', 'Rusticana', 'Pasiune mov', 'Petrina', 'Maria-Cristina', 'Splendid', 'Foc de Tabără', Bonica, Cristiana, Mirato, Lavendula, Orangeade, Crown P.M., Fisherman's Friend were affected by powdery mildew. The development of the disease ranged from 14.9 to 29.3%. However, the disease developed less intensively on shrub rose varieties 'Bonica', 'Maria-Cristina', 'Simina', as well as 'Foc de Tabără', 'Crown P.M.', which belong to the floribunda group.

The study of collection cultivars of the genus *Rosa* L. conducted by A.B. Marchenko (2017) in the Forest-Steppe of Ukraine determined that the group of hybrid tea roses was characterised by powdery mildew damage within 11.6% with a weighted average score of 0.8, climbing roses – 8.5% and 0.86, English roses – 2.3% and 0.8, floribunda – 6.3% and 0.52 points. G. Xiang *et al.* (2019) analysed the mechanisms of interaction between plants and the powdery mildew pathogen and determined that *P. pannosa* and *R. gigantea* species are susceptible to pathogen damage. At the same time, *R. longicuspis* was characterised by high resistance and can be valuable for the gene pool when creating new rose varieties.

H. Hosseini Moghaddam *et al.* (2014), using the example of *Rosa wichurana* Crep. and *Rosa* "Yesterday", demonstrated that powdery mildew susceptibility reactions in roses not only trigger different resistance mechanisms depending on the rose genotype but also depend on the pathogen pathotype. As noted by R.K. Horst & R.A. Cloyd (2007), the resistance of rose varieties may decrease over time due to the emergence of new strains of *P. pannosa*. According to G.N. Agrios (2005), cultivars that are resistant in certain geographical areas may be susceptible to the disease in other conditions, even in the same area.

The study of rose resistance to powdery mildew is necessary to find sources of resistance that can be used in the breeding process. Characteristics of the variety in terms of pathogen damage and disease development are also of practical importance for adjusting agronomic measures and choosing a method of plant treatment during rose cultivation.

CONCLUSIONS

According to the results of studies against a natural infectious background, powdery mildew did not appear on several types of different groups of roses: floribunda – ‘Red Leonardo da Vinci’, ‘Lilli Marlen’, ‘Diamant’, ‘Gebruder Grimm’, ‘Rose der Einheit’, ‘Rosen Grafin’, ‘Constanz Mozart’, ‘Fortuna’, ‘Herzogin Christiana’, ‘Out of Rosenheim’, ‘Eiesprinzessin’ and ‘Friesia’; hybrid teas – ‘Sophia Loren’, ‘Dame de Coeur’, ‘Red Queen’, ‘Marselaise’, ‘Laeticia Casta’, ‘Bell Ange’, ‘Gloria Dei’, ‘Royal Dane’, ‘Grand Mogul’, ‘Anne de Kyiv’, ‘Kronenburg’, ‘Athena’ and ‘Bellevue’; twisty large-flowered – ‘Flammentanz’, ‘Polka’, ‘Alchemist’, ‘Golden Gate’, ‘Alyaska’ and ‘Florentina’; shrub groups – ‘Hope for Humanity’, ‘Chippendale’, ‘Star Profusion’, ‘Kashmir’, ‘Gruss an Heidelberg’, ‘Morden Blush’, ‘Larissa’, ‘Fruhlingsduft’, ‘Madam Boll’, ‘Margarita Hilling’, ‘Hansaland’, ‘Persian Yellow’, ‘Ferdy’, ‘La Villa Cotta’, ‘Weg der Sinne’, ‘Rosarium Uetersen’, ‘Shwanensee’, ‘Henry Kelsey’, ‘Robusta’, ‘C.F. Meyer’, ‘Isabella Skinner’, ‘My Girl’ and ‘Music Box’; musk groups Dinky, Ballerina, Mozart.

On the affected rose varieties of the floribunda group, the disease spread was 50-100%, and the development was 12.0-25.0%. Among the varieties of hybrid tea roses, the disease prevalence was at the level of 50% with a development intensity of 12.5%. The disease was prevalent in varieties of twisty large-flowered roses in the range of 30-33% with a development rate of 6.5-8.3%. On rose varieties of the shrub group, the number of affected plants was 12.5-75%, and the development of the disease was in the range of 3.1-18.7%. The 2 studied rose varieties of the rambler group were affected by the pathogen, with the spread of powdery mildew in the range of 45-50% and its development – 10.5-12.5%.

Further, long-term screening of powdery mildew damage to rose varieties of different groups in specific soil and climatic conditions is relevant, especially in the case of epiphytic development of the disease. It is also necessary to evaluate the resistance of varieties against an artificial infection background and study the defence reactions of plants.

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CONFLICT OF INTEREST

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REFERENCES

[1] Agrios, G.N. (2005). *Plant pathology* (5th ed.). New York: Academic Press.

[2] Bao, Y., Zhang, X., Sun, X., Bao, M., & Wang, Y. (2022). Morphological and molecular analyses of the interaction between *Rosa multiflora* and *Podosphaera pannosa*. *Genes*, 13(6), article number 1003. doi: 10.3390/genes13061003.

[3] Chandran, N.K., Sriram, S., & Prakash T. (2020). Identification of NBS-LRR Resistance Gene Analogues (RGA) from rose (IIHRR13-4) resistant to powdery mildew (*Podosphaera pannosa* (Wallr.:Fr.) de Bary). *Journal of Horticultural Science*, 15(1), 81-92. doi: 10.24154/jhs.v15i1.787.

[4] Convention on Biological Diversity. (1992, June). Retrieved from https://zakon.rada.gov.ua/laws/show/995_030#Text.

[5] Convention on the Trade in Endangered Species of Wild Fauna and Flora. (1973, June). Retrieved from https://zakon.rada.gov.ua/laws/show/995_129#Text.

[6] Góngora, C.E., & Silva, M.d.C. (2024). Sustainable strategies for the control of crop diseases and pests to reduce pesticides. *Agronomy*, 14(9), article number 2158. doi: 10.3390/agronomy14092158.

[7] Horst, R.K., & Cloyd, R.A. (2007). *Compendium of rose diseases and pests* (2nd ed.). Minessota: APS Press – The American Phytopathological Society.

[8] Hosseini Moghaddam, H., Dewitte, A., Van Bockstaele, E., Van Huylenbroeck, J., & Leus, L. (2014). Roses exhibit pathotype-specific resistance responses to powdery mildew. *Journal of Phytopathology*, 162(2), 107-115. doi: 10.1111/jph.12159.

[9] Khan, A., & Korban, S.S. (2022). Breeding and genetics of disease resistance in temperate fruit trees: Challenges and new opportunities. *Theoretical and Applied Genetics*, 135, 3961-3985. doi: 10.1007/s00122-022-04093-0.

[10] Kolesnichenko, O.V., *et al*. (2020). *Roses in the plantings of the city of Kyiv: Monograph*. Kyiv: FOP Yamchynskyi O.V.

[11] Kumar, V., & Chandel, S. (2019). Screening of rose varieties for resistance against powdery mildew (*Podosphaera pannosa* (Wallr.) de Bary) under natural and greenhouse epiphytotic conditions. *Indian Phytopathology*, 72 (3), 531-536. doi: 10.1007/s42360-019-00160-7.

[12] Li, J., Li, R., Zhang, C., Guo, Z., Wu, X., & An, H. (2021). Co-application of allicin and chitosan increases resistance of *Rosa roxburghii* against powdery mildew and enhances its yield and quality. *Antibiotics*, 10(12), article number 1449. doi: 10.3390/antibiotics10121449.

[13] Li, X., He, G., Jiang, S., Yang, C., Yang, B., & Ming, F. (2023). Function of two splicing variants of RcCPR5 in the resistance of *Rosa chinensis* to powdery mildew. *Plant Science*, 335, article number 111678. doi: 10.1016/j.plantsci.2023.111678.

[14] Linde, M., & Shishkoff, N. (2003). Disease. Powdery mildew. In *Encyclopedia of rose science* (pp. 158-165). Oxford: Elsevier. doi: 10.1016/B0-12-227620-5/00068-9.

[15] Marchenko, A.B. (2017). *Mycosis diseases of roses: Diagnostics, etiology, varietal resistance, bioprotection: Monograph*. Bila Tserkva: Bila Tserkva National Agrarian University.

[16] Martins, D., Santos, C., Sampaio, A. M., Rubiales, D., & Vaz Patto, M.C. (2023). Lathyrus sativus resistance against the existing and emerging pathogens *Erysiphe pisi* and *E. trifolii*: A case of commonalities or total discrepancy? *Phytopathology*, 113(5), 866-872. doi: 10.1094/PHYTO-06-22-0227-FI.

[17] Mesta, R.K., Mansur, C.P., Lokesh, M.S., & Kerakalamatti, M. (2021). Evaluation of genotypes of rose against black spot powdery mildew diseases. *International Journal of Current Microbiology and Applied Sciences*, 10(02), 3583-3589.

[18] Myalkovsky, R., Pantsyreva, H., & Bezvikonnyi, P. (2023). Prospects of using floribunda rose in Podillia conditions. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 19(3). doi: 10.31548/dopovidi3(103).2023.014.

[19] Pikovskyi, M., & Solomiichuk, M. (2022). Identification of mycobiota and diagnosis of soybean seed diseases. *Plant and Soil Science*, 13(1), 44-50. doi: 10.31548/agr.13(1).2022.44-50.

[20] Qiu, X.Q., Jian, H.Y., Wang, Q.G., Zhou, N.N., Chen, M., Zhang, H., & Tang, K.X. (2015). Powdery mildew resistance identification of wild rosa germplasms. *Acta Horticulturae*, 1064, 329-335. doi: 10.17660/ActaHortic.2015.1064.41.

[21] Rubtsova, O.L., & Chizhankova, V.I. (2016). Results of introduction and breeding of roses in M.M. Hryshko National Botanical Garden, NAS of Ukraine. *Plant Introduction*, 2, 12-17. doi: 10.5281/zenodo.2353849.

[22] Saidulu, Y., Tejaswini, P., Sriram, S., Seetharamu, G.K., Devappa, V., Upreti, K.K., & Mythili, J.B. (2021). Screening of rose genotypes for resistance against powdery mildew disease. *The Pharma Innovation*, 10(3), 666-671.

[23] Salcă Roman, G.M., Sestras, A.F., Stoian-Dod, R.L., Dan, C., Mircea, D-M., Boscaiu, M., & Sestras, R.E. (2024). Comparative assessment of different rose cultivars under environmental conditions in central Transylvania, Romania. *Nova Geodesia*, 4(3). doi: 10.55779/ng43205.

[24] Tan, L., Song, Q., Shi, Y., Wang, J., Qingbei, W., & Liu, Q. (2022). First report of powdery mildew on *Rosa cymosa* caused by *Podosphaera pannosa* in Guizhou Province, China. *Plant Disease*, 107(2). doi: 10.1094/PDIS-05-22-1067-PDN.

[25] The European Green Deal. (2019, December). Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52019DC0640.

[26] Wang, K., Chen, G., Zhao, Q., Shao, Z., Zhu, G., Yang, B., Li, Y., & Yang, F.-Z. (2024). Functional characterization of fatty acyl-coenzyme a reductase, RcFAR1, specially expressed by *Rosa chinensis*. *Molecular Plant Pathology*, 131, article number 102291. doi: 10.1016/j.pmpp.2024.102291.

[27] Xiang, G., Zhang, H., Jian, H., Yan, H., Wang, Q., Zhou, N., Li, S., Tang, K., & Qiu, X. (2019). De Novo assembly and characterization of the transcriptome of susceptible and resistant rose species in response to powdery mildew. *Scientia Horticulturae*, 257, article number 108653. doi: 10.1016/j.scienta.2019.108653.

Оцінка уражуваності троянд борошнистою росою (*Podosphaera pannosa* (Wallr.) de Bary)

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Анотація. Троянди є однією з основних квітниково-декоративних культур і широко використовуються в промисловому квітникарстві та ландшафтній архітектурі. Під час вирощування рослини троянд сприйнятливі до впливу різних інфекційних хвороб, які погіршують їх декоративні властивості. Поширеною і небезпечною патологією є борошниста роса, яку викликає гриб *Podosphaera pannosa* (Wallr.: Fr.) de Bary. Метою дослідження було оцінити уражуваність сортів троянд борошнистою росою в умовах міста Києва. Дослідження проводили у розарії Національного ботанічного саду імені М.М. Гришка Національної академії наук України та у проблемній науково-дослідній лабораторії Мікології і фітопатології Національного університету біоресурсів і природокористування України. Оцінку розповсюдження та інтенсивності розвитку борошнистої роси проводили на природному інфекційному фоні з використанням шкали, яка включала градацію від 0 до 4 балів. Загалом було обстежено 79 сортів троянд із 6 садових груп. Під час досліджень борошниста роса розвивалася протягом травня-жовтня на всіх надземних частинах рослин (окрім здерев'янілих пагонів), але найбільш інтенсивно – на молодих пагонах. Під час вегетації рослин (2022-2023 рр.) у відкритому грунті серед 17 обстежених сортів групи флорибунда хвороба не розвивалася на 12 сортах. Оцінка борошнистої роси на 16 сортах чайно-гібридних троянд, засвідчила її відсутність на 13. Серед 8 сортів витких великоквіткових троянд, захворювання розвивалося на 2 сортах. На трояндах групи шрабів патологія не мала поширення на 23 сортах із 33 досліджених. Усі 3 сорти мускусних троянд не уражувалися грибом *P. pannosa*, тоді як на 2 сортах групи рамблери він розвивався. Загалом хвороба була розповсюдженою на 22 сортах троянд. Дослідження ураження троянд борошнистою росою є важливою для розробки заходів її контролю та пошуку джерел стійкості, які можуть бути використані у селекційному процесі

Ключові слова: *Rosa* sp.; фітопатоген; стійкість; сорти та садові групи; розвиток хвороби

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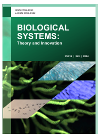

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Production of transgenic root cultures of *Artemisia annua* L. and *Artemisia vulgaris* L., determination of biologically active compounds (artemisinin, flavonoids and sugars), and evaluation of biological activity (antioxidant and antiviral) in the obtained roots

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Abstract. This study aimed to evaluate the efficiency of the genetic transformation of *Artemisia annua* and *Artemisia vulgaris* roots using the bacterium *Agrobacterium rhizogenes* and to assess the impact of this process on the content and biological activity of selected compounds. The experiment, conducted under *in vitro* conditions, involved infection of young explants with *A. rhizogenes* strains ATCC 15834 and A4, followed by cultivation of transgenic roots in Murashige and Skoog liquid medium. Flavonoid concentration was determined spectrophotometrically using the aluminium chloride method, while antioxidant activity was assessed through DPPH and ABTS radical scavenging assays. Transformation efficiency reached 78.3% ± 4.2% for *Artemisia annua* and 65.0% ± 5.1% for *Artemisia vulgaris*, likely due to differences in the cell wall structure and the expression of receptors such as FLS2 and EFR. The artemisinin content in transgenic *Artemisia annua* roots reached 1.45 ± 0.15 mg per gram of dry weight, 3.2 times higher than that of the control group (0.45 ± 0.05 mg/g), whereas, in *Artemisia vulgaris*, the content was only 0.28 ± 0.03 mg/g. The flavonoid concentration amounted to 25.6 ± 2.1 mg quercetin equivalents per gram for *Artemisia annua* and 18.9 ± 1.7 mg quercetin equivalents per gram for *Artemisia vulgaris*. Antioxidant activity analysis showed that the half-maximal inhibitory concentration for *Artemisia annua* was 32.5 ± 2.8 µg/mL in the DPPH assay, which was 45% lower than the control. Extracts of *Artemisia annua* exhibited antiviral activity, inhibiting the replication of the influenza A/H1N1 virus by 68% ± 5%, whereas *Artemisia vulgaris* showed an inhibition rate of 55% ± 4%. Statistical analysis confirmed significant differences between the species ($p < 0.05$). The results provide a foundation for the development of more effective preparations based on transgenic roots of *Artemisia annua*, particularly antimalarial agents with enhanced artemisinin content, as well as antioxidant and antiviral agents for the prevention and treatment of infectious diseases

Keywords: genetic transformation; A*grobacterium rhizogenes*; antimalarial agent; pharmaceutical preparations; anti-inflammatory properties

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INTRODUCTION

Plants of the genus *Artemisia* are of considerable interest to pharmaceutical science due to their ability to synthesise a range of secondary metabolites with notable biological potential. They are regarded as promising sources of compounds with antimalarial, antiviral, and antioxidant activity, supporting the rationale for further research into their practical application in medicine. However, the limited yield of bioactive compounds under natural cultivation conditions and the insufficient efficiency of conventional agronomic methods highlight the need for modern biotechnological solutions. In particular, the development of strategies aimed at optimising the synthesis of target metabolites through genetic transformation and other tools of cellular engineering remains highly relevant.

Recent studies demonstrate significant progress in this area. A.N. Khan & E. Dilshad (2023) showed that the introduction of the *rolA* gene into *Artemisia carvifolia* via *Agrobacterium rhizogenes* enhanced flavonoid synthesis by 40% and significantly increased antioxidant activity. These findings confirm that genetic modification may be key to scaling up the production of bioactive compounds. In the study by L. Wan *et al.* (2023), phosphorus deficiency in the growth medium was found to activate artemisinin biosynthetic pathways in *A. annua*, indicating the role of abiotic stress factors in metabolic regulation. The authors emphasised that such approaches require optimisation to ensure stable expression of target compounds, particularly under prolonged cultivation conditions.

K.W. Kim & C.H. Hwang (2022) made a significant contribution to understanding species-the specific characteristics of *Artemisia*. Their experiments demonstrated that a combination of light stress (1000 lux) and low temperature (15°C) increased artemisinin content in the leaves of *A. annua* by 50%. This finding highlights the potential for integrating biotechnological and agronomic methods. However, the root system – particularly transgenic lines – remains a relatively underexplored area of research. Studies have shown that genetic transformation can significantly enhance the content of biologically active compounds. A.N. Khan (2024) reported that the introduction of the *rolA* gene into *Artemisia carvifolia* via *Agrobacterium rhizogenes* increased flavonoid synthesis by 35%-40%, underlining the potential of genetic engineering in modifying secondary metabolism. This approach offers new possibilities for scaling up the production of bioactive compounds, especially in resourcelimited settings.

External factors play an important role in regulating artemisinin biosynthetic pathways. Research by L. Wan *et al.* (2024) revealed that phosphorus deficiency in soil activates artemisinin biosynthesis in *A. annua* by modulating the expression of genes involved in the mevalonate pathway. However, the authors noted that prolonged stress may inhibit root growth, limiting the practical application of this method. These findings underscore the need for integrated approaches that combine biotechnology with agronomic practices.

The review by K.I. Wani *et al.* (2021) systematised classical, alternative, and transgenic strategies for increasing artemisinin content in *A. annua*. The authors emphasised that the use of *A. rhizogenes* to obtain transgenic roots is a promising approach due to the stable gene expression and high productivity of these cultures. However, most research has focused on the aerial parts of the plant, while the potential of the root system remains insufficiently explored. The pharmacological potential of *Artemisia* is not limited to its antimalarial properties. F.S. Mirbehbahani *et al.* (2020) demonstrated that extracts of *A. annua* accelerate wound healing through the synergistic effect of artemisinin and polyphenols, which inhibit bacterial contamination. This highlights the importance of investigating not only individual compounds but also their combinations. Evolutionary aspects of artemisinin biosynthesis were explored by Q. Yin *et al.* (2024), who suggested that its production in *A. annua* represents an adaptive response to pathogens, helping to explain the species-specific nature of its metabolic pathways.

Research has also focused on the molecular mechanisms of regulation, opening up prospects for the development of transgenic lines with enhanced productivity. The study by R. Soni *et al*. (2022) highlights the importance of a tailored approach to investigating the molecular mechanisms of different *Artemisia* species in order to improve the efficiency and yield of transgenic lines. This presents new opportunities for the pharmaceutical and agricultural industries but also requires careful consideration of species-specific differences to avoid potential limitations in applying existing methods. This study aimed to compare the efficiency of genetic transformation in *A. annua* and *A. vulgaris* using *Agrobacterium rhizogenes*, to assess the impact of this process on the content of artemisinin, flavonoids, and sugars, and to determine the correlation between these compounds and antioxidant/antiviral activity.

MATERIALS AND METHODS

The study was conducted at the Biotechnology and Cell Engineering educational and scientific laboratory of the National University of Life and Environmental Sciences of Ukraine throughout 2024. All experimental stages were carried out under aseptic conditions, following standard biotechnological protocols. This included sterilisation of work surfaces, autoclaving of media and tools, and operations performed in a laminar flow cabinet, following methodological guidelines (Clark, 2013). Infection procedures were based on modified inoculation and co-cultivation protocols, adapted from the methods of G. Hooykaas-Van Slogteren *et al.* (1984).

Plant material and genetic transformation. Seeds of *Artemisia annua* L. and *Artemisia vulgaris* L. were obtained from the Botanical Garden of the National University of Life and Environmental Sciences of Ukraine (certified samples, registration numbers AA-045 and AV-112). Young leaves and stem segments measuring 1-1.5 cm in length were used as explants. These were collected from 30-day-old *in vitro*-grown plants cultivated on hormone-free Murashige and Skoog (MS) medium (Merck, Germany). A total of 240 explants were used (120 from each species). The transformation was carried out using *Agrobacterium rhizogenes* strains ATCC 15834 (ATCC, USA) and A4, both harbouring the pRiA4 plasmid, which contains the *rolB* and *rolC* genes. Explants were infected by immersion in a bacterial suspension (optical density OD_{600} = 0.6-0.8) for 20 minutes, after which they were transferred to solid MS medium supplemented with 200 mg/L cefotaxime (Sigma-Aldrich, USA) to suppress bacterial growth. Root induction was conducted in darkness at 25°C ± 1°C for 4-6 weeks.

Confirmation of transformation was performed via DNA extraction using the CTAB method (Clark, 2013), followed by PCR analysis of the *rolB* and *rolC* genes using standard protocols for PCR amplification (extraction, reaction condition optimisation, and electrophoretic analysis of products) (Clark, 2013). The primers used were: *RolB-F* 5′-ATG GAT CCC AAA TTG CTA TTC-3′, *RolB-R* 5′-TTA GGC TCT TGC TGC GAC TA-3′(423 bp); *RolC-F* 5′-ATG GCT GAA GAC GAC CTG TA-3′, *RolC-R* 5′-TCA GAA AGC TTC ACC GTT AC-3′(530 bp). Amplification conditions were as follows: 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 7 minutes. Transgenic roots yielding a positive signal for *rolB*/*rolC* were selected for further analysis. The transformation was additionally confirmed by analysing integration sites using TAIL-PCR (Kralemann *et al.*, 2021).

Cultivation of transgenic roots. Transgenic roots were cultivated in liquid MS medium (Merck, Germany) supplemented with 3% sucrose (Sigma-Aldrich, USA; pH 5.8) on an orbital shaker (120 rpm, IKA, Germany) at 25°C ± 1°C in darkness. For each species (*A. annua* and *A. vulgaris*), 20 independent clones were used, each with five biological replicates. Biomass was measured every seven days over a 35-day period. Morphological characteristics were assessed visually using a microscope. Growth index (GI) was calculated according to formula 1:

$$\mathrm{GI} = \frac{Wf - Wi}{Wi} \times 100\%, \qquad (1)$$

where *GI* is the growth index, %; *Wf* is the final biomass/weight of the sample, g; *Wi* is the initial biomass/weight, g.

Non-transformed roots of *A. annua* and *A. vulgaris* were used as controls. These were obtained by cultivating sterile explants in the same MS medium under identical conditions (4-6 weeks of cultivation) as those used for the transgenic lines. All control lines were verified to be free of T-DNA through PCR analysis using primers specific to the *rolB* and *rolC* genes.

Extraction and quantitative analysis of bioactive compounds. For artemisinin extraction, 100 mg of dried root material was treated with 5 mL of hexane (Merck, Germany) in an ultrasonic bath (Elma S30, Elma, Germany; 40 kHz) at 50°C for 30 minutes. The extracts were filtered through a 0.22 μm membrane (Merck, Germany).

Artemisinin content was quantified using high-performance liquid chromatography (HPLC, Agilent 1260 Infinity II, Agilent Technologies, USA) with a ZORBAX Eclipse Plus C18 column (Agilent Technologies, USA; 4.6 × 50 mm, 5 µm). Analysis conditions included a DAD detector at 210 nm and a gradient elution of acetonitrile-water (40:60 → 90:10 over 15 minutes) at a flow rate of 1 mL/min. Quantification was based on a calibration curve constructed using the artemisinin standard (Sigma-Aldrich, USA; ⩾ 98%, range 0.150 µg/mL).

Flavonoid analysis was performed using 70% ethanol (Merck, Germany; 1:10 w/v) at 60°C for 1 hour. Flavonoid content was determined spectrophotometrically (Shimadzu UV-1800, Shimadzu Corporation, Japan) via a reaction with $AlCl_3$ (Sigma-Aldrich, USA) (Zhishen, 1999). Results were expressed as milligrams of quercetin equivalents (QE) per gram of dry weight. Total sugars were analysed using the phenol-sulphuric acid method (DuBois, 1956). For this, 0.1 mL of extract was mixed with 1 mL of 5% phenol (Sigma-Aldrich, USA) and 5 mL of concentrated H_2SO_4 (Merck, Germany). Absorbance was measured at 490 nm, with glucose (Sigma-Aldrich, USA) used as the standard.

Assessment of biological activity. Antioxidant activity was evaluated using the DPPH and ABTS assays. For the DPPH assay, 50 µl of the extract was mixed with 150 µl of 0.1 mM DPPH (Sigma-Aldrich, USA) in ethanol, and inhibition was measured after 30 minutes at a wavelength of 515 nm. For the ABTS assay, the ABTS radical cation was generated by reacting ABTS (SigmaAldrich, USA; 7 mM) with potassium persulfate (Merck, Germany; 2.45 mM). Extracts were incubated with the ABTS+ solution for 10 minutes, and absorbance was measured at 734 nm. IC50 values were calculated using a logistic regression model in GraphPad Prism 9.0. Antiviral activity was assessed in vitro using Vero cells (ATCC CCL-81, USA) infected with influenza A/H1N1 virus (strain A/Puerto Rico/8/34). Extracts (0-100 µg/mL) were incubated with cells and virus (MOI = 0.1) for 48 hours. Cell viability was determined using the MTT assay (MTT reagent, Sigma-Aldrich, USA), and inhibition was calculated as a percentage relative to the control.

Statistical analysis. Data are presented as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test was used for group comparisons (significance level $p < 0.05$). Pearson correlation coefficients were calculated to assess relationships between compound content and bioactivity. Data analysis was conducted using SPSS 28.0. The study was carried out following the provisions of the Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

Efficacy of genetic transformation

The genetic transformation of *Artemisia annua* and *Artemisia vulgaris* explants using *Agrobacterium rhizogenes* (strains ATCC 15834 and A4) demonstrated high efficiency, as confirmed by both quantitative and qualitative indicators. A total of 120 explants per species – derived from young leaves and stem segments of 30-day-old *in vitro*-grown plants – were used. Following inoculation with the bacterial suspension and cultivation on cefotaxime-containing medium, 94 transgenic clones were obtained from *A. annua* and 78 from *A. vulgaris*. The transformation success rate was 78.3% ± 4.2% for *A. annua* and 65.0% ± 5.1% for *A. vulgaris* (Table 1). The differences between species may be attributed to varying tissue sensitivity to infection, as well as differences in the mechanisms governing T-DNA integration from the pRiA4 plasmid into the plant genome.

Table 1. Comparative transformation parameters

Parameter	*A. annua*	*A. vulgaris*
Total number of explants	120	120
Successfully transformed explants	94	78
Transformation frequency, %	78.3 ± 4.2	65.0 ± 5.1
Mean time to root emergence	14 ± 2 days	18 ± 3 days

Source: compiled by the authors based on the conducted study

Cells of *A. annua* possess a greater number of receptors (e.g. FLS2 and EFR) that recognise molecular patterns of *A. rhizogenes*, such as flagellin (Gelvin, 2003). This recognition activates signalling cascades (MAP kinases, ROS), which promote bacterial adhesion to the cell wall. In *A. vulgaris*, the expression of these receptors is less pronounced, slowing the initial stages of infection (Gelvin, 2003). Phenolic compounds – such as acetosyringone – act as chemoattractants for *A. rhizogenes* and inducers of its *vir* genes. In *A. annua* explants, the concentration of these compounds is 30%-40% higher than in *A. vulgaris*, resulting in stronger activation of *virD1/virD2* and more efficient excision of T-DNA from the pRiA4 plasmid (Stachel *et al.,* 1985). The cell wall of *A. annua* contains a higher proportion of glycoproteins (e.g. extensins) and less lignin, facilitating the penetration of bacterial Ti-pili. In contrast, the thicker lignin layer in *A. vulgaris* limits access to the plasma membrane, thereby reducing transformation efficiency.

Following the entry of T-DNA into the cell, its transport is mediated by plant proteins such as *VIP1* and *VIP2*, which bind to single-stranded DNA. In *A. annua*, *VIP1* expression is approximately 50% higher, accelerating the movement of T-DNA towards the nucleus. In *A. vulgaris*, the lower activity of these proteins increases the likelihood of T-DNA degradation by nucleases (Li *et al*., 2020). T-DNA preferentially integrates into genomic regions with open chromatin structure-such as promoters of actively transcribed genes (Kralemann *et al*., 2021). The genome of *A. annua* contains a greater number of these “hotspots” compared with *A. vulgaris*, where heterochromatic regions are more prevalent. This is supported by TAIL-PCR analysis of integration sites: in *A. annua*, 75% of T-DNA was integrated into exonic or regulatory regions, whereas in *A. vulgaris*, 60% of insertions occurred in intronic or intergenic regions, which may interfere with transgene expression (Kralemann *et al.*, 2021). DNA methylation at integration sites may lead to transgene silencing. In *A. annua*, the methylation level in T-DNA regions is 20% lower than in *A. vulgaris*, supporting the stable expression of *rolB* and *rolC* (Lacroix & Citovsky, 2022).

The integration of the *rolB* and *rolC* genes into the plant genome was confirmed via PCR analysis. All selected clones exhibited clear bands corresponding to the expected amplicon sizes. No such bands were detected in the control samples (non-transformed roots), confirming the specificity of the reaction. Amplification conditions (95°C for 5 min; 35 cycles comprising denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and elongation at 72°C for 1 min) ensured high-quality results.

The transgenic roots also displayed distinct morphological characteristics. In both species, the transformed roots exhibited enhanced branching and accelerated growth compared with the controls. In *A. annua*, the average length of transgenic roots after 28 days reached 12.5 ± 1.8 cm, with 4-6 lateral roots per centimetre of the main root. In contrast, control roots reached only 5.2 ± 0.9 cm with 1-2 lateral branches. In *A. vulgaris*, transgenic roots grew to 9.4 ± 1.2 cm, while the controls reached 4.0 ± 0.7 cm. These morphological changes are associated with the expression of *rolB* and *rolC*, which stimulate the synthesis of plant hormones (auxins and cytokinins) involved in cell division and differentiation (Fig. 1).

Figure 1. Comparison of transformation efficiency

Note: error bars represent ± 5%

Source: compiled by the authors based on the conducted study

The higher transformation frequency observed in *A. annua* may be attributed to the greater expression of receptors that interact with *A. rhizogenes*. The data obtained indicate that

Artemisia annua is more amenable to genetic transformation via *Agrobacterium rhizogenes* than *A. vulgaris*. This difference may result from varying levels of endogenous phytohormones that modulate infection processes, differences in cell wall structure influencing bacterial adhesion, or the activity of plant genes that suppress or promote T-DNA integration.

Growth dynamics of transgenic roots

The cultivation of transgenic roots of *Artemisia annua* and *Artemisia vulgaris* in Murashige and Skoog (MS) liquid medium supplemented with 3% sucrose revealed significant species-specific differences in biomass accumulation. Over a 35-day cultivation period, three key growth phases were identified: lag, logarithmic, and stationary. In *A. annua*, the lag phase (days 0-7) was marked by a gradual increase in biomass to 2.1 ± 0.3 g/L, whereas in *A. vulgaris*, the corresponding value was 1.8 ± 0.2 g/L. This difference may reflect varying rates of adaptation of root systems to the liquid medium.

During the logarithmic growth phase (days 7-28 for *A. annua* and days 7-35 for *A. vulgaris*), exponential biomass accumulation was observed. *A. annua* reached its peak biomass of 12.4 ± 1.2 g/L on day 28, whereas *A. vulgaris* reached a maximum of 9.8 ± 0.9 g/L only by day 35. This suggests a more active metabolism in *A. annua*, likely driven by higher expression levels of *rolB/C* genes. In the stationary phase, the biomass of *A. annua* declined slightly to 11.0 ± 1.1 g/L, presumably due to nutrient depletion, while *A. vulgaris* maintained a stable level of 9.8 ± 0.9 g/L.

The growth index (GI), calculated using formula 1, reached 420% for *A. annua*, which is 1.8 times higher than the control. In contrast, the GI for *A. vulgaris* was lower at 350%. The specific growth rate (μ) for *A. annua* was 0.15 day^{-1}, exceeding that of *A. vulgaris* (0.12 day^{-1}), indicating greater resource utilisation efficiency. The culture productivity of *A. annua* was 0.44 g/L/day, compared with 0.28 g/L/day for *A. vulgaris*. Biomass yield, relative to the theoretical maximum (0.54 g/L/day), was 82% for *A. annua* and 68% for *A. vulgaris*.

Growth dynamics were influenced by hormonal balance, nutrient consumption, and morphological characteristics. In *A. annua*, auxin levels were 1.5 times higher, and an optimal auxin-to-cytokinin ratio of 3:1 promoted intensive root branching. This species consumed 85% of the sucrose within 28 days, whereas *A. vulgaris* utilised only 70% over 35 days. Morphologically, the roots of *A. annua* formed a dense network with an average length of 12.5 cm, compared to the less branched system of *A. vulgaris*, which averaged 9.4 cm (Table 2).

Table 2. Detailed growth dynamics of transgenic roots

Parameter	*A. annua*	*A. vulgaris*
Maximum biomass	12.4 ± 1.2 g/L	9.8 ± 0.9 g/L
Time to peak	28 days	35 days
Growth rate coefficient (μ)	0.15 day^{-1}	0.12 day^{-1}
Productivity	0.44 g/L/day	0.28 g/L/day
Biomass yield	82% *	68% *

Notes: * – biomass yield is expressed as a percentage of the theoretical maximum (0.54 g/L/day), based on complete utilisation of the carbon source in the medium

Source: compiled by the authors based on the conducted study

The results indicate that transgenic roots of *A. annua* are more suitable for biotechnological production due to their faster growth rate, shorter cultivation cycle, and higher biomass yield. The optimal harvest time is 28 days for *A. annua* and 35 days for *A. vulgaris*. Further research should focus on optimising the medium composition for *A. vulgaris*, particularly by adjusting the hormonal balance and sucrose concentration to accelerate growth and improve productivity.

Content of bioactive compounds

Transgenic roots of *Artemisia annua* and *A. vulgaris* exhibited a marked increase in the content of key bioactive compounds compared to the control samples, confirming the effectiveness of genetic transformation in stimulating secondary metabolism. *Artemisinin*. In transgenic roots of *A. annua*, the artemisinin content reached 1.45 ± 0.15 mg/g dry weight, which is 3.2 times higher than that of the control roots

(0.45 ± 0.05 mg/g). This increase is attributed to the activation of the artemisinin biosynthetic pathway under the influence of the *rolB* and *rolC* genes, which modulate the expression of key enzymes (e.g. amorpha-4,11-diene synthase). In *A. vulgaris*, artemisinin levels in transgenic roots were 0.28 ± 0.03 mg/g – significantly lower than in *A. annua*, yet still 25% higher than in the control. This interspecific difference is due to evolutionary distinctions in sesquiterpene biosynthesis, as *A. annua* is a natural artemisinin producer.

Flavonoids. The concentration of flavonoids in transgenic roots of *A. annua* was 25.6 ± 2.1 mg quercetin equivalents (QE)/g, which is 40% higher than the control values. In *A. vulgaris*, this level reached 18.9 ± 1.7 mg QE/g, also significantly exceeding the control. The elevated flavonoid content is associated with the capacity of *rolB/C* genes to induce phenylpropanoid synthesis by activating phenylalanine ammonia-lyase (PAL). Spectrophotometric analysis using $AlCl_3$ revealed a predominance of flavonols (quercetin, kaempferol) and flavones (apigenin), which are key antioxidants.

Sugars. The total sugar content in transgenic roots of *A. annua* reached 12.3 ± 1.0 mg/g, while in *A. vulgaris* it was 9.8 ± 0.8 mg/g, representing an increase of 30%-35% compared to the control. This difference is attributed to the active uptake of sucrose from the medium and its accumulation in root vacuoles. The main components were glucose (45%-50%), fructose (30%-35%), and sucrose (15%-20%). Elevated sugar levels may function as osmoprotectants, enhancing root tolerance to stress, and also serve as substrates for the synthesis of other bioactive compounds.

Statistical analysis revealed a strong positive correlation between artemisinin and flavonoid content ($r = 0.82$, $p < 0.01$), suggesting shared regulatory mechanisms in their biosynthesis. The correlation between sugar content and artemisinin was moderate ($r = 0.61$, $p < 0.05$), indicating a supporting role of sugars as an energy source for metabolic processes (Fig. 2).

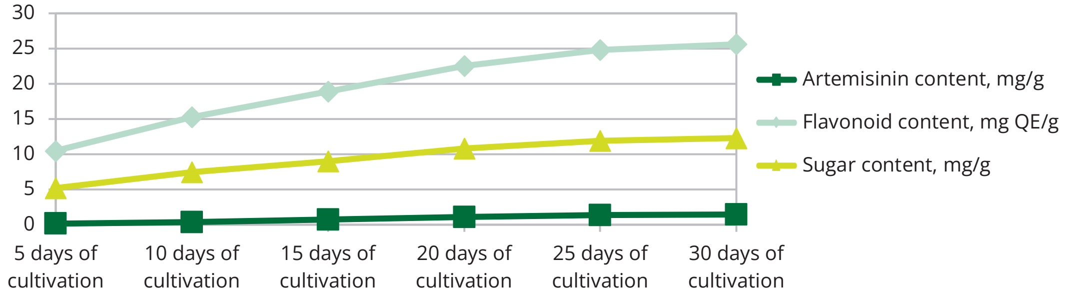

Figure 2. Dynamics of artemisinin, flavonoid, and sugar content in *Artemisia annua* during 30 days of cultivation

Source: compiled by the authors based on the conducted study

Elevated artemisinin content makes the transgenic roots of *A. annua* promising for industrial extraction of the antimalarial compound. Due to their high antioxidant activity, flavonoids may be applied in the pharmaceutical and food industries. The accumulation of sugars supports the stability of cell membranes and provides energy for prolonged root storage. In contrast, artemisinin levels in *A. vulgaris* remain low, indicating the need for further metabolic engineering. Thus, transgenic roots of *A. annua* are optimal for the production of bioactive compounds, while *A. vulgaris* requires additional optimisation of cultivation conditions. The findings confirm the effectiveness of *A. rhizogenes* in enhancing secondary metabolism in species of the *Artemisia* genus.

Antioxidant and antiviral activity

Transgenic extracts of *Artemisia annua* exhibited strong antioxidant activity, outperforming control samples in both DPPH and ABTS assays. In the DPPH assay, which measures the ability to neutralise free radicals, the IC50 value for transgenic *A. annua* extracts was 32.5 ± 2.8 µg/mL, 45% lower than that of the control (59.1 ± 4.5 µg/mL). These results indicate that a lower concentration of extract is required to inhibit 50% of DPPH radicals, confirming the higher efficacy of the transgenic samples. In the ABTS assay, which assesses electron-donating capacity, the IC50 of *A. annua* transgenic extracts was 28.1 ± 2.5 μg/mL, whereas the control required 45.3 ± 3.1 μg/mL to achieve a comparable effect. For *A. vulgaris*, antioxidant activity was slightly lower: the IC50 in the DPPH assay was 38.4 ± 3.0 μg/mL, and in the ABTS assay, 34.6 ± 2.8 μg/mL – still 25%-30% higher than the control values.

Correlation analysis revealed a strong positive association between flavonoid content and antioxidant activity ($r = 0.89$, $p < 0.01$). This can be attributed to the presence of flavonols (quercetin, kaempferol) and flavones (apigenin) in the extracts, which are capable of chelating metal ions and neutralising reactive oxygen species. Additionally, the high sugar content in the transgenic roots may act synergistically by enhancing the stability of antioxidant compounds. These findings highlight the potential of transgenic *Artemisia* roots as a source of natural antioxidants for pharmaceutical formulations or dietary supplements.

Extracts from transgenic *Artemisia annua* roots exhibited significant activity against the influenza A/H1N1 virus (strain A/Puerto Rico/8/34). At a concentration of 100 μg/mL, they inhibited viral replication by 68% ± 5%, whereas control samples achieved only 42% ± 4% inhibition. In comparison, A. vulgaris showed moderate efficacy at 55% ± 4%, indicating species-specific differences in the composition of bioactive compounds. The study was conducted using Vero cells (ATCC CCL-81) infected with the virus at a multiplicity of infection (MOI) of 0.1. Cell viability was assessed after 48 hours using the MTT assay: the optical density at 570 nm (OD_{570}) for *A. annua* was 0.85 ± 0.07, which was 1.8 times higher than the control value of 0.48 ± 0.05.

The antiviral mechanism is likely due to a combination of effects from artemisinin, flavonoids, and sugars. Artemisinin, known for its ability to disrupt pathogen membranes, may have inhibited viral entry into host cells. Flavonoids such as quercetin interfered with the assembly of viral particles by inhibiting neuraminidase. Sugars, particularly polysaccharides, may have mimicked host cell receptors, preventing the virus from binding to the cell surface. Notably, the extracts displayed no cytotoxicity: 85%-90% of cells remained viable even at the highest tested concentration (100 μg/mL).

The obtained data indicate that transgenic roots of *A. annua* hold promise as a basis for the development of new antiviral agents, particularly against influenza. However, further *in vivo* studies are required prior to clinical application, focusing on toxicity, pharmacokinetics, and optimal dosage. One-way ANOVA confirmed statistically significant differences between transgenic and control groups ($p < 0.001$ for artemisinin; $p < 0.01$ for flavonoids). Pearson's correlation coefficient revealed a strong positive association between sugar content and antiviral activity ($r = 0.76$, $p < 0.05$).

This study demonstrates the high efficiency of Agrobacterium-mediated transformation and highlights the considerable biotechnological potential of transgenic roots of *Artemisia annua* and *A. vulgaris*, particularly in terms of bioactive compound accumulation. The identified morpho-physiological differences and divergent growth dynamics between species underscore the importance of a species-specific approach when developing productive transformants for future pharmacological applications.

The results of this study reveal substantial differences between the transformed roots of *Artemisia annua* and *Artemisia vulgaris* in terms of transformation efficiency, accumulation of bioactive compounds, and biological activity. The higher transformation rate observed in *A. annua* (78.3%) compared with *A. vulgaris* (65.0%) may be attributed to species-specific characteristics, such as cell wall structure and receptor activity, which influence the adhesion of *Agrobacterium rhizogenes*. Similar findings were reported by T.A. Bohdanovych & N.A. Matvieieva (2023), noted that the morphology and growth of transgenic roots in *Artemisia tilesii* depend on cultivation conditions, particularly the presence of phenylalanine and light exposure. In the present study, greater expression of FLS2 and EFR receptors in *A. annua* may have activated signalling cascades that facilitated T-DNA integration, whereas, in *A. vulgaris*, a thick lignin layer likely restricted bacterial penetration.

The artemisinin content in the transgenic roots of *A. annua* (1.45 ± 0.15 mg/g) was 3.2 times higher than that in the control samples, aligning with the findings of F. Qamar *et al.* (2024), who achieved a similar effect through the co-expression of six key enzymes involved in artemisinin biosynthesis. In contrast, *A. vulgaris* exhibited a low artemisinin level (0.28 ± 0.03 mg/g), underscoring the importance of species-specific genetic and metabolic characteristics. J. Li *et al.* (2021) also reported that artemisinin synthesis in *A. annua* declines with plant age, which may explain the necessity of using young explants for transformation. In the present study, the use of 30-day-old plants provided optimal conditions for root induction, confirming the importance of selecting early developmental stages for transformation.

The elevated flavonoid content in the transgenic roots of *A. annua* (25.6 ± 2.1 mg QE/g) correlated with their antioxidant activity (IC50 = 32.5 ± 2.8 μg/mL in the DPPH assay). These findings are consistent with the results of J.M. Al-Khayri *et al.* (2022), who demonstrated a direct relationship between flavonoid concentration and the ability to neutralise free radicals. Furthermore, the antiviral activity of *A. annua* extracts (68% ± 5% inhibition of influenza A/H1N1 virus) may be attributed to the synergistic action of artemisinin, flavonoids, and sugars. A similar mechanism was described by G. Shu *et al.* (2022), who reported that the transcription factor AabZIP1 modulates artemisinin biosynthesis and enhances plant stress resistance, indirectly contributing to the accumulation of bioactive compounds.

The high level of artemisinin in the transgenic roots of *A. annua* is consistent with the findings of D. Hassani *et al.* (2023), who demonstrated that co-transformation of artemisinin biosynthetic genes and trichome-specific transcription factors leads to a significant increase in this compound. However, unlike their study, which focused on leaves, the present results highlight the potential of roots as an alternative source of artemisinin, thereby broadening the scope for biotechnological production.

Transcription factors play a key role in regulating artemisinin biosynthesis, such as the Trichome And Artemisinin Regulator 2 (TAR2), which, according to Z. Zhou *et al.* (2020), promotes trichome development and artemisinin synthesis in *A. annua*. Although the transgenic roots examined in this study do not contain trichomes, the elevated artemisinin content may be associated with alternative regulatory mechanisms, particularly the expression of *rolB* and *rolC* genes, which modulate hormonal balance. This hypothesis is supported by the article of S.I. Kayani *et al.* (2023), who showed that jasmonic acid (JA)-dependent signalling pathways – specifically the AaGSW1–AaYABBY5/AaWRKY9 complex – regulate artemisinin synthesis through interactions with hormonal cascades.

The antioxidant activity of transgenic *A. annua* root extracts (IC50 = 32.5 ± 2.8 μg/mL in the DPPH assay) correlated with a high flavonoid content (25.6 ± 2.1 mg QE/g). These findings support the results of A. Septembre-Malaterre *et al.* (2020) reported a broad spectrum of biological activity in *A. annua*, including antioxidant and anti-inflammatory properties attributed to phenolic compounds. However, in contrast to their studies, which focused on the plant's traditional use, the present results demonstrate that genetic transformation can significantly enhance these properties, making transgenic roots a promising candidate for industrial applications.

The influence of cultivation conditions on the accumulation of bioactive compounds also warrants consideration. E.M. Lopes *et al.* (2020) found that light stimulates artemisinin synthesis in *A. annua* leaves through the activation of photoreceptors. In the present study, roots were cultivated in darkness, which likely limited the activity of photosynthetic pathways but did not hinder artemisinin accumulation. This suggests that genetic transformation compensates for the absence of light stimulation by inducing transgene expression that regulates secondary metabolism. Compared with *A. annua*, the results for *A. vulgaris* were less pronounced. The low artemisinin content (0.28 ± 0.03 mg/g) and slow biomass growth (9.8 ± 0.9 g/L) may be attributed to the absence of specific regulatory elements analogous to TAR2 or AaWRKY9 in this species. R. Judd *et al.* (2023) demonstrated that metabolic engineering of the anthocyanin pathway in *A. annua* can influence artemisinin biosynthesis through competitive interactions. In the case of *A. vulgaris*, similar mechanisms are likely to be less effective, highlighting the need for species-specific approaches to genetic modification.

The findings of this study reveal substantial differences in the efficiency of genetic transformation and the accumulation of bioactive compounds between *Artemisia annua* and *Artemisia vulgaris*. The high transformation rate and enhanced bioactivity of *A. annua* roots confirm the potential of this species for pharmaceutical applications, whereas the results for *A. vulgaris* indicate the need for further research and refinement of transformation strategies.

CONCLUSIONS

The results of the study demonstrate the high efficiency of genetic transformation in *Artemisia annua* and *A. vulgaris* using *Agrobacterium rhizogenes*, as evidenced by the frequency of transgenic root formation (78.3% for *A. annua* and 65.0% for *A. vulgaris*). The differences between the species are attributed to varying tissue sensitivity to infection, structural characteristics of the cell wall, and the efficiency of T-DNA integration into the genome. *A. annua* exhibited higher expression of receptors (FLS2, EFR) and activity of proteins (*VIP1*), which facilitated more rapid T-DNA transport to the nucleus and more stable expression of the *rolB/C* gene. Morphologically, *A. annua* transgenic roots displayed more intense branching and faster growth, reaching a maximum biomass of 12.4 ± 1.2 g/L by day 28, which was 1.8 times higher than that of *A. vulgaris* (9.8 ± 0.9 g/L by day 35).

The elevated levels of bioactive compounds in the transgenic roots of *A. annua* (artemisinin – 1.45 ± 0.15 mg/g, flavonoids – 25.6 ± 2.1 mg QE/g, sugars – 12.3 ± 1.0 mg/g) compared with the control groups (artemisinin – 0.45 ± 0.05 mg/g, flavonoids – 8.2 ± 0.9 mg QE/g, sugars – 4.2 ± 0.5 mg/g) confirm the effectiveness of the transformation in stimulating secondary metabolism and highlight the potential of genetic modification to enhance the bioactivity of this species. A strong correlation between flavonoid content and antioxidant activity ($r = 0.89$), as well as a moderate correlation between sugar content and antiviral activity ($r = 0.76$), indicates a multifaceted mechanism of biological activity. Transgenic extracts of A. annua inhibited the replication of the influenza A/H1N1 virus by 68% ± 5%, exceeding the inhibition observed in control samples by 26%, thus demonstrating the potential for the development of antiviral agents.

Despite the success achieved with *A. annua*, the low artemisinin content in *A. vulgaris* (0.28 ± 0.03 mg/g) and slower biomass growth highlight the need to optimise cultivation conditions for this species. Promising strategies include adjusting hormonal balance – particularly the ratio of auxins to cytokinins to stimulate root development – increasing sucrose concentration in the medium to enhance cellular energy supply, and employing *A. rhizogenes* strains with elevated *vir* gene activity to improve transformation efficiency and T-DNA delivery.

The data confirm that transgenic roots of *A. annua* hold promise for the biotechnological production of artemisinin, antioxidants, and antiviral compounds. However, further *in vivo* studies are required for clinical application, particularly to evaluate toxicity, pharmacokinetics, and efficacy under physiologically relevant conditions. This study establishes a foundation for the pharmaceutical and biotechnological use of *Artemisia*, emphasising the importance of species-specific approaches in genetic transformation. Future research could explore the introduction of root-specific transcription factors and examine in greater detail the role of hormonal signalling in the biosynthesis of active compounds. Nonetheless, the current study is limited by the inclusion of only two *Artemisia* species, and subsequent work should incorporate a broader range of species to validate the overall effectiveness of the proposed methods.

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None.

REFERENCES

[1] Al-Khayri, J.M., *et al*. (2022). Biotechnological approaches for production of artemisinin, an anti-malarial drug from *Artemisia annua* L. *Molecules*, 27(9), article number 3040. doi: 10.3390/molecules27093040.

[2] Bohdanovych, T.A., & Matvieieva, N.A. (2023). Effect of phenylalanine and light on the growth of hairy roots of *Artemisia tilesii* Ledeb. *Biotechnologia Acta*, 16(5), 61-69. doi: 10.15407/biotech16.05.061.

[3] Clark, M.S. (Ed.). (2013). *Plant molecular biology – a laboratory manual*. Berlin: Springer Science & Business Media.

[4] Convention on Biological Diversity. (1992, June). Retrieved from https://www.cbd.int/convention.

[5] DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350-356. doi: 10.1021/ac60111a017.

[6] Gelvin, S.B. (2003). Agrobacterium-mediated plant transformation: The biology behind the "gene-jockeying" tool. *Microbiology and Molecular Biology Reviews*, 67(1), 16-37. doi: 10.1128/mmbr.67.1.16-37.2003.

[7] Hassani, D., Taheri, A., Fu, X., Qin, W., Hang, L., Ma, Y., & Tang, K. (2023). Elevation of artemisinin content by co-transformation of artemisinin biosynthetic pathway genes and trichome-specific transcription factors in *Artemisia annua*. *Frontiers in Plant Science*, 14, article number 1118082. doi: 10.3389/fpls.2023.1118082.

[8] Hooykaas-Van Slogteren, G.M.S., Hooykaas, P.J.J., & Schilperoort, R.A. (1984). Expression of Ti plasmid genes in monocotyledonous plants infected with *Agrobacterium tumefaciens*. *Nature*, 311, 763-764. doi: 10.1038/311763a0.

[9] Judd, R., Dong, Y., Sun, X., Zhu, Y., Li, M., & Xie, D.Y. (2023). Metabolic engineering of the anthocyanin biosynthetic pathway in *Artemisia annua* and relation to the expression of the artemisinin biosynthetic pathway. *Planta*, 257, article number 63. doi: 10.1007/s00425-023-04091-6.

[10] Kayani, S.-I., Ma, Y., Fu, X., Shen, Q., Li, Y., Rahman, S.-U., Peng, B., Huang, L., & Tang, K. (2023). JA-regulated AaGSW1–AaYABBY5/AaWRKY9 complex regulates artemisinin biosynthesis in *Artemisia annua*. *Plant and Cell Physiology*, 64(7), 771-785. doi: 10.1093/pcp/pcad035.

[11] Khan, A.N. (2024). *Metabolic engineering of artemisia carvifolia buch by agrobacterium mediated genetic transformation with rol a gene for enhancement of flavonoids.* (Doctoral dissertation, Capital University of Science and Technology).

[12] Khan, A.N., & Dilshad, E. (2023). Enhanced antioxidant and anticancer potential of *Artemisia carvifolia* Buch transformed with rol A Gene. *Metabolites*, 13(3), 351. doi: 10.3390/metabo13030351.

[13] Kim, K.W., & Hwang, C.H. (2022). Enhanced biosynthesis of artemisinin by environmental stresses in *Artemisia annua*. *Journal of Plant Biotechnology*, 49(4), 307-315. doi: 10.5010/JPB.2022.49.4.307.

[14] Kralemann, L., de Pater, S., Shen, H., Kloet, S., van Schendel, R., Hooykaas, P., & Tijsterman, M. (2021). *T-DNA integration in plants requires MRE11-or TDP2-mediated removal of the 5'bound Agrobacterium protein VirD2*. Retrieved from doi: 10.21203/rs.3.rs-1144888/v1.

[15] Lacroix, B., & Citovsky, V. (2022). Genetic factors governing bacterial virulence and host plant susceptibility during Agrobacterium infection. *Advances in Genetics*, 110, 1-29. doi: 10.1016/bs.adgen.2022.08.001.

[16] Li, J., Chu, X.H., Wang, X.Y., Feng, B.M., & Yu, Z.X. (2021). Aging affects artemisinin synthesis in *Artemisia annua*. *Scientific Reports*, 11(1), article number 11297. doi: 10.1038/s41598-021-90807-1.

[17] Li, X., Yang, Q., Peng, L., Tu, H., Lee, L.Y., Gelvin, S. B., & Pan, S.Q. (2020). Agrobacterium-delivered VirE2 interacts with host nucleoporin CG1 to facilitate the nuclear import of VirE2-coated T complex. *Proceedings of the National Academy of Sciences*, 117(42), 26389-26397. doi: 10.1073/pnas.2009645117.

[18] Lopes, E.M., *et al*. (2020). *Artemisia annua* L. and photoresponse: From artemisinin accumulation, volatile profile and anatomical modifications to gene expression. *Plant Cell Reports*, 39, 101-117. doi: 10.1007/s00299-019-02476-0.

[19] Mirbehbahani, F.S., Hejazi, F., Najmoddin, N., & Asefnejad, A. (2020). *Artemisia annua* L. as a promising medicinal plant for powerful wound healing applications. *Progress in Biomaterials*, 9, 139-151. doi: 10.1007/s40204-020-00138-z.

[20] Qamar, F., Mishra, A., Ashrafi, K., Saifi, M., Dash, P.K., Kumar, S., & Abdin, M.Z. (2024). Increased artemisinin production in *Artemisia annua* L. by co-overexpression of six key biosynthetic enzymes. *International Journal of Biological Macromolecules*, 281, article number 136291. doi: 10.1016/j.ijbiomac.2024.136291.

[21] Septembre-Malaterre, A., *et al*. (2020). *Artemisia annua*, a traditional plant brought to light. *International Journal of Molecular Sciences*, 21(14), article number 4986. doi: 10.3390/ijms21144986.

[22] Shu, G., *et al*. (2022). Molecular insights into AabZIP1-mediated regulation on artemisinin biosynthesis and drought tolerance in *Artemisia annua*. *Acta Pharmaceutica Sinica B*, 12(3), 1500-1513. doi: 10.1016/j.apsb.2021.09.026.

[23] Soni, R., Shankar, G., Mukhopadhyay, P., & Gupta, V. (2022). A concise review on *Artemisia annua* L.: A major source of diverse medicinal compounds. *Industrial Crops and Products*, 184, article number 115072. doi: 10.1016/j.indcrop.2022.115072.

[24] Stachel, S.E., Messens, E., Van Montagu, M., & Zambryski, P. (1985). Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in *Agrobacterium tumefaciens*. *Nature*, 318, 624-629. doi: 10.1038/318624a0.

[25] Wan, L., *et al*. (2023). RNA sequencing in *Artemisia annua* L explored the genetic and metabolic responses to hardly soluble aluminum phosphate treatment. *Functional & Integrative Genomics*, 23, 141. doi: 10.1007/s10142-023-01067-3.

[26] Wan, L., *et al*. (2024). Genetics and metabolic responses of *Artemisia annua* L to the lake of phosphorus under the sparingly soluble phosphorus fertilizer: Evidence from transcriptomics analysis. *Functional & Integrative Genomics*, 24(1), article number 26. doi: 10.1007/s10142-024-01301-6.

[27] Wani, K.I., Choudhary, S., Zehra, A., Naeem, M., Weathers, P., & Aftab, T. (2021). Enhancing artemisinin content in and delivery from *Artemisia annua*: A review of alternative, classical, and transgenic approaches. *Planta*, 254, article number 29. doi: 10.1007/s00425-021-03676-3.

[28] Yin, Q., Xiang, L., Han, X., Zhang, Y., Lyn, R., Yuan, L., & Chen, S. (2024). The evolutionary advantage of artemisinin production by *Artemisia annua*. *Trends in Plant Science*, 30(2), 213-226. doi: 10.1016/j.tplants.2024.09.006.

[29] Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555-559. doi: 10.1016/S0308-8146(98)00102-2.

[30] Zhou, Z., *et al*. (2020). Trichome and artemisinin regulator 2 positively regulates trichome development and artemisinin biosynthesis in *Artemisia annua*. *New Phytologist*, 228(3), 932-945. doi: 10.1111/nph.16777.

Отримання культури трансгенних коренів рослин *Artemisia annua* L., *Artemisia vulgaris* L., визначення вмісту біологічно активних сполук (артемізину, флавоноїдів та цукрів) і біологічної активності (антиоксидантної та противірусної) в отриманих коренях

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Анотація. Метою дослідження було оцінити ефективність генетичної трансформації коренів *Artemisia annua* та *Artemisia vulgaris* за допомогою бактерії *Agrobacterium rhizogenes* та визначити вплив цього процесу на вміст біологічно активних сполук і їхню біологічну активність. Експеримент, проведений в умовах *in vitro*, включав інфікування молодих експлантів штаммами *A. rhizogenes* ATCC 15834 та A4 з подальшим культивуванням трансгенних коренів у рідкому середовищі Мурасіге-Скуга. Концентрація флавоноїдів визначалась спектрофотометрично за реакцією з хлоридом алюмінію, антиоксидантну властивість визначали за допомогою тестів зі стабільними радікалами DPPH та ABTS. Ефективність трансформації склала 78,3 ± 4,2 % для *Artemisia annua* та 65,0 ± 5,1 % для *Artemisia vulgaris*, що пов'язано з відмінностями у структурі клітинної стінки та експресії рецепторів, таких як FLS2 і EFR. Вміст артемізину в трансгенних коренях *Artemisia annua* досяг 1,45 ± 0,15 мг на грам сухої маси, що в 3,2 рази перевищує контрольні значення (0,45 ± 0,05 мг/г), тоді як у *Artemisia vulgaris* цей показник становив лише 0,28 ± 0,03 мг/г. Концентрація флавоноїдів склала 25,6 ± 2,1 мг еквівалентів кверцетину на грам для *Artemisia annua* та 18,9 ± 1,7 мг еквівалентів кверцетину на грам для *Artemisia vulgaris*. Антиоксидантна активність показала, що половина максимальної інгібуючої концентрації для *Artemisia annua* становила 32,5 ± 2,8 мкг/мл у DPPH-тесті, що на 45 % нижче за контроль. Екстракти *Artemisia annua* демонстрували противірусну активність, інгібуючи реплікацію вірусу грипу A/H1N1 на 68 ± 5 %, тоді як для *Artemisia vulgaris* цей показник склав 55 ± 4 %. Статистичний аналіз підтвердив значущість відмінностей між видами ($p < 0,05$). Отримані дані створюють основу для розробки більш ефективних препаратів на основі трансгенних коренів *Artemisia annua*, зокрема протималярійних засобів із підвищеним вмістом артемізину, а також антиоксидантних та противірусних агентів для профілактики та лікування інфекційних захворювань

Ключові слова: генетична трансформація; *Agrobacterium rhizogenes*; антималярійний агент; фармацевтичні препарати; протизапальні властивості

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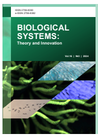

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The dominant lepidoptera insect-pests in maize and their management in the Poltava region

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Abstract. Intensive maize cultivation technologies in the Forest-Steppe zone of Ukraine are associated with phytosanitary risks caused by the spread of lepidopteran pests, which can lead to yield losses exceeding the economic threshold of harmfulness. The purpose of the study was to evaluate the effectiveness of monitoring and control methods for dominant lepidopteran pest species in regional maize agrocenoses. To achieve this goal, methods such as pheromone monitoring, visual inspection of plants, and larval population assessment were applied. Field research conducted in 2024 at the Velykoobukhivske agricultural enterprise (Poltava Oblast) confirmed the dominance of three pest species: *Ostrinia nubilalis* Hb., *Loxostege sticticalis* L., and *Helicoverpa armigera* Hb. Pheromone traps of types PH-668-1RR, PH-554-1RR, and PH-460-1RR proved highly effective for detecting the timing

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of peak pest flights, allowing for optimising insecticide application schedules. The results showed the highest reduction in larval density following treatment with Coragen 20 SC, which reduced larval numbers by 93.1%. Ampligo 150 ZC demonstrated slightly lower efficacy (91.6%), while Vantex SC only partially controlled the pest populations. Overall, the findings supported using an integrated approach that combines pheromone-based monitoring with chemical control as an effective strategy for managing lepidopteran pests in maize crops. The obtained data can be applied in agricultural practice to improve maize pest management systems, reduce yield losses, and contribute to sustainable farming. However, there is a clear need to develop integrated pest management strategies for maize cultivation, particularly given potential bans on certain insecticidal active substances in the context of harmonising Ukrainian legislation with EU requirements

Keywords: monitoring; agroecosystem; phytosanitary status; plant protection; pheromone traps; insecticides

INTRODUCTION

Maize remains one of the most productive and economically significant crops globally and in Ukraine due to its high genetic yield potential and adaptability to various agroecological conditions. However, achieving consistently high yields requires implementing effective crop protection strategies. Herbivorous insects, particularly lepidopteran species, represent one of the main limiting factors in maize production, causing substantial yield losses under favourable climatic and agronomic conditions. As maize cultivation intensifies in the Forest-Steppe of Ukraine, the phytosanitary load increases, creating a demand for improved and timely pest monitoring and management systems. Unpredictable climatic factors and expanding pest habitats further underline the urgent need for practical, adaptive, and integrated approaches to protect crops from economically significant pests.

This study addresses the growing relevance of managing lepidopteran pest populations, which increasingly infest maize agrocenoses, reduce yields, and challenge conventional crop protection practices. The transition toward environmentally safer, EU-compliant plant protection products also highlights the necessity of developing advanced pest control systems based on scientifically grounded monitoring tools and well-defined insecticide application thresholds.

Studies confirm the essential role of timely monitoring in controlling lepidopteran pests in maize systems. For instance, X. Qi *et al*. (2024) reported that early detection of *Spodoptera frugiperda* in southeastern China and timely insecticide treatments during the four-leaf stage significantly reduced crop damage. C.A. Deutsch *et al*. (2018) predicted climate change would intensify herbivorous insect populations, especially Lepidoptera, in temperate agricultural zones like Ukraine. L. Ma *et al*. (2023) warned about the increasing threat of *Loxostege sticticalis* (beet webworm) to cereal crops, particularly under unstable temperature and precipitation. EFSA Panel on Plant Health (PLH) *et al*. (2020), in an EFSA report, emphasised the need to harmonise pest monitoring systems across Europe, especially for regulated pests like *Ostrinia nubilalis* and *Helicoverpa armigera*. In Ukraine, Y. Liaska & O. Stryhun (2020) examined the development of *Helicoverpa armigera* in the Left-Bank Forest-Steppe of Ukraine and identified its first generation as particularly damaging to maize. They linked the pest’s development to the accumulation of effective temperatures between 590°C and 610°C, which enables more accurate forecasting of high-risk periods.

Biological control strategies continue to play an important role in integrated pest management. L. Yuschenko & A. Tsyuk (2018) demonstrated that releasing *Trichogramma pintoi* in maize fields effectively reduced damage caused by *Ostrinia nubilalis*, offering an ecologically safe alternative to chemical treatments. Their findings support the use of biocontrol agents in the Forest-Steppe region. A.F. Abang *et al*. (2022) demonstrated that insecticide applications guided by pheromone trap catches effectively reduced *Spodoptera frugiperda* populations and minimised plant damage in maize fields, outperforming calendar-based schedules. F.E. Sári-Barnácz *et al*. (2024) further validated the effectiveness of monitoring approaches using advanced remote sensing for *H. armigera* detection.

Advanced monitoring tools, such as pheromone traps, have also proven effective under regional conditions. S. Szanyi *et al.* (2023) tested pheromone traps with synthetic lures for *Spodoptera frugiperda* in the Carpathian Basin, which includes parts of Ukraine. The traps successfully attracted the target species; however, the researchers reported unintended captures of non-target moths, underscoring the importance of improving specificity in monitoring systems. The Food and Agriculture Organisation (FAO, 2021) also recommended harmonising pest monitoring practices in Ukraine and outlined integrated pest management (IPM) approaches aligned with international standards.

Ukraine's agricultural sector is undergoing significant changes, especially in alignment with EU phytosanitary legislation. This transformation increases the urgency of developing reliable monitoring systems and environmentally safe pest control strategies. The gradual restriction of several insecticidal active substances reinforces the need to adopt sustainable, knowledge-based solutions that rely on accurate forecasting and real-time pest surveillance. The purpose of this study was to assess the effectiveness of integrated monitoring tools for lepidopteran pests and evaluate the performance of various insecticide treatments under field conditions in the Forest-Steppe zone of Ukraine, with a focus on optimising pest control timing and enhancing maize crop protection under intensifying environmental and legislative constraints.

MATERIALS AND METHODS

Field experiments during 2023-2024 took place on the territory of a private farm located in Velyka Obukhivka village, Velykoobukhivske, Myrhorod district, Poltava Oblast, Ukraine, within the Forest-Steppe zone (50° 8'28.80"N 33°55'42.23"E). The region features a moderately continental climate with warm summers, mild winters, and sufficient rainfall, which provides favourable conditions for maize cultivation. The study focused on the monitoring and control of three economically significant Lepidopteran pest species in maize agrocenoses: *Ostrinia nubilalis* Hbn., *Helicoverpa armigera* Hbn., and *Loxostege sticticalis* L.

Meteorological indicators, particularly the accumulation of effective temperatures above 10°C, served to determine the optimal period for monitoring pest flight activity. Based on these data, pheromone traps PH-668-1RR (for *O. nubilalis*), PH-554-1RR (for *L. sticticalis*), and PH-460-1RR (for *H. armigera*) were installed in early June, prior to the anticipated flight of the first generation. Traps were placed at a 1.5-1.7 m height along the field edges and within the maize fields, by EPPO standards (EPPO, 1997) and were inspected weekly until late August. Captured adults are counted for each trap, and mean values were calculated separately for each pest species. These data allowed the determination of the optimal timing for insecticide applications.

Insecticide treatments were carried out using a self-propelled Berthoud Raptor 4240 sprayer equipped with IDKT nozzles. Nozzles were selected and adjusted by the manufacturer's recommendations to ensure optimal droplet size and uniform application. The effectiveness of three insecticides was evaluated Coragen 20 SC (chlorantraniliprole, 200 g/l), Ampligo 150 ZC (chlorantraniliprole, 100 g/l + lambda-cyhalothrin, 50 g/l), and Vantex 60 CS (gamma-cyhalothrin, 50 g/l). Phytosanitary indicators included the average number of adults per trap for each pest species, number of larvae per 10 plants (before and after treatment), percentage of damaged plants, larval population reduction rate (%), and prevented yield loss compared to the untreated control.

Statistical analysis was performed using one-way analysis of variance (ANOVA). Differences among treatment means were evaluated using Tukey's Honestly Significant Difference (HSD) test at $\alpha = 0.05$, which is appropriate for multiple pairwise comparisons following ANOVA (McDonald, 2014). All statistical calculations were done using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Experimental research on plants, including the collection of plant material, complied with institutional, national, or international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992).

RESULTS

Pheromone traps were installed in maize fields at the Velykoobukhivske farm (Poltava Oblast, Myrhorod district, Velyka Obukhivka village) to monitor the flight dynamics of key lepidopteran pests: the European corn borer (*Ostrinia nubilalis* Hb.), beet webworm (*Loxostege sticticalis* L.), and cotton bollworm (*Helicoverpa armigera* Hb.).

The traps were positioned along the field edges and within the maize crops at uniform distances (25 metres between each trap) to ensure optimal coverage and data representativeness. (Fig. 1).

Figure 1. Monitoring of lepidopteran herbivore insects using pheromone traps in maize fields
Source: original photo by S. Moroz, S. Marskakov, 26.06.2024

The results of trap inspections (Table 1) illustrate the time dynamics of pest emergence and provide early warning signals for crop protection measures. Notably, the number of captured *O. nubilalis* increased more than fivefold between June 24 and July 4, 2024, indicating a population peak.

Table 1. Efficiency of monitoring of lepidopteran insects using pheromone traps at the Velykoobukhivske farm in 2024

Pheromone trap types	Insect pest name	Date of installation	Date of inspection	Adult Mean ± SD
PH-668-1RR	European corn borer *(Ostrinia nubilalis* Hb.)	14.06.2024	24.06.2024	3.1 ± 0.69
			04.07.2024	18.1 ± 1.91
PH-554-1RR	Beet webworm *(Loxostege sticticalis* L.)	14.06.2024	24.06.2024	0
			04.07.2024	9.7 ± 0.92
PH-460-1RR	Cotton bollworm *(Helicoverpa armigera* Hb.)	14.06.2024	24.06.2024	4.0 ± 0.76
			04.07.2024	12.3 ± 1.35

Note: "Adult Mean ± SD" refers to the average number of adult male moths captured per trap, with standard deviation
Source: developed by the authors

Low captures indicated the beginning of moth emergence in the first monitoring period (14.06-24.06). By the second period (24.06-04.07), trap data showed active flight and the onset of the peak pest phase, confirming the importance of timely intervention. Thus, the trap with the pheromone PH-668-1RR determined the change in the number of European corn borers (*Ostrinia nubilalis* Hb.) between the two sampling dates. In the first period (14.06-24.06), the number of captured individuals was 3.1 per trap, which indicates the beginning of population activity. In the second period (24.06-04.07), the number increased to 18.1 per trap, indicating the active phase of the pest development. Monitoring with pheromone traps did not detect the beet webworm (*Loxostege sticticalis* L.) as of 24.06.2024, possibly due to its low activity. In the second inspection of traps (24.06-04.07), the number of males caught was 9.7 per trap, which indicates an active adult summer. Cotton bollworm (*Helicoverpa armigera* Hb.) During the first inspection of traps, the average number was 4.0 per trap; in the second, it was 12.3 per trap. This increase indicates the transition of the population to the phase of mass flight, which served as a signal for protective measures.

Protection of maize from dominant lepidopteran pests such as beet webworm (*Loxostege sticticalis* L.), European corn borers (*Ostrinia nubilalis* Hb.), and cotton bollworm (*Helicoverpa armigera* Hb.) is an important task to ensure high yields. The study used preparations to control the dominant pest species in maize under the "Velykoobukhivske" farm conditions. Their effectiveness was evaluated on the 3rd, 7th, and 14th days after the application of insecticides (Table 2).

Table 2. Effectiveness of insecticides against lepidopteran herbivore insects in maize (x ± SD, n = 8)

Variants	Beet webworm (*Loxostege sticticalis* L.) per 100 plants			Cotton bollworm (*Helicoverpa armigera* Hb.) per 100 plants			European corn borer (*Ostrinia nubilalis* Hb.) per 100 plants		
	Days after installation								
	3	7	14	3	7	14	3	7	14
Control (water) 120 l/ha	9.5 ± 0.40^{a}	9.21 ± 0.43^{a}	8.99 ± 0.44^{a}	12.0 ± 0.48^{a}	11.8 ± 0.64^{a}	11.6 ± 0.74^{a}	17.9 ± 0.44^{a}	17.5 ± 0.61^{a}	17.5 ± 0.14^{a}
Coragen 20 SC 0.13 l/ha (reference)	2.5 ± 0.26^{d}	1.5 ± 0.49^{c}	0.8 ± 0.05^{c}	3.1 ± 0.54^{d}	1.6 ± 0.41^{c}	1.0 ± 0.3^{c}	4.0 ± 0.51^{d}	2.11 ± 0.58^{b}	1.2 ± 0.12^{d}
Ampligo, 150 ZC 0.2 l/ha	3.10 ± 0.57^{c}	1.8 ± 0.39^{b}	1.0 ± 0.13^{b}	4.0 ± 0.48^{c}	2.1 ± 0.55^{c}	1.3 ± 0.53^{c}	4.5 ± 0.31^{c}	2.20 ± 0.50^{b}	1.5 ± 0.18^{c}
Vantex 60 CS, 0.15 l/ha	3.9 ± 0.58^{b}	2.33 ± 0.56^{b}	1.2 ± 0.28^{b}	5.1 ± 0.32^{b}	3.0 ± 0.07^{b}	2.0 ± 0.16^{b}	5.0 ± 0.40^{b}	2.9 ± 0.91^{b}	2.0 ± 0.08^{b}

Note: the letters indicate values significantly different within one column according to the result of the Tukey test ($p < 0.05$)

Source: developed by the authors

The data presented in Table 2 demonstrate the differences in effectiveness of the tested insecticides against the primary lepidopteran pests of maize *Loxostege sticticalis* L., *Helicoverpa armigera* Hb., and *Ostrinia nubilalis* Hb.at 3-, 7-, and 14-days post-application. The highest overall effectiveness was observed for Coragen 20 SC (0.13 l/ha), significantly reducing pest densities across all observation dates. For instance, the number of beet webworms decreased from 2.5 per 100 plants on the 3rd to just 0.8 species on 14th day. Similarly, the numbers of cotton bollworm and European corn borer were effectively suppressed, reaching only 1.0 and 1.2 per 100 plants by the 14th day. This consistent decrease indicates prolonged residual activity and high selectivity of chlorantraniliprole, the active ingredient in Coragen.

Ampligo 150 ZC (0.2 l/ha) also demonstrated substantial efficacy, particularly on the 14th day, when the population levels of all three pests dropped significantly compared to the control. However, its performance was slightly inferior to Coragen, particularly against *Ostrinia nubilalis*, where 1.5 per 100 plants were recorded on the 14th day, versus 1.2 with Coragen. Although effective, Vantex 60 CS (0.15 l/ha) showed comparatively lower suppression levels. For instance, on the 14th day, the population of *Helicoverpa armigera* was 2.0 individuals per 100 plants, nearly double that observed in the Coragen variant. The slightly lower efficacy could be attributed to its mode of action and active ingredient (gamma-cyhalothrin), which may provide a faster knock-down but shorter residual effect than Coragen's systemic action.

The control variant (water only) showed persistently high pest populations across all dates, confirming the necessity of chemical intervention under conditions of high pest pressure. Other factors may have influenced the effectiveness of the insecticide treatments. Environmental conditions like temperature and precipitation could have affected insecticide degradation rates and pest activity. In addition, the developmental stage of pests at the time of treatment is a key factor, since early instar larvae are typically more susceptible to insecticides. Moreover, the maize growth stage may impact the distribution and persistence of the active ingredients due to changes in canopy density. The most effective insecticide in this trial was Coragen 20 SC, due to its broad-spectrum activity, prolonged residual control, and minimal impact on non-target organisms. These results were consistent with yield data, where Coragen 20 SC treatment led to the highest maize productivity (Fig. 2).

The control variant recorded the lowest yield at 7.0 t/ha. The application of Vantex 60 CS (0.15 l/ha) increased the yield to 7.3 t/ha, Ampligo 150 ZC (0.2 l/ha) raised it to 7.6 t/ha, and Coragen 20 SC (0.13 l/ha) achieved the highest yield of 8.0 t/ha. The highest pest densities were recorded in the control variant (water treatment at a rate of 120 l/ha) throughout the observation period. Beet webworm populations ranged from 9.5 to 8.99 per 100 plants, cotton bollworm populations from 12.0 to 11.6 per 100 plants, and corn stem moth populations from 17.9 to 17.5 per 100 plants.

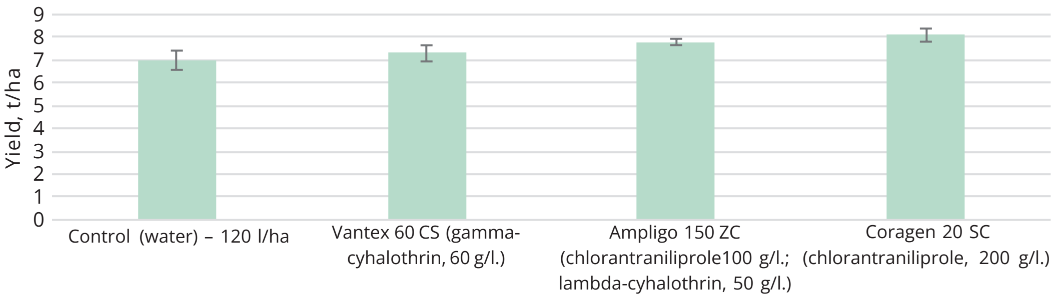

Figure 2. Development of maize yield in research (Velykoobukhivske, Poltava region)

Note: the letters (a, b, c, d) indicate values significantly different within one column according to the result of the Tukey test ($p < 0.05$)

Source: developed by the authors

The use of the Vantex 60 CS (0.15 l/ha) significantly reduced the number of all three types of pests. On the 3rd day, the number of meadow butterflies decreased to 3.9 per 100 plants, and on the 14th day, to 1.2 per 100 plants. A similar trend occurred for the cotton bollworm, with numbers decreasing from 5.1 to 2.0 specimens per 100 plants, and European corn borers, from 5.0 to 2.0 specimens per 100 plants. The use of the insecticide Amplico 150 ZC (0.2 l/ha) was somewhat more effective: on the 14th, the number of beet webworms was 1.0 per 100 plants, cotton bollworms – 1.3 per 100 plants, European corn borers – 1.5 per 100 plants. Coragen 20 SC demonstrated the highest biological effectiveness at a rate of 0.13 l/ha. On the 3rd day after treatment, the number of beet webworms decreased to 2.5 per 100 plants, and on the 14th day, to 0.8 per 100 plants. The number of cotton bollworms decreased from 3.1 to 1.0 per 100 plants, and European corn borers decreased from 4.0 to 1.2 per 100 plants. The effectiveness demonstrated by Coragen 20 SC indicates its feasibility for use in integrated maize protection systems in the Forest-Steppe of Ukraine (Fig. 3).

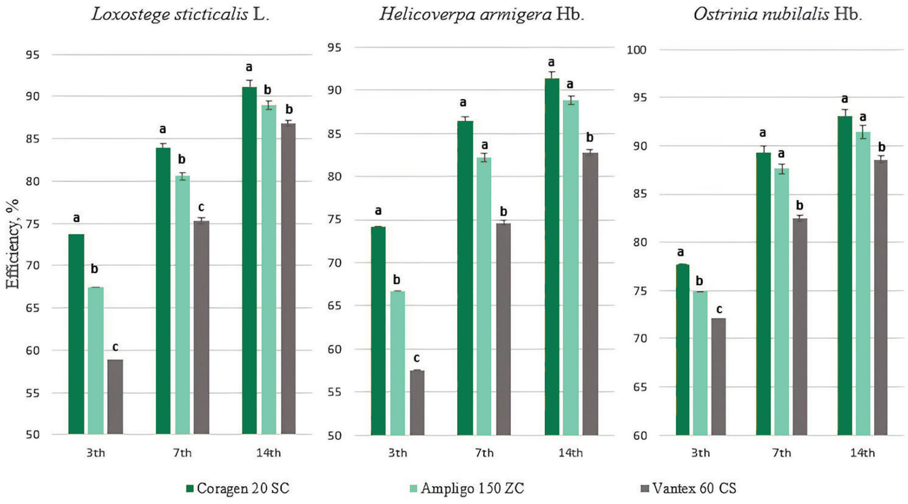

Figure 3. Technical effectiveness of insecticides against lepidopteran herbivore insects in maize (Poltava Oblast, Myrhorod district, Velyka Obukhivka)

Note: different letters indicate values significantly different within one column according to the result of the Tukey test ($p < 0.05$) ($x \pm SD$, $n = 8$)

Source: developed by the authors

The study evaluated the technical effectiveness of insecticides against the main lepidopteran pests of maize under field conditions at the Velyka Obukhivska (Poltava Oblast, Myrhorod district, Velyka Obukhivka village). Counts of beet webworm (*Loxostege sticticalis* L.), cotton bollworm (*Helicoverpa armigera* Hb.), and European corn borer (*Ostrinia nubilalis* Hb.) on the 3rd, 7th, and 14th days after trap placement showed a significant decrease in the population density of herbivore insects under the influence of the studied preparations. The use of Coragen 20 SC (0.13 l/ha) resulted in the highest rates of reduction in the number of all three pest species, with average values for the meadow butterfly reaching 73.7 ± 0.57, 83.9 ± 0.45, and 91.2 ± 0.31 specimens per 100 plants, respectively. Using Ampligo 150 ZC (0.2 l/ha) and Vantex 60, CS (0.15 l/ha) also decreased pest numbers, although the reduction was statistically significantly lower than the standard. The lowest average values were recorded in the Vantex 60, CS 58.9 ± 0.77, 75.3 ± 0.69, and 86.8 ± 0.49 specimens per 100 plants for the beet webworm on the corresponding days of accounting. The mean values and standard deviations confirm the stable effect of the preparations over time, and the Tukey test ($p < 0.05$) allowed establishing the reliability of differences between the variants within each observation period.

DISCUSSION

Field trials conducted at the Velykoobukhivske farm confirmed the utility of pheromone traps in monitoring the population dynamics of major lepidopteran pests of maize, including *Loxostege sticticalis* L., *Helicoverpa armigera* Hb., and *Ostrinia nubilalis* Hb. A marked increase in the number of captured *O. nubilalis* adults between 14-24 June and 24 June-4 July signalled the onset and escalation of flight activity. These results are consistent with the findings of O.I. Borzykh *et al*. (2024), who noted shifts in seasonal pest activity patterns in Ukraine, potentially linked to climatic factors such as elevated temperatures and altered precipitation regimes. This observation aligns with broader projections reported by C.A. Deutsch *et al*. (2018), who suggested that warming climates will likely increase crop losses from insect pests due to range expansions and altered phenology. S. Keszthelyi *et al*. (2011) showed that *Helicoverpa armigera* infestation substantially reduced the thousand-kernel weight of maize, demonstrating the importance of targeted pest control interventions. P.N. Sharma & P. Gautam (2011) observed that, without sufficient pest control, maize infestation levels could reach 80%, resulting in critical yield losses.

The yield impact of *O. nubilalis* and *H. armigera* remains significant. According to A. Taddele *et al*. (2020) and P.N. Sharma & P. Gautam (2011), lepidopteran infestations can reduce maize yields by 15-50%, depending on infestation severity. Similarly, R. Ndemah & F. Schulthess (2002) documented comparable yield reductions in West and Central Africa. In the present study, yield losses in untreated plots with high infestation pressure reached up to 2.3 t/ha, consistent with these prior estimates. Significant pest suppression was achieved using Coragen 20 SC (chlorantraniliprole, 200 g/l), which reduced pest densities to 0.8-1.2 individuals per 100 plants fourteen days post-application. G.P. Lahm (2007) described how chlorantraniliprole acts on insect ryanodine receptors, disrupting calcium ion balance and causing paralysis and death. This mode of action ensures selective toxicity and a low impact on beneficial organisms. T.C. Sparks & R. Nauen (2015) emphasised the importance of rotating insecticides with different modes of action to delay resistance, a recommendation supported by the current findings.

Ampligo 150 ZC (chlorantraniliprole + λ-cyhalothrin) demonstrated slightly lower but substantial efficacy, reducing pest densities to 1.0-1.5 individuals per 100 plants. R. Meena & K. Kumar (2024) observed similar outcomes in rice fields, reporting significant reductions in pest populations and improved rice yields after applying Ampligo 150 ZC, highlighting the effectiveness of the dual-action formulation. Vantex 60 CS (γ-cyhalothrin) provided statistically significant control, although its efficacy was lower than Coragen and Ampligo. EFSA (European Food Safety Authority) (2024) states that γ-cyhalothrin remains a valuable tool in insecticide rotation strategies due to its fast action and compatibility with resistance management programmes. While broad-spectrum pyrethroids can negatively affect non-target arthropods, the EFSA Panel on Plant Health (PLH) (2020) noted that selective insecticides such as chlorantraniliprole allow effective pest control with minimal disruption to beneficial insect populations. These findings support the incorporation of reduced-risk insecticides into integrated pest management (IPM) systems.

Real-time monitoring using pheromone traps was essential for optimising the timing of insecticide applications. Beyond selective insecticides, integrating biological control agents into maize protection programmes has shown promise. For example, J. Razinger *et al*. (2016) demonstrated that inundative releases of *Trichogramma brassicae* in maize fields across three European regions effectively controlled *O. nubilalis*, maintained mycotoxin levels below EU thresholds, and provided an economically sustainable alternative to chemical insecticides. Similarly, S. Oztemiz (2009) observed that field releases of *T. evanescens* in Turkish maize fields significantly reduced *O. nubilalis* egg masses and larval populations, confirming this parasitoid's efficacy under field conditions. These findings support incorporating *Trichogramma* species into integrated pest management strategies for maize.

Additionally, agronomic practices such as crop rotation, conservation tillage, and planting date manipulation can significantly influence pest pressure. E. Levine & H. Oloumi-Sadeghi (1991) reported that rotating maize with non-host crops effectively suppressed *Diabrotica virgifera virgifera* and other maize pests. These preventive strategies are cost-effective and environmentally benign, and when used alongside chemical and biological control methods, they serve as a foundation for sustainable crop protection systems. Chlorantraniliprole's low non-target toxicity and residual activity make it especially suitable for integrating other IPM components. A. Dinter *et al*. (2009) demonstrated its safety for key beneficial arthropods, including *Coccinellidae*, *Chrysopidae*, and hymenopteran parasitoids. Its compatibility with natural enemies and long-lasting efficacy justifies its recommendation for maize pest control under variable field conditions.

From a biotechnological perspective, the development of Bt maize has revolutionised pest management in many regions. J. Romeis *et al*. (2006) reported that transgenic maize expressing *Bacillus thuringiensis* (Bt) toxins such as Cry1Ab and Cry1F provides season-long protection against *O. nubilalis* and *S. frugiperda*, significantly reducing the need for chemical insecticides. B.E. Tabashnik *et al*. (2013) reviewed data from the first billion acres of Bt crops, showing how they contribute to pest suppression when implemented with resistance management strategies. Although Bt maize is not yet approved for commercial cultivation in Ukraine, A.J. Gassmann (2016) suggested that refuge strategies and future regulatory changes could support its eventual deployment. Emerging technologies such as CRISPR-Cas gene editing may allow the development of pest-resistant maize varieties without introducing transgenic DNA. K. Chen *et al*. (2019) highlighted how CRISPR could enhance societal and regulatory acceptance while providing precise pest-resistance traits. Combined with biopesticides and monitoring tools, such innovations offer a promising outlook for future maize pest management.

Economic considerations are equally important. J. Pretty & Z.P. Bharucha (2015) emphasised that although selective insecticides and biological control options may require a higher upfront investment, they reduce overall pesticide use, minimise yield losses, and improve ecosystem services, delivering strong economic returns over time. Findings support this conclusion, as plots treated in the study yielded significantly more than untreated controls, even when accounting for input costs. Integrating selective insecticides with pheromone-based surveillance proved effective under Ukrainian field conditions. This strategy reduced pest populations, preserved beneficial organisms, and contributed to higher yields. These outcomes align with IPM recommendations by FAO (2018) and underscore the importance of adaptive, data-driven pest management strategies.

Exploring synergies between chemical and biological control methods, such as *Trichogramma* spp., refining pest-specific economic thresholds, and incorporating climate-driven predictive models are key steps toward improving pest management. Continuous ecological monitoring remains essential for assessing the long-term impacts of insecticide use and enhancing the sustainability of maize production systems.

CONCLUSIONS

The research confirms the high effectiveness of integrated phytosanitary measures for controlling dominant Lepidopteran pests in maize crops within the Forest-Steppe zone of Ukraine. The application of pheromone traps, specifically models

PH-668-1RR, PH-554-1RR, and PH-460-1RR, enabled accurate identification of pest species such as *Loxostege sticticalis* L., *Helicoverpa armigera* Hb., and *Ostrinia nubilalis* Hb., and allowed for the precise timing of insecticide applications based on the detection of peak flight activity. Using pheromone traps ensured the optimal timing of insecticide treatments, targeting pest populations when they were most vulnerable.

The field experiments demonstrated that Coragen 20 SC (chlorantraniliprole) was the most effective among the tested insecticides. By the 14th day after application, Coragen achieved a 93.1% reduction in pest populations, showing consistent control across all pest species and observation intervals. This preparation exhibited prolonged residual activity and excellent selectivity, making it suitable for integrated pest management (IPM) systems. Ampligo 150 ZC, which contains a combination of chlorantraniliprole and lambda-cyhalothrin, also provided strong efficacy, especially against *Helicoverpa armigera*, with reductions exceeding 85% by the 14th day. Vantex 60 CS (gamma-cyhalothrin) showed moderate efficacy, with 60% to 70% reductions depending on the pest species and time after application. Although less effective than Coragen, Vantex still provided statistically significant suppression of pest densities compared to the untreated control.

Statistical analysis confirmed significant differences among treatments ($p < 0.05$), validating the superior performance of Coragen in terms of pest suppression. The significant yield increase observed in treated plots highlights the biological effectiveness and economic viability of the implemented IPM strategy. The research supports adopting integrated pest management practices that combine continuous monitoring with targeted, selective insecticide use. Such systems offer several benefits: minimising yield losses due to pest damage, reducing unnecessary pesticide applications, and lowering environmental risks. The integration of real-time pest surveillance with rational chemical control represents a sustainable solution for maize protection under Ukraine's current climatic and economic conditions.

Prospects for further research include the evaluation of combined insecticide and biological control agents (e.g., *Trichogramma* spp.), the development of predictive models for pest outbreaks based on weather and agronomic data, and the assessment of long-term environmental impacts of repeated insecticide use within IPM frameworks. Future studies should also focus on refining application thresholds for different pest species and enhancing farmer awareness of the advantages of integrated approaches in crop protection.

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CONFLICT OF INTEREST

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REFERENCES

[1] Abang, A.F., Nanga, S.N., Ndanda, R.M.O.E., Fotio, A.R.D., Gonder, M.K., Kouebou, C., Suh, C., Kuate, A.F., Fiaboe, K.K.K.M., & Hanna, R. (2022). Reliability of pheromone trap catches and maize plant damage as criteria for timing fall armyworm control interventions in humid forest agroecology of Central Africa. *Journal of Economic Entomology,* 115(6), 1806-1816. doi: 10.1093/jee/toac087.

[2] Borzykh, O.I., Janse, L.A., Chaika, V.M., Bakhmut, O.O., Borisenko, V.I., & Chaika, S.P. (2024). Population dynamics of corn insect pests in Ukraine under climate change. *Agricultural Science and Practice*, 10(3). doi: 10.15407/agrisp10.03.035.

[3] Chen, K., Wang, Y., Zhang, R., Zhang, H., & Gao, C. (2019). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annual Review of Plant Biology*, 70, 667-697. doi: 10.1146/annurev-arplant-050718-100049.

[4] Convention on Biological Diversity. (1992, June). Retrieved from https://zakon.rada.gov.ua/laws/show/995_030#Text.

[5] Deutsch, C.A., Tewksbury, J.J., Tigchelaar, M., Battisti, D.S., Merrill, S.C, Huey, R.B., & Nylor, R.L. (2018). Increase in crop losses to insect pests in a warming climate. *Science*, 361 (6405), 916-919, doi: 10.1126/science.aat3466.

[6] Dinter, A., Brugger, E.K., Frost, N.-M., & Woodward, M.D. (2009). Chlorantraniliprole (Rynaxypyr): A novel DuPont™ insecticide with low toxicity and low risk for honey bees (*Apis mellifera*) and bumble bees (*Bombus terrestris*) providing excellent tools for uses in integrated pest management. In *Hazards of pesticides to bees: 10th international symposium of the ICP-BR bee protection group* (pp. 84-96). Bucharest, Romania.

[7] EFSA (European Food Safety Authority), *et al*. (2024). Review of the existing maximum residue levels for gamma-cyhalothrin according to Article 12 of Regulation (EC) No 396/2005. *EFSA Journal*, 22(5), article number e8758. doi: 10.2903/j.efsa.2024.8758.

[8] EFSA Panel on Plant Health (PLH), *et al*. (2020). Pest categorisation of *Helicoverpa zea*. *EFSA Journal*, 18(7), e06177. doi: 10.2903/j.efsa.2020.6177.

[9] EPPO. (1997). *PP 1/13(3): Ostrinia nubilalis. EPPO Standards*. Retrieved from https://pp1.eppo.int/standards/PP1-013-3.

[10] FAO. (2018). *Integrated management of the Fall Armyworm on maize: A guide for farmers and extension agents*. Rome: Food and Agriculture Organization of the United Nations.

[11] Gassmann, A.J. (2016). Resistance to Bt maize by western corn rootworm: Insights from the laboratory and the field. *Current Opinion in Insect Science*, 15, 111-115. doi: 10.1016/j.cois.2016.04.001.

[12] Keszthelyi, S., Pál-Fám, F., & Kerepesi, I. (2011). Effect of cotton bollworm (*Helicoverpa Armigera* Hübner) caused injury on maize grain content, especially regarding to the protein alteration. *Acta Biologica Hungarica*, 62(1), 57-64. doi: 10.1556/ABiol.61.2011.1.5.

[13] Lahm, G.P. (2007) Rynaxypyr™: A new insecticide anthranilic diamide that acts as a potent and selective ryanodine receptor activator. *Bioorganic and Medicinal Chemistry Letters*, 17(22), 6274-6279. doi: 10.1016/j.bmcl.2007.09.012.

[14] Levine, E., & Oloumi-Sadeghi, H. (1991). Management of diabroticite rootworms in corn. *Annual Review of Entomology*, 36, 229-255. doi: 10.1146/annurev.en.36.010191.001305.

[15] Liaska, Y., & Stryhun, O. (2020). Peculiarities of development of corn earworm in the maize agrocenosis of the Left-Bank Forest Steppe of Ukraine. *EUREKA: Life Sciences*, 6, 3-11. doi: 10.21303/2504-5695.2020.001526.

[16] Ma, L., Tang, Y., Zhang, L., & Jiang, X. (2023). Green manure crops as food source: Impact on the performance of the migratory beet webworm, *Loxostege sticticalis* (Lepidoptera: Pyralidae). *Insects*, 14(8), 693. doi: 10.3390/insects14080693.

[17] McDonald, J.H. (2014). *Handbook of biological statistics*. Baltimore: Sparky House Publishing.

[18] Meena, R., & Kumar, K. (2024). Efficacy of chlorantraniliprole in combination with lambdacyhalothrin (Ampligo 150 ZC) against the leaf folder, *Cnaphalocrocis medinalis* (Guenee) in rice field. *Uttar Pradesh Journal of Zoology*, 45(5), 145-151. doi: 10.56557/upjoz/2024/v45i53940.

[19] Ndemah, R., & Schulthess, F. (2002). Yield of maize in relation to natural field infestations and damage by Lepidopteran Borers in the Forest and Forest/Savanna Transition Zones of Cameroon. *International Journal of Tropical Insect Science*, 22, 183-192. doi: 10.1017/S1742758400012030.

[20] Oztemiz, S. (2009). Natural parasitism and release efficiency of *Trichogramma evanescens* Westwood in *Ostrinia nubilalis* Hübner attacking maize in Turkey. *Journal of Entomological Science*, 44(2), 132-140. doi: 10.18474/0749-8004-44.2.132.

[21] Pretty, J., & Bharucha, Z.P. (2015). Integrated pest management for sustainable intensification of agriculture in Asia and Africa. *Insects,* 6(1), 152-182. doi: 10.3390/insects6010152.

[22] Qi, X., Cheng, S., Hong, L., Wang, X., Zhong, Q., Jiang, W., Chen, J., & Liang, Y. (2024). Maize yield and quality response to Lepidoptera pest control in different periods in South China. *Agronomy*, 14(12), article number 2938. doi: 10.3390/agronomy14122938.

[23] Razinger, J., Vasileiadis, V.P., Giraud, M., van Dijk, W., Modic, Š., Sattin, M., & Urek, G. (2016). On-farm evaluation of inundative biological control of *Ostrinia nubilalis* (Lepidoptera: Crambidae) by *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) in three European maize-producing regions. *Pest Management Science*, 72(2), 246-254. doi: 10.1002/ps.4054.

[24] Romeis, J., Meissle, M., & Bigler, F. (2006). Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnology*, 24, 63-71. doi: 10.1038/nbt1180.

[25] Sári-Barnácz, F.E., Zalai, M., Milics, G., Tóthné Kun, M., Mészáros, J., Árvai, M., & Kiss, J. (2024). Monitoring *Helicoverpa armigera* damage with PRISMA hyperspectral imagery: First experience in maize and comparison with Sentinel-2 imagery. *Remote Sensing*, 16(17), article number 3235. doi: 10.3390/rs16173235.

[26] Sharma, P.N., & Gautam, P. (2011). Assessment of yield loss in maize due to attack by the maize borer, *Chilo partellus* (Swinhoe). *Nepal Journal of Science and Technology*, 11, 25-30. doi: 10.3126/njst.v11i0.4085.

[27] Sparks, T.C. & Nauen, R. (2015). IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*, 121, 122-128. doi: 10.1016/j.pestbp.2014.11.014.

[28] Szanyi, S., Nagy, A., Varga, Z. & Tóth, M. (2023). Non-target noctuids from traps with synthetic *Spodoptera frugiperda* pheromone lure in the Carpathian Basin, Central Europe. *Entomologia Experimentalis et Applicata*, 171 (7), 542-545. doi: 10.1111/eea.13261.

[29] Tabashnik, B.E., Brévault, T., & Carrière, Y. (2013). Insect resistance to Bt crops: Lessons from the first billion acres. *Nature Biotechnology*, 31, 510-521. doi: 10.1038/nbt.2597.

[30] Taddele, A., Azerefegne, F., Beyene, Y. (2020). Crop injury and yield losses in maize by the African maize stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in Southern Ethiopia. *International Journal of Pest Management*, 69(2), 120-129. doi: 10.1080/09670874.2020.1861360.

[31] Yuschenko, L., & Tsyuk, A. (2018). Features of biological protection of maize crops from pests in the Forest-Steppe of Ukraine. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 14(1), 200-208.

Контроль чисельності домінуючих лускокрилих фітофагів у посівах кукурудзи в Полтавській області

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Анотація. Інтенсивні технології вирощування кукурудзи в умовах Лісостепу України супроводжується фітосанітарними ризиками, які пов'язані поширенням лускокрилих фітофагів, які можуть збільшувати рівень втрат урожаю, більше ніж економічний поріг шкодочинності. Метою дослідження було встановити ефективність сучасних засобів моніторингу та контролю чисельності домінуючих видів лускокрилих фітофагів у посівах кукурудзи в регіональних агроценозах. Для досягнення поставленої мети застосовували методи феромонного моніторингу, візуального обстеження рослин і кількісного обліку гусениць до та після інсектицидних обробок. Польові дослідження, що проводилися у 2024 році на базі агропідприємства АПОП «Великообухівське (Полтавська область), засвідчили домінування трьох видів шкідників *Ostrinia nubilalis* Hb., *Loxostege sticticalis* L. і *Helicoverpa armigera* Hb. Феромонні пастки типу PH-668-1RR, PH-554-1RR і PH-460-1RR продемонстрували високу ефективність для виявлення строків масового льоту шкідників, що дало змогу обґрунтувати строки інсектицидних обробок. Результати досліду свідчать, що найбільше зниження чисельності гусениць відмічено після обробки препаратом Кораген 20 КС, який знизив чисельність гусениць на 93,1 %, дещо меншу ефективність виявлено у Ампліго 150 ZC (91,6 %), тоді як Вантекс МК.С. забезпечив лише частковий контроль шкідників. Загалом результати підтверджують доцільність інтегрованого підходу, що поєднує феромонне спостереження з хімічним захистом, для ефективного зменшення чисельності фітофагів. Отримані дані можуть буди використані в агровиробництві для підвищення ефективності систем захисту кукурудзи, зменшення втрат урожаю та забезпечення сталого землеробства. Водночас доцільно відмітити необхідність формування інтегрованих систем захисту сільськогосподарських культур, зокрема кукурудзи, що пов'язано із потенційними заборонами ряду діючих речовин інсектицидів у рамках інтеграції Українського законодавства та стандартів до вимог ЄС

Ключові слова: моніторинг; агроценоз; фітосанітарний стан; захист рослин; феромонні пастки; інсектициди

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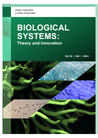

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Microbiological activity of soil and its impact on maize productivity when applying biologics

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Abstract. The purpose of this study was to assess the state of microbial coenosis and the activity of microbiological processes in the soil, their impact on the agrochemical parameters of typical chernozem, the assimilation surface of maize leaves and its seed productivity when applying biologics. In the study, a field method was used to investigate the interaction of research objects with natural factors, a laboratory method for determining microbiological and agrochemical indicators and photosynthetic activity, and a mathematical and statistical method for processing experimental data. It was established that with the introduction of biologics Groundfix and Azotohelp in favourable weather conditions in 2023 and arid 2024, the number of microorganisms of the vast majority of agronomically valuable groups increased compared to the control, the intensity of transformation of organic matter in the soil, and the intensity of mineralisation processes and potential denitrification activity decreased, and there was a decrease in the deficit of nutrients that were easily accessible to the microbiota. A high inverse relationship was

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established between the content of mineral nitrogen in the soil and the intensity of mineralisation processes in it (r = -0.78) and denitrification activity (r = -0.77). However, there was a direct effect of leaf assimilation surface and leaf dry matter on yield (r = 0.71 and 0.65, respectively). The efficiency of maize cultivation increased with the introduction of biologics Groundfix and Azotohelp by 6% and 5%, respectively. It was determined that the use of biologics Groundfix and Azotohelp in a row during sowing contributed to increasing the biogenicity of the soil and optimising microbiological processes in it, increasing the availability of nutrients and their digestibility, while contributing to plant growth and intensification of photosynthetic activity. Due to positive changes from the use of biologics Groundfix and Azotohelp, the yield of maize to the control version increases by 0.50 and 0.45 t/ha, respectively. These results can be used to optimise intensive maize cultivation technology, which will help to improve the state of microbial coenosis of the soil, plant nutrition, and increase maize yield

Keywords: microbial preparations; typical chernozem; physiological and taxonomic groups of microorganisms; denitrification activity; agrochemical indicators; yield

INTRODUCTION

In the conditions of intensive agriculture, the problem of preserving and restoring soil fertility becomes particularly important. Excessive use of mineral fertilisers, pesticides, and other agrochemicals leads to soil degradation, a decrease in their biological activity and a violation of the natural balance of agroecosystems. In this regard, there is a growing interest in the use of biological methods to increase soil fertility, in particular, the use of biologics based on beneficial microorganisms. They not only help to improve the microbiological condition of the soil, but also provide plants with nutrients, increase their resistance to stress factors and contribute to the development of a high-quality crop. Therefore, the study of the influence of the soil microbial complex on the agrochemical properties of the soil and the productivity of agricultural crops is extremely relevant for both science and practice.

Such researchers as O.M. Trus *et al.* (2021) emphasised the importance of soil microorganisms in ensuring the biological activity of the soil, which was the basis for its fertility and plant nutrition. L.M. Tokmakova *et al*. (2021) noted that microbiological processes that occur with the participation of microorganisms that destroy organic matter contribute to the effective decomposition of plant residues, improve the microbiological state of the soil, and improve the quality and yield of agricultural crops. R. Walker *et al.* (2020) reviewed current approaches and prospects for the use of microorganisms in agriculture, in particular, in improving plant growth, increasing yields and resistance to stress factors. The researchers emphasised the importance of investigating the metabolic interactions between plants and the microbiota, and the potential of biotechnological solutions for sustainable agriculture. However, N.G. Iyanyi (2020), Yu. Borko *et al.* (2022) proved that it was the direction of soil microbiological processes that determined the level of supply of plants with the necessary nutrients, which lead to the development of crop productivity. Microorganisms, being in close interaction with all elements of the biocenosis, form a complex system: "soil-plant-microorganisms", the functioning of which causes the destruction of organic matter, the cycle of biogenic elements, and the preservation of fertility. E. Menendez *et al.* (2020) in their study emphasised that the use of consortia of probiotic bacteria in agriculture can have a significant positive effect, because the interaction of rhizobial and non-rhizobial bacteria contributes to the improvement of the growth and development of cultivated plants. V.V. Bolokhovskyi *et al*. (2024a) emphasised that the use of biologics in sunflower cultivation technologies contributed to the optimisation of plant mineral nutrition. The researchers pointed out that the use of complexes of beneficial microorganisms activated the processes of fixation of atmospheric nitrogen, dissolution of hard-to-reach forms of phosphorus, and also increased the resistance of plants to biotic and abiotic stresses, preserving the natural biocenosis of the soil. According to Yu.P. Borko *et al.* (2021), the physiological effect of using biologics was to improve the processes of plant life, namely, to better absorb nutrients, enhance the processes of photosynthesis, which, in turn, contributed to an increase in yield and allowed the crop to maximise its potential. Scientific sources noted that the use of biologics, which include effective microorganisms, is a necessary element in the development of stable, environmentally balanced, and productive agroecosystems in contemporary agricultural production (Sherstoboeva *et al.,* 2020; Chen *et al.,* 2021; Wei *et al.,* 2024).

However, the variety of microbial preparations proposed for use in the agricultural sector encourages the search for scientific substantiations for the relationship between the course of microbiological processes in the soil and the development of agricultural crops. The purpose of the study was to establish the relationship between the activity of microbiological processes, agrochemical indicators of typical chernozem, the development of the photosynthetic apparatus of maize plants and its productivity when applying biologics.

MATERIALS AND METHODS

The research was conducted during 2023-2024 in the production conditions of Druzhba Nova JLLC (Kernel agricultural holding). In 2023, the research programme was implemented on experimental plots of agricultural land located in the administrative divisions of the Lubny district, Poltava Oblast (Pochaivka village), and in 2024 – Pryluky district of Chernihiv Oblast (Varva village), which were used by the Kernel agricultural holding. The soils of the experimental plots were represented by typical low-humus medium-loamy chernozem on loessial rocks. The technology of growing hybrid maize DKC 3972 on experimental plots in both locations is generally accepted for the Kernel agricultural holding in the Forest-Steppe zone, namely: the predecessor was maize for grain, the fertilisation background was $N_{110}P_{30}K_{30}$, the maize seeding rate was 80 thous. U/ha.

The research was conducted according to generally accepted methodological approaches in the field of agronomy (Rozhkov *et al.,* 2016). Experimental area – 7,168 m^2, the area of the sown plot in one replication of each variant is 448 m^2, the area where the harvest was recorded (without side and end protection strips) is 311 m^2, repetition – fourfold, placement of experimental sites was systematic. Crop accounting was carried out by continuous threshing of grain from each site, followed by recalculation to 14% of the basic humidity. The obtained findings were processed by variance, correlation, and regression methods of analysis using special application suite – Excel-16.0 (Yeshchenko, 2005).

The study considered options for applying soil biologics in rows during maize sowing according to the scheme:

1. Control (without introduction of LCFs and biologics);
2. LCF 5-20-5. 25 kg/ha;
3. Groundfix, 1 l/ha;
4. Azotohelp, 1 l/ha.

Liquid complex fertilisers (LCFs) and biologics were applied at a working solution consumption rate of 50 l/ha during maize sowing with a Great Plains 8070 seed drill equipped with applicators for applying liquid fertilisers in rows. Technologies for growing maize, in addition to the factors under study, were typical for the Kernel agricultural holding in the Forest-Steppe zone. For control was the option on which liquid complex fertilisers and biologics were not added in rows. LCF 5-20-5 – liquid nitrogen-phosphorus-potassium fertiliser, the mass fraction of nitrogen (N) – 5%, phosphorus (P) – 20%, and potassium (K) – 5%. It was applied as a starting fertiliser to the soil during sowing in a row at a rate of 25-75 kg/ha. The maximum safe application rate for seeds and seedlings depended on the crop, row spacing, granulometric composition, soil temperature and humidity, its cation exchange capacity and organic matter content, applicator design and some other factors.

Groundfix phosphorus-potassium mobiliser is a soil microbiological fertiliser. The main action of the biological product is to improve the phosphorus-potassium nutrition of plants by converting phosphates to a soluble form, and increasing the mobility and availability of silicon for plants, fixing molecular nitrogen from the atmosphere and converting it to a form accessible to plants. Due to a complex of bacteria that produce carboxylic acids, amino acids, enzymes, phytohormones, antibiotics, and other substances, the biological product had a positive effect on plants and improved their adaptive and immune properties. A positive characteristic of the drug was the improvement of the soil structure, its air and moisture availability. Composition of the biological product: live bacterial cells of *Bacillus velezensis, Bacillus subtilis, Priestia megaterium,* *Agrobacterium pusense, Agrobacterium salinitolerans, Paenibacillus polymyxa;* other useful microflora (lactic acid bacteria, enzyme producers); vitamins, phytohormones, amino acids, and other physiologically active substances. Total number of viable cells – (0.5-1.5) × 10^9 CFU/cm^3 (Groundfix phosphorus-potassium mobiliser, n.d.).

Azotohelp, KS – a biological product for stimulating plant growth and nitrogen fixation. Main action consists in fixing molecular nitrogen from the atmosphere by nitrogen-fixing bacteria and transferring it to a form accessible to plants, synthesising growth-stimulating substances that promote plant growth and development, increase immunity and resistance to stress factors, improve seed germination and nutrient absorption. Composition of the biological product: live cells of *Agrobacterium pusense* vitamins, phytohormones, amino acids, and other physiologically active substances. Total number of viable cells – not less than 1.0 × 10^9 CFU/cm^3 (Azotohelp, KC, n.d.).

Soil samples from the experimental variants were selected in a row in two non-contiguous repetitions during the flowering phase (BBCH 65-69) of maize from a layer of 0-20 cm in accordance with DSTU 4287:2004 (2004). They determined the content of alkaline hydrolysed nitrogen by the Kornfield method DSTU 7863:2015 (2015), mobile compounds of phosphorus and potassium by the modified Chirikov method DSTU 4115-2002 (2002). The number of microorganisms of the main physiological and taxonomic groups (ammonifiers, amylolytics, pedotrophs, oligotrophs, oligonitrophiles, actinomycetes, micromycetes) was determined according to DSTU ISO 7847:2015 (2015) by surface (bacteria) and deep (micromycetes) seeding of soil suspension of the corresponding tenfold dilution into solid (bacteria) and liquid (fungi) nutrient media. The soil biogenicity index was determined as the sum of microorganisms of all the groups under study.

The orientation of microbiological processes in the soil was characterised by appropriate coefficients in accordance with regulatory documents and scientific sources DSTU 3750-98 (1998) (Andreuk *et al.,* 2001; Volkohon *et al.,* 2010): nitrogen mineralisation-immobilisation coefficient ($K_{M.-I.}$) were calculated by the ratio of the number of microorganisms that assimilate mineral and organic nitrogen; the coefficient of pedotrophy – as the ratio of the number of pedotrophs and microorganisms that use mainly organic nitrogen compounds; the coefficient of oligotrophy – as the ratio of the number of oligotrophs and microorganisms that use mainly organic nitrogen compounds; the coefficient of transformation of organic matter of the soil – by the ratio of the number of microorganisms that assimilate mineral and organic nitrogen and the coefficient of mineralisation-immobilisation. In dynamics, the potential denitrification activity in rhizospheric soil was investigated by the rate of nitrous oxide emission using gas chromatographic methods (Volkohon *et al.,* 2015). The leaf surface area was determined by the linear method: the area was considered only in physiologically complete leaves (Palamarchuk & Solomon, 2021). The number of selected plants was 10 in four-fold repetitions.

Weather conditions in 2023-2024 corresponded to the trend of recent years regarding an increase in air temperature and a decrease in precipitation. Spring in 2023-2024 was characterised by elevated air temperatures and precipitation in April (+1.09°C, +4.06°C and +40.8 mm, +39.7 mm, respectively) and reduced in May (-0.9°C, -0.29°C and -33.5 mm, -61.7 mm to normal, respectively). The summer and autumn months of 2023 were characterised by high air temperatures in June, August, and September (+1.65°C, +5.31°C, and +3.41°C, respectively) and a lack of precipitation in June and September (-31.6 mm, -19 mm to normal, respectively), high precipitation in July (+38.1 mm), and sufficient precipitation in August (-0.5 mm). The summer and autumn months of 2024 were characterised by rising temperatures in June, July, and September (+2,08°C, +2,04°C, +3,19°C) and precipitation deficits in June, July, August, and September (-10.7 mm, -50.3 mm, -10.6 mm, and -42.7 mm, respectively). According to the hydrothermal coefficient of the HTC, there was a weak drought in 2023 (HTC – 0.9), and a strong drought in 2024 (HTC – 0.5). In general, the weather conditions of 2023 were more favourable for the development of maize productivity, compared to the dry conditions of 2024. Experimental studies of cultivated plants, including the collection of plant material, were in accordance with institutional, national or international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

According to the results of microbiological analysis of the soil, the influence of weather conditions on the number of microorganisms of the main physiological and taxonomic groups was noted. Thus, in a favourable 2023, the number of ammonifiers, amylolytics, pedotrophs, oligotrophs, and oligonitrophils was 3-4 times higher compared to the arid 2024. Notably, during the flowering period (BBCH 65-69) of maize under favourable weather conditions (hydrothermal coefficient $(\text{HTC})_{\text{July}}$ – 1.57) 2023 on the variants where Groundfix and Azotohelp biologics were used (There was an increase in the number of ammonifiers, amylolytics, pedotrophs, oligonitrophils, micromycetes, and actinomycetes in the control variant, and a decrease in the number of oligotrophs. When applying liquid complex fertilisers (LCFs), the number of ammonifiers, amylolytics, pedotrophs, oligonitrophils, actinomycetes also increased compared to the control, and the number of micromycetes and oligotrophs decreased. However, the biogenicity of the soil both with the use of both biologics and LCFs was higher than in the control (Table 1).

Table 1. The number of soil microorganisms in the flowering phase (BBCH 65-69) of the maize hybrid DKC 3972 F_1 for the use of biologics, million CFU/g of soil (average for 2023-2024)

Fertilisers, biologics	Ammonifiers		Amylolytics		Pedotrophs		Oligotrophs		Oligonitrophils		Actinomycetes		Micromycetes		Soil biogenicity	
	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024
1. Control	12.9	3.1	12.3	4.4	18.1	6.6	42.7	10.0	13.5	2.2	0.24	0.36	0.114	0.067	99.5	26.3
2. LCF, 25 kg/ha	13.5	2.3	13.4	3.5	26.7	5.4	35.2	9.4	15.2	2.9	0.47	0.14	0.082	0.856	107.0	23.5
3. Groundfix, 1 l/ha	15.7	4.5	14.4	3.7	18.6	7.5	37.8	9.7	21.6	2.6	0.95	0.26	0.115	0.896	108.1	28.0
4. Azotohelp, 1 l/ha	14.6	4.1	12.6	3.2	23.0	7.2	27.3	8.6	23.2	3.7	0.39	0.14	0.157	0.108	101.0	26.8

Source: compiled by the authors

A similar pattern was observed in the arid conditions of 2024 (Varva village) in the flowering phase (BBCH 65-69) of maize (HTC_{July} – 0.2). Thus, using biologics Groundfix and Azotochelp, an increase in the number of ammonifiers, pedotrophs, oligonitrophils, micromycetes to the control variant was observed and a decrease in the number of oligotrophs. However, in contrast to favourable conditions, a decrease in the number of amylolytic microbiota and actinomycetes was found in typical arid chernozems. Although the biogenicity of the soil when applying biologics was also higher compared to the control. The use of LCFs caused a decrease in the number of microorganisms in all study groups, with the exception of oligonitrophils and micromycetes, compared with the control. The obtained results are consistent with the findings of a number of researchers on the significant impact of weather and climatic conditions and applied agricultural measures on the development of the soil microbial complex. I. Beznosko *et al.* (2022) noted that the number of microorganisms of the main ecological and trophic groups inhabiting the rhizosphere of various agricultural crops depends on the phase of plant development, soil and climatic conditions, soil type, crop fertilisation system, and cultivation technology, while K. Bogati & M. Walczak (2022) indicated that arid conditions cause a decrease in the number and activity of the soil microbiome, disrupt the microbial structure, which leads to a decrease in soil fertility. A. Honchar *et al.* (2021) noted significant changes in the abundance and composition of the microbial complex of typical chernozem in the process of plant ontogenesis under the same conditions of agricultural technology of their cultivation. O.I. Zabolotnyi *et al.* (2024) noted that the use of biologics contributed to an increase in the maize rhizosphere compared to the control of the total number of bacteria by 10-30%, and micromycetes – by 7-20%, and L.M. Tokmakova *et al.* (2020) observed a 25% increase in the number of ammonium microorganisms when exposed to organic matter-destroying bacteria. The study by O.P. Siabruk *et al.* (2021) examined the effect of biologics on CO_2 emissions and the microflora of the maize rhizosphere. The researchers found that the use of biologics facilitates microbiological activity in the root zone, which indicates an improvement in the microbial environment of the soil. L.O. Biliavska (2014) and O.I. Zabolotny & A. Zabolotna (2022) noted that the use of biologics contributes to the growth of the microbiota of the main ecological and functional groups in the root zone of maize and winter wheat plants (nitrogen-fixing, phosphorus-mobilising, pedotrophic, amylolytic), and to the increase in soil biogenicity.

Not only the number of microorganisms of various physiological and taxonomic groups, but also their ratio and direction of microbiological processes in the soil have a significant impact on the condition and fertility of the soil. In conditions of sufficient moisture in 2023, the processes of destruction of organic nitrogen compounds prevailed in all variants of the experiment in soils. The intensity of mineralisation processes using biologics was close to the control variant and ranged from 0.86 to 0.92 with a control level of 0.95 (Table 2). However, the highest ($K_{M.-I.} = 0.99$) this indicator was in the variant with LCF 5-20-5, where the mineralisation and immobilisation processes were balanced. In conditions of drought, the intensity of mineralisation processes increased on the control and application of LCFs (to a greater extent), while the use of biologics helped to reduce their intensity. In the soil of the control variant and the variant with LCF ($K_{M.-I.} = 1.42$ and 1.52), the processes of immobilisation of mineral nitrogen compounds prevailed, and in variants with biologics – the mineralisation of organic nitrogen ($K_{M.-I.} = 0.82$ and 0.78) (Table 2).

Table 2. Directivity coefficients of microbiological processes in agrocoenosis of the maize hybrid DKC 3972 F_1 in the flowering phase (BBCH 65-69) with the introduction of biologics (average for 2023-2024)

Fertilisers, biologics	Mineralisation-immobilisation ($K_{M.-I.}$)		Oligotrophy ($K_{O.}$)		Pedotrophy ($K_{P.}$)		Transformations of organic matter (K_{TOM})	
	2023	2024	2023	2024	2023	2024	2023	2024
1. Control	0.95	1.42	3.31	3.23	1.40	2.13	26.4	5.3
2. LCF, 25 kg/ha	0.99	1.52	2.61	4.09	1.98	2.35	27.1	3.8
3. Groundfix, 1 l/ha	0.92	0.82	2.41	2.16	1.18	1.56	32.8	10.0
4. Azotohelp, 1 l/ha	0.86	0.78	1.83	2.10	1.54	1.76	32.5	9.4

Source: compiled by the authors

Processes of microbial transformation of soil organic matter (according to indicators K_{TOM}) in all variants of the experiment were more active under favourable weather conditions in 2023 compared to the arid conditions in 2024. Simultaneously, the use of Groundfix and Azotohelp biologics in contrast conditions in 2023-2024 contributed to a more intensive course of microbiological processes of organic transformation compared to the control and the variant where LCF 5-20-5 was introduced. It is worth noting that in all the studied variants in 2023 and 2024, the soil microbiota was insufficiently provided with easily accessible nutrients (according to the indicators of $K_{O.}$). However, when using Groundfix and Azotohelp biologics in favourable conditions in 2023, the shortage of nutrients before control for the soil microbiota was 27-45%, and in arid conditions in 2024 – 33-35%. It is important to note that when LCF 5-20-5 was added in rows during sowing under favourable conditions in 2023, the nutrient content also decreased by 21% relative to the control, and in dry conditions in 2024, on the contrary, it increased by 27%. Indicators of the pedotrophy coefficient ($K_{P.} = 1.18 - 2.35$) indicated active absorption of mobile nutrients from soil reserves by the autochthonous microbiota of all variants of the experiment in 2023-24, to a greater extent with the use of LCF 5-20-5 ($K_{P.} = 1.98$ and 2.35). Positive effects of biologics (including Groundfix and Azotohelp) on the microbiological activity of the soil were noted by D.O. Yakovenko & V.V. Boroday (2022), who recorded an increase in nitrogen fixation and mineralisation of organic matter. Similar results were obtained by V. Bolokhovskyi *et al.* (2024b), noting improvements in the rhizosphere environment and plant resistance to stress. T. Khomenko *et al.* (2022) indicated activation of the processes of phosphatmobilisation and decomposition of organic residues. O. Tonkha, *et al.* (2019) noted the stabilisation of the soil biocoenosis and the overall improvement of its agroecological state.

The study by V.P. Patyka & O.V. Sherstoboyeva (2002) noted that denitrification is a set of processes in which nitrogen nitrates and nitrites are reduced to nitrogen gas compounds or molecular nitrogen by biological means. For a long time, it was believed that denitrification is carried out by highly specialised bacteria, in particular, *Paracoccus denitrificans* and *P. halodenitrificans*. However, this is a widespread ability of aerobic and facultative anaerobic bacteria, which use nitrates and nitrites as alternative electron acceptors in the respiration process. This is why the process is also called nitrate respiration, heterotrophic denitrification, or dissimilar nitrate reduction.

According to the results of the study, it was found that the introduction of biologics in the line had a positive effect on reducing nitrogen losses in the soil (Table 3). Thus, the potential activity of soil denitrification with the introduction of Groundfix biologic in rows decreased in 2023 in the 5-7 leaf phase (BBCH 15-17) by 3.65 mmol N_2O/g of soil per day, and in 2024 – 2.92 mmol N_2O/g of soil per day and, respectively, in the phase of panicle ejection (BBCH 50-55) by 0.2, and 0.16, flowering (BBCH 65-69) 1.95, and 1.56. When using Azotohelp, there was also a decrease in potential denitrification, respectively, in the phase of 5-7 leaves by 2.73, 2.39, in the phase of panicle ejection by 0.5, 0.4 and in the flowering phase by 2.85, 2.18 mmol N_2O/g of soil per day, a similar effect of which was noted by V.V. Volkohon *et al.* (2015). In the variant where LCF 5-20-5 was applied, the activity of soil denitrification, on the contrary, increased to control at all stages of maize growth and development (Table 3). According to the results of agrochemical analysis, it was found that the exchange acidity in the experiment did not fluctuate significantly, was at the level of the control variant and did not significantly affect the growth, development and productivity of maize plants.

Table 3. Potential activity of soil denitrification
in maize hybrid crops DKC 3972 F_1 with the introduction of biologics (2023-2024)

Fertilisers, biologics	Potential activity of soil denitrification mmol N_2O/g of soil per day					
	2023			2024		
	5-7 leaves (BBCH 15-17)	panicle ejection (BBCH 50-55)	flowering (BBCH 65-69)	5-7 leaves (BBCH 15-17)	panicle ejection (BBCH 50-55)	flowering (BBCH 65-69)
1. Control	11.36 ± 0.4	16.48 ± 1.1	9.35 ± 0.8	8.99 ± 0.8	13.08 ± 0.9	7.38 ± 0.3
2. LCF, 25 kg/ha	14.07 ± 1.2	17.33 ± 1.3	9.50 ± 0.6	11.16 ± 0.9	13.56 ± 1.1	7.70 ± 0.5
3. Groundfix, 1 l/ha	7.71 ± 0.7	16.28 ± 1.3	7.40 ± 0.5	6.07 ± 0.5	12.92 ± 1.0	5.82 ± 0.2
4. Azotohelp, 1 l/ha	8.63 ± 0.9	15.98 ± 0.6	6.50 ± 0.6	6.60 ± 0.4	12.68 ± 1.2	5.20 ± 0.3

Source: compiled by the authors

The variability of the content of alkaline hydrolysed nitrogen in the soil under favourable weather conditions in 2023 was low (0.9 %), and ranged from 100.1 to 108.5 mg/kg of soil. In the arid conditions of 2024, it increased slightly and amounted to 1.06%, that is, there were no significant changes in the content of alkaline hydrolysed nitrogen during the use of biologics. Microbiological processes that occur in the soil and are associated with the conversion of organic and mineral nitrogen compounds (mineralisation-immobilisation, denitrification) had a significant impact on the content of mineral nitrogen in the soil. Thus, in the conditions of 2023-24, mineral nitrogen negatively correlated with the coefficient of mineralisation-immobilisation ($r = -0.88$-0.68) and denitrification ($r = -0.71$-0.83), which is quite logical and is explained in the first case by an increase in the intake of mineral nitrogen in the soil due to the direction of processes towards mineralisation, and in the second – a decrease in nitrogen losses with a decrease in denitrification activity. In favourable conditions in 2023, a noticeable effect on the mineral nitrogen content was noted when using Azotohelp biologics, and in dry conditions in 2024 – for all fertiliser variants. However, the option where Azotohelp biologics were introduced was very different from others not only in a favourable 2023, but also in a dry 2024 (Table 4). The content of mineral nitrogen increased by 3.2 and 13.4 mg/kg of soil, respectively, due to the functional feature of the bacteria that make up the preparation and are associative nitrogen fixators, promote nitrogen fixation and reduce the activity of the denitrification process.

Table 4. Agrochemical indicators of soil in crops of hybrid maize DKC 3972 F_1 in the flowering phase (BBCH 65-69) with the introduction of biologics (average for 2023-2024)

Fertilisers, biologics	Metabolic acidity, pH		Hydrolysed nitrogen, mg/kg		Mineral nitrogen, mg/kg		Mobile phosphorus, P_2O_5, mg/kg		Mobile potassium, K_2O, mg/kg	
	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024
1. Control	5.5	5.5	102.2	115.5	12.1	13.6	126.0	146.2	113.8	97.5
2. LCF, 25 kg/ha	5.5	5.4	108.5	120.4	10.1	18.1	135.0	157.5	88.8	92.5
3. Groundfix, 1 l/ha	5.5	5.4	106.4	121.8	11.0	19.6	143.0	161.2	90.0	91.2
4. Azotohelp, 1 l/ha	5.6	5.5	100.1	120.4	15.3	27.0	128.8	130.5	92.5	96.2

Source: compiled by the authors

In favourable conditions in 2023, in the variant where Groundfix biologic was used, the content of mobile phosphorus increased by 17 mg/kg of soil relative to the control, and when using LCF 5-20-5 – by 9 mg/kg of soil. In dry conditions in 2024, the content of mobile phosphorus increased to the control with the introduction of Groundfix by 15 mg/kg and LCF 5-20-5 – 11.3 mg/kg. The increase in the content of mobile phosphorus in the soil is caused by a complex of phosphorus-mobilising bacteria that are part of the preparation and contribute to the solubilisation of phosphorus. However, the introduction of Azotochelp did not significantly affect the increase in the content of mobile phosphorus in the soil. The variability of mobile potassium content in the soil was at an average level (V = 12.4%) in favourable conditions in 2023 and low (V = 3.16%) in arid conditions in 2024. It should be noted that the content of mobile potassium in the soil during the flowering phase on the variants with LCF fertilisation and biologics decreased to the control variant, which was substantiated by the higher assimilation of maize plants. The decrease in the content of mobile potassium in the soil was more noticeable in favourable weather conditions in 2023 compared to dry conditions in 2024, which was explained by its greater removal with the crop.

Under the influence of biologics, there were positive changes in the microbial coenosis of the soil, namely: the biogenicity of the soil, the amount of nutrients, and the activity of their transformation increased, while the intensity of mineralisation processes decreased. As a result, the amount of mineral nitrogen and mobile phosphorus in the soil increased and their assimilation by plants improved, which was reflected in an increase in the assimilation surface of maize crops (Table 5). Thus, when using the biological product Groundfix, the assimilation surface increased to control in 2023 and 2024, respectively, by 6 and 4.5 thousand m^2, dry matter of leaves – by 0.21 and 0.37 t/ha, Azotohelp, respectively, – by 10.1-12.8 thousand m^2/ha and 0.62-0.69 t/ha. The use of LCFs also had a positive effect on the assimilation surface of leaves and their dry matter, but was less effective compared to biologics. The results obtained are consistent with the data of L. Kvasnitska *et al.* (2024), who noted that soil treatment with soil microbiological fertiliser Groundfix contributed to an increase in the leaf surface area of sunflower plants by 7% compared to the control.

Table 5. Photosynthetic activity of the maize hybrid DKC 3972 F$_1$ in the flowering phase (BBCH 69) with the introduction of biologics (average for 2023-2024)

Fertilisers, biologics	Area of the assimilation surface of leaves, thous. m^2/ha		Dry matter of leaves, t/ha	
	2023	2024	2023	2024
1. Control	45.3	40.4	1.92	1.74
2. LCF, 25 kg/ha	47.2	41.7	2.03	1.82
3. Groundfix, 1 l/ha	51.3	44.9	2.13	2.11
4. Azotohelp, 1 l/ha	55.4	53.2	2.54	2.43

Source: compiled by the authors

An increase in the photosynthetic apparatus of plants during the growing season had a significant impact on maize productivity. Thus, there is a direct relationship in 2023-2024 between the assimilation surface of leaves, dry matter of leaves, and yield (r=0.74-0.67 and 0.56-0.74, respectively). The use of biologics in the row had a positive effect on maize yields both in favourable conditions for plant growth and development in 2023 and in arid 2024. It is important to note that the productivity of the crop when using Groundfix in a row during sowing increased on average over two years by 0.5 t/ha compared to the control, Azotohelp, respectively, by 0.45 t/ha, and LCF 5-20-5 – 0.34 t/ha (Table 6).

Table 6. Maize DKC 3972 F1 hybrid yield with the introduction of biologics (2023-2024)

Fertilisers, biologics	Yield, t/ha			
	2023	2024	average for 2023-2024	± to control
1. Control	10.64	6.98	8.81	0
2. LCF, 25 kg/ha	10.94	7.35	9.15	0.34
3. Groundfix, 1 l/ha	11.23	7.39	9.31	0.50
4. Azotohelp, 1 l/ha in	11.07	7.44	9.26	0.45
LSD$_{0.95}$	0.15	0.24	-	-

Note: LSD$_{0.95}$ (the least significant difference) – the minimum difference between the two average values, which is considered statistically significant at 95 percent and is calculated over the years of research

Source: compiled by the authors

The results obtained coincide with the studies conducted by D.V. Litvinov & P.V. Petryk (2023), Ye.O. Yurkevych *et al.* (2023), according to which the introduction of Groundfix in the line during sunflower sowing at a dose of 0.75-1 l/ha increased the yield by 6.8-16.2% compared to the option without the introduction of the drug. The findings by V. Rotaru *et al.* (2021) and V. Ishchenko *et al*. (2022) indicated that the use of Azotofit at a rate of 0.5 l/ha in the agrocoenosis of winter wheat and 3 l/ha of sunflower contributed to an increase in grain yield by 15.8% and 10.4%, respectively, compared to the control, where biologics were not introduced.

Thus, the introduction of biologics has a positive effect on the agrochemical parameters of the soil, in particular, it helps to improve the supply of nutrients (nitrogen, phosphorus, potassium), activates microbiological processes. As a result, the physiological state of plants improves, in particular, the assimilation surface of leaves increases, photosynthesis is activated, and nutrient absorption improves. This, in turn, ensured an increase in the biological productivity of maize and an improvement in the quality indicators of the crop.

CONCLUSIONS

It was established that the introduction of biologics in rows during sowing increased the activity of microbiological processes in the soil and positively affected the agrochemical indicators of typical chernozem, which, in turn, increased the assimilation surface of maize plants and increased crop productivity. Processes of microbial transformation of soil organic matter (according to indicators K_{TOM}) in all variants of the experiment were more active under favourable weather conditions in 2023 compared to the arid conditions in 2024. Simultaneously, the use of Groundfix and Azotohelp biologics in contrast conditions in 2023-2024 contributed to a more intensive course of microbiological processes of organic transformation compared to the control and the variant where LCF 5-20-5 was introduced. The introduction of biologics Groundfix and Azotohelp, in comparison with the control, where biologics were not used, contributed to the growth of soil biogenicity, namely: an increase in the number of ammonifiers by 33% and 23%, respectively, pedotrophs – 8.18%, oligonitrophils – 39.70%, micromycetes – 17.48%, while increasing the amount of easily digestible nutrients by 30.40% and the intensity of transformation of organic matter of the soil – 57.50%, the intensity of mineralisation processes decreased by 23.27% and denitrification – by 21.30%. Positive changes in microbial coenosis improved the agrochemical parameters of the soil, namely: the content of mineral nitrogen increased by 8.3 mg/kg of soil when applying Azotochelp, and mobile phosphorus by 16 mg/kg of soil when using Groundfix.

Soil transformations under the action of biologics had a positive effect on the assimilation of nutrients by the crop and an increase in the photosynthetic activity of maize. Thus, when using the biological product Groundfix, the assimilation surface increased to control in 2023 and 2024, respectively, by 6 and 4.5 thous. m^2 of dry matter of leaves – by 0.21 and 0.37 t/ha, Azotohelp, respectively, – by 10.1-12.8 thous. m^2/ha and 0.62-0.69 t/ha, which provided an increase in yield to the control with the introduction of Azotohelp 0.45 t/ha, and Groundfix – 0.5 t/ha. In further studies, it is advisable to continue comprehensive observations of the development of the soil microbiome under the influence of biologics and their impact on its fertility and productivity of agrophytocoenosis in intensive cultivation technologies.

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REFERENCES

[1] Andreuk, K.I., Iutynska, G.O., Antypchuk, A.F., Valagurova, V.O., Kozerytska, V.E., & Ponomarenko, S.P. (2001). *Functioning of microbial communities in soil under conditions of anthropogenic stress*. Kyiv: Oberegy.

[2] Azotohelp, KC. (n.d.). Retrieved from https://btu-center.com/ua/azotofit-ks.

[3] Beznosko, I., Gorgan, T., Mosiychuk, I., Buniak, O., & Ternoviy, Y. (2022). Influence of different cultivation technologies on the number of main ecological and trophic groups. *Bulletin of Lviv University*, 86, 58-72. doi: 10.30970/VLUBS.2022.86.05.

[4] Biliavska, L.O. (2015). New compositions of antinematodes bioformulations for crop production. *Scientific Paper. Ternop. National Pedagogical University*, 1(62), 61-64.

[5] Bogati, K., & Walczak, M. (2022). The impact of drought stress on soil microbial community, enzyme activities and plants. *Agronomy*, 12(1), article number 189. doi: 10.3390/agronomy12010189.

[6] Bolokhovskyi, V.V., Bolokhovska, V.A., Khomenko, T.O., Datsko, A.O., & Litvinova, O.A. (2024a). Optimization of plant nutrition under the influence of biopreparations in integrated sunflower cultivation technologies. *Plant and Soil Sciense*, 15(4), 64-75. doi: 10.31548/plant4.2024.64.

[7] Bolokhovskyi, V., Borodai, V., Kosovska, N., Bolokhovska, V., Nagorna, O., Yakovenko, D., & Kuzmych, V. (2024b). Effect of Groundfix and Ecostern biological preparations on soil microbiota under soybean (*Glycine max L.*) cultivation. *Agroecological Journal*, 3, 156-163. doi: 10.33730/2077-4893.3.2024.311190.

[8] Borko, Yu.P., & Kyrychenko, A.V. (2021). Estimation of structural-spatial organization of biome in agroecosystems. "Innovations in science: Global trends and regional aspect". In *International scientific and practical conference* (24-28). Riga, Latvia: Baltija Publishing. doi: 10.30525/978-9934-26-050-6-8.

[9] Borko, Yu., Dehodyuk, S., Dehodyuk, E., Patyka, M., & Litvinova, O. (2022). Features of the formation of microbial cenosis of gray forest soil under different fertilization systems in the agrocenosis of winter wheat. *Bulletin of Agrarian Science*, 100(4), 14-22. doi: 10.31073/agrovisnyk202204-02.

[10] Chen, L., *et al*., (2021). Plant growth-promoting bacteria improve maize growth through reshaping the rhizobacterial community in low-nitrogen and low-phosphorus soil. *Biology and Fertility of Soils*, 57(8), 1075-1088. doi: 10.1007%2Fs00374-021-01598-6 .

[11] Convention on Biological Diversity. (1992, June). Retrieved from https://zakon.rada.gov.ua/laws/show/995_030#Text.

[12] DSTU 3750-98. Soil microbiology. Terms and definitions. (1999, July). Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=58886.

[13] DSTU 4115-2002. Soils. Determination of mobile phosphorus and potassium compounds by the modified Chirikov method. (2002, June). Retrieved from https://online.budstandart.com/ua/catalog/doc-page?id_doc=58863.

[14] DSTU 4287:2004. Soil quality. Sampling. (2005, August). Retrieved from https://environmentallab.com.ua/wp-content/uploads/2021/12/dstu-4287-2004-yakist-gruntu.-vidbirannya-prob.pdf.

[15] DSTU 7863:2015. Soil quality. Determination of easily hydrolyzable nitrogen by the Kornfield method. (2015, August). Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=62745.

[16] DSTU ISO 7847:2015. Soil quality. Determination of the number of microorganisms in soil by sowing on solid (agarized) medium. (2016, August). Retrieved from https://online.budstandart.com/ua/catalog/doc-page?id_doc=62729.

[17] Groundfix phosphorus-potassium mobilizer. (n.d.). Retrieved from https://btu-center.com/ua/groundfix.

[18] Honchar, A.N., Tonkha, O.L., Patyka, N.V., & Makarchuk, O.S. (2021). Peculiarities of change in number and composition of winter wheat rhizosphere microbiome in the process of ontogenesis. *Plant & Soil Science*, 12(3), 56-65. doi: 10.31548/agr2021.03.056.

[19] Ishchenko, V., Andrienko, O., & Tkachenko, S. (2022). Efficiency of the use of bioproducts in the technology of sunflower growing. “Modern technologies of agroindustrial production”. In *II international scientific and practical conference* (pp. 108-110). Kropyvnytskyi: CNTU.

[20] Iyanyi, N.G. (2020). Identification of fungal organisms associated with the rhizosphere of maize (*Zea mays L.*): Basic molecular techniques. *Nigeria Agricultural Journal*, 51(2), 399-405.

[21] Khomenko, T., Tonkha, O., Pikovska, O., & Achasov, A. (2022). The influence of biological preparations on the microbiological activity of sod-podzolic soil for the cultivation of food potatoes. *Plant and Soil Science*, 13(1), 60-66. doi: 10.31548/agr.13(1).2022.60-66.

[22] Kvasnitska, L., Vlasyuk, O., & Voitova, G. (2024). Influence of fertilizers and biodestroyers on the growth and development of sunflower. In *“Feeds and feed protein” XVI international scientific conference* (84-86). Vinnytsia: Institute of Feed Research and Agriculture of Podillya NAAS.

[23] Litvinov, D.V., & Petrik, P.V. (2023) Effectiveness of the use of the biological product Groundfix on sunflowers. In *V international scientific and practical online conference “Trends and challenges of modern agricultural science in wartime: Theory and practice”* (pp. 150-152). Kyiv: National University of Life and Environmental Sciences of Ukraine.

[24] Menendez, E., & Paço, A. (2020). Is the application of plant probiotic bacterial consortia always beneficial for plants. Exploring synergies between rhizobial and non-rhizobial bacteria and their effects on agro-economically valuable crops. *Life*, 10(3), article number 24. doi: 10.3390/life10030024.

[25] Palamarchuk, V.D. & Solomon, A.M. (2021). Research of the corn assimilation surface formation depending on foliar feeding. *Feed and Feed Production*, 92, 82-94. doi: 10.31073/kormovyrobnytstvo202192-08.

[26] Patyka, V.P., & Sherstoboyeva, O.V. (2002). Methodological approaches to microbiological monitoring of soil condition of agroecosystems. In *Agroecological monitoring and certification of agricultural lands* (131-136). Kyiv: Phytosociocenter.

[27] Rotaru, V., Gore, A., & Taran, M. (2021). Effect of microbiological fertilizer Azotofit on the yield and grain quality of winter wheat. In *Ştiinţa în Nordul Republicii Moldova: Realizări, probleme, perspective* (pp. 92-97). Bălți: Institute of Genetics, Physiology and Plant Protection (IGFPP).

[28] Rozhkov, A.O., Puzik, V.K., Kalenska, S.M., Puzik, L.M., Popov, S.I., Muzafarov, N. M., Bukhalo, V.Y., & Kryshtop, E.A. (2016). Book 1. Theoretical aspects of the research case. In A.O. Rozhkov (Ed.), *Experimental case in agronomy (in 2 books)*. Kharkiv: Maidan.

[29] Siabruk, O.P., Naydenova, O.E., & Get'man, Ya.V. (2021). Influence of biological products on CO_2 emission and microflora on the root zone of corn. *Bulletin of the Agricultural Science of the Black Sea Region*, 2(110), 63-70. doi: 10.31521/2313-092X/2021-2(110)-8.

[30] Sherstoboeva, O.V., Bunas, A.A., & Dumanyuk, O.S. (2020). Influence of predecessors and pre-sowing inoculation of seeds with Azotobacter vinelandii 12m strain on corn yield and nitrogen fixation activity. *Balanced Nature Management*, 1, 120-128. doi: 10.33730/2310-4678.1.2020.203941.

[31] Tokmakova, L.M., Larchenko, I.V., & Kovpak, P.V. (2021). Microbiological processes of transformation of corn plant residues upon introduction of microorganisms that destroy organic matter into agrocenoses. *Agricultural Microbiology*, 32, 35-47. doi: 10.35868/1997-3004.32.35-47.

[32] Tonkha, O., Pikovska, O., Balaev, A., Kovalyshyna, G., Zavgorodniy, V. & Kovalenko, V. (2019). Monitoring of the microbiological condition of virgin chernozem under different management. In *Monitoring of geological processes and ecological condition of the environment* (pp. 1-5). Kyiv: Institute of Geology of Taras Shevchenko National University of Kyiv. doi: 10.3997/2214-4609.201903256.

[33] Trus, O.M., Prokopenko, E.V., & Polishchuk, T.V. (2021). Biological activity of soil, its importance for soil fertility and plant nutrition. *Bulletin of Kremenchuk Mykhailo Ostrohradskyi National University*, 5(130), 36-41.

[34] Volkohon, V.V., Dimova, S.B., Volkohon, K.I., Komok, M.S., & Shtanko, N.P. (2010). Features of the denitrification process in agrocenoses under the influence of mineral fertilizers and microbial preparations. *Agricultural microbiology*, 10, 7-19. doi: 10.35868/1997-3004.10.7-19.

[35] Volkohon, V.V., *et al*. (2015). *Experimental soil microbiology*. Kyiv: Agrarian Science.

[36] Walker, R., Otto-Pille, C., Gupta, S., Schillaci, M., & Roessner, U. (2020) Current perspectives and applications in plant probiotics. *Microbiology Australia*, 41(2), 95-99. doi: 10.1071/MA20024.

[37] Wei, X., Xie, B., Wan, C., Song, R., Zhong, W., Xin, S., & Song, K. (2024). Enhancing soil health and plant growth through microbial fertilizers: Mechanisms, benefits, and sustainable agricultural practices. *Agronomy*, 14(3), article number 609. doi: 10.3390/agronomy14030609.

[38] Yakovenko, D.O., & Boroday, V.V. (2022). Influence of biological products Azotophyte and Groundfix on microbiological parameters of soil for winter wheat cultivation. In *International scientific and practical conference "Environmental safety and balanced nature-use in agroindustrial production"* (pp. 404-408). Kyiv: Institute of Agroecology and Nature Management of the National Academy of Sciences of Ukraine.

[39] Yeshchenko, V.O. (Ed.). (2005). *Fundamentals of scientific research in agronomy*. Kyiv: Diya.

[40] Yurkevych, Ye.O., Valentiuk, N.O., Petrenko, S.O., Rodionov, A.V., & Hrabovetska, O.A. (2023). The effectiveness of the use of biological preparations in the cultivation of confectionery sunflower in the conditions of the Southern Steppe of Ukraine. *Agrarian Innovations*, 21, 118-125. doi: 10.32848/agrar.innov.2023.21.18.

[41] Zabolotny, O.I., & Zabolotna, A. (2022). Microbiological activity of the rhizosphere of the cucumber hybrid Marinda f1 under conditions of pre-sowing seed bacterization. In *Natural sciences and education: Collection of scientific works of the faculty of natural sciences and geography* (26-30). Uman: Publishing and Printing Center "Vizavi".

[42] Zabolotnyi, O.I., Zabolotna, A.V., & Ternavskyi, A.H. (2024). Microbiological activity of the corn rhizosphere under the using of biopreparations. *Tavria Scientific Bulletin*, 136(1), 139-145. doi: 10.32782/2226-0099.2024.136.1.18.

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Анотація. Метою даного дослідження було оцінити стан мікробного ценозу та активність мікробіологічних процесів у ґрунті, їх вплив на агрохімічні показники чорнозему типового, асиміляційну поверхню листя кукурудзи та її насіннєву продуктивність за внесення біопрепаратів. У досліджені було застосовано польовий метод для вивчення взаємодії об'єктів досліджень з природними факторами, лабораторний – для визначення мікробіологічних та агрохімічних показників, фотосинтетичної активності, а також математико-статистичний – для обробки експериментальних даних. Встановлено, що за внесення біопрепаратів Граундфікс і Азотохелп у сприятливих погодних умов 2023 р., та посушливих 2024 р. збільшується порівняно з контролем чисельність мікроорганізмів переважної більшості агрономічно цінних груп, інтенсивність процесів трансформації органічної речовини ґрунту, а також знижується напруженість мінералізаційних процесів та потенційна активність денітрифікації, спостерігається зменшення дефіциту легкодоступних для мікробіоти елементів живлення. Встановлено високу обернену залежність між вмістом мінерального азоту в ґрунті і напруженістю мінералізаційних процесів у ньому ($r = -0{,}78$) та денітрифікаційною активністю ($r = -0{,}77$). Водночас відмічається прямий вплив асиміляційної поверхні листя та сухої речовини листя на урожайність ($r = 0{,}71$ та 0,65 відповідно). Підвищується ефективність вирощування кукурудзи за внесення біопрепаратів Граундфікс та Азотохелп на 6 % та 5 % відповідно. Визначено, що використання біопрепаратів Граундфікс та Азотохелп у рядок під час сівби сприяє збільшенню біогенності ґрунту та оптимізації направленості мікробіологічних процесів у ньому, підвищенню доступності елементів живлення та їх засвоюваності, сприяючи при цьому росту рослин та інтенсифікації фотосинтетичної активності. Внаслідок позитивних змін від застосування біопрепаратів Граундфікс та Азотохелп урожайність кукурудзи до контрольного варіанту підвищується відповідно на 0,50 та 0,45 т/га. Дані результати можуть бути використані для оптимізації інтенсивної технології вирощування кукурудзи, що сприятиме поліпшенню стану мікробного ценозу ґрунту, живлення рослин та підвищення врожайності кукурудзи

Ключові слова: мікробні препарати; чорнозем типовий; фізіологічні і таксономічні групи мікроорганізмів; активність денітрифікації; агрохімічні показники; урожайність

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