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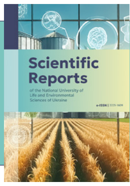

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Effect of mineral nutrition and nitrogen-fixing agent on maize yield in the Central Forest-Steppe of Ukraine

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Abstract. In modern agricultural production, there is an increasing demand for the use of biopreparations, particularly nitrogen-fixing agents, which can reduce the need for mineral fertilisers while enhancing maize yields. This study aimed to determine the effects of different fertilisation

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strategies and plant densities on the morphological characteristics and yield of maize under the agroecological conditions of the Central Forest-Steppe of Ukraine. Field experiments were conducted during 2023-2024 under the soil and climatic conditions of a farm in the Kyiv Region, using the maize hybrid P9255 (FAO 330). The area of each experimental plot was 33 m^2, with four replications and a sequential multilevel arrangement. The application of a nitrogen-fixing biological agent in combination with mineral fertilisers had a positive effect on plant growth. In particular, plant height was 1.7%-5.1% greater than in the treatments where only mineral fertilisers were applied. An evaluation was carried out to determine the effectiveness and impact of optimising the fertilisation system on the morphological characteristics and productivity of maize plants through the use of a biological preparation in combination with mineral fertilisers at various application rates. Overall, the application of mineral fertilisers alone resulted in a 14.5%-22.5% increase in grain yield compared to the control. The highest yield, reaching 12.94 t/ha, was recorded under the $N_{150}P_{120}K_{120}$ treatment at a plant density of 70,000 plants/ha. In treatments combining mineral fertilisers with the biological agent, grain yield was 12.2% 21.2% higher than in the corresponding control variants. The highest average grain yield, amounting to 13.38 t/ha, was achieved with the $N_{150}P_{120}K_{120}$ + nitrogen-fixing agent treatment at a plant density of 70,000 plants/ha. The application of the biological preparation in combination with mineral fertilisers produced a 3.6% (or 0.43 t/ha) higher grain yield compared to treatments without the biological agent

Keywords: biological preparation; plant density; plant height; mineral fertilisers; grain yield

Introduction

Improving the efficiency of resource use in crop production is one of the key objectives of modern agriculture. In the context of climate change, the limited availability of mineral fertilisers, and the growing need for environmentally sustainable technologies, the adoption of alternative or combined approaches to crop nutrition, including the use of biological agents, is becoming increasingly important. Maize (*Zea mays* L.) is one of the leading food and fodder crops, occupying a prominent position in the structure of grain production in Ukraine. Its high sensitivity to mineral nutrition makes it a suitable model crop for evaluating fertiliser efficiency. The combination of mineral and biological nitrogen sources may serve as a tool for increasing yields while reducing dependence on conventional agrochemicals (Mokrienko & Kornienko, 2024).

Maize is cultivated in most countries around the world. According to N. Tanklevska *et al*. (2020), approximately half of global maize production is concentrated in the USA and China, which are also the largest consumers and processors of maize products. Ukraine accounts for around 3.2% of global maize grain production. At the same time, maize represents nearly 50% of Ukraine's total grain output, making it a strategically important crop for ensuring the sustainable development of the national agricultural sector. According to B. Kamenshchuk (2020), maize yield and production volumes can be increased by improving cultivation technologies through the realisation of genetic potential, the introduction of new high-yielding hybrids, the use of modern plant protection products, and the optimisation of mineral nutrition.

Maize has the potential to produce high grain and green mass yields on all soil types suitable for the cultivation of major agricultural crops. Optimal conditions for maize growth and development are found on fertile soils that are well-supplied with moisture and free from waterlogging. According to O.I. Zinchenko *et al.* (2001), the presence of mineral nutrients in the soil, along with a near-neutral or slightly acidic pH level, is are important factor for the robust development of the root system and the overall health of the crop. For grain maize production, chernozem and dark chestnut soils with high fertility are considered optimal.

At present, agricultural production in Ukraine is experiencing rising costs for various technological inputs, including fuel and lubricants, seeds, pesticides, and particularly mineral fertilisers. This situation necessitates the development of improved cultivation and fertilisation technologies for maize. The combined use of mineral fertilisers and biological agents has a beneficial effect on soil properties, helping to maintain soil fertility and facilitate the mobilisation of phosphorus and potassium from insoluble compounds into plant-available forms. This enhances the efficiency of mineral fertilisers, improves nutrient uptake, and increases the resilience of crops to adverse environmental conditions, ultimately contributing to higher yields. Research on the application of biological preparations, particularly nitrogen-fixing agents, is of increasing relevance. These agents positively influence crop productivity and can reduce the need for nitrogen fertilisers (Hovenko, 2022).

Researchers in various countries are studying biological preparations based on the nitrogen-fixing endophytic bacterium *Methylobacterium symbioticum*. According to R. Torres Vera *et al.* (2024), this bacterium interacts with the host plant by supplying it with nitrogen from the atmosphere. When applied to the plant in the form of an aqueous solution, colonisation by naturally occurring bacteria takes place, and they continue to coexist with the plant throughout the growing season. As a result, crops may receive an additional nitrogen supply of 20-30 kg/ha or more. In particular, M.Â. Rodrigues *et al.* (2024) evaluated nitrogen fixation in maize plants treated with *Methylobacterium symbioticum* during 2021 and 2022. The additional nitrogen provided reached 58.8 kg/ha and 14.5 kg/ha, respectively. According to their findings, further research should focus on identifying which crops and growing conditions allow nitrogen-fixing agents to be most effective in enhancing nitrogen fixation and improving plant productivity.

One of the key morphobiological traits influencing plant growth and development is stem height. This characteristic is particularly important in maize, where it has both biological and technological significance and a considerable impact on crop productivity and yield. Based on three years of research, I.I. Senyk *et al.* (2023) established a direct correlation between plant height in maize and the rate of mineral nitrogen application. The optimisation of nutritional conditions through balanced rates of mineral fertilisers combined with biological preparations leads to more intensive stem growth and enhanced leaf area development, which positively affects overall crop yield.

Therefore, investigating the effectiveness of combining mineral fertilisers with biological preparations in maize fertilisation systems is a crucial research objective. Such approaches create favourable conditions for plant growth and development, ultimately contributing to higher productivity, which remains a highly relevant goal.

This study aimed to examine the effects of fertilisation system optimisation and plant density on the morphological characteristics and productivity of maize crops under the conditions of the Central Forest-Steppe of Ukraine.

Materials and Methods

Field research were carried out during 2023–2024 following standard methodologies developed by A.P. Lisoval (2001), S.O. Trybel *et al.* (2001), and V.O. Ushkarenko *et al.* (2019). The study was conducted under the soil and climatic conditions of the Liubaretske agricultural limited liability company (ALLC) in the Boryspil District of Kyiv Region (coordinates: 50°13’19.8”N, 31°09’49.6”E), using the maize hybrid P9255 (FAO 330). The experimental field is characterised by leached, low-humus chernozem soil with a humus content of 3.8%. The content of available nutrients in the soil was as follows: NO_3 –

2.05 mg/100 g of soil, P_2O_5 – 7.3 mg/100 g, K_2O – 14.0 mg/100 g. The soil pH was 6.2, and the bulk density was 1.2 g/cm^3. Each experimental plot had an area of 33 m^2. The experiment was arranged in four replications using a sequential multi-tier layout. The field trial design included variants with different application rates of mineral fertilisers, as well as treatments with the biological preparation–Blue N, based on the nitrogen-fixing endophytic bacterium *Methylobacterium symbioticum*. Control treatments included unfertilised plots and plots treated with the biological preparation alone, all tested under three different plant densities (Table 1).

Table 1. *Field experiment design*

Variant	Mineral fertiliser application rate, kg/ha active ingredient	Biopreparation rate, kg/ha	Plant density, thousand plants/ha		
Control (no fertiliser)	–	–	60	70	80
NPK fertiliser + urea	$N_{60}P_{45}K_{45}$	–	60	70	80
NPK fertiliser + urea	$N_{90}P_{60}K_{60}$	–	60	70	80
NPK fertiliser + urea	$N_{120}P_{90}K_{90}$	–	60	70	80
NPK fertiliser + urea	$N_{150}P_{120}K_{120}$	–	60	70	80
Control (no fertiliser) + nitrogen fixer	–	0.333	60	70	80
NPK fertiliser + urea + nitrogen fixer	$N_{60}P_{45}K_{45}$	0.333	60	70	80
NPK fertiliser + urea + nitrogen fixer	$N_{90}P_{60}K_{60}$	0.333	60	70	80
NPK fertiliser + urea + nitrogen fixer	$N_{120}P_{90}K_{90}$	0.333	60	70	80
NPK fertiliser + urea + nitrogen fixer	$N_{150}P_{120}K_{120}$	0.333	60	70	80

Source: developed by the authors

Mineral fertilisers were applied to the soil at rates specified in the experimental design. NPK fertiliser ($N_{15}P_{15}K_{15}$) was used as the base fertiliser, supplemented with urea (N_{46}). The fertilisers were broadcast and incorporated into the soil using a cultivator to a depth of 10-12 cm. After emergence, when the plants reached the 5-6 leaf stage, plots in the relevant treatments were sprayed with the biological preparation at the recommended application rate of 333 g/ha. The working solution volume used was 250 L/ha. The study was conducted in compliance with the Convention on Biological Diversity (1992).

Results

The research area is characterised by unstable and insufficient precipitation. The average annual relative humidity is 76%, with fluctuations ranging from 36% to 99%. In 2023, total precipitation during the growing season reached 375.4 mm, exceeding the long-term average by 9.4%. The hydrothermal coefficient (HTC) for that year was 1.23 (Table 2).

Table 2. *Meteorological conditions during the growing season, Boryspil District*

Indicator	2023	2024	Long-term average
Sum of active temperatures (April-September), °C	3,063.6	3,412.5	2,825.0
Sum of effective temperatures (> 10°C), April-September	1,463.6	1,715.4	1,300.0
Total precipitation, April-September, mm	375.4	329.2	343.0
HTC	1.23	0.96	1.21

Source: compiled by the authors based on measurements from the i-Metos online weather station

In 2024, the total precipitation was only 329.2 mm, amounting to 95.9% of the long-term norm. The HTC for that year was 0.96, indicating mild drought conditions. In 2023, between June and the first ten-day period of July, 116.8 mm of rainfall (31.1% of total growing season precipitation), while in 2024, this figure was 102.6 mm (31.2%), suggesting that maize plants received adequate moisture during the pre-flowering period. Overall, an analysis of weather data for 2023-2024, compared with long-term averages, indicates that the amount of rainfall during the growing season is generally sufficient to support high crop yields, particularly for maize.

In 2023 and 2024, a gradual increase in the sums of active (SAT) and effective temperatures (SET) was observed. The effective temperature sums exceeded the long-term average by 163.6°C-415.4°C. Such an increase in heat accumulation may accelerate the growth and development of agricultural crops, leading to earlier maturation. This was evident in 2024, when maize harvesting began in late August – approximately two weeks earlier than usual – at grain moisture levels ranging from 14% to 25%, depending on the maturity group of the hybrids.

The application of mineral fertilisers improved nitrogen nutrition in maize plants, resulting in more vigorous growth and development. Plant height was measured during the flowering stage, which occurred in the first and second ten-day periods of July, allowing for the assessment of the influence of the studied factors on this trait. It was found that applying the lowest rate of mineral fertilisers increased the height of hybrid P9255 plants by an average of 3.7% compared to the control (Fig. 1).

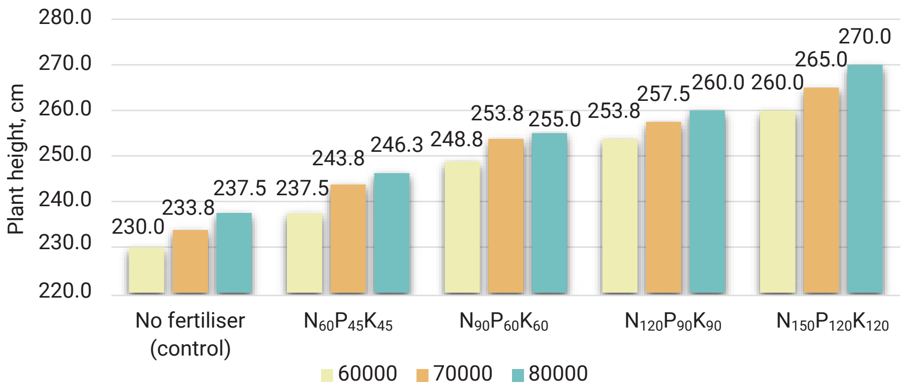

Figure 1. *Maize plant height during the flowering stage depending on plant density and fertilisation background (mineral fertilisers)*

Source: developed by the authors based on research results

The application of the highest rates of mineral fertilisers resulted in an average increase in plant height of 13.4% compared to the control. As plant density increased, a gradual rise in height was observed due to intensified competition between plants, provided that there was no moisture deficit. Specifically, at a plant density of 70,000 plants/ha, the average plant height exceeded that at 60,000 plants/ha by 1.9%, and at 80,000 plants/ha by 3.1%. Measurements of plant height in treatments where mineral fertilisers were used in combination with foliar application of a biopreparation revealed that, under minimum fertiliser application rates, plant height on average exceeded the control by 3.1% (Fig. 2). The use of maximum fertiliser rates led to an average increase in plant height of 15.5% compared to the control. The highest average plant height values were recorded in the treatments $N_{120}P_{90}K_{90}$ + nitrogen fixer (255-266 cm) and $N_{150}P_{120}K_{120}$ + nitrogen fixer (265-284 cm).

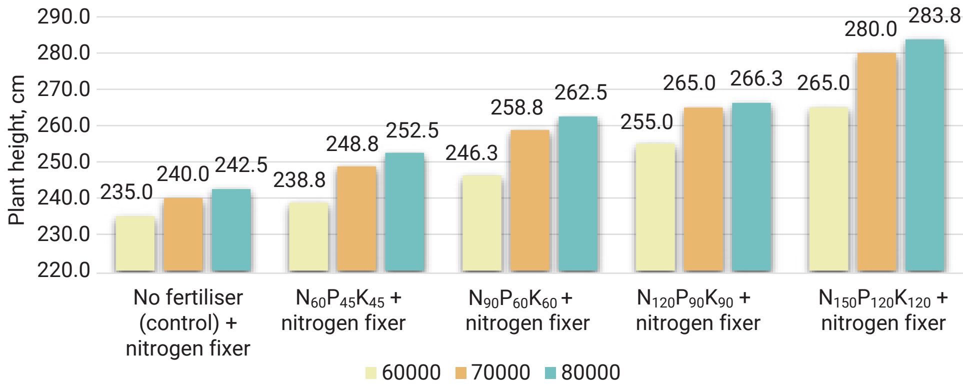

***Figure 2.** Maize plant height during the flowering stage depending on plant density and fertilisation background (mineral fertilisers + biopreparation)*

Source: developed by the authors based on research results

The average height of hybrid P9255 plants was 4.2% greater at a plant density of 70,000 plants/ha and 5.4% greater at 80,000 plants/ha compared to the respective values recorded at 60,000 plants/ha. Following the foliar application of the nitrogen-fixing agent, the average plant height exceeded that of treatments with mineral fertilisers alone by 1.7%-5.1%. This outcome is primarily attributed to the improved uptake of mineral nutrients by the plants, particularly nitrogen. A strong positive correlation (r = 0.98) was observed between the rate of nitrogen application and plant height.

Harvesting of maize grain from the experimental plots was carried out in mid-September 2023 and in late August 2024. Enhanced nitrogen availability contributed to more vigorous plant growth and development, which in turn promoted higher productivity compared to the control. The highest grain yields were recorded in the $N_{120}P_{90}K_{90}$ and $N_{150}P_{120}K_{120}$ treatments at a plant density of 70,000 plants/ha, reaching 12.71 and 12.94 t/ha, respectively (Fig. 3). A strong positive correlation (r = 0.92) was identified between nitrogen application rates and yield across different plant densities. Overall, treatments with mineral fertilisers produced maize yields 1.56-2.38 t/ha (14.5%-22.5%) higher than the control.

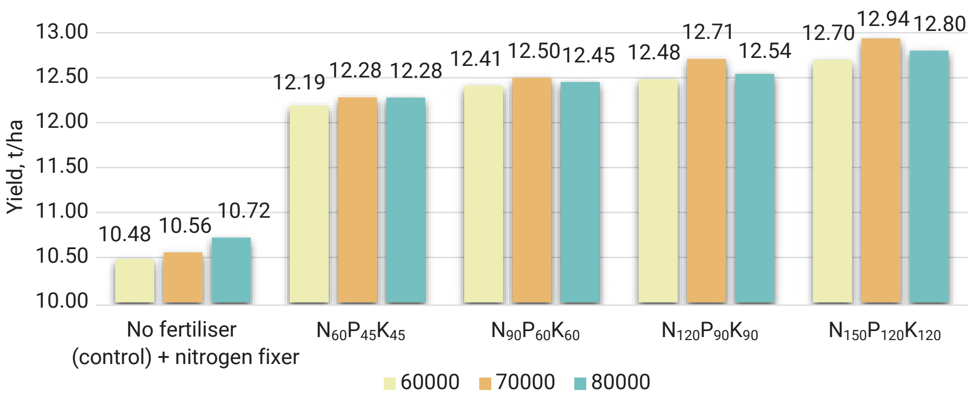

***Figure 3.** Maize yield depending on plant density and mineral fertiliser application rates*

Source: developed by the authors based on research results

Treatments combining mineral fertilisers with foliar application of a nitrogen-fixing agent supported vigorous plant growth and development, resulting in high productivity (Fig. 4). In these treatments, maize grain yields were 1.38-2.30 t/ha (12.2%-21.2%) higher than in the control. The highest average grain yield, reaching 13.38 t/ha, was recorded in the $N_{150}P_{120}K_{120}$ + nitrogen fixer treatment at a plant density of 70,000 plants/ha.

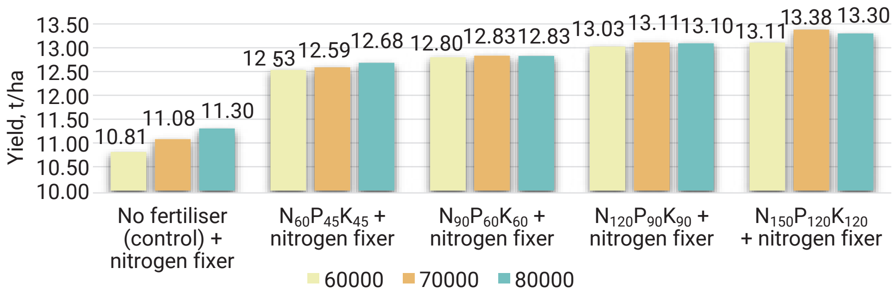

Figure 4. *Maize yield depending on plant density and mineral fertiliser application rates with nitrogenfixing agent*

Source: developed by the authors based on research results

Maize responded positively to the inclusion of the nitrogen-fixing agent in the cultivation technology, which enhanced nitrogen uptake. A strong positive correlation (r = 0.95) was observed between nitrogen application rates and crop yield across different plant densities. On average, grain yield was 3.6% or 0.43 t/ha higher compared to treatments without foliar application of the biopreparation.

Similar studies have been conducted by other researchers. Polish authors A. Zapałowska & W. Jarecki (2024) reported a significant effect of mineral fertilisation on maize growth parameters, including plant height, ear length, number of kernels per ear, and 1,000-grain weight. Ukrainian researchers I.I. Senyk *et al.* (2023) established a direct correlation between maize plant height and the application rate and form of mineral nitrogen fertilisers. Under an identical fertiliser rate of $N_{210}P_{60}K_{60}$, the tallest plants were observed with anhydrous ammonia (268.9 cm at the milk-wax ripeness stage), followed by urea (266.0 cm), and UAN-32 (251.5 cm). M.I. Dudka *et al.* (2020) demonstrated that foliar feeding with a mixture of urea and chelated micronutrients increased plant height by 6-7 cm, and by up to 10 cm when applied twice – during the 5-6 and 8-9 leaf stages. In terms of yield, a single application increased maize grain output by 0.30-0.34 t/ha, while two applications raised it by 0.41 t/ha.

According to O.I. Tsyliuryk *et al.* (2024), under the conditions of the Northern Steppe of Ukraine, the maximum height of maize plants, regardless of the hybrid maturity group, was observed at a plant density of 30,000 plants/ha. A gradual increase in plant density to 60,000 plants/ha led to a decrease in plant height from 210-253 cm to 202-236 cm. This reduction was attributed to competitive interactions among plants for essential resources such as water, nutrients, and light. The application of mineral fertilisers, particularly at a rate of $N_{60}P_{60}K_{60}$, enabled an increase in plant height by 6-25 cm compared with unfertilised treatments. Research by O.V. Sydyakina & M.O. Ivaniv (2020) found that the application of growth stimulants resulted in increased plant height by 3-6 cm at harvest, depending on the mineral nutrition background. The use of mineral fertilisers at a rate of $N_{180}P_{90}$, combined with foliar application of a growth stimulant, resulted in a grain yield increase of 3.8 t/ha. Y.I. Tkalich *et al.* (2023) treated maize crops with plant growth regulators at the 3-5 leaf stage, which contributed to a 5-7 cm (2.1%-2.8%) increase in plant height and a 1.23 t/ha (18.7%) increase in grain yield. O.I. Len *et al.* (2021) studied the combined effect of applying mineral fertilisers at a rate of $N_{45}K_{40}K_{60}$ and foliar feeding at the 5-6 leaf stage with urea (N_{15}) and a water-soluble multimineral micronutrient fertiliser. The resulting yield increase in hybrid grain compared with the control ranged from 1.06 to 1.20 t/ha, or 18.9%-19.2%.

Researchers I.M. Didur & V. Tsyhanskyy (2017), as well as V. Tsyhanskyy (2017), established that foliar feeding with biological preparations creates optimal conditions for the uptake of mineral nutrients and maximises the biological potential of maize plants. Experiments conducted by R. Singh *et al.* (2024) focused on the interaction between nitrogen, phosphorus, and potassium fertilisers, which significantly influence the absorption of micronutrients by maize plants and their availability in the soil. The application of increased nitrogen rates raises the concentration of micronutrients in maize grain.

T. Marchenko (2019) reported a strong correlation between plant height, ear placement height, leaf surface area, and grain yield in hybrids. According to L. Shevchenko & L. Tokmakova (2018), the use of growth stimulants through seed bacterisation and surface treatment of vegetative plants at the 7-9 leaf stage nearly doubles the net photosynthetic productivity and prolongs the photosynthetic activity of maize plants. V. Kaminskyi & N. Asanishvili (2020) highlighted the potential and experimentally confirmed the effectiveness of improving photosynthetic performance and the overall productivity of maize through the development of agrocenoses with optimal morphostructural parameters and functional characteristics, achieved by implementing advanced cultivation technologies.

According to D.A. López-Carmona *et al.* (2019), the application of biological preparations promotes the development of the root system and enhances symbiosis with nitrogen-fixing bacteria and mycorrhizae. This, in turn, improves maize plants' utilisation of soil moisture, leading to increased crop productivity and enhanced grain quality, particularly with higher levels of protein, starch and fat. Polish researchers M. Niewęgłowski *et al.* (2024) and A. Zapałowska & W. Jarecki (2024) found that the application of mineral fertilisers and biological stimulants increased the protein content in the grain of the maize hybrids studied, compared with the control. An increase in starch and fat content in the grain was also observed, although the differences were not statistically significant. At the same time, the influence of moisture availability must be taken into account. Research by N. Wang *et al.* (2025) showed that limited water supply reduced grain yield, dry matter accumulation, nitrogen and water uptake and use efficiency, and negatively affected most soil properties compared with plots supplied with sufficient water.

Thus, determining the effectiveness of mineral fertilisers in combination with modern biological preparations in maize nutrition systems remains a complex and highly relevant task. The effectiveness of nitrogen-fixing agents in increasing maize grain yield is a key component of cultivation technologies in the context of climate change and rising mineral fertiliser costs. The conducted studies have demonstrated the impact of optimising fertiliser regimes on the formation of optimal morphostructural plant characteristics and maize yield under varying plant densities in conditions of unstable and limited moisture availability.

Conclusions

It was established that optimising maize nutrition – specifically, combining mineral fertilisers with the biopreparation Blue N – had a positive effect on crop functioning. This approach contributed to an increase in plant height, enhanced accumulation of organic matter, and, consequently, higher yield levels. The height of P9255 maize plants under the application of the biological preparation was, on average, 1.7%-5.1% greater than in treatments where only mineral fertilisers were used.

With increasing plant density, a gradual rise in maize height was observed, attributed to intensified competition among plants. In treatments with mineral fertilisers alone, plant height at a density of 70,000 plants/ha was 1.9% higher, and at 80,000 plants/ha – 3.0% higher than at 60,000 plants/ha. When mineral fertilisers were combined with foliar application of a nitrogen-fixing agent, plant height at a density of 70,000 plants/ha exceeded that at 60,000 plants/ha by 4.7%, and at 80,000 plants/ha – by 6.0%

On average, over the period 2023-2024, the application of mineral fertilisers alone resulted in a 14.5%-22.5% increase in maize grain yield compared with the control. The highest yield, reaching 12.94 t/ha, was recorded under $N_{150}P_{120}K_{120}$ treatment with a plant density of 70,000 plants/ha. In treatments where mineral fertilisers were combined with a biological agent, grain yield increased by 12.2%-21.2% compared with the corresponding control plus nitrogen-fixing agent. The treatment $N_{150}P_{120}K_{120}$ + nitrogen fixer at a plant density of 70,000 plants/ha provided the highest average grain yield, reaching 13.38 t/ha.

Future research should focus on identifying agricultural crops that, in association with *Methylobacterium symbioticum*, will facilitate efficient atmospheric nitrogen assimilation and contribute to improved crop productivity. Additionally, it is necessary to assess the economic and energy efficiency of optimising nitrogen nutrition in agricultural crops through the use of nitrogen-fixing biopreparations.

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Conflict of Interest

None.

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Вплив мінерального живлення та азотфіксатора на урожайність кукурудзи в Центральному Лісостепу України

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Анотація. У сучасному аграрному виробництві зростає потреба у застосуванні біопрепаратів, зокрема азотфіксаторів, які дозволяють зменшити використання мінеральних добрив і водночас підвищити урожайність кукурудзи. Метою дослідження було встановлення впливу різних варіантів оптимізації удобрення та густоти стояння рослин на морфологічні характеристики та урожайність кукурудзи в агроекологічних умовах Центрального Лісостепу України. Польові дослідження проведено впродовж 2023-2024 рр. в ґрунтово-кліматичних умовах господарства в Київській області на посівах кукурудзи гібриду P9255 (ФАО 330). Розмір дослідної ділянки був 33 м^2, повторність 4-кратна, розміщення ділянок послідовне багатоярусне. Використання біологічного препарату азотфіксатора в системі удобрення кукурудзи у поєднанні з мінеральними добривами позитивно впливало на ріст рослин. При цьому висота рослин кукурудзи на 1,7-5,1 % перевищувала відповідний показник варіантів із внесенням лише мінеральних добрив. Проведено оцінку ефективності та визначено вплив оптимізації системи удобрення на морфологічні ознаки та продуктивність рослин кукурудзи за рахунок застосування біопрепарату у поєднанні із мінеральними добривами за різних норм їх внесення. Загалом, за застосування лише мінеральних добрив одержано урожайність зерна кукурудзи на 14,5-22,5 % вищу, порівняно з контролем. Найбільшу урожайність, яка сягала 12,94 т/га, отримано у варіанті $N_{150}P_{120}K_{120}$ за густоти стояння 70 тис./га. У варіантах із застосуванням мінеральних добрив у поєднанні з біопрепаратом одержано урожайність зерна кукурудзи на 12,2-21,2 % вищу, ніж на відповідному контролі. Найбільший середній показник урожайності зерна, який сягав 13,38 т/га, забезпечив варіант $N_{150}P_{120}K_{120}$ + азотфіксатор за густоти стояння рослин на рівні 70 тис./га. При застосуванні біопрепарату у поєднанні з мінеральними добривами одержано на 3,6 % або 0,43 т/га вищу урожайність зерна культури, ніж у варіантах без його внесення

Ключові слова: біопрепарат; густота стояння; висота рослин; мінеральні добрива; урожайність зерна