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ORIGINAL RESEARCH

# Effect of fertilization and microbial preparations on productivity of chickpea (*Cicer arietinum* L.)

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## Abstract

The aim of the study was to optimize the nutritional status and increase the productivity of chickpeas through the application of mineral and organic-mineral fertilizers as well as microbial preparations based on nitrogen-fixing, phosphorus-mobilizing, and potassium-mobilizing microorganisms. The research was conducted in 2019–2021 in the Poltava Research Agricultural Station (Ukraine). NPK fertilizers were applied at a dose, of 20, 80, and 80 kg ha$^{-1}$, respectively. Before sowing, the seeds were inoculated with the microbial preparation BiNitro Chickpea (BN) and Biofosforyn (BF). Foliar feeding was carried out with the microfertilizer Freya–Aqua™ C(12) Legumes (F). The development of chickpea leaf area and the intensity of organic matter production were largely determined by the supply of the main macronutrients to the plants. The introduction of NPK significantly increased leaf area and net photosynthetic productivity, while the effect of BN, BF, and F on the values of these indices was not significant. Their effect was manifested during the accumulation of aboveground biomass and the formation of yield structure elements. An increase in the seed yield was shown in relation to the application of NPK, microbial preparations, and micronutrient fertilizers. Regardless of the NPK, the highest yield was shown in the variant of the combination of BN+BF+F.

## Keywords

chickpea; microbial preparation; microfertilizer; mineral fertilizer; seed yield; yield structure

## 1. Introduction

One of the ways to supply the food and fodder sector with sources of plant protein in conditions of increasing average daily air temperature and duration of dry periods is to increase the area sown and the level of productivity of leguminous crops, which are characterized by high adaptability to the effects of unfavorable environmental factors. A valuable representative of this group of crops is chickpea (*Cicer arietinum* L.), the seeds of which contain approximately 19.3–25.4% of protein, which is well-balanced in terms of the amino acid composition (Erman et al., 2011), 60–70% of carbohydrates, 5–7% of fat, vitamins, trace elements, carotenoids, organic acids, and biologically active compounds (Jukanti et al., 2012).

Chickpeas rank third in terms of globally sown area after soya and beans (Thangwana & Ogola, 2012). In terms of legume seed production, its share is about 20%, or an average of 13.1 million tons (Ouji et al., 2016). The widespread distribution of chickpea cultivation worldwide is linked, first and foremost, to the biological properties of the plant, which confer its high adaptability to different agroclimatic growth conditions. The chickpea is a fairly drought-tolerant and heat-tolerant crop due to its well-developed root system capable of overcoming mechanical obstacles and the increased osmotic pressure in the plant cells, thus strongly retaining water and reducing evaporation (Kaushal et al., 2013).

As a legume, chickpeas have the ability to enter into a symbiotic relationship with root-nodule bacteria of the genus *Mesorhizobium*, such as *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum*. Thanks to the functioning of the symbiotic nitrogen-fixing system, chickpea plants can meet around 70% of their nitrogen requirements. The reduced form of nitrogen is further used by plants in the synthesis of proteins and nucleic acids, which are the most important building and signaling substances in cells, and chlorophyll molecules, which enable the plant to transfer solar energy through photosynthesis into energy that can be used by plants (Esfahani et al., 2014).

A promising environmentally friendly and ecologically safe method of growing legumes and increasing their yield is the use of microbial preparations based on nitrogen-fixing microorganisms, which promote the emergence of a more developed symbiotic apparatus, increasing its efficiency and accelerating the natural process of biological $N_2$ fixation to meet the nutritional needs of plants for this element and restore soil fertility. This also reduces dependence on chemical fertilizers (Achmad et al., 2019; Dzida et al., 2023; Makhlouf et al., 2023; Saeed et al., 2021). Nevertheless, proper management of plant nitrogen supplies through the use of effective rhizobial inoculants and the application of a starter nitrogen dose can increase the productivity level in legumes (Salvagiotti et al., 2008).

There are scientific reports showing that, during the period of root colonization by rhizobia and the onset of biological nitrogen fixation, young plants may need a small amount of mineral nitrogen to achieve sufficient vegetative growth. On the other hand, inappropriate use of starter nitrogen may have a negative effect on the establishment of symbiosis between rhizobium bacteria and legumes. When there is a high supply of mineral nitrogen to the plant, lentils tend to use nitrogen from the soil rather than from the atmosphere (Jinwen et al., 2016).

One of the important factors determining the establishment and functioning of legume symbiosis with rhizobium bacteria is the supply of phosphorus to plants, which affects the size of the symbiotic apparatus and the intensity of metabolic processes in the nodules (Zaheer et al., 2019). Research has shown that even a slight deficiency of this element in the soil can lead to inhibition of the activity of the nitrogen-fixing enzyme nitrate reductase in the nodules (Li et al., 2021). According to Khan et al. (2009), crop inoculation can increase phosphorus assimilation by 40 to 50 $P_2O_5$ kg ha and increase yield by 10–20%.

Potassium is a component of enzymes that determine the direction and intensity of major physiological and biochemical processes in plants, such as photosynthesis, respiration, protein synthesis, and accumulation of symbiotically fixed nitrogen. It thus promotes the efficient use of water by plants and increases plant resistance to heat stress, drought, and damage from diseases and pests. In addition, potassium deficiency hinders nitrogen uptake and consequently inhibits the growth of the assimilative surface area of leaves and reduces nitrate uptake and transport (Goud et al., 2014).

A prospective method to promote the growth and development of agricultural crops is the use of microbial preparations based on nitrogen-fixing and phosphorus- and potassium-mobilizing microorganisms. Their use promotes the improvement of the supply of nitrogen and soluble forms of phosphorus and potassium to plants thus increasing plant productivity. Biofertilizers enhance soil fertility by fixing atmospheric nitrogen both with and without plant roots, solubilizing insoluble soil phosphates, and generating plant growth chemicals in the soil. Vegetative growth and yield improve after legume seed plants are inoculated with associative $N_2$-fixing bacteria (Fasusi et al., 2021; Mohanty et al., 2021; Santoyo et al., 2021; Sayed & Ouis, 2022). In the study conducted by Meena et al. (2020), the use of biofertilizers contributed to the release of growth hormones into the rhizosphere, increasing the intensity of cell division, which could explain the increase in leaf area and photosynthetic productivity. It has also been determined that the use of microbial inoculants increases the availability of nutrients and the level of their absorption by plants and activates the physiological and biochemical processes that underlie high yields (Bertola et al., 2019).

All biochemical reactions in the plant organism require micronutrients, which are structural components of enzymes, hormones, and vitamins. They play an important role in the synthesis of proteins, nucleic acids, and photosynthetic pigments and in the structure and functional integrity of cell membranes (Dass et al., 2022; Elham et al., 2022). Numerous studies have shown that the application of humic substances at low concentrations increases nutrient uptake and, by inducing carbon and nitrogen metabolism, contributes to the growth of the root system and above-ground parts of plants (Canellas et al., 2020).

An effective method to increase the mineral supply to plants is foliar feeding. The application of foliar fertilizers, especially during critical periods of plant development, can increase seed yield by 12–25% (Hu et al., 2008). Foliar fertilization effectively delays leaf senescence and extends the duration of photosynthetic activity, which enables the duration of the formation of the generative parts of the plant to be extended, increases the number of pods, and improves seed quality (Das & Jana, 2016).

High yields of chickpeas can only be achieved in good environmental conditions (soil and weather) and in conditions of an optimal supply of nutrients essential for plant growth and development. Even temporary changes and disturbances in plant growth processes can lead to changes in yield formation processes. It is therefore important to improve conditions for plant growth and development during the growing season by optimizing plant nutrition. Currently, the use of microbiological preparations in the technological process of chickpea cultivation is a relatively promising approach that improves plant nutrition and increases its productivity. In this regard, improving the fertilizer system through the integrated use of mineral fertilizers, micronutrient fertilizers, and microbiological preparations based on nitrogen-fixing and phosphorus- and potassium-mobilizing microorganisms is relevant.

The purpose of the study was to determine the effect of mineral fertilizers, microfertilizers, and microbiological preparations based on nitrogen-fixing and phosphorus- and potassium-mobilizing microorganisms and their combinations on chickpea yield formation.

The hypothesis was that the use of mineral fertilizers and micronutrient fertilizers will improve the nutritional status of plants and will have a positive effect on the formation of productivity elements and increase the yield of chickpea seeds.

## 2. Material and methods

### 2.1. Experimental conditions and treatments

The research was conducted in 2019–2021 in the conditions of a two-factor field experiment in the M. I. Vavylov Poltava Research Agricultural Station of the Institute of Pig Breeding and Agroindustrial Production of the National Academy of Agricultural Sciences. The geographical location of the study area is 49.55° north latitude and 34.78° east longitude. The average height above sea level is 175 m.

The soil in the experimental field is a typical low-humus, heavy, clayey (loamy) chernozem with humus content of 5.15% (in the 0–20 cm layer); available macroelements include (mg $kg^{-1}$ soil) nitrogen—162, phosphorus—150, and potassium—208, $pH_{KCl}$ is 5.8, and soil EC is 83.2 μS $sm^{-1}$.

The meteorological conditions during the growing seasons in the years of the study were quite variable (Figure 1, Figure 2). In 2019, the uneven distribution of precipitation and the reduced amount of precipitation in the period April–August by 64.6 mm, compared to the long-term average, were combined with the increased values of the average daily air temperature. The amount of precipitation during the growing seasons in 2020 and 2021 was 258.5 mm and 327.0 mm, respectively, which exceeded the long-term average by 5.2% and 33.1%, respectively. At the same time, the average air temperature in the summer months was higher than the long-term average, especially in 2021.

The experiment was conducted according to a randomized block (split-plot) scheme in four replicates. The experimental design included the following factors: A – mineral

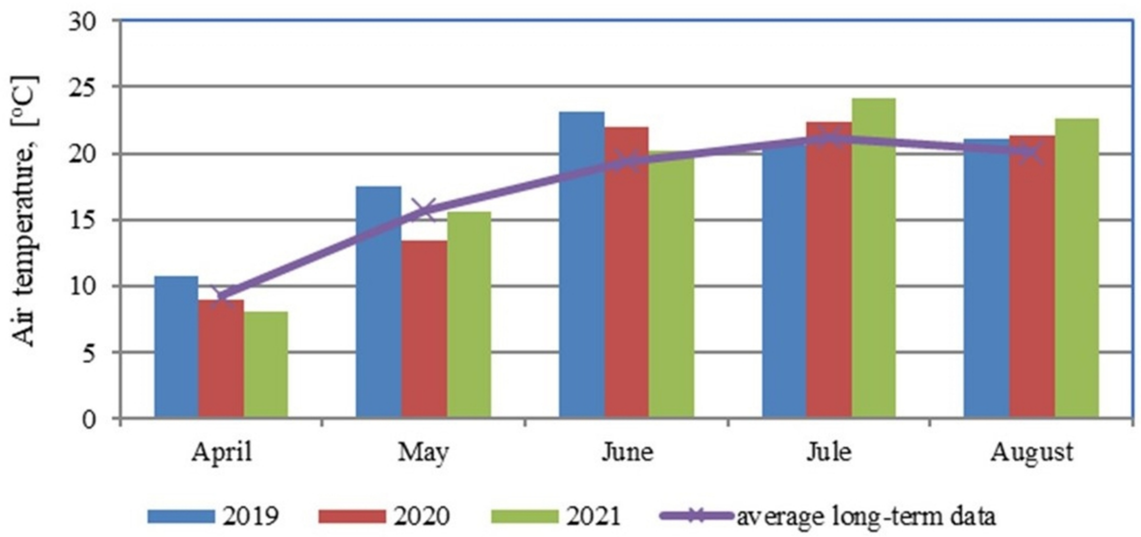


**Figure 1** Air temperature during the growing season of 2019–2021 according to the meteorological post of the Poltava State Agricultural Experiment Station.

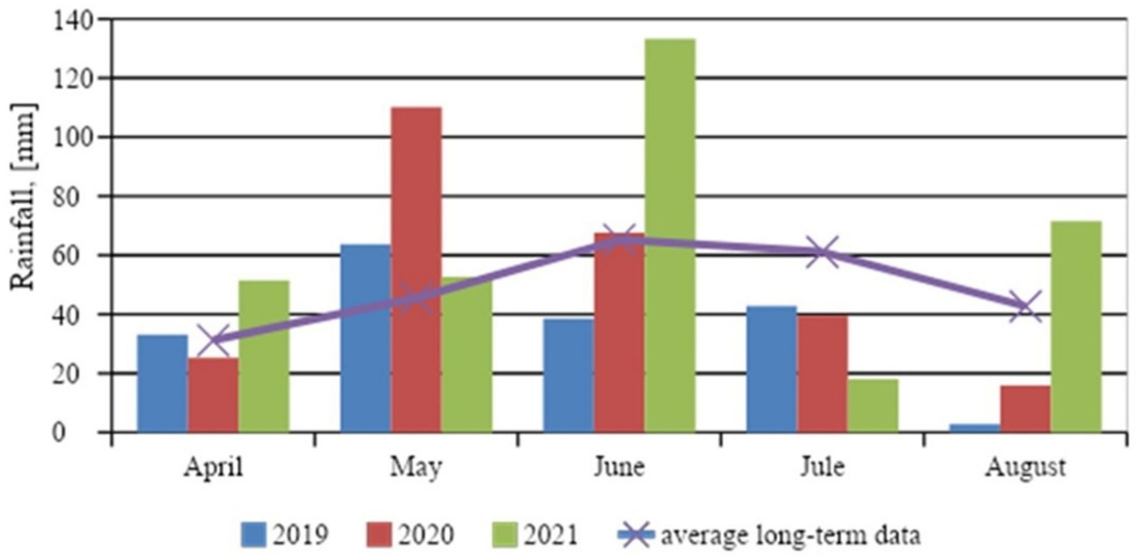


**Figure 2** Rainfall during the growing seasons of 2019–2021 according to the meteorological post of the Poltava State Agricultural Experiment Station.

fertilization: $N_0P_0K_0$, application of $N_{20}P_{80}K_{80}$; B – treatment with different combinations of microbial preparations and microfertilizer (Table 1).

The plot area was 27 $m^2$ (harvested area of 25 $m^2$). The plot consisted of 6 rows that were 45 cm wide. The forecrop of chickpea was winter wheat.

Phosphorus fertilization in the form of ammophos (nitrogen content 12%, phosphorus 52%) and potassium fertilization in the form of potassium chloride (potassium content 60%) were applied in autumn at a dose of 80 kg $ha^{-1}$ $P_2O_5$ and 80 kg $ha^{-1}$ $K_2O$ before the main soil cultivation. Nitrogen fertilizers in the form of ammonium nitrate (34.5%) were applied to the pre-sowing crop at a dose of 20 kg $ha^{-1}$.

Chickpeas were sown in the third ten days of April (between 22 and 25 April).

The seeds were sown at a density of 40 germinating seeds per $m^2$ to a depth of 6–8 cm. Before sowing, the seeds were inoculated with a microbial preparation based

**Table 1** Design of the experimental treatments.

| First factor | Fertilization |
|---|---|
| NF | $N_0P_0K_0$ |
| NPK | $N_{20}P_{80}K_{80}$ |
| **Second factor** | **Treatment** |
| K | Control (without plant nutrition) |
| BN | BiNitro Chickpea (2.0 l $t^{-1}$) |
| BN+BF | BiNitro Chickpea (2.0 l $t^{-1}$) + Biofosforyn (2.0 l $t^{-1}$) |
| F | Freya–Aqua™ C(12) Legumes (2.0 l $ha^{-1}$) |
| BN+F | BiNitro Chickpea (2.0 l $t^{-1}$) + Freya–Aqua™ C(12) Legumes (2.0 l $ha^{-1}$) |
| BN+BF+F | BiNitro Chickpea (2.0 l $t^{-1}$) + Biofosforyn (2.0 l $t^{-1}$) + Freya–Aqua™ C(12) Legumes (2.0 l $ha^{1}$) |

on nitrogen-fixing microorganisms BiNitro Chickpea (2.0 l $t^{-1}$) containing nitrogen-fixing nodule bacteria *Mesorhizobium ciceri* strain MC 285 with a titer of at least 2 × 109 CFU/ml and products of their metabolism: phytohormones, amino acids, vitamins and its complex or a microbial preparation Biofosforyn (2.0 l $t^{-1}$) based on live cells and spores of the bacterium *Bacillus megaterium* strain BM 206 with a titer of at least 5 × 108 CFU/ml and their metabolic products: phytohormones of the auxin, gibberellin, and cytokinin series, amino acids, and vitamins. Foliar feeding of the chickpea plants was carried out at the beginning of the budding stage with the microfertilizer Freya–Aqua™ C(12) Legumes (2.0 l $ha^{-1}$), which is a concentrated solution of humic substances with trace elements for legumes (N, P, K, S, Cu, Fe, Zn, Mn, B, Mo, Co, Ni). The seed inoculation and foliar feeding of the plants were carried out according to the experimental scheme. All other agrotechnical techniques were carried out according to the recommended agrotechnology protocols for chickpea cultivation.

### 2.2. Leaf area and above ground dry mass

The leaf area was determined with the excision method (Nichiporovich, 1969) at the flowering stage (BBCH 71–74, R4). To determine this indicator, 10 plants were taken from each plot. Leaves from each plant were plucked and weighed to the second decimal point, and incisions were made with a special key of a certain diameter. Knowing the weight and area of the incisions as well as the total weight of the leaf, the leaf area was determined using the formula:

$$S = P * \ S_1 * nP_1$$

where:

$S$ — total leaf area, $cm^2$; $S_1$ — area of one section, $cm^2$; $P$ — total leaf weight, g; $n$ — number of slices; $P_1$ — weight of sections, g

Once the leaf area of each plant was determined, the average leaf area for each variant of the experiment was calculated by taking the value of the average leaf area of one plant and multiplying it by the number of plants per square meter. The result was multiplied by 10,000 to convert it to an average leaf area per hectare.

To determine the dry weight of the plants, the selected test samples were oven-dried at 105 °C to constant weight.

### 2.3. Net photosynthetic productivity (NPP)

The Net Photosynthetic Productivity (NPP) (g $cm^{-2}$ $day^{-1}$) was determined using to the following formula (Nichiporovich, 1969):

NPP = $(M_2 - M_1)$ / $[0.5 - (S_1 + S_2) - T]$, where $M_1$, $M_2$ are the mass of completely dry plants per unit area (g) at the beginning and end of a certain period; $S_1$, $S_2$ are the area of the leaf apparatus in the designated periods ($cm^2$); T is the duration of the period (days).

NPP acts as a widely used measure of plant photosynthetic efficiency, which expresses dry biomass gain per unit leaf area and is a complex physiological variable related to the rate of photosynthesis and respiration.

### 2.4. Yield and its components

In order to carry out a yield structure element analysis and to determine the size of the structural components of the chickpea harvest, such as the total number of pods and kernels on the plants, 15 plants from each plot were selected before harvesting (Hrytsaienko et al., 2003). Harvesting was conducted from each plot separately at the full maturity stage of chickpea (R-12) in the third ten days of August. During harvesting, samples were taken to determine the seed moisture and weight of 1,000 seeds.

### 2.5. Statistical analyses, data processing and analysis

Data were statistically analyzed using the analysis of variance (ANOVA) method for a two-factor split-plot experiment. Since similar results were obtained in each year of the study, a pooled analysis was performed and the results are presented as averages over the 3 years. The averages were compared using the Tukey Test at a significance level of 0.05.

## 3. Results

### 3.1. Leaf area (LA)

The results show a significant positive effect of mineral fertilization (NPK) on the development of the leaf area of chickpea plants (Figure 3A). Its increase after the application of the NPK variants relative to the control was significant and averaged 11.1%. The chickpea leaf area, depending on the microbial preparations and microfertilizers applied, increased from 29.1 thousand $m^2$ $ha^{-1}$ in the control variant (K) to 31.9 thousand $m^2$ $ha^{-1}$ in the variant combining seed inoculation and foliar plant nutrition (BN+BF+F). There was a positive trend towards an increase in chickpea leaf area under the influence of the applied microbial preparations and microfertilizers, but the differences were not statistically significant (Table 2).

**Table 2** Results (*p*-values) of a two-way ANOVA of the effect of NPK fertilization, seed inoculation, foliar plant application, and their interaction on the morphological features of plants, physiological traits, yield components, and yield.

| Treatment | LA | NPP | AGDM | NP | NS | TSW | SY |
|---|---|---|---|---|---|---|---|
| NPK | 0.0000 | 0.0072 | 0.0000 | 0.0001 | 0.0000 | 0.0000 | 0.0000 |
| Treatments | 0.1282 | 0.1733 | 0.0001 | 0.0041 | 0.0015 | 0.0001 | 0.0006 |
| NPK × Treatments | 0.9949 | 0.9721 | 0.9362 | 0.9911 | 0.9023 | 0.9446 | 0.9991 |

Leaf area **(LA)**, net photosynthetic productivity **(NPP)**, above ground dry mass of plant **(AGDM)**, number of pods per plant **(NP)**, number of seeds per plant **(NS)**, 1,000-seed weight **(TSW)**, seed yield **(SY)**.

### 3.2. Net photosynthetic productivity (NPP)

The results presented in this study indicate a significant positive effect of the NPK mineral fertilization on the intensity of organic matter production per unit area of chickpea leaves (Figure 3B). In the NPK-applied sites, the increase in net photosynthetic productivity (NPP) was 2.8% compared to the variant without mineral fertilization (NF). The application of the microbial and micronutrient preparations had no significant effect on the value of NPP. The increase in the value of this indicator compared to the control in the variants of application of the microbial preparations, microfertilizers, and their combination was about 1.0%, which was within the statistical error.

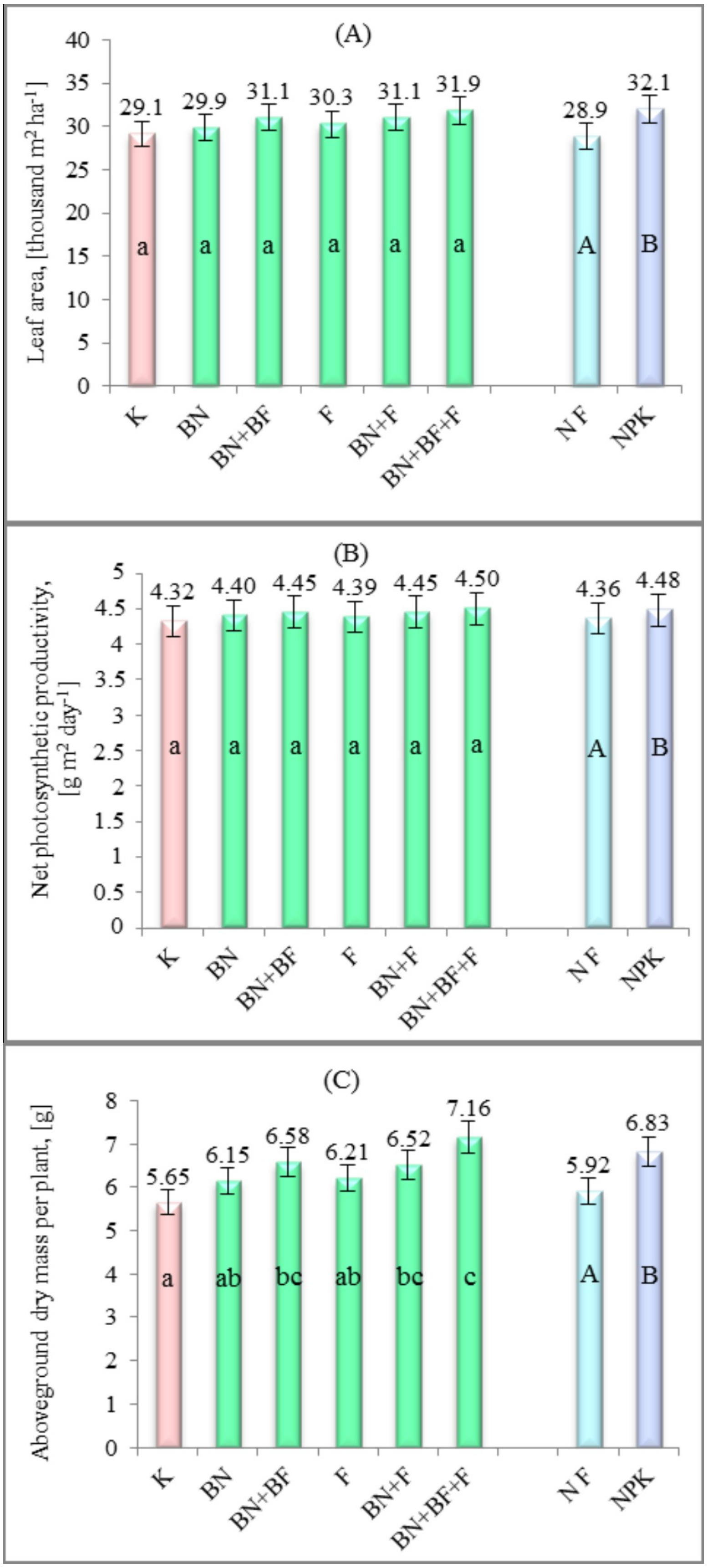


**Figure 3** **(A)** Leaf area (LA), **(B)** net photosynthetic productivity (NPP), and **(C)** aboveground dry mass per plant (AGDM) of chickpea depending on mineral fertilization, seed inoculation, foliar application, and their combination; K — control object, BN — BiNitro Chickpea; BN+BF — BiNitro Chickpea+Biofosforyn; F — Freya–Aqua™ C(12) Legumes; BN+F — BiNitro Chickpea+Freya–Aqua™ C(12) Legumes; BN+BF+F — BiNitro Chickpea+Biofosforyn+Freya–Aqua™ C(12) Legumes; NF — $N_0P_0K_0$; NPK — $N_{20}P_{80}K_{80}$.

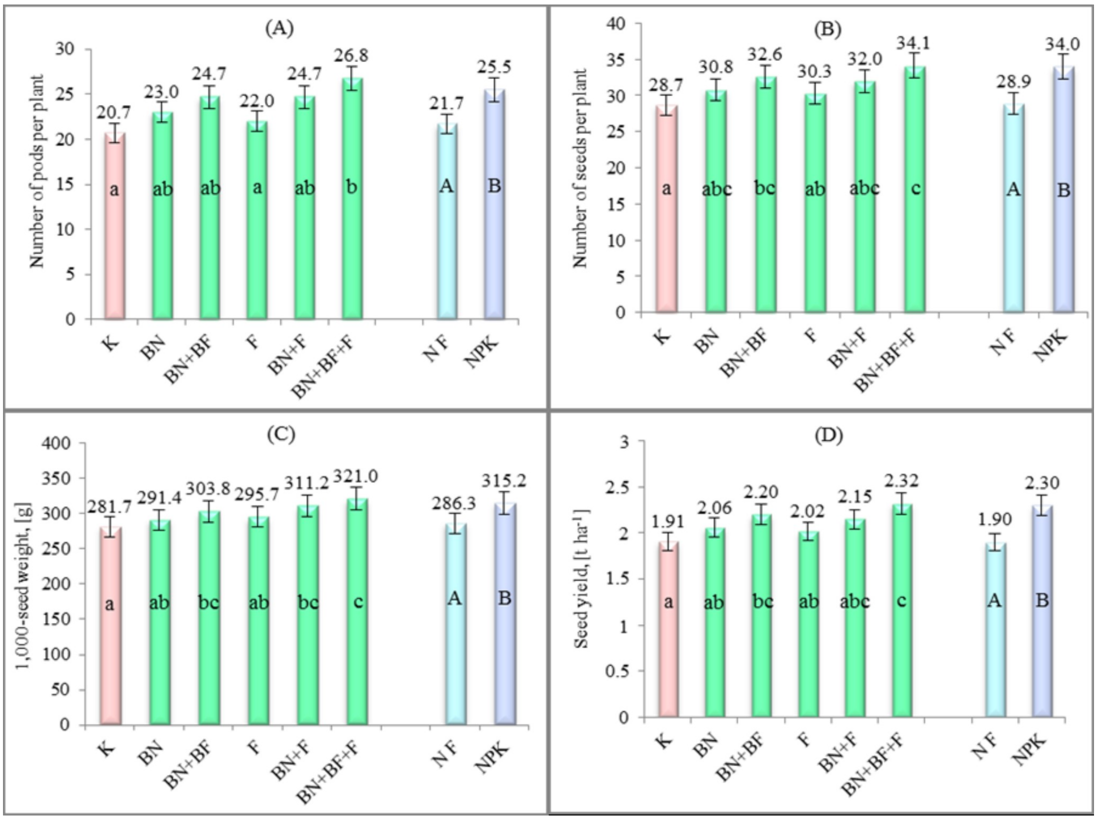


**Figure 4** **(A)** Number of pods per plant (NP), **(B)** number of seeds per plant (NS), **(C)** 1,000-seed weight (TSW), and **(D)** seed yield (SY) of chickpea depending on mineral fertilization, seed inoculation, foliar application, and their combination; K — control object, BN — BiNitro Chickpea; BN+BF — BiNitro Chickpea+Biofosforyn; F — Freya–Aqua™ C(12) Legumes; BN+F — BiNitro Chickpea+Freya–Aqua™ C(12) Legumes; BN+BF+F — BiNitro Chickpea + Biofosforyn + Freya–Aqua™ C(12) Legumes; NF — $N_0P_0K_0$; NPK — $N_{20}P_{80}K_{80}$.

### 3.3. Above ground dry mass (AGDM)

The study recorded a significant positive effect of the mineral fertilizers on dry matter accumulation by chickpea plants (Figure 3C). In the NPK-applied variants, the aboveground dry matter per plant increased by an average of 15.4% compared to sites where no mineral fertilizer was applied. Regardless of the NPK fertilization, the combined application of the BN+BF inoculants contributed to a significant increase in the dry weight of the aboveground part of the plant (by 16.5%) compared to the control. In the variant combining seed inoculation with the microbial preparation BN and foliar feeding of plants with micronutrient F, the dry weight per plant was 6.52 g, which was significantly higher than in the control (by 15.4%). The BN+BF+F combination was the most effective in terms of dry biomass accumulation by the chickpea plants. In this variant, AGDM reached a maximum value of 7.16 g per plant, which was significantly higher than in the control (by 26.7%).

### 3.4. Number of pods per plant (NP)

The study showed a significant positive effect of the mineral fertilizers, microbial preparations, and their combinations on the formation of chickpea yield structure elements. The application of NPK led to a significant increase in the NP (by 17.5% on average) compared to variants NF (Figure 4A). The applied microbial and microfertilizer preparations changed the NP to between 20.7 and 26.8 pods per plant, but a significant increase of 29.5% compared to the control was recorded only in the variant with the combination of seed inoculation with the complex of microbial preparations (BN+BF) and foliar feeding of plants with microfertilizer F compared to the control (by 26.7%).

### 3.5. Number of seeds per plant (NS)

The increase in the NP on the plants contributed to an increase in the NS, as it is one of the most important elements determining yield (Figure 4B). In the NPK application variants, the NS increased by 17.6% compared to the NF object. The microbial and microfertilizer application variants revealed a tendency to increase the NS, but a significant increase in the NS, compared to the control, was observed in the BN+BF and BN+BF+F variants, i.e. by 13.6% and 18.8%, respectively.

### 3.6. 1,000-seed weight (TSW)

The improvement in plant nutritional status as a result of the NPK application contributed to an increase in 1,000-seed weight (Figure 4C). It was most pronounced in the NPK application variants, where the increase in this trait relative to the NF object averaged 10.1%. The seed inoculation with the BN+BF and BN+F preparations contributed to a significant increase in 1,000-seed weight of 7.8% and 10.5%, respectively, relative to the control. In the variant of the combined application of the microbial preparations and the BN+BF+F microfertilizer, the weight of 1,000 seeds was the highest (332.6 g) and exceeded the control by 13.9%.

### 3.7. Seed yield (SY)

The results showed a significant positive effect of NPK on chickpea SY (Figure 4D). Compared to the NF object, the NPK-fertilized chickpea had on average 21.0% higher SY. An increase in SY was also shown in relation to the application of microbial preparations and micronutrient fertilizers and their combinations. Regardless of the NPK fertilization, a significant increase in the yield (15.2%) compared to the control was shown in the BN+BF seed inoculation variant. Along with the improvement in the plant nutrition regime, the productivity of chickpea also increased significantly in the variant of the combination of seed inoculation with the BN+BF complex and foliar feeding of F plants, reaching the maximum value (2.32 t $ha^{-1}$). In the other variants, the yield increase, compared to the control, was not statistically confirmed.

### 3.8. Relationships between morphological features of plants (LA, AGDM), physiological traits (NPP), yield components (NP, NS, TSW), and yield (SY)

The yield is the result of the interrelated physiological processes occurring in plants under the influence of environmental factors. The results of the study showed a significant positive influence of plant structure elements (LA, AGDM), yield structure (NP, NS, TSW), and physiological traits (NPP) on the formation of chickpea SY. The size of the LA as an absorber of solar radiation energy and a source of photoassimilates (carbohydrates) necessary for the formation and development of generative organs and fruit played an important role in shaping SY. This is indicated by a strong positive correlation ($r$ = 0.90) between LA and SY (Figure 5A). The efficiency of the plant's conversion of solar radiation energy into biomass also had a significant positive effect on the yield. This was confirmed by the positive correlation ($r$ = 0.72) between the NPP index, expressing the amount of organic matter synthesized per unit of LA in a given time, and SY (Figure 5B). The organic compounds synthesized by the leaves were further utilized by the plants to sustain their vital function and contributed to the formation of the yield. This was evidenced by the strong positive correlations between SY and AGDM ($r$ = 0.99) and TSW ($r$ = 0.92) (Figure 5C, D). SY was also significantly positively correlated with NP ($r$ = 0.83) and NS ($r$ = 0.87) (Figure 5E, F).

## 4. Discussion

### 4.1. Leaf area (LA), net photosynthetic productivity (NPP), above ground dry mass (AGDM)

In our study, we found a significant positive effect of nitrogen, phosphate, and potassium fertilizers on chickpea leaf area size and photosynthetic productivity. The positive effect of nitrogen fertilizers on the development of leaf area and photosynthetic

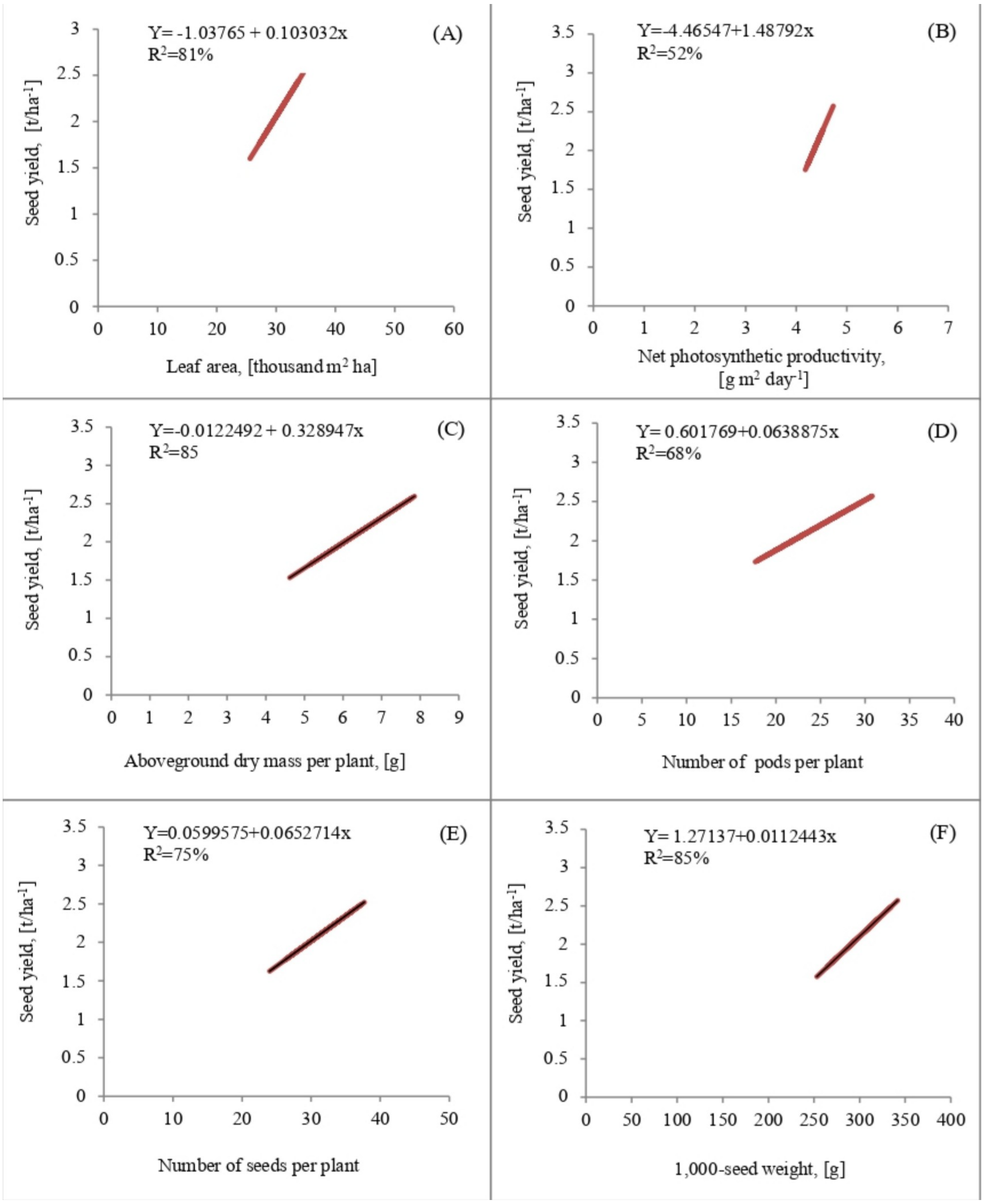


**Figure 5** Relationships between the seed yield of chickpea and (**A**) leaf area (LA), (**B**) net photosynthetic productivity (NPP), (**C**) aboveground dry mass per plant (AGDM), (**D**) number of pods per plant (NP), (**E**) number of seeds per plant (NS), and (**F**) 1,000-seed weight (TSW).

activity of plants, contributing to the vigorous growth of their vegetative parts, has been confirmed by Caliskan et al. (2008). A similar trend was observed by Abayomi et al. (2008) in cowpea (*Vigna unguiculata* (L.) Walp.). The authors found that N fertilization delayed leaf ageing and increased leaf number and LAI. On the other hand, Escalante-Estrada et al. (2014) showed that the number of leaves on the plants and the rate of appearance of new leaf blades were determined by the supply of mineral nutrients, among which nitrogen delayed leaf ageing and prolonged the active period of the leaves and the production of more photoassimilates, which were further directed to the seeds. Other researchers have also reported an increase in leaf area size as a result of an increase in the number of leaves on plants with an increase in the nitrogen fertilizer dose (Namvar et al., 2011).

Phosphorus application stimulates plant growth, root system activity, and nodule formation. In a study conducted by Choudhary et al. (2008), it was found that phosphorus application increased the leaf area of soybean (*Glycine max* L. Merr.) and mung bean (*Vigna radiata* L.) by 64% and 90%, respectively, compared to the unfertilized control, which showed a decrease in photosynthesis and nitrogen fixation due to a decrease in nitrogenase activity induced by phosphorus deficiency.

In our study, the application of NPK significantly enhanced the size of the leaf surface, compared to the control. At the same time, there was a positive trend towards an increase in the leaf surface area of chickpea under the influence of the microbial preparations and microfertilizers, but the differences were not statistically confirmed. The size of the leaf area and the duration of its active functioning determine the extent to which photosynthetically active radiation (PAR) is absorbed by crop plants and converted into organic compounds. The intensity of organic matter production per unit LA per day reflects the NPP. The results of our study showed a significant positive effect of NPK on LA formation and an increase in the intensity of organic compound synthesis, while the variants of microbial and microfertilizer application and their combinations also showed such a trend. Thalooth et al. (2006) found a significant positive effect of foliar fertilization with Zn, K, and Mg on the biomass production in mung bean plants (*Vigna radiata* L.). The researchers explained this effect as the stimulating effect of trace elements on plant metabolism and biological activity, which was manifested by increased enzymatic activity, leaf blade area, and photosynthetic pigment concentrations. Other researchers showed a positive effect of foliar fertilization with Zn, Mn, and Fe on shaping plant productivity, which they explained as the stimulating effect of these nutrients on metabolism and biological activity, photosynthetic pigment synthesis processes, and the activity of enzymatic systems controlling plant growth processes (Kassab, 2005).

According to Sogut (2006), the advantage of symbiotically fixed nitrogen over mineral nitrogen is explained by the fact that symbiotic nitrogen is already in an organic reduced form and is therefore more readily available to plants, whereas, in the absence of symbiotic N, the plant expends a lot of energy to take up nitrate and reduce it to $NH_3$. In our study, the combined application of inoculants based on nitrogen-fixing and phosphorus-mobilizing bacteria contributed to a significant increase in the dry weight of the aboveground part of plants (by 16.5%), compared to the control object. The efficacy of inoculation of chickpea seeds with the nitrogen-fixing *Mesorhizobium* bacteria, which we have demonstrated, is in line with research conducted by other scientists. According to Albayrak et al. (2006), the positive effect of the microorganisms on the plant growth process is also due to the fact that *Rhizobium* bacteria synthesize the phytohormone auxin as a secondary metabolite in inoculated plants, which promotes the development of the root system and stimulates leaf area growth. In turn, a strong root system has the ability to increase the water and mineral supply to plants and, consequently, increase plant yield. In a study carried out by Khaitov et al. (2016), inoculation of chickpea plants with *Rhizobium* strains significantly increased the dry weight of shoots, dry weight of roots, and dry weight of nodules by 17%, 12%, and 20%, respectively, compared to non-inoculated plants. The shoot length, root length, shoot dry weight, and root dry weight of inoculated plants increased by 52%, 43%, 36%, and 64%, respectively, compared to control plants.

The effectiveness of inoculation of chickpea seeds with a complex of nitrogen-fixing and phosphorus-mobilizing bacteria recorded in our study is in line with a study reported by Wani et al. (2007), which determined the effect of applying *Mesorhizobium* strains and their combination with *Pseudomonas* and *Bacillus* bacteria in chickpea cultivation. According to the results, the application of *Mesorhizobium* significantly ($p \leq 0.05$) increased total plant dry matter accumulation by 160% and 115%, compared to the control without inoculation, at 90 and 145 days after sowing, respectively. The single inoculation with *Pseudomonas* or *Bacillus* had no significant effect, while the co-inoculation showed a tendency to increase biomass accumulation. The positive effect of inoculation with such microorganisms may occur because soluble phosphorus compounds, formed as a result of the vital activities of phosphorus-mobilizing microorganisms, are utilized not only by plants but also by nitrogen-fixing soil microorganisms, which thus exhibit higher vital activity; therefore, it is advisable to apply them in a complex.

### 4.2. Seed yield and elements of yield structure

The NPK mineral fertilization significantly influenced the yield and the elements of the yield structure of chickpea. The results of our study showed that the application of $N_{20}P_{80}K_{80}$ led to a significant increase in the NP, NS, TSW, and SY values compared to the $N_0P_0K_0$ variants. Our results are in line with data from the study conducted by Sahu et al. (2020), which showed positive effects of NPK application on plant growth, number of pods and seeds per plant, and seed yield in chickpea. The application of the fertilizer increased the seed yield by 4.8% at the $N_{10}P_{20}K_{10}$ dose and by 13.3% in the double dose ($N_{20}P_{40}K_{20}$) compared to the $N_0P_0K_0$ treatment. The authors showed positive effects of the mineral nitrogen application on plant growth and development, dry matter formation, and crop yield even in dry climates. In our research, the weather conditions in 2019 were also not very favorable for chickpea cultivation due to the low rainfall in June, July, and August (Figure 2). However, the chickpea yield was only slightly lower than in 2020 and 2021 due to the very good soil conditions (low-humus, heavy, clayey (loamy) chernozem with humus content of 5.15%), which retained moisture for a long time. In a meta-analysis of studies from 1966–2006, Salvagiotti et al. (2008) showed that mineral nitrogen application increased plant height, dry matter production, number of lateral branches, and number of pods and seeds, and ultimately increased the biological productivity of soybean (a mean linear increase of 0.013 mg seed yield per kg increase in N accumulation in aboveground biomass). Other studies indicate an increase in plant nitrogen uptake due to improved plant phosphorus nutrition. The results reported by Dotaniya et al. (2014) showed that increased P application improved total plant uptake of nitrogen and phosphorus. On the other hand, Rajput (2018) showed that potassium fertilization had a significant positive effect on plant height, number of pods per plant, 1,000-seed weight, and seed yield. In addition, potassium fertilization increased the intensity of plant uptake of N and P. At the same time, the highest indicators of total biomass, plant pods, weight of 1,000 seeds, and seed yield were noted in the variant of combined application of N, P, and K.

However, not only the amount of the fertilizer applied but also the proportion of the fertilizer has an important influence on the development of chickpea productivity. Saeed et al. (2004) showed that the highest level of chickpea seed yield (average 2.37 t $ha^{-1}$) was recorded in the $N_{35}P_{87,5}K_{100}$ application variant. Increasing or decreasing the rate of individual mineral fertilizers resulted in a decrease in chickpea seed yield. On the other hand, Shan et al. (2016) showed a significant increase in the seed yield and 1,000-seed weight of chickpea under mineral fertilizer application at a concentration of $N_{30}P_{60}K_{30}$ (5.46 t $ha^{-1}$ and 237 g, respectively), compared to $N_{30}P_{60}K_{00}$ (4.26 t $ha^{-1}$ and 230 g, respectively).

In our study, the microfertilizer application (F) significantly increased the NP, NS, TSW, and SY of chickpea compared to the control object. Foliar spray of nutrients is the best method of fertilizer application to control their losses from the soil and make them more easily available to the plant and thus increase the quantity and quality of the yield. Mehboob et al. (2022) showed that foliar fertilization of chickpea plants with boron contributed to an increase in the number and size of leaves on plants, lateral branching, pods on plants, 1,000-seed weight, and seed yield. The results obtained by Sahare et al. (2019) showed an increase in plant height, aboveground weight, number of first and second order branches, number of pods on plants, and seeds in pods. The seed yield was increased from 0.84 to 1.82 t $ha^{-1}$ for a combination of mineral fertilizer and foliar feeding of plants with $N_{20}P_{40}K_{20}S_{20}$ + Mo + B + 2% DAP spray. According to the conclusions formulated by Srivastava et al. (1997), foliar application of boron increases flowering, which in turn has a beneficial effect on the number of pods. A deficiency of this element, on the other hand, has a negative effect on the generative phase of the plant, which manifests itself as increased flower shedding and a corresponding decrease in the number of pods. The high effectiveness of combining mineral fertilizers and foliar feeding of plants with microfertilizers was also reported by Gul et al. (2011).

In our study, we noted a positive effect of the seed treatment with the microbial preparations and the complex application of the microbial preparations and micro-fertilizers on the structure elements and yield of chickpea. Microbial preparations can improve the biological properties of soil by raising nutrient uptake. In a study conducted by Mohammadi et al. (2010), the combined application of nitrogen-fixing and phosphorus-mobilizing microorganisms increased the molecular nitrogen-fixing capacity of chickpea, resulting in a better seed nitrogen supply and improved seed quality indices. Improved quantitative and qualitative parameters of chickpea yield under the combined application of nitrogen-fixing and phosphorus-mobilizing microorganisms have also been reported by other authors (Rudresh et al., 2005). Kheroar et al. (2018) observed that the use of biofertilizers enhanced plant stress tolerance, increased root and shoot biomass, and increased the number of productive tillers, rice grain weight, nutrient availability, and uptake by plants, all of which have a direct impact on grain yield. Additionally, in a study reported by Meena et al. (2020), a combination of mineral fertilizer application and a comprehensive application of microbial preparations proved to be the most effective in shaping chickpea productivity. The results of research carried out by Kumari et al. (2019) showed that the combined application of Zn, B, and Mo and *Rhizobium* inoculation exerted a beneficial effect on the number of pods per plant, but no significant result was observed in the number of seeds per pod.

## 5. Conclusions

The results of the research indicate that the use of the $N_{20}P_{80}K_{80}$ fertilizers had the greatest impact on the productivity of chickpea. The lack of fertilization with macronutrients resulted in a significant decrease in the value of all tested indicators.

The addition of microelements and microbiological preparations based on nitrogen-fixing microorganisms and phosphorus- and potassium-mobilizing microorganisms improved chickpea productivity indices, increasing the yield structure elements (NP, NS, TSW) and, consequently, chickpea seed yield (SY). On the other hand, the size of leaf area (LA) and net photosynthetic productivity (NPP) was significantly determined only by the supply of the main macronutrients to the plants.

Irrespective of the NPK used, the amount of aboveground biomass, chickpea productivity indices (NP, NS, TSW), and SY had the highest values when the complex BiNitro Chickpeas+Biofosforyna+Freya–Aqua™ C(12) Legumes was used.

A significant impact of all the studied indices (LA, AGDM, NP, NS, TSW, NPP) on the yield of chickpea seeds was also demonstrated, as evidenced by the strong positive correlations and the high values of regression coefficients.

## Data availability statement

The data presented in this study are available upon request from the first author.

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