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Advanced two-component microbial inoculant development for enhancing the productivity of agricultural crops*

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Abstract

The present study investigates the effectiveness of microbiological preparations in enhancing growth and productivity of key agricultural crops, including barley, maize, sunflower, and root crops (table beet, sugar beet, and potato) under field conditions. The experimental design included four replicates per treatment, with statistical analysis. For maize, the application of microbial consortia resulted in significant improvements in biometric and yield parameters compared to the untreated control. The most pronounced effect was observed with Preparation No.1 (*Azotobacter chroococcum* MDC 6111 in combination with *Pseudomonas tritici* MDC 9168), which increased plant height by 31.4%, ear mass by 63.2%, and 1000-grain weight by 35.1%, corresponding to a total yield increase of 35.1%. In sunflowers, similar trends were detected. Preparation No.1 led to a 24.8% increase in plant height and a 56.1% increase in 1000-seed weight. Preparation No.3 (*Azotobacter chroococcum* MDC 6111) also demonstrated a notable positive effect (yield increase of 42.3%), while Preparation No.2 (commercial mineral fertilizer "Nitroammophoska") showed only marginal improvement. Regarding root crops, Preparation No.1 markedly enhanced productivity. In table beet, yield reached 14.51 ± 0.91 kg/10 m² versus 5.89 ± 0.42 kg/10 m² in the control. Sugar beet yield increased by 2.4-fold, and potato yield by 27.9%. Preparation No.2 showed moderate effectiveness in beets. These findings emphasizing the role of rhizosphere microorganisms in nutrient mobilization, hormonal regulation, and stress resistance. The study supports the use of microbial inoculants, particularly Preparation No.1, as a sustainable tool to improve crop productivity in diverse agroecosystems.

Keywords

Azotobacter chroococcum, crops, field trials, microbial inoculants, nitrogen fixation, plant growth-promoting rhizobacteria, *Pseudomonas tritici*, sustainable agriculture

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Introduction

Contemporary agriculture is increasingly focused on developing sustainable alternatives to conventional intensification strategies, which primarily rely on mineral fertilizers and synthetic plant growth regulators. The growing environmental challenges, such as soil degradation, contamination of water resources, and loss of biodiversity, underscore the urgent need to transition toward environmentally safe, resource-efficient agronomic practices. One promising avenue in this context is the use of biological products based on beneficial microorganisms with multifunctional plant growth-promoting properties (Kumar and Verma 2022; Sarkar et al. 2022).

Of particular interest are microbial inoculants that include free-living and associative nitrogen-fixing (NF) bacteria, phosphate-solubilizing (PS) organisms, phytohormone producers, and antagonistic strains capable of suppressing phytopathogens. These microbial formulations can positively influence plant physiological processes, improve nutrient availability, and reduce phytosanitary pressure by modulating the microbial environment in the rhizosphere (Bashan et al. 2014; Goswami et al. 2023; Goginyan et al. 2025).

Among the most widely studied microbes is *Azotobacter chroococcum*, a free-living, aerobic nitrogen fixer capable of converting atmospheric nitrogen into bioavailable forms independently of symbiotic associations. In addition to nitrogen fixation, this species produces B-group vitamins, auxins, gibberellins, and other phytohormones. Furthermore, it synthesizes extracellular polysaccharides that enhance soil structure and root colonization, contributing to improved root development and plant resilience (Ivanova et al. 2020; Wu et al. 2022). Its metabolic activity is also associated with soil remediation and stabilization of microbial communities in the rhizosphere.

Equally important are strains from the genus *Pseudomonas*, which are characterized by strong antagonistic activity against a broad range of phytopathogenic fungi and bacteria. This is primarily due to the synthesis of bioactive compounds such as phenazines, pyrrolnitrin, siderophores, hydrogen cyanide, and hydrolytic enzymes (Kalayu 2019; Haas and Défago 2005). In addition to disease suppression, *Pseudomonas* enhances plant growth, induces systemic resistance, and increases tolerance to abiotic stresses such as drought, salinity, and temperature fluctuations (Mercado-Blanco and Bakker 2007).

The development of microbial consortia combining functionally diverse strains offers a synergistic approach to promoting plant growth and health. Co-inoculation of *Azotobacter chroococcum* and *Pseudomonas chlororaphis* has been shown to improve nutrient uptake, modulate hormonal balances, and enhance biocontrol efficacy. Several studies report that such multifunctional formulations can significantly improve yield and crop quality across a variety of plant species, including cereals, industrial crops, and vegetables (Vessey 2003; Kloepper et al. 2004; Ahmad et al. 2021).

However, despite growing empirical evidence, several challenges remain. These include the optimization of strain compatibility, formulation stability, resistance to environmental stressors, and consistency of performance under different soil and climatic conditions. Furthermore, there is a critical need to develop scientifically based application protocols that account for crop developmental stages, soil properties, crop rotation systems, and regional agroecological conditions. These considerations are particularly important in the face of climate change and the growing demand for adaptive and resilient agricultural practices.

The primary aim of this research was to evaluate the effectiveness of microbiological preparations based on free-living NF bacteria and PS bacteria in promoting plant growth and enhancing crop productivity under field conditions. The study focused on assessing crop-specific responses of spring barley, maize, sunflower, as well as root crops such as table beet, sugar beet, and potato. Three different preparations were compared to identify the most effective in improving plant biometric traits and yield parameters. Ultimately, this research sought to determine the potential of the most effective formulations for use in sustainable and organic farming systems, thereby offering environmentally friendly alternatives to chemical fertilizers.

Materials and methods

Strain characteristics, biological properties, and cultivation conditions

The ***Azotobacter chroococcum*** MDC* 6111 strain (Beijerinck 1901) was isolated from the wheat rhizosphere (chestnut soils, Mkhchyan village, Artashat region, 845 m a.s.l., Armenia). The cells are Gram-negative (GN), highly motile; in young cultures, they appear as rods, later becoming oval or coccoid, occurring singly, in pairs, or in short chains. Cell diameter reaches up to 2.1 μm. The strain is aerobic. In young cultures, growth is homogeneous, while in mature cultures, it becomes granular. Under unfavorable conditions, it forms cysts surrounded by mucilage. The strain exhibits strong pigment production. The optimal temperature for growth is 27–28 °C; the optimal pH is 7.0–7.5, with a growth range between pH 4.8 and 8.5. The average colony-forming units per milliliter (CFU/mL) is 3×10^9. The strain assimilates: glucose, sucrose, lactose, arabinose, xylose, fructose, galactose, raffinose, cellobiose, rhamnose, mannose, maltose, dextrin, mannitol, glycerol, dulcitol, sorbitol, pyruvic, fumaric, succinic, oxalic, and maleic acids. It does not assimilate inulin, phthalic, stearic, benzoic, or salicylic acids. It reduces nitrates. It does not liquefy gelatin or agar, does not produce gas, and hydrolyzes milk.

* **MDC**, Microbial Depository Center – the Division of the "Armbiotechnology" Scientific and Production Center of the National Academy of Sciences of the Republic of Armenia – acronym of the National Culture Collection of Non-pathogenic Microorganisms

The strain grows weakly on potato medium. It fixes 13.5 mg of nitrogen per 1 g of consumed glucose (Goginyan et al. 2025), whereas most *A. chroococcum* strains assimilate no more than 10 mg of molecular nitrogen per 1 g of consumed carbon source (Dasgupta et al. 2021).

Storage conditions: nutrient medium (Ashby medium, g/L): K_2HPO_4 – 0.2; $MgSO_4$ – 0.2; K_2SO_4 – 0.1; NaCl – 0.2; $CaCO_3$ – 3.0; sucrose – 15.0; agar – 14.0. Subculturing is carried out every 6 months. Sterilization: 121 °C for 15 minutes (Subbarao 1977; Harutyunyan et al. 2024).

***Pseudomonas tritici* MDC 9168** (Girard et al. 2022). Originally isolated from the rhizosphere of sugar beet and initially deposited as *Pseudomonas chlororaphis* subsp. *chlororaphis*. It is an obligatory aerobe. The cells are motile, GN, short oval rods, non-spore-forming, polymorphic, measuring 1.5 × 2.0 µm. On solid media, the strain forms small, round colonies with smooth edges, flat and transparent, with a yellowish pigmentation. The optimal growth temperature is 30 °C, with a growth range of 15–37 °C. It does not fix molecular nitrogen. It solubilizes phosphates, approximately 280 mg/L of $Ca_3(PO_4)_2$ per day. It does not degrade cellulose but hydrolyze starch. The strain ferments mannitol, sucrose, glycerol, maltose, and salicin. It utilizes ammonium and nitrate salts as sole nitrogen sources. It assimilates various sugars (galactose, glucose, xylose, maltose, mannitol, rhamnose, sucrose, trehalose, fructose) with acid production. It is resistant to antibiotics such as cephalexin, cefazolin, amoxicillin, ampicillin, and penicillin.

Cultivation media for *Pseudomonas*: Fish-based medium (g/L): Fish paste – 20.0; Yeast extract – 5.0; Agar – 20.0. pH 6.8–7.0. Sterilization: 121 °C for 20 minutes. Legume extract medium: Boil 50 g of pea seeds in 1 L of water for 40 minutes and filter. Add 10.0 g of sucrose/glucose/mannitol, 1.0 g K_2HPO_4, and bring to 1 L total volume. pH 6.8–7.0 Sterilization: 121 °C for 20 minutes (Goginyan et al. 2024).

Molecular genetic analysis of microbial strains to clarify their taxonomic affiliation was carried out using Pacific Biosciences technology (SMRT PacBio – Single Molecule Real-Time sequencing) in collaboration with Novogene (Oxford, UK).

Determination of phosphatase activity

Phosphatase activity of microorganisms (alkaline and acid) was determined according to the methods described by (Tabatabai and Bremner 1969; Moscatelli et al. 2005), using para-nitrophenyl phosphate (pNPP) as the substrate. This compound is hydrolyzed by the corresponding enzymes to form para-nitrophenol (pNP). The amount of pNP produced is directly proportional to the enzymatic activity and is measured spectrophotometrically at 405 nm. Enzyme activity was calculated using the following equation [1]:

$$A = C \times V_t / t \times V_s \quad [1],$$

where:
A – phosphatase activity (µmol pNP/mL/h);
C – concentration of pNP determined from the standard curve (µmol/mL);
V_t – total volume of the reaction mixture (mL);
t – incubation time (h);
V_s – volume of the sample (mL).

Nitrogenase activity was determined using the acetylene reduction assay (ARA) according to the methods described by (Hardy et al. 1968; Herridge et al. 2008).

Co-cultivation conditions of strains in a 10 L fermenter: Working volume – 6.0 L; Volume of nutrient medium – 5.4 L; Inoculum volume – 0.4 L for *Azotobacter chroococcum* and 0.2 L for *Pseudomonas tritici*; Stirrer speed – 800 rpm; Cultivation temperature – 28–30 °C; Aeration (air supply rate) – 4.0 L/min; Cultivation period – 36 h; Total microbial titer at the end of fermentation (CFU/mL): 1.75×10^9; Residual sugar after 36 h – 0.08%; pH – 6.8–7.2; Foaming – low. The composition of the fermentation medium is presented in Table 1.

Table 1. Composition of the universal medium for co-cultivation (g/L) in modification (Serazetdinova et al. 2025).

Component	Concentration	Purpose
Sucrose	10.0	Carbon source
K_2HPO_4	1.0	Buffer and phosphorus source
KH_2PO_4	0.5	Buffer and phosphorus source
$MgSO_4 \times 7H_2O$	0.2	Magnesium source
NaCl	0.2	Osmotic balance
$CaCl_2 \times 2H_2O$	0.1	Calcium source
$FeSO_4 \times 7H_2O$	2 mg	Micronutrient
$MnSO_4 \times H_2O$	1 mg	Micronutrient
Na_2MoO_4	0.1 mg	Nitrogenase cofactor
Yeast extract	0.5	Source of B vitamins
L-Tryptophan	0.05	Auxin synthesis stimulation
Medium pH	7.0–7.2	Optimal for both microorganisms

The study focused on agricultural crops of high agronomic and economic importance, including:

- Barley (*Hordeum vulgare* L.) – a cereal crop widely used in food, feed, and brewing industries. It is characterized by a short growing season and high responsiveness to microbiological growth stimulants.
- Maize (*Zea mays* L.) – a staple food and forage crop, sensitive to nitrogen and phosphorus nutrition, making it a suitable model for evaluating the effectiveness of NF and PS bioformulations.
- Sunflower (*Helianthus annuus* L.) – an oilseed crop with high phosphorus demand and sensitivity to biotic and abiotic stressors. It is used to assess the bioprotective and phytostimulatory effects of microbial consortia.
- Sugar beet (*Beta vulgaris* subsp. *vulgaris* var. *altissima*) – a root crop grown for industrial purposes, highly dependent on soil quality and mineral nutrition, particularly during periods of active growth.
- Potato (*Solanum tuberosum* L.) – a key food crop requiring balanced phosphorus and nitrogen nutrition.

Due to its high susceptibility to phytopathogens, it serves as an effective model for assessing the antagonistic properties of tested bacterial strains.

Pre-sowing treatment of plants with biopreparations in field trials

Pre-sowing treatment of agricultural crops (barley, maize, sunflower, common beet, sugar beet, potato) was carried out using a biopreparation based on strains of *Azotobacter chroococcum* MDC 6111 and *Pseudomonas tritici* MDC 9168 (hereinafter referred to as "Preparation No. 1"). The following controls were also used: untreated plants; commercial mineral fertilizer "Nitroammophoska" (Preparation No. 2); a biopreparation based solely on *Azotobacter chroococcum* MDC 6111 (Preparation No. 3).

All microbial preparations were applied at a rate of 2 L/ha of suspension, with a concentration of 1 billion microbial cells per mL.

The mineral fertilizer "Nitroammophoska" was applied to the recommended rate (barley – 120 kg/ha; maize and sunflower – 200 kg/ha; root crops – 150 kg/ha) for 1 hectare of land (GOST 19691-84; GOST R 58682-2019; Stepanov 2013). Seeds and seedlings of the plants were treated with 1–2-day-old bacterial strain suspensions (titer 10^8–10^{10} CFU/mL, exposure time 40 minutes). Potato tubers (2–3 per hole) were planted in holes with a perlite cushion (about 50 g), previously moistened with the corresponding biopreparation suspensions. The planting distance for maize and sunflower seeds was 30 cm, and for root crops, including potatoes, it was 60 cm.

Pre-sowing agrochemical soil analysis

The pre-sowing agrochemical analysis revealed that the soil was classified as light loamy in terms of mechanical composition and belonged to the brown semi-desert type. The soil was characterized by a relatively low organic matter content (2.73%), slight salinity (0.092%), low concentrations of carbonates (0.67 meq/100 g) and sulfates (0.07 meq/100 g), as well as varying levels of essential nutrients typical for this soil type: available nitrogen forms (NO_3^-) – 30.2 meq/100 g, phosphorus (PO_4^{3-}) – 14.0 meq/100 g, and potassium (K^+ – 14.7 meq/100 g (Goginyan et al. 2025).

Data collection and evaluation: germination, biometric parameters, and crop yield

During the field trials, the effects of bacterial preparations on plant growth, development, and productivity were assessed. The evaluated parameters included field germination rate, biometric characteristics, yield structure elements, and overall crop yield. The experimental methodology adhered to standard agronomic practices and regulatory norms (GOST 12038-84) (Table 2).

Table 2. List of recorded indicators.

Indicator	Units	Assessment Stage	Method
Field germination	%	Day 10 after sowing	Manual counting
Plant height	cm	Tillering, heading	Measurement with ruler
Number of shoots	pcs/plant	Tillering	Manual counting
Weight of 1000 grains	g	Full maturity	Weighing
Number of grains per spike	pcs	Full maturity	Manual counting
Yield	c/ha	Harvest	Weighing and recalculation

Germination was assessed on days 10–12 after sowing within an area of 1 m² in three replicates. The value was calculated using formula [2]:

$$\text{Germination rate (\%)} = (\text{number of germinated seedlings} / \text{numbers of sown seeds}) \times 100 \quad [2]$$

Biometric parameters of the plants were assessed during the tillering, stem elongation, and heading stages. Measurements included plant height (cm), the number of productive tillers, and other relevant traits (Hunt 1990).

At the full **maturity stage**, detailed measurements were taken: the number of ears per 1 m², the number of grains per ear, the weight of 1000 grains (g), and the grain weight from the sampled area (converted to c/ha).

Grain yield was determined by weighing the threshed grain from 1 m² plots (in three replicates) and converting the result to a per-hectare basis, taking into account a standard grain moisture content of 14% (Dospekhov 1985). Calculations were performed using formula [3].

$$\text{Yield (q/ha)} = (\text{Area (m}^2) / \text{Grain weight (g)}) \times 0.1 \quad [3]$$

Statistical analysis

The experimental data were statistically analyzed using analysis of variance (ANOVA) and the Student's *t*-test at a significance level of $p < 0.05$. Statistical computations were performed using Microsoft Excel, following the methodologies described by Steel and Torrie (1980) and Dospekhov (1985).

Results

Effect of microbiological preparations on the growth and productivity of spring barley

Field trials demonstrated a statistically significant effect of microbiological preparations on the growth and productivity of spring barley (*Hordeum vulgare* L., local cultivar). The results are presented in Table 3. According to one-way analysis of variance, the differences between treatments were statistically significant for all studied traits (yield, plant height, spike length, 1000-grain weight, and number of grains per spike) at a significance level of $p < 0.05$.

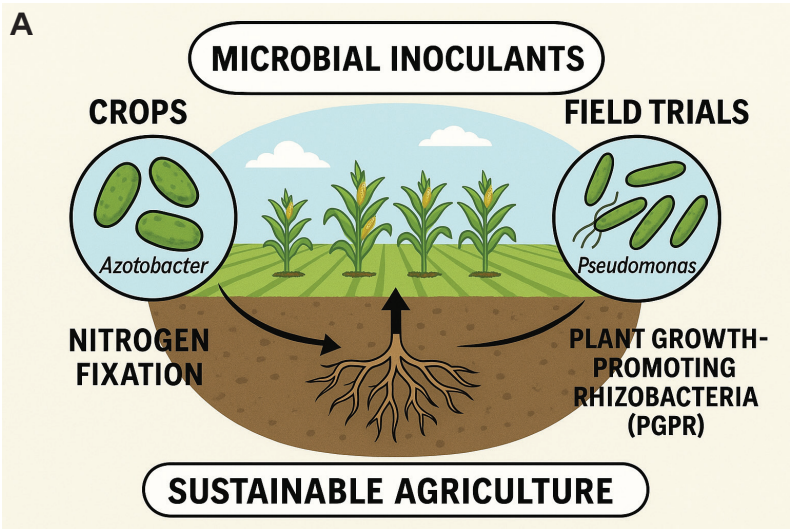

Figure 1. Descriptive scheme of the experimental setup (**A**); Stages of the experimental study (**B**).

Table 3. Effect of microbiological preparations on the growth and productivity of spring barley (mean ± standard deviation, *n* = 4).

Treatment	Plant height, cm	Spike length, cm	Grains per spike	Yield, kg/10 m²	Increase, %	1000-grain weight, g
Control	64.3 ± 1.9	7.7 ± 0.3	13.8 ± 0.6	0.86 ± 0.06	100%	51.0 ± 1.2
Preparation No.1	82.5 ± 2.2	8.9 ± 0.4	30.7 ± 1.0	1.49 ± 0.07	173.7%	61.4 ± 1.5
Preparation No.2	70.0 ± 1.8	8.4 ± 0.3	16.3 ± 0.9	1.25 ± 0.06	145.3%	54.4 ± 1.3
Preparation No.3	73.3 ± 2.0	8.7 ± 0.4	21.4 ± 1.1	1.39 ± 0.07	161.6%	57.2 ± 1.4

Notes. Control: untreated plants. Preparation No.1: *Azotobacter chroococcum* MDC 6111 in combination with *Pseudomonas tritici* MDC 9168. Preparation No.2: commercial mineral fertilizer "Nitroammophoska". Preparation No.3: based solely on *Azotobacter chroococcum* MDC 6111.

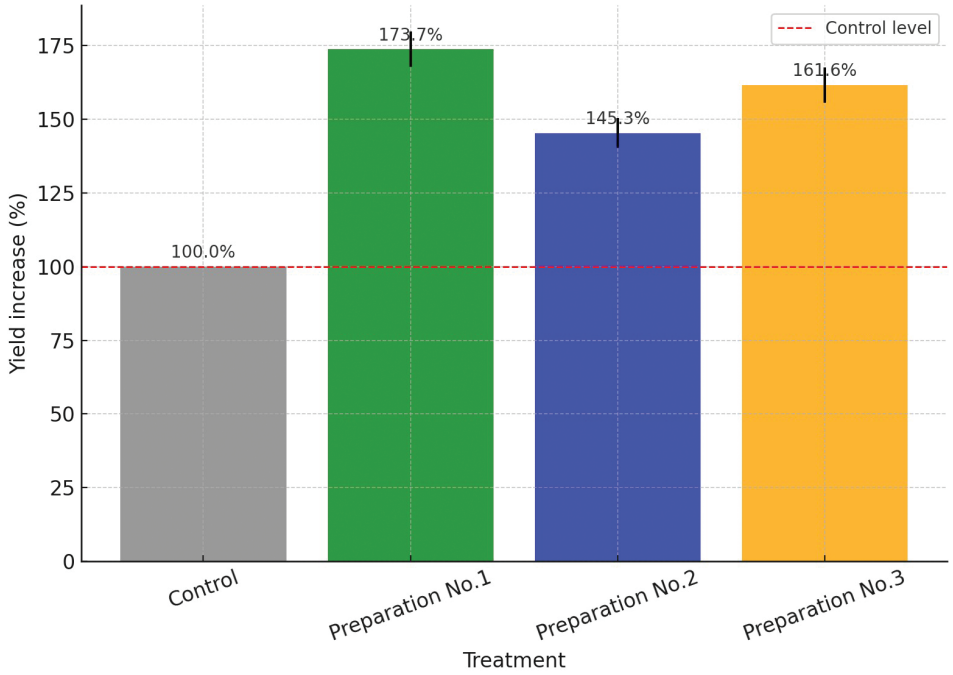

Figure 2. Effect of preparations on barley yield. The Graph clearly shows that all three preparations significantly increased barley yield compared to the untreated control. Among them, Preparation No.1 was the most effective, followed by Preparation No.3, and then Preparation No.2.

The highest yield increase compared to the control was observed with Preparation No.1 – 1.49 ± 0.07 kg/10 m², corresponding to a 73.7% increase (Fig. 2). A substantial enhancement was also recorded in the number of grains per spike (30.7 ± 1.0) and 1000-grain weight (61.4 ± 1.5 g). Plant height in this variant reached 82.5 ± 2.2 cm, which significantly exceeded the control value of 64.3 ± 1.9 cm ($p < 0.05$).

The application of Preparation No.3 resulted in a yield of 1.39 ± 0.07 kg/10 m², 61.6% higher than in the control. Productivity parameters also improved: the 1000-grain weight reached 57.2 ± 1.4 g, and the number of grains per spike increased to 21.4 ± 1.1. Plant height was 73.3 ± 2.0 cm. The differences in key traits were statistically significant ($p < 0.05$).

Preparation No.2 showed a moderate effect on crop productivity, with a yield of 1.25 ± 0.06 kg/10 m², representing a 45.3% increase over the control. The number of grains per spike increased to 16.3 ± 0.9, the 1000-grain weight to 54.4 ± 1.3 g, and plant height to 70.0 ± 1.8 cm. Despite the relatively modest results compared to Preparations No.1 and No.3, Preparation No.2 still produced statistically significant improvements in all measured traits.

The control treatment (no application) showed the lowest values for all parameters: yield – 0.86 ± 0.06 kg/10 m², 1000-grain weight – 51.0 ± 1.2 g, number of grains per spike – 13.8 ± 0.6, and plant height – 64.3 ± 1.9 cm.

Thus, the use of microbiological preparations, particularly Preparation No.1, positively influenced the morphometric and productivity parameters of spring barley. These findings confirm the effectiveness of microbial formulations containing NF and plant growth-promoting rhizobacteria (PGPR) in enhancing cereal crop yields.

Effect of microbiological preparations on the growth and productivity of maize (Hybrid Erika F1)

Field trials revealed a pronounced positive effect of microbiological preparations on the growth and productivity of maize hybrid Erika F1. Summarized data are presented in Table 4. All treatments with microbial preparations demonstrated improvements in biometric and yield-related parameters compared to the untreated control.

The most effective treatment was Preparation No.1. In this variant, the average plant height reached 135.8

Table 4. Effect of microbiological preparations on the productivity of maize (Erika F1 hybrid, mean ± standard deviation, $n = 4$).

Treatment	Plant height, cm	Ear length, cm	Ear weight, g	1000-kernel weight, g	Yield increase, %
Control	103.3 ± 5.2	15.2	152 ± 7.6	244.2 ± 12.2	100%
Preparation No.1	135.8 ± 6.8	16.1	248 ± 12.4	330.0 ± 16.5	135.1%
Preparation No.2	115.0 ± 5.8	15.6	177 ± 8.9	260.0 ± 13.0	106.5%
Preparation No.3	122.5 ± 6.1	16.0	220 ± 11.0	290.0 ± 14.5	118.8%

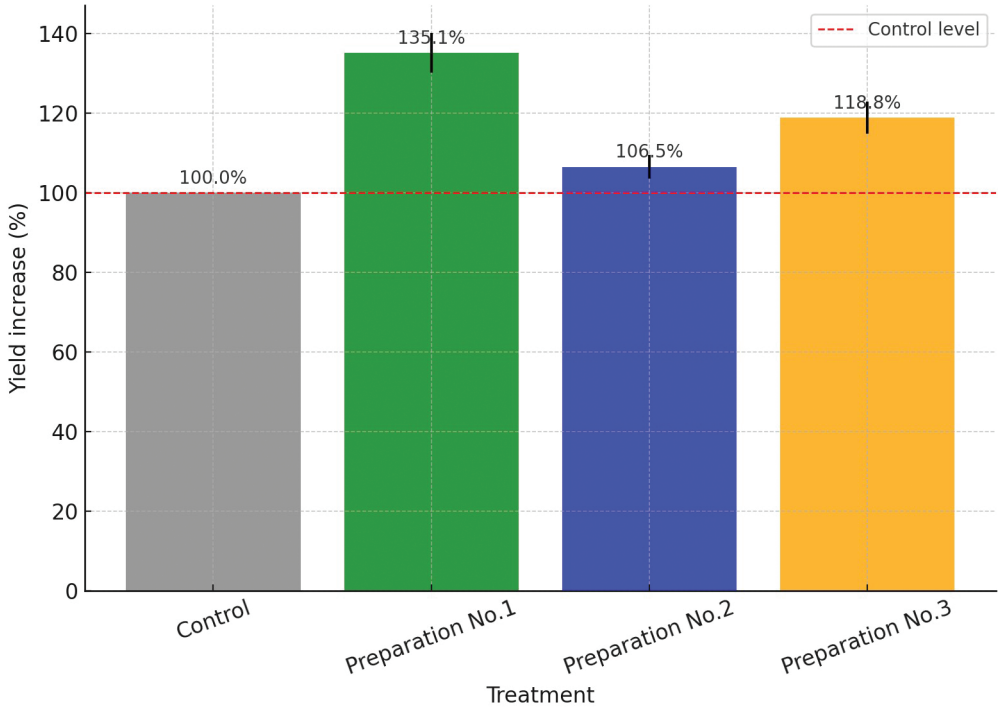

Figure 3. Effect of preparations on maize yield. Preparation No.1 is the best candidate for further application and scale-up. Preparation No.3 is also promising, while Preparation No.2 shows limited benefit.

± 6.8 cm, compared to 103.3 ± 5.2 cm in the control. The average ear weight was 248 ± 12.4 g, significantly exceeding that of the control (152 ± 7.6 g). The 1000-kernel weight increased to 330.0 ± 16.5 g versus 244.2 ± 12.2 g in the untreated group. The overall yield increase reached 135.1% (Fig. 3), indicating high physiological activity of the microbial strains in the preparation likely due to enhanced nutrient uptake and stimulation of phytohormone synthesis.

Preparation No.3 also demonstrated a notable positive effect: plant height reached 122.5 ± 6.1 cm, ear weight – 220 ± 11.0 g, 1000-kernel weight – 290.0 ± 14.5 g, and yield increase – 118.8%. These results support its consideration as an effective tool for increasing maize productivity.

A more moderate, yet consistent, improvement in indicators was observed with Preparation No.2. Plant height reached 115.0 ± 5.8 cm, ear weight – 177 ± 8.9 g, 1000-kernel weight – 260.0 ± 13.0 g, and yield increase – 106.5%. Although the improvements were relatively modest, the effect was still clearly positive.

The control variant, without any microbial treatment, exhibited the lowest values across all parameters: plant height – 103.3 ± 5.2 cm, ear length – 15.2 cm, ear weight – 152 ± 7.6 g, and 1000-kernel weight – 244.2 ± 12.2 g.

The experimental results align with findings from other studies emphasizing the role of rhizosphere microorganisms in promoting plant growth, enhancing stress tolerance, and improving productivity through the production of auxins, siderophores, enzymes, and other biologically active compounds (Vessey 2003; Bhardwaj et al. 2014).

Thus, microbiological preparations, particularly Preparation No.1, demonstrate high potential as biologically active agents for sustainably increasing maize yield and can be recommended for large-scale application in crop production systems.

Effect of microbiological preparations on the growth and productivity of sunflower

Field trials were conducted to evaluate the effectiveness of various microbiological preparations on the productivity of sunflower cultivar Maslyanka. The results are summarized in Table 5.

Table 5. Effect of microbiological preparations on sunflower productivity (cv. Maslyanka, mean ± standard deviation, *n* = 4).

Treatment	Plant height, cm	1000-seed weight, g	Yield increase, %
Control	134.6 ± 5.2	49.9 ± 2.1	100.0
Preparation No.1	168.0 ± 6.0	77.9 ± 3.2	156.1
Preparation No.2	137.0 ± 5.5	50.8 ± 2.3	101.8
Preparation No.3	160.7 ± 5.8	71.0 ± 3.1	142.3

Analysis of variance revealed a statistically significant effect of the microbial treatments on plant height ($F = 35.22$; $p < 0.001$) and 1000-seed weight ($F = 29.87$; $p < 0.001$), indicating substantial differences between the control and treatment groups.

Application of Preparation No.1 resulted in the highest plant height – 168.0 ± 6.0 cm – significantly exceeding the control value (134.6 ± 5.2 cm, $p < 0.01$, Student's

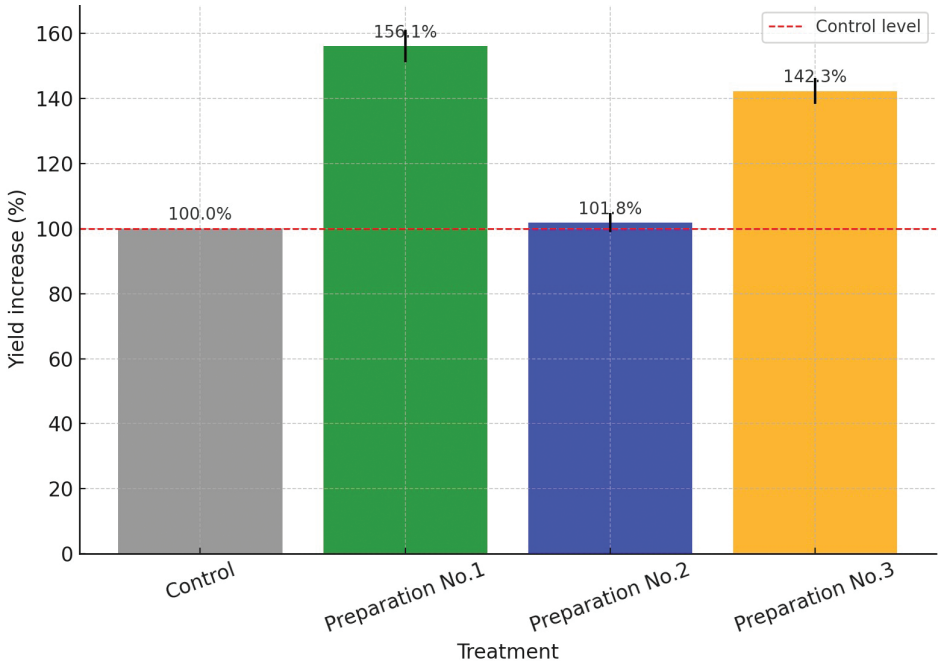

Figure 4. Effect of preparations on sunflower yield. For sunflower (cv. Maslyanka), Preparation No.1 is the most promising, leading to a 56% yield increase compared to control, followed by Preparation No.3 (+42%). Preparation No.2 had negligible effect.

t-test) (Fig. 4). The 1000-seed weight reached 77.9 ± 3.2 g versus 49.9 ± 2.1 g in the control ($p < 0.01$), leading to a 56.1% yield increase compared to the untreated group. This effect is likely attributable to rhizospheric microorganisms that enhance mineral nutrient uptake, stimulate the synthesis of auxins and cytokinins, and improve plant stress tolerance.

Preparation No.3 also demonstrated high efficacy, increasing plant height to 160.7 ± 5.8 cm and 1000-seed weight to 71.0 ± 3.1 g. The resulting yield increase was 42.3% compared to the control, which was statistically significant ($p < 0.05$).

Preparation No.2 exhibited moderate effectiveness. Plant height in this treatment reached 137.0 ± 5.5 cm, with a 1000-seed weight of 50.8 ± 2.3 g and a yield increase of 1.8%. Despite a trend toward increased productivity, the differences compared to the control were not statistically significant ($p > 0.05$), indicating low biological activity of the applied strain or its incompatibility with the specific agroecosystem conditions.

The control group (without microbial inoculation) exhibited the lowest values across all measured parameters, emphasizing the importance of biological inoculation in sunflower cultivation technologies.

These findings confirm the high effectiveness of Preparations No.1 and No.3 in enhancing sunflower productivity through stimulation of plant growth, formation of heavier seeds, and activation of plant metabolic processes (Vessey 2003; Bhardwaj et al. 2014).

Effect of microbiological preparations on the growth and productivity of root crops

field trials evaluating the influence of microbiological preparations on the productivity of root crops revealed a pronounced stimulating effect, particularly of Preparation No.1, on the yield of table beet, sugar beet, and potato (Table 6).

The yield of table beet (cv. Detroit) treated with Preparation No.1 reached 14.51 ± 0.91 kg/10 m², which was significantly higher than the control (5.89 ± 0.42 kg/10 m²; $p < 0.01$, *t*-test) (Fig. 5). The relative yield increase was 146.3%. One-way analysis of variance confirmed a statistically significant effect of the "preparation" factor on yield ($F(2, 9) = 85.4$, $p < 0.001$), with the most pronounced effect observed in the Preparation No.1 treatment. This may be attributed to the active role of rhizospheric

Table 6. Effect of microbiological preparations on root crop yield (mean ± standard deviation, $n = 4$).

Treatment	Table beet (cv. Detroit)		Sugar beet		Potato	
	Yield, kg/10 m²	Increase, %	Yield, kg/10 m²	Increase, %	Yield, kg/10 m²	Increase, %
Control	5.89 ± 0.42	100	4.49 ± 0.38	100	17.20 ± 1.10	100
Preparation No.1	14.51 ± 0.91	246.3	10.33 ± 0.62	240.0	22.00 ± 1.24	127.9
Preparation No.2	9.21 ± 0.56	156.4	5.06 ± 0.40	112.0	n/a	n/a
Preparation No.3	n/a	n/a	n/a	n/a	n/a	n/a

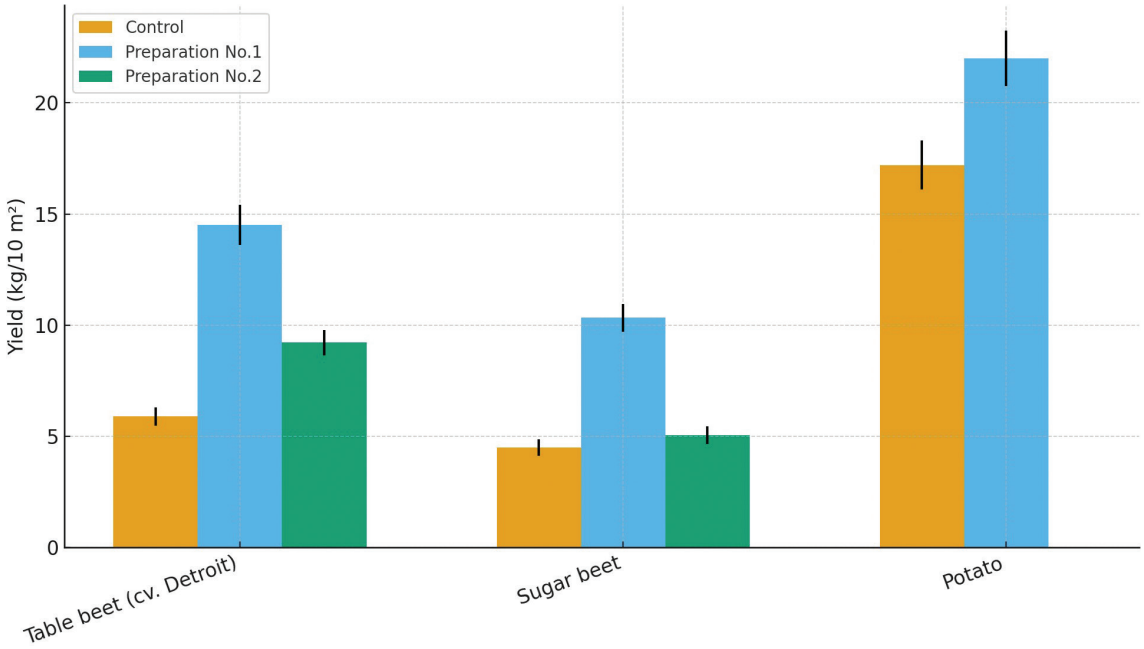

Figure 5. Effect of preparations on root crop yield. Beets responded strongest to microbiological preparations, especially to Preparation No.1, which suggests that root crops with high carbohydrate content may benefit more from microbial inoculants that enhance nutrient uptake or root health. Potato showed a smaller relative increase, which may indicate that tuber crops are less responsive to these particular microbial strains or that their baseline yield is already high. Variation between preparations demonstrates the importance of strain–host specificity: not all microbial inoculants are equally effective for all crops.

microorganisms in nutrient mobilization and stimulation of photosynthetic activity.

A similar trend was observed for sugar beet, with the highest yield (10.33 ± 0.62 kg/10 m²) recorded in the Preparation No.1 treatment, which was 2.4 times higher than the control (4.49 ± 0.38 kg/10 m²; $p < 0.01$). Preparation No.2 also demonstrated a positive, albeit less pronounced, effect (5.06 ± 0.40 kg/10 m²), but the difference from the control was statistically insignificant ($p > 0.05$). These results suggest that Preparation No.1 may enhance sugar and biomass accumulation in root crops, which is critical for increasing sugar yield per unit area.

In potato, yield under Preparation No.1 reached 22.00 ± 1.24 kg/10 m², significantly exceeding the control value of 17.20 ± 1.10 kg/10 m² ($p < 0.05$), corresponding to a 27.9% yield increase. This effect may be associated with improved uptake of nitrogen, phosphorus, and other macronutrients, as well as the synthesis of microbial phytohormones that stimulate root crop growth.

Preparation No.2, while producing a statistically significant yield increase in table beet ($p < 0.05$), was less effective compared to Preparation No.1. For sugar beet, the observed effect was not statistically significant, and no data was available for potato, making it difficult to draw conclusions regarding the universality of this preparation.

Preparation No.3 was not tested in this experimental cycle.

Thus, the field trial results clearly indicate the high effectiveness of Preparation No.1 in enhancing the yield of root crops. Statistical analysis confirms the reliability of the observed differences, allowing for the recommendation of this preparation for use in sustainable agriculture, including organic and biologized farming systems.

Discussion

The outcomes of the present field trials clearly demonstrate the pronounced efficacy of microbiological preparations, particularly two-component Preparation No.1 containing *Azotobacter chroococcum* and *Pseudomonas tritici* strains, in enhancing the yield performance of root crops, including table beet (*Beta vulgaris* L. var. *conditiva*), sugar beet (*B. vulgaris* L. var. *altissima*), and potato (*Solanum tuberosum* L.). These findings are in line with previous studies that have reported the positive influence of PGPR on biomass accumulation, root system development, and final crop yield (Vessey 2003; Hayat et al. 2010; Bhardwaj et al. 2014).

In the case of table beet, the application of Preparation No.1 led to a statistically significant yield increase of 146.3% relative to the control. Similar effects have been reported by Singh et al. (2016), where inoculation with *Azospirillum* and *Bacillus* strains increased root biomass and sugar accumulation in *Beta vulgaris* cultivars by 40–100%. The enhanced performance observed in our study may be attributed to synergistic microbial interactions that improve nutrient solubilization, especially phosphorus, and stimulate phytohormonal activity (auxins, gibberellins, cytokinins), resulting in enhanced root elongation and photosynthetic efficiency (Calvo et al. 2014).

The yield of sugar beet under the influence of Preparation No.1 increased by 2.4 times compared to the control, which is consistent with data from Rahmoune et al. (2017), who showed that co-inoculation with NF and PS bacteria increased sugar beet yield by 150–220%, depending on soil fertility. Our results not only confirm these findings but also suggest a potentially greater benefit when microbial consortia are tailored to specific crop needs and edaphic conditions. The relatively weaker effect of Preparation No.2, which did not lead to a statistically significant yield increase, highlights the importance of strain compatibility and adaptation to the rhizosphere environment, as suggested by Bashan et al. (2014).

Regarding potato, the yield gain of 27.9% in the Preparation No.1 treatment is comparable to results reported by Egamberdiyeva (2007), who observed 20–35% increases in tuber yields using *Azotobacter* and *Pseudomonas*-based biofertilizers. Moreover, Malusá et al. (2012) emphasized the role of microbial phytohormones in promoting tuber formation and early shoot emergence, aligning well with the observed growth stimulation in our trials. The absence of yield data for potato under Preparation No.2 application limits direct comparison, although the lower efficacy seen in beet crops suggests it may be less suitable for potato cultivation.

It is worth noting that Preparation No.3 was not tested on root crops in this trial cycle. However, its effectiveness on sunflower in the same experimental setup suggests its potential value and warrants further investigation. The differential performance of microbial formulations across crop species underscores the importance of host-microbiome specificity and supports the concept of designing crop-adapted bioinoculants, as emphasized by Compant et al. (2010) and Berg et al. (2013).

When compared to commercially available biofertilizers, our Preparation No.1 demonstrated yield responses that are equal to or greater than reported benchmarks. For instance, Rhizobium- and *Azotobacter*-based formulations such as BioGro (Bio-Gro, Inc., USA), Biomax (Agro-K, USA), and Azogrow (Migrow Agro Products, India) have been reported to increase root and tuber yields by 20–60% depending on crop and environmental conditions. Similarly, commercial inoculants containing *Bacillus amyloliquefaciens*, *Pseudomonas fluorescens* or *Bacillus subtilis* (e.g., FZB42 and RhizoVital (ABiTEP GmbH, Germany)) typically enhance vegetable crop yields in the range of 15–50% (Chowdhury et al. 2015). In this context, the >140% yield increases observed for beets with Preparation No.1 far exceeds the average performance of many single-strain or dual-strain commercial products, highlighting the advantage of well-selected, synergistic consortia. The only comparable yield boosts (>100%) have been reported in some optimized consortia-based inoculants developed for sugarcane and maize (Verma et al. 2010; 2014), further confirming that multi-strain approaches can substantially outperform standard inoculants.

In conclusion, the present findings confirm that microbiological inoculants, especially Preparation No.1, can serve as highly effective tools for improving the productivity of root crops under field conditions. These results contribute to the growing body of evidence supporting the use of biofertilizers in sustainable and organic agriculture. Moreover, they reinforce the need for further research on the optimization of microbial consortia tailored to crop genotype and environmental conditions to ensure consistent and reproducible outcomes, as well as systematic benchmarking against existing commercial products to highlight comparative advantages and support broader adoption.

Conclusion

The results of the field trials clearly demonstrate the significant potential of microbial preparations, particularly the multi-strain formulation "Preparation No. 1," in enhancing the growth and productivity of root crops such as table beet (*Beta vulgaris* L. var. *conditiva*), sugar beet (*Beta vulgaris* L. var. *altissima*), and potato (*Solanum tuberosum* L.). Application of this bioformulation led to statistically significant increases in yield across all tested crops, with the most pronounced effects observed in table and sugar beet, where yield gains exceeded 140% relative to the untreated control.

The observed effects are likely associated with the synergistic action of NF, PS, and PGPR included in the formulation, which improve nutrient availability, stimulate root development, and enhance stress tolerance. In particular, the combined activity of *Azotobacter chroococcum* and *Pseudomonas tritici* may underlie the improved nutrient assimilation and suppression of soil-borne pathogens, resulting in healthier plants and improved biomass accumulation.

While these findings are highly encouraging, it is essential to recognize that the results were obtained under specific soil and climatic conditions, which can strongly influence the effectiveness of microbial inoculants. The composition of indigenous soil microbiota, organic matter content, pH, temperature, and rainfall patterns are all known to modulate the performance of bioformulations. Consequently, the results should be interpreted with caution in terms of their broader applicability to other agro-ecological zones.

To build on the promising results of this study, several future research directions should be pursued. First, multi-season and multi-location field trials are needed to confirm the reproducibility of the yield-enhancing effects under diverse soil types, climatic conditions, and agricultural practices. Second, detailed soil microbiome impact studies are required to assess how microbial inoculants interact with native microbial communities, and whether their introduction leads to beneficial synergistic effects or unintended shifts in soil biodiversity. Third, long-term sustainability assessments should be undertaken to evaluate whether repeated application of microbial preparations continues to support crop

productivity without diminishing returns or negative impacts on soil fertility. The studies should also examine the cumulative effects on soil organic matter, nutrient cycling, and resilience to abiotic stresses such as drought or salinity.

In summary, the current findings provide robust evidence that microbial preparations, particularly multi-strain consortia, represent a highly promising component of sustainable agricultural systems. By improving nutrient use efficiency, promoting plant health, and potentially reducing reliance on chemical fertilizers, these bioformulations can contribute to both environmental sustainability and economic profitability. Nevertheless, comprehensive and long-term investigations are necessary to validate their performance under varying conditions, optimize their formulations, and integrate them effectively into modern crop management strategies.

Author contributions

All authors have accepted responsibility for the entire content of this manuscript and consented to its submission to the journal, reviewed all the results and approved the final version of the manuscript. SH – data curation, formal analysis, investigation, methodology; TS – formal analysis, investigation, methodology; GK – formal analysis, investigation; MK – formal analysis; AK – writing – review and editing, funding acquisition, validation; OP – data curation, validation; VB – writing – review and editing, investigation, methodology; AT – resources, software; VG – conceptualization, writing – original draft, data curation, funding acquisition, investigation, methodology, project administration, supervision.

Conflict of interest

Authors state no conflict of interest.

Data availability statement

All data generated or analyzed during this study is included in this published article.

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