

APPLICATION OF BIOINOCULANTS FOR OPTIMIZING THE USE OF MINERAL FERTILIZERS IN THE PRODUCTION OF SILAGE CORN*

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Abstract

Maize is one of the most important agricultural crops globally and in Bosnia and Herzegovina, where cereals are cultivated on 204,368 ha, of which 57% (116,490 ha) is dedicated to maize production. The application of plant growth-promoting rhizobacteria (PGPR), such as the genera *Pseudomonas* and *Bacillus*, represents a sustainable alternative to mineral fertilizers, particularly in phosphorus-deficient soils commonly found in Bosnia and Herzegovina. A field experiment with ten fertilization treatments, arranged in a randomized block design with three replications, was conducted to evaluate the effects of seed inoculation, organic fertilization, and mineral fertilization on silage maize production. Grain yield, ear characteristics, and nutrient concentrations in maize leaves and grain were determined using standard analytical methods.

The highest grain yield (15.2 t ha⁻¹) was achieved with the combined application of organic fertilization (30 t ha⁻¹) and bacterial inoculation, with a statistically significant difference compared to the control treatment. The applied fertilization treatments improved nutrient uptake and increased the content of protein, nitrogen, phosphorus, and magnesium in the grain, while zinc concentration generally decreased, indicating an antagonistic relationship between phosphorus and zinc.

The obtained results confirm the potential of combining rhizobacterial inoculation and organic fertilization to enhance maize productivity and improve plant nutrient status.

Keywords: *maize, fertilization, plant growth-promoting rhizobacteria (PGPR), bioinoculants, yield*

INTRODUCTION

Maize (*Zea mays* L.) is an annual crop species belonging to the grass family (Poaceae), characterized by the highest genetic yield potential among cereal crops and by its extensive utilization in human and animal nutrition, as well as in the processing industry (Kovačević and Rastija, 2014). Due to its considerable agricultural and economic importance, continuous efforts are being made to improve maize production through the implementation of advanced agronomic practices.

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Maize is characterized by a robust aboveground biomass and a well-developed root system, requiring adequate nutrition and sufficient availability of essential plant nutrients. In particular, nitrogen (N), phosphorus (P), and potassium (K) play a crucial role in achieving optimal growth and yield. The average nutrient requirements for optimum maize production are estimated at 250–300 kg N ha⁻¹, 100–120 kg P₂O₅ ha⁻¹, and 280–300 kg K₂O ha⁻¹ (Marschner, 2012).

Under local production conditions, the majority of these nutrients are supplied through mineral fertilizers. However, the economic crisis caused by the war in Ukraine has resulted in an approximately 100% increase in mineral fertilizer prices, thereby imposing additional challenges on agricultural production and significantly increasing production costs for farmers.

In order to reduce production costs and mitigate the negative environmental impacts associated with the excessive use of mineral fertilizers, increasing attention has been directed toward the application of sustainable technologies, innovative practices, and environmentally friendly approaches. One such approach involves seed inoculation with plant growth-promoting rhizobacteria (PGPR). In particular, bacterial genera such as *Pseudomonas* and *Bacillus* have demonstrated significant potential in enhancing phosphorus solubilization in soil, thereby improving its availability to plants. To reduce dependence on mineral fertilizers, organic fertilizers and plant growth-promoting rhizobacteria (PGPR) can be utilized (Khan, 2005).

These rhizosphere-colonizing microorganisms enhance plant growth and nutrient availability (Kloepper & Schroth, 1978; Gray & Smith, 2005). Important PGPR include nitrogen-fixing genera such as *Rhizobium*, *Azotobacter*, *Bacillus*, and *Azospirillum*, while phosphorus-solubilizing bacteria mainly belong to the genera *Bacillus* and *Paenibacillus* (Brown, 1974). PGPR bacteria contribute to sustainable agricultural production by stimulating plant growth, enhancing nutrient uptake, and protecting plants against diseases (Shippers *et al.*, 1995; Dobbelaere *et al.*, 2003; Cakmakci *et al.*, 2006). They can also improve plant tolerance to abiotic stresses and increase nutrient availability through nitrogen fixation and phosphorus solubilization (Mrkovački & Bjelić, 2011). Several bacterial genera are capable of solubilizing insoluble phosphorus compounds, thereby increasing phosphorus availability to plants (Rodríguez & Fraga, 1999).

Phosphorus availability in many agricultural soils of Bosnia and Herzegovina is generally low and strongly influenced by soil properties and environmental conditions. Moreover, plants utilize only about 20% of phosphorus applied through mineral fertilizers, highlighting the need for alternative strategies to improve phosphorus use efficiency.

According to data provided by the Food and Agriculture Organization (FAO), the production areas and average maize yields in the countries of the region, Europe, and worldwide are presented in Table 1.

Table 1. Maize production areas and average yields in the region, Europe, and worldwide (FAOSTAT)

Country/Region	2023.		2024.	
	ha	t ha ⁻¹	ha	t ha ⁻¹
Bosnia&Herzegovina	102 823	6.97	121 966	6.25
Serbia	922 980	7.18	987 492	5.17
Croatia	269 090	7.40	273 160	7.57
Europe	8 298 960	7.35	8 673 210	6.79
Worldwide	211 099 135	5.87	209 954 361	5.80

In 2024, major cereal crops in Bosnia and Herzegovina were cultivated on a total area of 121 966 ha, with an overall production of 6.25 t ha⁻¹ of grain. Within the total cereal cropping structure, approximately 57% of the cultivated area was occupied by maize. Regarding cereal production, a total of 7 622 875 t of maize grain was produced (FAOSTAT).

According to Lončarić *et al.* (2015), based on wheat production in Croatia, mineral fertilizers account for 18.77–38.66% of the total production costs, depending on soil fertility status.

The aim of this study was to evaluate the effectiveness of bacterial inoculant application in silage maize production at KJP Poljoprivredno dobro Butmir. The use of these bioinoculants is expected to reduce the dependence on mineral fertilizers, increase silage maize yield and quality, and provide significant economic and environmental benefits

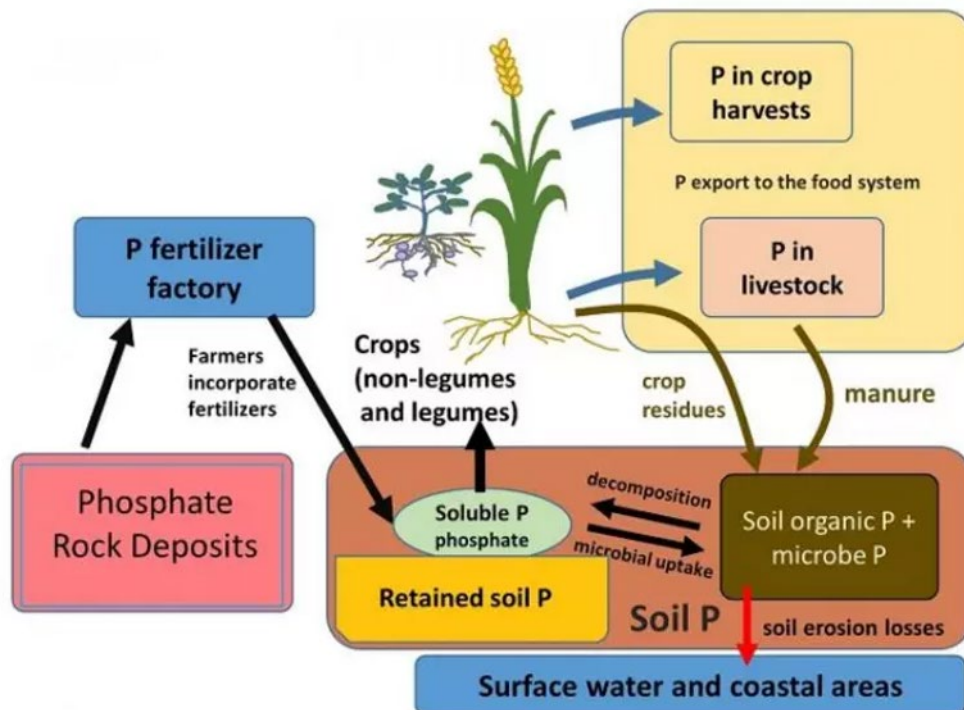


Figure 1. Phosphorus cycling in soil (source: https://serc.carleton.edu/integrate/teaching_materials/food_supply/student_materials/1176)

MATERIALS AND METHODS

The first phase of the study involved the inoculation of maize seeds with bacterial strains belonging to the genera *Pseudomonas* and *Bacillus*. Seed inoculation was performed at University of Sarajevo - The Institute for Genetic Engineering and Biotechnology (INGEB).

The second phase of the study included soil sampling on the production fields of the agricultural farm “PD Butmir”, and based on the agrochemical soil analysis, the most suitable location for the experimental plots was selected. KJP Poljoprivredno dobro Butmir was established in 1893 and is recognized as the oldest agricultural enterprise in Sarajevo. Today, as the Cantonal Public Company “Poljoprivredno dobro Butmir”, it operates a dairy farm with approximately 500 milking cows, for whose feeding nearly 200 ha of silage maize are cultivated annually.

Following soil preparation, the experiment was established according to a randomized block design with three replications. Each treatment was arranged in plots of 10 m² (5 m × 2 m). A total of 70 maize seeds were sown in each plot (7 rows × 10 seeds per row).

The maize hybrid used for sowing was BC 415 (FAO maturity group 400), which is well adapted to the agroecological conditions of the experimental area. Sowing was performed at a spacing of 70 cm × 20 cm. The experimental plots were sown on May 11, 2023. The experiment was established later than the optimal maize sowing period due to continuous rainfall during April and May. Mineral fertilizers (NPK and CAN), organic fertilizer (cattle manure), and bacterial inoculants were used in the experiment. The applied quantities are presented in Table 2.

Table 2. Fertilizer application rates used in the experiment

DOSE	ORGANIC FERTILIZER (t ha ⁻¹)	NPK (kg ha ⁻¹) (7:20:30)	CAN (kg)
I	10	300	367
II	20	450	563
III	30	600	733

The experimental setup included the following treatments:

1. Control treatment without fertilization
2. Organic fertilization treatment (III) + NPK (Dose 1)
3. Organic fertilization treatment (III) + NPK (Dose 2)
4. Organic fertilization treatment (III) + NPK (Dose 3)
5. NPK treatment (Dose 1) + inoculant (*Pseudomonas*, *Bacillus*)
6. NPK treatment (Dose 2) + inoculant (*Pseudomonas*, *Bacillus*)
7. NPK treatment (Dose 3) + inoculant (*Pseudomonas*, *Bacillus*)
8. Organic fertilization treatment (I) + inoculant (*Pseudomonas*, *Bacillus*)
9. Organic fertilization treatment (II) + inoculant (*Pseudomonas*, *Bacillus*)
10. Organic fertilization treatment (III) + inoculant (*Pseudomonas*, *Bacillus*)

During the experiment, standard agronomic practices were applied. Upon completion of the trial, plant material samples were collected, including samples of green silage biomass at the stage of silage maturity, as well as leaf and grain samples at technological maturity.

After sampling, the material was prepared for laboratory analysis through several procedures, including washing leaf samples with deionized water, drying the leaves both in a drying oven and under ambient air conditions, weighing, and preparation for grinding. Plant material analyses included the determination of silage biomass yield and grain yield, while chemical analyses included the determination of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), and protein content.

The following methods were used in this study:

- determination of soil pH using a pH meter (ISO 10390, 1994),
- determination of organic carbon in soil by sulfochromic oxidation (ISO 14235, Geneva, 1998),

- determination of readily available phosphorus and potassium in soil samples using the ammonium lactate (AL) method (Egner *et al.*, 1960),
- determination of total nitrogen content in soil by the Kjeldahl method (Čivić *et al.*, 2004),
- determination of protein content multiplied by a factor of 6.25 (Čivić *et al.*, 2004),
- determination of protein content, calculated by multiplying the total nitrogen content by a factor of 6.25
- extraction of phosphorus and potassium from plant material using nitric and sulfuric acid (2.5:1 ratio), according to the method described by Lisjak *et al.* (2009),
- determination of phosphorus and potassium content in plant material samples, as well as total and available forms of nutrients in soil samples, using flame atomic absorption spectrophotometry on a Shimadzu 7000 AA instrument (ISO 11047, 1998).

RESULTS AND DISCUSSION

In accordance with the research topic, “Application of bioinoculants for optimizing mineral fertilizer use in silage maize“, and the defined objectives, statistical data analysis was performed using the SPSS statistical software package. The results are presented in this chapter.

Based on the physical and chemical characteristics, the soil on which the experiment was established was classified as clay loam of alluvial origin, with nearly equal proportions of sand, silt, and clay. The soil exhibited a slightly acidic reaction (pH 6.6). It was moderately humus-rich (2.9%), with a low content of plant-available phosphorus (12 mg 100 g⁻¹ soil) and a medium content of plant-available potassium (20 mg 100 g⁻¹ soil).

Maize grain yield and ear length

For comparison of the mean values of yield and ear length, Student’s T-test was applied, by which the experimental treatments were compared with the control treatment. The results are presented in Table 3.

Table 3. Average maize grain yield and ear length under different fertilization treatments

Fertilization treatment	Yield (t ha ⁻¹)	Ear length (cm)
Control treatment	9.66	18.53
NPK 1+ Organic	12.18*	19.90*
NPK 2+ Organic	11.43*	18.2
NPK 3+ Organic	13.19**	18.41
NPK 1+ Inoculated	8.69	16.43
NPK 2+ Inoculated	10.97	16.1
NPK 3+ Inoculated	12.83**	22.05*
Organic 1+ Inoculated	11.91*	20.64*
Organic 2+ Inoculated	11.12*	20.48*
Organic 3+ Inoculated	15.21***	21.41*

*Statistically significantly higher maize grain yield and ear length

From the table above, the following conclusions can be drawn. The effects of fertilization and inoculation on maize grain yield were statistically significant compared to the control treatment. The highest grain yield was recorded in treatment 10, where 30 t ha⁻¹ of cattle manure was applied in combination with inoculation, resulting in a yield of 15.2 t ha⁻¹. This treatment showed a highly statistically significant difference compared to the control. This response suggests that the yield increase resulted not only from an additional nutrient supply but also from enhanced biological activity in the rhizosphere, leading to improved nutrient availability and uptake. Similar findings have been reported by Zuluga *et al.* (2024), who emphasized that the integration of organic amendments with PGPR provides more stable field performance than microbial inoculants applied alone.

The lowest grain yields were observed in treatments 5 and 6, which involved the combination of mineral NPK fertilizer and inoculation, specifically at rates of 300 kg NPK + 367 kg CAN and 450 kg NPK + 563 kg CAN, respectively. In these treatments, yield differences compared to the control were not statistically significant. In all other treatments, yield differences relative to control were either statistically significant or highly significant.

Based on these results, it can be concluded that fertilization strategies combining organic fertilizers with inoculation may represent a viable alternative to the combined use of mineral (NPK) and organic fertilizers. Such an approach has the potential to reduce the application of mineral fertilizers, thereby lowering production costs and exerting a positive environmental effect.

Regarding maize ear length, the results presented in Table 3 show that average ear length ranged from 16.1 to 22.05 cm. In the control treatment without fertilization, ear length was 18.53 cm. All fertilization treatments resulted in higher mean ear length compared to the control, except variant 5 and 6. However, statistical analysis indicated significant differences in ear length in five treatments, namely: NPK 1 + organic

fertilization, NPK 3 + inoculation, Organic 1 + inoculation, Organic 2 + inoculation, and Organic 3 + inoculation treatments. Ear length followed the same trend as grain yield, with the greatest values recorded in treatments combining bacterial inoculation with farmyard manure. Since ear length is closely associated with kernel number and final grain yield, these results indicate that improved nutrient availability during vegetative growth positively influenced reproductive development. The enhanced ear development is likely attributable to improved nutrient uptake and PGPR-induced stimulation of plant growth regulators (Kaushal *et al.*, 2023).

Effect of different fertilization treatments on the content of individual elements in maize leaves and grain

Following the measurements of maize grain yield and ear length, the percentage content of individual elements in maize leaves and grain was also determined for each fertilization treatment. The elements analyzed included nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and zinc (Zn), and protein content. For the comparison of mean values, Student's t-test was applied, whereby the experimental treatments were compared with the control treatment. The results are presented in Table 4.

Table 4. Effect of different fertilization treatments on the content of individual elements in maize leaves

Fertilization treatments	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (mg kg ⁻¹)
Control treatment	1.82	0.09	1.52	0.67	0.05	43.32
NPK 1+ Organic	1.73	0.10	1.53	0.69*	0.06	31.35*
NPK 2+ Organic	2.14*	0.13	1.53	0.70*	0.06	30.68*
NPK 3+ Organic	2.24*	0.18*	1.62*	0.76*	0.07	45.47
NPK 1+ Inoculated	2.62*	0.14	1.52	0.64	0.07	36.31*
NPK 2+ Inoculated	2.97*	0.18*	1.58*	0.77*	0.08	31.65*
NPK 3+ Inoculated	2.55*	0.18*	1.62*	0.71*	0.08	30.24*
Organic 1+ Inoculated	2.25*	0.16*	1.57	0.68*	0.08	32.50*
Organic 2+ Inoculated	1.81	0.12	1.55	0.50	0.08	23.87*
Organic 3+ Inoculated	2.32*	0.16*	1.56	0.80*	0.08	30.31*

* Statistically significant difference

After examining the nutrient content in maize leaves, the same elements were also measured in maize grain. In addition to these elements, the protein content was also determined in the maize grain. The results of these measurements are presented in Table 5.

Table 5. Effect of different fertilization treatments on the content of individual elements and protein in maize grain

Fertilizer treatments	Proteins	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (mg kg⁻¹)
Control treatment	6.56	1.05	0.17	0.29	0.02	0.50	54.5
NPK 1+ Organic	7.36*	1.18*	0.21*	0.31	0.02	0.50	56.38
NPK 2+ Organic	8.42*	1.35*	0.23*	0.31	0.02	0.59*	40.69*
NPK 3+ Organic	9.48*	1.52*	0.33*	0.35	0.02	0.60*	51.94
NPK 1+ Inoculated	10.52*	1.68*	0.31*	0.30	0.03	0.59*	40.60*
NPK 2+ Inoculated	7.06*	1.13*	0.23	0.31	0.03	0.64*	53.98
NPK 3+ Inoculated	9.36*	1.54*	0.27*	0.40	0.03	0.68*	56.86
Organic 1+ Inoculated	9.19*	1.47*	0.27*	0.29	0.03	0.50	54.78
Organic 2+ Inoculated	8.04*	1.29*	0.24*	0.30	0.03	0.68*	63.11*
Organic 3+ Inoculated	8.92*	1.43*	0.24*	0.30	0.03	0.68*	46.69*

**Statistically significant difference*

The data indicates that the effect of fertilization on nitrogen content in maize leaves was significant in all treatments compared to the control, except in the NPK1 + organic and Organic2 + inoculation treatments.

Table 5 shows that the effect of fertilization on protein and nitrogen content in maize grain was statistically significantly higher in all treatments compared to the control.

The effect of fertilization on phosphorus content in maize leaves indicates that the NPK3 + organic, NPK2 + inoculation, NPK3 + inoculation, Organic1 + inoculation, and Organic3 + inoculation treatments had a statistically significant effect. The remaining treatments showed higher phosphorus content in leaves compared to the control, but these differences were not statistically significant. It can be concluded that seed inoculation with these bacterial strains had a positive effect on phosphorus uptake, as four out of six inoculated treatments showed a statistically higher phosphorus content in maize leaves. Bioinoculation also increased nitrogen accumulation in both leaves and grain, which was accompanied by higher grain protein content. These results suggest improved nitrogen use efficiency through enhanced root growth and greater microbial activity in the rhizosphere, particularly when bioinoculants were combined with organic fertilization. Similar responses have been reported in previous studies evaluating PGPR-based fertilization strategies.

The effect of fertilization on phosphorus content in maize grain shows that all fertilization treatments resulted in a statistically significantly higher phosphorus content compared to the control treatment without fertilization.

Potassium content in maize leaves was higher in all experimental treatments compared to the control; however, only three treatments showed a statistically significant increase: NPK3 + organic, NPK2 + inoculation, and NPK3 + inoculation.

Potassium content in maize grain ranged from 0.29 to 0.40%, but no statistically significant differences were observed among treatments.

Calcium content in maize leaves was significantly higher in all treatments compared to the control, except in the NPK1 + inoculation and Organic2 + inoculation treatments. Calcium content in maize grain ranged from 0.02 to 0.03, with no statistically significant differences compared to the control. Most inoculated treatments also showed increased phosphorus concentrations in leaves and grain, confirming the ability of *Pseudomonas* and *Bacillus* to improve phosphorus availability through phosphate solubilization. In contrast, potassium concentrations were only slightly affected, indicating that the principal nutritional benefits of the applied bioinoculants were associated with improved nitrogen and phosphorus acquisition rather than potassium uptake. The treatments significantly influenced calcium accumulation in maize leaves but not in grain, whereas the opposite trend was observed for magnesium. Increased magnesium concentration in grain may reflect improved nutrient uptake associated with enhanced root activity and greater mobility of this element within the plant.

Unlike the macronutrients, zinc concentrations tended to decrease in treatments with higher phosphorus accumulation, reflecting the well-known antagonistic interaction between phosphorus and zinc. Although no negative effects on grain yield were observed, these results suggest that zinc nutrition should be monitored when bioinoculants are used to increase phosphorus availability over prolonged periods.

Magnesium content in maize leaves was higher in all treatments compared to the control; however, none of the differences were statistically significant. In maize grain, magnesium content was statistically significantly higher in all treatments compared to the control, except in the NPK1 + organic and Organic1 + inoculation treatments.

Zinc content in maize leaves was statistically significantly lower in all treatments compared to the control, except in the NPK3 + organic treatment, which showed a higher value than the control, although this difference was not statistically significant. Zinc content in maize grain was statistically significantly lower in four treatments compared to the control (NPK1 + organic, NPK1 + inoculation, Organic2 + inoculation, and Organic3 + inoculation).

These results may be explained by the fact that the applied fertilization treatments had a positive effect on phosphorus and calcium uptake, while simultaneously reducing zinc uptake, as these elements are known to exhibit antagonistic interactions with zinc. Considering the importance of zinc in animal nutrition and the observed reduction in its content in both leaves and grain due to the antagonistic effect of phosphorus, it is recommended that additional zinc fertilization be applied in maize production.

CONCLUSION

From the perspective of sustainable agriculture, the results of this study are of particular importance, as they demonstrate that the combined application of farmyard manure and PGPR bacteria can represent an effective strategy for improving nutrient use efficiency and reducing dependence on mineral fertilizers. Such an approach contributes to the preservation of soil fertility, enhances the biological activity of the rhizosphere, and reduces the environmental burden of agricultural production, in line with contemporary concepts of sustainable soil resource management.

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PRIMJENA BIOINOKULANATA ZA OPTIMIZACIJU UPOTREBE MINERALNIH GNOJIVA U PROIZVODNJI SILAŽNOG KUKURUZA

Sažetak

Kukuruz je jedna od najznačajnijih poljoprivrednih kultura u svijetu i Bosni i Hercegovini, gdje se žitarice uzgajaju na površini od 204.368 ha, od čega se 57% (116.490 ha) odnosi na proizvodnju kukuruza. Primjena biljnih rizobakterija koje potiču rast biljaka (PGPR), kao što su rodovi *Pseudomonas* i *Bacillus*, predstavlja održivu alternativu mineralnim gnojivima, posebno na tlima siromašnim fosforom koja su česta u Bosni i Hercegovini. Poljski ogled sa deset varijanti gnojidbe, postavljen prema slučajnom bloknom rasporedu u tri ponavljanja, proveden je s ciljem procjene uticaja inokulacije sjemena, organske i mineralne gnojidbe na proizvodnju silažnog kukuruza. Prinos zrna, karakteristike klipa i koncentracije hraniva u listu i zrnu kukuruza određeni su primjenom standardnih analitičkih metoda.

Najveći prinos zrna (15,2 t ha⁻¹) ostvaren je kombiniranom primjenom organske gnojidbe (30 t ha⁻¹) i bakterijske inokulacije, pri čemu je utvrđena statistički značajna razlika u odnosu na kontrolni tretman. Primijenjeni tretmani gnojidbe poboljšali su usvajanje hraniva i povećali sadržaj proteina, dušika, fosfora i magnezija u zrnu, dok se koncentracija cinka uglavnom smanjila, što ukazuje na antagonistički odnos između fosfora i cinka. Dobiveni rezultati potvrđuju potencijal primjene kombinacije rizobakterijske inokulacije i organske gnojidbe za povećanje produktivnosti kukuruza i poboljšanje statusa hraniva u biljci.

Ključne riječi: kukuruz, gnojidba, bakterije koje potiču rast biljaka (PGPR), bioinokulanti, prinos