THE USE OF AZOTOBACTER IN ORGANIC MAIZE PRODUCTION

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Abstract: *Maize is an important field crop which is mainly grown in the system of conventional production accompanied by the use of mineral fertilizers. Organic maize production is not widespread but it has been getting more attention recently. The aim of the research was to determine the effect of azotobacter on the microbiological activity in the rhizosphere and on the grain yield of various maize hybrids grown in the system of organic production. The experiment was conducted in field conditions at the Institute of Field and Vegetable Crops, Department of Organic Agriculture and Biodiversity in Backi Petrovac. Four maize hybrids (ZP 555 su, NS 609b, 620k and NS 6030) and three concentrations of Azotobacter chroococcum were used. The grain yield of maize was determined at the end of the vegetation period. The number of azotobacter, the number of aminoheterotrophs and dehydrogenase activity in the rhizospheric soil were determined, too. The grain yield increased in the variants with azotobacter. The greatest increase in the grain yield was recorded in ZP 555 su hybrid and it amounted to 1000 kg/ha. In NS 6030 hybrid, the increase in yield amounted to 280 kg/ha and in 620k it amounted to 450 kg/ha. In NS 609b hybrid, there was no effect. Azotobacter could increase maize yield by stimulating processes such as seed germination, resistance of seedlings to stress conditions, nitrogen fixation and production of phytohormones. The use of azotobacter did not significantly affect the number of azotobacter or aminoheterotrophs. Dehydrogenase activity increased in the variants with azotobacter. A positive correlational interdependence between dehydrogenase activity and yield indicates the existence of an active microbial population in the maize rhizosphere. In order to achieve the best effects of inoculation, it is necessary to learn about and follow the dynamics of the applied microorganisms in field conditions The paper presents preliminary results of the research conducted during one year. Therefore, no final conclusions can be drawn. Still, the results indicate in which direction further research should be done.*

Key words: *Azotobacter chroococcum, maize, yield, number of microorganisms.*

INTRODUCTION

Maize is an important agricultural crop typical of the temperate climatic zone. It is grown over large areas because it is used in human nutrition and in the production of fodder. The grain yield of maize depends on the genetic potential of a hybrid, soil characteristics, agrotechnical measures and climatic factors (JOCKOVIĆ et al., 2010; JELIČIĆ et al., 2006).

Conventional agricultural production implies large amounts of mineral fertilizers which increases production costs and causes pollution of the environment (DAI et al., 2004). Plant growth in the system of organic production includes only the use of controlled organic fertilizers and biofertilizers. Biofertilizers are microbiological fertilizers which contain specific species of microorganisms and are used for stimulating microbiological processes in which plant nutrients are released. For instance, *Pseudomonas fluorescens* and *Bacillus subtilis* are growth promotors whereas *Bacillus megatherium var.phosphaticus* mineralizes organic phosphorus compounds. Mycorrhizae provide plants with phosphorus and water, whereas

symbiotic and free-living nitrogen-fixers fix elementary nitrogen and increase the amount of nitrogen in soil (TURAN et al.,2006**,** HERNANDEZ et al., 2004; IDRISS et al., 2002). As maize requires about 120 to 180 kg N/ha for its growth, development and optimum yield, the use of nitrogen-fixing bacteria is an alternative to mineral nitrogen fertilizers in organic maize production (GOVEDARICA et al., 1998; WU et al. 2005, EBRAHIMI et al. 2007). The use of these can replace 30-50% of the total amount of mineral nitrogen (MILOŠEVIĆ et al., 2001).

The aim of the research was to determine the effect of different concentrations of *Azotobacter chroococcum* on the microbiological activity in the rhizosphere and on the grain yield of different maize hybrids grown in the system of organic production.

MATERIAL AND METHODS

The experiments were conducted in field conditions in the experimental field of the Institute of Field and Vegetable Crops, Department of Organic Agriculture and Biodiversity, the locality of Backi Petrovac. A two-level factorial split-plot experiment was set where the first factor was the maize hybrid and the second factor was the amount of *Azotobacter chroococcum* inoculum.

The maize hybrids were ZP 555 su, NS 609b, 620k and NS 6030. Seed inoculation was performed before sowing, introducing 100ml, 75ml and 50ml of *Azotobacter chroococcum* into 1000 grains of maize. The *Azotobacter chroococcum* titer was 10^8 CFU/ml. The effect of inoculation was determined at the end of the vegetation period.

The grain yield of NS 609b, 620k and NS 6030 maize hybrids was calculated at 14% moisture whereas in ZP 555 hybrid, the yield was calculated at the stage of 30% of dry matter.

Microbiological research included investigating the number of azotobacter and aminoheterotrophs in the rhizosphere. The number of microorganisms was determined by the method of agar plates (TROLLDENIER, 1996) by introducing a diluted soil suspension into the proper media (Hi media) and counted per one gram of absolutely dry soil. Dehydrogenase activity was determined spectrophotometrically (LENHARD, 1956 and THALMANN, 1968).

The statistical data processing included the correlation analysis.

RESULTS AND DISCUSSIONS

A large number of living cells of *Azotobacter chroococcum* was introduced into the soil by means of inoculation. However, their number in the rhizosphere of the ivestigated maize hybrids remained the same or decreased (Table 1) which points to their lower adaptability in comparison with the indigenous population of azotobacter.

Table 1.

The effect of inoculation on the number of azotobacter (log No)

Variant	Maize hybrids			
	ZP 555 su	620k	NS 609b	NS 6030
Control	2.76	3.60	3.56	2.80
100 ml *A.chroococcum*	2.69	3.63	3.73	2.88
75 ml *A.chroococcum*	2.60	3.50	3.67	2.76
50 ml *A.chroococcum*	3.60	3.77	3.62	2.91

The table shows mean values (arithmetic mean of four repetitions).

The number of aminoheterotrophs in the inoculated variants was also similar to the number of these in the control (Table 2).

According to many authors, introduced microorganisms change the microbial population in soil. Certain groups of microorganisms can be either stimulated or inhibited or there may be no effect at all on the structure of the indigenous population (SCHWIEGER and TEBBE, 2000; BACILIO-JIMENEZ et al., 2001; DOBBELAERE et al., 2003). PANDEY et al.(1998)

mentioned that introducing *Azotobacter chroococcum* and *Azospirillum brasilense* into soil under maize stimulated the growth of actinomycetes and free-living nitrogen-fixers.

Table 2.

The effect of inoculation on the number of aminoheterotrophs (log No)

Variant	Maize hybrids			
	ZP 555 su	620k	NS 609b	NS 6030
Control	7.01	7.28	6.95	7.00
100 ml *A.chroococcum*	7.28	7.17	7.06	5.23
75 ml *A.chroococcum*	7.34	6.81	7.24	7.27
50 ml *A.chroococcum*	7.18	7.17	7.13	7.20

The table shows mean values (arithmetic mean of four repetitions).

This research focused on following the change in number of only two groups of microorganisms which is certainly not an indicator of total microbiological activity of soil. A more realistic indicator is dehydrogenase activity which points to the intensity of oxidation-reduction, i.e. the intensity of metabolic activity of microorganisms. In this research, dehydrogenase activity increased in all the variants where azotobacter was applied (Table 3). These results are in accordance with the research by MAWDSLEY and BURNS (1994) who mentioned that inoculating maize with bacteria results in an increased number of microorganisms and enzymatic activity in rhizospheric soil.

Table 3.

The effect of inoculation on dehydrogenase activity (μg TPF/10g of soil)

Variant	Maize hybrids			
	ZP 555 su	620k	NS 609b	NS 6030
Control	730.25	945.00	877.50	942.00
100 ml *A.chroococcum*	724.50	1244.00	1018.50	943.00
75 ml *A.chroococcum*	1000.75	985.00	914.75	950.00
50 ml *A.chroococcum*	962.75	1063.00	949.00	1055.50

The table shows mean values (arithmetic mean of four repetitions).

The use of azotobacter had a positive effect on the grain yield of maize. In the variants where azotobacter was applied, the grain yield increased in three maize hybrids (Table 4). In ZP 555 su, the yield increased by 1000 kg/ha, in NS 6030 by 280 kg/ha and in 620k by 450 kg/ha. In NS 609b hybrid, the inoculation did not have any effect.

Table 4.

The effect of inoculation on the grain yield of maize (t/ha)

Variant	Maize hybrids			
	ZP 555 su	620k	NS 609b	NS 6030
Control	12.27	4.27	8.88	10.59
100 ml *A.chroococcum*	13.32	4.97	8.39	10.90
75 ml *A.chroococcum*	13.24	4.89	8.87	10.75
50 ml *A.chroococcum*	13.31	4.30	8.92	10.96

The table shows mean values (arithmetic mean of four repetitions).

HAJNAL-JAFARI (2010) and JARAK et al.(2011) arrived at similar conclusions concerning the use of free-living and associative nitrogen-fixing bacteria in maize production. SHAUKAT et al. (2006) and EGAMBERDIYEVA (2007) stated that biofertilizers increase maize yield by stimulating processes such as seed germination, resistance of seedlings to stress conditions, nitrogen fixation and production of phytohormones.

Correlation analysis showed a significant positive interdependence between certain indicators of microbiological activity in the rhizosphere and grain yield of the investigated

maize hybrids, especially in ZP 555 su hybrid (Table 5). This hybrid is characterized by shorter vegetation period and it was harvested at the stage of 30% of dry matter in the grain. At that stage, the root is still very active, microbiological processes are intensive which causes a greater dependence between the plant and microorganisms. Aminoheterotrophs decompose proteins and provide the plant with nitrogen. A positive correlational interdependence between dehydrogenase activity and yield indicates the existence of an active microbial population in the maize rhizosphere.

Table 5.

The coefficients of correlation between indicators of microbiological activity and grain yield of maize hybrids

Microbiological parameters	Maize hybrids			
	ZP 555 su yield	620k yield	NS 609b yield	NS 6030 yield
Azotobacter	0.255	-0.562	-0.798	0.773
Aminoheterotrophs	0.873	-0.603	0.174	-0.328
Dehydrogenase activity	0.520	0.560	-0.839	0.650

In order to achieve the best effects of inoculation, it is necessary to learn about and follow the dynamics of the applied microorganisms in field conditions. This issue has been getting more attention in organic agricultural production. This paper shows preliminary results of the research conducted during one year. Therefore, no final conclusions can be drawn but the results are an important indicator of the direction in which further research should be done.

CONCLUSIONS

The use of *Azotobacter chroococcum* did not affect the number of azotobacter or aminoheterotrophs. Dehydrogenase activity increased in all the variants where azotobacter was applied. The grain yield increased in three maize hybrids (ZP 555 su, 620k and NS 6030). The best results were recorded in ZP 555 su hybrid, with an increase in yield amounting to 1000 kg/ha.

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BIBLIOGRAPHY

1. BACILIO-JIMENEZ M, AGILAR-FLORES S, DEL VALLE MV, PEREZ A, ZEPEDA A, ZENTENO E (2001) - Endophytic bacteria in rice seeds inhibit early colonization of roots by *Azospirillum brasilense*. Soil Biol. Biochem. 33: 167-172.
2. DAI J, BECQUER T, ROUILLER J, REVERSAT H, BERNHARD G, LAVELLE F (2004) - Influence of heavy metals on C and N mineralization and microbial biomass in Zn-, Pb-, Cu- and Cd-contaminated soils. Applied Soil Ecology 25: 99-109.
3. DOBBELAERE S, VANDERLEYDEN J, OKON Y (2003) - Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit. Rev. Plant Sci. 22: 107-149.
4. EBRAHIMI S, NEJAD HI, RAD AHS, AKBARI GA, AMIRY R, SANAVY SM (2007) - Effect of *Azotobacter chroococcum* Aplication on Quantity and Quality Forage of Rapeseed Cultivars. Pakistan Journal of Biological Sciences 10 (18): 3126-3130.
5. EGAMBERDIYEVA D (2007) - The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. App. Soil. Ecology 36: 184-189.
6. GOVEDARICA M, JELIČIĆ Z, JARAK M, MILOŠEVIĆ N, STOJNIĆ N, RAŠKOVIĆ D (1998) - Značaj asocijativne azotofiksacije u proizvodnji kukuruza. Zbornik naučnih radova Instituta

PKB Agroekonomik 4 (1): 95-102.
7. HAJNAL-JAFARI T (2010) - Uticaj inokulacije na prinos i mikrobiološku aktivnost u zemljištu pod usevom kukuruza. Doktorska disertacija. Poljoprivredni fakultet, Novi Sad.
8. HERNANDEZ ME, KAPPLER A, NEWMAN DK (2004) - Phenazines and other redoxe-active antibiotics promote microbial mineral reduction. Applied Environmental Microbiology 70 (2): 921-928.
9. IDRISS EE, MAKAREWICZ O, FAROUK A, ROSNER K, GREINER R, BOCHOW H, RICHTER T, BORRIS R (2002) - Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant growth promoting effect. Microbiology 148: 2097-2109.
10. JARAK M, JELIČIĆ Z, KUZEVSKI J, MRKOVAČKI N, ĐURIĆ S (2011) - The use of azotobacter in maize production: The effect on microbiological activity of soil, early plant growth and grain yield. Contemporary Agriculture 60: 80-85.
11. JELIČIĆ Z, ERIĆ M, PAVLOVIĆ A, KAČAREVIĆ A (2006) - Produktivne mogućnosti hibrida kukuruza instituta PKB Agroekonomik u makroogledima u 2005. Godini. Zbornik naučnih radova 12 (1-2): 23-31.
12. JOCKOVIĆ Đ, STOJAKOVIĆ M, IVANOVIĆ M, BEKAVAC G, POPOV R, ĐALOVIĆ I (2010) - NS maize hybrids – today and tomorrow. Field Veg. Crop Res. 47 (1), 325–333.
13. LENHARD G. (1956) - Die dehydrogenase aktivitat das bodeusald mass fur die mikroorganizmentatigk eit im boden. Z. Pflansenern. Dung Bodenkude. 73: 1-11.
14. MAWDSLEY JL, BURNS RG (1994) - Inoculation of plants with a *Flavobacterium* species results in altered rhizosphere enzyme activities. Soil Biol. Biochem. 26: 871-882.
15. MILOŠEVIĆ N, GOVEDARICA M (2001) - Mogućnost primene biofertilizatora u proizvodnji ratarskih neleguminoznih biljaka. Zbornik radova Instititа za ratarstvo i povrtarsvo 35: 53-65.
16. PANDEY A, SHARMA E, PALNI LMS (1998) - Influence of bacterial inoculation on maize in upland farming systems of the Sikkim Himalaya. Soil Biol. Biochem. 30: 379-384.
17. SCHWEIGER F, TEBBE CC (2001) - Effect of field inoculation with *Sinorhizobium meliloti* L33 on the composition of bacterial communities in rhizosphere of a target plant (*Medicago sativa*) and a non-target plant (*Chenopodium album*)-Linking of 16S rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultured bacteria. Appl. Environ. Microbiol. 66: 3556-3565.
18. SHAUKAT K, AFFRASAYAB S, HASNAIN S (2006) - Growth responses of *Triticum aestivum* to plant growth promoting rhizobacteria used as a biofertilizer. Res. J. Microbiol. Vol 1(4): 330-338.
19. THALMANN A. (1968) - Zur methodik des bestmmung des dehydrogenase activitat im bodenmittels TTC. Landw. Forch. 21: 249-258.
20. TROLLDENIER G. (1996) - Plate Count Technique. In Methods in Soil Biology. Ed.Franz Schinner, Ellen Kandeler, Richard Ohlinger, Rosa Margesin. Springer-Verlag Berlin Heildeberg., pp. 20-26.
21. TURAN M, ATAOGLU N, SAHIN F (2006) - Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. Sustanable Agriculture 28: 99-108.
22. WU SC, CAO ZH, LI ZG, CHEUG KC, WONG MH (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125: 155-166.