



## Effect of Co-Inoculation with Different Groups of Beneficial Microorganisms on the Microbiological Properties of Soil and Yield of Maize (*Zea mays* L.)

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**Summary:** The effect of co-inoculation on the microbiological properties of rhizospheric soil and yield of maize was investigated in field conditions. The total number of microorganisms (TNM) was largest in variant where Plant Growth Promoting Bacteria (PGPB), Phosphorus Solubilizing Bacteria (PSB) and Arbuscular Mycorrhiza (AM) were applied. TNM was  $418.10 \times 10^7$  /g soil. The largest number of fungi was achieved in the variant with PGPM ( $29.65 \times 10^4$  /g soil). The joint use of PGPB, PSB and AM resulted in the largest number of phosphominerallizers (PM) ( $31.67 \times 10^4$  /g soil). Dehydrogenase activity (DHA) increased only in the variant where all the microorganisms were introduced ( $924 \mu\text{g TPF}/10 \text{ g soil}$ ). The co-inoculation led on average to the increased activity of acid phosphatase (ACP) and alkaline phosphatase (ALP). The highest yield of maize was achieved in the variant with PGPB (15.33 t/ha). Correlation analysis proved a high degree of interdependence between microbiological activity and maize yield.

**Key words:** arbuscular mycorrhizae, enzymatic activity, inoculation, microorganisms, maize, PGPB, PSB, soils, yield

### Introduction

It is necessary to provide maize with adequate amounts of macroelements for optimum growth and development, as well as achievement of high yield. A part of nutrients is provided by application of microbial inoculants (Dobbelaere et al. 2003). Inoculants are produced from different kinds of microorganisms, PGPB among others. PGPB, directly or indirectly, affect the growth and development of plants and the amount of yield through various mechanisms: associative nitrogen fixation, production of phytohormones, ACC (l-aminocyclopropane-l-carboxylate) deaminase activity, production of phenazine and exopolysaccharides (Alami et al. 2000, Idriss et al. 2002, Ryu et al. 2003, Hernandez et al. 2004, Egamberdiyeva, 2007). Phosphominerallizers increase the supply of phosphorus by means of mineralization of organic phosphorus compounds. The important phosphominerallizing bacteria is *Bacillus*. Arbuscular mycorrhiza (AM) improve the physical properties of soil, soil aeration and

water dynamics (Rillig et al. 2002). AM also enable release of phosphorus from inaccessible compounds (Whitelaw 1999, Harrier et al. 2003, Vessey et al. 2004). By means of inoculation, a large amount of exogenic microorganisms is introduced into the soil and they affect the indigenous microbial population. At the same time, the inoculants are exposed to the influence of resident microorganisms (Bolton et al. 1991, De Leij et al. 1995, Natsch et al. 1997). As microorganisms have an important role in agricultural production, it is necessary to observe changes in the microbiological activity in soil which are the result of different agrotechnical measures and inoculation itself.

The aim of this investigation was to determine the effect of co-inoculation with PGPM (*Azotobacter chroococcum*, *Azospirillum lipoferum*), PSB (*Bacillus megaterium*) and AM (*Glomus intraradices*) on the microbiological properties of soil as well as on the yield of maize (*Zea mays* L.)

### Materials and Methods

The cultures of bacteria *A. chroococcum*, *A. lipoferum*, *B. megaterium* and fungus *G. intraradices* were taken from the collection of the Department of Microbiology, Faculty of Agriculture, University of Novi Sad.

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*A. chroococcum* and *A. lipoferum* were grown in a liquid medium by Fjodorov (Anderson 1965). *B. megaterium* was grown in a liquid mesopeptonic medium. Inoculum of AM fungi was multiplied and sustained by the open-pot culture method (Gilmore 1968).

The field experiment was carried out in chernozem soil having the following characteristics: pH in H<sub>2</sub>O 7.67, pH in KCL 6.90, %N 0.17, % humus 2.66, % CaCO<sub>3</sub> 0.98, P<sub>2</sub>O<sub>5</sub> 16.28 mg/100g of soil, K<sub>2</sub>O 33.52 mg/100g of soil. The experiment was conducted in three repetitions. The size of the experimental plot was 5m<sup>2</sup>. The seeding was done in the optimum period (April). Before seeding, the maize (*Z. mays*) seed (hybrid NSSC 640, Institute of Field and Vegetable Crops, Novi Sad, Serbia) was inoculated with 150 ml of the liquid inoculum (10<sup>8</sup> CFU/ml and 100 g of AM inoculum containing mycelia and spores). The scheme of inoculation was the following: 1) control, 2) *A.chroococcum*+*A. lipoferum*, 3) *A.chroococcum*+*A.lipoferum*+*B. megaterium*, 4) *A.chroococcum* + *A.lipoferum* + *B.megaterium* + *G.intraradices*.

Irrigation was carried out in April in the period of 14 days. Digging was carried out in the phase of 6-8 leaves. Soil samples for microbiological analyses were taken from the rhizosphere at the end of the vegetation period. The yield of maize with 14% grain moisture was determined after harvesting.

The number of microorganisms was determined by the method of agar plates (Trolldenier 1996). The TNM was determined in an agarized soil extract :10<sup>-7</sup> dilution (Wollum 1982). The number of fungi was determined in Czapek's agar:10<sup>-4</sup> dilution (Parkinson 1982) whereas the number of PM was determined in the Menkina medium:10<sup>-4</sup> dilution (Niewolak 1980). All groups of microorganisms were incubated at the constant temperature of 28°C.

The period of incubation for the TNM lasted seven days, five days in fungi and PM.

DHA was determined by the spectrophotometric method (Lenhard 1956) modified by Thalmann (1968). The activity of ACP and ALP was determined spectrophotometrically by Flint (1974).

The statistical data analysis included analysis of variance for determining LSD on the significance level of 0.05 and correlation analysis. The analyses were performed using software STATISTICS 10.0.

## Results and Discussion

Inoculation affected the number of individual groups of microorganisms and enzymatic activity in different ways (Tables 1 and 2). The TNM increased in all inoculated variants. Fisher's LSD test showed significant differences between the control and variant 4 (PGPB+PSB+AM). A statistically significant difference in the number of fungi was observed between the control and variant 2 (PGPB), and between variant 2 (PGPB) and variant 3 (PGPB+PSB). In the variants with inoculation the number of PM increased, but there were no significant differences between the control and the examined variants.

On average, DHA decreased in variants with inoculation. DHA increased only in variant 4 (PGPB+PSB+AM), although the increase was not statistically significant in comparison with the control. The activity of this enzyme significantly decreased in variant 2 (PGPB).

The co-inoculation on average led to an increase in ACP. However, there was no statistically significant difference between the control and the variants of the experiment nor between individual variants. Likewise, there was no statistically significant difference in the ALP between the control and the inoculated variants.

Table 1. The number of microorganisms in rhizospheric soil  
Tabela 1. Brojnost mikroorganizama u rizosfernom zemljištu

Variants	TNM (10 <sup>7</sup> /g soil)	Fungi (10 <sup>4</sup> /g soil)	PM (10 <sup>4</sup> /g soil)
1	221.61 <sup>b</sup>	11.96 <sup>b</sup>	11.16 <sup>a</sup>
2	326.15 <sup>ab</sup>	29.65 <sup>a</sup>	22.63 <sup>a</sup>
3	244.67 <sup>ab</sup>	11.16 <sup>b</sup>	11.95 <sup>a</sup>
4	418.10 <sup>a</sup>	23.76 <sup>ab</sup>	31.67 <sup>a</sup>
Average 2-4	329.64	21.45	22.08

The table shows mean values (arithmetic mean of three repetitions). There is no statistically significant difference between mean values in each column with the same letter in superscript on the basis of LSD test ( $p < 0.05$ )

Key: (1) control, (2) *A.chroococcum*+*A.lipoferum*, (3) *A.chroococcum*+*A.lipoferum*+*B.megaterium*, (4) *A.chroococcum*+*A.lipoferum*+*B. megaterium*+*G.intraradices*.

Inoculation had a statistically significant effect on the grain yield of maize (Table 3). The highest yield was achieved in variant 2 (PGPB) and variant 4 (PGPB+PSB+AM). A statistically significant difference was observed between variant 2 (PGPB) and variant 3 (PGPB+AM).

Correlation analysis proved the existence of a high degree of interdependence between the investigated parameters of microbiological activity and grain yield of maize (Table 4). A strong correlational interdependence was observed between the TNM and fungi ( $r=0.752$ ), PM

Table 2. Enzymatic activity in rhizospheric soil  
Tabela 2. Enzimatska aktivnost u rizosfernom zemljištu

Variants	DHA ( $\mu\text{g TPF}/10\text{ g soil}$ )	ACP ( $\mu\text{mol pPN}/\text{s}/\text{dm}^3$ )	ALP ( $\mu\text{mol pPN}/\text{s}/\text{dm}^3$ )
1	832 <sup>b</sup>	2.93 <sup>a</sup>	3.00 <sup>a</sup>
2	685 <sup>a</sup>	3.33 <sup>a</sup>	4.07 <sup>a</sup>
3	831 <sup>b</sup>	2.39 <sup>a</sup>	3.87 <sup>a</sup>
4	924 <sup>b</sup>	3.9 <sup>a</sup>	4.24 <sup>a</sup>
Average 2-4	813.33	3.21	4.06

The table shows mean values (arithmetic mean of three repetitions). There is no statistically significant difference between mean values in each column with the same letter in superscript on the basis of LSD test ( $p < 0.05$ )

Key: (1) control, (2) *A.chroococcum*+*A.lipoferum*, (3) *A.chroococcum*+*A.lipoferum*+*B.megaterium*, (4) *A.chroococcum*+*A.lipoferum*+*B.megaterium*+*G.intraradices*.

Table 3. Grain yield of maize (t/ha)  
Tabela 3. Prinos zrna kukuruza (t/ha)

Variants	Grain yield (t/ha)
1	13.64 <sup>ab</sup>
2	15.33 <sup>a</sup>
3	12.62 <sup>b</sup>
4	14.13 <sup>ab</sup>
Average 2-4	14.03

The table shows mean values (arithmetic mean of three repetitions). There is no statistically significant difference between mean values in each column with the same letter in superscript on the basis of LSD test ( $p < 0.05$ )

Key: (1) control, (2) *A.chroococcum*+*A.lipoferum*, (3) *A.chroococcum*+*A.lipoferum*+*B.megaterium*, (4) *A.chroococcum*+*A.lipoferum*+*B.megaterium*+*G.intraradices*.

Table 4. Correlation coefficients between the investigated parameters  
Tabela 4. Koeficijenti korelacije između ispitivanih parametara

	TNM	fungi	PM	DHA	ACP	ALP	Yield
TNM	-						
Fungi	0.752*						
PM	0.996*	0.789*					
DHA	0.274	-0.413	0.229				
ACP	0.896*	0.749*	0.926*	0.226			
ALP	0.801*	0.670*	0.767*	0.003	0.475		
yield	0.517*	0.920*	0.582*	-0.557	0.679*	0.326	-

Correlation coefficients are the result of the average values for every examined parameter on the level of significance 0.05

( $r=0.996$ ), ACP ( $r=0.896$ ) and ALP ( $r=0.801$ ). There was a positive correlational interdependence between the number of fungi and ACP ( $r=0.749$ ), and between the number of fungi and ALP ( $r=0.670$ ). The number of PM is in positive correlational interdependence with the activity of ACP ( $r=0.926$ ) and ALP ( $r=0.767$ ). The correlation analysis showed a positive effect of the TNM, number of fungi and PM, and ACP on the grain yield of maize.

The introduced microorganisms can affect the structure of the indigenous microbial population in different ways (Nacamulli et al. 1997). Some microorganisms are stimulated, some are inhibited and in some microorganisms there is no change (Schwieger & Tebbe 2000, Bacilio-Jimenez et al. 2001).

In this research three different inocula containing two or more microorganisms were used. In the inoculated variants, there was an increase in the total number of microorganisms, the number of fungi and phosphom mineralizers. It is assumed that the total microbial biomass in rhizospheric soil increased as the result of inoculation. However, the inoculation did not lead to an increase of dehydrogenase activity in rhizospheric soil. This points to the fact that the indigenous microbial population had a greater metabolic activity, i.e. that the introduction of microorganisms caused a change in oxidoreduction reactions. It is consistent with the results of Requena et al. (1997, 2001) and recent works of Andronov et al. (2009) and Gamalero et al. (2009). Averagely higher phosphatase activity in the inoculated variants points to intensification of the process of mineralization of phosphorus compounds and better provision of the plant with inorganic phosphorus. The results of this research showed that the co-inoculation of the maize seed had a positive or

stimulating effect on the yield of the plant. The grain yield increased in the variant with the applied PGPR and in the variant with PGPR, PSB and mycorrhiza. Such positive effect may be the result of stimulation of seed germination, the resistance of seedlings to stress, production of active substances-growth promoters, associative nitrogen fixation (Egamberdiyeva, 2007). There are many different bacteria which promote plant growth. They include *Azotobacter sp.*, *Azospirillum sp.*, *Pseudomonas*, *Bacillus sp.* and many others (Turan et al. 2006). When PGPB and mycorrhiza are applied together, a mutualistic relationship forms in which PGPB induces the formation of mycorrhizal association with the plant by means of stimulating the growth of hyphae (Dunstan et al. 1998, Tiwari et al. 2004). Mycorrhizae enable better water provision and availability of plant nutrients and induce the protection mechanism of the plant against pathogens (Smith et al. 2001, Marulanda et al. 2003). Vivas et al. (2003, 2006) and Artursson et al. (2006) explain that the positive effect of co-inoculation with PGPR and AM on the plant yield could be the result of the fact that the bacteria help the release of nutrients after decomposing the mycelium of a mycorrhiza. Yazdani et al. (2009), Gholami et al. (2009), and Zaidi et al. (2006) reached similar conclusions.

The correlation analysis showed that fungi and phosphom mineralizers are important producers of acid and alkaline phosphatase. Tabatabai (1994) also mentioned microorganisms as important producers of phosphatase. The results of the research conducted by Dick (1996), Hojati and Nourbakhsh (2006) were similar to the results in this research. They investigated enzymatic activity and microbial biomass in soil and confirmed a positive correlation between the microbial biomass of carbon and L-glutaminase activity, alkaline phosphatase, arylsulphatase and  $\beta$ -glucosidase.

## Conclusions

The results of the present research point to the existence of a developed and active microbial population in the rhizospheric soil which formed and survived after the introduction of the inoculants. The success of inoculation is influenced by the environmental conditions especially when the experiments are carried out in the field. However, what is of greatest importance is the right choice of microorganisms. In our opinion, the choice should lean towards local isolates of microorganisms.

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## Uticaj združene inokulacije sa različitim grupama korisnih mikroorganizama na mikrobiološka svojstva zemljišta i prinos zrna kukuruza (*Zea mays* L.)

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**Izvod:** Uticaj združene inokulacije na mikrobiološka svojstva rizosfernog zemljišta i na prinos zrna kukuruza je ispitivan u poljskim uslovima. Najveći ukupan broj mikroorganizama je bio u varijanti gde su primenjeni mikroorganizmi-stimulatori biljnog rasta (PGPB), fosfomobilizatori i mikorizna gljiva i iznosio je  $418,10 \times 10^7$  /g zemljišta. Najveći broj gljiva je dobijen u varijanti sa PGP bakterijama ( $29,65 \times 10^4$ /g zemljišta). Zajedničkom primenom PGPB, fosfomobilizatora i mikorize povećan je broj fosfomineralizatora ( $31,67 \times 10^4$ /g zemljišta). Dehidrogenazna aktivnost je povećana samo u varijanti gde su primenjeni svi ispitivani mikroorganizmi ( $924 \mu\text{g TPF}/10$  g zemljišta). U proseku je povećana aktivnost kisele i alkalne fosfataze, ali ono nije statistički značajno. Najveći prinos zrna kukuruza je dobijen u tretmanu sa mikroorganizmima-stimulatorima rasta ( $15,33$  t/ha). Korelacionom analizom je utvrđen visok stepen međuzavisnosti između ispitivanih parametara mikrobiološke aktivnosti i prinosa zrna kukuruza.

**Ključne reči:** arbuskularna mikoriza, enzimatika aktivnost, fosfomobilizatori, inokulacija, kukuruz, mikroorganizmi, PGPB, prinos, zemljišta