ORIGINAL SCIENTIFIC PAPER

Evaluation of NPK fertilizers and bacterial inoculants influence on soil dehydrogenase activity and microbial biomass and yield of maize

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Abstract

The aim of this study was to evaluate the influence of an application of different rates of mineral fertilizers and their combination with associative N-fixing *Klebsiella planticola* and *Enterobacter* spp. and sampling period on microbial biomass carbon and dehydrogenase activity in Cambisol and grain yield of maize.

The values of the studied parameters of the soil microbial activity were significantly higher in the variants where combination of bacterial inoculants and lower rates of mineral fertilizers was applied, as well as in the second sampling period. Mentioned combination also resulted in higher grain yield of maize comparing to the application of lower rates of the pure NPK nutrients.

Key words: microbial biomass, dehydrogenase activity, NPK fertilizers, N-fixing bacteria, maize

Introduction

The studies in the field of fertilization are mostly focused on the increase of the yield of crops whereas the traits of the cumulative effect of fertilizers (the change of biological and chemical soil properties, the content of biogenic elements and heavy metals etc.) have often been disregarded. Regardless of its major role in crop productivity and soil fertility, the application of mineral fertilizers (particularly nitrogen) may induce a series of negative consequences, from the microbiological, economic and ecological aspects (Acosta-Martinez and Tabatabai, 2000).

The coefficient of nitrogen utilization by crops is low (30-60%) and is being reduced even more with the increase of nitrogen fertilizers rates. The problems concerned can be overcome by partial replacement of these fertilizers by application of microbial inoculants, in order to inhibit or stimulate certain cellular processes, including mineralization ones, thus leading to the improvement of physico-chemical and biological soil properties. (Milošević et al., 2003).

Having in mind the above mentioned, the aim of this investigation was to examine the influence of different rates of mineral fertilizers [composite NPK (15:15:15)] and their combination with selected soil bacterial inoculants, and sampling period on microbial biomass carbon and dehydrogenase activity in Cambisol and grain yield of maize.

Material and methods

The investigation was conducted on Mladenovac experimental station of Institute of Soil Science, located 55 km from Belgrade in Serbia, during 2006. Mean monthly temperature and precipitation summ for the investigated period are presented in Table 1.

The studied soil type was Cambisol. The experiment was set up in a randomized block design with three

Proceedings. 46th Croatian and 6th International Symposium on Agriculture. Opatija. Croatia (645-649)

replicates, based on the following variants: control (Ø, non-fertilized soil); 60 kg·ha^{-1} N and P_2O_5, and 40 kg K_2O ha^{-1} (N1); 120 kg·ha^{-1} N, P_2O_5 and K_2O (N2); *Enterobacter* sp. strains + 60 kg·ha^{-1} N and P_2O_5, and 40 kg K_2O ha^{-1} (ES+N1); *Enterobacter* sp. strains + 120 kg·ha^{-1} N, P_2O_5 and K_2O (ES+N2); *Klebsiella planticola* + 60 kg·ha^{-1} N and P_2O_5, and 40 kg K_2O ha^{-1} (KP+N1); *K. planticola* + 120 kg·ha^{-1} N, P_2O_5 and K_2O (KP+N2). Maize (hybrid ZP-341, FAO 300) was used as a test plants.

Nitrogen fertilizer was applied in the form of urea with 46% N, phosphorus - in the form of monoammonium phosphate with 52% P_2O_5 and 11% N, and potassium - as 40% potassium salt (KCl).

Table 1. Mean monthly temperature and precipitation summ for the study year.

Month	Year 2006		Mean 1990-2006	
	Temperature (°C)	Precipitation (mm)	Temperature (°C)	Precipitation (mm)
January	-0.5	43.2	1.8	41.9
February	1.9	59.1	3.7	36.8
March	6.5	104.4	8.0	42.8
April	13.7	97.0	12.8	54.6
May	17.4	42.3	18.2	51.4
June	20.2	137.8	21.6	94.8
July	24.7	23.3	23.2	66.1
August	20.9	120.6	23.1	60.1
September	19.2	24.3	17.6	63.8
October	15.2	20.9	13.1	53.8
November	8.9	24.5	7.4	55.6
December	4.3	51.9	2.3	61.5
Mean	12.8	-	12.7	-
Total	-	749.3	-	683.2

The pure culture of an associative N-fixing bacterium *K. planticola* was obtained from the stock culture of the Microbiology Laboratory of Faculty of Agronomy (Čačak, Serbia), while the *Enterobacter* strains (KG-75 and KG-76) were obtained from the stock culture of the Microbiology Laboratory in the Center for Small Grains (Kragujevac, Serbia), where they have been isolated from the rhizosphere of wheat.

Pure liquid inoculums of *K. planticola* and *Enterobacter* spp. were made using fermentors with suitable nutrient broth and incubated with aeration for 48 h at 28°C ± 1. The inoculation of the soil under young, 2-3 leaves formed plants of maize, was carried out using plastic haversack sprinkler with 300.00 cm^3/m^2 of diluted liquid bacterial inoculum, previously made by adding the tap water in the pure bacterial liquid inoculum.

For the purpose of microbiological analyses the soil samples were taken three times during vegetation period of maize (intensive plant growth stage - 7-8 leaves, milk-waxy maturity stage, full grain maturity stage), from the plough layer (0-20 cm).

The following soil chemical parameters were analyzed: soil acidity - potentiometrically, using glass electrode pH meter; available phosphorus and potassium - spectrophotometrically and flame-photometrically, using Al-method by Egner-Riehm; humus content, using Tiurin's method, modified by Simakov; soil total N, using elemental CNS analyzer, Vario model EL III (Džamić et al., 1996; Nelson and Sommers, 1996).

Microbial biomass carbon (MBC) was measured using the chloroform fumigation incubation method, based on CO evolution (Jenkinson and Powlson, 1976). Soil dehydrogenase activity (DHA) was assayed under standard conditions (24 hours of incubation at 30°C ± 1) by measuring the intensity of the red-coloured triphenyl formazan extinction, formed by reduction of 2, 3, 5 - triphenyltetrazolium chloride, spectrophotometrically (Thalmann, 1968).

The microbiological data obtained were analyzed by the method of the analysis of variance, using SPS Statistica 6.0 Software. The significance of the differences between the study factors was compared by the LSD test at $P < 0.05$ and $P < 0.01$. The grain yield of maize was calculated at 14% moisture.

Results and discussion

The main chemical characteristics of the study soil sampled before the trial was set up are presented in Table 2. The soil is characterized by acid reaction, high available potassium and medium available phosphorus, humus and total nitrogen supply.

Table 2. Main chemical characteristics of the studied Cambisol.

Parameter		Mean	Standard deviation	Range
pH	nKCl	4.06	0.05	4.00-4.10
	H_2O	4.90	0.03	4.87-4.92
P_2O_5 (mg 100 g^{-1})		15.73	0.31	15.51-16.09
K_2O (mg 100 g^{-1})		25.30	0.30	25.08-25.65
Humus (%)		2.19	0.01	2.18-2.19
Total N (%)		0.136	0.005	0.132-0.141

The values of MBC and DHA in the soil depended on the fertilization variant used, as well as the sampling period studied (Tables 3 and 4). The analysis of the experimental data showed that the highest and statistically highly significant ($P < 0.01$) level of DHA and MBC inhibition in the soil was determined in the variant with high rates of NPK nutrients (N2) during all studied vegetation periods of maize. Opposite to this, the highest and statistically highly significant ($P < 0.01$) stimulation of DHA and MBC in the soil was affected by applied combination of the microbial inoculants used and low rates of NPK fertilizers (variants ES+N1 and KP+N1).

Table 3. The effect of fertilization variant (A) and sampling period (B) on average dehydrogenase activity (µg TPF·10 g^{-1} of an air-dry soil) in Cambisol under maize

Variants (A)		Ø	N1	N2	KP+N1	KP+N2	ES+N1	ES+N2	$\bar{X}$ B
Sampling period (B)	I	83.43	82.70	39.30	100.03	49.20	108.70	51.67	73.58
	II	93.47	90.13	39.07	126.27	60.07	128.13	62.33	85.64
	III	90.70	84.17	34.67	112.87	51.50	115.33	55.20	77.78
$\bar{X}$ A		89.20	85.67	37.68	113.06	53.59	117.39	56.40	79.00
LSD			*A*				*B*		
0.05			2.33				1.22		
0.01			3.09				1.62		

Table 4. The effect of fertilization variant (A) and sampling period (B) on average microbial biomass C (mg·kg^{-1} of an absolutely dry soil) in Cambisol under maize

Variants (A)		Ø	N1	N2	KP+N1	KP+N2	ES+N1	ES+N2	$\bar{X}$ B
Sampling period (B)	I	54.90	48.28	22.83	77.56	29.63	93.94	35.03	51.74
	II	62.40	53.86	38.17	98.65	39.56	113.99	40.96	63.94
	III	56.64	50.89	19.69	85.05	37.65	103.35	39.91	56.17
$\bar{X}$ A		57.98	51.01	26.90	87.09	35.61	103.76	38.63	57.28
LSD			*A*				*B*		
0.05			10.33				5.40		
0.01			13.74				7.18		

Similar to our studies, results of the previous studies indicate that long-term application of high doses of mineral fertilizers significantly decrease microbiological activiity in soil by decreasing soil pH and increasing N content in soil (Aciego Pietri and Brookes, 2008; Wang et al., 2009). Other findings (Csitári and Hoffmann, 2005) point out that different fertilizer treatments influence soil biological parameters significantly, but there is no linear correlation between them and the quantity of fertilizer active agents. Stimulative effects of the combine usage of the associative N-fixing bacteria and low rates of NPK nutrients on soil MBC and DHA were also reported in previous studies (Raičević et al., 2006).

According to our studies, the values of MBC and DHA depended significantly on sampling period studied. The highest values of MBC and DHA in the soil were determined in the second sampling period, which was statistically highly significantly more ($P < 0.01$) comparing to the other two vegetation periods of maize. The highest values of the studied parameters of soil fertility in the second sampling period is, probably, due to a better distribution of precipitation during summer in the year studied. Similar to this, Nagaraja et al. (2002) reported that an increase in soil moisture status during the wet periods of the year resulted in higher biological activity.

Concerning the grain yield of maize (Figure 1), the highest increase was obtained by combined application of microbial inoculants used and high rates of mineral NPK fertilizers, although it should be noted that with combined usage of microbial inoculants and low rates of mineral NPK fertilizers was obtained higher yields comparing to the application of lower rates of mineral NPK fertilizers in conditions of agricultural production typical for this study. Similar results for both constatations were also obtained in previous researches (Dobbelaere et al., 2001; Dalla Santa et al., 2004).

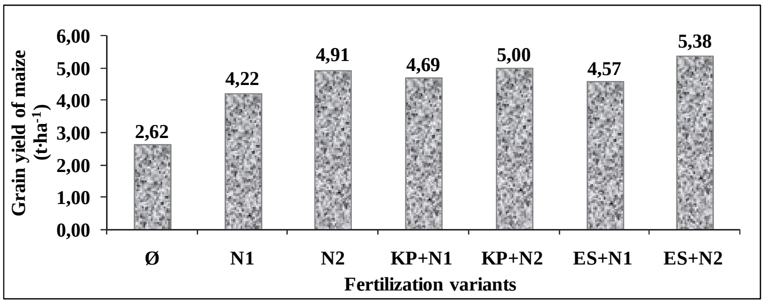

Figure 1. The effect of the fertilization variants on the grain yield of maize ($t \cdot ha^{-1}$).

Conclusions

The studies indicate that bacterial inoculants can be used to supplement the use of urea-N, reducing the amounts of NPK fertilizers used and positively affecting on dynamics and direction of the soil microbial processes. The combined usage of microbial inoculants and low rates of mineral fertilizers also resulted in higher maize yields comparing to the application of lower rates of the pure NPK nutrients in the studied agroecological conditions.

Acknowledgement

The authors are grateful to Ministry of Science of Republic of Serbia for financial support.

References

Aciego Pietri J.C. and Brookes P.C. (2008). Relationships between soil pH and microbial properties in a UK arable soil. Soil. Biol. Biochem., 40:1856-1861.

Acosta-Martinez V. and Tabatabai M.A. (2000). Enzyme activities in a limed agricultural soil. Biol. Fertil. Soils, 31: 85-91.

Csitári G. and Hoffmann S. (2005). Comparative study on soil biological parameters at a long-term field experiment. Arch. Agron. Soil Sci., 51: 563-569.

Dalla Santa O.R., Soccol C.R., Ronzelli Junior P., Fernández Hernández R., Michelena Alvarez G.L., Dalla Santa H.S. and Pandey A. (2004). Effects of inoculation of Azospirillum sp. in maize seeds under field conditions. J. Food Agr. Environ., 2:238-242.

Dobbelaere S., Croonenborghs A., Thys A., Ptacek D., Vanderleyden J., Dutto P., Labandera-Gonzalez C., Caballero-Mellado J., Aguirre J.F., Kapulnik Y., Brener S., Burdman S., Kadouri D., Sarig S. and Okon Y. (2001). Responses of agronomically important crops to inoculation with Azospirillum. Aust. J. Plant Physiol., 28:871-879.

Džamić R., Stevanović D. and Jakovljević M. (1996). Praktikum iz agrohemije. Poljoprivredni fakultet, Beograd, Srbija.

Jenkinson D.S. and Powlson D.S. (1976). The effects of biocidal treatments on metabolism in soil - V: A method for measuring soil biomass. Soil Biol. Biochem., 8:209-213.

Milošević N., Govedarica M., Jeličić Z., Protić R., Kuzevski J. and Krstanović S. (2003). Microbial inoculants as biofertilizers: testing, potential and important factor for sustainable agriculture. In: Komnenić V. (ed) Proceedings of research papers, PKB Institute Agroekonomik, Belgrade, Serbia, 9:89-98.

Nagaraja M.S., Parama V.R.R., Srinivasamurthy C.A., Siddaramappa R., Suseela Devi L. and Lalitha B.S. (2002). Soil biological processes: Seasonal changes in natural and man made ecosystems. In: IUSS (ed) Proceedings of the 17th World Congress of Soil Science, Symposium No 9, Kasetsart University, Bangkok, Thailand, 401-410.

Nelson D.W. and Sommers L.E. (1996). Total carbon, organic carbon, and organic matter. In: Sparks D.L. (ed) Methods of Soil Analysis, Part 3, SSSA, Madison, Wiskonsin, 961-1010.

Raičević V., Jakovljević M., Kiković D., Vasić G., Lalević B., Antić-Mladenović S. and Mićanović D. (2006). Importance and possibilities of biofertilization in modern agricultural production. Zemlj. biljka, 55:195-202.

Thalmann A. (1968). Zur Methodik der Bestimmung der Dehydrogenaseaktivität im Boden mittels Triphenyltetrazoliumchlorid (TTC). Landwirtsch. Forsch., 21:249-258.

Wang H., Mo J., Lu X., Xue J., Li J. and Fang Y. (2009). Effects of elevated nitrogen deposition on soil microbial biomass carbon in major subtropical forests of southern China. Front. Forest. China, 4:21-27.

sa2011_0513