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Aleksandra B. Stanojković[1], *Dragutin A. Đukić*[2], *Leka G. Mandić*[2], *Bogić M. Miličić*[1]

[1] Institute of Soil Science, Teodora Drajzera 7, 11000 Belgrade, Serbia
[2] Faculty of Agronomy, Cara Dušana 34, 32000 Čačak, Serbia

THE INFLUENCE OF MINERAL AND BACTERIAL FERTILIZATION ON THE NUMBER OF FUNGI IN SOIL UNDER MAIZE

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 the number of fungi in Cambisol and grain yield of maize. The investigation was conducted on Mladenovac experimental station and in the Laboratory of Institute of Soil Science, Belgrade, during 2006. Unfertilized soil was used as the control soil. Each of the studied variants was carried out in three replications. The effect of the studied fertilizers was determined three times during the maize growing season, the number of fungi being determined by indirect dilution method on Czapek nutritive medium. The results of the study showed that all fertilization variants studied influenced, more or less, fungal growth in the study soil. However, the applied high content of mineral nitrogen, phosphorus and potassium, as well as their combination with bacterial inoculants brought about the highest increase in the number of fungi during all studied vegetation periods of maize. The highest increase in the number of soil fungi was registered in the second sampling period. The highest increase in the grain yield of maize was obtained by combined application of microbial inoculants and high rates of mineral NPK fertilizers.
KEY WORDS: fungi, maize, N-fixing bacteria, NPK fertilizers, soil

INTRODUCTION

The studies in the field of fertilization are mostly focused on the increase of the crops yield 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 their major role in crop productivity and soil fertility, the application of mineral fertilizers (particularly nitrogen) may induce a series of negative consequences from microbiological, economic and ecological aspects (Acosta – Martinez and Tabatabai, 2000). 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 (M i l o š e v i ć et al., 2003).

The incorporation of higher rates of mineral fertilizers into soil, acid ones in particular, and their long-term usage is depressing for the majority of microorganisms, except for fungi (J e m c e v and Đ u k i ć , 2000; P e š a k o v i ć , 2007). With regard to the predominance of fungi in acid soils, it has also been suggested that their population number rises with more intensive application of the stated fertilizers. A large number of authors addressing this issue account for this rise in population density and activity of the majority of microorganisms in soil by limiting the C: N relation and the intensification of the mineralizing processes therein, as well as by the re-distribution within the complex of microbial cenoses in favor of soil fungi (A c o s t a – M a r t i n e z and T a b a t a b a i , 2000; Z h a n g and W a n g , 2006; P e š a k o v i ć et al., 2008).

Fungi perform important functions in the soil related to nutrient cycling, disease suppression and water dynamics, all of which help plants become healthier and more vigorous. Along with bacteria, fungi are important decomposers of hard to digest organic matter. They use nitrogen in the soil to decompose residues rich in woody carbon and low in nitrogen, and to convert the nutrients in the residues into forms that are more accessible for other organisms (J e n k i n s , 2005).

Given the fact that soil fungi have evolved a complex enzymatic system that helps them transform chemical compounds that are not easily degradable (M a n d i ć, 2002), the underlying assumption of this study was that a change in their number may be used as a reliable indicator of soil biogeny.

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 the number of fungi in Cambisol and grain yield of maize.

MATERIALS 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 sum 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 replicates, based on the following variants: control (Ø, non-fertilized soil); 60 $kg \cdot ha^{-1}$ N and P_2O_5, and 40 $kg \cdot ha^{-1}$ K_2O (N1); 120 $kg \cdot ha^{-1}$ N, P_2O_5 and K_2O (N2); *Enterobacter* sp. strains + 60 $kg \cdot ha^{-1}$ N and P_2O_5, and 40 $kg \cdot ha^{-1}$ K_2O (ES+N1); *Enterobacter* sp. strains + 120 $kg \cdot ha^{-1}$ N, P_2O_5 and K_2O (ES+N2); *Klebsiella planticola* + 60 $kg \cdot ha^{-1}$ N and P_2O_5, and

Tab. 1 – Mean monthly temperature and precipitation sum for the study year (Belgrade Weather Bureau)

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

40 kg·ha^{-1} K_2O (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 plant.

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).

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 ± 2. The inoculation of the soil under young formed plants of maize with 2-3 leaves 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 into pure bacterial liquid inoculum.

The preliminary observation of the soil studied included the analyses of the following soil chemical parameters: soil acidity – potentiometrically, using glass electrode pH meter; available phosphorus and potassium – spectrophotometrically and flame-photometrically, respectively, using Al-method by Egner-Riehm; humus content, after Tiurin's method, modified by S i m a k o v (Dž a m i ć et al., 1996); soil total N, using elemental CNS analyzer, Vario model EL III (N e l s o n and S o m m e r s, 1996).

The samples subjected to microbiological analyses were taken three times during the vegetation period of maize (intensive plant growth stage – 7-8 leaves, milk-waxy maturity stage, full grain maturity stage), from the plough soil layer (0-15 cm), using method of the scattered sampling according to V o j i - n o v i ć et al. (1966).

The number of soil fungi was determined on Czapek nutritive medium, using indirect dilution method, by inoculation of the nutritive medium with decimal dilution of certain amount of soil suspension (S a r i ć, 1989). The duration of incubation was 5 days at 28°C ± 2. The analyses were performed in three replications, whereby the number of fungi was calculated on 1.0 g of absolutely dry soil.

The obtained microbiological data were analyzed by the method of variance analysis, using SPS Statistica 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

1. Chemical properties of the study soil

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

Tab. 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

2. Studying the effect of applied fertilizers on average number of soil fungi

The obtained experimental data on the average number of soil fungi inferred that the presence of this group of microorganisms in Cambisol depended on the fertilization variant used, as well as the studied sampling period (Table 3). It was determined that all fertilization variants studied stimulated the growth of fungi in the study soil. The highest and statistically highly significant ($P < 0.01$) stimulation of the growth of soil fungi was determined in the variant with high rates of NPK nutrients (N2) during all studied vegetation periods of maize, as well as in the variants where combination of the microbial inoculants and high rates of NPK fertilizers (variants ES+N2 and KP+N2) were applied. This trend was notably observed in the second sampling period of the maize growing season, which was characterized by higher temperatures and moisture rates (Table 1). This was also determined by other authors (J e m c e v and Đ u k i ć , 2000). The fertilization variant x vegetation period interaction (A x B) during the study year showed that statistically highly significant stimulating effects of the variants with high rates of NPK nutrients on the growth of soil fungi did not significantly varied between the studied vegetation periods ($P > 0.05$), although they were more pronounced in the second sampling period.

Tab. 3 – The effect of fertilization variant (A) and sampling period (B) on average number of fungi (10^4 g^{-1} of an absolutely dry soil) in Cambisol under maize

Variant (A)		Ø	N1	N2	KP+N1	KP+N2	ES+N1	ES+N2	X̄B
Sampling period (B)	I	13.97	30.45	52.55	24.18	38.67	24.27	37.91	31.71
	II	15.73	32.48	54.88	27.24	38.94	26.70	38.48	33.49
	III	13.64	30.00	50.03	20.24	33.61	19.12	34.88	28.79
X̄A		14.45	30.98	52.49	23.89	37.07	23.36	37.09	31.33

LSD	A	B	A x B
0.05	5.94	3.10	10.29
0.01	7.90	4.13	13.69

Generally speaking, the rise in fertilizer rate was accompanied by the rise in the number of soil fungi, which, to a certain degree, may be considered positive. However, over-activation of fungi may be damaging, as the processes directed towards establishing of the disturbed balance lead to the weakening of physical, chemical and biological properties of soil (M a n d i ć et al., 2004) and the incidence of toxic fungi (M i l o š e v i ć et al., 1993), whereby *Penicillium* species assume predominance (M a n d i ć , 2002; P e š a k o v i ć , 2007; P e š a k o v i ć et al., 2009).

3. Studying the effect of applied fertilizers on the yield of maize

The analysis of the grain yield of maize (Figure 1) showed that the highest increase was obtained by using combined application of bacterial inoculants and high rates of mineral NPK fertilizers, although it should be noted that with combined usage of bacterial inoculants and low rates of mineral NPK fertilizers higher yields were obtained, in comparison to the application of lower rates of mineral NPK fertilizers in the conditions of agricultural production typical for this study. Similar results for both constatations were also obtained in previous researches (D o b b e l a e r e et al., 2001; D a l l a S a n t a et al., 2004).

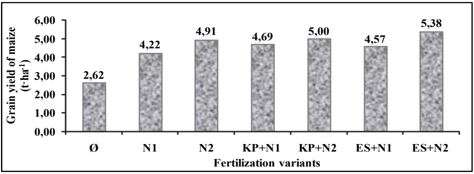

Fig. 1 – The effect of the fertilization variants on the grain yield of maize ($t \cdot ha^{-1}$)

CONCLUSIONS

The results of the study on the effects of an application of different rates of mineral fertilizers and their combination with associative N-fixing *Klebsiella planticola* and *Enterobacter* spp. on the number of fungi in Cambisol and grain yield of maize infer the following:

- the number of the studied group of microorganisms depended on the type and rate of fertilizers used, as well as the sampling period studied;
- the applied fertilizers brought about an increase in the number of fungi, particularly in the variants that included high content of nitrogen, phosphorus and potassium;
- the highest number of soil fungi was registered in the second sampling period;
- the highest increase in the grain yield of maize was obtained by combined application of bacterial inoculants and high rates of mineral NPK fertilizers.

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УТИЦАЈ МИНЕРАЛНИХ И БАКТЕРИЈСКИХ ЂУБРИВА НА БРОЈНОСТ ГЉИВА У ЗЕМЉИШТУ ПОД УСЕВОМ КУКУРУЗА

Александра Б. Станојковић[1], Драгутин А. Ђукић[2], Лека Г. Мандић[2], Богић М. Миличић[1]

[1] Институт за земљиште, Теодора Драјзера 7, 11000 Београд, Србија
[2] Агрономски факултет, Цара Душана 34, 32000 Чачак, Србија

Резиме

Циљ истраживања је био да се испита утицај примене различитих доза минералних ђубрива и њихових комбинација са асоцијативним азотофиксирајућим бактеријама *Klebsiella planticola* и *Enterobacter* spp., као и фазе узимања узорака на бројност гљива у земљишту типа гајњача и принос зрна кукуруза. Истраживања су извођена на експерименталном Огледном пољу Института за земљиште у Младеновацу, и у Лабораторији Института за земљиште у Београду, током 2006. године. Као контрола коришћено је неђубрено земљиште. Свака од анализираних варијаната била је заступљена у три понављања. Ефекат примењених ђубрива одређиван је три пута током вегетације кукуруза, а праћен је путем утврђивања бројности гљива индиректном методом агарних плоча на Чапековој хранљивој подлози. Резултати истраживања су показали да су све испитиване варијанте ђубрења у мањој или већој мери стимулисале развој гљива у проучаваном типу земљишта, али је примењена висока доза минералног азота, фосфора и калијума, као и њена комбинација са тестираним бактеријским инокулантима, условила највеће повећање бројности гљива током целог вегетационог периода кукуруза. Највећа бројност земљишних гљива је утврђена у другој фази узимања узорака. Највеће повећање приноса зрна кукуруза забележено је на варијантама на којима је примењена комбинација бактеријских инокуланата и високих доза NPK ђубрива.