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MINERAL AND BACTERIAL FERTILISATION EFFECT ON THE NUMBER OF FUNGI IN SOIL UNDER WINTER WHEAT AND THE YIELD OF WHEAT

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Abstract. In 2007, the influence of the sampling period and different rates of applied mineral fertilisers and their combinations with bacterial inoculants (nitrogen (N)-fixing *Klebsiella planticola* and *Enterobacter* sp.) on the number of fungi in Eutric cambisol and the yield of winter wheat was evaluated in this study. The following variants were studied: control (non-fertilised soil); 60 kg ha^{-1} N and P_2O_5, and 40 kg ha^{-1} K_2O (N1, low rates of mineral fertilisers); 120 kg ha^{-1} N, P_2O_5 and K_2O (N2, high rates of mineral fertilisers); *Enterobacter* sp. strains + N1; *Enterobacter* sp. strains + N2; *K. planticola* + N1; *K. planticola* + N2. The effect of the studied fertilisers was determined two times over the wheat growing season, the number of fungi being determined by indirect dilution method on Chapek nutritive medium. The results of the study showed that the applied high content of mineral fertilisers, as well as their combination with bacterial inoculants used, brought about the highest increase in the number of fungi during studied vegetation periods of wheat, as well as the highest increase in the grain yield of wheat. The highest number of soil fungi was registered in the second sampling period.

Keywords: fungi, Eutric cambisol, wheat, N-fixing bacteria, mineral fertilisers.

AIMS AND BACKGROUND

Considering that soil fungi have evolved a complex enzymatic system that helps them transform chemical compounds that are not easily degradable[1], the underlying assumption of this study was that a change in their number may be used as a reliable indicator of soil biogeny. Thus, the aim of this investigation was to examine the influence of different rates of mineral fertilisers (composite NPK (15:15:15)) and their combination with selected soil bacterial inoculants, and sampling period on the number of fungi in Eutric cambisol and grain yield of wheat.

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The studies in the field of fertilisation are mostly focused on the increase of the crops yield whereas the traits of the cumulative effect of fertilisers (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 fertilisers (particularly nitrogen) may induce a series of negative consequences, from microbiological, economic and ecological aspects[2,3].

Nitrogen input for agricultural systems is important for their sustainability. However, when N inputs are very high, the excess can contribute to greater agricultural N losses that impact soil and groundwater quality[4]. The problems concerned can be overcome by partial replacement of mineral fertilisers by application of microbial inoculants, in order to inhibit or stimulate certain cellular processes, including mineralisation ones, thus leading to the improvement of physicochemical and biological soil properties[5]. Particularly important in this respect are diazotrophs, being nitrogen-fixing organisms and producers of growth stimulators, vitamins and antibiotics[6].

The incorporation of higher rates of mineral fertilisers into soil, acid ones in particular, and their long-term usage is depressing for the majority of microorganisms accept for fungi[7,8]. With regard to the predominance of fungi in acid soils, it is also suggested that their population number rises with more intensive application of the stated fertilisers. 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 of the C:N relation and the intensification of the mineralising processes therein, as well as by the re-distribution within the complex of microbial cenoses in favour of soil fungi[9,10].

Fungi perform important functions within the soil in relation 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 woody carbon-rich residues low in nitrogen and convert the nutrients in the residues to forms that are more accessible for other organisms[11]. Regarding their role in the soil system, it could be said that they present a reliable indicator of the soil biological productivity.

EXPERIMENTAL

The investigation was conducted on the Mladenovac experimental station of Institute of Soil Science, located 55 km of Belgrade in Serbia, during the year 2007. Mean monthly temperature and precipitation sum for the investigated period are presented in Table 1. The studied soil type was Eutric cambisol. The experiment was set up in a randomised block design with 3 replicates, based on the following variants: control (Ø, non-fertilised soil); 60 kg ha^{-1} N and P_2O_5, and 40 kg K_2O ha^{-1} (N1, low rates of mineral fertilisers); 120 kg ha^{-1} N, P_2O_5 and K_2O (N2, high rates of mineral fertilisers); *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). Winter wheat (cv. Evropa 90) was used as a test plant.

Table 1. Mean monthly air temperature and precipitation summ for the study year (the Belgrade Weather Bureau)

Month	Year 2007		Mean 1990–2007	
	temperature (°C)	precipitation (mm)	temperature (°C)	precipitation (mm)
January	7.6	49.3	1.8	41.9
February	7.2	56.0	3.7	36.8
March	10.2	99.6	8.0	42.8
April	**14.9**	**3.8**	12.8	54.6
May	19.5	79.0	18.2	51.4
June	**23.8**	**107.6**	21.6	94.8
July	25.8	17.5	23.2	66.1
August	24.2	72.5	23.1	60.1
September	16.2	84.1	17.6	63.8
October	11.8	103.6	13.1	53.8
November	5.2	131.5	7.4	55.6
December	1.1	34.5	2.3	61.5
Mean	14.0	–	12.7	–
Total	–	839.0	–	683.2

Nitrogen fertiliser 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 (Cacak, 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 were isolated from the rhizosphere of wheat.

Pure liquid inoculums of *K. planticola* and *Enterobacter* sp. 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, 2–3 leaves formed plants of wheat, 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.

The preliminary observation of the soil studied included the analysis 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, using the Tiurin method, modified by Simakov[12]; soil total N, using elemental CNS analyser, Vario model EL III (Ref. 13).

For the purpose of microbiological analyses the soil samples were taken in the beginning and at the end of the plant growth, from the plough soil layer (0–20 cm), using the method of scattered sample[14].

The number of soil fungi was determined on Chapek nutritive medium, using indirect dilution method, by an inoculation of the nutritive medium with decimal dilution of the certain amount of soil suspension[15]. 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 of an absolutely dry soil.

The microbiological data obtained were analysed by the method of the analysis of variance, 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 wheat was calculated at 14% moisture.

RESULTS AND DISCUSSION

Chemical properties of the study soil. The main chemical properties of the study soil are presented in Table 2.

Table 2. Main chemical characteristics of the studied Eutric cambisol

Statistical parameters	pH		P_2O_5	K_2O	Humus	Total N
	H_2O	1M KCl	(mg 100 g^{-1})		(%)	
Mean	4.90	4.06	15.7	25.3	2.19	0.136
Standard deviation	0.03	0.05	0.3	0.3	0.01	0.005
Range	4.87–4.92	4.00–4.10	15.5–16.1	25.1–25.7	2.18–2.19	0.132–0.141

The soil is characterised by acid reaction, high available potassium and medium available phosphorus, humus and total nitrogen supply.

Effect of applied fertilisers on average number of soil fungi. The obtained experimental results on the average number of soil fungi inferred that the presence of this group of microorganism in Eutric cambisol depended on the fertilisation variant used, as well as the sampling period studied (Table 3). It was determined that all fertilisation variants studied influenced more or less stimulating on the fungi growth 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 studied vegetation periods of wheat, as well as in the variants where combination of the microbial inoculants used and high rates of NPK fertilisers (variants ES+N2 and KP+N2) was applied. This trend was notably observed in the second sampling period of the wheat growing season, the growing of which was favoured by higher temperatures and moisture rates alike (Table 1), which is also determined by other authors[7]. The fertilisation variant × vegetation period interaction (A × B) during the study year showed that statistically highly significant stimulative effects of the variants with high rates of NPK nutrients on the growth of soil fungi did not statistically significantly varied between the studied two vegetation periods ($P > 0.05$), although they were more pronounced in the second sampling period.

Table 3. Effect of fertilisation variant (A) and sampling period (B) on average number of fungi (10^4 g^{-1} of an absolutely dry soil) in Eutric cambisol under winter wheat

Variants (A)		Ø	N1	N2	KP+N1	KP+N2	ES+N1	ES+N2	$\overline{X}B$
Sampling	I	16.36	36.09	57.52	29.88	46.58	29.09	47.03	37.51
period (B)	II	23.18	40.09	65.03	34.27	51.12	33.97	49.12	42.40
$\overline{X}A$		19.77	38.09	61.27	32.08	48.85	31.53	48.08	39.95

LSD	A	B
0.05	12.77	4.71
0.01	16.84	6.81

LSD – least significant differences.

Generally speaking, the rise in fertiliser rate was accompanied by the rise in the number of soil fungi, which, by 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[16], and the incidence of toxic fungi[17], whereby *Penicillium* species assume predominance[1,8,18].

Effect of applied fertilisers on the yield of winter wheat. The analysis of the yield of wheat grain showed the noticeable yield differences between the applied fertilisation treatments (Fig. 1).

The highest increase in yield was obtained by combined application of bacterial inoculants used and high rates of mineral NPK fertilisers, although it should be noted that with combined usage of bacterial inoculants and low rates of mineral NPK fertilisers were obtained higher yields comparing to the application of low rates of the pure mineral nutrients (60 kg ha^{-1} N and P_2O_5, and 40 kg K_2O ha^{-1}) in conditions of agricultural production typical for this study. Similar results were obtained in the study of other authors[19], in which a significant interaction effect of nitrogen fertilisers and microbial inoculation on wheat yield compared to the unfertilised variants was also found.

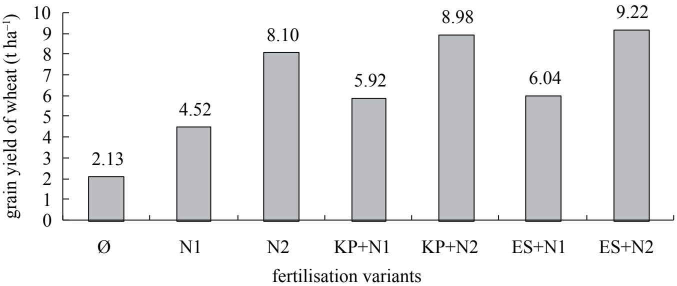

Fig. 1. Effect of fertilisation variants on the grain yield of winter wheat (t ha^{-1})

The character of the effects of the applied fertilisers on the yield of crop also depended on the weather conditions specific to each year of study. Specifically, good distribution of rainfall and temperature during 2006/2007 contributed largely to the achieved high yields of wheat in the investigated agro-ecological conditions, as indicated by other authors[20].

CONCLUSIONS

The results of the study of the effects of an application of different rates of mineral fertilisers and their combination with associative N-fixing *Klebsiella planticola* and *Enterobacter* sp. on the number of fungi in Eutric cambisol and grain yield of winter wheat infer the following:

– the number of the studied group of microorganisms depended on the type and rate of fertilisers used, as well as the sampling period studied;

– the applied fertilisers 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 wheat was obtained by combined application of bacterial inoculants used and high rates of mineral NPK fertilisers.

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