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Evaluation of the chemical composition and yield of crops as influenced by bacterial and mineral fertilization

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Abstract

The aim of this study was to evaluate the influence of an application of different rates of composite mineral fertilizers and their combination with bacterial inoculants (N-fixing Klebsiella planticola and Enterobacter spp.) on chemical composition and yield of the grain of maize and wheat cultivated in maize - winter wheat - maize rotation system on eutric cambisol type of soil during the three growing seasons: 2006, 2007 and 2008. Unfertilized soil was used as a control. The contents of nitrogen, phosphorus, potassium and crude proteins in grain samples were determined. Measuring of the yield of crops was carried out at the end of the vegetation. The results of the study showed that an application of high rates of composite mineral fertilizers and their combination with bacterial inoculants resulted in increased contents of nitrogen, phosphorus, potassium and proteins in the grains of both crops studied, which was noticeably observed in the milk-waxy maturity stage of maize in 2006 and 2008. The highest increase in the yield of the crops studied was obtained by the same mentioned treatments, although the combination of bacterial inoculants and lower rates of mineral fertilizers resulted in higher yields comparing to the application of lower rates of the pure mineral nutrients. The yield of maize was noticeably lower in the third year of the study than in the first.

Key words: Composite mineral fertilizers, bacterial inoculants, maize, wheat, chemical composition, yield, eutric cambisol.

Introduction

Wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) are two major crops in agricultural production in Serbia. The crop rotation of wheat and maize is a particularly dominant cropping system. Generally speaking, in this country the cultivation of wheat occupies an area of 600,000 to 900,000 ha with an average yield of 3.92 to 6.42 $t{\cdot}ha^{-1}$ [1], while the maize cultivation occupies an area of about 1.300.000 ha with an average yield of 5.00 to 6.00 $t{\cdot}ha^{-1}$ [2].

Increasing the yield and improving the quality of crops have been the challenges for sustainable agriculture [3, 4]. The yield of maize and wheat, in addition to varietal characteristics, largely depends on the tillage, chemical, physical and microbiological

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properties of the soil [5, 6]. Fertilization, among other factors, was one of the reasons that pushed crop production [7], 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, increased use of mineral fertilizers (particularly nitrogen) in agricultural production has however raised concerns, because the nitrogen surplus is at risk of leaving the plant-soil system and thereby causing environmental contamination. This is in addition to increased costs associated with the manufacture and distribution of nitrogen (N) fertilizer [8, 9]. Somewhat safer are phosphorous (P) fertilizers which, applied at higher rates, lead to undesirable accumulation of a series of the other elements in soil, such as stable strontium, natural radioactive compounds of uranium, radium and thorium. The damaging effect of potassium (K) fertilizers is reflected in the fact that these introduce a large quantity of chlorine which can cause a series of undesirable effects (e.g. chlorine effect) both in water and soil. The intensity of the stated processes is governed by the type and rate of applied fertilizers [10]. Thus, sustainable agriculture in Serbia should not be only a steady and substantial increase in crop yields, but also the management and conservation of soil and water. 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 [11].

Many studies have been conducted to understand and evaluate the effects of environmental factors and agronomic practices on the chemical constituents and nutritive value of the grain and its anatomical parts. According to them [12], when analyzing the impact of fertilization in a system (e.g. a crop sequence), the amount of nutrients returned with plant residues has an important role (e.g. N fertilization strongly influences the quality and quantity of protein in wheat).

The plant production systems, type and rate of applied fertilizers and climate characteristics affect greatly on intensity of the N, P and K uptake by agricultural crops and their yield. Thus, 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 on chemical composition and yield of the grain of maize and wheat cultivated in crop rotation system on eutric cambisol type of soil.

Material and methods

Study area

The investigation was conducted on Mladenovac experimental station of Institute of Soil Science, located 55 km south-east from Belgrade in Serbia, in the period 2006-2008. Mean monthly air temperatures and precipitation sums for the investigated period are presented in Table 1. Years 2007 and 2008 were warmer than 2006, due to a 2-3 ^{o}C higher temperature in May, June and August. The lowest precipitation sum was registered in 2008, and the highest - in 2007.

Table 1. Mean monthly air temperatures (°C) and precipitation sum (mm) for the period 2006 – 2008.

Month	Year						Mean 1990-2008	
	2006		2007		2008			
	Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)
January	-0.5	43.2	7.6	49.3	3.2	44.6	1.8	41.9
February	1.9	59.1	7.2	56.0	6.3	8.3	3.7	36.8
March	6.5	104.4	10.2	99.6	9.1	79.7	8.0	42.8
April	13.7	97.0	***14.9***	***3.8***	13.8	34.9	12.8	54.6
May	17.4	42.3	19.5	79.0	19.3	60.6	18.2	51.4
June	***20.2***	***137.8***	***23.8***	***107.6***	***23.0***	***43.3***	21.6	94.8
July	24.7	23.3	25.8	17.5	23.7	53.0	23.2	66.1
August	***20.9***	***120.6***	24.2	72.5	***24.0***	***45.6***	23.1	60.1
September	19.2	24.3	16.2	84.1	17.0	68.5	17.6	63.8
October	***15.2***	***20.9***	11.8	103.6	***14.8***	***18.4***	13.1	53.8
November	8.9	24.5	5.2	131.5	9.1	51.0	7.4	55.6
December	4.3	51.9	1.1	34.5	4.6	79.0	2.3	61.5
Mean	12.8	-	14.0	-	14.0	-	12.7	-
Total	-	749.3	-	839.0	-	586.9	-	683.2

Field trial

The studied soil type was eutric cambisol. The experiment was set up in a randomized block design on 10 × 6 m^2 plot size, 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 K_2O ha^{-1} (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 K_2O ha^{-1} (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 40 kg K_2O ha^{-1} (KP+N1); *Klebsiella planticola* + 120 $kg{\cdot}ha^{-1}$ N, P_2O_5 and K_2O (KP+N2). Maize (hybrid ZP-341, FAO 300) in 2006 and 2008, and winter wheat (cv. Evropa 90) in 2007, were included in the trial.

Applied NPK fertilizers and soil bacterial inoculation

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

The pure culture of an associative N-fixing bacterium *Klebsiella planticola* (strain TSHA-91), cultivated on the slanting nutrient medium for 24 h at 28°C ± 1, was obtained from the stock culture of the Microbiology Laboratory of Faculty of Agronomy (Čačak, Serbia). The medium had the following chemical composition: peptone 1 - 1.20 g; K_2HPO_4 - 0.50 g; KH_2PO_4 - 0.30 g; $MgSO_4$ - 0.10 g; $CaCl_2$ - 0.03 g; sucrose - 6.00 g; $(NH_4)_2SO_4$ - 0.14 g; yeast extract - 0.10 g; agar - 16.00 g; distilled deionized water – 1.00 dm^3; pH 7.3. Associative N-fixing *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. The pure culture of these strains were cultivated on the slanting nutrient medium (MPA, Torlak, Belgrade) for 48 h at 28°C ±

1. The medium had the following chemical composition: peptone 1 – 15.00 g; meat extract - 3.00 g; NaCl - 5.00 g; K_2HPO_4 - 0.30 g; agar - 18.00 g; distilled deionized water – 1.00 dm^3; pH 7.3.

18.00 dm^3 of the pure liquid inoculum of *K. planticola* (100-300 x 10^7 cells per 1.0 cm^3 of inoculum), as well as 18.00 dm^3 of the pure liquid inoculum of *Enterobacter* strains (100-180 x 10^7 cells per 1.0 cm^3 of inoculum) 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 and wheat, was carried out using plastic haversack sprinkler with 300.00 cm^3/m^2 of diluted liquid bacterial inoculum, previously made by adding 32.00 dm^3 of the tap water in 18.00 dm^3 of the pure bacterial liquid inoculum.

Soil analysis

The preliminary observation of the soil studied included the analysis of the following soil chemical parameters: soil acidity - potentiometrically, using glass electrode; available phosphorus and potassium - spectrophotometrically and flame-photometrically, respectively, using Al-method by Egner-Riehm [13], where 0.1M lactate (pH = 3.7) was used as an extract; humus content, using Tiurin's method, modified by Simakov [14]; soil total nitrogen, using elemental CNS analyzer, Vario model EL III [15].

Plant analysis

In the milk-waxy maturity stage (vegetation stage I) and full grain maturity stage (vegetation stage II) of maize, as well as in the full grain maturity stage (vegetation stage II) of winter wheat the grains were taken and weighed before and after drying at 105°C. For all the plant samples from all the variants studied the chemical analyses of the grains were done. The contents of phosphorus (P) and potassium (K) in grain samples were determined by so called “wet” combustion, i.e. they were heated to boiling with the mixture of concentrated acids: H_2SO_4 and $HClO_4$. In the obtained solution, phosphorus was determined by spectrophotometer with molybdate, and potassium – by flame emission photometry [13]. The content of nitrogen (N) was analyzed on elemental CNS analyzer, Vario model EL III [15], while the content of the crude proteins in dry matter was calculated on the basis of N content, using the following formula: crude proteins (%) = N (%) x 6.25 (factor for conversion of nitrogen content to crude protein) [16].

The harvesting and measuring of the yield of maize and wheat were carried out at the end of the vegetation, in the full grain maturity stage of the crops. Maize was harvested in the first decade of October in 2006 and 2008, while the harvesting of wheat was done in the third decade of June in 2007. The data on the grain yield were adjusted to 14% moisture content.

Data analysis

Statistical analysis of the results obtained from the chemical analyses of the grains was performed using SYSTAT - 16 software [17]. The statistical significance (P-value) in effects of different fertilization variants on all the variables tested was determined using Analysis of Variance (ANOVA) method. The effects of applied fertilizers on the chemical composition and yield of maize and wheat were evaluated using Duncan’s Multiple Range Test (DMRT) at P = 0.05 and P = 0.01.

Results and discussions

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.

Table 2. Main chemical characteristics of the studied eutric 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

Studying the effect of applied fertilizers on the chemical composition of grain

By analyzing the dynamics of accumulation of nitrogen, phosphorus, potassium and proteins in maize and wheat grains during the three-year study (Tables 3, 4 and 5) it was determined that the grain chemical composition depended on the fertilization variant used, as well as the vegetation period of maize studied.

Table 3. The effect of the fertilization variants on the average chemical composition of the maize grain (in %) during 2006.

Fertilization variant	Total N (%)		P_2O_5 (%)		K_2O (%)		Proteins (%)	
	Vegetation stage							
	I	II	I	II	I	II	I	II
Ø	1.24 g	1.00 g	1.10 g	0.86 f	0.31 g	0.22 f	7.75 g	6.25 g
N1	2.45 f	1.23 f	1.22 f	0.99 e	0.59 f	0.50 e	15.31 f	7.69 f
N2	2.61 c	1.51 c	1.41 c	1.19 c	0.82 c	0.73 c	16.31 c	9.44 c
KP+N1	2.52 d	1.33 d	1.28 d	0.06 d	0.61 e	0.51 e	15.75 d	8.32 d
KP+N2	2.83 a	1.61 b	1.65 a	1.35 b	0.90 b	0.81 b	17.69 a	10.06 b
ES+N1	2.49 e	1.29 e	1.22 e	1.00 e	0.62 d	0.53 d	15.56 e	8.06 e
ES+N2	2.63 b	1.65 a	1.63 b	1.41 a	0.91 a	0.86 a	16.44 b	10.31 a
P value	***	***	***	***	***	***	***	***
LSD (0.05)	0.074	0.003	0.005	0.003	0.003	0.006	0.012	0.006
LSD (0.01)	0.010	0.005	0.006	0.005	0.007	0.009	0.017	0.008

DMRT was used to compare different variants at $P = 0.05$ and $P = 0.01$; *** indicates statistical significant differences at the $P<0.05$, $P<0.01$ and $P<0.001$ levels, respectively; LSD indicates least significant differences.

Table 4. The effect of the fertilization variants on the average chemical composition of the wheat grain (in %) in 2007.

Fertilization variant	Total N (%)	P_2O_5 (%)	K_2O (%)	Proteins (%)
	Vegetation stage II			
Ø	1.31 g	1.41 f	0.24 f	8.19 g
N1	1.49 f	1.54 e	0.41 e	9.31 f
N2	1.72 c	1.66 c	0.71 b	10.75 c
KP+N1	1.59 d	1.57 d	0.52 d	9.94 d
KP+N2	1.88 b	1.68 b	0.87 a	11.75 b
ES+N1	1.58 e	1.58 d	0.55 c	9.88 e
ES+N2	1.89 a	1.76 a	0.87 a	11.81 a
P value	***	***	***	***
LSD (0.05)	0.005	0.023	0.012	0.012
LSD (0.01)	0.007	0.032	0.017	0.016

DMRT was used to compare different variants at P = 0.05 and P = 0.01; *** indicates statistical significant differences at the P<0.05, P<0.01 and P<0.001 levels, respectively; LSD indicates least significant differences.

Application of high rates of mineral NPK fertilizers and their combination with bacterial inoculants has caused a significant increase in the share of nitrogen, phosphorus, potassium and protein in the grains of both crops studied compared to the other tested variants. This trend was notably observed in the milk-waxy maturity stage of maize, the vegetation period in which the growth of crops and accumulation of nutrients are the most intensive [18].

Hence, the excess of microbiologically fixed nitrogen, with higher amounts of mineral nitrogen, influenced positively on the accumulation of the stated elements and compounds in the study plant material, which is in accordance with previous researches [19, 20]. According to these studies, microbial inoculation of seeds, combined with different rates of mineral NPK fertilizers, significantly increases both the content of nitrogen and phosphorus in plants.

Table 5. The effect of the fertilization variants on the average chemical composition of the maize grain (in %) during 2008.

Fertilization variant	Total N (%)		P_2O_5 (%)		K_2O (%)		Proteins (%)	
	Vegetation stage							
	I	II	I	II	I	II	I	II
Ø	1.85 g	1.26 e	1.13 f	0.89 f	0.34 e	0.24 e	11.56 e	7.88 g
N1	2.52 f	1.55 c	1.25 e	1.02 e	0.58 d	0.49 d	15.75 d	9.69 d
N2	2.88 c	1.67 b	1.44 c	1.21 c	0.83 b	0.72 b	18.00 a	10.44 c
KP+N1	2.78 d	1.51 d	1.30 d	1.09 d	0.64 c	0.54 c	17.38 b	9.44 f
KP+N2	2.99 a	1.71 a	1.58 a	1.25 a	1.02 a	0.92 a	18.69 a	10.69 a
ES+N1	2.71 e	1.51 d	1.24 e	1.02 e	0.64 c	0.53 c	16.94 c	9.44 e
ES+N2	2.90 b	1.68 b	1.55 b	1.23 b	1.02 a	0.92 a	18.13 a	10.50 b
P value	***	***	***	***	***	***	***	***
LSD (0.05)	0.005	0.017	0.005	0.005	0.009	0.019	0.381	0.020
LSD (0.01)	0.007	0.024	0.007	0.007	0.012	0.027	0.529	0.029

DMRT was used to compare different variants at P = 0.05 and P = 0.01; *** indicates statistical significant differences at the P<0.05, P<0.01 and P<0.001 levels, respectively; LSD indicates least significant differences.

Studying the effect of applied fertilizers on the yield of maize and wheat

The analysis of the yield of maize and wheat grain (based on Duncan's test) showed the significant yield differences between the applied fertilization treatments (Table 6).

The highest increase in yield was obtained by combined application of bacterial inoculants used and high rates of mineral NPK fertilizers for both crops studied. In addition, it should be noted that with combined usage of bacterial inoculants and low rates of mineral NPK fertilizers were obtained higher yields comparing to the application of lower rates of the pure mineral NPK nutrients. Similar results were obtained in the previous study [20], in which it was determined significantly higher maize yield in treatments that were treated with microbial fertilizer and high rates of mineral nitrogen (150 $kg \cdot ha^{-1}$). Other authors [21] also found a significant interaction effect of nitrogen fertilizers and microbial inoculation on wheat yield compared to the unfertilized variants.

The character of the effects of the applied fertilizers on the yield of crops depended on the weather conditions specific to each year of study. Specifically, the yield of maize, for most of the variants, was noticeably lower in the third year of the study than in the first (Table 6), which is likely due to unfavorable weather conditions during the maize growing period in 2008. This is consistent with some previous results [22], which point out at high correlation relationship between temperature and precipitation and yield of maize. In addition, in 2008 was also observed noticeably less interactive effects of microbiological and lower rates of mineral fertilizers in relation to their effects in 2006.

Table 6. The effect of the fertilization variants on the average yield of maize and wheat grain

(in $t \cdot ha^{-1}$) during the three-year study.

Fertilization variant	Grain yield ($t \cdot ha^{-1}$)					
	Maize (year 2006)		Wheat (year 2007)		Maize (year 2008)	
Ø	2.627	g	2.133	g	2.174	f
N1	4.221	f	4.816	f	2.977	e
N2	4.905	c	8.100	c	3.903	b
KP+N1	4.688	d	5.921	e	3.557	c
KP+N2	4.993	b	8.976	b	4.355	a
ES+N1	4.568	e	6.043	d	3.186	d
ES+N2	5.374	a	9.216	a	4.355	a
P value	***		***		***	
LSD (0.05)	0.006		0.001		0.046	
LSD (0.01)	0.008		0.002		0.064	

DMRT was used to compare different variants at P = 0.05 and P = 0.01; *** indicates statistical significant differences at the P<0.05, P<0.01 and P<0.001 levels, respectively; LSD indicates least significant differences.

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 [23].

Conclusions

The present study demonstrated the significant positive effects of combined application of bacterial inoculants used and high and low rates of the composite mineral fertilizers on the yield of maize and wheat. The same results were obtained regarding the effects of the mentioned applied combinations on the contents of nitrogen, phosphorus, potassium and proteins in the grains of both crops studied, which was noticeably observed in the milk-waxy maturity stage of maize. These data suggest that the studied bacterial inoculants (*Klebsiella planticola* and *Enterobacter* spp.) can be used in further investigations as the potential agents of new biofertilizers for improved maize and wheat production.

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