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CHEMICAL COMPOSITION AND YIELD OF MAIZE GREEN BIOMASS AS AFFECTED BY BACTERIAL AND MINERAL FERTILIZATION

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Abstract: The purpose of the 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 maize green biomass (without spikes) on acid Eutric Cambisol during the two growing seasons: 2006 and 2008. Unfertilized soil was used as a control. The contents of nitrogen, phosphorus, potassium and crude proteins in biomass samples were determined three times during the maize vegetation season, as follows: stage of intensive growth, milk-waxy maturity stage and full maturity stage. Measuring of the green biomass yield was carried out at the end of the vegetation. The results of the study showed that the use of high rates of composite mineral fertilizers and their combination with bacterial inoculants resulted in increased contents of nitrogen, phosphorus, potassium and crude proteins in the maize biomass during the both study years, which was noticeably observed in the stage of intensive growth. The highest increase in the biomass yield 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 data suggest that the studied bacterial inoculants can be used in further investigations as the potential agents of new biofertilizers for improved maize production and other agriculture crops in animal nutrition.

Key words: maize green biomass, yield, chemical composition, composite mineral fertilizers, bacterial inoculants, Eutric Cambisol

Introduction

Along with wheat, maize (*Zea mays* L.) represents a major crop in agricultural production in Serbia, where its cultivation occupies an area of about 1.300.000 ha with an average yield of 5.00 to 6.00 t ha^{-1} *(Jocković et al., 2005)*. The great importance of maize stems primarily from the diversity of its use, yield potential, opportunities in achieving high yields of grain and silage and in conditions without irrigation, but also from the fact that it is the basic ingredient in livestock feed. According to the quantity of organic matter produced per hectare, together with sugar beet the maize occupies first place in agriculture production, surpassing all other cultivated plant species *(Latković, 2010)*. In livestock feed the whole maize plant or its parts can be used in ripe or green state. Grain is an important concentrated livestock feed, especially for fattening. The whole plant is used for making silage as a high quality food and provides more fodder units than any other plant *(Milosavljević et al., 2010)*.

Increasing the yield and improving the quality of maize crops have been the challenges for sustainable agriculture *(Yu-Kui et al., 2009; Abumhadi and Atanassov, 2010)*. The yield of maize, cultivated for different purposes, in addition to varietal characteristics, largely depends on the climate characteristics (rainfall and temperature regimes particularly during the summer seasons), tillage, chemical, physical and microbiological properties of the soil *(Jeličić et al., 1997; Protić et al., 2004; Mandić et al., 2016)*. Fertilization, among other factors, was one of the reasons that pushed crop production *(Salvagiotti et al., 2010)*, 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. 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. 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 *(Acosta-Martinez and Tabatabai, 2000; Alizadeh and Ghadeai, 2006)*. Consequently, 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 physical, chemical and biological soil properties *(Milošević et al., 2003; Pešaković et al., 2008)*.

Regarding the preceding comments, the main purpose of this research was to evaluate the influence of different rates of composite mineral NPK fertilizers

(15:15:15) and their combination with selected soil bacterial inoculants on chemical composition and yield of the maize green biomass cultivated 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, during 2006 and 2008. Mean monthly air temperatures and precipitation sums for the investigated period are presented in Figure 1. Year 2008 was warmer than 2006, due to a 2-3 °C higher temperature in May, June and August. This year was also lower in precipitation sum comparing to 2006. According to data in climate diagram, distribution of rainfall in 2006 was favorable for maize growing than in 2008 because of the drought periods preceding the rainy season, and during the summer months of June and July there were more than 100 mm of the rainfall.

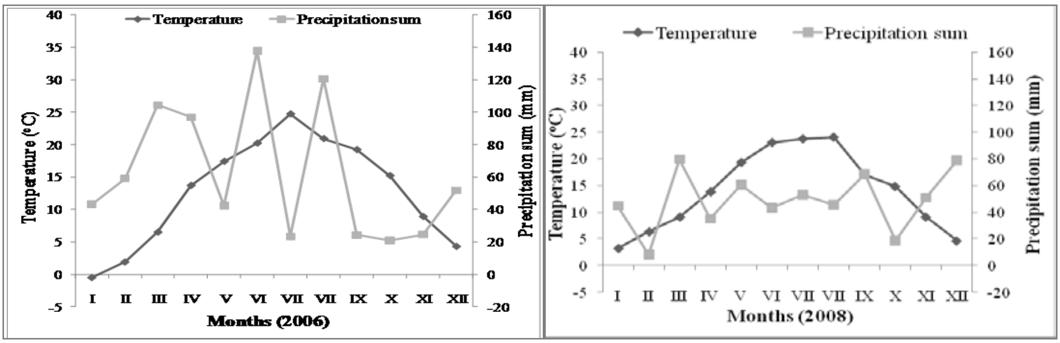

Figure 1. Climate diagram according to Walter for 2006 and 2008 for the study locality

Field trial

The soil type studied in present research was Eutric Cambisol *(WRB, 2014)*. The experiment was set up in a randomized block design on 9 × 6 m^2 plot size, with three replications, 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); *Klebsiella planticola* + 120 kg ha^{-1} N, P_2O_5 and K_2O (KP+N2). Maize (hybrid ZP-341, FAO 300) in 2006 and 2008, was used as a test plant in the trial.

Mineral fertilization and soil bacterial inoculation

Composite NPK mineral fertilizer in relation 15:15:15 was applied in the trial. Nitrogen (N) fertilizer was applied in the form of urea with 46% N, phosphorus (P) – in the form of monoammonium phosphate (MAP) with 52% P_2O_5 and 11% N, and potassium (K) – as a 40% potassium salt (KCl). The established amounts of mineral fertilizer have been applied in the spring of 2006 and 2008, before sowing the maize.

The pure culture of an associative N-fixing bacterium *Klebsiella planticola* (strain TSHA-91) was obtained from the stock culture of the Microbiology Laboratory of Faculty of Agronomy (Čačak, Serbia) and cultivated on the slanting nutrient medium for 24 h at 28°C ± 1. Chemical composition of the medium was as follows: 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. The pure culture of associative N-fixing *Enterobacter* strains KG-75 and KG-76 were obtained from the stock culture of the Microbiology Laboratory of the Center for Small Grains (Kragujevac, Serbia), where they have been isolated from the rhizosphere of wheat. These strains were cultivated for 48 h at 28°C ± 1 on the slanting nutrient medium (MPA, Torlak, Belgrade) with 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.

The pure liquid inoculum of *K. planticola* (100-300 x 10^7 cells per 1.0 cm^3 of inoculum) in amount of 18.00 dm^3, as well as the pure liquid inoculum of *Enterobacter* strains (100-180 x 10^7 cells per 1.0 cm^3 of inoculum) in the same amount, were made using fermentors with suitable nutrient broth and incubated with aeration for 48 h at 28°C ± 1.

The bacterial inoculation of the soil 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. The inoculation was performed when the maize was in the stage of 2-3 formed leaves.

The method of mineral fertilization and soil bacterial inoculation used in this study was previously described *(Stanojković et al., 2012)*.

Soil preparation and analysis

The samples of soil were air-dried, crushed and passed through a sieve with a diameter of ≤ 2 mm. The preliminary analysis of the study soil included the following chemical parameters: soil acidity (pH in H_2O and 1M KCl, v/v - soil:H_2O=1:5, soil:1M KCl=1:5) was analyzed potentiometrically, using glass electrode *(SRPS ISO 10390, 2007)*; total nitrogen (N) was analyzed on elemental CNS analyzer Vario EL III *(Nelson and Sommers, 1996)*; available phosphorus (P_2O_5) and potassium (K_2O) were analyzed by Al-method according to Egner-Riehm *(Riehm, 1958)*, where K_2O was determined by flame emission photometry and P_2O_5 by spectrophotometer after color development with ammonium molybdate and stannous chloride; humus content was determined using Tiurin's method, modified by Simakov *(Ostrowska et al., 1991)*.

Plant analysis

The maize biomass without spikes was taken in three stages of the plant: intensive growth (vegetation stage I), milk-waxy maturity stage (vegetation stage II) and full maturity stage (vegetation stage III). The samples of the plant material was then weighed before and after drying at 105°C. For all the plant samples from all the variants studied the chemical analyses of the maize biomass were done. The contents of phosphorus (P) and potassium (K) were determined by so called “wet” combustion, i.e. they were heated to boiling with the mixture of concentrated sulfuric (H_2SO_4) and perchloric ($HClO_4$) acids. In the obtained solution, P was determined by spectrophotometer with molybdate, and K – by flame emission photometry *(Jakovljević et al., 1985)*. The content of nitrogen (N) was analyzed using elemental CNS analyzer, Vario model EL III *(Nelson and Sommers, 1996)*, while the content of crude proteins was calculated on the basis of N content according to *Licitra et al. (1996)*, using the following formula: crude proteins (%) = N (%) x 6.25 (factor for conversion of nitrogen content to crude protein).

Maize harvest was performed manually from each plot in the full maturity stage, when the dry matter was 20-25% during the first decade of October in 2006 and 2008. Plants from each plot were cut on height 20 cm at harvest time and biomass yield was measured. The yield was converted into t ha^{-1}.

Data analysis

The obtained data on soil properties were presented as arithmetic means of three replicates, standard deviation values and intervals. The effects of different fertilization variants on all the variables tested were evaluated using Analysis of

Variance (SPSS 20.0, Chicago, USA), followed by Duncan's Multiple Range Test (DMRT). Significant differences between means were tested by the LSD test 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 1. According to the reference values *(Šestić et al., 1969)*, the soil is characterized by acid reaction, high available potassium and medium available phosphorus, humus and total nitrogen supply.

Table 1. Main chemical characteristics of the studied Eutric Cambisol

Chemical parameter	Value (means ± standard deviation)	Intervals
pH in H_2O	4.90±0.03	4.87-4.92
pH in 1M KCl	4.06±0.05	4.00-4.10
Total N (%)	0.136±0.005	0.132-0.141
Humus (%)	2.19±0.01	2.18-2.19
Available P_2O_5 (mg $100g^{-1}$)	15.73±0.31	15.51-16.09
Available K_2O (mg $100g^{-1}$)	25.30±0.30	25.08-25.65

Effect of applied fertilizers on the chemical composition of green biomass

By analyzing the dynamics of accumulation of nitrogen, phosphorus, potassium and proteins in maize biomass during 2006 and 2008 (Tables 2 and 3) it was determined that the biomass chemical composition depended on the fertilization variant used, as well as the vegetation period of maize studied.

Table 2. Effect of the fertilization variants on chemical composition of the maize biomass during 2006

Variant	Vegetation stage	Total N (%)	Crude proteins (%)	P_2O_5 (%)	K_2O (%)
Ø	I	2.799±0.001g	17.491±0.002g	1.142±0.002f	2.413±0.002g
	II	0.295±0.001g	1.840±0.001g	0.888±0.002e	1.344±0.002g
	III	0.153±0.003f	0.958±0.005g	0.765±0.002f	1.205±0.005g
N1	I	3.903±0.002f	24.386±0.002f	1.262±0.002e	2.533±0.003f
	II	0.554±0.002f	3.457±0.002f	1.005±0.003d	1.943±0.002f
	III	0.378±0.001d	2.370±0.001e	0.882±0.003e	1.712±0.011f
N2	I	4.964±0.002c	31.022±0.005c	1.453±0.003c	3.040±0.008c
	II	0.770±0.001c	4.807±0.002c	1.188±0.029b	2.716±0.006c
	III	0.711±0.002c	4.456±0.005c	1.071±0.002c	2.553±0.003c
KP+N1	I	4.422±0.002d	27.622±0.003d	1.326±0.002d	2.917±0.001d
	II	0.671±0.002d	4.202±0.001d	1.071±0.002c	2.150±0.003e
	III	0.378±0.003d	2.383±0.003d	0.948±0.002d	1.942±0.002d
KP+N2	I	5.439±0.002b	33.993±0.004b	1.689±0.008a	3.283±0.003a
	II	0.913±0.002b	5.713±0.002b	1.437±0.002a	2.860±0.002a
	III	0.778±0.002b	4.589±0.003b	1.241±0.002b	2.609±0.008a
ES+N1	I	4.111±0.001e	25.699±0.002e	1.264±0.002e	2.854±0.001e
	II	0.592±0.002e	3.713±0.003e	1.006±0.007d	2.155±0.002d
	III	0.333±0.003e	2.090±0.001f	0.881±0.002e	1.886±0.005e
ES+N2	I	5.563±0.001a	34.776±0.002a	1.673±0.001b	3.265±0.002b
	II	1.3033±0.002a	8.138±0.002a	1.422±0.003a	2.847±0.002b
	III	0.856±0.002a	5.357±0.002a	1.291±0.002a	2.591±0.002b
P value	I	***	***	***	***
LSD (0.05)		0.0026	0.005	0.006	0.006
LSD (0.01)		0.0037	0.007	0.008	0.009
P value	II	***	***	***	***
LSD (0.05)		0.003	0.003	0.019	0.005
LSD (0.01)		0.004	0.004	0.027	0.006
P value	III	***	***	***	***
LSD (0.05)		0.005	0.006	0.003	0.010
LSD (0.01)		0.007	0.008	0.004	0.014

LSD indicates least significant differences 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; DMRT was used to compare different variants at P≤ 0.05, where values followed by the same letter in a column are not significantly different.

Table 3. Effect of the fertilization variants on chemical composition of the maize biomass during 2008

Variant	Vegetation stage	Total N (%)	Crude proteins (%)	P_2O_5 (%)	K_2O (%)
Ø	I	2.828±0.002g	17.676±0.021g	1.165±0.004f	2.433±0.006f
	II	0.505±0.009g	3.211±0.010g	0.913±0.003e	1.366±0.005f
	III	0.429±0.025f	2.755±0.001g	0.782±0.004f	1.228±0.002g
N1	I	4.366±0.005f	27.295±0.005f	1.285±0.001e	2.555±0.005e
	II	0.613±0.015f	3.736±0.032f	1.032±0.001d	1.964±0.004e
	III	0.495±0.003e	3.072±0.002f	0.902±0.002e	1.741±0.003f
N2	I	6.173±0.006c	38.555±0.051c	1.477±0.012c	3.065±0.003b
	II	1.131±0.001c	7.066±0.004c	1.225±0.002b	2.745±0.004c
	III	0.716±0.014c	4.534±0.002c	1.095±0.001c	2.577±0.002c
KP+N1	I	5.524±0.022e	34.446±0.005e	1.353±0.006d	2.944±0.003c
	II	0.896±0.004d	5.552±0.045d	1.096±0.002c	2.175±0.004d
	III	0.587±0.004d	3.686±0.002d	0.968±0.002d	1.967±0.003d
KP+N2	I	6.943±0.002a	43.395±0.010a	1.716±0.002a	3.306±0.002a
	II	1.435±0.003a	8.970±0.026a	1.350±0.026a	2.886±0.003a
	III	1.025±0.001a	6.416±0.001a	1.136±0.003a	2.636±0.004a
ES+N1	I	6.001±0.002d	37.537±0.032d	1.288±0.001e	2.856±0.040d
	II	0.733±0.002e	4.595±0.003e	1.033±0.003d	2.176±0.003d
	III	0.533±0.028e	3.416±0.001e	0.904±0.004e	1.910±0.010e
ES+N2	I	6.632±0.001b	41.476±0.032b	1.693±0.003b	3.288±0.003a
	II	1.3033±0.002b	8.133±0.003b	1.345±0.002a	2.872±0.001b
	III	0.946±0.040b	6.063±0.004b	1.118±0.002b	2.616±0.002b
P value	I	***	***	***	***
LSD (0.05)		0.015	0.048	0.009	0.027
LSD (0.01)		0.021	0.066	0.013	0.037
P value	II	***	***	***	***
LSD (0.05)		0.012	0.041	0.018	0.005
LSD (0.01)		0.0017	0.057	0.025	0.006
P value	III	***	***	***	***
LSD (0.05)		0.037	0.004	0.005	0.008
LSD (0.01)		0.052	0.005	0.006	0.010

LSD indicates least significant differences 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; DMRT was used to compare different variants at P≤ 0.05), where values followed by the same letter in a column are not significantly different.

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 crude proteins in the maize biomass compared to the other tested variants. This trend was noticeably observed in the stage of maize intensive growth, the vegetation period in which the accumulation of nutrients is the most intensive *(Čurić, 1982)*.

Hence, the excess of microbiologicaly 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 *(Pandey et al., 1998; Dalla Santa et al., 2004)*. 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.

Effect of applied fertilizers on the yield of green biomass

The analysis of the yield of maize green biomass (based on Duncan's test) showed highly significant yield differences between the applied fertilization treatments (Table 4). The highest increase in yield was obtained by combined application of bacterial inoculants used and high rates of mineral NPK fertilizers for both study years. 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 *(Dalla Santa et al., 2004)*, 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 ha^{-1}). Other authors *(El-Sirafy et al., 2006)* also found a significant interaction effect of nitrogen fertilizers and microbial inoculation on crops yield compared to the unfertilized variants.

Table 4. The effect of the fertilization variants on the yield of maize biomass in the study years

Fertilization variant	Maize biomass yield (t ha^{-1})	
	Year 2006	Year 2008
Ø	4073±63g	3373±19g
N1	5140±61f	4464±25f
N2	13242±70c	12074±78c
KP+N1	7083±34d	5954±51d
KP+N2	16736±44a	12215±48b
ES+N1	5885±46e	5485±66e
ES+N2	16454±51b	12721±34a
P value	***	***
LSD (0.05)	94.38	87.66
LSD (0.01)	130.99	121.67

LSD indicates least significant differences 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; DMRT was used to compare different variants at P≤ 0.05), where values followed by the same letter in a column are not significantly different.

The character of the applied fertilizers effects on the yield of maize biomass also 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 2008 than in 2006 (Table 4), which is likely due to unfavorable weather conditions during the maize growing period in 2008. This is consistent with some previous results *(Josipović et al., 2005; Maklenović et al., 2009)*, 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.

Conclusion

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 green biomass (without spikes) for both study years. The same results were obtained regarding the effects of the mentioned applied combinations on the contents of nitrogen, phosphorus, potassium and crude proteins in the maize biomass, which was noticeably observed in the stage of the maize intensive growth. 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 production and other agriculture crops in animal nutrition.

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Procena uticaja bakterijske i mineralne fertilizacije na hemijski sastav i prinos zelene biomase kukuruza

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Rezime

Cilj ovog istraživanja je bio da se proceni uticaj primene različitih doza kompleksnih mineralnih đubriva i njihovih kombinacija sa bakterijskim inokulantima (azotofiksirajuće bakterije *Klebsiella planticola* i *Enterobacter* spp.) na hemijski sastav i prinos zelene biomase kukuruza na kiselom eutričnom kambisolu tokom dve vegetacione sezone: 2006 i 2008. Neđubreno zemljište je služilo kao kontrola. Sadržaj azota, fosfora, kalijuma i sirovih proteina u uzorcima biomase su određivani tri puta tokom vegetativne sezone kukuruze, i to: u fazi intenzivnog porasta, fazi mlečno-voštane zrelosti i fazi pune zrelosti. Merenje prinosa zelene biomase obavljeno je krajem vegetacije. Rezultati istraživanja su pokazali da je primena visokih doza kompleksnih mineralnih đubriva i njihova kombinacija sa bakterijskim inokulantima uticala na povećanje sadržaja azota, fosfora, kalijuma i sirovih proteina u biomasi kukuruza tokom obe godine istraživanja, što je naročito izraženo u fazi njegovog intenzivnog porasta. Najveći porast prinosa biomase je dobijen na istim navedenim varijantama, a isto tako je i primena kombinacije bakterijskih inokulanata i manjih doza mineralnih đubriva rezultirala većim prinosima u odnosu na primenu manjih doza čistih mineralnih hraniva. Dobijeni podaci ukazuju da se ispitivani bakterijski inokulanti mogu koristiti u daljim istraživanjima kao potencijalni agenti novih biofertilizatora u cilju poboljšanja proizvodnje kukuruza i drugih poljoprivrednih kultura u ishrani životinja.

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