









# **Book of Proceedings**

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"GLOBAL CHALLENGES THROUGH THE PRISM OF RURAL DEVELOPMENT IN THE SECTOR OF AGRICULTURE AND TOURISM"



Šabac, Serbia 10<sup>th</sup> May, 2024

## "GLOBAL CHALLENGES THROUGH THE PRISM OF RURAL DEVELOPMENT IN THE SECTOR OF AGRICULTURE AND TOURISM"

### **Book of Proceedings**

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#### EFFECTIVE MICROORGANISMS IN SUSTAINABLE MAIZE PRODUCTION

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#### **ABSTRACT**

Conventional food production has led to a number of problems in the environment, especially soil degradation. The sustainable method of production is also acceptable for rural areas. The goal of the work was to determine the dynamics of changes in the abundance of significant groups of microorganisms in the maize rhizosphere after the application of preparations with effective microorganisms. The experiment was set up in 2018 in the Mačva region. There were three in the research: A: different amounts of mineral nitrogen 160 and 120 kg·Nha-1, B: three hybrids ZP 427, ZP 548 and ZP 648 and factor C treatment with effective microorganisms in the preparation EM Aktiv. The preparation was applied to the soil before sowing at 20 l·ha-1 and twice during the growing season at 6 l·ha-1. The total number of microbes (10<sup>7</sup>) and the number of fungi (10<sup>4</sup>) were determined as important parameters that are important for acidic soils. Agroecological conditions were relatively optimal, while agrochemical analyzes of the soil showed that the soil had an acidic reaction. In such conditions, the application of EM technology had positive results on the examined parameters. The tested factors had a statistically significant effect on the number of tested groups of microbes compared to the control. The number of examined parameters was higher at 120 kg N·ha<sup>-1</sup> and in the rhizosphere of hybrid ZP 427. Treatment with EM did not have a statistically significant effect on the increase of nitrogen content in the grains of the examined hybrids.

**Keywords**: maize, total number of microbes, fungi, effective microorganisms.



#### INTRODUCTION

Declining soil fertility is one of the most serious problems facing humanity as it tries to feed a growing population. In the soils of Vojvodina, a decrease in humus content from 0.5% to 1.0% was determined, which represents an extremely high decrease, and is the result of intensive processing with the use of mineral fertilizers and the neglect of the application of organic fertilizers (Vasin, 2012). The introduction of high doses of mineral fertilizers worsens the agrochemical properties of the soil as well as the accessibility of microelements (Zn, B, Cu, Mo), which noticeably lowers the quality of plant products. Soil is a hard-to-renew natural resource and all important international declarations on the use of nature emphasize the importance of soil as a universal good of humanity that must be protected by the joint action of science, politics and society as a whole, and in 2015. year according to the decision of the FAO, soil was declared a non-renewable resource (Manojlović et al., 2021).

The constant threat of degradation and loss of fertile soil prioritizes the development of strict soil protection directives promoted by the European Union. The European Green Directive has a series of requirements in food production aimed at reducing the emission of gases that affect the increase in climate change. as well as standards that define a product with a high biological value. This motivated the scientific and professional public to find new directions in food production by introducing ecologically acceptable inputs that would replace chemical preparations in nutrition to protect plants, maintain plant yields, preserve potential soil fertility and resources important for the survival of humanity.

One of the important directions is sustainable systems of agricultural production. The concept of sustainable agriculture defines a production process that must be safe for the environment, and in which a high-quality and safe final product is obtained (Figure 1). In the framework of sustainable agriculture, integral and organic are distinguished. Both systems are based on methods aimed at preserving and improving soil fertility. Therefore, research into the application of different groups of microorganisms and the interaction between microorganisms and ecosystems helped to determine their role in order to achieve the goals of sustainable agriculture.

Figure 1. Components of intensification of sustainable and conventional agriculture. and sustainable environment

#### Sustainable Intesification

Microbial biotechnology Green tehnology Organic fertilizer High soil biodiversity Save land High yield

### Agricultural Intensification Sustainable Environment Synthetic fertilizer Organic fertilizer

Synthetic fertilizer Nitrification Inhibitor Low soil biodiversity Low soil quality High yield Organic fertilizer
High soil biodivesity
High soil quality
Save land
Low yield

(https://www.organic-world.net/yearbook/yearbook-2020.html)



Maize is called yellow gold because of its role in human and animal nutrition. in the production of food and non-food products. According to Dowswell et al., (2019), 20 million tons of maize is used for starch, 10 million tons for ethanol fuel production, 3 million for grains and baked goods, 0.7 million for grain and hybrid seed sales. As a result of its low price compared to other crops. maize is used in large quantities in the diet of domestic animals. Maize is an important raw material for the production of bioenergetics, paper, packaging and many additives.

Together with wheat and rice. it occupies the largest area in the world. In Serbia. it is cultivated on more than one million hectares per year (https://gain.fas.usda.gov/Belgrade\_Serbia). Serbia ranks fifth in maize production out of 47 CEFTA countries and most countries in the EU and CIS (Commonwealth of Independent States). The necessity to intensify its production in a sustainable way is of utmost importance. Consequently, to reduce the use of synthetic fertilizers, inoculation with different plant growth-promoting microbes as a whole or together with manure would be crucial to improve maize productivity and promote its sustainable production.

The application of different groups of microorganisms is a biotechnological alternative for increasing crop productivity, increasing the availability of nutrients. and reducing the use of synthetic fertilizers. Previous research has shown that thanks to the properties of symbiotic and free-living microorganisms, the problem of availability of nutrients can be solved, the resistance of plants to drought and infection by phytopathogenic organisms can be increased. Zheng et al. (2018) determined that the application of *Bacillus subtilis* can affect the physical, chemical and hydrological characteristics of the rhizosphere. thereby improving the drought resistance of plants in the long term. Wang et al. (2018) established the significant role of *Streptomyces albireticul* and *Streptomyces alboflavus* as biocontrol agents.

In the last few years, there has been an increasing number of researches related to the application of preparations with a mix of different groups of microorganisms. In practical application and scientific literature, they are known as multiple preparations with effective microorganisms that are the basis of EM biotechnology in sustainable food production. Such preparations can be used for the treatment of seeds and planting material, during the growing season, as well as on the soil (Kołodziejczyk 2014). Such diverse potential use of effective microorganisms is due to the high enzymatic specificity of microorganisms, which allows them to survive in different environments.

The aim of the work was to determine the influence of the application of effective microorganisms on the change in the abundance of significant groups of microorganisms in the maize rhizosphere and nitrogen synthesis in the grain.

#### MATERIAL AND METHODS OF WORK

Experimental research was conducted in 2018 in the municipality of Vladimirci, Mačva region. The area of the elementary plot is  $14\ m^2$ . The plots were laid out according to the plan of divided plots in four repetitions. The pre-crop was wheat. All agrotechnical mea sures were applied in optimal terms



<u>Factor A</u>: Fertilization in the fall is plowed with 30 t ha<sup>-1</sup> of manure and complete mineral NPK fertilizer formulation 15:15:15. Before sowing, fertilizer was applied with mineral nitrogen fertilizer AN 36% so that N1 - 160 kg ha<sup>-1</sup> and N2 -120 kg ha<sup>-1</sup> were provided for plant nutrition.

<u>Factor B</u> Hybrids: Three hybrids were sown, of the toothed yellow grain type, selected by the Zemun Polje Maize Institute ZP 427 (FAO 400); ZP 548 (FAO 500); ZP 687 (FAO 600).

<u>Factor C</u> Treatments: EM Aktiv, a liquid preparation of microbiological origin. was used in the experiment. The preparation is a mixture of a large group of effective microorganisms (photosynthetic bacteria, sulfate-reducing bacteria, lactic-acid fermentation bacteria, yeasts, actinomycetes and fungi) and sugarcane molasses with the addition of microelements. The preparation represents a variant of EM technology that is part of the research of Japanese researchers Higa (1994). There were two variants in the experiments: EM 0 without the preparation -control and variant EM 1 with preparation. The preparation EM Aktiv was applied: to the soil 7 days before sowing in the amount of 30 l ha<sup>-1</sup> with water in a ratio of 1:10. During the growing season. it was applied foliarly twice in the amount of 6 l ha<sup>-1</sup> with water in a ratio of 1:100. The first treatment was in the phenological phase of 5-7 leaves, and the second after 15 days.

Before sowing, soil samples were taken to determine basic agrochemical analyses. In the stage of technological maturity of maize, samples were taken from the rhizosphere of plant roots and the total number of microorganisms was determined on soil agar  $10^{-7}$ , the number of ammonifiers on mesopeptone agar  $10^{-7}$  and fungal dilution on Čapek's agar  $10^{-4}$ . All laboratory research was carried out in the laboratories of the Institute for Crops and Vegetables in Novi Sad.

For the successful production of maize. optimal agrometerological conditions are necessary for all phenological stages of maize development. Figure 2 shows that the average air temperature in the growing season was 19.1°C, and the total amount of precipitation was 426.4 mm. The measured temperature values are higher by 1.3 °C and precipitation by 13.6 mm less than the multi-year average (1964-2018).

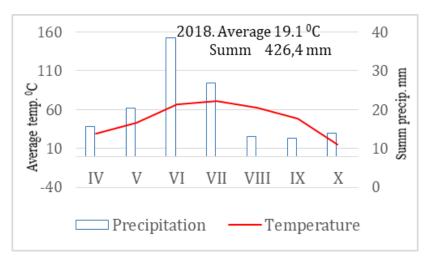


Figure 2. Average air temperature (°C) and sum precipitation (mm)



#### RESULTS AND DISCUSSION

Based on the results shown in Table 1, it can be seen that the soil has a very acidic reaction (rN 4.01-4.17) in KCl. The read value of the CaCO<sub>3</sub> content classifies the examined soil in the carbonate-free class. According to the humus content, the studied soil belongs to the class of low humus soils. According to the content of readily available phosphorus, the soil belongs to the group of low-provided soils, and according to the content of readily available potassium to the group of high-provided soils. Acidic soils occupy 30% of the total world surface (Esvaran et al., 1997). In Serbia, more than 60% of arable land has acidic soils.

Table 1. Agrochemical properties of soil

рН		CaCO3	Humus	Total N	AL-P <sub>2</sub> O <sub>5</sub>	AL-K <sub>2</sub> O
KCl	H <sub>2</sub> O	%	%	%	mg 100 g <sup>-1</sup>	
4.01-4.17	5.15-5.45	0.00	1.86-2.30	0.158-0.184	3.1-6.6	12.74-37.79

The number of systematic groups and the value of dehydrogenase enzymes are used as the only indicators of general microbiological activity. Soils with a high humus content have a higher content of heterotrophic microorganisms. At the same time, their oxidation-reduction ability, i.e. electron transfer processes, as well as the mineralization of fresh organic matter and humus, is greater. It is a good indicator that such soils are suitable for plant production.

In addition to these basic representatives of soil biogenicity, for a detailed assessment of soil condition, damage, toxicosis, etc. the enzymatic activities of microorganisms that produce phosphatase, catalase, peroxidase, lignases, lipases and others are used. Each of these produced enzymes and microorganisms is a good indicator of the presence of substances such as lipids, lignin, organic compounds of phosphorus and others in the soil.

Microorganisms in the soil are very heterogeneous and the most numerous group of organisms. Compared to other organisms, a large part of soil biomass is made up of bacteria and fungi. These groups of microorganisms have a central role in the metabolic activity of the soil, because they participate in it with 60-90%. Many processes important for maintaining soil fertility are the result of their life activity.

The total number of microorganisms represents one of the important parameters of soil biogenicity because they represent a large group of microorganisms that participate in the processes of humification and dehumification, as well as nitrogen fixation. In their metabolic activities, they release certain nutrients found in organic matter (N, P, S, C), influence the nutrition of plants with the products of their life activity and thus participate in the creation and maintenance of soil fertility, growth, yield and health plants. The total number of microorganisms is taken as a reliable indicator when assessing the condition of the soil. A greater number of total microflora is not an indicator of a greater effective production capacity of the soil, because it depends on a number of other factors, but it certainly represents a greater potential for the formation of organic matter in the soil.

The total number of microorganisms in the study was average  $350.38 \times 10^7$  (Table 2).



N (kg.ha <sup>-1</sup> )	Hybrides (B)	EM (C)		<del></del>	<del></del> .		LSD 0.05
(A)		EM <sub>0</sub>	EM <sub>1</sub>	$-\overline{\mathbf{X}}\mathbf{A} \times \mathbf{B}$	$\overline{\mathbf{X}}$ A	Factor	LSD 0.01
160	427	205.62	263.34	234.48		A**	13.13 10.24
	548	289.65	229.51	259.58	258.67	B**	9.74
	684	293.53	270.39	281.96	_		14.18
	$\overline{\mathbf{X}}$ A×C	262.93	254.41			C**	17.07
120	427	376.41	640.72	508.56	442.11	C	22.77
	548	417.41	346.36	382.38		A x B**	13.78
	684	361.92	508.79	435.35	442.11		20.05
	$\overline{\mathbf{X}}$ A×C	385.57	498.63		-	A C*	24.13
<b>X</b> B × C	427	291.01	452.03	371.52		A x C*	32.20
	548	354.02	287.93	320.98	_	B x C**	29.56
	684	327.72	389.95	359.65	$\overline{\mathbf{X}}$ B		39.43
	$\overline{\mathbf{X}}$ C	324.25	376.52			A x B x C**	41.80 55.77
		Average			350.38	CV %	16.77

Table 2. Total number of microorganisms (x 10<sup>7</sup> CFU g<sup>-1</sup> absolutely dry soil)

Numerous factors in the environment affect microbiological activity. Some of them are called modulators, as opposed to those that are called resources and are important for growth. The modulators include pH, temperature, salinity, while the modulators are the availability of carbon and nitrogen. Different amounts and types of fertilizers have different effects on the diversity of microorganisms. A greater amount of ammonia nitrogen leads to greater acidification of the environment, which can negatively affect a greater number of bacteria, especially genera that participate in the nitrogen cycle. In the conducted research, with a lower amount of mineral nitrogen, a 70.91% higher total number of microorganisms was found. The highest total number of microorganisms was determined in the rhizosphere of hybrid ZP 427. The difference in the total number of microorganisms in relation to the state in the rhizosphere of hybrid ZP 548 was at a significance level of p<0.01, and in relation to hybrid ZP 684 at a significance level of p<0.05.

Treatment with effective microorganisms (EM) had a significant effect on the change in the total number of microorganisms. The difference of 16.12% was highly statistically significant compared to the control variant. In the interaction of hybrids and treatments, the highest total number of microbes was in the rhizosphere of hybrid ZP 427 (452.03 x  $10^7$ ), and in the interaction of the amount of mineral nitrogen and tremane, the highest total number of microbes was at a lower amount of mineral nitrogen and the hybrid ZP 427 (640.72 x  $10^7$ ).

The interaction between plants and microorganisms is becoming increasingly important in sustainable agriculture systems. primarily in order to transform and mobilize nutrients from the soil.

According to a number of authors. it was established that maize can significantly influence the composition and abundance of microorganisms in the rhizosphere with its root secretions. In research by Kandeler et al. (2002) determined that the diversity of the bacterial population and their activity in the rhizosphere vary under the influence of maize root exudates.



According to the data in the literature, it was determined that representatives of different genera are represented in the maize rhizosphere *Pantoea. Bacillus. Burkholderia* and *Klebsiella* (Ikeda et al., 2013). Roesch et al. (2008) found a greater number of different genera in the rhizosphere of maize roots, as well as the presence of certain types of bacteria in the tissue of roots and plants grown in field conditions. Also, many studies have shown that the application of different groups of microorganisms has a significant impact on the dynamics of changes in the total number of microbes and individual groups in the rhizosphere of different plant species (Cvijanović et al., 2007).

As soil dwellers, fungi build significant biomass in the soil, which can amount to up to 20 l th<sup>-1</sup>. They are regular inhabitants of all types of agricultural land, but are most dominant in soils rich in organic matter and in acidic reaction environments.

Based on the results presented in Table 3, it can be seen that the hybrids did not have a statistically significant effect on the development of fungi, while the interaction of fertilization and hybrids at the significance level of p<0.05 influenced the differences in the number of fungi. Other examined factors had a highly significant influence on the dynamics of changes in the number of fungi.

The development of fungi in the soil is simultaneously influenced by several factors, of which the amount of organic matter, the concentration of hydrogen ions, humidity, temperature, etc. are particularly significant. Fungi participate in the biodegradation of cellulose, hemicellulose and lignin in acidic soils because they have a highly developed enzyme system. This is very significant from the aspect of creating organic matter in the soil because fungi are more active in acidic environments than bacteria. Decomposition of cellulose, which makes up 50% of fresh organic matter, is done by fungi.

The average number of fungi was  $73.73 \times 10^4$  (Table 3). At a lower amount of mineral nitrogen. 17.94% more fungal cells were found. Treatment with EM significantly increased the number of fungi by 8.52%. In the interaction between hybrid and treatment, the highest number of fungi was found in the rhizosphere of hybrid ZP 427 (92.16 x  $10^4$ ) during treatment with EM. In the interaction between fertilization and treatments, the highest number of fungi was determined in the rhizosphere of the ZP 427 hybrid ( $133.25 \times 10^4$ ), with a lower amount of mineral nitrogen.



Table 3. The total number of fungi (x 10<sup>4</sup> CFU g<sup>-1</sup> absolutely dry soil)

N (kg.ha <sup>-1</sup> )	Hybrides EM (C)		(C)	<b>▼</b> . p	₹.	Factor	LSD 0.05
(A)	(B)	EM <sub>0</sub>	EM <sub>1</sub>	$\overline{\mathbf{X}}$ A×B	$\overline{\mathbf{X}}$ A		LSD 0.01
160	427	101.12	51.08	76.11			7.77
	727	101.12	31.00	70.11		A**	8.55
	548	44.05	75.41	59.73	67.67	В	4.48
	684	91.89	42.46	61.17	_		6.28
	$\overline{\mathbf{X}}$ A×C	79.02	56.32			C**	6.19
120	427	23.6	133.25	78.42	79.81	C	8.22
	548	70.1	85.78	77.94		A x B*	7.76
	684	93.54	72.53	83.03	7 7.01		10.88
	$\overline{\mathbf{X}}$ A×C	62.41	97.19	79.81		A x C**	10.73
<b>X</b> B × C	427	62.36	92.16	77.26	<b>X</b> B	AXC	14.24
	548	57.07	80.59	68.83		B x C** A x B x C*	10.73
	684	92.71	57.49	75.10			14.24
	<b>X</b> €	70.72	76.75	_			18.58
		70.72	70.73		_		24.66
	Average			73.73		CV %	27.78

The nitrogen content in maize kernels is very significant, because nitrogen is the main element in the composition of raw proteins. The nitrogen content in maize kernels and other parts of the plant is different and depends on the genotype of the plant, the amount of macro and micro nutrients. Nitrogen in the grain originates from nitrogen taken from the soil during grain filling and from nitrogen transported from the vegetative organs into the grain. The average nitrogen content in the grain of the examined hybrids was 1.43% (Table 4).

Table 4. Nitrogen content in the grain of the examined hybrids (%)

	U		O		, ,	
) Hybrides EM(C) VALUE		<b>▼</b> 4D	₹ ^	Fastan	LSD 0.05	
(B)	$EM_0$	$EM_1$	<b>A</b> A×B	<b>A</b> A	Factor	LSD 0.01
427 1.	1 29	1 34	1.34	1.44	A**	0.017
	1.27	1.54				0.019
548	1.35	1.46	1.43		B**	0.019
684	1.57	1.60	1.59			0.026
$\overline{\mathbf{X}}$ A×C	1.40	1.47			C	0.024
427	1.25	1.39	1.32	1.50	C	0.036
548	1.46	1.46	1.46		A x B**	0.033
684	1.83	1.60	1.71			0.046
$\overline{\mathbf{X}}$ A×C	1.51	1.48			A ** C	0.041
427	1.27	1.36	1.34	l3 	AXC	0.055
548	1.40	1.46	1.43		ВхС	0.041
684	1.70	1.60	1.65			0.055
<b>▼</b> C 1.46	1 4.6	1 47			A x B x C*	0.072
ΛC	1.40	1.47				0.095
	Average	1.46	CV %	3.84		
	(B)  427  548  684 $\overline{X}$ A×C  427  548  684 $\overline{X}$ A×C  427  548	Hybrides     EM       (B) $EM_0$ $427$ $1.29$ $548$ $1.35$ $684$ $1.57$ $\overline{X}$ A×C $1.40$ $427$ $1.25$ $548$ $1.46$ $684$ $1.83$ $\overline{X}$ A×C $1.51$ $427$ $1.27$ $548$ $1.40$ $684$ $1.70$ $\overline{X}$ C $1.46$	Hybrides $EM (C)$ (B) $EM_0$ $EM_1$ 427         1.29         1.34           548         1.35         1.46           684         1.57         1.60 $\overline{X}$ A×C         1.40         1.47           427         1.25         1.39           548         1.46         1.46           684         1.83         1.60 $\overline{X}$ A×C         1.51         1.48           427         1.27         1.36           548         1.40         1.46           684         1.70         1.60	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$



The examined factors and their interactions had a different effect on the nitrogen content in the grain. Fertilization and traits of hybrids had a highly significant effect on nitrogen content in grain, while treatments and the interaction of treatments with hybrids and fertilization had no statistically significant effect on this trait. When fertilizing with 120 kg ha<sup>-1</sup>, the nitrogen content in the grain was 4.16% higher than when a higher amount of mineral nitrogen was applied. The hybrid ZP 684 had the highest nitrogen content in the grain.

In the literature, there is relatively little data on the impact of EM application on the chemical composition of maize grains. However, in research on the impact of foliar application of effective microorganisms, a significant impact on the increased content of protein and oil in soybeans (Cvijanović et al., 2020) and protein in wheat was found (Cvijanović et al., 2022).

The use of effective microorganisms can have a positive effect on the quality of soil and products. which is also very important from the aspect of tourism development. By preserving the gene pool. the vitality of environmental elements. obtaining products with higher nutritional properties can be an advantage in tourism offers and the development of rural areas.

#### **CONCLUSIONS**

Based on the presented results. it can be concluded that the application of preparations with effective microorganisms is desirable because in conditions that are unfavorable for plant development and the diversity of the microbial population in the soil. they contribute significantly to the increase in abundance. They can also improve the nutritional value of the fruits.

Application of such products can be included in a sustainable production system. The recommendation would be that they become part of mandatory agrotechnical measures.

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