Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2024, Vol. 8 (4), 324-335
https://doi.org/10.29329/ijiaar.2024.1109.5

Original article

Influence of Effective Microorganisms and Mineral Fertilizers on Soil Biogenicity Parameters and Soybean Yield

Gorica Cvijanović [a], Vojin Đukić [b], Marija Bajagić [a, *], Zlatica Mamlić [b], Biljana Šević [c], Vojin Cvijanović [d] & Aleksandra Ivetić [d]

[a] University of Bijeljina, Faculty of Agriculture, Bijeljina, Bosnia and Herzegovina
[b] Institute of Field and Vegetable Crops, Novi Sad, Serbia
[c] Institute of Vegetable growing, Smederevska Palanka, Serbia
[d] Institute for Science Application in Agriculture, Belgrade, Serbia

Abstract

The research aimed to determine the effect of the application of effective microorganisms (EM) on the basic microbiological parameters of soil biogenicity and the height of soybean yield. The research was conducted in the period from 2016-2018. Factor A is the years 2016-2018; factor B soybean genotype Galina, Sava, Rubin and factor C application of EM: variant 1 - control, variant 2 - EM in the soil 20 lha-1 and foliar treatment in the phase of plant development from three to four trefoils and the budonization phase, (5 lha- 1); variant 3 - NPK fertilizer (8:15:15), 300 kgha-1, and variant 4 - EM in the soil 20 lha-1 and foliar treatments in the stage of plant development from three to four trefoils and the budonization stage + NPK of 300 kgha- 1. During full flowering, the basic parameters of soil biogenicity, the total number of microorganisms (TNB), the number of azotobacter (AZB) and actinomycetes (ACT) were determined. At the end of the growing season, the grain yield was measured. The results showed that factor A had a very significant influence on all the examined traits. Application of EM had a significant impact on all investigated parameters. The parameters of soil biogenicity in Variant 4 EM+NPK were statistically significantly (p<0.01) higher than the control and Variant 3. Variant 2 had a greater number p<0.01 compared to the control, while in relation to the long varieties, the significance was p<0.05. Factor C significantly influenced the examined parameters. The highest number of all tested parameters was determined in the Rubin genotype's rhizosphere. The obtained results were compatible with the yield level. The variety Rubin had the highest grain yield of 4105.03 kgha-1. Variant 2 with EM increased the yield by 13.29% compared to the control, which was at the level (p<0.05), and with the application of EM+NPK variant 4, the yield was higher by 15.95%, which was at the (p<0.01) level of significance.

Keywords: Effective Microorganisms, Soybean, Soil Biogenicity, Yield.

Received: 26 July 2024 * **Accepted:** 03 December 2024 * **DOI:** https://doi.org/10.29329/ijiaar.2024.1109.5

* **Corresponding author:**

Marija Bajagić is an assistant professor at the Faculty of Agriculture, University of Bijeljina, Bosnia and Herzegovina. Her research interests include the Field crops, Biotechnology and Organic agriculture. She has lived, worked, and studied in Belgrade, Serbia.
Email: bajagicmarija@yahoo.com

INTRODUCTION

The development of sustainable agriculture intensifies research into the application of various groups of microorganisms that play a role in the preventive protection and supplementary nutrition of plants. In modern research, preparations with different groups of microorganisms are increasingly being used to provide good conditions for achieving stable yields while preserving the quality of the soil. Cho and Koyama (1997) reported that microorganisms are small units of life that are too small; they are found everywhere in nature and play an important role in maintaining the ecological balance. Effective microorganisms are mixed cultures of beneficial organisms found in nature that can be applied as inoculants to increase the microbial diversity of soil ecosystems.

The application of effective microorganisms (EM) is today the subject of many researches in the world (Higa, 2001; Sabeti et al., 2017). Effective microorganisms (EM) represent a good combination of free aerobic and anaerobic microscopic organisms found in nature. The main types of microorganisms that are represented in preparations with EM are lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus casei*, *Streptococcus lactis*), photosynthetic bacteria (*Rhodopseudomonas palustris and Rhodobacter sphaeroides*), yeasts, actinomycetes and various types of fungi. These types of microorganisms produce a large amount of amino acids, enzymes, hormones, antibiotics, vitamins and other bioactive compounds. These substances are directly taken up by plants (Kim and Lee, 2000; Ranjith et al., 2007) and can be substrates for bacteria and for increasing the diversity of microflora in the soil.

Himangini et al. (2019) noted that the presence or absence of these beneficial microorganisms in any soil system precisely differentiates "living" from "dead soil." A healthy soil ecology has the ability to protect plants from soil-borne diseases caused by pathogenic microorganisms and parasites. The soil system offers this protection through a balanced relationship between pathogenic and billions of beneficial microorganisms working together in synergy.

Using effective microorganisms (EM) can reduce the amount of chemical fertilizers, increase plant yield parameters, as well as the amount of biologically active substances in fruits and seeds. According to the research of Sabeti et al. (2017) the application of EM in the soil affected the reduction of the negative effect of salinity on the morphology of corn roots. They also determined that applying EM to the soil and foliar treatment of sweet corn can increase root dry mass, root volume and shoot length.

According to Javid (2006) foliar application of EM with the use of NPK fertilizers in the production of peas (*Pisum sativum* L.) increases the number of nodules on the roots of peas by 217%, and the biomass of nodules by 167%. Also, it was determined that fertilization with effective microorganisms and NPK fertilizers increased grain yield by 126%, and in combination with organic fertilizer, the increase in grain yield was 145%. The same author determined that by applying EM in wheat production, grain yield can be increased by 27% compared to production where only chemical fertilizers were applied. Similarly, Xiaohou et al. (2001) state that spraying with effective microorganisms can increase the yield and quality of various crops. Adding photosynthetic bacteria to the soil increases the content of other effective microorganisms, e.g. mycorrhizal fungi. The increase of mycorrhizal fungi in the rhizosphere is due to more available nitrogenous compounds (amino acids) which are secretions of photosynthetic bacteria. These fungi can coexist with nitrogen-fixing bacteria, increasing nitrogen-fixing ability and grain yield.

Soybean is a plant species that has extremely great economic and agrotechnical importance, so it has been the focus of many researches in recent decades. The application of symbiotic bacteria from the Rhizobium family when growing soybeans has become a mandatory measure. Bacterial species such as (*Bradyrhizobium japonicum*, *Bradyrhizobium elkani* and *Sinorhizobium fredii*) in the process of biological nitrogen fixation are capable of providing up to 70% of the nitrogen required for optimal soybean production (Martinez Romero and Caballero Mellado, 1996). The other 30% of nitrogen needs must be provided in a way that respects the principles of good agricultural practice and preserves the quality of the soil. One of the basic conditions for good soil quality is an increase in the diversity of the soil microbial population. By increasing the total number of microorganisms as well as certain physiological groups, good conditions are created for achieving a high and high-quality yield.

The main goal of the work was to determine the impact of EM application on soil biogenicity parameters in the soybean rhizosphere and the height of grain yield in three-year research.

MATERIALS and METHODS

Experimental research and planting

The research was carried out in the period 2016-2018 on chernozem type soil. The experiment was set up according to the design of a split-plot experiment on an area of 2475 m^2 on the experimental field Rimski Šančevi at the Institute of Field and Vegetable Crops in Novi Sad. The size of the basic plot was 15 m^2 (six rows of soybeans with an inter-row distance of 50 cm and a row length of five meters). For the analysis of the examined parameters, four central rows were used. In the experiment, 3 factors were set in four repetitions. All agrotechnical measures were implemented in the optimal terms and correctly.

Factor A: years 2016-2018 (2016 was the most optimal weather conditions for soybean production; 2017 was a year with a pronounced dry period and very high temperatures, which is unfavorable for soybean production, and 2018, which was favorable for soybean production).

Factor B: genotypes of different ripening groups of soybeans, selection of the Institute of Crop and Vegetable Production Novi Sad (1. Galina, 0 ripening group, length of the vegetation period of 119 days, 2. Sava, I group of ripening, length of the vegetation period of 123 days, 3. Rubin, II ripening group length of vegetation period 133 days)

Factor C: fertilization with the following variants:

1. Control, i.e. variant without application of NPK fertilizers and EM Aktiv preparation,

2. Variant with the application of effective microorganisms (EM) in a liquid preparation with the commercial name EM Aktiv (application to the land before sowing in the amount of 20 liters per hectare and two foliar treatments in the phase of plant development from three to four trefoils and the budonization phase, i.e. the appearance of buds on soybean tree in the amount of 5 lha^{-1}).

3. Variant with the application of NPK fertilizer during the basic tillage in autumn, fertilizer formulation 8:15:15, quantity 300 $kgha^{-1}$.

4. Variant with the application of NPK fertilizer, formulation 8:15:15, in the amount of 300 $kgha^{-1}$, during the basic cultivation of the soil in autumn and the application of effective microorganisms in the form of the preparation EM Aktiv 5 lha^{-1} (incorporation into the soil before sowing in the amount 20 liters per hectare and two foliar treatments during the growing season - at the stage of plant development of three to four trefoils and in the budonization phase, before the flowering of soybeans in the amount of five liters per hectare.

The sampling

In the phenophase of full flowering - R_2, rhizosphere soil samples were taken 0.5 cm from the central root and root hairs. Samples were taken from the rhizosphere of three plants from the central rows. Microbiological analyzes were carried out in the Department of Microbiology, Department of Soybean at the Institute of Field and Vegetable Crops in Novi Sad. In the samples, the total number of bacteria (TNB x 10^{-7}g soil), azotobacter (AZB x 10^{-2} g soil), and actinomycetes (ACT x 10^{-4} g soil) were determined by the method of agar plates. Appropriate nutrient media were used (Hi Media Laboratories Pvt. Limited, Mumbai, India): nutrient agar for the total number of bacteria, synthetic agar for the number of actinomycetes, and azotobacter medium with mannitol for the number of azotobacter.

At the end of the growing season, the harvest was carried out and the grain yield was measured and converted to 14% moisture.

Weather conditions

During the growing season, soybeans require higher amounts of moisture in the first stages of development, and in the later stages from flowering to ripening, a good distribution of precipitation is necessary. Data on temperatures and precipitation were taken from the website of the Republic Hydrometeorological Institute for the meteorological station "Rimski Šančevi" (http://www.hidmet.gov.rs/ciril/meteorologija/klimatologija_godisnjaci.php).

Based on the measurements, it was determined that the mean monthly temperatures for the growing season of soybeans in the research years had higher values by 1.1°C in 2016, 1.6°C in 2017 and 2.5°C in 2018. compared to the multi-year average (18.1°C) (Table 1). Precipitation was higher in 2016 by + 75.6 lm^{-2} and in 2018 by + 60.9 lm^{-2} compared to the multi-year average (375.0 lm^{-2}), while in 2017 a deficit was recorded - 58.5 lm^{-2} of precipitation during the soybean vegetation period. The highest amount of precipitation per month was in 2016 (the most favorable conditions for soybean production).

Table 1. Average monthly temperatures (°C) and total precipitation (lm^{-2}) for the soybean growing season 2016-2018.

Month	Average monthly temperatures (°C)			Long-term average 1964-2015	Average monthly precipitation (lm^{-2})			Long-term average 1964-2015
	2016	2017	2018		2016	2017	2018	
April	14.2	11.4	17.2	11.7	74.5	57.0	49.0	46.9
May	16.9	17.6	20.4	17.0	85.0	82.9	64.2	67.1
Jun	21.7	23.2	21.5	20.0	143.2	65.7	163.2	86.5
July	22.8	24.3	22.0	21.7	68.4	12.0	81.2	67.4
August	21.1	24.8	24.0	21.2	45.8	17.4	51.2	59.3
September	18.5	16.9	18.5	16.9	33.7	81.5	27.1	47.8
Average/Sum	**19.2**	**19.7**	**20.6**	**18.1**	**450.6**	**316.5**	**435.9**	**375.0**

Source: http://www.hidmet.gov.rs/ciril/meteorologija/klimatologija_godisnjaci.php

Statistical analysis

The data were statistically processed using STATISTICA 10 software. The significance of the difference between the applied treatments was determined using Fisher's LSD test.

RESULTS and DISCUSSION

The EM concept is based on the inoculation of mixed cultures of beneficial microorganisms into the soil where they shift the microbiological balance and create an environment that is favorable for plant growth and health (Goessler and Kuehenelt, 2002). By introducing a large group of EM into the soil, their dominance over the autochthonous population is enabled, and the microbial-ecological balance in the soil is encouraged.

The dynamics of the total number of microorganisms and certain systematic and physiological groups is a variable value and depends on a number of factors (soil structure, presence of toxicants and anthropogenic influence). In agricultural soils, fertilizers greatly affect the function and structure of the microbial community (Cinnadurai et al., 2013), organic fertilizers significantly affect the accumulation of bacteria and the increase of biomass in the soil (Murugan and Kumar, 2013), while mineral fertilizers significantly affect the reduction of activity and abundance in the microbial community (Cvijanović et al., 2007).

Analysis of variance showed that the main sources of variation (year, genotype and fertilization) as well as their interactions had a significant impact on the examined parameters of soil biogenicity. The year as a factor had a statistically significant effect on the investigated parameters at the level of $p<0.01$.

In 2017, the statistically lowest abundance of TNB (97.36 x10^{-7}) (Table 2) AZB (125.79 x 10^{-2}) (Table 3) as well as actinomycetes (22.06 x 10^{-4}) (Table 4). If 2016 is taken as a reference year, because it was the most favorable for soybean production, a significant decrease in the number of microbes in 2017 was determined (TNB by 59.64%, AZB by 11.5%, ACT by 55.33%), because it was the biggest rainfall deficit. In 2018, the TNB was determined to be 159.16 x 10^{-7}, which was statistically significantly less than in 2016, although the most rain fell in June. The number of AZB was the highest in 2018 (150.76 x 10^{-2}), which was a statistically significantly higher number only compared to 2017 (142.12 x 10^{-2}), while compared to 2016 (142.12 x 10^{-2}) no significant difference was found. This ratio of the abundance of AZB in 2016 and 2018 is assumed to be the result of approximately the same values of measured temperature and amount of precipitation in June, when soil samples were taken. Unfavorable agrometeorological conditions in 2017 had a significant impact on the smaller number of ACT. The number of ACT in 2017 (22.06 x 10^{-4}) was statistically significantly lower ($p<0.01$) compared to 2016 (49.38 x 10^{-4}) and 2018 (43.87 x 10^{-4}). The number of ACT in 2016 and 2018 was different, but the differences were not statistically significant.

Factor C significantly influenced the change in abundance in the soybean rhizosphere. There were statistically significant differences between the variants where EM was applied in relation to the control and the application of NPK, while there was no statistical significance between the variants with EM. On average for all three years of research, TNB in the control was 136.85 x 10^{-7}, and with the application of NPK 126.03 x 10^{-7}. Both variants had a statistically significantly lower abundance than when applying EM in variant 2 (208.56 x 10^{-7}) and in variant 3 (197.91 x 10^{-7}). The number of AZB applications of EM in variant 2 (150.84 x 10^{-2}), as well as variant 3 with NPK (151.43 x 10^{-2}) was statistically highly significantly higher compared to the control (123.67 x 10^{-2}) ($p<0.01$). Variant 4 (143.78 x 10^{-2}) increased the number of Azotobacter compared to the control to a significance level of $p<0.05$, while in the comparison of variants 2 and 3 the number was lower but not statistically significant. The number of ACT on average for all years of research was the highest when applying EM in variant 2 (42.40 x 10^{-4}), which was statistically significant at the $p<0.05$ level). Other variants did not have a statistically significant effect on the change in the number of ACT.

Similar results were published by Cvijanović (2007) when examining the application of the mixture cultures of *Azotobacter chroococcum*, *A. vinelandi*, *Derxia sp.*, *Bacillus megatherium*, *B. licheniformis* and *B. subtilis* increases soil biogenity (total number of microorganisms and oligonitrophiles) and the number of the total number of microorganisms, number of oligonitrophiles, actinomycetes, azotobacter and fungi during the plant growing season. Cvijanović et al. (2018) confirmed that the application of EM can increase the abundance of the total number of microorganisms and azotobacter in the rhizosphere of wheat.

Table 2. Total number of microorganisms (TNB x 10^{-7} g soil)

Year (A)	Genotipe (B)	Fertilizer (C) 1.Control	2.EM Activ	3. NPK	4.EM Activ + NPK	x̄ AxB	x̄ A
2016	Galina	208.24	297.61	186.27	275.26	241.85	241.26
	Sava	198.63	264.55	172.19	272.82	227.02	
	Rubin	220.07	312.64	195.45	291.43	254.90	
	x̄ AxC	208.98	291.60	184.63	279.84		
2017	Galina	56.25	132.47	72.73	119.46	95.23	97.36
	Sava	68.49	129.61	65.09	120.77	95.99	
	Rubin	62.35	138.86	80.22	126.05	100.87	
	x̄ AxC	62.36	133.67	72.68	122.09		
2018	Galina	126.53	198.81	118.54	184.47	157.09	159.16
	Sava	130.67	185.49	122.22	167.11	151.37	
	Rubin	144.28	205.17	126.40	200.59	169.11	
	x̄ AxC	133.83	163.16	122.39	184.06	x̄ B	
x̄ BxC	Galina	130.34	209.63	125.84	193.06	164.71	
	Sava	132.59	197.65	119.83	186.90	159.24	
	Rubin	142.23	218.89	134.02	206.02	175.29	
	x̄ C	135.05	208.72	126.59	195.51		
		Average 2016-2018				**166.41**	
LSD	**A**	**B**	**C**	**A x B**	**A x C**	**B x C**	**AxBxC**
1 %	68.73	20.64	64.37	29.62	66.50	49.62	66.12
5 %	50.24	14.14	48.54	22.45	47.92	36.18	48.06

Table 3. The abundance of Azotobacter in the soybean rhizosphere (AZB x 10^{-2} g soil)

Year (A)	Genotipe (B)	Fertilizer (C) 1.Control	2.EM Activ	3.NPK	4.EM Activ NPK	x̄ AxB	x̄ A
2016	Galina	152.43	136.92	140.65	150.88	145.22	142.12
	Sava	86.25	145.38	97.36	201.24	132.56	
	Rubin	161.57	129.55	232.46	154.76	169.59	
	AxC	133.42	137.28	156.82	168.96		
2017	Galina	64.29	85.60	58.80	64.54	68.31	125.79
	Sava	116.24	92.49	108.28	145.81	115.71	
	Rubin	171.28	234.51	199.28	168.33	193.35	
	AxC	117.27	144.06	122.12	126.23		
2018	Galina	136.12	127.37	128.63	97.26	122.35	150.76
	Sava	100.60	132.54	192.53	167.69	148.34	
	Rubin	124.20	253.66	204.95	143.50	181.58	
	AxC	120.30	171.19	175.37	136.15	x̄ B	
x̄ BxC	Galina	117.61	116.63	109.36	104.23	111.95	
	Sava	101.03	123.47	132.72	171.58	132.25	
	Rubin	152.35	205.91	212.23	155.53	181.51	
	C	123.67	150.84	151.43	143.78		
		Average 2016-2018				**141.90**	
LSD	**A**	**B**	**C**	**A x B**	**A x C**	**B x C**	**Ax B xC**
1 %	24.8	44.86	25.17	40.29	27.44	37.61	34.82
5 %	17.52	33.18	18.64	29.71	20.29	27.78	25.72

The use of effective microorganisms (EM) as soil inoculants is based on the principles of natural ecosystems that are maintained such as: greater diversity and abundance of organisms, greater interaction and a more stable ecosystem. Thanks to them, a small contribution is made to apply these principles in agricultural soils and to change the microbiological balance in favor of increased growth, production and protection of plants (Higa, 1991). Effective microorganisms are not a substitute for other agrotechnical measures related to plants and soil. If used appropriately, they can significantly increase the beneficial effects of those measures (Higa and Wididana, 1991) and represent an additional dimension for optimal production.

Plant-microbe interactions through different mechanisms can positively affect plant growth (Moulin et al., 2001). Different types of organism's complement each other and are in a mutually beneficial relationship with plant roots in the soil ecosystem (Sun et al., 2014). Effective microorganisms increase soil fertility and promote growth, flowering, fruit development, ripening, crop yields and crop quality.

The effects of plant species are important variables in determining the bacterial composition of the rhizosphere. Plants, with their secretions, have a significant influence on the interaction with microbes from soil niches. Excretions contain sugars and acids, and the number and diversity of the rhizosphere microflora depends on their quantity. Depending on the mass of the roots, the amount of exudates that the plants secrete into the environment also depends. On average for all years of research and applied varieties, the highest total number was found in the rhizosphere of the Rubin variety (factor B) (175.29×10^{-7}), which is of the II ripening group and has the largest green mass. Compared to UB in the rhizosphere of the Sava variety (159.24×10^{-7}), the Rubin variety had a statistically significantly higher UB ($p<0.05$), while there was no statistically significant difference compared to the Galina variety. On average for the entire research period, the interaction B x C had the highest UB in the variety Rubin in fertilization variant 2 with EM (218.89×10^{-7}), which was also an increase of 53.89% compared to the control (136.89×10^{-7}). In variant 4, the increase was by 44.84%.

If it is observed by the years of research, in 2017 the biggest increase was determined by the application of EM in variant 2 (49.60%) and variant 4 (33.90%) in 2017. The obtained results are a significant indicator that the application of EM can reduce the negative impact of agroclimatic conditions. On average, the control variant had the lowest total number of microorganisms

The influence of the variety on the abundance of Azotobacter was greatest in the rhizosphere of the Rubin variety (181.51×10^{-2}). The determined abundance on average for all varieties in the 2016-2018 research period was statistically significantly higher ($p<0.01$) than in the Galina variety (111.95×10^{-2}) and the Sava variety (138.86×10^{-2}).

The abundance of AZ was the highest in the rhizosphere of the Rubin cultivar using NPK variant 3 (2012.23×10^{-2}), which is 39.30% more than the control (152.35×10^{-2}). EM in variant 2 (205.91 x

10^{-2}), increased the yield of AZ by 35.15% compared to the control, while variant 4 (155.53 x 10^{-2}) had the lowest percentage increase of 2.08% compared to control (155.53 x 10^{-2}).

In the interaction between variety and fertilization by year of research, it was determined that in 2016, the variety Rubin had the highest number with variant 3 (156.82 x 10^{-2}), in 2017 with variant 2 (234.51 x 10^{-2}). In 2018, the highest abundance of AZ was in the rhizosphere of the Rubin variety in variant 2 (253.66 x 10^{-2}).

The variety Rubin had the highest number of ACT in the rhizosphere (43.49 x 10^{-4}), which was statistically significant ($p<0.05$) with the number of ACT in the rhizosphere of the Sava variety (37.54 x 10^{-4}), but it was not significant with by the number of ACT in the rhizosphere of the Galina variety (39.41 x 10^{-4}). On average for all variants in the research, the Rubin variety had the highest value in 2018 in variant 2 with EM (56.86 x 10^{-4}) and the Galina variety in variant 4 (52.60 x 10^{-4}).

Table 4. Number of actinomycetes (ACT x 10^{-4} g soil)

Year (A)	Genotipe (B)	Fertilizer (C)					
		1.Control	2.EM Activ	3.NPK	4.EM Activ + NPK	X̄ AxB	X̄A
2016	Galina	48.23	52.23	45.50	50.97	49.01	49.38
	Sava	45.00	50.68	46.44	47.01	47.28	
	Rubin	52.82	54.00	49.50	51.17	51.87	
	X̄ AxC	48.68	52.30	47.15	49.72		
2017	Galina	15.40	20.66	17.39	21.23	18.67	22.06
	Sava	25.09	23.39	19.59	22.64	22.68	
	Rubin	24.36	27.38	22.44	25.20	24.85	
	X̄ AxC	21.62	23.81	19.81	23.02		
2018	Galina	48.67	50.20	46.98	52.60	49.61	48.65
	Sava	39.52	46.30	41.28	43.27	42.59	
	Rubin	50.94	56.86	51.62	55.63	53.76	
	X̄ AxC	46.37	51.12	46.63	50.50	X̄ B	
X̄ BxC	Galina	35.43	41.01	36.62	41.60	39.41	
	Sava	36.63	40.12	35.77	37.64	37.54	
	Rubin	42.71	46.08	41.19	44.00	43.49	
	X̄ C	38.25	42.40	37.86	41.03		
		Average 2016-2018				40.14	
LSD	A	B	C	A x B	A x C	B x C	AxBxC
1 %	21.77	6.36	5.26	8.18	6.11	8.03	9.33
5 %	16.34	4.73	3.89	6.08	4.50	5.93	6.62

The obtained results are compatible with the results of Sangakkara and Higa (1994) who studied the effect of EM on rhizobacteria (ie nodulation in beans) on soils with low and high rhizobacteria populations. The application of EM significantly increased the number of bacteria in the soil. Javaid et al. (2000) found a significant increase in the mycorrhizal colonization of *Vigna radiata* due to the application of beneficial microorganisms. For populations of beneficial microorganisms to be effective after inoculation, their initial population in the soil must be at a certain critical level. This ensures that the amount of bioactive substances they produce is sufficient to achieve the desired effect in production and/or protection in plants.

The average soybean yield in 2016 was 4700.32 kgha^{-1}, and in 2018 it was 4411.05 kgha^{-1}, and these values were statistically significantly higher than in 2017 (2993.14 kgha^{-1}) (Table 5).

Observing the average values for grain yield by individual varieties, it can be seen that the highest value was recorded for the soybean variety Rubin (4105.03 kgha^{-1}), which is statistically very significant ($p<0.01$) higher value compared to the yields of Sava varieties (3940.16 kgha^{-1}) and Galina (3999.12 kgha^{-1}). The average yield by fertilization variants shows that the highest value was recorded on the variant with the application of EM variant 4 (4240.32 kgha^{-1}), which is statistically very significant ($p<0.01$) a higher value compared to the control variant of the experiment (3657, 01 kgha^{-1}) by 15.95%. On variant 2 with the application of EM, the yield was (4143.23 kgha^{-1}), which is a significant increase ($p<0.05$) by 13.29% compared to the control. The application of NPK (4076.56 kgha^{-1}) did not statistically significantly increase the yield compared to the control. The obtained results are compatible with the number of microorganisms.

Table 5. Effect of EM on grain yield (kgha^{-1})

Year (A)	Genotipe (B)	Fertilizer (C) 1.Control	2.EM Activ	3.NPK	4.EM Activ + NPK	$\bar{x}$ AxB	$\bar{x}$ A
2016	**Galina**	4098.75	4461.94	4485.95	4597.14	4410.95	4700.32
	Sava	4223.51	4696.34	4658.83	4792.95	4592.91	
	Rubin	4651.84	5240.94	4963.52	5332.10	5097.10	
	$\bar{x}$ AxC	4324.70	4799.74	4769.43	4907.40		
2017	**Galina**	2442.34	2911.91	2796.86	2902.38	2763.37	2993.14
	Sava	2604.15	2799.39	2941.24	3067.14	2852.98	
	Rubin	2724.15	3610.89	3462.63	3654.63	3363.07	
	$\bar{x}$ AxC	2590.21	3107.40	3066.91	3208.05		
2018	**Galina**	3756.12	4185.37	4122.59	4228.10	4073.04	4411.05
	Sava	4060.04	4439.75	4433.50	4565.09	4374.59	
	Rubin	4352.21	4942.55	4823.96	5023.33	4785.51	
	$\bar{x}$ AxC	4056.56	4522.56	4460.02	4605.51	**$\bar{x}$ B**	
$\bar{x}$ BxC	**Galina**	3432.40	3853.07	3801.80	3909.21	3999.12	
	Sava	3629.23	3978.49	4011.19	4141.73	3940.16	
	Rubin	3909.40	4598.13	4416.70	4670.02	4105.03	
	$\bar{x}$ C	3657.01	4143.23	4076.56	4240.32		
		Average 2016-2018				**4118.17**	
LSD	**A**	**B**	**C**	**A x B**	**A x C**	**B x C**	**AxBxC**
1 %	536.42	397.48	552.14	612.23	572.61	607.08	682.01
5 %	380.20	288.69	398.47	446.82	408.16	440.22	524.62

Conclusion

Application of effective microorganisms can increase the total number of microorganisms, the abundance of azotobacter and actinomycetes. Increases in abundance are at the level of statistical significance $p<0.01$ compared to the control and application of only NPK fertilizers. Differences in the number of tested groups of microorganisms when applying EM and EM + NPK were not at the level of statistical significance. The highest percentage increase in the examined parameters was in 2017, which had the largest water deficit. The Rubin variety had the highest number of examined groups of microorganisms on average for the research period. Applying EM can influence a statistically significant increase in the number of microorganisms in conditions when agroclimatic conditions are unfavorable for soybean cultivation. By applying EM, the yield of grain can be increased by 13.29% by applying only EM, and in combination with NPK the increase was 15.95%.

REFERENCES

Cinnadurai, C., Gopalaswamy, G., & Balachandar, D. (2013). Diversity of cultivable *Azotobacter* in the semi-arid alfisol receiving long-term organic and inorganic nutrient amendments. *Ann Microbiol*, 1 63, 1397-1404.

Cho, H.K., & Koyama, A. (1997). *Korean natural farming: indigenous microorganisms and vital power of crop/livestock*. Korean Natural Farming

Cvijanović, G., Milošević, N., & Jarak, M. (2007). The Importance of Diazotrophs as Biofertilisers in the Maize and Soybean Production. *Genetika - Genetics*, *39* (3), 395

Cvijanović, G., Đurić, N., Marinković, J., Đukić, V., Dozet, G., Petrović, G., & Cvijanović, M. (2017). The influence of different ways of feeding wheat on rhizosphere microflora and wheat yield. *Proceedings of the PKB Agroeconomics Institute*, *23*(1-2), 95-104.

Fehr, W.R. & Caviness, C.E. (1977). *Stages of soybean development*. Iowa Agric. and Home Econ. Exp. Stn. Spec. Rep. 80.

Higa T. (1991). *Effective microorganisms*: A biotechnology for mankind.p. 8-14. In J.F. Parr, S.B. Hornick, and C.E. Whitman (ed.) Proceedings of the First International Conference on Kyusei Nature Farming. U.S. Department of Agriculture, Washington, D.C., USA.

Higa, T. & Wididana, G.N. (1991). *Changes in the soil microflora induced by Effective Microorganisms*., 153-162. In J.F. Parr, S.B. Hornick, and C.E. Whitman. (ed.) Proceedings of the First International Conference on Kyusei Nature Farming. U.S. Department of Agriculture,

Higa, T. (2001). Effective Microorganisms in the context of Kyusei Nature Farming: a technology for the future. Paper presented at: *Sixth International Conference on Kyusei Nature Farming Pretoria*, South Africa.

Himangini, J., Somduttand, Piyush, C., & Mundr, S.L. (2019). Role of Effective Microorganisms (EM) in Sustainable Agriculture *Int. J. Curr. Microbiol. App. Sci*, 8(3), 172-181.

Goessler, W. & Kuehenelt, D. (2002). *Analytical methods for the determination of arsenic and arsenic compounds in the environment*. In: William T, Frankenberger Jr, 27-50.

Jahanban, L. & Lotfifar, O. (2012). Study of the effective organism (EM) application effect on efficacy of chemical and organic fertilizers in corn cultivation (*Zea maizs*. C704). *Plant Production Technology*, 11, 43-52.

Javaid, A. (2006). Foliar application of effective microorganisms on pea as an alternative fertilizer. *Agron Sustain Dev*, 26, 257–262.

Javaid, A., Bajwa, R., Rabbani, N., & Uzma, M. (2000). EM and VAM Technology in Pakistan. IX: Effect of EM application on growth, yield, nodulation and VA mycorrhizal colonization in *Vigna radiata* (L) Wilczek. *Pak J Biol Sci,* 3, 694-698.

Kim, J.K. & Lee, B.K. (2000). Mass production of *Rhodopseudomonas palustris* as diet for aquaculture. *Aquacult Eng,* 23, 281-293.

Ranjith, N.K., Sasikala, C., & Ramana, C.V. (2007). Catabolism of l-phenylalanine and l-tyrosine by *Rhodobacter sphaeroides* OU5 occurs through 3, 4- dihydroxyphenylalanine. *Res. Microbiol*., 158, 506–511.

Martinez-Romero, E. & Caballero-Mellado, J. (1996). Rhizobium phylogenies and bacterial genetic diversity. *Critical Rev. Plant Sci*., 15, 113-140.

Moulin, L., Munive, A., Dreyfus, B., & Boivin-Masson, C. (2001). Nodulation of legumes by members of the beta-subclass of Proteobacteria. *Nature*, 411: 948–950

Murugan, R. & Kumar, S. (2013). Influence of long-term fertilisation and crop rotation on changes in fungal and bacterial residues in a tropical ricefield soil. *Biol Fertil Soils*, 49, 847-856.

Priyadi, K., Hadi, A., Siagian, T.H., Nisa, C., Azizah, A., Raihani, N., and Inubushi, K. (2005). Effect of soil type, applications of chicken manure and effective microorganisms on corn yield and microbial properties of acidic wetland soils in Indonesia. *Soil Science & Plant Nutrition* 51, 689-691.

Sabeti, Z., Armin, M., & Vaezi, K.M.R. (2017). Investigation of effective microorganisms application method on alleviation of salt stress effects on root morphology of sweet corn. *Farming and vegetable growing*, *54*(2), 48-55. https://doi.org/10.5937/ratpov54-12469

Sangakkara, U.R. & Higa, T. (1994). Effect of EM on the growth and yield of selected food crops in Sri Lanka. In: Parr JF, Homick SB, Simpson ME (eds) *Proceedings of the 2nd International Conference on Kyusei Nature Farming*, USDA, Washington, DC, 118–124.

Sun, P.F., Fang, W.T., Shin, L.Y., Wei, J.Y., Fu, S.F., & Chou, J.Y. (2014). Indole-3- Acetic Acid-producing yeasts in the phyllosphere of the carnivorous plant *Drosera indica* L. *Plos one 9*(12), e114196, https://doi.org/10.1371/journal.pone.0114196.

Xiaohou, S., Diyou, L., Liang, Z., Hu, W., & Hui, W. (2001). Use of EM-tech-nology in agriculture and environmental management in China, *Nat. Farm. Environ*. 2, 9-18.