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EM BIOTECHNOLOGY - THE FUTURE IN SUSTAINABLE AGRICULTURAL PRODUCTION

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Abstract: Due to environmental degradation environment, the consequences of climate change, and the need to produce food with high nutritional value, there is an increasing number of research whose results highlight the importance of applying a large group of microorganisms known as effective microorganisms or EM biotechnology. Biopreparations containing EM are widely used in sustainable agriculture. This versatile potential for using effective microorganisms results from their high enzymatic specificity, which enables them to survive in different environments. EM do not have a withdrawal period, making them suitable for use in sustainable agricultural production systems. The use of EM preparations has been intensive worldwide since the1980s. In Serbia, research is being conducted on the application of biopreparations with EM in sustainable agricultural production systems in both farming and vegetable growing. The aim of the research is to highlight the importance of introducing this group of microorganisms into integrated wheat production. Wheat is one of the most important plant species in human nutrition. This paper presents the results of the impact of applying biological preparations with EM on soil biogenicity parameters in the rhizosphere and the productive properties of different wheat varieties. Based on the presented results, it can be concluded that EM biotechnology is a safe way to fulfill the principles of sustainable agricultural production in this case of wheat.

Keywords: Effective EM microorganisms, Sustainable agriculture, Wheat

INTRODUCTION

Management strategies in sustainable agricultural systems aim to reduce the input of chemical fertilizers, pesticides and energy demand in general, the main goal of which is to protect the environment and reduce the impact on climate change. In systems of sustainable agricultural production, it is necessary to apply diversified crop rotations, integration of livestock and crop production, a greater share of legumes such as seed crops, intercrops or intercrops in rotation, organic fertilization and integrated pest and disease management. Knowing that natural resources are limited, it is necessary to develop sustainable methods of food production to ensure food security for an ever-growing population (Gabardo et al., 2020; Macoski et al., 2021; Clock et al., 2021). Microbes have a significant place in sustainable methods aimed at promoting environmental protection, agricultural biotechnology and more efficient treatment of agricultural and municipal waste. In order to stimulate natural biological cycles (cycles of biogenic elements) in practice, as a mandatory measure, different groups of microbes are used as biofertilizer.

The group of biofertilizers includes microbes that have the ability to fix elemental nitrogen (*Rhizobium*, *Azotobacteraceae*), then those that release phosphorus (*Bacillus megaterium vr. phosphaticus*) and potassium (*Bacillus circulans*) into the soil from organic matter, making these elements available to plants, and groups of microbes that prevent the development of phytopathogenic microorganisms (Boligłowa et al., 2005, 2008).

In the modern directions of agricultural production, the most important role is played by inoculants with microbes that can be used as biofertilizers, biostimulators and biopesticides. In this sense, a new concept is emerging EM biotechnology with effective microorganisms (EM).

Effective microorganisms are mixed cultures consisting of photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes and fermentation fungi. The concept of EM biotechnology was developed by Professor Thibault Higa in the 1980s as a need to reduce organic waste in the environment and protect the soil (Higa, 1994). These microbes are classified into large functional groups such as: photosynthetic bacteria, lactic acid bacteria, yeast group, actinomycete group and fungi. This technology is part of the National Program in 140 countries in the world, where they have proven to be the most appropriate way to replace traditional measures in plant nutrition and protection and improving soil quality. Thanks to EM biotechnology, farmers around the world can increase the quantity and quality of crops without impacting the environment (Paśmionka et al., 2015; Vaitkevičienė et al., 2021; Bajagić et al., 2024). Interest in the use of EM in agricultural practices has grown significantly in recent years both in promoting plant growth and in the biological control of plant pests and diseases. They also represent potential substitutes for chemical products and therefore can contribute to the preservation of the environment (Domenico, 2019). They have found their application in areas of agriculture such as soil regeneration, crop production, livestock production, agri-food industry and storage. Such a versatile potential of using effective microorganisms is a consequence of the high enzymatic specificity of microorganisms, which allows them to survive in different environments. The use of EM biorechnology in farming meets Global GAP standards, preserves soil quality and respects the principles of economy and ecology that are part of the EU National Program for Sustainable Development.

Concerns about environmental problems, climate change created by agricultural production processes, are increasing the need for research that will indicate the real benefits of EM for plants and soil (Golec et al., 2007; Mayer et al., 2010). The lower efficiency of chemical products, the greater occurrence of resistant diseases and pests, and the rising costs of production have driven the market for biological inputs (De Melo et al., 2021). New research results are always needed and have helped improve production, formulation, storage and field application techniques, both small and large scale. The use of preparations with EM has long been tested in Japan, China, Malaysia, Russia, Poland, the Czech Republic, Romania, while the biggest user of preparations from EM biotechnology is Brazil, where effective microorganisms have proven to be the most suitable way to replace traditional management methods with natural methods. They have found application in the form of intercrop spraying, after harvest residues, manure mineralization, for the treatment of seeds, tubers and rhizomes, and directly or foliarly on plants (Pszczółkowski et al., 2018; Wu et al., 2013; Cvijanović et al., 2024). Significant production effects are also observed when using preparations with EM for the preparation of high-quality fertilizers, compost and plant extracts as strong nutrients (Huk, 2009).

Advantages of Applying EM Biotechnology in Plant Production

Effective microorganisms play a major role in the preparation of manure. Manure is a mixture of substances of organic origin from plants and animals. In its fresh state, it is not important for use but must be subjected to the fermentation process. Adding EM directly to the manure prevents the spread of unpleasant odors of ammonia and trimethylamine, which are produced in the fermentation process, and reduces the presence of harmful insects. Gunawan et al. (2020) determined that the application of EM in the preparation of poultry manure can reduce the ripening time by 21 days, and that it affects the increase in the morphological characteristics of amaranth *(Amaranthus hybridus)*. Hamad et al. (2020) showed that adding preparations with EM in a concentration of 1% reduces the growth of pathogenic bacteria (*S. aureus* and *E. coli*). Safwat et al. (2018) determined that in the presence of EM in a concentration of 0.5-1%, the growth of pathogenic bacteria such as *Bacillus subtilis, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Streptococcus faecalis, Aspergillus flavus, Aspergillus niger, Candida albicans* and *Candida parapsilosis*. The primary role of EM in inhibiting the growth of pathogenic bacteria has not been precisely determined, but it is assumed to arise as a result of competition in the food medium with pathogenic organisms. Then the products of EM metabolism such as lactic acid act as sterilizers that inhibit harmful microbes (Vicente et al., 2007; Safwat et al., 2018). Reddy and Giller (2008) found that adding EM in squash and legume production can successfully control insects. The application of EM by spraying plants over the leaves several times during the growing season affects the increase in the nutritional properties of the fruits of lettuce, corn, wheat, and soybean plants (Stojanović et al., 2020: Stepić et al., 2022; Cvijanović et al., 2022a). Effective microorganisms are important in composting organic waste. Bastami et al. (2016) determined that during composting of organic waste at the beginning of the process, the temperature and the content of macronutrients NPK increase in the pile, which indicates a faster activity of total microbes. Gases such as methane, nitrogen and carbon oxides are released during these processes. The results of Joung-Soo's research (2015) showed that by adding EM to the composting mixture, the ammonia removal efficiency was 55.9%, which is very important from the aspect of reducing the production of greenhouse gases.

Considering the necessity of development and expansion of food production according to the principles of sustainable production, EM biotechnology offers the possibility of developing ecological methods of growing plants.

For the last few years, research has been conducted on the effect of applying EM biotechnology preparations in plant production in agroecological conditions in Serbia. The aim of the work is to point out the importance of the introduction of this group of microbes in the production of wheat, which is one of the most important plant species in human nutrition.

MATERIAL AND METHODS

The paper will present the results of the three-year research on the location of Padinska Skela (ΨN 44^0 56', λE 25^0 28') region Banat. The area of the experimental plot was 576 m^2, and the elementary plot was 5 m^2. The plots were laid out according to the plan of divided plots in four repetitions. The first crop was corn. All agrotechnical measures were applied in optimal terms. In the experiment, a liquid preparation with effective microorganisms EM Aktiv (trade name) was used (Figure 1). The preparation is registered and certified for organic production in Serbia and the EU. On the website of the Ministry of Agriculture, Water Management and Forestry of the RS, the preparation EM Aktiv is on the list of permitted and registered means for plant nutrition and soil improvers that can be used in organic agriculture (www.minpolj.gov.rs/organska/).

Figure 1. Application of preparations with EM in the foliar wheat crop

Different varieties of wheat (domestic and foreign) were used for sowing. Microbiological methods were used to determine the basic parameters of soil biogenicity (total number of microorganisms and abundance of azotobacter) in the rhizosphere of wheat varieties Talas, Simonida, NS-40 and Sirtaki. The total number of microorganisms was determined on soil agar dilution 10^{-7}, and the number of azotobacter on Fyodor's medium by the method of fertile drops dilution 10^{-1}. Generative properties, weight of 1000 grains and yield, were determined in Ratarica, Pobeda, Nogal and Apache varieties. The characteristics of the soil are given in Table 1. The soil is slightly alkaline in KCl to slightly alkaline reaction in H_2O. According to the content of $CaCO_3$, it belongs to the low carbonate group. Medium is provided in humus, and the content of phosphorus and potassium is higher than the optimal values (15-20 mg/100 g optimal for agricultural production).

Table 1. Agrochemical properties of soil on experimental plots

pH		$CaCO_3$ (%)	Hummus (%)	Total inorganic nitrogen mg /kg	P_2O_5 mg/100 g	K_2O mg/100 g
KCl	H_2O					
6.23	7.60	1.07	2.33	6.98	21.45	34.58

RESULTS AND DISCUSSION

The foundation that maintains the food chain is the soil in which different types of microbes live. Based on this concept, it was determined that the use of EM contributes to the natural strengthening of the productive capacity of the soil. Souza et al. (2015) state that EM biotechnology can be used successfully in soil revitalization, because they "clean" it from various toxicants and make the soil richer in vital energy. By applying beneficial microorganisms, the soil retains more water, which means more successful plant production in periods of drought or when growing on sandy soil. This improvement is a consequence of the increase of organic matter in the soil, the reduction of porosity, due to microbial activity, and the ionic balance, which favors the interaction of surface charges of the physical structure of the soil with ionic charges (Hoyos et al., 2008). When they are introduced into the soil, directly or through seeds, they have a positive effect on increasing the microbial biodiversity in the soil, whereby the nutrients from the soil become more quickly available to the plants, because they accelerate the mineralization processes of organic matter. Microbial preparations with EM, in addition to living cells of different groups of microbes, contain amino and humic acids, vitamins, hormones, enzymes and antibiotics that are the result of metabolic processes of the microbes themselves.

On the plot where soil biogenicity parameters were determined, basic fertilization with complex NPK fertilizer in the amount of 400 kg ha^{-1} (15:15:15) was applied in the autumn before sowing. In the spring, supplementary fertilization was carried out with organic pelleted poultry manure in the amount of 1750 kg ha^{-1} (4:4:4) (F_1). Poultry manure was treated with a biopreparation that was diluted with water in a ratio of 1:10 (F_2). In the control variety (F_0), top dressing was with 150 kg ha^{-1} Urea (46 N%). The basic indicators of the condition of the soil, the total number of microorganisms (TNM) and the abundance of azotobacter (Az) in the rhizosphere of wheat in the earing phenophase, were monitored. According to Mićanović (1997), in the rhizosphere of wheat, depending on the genotype of the plants, the largest number of microorganisms was found in the wheat heading stage. The total number of microorganisms in the soil has long been taken as a reliable indicator when assessing the condition of the soil. The abundance and composition of the total microflora is higher in soils with a higher content of organic matter and optimal agrochemical properties. The tested varieties had a high influence on the variability of the total number of microorganisms in the soil, and the differences obtained were statistically very significant. 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 efficiency of applied EM was statistically highly significant (Table 2). The total number of microorganisms in the F_2 variant was 75.26% higher than in the F_1 variant and 139.47% higher than in the F_0 control. The results indicate that the autochthonous microbial population has been activated. It is assumed that there was a competition for food of introduced EM microorganisms with the autochthonous population in the rhizosphere microbiome, but also that there was a greater production of substances of microbiological origin that can be food for different types of microbes.

Table 2. The total nomber microorganisms in the rhizosphere of tested wheat varieties depending on the method of fertilization CFU ml^{-7} g^{-1} of absolutely dry soil

Variety (A)	**Fertility B**			**□A**	**Factor**	**LSD**	
	F_0	**F_1**	**F_2**			**5%**	**1%**
Talas	34.52	66.94	70.61	57.35			
Simonida	37.84	60.56	86.65	61.68	A**	1.39	1.89
NS 40S	28.01	60.63	88.53	59.05	B**	4.20	6.63
Sirtaki	37.44	53.37	84.24	58.35	A x B**	12.40	13.27
□ B	**34.45**	**60.38**	**82.50**	**59.11**	CV (%) = 2.33		

Azotobacter is a very important group of bacteria that fix atmospheric nitrogen and translate it into forms available to plants. This group of bacteria is very sensitive to all changes that occur in the soil and reacts with its abundance and life activity. Okon and Kapulnik (1986) determined that some species of Azotobacter can be found on the roots of wheat in the early stages of development, even in the tissue of the roots of wheat at the site of formation of the knot of bokorenia. That is why it is very important to apply methods that increase the number and activity of this group of bacteria. The variability of the number of Azotobacter was highly significant in varieties, in the application of fertilizers and in the relationship between these two factors. A statistically significant increase in Azotobacter was determined by EM application from 21.78% compared to the control, to 5.44% compared to the variant without EM application (Table 3).

Table 3. The abundance *Azotobacter* in the rhizosphere of tested wheat varieties depending on the method of fertilization CFU ml^{-1} g^{-1} of absolutely dry soil

Variety (A)	**Fertility B**			□ **A**	**Factor**	**LSD**	
	F_0	F_1	F_2			**5%**	**1%**
Talas	113,6	151.6	173.7	146.3			
Simonida	142.9	157.7	167.3	162.5	A**	3.75	5.10
NS 40S	116.6	133.3	123.8	124.6	B**	3.25	4.41
Sirtaki	89.26	91.4	98.26	92.98	A x B**	6.50	8.83
□ **B**	**115.59**	**133.50**	**140.77**	**129.95**	CV (%) = 2.89		

In the second part of the experiment, where the varieties Ratarica, Pobeda, Nogal and Apache were grown, EM was applied twice during the growing season (in the phenophase of budding and before earring). Fertilization with 400 kg ha^{-1} NPK (15:15:15) before sowing in autumn was carried out as a source of nutrients for the plants. In the spring, during the budding phenophase, top dressing with urea (46%) was carried out, namely 150 kg ha^{-1} (N_0), 150 kg ha^{-1} (N_1) and 100 kg ha^{-1} (N_3). In variants N_2 and N_3, EM was foliarly applied in the amount of 6 l ha^{-1}. The productive properties of plants with a weight of 1000 grains and grain yield were determined. In wheat production, it is very important to achieve a large weight of 1000 grains. If the weight of 1000 grains are greater, the germination, budding, longer ears and yield are higher. With an increase in the weight of 1000 grains, the resistance of the variety to unfavorable agrometeorological conditions increases, and the quality of grinding wheat grains is better. Using EM, a significant increase in the weight of 1.000 grains ($p<0.05$) was determined compared to the control and the variant in which the amount of urea was reduced. However, no statistically significant difference was found between the control N_0 and the variant N_2. The weight of 1000 grains were higher than the control by 1.83% and compared to the N_2 variant by 1.68% (Table 4).

Table 4. The influence of EM on the weight of 1000 grains (g)

Variety (A)	**Fertility B**			□ **A**	**Factor**	**LSD**	
	N_0	N_1	N_2			**5%**	**1%**
Ratarica	41.65	41.17	41.87	41.56			
Pobeda	43.18	44.10	43.58	43.62	A**	1.13	1.72
Nogal	37.43	38.99	38.58	38.33	B*	0.50	1.07
Apač	39.04	40.04	39.53	39.53	A x B*	1.20	1.56
□ **B**	**40.33**	**41.07**	**40.39**	**40.76**	CV (%) = 7.99		

The obtained results are compatible with the results of Jalil et al. (2017) who show that it is possible, depending on the characteristics of the variety, to reduce the amount of mineral nitrogen by using biopreparations with effective microorganisms. Different types of microorganisms that make up EM produce different substances such as: organic acids, plant hormones (gibberellins, auxins and cytokinins), vitamins, antibiotics and polysaccharides. All these substances directly or indirectly have a positive effect on plant growth. They improve the photosynthetic capacity, applied foliar has the effect of supplementary fertilization, and eliminate the use of pesticides and increases the resistance of plants to oxidative stress, which has a positive effect on grain yield. According to the research conducted, it was determined that the yield increase in the N1 variant ranged from 6.84% (N_0) to 2.43% (N_2), which was at the level of ($p<0.01$) (Table 5).

Table 5. Yield of wheat grain (kg ha^{-1})

Variety (A)	Fertility B			□ A	Factor	LSD	
	N_0	**N_1**	**N_2**			**5%**	**1%**
Ratarica	6.220	6.447	6.534	6.400			
Pobeda	6.340	6.852	6.452	6.548	A*	0.66	0.96
Nogal	5.877	6.241	5.957	6.025	B*	0.23	0.36
Apač	6.269	6.857	6.826	6.650	A x B	0.44	0.64
□B	**6.176**	**6.599**	**6.442**	**6.405,7**	CV (%) = 11.47		

With the use of microbiological agents as growth stimulators and agents in the biological control of diseases and pests in crops, we are in a new era of food production. EM biotechnology can contribute to intensive use, especially in sustainable production systems as well as in drought conditions. During the implementation of biocontrol measures and/or promotion of plant growth, favoring the preservation of the environment, they have been identified as a viable alternative for ecologically and economically sustainable systems of agricultural production. Knowing all the benefits of EM for plants, research is needed to demonstrate its effectiveness in different ways of application and in different agroecological conditions.

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