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INFLUENCE OF EFFECTIVE MICROORGANISMS ON BIOACTIVE SUBSTANCES IN DIFFERENT PLANT SPECIES

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Abstract

Due to the pronounced food needs of the growing population, inputs in agricultural production in the form of fertilizers, pesticides and mechanization of agriculture have increased, which has led to higher yields, but also numerous problems in the environment. Although agriculture is highly dependent on climate conditions, it also has a significant role in changing them. All of this has influenced the introduction of measures to replace chemical inputs and protect the health of people and ecosystems. One of the measures is the introduction of different microbiological products into the production technology. In order to test the effect of effective microorganisms in the preparation (EM Aktiv (trade name) on the characteristic bioactive substances of certain plant species, research was conducted on different genotypes: lettuce on the total antioxidant potential and vitamin C, wheat on the grain protein content and corn on the nitrogen content. Research has been carried out for many years in different agro-climatic conditions. Using preparations with effective microorganisms, it is possible to increase the content of biologically active substances in agricultural and vegetable crops and alleviate the stress of plants in unfavorable agro-meteorological conditions.

Key words: *effective microorganisms, lettuce, bean, wheat, corn, bioactive substances.*

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Introduction

Agricultural production has come a long way in development. In the 20th century, it experienced its two major transformations: the industrial and genetic revolutions. The result is the production of large amounts of food and a negative impact on ecosystem health and climate change.

Agricultural production has entered a major technological transformation. Taking into account the need to protect the basic resources for food production, reduce the production of greenhouse gases and produce food with high nutritional value, the concept of measures that can meet the requirements of sustainable food production is being developed. Sustainable food production implies changes in the plant production system. There is a growing need for the inclusion of biological agents in the control and protection of plants from waterborne diseases, as well as in plant nutrition. Knowing that natural resources are limited, sustainable methods of food production are essential to ensure food security for an ever-growing population.

In sustainable agricultural production, the application of so-called EM biotechnology preparations is of increasing importance. Currently, EM biotechnology is used in areas of agriculture such as environmental protection, soil regeneration, crop production, livestock production, agri-food industry and storage. The basis of this technology is a large group of microorganisms that are called effective microorganisms in the professional public. EM preparations contain a mixture of active strains of lactic acid bacteria, photosynthetic bacteria, actinomycetes, fungi, and yeasts in a medium made from sugar cane molasses that ensures low pH values. These groups of microorganisms have different functions, and there is a constant exchange of nutrients between them, which promotes their symbiosis for which there is no withdrawal period (Higa and Parr, 1994). They make a significant contribution in improving soil structure, stimulating growth and preventive plant protection, in livestock production, in agricultural and food technology for biological waste disposal (Gałązka et. al., 2015; Van Vliet et al., 2005).

EM biotechnology in agricultural plant production

In agricultural plant production, EM preparations can be applied within all technological measures of production. By applying them to the soil, soil regeneration is encouraged, sources of infection are eliminated or reduced, Higa (1998), and according to Cvijanović et al. (2019) encourage the development

of an indigenous soil microbiome. They participate in the synthesis of humus components, mineralization of organic plant residues and fertilizers in the soil, thereby releasing macro and macro nutrients for the plant. They produce plant hormones (gibberellins, auxins and cytkonins), vitamins, organic acids, antibiotics, polysaccharides that stimulate plant growth (Souza et al., 2015). They are recommended for the treatment of seeds, seedlings, tubers, foliar treatment of plants in vegetation, on stubble before plowing, in the production of high-quality biofertilizer from manure (Pszczółkowski and Sawicka, 2018). In addition, the application of EM in the process of composting solid communal and agricultural waste produces a high-quality soil regenerator and biofertilizer. The advantage of using these preparations is that they do not have a withdrawal period Higa et al., (1994), and they can be used in all physiological stages of plant development through flower leaves and fruits. The direct effect of the use of these preparations is in increasing the yield and biological values of the fruits of the plants (Sawick et al., 2019).

Today, in more than 140 countries on different continents, preparations with effective microorganisms are used in the development of methods for plant biodynamic and organic production (Pszczółkowski et al. 2023). Preparations with effective microorganisms have been tested in Japan, China, Malaysia, Russia, Poland, the Czech Republic, Romania and others. In Europe, this technology is increasingly prevalent at the level of individual farms, especially organic farms (Wu et al., 2013). In Serbia, research is being conducted on the impact of EM Aktiv preparations with effective microorganisms in the production of various plant species. The groups of microorganisms present in EM preparations are isolated from natural habitats. Depending on the origin of the insulation, the composition of the EM preparation also depends. Iriti et al. (2019) point out that lactic acid bacteria mostly predominate. These groups of bacteria have important biotechnological functions because they produce lactic acid that mineralizes organic forms of phosphorus, which is an important nutrient for plant nutrition.

Mechanisms of action of effective microorganisms on plant properties can be direct and indirect. Direct mechanisms imply the participation of microorganisms in the circulation of nutrients, production and synthesis of plant hormones. Indirect mechanisms include the synthesis of antibiotics, the production of sideraphores and enzymes. Direct and indirect mechanisms have a positive effect on seed germination, increasing plant resistance to stress caused by abiotic conditions and the attack of phytopathogenic organisms, in-

creasing plant biomass, increasing the morphological characteristics of plants important for yield, as well as the nutritional properties of fruits.

The use of effective microorganisms as a supplement or replacement for mineral fertilizers is an environmentally acceptable way of food production. Considering the growing demands of the market for food without residues of harmful active substances and with an increased content of bioactive substances, it justifies the application of effective microorganisms.

The influence of the application of effective microorganisms on the bioactive components in fruits

In the research conducted, a preparation with effective microorganisms EM Aktiv was used. EM Aktiv is a yellow-brown liquid with a pH of 3.0–3.5 that includes many strains of effective microorganisms. The microbiological composition of EM Aktiva is protected by patent law and is a trade secret. Therefore, no detailed information is given on the detailed composition of the preparation. This formulation is based on effective microorganism (EM) technology, which involves the use of a mixture of beneficial microorganisms such as different strains of lactic acid bacteria, yeast and other microorganisms to improve soil health, plant growth and overall ecosystem balance.

In several years of research on the application of EM Active in the production of different genotypes of lettuce (*Lactuca sativa* L.), increases in secondary metabolites in lettuce leaves were determined. The value of lettuce, as a low-calorie leafy vegetable, is related to the content of various biomolecules such as vitamins, terpenoids, carotenoids, polyphenols including phenolic acids and flavonoids. Determination of total antioxidant activity is one of the most important parameters from the aspect of food quality. EM active was applied in the production of different genotypes of lettuce in the spring, autumn and winter seasons. The preparation was applied to the soil before planting 4 times during the growing season in the recommended concentration. Antioxidant activity in lettuce leaves was increased by 63-68% on average, depending on the lettuce genotype and growing season, while the increase in vitamin C was 54-56% compared to the control variant. The application of the preparation led to a significantly higher content of vitamin C in spring and winter, while the content of vitamin C was significantly reduced in autumn. Such a response suggests the importance of applying the preparation in combination with varieties and growing season (Stojanović et al., 2022).

Considering the importance of beans in human nutrition, research was conducted on the application of preparations in the production of beans in the period from 2016-2017 in the Bačka Topola region of Vojvodina. Beans (*Phaseolus vulgaris*) are traditionally represented in the diet of people in Serbia. The annual consumption of beans per capita is 5.4 kg. Beans have a high energy value; they contain almost all essential amino acids. In addition, it contains lecithin, as well as potassium, calcium, iron, phosphorus, magnesium, zinc and sodium. It is an excellent source of protein, and unlike other sources, beans are low in fat, so they do not contain saturated fatty acids or cholesterol. As for vitamins, it is an excellent source of folic acid, vitamins B6, K, riboflavin (B2) and B3. Research was conducted with two varieties of beans (Maxa white and Zlatko yellow). The preparation EM Aktiv was introduced into the soil seven days before the beans were planted. Bean seeds were inoculated with compatible strains of nitrogen-fixing bacteria of the genus *Rhizobium*. During the growing season, two treatments with EM Active were performed (in the phenophase of 3-4 leaves and before flowering). An increase in protein content in the grain of both varieties of beans in different agrometeorological conditions was determined from 5.92-9.54% (Table 1).

Table 1. The content of total proteins (%) in the grain of different varieties of beans

Beans	Method of production	2014	2015	2016	Average	Deviation (%)
Maksa	Control	21,97	18,37	19,97	20,10	100
	EM Aktiv	23,26	19,45	21,15	21,29	5,92
Zlatko	Control	20,37	17,03	18,52	18,64	100
	EM Aktiv	22,32	18,67	20,29	20,42	9,54

Source: Dozet et al., 2021

Research on the application of EM actives in the wheat crop (*Triticum spp*.) was conducted in the period 2017-2019 at the location of Vojvodina-Padinska Skela. Nutritional and technological quality, price and adaptability to different environmental conditions have influenced that wheat is one of the main cereals in the world, in terms of production and consumption. Intensive urban, industrial technological development has influenced the mixing of modern and traditional sociological cultures and ways of eating, which contributed to increasing the use of wheat in the diet even in countries where wheat was not traditionally used. The consumption of wheat varies by region, but for the areas of the Republic of Serbia it is around 120 kg per inhabitant per year.

Along with corn and rice, wheat is the main source of plant proteins in human food. The protein content in wheat grain ranges from 8 to 11% in bread wheat and from 10 to 15% in durum wheat. Protein content is determined by both genetic and environmental factors (Altenbach, 2012).

Considering that wheat is present in the production of flour, the quality of flour and bread depends on the protein content. In addition to breeding as a method for improving the amount of protein in wheat grain, the application of different methods also has a significant effect.

In research on the application of the preparation EM Aktiv in wheat production, an increase in protein content in the grain of bread wheat genotypes (Ratarica and Pobeda) was determined. Plant nutrition was provided with 400 kg ha^{-1} NPK (15:15:15) in autumn, and 100 kg ha^{-1} Urea (N 46%) in the spring. The preparation was applied twice at 7 l ha^{-1} during the growing season (phenophase of leafing and flowering) as a supplemental nutrition. An increase in the content of total proteins was determined on average for all three years from 1.56% in the Ratarica variety to 3.98% in the Pobeda variety. The protein content was different depending on the agrometeorological conditions, but in each year of the research, an increase in the protein content was determined, and it can be concluded that the application of the preparation alleviated plant stress caused by abiotic factors (Table 2).

Table 2. The content of total proteins (%) in the grain of different genotypes of bread wheat

Bread wheat	Method of production	2017	2018	2019	Average	Deviation (%)
Ratarica	Control	13,02	13,79	13,40	13,40	100
	EM Aktiv	13,17	13,88	13,79	13,61	1,56
Pobeda	Control	13,19	13,40	13,29	13,29	100
	EM Aktiv	13,78	13,86	13,82	13,82	3,98

Source: *Cvijanović et al., 2022*

Maize (*Zea mays*) is a very dominant crop in the food industry, and in recent years also in the production of bioenergy. Predictions show that by 2025, corn production in the world will increase significantly, while the need for this crop will double in developing countries by 2050 (Rosegrant et al., 2008). Large amounts of nitrogen are necessary to achieve the genetic potential of maize fertility. In the literature, a large number of works can be found in which the influence of N in increasing yields and improving yield compo-

nents has been examined and confirmed. The amount of applied nitrogen has a linear correlation with the morphological characteristics of maize. That the application of nitrogen is specific is shown by the results of Latković et al., (2010), who determined that different doses of N (40, 80 and 120 kg ha^{-1}) influenced the increase in yield with increasing doses. But the question of economic and ecological justification arises. That is why, in addition to breeding, technologies are constantly being researched that would maintain production with the least negative impact on the environment. Previous research with the application of different groups of microorganisms has given positive results in terms of maintaining the biogenicity of the soil and the height of the yield (Cvijanović et al. 2019).

Considering the increasing presence of maize in human nutrition, research was conducted on the impact of EM Active on the nitrogen content in the grain of various maize hybrids grown in the Valjevo region in the period 2017-2018. Agrometeorological conditions in 2017 were unfavorable because there was a pronounced drought, while 2018 was more favorable for maize production. The application of the preparation was in two variants. In variant EM1, the preparation was applied twice during vegetation (in the phenophase of 5-7 leaves and after 15 days) 6 l ha^{-1}, and in variant EM2 it was applied to the soil 7 days before sowing (30 l ha^{-1}) and twice in during the growing season (in the phenophase of 5-7 leaves and after 15 days).

Nitrogen content was the interaction of hybrids and agrometeorological conditions in the examined years. In the test, both variants of the new preparation gave positive results of nitrogen content compared to the control. In conditions of drought, the application of EM active in both variants, an increase of nitrogen in the grain of 1.31-3.43% was determined, while in the year that was more favorable for maize production, the increase was 3.58-18.19% (Table 3). And in these researches, it was shown that in conditions of unfavorable agrometeorological conditions, the application of preparations with effective microorganisms is necessary and acceptable.

Table 3. Nitrogen content in the grain of different maize genotypes

Years	Hybrids	EM_0 (control)	EM_1	EM_2
2017	ZP427	1.297	1.116	1.514
	ZP548	1.293	1.709	1.444
	ZP 684	1.519	1.427	1.205
	Prosek	1.370	1.417	1.388
	Deviation (%)	100	3.43	1.31
2018	ZP427	1.285	1.376	1.849
	ZP548	1.454	1.345	1.304
	ZP 684	1.449	1.616	1.798
	Prosek	1.396	1.446	1.650
	Deviation (%)	100	3.58	18.19

Source: *Stepić et al., 2022*

Conclusion

Conducted research shows that there is a strong positive relationship between the tested characteristics of the fruits and the application of preparations with effective microorganisms.

The genotypes of the investigated plant species as well as the agrometeorological conditions, which significantly modified the content of the investigated traits, had a significant influence on the content of the investigated traits. In unfavorable agrometeorological conditions, it is possible to achieve a higher quality product by using effective microorganisms.

Further research should be focused on the examination of the combitable relationships of the prepastor with the genotypes of the plant species. It is also important to point out that agriculture and tourism are connected and mutually conditioned. The renaming of such preparations in rural households that are involved in some form of tourism could significantly contribute to the improvement of households.

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