

POSSIBILITY OF WHEAT PRODUCTION IN AN INTEGRAL SYSTEM

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ABSTRACT

The aim of this study was to determine the influence foliar application of the microbiological preparation (EM Aktiv) with a mixture of different groups of microorganisms (on different genotypes of wheat in sustainable agricultural production on the weight of 1000 grains and grain yield.) The research was conducted in the period 2016/2017-2018/2019 (factor A) in the region of Vojvodina, Serbia, which includes four varieties of wheat (factor B). Factor C three variants of nitrogen are provided for wheat nutrition: 129, 106 and 83 kg·ha⁻¹. During the vegetation, applications were performed with the microbiological preparation "EM Aktiv": F₀ 129 kg·ha⁻¹ without EM, F₁ 129 kg·ha⁻¹ + 1 x EM; F₂ 106 kg·ha⁻¹ + 2 x EM; F₃ 83 kg·ha⁻¹ + 3 x EM. At the end of the growing season, the weight of 1000 grains and the grain yield were measured. Factors A and B had an effect on the increase of the tested traits $p < 0.01$. The highest values were recorded in 2018/2019. Factor C affected an increase in the weight of 1000 grains from 0.14% (F₃) to 1.91% (F₁). The differences in grain yield were significantly influenced by treatments (C) ($p < 0.05$). The increase was from 1.91 % (F₃) to 4.76 % (F₁).

Key words: wheat, effective microorganisms, weight of 1000 grains, yield.

INTRODUCTION

At the beginning of the 21st century, great demands were placed on agricultural production. It is essential to meet the food needs of the growing population. The Food and Agriculture Organization of the United Nations (FAO) reports that compared to 2010 levels, global food production needs to increase by 70% by 2050 to feed the world's population, which is expected to reach between 9.4 and 10.2 billion (FAO 2009). According to Kovačević, (2011), food production in the world is 3%, and the rate of population growth is about 2%, so it can be said that the increase in food production follows the increase in population. This goal needs to be achieved in conditions where there is no increase in arable land, when the demand for water is increasing, in conditions of climate change, and even when soil health is under pressure. Since the previous concept of agricultural production had a lot of negative consequences on the environment and food quality, sustainable food production systems were introduced at the end of the last century.

This conditioned the development of new directions in food production such as sustainable and precise/smart agriculture. The concept of precision agriculture is based on the use of the Internet and information technologies that enable the prediction of demands from the environment, which are often complex and unknown, the introduction of digitization, robotics, and the use of GPS systems (Karadžić & Babić, 2005). This form of food production is not fast to be accepted, especially in rural areas, and sustainable agriculture is more acceptable. The term sustainable agriculture means an integrated system of plant and livestock production that will, in the long term: meet people's needs for food, improve the quality of the environment and preserve basic natural resources that are irreplaceable in food production (land, water, air and biodiversity).

Sustainable production must meet the basic standards set by the International Movement for Sustainable Production Management through low-input production. This form of production has its own subsystem, integral production, which is the most acceptable in the world. Integral production implies the application of modern agrotechnical measures, the use of integral methods in plant protection, the use of microbiological fertilizers, which can influence the reduction of nitrogenous mineral fertilizers, which results in obtaining products that meet standards in terms of quality and food safety. The use of preparations with different types of microorganisms (biofertilizers and biopesticides) is increasingly prevalent in plant production (Sharma et al., 2012; Molla et al., 2012). In the last few years, there has been an increasing number of researches related to the interactions of plants and certain groups of microorganisms with the aim of ecologically and economically profitable production (Cvijanović et al., 2012). For this purpose, symbiotic and associative groups of microorganisms are used in the system of growing leguminous and non-leguminous plant species. Microorganisms that are part of microbiological fertilizers produce growth substances, polysaccharides, vitamins, enzymes, amino acids, etc. compounds. These compounds stimulate the growth and development of plants, which has a positive effect on the yield and quality of the yield. The use of microbial inoculants in agriculture has increased significantly over the last two decades (Hayat et al., 2010). Microbial inoculants that act as biofertilizers are considered biostimulants. Biofertilizers are biological products containing live microorganisms that, when applied to seeds, plant surfaces or soil, promote growth by several mechanisms such as increasing nutrient supply, increasing root biomass or root surface area, and increasing plant nutrient uptake capacity (Vessey, 2003; Aloni et al., 2006). Application of microbiological fertilizers in sustainable production systems is imperative in the production of healthy food, because they can replace different amounts of mineral fertilizers. Application of preparations with effective microorganisms that represent a large group of different types of microorganisms can be used in conventional, organic and integral agricultural production and their use is recognized as part of the strategy of sustainable agricultural production. Many studies have shown that by applying preparations with effective microorganisms in combination with mineral and organic fertilizers, the use of mineral fertilizers and pesticides can be reduced. The joint application of NPK fertilizers, manure and effective organisms in the wheat and rice cultivation system resulted in more straw and a higher grain yield, compared to the application of only mineral and organic fertilizers (Hussain et al., 2000). Wheat (*Triticum aestivum*) has an important place in human nutrition. As a basic bread crop in developed countries, it is represented by 53%, and in underdeveloped countries by 85% of the total world production (Pena, 2007). That is why wheat has an emphasized social function, because it is a basic part of the diet of the lower layers of the population. The global wheat market has been changing for the past few years. In the period 2010-2018, wheat production in the world took place on 20-27% of the world's arable land with an average yield of 3.28 t ha⁻¹.

Wheat has the greatest requirements for achieving the genetic potential of fertility for nitrogen ($100\text{-}200\text{ kg}\cdot\text{ha}^{-1}\text{ N}$), phosphorus ($50\text{-}130\text{ kg}\cdot\text{ha}^{-1}\text{ P}_2\text{O}_5$), potassium ($40\text{-}100\text{ kg}\cdot\text{ha}^{-1}\text{ K}_2\text{O}$), calcium, sulfur and magnesium. Nitrogen is of primary importance in the nutrition of wheat and is necessary in the synthesis of nucleic acids and proteins. Nitrogen fertilizers have the greatest importance for the growth and productivity of plants and the quality of wheat grains (Kichey et al., 2007). Nitrogen deficiency has a negative effect on the formation of morphological characteristics of plants, which significantly determine the yield per unit area. About 68% of applied nitrogen fertilizer is incorporated into wheat plants, 18% remains in the surface layer of the soil while the remaining 14% is denitrified and leached from the soil (Barraclough et al., 2010). In previous research, it was determined that wheat has an associative relationship with free-living diazotrophs in the soil from the family *Enterobacteriaceae*, *Pseudomonaceae*, *Azotobacteraceae*. It was found that a proteinaceous fraction of wheat root exudates is capable of attracting bacteria and increasing their nitrogen-fixing ability. According to Galkin et al., (1989), this trait has a pronounced variability with the wheat genotype. According to research by Cvijanović et al. (2007) in the wheat seed inoculation system with associative diazotrophs (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Beijerinckia Derx* and *Klebsiella planticola*) the grain yield can be increased by 11.64% and the protein content in the grain by 6.40%. Research in this area has shown that a proteinaceous fraction of wheat root exudates is capable of attracting bacteria of the *Enterobacteriaceae*, *Pseudomonaceae*, *Azotobacteraceae* families and increasing their nitrogen-fixing ability, which makes it possible to increase the yield of wheat and reduce the amount of mineral nitrogen fertilizers. According to research by Andres et al., (2009) inoculating wheat seeds with species from the genera *Azospirillum* and *Pseudomonas* can increase root biomass by 40% and grain yield by 16%. Considering the presence of wheat in people's diet with the development of various movements for functional and highly nutritious food in recent decades, various researches have been conducted. Therefore, the goal of the work was to examine the impact of the application of effective microorganisms and different amounts of mineral nitrogen in the integral system of cultivation of different genotypes of wheat.

MATERIALS AND METHODS

Experimental research was carried out in 2016/2017-2018/2019 in Banat, Vojvodina ($\Psi\text{N } 44^{\circ} 56'$, $\lambda\text{E } 25^{\circ} 28'$). The area of the elementary plot was 5 m^2 . Plots were laid out according to a split-plot design in four replications with three factors. Sowing was carried out in a density of $400\text{ grains}\cdot\text{m}^{-2}$

- **Factor A** - Years of research
- **Factor B** - Wheat varieties: 1. PKB Ratarica late winter variety type $\text{B}_1\text{-A}_2$. 2. NS Pobeda sredenje late variety type $\text{A}_1\text{-A}_2$. Both varieties were created in Serbia. 3. Noyal early variety type A_2 . 4. Apache mid-late variety type B_1 , both varieties are from France.
- **Factor C** - Fertilization: To ensure proper nutrition of wheat in the basic tillage, mineral complex fertilizer NPK formulation 15:15:15 was used. In autumn, $400\text{ kg}\cdot\text{ha}^{-1}$ of NPK 15:15:15 was added to the soil, in the amount calculated for the area of the experimental plot. In the spring, wheat was fed with nitrogen fertilizer Urea 46% N in the amount that provided: 129, 106, and $83\text{ kg}\cdot\text{ha}^{-1}\text{ N}$, $60\text{ kg}\cdot\text{ha}^{-1}\text{ P}_2\text{O}_5$ and $60\text{ kg}\cdot\text{ha}^{-1}\text{ K}_2\text{O}$.

During the growing season, foliar applications were carried out with the microbiological preparation EM Aktiv, which contains a mixture of over 80 different types of effective

microorganisms (*Lactobacillus plantarum*, *Lactobacillus casei*, *Streptococcus lactis*, *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*, *Saccharomyces carevisiae*, *Streptomyces albus*, *Streptomyces griseus*, *Aspergillus oryzae*, *Mucor hiemalis*, *Bacillus subtilis*, *Bacillus megaterium*, *Azotobacter sp.*). Application of the preparation was in the amount of 6 l·ha⁻¹ in different phenophases of plant development. This is how it was achieved:

F₀ – 129 kg·ha⁻¹ N without applying EM Aktiv – control

F₁ – 129 kg·ha⁻¹ N + EM Aktiv 1 x (in the phenophases of stem extension)

F₂ – 106 kg·ha⁻¹ N + EM Aktiv 2 x (in the phenophases of stem extension and flowering)

F₃ – 83 kg·ha⁻¹ N + EM Aktiv 3 x (in the phenophases of stem extension, flowering and grain filling - ripening)

The obtained results were statistically processed using the analysis of variance method as a split-plot experiment in the DSAASTAT program. The significance of differences between treatments was tested by the LSD test at the significance level of p<0.01 and p<0.05.

Agrometeorological conditions: The average value of mean monthly temperatures and total precipitation were measured in the period from October to July. In 2016/2017 and 2018/2019, the average air temperature was 10.9°C, while in 2017/2018, the average monthly air temperature was 9.73°C. The precipitation totals in 2016/2017 and 2018/2019 were very similar (536.7 and 537.3 mm). In 2017/2018, the amount of precipitation was less, 482.3 mm. In addition to the amount of precipitation, the distribution of precipitation is very important. In 2018, a large amount of rain fell in June, which remained on the soil surface and significantly affected the observed parameters (Table 1).

Table 1. Average mean monthly temperatures (°C) and total precipitation (mm) for the research period

Year	Average		Month										Average
	Sum	X	XI	XII	I	II	III	IV	V	VI	VII		
2017	°C	10.8	6.5	2.8	0.2	7.3	7.8	13.9	16.3	21.5	22.6	10.9	
	mm	70.6	50.8	10.8	46.5	46.4	78.8	34.4	74.4	89.2	34.8	536.7	
2018	°C	9.6	5.9	-0.6	-5.1	3.3	9.9	11.1	17.2	22.5	23.5	9.73	
	mm	70.7	75.0	4.6	18.6	26.9	22.0	46.2	71.6	106.5	40.9	482.3	
2019	°C	11.0	6.3	3.3	3.1	1.4	5.2	16.5	19.8	21.1	22.1	10.9	
	mm	57.0	48.1	40.6	39.2	47.2	58.2	29.4	80.1	70.1	66.7	537.3	

RESULTS AND DISCUSSION

The mass of 1000 grains is defined as the absolute mass of absolutely dry and undamaged grains. The weight of 1000 grains is used as a measure of quality, because with the same grain size, the heavier will indicate the possibility of greater utilization in flour. The mass of 1000 grains can vary between varieties of the same plant species, depending on agrometeorological conditions and the amount of applied fertilizers. Based on the obtained results, the average weight of 1000 grains was 40.57 g (Table 2). The years of examination (A) had a highly significant effect. The highest mass of 1000 grains was in 2018/2019 (44.34 g), which is 9.13% more than in 2016/2017 and 20.68% more than in 2017/2018. The schedule of precipitation had a significant impact, as

well as the large amount of precipitation in June 2018 (106.5 mm), which affected the laying of plants and retention of water on the surface.

Varietal specificity (B) had a highly significant effect on the differences in the mass of 1000 grains. The varieties PKB Ratarica (41.82 g) and NS Pobeda (43.73 g) had a greater mass of 1000 grains than the varieties Nogal (37.30 g) and Apache (39.43 g). The determined differences were significant at the $p < 0.01$ level. Fertilization and treatments (C) affected the increase in the mass of 1000 grains, but the differences were not statistically significant. The increase in the weight of 1000 grains was from 0.14% in treatment F_3 to 1.91% in treatment F_1 . It can be said that by applying a microbiological preparation several times in the vegetation, the amount of mineral nitrogen can be reduced, without large differences in the mass of 1000 grains. Similar results were obtained by Cvijanović et al. (2011) in three-year research on foliar application of different types of diazotrophs (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Beijerinckia derox*, *Klebsiella planticola*) in wheat production.

Table 2. Mass of 1000 grains (g) depending on the applied factors

Year (A)	Genotypes (B)	Fertilization and treatments (C)				□ A x B	□ A
		F ₀	F ₁	F ₂	F ₃		
2016/2 017	PKB	42.	41.	42.	42.	41.75	40.
	Ratarica	00	00	00	00		
	NS Pobeda	42.	43.	41.	43.		
	Nogal	39.	39.	38.	38.		
	Apach	50	50	00	50		
□ A x C		40.	40.	40.	40.		63
		75	75	13	88		
2017/2 018	PKB	37.	36.	38.	38.	37.60	36.
	Ratarica	63	35	43	00		
	NS Pobeda	40.	41.	42.	39.		
	Nogal	05	25	60	18		
	Apach	33.	32.	33.	31.		
□ A x C		36.	36.	37.	36.		74
		51	38	91	16		
2018/2 019	PKB	45.	46.	47.	45.	46.10	44.
	Ratarica	33	17	30	60		
	NS Pobeda	47.	47.	48.	48.		
	Nogal	48	98	68	58		
	Apach	15	38	62	33		
□ A x C		43.	45.	44.	44.		34
		72	30	19	14		
□ B x C	PKB	41.	41.	42.	41.	41.82	B
	Ratarica	65	17	58	87		
	NS Pobeda	43.	44.	44.	43.		
	Nogal	18	08	09	58		
	Apach	37.	37.	37.	36.		
□ C		40.	40.	40.	40.		34
		33	81	74	39		
Average 2017-2019							40.
							57

	A	B*	A x B	C	A x C*	B x C	A x B x C
LSD 0.05	1. 44	1.9 7	3.41	0.71	1.23	1.42	2.4 5
LSD 0.01	1. 63	2.6 6	4.61	0.94	1.62	1.87	3.2 5

The fertility of wheat genotypes is a complex property, the realization of which depends on the expression of a large number of quantitative properties, as its components. Wheat grain yield is influenced by various factors, primarily genotype characteristics, soil fertility and applied agro technical measures. According to Araus et al., (2004) selections and breeding contribute 28-50% to the increase in yield of created wheat varieties, while 50-72% is the result of improved agronomic measures in wheat production technology. Based on the analysis of world trends, the increase in wheat yields during the 20th century was the result of intensification of production. Seibutis et al., (2009) pointed out that the grain yield of winter wheat has a significant influence on the wheat cultivation system.

The average yield of wheat grains was $6.48 \text{ t}\cdot\text{ha}^{-1}$ (Table 3). Agrometeorological conditions (A) had a statistically significant ($p < 0.05$) influence on the realized differences in grain yield. The highest yields were in 2018/2019 ($6.73 \text{ t}\cdot\text{ha}^{-1}$), which is 3.85% more than in the first year. Such differences were significantly influenced by average temperatures and total precipitation. In 2016/2017, average monthly temperatures and precipitation in the period April-June were lower than in 2018/2019. year. In 2018, a smaller amount of precipitation was determined (485.3 mm), as well as a large amount of precipitation in June, and grain yields were lower by 8.19%. The obtained results are comparative with the results of Mladenov et al., (2014) who determined that the decrease in yield of wheat as a result of rain ($107.6 \text{ l}\cdot\text{m}^{-2}$) in the full maturity phase is due to grain shedding or the activation of physiological processes in the grain and concluded that every $1\cdot\text{m}^{-2}$ affects the decrease in yield from $10 \text{ kg}\cdot\text{ha}^{-1}$ (0.12%), respectively $10 \text{ l}\cdot\text{m}^{-2}$ reduces the yield by $100 \text{ kg}\cdot\text{ha}^{-1}$ or 1.2%.

The height of the yield certainly depends on the characteristics of the variety (B). In the conducted research, a statistically significant difference in grain yield was found in the tested wheat genotypes. The variety PKB Ratarica had the highest grain yield $7.02 \text{ t}\cdot\text{ha}^{-1}$. Fertilization and treatments (C) had a statistically significant ($p < 0.05$) effect on wheat grain yield. The highest yield ($6.62 \text{ t}\cdot\text{ha}^{-1}$) was found in treatment F_2 , where $106 \text{ kg}\cdot\text{ha}^{-1}$ N and two foliar treatments with effective microorganisms were applied. Compared to control F_0 , the difference was 5.24%, and compared to treatments F_1 by 0.45% and treatment F_3 by 3.27%. The interaction B x C had a statistically highly significant influence ($p < 0.01$) on the height of grain yield. The highest yield was in the PKB Ratarica variety in the F_2 treatment ($7.21 \text{ t}\cdot\text{ha}^{-1}$). The obtained results are comparable to the research of Roljević, (2014), where it was determined that the use of a microbiological preparation in the organic production of alternative types of grain can increase the grain yield by 12-26%, and in combination with the use of fertilizers, the increase in grain can be 33-45% in compared to control. It can be said that the application of plants with effective microorganisms had a significant impact on the properties tested. In general, the morphology of plants during the vegetative phase is characterized by the formation of larger leaves. This affects the surface area available for microbial colonization and could be one reason why microbial communities differ between plant species. Similarly, if microbiological preparations are applied in the earlier stages of plant development, when the leaves are small, the number of microbes increases. Although the plant habitus cannot be considered a homogeneous environment, the different spheres can be distinguished according to the number of photosynthetic products on the

leaves and the number of microbes. Likewise, depending on the shape, structure and position of the leaf, there are large fluctuations in the number and type of microbial phyllosphere population. The phyllosphere is normally rich in a large number of microbes, including bacteria, fungi, yeast and protozoa (Lindow & Brandl, 2003; Leveau, 2006). Bacteria are the most abundant members of the phyllosphere community and have been shown to colonize leaves at densities of up to 10^8 cells cm^{-2} (Leveau, 2006). It is assumed that the foliar application of a large group of effective microorganisms additionally increases their number and biochemical activity, which has a significant impact on the development of plants, because the process of photosynthesis is intensified, and thus the synthesis of organic matter. In the last few years, in order to increase the yield of wheat and protect the environment, research has been included in the application of different groups of microorganisms that participate in the cycle of nitrogen, phosphorus, mineralization of organic matter, and the production of substances that are growth promoters. Effective microorganisms are producers of auxin, an indole 3-acetic acid that plays a key role in plant growth, developing many functions including cell elongation and division.

Table 3. The yield of wheat grains depending on the applied factors ($\text{t} \cdot \text{ha}^{-1}$)

Year (A)	Genotypes (B)	Fertilization and treatments (C)				□ A x B	□ A
		F ₀	F ₁	F ₂	F ₃		
2016/2017	PKB Ratarica	5.54	5.44	6.10	6.08	5.79	6.48
	NS Pobeda	6.46	7.13	6.62	6.17	6.59	
	Nogal	6.29	6.85	6.64	6.52	6.57	
	Apach	6.63	6.85	7.38	7.07	6.98	
□ A x C		6.23	6.57	6.69	6.46		
2017/2018	PKB Ratarica	5.59	5.44	5.25	6.08	5.59	6.22
	NS Pobeda	6.28	6.88	6.18	5.65	6.25	
	Nogal	5.88	6.67	6.10	6.19	6.21	
	Apach	6.63	6.82	6.78	7.13	6.84	
□ A x C		6.09	6.45	6.08	6.26		
2018/2019	PKB Ratarica	6.04	5.63	7.14	6.12	6.23	6.73
	NS Pobeda	6.28	6.91	7.01	6.43	6.66	
	Nogal	6.67	7.26	6.73	6.45	6.78	
	Apach	7.24	7.18	7.47	7.06	7.23	
□ A x C		6.56	6.75	7.09	6.51		
□ B x C	PKB Ratarica	6.83	6.95	7.21	7.09	7.02	□ B
	NS Pobeda	6.34	6.98	6.60	6.08	6.50	
	Nogal	5.72	5.50	6.16	6.09	5.87	
	Apach	6.28	6.93	6.49	6.38	6.52	
□ C		6.29	6.59	6.62	6.41		
Average 2017-2019							6.48
	A*	B**	A x B	C*	A x C	B x C**	A x B x C
LSD 0.05	0.50	0.28	0.58	0.25	0.44	0.50	0.87
LSD 0.01	0.59	0.38	0.65	0.33	0.58	0.66	1.15

CONCLUSIONS

In the integral production of wheat, agrometeorological conditions and genotype have a statistically significant influence on the weight of 1000 grains and the height of the grain yield. Fertilization in combination with foliar treatments on average for all investigated factors had an effect on increasing the value of the investigated traits. The mass of 1000 grains with a decrease in the amount of nitrogen and an increase in the number of foliar treatments was higher than the control, but without statistical significance, while the grain yield was higher at the level of $p < 0.05$ significance. The general conclusion is that by applying effective microorganisms in wheat supplementary nutrition, stable grain yields can be achieved in an integral system with the application of $106\text{--}129 \text{ kgN}\cdot\text{ha}^{-1}$ and foliar treatment with effective microorganisms.

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