Original paper DOI:10.5937/ZemBilj2402038B

Application of effective microorganisms to reduce mineral nitrogen for feeding wheat

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ABSTRACT

The key to future agricultural production is an ecological approach in order to obtain health-safe food. In this regard, the application of effective microorganisms (EM) in all production systems is a new technological measure. The goal of the research is to determine how the application of EM and different amounts of nitrogen fertilizer affect the yield parameters of bread wheat plants and soil microbial parameters in the rhizosphere. The research was carried out in the field in the period 2017-2019 (factor A) in Serbia, by growing the bread wheat variety Ratarica, in two different sowing densities (400 and 500 grains per m^2) (factor B). Factor C, fertilization treatments: F1 control = NPK (15:15:15) 400 kg ha^{-1} + 150 kg ha^{-1} UREA (46% N); F2 = F1 + 1 foliar treatment with EM preparation; F3 = NPK + 100 kg ha^{-1} UREA + 2 foliar treatments; F4 = NPK + 50 kg ha^{-1} UREA + 3 foliar treatments. The increase in soil microbial parameters was recorded in the F3 treatment (total number of microorganisms by 5.49% and the number of *Azotobacter* by 9.00%) compared to the control ($p<0.01$). The yield of grains was the highest in the F2 treatment (by 5.08%) compared to the control. It is concluded that in sustainable bread wheat production systems, with the application of EM, the amount of mineral nitrogen in the feed can be reduced by 25-30 kg ha^{-1}.
Key words: wheat; fertilizer; effective microorganisms; soil biogenicity; yield

Introduction

At the beginning of the 21st century, agriculture experienced a major technological transformation with the introduction of green directives. The European Green Directive has a series of requirements in food production aimed at reducing the emission of gases that affect the increase in climate change, as well as standards that define a product with a high biological value. Slogans such as Zero Residues, Zero Waste and Zero Kilometers represent a manifesto of a new food concept. This motivated the scientific and professional public to find new directions in food production by introducing ecologically acceptable inputs that would replace chemical preparations in nutrition and plant protection. It has long been known that the introduction of certain groups of microorganisms that are individually compatible with plant species such as rhizobium and legumes reduces the amount of mineral nitrogen in production.

Wheat has great agrotechnical importance in crop rotation. It has a pronounced associative relationship with nitrogen-fixing soil bacteria of the genus *Azotobacter, Agrobacterium, Azospirillum, Beijerinckia*. Numerous studies show that by using different groups of microorganisms as biostimulators in wheat production, it is possible to increase the root mass Naiman, (2009), yield components, grain nutritional properties (Cvijanović et al. 2008; Hijri and Boi, 2018). There are increasing demands to introduce preparations in sustainable agricultural production systems that are the result of EM biotechnology, which is based on a large group of effective microorganisms that have attracted the attention of the scientific and professional public. The potential of the application of effective microorganisms is great for the stimulation of seeds, seedlings, plants and soil regeneration. Mucus and other substances released by EM attach humus and mineral particles, forming clods of the soil, which improves its structure. Microorganisms also produce bioactive substances useful to plants, such as hormones and growth stimulators. Pszczółkowski et al. (2023) states that EM can produce hormones and growth stimulators that act on the cell division of microorganisms and can change the structure of the rhizosphere microbial community and stimulate plant growth. They influence the increase of plants' resistance to stress and the content of bioactive components in the fruits. EM biotechnology is currently used in 140 countries on most continents because effective microorganisms have been shown to be most suitable for sustainable food production systems (Sumbul et al. 2020; Cvijanović et al. 2022;). Zhang et al. (2022) explain the technological progress of applying EM in unfavorable climatic conditions, because they increase the resistance of plants to stress.

The application of EM biotechnology in the future will have an increasing application in the production of crops that occupy the world's largest areas. The goal of the work was to determine the influence of the application of effective microorganisms on the number of basic parameters of soil biogenicity in the rhizosphere of wheat and the height of the grain yield.

Material and methods

Plant material - The culture used in the research is the wheat variety Ratarica, selected by the Institute of PKB Agroeconomics, Serbia. It belongs to the mid-late winter bread variety, whose main morphological characteristic is the retention of the green color of the top leaf until the beginning of the harvest. Also, it has a great power of budding, and the ear is axisless, whitish in color when ripe. Ratarica is a high-yielding variety with a genetic potential for yield in poor conditions (5 to 11 t ha^{-1}), which implies a pronounced tolerance to lodging, drought, stress conditions and grain stiffness.

Research setting and location - The experimental research was conducted in an open field on the experimental plot of the Institute of PKB Agroeconomics, Serbia (ΨN 440 56'; λE 250 28'). The trial was set up in a randomized design with 4 replications. The total area of the sample was 84 m^2, the area of the elementary plot was 5 m^2 (the total number of plots was 32). Agrotechnical measures, typical for sustainable production, were carried out in optimal terms. The previous crop was corn. Sowing was carried out in optimal terms according to the manual sowing system. The protective insulating belt was wheat of the same variety. Three factors were included in the research: Factor Y - year of research, Factor D - Density of seed sowing and Factor F – Fertilization

Agroclimatic conditions - At the meteorological station at the PKB Agroeconomics Institute, Belgrade, Serbia, data on the sum of precipitation and temperature were monitored and collected for research in production years 2016/2017, 2017/2018 i 2018/2019 (Table 1).

Table 1. Average values of mean monthly air temperatures (°C) and sum of monthly precipitation (mm)

Average values of mean monthly air temperatures (°C)											
Year	Monthly										Average
	X	XI	XII	I	II	III	IV	V	VI	VII	
2017	10,8	6,5	2,8	0,2	7,3	7,8	13,9	16,3	21,5	22,6	10,9
2018	9,6	5,9	-0,6	-5,1	3,3	9,9	11,1	17,2	22,5	23,5	9,73
2019	11,0	6,3	3,3	3,1	1,4	5,2	16,5	19,8	21,1	22,1	10,9
Sum of monthly precipitation (mm)											
Year	Monthly										Average
	X	XI	XII	I	II	III	IV	V	VI	VII	
2017	70,6	50,8	10,8	46,5	46,4	78,8	34,4	74,4	89,2	34,8	536,7
2018	70,7	75,0	4,6	18,6	26,9	22,0	46,2	71,6	106,5	40,9	482,3
2019	57,0	48,1	40,6	39,2	47,2	58,2	29,4	80,1	70,1	66,7	537,3

Density of seed sowing - Examination of the sowing density is an important parameter because in this way a high degree of the genetic potential of the fertility of the variety is achieved in different growing conditions. That is why the experiment was sown in two different seed densities: 400 and 500 grains per m^2.

Fertilization - Fertilization was in 4 variants in the form of basic and supplementary feeding (Table 2). The basic NPK complex fertilizer (15:15:15) was introduced in autumn in quantity 400 kg NPK ha^{-1}. Spring feeding of wheat was with mineral nitrogen UREA 46% N in combination with effective microorganisms in the preparation EM Aktiv (trade name). Microbiological preparation EM Aktiv is a liquid preparation, which consists of effective microorganisms (EM), a mixture of different types of microorganisms of photosynthetic bacteria *(Rhodopseudomonas palustris, Rhodobacter sphaeroides)*, milk bacteria *(Lactobacillus plantarum, L. casei, Streptococcus lactis)*, yeasts *(Saccharomyces cerevisiae, Candida utilis)*, actinomycetes *(Streptomyces albus, Streptomyces griseu)* and fungi *(Aspergillus oryzae* и

Mucor hiemalis). EM Aktiv was applied foliarly in different phenophases of plant development, in an amount of 6 l ha^{-1} with water in a ratio of 1:100.

Table 2. Variants of fertilization on the experimental plot

Fertilization variants	Before sowing NPK (kg ha^{-1})	UREA (46% N)	Microbiological fertilizer	Total nutrients N : P_2O_5 : K_2O
F 1	400	150	Control	129 : 60 : 60
F 2	400	150	Phase of stem extension	129 : 60 : 60
F 3	400	100	Phase of stem extension Phase od flowering	106 : 60 : 60
F 4	400	50	Phase of stem extension Phase od flowering Phase of ripening	83 : 60 : 60

Sampling - The harvest was carried out with a combine adapted for harvesting on smaller plots, and the grain yield was pre-parched at 14% moisture and expressed in t ha^{-1}. In order to determine soil microbial properties, samples of rhizosphere soil were taken in the phenophase of technological maturity . The abundance of tested microbial groups were determineted on appropriate nutritive media by a soil dilution plate method. . The total number of microorganisms, was determineted on soil agar (Pochon and Tardeaux, 1962) at a dilution of 10^{-7}. The abundance of *Azotobaceter* was determined on a nitrogen-free medium (Fyodorov's medium) using the method of 25 fertile drops (Anderson, 1978), from a 10^{-1} dilution. Plates were incubated at the temperature of 28℃. The incubation period for the total number of microorganisms was seven days and for *Azotobacter* 48 hours. After incubation, the colonies were counted and the average number of colony forming units (CFU) was calculated at gram of absolutely dry soil (Wollum, 1982).

Statistical analysis - Data were subjected to analysis of variance and regression. For the qualitative factor, means were compared using three-factor ANOVA and LSD test with 1% and 5% significance. All analyzes and tests were performed using the statistical program DSAASTAT (Perugia, Italy).

Results and discussion

Agrometeorological conditions in 2017 and 2019 were uniform and relatively optimal for wheat development. Average daily temperatures and total precipitation were higher than in 2018. In addition, in 2018, a large amount of precipitation (106.5 mm) fell in June, and high temperatures shortened and hindered the grain pouring process, which had a significant impact on the examined parameters. Considering that the precipitation was torrential, the water lay on the surface, which had a significant impact on the composition of the microbiome in the soil.

Total number of microorganisms - Microorganisms includes bacteria, fungi, algae, protozoa and nematodes, because their metabolism is related to the flow of energy and the circulation of elements in ecosystems. Their dynamics, spatial distribution and variation is dependent on numerous of factors (soil properties, temperature and water, concentration of gases and heavy metals, chemical toxicants, nitrogen and phosphorus content). Also, the abundance depends on the genetic and variability of the plant, which is the result of its compatibility with microorganisms and applied agrotechnical measures. One of the key importance is the influence of plants, which refers to the release of various secretions through the roots into the soil (Vives-Peris et al., 2020). Secretions are rich in sugar and acid molecules that directly affect the number and diversity of the microbial population, because they are attracted to them, and the microorganisms break down those molecules.

The average total number of microorganisms was 210.74×10^7 (Table 3). The favorable relationship between temperature and precipitation in 2017 and 2019 had a positive effect on the microbial community. The highest total number of microorganisms was recorded in 2017 (263.58×10^7). Compared to 2019, it was higher only by 0.73%, because the production conditions were similar. However, compared to 2018, the total number of microorganisms was higher by 146.61%. From the obtained results, it can be concluded that the total number of microorganisms reacts significantly to unfavorble agro-climatic conditions compare the optimal. The obtained results are in agreement with Donn et al. (2015), which confirms that during each vegetative season of growing wheat, there are variations in rhizosphere biogenicity. In all three reserach years, the total number of microorganisms was significantly higer at the sowing density of 500 grains per m^2, compared to the sowing density of 400 grains per m^2 (by 19,67%, on average). The obtained results, showed that sowing at a narrow row spacing , the amount of root exudates increases, which is the trigger for the development of the higher number of bacteria. Ayazet al., (2023) determined that the number of plants and root mass directly affect the amount of secretion released into the environment, which implies from the obtained results that the total number of microorganisms increased in the sowing of a higher number of plants per unit area.

The highest total number of microorganisms was recorded in the F3 treatment (216.67×10^7), which was 5.49% higher than in the control (F1) and 1.04% and 3.94% higher than in the F2 and the F4 treatments, respectively. The obtained results are very significant because with reduced amounts of mineral nitrogen and an increased number of treatments with EM Active, stability in the microbiome can be achieved. The above implies that the amount of mineral nitrogen of about 25-30 kg ha^{-1} can be replaced if a preparation with effective microorganisms is used. The obtained results are compatible with the results of Abduladin et al. (2020) who found that in the soybean rhizosphere after the application of effective microorganisms, the total number of bacteria increased by 44.76% to 54.55%, depending on the amount of applied NPK fertilizer. Also, they point out that the results of the research years showed that the impact of EM preparations was the highest in the unfavorable agro-climatic conditions, which gives an advantage to the use of these preparations.

Table 3. Influence of the tested factors on the total number of microorganisms (×10^7 CFU of absolutely dry soil)

Year (Y)	Density of seed sowing (D)	Fertilization (F)				x̄ Y x F	x̄ Y
		F1	F2	F3	F4		
2017	400	265.67	208.67	282.67	253.00	252.50	263.58
	500	221.67	319.67	299.67	257.67	274.67	
	x̄ D x F	243.67	264.17	291.17	255.33		
2018	400	132.67	77.67	32.67	55.00	74.50	106.88
	500	157.67	140.33	102.67	156.33	139.25	
	x̄ D x F	145.17	109.00	67.67	105.67		
2019	400	232.33	208.67	282.67	270.67	248.58	261.75
	500	222.33	319.67	299.67	258.00	274.92	
	x̄ D x F	227.33	264.17	291.17	264.33		
x̄ D x F	400	210.22	165.00	199.33	192.89	x̄ D	191.86
	500	200.56	259.89	234.00	224.00		229.61
	x̄ F	205.39	212.44	216.67	208.44		
Average 2017-2019							210.74

LSD	Y**	D**	F*	YxD**	YxF**	DxF**	YxDxF**
p<0.05*	0.73	0.62	0.72	1.07	1.025	1.02	1.77
p<0.01**	0.97	0.93	0.97	1.62	1.68	1.37	2.37

Total number of *Azotobacter* - Bacteria belonging to the genus *Azotobacter* are responsible for the fixation of atmospheric nitrogen, which they reduce to molecules available for plants (Sumbul et al. 2020). According to Gothandapani, Sekar, Padaria (2017) numerous researches on the impact of *Azotobacter* in agriculture is related to their activity and production of plant growth stimulants, which further directly affects the yield quality and quantity.

The average number of Azotobacter was 103.35×10^{-1} (Table 4). In years with a more favorable distribution of precipitation and a better air regime in the soil, the abundance of azotobacter was higher. The highest number was determined in 2019, which is 27.05% higher than in 2017 and 74.22% higher than in 2018. As for the sowing density, it can be observed that at a density of 500 grains m^2, the average number of *Azotobacter* for all three years was higher by 65.28% than at a density of 400 grains m^2.

Table 4. The influence of the tested factors on the number of Azotobacter (×10 CFU g^{-1} of absolutely dry soil)

Year (Y)	Density of seed sowing (D)	Fertilization (F) F1	F2	F3	F4	x̄ Y x F	x̄ Y
2017	400	78.17	94.67	80.67	58.17	77.92	103.35
	500	127.17	112.67	143.17	132.17	128.79	
	x̄ D x F	102.67	103.67	111.92	95.17		
2018	400	71.33	79.67	66.67	73.67	72.83	75.38
	500	89.67	95.67	63.67	62.67	77.92	
	x̄ D x F	80.50	87.67	65.17	68.17		
2019	400	85.00	109.67	94.67	42.67	83.00	131.33
	500	164.67	129.67	222.67	201.67	179.67	
	x̄ D x F	124.83	119.67	158.67	122.17		
x̄ D x F	400	78.17	94.67	80.67	58.17	x̄ D	77.92
	500	127.17	112.67	143.17	132.17		128.79
	x̄ F	102.67	103.67	111.92	95.17		
Average 2017-2019							103.35

LSD	Y**	D**	F*	YxD*	YxF**	DxF**	YxDxF^{ns}
p<0.05*	17.67	11.11	10.50	19.25	38.97	31.82	55.11
p<0.01**	23.59	16.84	15.17	29.17	52.26	42.67	73.90

The tested fertilization treatments had an effect on the change in the number of *Azotobacter* at the significance level of p<0.05. In the F3 tretment, the number of azotobacter was higher by 9.01% compared to the control, and by 7.95% and 17.60% compared to the F2 and F4 treatment, respectively. Kurrey et al., (2018) investigated the effect of different doses of microbial inoculum in onion production and obtained positive results in increasing the content of phosphorus, organic carbon, nitrogen, potassium and sulfur. The research of Ebrahim et al. (2023) showed that six different biofertilizer treatments (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Flavobacterium* F-40, *Bacillus megaterium*, *Pseudomonas* *fluorescens* and *Rhizophagus irregularis*) in the production of *Zygophyllum eurypterum* seedlings, had a positive effect on the abundance of all fungi, as well as on the morphological and productive properties of the examined plant. The results of Abdel Latef et al. (2021) showed that the application of *Azotobacter chroococcum* and *Alcaligenes faecalis* in the cultivation of canola plants (*Brassica napus*, L.) led to an increase in the concentration of macro and microelements needed by the plant.

The yield of wheat grains is the result of many physiological changes during the development of plants, which are under the direct influence of many factors. The yield of wheat grains is influenced by the characteristics of the variety, climatic factors, biogenicity of the soil and agrotechnical measures.

For the test period 2017-2019, the average yield of 6.00 t ha^{-1} of wheat grains was achieved (Table 5). In 2017, d grain yield was 5.84 t ha^{-1}, while in 2019 the highest yield of 6.53 t ha^{-1} was recorded. The lowest yield was 5.62 t ha^{-1} in 2018, which is primarily the result of unfavorable agrometeorological conditions, because in conditions of uneven distribution of precipitation, the full effect of the applied preparation is missing. According to Malha et al. (2021) the impact of climate conditions in terms of increased rainfall and high temperatures can negatively affect wheat production. Liu et al. (2016) state that higher amounts of precipitation affect yield reduction by up to 7%, which is correlated with the obtained results. In addition, the low yield in 2018 can be explained as a consequence of the lowest total number of microorganisms and *Azotobacter*. The sowing density had no significant effect on the obtained average grain yields However, at a higher seeding density, the grain yield was higher by 2.53% compared to the grain yield at a lower seeding density.

Based on the obtained results, the highest grain yield (6.20 t ha^{-1}) was achieved on F2 tretment. Compared to treatments F1, F3, and F4 the grain yield was higher by 5.08%, 1.47%, and 7.26% respectively. The obtained results indicate a significant contribution of the application of effective microorganisms with a smaller amount of mineral nitrogen in the wheat crop. The application of EM preparation to the soil in combination with manure or the recommended dose of mineral fertilizer resulted in an increase in mung bean yield (Higa et al., 2003). Also, Bajagić et al. (2023) reported that the application of EM Aktiv preparation increased the the soybean yield by 21.52% compared to the control, on average for two years and two soybean varieties.

Table 5. Influence of examined factors on grain yield (t ha^{-1})

Year (Y)	Density of seed sowing (D)	Fertilization (F)				x̄ Y x F	x̄ Y
		F1	F2	F3	F4		
2017	400	5.80	6.09	5.99	5.67	5.89	5.84
	500	5.54	6.08	6.10	5.44	5.79	
	x̄ D x F	5.67	6.08	6.05	5.56		
2018	400	5.97	5.97	5.21	5.42	5.64	5.62
	500	5.59	6.08	5.25	5.44	5.59	
	x̄ D x F	5.78	6.02	5.23	5.43		
2019	400	6.04	6.12	7.14	5.63	6.23	6.53
	500	6.45	6.85	6.97	7.06	6.83	
	x̄ D x F	6.25	6.48	7.06	6.34		
x̄ D x F	400	5.94	6.06	6.11	5.57	x̄ D	5.92
	500	5.86	6.33	6.11	5.98		6.07
	x̄ F	5.90	6.20	6.11	5.78		
Average 2017-2019							6.00
LSD	Y*	D^{ns}	F*	YxD^{ns}	YxF*	DxF^{ns}	YxDxF^{ns}
p<0.05*	0.66	0.43	0.31	0.74	0.54	0.44	0.76
p<0.01**	0.88	0.61	0.41	1.06	0.72	0.58	1.01

Conclusion

Based on the obtained results, it can be concluded that in the bread wheat production system of the Ratarica variety, the influence of agro-meteorological conditions, the density of sowing and fertilization with effective microorganisms in combination with different amounts of mineral nitrogen had a very significant impact on the rizosphere microbial community. An increase in the number of basic parameters of soil biogenicity (total number of microorganisms and number of azotobacter) was determined. The highest effect was reached with the amount of mineral nitrogen lower by 25-30 kg ha^{-1} than recommended, and with the application of effective micro-fertilizers twice in the vegetation period. At a density of 500 grains per m^2, higher values of all investigated parameters were determined. In general, by applying preparation with effective microorganisms, stable production of bread grain can be achieved in sustainable production systems. This method of production affects the protection of the basic elements of the environment important for food production.

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Original paper DOI:10.5937/ZemBilj2402038B

Primena efektivnih mikroorganizama u cilju smanjenja mineralnog azota u prihrani pšenice

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IZVOD

Ključ za buduću poljoprivrednu proizvodnju jeste еколошки приступ u cilju dobijanja zdravstveno bezbedne hrane. S tim u vezi primena efektivnih mikroorganizama (EM) u svim sistemima proizvodnje je nova tehnološka mera. Cilj istraživanja je da se utvrdi na koji način primena EM i različite količine azotnog đubriva utiču na parametre prinosa biljaka hlebne pšenice i parametre biogenosti zemljišta u rizosferi biljaka. Istraživanja su bila na terenu u periodu 2017-2019, (faktor A) u Srbiji, gajenjem hlebne sorte pšenice, u dve različite gustine setve (400 i 500 zrna po m^2) (faktor B). Faktor C tretmani đubrenja: F1 kontrola = NPK (15:15:15) 400 kg ha^{-1} + 150 kg ha^{-1} UREA (46% N); F2 = F1 + 1 folijarni tretman sa EM preparatom; F3 = NPK + 100 kg ha^{-1} UREA + 2 folijarna tretmana; F4 = NPK + 50 kg ha^{-1} UREA + 3 folijarna tretmana. Povećanje parametara biogenosti zemljišta bilo je pri tretmanu F3 (ukupan broj mikroorganizama za 5,49% i brojnost *Azotobacter* za 9.00%) u odnosu na kontrolu ($p<0.01$). Prinos zrna bio je najveći pri tertamanu F2, 5.08% veći u odnosu na kontrolu. Zaključuje se da se u održivim sistemima proizvodnje hlebne pšenice uz primenu efektivnih mikroorganizama može smanjiti količina mineralnog azota u prihrani za 25-30 kg ha^{-1}.

Ključne reči: pšenica, đubrenje, efektivni mikroorganizmi, biogenost zemljišta, prinos.

Received 11.10.2024
Revised 01.11.2024
Accepted 18.11. 2024