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Effect of application of microbiological preparation and different nitrogen fertilisation on wheat yield elements

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Abstract: Two-year experiments were conducted on two different soil types during 2021/22 and 2022/23 to study the impact of microbiological preparations, Mycor-FAZOS and Vitality-FAZOS, on the yield and quality elements of winter wheat cultivar Maja (Agrigenetics). The experiment was set up in a split-block design with four repetitions across two soil types, with 12 different variants on each soil type. The base plot area was 40 m^2, and the effective plot area was 27 m^2. The research factors included: A (soil type): A1 – gley soil; A2 – eutric cambisol; B (disease control): B1 – control; B2 – chemical pesticides; B3 – biopreparates (seed treatment + foliar treatment) and C (nitrogen fertilisation): C1 – based on soil analysis; C2 – 70% of recommended application. Variables included grain yield (t/ha), grain protein content (%), hectoliter weight (kg), and plant height (cm). The highest average grain yield was achieved with variant B3C2, with a 12.50% increase compared to variant B2C1. Differences between these variants included 6.0% for protein content, 5.97% for hectoliter weight, and 7.93% for plant height. It was observed that Mycor-FAZOS seed treatment promoted root development, resulting in healthier, taller, more robust plants with a more uniform growth and darker colour than untreated seed variants (indicating increased photosynthesis index). The protection achieved with Vitality-FAZOS biopreparations (fungicidal microorganisms synthesising growth hormones) ensured healthier wheat plants, leading to higher seed yield and better quality parameters.

Keywords: *Triticum aestivum* L; biopreparation; fertilisation; protection; yield elements

Wheat is the most important bread grain in the Republic of Croatia (Croatian Encyclopedia 2013–2025). Other small grains are minimally used. Wheat's prevalence in crop rotations has led to the intensification of diseases and pests specific to it, necessitating adequate protection. Chemical pesticides and fertilisers contribute to environmental pollution, the development of resistant pests, and serious health issues. Chemical substances containing heavy metals contaminate soil and may affect human health due to residues in the final product. Similar issues arise with mineral fertilisers, especially phosphorus-based ones (Verkleij 1993, Defarge et al. 2018).

Furthermore, heavy metals and metalloids (e.g., Cr, Mn, Co, Ni, Cu, Zn, Cd, Sn, Hg, Pb) exhibit toxicity to humans and animals, negatively impacting plant growth, food safety, and soil microflora. Chemical pesticides are the main sources of heavy metals in agricultural soil (Ross 1994, Repetto and Baliga 1997, Wei and Yang 2010, Tóth et al. 2016, Marrugo-Negrete et al. 2017). Their

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health risks include cardiovascular disease, neurological issues, childhood autism, and various psychosomatic disorders (Geier et al. 2012, Yousef et al. 2013, Chen et al. 2016, Ijomone et al. 2020, Gianì et al. 2021).

Microbiological and organic fertilisers are viable alternatives. Increasingly, beneficial microorganisms are employed to combat plant diseases and absorb atmospheric nitrogen, dissolve rock phosphates, and synthesise growth hormones (Seeburg et al. 1978, Sharma and Kaur 2017, Khoshru et al. 2023). In this study, Mycor-FAZOS, a biopreparation containing *Glomus* (*G. aggregatum*, *G. clarum*, *G. intraradices*, *G. versiforme*) and *Gigaspora* (*G. rosea*, *G. albida*, *G. margarita)* mycorrhizal fungi and nitrogen-fixing bacteria *Azotobacter chroococcum* and *Azospirillum brasilense*, was used for seed treatment. Vitality-FAZOS, a foliar treatment containing four *Trichoderma* species (*T. harzianum*, *T. viride*, *T. virens*, *T. asperellum*), was applied. *Trichoderma* is noted for its fungicidal effects and ability to synthesise growth hormones and increase the photosynthesis index (Zin and Badaluddin 2020, Illescas et al. 2021, Tyśkiewicz et al. 2022). All microorganisms used were isolated from soils in Croatia.

Biopreparations are recognised and have an eco certificate (Austria Bio Garantie, www.inputs.eu). For the above reasons, we used biopreparations containing pure cultures of microorganisms isolated from our soil to protect wheat, whose activity was confirmed in laboratory and field conditions.

MATERIAL AND METHODS

The research was conducted on Gley soil and Eutric cambisol in 2021/22 and 2022/23 (Table 1).

The experiment was set up as a split-block design with four repetitions on each soil type and 12 different variants per soil type. The base plot size was 40 m^2 (4 m wide by 10 m long), while the effective plot was 27 m^2.

Table 1. Chemical properties of tested soil

Ploughing layer (0–30 cm)	Soil type	
	Gley soil	Eutric cambisol
pH_{H_2O}	7.54	5.87
pH_{KCl}	6.71	5.02
Organic carbon (%)	2.01	1.28
N (mg/kg)	18	13
P (mg/kg)	103.33	88.68
K (mg/kg)	182.60	165.42

Soil samples were collected for microbiological analysis following the removal of the preceding crop, in our case, maize (Table 2).

Selective nutrient media were used to determine the total number of microorganisms. The methods used are dilution plating and culturing (Tunlid et al. 1989, Boehm et al. 1993).

The research factors were: 1. Soil type: (1) Gley soil and (2) Eutric cambisol; 2. Disease control: (1) control, (2) chemical pesticides and (3) biopreparates (seed + foliar treatment); 3. Nitrogen fertilisation: (1) Based on soil analysis – 100% and (2) 70% of the recommended dose

Base fertilisation on Gley soil was 34.9 kg P/ha, 49.8 kg K/ha, and 50 kg N/ha. Nitrogen topdressing was 30 kg N/ha first, followed by 20 kg N/ha, totalling 100 kg N/ha over the growing season.

On Eutric cambisol, base fertilisation was 52.4 kg P/ha, 83 kg K/ha, and 80 kg N/ha, with topdressing of 40 kg N/ha first, followed by 35 kg N/ha, totalling 155 kg N/ha.

Sowing was performed within optimal windows. Wheat (cv. Maja AG) on Gley soil was sown on October 17, 2021, and October 22, 2022, and on Eutric cambisol on October 20, 2021, and October 26, 2022. Just before sowing, seeds were treated with a biopreparation containing seven strains of *Glomus* and *Gigaspora* fungi (7 × 10^8 CFU (colony-forming unit)/g) and nitrogen-fixing bacteria *Azotobacter chroococcum* (4 × 10^8 CFU/g) and *Azospirillum brasilense* (2 × 10^8 CFU/g) at a rate of 0.50 kg/ha (variant B2).

Seed sufficient for one hectare and 0.5 kg of bio preparation were mixed in a mixer and then transferred to the seeder. The volcanic rock Teralite, in powder form, was used as a carrier for microorganisms.

Disease protection treatments were carried out on April 19, 2022, and April 23, 2023, on both soil types using either fungicides (variant C2) Revystar (0.6 L/ha) + Flex (0.2 L/ha) or the microbiological preparation Vitality-FAZOS with four *Trichoderma* species (7 × 10^9 CFU/mL) at 1 L/ha (variant C3). The second protection treatment was performed on May 23, 2022, and May 29, 2023, with the same products.

Table 2. Microbiological properties of soil

Soil type	Bacteria	Fungi	Actinomycetes
	(log CFU/g)		
Gley soil	8.69	4.22	6.38
Eutric cambisol	6.07	6.54	4.70

CFU – colony-forming units

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At the end of June, the length of the plants was measured, and after harvesting, the yield, hectoliter weight, protein content in the grain and plant height were determined. The protein content was determined using the Infratec Grain Analyser (near infrared spectroscopic method).

Each of the four species of *Trichoderma* was grown individually on solid nutrient medium and transferred to sterile bottles with liquid nutrient medium, where incubation continued for another 72 h. All four species were transferred together, so the stated amount refers to all species. The treatment was done by suspending 1 L per ha of Vitality-FAZOS biopreparation in 200 L of water. Since *Trichoderma* spp. was in a liquid nutrient medium, there was no problem with its adhesion to the above-ground mass of the plant. Chemical fungicides were used at recommended doses.

Figures 1 and 2 show the monthly average amount of precipitation (mm), the monthly average temperature (°C) during the research, and the long-term average. Compared to the first year of the study, precipitation and temperature values distribution were more suitable for wheat growth in the second year of the field trial.

The data obtained were processed using statistical methods (analysis of variance, in the statistical program SAS 9.3, London, UK).

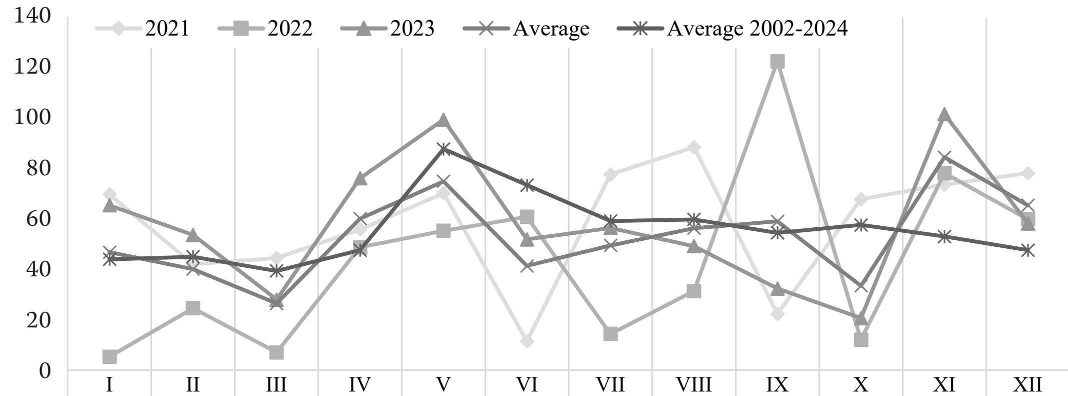
RESULTS AND DISCUSSION

Grain yield. The highest average wheat grain yield (t/ha) in the first year on Gley soil was achieved with lower nitrogen fertilisation, seed treatment, and biopreparation-based disease protection (8.62 t/ha). This yield was statistically significantly higher ($P < 0.01$) than all other variants (Table 3). The highest average yield using biopreparations was 11.23% higher compared to the variant of untreated seeds with protection from chemical fungicides and reduced nitrogen fertilisation.

In the second year of research, the same variant also achieved the highest average result (9.18 t/ha). All other variants achieved a statistically significantly lower yield of wheat grains ($P < 0.01$). In the second research year, the difference between the best variant treated with biopreparations, including a lower fertilisation rate and the best variant with chemical protection, including reduced nitrogen fertilisation, was 14.32%.

On the Eutric cambisol in the 1st year of the study, the highest average yield was obtained with lower nitrogen fertilisation and the application of biopreparations (6.91 t/ha). However, there was no statistically significant difference between the variants treated with chemical fungicides and those with lower inputs of nitrogen fertilisers (6.77 t/ha). In the second year of the research, the highest average yield was obtained in the same cultivar (8.24 t/ha) and was 6.19% higher than the highest average yield treated with chemical fungicides, with lower fertilisation with nitrogen fertilisers (7.76 t/ha).

It is evident that there is a smaller difference between the treatment with chemical fungicides and biopreparations in both treated and untreated seed variants with Eutric cambisol. The reason for this



	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2021	69.6	41.9	44.4	55.7	70.1	11.4	77.5	88.3	22.2	67.7	73.5	78
2022	5.3	24.5	7	48.6	55.2	60.7	14.3	31.3	122.3	12	78	59.8
2023	65.3	53.5	27.9	76	99.2	51.8	56.3	49.1	32.3	20.6	101.4	58.1
Average	46.7	40	26.4	60.01	74.8	41.3	49.4	56.2	58.9	33.4	84.3	65.3
Average 2002-2024	43.9	44.9	39.3	47.5	87.6	73.3	59.1	59.7	54.4	57.5	52.9	47.5

Figure 1. Monthly amounts of precipitation (mm)

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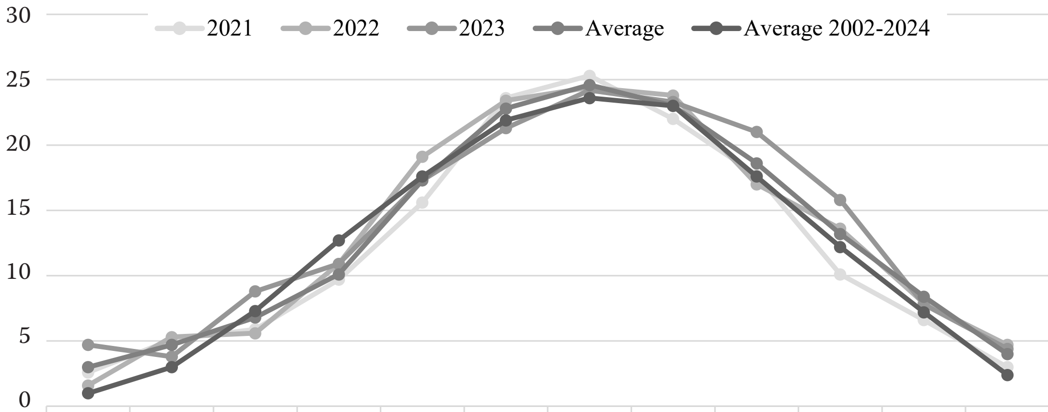

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2021	2.6	5.1	5.9	9.7	15.6	23.6	25.3	22	17.7	10.1	6.6	3
2022	1.6	5.3	5.6	10.9	19.1	23.4	24.4	23.8	17	13.6	7.9	4.7
2023	4.7	3.8	8.8	10.9	17.3	21.3	24.2	23.3	21	15.8	7.8	4.4
Average	3	4.7	6.8	10.1	17.3	22.8	24.6	23	18.6	13.2	8.4	4
Average 2002-2024	1	3	7.3	12.7	17.6	21.9	23.6	23	17.6	12.2	7.2	2.4

Figure 2. Monthly air temperatures (°C)

is probably a significantly lower pH, and therefore a different qualitative and quantitative composition of soil microorganisms (Tables 1 and 2).

Cvijanović et al. (2008) treated wheat seeds with the beneficial bacteria *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Beijerinckia Derxii* and *Klebsiella planticola*, achieving an increase in wheat yield by 11.64%. De Santis et al. (2022) treated wheat seeds with mycorrhizal fungi, which led to a general improvement in yield (24%) an increase in protein content (16%), and an improvement in gluten quality, with an increased proportion of HMW-GS from +17% to +92%.

In their research, Salmabi et al. (2018) obtained an increase in wheat yield by 11% using small concentrations of several types of microorganisms (*Azotobacter* sp., *Agrobacterium* sp., *Bacillus* sp., *Pseudomonas* sp.,

Table 3. Wheat grain yield (t/ha) during the two-year investigation (2022, 2023)

Seed and foliar treatment	Gley soil			Eutric cambisol			Total average
	nitrogen fertilisation						
	100%	70%	average	100%	70%	average	
2022							
A	5.13	5.49	5.31	4.36	4.55	4.46	4.89
B	7.30	7.75	7.53	6.53	6.77	6.65	7.09
C	8.17**	8.62**	8.40**	6.69	6.91	6.80	7.60**
Average	6.87	7.25	7.08	5.86	6.08	5.97	6.53
$LSD_{0.05}$	0.32	0.28	0.31	0.25	0.29	0.28	0.29
$LSD_{0.01}$	0.5	0.55	0.58	0.49	0.57	0.54	0.58
2023							
A	5.75	5.78	5.77	4.96	5.27	5.11	5.44
B	7.86	8.03	7.95	7.60	7.76	7.68	7.82
C	8.67**	9.18**	8.93**	7.87*	8.24**	8.06**	8.50**
Average	7.43	7.66	7.55	6.81	7.09	6.95	7.25
$LSD_{0.05}$	0.26	0.32	0.30	0.23	0.19	0.20	0.25
$LSD_{0.01}$	0.55	0.60	0.58	0.45	0.35	0.37	0.48

Disease protection: A – control; B – treatment with chemical fungicides; C – treatment with biopreparations. *LSD* – least significant difference

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Trichoderma sp., *Azospirillum* sp., *Rhizophagus* sp.). In their research results, Tiwari et al. (2022) point out that they suppressed the pathogen *Bipolaris sorokiniana* by using *Trichoderma* spp. in wheat culture.

Covacevich et al. (2025) treated seeds with mycorrhizal fungi and the beneficial fungus *Trichoderma* spp. to control pathogenic nematodes and obtained excellent results. However, we sprayed the aboveground mass with the biopreparation Vitality-FAZOS on the same dates as we treated with chemical fungicides. In addition to exploiting the antagonism between *Trichoderma* spp. and pathogenic fungi, this beneficial fungus also promoted the growth of the aboveground mass, which ultimately led to an increase in yield.

These studies confirm that biostimulants can stimulate plant growth and yield even when used in small amounts, so they can be used complementarily. For this reason, it is also possible to reduce mineral fertilisation and replace chemical preparations with biopreparations for the treatment of seeds and aboveground parts of the plant.

Grain protein content. The highest average protein content in wheat grain (%) in both years of research and on both soil types was obtained in the variant of treated seeds and with protection against diseases using biopreparations (Table 4). All other variants achieved statistically very significantly lower yields of wheat grains ($P < 0.01$).

The average two-year protein content in wheat grains, in the cultivar mentioned above, on Gley soil was 14.99% and was 2.18% higher than on Eutric cambisol (14.67%). However, compared to the variant with protection from chemical fungicides (14.45%), the difference was 3.74% on the Gley soil. On the Eutric cambisol, the difference mentioned was 4.86%.

The highest average protein content in the grain obtained in the variant of treated seeds and with protection from diseases by the application of biopreparations on the Gley soil was 15.84% higher compared to the control variant. On Eutric cambisol, this difference was 14.07%.

The research results show that numerous authors obtained significantly higher protein content in wheat grain after seed treatment with beneficial bacteria (Donn et al. 2015, Salmabi et al. 2018, Boleta et al. 2020, Moradgholi et al. 2021, Cvijanović et al. 2022). Perelló et al. (2003) used the fungi *Trichoderma koningii* to protect against diseases in Australia, China, and America, and they obtained an increase in all investigated parameters of grain yield and quality.

Akbar et al. (2023), using mycorrhizal fungi to inoculate wheat seeds, reported that the protein content increased to 24.2%. Concentrations of zinc, iron, phosphorus and potassium were also increased to 14.80, 24.00, 21.00, and 30.90% in wheat grain. Improvement

Table 4. Protein content in wheat grain (%) during the two-year investigation (2022, 2023)

Seed and foliar treatment	Gley soil			Eutric cambisol			Total average
	nitrogen fertilisation						
	100%	70%	average	100%	70%	average	
2022							
A	12.72	13.08	12.90	12.50	12.83	12.67	12.78
B	14.11	14.39	14.25	13.67	13.94	13.81	14.03
C	14.48**	14.90**	14.69**	14.21**	14.53**	14.37**	14.53**
Average	13.77	14.12	13.95	13.46	13.77	13.61	13.78
$LSD_{0.05}$	0.12	0.17	0.15	0.19	0.17	0.19	0.18
$LSD_{0.01}$	0.20	0.32	0.28	0.36	0.33	0.37	0.35
2023							
A	13.17	12.80	12.99	12.69	12.88	12.79	12.89
B	14.29	14.50	14.40	13.90	14.03	13.97	14.19
C	14.57**	15.07**	14.82**	14.29**	14.80**	14.55**	14.69**
Average	14.01	14.12	14.07	13.63	13.90	13.77	13.92
$LSD_{0.05}$	0.14	0.18	0.16	0.13	0.20	0.17	0.17
$LSD_{0.01}$	0.27	0.34	0.30	0.25	0.38	0.31	0.33

Disease protection: A – control; B – treatment with chemical fungicides; C – treatment with biopreparations; *LSD* – least significant difference

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effects were also observed on soil fertility, such as the percentage of soil organic carbon, available phosphorus, and potassium increasing by 23.9, 35.8, and 64.7%, depending on the soil properties tested.

Although most research is based on the introduction of effective microorganisms into seeds or the soil, more and more research, as well as the application of microorganisms itself, is focused on foliar treatment to protect against diseases and pests or increase the photosynthesis index.

The authors (Ruth et al. 2011, Ezawa et al. 2018, El-Saadony et al. 2022, Weng et al. 2022) state that due to the huge mass of hyphae of mycorrhizal fungi that intertwine the roots of the plant, pathogenic fungi do not find an infectious site. They also have an antagonistic effect on pathogens in the rhizosphere of plants with their escudates. These substances are strong enough to solubilise rock phosphates, which become available to plants.

Hectoliter weight. According to the results of the wheat yield parameter, the highest average hectoliter weight (kg/hL) in both years of research and on both types of soil was obtained in the variant of treated seeds and with protection from diseases by the use of biopreparations (Table 5). All other varieties achieved a statistically significantly lower hectolitres weight ($P < 0.01$).

The average hectoliter weight of the mentioned variant on gley soil during the two-year study was 82.92 kg/hL, 5.32% higher than the chemical protection against disease variant (78.73 kg/hL) and 12.02% higher than the control variant. These differences on the Eutric cambisol amounted to 2.30% (untreated seed variant with biological protection against disease) or 12.37% (control variant).

In the results of their research, the authors Denardi Munareto et al. (2019) using the associative nitrogen bacteria *Azospirillum brasilense* in wheat, obtained an increase in all research elements, including hectoliter weight by 7.3–11.4%. The authors of Choudhary et al. (2022) used the non-symbiotic nitrogen bacterium *Azotobacter chroococcum* and achieved an increase in hectoliter weight by 6.5%. Using the beneficial fungus *Trichoderma* spp., Mutlu et al. (2021) obtained an increase in all elements of yield and quality of barley, including the hectoliter weight by 4.8–8.5%.

Plant height. The highest average height of a wheat plant (cm) during the two-year study on Gley soil (Table 6) was obtained with seed treatment and protection against diseases using biopreparations (85.60 cm). All other variants achieved a statistically significant wheat plant height ($P < 0.01$). The highest average height of a wheat plant with seed treatment and protection against disease using biopreparations

Table 5. Hectoliter weight (kg/hL) during the two-year investigation (2022, 2023)

Seed and foliar treatment	Gley soil			Eutric cambisol			Total average
	nitrogen fertilisation						
	100%	70%	average	100%	70%	average	
2022							
A	73.20	73.96	73.58	72.14	72.23	72.19	72.89
B	77.24	78.90	78.07	76.08	76.53	76.31	77.19
C	80.13**	81.94**	81.04**	78.55**	79.29**	78.92**	79.98**
Average	76.86	78.27	77.57	75.59	76.02	75.81	76.69
$LS_{D0.0}5$	0.43	0.56	0.50	0.39	0.51	0.44	0.47
$LSD_{0.01}$	0.79	0.94	0.91	0.76	0.94	0.81	0.86
2023							
A	73.30	74.08	73.69	72.48	73.17	72.83	73.26
B	78.19	78.55	78.37	77.12	77.48	77.30	77.84
C	81.84**	83.90**	82.87**	80.05**	81.93**	80.99**	81.93**
Average	77.78	78.84	78.31	76.55	77.53	77.04	77.68
$LSD_{0.05}$	0.37	0.53	0.44	0.34	0.49	0.41	0.43
$LSD_{0.01}$	0.72	1.01	0.82	0.61	0.90	0.80	0.81

Disease protection: A – control; B – treatment with chemical fungicides; C – treatment with biopreparations; *LSD* – least significant difference

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Table 6. Plant height (cm) during the two-year investigation (2022, 2023)

Seed and foliar treatment	Gley soil			Eutric cambisol			Total average
	nitrogen fertilisation						
	100%	70%	average	100%	70%	average	
2022							
A	74.12	75.98	74.70	72.26	71.98	72.12	73.41
B	78.90	79.83	79.37	74.10	76.03	75.07	77.22
C	82.55**	84.10**	83.34**	80.45**	81.57**	81.01**	82.18**
Average	78.52	79.74	79.13	75.60	76.53	76.07	77.59
$LSD_{0.05}$	0.43	0.56	0.50	0.39	0.51	0.44	0.47
$LSD_{0.01}$	0.79	0.94	0.91	0.76	0.94	0.81	0.86
2023							
A	76.38	77.13	76.76	75.30	76.10	75.70	76.23
B	80.63	83.40	82.02	78.09	80.77	79.43	80.73
C	85.00**	87.10**	86.05**	82.14**	83.65**	82.90**	84.48**
Average	80.67	82.54	81.61	78.51	80.17	79.34	80.48
$LSD_{0.05}$	0.37	0.53	0.44	0.34	0.49	0.41	0.43
$LSD_{0.01}$	0.72	1.01	0.82	0.61	0.90	0.80	0.81

Disease protection: A – control; B – treatment with chemical fungicides; C – treatment with biopreparations; *LSD* – least significant difference

was 1.05% higher compared to the variant of wheat protection with chemical fungicides (81.62 cm).

On Eutric cambisol, the highest average wheat plant height (cm) during the two-year study was also obtained with seed treatment and disease protection using biopreparations (82.61 cm). At the same time, all other varieties achieved a statistically significant wheat plant height ($P < 0.01$). The highest average height of a wheat plant with seed treatment and protection against disease using biopreparations was 1.05% higher than the variant of protection with chemical fungicides (78.40 cm).

By treating wheat seeds with endomycorrhizal fungi, Al-Karaki et al. (2004) obtained an increase in plant height by 1.7% to 4.3% depending on the wheat cultivar. Mahato et al. (2018) state that they obtained an increase in the height of wheat plants by 4.6% using the beneficial bacteria *Trichoderma viride* by treating the soil with an inoculum suspension in water. The authors state that in the research results, the lowest values of the investigated parameters were obtained in the combination of *T. viride* and mineral NPK fertiliser, which they explain by a type of antagonism between this beneficial fungus and inorganic fertiliser.

According to the results during this two-year research (2021/2022, 2022/23) the highest average yield of wheat grain (t/ha), protein content in wheat grain (%), hectoliter weight (kg/hL) and average height of a wheat plant (cm) during a two-year study on gley soil was obtained with seed treatment with mycorrhizal fungi and non-symbiotic nitrogen bacteria and protection against diseases also by using biopreparations.

The obtained differences between yield elements and wheat quality between soil types (Gley soil, Eutric cambisol) can be explained by different pedo-physical and chemical-microbiological soil properties. Namely, Gley soil has much better listed properties compared to Eutric cambisol. Also, weather conditions during the second year of research were more favourable for wheat growth than the first year.

Furthermore, we can replace chemical fungicides with microbiological ones, which synthesise growth hormones in addition to their protective effect. These increase the photosynthesis index and contribute to plant development, which is reflected in yield elements. As a result, we get healthy plants and soil without pesticide residues, which represent a major health and environmental problem.

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