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PRELIMINARY DETERMINATION OF THE POSSIBILITY OF REDUCING NITROGEN FERTILIZATION UNDER THE INFLUENCE OF BACTERIAL FORMULATIONS IN THE CULTIVATION OF *TRITICUM AESTIVUM* L.

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Abstract. The use of microorganisms in agriculture is attracting increasing interest. Thus, the state of knowledge on this issue and biotechnological advances are significantly increasing. A field research was conducted during the 2022–2023 growing season to evaluate the influence of the combined application of bacterial formulation and mineral nitrogen fertilization on grain and straw yield of winter wheat (*Triticum aestivum* L.) and the structure of the obtained yield. The factors of the conducted field experiment were bacterial formulations: control (no application of bacterial formulations), I – *Azotobacter and Arthrobacter*, II – *Bacillus subtillis, Bacillus megaterium, Bacillus azotofixans*, III – *Bacillus azotofixans*; B – mineral nitrogen fertilization: control (no mineral nitrogen fertilization), 43 kg $N \cdot ha^{-1}$, 86 kg $N \cdot ha^{-1}$, 130 kg $N \cdot ha^{-1}$. Research was conducted demonstrating the most favorable effects after applying a bacterial formulation containing *Azotobacter* and *Arthrobacter bacteria*. It was statistically significant increase in grain yield by an average of 17%, number of ears per m^2 by 15%, ear length by 5% and thousand grain weight by 3% The application of other formulations also caused an increase in the analyzed traits, but they were lower than those given for *Azotobacte*r and *Arthrobacter* bacteria. A gradual increase in the level of mineral nitrogen fertilization also had a positive effect on the analyzed traits. The research conducted in the field found a possible reduction in mineral nitrogen fertilization under the conditions of the conducted experiment by about 33% without yield losses when using a bacterial formulation containing *Azotobacter* and *Arthrobacter*. Thus, it is appropriate to recommend the use of these bacteria in winter wheat cultivation, but due to the possible variable effectiveness of application under different soil and climatic conditions, the presented research should be continued in different areas both in wheat and other crops.

Key words: winter wheat, mineral fertilization, bacterial formulation, grain yield.

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INTRODUCTION

The results obtained from wheat cultivation depend largely on the selection of the variety for the given conditions, tillage, and to the greatest extent on fertilization and weather conditions during the growing season (Morgounov et al. 2014; Peng et al. 2020; Hlisnikovský et al. 2023). According to Lollato et al. (2020), the most important macronutrient affecting wheat yield and quality is nitrogen. The continuous growth of the world's population, and thus the need to increase food production, is causing an increase in the use of nitrogen fertilizers worldwide (Zhang and Zhang 2007). However, the use of mineral fertilizers negatively affects the environment. The use, especially excessive use of nitrogen fertilizers can cause groundwater pollution (Mahvi et al. 2005) and according to Spiertz (2009) in Europe, agriculture is responsible for 40 to 80% of the nitrogen load entering surface water. Also as reported by Ghimire et al. (2023), excessive and unsustainable use of fertilizers contributes to greenhouse gas (N_2O) emissions. It should also be noted that high doses of nitrogen in wheat cultivation can cause a reduction in the yield obtained and its quality due to lodging and changes in plant metabolism (Kong et al. 2017; Khan et al. 2020). In addition, according to data presented by Omara et al. (2019), the worldwide efficiency of nitrogen use from mineral fertilizers is only about 35%. The use of nitrogen fertilizers is also one of the main cost-intensive components in grain cultivation (Herrera et al. 2016). According to data presented by Hlisnikovský et al. (2023), over the past 10 years or so, the price of nitrogen fertilizers has increased, depending on the type, from 84 to 150%. For the reasons given, it is necessary to look for solutions that would make it possible to reduce the use of mineral nitrogen fertilizers without adversely affecting the grain yields obtained and their quality.

One way to reduce the use of nitrogen fertilizers could be the use of microorganisms in agriculture. According to Herrera et al. (2016), several microorganisms are currently being used in agriculture, but there are still doubts about their effectiveness and stability over time and changing climatic conditions. It has been demonstrated that rhizospheric microorganisms which are referred to as plant growth-promoting bacteria (PGPR) include *Azospirillum, Azotobacter, Pseudomonas, Acetobacter, Serratia, Bacillus*, and *Burkholderia* can cause an increase in plant yield and improve the quality of yields obtained (Herrera et al. 2016). These bacteria affect plant nutrition through non-symbiotic nitrogen fixation, increasing the availability of nutrients in the rhizosphere such as phosphorus, and increasing root surface area through the production of indoleacetic acid IAA, cytokinin, gibberellin, among others (Dal Cortivo et al. 2017). Nitrogen-fixing bacteria and phosphate-solubilizing bacteria tend to convert atmospheric nitrogen into a plant-available form, produce enzymes, and dissolve insoluble phosphate from organic and inorganic sources (Ahmad et al. 2017).

The mechanism of nitrogen delivery to the plant is the same in all free-living bacteria. Non-symbiotic bacteria carry out biological nitrogen fixation only during growth and assimilate nitrogen for the metabolism of their cells, without releasing the excess into the environment. Only after cell death is the plant or soil enriched with this element (Górski et al. 2023). The mechanism by which phosphorus is dissolved into plant-available forms by phosphorus-releasing bacteria is the production of mineral solubilizing compounds such as organic acids, siderophores, protons, hydroxyl ions and CO_2 (Sharma et al. 2013). Currently, there is a growing interest in applying PGPR to cereal crops. A number of studies (Turan et al. 2012; Kumar et al. 2014; Game et al. 2020; Gayatri et al. 2022) have demonstrated an increase in yield and quality in cereal crops following the application of bacterial formulation. However, several factors, such as the genotype of the plant, the species and strain of bacteria, and agricultural practices, can affect the response of plants and the success of bacterial formulation application (Tahir et al. 2015). Thus, there are several doubts about the efficacy and stability of positive effects on the performance of bacterial formulations over time and varying climatic conditions (Herrera et al. 2016).

For these reasons, a field research was undertaken with the aim of evaluating the possibility of reducing mineral nitrogen fertilization under the influence of bacterial formulation application in winter wheat cultivation. The research hypothesis assumed that the use of bacterial formulation would reduce the amount of mineral fertilizer without negatively affecting winter wheat yields and yield structure.

MATERIALS AND METHODS

The field experiment was conducted in Poland during the 2022–2023 growing season near the village of Wierzbowo (52°57′50″N 20°43′30″E) in Mazowieckie Voivodship on soil of class IIIa of the good wheat complex – according to the Polish soil classification. The soil on which the experiment was carried out was characterized by a neutral pH (pH 6.5) with an organic carbon content of 2.24% d.m. The content of available mineral elements in the soil was 12.8 mg P·100 g^{-1} soil, 50.6 mg K·100 g^{-1} soil, 12.5 mg Mg·100 g^{-1} soil. The nitrogen content before the establishment of the experiment was as follows: in the 0–30 cm layer 5.02 mg N·kg^{-1} soil, in the 30–60 cm layer 2.83 mg·kg^{-1} soil. Weather conditions during the implementation of the field experiment were obtained from the meteorological station belonging to the Ignacy Mościcki State University of Applied Sciences in Ciechanów. The course of weather conditions is shown in Fig. 1.

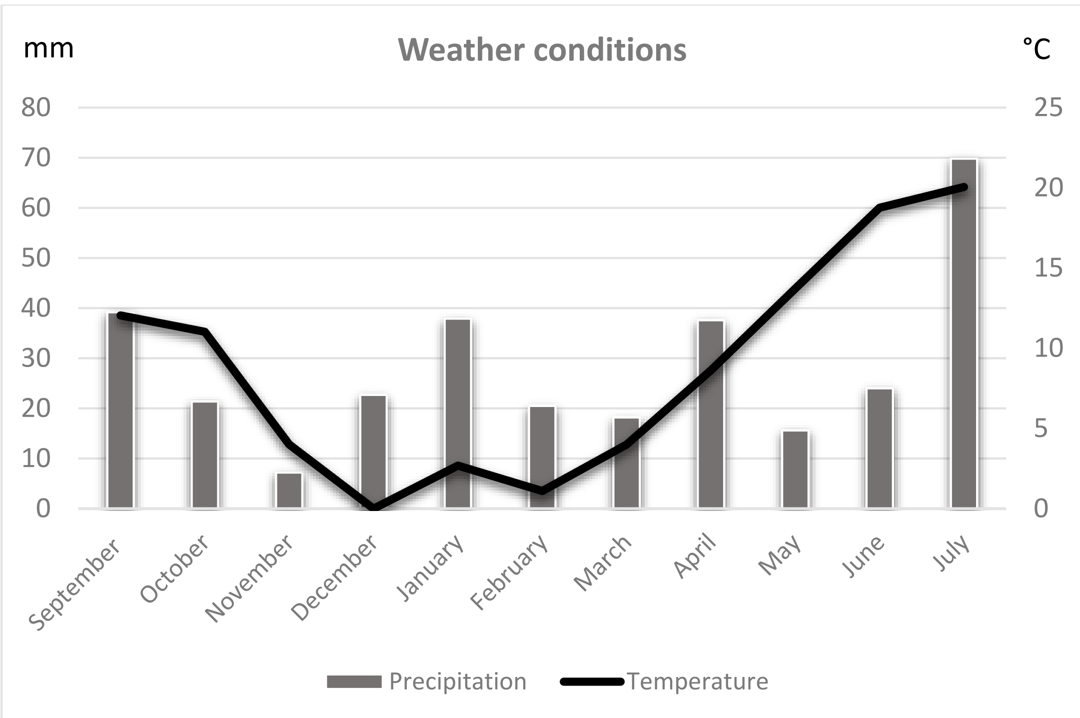

Fig. 1. Weather conditions during the growing season of winter wheat

Weather conditions during field research varied. The average temperature during the analyzed period was characteristic of the climatic zone of the research site. A gradual decrease in temperature was observed from September to December. Then from December to July an increase in temperature was observed. On the other hand, the precipitation occurring during the analyzed period was erratic. In the months of September, January and April, a precipitation sum of about 40 mm was recorded, while in July it was about 70 mm. In the remaining months, precipitation was about 20 mm, except in December where a precipitation sum of less than 10 mm was recorded.

The field experiment was established in a randomized block design with three repetitions. The following experimental factors were analyzed: bacterial formulation: control (no application of bacterial formulation), I – *Azotobacter* and *Arthrobacter*, II – *Bacillus subtilis*, *Bacillus megaterium, Bacillus azotofixans*, III – *Bacillus azotofixans*; and mineral nitrogen fertilization: control (no mineral nitrogen fertilization), 43 kg N·ha^{-1}, 86 kg N·ha^{-1}, 130 kg N·ha^{-1}.

The area of one experimental plot was 20 m^2. The forecrop for the winter wheat crop was rapeseed (*Brassica napus* L. *var. napus*) and charlock (*Sinapis* L.). The rapeseed residue was mixed into the soil with a disc aggregate to control weeds and break up the crop residue. A chisel cultivator, without plowing, was used to prepare the soil before sowing. Sowing of winter wheat was carried out on 26.09.2022 with a grain drill, at a depth of 3 cm with a row spacing of 12 cm. The sowing rate of winter wheat Euforia variety was 170 kg·ha^{-1} (400 grains/m^2). At the same time as sowing, fertilization was applied at a rate of 300 kg·ha^{-1} with a compound fertilizer composed of 40% SO_3, 30% CaO and 10% SiO_2. During winter wheat vegetation on 04.05.2023, a growth regulator containing 750 g/l chlormequat chloride was applied at a rate of 1.5 L ha^{-1}. During the experiment, full herbicide and fungicide protection was applied in accordance with the agricultural practice of the experimental area. Full mineral fertilization with nitrogen was applied at three doses. The first dose was applied on 17.10.2022 at 20 kg N·ha^{-1} in the form of ammonium nitrate 32%. The second dose was applied on 06.04.2023 in the amount of 80 kg N·ha^{-1} also in the form of ammonium nitrate 32%. The third dose on 10.05.2023 consisted of a foliar spray containing 14.85% N and urea 46%. In total, the third dose provided 30 kg N·ha^{-1}. The total full mineral fertilization with nitrogen was 130 kg N·ha^{-1}, which was taken as 100% fertilization. Accordingly, on subsequent experimental sites, mineral nitrogen fertilization was reduced to about 67% – 86 kg N·ha^{-1}, and about 33% – 43 kg N·ha^{-1}. The application of bacterial formulation was carried out on 19.04.2023 on a warm, cloudy day at a temperature of about 20°C. Bacterial formulation I containing *Azotobacter* and *Arthrobacter* bacteria (10^9 CFU·g^{-1}) was applied at a rate of 0.1 kg/300 L water·ha^{-1}. Bacterial formulation II containing *Bacillus subtillis, Bacillus megaterium, Bacillus azotofixans* (10^9 CFU·g^{-1}) was applied at a rate of 1 kg/300 L water·ha^{-1}. On the other hand, spraying with formulation III, which included *Bacillus azotofixans* bacteria (10^9 CFU·g^{-1}), was applied at a rate of 1 kg/300 L water ha^{-1}. The doses of the applied bacterial formulations were based on the manufacturer's recommendations. Harvesting of winter wheat was carried out on 25.07.2023 using electric shears from an area of 1 m^2 from each experimental plot. In the obtained experimental material, the number of ears per m^2, ear length, grain yield and straw yield were evaluated. In the obtained grain yield, the weight of 1000 grains was evaluated.

The obtained results were subjected to statistical analysis by ANOVA. The significance of sources of variation was tested using Fisher-Snedecor's F test ($F \leq 0.05$), and differences between the compared averages were verified using Tukey's HSD test ($p \leq 0.05$). The strength of the relationship between grain yield and yield structure was assessed by calculating Pearson correlation coefficients. All calculations were performed using Statistica version 13.3 (Hamburg, Germany).

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) carried out for the experiment factors researched and their interactions are shown in Table 1. Significant effects of the use of bacterial formulation and nitrogen fertilization on grain yield, straw yield, number of ears per m^2, ear length and thousand grain weight were demonstrated. The interaction of experimental factors on the analyzed traits was also revealed, with the exception of ear length.

Table 1. Analysis of variance (ANOVA) for impact of the analyzed factors of the experiment on the effects of winter wheat cultivation

Source of variation	Parameter				
	grain yield	straw yield	number of ears per m^2	ear length	1000 grain weight
Bacterial formulations (B)	<0.01	<0.05	<0.01	<0.01	<0.01
Mineral nitrogen fertilization (M)	<0.01	<0.01	<0.01	<0.01	<0.01
B × M	<0.05	<0.05	<0.05	ns	<0.05

ns – not significantly.

The highest yield of winter wheat was found after application of formulation I, but it did not differ significantly from the yield obtained after application of formulation II (Table 2).

Table 2. Winter wheat grain yield ($t \cdot ha^{-1}$) as affected by the application of bacterial formulation and mineral nitrogen fertilization

Bacterial formulations	Mineral nitrogen fertilization ($kg\ N \cdot ha^{-1}$)				Means
	0	43	86	130	
Control	5.83^{a}	6.88^{ab}	7.92^{b}	9.11^{c}	7.43^{A}
I	7.67^{a}	7.96^{a}	9.07^{b}	10.15^{b}	8.71^{C}
II	7.27^{a}	7.88^{ab}	8.93^{bc}	9.76^{c}	8.46^{BC}
III	7.02^{a}	7.56^{ab}	8.64^{bc}	9.72^{c}	8.24^{B}
Means	6.95^{A}	7.57^{B}	8.64^{C}	9.68^{D}	–

Control – no bacterial formulations, I – *Azotobacter* and *Arthrobacter*, II – *Bacillus subtillis*. *Bacillus megaterium*, *Bacillus azotofixans*, III – *Bacillus azotofixans*. Values in verse for the interaction followed by the same small letter (a, b, c) do not differ significantly at p ≤ 0.05. Means for the bacterial formulations in a column followed by the same capital letter (A, B, C) do not differ significantly at p ≤ 0.05. Means for the mineral nitrogen fertilization in verse followed by the same capital letter (A, B, C, D) do not differ significantly at p ≤ 0.05.

There were also no significant differences in average yield between formulation II and III. Compared to control objects without bacterial formulation, there was an 11% (0.80 $t \cdot ha^{-1}$)

increase in yield after application of formulation III, 14% (1.3 t·ha^{-1}) increase in yield of formulation II and 17% (1.28 t·ha^{-1}) increase in yield of formulation I. Nitrogen fertilization also significantly differentiated wheat yields. The lowest yield was obtained on control objects in the absence of nitrogen fertilization. The application of fertilizer at 43 kg N·ha^{-1} increased yield by 9% (0.62 t·ha^{-1}), 86 kg N·ha^{-1} by 24% (1.69 t·ha^{-1}), and 130 kg N·ha^{-1} by 39% (2.74 t·ha^{-1}), respectively, compared to the control objects. The interaction of bacterial formulation × nitrogen fertilization in the case of application of formulation I demonstrated that there were no significant differences between the control object and the object with fertilization at 43 kg N·ha^{-1} and between fertilization at 86 and 130 kg N·ha^{-1}. The application of formulation II and III achieved the highest yield with the highest nitrogen fertilization analyzed. However, no significant difference was found between the highest fertilization and the amount of 86 kg N·ha^{-1}. Similarly, no significant differences were found between the objects with fertilizations of 43 and 86 kg N·ha^{-1}.

The highest straw yield was obtained after the application of formulation II, but it did not differ from the straw yield obtained with the other bacterial formulations (Table 3).

Table 3. Winter wheat straw yield (t·ha^{-1}) as affected by the application of bacterial formulation and mineral nitrogen fertilization

Bacterial formulations	Mineral nitrogen fertilization (kg N·ha^{-1})				Means
	0	43	86	130	
Control	7.87^{a}	8.29^{ab}	9.12^{bc}	9.56^{c}	8.71^{A}
I	8.05^{a}	8.85^{ab}	9.56^{b}	9.61^{b}	9.02^{AB}
II	8.62^{a}	8.79^{a}	9.52^{a}	9.58^{a}	9.13^{B}
III	8.19^{a}	8.26^{a}	9.21^{ab}	9.57^{b}	8.81^{AB}
Means	8.18^{A}	8.55^{A}	9.35^{B}	9.58^{B}	–

Control – no bacterial formulations, I – *Azotobacter* and *Arthrobacter*, II – *Bacillus subtillis*. *Bacillus megaterium*, *Bacillus azotofixans*, III – *Bacillus azotofixans*. Values in verse for the interaction followed by the same small letter (a, b, c) do not differ significantly at $p \leq 0.05$. Means for the bacterial formulations in a column followed by the same capital letter (A, B) do not differ significantly at $p \leq 0.05$. Means for the mineral nitrogen fertilization in verse followed by the same capital letter (A, B) do not differ significantly at $p \leq 0.05$.

In addition, there were no significant differences between the control objects without bacterial formulation and formulation I and III. With respect to the control object without bacterial formulations, the application of formulation III resulted in an increase in straw yield by about 1% (0.10 t·ha^{-1}), formulation I by 4% (0.31 t·ha^{-1}), and formulation II by 5% (0.42 t·ha^{-1}). The highest straw yield was obtained on the objects where nitrogen fertilization was applied at 86 and 130 kg N·ha^{-1}, while the lowest straw yield was obtained on the control objects and when fertilization was applied at 43 kg N·ha^{-1}. The interaction of bacterial formulation x nitrogen fertilization demonstrated that the application of formulation I and the application of mineral fertilization obtained straw yields that were not significantly different. The lowest straw yield on the sites with this formulation was obtained on the sites without mineral fertilization. In addition, no significant differences were found between the control objects and the objects where fertilization was applied at 43 kg N·ha^{-1}. In the case of application of formulation II, no significant differences were found between all the analyzed objects. The application of formulation III yielded the highest straw yield when fertilization was applied at 86 and 130 kg N·ha^{-1}. The lowest straw yield when this formulation was applied was obtained on control objects, but no statistically significant differences were found between this object and the application of fertilization at 43 and 86 kg N·ha^{-1}.

The highest number of ears per m^2 was obtained on sites where formulation I was applied (Table 4).

Table 4. Number of ears per m^2 (pcs.) of winter wheat as affected by the application of bacterial formulation and mineral nitrogen fertilization

Bacterial formulations	Mineral nitrogen fertilization (kg N·ha^{-1})				Means
	0	43	86	130	
Control	564^{a}	592^{ab}	644^{b}	684^{b}	621^{A}
I	656^{a}	684^{ab}	712^{b}	812^{c}	716^{C}
II	632^{a}	660^{a}	708^{a}	792^{b}	698^{BC}
III	616^{a}	656^{a}	672^{a}	760^{b}	676^{B}
Means	617^{A}	648^{B}	684^{C}	762^{D}	–

Control – no bacterial formulations, I – *Azotobacter* and *Arthrobacter*, II – *Bacillus subtillis*. *Bacillus megaterium*, *Bacillus azotofixans*, III – *Bacillus azotofixans*. Values in verse for the interaction followed by the same small letter (a, b, c) do not differ significantly at $p \leq 0.05$. Means for the bacterial formulations in a column followed by the same capital letter (A, B, C) do not differ significantly at $p \leq 0.05$. Means for the mineral nitrogen fertilization in verse followed by the same capital letter (A, B, C, D) do not differ significantly at $p \leq 0.05$.

In addition, there were no significant differences between formulation I and II, and between formulation II and III. Compared to the control objects, the application of formulation I increased the number of ears per m^2 by 15% (95 pcs.), formulation II by 12% (77 pcs.), and formulation III by 9% (55 pcs.). The lowest number of ears per m^2 was obtained on objects where no nitrogen fertilization was applied, while the highest on objects where 130 kg N·ha^{-1} was applied. Compared to control objects, fertilization with 43 kg N·ha^{-1} demonstrated an increase in the number of ears per m^2 by 5% (31 pcs.), 86 kg N·ha^{-1} by 11% (67 pcs.), and 130 kg N·ha^{-1} by 24% (145 pcs.). The interaction of bacterial formulation × nitrogen fertilization for formulation I demonstrated the highest number of ears per m^2 with the highest nitrogen fertilization. While the lowest on the object without nitrogen fertilization, there was also no significant difference between this object and the object with fertilization of 43 kg N·ha^{-1}. There were also no significant differences between the objects with fertilization of 43 and 86 kg N·ha^{-1}. On the objects with formulation II and III, the highest number of ears per m^2 was found at the highest nitrogen fertilization. On the objects with these formulations, no significant differences were found between the control objects and those on which fertilization was applied at 43 and 86 kg N·ha^{-1}.

The longest wheat ears were found on objects on which formulation I was applied, while the shortest were found on objects without bacterial formulation (Table 5).

Table 5. Winter wheat ear length (cm) as affected by the application of bacterial formulation and mineral nitrogen fertilization

Bacterial formulations	Mineral nitrogen fertilization (kg N·ha^{-1})				Means
	0	43	86	130	
Control	8.6	9.1	9.7	10.0	9.4^{A}
I	9.1	9.6	10.1	10.3	9.8^{C}
II	8.9	9.5	9.8	10.0	9.6^{B}
III	9.0	9.5	10.0	10.1	9.6^{B}
Means	8.9^{A}	9.4^{B}	9.9^{C}	10.1^{D}	–

Control – no bacterial formulations; I – *Azotobacter* and *Arthrobacter*, II – *Bacillus subtillis*. *Bacillus megaterium*, *Bacillus azotofixans*, III – *Bacillus azotofixans*. Means for the bacterial formulations in a column followed by the same capital letter (A, B, C) do not differ significantly at $p \leq 0.05$. Means for the mineral nitrogen fertilization in verse followed by the same capital letter (A, B, C, D) do not differ significantly at $p \leq 0.05$.

In addition, there were no significant differences in ear length between the objects on which formulation II and III were applied. Application of formulation II and III increased ear length by 2%, formulation, while formulation I increased ear length by 4% compared to objects without bacterial formulation. Mineral nitrogen fertilization also significantly affected the length of wheat ears. The shortest ears were obtained on objects without fertilizer application, while the highest ones were obtained with the highest of the analyzed nitrogen fertilizers, i.e. 130 kg N·ha^{-1}. With respect to the control objects, fertilization at 43 kg N·ha^{-1} caused an increase in ear length by 6%, 86 kg N·ha^{-1} by 11%, while 130 kg N·ha^{-1} by 14%.

Field research demonstrated the highest 1000 grain weight of winter wheat after application of formulation I (Table 6).

Table 6. 1000 grain weight (g) of winter wheat as affected by the application of bacterial formulation and mineral nitrogen fertilization

Bacterial formulations	Mineral nitrogen fertilization (kg N·ha^{-1})				Means
	0	43	86	130	
Control	46.25^{a}	49.68^{b}	51.53^{bc}	52.18^{c}	49.91^{A}
I	49.03^{a}	50.51^{a}	52.94^{b}	53.97^{b}	51.61^{B}
II	48.61^{a}	50.03^{ab}	52.07^{bc}	52.61^{c}	50.83^{B}
III	48.92^{a}	50.06^{a}	52.84^{b}	53.32^{b}	51.29^{B}
Means	48.20^{A}	50.07^{B}	52.35^{C}	53.02^{C}	–

Control – no bacterial formulations, I – *Azotobacter* and *Arthrobacter*, II – *Bacillus subtillis*. *Bacillus megaterium*, *Bacillus azotofixans*, III – *Bacillus azotofixans*; values in verse for the interaction followed by the same small letter (a, b, c) do not differ significantly at $p \leq 0.05$. Means for the bacterial formulations in a column followed by the same capital letter (A, B) do not differ significantly at $p \leq 0.05$. Means for the mineral nitrogen fertilization in verse followed by the same capital letter (A, B, C) do not differ significantly at $p \leq 0.05$.

However, the obtained weight of 1000 grains after the application of this formulation did not differ significantly between the results obtained from other experimental objects where bacterial formulation was used. Significantly the lowest 1000 grain weight was obtained no objects on which bacterial formulation was not applied. However, the revealed average increase in the weight of 1000 grains between the objects on which bacterial formulation was applied and the control objects was small, amounting to only between 3 and 4%. The application of nitrogen fertilization also significantly differentiated the weight of 1000 grains of wheat. The lowest weight of 1000 grains was revealed on objects where no mineral nitrogen fertilization was applied, higher by 4% on objects where fertilization was applied at 43 kg $N \cdot ha^{-1}$, and significantly highest on objects where fertilization was applied at 86 and 130 kg $N \cdot ha^{-1}$ (an increase of 9 and 10%, respectively, compared to control objects). The field experiment also demonstrated the interaction of bacterial formulation x mineral fertilization in relation to the weight of 1000 grains. With the application of formulation I and III, the lowest weight of 1000 grains was revealed on the objects without mineral nitrogen fertilization and with fertilization of 43 kg $N \cdot ha^{-1}$. Significantly higher values not statistically different were obtained with fertilization of 86 and 130 kg $N \cdot ha^{-1}$. In contrast, the application of formulation II demonstrated the lowest weight of 1000 grains on the objects without nitrogen fertilization. However, the value obtained was not significantly different from the object with mineral nitrogen fertilization at 43 kg $N \cdot ha^{-1}$. In contrast, the highest value of 1000 grain weight was obtained on objects where the highest mineral nitrogen fertilization was applied. However, in this case, no statistically significant differences were revealed with the object on which fertilization was applied at 86 kg $N \cdot ha^{-1}$.

Pearson correlation analysis was conducted and demonstrated a highly significant relationship ($p < 0.01$) between the analyzed parameters (Table 7).

Table 7. Correlation coefficients (n = 48) between winter wheat grain yield and yield structure

Parameter	Grain yield	Straw yield	Number of ears per m^2	Ear length
Straw yield	0.8210	–	–	–
Number of ears per m^2	0.8549	0.7928	–	–
Ear length	0.8482	0.8224	0.7611	–
1000 grain weight	0.8459	0.8220	0.7492	0.8814

The analysis demonstrated the highest correlation between grain yield and the number of ears per m^2. However, a highly significant correlation occurred between yield and ear length and 1000 seed weight. A slightly lower correlation value was obtained between grain yield and straw yield of winter wheat. Straw yield, on the other hand, was also highly significantly correlated with the other parameters of wheat yield structure, as were the other parameters analyzed.

DISCUSSION

Nitrogen is an essential macronutrient for plants because it is a major part of proteins and nucleotides. Producing 1 kg of dry biomass requires plant roots to take up between 20–50 g of N (Shahzad and Ahmad 2019). The natural supply of nitrogen is insufficient to meet the plants' needs for optimal yield (Ahmad et al. 2015). Therefore, it is important to supply nitrogen from other sources.

Thus, in a study conducted, the application of bacterial formulation containing *Bacillus subtillis, Bacillus megaterium, Bacillus azotofixans, Azotobacter* and *Arthrobacter* which increase nitrogen availability in the soil as well as increasing mineral fertilization resulted in an increase in winter wheat yield. Also, research by Hafez et al. (2019) in which bacterial formulations containing *Azospirillum* and *Azotobacter* were used demonstrated an increase in wheat yields received compared to control objects. Similarly to the presented study, the application of *Paenibacillus azotofixans* + *Bacillus megaterium* + *Bacillus subtilis* in the study of Stępień et al. (2022) resulted in an increase in wheat yields. The percentage increase in wheat yield obtained in the cited experiment was about 3 percentage points higher than in the authors' own study. The reason for the increase in wheat yield can be linked to the increased availability of nutrients in the soil as a result of bacterial formulation (El-Sorady et al. 2022). In addition, the use of bacterial formulation results in more intensive development of plant root surfaces (Vafa et al. 2021). It contributes to more intensive absorption of water and nutrients (Rostami and Mohammadi 2020). However, in our own study, nitrogen fertilization had a greater influence on wheat grain yield than the use of bacterial formulation. Nitrogen fertilization resulted in an increase in yield between the control and full fertilization facilities by nearly 40%, while the most favorable bacterial formulation in this study resulted in an increase of less than 20%.

According to Vafa et al. (2021), the use of biofertilizers can lead to increased photosynthesis as a result of the aforementioned increase in water and nutrient uptake, which consequently leads to an increase in plant height. Ghanbarzadeh et al. (2019) point to the production of phytohormones and improved N availability in the soil as a possible reason for increased plant height as a result of the use of bacterial formulation. A research by El-Sorady et al. (2022) and Jiriaei et al. (2014) also demonstrated an increase in the number of ears per m^2 as a result of bacterial formulation and mineral fertilization. Also, Tavakoli and Jalali (2016) indicate a positive effect of the combined application of bacterial formulation and nitrogen fertilizer on the number of wheat ears per m^2, which again is attributed to better root system development and higher nutrient uptake. This finding is confirmed by the results of our own research, in which both bacterial formulation and nitrogen fertilization resulted in an increase in the number of ears per m^2.

On the other hand, the increase in wheat straw yield obtained in our own study can be attributed precisely to the increased number of ears per m^2 and presumably to the greater plant height. According to Chandran et al. (2021), PGPR bacteria directly increase plant growth through the production of phytohormones such as auxins, cytokinins, gibberellins and ethylene which play an important role in root revitalization. Damam et al. (2016) report that phytohormones are the most important growth regulators because of the activation of plant metabolisms and simulation of defense processes. PGPR bacteria can also indirectly improve plant growth by preventing pathogenic bacteria and fungi as a result of producing antimicrobial metabolites such as chitinase, protease and lipase. All these aspects positively affect both plant yield and yield structure.

Research conducted by El-Sorady et al. (2022) and Gayatri et al. (2022) demonstrated an increase in the weight of 1000 grains after the application of bacterial formulations based on phosphate-solubilizing bacteria and nitrogen-fixing bacteria. Research by Harahap et al. (2023) on the application of biofertilizers in combination with mineral fertilization in rice cultivation also demonstrated an increase in 1000 grain weight. The increase in the weight of 1000 grains can be attributed to the increased development of the root system, which plays a key role in the absorption and transfer of nutrients to the reproductive parts of the plant crown, resulting in improved yield parameters (İpek 2019). This relationship is confirmed by the authors' own results.

On the basis of the Pearson correlation carried out, it can be concluded that the obtained grain yield depended to the greatest extent on the number of ears per m^2. However, the obtained correlation values between the other analyzed parameters of the yield structure were also highly significant. On the other hand, the correlation values of grain yield with yield structure parameters obtained by other authors detected the highest correlation between yield and the weight of 1000 grains (El-Sorady et al. 2022; Yousefian et al. 2021). According to Wang et al. (2021), the yield traits that most significantly affect yield are the weight of 1000 seeds, the number of grains per ear and the number of ears per area. However, analyzing research conducted by other authors, the highest correlation coefficients between grain yield and yield traits are different (Al-Tabbal and Al-Fraihat 2012; Singh et al. 2015; Wiegmann et al. 2019). Significant influence on the obtained correlation values may have soil and climatic conditions of the cultivation (Górski et al. 2023). According to Levakova (2022), an increase or decrease in one of the yield parameters can be compensated by another depending on the climatic conditions of the research conducted. The authors' field experiment on combining mineral nitrogen application with bacterial formulation demonstrated a possible reduction in mineral fertilization nitrogen by 33% without a statistically significant reduction in winter wheat yield. Research by Fukami et al. (2016) on the application of *Azospirillum brasilense* bacteria to corn revealed a reduced need for nitrogen fertilization by 25%, while Oliveira et al. (2017) demonstrated a possible reduction in fertilization by up to 80%. On the other hand, Volkogon et al. (2022) found that the use of bacterial formulation based on the same bacteria can offset the mineral fertilization of N60P60K60 in barley cultivation. Thus, it is reasonable to assume that the use of bacterial formulation may show variable effectiveness depending on soil and climatic conditions and the species of crops grown. However, both the presented results of the authors' own research and those presented in the available literature unanimously indicate positive effects of introducing bacterial formulation in agriculture.

CONCLUSIONS

The use of bacterial formulation in combination with mineral nitrogen fertilization has a positive effect on grain and straw yield of winter wheat and the structure of the yield obtained. The most favorable effects in the presented study were obtained from the application of *Azotobacter* and *Arthrobacter* both with full and limited mineral nitrogen fertilization. In addition, the use of bacterial formulations allowed to reduce fertilization by about 33%, which is important for both economic and environmental reasons. Thus, it can be concluded that bacterial formulations containing these bacteria should be particularly recommended for widespread agricultural practice in winter wheat cultivation. Preliminary field research has demonstrated the positive effect of the combination of bacterial formulation × mineral fertilization. However, this type of research should be continued both with the microorganisms in question for different field crops and under different climatic conditions, as the effectiveness of their application may be largely dependent on these parameters.

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WSTĘPNE OKREŚLENIE MOŻLIWOŚCI REDUKCJI NAWOŻENIA AZOTOWEGO POD WPŁYWEM PREPARATÓW BAKTERYJNYCH W UPRAWIE *TRITICUM AESTIVUM* L.

Streszczenie. Stosowanie mikroorganizmów w rolnictwie wzbudza coraz większe zainteresowanie. Tym samym stan wiedzy dotyczący tego zagadnienia oraz postęp biotechnologiczny znacznie wzrastają. Przeprowadzono badania polowe w sezonie wegetacyjnym 2022–2023, które miały na celu ocenę wpływu łącznego stosowania preparatów bakteryjnych oraz nawożenia mineralnego azotem na plon ziarna i słomy pszenicy ozimej (*Triticum aestivum* L.) oraz strukturę uzyskiwanego plonu. Czynnikami prowadzonego doświadczenia polowego były preparaty bakteryjne: kontrola (brak stosowania preparatów bakteryjnych), I – *Azotobacter* i *Arthrobacter*, II – *Bacillus subtillis, Bacillus megaterium, Bacillus azotofixans*, III – *Bacillus azotofixans*; oraz nawożenie mineralne azotem: kontrola (brak nawożenia mineralnego azotem), 43 kg $N \cdot ha^{-1}$, 86 kg $N \cdot ha^{-1}$, 130 kg $N \cdot ha^{-1}$. Przeprowadzone badania wykazały najkorzystniejsze efekty po zastosowania preparatu bakteryjnego zawierającego bakterie *Azotobacter* i *Arthrobacter*. Wykazano istotny statystycznie wzrost plonów ziarna średnio o 17%, liczby kłosów na m^2 o 15%, długości kłosa o 5% oraz masy tysiąca ziaren o 3%. Zastosowanie pozostałych preparatów również powodowało wzrost analizowanych cech, lecz były one niższe od podanych dla bakterii *Azotobacter* i *Arthrobacter*. Stopniowy wzrost poziomu nawożenia mineralnego N również wpływał pozytywnie na analizowane cechy. Przeprowadzone badania polowe pozwoliły stwierdzić możliwą redukcję nawożenie mineralnego w warunkach prowadzonego doświadczenia N o około 33% bez strat w plonach przy zastosowaniu preparatu bakteryjnego zawierającego *Azotobacter* i *Arthrobacter*. Należy więc zalecać stosowanie tych bakterii w uprawie pszenicy ozimej, jednak ze względu na możliwą zmienną skuteczność stosowania w różnych warunkach glebowo klimatycznych należy kontynuować przedstawione badania na różnych obszarach zarówno w uprawie pszenicy, jak i innych roślin.

Słowa kluczowe: pszenica ozima, nawożenie mineralne, preparaty bakteryjne, plon ziarna.