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The Influence of Different Nitrogen Fertilizer Rates, Urease Inhibitors and Biological Preparations on Maize Grain Yield and Yield Structure Elements

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Abstract: The field experiment was performed in 2019–2021 at the Experimental Station of Vytautas Magnus University Agriculture Academy (54°52′ N, 23°49′ E). The soil of the experimental field was Endohipogleyic-Eutric Planasol. The studied factors were: Factor A—different nitrogen fertilizer rates: (1) 100 kg N ha^{-1}; (2) 140 kg N ha^{-1}; (3) 180 kg N ha^{-1}; Factor B—the use of urease inhibitors (UI) and biological preparations (BP): (1) urease inhibitors (UI) and biological preparations (BP) were not used; (2) Urease inhibitor (UI ATS)—ammonium thiosulfate—[$(NH_4)_2S_2O_3$ 12-0-0-26S]; (3) Urease inhibitor (UI URN)—N-butyl-thiophosphorus triamide (NBPT) and N-propyl-thiophosphorus triamide (NPPT); (4) Biological preparation (BP HUM)—suspension of humic and fulvic acids; (5) Biological preparation (BP FIT)—*Ascophyllum nodosum* suspension. Our studies showed that the highest yield of maize grain (8.9–12.0 t ha^{-1}) was obtained by fertilizing with N180 and using the urease inhibitor ammonium thiosulfate (ATS). ATS significantly increased corn grain yield in all backgrounds of nitrogen fertilization. The investigated urease inhibitors and biologics had a higher and more significant ($p < 0.05$) effect on maize grain yield when fertilized with N_{100} nitrogen. The increase in nitrogen fertilizer rates had an effect on maize grain yield, with the largest increase in yield being found in the increase in nitrogen rate from N_{100} to N_{140}, and the increase in rate to N180 was less effective. The maximum mass of 1000 grains (323.5 g) was determined in 2019 by fertilization with N180 and use of the urease inhibitor UI URN. The urease inhibitor UI ATS was more effective when fertilized with lower rates of N_{100} and N_{140}. Positive, moderate, strong and very strong, statistically significant correlations (r^2 = 0.48–0.91) were most often found between the latter indicators and nitrogen fertilizer rates throughout the study year. The largest amount of grain (497 units) in the cob was determined in 2019, using fertilization with N_{140} and UI ATS, but no significant differences were found between the different fertilizer rates and the tested preparations. These results suggest that urease inhibitors and biologics can reduce dependence on nitrogen fertilizers and increase maize yield, a technology that should be practiced by maize growers.

Keywords: *Zea mays* L.; N-fertilization; N reduction; urease inhibitors; bio-preparations; productivity

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1. Introduction

Recently, the popularity and importance of maize have increased [1,2]. This is related to the physiological properties of these plants. Maize has mainly been grown for silage, but the areas of maize grown for grain have been expanding [3]. To expand the cultivation of maize for grain, it is important to develop a technology that uses sustainable technological and biological advances. Despite the production potential of maize developed by breeders, this is not yet fully exploited [4]. This highlights the lack of knowledge and skills needed to carry out the work involved in the cultivation technology, and the importance of punctuality and accuracy in the work [5]. Maize, like other cereals, requires careful work. A key aspect of this work is to learn and implement a new maize-growing technology, considering the effects of fertilizers and their combinations on crop structure. The influence of fertilization is very important in the cultivation of maize, as it determines the number of plants per unit area, i.e., one of the factors influencing the grain yield structure [6]. The influence of nitrogen fertilization is particularly high in the early stages of development when meteorological conditions in the early stages of growth might cause stress to maize [7]. Properly selected fertilizers and their combinations at the beginning of plant vegetation positively affect their yield and yield structure [8].

Maize is a fast-growing plant. Its need for essential nutrients is high, and the lack of nutrients in any plant can slow its growth and reduce productivity [9]. In maize cultivation, nutrient deficiencies at key growth stages can affect plant productivity [10].

Nitrogen is one of the most important nutrients for plant growth. However, intensive agricultural activities (intensive tillage, non-compliance with crop rotation, destruction of soil structure) reduce the total nitrogen content in the topsoil, especially in dry weather [11]. To maximize crop productivity, growers usually compensate for nutrient deficiencies with mineral fertilizers. Selecting fertilizers that do not match the plant yields results in excessive nitrogen levels, which can lead to undesirable consequences for the environment, usually an increased risk of nitrate leaching from the root zone under heavy rainfall during plant vegetation [12–14]. Excessive nitrogen use also reduces nitrogen efficiency and increases the cost of plant cultivation. To achieve sustainable agricultural development, it is necessary to develop a scientific nitrogen management strategy to improve nitrogen utilization efficiency and reduce nitrate leaching losses while maintaining a high grain yield potential [15].

Researchers recommend paying attention to the fact that maize causes some stress on the soil due to the high demand for nutrients, as unreasonable rates of nitrogen fertilizers are used to achieve high plant productivity. Nitrogen is one of the major nutrients for plants when intensifying crop production [16]. The proper and rational use of nitrogen not only ensures the stability of crop yields but also improves the biological and technological value of crops, changes the chemical composition of the soil, and can negatively affect the natural environment [17]. During the cultivation of maize, nitrogen should be used at higher rates but without compromising environmental requirements [18].

In maize, the main component of the grain crop–the cob–begins to form in the three-leaves stage (BBCH 13) and continues until the five-leaves stage (BBCH 15) [18]. Ensuring an optimal level of plant growth, including the availability of nutrients, guarantees the realization of harvest potential. Maize has a naturally high absorption capacity for nutrients that are used regularly [18]. However, high yields cannot be expected when bypassing natural soil fertility without regulating soil pH. Therefore, doses of mineral fertilizers, including nitrogen, should meet nutritional needs, considering the number of potential components taken from the soil [19].

The implementation of optimal fertilization technologies must include the right source of nutrients, the right rate, the right time, and the place of release. Such an approach can increase the efficiency of crop nitrogen use and reduce N_2O emissions [20]. From an agroecological perspective, high N_2O emissions from maize crops are due to discrepancies between nitrogen content and demand in maize crops, as fertilizers are usually applied well before rapid plant growth occurs [21,22]. Urease inhibitors reduce the activity of the urease enzyme and, therefore, slow the hydrolysis of urea, leading to a decrease in the volatilization of ammonia (NH_3) from soils [23,24].

If soils in cultivation regions are described as having low organic matter reserves and a low pH, Majidian, et al. [25] found that high maize productivity can be obtained using mineral and organic fertilizers. Adhikary, et al. [26] argue that the addition of organic fertilizers to soils rich in organic matter can increase the content of soil nutrients and provide plants with the necessary nutrients, but the nutrient supply may take longer due to the slow decomposition and excretion of nutrients into the soil. Plant nutritionists argue that most agricultural soils have the hardest time maintaining the optimal concentration of nutrients available to plants. In soil, organic matter is a key component in regulating chemical, physical, and biological processes [27]. Humic substances can chelate plant nutrients and reduce their leaching, thus facilitating their uptake by plants. However, the positive effect of humic substances is reduced when the organic matter content in the soil is too low or too high [28]. The growing use of humic substances as plant-growth promoters has attracted attention due to their effects on nutrient utilization efficiency, crop quality, and protection against abiotic stress [29,30]. Abiotic stress is one of the most important factors limiting food safety and plant productivity. The growing number of extreme events in many regions highlights the importance of crop protection for an economy based on agricultural production [30]. The application of measures to reduce plant stress is a promising way of managing the adaptation of plants to adverse environmental conditions by activating their mechanisms of adaptation to environmental factors [31]. Humic acids can be used as natural compounds to manage plant stress and increase nutrient uptake efficiency. The treatment of plants with humic acids has been shown to improve their resistance to major abiotic stresses, including salts, drought, and the impact of heavy metal toxicity [32].

Plant biostimulants are substances that can improve crop productivity and quality, increase the availability of nutrients in the soil, improve the efficiency of plant nutrient use, and promote the decomposition and humification of organic matter in the soil [33]. Recently, biostimulants have become widely used in traditional crop production because they can improve plant productivity and quality, as well as respond to economic and production sustainability requirements [34]. Maize biostimulants have attracted the attention of maize growers because of their effectiveness in promoting morphological, physiological, and biochemical processes in crops [35].

One of the ways of controlling the processes that take place in plants and increase the efficiency of mineral fertilizers is the use of seaweed extracts, which are naturally occurring substances. Recently, the use of these biostimulants has been growing rapidly [36]. *Ascophyllum nodosum, Fucus spp., Laminaria spp., Sargassum spp., Turbinaria spp.* and *Ecklonia maxima* are important brown seaweeds that are partially or completely used as an alternative to chemical fertilizers [37,38]. The potential benefits of these biostimulants in stimulating plant growth and increasing crop yields have provided an incentive to develop crop technologies that use water and nutrients more efficiently. Positive effects have been attributed to plant growth regulators and, possibly, trace elements that promote root growth, mineral uptake, photosynthetic capacity, and stress resistance [39]. Biostimulants can improve the ability of plants to use and absorb nutrients, improve their growth, and positively affect the quality of the final product [40]. Biostimulants can also increase plant resistance to various types of abiotic stress, the ability to cope with adverse conditions, and maintain productivity [41]. In addition, the use of biostimulants can also significantly reduce the cost of fertilizers, which reduces the environmental impact of farming technologies [42].

It is likely that the use of urease inhibitors and biological preparations will allow for more efficient use of nitrogen fertilizers to achieve optimal yields of maize grains.

The aim of the study was to investigate the influence of different nitrogen fertilizer rates, urease inhibitors, and biological preparations on maize grain yield and yield structure elements.

2. Materials and Methods

2.1. Research Location and Arrangement of the Experiment

The field experiment was performed in 2019–2021 at the Experimental Station of Vytautas Magnus University Agriculture Academy (54°52′ N, 23°49′ E). The soil of the experimental field was *Endohipogleyic-Eutric Planasol*, according to the World Reference Base (WRB) classification, with medium loam on sandy light loam [43]. The plowing layer was 23–27 cm thick. The soil was neutral (pH~6.7), with medium humus content ~2.86%, medium potash content ~154 mg kg^{-1} and high phosphorus content ~266 mg kg^{-1}. There were 45 fields in the field experiment, each with an initial (gross) area of 66 m^2 (width 5.5 m, length 12 m). The area of the accounting (net) field was 45 m^2 (width 4.5 m, length 10 m). The field experiment was performed in 3 iterations; the fields in the iteration blocks were arranged randomly. The studied factors were: Factor A—different nitrogen fertilizer rates: (1) 100 kg N ha^{-1}; (2) 140 kg N ha^{-1}; (3) 180 kg N ha^{-1}; Factor B—the use of urease inhibitors (UI) and biological preparations (BP): (1) Urease inhibitors (UI) and biological preparations (BP) were not used; (2) Urease inhibitor UI ATS was used; (3) Urease inhibitor UI URN was used; (4) Biological preparation BP HUM was used; (5). Biological preparation BP FIT was used. In 2019, maize was sown on 23 April, in 2020—on 27 April—and in 2021—on 11 May. The early maturing (short, about 90 days vegetation period) hybrid maize variety P7326 (selection company DuPont Pioneer) was chosen for the experiment. Seed rate was 80,000 seeds ha^{-1}; spacing–75 cm.

PK fertilizers were spread in all fields and applied before maize sowing: phosphorus fertilizer (double superphosphate Ca $(H_2PO_4)_2H_2O$ was selected), fertilization rate—60 kg ha^{-1} P_2O_5; potassium fertilizer selected was potassium chloride KCl, fertilization rate—60 kg ha^{-1} K_2O. Maize crop at BBCH stage 16 was sprayed with herbicide containing the active substances mesotrione 75 g L^{-1} + nicosulfuron 30 g L^{-1}–1.0 L ha^{-1}.

The characteristics of the use of the researched factors A and B in the field experiment are the following:

Factor A—different rates of nitrogen (N) fertilizer:

1. (N_{100}) 238 L ha^{-1} KAS-32 (solution of urea $CO(NH_2)_2$ and ammonium nitrate NH_4NO_3) was applied to the soil surface immediately after sowing;
2. (N_{140}) 333.2 L ha^{-1} KAS-32 (solution of urea $CO(NH_2)_2$ and ammonium nitrate NH_4NO_3) was applied to the soil surface immediately after sowing;
3. (N_{180}) 428.4 L ha^{-1} KAS-32 (solution of urea $CO(NH_2)_2$ and ammonium nitrate NH_4NO_3) was applied to the soil surface immediately after sowing.

Factor B–use of urease inhibitors (UI) and biological preparations (BP):

1. (Without UI and BP) urease inhibitors and biological preparations not used;
2. (UI ATS) urease inhibitor—ammonium thiosulfate [ATS, $(NH_4)_2S_2O_3$ 12-0-0-26S] (10% irrigated with KAS-32: in fields fertilized with N_{100}–23.8 L ha^{-1}; in fields fertilized with N_{140}–33.3 L ha^{-1}; in fields fertilized with N_{180}–42.8 L ha^{-1};
3. (UI URN) urease inhibitor—N-butyl-thiophosphoric triamide (NBPT) 188 g L^{-1} and N-propyl-thiophosphoric triamide (NPPT) 87 g L^{-1} (1.0 L ha^{-1} irrigated with KAS-32);
4. (BP HUM) biological preparation—15% suspension of humic and fulvic acids, pH 4–5 (1.0 L ha^{-1} irrigated together with KAS-32);
5. (BP FIT) biological preparation—20% suspension of *Ascophyllum nodosum* (0.6 L ha^{-1} sprayed on maize at stage BBCH 26).

When the maize reached physiological maturity, i.e., when a black dot appeared on the point of attachment of the grain to the cob, samples were taken from each field by random sampling of 10 plants (30 from each treatment, 450 plants in total). Samples were taken to determine the elements of maize grain yield and yield structure according to the methodology for conducting field experiments [44]. Harvested cobs were dried in the dryer, peeled, and threshed, the grains were weighed. and the following parameters were calculated: grain yield (t ha^{-1}), number of grains in the cob (units), and 1000-grain weight (g).

2.2. Statistical Analysis

Statistical data analysis was performed using computer programs ANOVA and STAT from the statistical software package SELEKCIJA. The research data were statistically evaluated using two-way analysis of variance. The significance of the differences between the variants was assessed using the F-criterion and the LSD test. Significant interactions between the studied factors were identified; therefore, the averages were not presented when analyzing the research data. The differences between the means of the variants without the same letters (a, b, c) are significant ($p < 0.05$). The standard error of the means is indicated by whiskers. Correlation-regression analysis revealed correlations between nitrogen fertilizer rates (x–kg ha^{-1}) and maize grain yield (Y_1) and yield structure elements (Y_2—1000-grain weight in grams, Y_3—number of grains in the cob in units). The statistical reliability of the regression coefficient was determined according to Fisher's F-criterion, and the correlation was determined according to Student's (*t*): $p \leq 0.05$—dependence was statistically significant at the 95% level of probability; $p \leq 0.01$—dependence was statistically significant at the 99% level of probability [45].

2.3. Weather Conditions

The average air temperature in May 2019 was close to the climate normal (CN); precipitation was 48% of the climate normal (Figure 1). Moisture deficiency may have affected plant development in the early stages of growth and development. In May 2020, the air temperature was below the CN and the precipitation exceeded the CN by 51%. May was not conducive to maize growth in both years studied. The temperature in July 2019 was slightly below the CN and the precipitation was below the CN. In April 2021, the temperature was lower than the CN, not allowing the ground to warm up. As a result, the sowing had to be postponed to the beginning of May. The temperature in May was below the CN as well but slightly higher than the average temperature in May 2020. However, precipitation was well above the CN. In June 2021, the air temperature was similar to that of June 2019 and 2020, with precipitation being 1.9 times lower than the CN. The temperature in July was above the CN by 3.9 °C and the precipitation was 2 times lower than the CN.

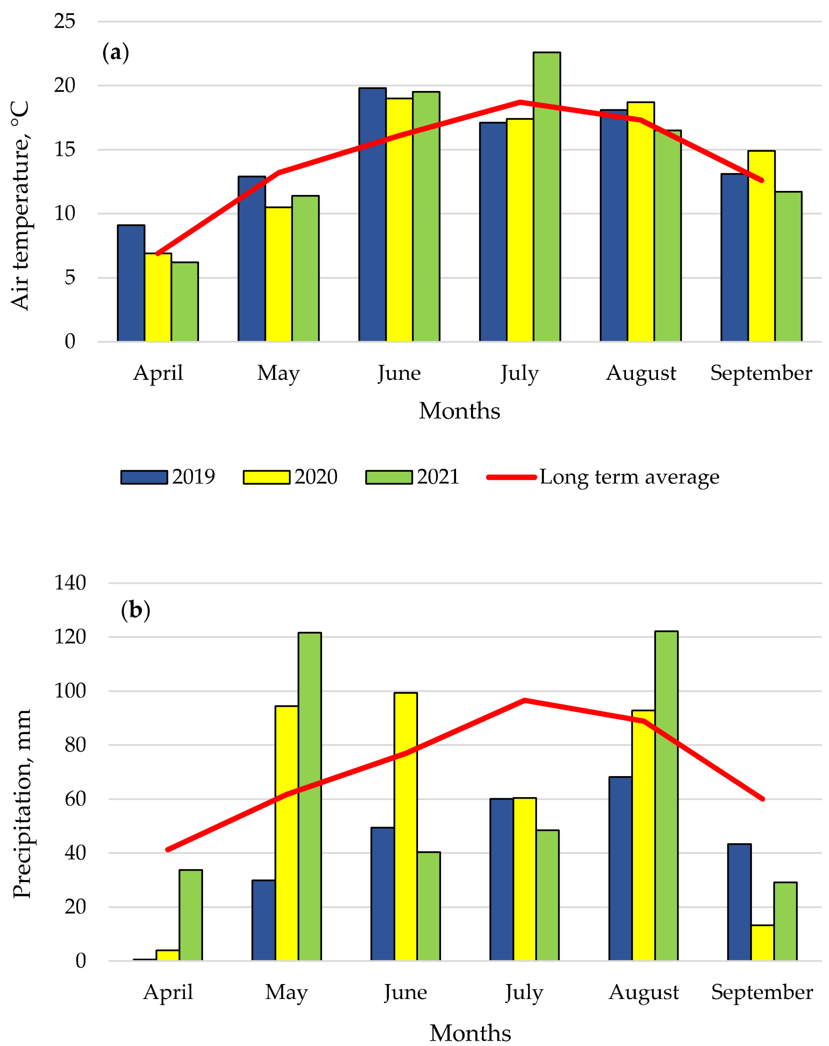

Figure 1. Meteorological conditions (air temperature °C (**a**), precipitation in mm (**b**)) in the year of the experiment (2019–2021), Kaunas Meteorological Station.

In August–September, the weather temperatures were similar in all survey years and met or slightly exceeded the CN, differing only in the amount of precipitation: in 2019, precipitation was lower than the CN, but the distribution was more even, resulting in favorable conditions for plant fertilization, grain formation, and maturity; in August 2020, the precipitation was higher than the CN, and in September 2021, precipitation was 1.4 times higher than the CN. In September 2020, precipitation was 4.5 times lower than the CN, and in 2021, it was two lower than the in 2021. It can be assumed that the high precipitation in August may have contributed to the slower grain maturity.

3. Results

3.1. The Influence of the Studied Factors on Maize Grain Yield

An interaction was established between nitrogen fertilizer rates and urease inhibitors. Our studies has shown that nitrogen fertilizer rates had a positive effect on maize grain yield (Figure 2). A significant increase in grain yield was observed when fertilizing with the lowest N_{100} and the highest N_{180} rates. The difference in grain yield between N_{100} and N_{180} fertilization rates was 1.3 t ha^{-1} in 2019, 1.7 t ha^{-1} in 2020, and 1.4 t ha^{-1} in 2021. The largest increase in grain yield in all study years was observed when increasing the fertilizer rate from N_{100} to N_{140}. Increasing the fertilizer rate from N_{140} to N_{180} resulted in a non-significant increase in maize grain yield.

To increase the efficiency of nitrogen fertilizer, UI ATS, and UI URN were used. The data show that the year in which UI ATS were used resulted in significantly higher grain yield variability. The highest yield of maize grain (12.0 t ha^{-1}) using UI ATS was determined in 2019 with N_{180} fertilization, which is significantly higher, at 1.9 t ha^{-1}, compared to the yield with N_{180} without urease inhibitor. In 2020 and 2021, the highest maize grain yields (11.2 and 8.9 t ha^{-1}, respectively) were determined using N_{180} fertilization and UI ATS. Using UI ATS and increasing the rate of nitrogen fertilization from N_{100} to N_{140} resulted in the biggest significant differences in yield supplement, and increasing the rate of fertilization from N_{140} to N_{180} did not result in a significant increase in grain yield.

When using UI URN, the highest yield (11.6 t ha^{-1}) was determined by fertilizing with N_{180}, which was significantly (1.5 t ha^{-1}) higher compared to fertilization with the same fertilizer rate without UI URN. The utilization of UI URN allowed the same trend of yield changes to be maintained as the utilization of UI ATS, with a substantial increase in grain yield throughout the study year when increasing fertilization rate from N_{100} to N_{140}. In this case, the year 2020 stood out, with a substantial yield difference between N_{140} and N_{180}.

Using the humic acid preparation BP HUM, the highest grain yield (10.8 t ha^{-1}) was determined in 2019 when fertilizing with N_{180}. Compared to fertilization without biological preparation, the yield significantly increased—by 0.7 t ha^{-1}. With the use of BP HUM and fertilization with N_{100}, the grain yield increased significantly compared to N_{100} without biological preparation. The year 2021 stood out in terms of N_{180} fertilization yield, and BP HUM did not significantly differ from N_{180} without BP HUM. When using the humic acid preparation in 2019, significant changes in yield were observed with N_{100} and N_{180} fertilization, and no significant differences were found among the specific fertilization rates (N_{100}–N_{140}–N_{180}). The effectiveness of the humic acid preparation is unquestionable, as the data presented in the Figure 2 show that the yield was significantly increased by 0.9–1.1 t ha^{-1} using BP HUM and N_{100} fertilization, compared to the fertilization without BP, depending on the year's conditions. The fertility of N_{140} and N_{180} grains significantly increased by 0.4–1.5 t ha^{-1} and 1.0–2.0 t ha^{-1}, respectively.

BP FIT had no significant effect on yield in maize that was fertilized differently, with nitrogen, in 2019. In the year 2020, which was less favorable for maize-growing, the utilization of BP FIT significantly increased the yield of maize grain when increasing the fertilizer rate from N_{100} to N_{140}, and no significant change in yields was observed between N_{140} and N_{180}. In 2021, a significant increase (0.9 t ha^{-1}) in grain yield was also found when comparing N_{100} and N_{140} fertilization rates. Comparing the efficiency of BP FIT in maize fertilized with different rates, it was found that fertilization with N_{100} increased the yield of maize by 1.1–1.6 t ha^{-1}, depending on the year's conditions. The significant influence of the tested preparation on the yield was determined when fertilizing with the highest N_{180} rate. This was 0.6–1.7 t ha^{-1} higher compared to fertilization without BP.

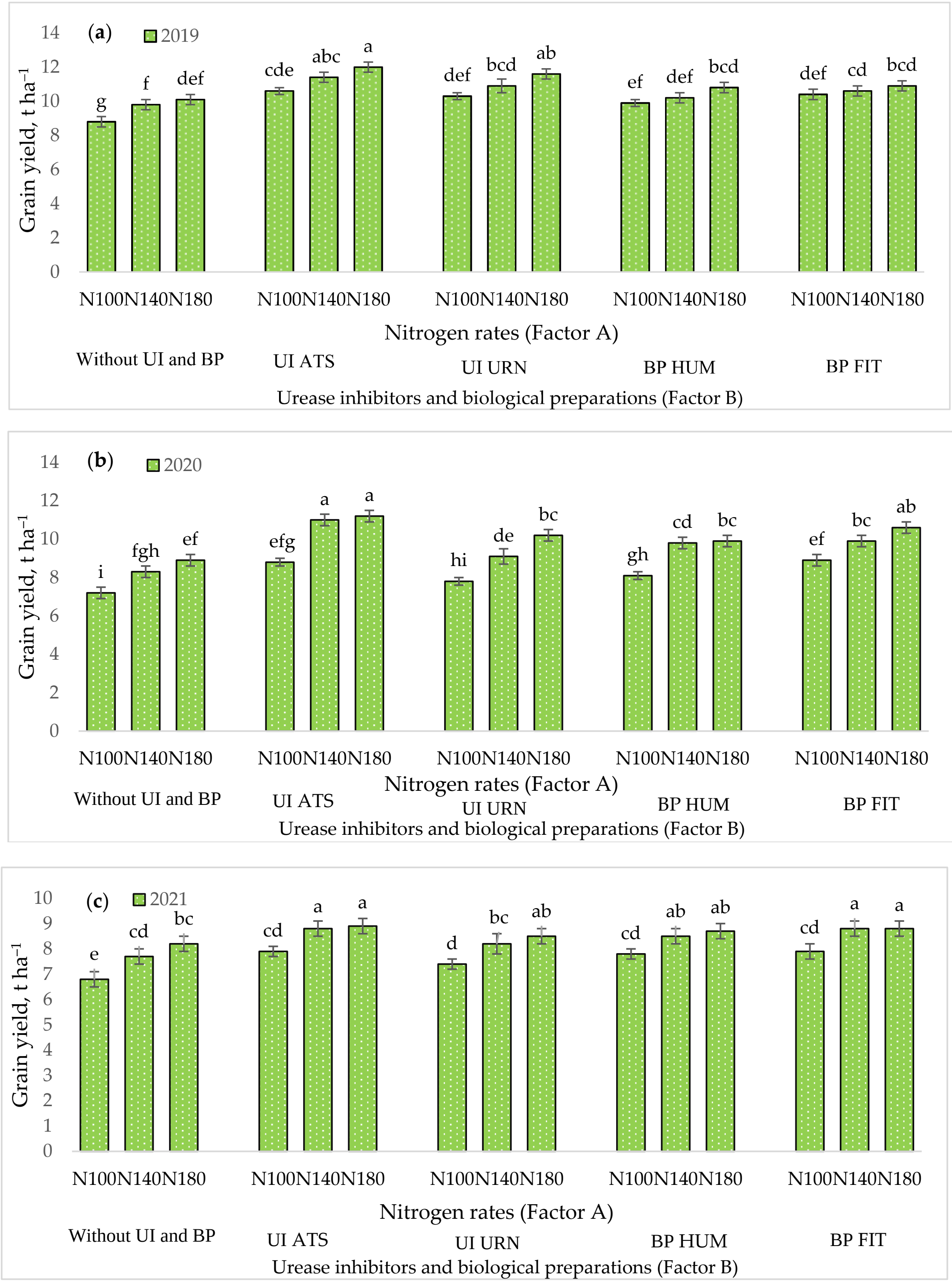

Figure 2. The influence of different nitrogen fertilizer rates, urease inhibitors, and biological preparations on maize grain yield, 2019 (**a**); 2020 (**b**); 2021 (**c**). UI—urease inhibitor; ATS—ammonium thiosulfate; URN—N-butyl-thiophosphoric triamide (NBPT) and N-propyl-thiophosphoric triamide (NPPT); BP—biological preparation; HUM—suspension of humic and fulvic acids; FIT—suspension of *Ascophyllum nodosum*. The differences between the means of the treatments with the different letters are significant ($p < 0.05$). Vertical dashes indicate the mean error.

3.2. The Influence of the Studied Factors on the 1000-Grain Weight of Maize

After harvesting the maize and estimating the 1000-grain weight, it was found that when fertilizing grain with different rates of nitrogen fertilizers, without using UI and BP, the highest 1000-grain weight (301.3 g) was obtained in 2019, when fertilizing with N_{180} (Figure 3). It was found that increasing the fertilization rate from N_{100} to N_{140} significantly increased the 1000-grain weight by 20.9 g, and increasing the fertilization rate to N_{180} did not result in a significant difference compared to N_{140}. In 2020 and 2021, the maize's 1000-grain weight was lower than it was in 2019. In 2020, a significantly larger difference was found after fertilizing maize with N_{180} compared to N_{140} and N_{100}. In 2021, as in previous research years, no significant difference was found between N_{100} and N_{140}, and a significantly larger difference was found with N_{180} fertilization.

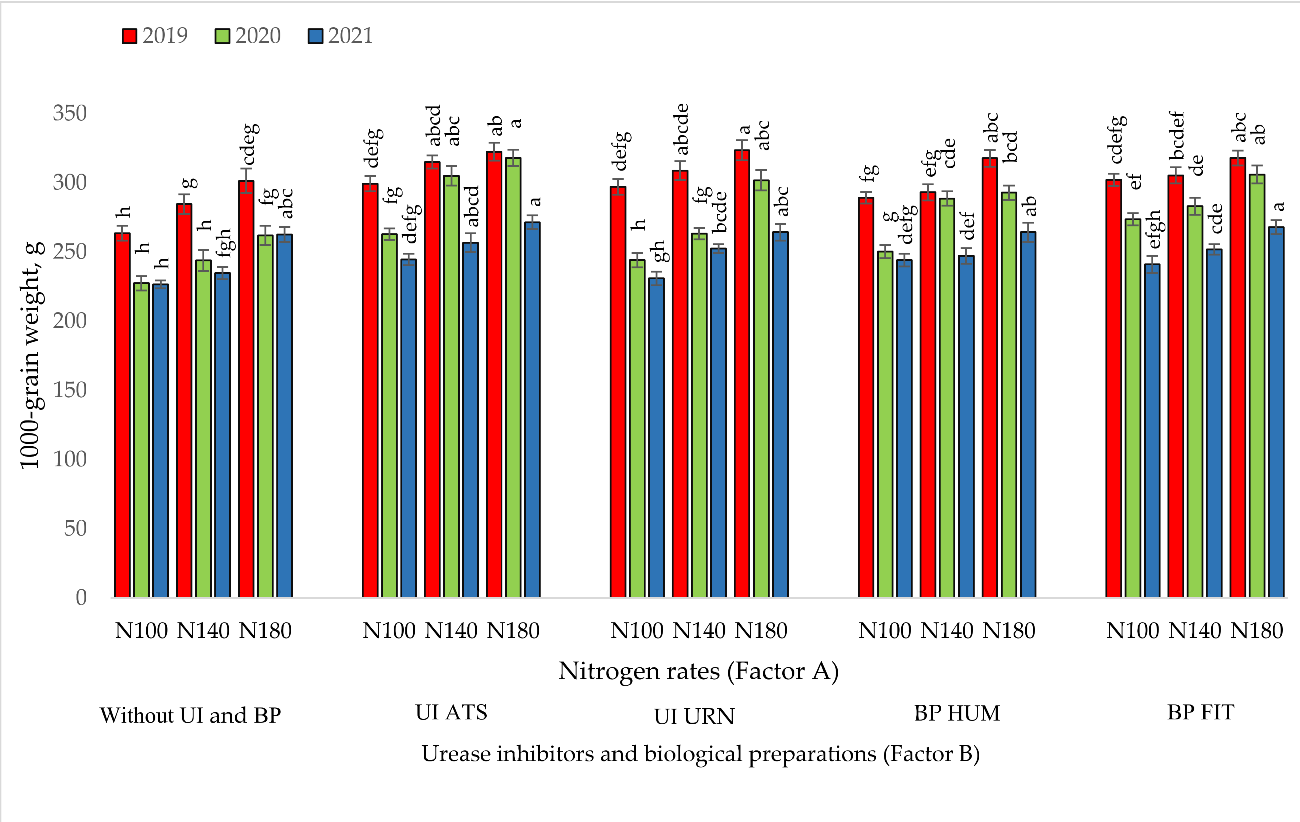

Figure 3. The influence of different nitrogen fertilizer rates, urease inhibitors, and biological preparations on 1000-grain weight of maize, 2019–2021. UI—urease inhibitor; ATS—ammonium thiosulfate; URN—N-butyl-thiophosphoric triamide (NBPT) and N-propyl-thiophosphoric triamide (NPPT); BP—biological preparation; HUM—suspension of humic and fulvic acids; FIT—suspension of *Ascophyllum nodosum*. The differences between the means of the treatments with the different letters are significant ($p < 0.05$). Vertical dashes indicate the mean error.

Using the UI ATS in 2019, a significant increase in 1000-grain weight was found in all fertilization cases compared to fertilization without UI. When assessing the influence of UI on the maize's 1000-grain weight, a significant increase was found compared with N_{100} and N_{180} fertilization rates. After fertilizing maize with N_{100} and using UI ATS, the 1000-grain weight was found to be 2 g lower compared to N_{180} fertilizer without UI. In the less favorable years for maize growth (2020 and 2021), the significant effect of UI ATS on the 1000-grain weight was found after fertilization with N_{140} and N_{180}, and no significant difference was found when comparing N_{140} and N_{180} fertilizer rates.

In 2021, the highest 1000-grain weight (271.5 g) was determined when fertilizing with N_{180}. Comparing the fertilization rates N_{140} and N_{180} with N_{100}, a significant difference was found, but no significant differences were found between N_{140} and N_{180} rates, as in previous years. The study of the influence of UI URN on the 1000-grain weight of maize revealed that, in 2019, the increase in fertilization rates had a positive effect on the increase in 1000-grain weight, but no significant difference was found between N_{140} and N_{180} fertilization rates. In 2020, positive significant differences were found between all fertilization rates. It can be assumed that UI ATS allowed plants to absorb nutrients more efficiently in the years that were less favorable for plant growth. The year 2021 was also not favorable for maize; the significant influence of the inhibitor when increasing the fertilizer rates was revealed, but no significant difference was found between N_{140} and N_{180} fertilization rates. Using UI (ATS and URN), the maize's 1000-grain weight at N_{100} fertilization was found to be slightly lower, depending on the year, compared to the N_{180} fertilization rate without UI.

The study of the influence of BP FIT on the maize's 1000-grain weight revealed that, in 2019, the preparation did not have a significant effect on this indicator. Comparing the maize's 1000-grain weight, treated with BP FIT and unprocessed, the data show that, in 2019, no significant difference was found with N_{180} fertilization and positive significant differences were found with N_{100} and N_{140} fertilization. In 2020, the efficiency of BP FIT was demonstrated by fertilizing with N_{180} nitrogen fertilizer rate, and no significant differences were found between N_{100} and N_{140} fertilizer rates. The same trends continued in 2021—1000-grain weight differed positively and significantly with the use of BP FIT for maize fertilization with N_{180}.

3.3. The Influence of the Studied Factors on the Number of Maize Grains in the Cob

In the presented data, the researched preparations and the different rates of nitrogen fertilization did not have a significant effect on the number of maize grains in the cob in most cases (Figure 4). In 2019, in none of the studied cases did the UI and BP have a significant effect on the changes in the number of grains. In 2020 and 2021, the same trends remained. In several cases, it was identified that the differences were significant. When fertilizing with nitrogen without UI and BP in 2021, the number of grains in the cob was significantly lower compared to 2019 and 2020. In addition, a significantly lower number of grains in the cob was found in 2021 when using UI ATS and fertilizing with N_{100}. When fertilizing with N_{140} and using the UI ATS, the number of grains was significantly higher compared to the fertilization rate of N_{100}.

3.4. Correlation-Regression Analysis

In 2019, in most cases positive, strong and statistically significant correlations were found between nitrogen fertilizer rates (x) and maize grain yield (Y_1); between nitrogen fertilizer rates (x) and 1000-grain weight of maize (Y_2) (Table 1). In 2020, nitrogen fertilizer rates positively strongly correlated with maize grain yield in all cases with r^2 = 0.69–0.89, $p < 0.01$. In 2021, the following statistically significant correlations between the same parameters were observed: when UI and BP were not used—r^2 = 0.82, $p < 0.01$. In the cases when UI was used correlations were weaker—r^2 = 0.57–0.71, or when BP applied—r^2 = 0.59–0.71.

Correlation-regression analysis showed that nitrogen fertilizer rates had positive strong influence on 1000-grain weight (Y_2) in all cases in 2020, when $p < 0.01$: r^2 = 0.69–0.87. In 2021, positive, strong and statistically significant correlations were found in most cases as well at $p < 0.01$: r^2 = 0.67–0.9, except when BP HUM was used—r^2 = 0.51, $p < 0.05$). No correlation was found between nitrogen fertilizer rates and maize grain yield; also between nitrogen fertilizer rates and 1000-grain weight of maize in the fields where BP FIT was used. Correlation-regression analysis between nitrogen fertilization rates (x) and Y_3—the number of grains in the cob—as well as the assessment of the data of individual research years, did not reveal any statistically significant dependencies.

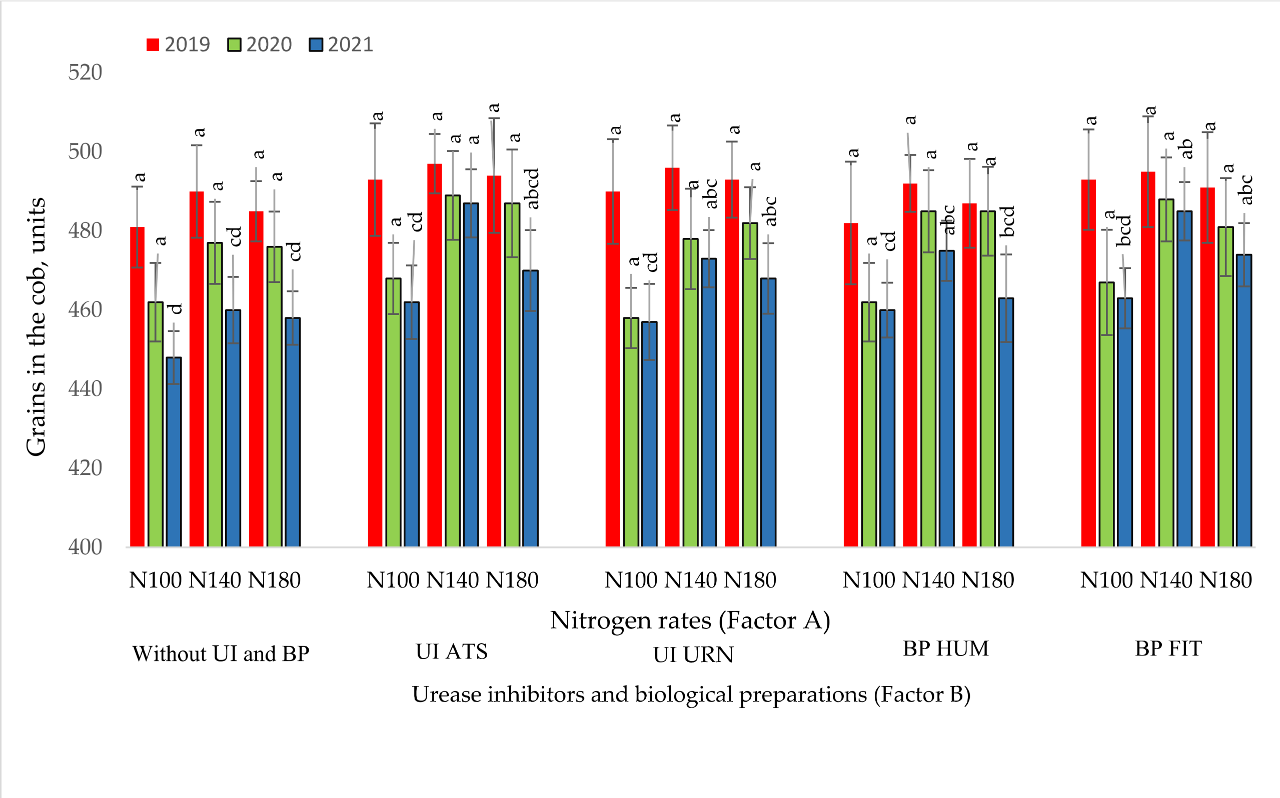

Figure 4. The influence of different nitrogen fertilizer rates, urease inhibitors, and biological preparations on the number of maize grains in the cob, 2019–2021. UI—urease inhibitor; ATS—ammonium thiosulfate; URN—N-butyl-thiophosphoric triamide (NBPT) and N-propyl-thiophosphoric triamide (NPPT); BP—biological preparation; HUM—suspension of humic and fulvic acids; FIT—suspension of *Ascophyllum nodosum*. The differences between the means of the treatments with the different letters are significant ($p < 0.05$). Vertical dashes indicate the mean error.

Table 1. Dependence of maize grain yield and yield structure elements (y) on different rates of nitrogen fertilization (factor A: x–kg ha^{-1}), 2019–2021.

Dependent Variables y	UI ir BP (Factor B)	Year	Regression Equation	r	r^2	p
y_1 Grain yield, t ha^{-1}	Without UI and BP	2019	y= 7.13 + 0.02x	0.80	0.65	$p < 0.01$
		2020	y = 5.12 + 0.02x	0.89	0.79	$p < 0.01$
		2021	y = 5.12 + 0.02x	0.90	0.82	$p < 0.01$
	UI ATS	2019	y = 8.91 + 0.02x	0.84	0.71	$p < 0.01$
		2020	y = 6.18 + 0.03x	0.84	0.70	$p < 0.01$
		2021	y = 6.83 + 0.01x	0.76	0.57	$p < 0,01$
	UI URN	2019	y = 8.67 + 0.02x	0.77	0.59	$p < 0.05$
		2020	y = 4.69 + 0.03x	0.94	0.89	$p < 0.01$
		2021	y = 6.06 + 0.01x	0.84	0.71	$p < 0.01$
	BP HUM	2019	y = 8.78 + 0.01x	0.69	0.48	$p < 0.05$
		2020	y = 6.23 + 0.02x	0.83	0.69	$p < 0.01$
		2021	y = 6.63 + 0.01x	0.84	0.71	$p < 0.01$
	BP FIT	2019	-	-	-	$p > 0.05$
		2020	y = 6.90 + 0.02x	0.86	0.74	$p < 0.01$
		2021	y = 6.84 + 0.01x	0.77	0.59	$p < 0.05$

Table 1. *Cont.*

Dependent Variables y	UI ir BP (Factor B)	Year	Regression Equation	*r*	r^2	*p*
y_2 1000-grains weight, g	Without UI and BP	2019	y = 217.2 + 0.47x	0.83	0.69	$p < 0.01$
		2020	y = 184.1 + 0.43x	0.83	0.69	$p < 0.01$
		2021	y = 178.1 + 0.45x	0.89	0.79	$p < 0.01$
	UI ATS	2019	y = 271.7 + 0.29x	0.76	0.57	$p < 0.05$
		2020	y = 198.5 + 0.69x	0.90	0.82	$p < 0.01$
		2021	y = 210.5 + 0.34x	0.82	0.68	$p < 0.01$
	UI URN	2019	y = 263.5 + 0.33x	0.76	0.57	$p < 0.05$
		2020	y = 168.7 + 0.72x	0.93	0.87	$p < 0.01$
		2021	y = 190.8 + 0.42x	0.88	0.78	$p < 0.01$
	BP HUM	2019	y = 250.1 + 0.36x	0.79	0.62	$p < 0.05$
		2020	y = 202.5 + 0.53x	0.85	0.73	$p < 0.01$
		2021	y = 213.3 + 0.28x	0.72	0.51	$p < 0.05$
	BP FIT	2019	-	-	-	$p > 0.05$
		2020	y = 230.7 + 0.413x	0.70	0.84	$p < 0.01$
		2021	y = 206.5 + 0.33x	0.83	0.70	$p < 0.01$
y_3 Quantity of grain in the cob, units	Without UI and BP UI ATS; UI URN; BP HUM; BP FIT	2019 2020 2021	-	-	-	$p > 0.05$

Note. *r*—Correlation coefficient, r^2—Coefficient of determination, *p*—Probability level.

4. Discussion

Growers often use a lot of fertilizers in crop production technologies to increase plant productivity. Improper use of nitrogen fertilizers, regardless of the consequences for the environment, carries a high risk of nitrate leaching outside the root zone in the event of heavy rainfall during plant growth.

Many crops are highly responsive to the use of nitrogen fertilizers. Nitrogen fertilizers are widely accepted as the main nutrient for the yield of maize grain, but economic returns are not always obtained. Recommendations for the use of nitrogen fertilizers need to consider grower-independent variables, yield forecasts, economic returns, technological level, and key soil properties [46]. After performing the experiment in 2019–2021, it was found that, by increasing the rate of nitrogen fertilizers from N_{100} to N_{180} without the use of additional measures, the yield of maize grain increased by 1.3–1.7 t ha^{-1} in different years. The largest increase (1.7 t ha^{-1}) was observed in 2020. The data show that the increase in nitrogen fertilizer rates was not effective enough in conditions that were less favorable for maize growth (in 2020 and 2021). Gao, et al. [15], Zhang, et al. [47] argue that excessive nitrogen utilization also reduces nitrogen use efficiency and increases costs. Sustainable agricultural development requires the development of a scientific nitrogen management strategy to improve nitrogen efficiency and reduce losses while maintaining a high potential for grain yields.

Sanz-Cobena, et al. [48] aimed to develop sustainable and environmentally friendly nitrogen utilization practices to reduce nitrogen loss and increase plant productivity. According to He, et al. [49], such practices include the use of urease and nitrification, inhibitors, sulfur elements and polymers, the removal of plant residues, and the use of biochar. Globally, increases in plant productivity are directly proportional to the use of synthetic fertilizers, although higher rates of nitrogen fertilizers significantly reduce nitrogen efficiency [50,51]. Our study is in line with other researchers—the substantially highest increase in maize grain yield in the experimental year was found by increasing the rate of nitrogen fertilization from N_{100} to N_{140}, and further increases to N_{180} were ineffective. In the first case, the yield of 1 kg of nitrogen grain increased by 0.03–0.04 t ha^{-1} depending on the year, and by increasing the fertilizer rate from N_{140} to N_{180}, the yield of one kilogram of nitrogen increased by 0.008–0.02 t ha^{-1}. Bogucka, et al. [52] found that the optimal rate of nitrogen fertilization when cultivating maize for grain was N_{150}. Higher rates of nitrogen fertilization did not significantly increase grain yields. Gołebiewska, et al. [53] showed that increasing the nitrogen rate to N_{150} could increase the yield of maize grains, and a further increase in fertilization rates substantially increased the yields. A similar trend was observed by Pizolato, et al. [54] and Silva, et al. [55].

Higher-efficiency fertilizers coated with urease or nitrification inhibitors can be used to reduce nitrogen loss in the soil [56]. Urease inhibitors reduce the activity of the urease enzyme, thereby slowing the hydrolysis of ammonium nitrogen and reducing the evaporation of ammonia (NH_3) from the soil [57]. Researchers suggest that one of the most promising new ways to reduce nitrogen loss is to use urease or nitrification inhibitors together with nitrogen fertilizers [58]. The use of inhibitors with nitrogen fertilizers has been a very effective tool in reducing nitrogen fertilizer losses and increasing plant productivity [59]. Dawar, et al. (2021) found that the use of inhibitors increased maize grain yield by 10–36%.

In our experiment in 2019–2021, it was found that fertilization with nitrogen fertilizers, in combination with the urease inhibitor ATS, significantly increased the yield of maize grain compared to fertilization with nitrogen fertilizers without a urease inhibitor. In 2019, when fertilizing with N_{100} and using the urease inhibitor ATS, the grain yield was 0.5 t ha^{-1} higher compared to N_{180} without urease inhibitor, and in 2020 and 2021, it ranged from 0.1 to 0.3 t ha^{-1} lower.

Analyzing the yield of maize grain, it can be stated that, by using UI ATS and fertilizing with the lowest (N_{100}) nitrogen fertilizer rate, 1 kg of nitrogen formed 0.1–0.08 t ha^{-1} grain yield, and using nitrogen fertilizer (N_{180}) without an inhibitor led to a grain yield of 0.05–0.06 t ha^{-1}. Summarizing the maize grain yields, it can be argued that the use of urease inhibitors results in higher plant productivity, with a 1.8 times lower fertilizer rate. In the experiment, it was found that the use of BP HUM and fertilization of maize with low rates of nitrogen (N100) resulted in a significant increase in yield in all study years compared to fertilization without biological preparation. Naveed, et al. [60] note that no significant differences were found in the increase in the fertilizer rate in years that were favorable for maize growth, but in less favorable years, the biological preparation led to the largest increase in grain yield (9.4%) compared to the control. Nutrient efficiency when using humates can increase the yield of maize grains. Several studies have shown that potassium humate releases nutrients slowly and can reduce nitrogen losses [61].

Nitrogen nutrition of plants determines grain yield and is one of the indicators influencing the condition of maize during the growing season. The choice of seaweed extract (BP FIT) in maize-growing technology resulted in significantly higher yields of maize grain compared to fertilization without seaweed. Only in the year 2019, which was favorable for maize, were no significant changes observed with the N_{180} fertilization. Latique, et al. [62] indicated that seaweed extract may be a potential factor influencing chlorophyll in plants. The use of seaweed also increased the chlorophyll content in other crops (tomatoes, wheat, barley). Alam, et al. [63] argue that *Ascophyllum nodosum* extract can increase the soil microbial activity in the root zone, affecting plant growth and productivity. Chen, et al. [64] indicate that the growth of maize seedlings was strongly stimulated by the use of seaweed fertilizers, and that the height of the seedlings and the green mass of the aboveground part significantly increased.

Our research showed that the tested preparations affected indicators of the 1000-grain weight of maize. The significant effect of the urease inhibitor ATS was revealed. Other studied biological preparations significantly increased the 1000-grain weight when fertilizing at the rate of N_{100}, and this indicator tended to increase with higher fertilization rates. A similar trend was confirmed by Khaliq, et al. [65], Sharar, et al. [66], and Michalski, et al. [67].

5. Conclusions

The field experiments of maize fertilization combined with urease inhibitors and biological preparations were carried out for three years. The increase in nitrogen fertilizer rates had an impact on maize grain yield and crop yield structure elements. Positive, moderate, strong, and very strong statistically significant correlations were found (r^2 = 0.48–0.91). The use of urease inhibitors and biological preparations increased the yield of maize grain in all investigated nitrogen fertilizer rates. The highest (12.0 t ha^{-1}) grain yield was determined in 2019 when fertilizing maize with N_{180}, in combination with the urease inhibitor ATS. In the experimental years of 2020 and 2021, the highest grain yields were also found when fertilizing with the highest rate of nitrogen and using ammonium thiosulfate (11.2 and 8.9 t ha^{-1}, respectively). The urease inhibitor ammonium thiosulfate significantly increased the yield of maize in both years compared to untreated maize. The combined use of nitrogen fertilizers and urease inhibitors and biological preparations would be an effective way to reduce nitrogen loss and increase maize productivity. An interaction was identified between nitrogen fertilizer rates and urease inhibitors. However, to achieve the economical and sustainable use of nitrogen fertilizers, urease inhibitors and biological preparations, different rates of nitrogen fertilizer, urease inhibitors and biological preparations need to be tested at different sites before they can be commercialized under different field conditions.

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