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Production and environmental aspects of the application of biostimulators Asahi SL, Kelpak SL and stimulator Tytanit with limited doses of nitrogen

Produkcyjne i środowiskowe aspekty stosowania biostymulatorów Asahi SL i Kelpak SL oraz stymulatora Tytanit przy ograniczonych dawkach azotu

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Słowa kluczowe: pszenica ozima, biostymulatory, nawożenie azotem, masa korzeni, zawartość chlorofilu, wielkość plonu.

Abstract

An effective way to reduce nitric oxide emissions may be to reduce the level of nitrogen fertilization. Specialized formulations such as biostimulators are a good alternative to conventional mineral fertilization. The aim of the study was to determine the effect of the application of biostimulation agents (Asahi SL, Kelpak SL, Tytanit) applied against the limited dose of nitrogen (75 kgN·ha-1) on winter wheat 'Muszelka', their effect was measured on the parameters: grain yield, protein content, amount of chlorophyll and root mass. No statistically significant changes in yields were observed, but after application of the Asahi SL and Kelpak SL, considerably higher values were found as compared to the controls. It should be emphasised that the applied formulations significantly affected the root mass and chlorophyll content of the leaves. Especially in the case of roots of the plants where the biostimulator Asahi was used in the phase BBCH 37, Kelpak in BBCH 23 and fertilizer Tytanit in BBCH 37. And in the case of chlorophyll objects, where Tytanit was applied in BBCH 37 and 69. In the study, significant correlation was found only between the chlorophyll content and the root mass (r = 0.54).

Streszczenie

Skutecznym sposobem ograniczenia emisji tlenku azotu może być zmniejszenie poziomu nawożenia azotem, w związku z tym stosowanie specjalistycznych preparatów, takich jak biostymulatory, które stanowią dobrą alternatywę dla konwencjonalnego nawożenia mineralnego. Celem badań było określenie wpływu aplikacji preparatów biostymulujących (Asahi SL, Kelpak SL, Tytanit) zastosowanych na tle ograniczonej dawki azotu (75 kgN·ha-1) na wielkość plonu, zawartość białka ziarna pszenicy ozimej odmiany „Muszelka" oraz ilości chlorofilu i masy korzeniowej. W przeprowadzonych badaniach nie zostały potwierdzone zmiany statystyczne plonu, jednak znaczne wyższe jego wartości stwierdzono po aplikacji biostymulatorów Asahi SL oraz Klepak SL w porównaniu do kontroli. Należy podkreślić, że zastosowane preparaty wpływały istotnie na wyraźny wzrost zawartość masy korzeni oraz chlorofilu w liściach, dotyczyło to w przypadku korzeni: obiektów gdzie zastosowano biostymulator Asahi w fazie BBCH 37, Klepak w fazie 23 i nawóz Tytanit w fazie BBCH 37,a w przypadku chlorofilu obiektów gdzie aplikowano Tytanit w fazach BBCH 37 i 69. Stwierdzono istotne dodatnią korelacje jedynie pomiędzy pomiędzy zawartością chlorofilu a masą korzeni (r=0,54).

1. INTRODUCTION

Anthropogenic emission of nitrogen compounds causes disturbance in the environment. Ammonia and nitrogen oxides intensify eutrophication and acidification of the environment, and contribute to the greenhouse effect as well [Sapek 2008]. According to a research conducted by the National Centre for Emissions Balancing and Management [2014], Polish agriculture is a significant emitter of N_2O (81.27 thousand tons). Consequently, the possibility of reducing the emission of this gas from agricultural sources, mainly from cultivated soils, should be sought. An effective way to reduce N_2O emissions may be to reduce nitrogen fertilization [Kaiser and Ruser 2000, Jungkunst et al. 2006]. According to the literature data, the measurable effect of N_2O emission from agricultural soils can only occur when nitrogen doses are reduced, and soil treatment is regulated [Sosulski et al. 2015]. This can also happen by using products such as biostimulators (growth stimulators or phytostimulators).

The intensification of agricultural production has adversely affected the fertility of the soil and thus, the environment. In addition, adverse biotic and abiotic conditions in crops can cause stressful reactions in plants, which can adversely affect their productivity. As a result, farmers increasingly seek safe and natural methods to improve soil productivity, and protect it from degradation. In modern cereal cultivation technology, the application of biostimulators has become a common practice in Poland. Their rational use is currently one of the main factors of the environment and soil productivity. As past researches have

Table 1. Scheme of experience of wheat variety 'Muszelka' — dose and development phase in which the formulations were applied.

Object- objekt	Agent- preparat	Rate- dawka	Developmental phase- faza rozwoju	BBCH phase- Skala Zadoksa
1	Kontrola '0'	'0'		
2	Tytanit (T1)	0,2 ha^{-1}	tillering- krzewienie	BBCH 23
3	Tytanit (T2)	0,2 + 0,2l·ha^{-1}	stem elongation- strzelanie w źdźbło	BBCH 37
4	Tytanit (T3)	0,2 + 0,2 + 0,2l·ha^{-1}	end of flowering- po kwitnieniu	BBCH 69
5	Asahi SL (A1)	0,6l·ha^{-1}	tillering- krzewienie	BBCH 23
6	Asahi SL (A2)	0,6 + 0,6l·ha^{-1}	stem elongation- strzelanie w źdźbło	BBCH 37
7	Kelpak SL (K)	2,0l·ha^{-1}	tillering- krzewienie	BBCH 23

shown, their task is to accelerate and control life processes and to increase plant resistance to stress.

These stimulators are safe for the environment and can in part complement the activity of chemical means of plant production including fertilization. Biostimulators are biologically active substances and are manufactured from the extracts from marine organisms (algae and seaweed), microorganisms, phytohormones and micro- and macroelements (e.g., titanium). In order to increase the production, potential biostimulators activate the metabolism and chemical reactions throughout the plant. Their main task is to improve the quantitative and qualitative parameters of crops as well as the amount of chlorophyll and root mass, which supports the uptake of nutrients from the soil, and thereby affects the reduction of their losses from the soil environment, in particular nitrogen [Jankowski and Dubis 2008, Kozak et al. 2008, Matysiak et al. 2011, Pacholczak et al. 2013, Jankowski et al. 2014]. In light of the current research and agricultural practice, it has been proven that the use of supporting preparations helps to minimize intensive mineral fertilization, in particular the doses of nitrogen.

The aim of the study was to determine the effect of the application of biostimulation agents (Asahi SL, Kelpak SL, Tytanit) applied against the limited dose of nitrogen (75 kgN·ha-1) on winter wheat 'Muszelka'. Measuring grain yield, protein content, amount of chlorophyll and root mass.

2. MATERIAL AND METHODS

The source material includes the results from the field experiments conducted in the years 2014—2015 at the Research Station of the Faculty of Agriculture and Biotechnology in Mochełek, the University of Technology and Life Sciences. The experiments were carried out on the luvisols, which are classified as Class IVa — average quality arable soil. This region is characterized by average air temperatures of 7.7 °C and mean of summer months (IV-IX) is 14 °C, while the average annual precipitation is low and is 432 mm.

The study was conducted on the basis of a one-factorial field experiment, where three stimulating agents were applied in different doses and developmental phases. Depending on the formulation (BBCH 23, 37, 69) and the control (n = 7), against the limited mineral fertilization. The following stimulant agents were used: Tytanit — mineral fertilizer, also classified as mineral growth stimulants; Asahi SL — plant growth and yield stimulator, biostimulant based on three nitrophenols; Kelpak SL — growth regulator, algae extract containing plant hormones: auxins, cytokines.

The test plant was winter wheat variety 'Muszelka', which is characterized by excellent prolificacy, for which the forecrop was winter oilseed rape. Wheat was sown on 24.09.2014 in the amount of 280 kg·ha^{-1}. Harvest was made at full maturity of grain in the second decade of July. Fertilization of NPK was applied on all objects including control at the following doses: 75 kg N·ha^{-1} (20 kg·ha^{-1} in autumn, 50 kg·ha^{-1} in spring in the form of ammonium nitrate and 5 kg·ha^{-1} in the form of urea for leaf sprays), 30 kg P·ha^{-1} and 66 kg K·ha^{-1}. The cultivation of wheat has been done according to the rules of proper agricultural technology recommended for this plant.

During the vegetation period, following measurements were taken on the representative plants under the effect of stimulant: content of chlorophyll in leaves and root weight of wheat. These studies were conducted two weeks after the application of biostimulants used in particular phases of phenological development. The harvest of winter wheat 'Muszelka' yield from each plot was made in the second decade of July in the full maturity of grain (BBCH 89).

The representative samples of plant material were collected, and following parameters were determined: total protein content in grain using whole grain analyser Infratec 1241; chlorophyll content in leaves using a Minolta-type apparatus. The individual parameters were determined according to the applicable standards for specialized devices with international ICC standards.

The results obtained were statistically analysed by one-way analysis of variance according to a model compatible with the experience system, evaluating the significance of differences in the Tukey test with the significance level $\alpha = 0.05$, and calculating the correlation between the tested parameters using Statistica 10.0.

3. RESULTS AND DISCUSSION

It was found that the stimulants used in the study significantly increased the protein content in grain of winter wheat, as well as the amount of chlorophyll and the root mass, but did not significantly differentiate the yield (Table 2).

Despite the lack of significant influence on the yield of winter wheat 'Muszelka', after applying the agents in different phenological

Table 2. Mean values of selected winter wheat grain parameters from two years of research (2014—2015), depending on the application of stimulants against fertilization.

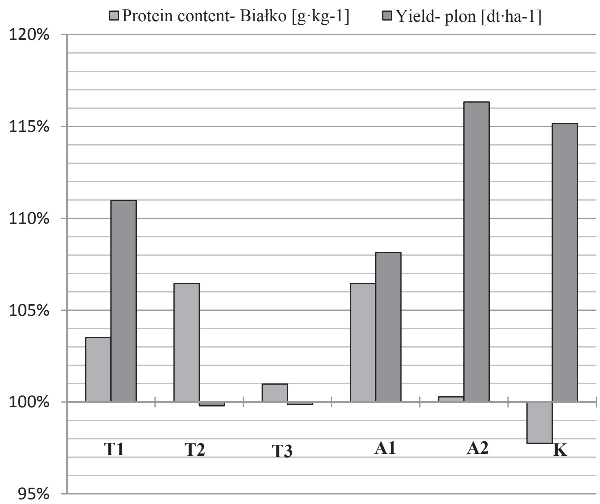
Fertilization variants - warianty nawozowe						
„0"	T1	T2	T3	A1	A2	K
Yield - plon [t·ha^{-1}]						
4,82$^{n.i.}$	5,35$^{n.i.}$	4,81$^{n.i.}$	4,81$^{n.i.}$	5,21$^{n.i.}$	5,6$^{n.i.}$	5,55$^{n.i.}$
Protein content - zawartość białka ogólnego [g·kg^{-1}]						
118,8^{a}	123^{a}	126,5^{ab}	120^{a}	126,5^{ab}	119,2^{a}	116,2^{a}
Weight of roots - masa korzeni [g]						
4,74^{a}	6,03^{b}	10,27^{ba}	12,35^{abc}	6,52^{abc}	12,19^{abc}	12,5^{abc}
Chlorophyll content – zawartość chlorofilu [SPAD]						
553,33^{a}	574,5^{b}	619^{abcd}	627,67^{abcd}	563,33^{a}	593,5^{abc}	585,17^{ab}

'0'- control- kontrola;
The application of a stimulant- zastosowanie preparatu: T1- Tytanit used in the stage BBCH 23, T2- Tytanit used in the stage BBCH 37, T3- Tytanit used in the stage BBCH 69, A1- Asahi SL used in the stage BBCH 23, A2- Asahi SL used in the stage BBCH 37, K- Kelpak SL used in the stage 23;
n.i.- non-significant differences- różnice nieistotne;
a, b, … - mean values marked with different letters in line differ significantly depending on the fertilization variants;
a, b, … - wartości średnie oznaczone różnymi literami w wierszu różnią się istotnie w zależności od zastosowanych wariantów nawozowych.

phases against mineral fertilization, its size was varied and ranged from 4.81 t·ha^{-1} to 5.6 t·ha^{-1}, with an average of 5.16 t·ha^{-1}. The highest average yield from years of study was obtained at the object where biostimulant Asahi SL was applied in phase BBCH 37, and the lowest after application of Tytanit in phase BBCH 37 and 69 (Table 2). However, it should be noted that the average grain yield after the application of Asahi SL (BBCH 37), Kelpak SL (BBCH 23) Tytanit (BBCH 23) and Asahi SL (BBCH 23) were higher compared to the control by 16 %, 15 %, 11% and 8%, respectively (Figure 1). Consequently, it can be considered that the biostimulants used against limited nitrogen dose produced higher or comparable yields, irrespective of the time of their application compared to the yields obtained from control crops. This confirms the hypothesis that the reduction of the nitrogen dose, together with the application of the formulation, does not affect the grain yield and in some cases, may even contribute to their increase. The influence of biostimulators on wheat grain yields was also obtained by other authors in their research [Kozak et al. 2008, Kotwica et al. 2014, Kierzek et al. 2015]. Accordingly, the combined use of a limited dose of nitrogen (75 kg N·ha^{-1}) and biostimulant agents resulted in higher yields compared to the cited literature (80-160 kg N·ha^{-1}) [Truba et al. 2012, Knapowski et al. 2016] and this causes a higher intake of nutrients from the soil.

The used agents significantly impacted the total protein content in winter wheat grains, which was within the range of 116.2 g·kg^{-1} to 126.5 g·kg^{-1}, with an average content of 121.45 g·kg^{-1} (tab. 2). The results obtained are consistent with those reported in the literature for winter wheat, and are within the range of 90 to 130 g·kg^{-1} [Wróbel, Woźniak 2008, Knapowski et al. 2010].

After the application of the tested stimulant agents, there was generally a significant increase in the mean total protein content



for explanations, see Table 2 - objaśnienia pod tabelą 2

Figure 1. Protein content and yield of winter wheat variety 'Muszelka' after application of the tested agents depending on the time of their use by BBCH scale, (as 100% — control).

in grain of winter wheat compared to the value obtained on the control object, except the object where Kelpak SL was applied. The highest content of this parameter was obtained after the application of Asahi SL in BBCH 23 and Tytanit in stage BBCH 37; they were significantly higher by 6.5% compared to the control. The higher values of total protein content in wheat grains collected from the facilities, where these biostimulators were applied may indicate that they have a significant effect on the higher nitrogen uptake from the soil and on its proper metabolism in the plant. These results confirm the thesis by Czubiński [2011] that states the biostimulating agents are substances that affect the changes in biochemical processes in the plant, increase the natural reactions in plant cells. Also, Kowalska et al. [2012] confirms that they increase the intensity of nutrient uptake by crops by improving the existing mechanisms in plants.

The biostimulators significantly determined the amount of chlorophyll in wheat leaves, which was examined two weeks after the application of stimulators used within phenological phases. It has been found that the amount of chlorophyll increased for all the agents used in relation to control (Table 2). Significant increase in the amount of chlorophyll only by 3% occurred after the application of Tytanit in phase BBCH 23. However, in the phases BBCH 37 and BBCH 69, the amount of chlorophyll increased by 12% and 13% respectively (Figure 2), which did not respond with the grain yield (Table 2). A similar effect on the amount of chlorophyll in leaves, but slightly smaller, was found in case of Asahi SL, where growth was observed after the application in BBCH 23 only by 2%, but in phase BBCH 37 by up to 7%, compared to control. Where the stimulator Kelpak SL was applied in BBCH 23, the amount of chlorophyll was 6% higher compared to the control (Table 2.). It can be concluded that the single application of the Kelpak SL in phase BBCH 23 was most effective for the amount of chlorophyll in this phase, also compared to all tested biostimulation agents (Figure 2). It should be noted that the other two stimulators: Tytanit and Asahi SL, applied in the following phases, significantly affected the increase in the amount of chlorophyll content obtained after the phase BBCH 23 compared to the control.

Similar results were obtained by Thirumaran et al. [2009] and Matysiak et al. [2012], showing the effect of Ecklonia maxima algae on chlorophyll content. Also, the positive effect of biostimulants on the content of the chlorophyll in the leaves of plants was found by Przybysz et al. [2008] and by Joubert and Lefranc [2008].

The root mass of the winter wheat 'Muszelka', determined on the basis of representative samples, was characterized by a significant increase and ranged from 4.74 g to 12.5 g. The one-time application of the Kelpak SL increased the tested parameter by 164% compared to the control (Figure 2). The three-time application of Tytanit in the studied phenological phases (BBCH 23, 37 and 69) showed a significant increase in the root mass by 27%, 116.6% and 160.5% respectively, as compared to the control. While the double use of Asahi SL in BBCH 23 and BBCH 37 increased the weight of the roots of wheat respectively by 37.5%, and 157% (Table 2). In summary, the application of biostimulators with a reduced dose of nitrogen increased the mass of roots almost 2 or 3 times (Figure 2). Literature sources does not provide conclusive opinion about impact of biostimulators on root mass. For example, Szymczak - Nowak [2009] has a beneficial effect of algae preparations on the increase in root mass, which was confirmed in our study. In contrast, Kołodziej [2004] did not find any increase in root mass after using stimulants.

The correlation values for the parameters studied in winter wheat were also calculated. The significantly positive correlation was found only between the mass of roots and the amount of chlorophyll in leaves (r = 0.54). For this relationship, linear regression equations were calculated and presented graphically

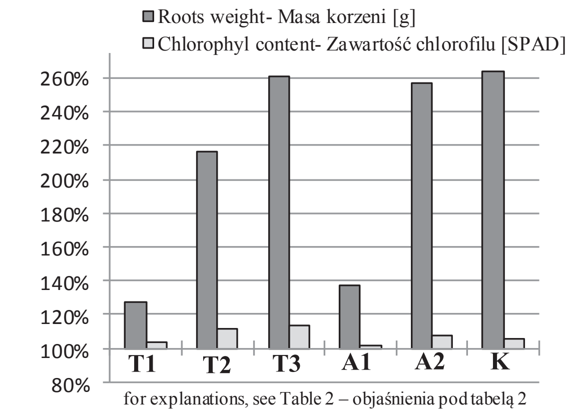


Figure 2. Content of root mass and chlorophyll in leaf of winter wheat 'Muszelka' after the application of the tested agents depending on the time of their use by BBCH scale, (as 100% — control).

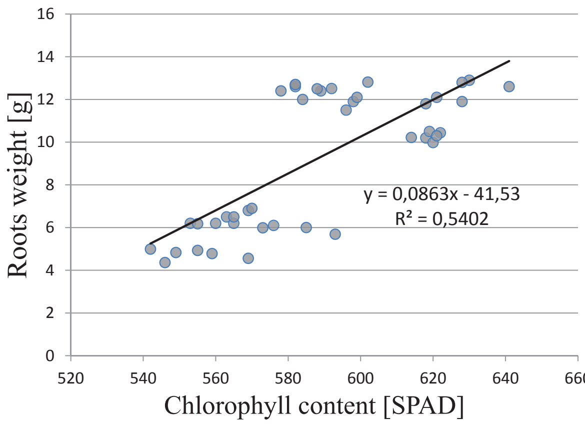


Figure 3. Correlation between root mass [g] and chlorophyll content [SPAD] in winter wheat.

(fig.3), indicating that with increasing root mass chlorophyll content increases. Also, Ibragimova et al. [2008] and Grzyś [2012] in their research showed a significant positive correlation between these parameters after the application of biostimulators. From a number of scientific studies and from the information obtained from the manufacturers, it is clear that the use of biostimulators has a positive effect on the size and quality of the crop and on the growth of the root system. Therefore, on the efficient extraction of nutrients from the soil [Kołodziej 2004, Ibragimova et al. 2008, Kotwica et al. 2014], the application of these stimulators also affects the activity of soil microorganisms, which affects the better use of soil and fertilizer nitrogen. It should be emphasized that the nitrogen not collected by crop plants or soil microorganisms is subject to many processes, as a result of which, a significant part is losses by emission and leaching. This may also lead to the disturbance of microbiological balance and, consequently to soil degradation [Sosnowski et al. 2012].

These studies have shown that the use of biostimulation agents in the era of intensive cultivation of plants may be an effective way to reduce the use of chemicals (fertilization), including the reduction of nitrogen doses.

4. CONCLUSIONS

1. It was found that the applied biostimulation formulas Asahi SL, Kelpak SL and Tytanit, against the reduced dose of nitrogen (75 kgN·ha^{-1}), did not significantly differentiate the grain yield of winter wheat 'Muszelka'. The highest, but statistically unproven, yields were observed after the application of Asahi SL in phase BBCH 37 and Kelpak in phase BBCH 23.
2. The used stimulators significantly differentiated the amount of chlorophyll in wheat leaves and the root mass, their application in the tested phenotypic phases in scale BBCH influenced the increase of these parameters relative to the control. The highest values were found for chlorophyll after the application of Tytanit in the phase BBCH 69, and in the case of root mass after the application of stimulator Kelpak in BBCH 23.
3. Total protein content in wheat was significantly increased by the applied biostimulants, the highest average content found after the application of Asahi SL in phase BBCH 37 and Tytanit in BBCH 23, and the lowest was obtained on the control.
4. The study leads to the conclusion that the use of biostimulants against the limited dose of nitrogen appears to be ecologically beneficial. Most of all, they affect the stabilization of yield, the quality of plants, the amount of chlorophyll, the mass of roots and thus a higher download of nitrogen. It can be assumed that these stimulants can contribute to the reduction of nitrogen oxide emissions from agricultural soils.

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