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Yield and Macronutrient Accumulation in Grain of Spring Wheat (*Triticum aestivum* ssp. *vulgare* L.) as Affected by Biostimulant Application

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Abstract

Plant growth and development, resistance to stress, nutrient uptake and yield can be supported by the use of biostimulants. The aim of this study was to determine the influence of the seaweed biostimulant Kelpak and its combined application with the preparation Lithovit on yield components, grain yield and accumulation of macroelements in spring wheat grain. The study was based on field experiments located in Poland, (53°13'N; 17°51'E), conducted in 2010-2011 on a typical Alfisol (USDA). In the one-factorial field experiment with the spring wheat (*Triticum aestivum ssp. vulgare*) cultivar Katoda, we compared the effects of the application of the biostimulant Kelpak (seaweed extract) (T1) alone at a dose of 2 l ha^{-1}, to a mixture of two preparations, Kelpak 1.5 l ha^{-1}+Lithovit (finely milled limestone) 1.5 kg ha^{-1} (T2) with the control (without preparations) (T3). The study showed that foliar application of the biostimulant Kelpak at shooting increased spring wheat yield, but there was no additional increase in yield after its combined application with Lithovit. Application of the biostimulant Kelpak increased the number of generative tillers, and the combined application of prepared Kelpak and Lithovit had a favourable effect on the number of grains per ear. Application of biostimulant Kelpak and its mixture with Lithovit caused increased accumulation of N, P and K in grain.

Keywords: Grain yield; Straw weight; Harvest index; Macronutrient content; Nutrient accumulation

Introduction

Biostimulants are organic substances which, even when used in small amounts, stimulate growth and development of plants, and this response cannot be attributed to traditional nutrients [1]. Favourable effects of the application of extracts from algae indicate the benefit of their use in cultivation of horticultural and agricultural crops [2-4]. Seaweed biostimulants contain many active substances, including growth hormones: auxins, cytokinins and also polyamines and brassinosteroids [5,6]. Auxins have an effect on root formation, cytokinins on stem elongation, and polyamines on growth and development [7]. Due to the presence of phytohormones, marine algae-based seaweed extracts can increase plant biomass and reproductive yields [8,9]. In marine algae preparations, the presence of alginate, fucans, laminaran were also found, which are necessary in defensive activity of plants against diseases and pests [3,4,10]. Active substances of algae extracts applied in field crops can also reduce the effect of drought stress [11] or P and K deficiency [12]. Algae biostimulants may be used alone or combined with synthetic preparations and growth regulators [1]. They are applied mostly on leaves and may be used several times during the growth period [4]. The effect of such preparations is dependent on the time of their application and the dose [13,14].

One of the seaweed biostimulants is Kelpak, which is obtained from marine alga (*Ecklonia maxima* Osbeck), belongs to the class of brown algae (*Phaeophyta*), and is harvested on the coast of Africa. Kelpak contains phytohomones: auxins and cytokinins (11 and 0.031 mg l^{-1}, respectively), alginians, amino acids, as well as small amounts of macro and microelements [5]. In studies of cereal crops, the application of seaweed biostimulants caused root and shoot growth [15-17]. Increased accumulation of macro- and micro-nutrients was also indicated [18-20]. In another group of biostimulants is Lithovit, which is a finely milled limestone (a particle size smaller than the size of stomata) consisting mainly of (Ca, Mg)-CO_3 as well as different micronutrients (Mn, Cu, Zn, Ni, Fe) relevant for plant physiology (Patent DE202006011165 U1). However, there are no of recommendations for dose and time of biostimulator Kelpak and Lithovit application in spring wheat. According to Sharma et al. [1] further studies concerning the use of biostimulants in cereals crop should be focused on the evaluation of crop trial protocols to reduce the impact of biotic and abiotic stresses as well as assessing synergistic responses to mixtures including macroalgal extracts and other biostimulants.

The aim of this study was to determine the influence of the seaweed biostimulant Kelpak and its combined application with the preparation Lithovit on yield components, grain yield and accumulation of macroelements in spring wheat grain yield.

Materials and Methods

Experimental site

The study was based on field experiments located in Poland, (53°13'N; 17°51'E), conducted in 2010-2011 on a typical Alfisol (USDA). The topsoil at the experimental site was characterized by medium content of available potassium 95-150 mg kg^{-1} and phosphorus 190-210 $mgkg^{-1}$ (both determined with Egner-Riehm method), very low content of magnesium <20.0 mg kg^{-1} (Schetschabel method), and was slightly acidic (pH in 1M KCL 5.7-6.1) (with the use of potentiometry). The content of total nitrogen 0.69-0.75 g kg^{-1} and organic carbon 7.55-7.80 g kg^{-1} in soil was relatively low.

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Experimental design

This study was based on a strict, one-factorial field experiment with the spring wheat (*Triticum aestivum ssp. vulgare*) cultivar Katoda, in which the effects of the application of the biostimulant Kelpak (T1) alone in a dose of 2 l ha^{-1}, or a mixture of two preparations: Kelpak 1.5 l ha^{-1}+Lithovit 1.5 l ha^{-1} (T2), were compared with the control (with no preparations) (T3). Preparations were applied at the shooting stage (4-5 leaves), after dissolving in water (300 l ha^{-1}).

Crop management

The spring wheat was sown 02-04 of April at a density of 500 no m^2 (at amount of 230 kg ha^{-1}) on plots with an area of 12 m^2, in four replications. Presowing fertilization of 31 kg ha^{-1} P (superphosphate), 66 kg ha^{-1} K (potassium chloride) and 80 kg ha^{-1} N (ammonium nitrate) was applied. At the beginning of the shooting state, the other rate of N (ammonium nitrate) was applied at a rate of 60 kgha^{-1} N. For weed control, triasulfuron 118.6 gha^{-1}+dicamba 7.4 gha^{-1} was applied at BBCH 22-24. To protect against diseases, epoxiconazole 93 g ha^{-1}+fenpropimorph 300 g ha^{-1}, and metrafenone 112.5 g ha^{-1} were applied at BBCH 34-39 and fusilazole 125 g ha^{-1}+karbendazim 250 g ha^{-1} at BBCH 51-59. Pest control was performed as a single application at BBCH 59, using dimethoat 200 g ha^{-1}. Wheat harvest was performed over the first ten days of August with the plot combine harvester Wintersteiger.

Sampling, measurement and chemical analysis

The number of generative tillers in the area of 1 m^2 was calculated at the flowering stage (BBCH 75). The number of grains per ear was determined at the full maturity stage on 30 randomly selected ears from each plot. The grain yield was determined directly after harvest and straw yield 7 days after. Presented grain and straw yields were calculated per the set humidity 14%. The 1000-kernel weight was assessed one month after harvest based on 200 grains from each plot. Harvest index was calculated for each plot as dry matter of grain yield divided by the sum of dry matter of grain yield and straw yield. Wheat grain was ground prior to chemical analyses. Mineralization was obtained by wet burning of fragmented material with perhydrol and sulfuric acid. Analyses were made with the following methods: total nitrogen content with the Kjeldahl method, phosphorus content with the molybdenum-vanadium test, and potassium with flame photometry. N, P and K uptake was calculated for each plot as the product of grain dry mass yield and the contents of individual macroelements.

Statistical analysis

The obtained results were analysed statistically using the statistical program Analysis of Variance for orthogonal experiments by the University of Technology and Life Sciences in Bydgoszcz, Poland. The differences between values were verified with Tukey's test on the significance level $P \leq 0.05$.

Results and Discussion

The weather conditions in the study region were not very favorable for spring wheat (Table 1). The average precipitation of 275.1 mm from March to August limits plant growth and yield. Thermal conditions are mostly favorable, but periodic heat waves in conditions of low total precipitation may enhance drought stress. The course of the weather conditions was different in the successive years of the study (Table 1). In 2010, from March to August, the total precipitation was higher by 18.3% than in 2011. In 2010, during the period from sowing (April) to the flag leaf stage (May), rainfalls were higher than in 2011, but at ear formation and flowering stages (June), there was only 18.1 mm of rainfall. The amount of rainfall during kernel development and maturation (July-August) did not limit plant growth. The years 2010 and 2011 were slightly warmer than the long-term average. In 2010 it was cooler in the successive months of growth from April to June than in 2011. In contrast, July and August were cooler in 2011.

In the present study, the number of generative shoots was on average 492 pieces m^{-2} and was similar in both years of the study. In 2010 and on average from the two years of the study, the application of the biostimulant Kelpak caused an increase in the number of generative shoots as compared with the control (Table 2). Increased wheat tillering after the use of algae extracts was noted also in another study [14,20]. In the study by Kumar and Sahoo [15], a positive effect of soaking seeds in an extract from algae was proved both for the number of branches and for the tiller length.

In our study of 2011, the number of grains per ear was higher, and the thousand grain weight was almost two times lower, as compared to 2010 (Table 3). In 2011 as well as on average in the long-term period, an increase in the number of grain per ear was observed after the combined application of Kelpak and Lithovit. A favorable effect of algae extracts on the yield components of spring wheat was also reported by Beckett and van Staden [21]. In this study the preparation applied at the 1-4 leaf stage in conditions of K deficit (moderate and heavy) increased both the number of grains per ear and the weight of a single grain. Zodape et al. [14] also indicated an increase in the 1000-kernel weight of wheat grains, but they did not find an effect on the number of grains per ear. In the study by Matysiak and Adamczewski [22], the 1000-kernel weight of spring wheat grain was higher after the application of the biostimulant Kelpak in a dose of 1.5 l ha^{-1} as compared with the variant without the application.

The spring wheat grain yield of 3.5 t ha^{-1} obtained in the study (Table 3) was similar to that obtained in the study by Matysiak and Adamczewski [22]. In the present study, despite different weather conditions, no considerable differences in yield were recorded in the harvest years 2010 and 2011. The applied biostimulants had a significant effect ($P \leq 0.05$) on grain yield quantity. In 2010 and 2011, after the application of the biostimulant Kelpak, the spring wheat grain was higher than in the control. After the combined application of the preparations Kelpak and Lithovit the yield was similar to that obtained in the treatment with Kelpak alone and to the control. On average of the two years of the study, the application of the seaweed extract alone as well as its combined application with the biostimulant Lithovit brought a significant increase ($P \leq 0.05$) in grain yield as compared with the variant without the application. This increase was obtained due to the increase in the number of generative shoots after the application of biostimulant Kelpak alone or as a result of an increase in the number of grains per ear, after combined application of Kelpak and Lithovit (Table 2). Similarly, Zodape et al. [14] indicate an increase in the number of generative shoots and thousand seed weight and the wheat grain yield after the application of an algae preparation, whereas this response was dependent on the dose applied. Shah et al. [20], when applying an algae extract, obtained an increase in wheat yield by 2.5-10%, depending on the dose. Favorable effect of the biostimulant Kelpak was also shown in another study by Matysiak and Adamczewski [22] when the application of doses 1.5 or 2 l ha^{-1} at the shooting stage resulted in an increase in the wheat grain yield by 13% as compared with the control. In contrast, the study by Beckett and van Staden [21], Kelpak did not affect the weight of grains per ear, in

Month	Precipitation (mm)			Air temperature °C		
	2010	2011	1949-2011	2010	2011	1949-2011
March	28.6	11.7	24.5	2.4	2.2	1.9
April	33.8	13.5	27.4	7.8	10.5	7.4
May	92.6	38.4	43.2	11.5	13.5	12.7
June	18.1	100.8	53.7	16.7	17.7	16.3
July	107.4	132.5	73.1	21.6	17.5	18
August	150.7	67.7	53.2	18.4	17.7	17.5
Total/mean	431.2	364.6	275.1	13.1	13.2	12.3

Table 1: Weather conditions at experimental locality.

Year	Treatment			Mean	LSD
	Control	Kelpak 2 l ha^{-1}	Kelpak 1.5 l ha^{-1} +Lithovit 1.5 kg ha^{-1}		
Number of generative tillers [no m^{-2}]					
2010	471.0	529.0	464.7	488.2	54.3
2011	496.0	498.5	493.0	495.8	ns
Mean	483.5	513.8	478.8	492.0	30.0
Number of grain per ear [no]					
2010	19.1	19.3	19.8	19.4	ns
2011	35.0	35.3	37.5	35.9	2.16
Mean	27.1	27.3	28.7	27.7	1.05
1000-kernel weight [g]					
2010	50.2	50.4	49.5	50.0	ns
2011	26.3	26.1	26.6	26.3	ns
Mean	38.3	38.3	38.1	38.2	ns

Table 2: Effect of biostimulant application on number of generative tillers, number of grain per ear and 1000-kernel weight of spring wheat.

conditions of the optimal K, but it significantly ($P \leq 0.05$) increased the yield in conditions of stress caused by K deficit. This increase resulted from increasing the number of grains per ear and the weight of a single grain. This study also indicated an increase in the leaf area and root weight after the application of Kelpak in conditions of K deficit. Also a significant ($P \leq 0.05$) influence of Lithovit on foliar area growth and development was shown [23].

In the present study, the straw weight in 2010, characterized by high total precipitation at the shooting stage (May), was two times higher than in 2011. The application of biostimulant Kelpak in 2010 or its combined application with Lithovit in 2011 caused a significant reduction ($P \leq 0.05$) in straw weight as compared with the control. Straw weight, on average in the two years of the study, was lower after the application of the seaweed extract alone in comparison with the variant with no preparations. Literature reports show an increase in the number of shoots per plant and their height after the application of seaweed extracts [15,20,24]. In our study of spring wheat the reduction of straw weight after the application of biostimulants resulting from different distribution of assimilates than in the control. An increased accumulation of assimilates after the application of biostimulant was noted only in the grain. Because of this, both in successive years of the study and on average from the years, the harvest index was higher in the control as compared with the treatments with biostimulant Kelpak as well as Kelpak+Lithovit.

Significant effect ($P \leq 0.05$) of the studied preparations on the average content from two years of the study of N, P, K Mg in spring wheat grain was indicated (Table 4). Wheat grain contained less N and Mg after the application of Kelpak as compared with the control. The

Year	Treatment			Mean	LSD
	Control	Kelpak 2 l ha^{-1}	Kelpak 1.5 l ha^{-1} +Lithovit 1.5 kg ha^{-1}		
Grain yield [t ha^{-1}]					
2010	3.27	3.56	3.54	3.45	0.272
2011	3.46	3.65	3.62	3.57	0.171
Mean	3.36	3.60	3.58	3.51	0.210
Straw weight [t ha^{-1}]					
2010	6.53	5.88	6.29	6.23	0.472
2011	3.09	2.95	2.88	2.97	0.173
Mean	4.81	4.42	4.59	4.60	0.380
Harvest index [%]					
2010	33.4	37.7	36.1	35.7	1.85
2011	52.8	55.3	55.8	54.6	1.66
Mean	43.1	46.5	45.9	45.1	2.1

Table 3: Effect of biostimulant application on grain yield, weight of straw and harvest index of spring wheat.

Year	Treatment			Mean	LSD
	Control	Kelpak 2 l ha^{-1}	Kelpak 1.5 l ha^{-1} +Lithovit 1.5 kg ha^{-1}		
N					
2010	1.66	1.62	1.64	1.64	ns
2011	2.41	2.30	2.36	2.35	ns
Mean	2.03	1.96	2.00	1.99	0.047
P					
2010	0.299	0.296	0.306	0.300	ns
2011	0.371	0.369	0.369	0.370	ns
Mean	0.335	0.333	0.337	0.335	0.003
K					
2010	0.187	0.199	0.195	0.193	ns
2011	0.498	0.490	0.485	0.491	ns
Mean	0.342	0.345	0.340	0.342	0.003
Mg					
2010	0.136	0.130	0.129	0.131	ns
2011	0.183	0.183	0.186	0.184	ns
Mean	0.160	0.157	0.158	0.158	0.003

Table 4: Effect of biostimulant application on macronutrient content in spring wheat grain.

content of P after the application of seaweed extract was lower than after the combined application of Kelpak and Lithovit. Wheat grain contained more K when only biostimulant Kelpak was applied, as compared with its combined application with the preparation Lithovit, as well as in relation to the control. N, P and K uptake in wheat grain, on average in the two-year period of the study, was higher both after the application of the biostimulant Kelpak and its combined application with the Lithovit compared with the control (Table 5). For N and K such relationship was also shown in 2010. The use of biostimulants did not affect Mg uptake in wheat grain. Shah et al. [20] report an increase in K and N uptake in some variants of seaweed extract application and mostly the lack of effect on P uptake. Zodape et al. also indicated an increasing accumulation of N, P, K under the influence of the seaweed biostimulant, particularly at the highest dose. Similarly, in the study by Beckett and van Staden [18], liquid feed (hydroponic) improved the yield of nutrient stressed wheat allowing better nutrient uptake.

Base on the study we can concluded that foliar application of the biostimulant Kelpak (seaweed extract) at the shooting stage increased spring wheat yield, but there was no additional growth in yield after

Year	Treatment			Mean	LSD
	Control	Kelpak 2 l ha^{-1} BBCH 22	Kelpak 1.5 l ha^{-1} +Lithovit 1.5 kg ha^{-1}		
N					
2010	45.9	49.5	49.9	48.4	2.84
2011	71.6	72.1	73.5	72.4	ns
Mean	58.8	60.8	61.7	60.4	1.375
P					
2010	8.37	9.06	9.31	8.91	ns
2011	11.0	11.6	11.7	11.4	ns
Mean	9.7	10.31	10.5	10.2	0.595
K					
2010	5.26	6.08	5.94	5.76	0.661
2011	14.8	15.3	14.8	15.0	ns
Mean	10.0	10.7	10.4	10.4	0.320
Mg					
2010	3.82	3.96	3.92	3.90	ns
2011	5.43	5.72	5.80	5.65	ns
Mean	4.63	4.84	4.86	4.78	ns

Table 5: Effect of biostimulant application on accumulation of macronutrient in spring wheat grain.

its combined application with Lithovit (finely milled limestone). Application of the biostimulant Kelpak increased the number of generative shoots and combined use of prepared Kelpak and Lithovit had a favorable effect on the number of grains per spring wheat spike. Application of the biostimulant Kelpak as well as its mixture with the prepared Lithovit caused increased accumulation of N, P, and K in wheat grain.

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