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EFFECT OF BIOSTIMULANT APPLICATION IN CULTIVATION OF SPRING BARLEY

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ABSTRACT

Background. Biostimulants support plant growth and development, induce increased resistance to stress and may have a favourable effect on yields of agricultural crops and vegetables.

Material and methods. The strict field experiment was conducted over the years 2010-2011, at the Research Station of the Faculty of Agriculture and Biotechnology in Mochełek (53°13’ N; 17°51’ E). The aim of this study was an assessment of the effect of biostimulants on yield components, grain and straw yield, root weight and the accumulation of NPK and Mg in spring barley grain. The one-factor experiment with spring barley cv. Nuevo aimed to study the effect of the seaweed biostimulant Kelpak (at a rate of 2 $dm^3 \cdot ha^{-1}$) and its combined application with the preparation Lithovit (at rates of 1.5 $dm^3 \cdot ha^{-1}$ + 1.5 $kg \cdot ha^{-1}$, respectively) on the biometric traits of plants and on grain yield.

Results. The foliar application of the biostimulant Kelpak at a rate of 2 $dm^3 \cdot ha^{-1}$ at the tillering (4-5 leaf stage) or the combined application of the preparations Kelpak 1.5 $dm^3 \cdot ha^{-1}$ and Lithovit 1.5 $kg \cdot ha^{-1}$ had a favourable effect on grain yield. The application of only Kelpak caused an increase in the fresh weight of spring barley roots, and the combined use of Kelpak and Lithovit had a favourable effect on straw weight. In both treatments, thousand grain weight was higher than in the control. After the combined application of both preparations the barley grain was characterized by the highest protein concentration. The application of only Kelpak resulted in an increased accumulation of N, P, K and Mg in barley grain. The combined application of Kelpak and Lithovit caused an increase in the accumulation of N and Mg in grain.

Conclusion. The beneficial effect on yield of the biostimulant Kelpak at a rate of 2 $dm^3 \cdot ha^{-1}$ or the combined application of preparations Kelpak 1.5 $dm^3 \cdot ha^{-1}$ and Lithovit 1.5 $kg \cdot ha^{-1}$ at the tillering (4-5 leaf stage) justifies the application of those preparations in the cultivation of spring barley.

Key words: crude fibre, grain yield, macroelements accumulation, root weight, straw weight, total protein

INTRODUCTION

Biostimulants are substances that when used in small amounts stimulate plant growth, which cannot be attributed solely to the application of the basic nutrients (Sharma *et al*., 2014). The favourable effect of biostimulants has been indicated in the cultivation of many field crops (Khan *et al*., 2009; Craigie, 2011; Calvo *et al*., 2014). Preparations derived from marine algae are one of the most important groups of biostimulants. They contain many active substances, including growth hormones, auxins, cytokinins, as well as polyamides and brassinosteroids (Stirk and van Staden, 2014; Stirk *et al*., 2014). Thanks to the presence of phytohormones, marine algae extracts can cause an increase in the biomass and yield of agricultural crops and vegetables (Rayorath *et al*., 2008; Kurepin *et al*., 2014). Other active substances

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(alginates, fucoidan or laminarin), which have also been found in seaweed preparations, are necessary in the defensive activity of plants against diseases and pests (Khan *et al*., 2009; Craigie, 2011; Stadnik and de Freitas, 2014). The active ingredients of seaweed extracts applied to cultivated crops can also alleviate plant response to drought stress (Zhang and Ervin, 2004) or nutrients deficiency (Papenfus *et al*., 2013). Seaweed extracts are mostly used in the form of foliar application and can be applied several times during the growing period (Khan *et al*., 2009; Sharma *et al*., 2014). The effect of those preparations is dependent on the time of their application and the rate (Matysiak and Adamczewski, 2006; Kumar and Sahoo, 2011).

In studies concerning cereal crops, the application of seaweed preparations stimulated both the growth of roots and the underground parts (Nelson and van Staden, 1986; Steveni *et al*., 1992; Zodape *et al*., 2009). Other studies indicate increased accumulation of macro- and microelements in plants (Beckett and van Staden, 1990; Shaaban *et al*., 2010; Shah *et al*., 2013). Further studies should be aimed at optimization of methods for the use of biostimulants and assessment of the effects of combined use of different preparations in the reduction of biotic and abiotic stresses and the increase in field crop yields (Sharma *et al*., 2014).

The aim of this study was to assess the effect of the application of the biostimulant Kelpak, as well as its combined application with the preparation Lithovit, on yield components, root weight, grain and straw yield, as well as the accumulation of macroelements in spring barley grain.

MATERIAL AND METHODS

The study was conducted based on a field experiment located in Mochełek near Bydgoszcz (53°13’ N; 17°51’ E), in luvisol of quality class IV a, formed from sandy loam. The arable layer at the place of the study was characterized by a low to average content of available K (78.9-124.5 mg·kg^{-1}) and an average to high content of available P (83.6-92.4 mg·kg^{-1}), very low content of Mg (<20.0 mg·kg^{-1}) and slightly acid reaction (pH in 1M KCL 5.7-6.1). The content of total nitrogen in the soil was 0.69-0.75 g·kg^{-1}, and of organic carbon 7.55-7.80 g·kg^{-1}.

The strict one-factor field experiment with spring barley (*Hordeum vulgare* L.) cultivar Nuevo was conducted over the years 2010-2011. It compared the effects of application of the biostimulant Kelpak alone (T1) at a rate of 2 dm^3·ha^{-1}, and a mixture of two preparations, Kelpak 1.5 dm^3·ha^{-1} + Lithovit 1.5 kg·ha^{-1} (T2) with the control (without preparations) (T3). Kelpak is obtained from the marine macro-alga (*Ecklonia maxima* Osbeck) belonging to the class of brown algae (*Phaeophyta*), harvested of the coasts of Africa. Kelpak contains phytohormones: auxins and cytokinins (11 and 0.031 mg·dm^{-3}, respectively), alginates, amino acids as well as small amounts of macro- and microelements (Stirk *et al*., 2014). Lithovit is finely ground limestone (with particles smaller than the openings in the leaf stomata) containing mostly (Ca, Mg)CO_3 as well as microelements (Mn, Cu, Zn, Ni, Fe) that are essential for plant development (Patent DE202006011165 U1). The preparations were applied on leaves, at the spring barley tillering stage (4-5 leaf stage), after dissolving in water (300 dm^3·ha^{-1}).

Spring barley was sown on 02 and 05 April, in 2010 and 2011, respectively, at a density of 360 pcs.·m^{-2}, on plots with an area of 12 m^2, in four replications. Prior to sowing, mineral fertilization was applied at rates: 31 kg·ha^{-1} P, 66 kg·ha^{-1} K and 80 kg·ha^{-1} N. At the beginning of the shooting stage, N (ammonium nitrate) was applied again at a rate of 30 kg·ha^{-1} N. For weed control, we applied triasulfuron 118.6 g·ha^{-1} + dicamba 7.4 g·ha^{-1} at BBCH 22-24. To protect against diseases, epoxiconazole 93 g·ha^{-1} + fenpropimorph 300 g·ha^{-1}, metrafenone 112.5 g·ha^{-1}, at BBCH 34-39 and flusilazole 125 g·ha^{-1} + carbendazim 250 g·ha^{-1} at BBCH 51-59 were applied. Pest control was performed once, using dimethoate 200 g·ha^{-1} at the stage BBCH 59. Barley harvest was performed in the first ten days of August with a plot combine harvester Wintersteiger.

At the flowering stage (BBCH 65) generative shoots in an area of 1 m^2 were counted, and the root weight was determined on 20 consecutive plants in a row. The number of grains per spike was determined at the full maturity stage on 30 randomly selected spikes from each plot. The quantity of grain yield was determined after the harvest, and straw weight after seven days from threshing. The presented grain and straw yields were expressed for a fixed humidity of 14%. Thousand grain weight was determined 30-40 days after the harvest, based on 200 grains from each plot. Harvest index was calculated for each plot, as the ratio of grain weight to the sum of grain and straw weight. Barley grain was ground prior to chemical analyses. Mineralization was performed by wet combustion of fragmented material with hydrogen peroxide and sulphuric acid. The total protein content was calculated based on the content of N determined with the Kjeldahl method. Crude fibre content was determined with the modified Henneberg and Stohmann method. The vanadium-molybdenum method was used to determine P content and flame photometry for K. The content of Mg was determined with colorimetry using Titanium Yellow. The uptake of N, P, K and Mg presented in the study is the product of dry matter yield of grain and contents of individual macroelements.

The obtained results were subjected to statistical verification by the analysis of variance. The significance of differences between means was determined by Tukey's confidence half-interval, at the significance level α = 0.05.

RESULTS AND DISCUSSION

The weather conditions were varied in the years of the study (Fig. 1). The year 2010 was more favourable for the growth of spring barley. In 2010, in the period of intensive plant growth in April and May, the air temperatures were moderate and there were heavy rainfalls (a total of 126 mm). In 2011 in the same period it was much warmer, and the total precipitation amounted to only 51.9 mm. Intensive rainfall in June 2011 did not compensate for the earlier water deficit in spring.

The number of generative shoots of spring barley in 2010 and 2011, as well as on average from the two years of the study, after the use of the biostimulant Kelpak and after its combined application with the preparation Lithovit was similar to that found in the control treatment (Table 1). In studies of wheat, which is characterized by weaker tillering than barley, a seaweed biostimulant increased the rate of tillering (Kumar and Sahoo, 2011; Shah *et al*., 2013; Szczepanek and Grzybowski, 2016).

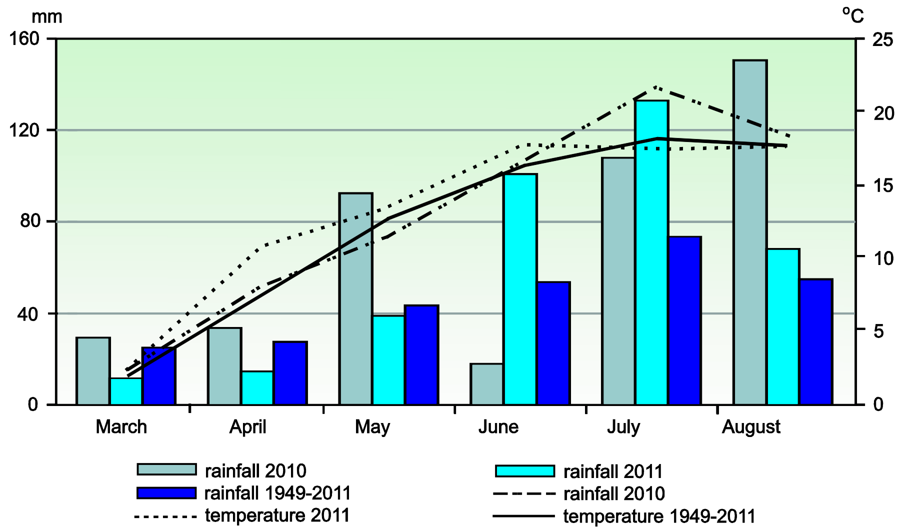

Fig. 1. Weather conditions in the study area

Table 1. Effect of biostimulant application on yield structure components and root weight of spring barley

Year	Treatment			Mean	$LSD_{\alpha=0.05}$
	Control	Kelpak 2 $dm^3 \cdot ha^{-1}$	Kelpak 1.5 $dm^3 \cdot ha^{-1}$ + Lithovit 1.5 $kg \cdot ha^{-1}$		
Number of generative shoots, pcs. m^{-2}					
2010	833.0	890.0	782.8	835.3	ns
2011	892.8	902.0	946.5	913.8	ns
Mean	862.9	896.0	864.6	874.5	ns
Number of grains per spike, pcs.					
2010	23.0	23.8	24.6	23.8	ns
2011	16.0	16.7	16.4	16.3	ns
Mean	19.5	20.2	20.5	20.1	ns
TGW, g					
2010	33.0	36.1	34.6	34.6	1.41
2011	45.2	46.0	46.7	46.0	1.16
Mean	39.1	41.1	40.6	40.3	0.68
Fresh root weight of 20 plants, g					
2010	23.6	30.8	31.6	28.6	ns
2011	19.8	26.0	23.0	23.0	ns
Mean	21.6	28.4	27.2	25.8	6.62

ns – non-significant differences

The number of grains per spike in 2010 was 46% higher than in 2011 (Table 1), which can be explained by the favourable weather conditions during formation of this yield structure component (Fig.1). In 2010 and 2011, as well as on average from the two years of the study, no significant effect of the studied preparations on the number of grains per spike was shown.

In 2011, with a smaller number of grains per spike, the thousand grain weight was higher as compared with 2010 (Table 1). In 2010, after the application of the biostimulant Kelpak, the thousand grain weight was significantly higher than after the application of both preparations, and that, in turn, was higher than in the control treatment. In 2011 only the combined application of the biostimulant Kelpak with the preparation Lithovit significantly increased the thousand grain weight as compared with the control. On average from the two years of the study, the thousand grain weight after treatment with the biostimulant Kelpak alone and after the application of both preparations was higher than in the control. In studies concerning spring wheat the favourable effect of algae extract on the thousand grain weight has also been found (Beckett and van Staden, 1989; Szczepanek and Grzybowski, 2016; Zodape *et al*., 2009).

Fresh root weight in 2010 was higher by 24.3% as compared with this trait in 2011 (Table 1). In 2010 and 2011, after the application of Kelpak, and after its combined application with Lithovit, the fresh root weight was not significantly different to that found in the control. On average from the two years, only in the case of application of the preparation Kelpak alone at the rate of 2 $dm^3 \cdot ha^{-1}$ was the fresh weight of barley roots higher as compared with the control. In the study by Steveni *et al*. (1992) barley in hydroponic cultivation responded with an increase in root weight to the extract from the marine macro-alga *Ascophyllum nodosum*. The stimulation of root growth could be affected by auxins contained in the preparation Kelpak (Tarakhovskaya *et al*., 2007).

Grain yield of the spring barley in 2011 was lower by 46.3%, as compared with the yield obtained in 2010 (Table 2). The increased yield in 2010 may be explained by the favourable weather conditions during the intensive growth phase of barley (Fig. 1). The number of grains per spike in that year was 1.46 times higher than in 2011 (Table 1). In 2010, after the application of the biostimulant Kelpak alone in a dose of 2 $dm^3 \cdot ha^{-1}$the grain yield was significantly higher than after its application in a dose of 1.5 $dm^3 \cdot ha^{-1}$with the preparation Lithovit in a dose of 1.5 $kg \cdot ha^{-1}$. In the study by Matysiak and Adamczewski (2006), the application of a higher dose of the biostimulant Kelpak also gave better results. In the study by these authors, Kelpak was applied at the barley shooting stage, and the increase in yield as compared with the control treatment amounted to 8% at a dose of 1.5 $dm^3 \cdot ha^{-1}$ and 11% at a dose of 2 $dm^3 \cdot ha^{-1}$. In the present study, in 2010, as well as on average over the 2 year period, the grain yield under both variants of biostimulant applications was higher than in the control treatment. Similar results were obtained in a study concerning spring wheat (Szczepanek and Grzybowski, 2016). The plants treated with the algae biostimulant Kelpak in a dose of 2 $dm^3 \cdot ha^{-1}$. Zodape *et al*. (2009) explained the increase in wheat grain yield after the application of algae extract as mostly connected with an increase in weight of the plant root system.

In the present study, in 2010 the straw weight, like the grain yield, was higher (by 27.8%) as compared with 2011 (Table 2). On average in the two years of the study, straw yield was the highest after the combined application of the biostimulant Kelpak with the preparation Lithovit. Studies of wheat have indicated that plant growth stimulation in the form of an increase in the number of shoots per plant and their height appears after the application of algae extracts (Zodape *et al*., 2009; Shah *et al*., 2013). In 2011, as well as on average from the two years of the study, after the application of the biostimulant Kelpak alone the harvest index was higher than in the control treatment (Table 2). This indicates a favourable distribution of assimilates, favouring production of the main crop mass (grain) at the expense of the additional crop mass (straw).

Table 2. Effect of biostimulant application on grain yield, straw weight and harvest index of spring barley

Year	Treatment			Mean	$LSD_{\alpha=0.05}$
	Control	Kelpak 2 $dm^3 \cdot ha^{-1}$	Kelpak 1.5 $dm^3 \cdot ha^{-1}$ + Lithovit 1.5 $kg \cdot ha^{-1}$		
Grain yield, $Mg \cdot ha^{-1}$					
2010	6.25	6.55	6.45	6.42	0.088
2011	3.25	3.67	3.42	3.45	0.279
Mean	4.74	5.11	4.94	4.93	0.047
Straw weight, $Mg \cdot ha^{-1}$					
2010	6.19	6.47	6.37	6.34	ns
2011	4.95	4.64	5.28	4.96	ns
Mean	5.57	5.55	5.83	5.65	0.244
Harvest index, %					
2010	50.2	50.3	50.3	50.3	ns
2011	39.7	44.3	39.4	41.1	4.20
Mean	45.0	47.3	44.8	45.7	1.05

In 2010, after the combined application of biostimulants Kelpak and Lithovit total protein content in the spring barley grain was higher than in the control treatment (Table 3). Contents of crude fibre, on average from the two years of the study after the application of the biostimulant Kelpak alone or in a mixture with the preparation Lithovit was significantly lower as compared with the control treatment.

In the present study, the mean accumulation of N in the grain of spring barley in 2010 was higher (by 43.3%), as compared with 2011 (Table 4). In 2010, after the combined application of the biostimulant Kelpak and Lithovit the accumulation of N was higher than in the control treatment. In 2011 no statistical effect of preparations on the trait in question was found. On average from the two years of the study, N accumulation in the treatment with the biostimulant from algae, as well as after the application of both preparations (Kelpak + Lithovit) was higher than in the control treatment. The mean accumulation of P in spring barley grain, like for N in 2010, was higher (by 37.2%) as compared with 2011. In 2011, as well as on average from the two years, only after treatment with the biostimulant Kelpak in a dose of 2 $dm^3 \cdot ha^{-1}$ was the accumulation of P significantly higher than in the control treatment. The mean accumulation of K, was higher (by 40.8%) in 2011 as compared with 2010. On average from the two years of the study, K accumulation after treatment with the biostimulant Kelpak was higher than in the control treatment. Mg accumulation in 2011 was lower by 26.4%, as compared with 2010. In 2010 and 2011, after the application of the biostimulant Kelpak, Mg accumulation was significantly higher than in the control treatment. On average from the two years of the study, Mg accumulation in the treatment with the biostimulant Kelpak alone was significantly higher than that found in the treatment where Kelpak + Lithovit were applied, and that, in turn, was higher than in the control treatment. The study concerning the response of wheat to the application of biostimulants from algae by Shah *et al*. (2013) indicated an increase in the uptake of K and N in some variants of application and mainly a lack of effect in the uptake of P. However, Zodape *et al*. (2009) recorded an increase in the accumulation of N, K and P after application of a seaweed biostimulant, particularly at the highest dose.

Table 3. Effect of biostimulant application on total protein content and crude fibre content in grain of spring barley

Year	Treatment			Mean	$LSD_{\alpha=0.05}$
	Control	Kelpak 2 $dm^3 \cdot ha^{-1}$	Kelpak 1.5 $dm^3 \cdot ha^{-1}$ + Lithovit 1.5 $kg \cdot ha^{-1}$		
Total protein content, %					
2010	8.63	8.88	9.75	9.06	0.888
2011	11.8	11.6	12.1	11.8	ns
Mean	10.2	10.3	10.9	10.4	0.413
Crude fibre content, %					
2010	5.01	4.51	4.57	4.70	ns
2011	3.96	3.82	3.72	3.83	ns
Mean	4.49	4.17	4.14	4.26	0.265

ns – non-significant differences

Table 4. Effect of biostimulant application on accumulation of macroelements in grain of spring barley, kg·ha^{-1}

Year	Treatment			Mean	$LSD_{\alpha=0.05}$
	Control	Kelpak 2 dm^3·ha^{-1}	Kelpak 1.5 dm^3·ha^{-1} + Lithovit 1.5 kg·ha^{-1}		
			N		
2010	74.1	80.1	86.2	80.1	8.12
2011	52.5	58.8	56.6	55.9	ns
Mean	63.3	69.4	71.4	68.0	3.93
			P		
2010	14.8	16.6	15.0	15.5	ns
2011	10.5	12.7	10.9	11.3	1.36
Mean	12.6	14.6	12.9	13.4	1.185
			K		
2010	10.0	10.3	10.6	10.3	ns
2011	13.9	15.5	14.3	14.5	ns
Mean	12.0	12.9	12.4	12.4	0.863
			Mg		
2010	7.03	7.66	7.47	7.39	0.45
2011	5.09	5.76	5.47	5.44	0.625
Mean	6.06	6.71	6.47	6.42	0.218

ns – non-significant differences

CONCLUSIONS

1. Foliar application of the biostimulant Kelpak in a dose of 2 dm^3·ha^{-1} or its combined used with the preparation Lithovit in a doses of 2 dm^3·ha^{-1} and 1.5 kg·ha^{-1}, respectively at the tillering stage of spring barley had a favourable effect on the quantity of grain yield.
2. Application of the biostimulant from algae caused an increase in the fresh root weight of spring barley, and the combined use of the preparations Kelpak and Lithovit had a favourable effect on straw weight. In both treatments, the thousand grain weight was higher than in the control treatment.
3. The application of the biostimulant Kelpak caused an increase in the accumulation of N, P, K and Mg in barley grain. The combined application of preparations Kelpak and Lithovit caused an increase in protein concentration and in the accumulation of N and Mg in grain.

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EFEKT APLIKACJI BIOSTYMULATORÓW W UPRAWIE JĘCZMIENIA JAREGO

Streszczenie

Biostymulatory wspomagają wzrost i rozwój roślin, powodują zwiększenie odporności na stres i mogą mieć korzystny wpływ na plonowanie roślin rolniczych jak i warzywnych. Celem pracy była ocena wpływu biostymlatorów na komponenty plonowania, plon ziarna i słomy, masę korzeni oraz akumulację NPK i Mg w ziarnie jęczmienia jarego. Ścisłe doświadczenie polowe prowadzono w latach 2010-2011, w Stacji Badawczej Wydziału Rolnictwa i Biotechnologii w Mochełku (53°13'N; 17°51'E). W doświadczeniu jednoczynnikowym z jęczmieniem jarym odmiany Nuevo badano wpływ biostymulatora Kelpak (w dawce 2 $dm^3 \cdot ha^{-1}$) lub łącznej jego aplikacji z preparatem Lithovit (odpowiednio w dawkach 1.5 dm^3 + 1.5 $kg \cdot ha^{-1}$) na cechy biometryczne roślin i plon ziarna. Nalistna aplikacja biostymulatora Kelpak w dawce 2 $dm^3 \cdot ha^{-1}$ w fazie krzewienia (fazie 4-5 liści) lub łączne zastosowanie preparatów Kelpak 1.5 $dm^3 \cdot ha^{-1}$ i Lithovit 1.5 $kg \cdot ha^{-1}$ miało korzystny wpływ na plon ziarna. Aplikacja biostymulatora Kelpak w dawce 2 $dm^3 \cdot ha^{-1}$

spowodowała zwiększenie świeżej masy korzeni jęczmienia jarego, a łączne zastosowanie obu preparatów miało korzystny wpływ na masę słomy. W obu wariantach aplikacji masa tysiąca nasion była większa niż w obiekcie kontrolnym. Największą koncentracją białka charakteryzowało się ziarno jęczmienia po łącznym stosowaniu obu preparatów. Zastosowanie biostymulatora Kelpak spowodowało zwiększenie akumulacji N, P, K i Mg w ziarnie jęczmienia. Łączna aplikacja preparatów Kelpak i Lithovit spowodowała zwiększenie akumulacji N i Mg w ziarnie. Korzystny wpływ biostymulatora Kelpak w dawce 2 $dm^3 \cdot ha^{-1}$ lub łącznej aplikacji preparatów Kelpak 1.5 $dm^3 \cdot ha^{-1}$ i Lithovit 1.5 $kg \cdot ha^{-1}$ w fazie krzewienia (4-5 liści) uzasadnia stosowanie tych preparatów w uprawie jęczmienia jarego.

Słowa kluczowe: akumulacja makroskładników, białko ogółem, masa korzeni, masa słomy, plon ziarna, włókno surowe