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EFFECT OF THE EXTRACT FROM *ECKLONIA MAXIMA* ON SELECTED MICRO-AND MACROELEMENTS IN AERIAL BIOMASS OF HYBRID ALFALFA

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Abstract

In order to determine the effect of a phytohormonal biostimulator on the evolution of morphological traits of hybrid alfalfa, a field experiment consisting of 6 m^2 plots cropped with this plant was set up in 2009. Each experimental variant was carried out in a triplicate. The experiment was run for three years (2009-2011). The experimental factor was an extract from *Ecklonia maxima*, sold under the commercial name of Kelpak SL. This product contains 11 mg dm^{-3} of auxin and 0.03 mg dm^{-3} of cytokines. In the experiment, it was used on all re-growths as a spray in a dose of 2 dm^{-3} ha^{-1} of the formula diluted in 350 dm^3 of water. The analyzed factors were the concentrations of macroelements: phosphorus, potassium, calcium, magnesium (in g kg^{-1} d.m.), and trace elements: manganese, zinc, copper, molybdenum (in mg kg^{-1} d.m.) in the aerial biomass of alfalfa plants. The ratios of Ca: P and K: (Ca + Mg) were also calculated. The results were analyzed statistically using the analysis of variance and the mean values were compared with the Tukey's test. The application of the seaweed extract led to an increase in phosphorus, potassium, zinc and manganese in alfalfa aerial biomass. The content of magnesium, calcium, copper and molybdenum did not change significantly in response to the analyzed extract. The modifications in the content of macroelements led to a decrease in the calcium to phosphorus ratio and an increase in the potassium-calcium ratio.

Keywords: seaweed extract, microelements, macroelements, macroelemental ratio, aerial biomass, alfalfa.

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WPŁYW EKSTRAKTU Z *ECKLONIA MAXIMA* NA ZAWARTOŚĆ WYBRANYCH MIKRO- I MAKROELEMENTÓW W BIOMASIE NADZIEMNEJ LUCERNY MIESZAŃCOWEJ

Abstrakt

W celu określenia wpływu biostymulatora fitohormonalnego na kształtowanie się cech morfologicznych lucerny mieszańcowej, założono doświadczenie polowe w 2009 roku. Powierzchnia poletka wynosiła 6 m^2. Każdą kombinację doświadczalną wykonano w trzech powtórzeniach. Doświadczenie użytkowano przez trzy lata (2009-2011). Czynnikiem doświadczenia był ekstrakt z *Ecklonia maxima* występujący pod nazwą Klepak SL. Preparat zawierał 11 mg dm^{-3} auksyn i 0,03 mg dm^{-3} cytokinin. Był on stosowany w na wszystkie odrosty w formie oprysku w dawce 2 dm^{-3} ha^{-1} preparatu rozcieńczonego w 350 dm^3 wody. Badane cechy to: zawartość makroelementów – fosforu, potasu, wapnia, magnezu (g kg^{-1} s.m.) i mikroelementów – manganu, cynku, miedzi, molibdenu (mg kg^{-1} s.m.) w biomasie nadziemnej roślinnej lucerny. Wyliczono również stosunek Ca:P oraz K:(Ca+Mg). Wyniki opracowano statystycznie, stosując analizę wariancji oraz $NIR_{0,05}$ wg testu Tukeya. Oprysk ekstraktem z wodorostów wpłynął na wzrost zawartości fosforu, potasu, cynku i manganu w biomasie nadziemnej lucerny. Zawartość wapnia, magnezu, miedzi i molibdenu nie zmieniła się znacząco pod wpływem czynnika badawczego. W konsekwencji zróżnicowania zawartości makroelementów pod wpływem ekstraktu nastąpił spadek wartości stosunku wapnia do fosforu i wzrost stosunku potasu do wapnia i magnezu.

Słowa kluczowe: ekstrakt z wodorostów, mikroelementy, makroelementy, stosunek makroelementów, biomasa nadziemna, lucerna.

INTRODUCTION

According to Bai et al. (2007) and Abou El-Yazied et al. (2012), seaweeds are one of the most important marine resources of the world. They are used as human food, animal feed and raw materials in many industries. They are also used as fertilizers for agricultural and horticultural crops (Temple et al. 1988, Beckett et al. 1994, Ouedraogo et al. 2001, Bai et al. 2007, Zodapea et al. 2009, Nour et al. 2010, Zodape et al. 2010, Abou El-Yazied et al. 2012). Better production results were obtained in production of agricultural crops after applying these extracts than mineral fertilizers (Aitken, Senn 1965). Owing to the presence of minerals and hormonal substances (Moller, Smith 1998, 1999), they cause an increase in plant resistance to stress and disease (Verkleij 1992). The chemical composition of extracts from seaweeds is complex and largely dependent on the method of seaweed harvest. According to Bai et al. (2007), the industrial production of extracts relies on the following species: *Enteromorpha intestine*, *Cladophora dalmatica*, *Ulva lactuca*, *Corralina mediterranea*, *Ascophyllum nodosum* and *Ecklonia maxima*. Studies on the use of extracts from seaweeds have been carried out since 1965 (Aitken, Senn 1965). Today, these preparations are seen as a new generation, natural organic fertilizer (Zodape et al. 2010, Abou El-Yazied et al. 2012). Plant protection based on extracts from seaweeds is gaining popularity, which has

stimulated the development of a large number of products now available on the European and American markets (Singh, Chandela 2005, Thirumaran et al. 2009, Nour et al. 2010, Sridhar, Rengasamy 2010, Zodape et al. 2010, Abou El-Yazied et al. 2012). It has been proven that plants treated with seaweed extract are more resistant to drought, soil deficiency of nutrients and salinity. In addition, it effectively reduces the stress associated with plant pruning. The beneficial effect of biostimulator phytohormones is primarily reflected by increased yields (Temple, Bomke 1989, Liu 1991, Verkleij 1992, Mostafa, Zheekh 1999, Zodape 2001, Matysiak, Adamczewski 2006, Matysiak et al. 2012). The application of these products also affects the content of micro- and macronutrients in plant material (Rathore et al. 2009, Zodapea et al. 2009, Abou El-Yazied et al. 2012). In literature, the effect of extracts on cereals and vegetables has already been presented but there are no studies on the application of such formulas on hybrid alfalfa.

This study was undertaken to determine the effect of extract from *Ecklonia maxima* on the content of selected micro- and macroelements in aerial biomass of hybrid alfalfa. The experiment was to elucidate whether an annual application of seaweed extract on alfalfa for three years would affect the content of P, K, Mg, Ca, Mn, Zn, Cu and Mo in the plant material.

MATERIAL AND METHODS

In 2009, a field experiment with hybrid alfalfa (variety Tula) was set up on an experimental field of the Department of Meadow Science and Grassland Management. The soil belonged to the type Anthrosols, and sub-type Hortisole type, developed formed from weak loamy sand. A soil analysis, performed at the Regional Chemistry Station in Wesoła, showed that the soil was neutral (pH KCl = 7.2), very rich in humus (3.78%), and contained the following amount of available phosphorus (P_20_5) 900 mg kg^{-1} and magnesium (Mg) 84 mg kg^{-1}, while the average amount of total nitrogen N was 1.8 g kg^{-1} and absorbable potassium (K_20) 190 mg kg^{-1}. The plot size was 6 m^2.

Before sowing and in the second and third year of crop cultivation, fertilization was applied introducing to soil 45 kg P ha^{-1} and 100 kg K ha^{-1}. In April 2009, alfalfa seeds were sown in the amount of 12 kg ha^{-1} (600 units m^{-2} assuming 100% germination), at a depth of about 1 cm. During the shoot formation stage, the biostimulator Kelpak SL, containing natural plant hormones such as auxin (11 mg dm^{-3}) and cytokines (0.03 mg dm^{-3}), was applied. Kelpak Sl is made from the brown alage *Ecklonia maxima* (Temple et al. 1988). The formula was applied sprayed on all re-growths. The experimental variants were as follows: A1 – control (no extract), A2 – extract applied in a dose of 2 dm^{-3} ha^{-1} of the formula diluted in 350 dm^3 of water. Each treatment was carried out in a triplicate. During the whole experiment (2009-2011), three cuts of alfalfa were harvested in the early flowering stage.

The content of macroelements in the biomass was determined in all the cuts over the three years. The following analytical methods were used: P – flow spectroscopy, K – emissions by flame spectroscopy, Ca and Mg – atomic absorption spectroscopy. Furthermore, based on the the content of macroelements, the following ratios were calculated: Ca: P and K: (Ca + Mg). Additionally, Mn, Zn, Cu and Mo were assayed via atomic absorption spectrometry.

The results were evaluated statistically with analysis of variance for multivariate experiments. Differences between mean values were verified with the Tukey's test at $p \leq 0.05$.

The meteorological data documenting the research period were obtained from the Hydrological and Meteorological Station in Siedlce. In addition, for determination of the temporal variability of meteorological elements and their influence on plant growth, the Sielianinov's hydrothermic rate was calculated.

The data presented in Table 1 show that the most favorable rainfall distribution, with optimum air temperatures, occurred in 2009 and 2011. There were no months with drought and severe drought in none of these years.

Table 1

Values of Sielianinov's hydrothermical index of (K) in individual months of vegetation

Year	Month						
	Apr	May	June	July	Aug	Sept	Oct
2009	1.03	2.24	1.03	1.26	1.36	1.01	1.73
2010	0.40	2.21	1.19	1.18	1.79	2.81	0.53
2011	1.10	0.89	0.72	2.19	0.84	0.78	0.94

$K < 0.5$ – severe drougth; 0.51-0.69 – drought; 0.70-0.99 – weak drought; K > 1 – no drought

RESULTS AND DISCUSSION

Application of the extract from *Ecklonia maxima* in the cultivation of hybrid alfalfa resulted significantly increased the phosphorus content (by over 10%) and potassium (by over 15%) – Table 2. The statistical analysis showed that the content of these elements also depended on the year. Regardless of the experimental factor, phosphorus was the lowest in the second year of cultivation (2.8 g kg^{-1} d.m.) and the highest one in the first year (3.1 g kg^{-1} d.m.). However, the potassium content increased with plant age. It is noteworthy that the annual application of the extract did not influence significantly the content of magnesium or calcium in the tested plant material. This is supported by results reported by Abou El-Yazied et al. (2012),

Table 2

Effect of the extract from *Ecklonia maxima* on the content of selected macroelements (g kg^{-1} d.m.) in aerial biomass of hybrid alfalfa in each year (means for cuts)

Macroelements	Years	Extract		Mean
		A1 (control)	A2 (factor)	
P	2009	2.70 *Bb*	3.40 *Aa*	3.10 *A*
	2010	2.70 *Bb*	2.90 *Ba*	2.80 *B*
	2011	3.00 *Aa*	3.00 *Ba*	3.00 *AB*
	mean	2.80 *b*	3.10 *a*	
K	2009	13.4 *Bb*	16.5 *Ba*	15.0 *B*
	2010	17.4 *Ab*	18.6 *Aa*	18.0 *A*
	2011	16.3 *Ab*	19.5 *Aa*	17.9 *A*
	mean	15.7 *b*	18.2 *a*	
Mg	2009	2.80 *Aa*	3.00 *Aa*	2.90 *A*
	2010	2.70 *Aa*	2.70 *Aa*	2.70 *A*
	2011	2.60 *Aa*	2.60 *Aa*	2.60 *A*
	mean	2.70 *a*	2.80 *a*	
Ca	2009	14.3 *Aa*	14.2 *Aa*	14.3 *A*
	2010	14.7 *Aa*	14.8 *Aa*	14.8 *A*
	2011	14.8 *Aa*	14.2 *Aa*	14.5 *A*
	mean	14.6 *a*	14.4 *a*	

mean values marked with the same small letter do not differ significantly
mean values marked with the same capital letter do not differ significantly

but does not correspond to the results obtained by Zodapea et al. (2009), who found a significant increase in the content of these macronutrients in cereal grains.

Many authors (Featonby-Smith, Van Staden, 1983, Verkleij 1992, Galbiattia et al. 2007) have shown beneficial effects of seaweed extracts as natural regulators, which increased yield, improved plant vigor and the strengthened the ability to resist unfavourable environmental conditions. Application of the extract as an organic biostimulator has quickly become common practice in horticulture because of the beneficial production effects (Verkleij 1992, Crouch, Van Staden 1993). According to Sanderson and Jameson (1986) or Stirk and Van Staden (1997), the main ingredients of extracts affecting the plants are cytokinins and auxins, which are found in the composition of most seaweed concentrates.

These hormones induce many processes connected with cytological and histological aspects of plants and have an influence on the content of some macronutrients (Wierzbowska, Bowszys 2008). The research of Abou El-Yazied et al. (2012) on effects of seaweed extracts on the quality of beans showed that their application during two growing seasons resulted in higher phosphorus and potassium concentrations in leaves against the control.

The same trend is observed in the magnesium content. Similar results are presented by Pise and Sabale (2010). An increase in the phosphorus

and potassium content in plant material after spray application of seaweed extract was found by Shehata et al. (2011). Also Nour et al. (2010), who studied the effect of seaweed extract spray on the chemical composition of tomato, showed that most K and P occurred in vegetables grown on the treated plots.

Studies on soybean (Rathore et al. 2009) regarding the response of this plant to different concentrations of seaweed extracts showed a significant increase in the P and K content after extract application, regardless of the concentration. Zodapea et al. (2009), who used 1% spray from an extract of *Kappaphycus alvarezii* on *Triticum aestivum,* found the grains to contain higher concentrations of K (over 15% increase), P (18%), ca (45%) and Mg (28%). Higher levels of macronutrients in plants after application of extracts were also reported by Beckett et al. (1994) and zahid (1999).

The references on the nutritional value of feeds emphasize the importance of quantitative ratios between minerals as a quality parameter (Staniak 2004, Jankowska-Huflejt, Wróbel 2008, Nowak et al. 2008). According to Staniak (2004) or Wierzbowska and Bowszys (2008), it is important to determine the ratios of Ca:P and K:(Ca+Mg). Herein, the application of *Ecklonia maxima* extract significantly narrowed the calcium to phosphorus ratio from 5.22 to 4.67 (Table 3), regardless of the research year. However, it was still above the norm, which should be from 1.8 to 2.1 in feed for ruminants, as given by Jankowska-Huflejt and Wróbel (2008).

Table 3

Macronutrient ratios in aerial biomass of hybrids alfalfa depending on the extract of *Ecklonia maxima* and the growing year (mean for cut)

Ratio	Years	Extract		Mean
		A1 (control)	A1 (control)	
Ca : P	2009	5.30 *Aa*	4.18 *Ba*	4.74 *B*
	2010	5.44 *Aa*	5.10 *Ab*	5.27 *A*
	2011	4.93 *Ba*	4.73 *Aa*	4.84 *B*
	mean	5.22 *a*	4.67 *b*	-
K : (Ca + Mg)	2009	0.78 *Bb*	0.97 *Ca*	0.87 *B*
	2010	1.00 *Aa*	1.05 *Ba*	1.03 *A*
	2011	0.94 *Ab*	1.16 *Aa*	1.05 *A*
	mean	0.91 *b*	1.06 *a*	-

mean values marked with the same small letter do not differ significantly
mean values marked with the same capital letter do not differ significantly

The biomass examined in the experiment had a very high content of potassium in relation to animal nutrition standards (Jankowska-Huflejt, Wróbel 2008). It largely influenced the K: (Ca+Mg) ratio, which reached 0.91 in the plant material collected from the control crops. Moreover, spraying alfalfa with the extract caused a statistically significant increase

by over 16%. In the subsequent years an increase in potassium content (Table 2) was observed, which further increased the K:(Ca+Mg) ratio to a value above 1 (Table 3).

Spraying hybrid alfalfa plants with the seaweed extract has led to a significant increase in the content of Mn and Zn in aerial plant biomass (Table 4).

Table 4

Effect of the *Ecklonia maxima* extract on the content of selected microelements (mg kg^{-1} d.m.) in aerial biomass of hybrid alfalfa in each year (means for cuts)

Microelements	Years	Extract		Mean
		A1 (control)	A2 (factor)	
Mn	2009	50.1 *Aa*	51.2 *Aa*	50.7 *A*
	2010	49.7 *AB*a	50.8 *Aa*	50.3 *A*
	2011	45.5 *Bb*	53.1 *Aa*	49.3 *A*
	mean	47.8 *b*	51.7 *a*	
Zn	2009	22.6 *Ab*	28.0 *Aa*	25.3 *A*
	2010	24.1 *Aa*	26.3 *Aa*	25.2 *A*
	2011	24.4 *A*	28.1 *Aab*	26.3 *A*
	mean	23.7 *b*	27.5 *a*	
Cu	2009	7.00 *Aa*	6.95 *Aa*	6.98 *A*
	2010	6.01 *Ba*	5.79 *Ba*	5.90 *B*
	2011	6.16 *Ba*	5.83 *ABa*	6.00 *AB*
	mean	6.39 *a*	6.19 *a*	
Mo	2009	0.51 *Aa*	0.48 *Aa*	0.50 *A*
	2010	0.47 *Ab*	0.56 *Aa*	0.52 *A*
	2011	0.49 *Aa*	0.52 *Aa*	0.51 *A*
	mean	0.49 *a*	0.52 *a*	

mean values marked with the same small letter do not differ significantly
mean values marked with the same capital letter do not differ significantly

The manganese content increased from 47.5 on control plots to 53.1 mg kg^{-1} on the treatment with the formula, which mean an over 7.5% increase. For zinc, an analogous increase was as high as over 13%. Regardless of the years the statistical evaluation did not find significant differentiation among the mean values after the application of the seaweed extract with respect to the content of copper and molybdenum. In addition, only one of the analyzed micronutrients, namely copper, was significantly differentiated in the biomass of alfalfa. The highest amount of this element was observed in the material collected in the first year. Higher content of some microelements was also found in the study of Zodape et al. (2009). According to these authors, application of the extract from *Kappaphycus alverezii* on cereals, regardless of the preparation concentration, resulted in an increase of zinc by 4.9%, and manganese by 9.42%, as well as molybdenum by16% in wheat grain. However, in their experiment, the copper content did not change under the influence of the extract.

CONCLUSIONS

1. Application of an extract from *Ecklonia maxima* in the cultivation of hybrid alfalfa resulted in a statistically significant increase in the content of phosphorus and potassium in aerial parts of this plant.

2. The content of calcium and magnesium in the biomass of alfalfa did not undergo significant differentiation under the influence of the seaweed extract during the research years.

3. Both the Ca : P and K : (Ca + Mg) ratios changed significantly as a result of the extract application. The calcium to phosphorus ratio was reduced by 10%, while that of potassium to calcium and magnesium increased by 16%.

4. Spraying with the seaweed extract resulted in an increase in the content of manganese and zinc in dry matter of alfalfa. Copper and molybdenum did not change significantly in repsponse to the extract.

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