

# Effect of *Ecklonia maxima* extract on photosynthesis activity and chlorophyll content of *Medicago* ×*varia* Martyn leaves

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Received: 8 October 2018; Accepted: 6 February 2019; doi:10.4067/S0718-58392019000200257

# ABSTRACT

Recently, there is a big interest in science to use seaweed extract in agricultural practice. Therefore, the aim of this paper was to determine the effect of foliar application of *Ecklonia maxima* extract on photosynthetic activity and chlorophyll content of *Medicago* ×*varia* Martyn leaves. The experimental factor was a commercial product containing *E. maxima* extract (Em). The following parameters were determined: maximum photosystem II efficiency ( $F_v/F_m$ ) in the dark-adapted state, actual photosystem II efficiency in the light-adapted state ( $\Delta F/F_m$ ), the photochemical quenching coefficient ( $q_N$ ), chlorophyll content determination. The Fisher-Snedecor test was used to determine whether the impact of experimental factor was significant, while the value of the HSD<sub>0.05</sub> was calculated with Tukey's test. As a reaction to the particular conditions during the experiment alfalfa treated with Em had a better efficiency of the photosynthetic apparatus in relation to the control to 0.635. The value of the actual efficiency of photosystem II ( $\Delta F/F_m$ ) determined under the same conditions turned out to be about 20% higher for plants treated with the extract. Chlorophyll a and b concentration, as a response to the extract, increased by 12% and 16.7%, respectively. The experiment showed that periods of rainfall shortages did not reverse the effect of the extract on plants, with an increase in chlorophyll a and b content during the growing seasons with dry and extremely dry months.

Key words: Chlorophyll fluorescence, *Ecklonia maxima*, marine algae extract, photochemical quenching coefficient, seaweed.

# INTRODUCTION

In available literature there have often been opposing accounts concerning foliar and soil application of seaweed extracts. Some authors (Koleska et al., 2017) have suggested that those extracts do not have any effect on plant growth. On the other hand, some well-documented studies on commercial use of *Ecklonia maxima* (Digruber et al., 2018) have claimed that its extracts enhance growth and yields of crops, prevent pests infestation, and improve the overall quality of plant products. These positive effects have been assigned mainly to the fact that such extracts contain plant hormones. Due to the content of hormones from auxin and cytokinin groups, those extracts, among other things, affect the regulation of several physiological processes (Mattner et al., 2013; Digruber et al., 2018). They are used in small doses because their active compounds are effective in low concentration. In the present experiment a product containing *E. maxima* (Osbeck) Papenfuss extract under the trade name of Kelpak was used. It is produced by Kelp Products (Pty) Ltd. in the South African city of Simons (Digruber et al., 2018). A number of studies (Sosnowski et al., 2014; 2016a; 2016b; 2017) have indicated that plants treated with Kelpak respond with an increased yield and higher concentration of organic compounds (protein, fibre, amino acids) and minerals in DM. In addition, this type of growth regulators contribute to the limiting

of plant stress, at the same time improving the intensity of physiological processes, including photosynthesis, and, as a consequence, yields and quality of crops improve too (Rathore et al., 2009; Sosnowski et al., 2017).

Photosynthesis, the intensity of which depends on very complex internal and external conditions, belongs to basic physiological processes in plants (Ilieva and Vasileva, 2013; Kocira et al., 2015; Zhao et al., 2016; Li et al., 2017; Michalek et al., 2018). A new method for its evaluation is chlorophyll fluorescence (CF) analysis, which, to a large extent, replaces conventional measurements of photosynthesis intensity. Chlorophyll fluorescence analysis is a highly sensitive attempt to measure photosynthetic efficiency of plants. It is especially useful in situations when the impact of environmental factors on plants is multifaceted, and, being completely non-invasive, it allows studying photosynthesis in vivo. In plant biomass production, the amount of photosynthetically active radiation (PAR) absorbed by plants and its conversion into DM are key factors. Usually, about 70%-80% of such radiation is absorbed by leaves, while the rest passes through them or is reflected. Energy absorbed this way can be used to convert  $CO_2$  and water into glucose, or is lost as fluorescent radiation. There is a close relationship between the amount of fluorescence and the intensity of photosynthesis, and CF can be a measure of the state and efficiency of plant photosynthetic apparatus (Nishiyama et al., 2006; Yokoya et al., 2007; Przybysz et al., 2014; Pokluda et al., 2016; Zhao et al., 2016; Li et al., 2017).

The aim of this paper was to determine the effect of foliar application of *Ecklonia maxima* extract on photosynthetic activity and chlorophyll content of *Medicago ×varia* leaves. The study was based in a 3 yr experiment to study the effect of two doses of the product on chlorophyll a and b content and on photosynthetic activity defined by measurements of chlorophyll fluorescence induction in consecutive harvests.

## **MATERIALS AND METHODS**

#### **Experimental conditions**

The research was based on a field experiment carried out at the experimental facilities of the University of Natural Sciences and Humanities in Siedlce ( $52^{\circ}10'03''$  N;  $22^{\circ}17'24''$  E), Poland, between 2014 and 2016. In April 2014, alfalfa (*Medicago ×varia* Martyn) seeds 'Comet' were planted on 6 m<sup>2</sup> plots at a depth of about 1 cm and at a seeding dose of 12 kg ha<sup>-1</sup> (600 plants m<sup>-2</sup>, assuming 100% germination). Made up of loamy sand the soil was of the anthropogenic order, culture earth type, and the subtype of hortisole, according to the Polish classification system (Technosols according to the FAO). The C content (C<sub>org</sub>) in soil organic compounds was 13.50 g kg<sup>-1</sup> DM, with total N of 1.30 g kg<sup>-1</sup> DM, and the C:N ratio 10.4:1. The pH of 6.8 was close to neutral. In addition, the soil had high content of absorbable forms of P and Mg, but absorbable forms of K were within the limits of the medium amount. Due to the high content of nutrients, mineral fertiliser was not used either before or during the growing season. The experiment was carried out in a split-plot design with three replicates. Plants samples were collected between 2014 and 2016. The experimental factor was a commercial product (Kelpak SL; Kelp Products [Pty] Ltd., Simons, South Africa) containing *E. maxima* extract. The chemical composition of the preparation according to the producer is presented in Table 1.

This product was applied as a spray to alfalfa plots twice during each growing cycle of alfalfa; the first treatment after the development of the first internode, the other when the first flower buds were visible outside leaves. For the first

Organic matter	Content	Phytohormones	Content
	%		mg L-1
Carbohydrates	31	Auxins	11
Alginic acid	13	Cytokinins	0.03
Total amino acid	7		
Manitol	4		
Macroelements			Microelements
N	4.10	Fe	140
$P_2O_5$	2.10	Zn	70
$P_2O_5$ $K_2O$	2.10 5.01	Zn Mn	70 12
P <sub>2</sub> O <sub>5</sub> K <sub>2</sub> O Ca	2.10 5.01 0.24	Zn Mn B	70 12 55
P <sub>2</sub> O <sub>5</sub> K <sub>2</sub> O Ca S	2.10 5.01 0.24 3.51	Zn Mn B I	70 12 55 28

Table 1.	Chemical	and bioc	hemical	composition	of Eckla	onia i	maxima	extract.
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application 2 L ha<sup>-1</sup> *E. maxima* extract dissolved in 350 L water was used. Treatments were control (C; sprayed with water) and *E. maxima* extract (Em).

Alfalfa was harvested three times each growing season, when 40%-50% of inflorescences blossomed: H1 (harvest 1) carried out in spring in the first half of June; H2 (harvest 2) carried out in summer in the second half of August; and H3 (harvest 3) carried out in autumn in the first half of October. Tillage was done maintaining good agricultural practices. Pest infestation was nonsignificant, and therefore pesticides were not used, with manual weeding done to remove unwanted plants.

### **Chlorophyll content determination**

Chlorophyll a and b content was determined with the Khaleghi et al. (2012). Plant material was collected from each plant at the full flowering stage (40%-50% flowers open). As for the photosynthetic pigments, the optical density of supernatants was determined with the spectrophotometer (Bio-RAD SmartSpec Plus, Hercules, California, USA) at 440, 465, and 663 nm. Next, the results (w/v) were calculated according to the following formulas:

Chlorophyll a content = [12.7(E663) - 2.69(E645)]Chlorophyll b content = [22.9(E645) - 4.68(E663)]Chlorophyll a + b content = [20.2(E645) - 8.02(E663)]

where *E* is extinction at a particular wavelength; *v* is amount of 80% acetone (cm<sup>3</sup>) used for extraction; *w* is sample weight (g).

## Photosynthetic activity determination

The photosynthetic activity of alfalfa leaves was measured during each regrowth. On the  $7^{\text{th}}$  day after the second application of *E. maxima* extract, 10 randomly selected leaves from each plot were used for the determination.

Photosynthetic activity of plants was determined by the measurement of chlorophyll fluorescence induction using a fluorometer (PAM 2000, Heinz Walz GmbH, Effeltrich, Germany). The following parameters were determined: Maximum photosystem II efficiency ( $F_v/F_m$ ) in the dark-adapted state (Khaleghi et al., 2012); actual photosystem II efficiency in the light-adapted state ( $\Delta F/F_m$ ); photochemical quenching coefficient ( $q_P$ ); and non-photochemical quenching coefficient ( $q_N$ ) (Khaleghi et al., 2012).

All measurements were recorded during the growing season, using well developed alfalfa leaves in six replicates. By taking the measurements a 2030-B clip holder (also Hainz Walz), a light emitting diode at 650 nm, and standard intensity of 0.15  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> photosynthetically active radiation (PAR) were used. In the dark-adapted state leaves were kept in darkness for 15 min.

#### Weather conditions

Sielianinov's hydrothermal coefficient was calculated in order to determine temporal variation of meteorological conditions and their effects on vegetation. The hydrothermal coefficient (K) was calculated on the basis of monthly sums of precipitation (P) and a sum of monthly air temperatures (t), using the following formula (Radzka et al., 2015):

$$K = P/0.1\Sigma t$$

[1]

Values of K are presented in Table 2. It was assumed that extreme conditions took place when its value was below 0.7 and above 2.5 (Radzka et al., 2015). Optimal temperature and moisture conditions were only in April 2014 and in

Table 2. The value of Sielianinov	's hydrothermal coefficient	(K) in the growing season.
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Year	April	May	June	July	August	September	October
2014	1.36	1.87	1.64	0.59	1.92	0.64	0.12
	(o)	(mw)	(mw)	(sd)	(mw)	(sd)	(ed)
2015	1.22	2.63	0.87	1.08	0.18	1.46	1.94
	(md)	(sw)	(d)	(md)	(ed)	(o)	(mw)
2016	1.89	0.82	1.02	2.15	1.05	0.86	3.65
	(mw)	(d)	(md)	(w)	(md)	(d)	(ew)

 $K \le 0.4$  extreme drought (ed);  $0.4 < K \le 0.7$  severe drought (sd);  $0.7 < K \le 1.0$  drought (d);  $1.0 < K \le 1.3$  moderate drought (md);  $1.3 < K \le 1.6$  optimal (o);  $1.6 < K \le 2.0$  moderately wet (mw);  $2.0 < K \le 2.5$  wet (w);  $2.5 < K \le 3.0$  severely wet (sw); K > 3.0 extremely wet (ew).

September 2015. In the remaining months of the growing periods they were not as favorable, varying from extremely dry in August 2015 to extremely wet in October 2016. Throughout the experiment the best conditions were at the beginning of each growing season. It can be concluded that the most difficult situation for plants was in 2015, when, apart from May and the end of the growing season, the weather ranged from moderately dry to extremely dry. The growing season of 2016 was characterized by a lack of periods with extreme droughts, when wet and quite wet spells prevailed.

#### Statistical analysis

The results of the research were processed statistically using ANOVA for repeated (3 yr), multi-factor, and recurrent measurements (three harvest in a growing season). The Fisher-Snedecor test was used to determine whether the impact of experimental factor was significant, while the value of the HSD<sub>0.05</sub> was calculated with Tukey's test. The Statistica program version 12.0 (Dell Inc., Tulsa, Oklahoma, USA) was applied for all other calculations. Means in the tables marked with the same letters in lines/columns do not differ significantly.

# **RESULTS AND DISCUSSION**

#### Effect of Ecklonia maxima extract on photosynthesis activity of alfalfa leaves

The experiment indicated that *E. maxima* extract had multi-directional effect on photosynthetic activity of the plants, as evidenced by higher values of such parameters as chlorophyll fluorescence induction and chlorophyll content in relation to the control (Tables 3-8). Measurement of CF made on hybrid alfalfa leaves showed that the maximum photochemical efficiency of photosystem II (PSII) ( $F_v/F_m$ ) determined after dark adaptation, as the average for all harvests (H1, H2, H3) in all growing seasons, underwent significant substantial differentiation on plots with the extract (Table 3). Its application caused a 12.4% increase of the  $F_v/F_m$  parameter, from 0.565 on the control to 0.635 on units with the extract. The value of the actual efficiency of PSII ( $\Delta F/F_m$ ) determined under the same conditions turned out to be about 20% higher (Table 4) for plants treated with the extract. It should therefore be noted that the growth of those parameters indicated a growth of the demand for products which constitute photosynthetic efficiency, and it also indicated a lack of interference in the process of plant growth and development (Khaleghi et al., 2012).

In addition, literature has shown (Khaleghi et al., 2012; Laisk et al., 2014) that an increasing  $F_v/F_m$  value means higher PSII activation because of a lack of photoinhibition in plant cells deficient in N; that is, the energy consumed to transport

Extract	Harvest	2014	2015	2016	Mean
Control	H1	0.601Aa	0.600Aa	0.549Aa	0.583A
	H2	0.550Ba	0.544Ba	0.550Aa	0.548A
	H3	0.597ABa	0.546Ba	0.544Aa	0.562A
Em	H1	0.634Aa	0.621Aa	0.619Aa	0.628A
	H2	0.667Aa	0.639Aab	0.626Ab	0.644A
	H3	0.632Aa	0.658Aa	0.620Ab	0.637A
Mean for e	extract effect				
Control		0.583Ba	0.563Ba	0.548Ba	0.565B
Em		0.644Aa	0.639Aa	0.622Aa	0.635A
Mean for h	narvest effect				
H1		0.618Aa	0.611Aa	0.584Aa	0.604A
H2		0.609Aa	0.592Aa	0.588Aa	0.596A
H3		0.615Aa	0.602Aa	0.582Aa	0.600A
Mean		0.614a	0.602a	0.585a	

Table 3. The effect of <i>Ecklonia maxima</i> extract (Em) on maximu	m photosystem II efficiency (F <sub>v</sub> /F <sub>m</sub> ) of alfalfa leaves.
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Means in lines with the same lower-case do not differ significantly. Means in columns with the same uppercase do not differ significantly.

H1: Harvest 1 carried out in spring in the first half of June; H2: harvest 2 carried out in summer in the second half of August; H3: harvest 3 carried out in autumn in the first half of October.

Table 4. The effect of *Ecklonia maxima* extract (Em) on actual photosystem II efficiency  $(\Delta F/F_m)$  of alfalfa leaves.

			Study year				
Extract	Harvest	2014	2015	2016	Mean		
Control	H1	0.381Ab	0.392Aab	0.418Aa	0.397A		
	H2	0.398Ab	0.381Ab	0.432Aa	0.404A		
	H3	0.391Ab	0.381Ab	0.430Aa	0.401A		
Em	H1	0.458Ab	0.571Aa	0.537Aa	0.531A		
	H2	0.410Ab	0.435Bb	0.541Aa	0.462B		
	H3	0.435Ab	0.407Bb	0.572Aa	0.470B		
Mean for e	extract effect						
Control		0.390Ba	0.385Ba	0.437Ba	0.404B		
Em		0.434Ab	0.471Aab	0.550Aa	0.485A		
Mean for h	narvest effect						
H1		0.420Ab	0.482Aa	0.478Aa	0.460A		
H2		0.404Ab	0.408Ab	0.487Aa	0.433B		
Н3		0.413Ab	0.394Bb	0.501Aa	0.436B		
Mean		0.412b	0.428b	0.489a			

Means in lines with the same lower-case do not differ significantly. Means in columns marked with the same uppercase do not differ significantly.

H1: Harvest 1 carried out in spring in the first half of June; H2: harvest 2 carried out in summer in the second half of August; H3: harvest 3 carried out in autumn in the first half of October.

electrons is not reduced. At the same time, an increased activity of reaction centres of PSII cells containing N means a high activity of photosynthetic apparatus and high efficiency of light energy conversion (Nishiyama et al., 2006). Algae extract application to hybrid alfalfa might have caused better nutrition of plant cells with N, as evidenced by increases in photosynthetic parameters such as maximum  $(F_v/F_m)$  and actual  $(\Delta F/F_m)$  efficiency of PSII of leaves.

The extract increased the value of the non-photochemical quenching coefficient (qN); as the data from Table 5 indicates it increased by 26% in relation to the control. The experiment did not show significant impact of the extract on the decline or increase of the coefficient of photochemical quenching ( $q_P$ ) of leaves (Table 6). Many studies (Laisk et al., 2014; Janka

			Study year		
Extract	Harvest	2014	2015	2016	Mean
Control	H1	0.106ABa	0.105Aa	0.103Aa	0.105A
	H2	0.115Aa	0.112Aa	0.109Aa	0.112A
	H3	0.103Ba	0.108Aa	0.102Aa	0.104A
Em	H1	0.139ABa	0.136Ba	0.122Ab	0.132AB
	H2	0.157Aa	0.163Aa	0.132Ab	0.151A
	H3	0.126Ba	0.117Ba	0.120Aa	0.121B
Mean for o	extract effect				
С		0.108Ba	0.108Ba	0.105Ba	0.107B
Em		0.141Aa	0.139Aa	0.125Ab	0.135A
Mean for l	harvest effect				
H1		0.123ABa	0.121ABa	0.113Ab	0.119B
H2		0.136Aa	0.138Aa	0.121Ab	0.132A
H3		0.115Ba	0.113Ba	0.111Aa	0.113B
Mean		0.125a	0.124a	0.115b	

Table 5. The effect of *Ecklonia maxima* extract (Em) on the non-photochemical quenching coefficient  $(q_N)$  of alfalfa leaves.

Mean in lines with the same lower-case do not differ significantly. Mean in columns with the same uppercase do not differ significantly.

H1: Harvest 1 carried out in spring in the first half of June; H2: harvest 2 carried out in summer in the second half of August; H3: harvest 3 carried out in autumn in the first half of October.

Table 6. The effect of *Ecklonia maxima* extract (Em) on the photochemical quenching coefficient (q<sub>P</sub>) of alfalfa leaves.

Extract	Harvest	2014	2015	2016	Mean
Control	H1	0.518ABa	0.515ABa	0.550ABa	0.528AB
	H2	0.623Aa	0.600Aa	0.551Ab	0.591A
	H3	0.456Ba	0.451Ba	0.498Ba	0.468B
Em	H1	0.602Aa	0.608Aa	0.551Ab	0.587A
	H2	0.627Aa	0.591Aa	0.561Ab	0.593A
	H3	0.487Ba	0.451Ba	0.508Aa	0.482B
Mean for e	extract effect				
Control		0.532Aa	0.522Aa	0.533Aa	0.529A
Em		0.572Aa	0.550Aa	0.540Aa	0.554A
Mean for h	narvest effect				
H1		0.560Ba	0.562Aa	0.551Aa	0.558AB
H2		0.625Aa	0.596Aa	0.556Ab	0.592A
H3		0.472Ca	0.451Ba	0.503Aa	0.475B
Mean		0.552a	0.536a	0.537a	

Means in lines with the same lower-case do not differ significantly. Means in columns marked with the same uppercase do not differ significantly.

H1: Harvest 1 carried out in spring in the first half of June; H2: harvest 2 carried out in summer in the second half of August; H3: harvest 3 carried out in autumn in the first half of October.

et al., 2015) have pointed out to a very large impact of genetic factors of the values on fluorescence parameters, which may explain a lack of  $q_P$  diversification as a response to extract application.

It was also found that the value of some parameters was dependent on weather conditions. The non-photochemical quenching coefficient ( $q_N$ ) and  $q_P$ , both taken as the average for extract doses and growing seasons ( $q_P - 0.592$ ;  $q_N - 0.132$ ), were the highest in plants collected in summer (H2). As the weather data indicate (Table 2), there were dry and wet periods during summer seasons, with an exception of the very dry summer of 2015. A periodic lack of rainfall with sunny warm weather did not cause a reduction in the efficiency of primary reaction of photosynthesis, and non-cyclic electron transport went well under these conditions.

#### Effect of Ecklonia maxima extract on chlorophyll content in alfalfa leaves

In relation to the control there was a significant 12% increase in chlorophyll a content in alfalfa leaves as a response to *E. maxima* extract (Tables 7). As an average for all growing seasons and harvests chlorophyll concentration was 209 mg 100 g<sup>-1</sup> fresh weight on control units and 234 mg 100 g<sup>-1</sup> fresh weight on units with the extract.

A much larger difference of 16.7% was recorded for the concentration of chlorophyll b (Table 8). According to literature (Yokoya et al., 2007; Zhao et al., 2016), those photosynthetic pigments are responsible for the collection and transfer of light absorbed to photosynthetic reaction centres, and their concentration affects photosynthesis efficiency. Thus, increasing chlorophyll content may be one of the causes of an increase in photosynthetic activity (Zhao et al., 2016). Correspondingly, in the present experiment the increase in the concentration of photosynthetic pigments, as a response to the extract, increased photosynthetic activity.

It was observed that the content of the pigments in alfalfa leaves was also dependent on weather conditions. The lowest chlorophyll a and b content in plants was in 2014 and 2015 when, as can be seen from the distribution of Sielianinov's hydrothermal coefficient values (Table 2), there was rainfall deficiency in the summer and autumn. Then, there were extremely dry months of October 2014 and August 2015, and very dry months of July and September 2014. Drought stress during those periods caused a significant decrease in the content of those pigments in alfalfa leaves. The lowest chlorophyll a content (206 mg 100 g<sup>-1</sup> fresh weight) was recorded in 2014, when it was about 17.5% lower than in 2016. In turn, the content of chlorophyll b in leaves decreased most strongly during the drought in 2015, when it was 15.3% lower than in 2016.

Other authors have dealt with the effect of drought stress on chlorophyll a and b content in crops. Kiani et al. (2008) demonstrated that the content of chlorophyll a and b in sunflower (*Helianthus annuus* L.) leaves decreased with increasing

			Study year		
Extract	Harvest	2014	2015	2016	Mean
		mg	g 100 g-1 fresh weig	ght	
Control	H1	191Ba	210Ba	209Ba	203B
	H2	164Bb	237Aa	258Aa	220A
	H3	198Ab	169Cc	248Aa	205B
Em	H1	221Ba	234Ba	231Aa	229B
	H2	194Cb	260Aa	249Aab	235A
	H3	268Aa	188Cb	257Aa	238A
Mean for e	extract effect				
Control		184Bc	205Bb	238Aa	209B
Em		228Ab	227Ab	246Aa	234A
Mean for h	narvest effect				
H1		206Bb	222Ba	220Ba	216A
H2		179Cb	249Aa	254Aa	227A
H3		233Ab	179Cc	253Aa	223A
Mean		206b	217b	242a	

Table 7. The effect of Ecklonia maxima extract (Em) on chlorophyll a content in alfalfa leaves.

Mean in lines marked with the same lower-case do not differ significantly. Mean in columns marked with the same uppercase do not differ significantly.

Table 8. The effect of Ecklonia maxima extract (Em) on chlorophyll b content in alfalfa leaves.

Extract	Harvest	2014	2015	2016	Mean		
		mg 100 g <sup>-1</sup> fresh weight					
Control	H1	86Ba	88ABa	97Bb	90B		
	H2	81Bb	99Ab	106Aa	95AB		
	H3	111Aa	80Bb	114Aa	102A		
Em	H1	109Ba	97Bb	111Ba	106A		
	H2	115Aa	120Aa	127Aa	111A		
	H3	118Aa	90Bb	119ABa	109A		
Mean extra	act effect						
Control		93Bb	89Bb	106Ba	96B		
Em		114Aa	102Ab	119Aa	112A		
Mean harv	vest effect						
H1		98Bb	93Bb	104Ba	98A		
H2		98Bb	110Aa	117Aa	108A		
H3		115Aa	85Cb	117Aa	105A		
Mean		104ab	98b	113a			

Means in lines with the same lower-case do not differ significantly. Means in columns with the same uppercase do not differ significantly.

water deficit. Similar results were obtained by Vasileva and Ilieva (2012). A chlorophyll content decrease in drought conditions has been also reported in grasses (*Lolium perenne*, *Poa pratensis*), legumes (*Medicago lupulina*, *Lotus corniculatus*) (AbdElgawad et al., 2015), cotton (*Gossypium hirsutum*) (Massacci et al., 2008) and olives (*Olea europaea*) (Arji and Arzani, 2008).

However, in the present experiment this content was significantly higher in growing seasons with extremely dry periods. In 2014 the content of chlorophyll a was 184 mg 100 g<sup>-1</sup> fresh weight in the control unit and 228 mg 100 g<sup>-1</sup> fresh weight in units with the extract. In 2015 the corresponding figures were 205 and 227 mg 100 g<sup>-1</sup> fresh weight, while in the case of chlorophyll b in 2014 the content ranged from 93 mg 100 g<sup>-1</sup> fresh weight in the control to 114 mg 100 g<sup>-1</sup> fresh weight on units with the extract, and in 2015 from 89 mg 100 g<sup>-1</sup> fresh weight to 102 mg 100 g<sup>-1</sup> fresh weight,

respectively. In conclusion, it should be noted that treating hybrid alfalfa with *E. maxima* extract resulted in an increase in the concentration of chlorophyll a and b in the leaves even in years with periodic shortages of rainfall.

# CONCLUSIONS

As a reaction to the particular conditions during the experiment *Medicago* ×*varia* treated with *Ecklonia maxima* extract had a better efficiency of the photosynthetic apparatus in relation to the control, with higher values of maximum ( $F_v/F_m$ ) and actual ( $\Delta F/F_m$ ) photosystem II efficiency and of the non-photochemical quenching coefficient ( $q_N$ ). Algae extract used on hybrid alfalfa leaves increased plant photosynthetic activity resulting from an increase in the content of chlorophyll pigments. Chlorophyll a and b concentration, as a response to the extract, increased by 12% and 16.7%, respectively. The experiment showed that periods of rainfall shortages did not reverse the effect of the extract on plants, with an increase in chlorophyll a and be content during the growing seasons with dry and extremely dry months.

## ACKNOWLEDGEMENTS

The research carried out the theme Nr 357/13/S was financed by the science grant of the Ministry of Science and Higher Education.

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