Journal of Soil Science and Plant Nutrition, 2017 , 17 (3), 770-780
RESEARCH ARTICLE

The effect of *Ecklonia maxima* extract on *Medicago* x *varia* T. Martyn biomass

Jacek Sosnowski*, Kazimierz Jankowski, Elżbieta Malinowska, Milena Truba

*Siedlce University of Natural Sciences and Humanities, Department of Grassland and Landscape Architecture Development, Institute of Agronomy, Poland *Corresponding author: jacek.sosnowski@uph.edu.pl*

Abstract

In 2013 a field experiment was set up in order to determine the effect of *Ecklonia maxima* extract on the development of the aboveground biomass of alfalfa (*Medicago* X *Varia* T. Martyn). The main experimental factor was a biostimulant based on the extract containing plant hormones. The extract was applied to the experimental units in the form of spraying. The following features were determined in the experiment: plant density, the number of shoots per square meter, dry matter weight per square meter, and the ratio of leaves to the entire biomass. The results were processed statistically with the analysis of variance, while differences between means were verified with Tukey's test ($p \leq 0.05$). Furthermore, the Pearson linear correlation coefficient and the coefficient of determination were calculated in order to determine the relationship between the number of plants and shoots per area unit and biomass weight. Extract application led to an increase in shoot length and density, and the weight of dry matter in individual harvest, but it did not affect plant density and the ratio of alfalfa leaves. However, a decrease of plant density in consecutive years was observed.

Keywords: Alfalfa, seaweed extract, biomass, shoots, biostimulant

1. Introduction

Literature has dealt with positive reaction of plants to the application of seaweed products, and it has been pointed out that the effect of the extracts is related to the species and variety of plants treated (Sultana *et al*., 2005). There have been many publications on extracts improving productivity and health of crops and reducing the use of fertilizers and pesticides (El-Yazied *et al*., 2012; Verkleij, 1992; Zodape 2001; Zodape *et al*., 2009; Sosnowski *et al*., 2013a,b; Sarawaneeyaruk *et al*., 2015). Plants treated with such substances are characterized by higher yields, resulting mainly from an increase in resistance to unfavourable environmental factors (drought, frost), pathogens, and pests, and more intense uptake of nutrients from the soil (Bai, 2007). That is why studying the effects of *Ecklonia maxima* extracts in the cultivation of alfalfa seemed to be important.

Alfalfa is one of the most common plants cultivated in the world, and its area is about 32 million hectares. Biological properties of the species, especially its tolerance to salinity and unfavourable moisture conditions, decide about its importance (Benabderrahim *et al*., 2012; Hattab *et al*., 2014). Lucerne is a long-day thermophilic plant, with normal development occurring at 20°C, but higher temperatures and good water conditions result in faster growth and earlier flowering. Due to the high content of vitamins, iron, and potassium lucerne is recommended to patients with anaemia and vitamin deficiency. However, long-term use of alfalfa is not completely secure. Due to the high content of vitamins, longer treatment may result in their accumulation, and after 2-3 months there should be a break. Those who take medication reducing blood clotting should not be treated with alfalfa products because of the high content of vitamin K. The discovery of toxic protein amino acid L-canavanine in the plant and in seeds was the reason why there was a considerable reduction in the use of alfalfa in therapy. This amino acid causes severe autoimmune reactions in animals and humans (Liang *et al*., 2011; Vasileva and Ilieva, 2011**)**. Despite this, on 13 of October 2009 the Commission of the European Communities approved the marketing of alfalfa leaf extract as a novel food ingredient in the Regulation (EC) No 258/97 of the European Parliament and of the Council. According to this decision, alfalfa protein concentrate with a specific amount of chemical compounds, including L-canavanines, can be used as a component of dietary supplements, at a daily dose of consumption not exceeding 10 g.

The aim of this study was to determine the effect of *Eclonia maxima* extract on the development of the aboveground biomass of alfalfa. The number of plants and shoots per unit area, the share of leaf blade, and the weight of biomass were determined in the experiment.

2. Materials and Methods

In 2013, a field study with *Medicago x varia* T. Martyn cv. 'Tula' on the experimental plots of the Department of Grassland and Landscape Architecture Development in Siedlce was established (52.169 °N, 22.280 °E). The soil there is of the culture earth order, hortisole type, formed from loamy sand. The analysis performed at the Regional Chemical Station in Wesoła found that that it was of neutral pH (pH in 1 N KCl = 7.2), with a high amount of humus (3.78%), available phosphorus (P_20_5 - 900 mg kg^{-1}) and magnesium (Mg - 84 mg kg^{-1}), and the average concentration of total nitrogen (N - 1.8 g kg^{-1}) and potassium (K_20 - 190 mg kg^{-1}). A single plot area was 6 m^2.

Before sowing, in the first year, and in the second and third year phosphorus was applied in an amount of 45 kg ha^{-1}, and potassium at the rate of 100 kg ha^{-1}. Alfalfa seeds were sown in April 2013 with a seeding rate of 12 kg ha^{-1} (600 seeds per square meter, assuming 100% germination), at a depth of about 1cm. Biostimulant Kelpak SL consisting of natural plant hormones, such as auxin (11 mg dm^{-3}) and cytokinin (0.03 mg dm^{-3}), was used in the experiment. It is an extract form brown algae (*Ecklonia maxima*), chemical and biochemical composition of which is presented in Table 1. During each growth cycle of alfalfa, the extract was applied once, during the six-true-leaf and early-bud stage, in the form of a spray. There were the following experimental units: A1-control (without biostimulant, with plants sprayed with water only), A2 – with biostimulant applied at a dose of 2 dm^3 ha^{-1} diluted in 350 L. of water. The experiment was replicated three times. Each year alfalfa was harvested three times (B1, B2, B3) in the early stage of flowering, with 40-50% of flowers formed. The following features were determined: the number of plants per square meter, the number of shoots per square meter, the percentage of leaves in the aboveground biomass, and dry matter yield [kg DM m^{-2}].

Table 1. Chemical and biochemical composition of *Ecklonia maxima* extract.

Organic matter	Content	Growth regulators	Content
Carbohydrates	31%	auxin	11 mg·dm^{-3}
Alginic acid	13%	cytokinin	0.03 mg·dm^{-3}
Total amino acid	7%		
Manitol	4%		
Macro elements		Micro elements	
N	4.10%	Fe	140 ppm
P_2O_5	2.10%	Zn	70 ppm
K_2O	5.01%	Mn	12 ppm
Ca	0.24%	B	55 ppm
S	3.51%	I	28 ppm
Mg	0.49%		

The results were processed statistically using the analysis of variance and Tukey's test. Furthermore, in order to determine the relationship between the number of plants per square meter and the amount of biomass [kg DM m^{-2}], but also between the number of shoots per square meter and the amount of biomass [kg DM m^{-2}], the Pearson correlation coefficient (r), and the coefficient of determination (R^2) were calculated. Throughout the experiment Meteorological data were obtained from the Hydrological and Meteorological Stations in Siedlce. In order to determine temporal variability of meteorological conditions and their influence on plant growth, Sielianinov's hydrothermal coefficient was calculated (Skowera, 2014). The data presented in Table 2 show that the most favourable distribution and the amount of rainfall, with optimum air temperatures within the growing period, were in 2013 and 2015. In those years there were no months with drought or severe drought.

Table 2. Sielianinov's hydrothermal index (K) in the growing seasons.

Year	Month						
	Apr.	May	June	July	Aug.	Sept.	Oct.
2013	1.03	2.24	1.03	1.26	1.36	1.01	1,73
2014	0.40	2.21	1.19	1.18	1.79	2.81	0.53
2015	1.10	0.89	0.72	2.19	0.84	0.78	0.94

K < 0.5 – severe drought; 0.51 – 0.69 - drought; 0.70 – 0.99 – moderate drought; K > 1 – no drought

3. Results

The effects of the biostimulant on plant density per square meter in subsequent years are presented in Table 3. The extract application did not result in a significant increase in plant density, which ranged, on average, from 133 per square meter in 2015 to 185 plants per square meter in 2013. Similarly, statistical analysis showed no significant differences in plant density between all three grass harvests in a year. The important process noted in the experiment was the falling number of plants in the successive years. The study indicates that the largest density of alfalfa plants (185 per m^{-2}) was in the first year. In subsequent years it fell significantly, with 133 plants per m^2 in the third year. It is suggested by other publications that plant density can be affected by the weather. In the second year there was a severe spring drought (Table 2), resulting in water deficit during the development of vegetative organs before the first harvest, which led to weakening and loss of plants.

It was also found that the shoot number per square meter had the biggest impact on the yield. The plots where the biostimulant was used (Table 4) had a higher (the average of 5%) shoot density than the control unit. There was an extensive change of this feature throughout the experiment. The most shoots (642 per m^{-2}) developed in 2015, but the least (555 per m^{-2}) in 2013.

Table 3. The number of plants per square meter.

	Cut (B)	Study year (C)			Mean
		2013	2014	2015	
A1 Control	B1	197 Aa	152 Ab	130 Ab	160 A
	B2	182 ABa	137 Ab	129 Ab	150 A
	B3	154 Ba	131 Aab	112 Ab	132 A
A1		178 a	140 b	124 b	147 A
A2 Biostimulant	B1	197 Aa	166 Ab	131 Ac	165 A
	B2	194 Aa	156 Ab	147 Ab	165 A
	B3	185 Aa	153 Ab	145 Ab	161 A
A2		192 a	158 b	131 b	164 A
Mean	B1	197 Aa	159 Ab	131 Ab	162 A
	B2	189 Aa	146 Ab	138 Ab	158 A
	B3	170 Aa	143 Ab	129 Ab	147 A
Mean		185 a	149 b	133 b	

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

Table 4. The number of shoots per square meter.

	Cut (B)	Study year (C)		
		2013	2014	2015
A1 Control	B1	587 Aab	550 Ab	662 Aa
	B2	562 Aab	549 Ab	637 Aa
	B3	552 Aab	521 Ab	591 Aa
A1		567 ab	540 b	630 a
A2 Biostimulant	B1	611 Aab	553 Ab	661 Aa
	B2	594 Ab	579 Ab	658 Aa
	B3	589 Aab	575 Ab	643 Aa
A2		598 ab	569 b	654 a
Mean	B1	599 Aab	552 Ab	662 Aa
	B2	576 Aab	564 Ab	648 Aa
	B3	571 Aab	548 Ab	617 Aa
Mean		582 ab	555 b	642 a

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

There were no significant differences in shoot density between growth cycles throughout the same growing season. It should be noted, however, that the density in each growth was high (from 604 to 579 shoots m^{-2}).

One of the biological indicators of the quality of plant material is the percentage of leaves in the biomass (Table 5).

The highest rate of leaves was in the third year (the average of 51.4%). It should be noted that the biostimulant did not cause any significant differences between leaf rates in consecutive years. However, harvest time had a significant influence on this feature. Most leaves were obtained in the first harvest (average 52.1%), and the least in the third one (average 36.6%).

A percentage of leaves in the biomass is an important indicator of the forage value. Leaves contain more protein, vitamins, carotene, phosphorus, calcium magnesium, and microelements than other parts of a plant.

An increased amount of leaves in biomass can be obtained cutting higher layers of alfalfa. Layer harvesting also allows fractionation of raw material for the production of protein concentrates, vitamins, carotene, and high-grade dried forage. This harvesting also facilitates proper growth of alfalfa.

Table 5. The percentage of leaves in the aboveground biomass.

	Cut (B)	Study year (C)			Mean
		2013	2014	2015	
A1 Control	B1	51.6 Ab	46.2 Ab	60.1 Aa	52.6 A
	B2	41.7 Bb	47.1 Aab	50.9 Ba	46.6 B
	B3	33.7 Cb	31.9 Bb	43.2 Ca	36.3 B
A1		42.3 b	41.7 b	51.4 a	45.1 A
A2 Biostimulant	B1	47.8 Ab	50.2 Aab	56.7 Aa	51.6 A
	B2	41.5 ABb	46.3 Ab	55.7 Aa	47.8 AB
	B3	35.9 Bb	32.7 Bb	42.2 Ba	36.9 B
A2		41.7 b	43.1 b	51.5 a	45.4 A
Mean	B1	49.7 Ab	48.2 Ab	58.4 Aa	52.1 A
	B2	41.6 Bb	46.7 Ab	53.3 Aa	47.2 B
	B3	34.8 Cb	32.3 Bb	42.7 Ba	36.6 C
Mean		42.0 B	42.4 B	51.4 A	

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

Compared to the control, extract application caused more than a 17% increase of biomass (Table 6). The linear analysis of relationship between the plant and shoot number and the size of biomass (Table 7) showed a significant positive correlation between these features. The number of alfalfa shoots in more than 63% (R^2 = 63.7%) determined the weight of the biomass, but the plant number only in 51% affected it.

Table 6. Dry matter yield per square meter.

	Cut (B)	Study year (C) 2013	2014	2015	Mean
A1 Control	B1	0.19 Aa	0.18 Aa	0.19 Aa	0.19 A
	B2	0.19 Aa	0.18 Aa	0.19 Aa	0.19 A
	B3	0.19 Aa	0.18 Aa	0.18 Aa	0.19 A
A1		0.19 A	0.18 A	0.19 A	0.19 B
A2 Biostimulant	B1	0.23 Aa	0.17Aa	0.23 Aa	0.21 A
	B2	0.23 Aa	0.23 Aa	0.23 Aa	0.23 A
	B3	0.20 Aa	0.21 Aa	0.21 Aa	0.21 A
A2		2.23 Aa	0.20 Aa	0.23 Aa	0.21 A
Mean	B1	0.21 Aa	0.19 Aa	0.21 Aa	0.20 A
	B2	0.21 Aa	0.20 Aa	0.21 Aa	0.20 A
	B3	0.20 Aa	0.19 Aa	0.20 Aa	0.20 A
Mean		0.20 A	0.20 A	0.20 A	

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

Table 7. Correlation between morphological characteristics of alfalfa

Morphological characteristics	x- biomass in kg DM m^{-2} r (R^2)*	The regression equation
y_1 - Number of plants per m^2	7.16 (51.3%)	$y = 0.2745+0.0699 \cdot x$
y_2 - Number of shoots per m^2	7.98 (63.7%)	$y = 1.8952+0.1592 \cdot x$
significant for p = 0.05, N = 54		

*r - correlation coefficient, R^2 - coefficient of determination

4. Discussion

A lot of studies have dealt with a positive effect of algae on germination, root mass, weight of the aboveground plant, the size of the photosynthetic apparatus, flowering, fruit size, the content of trace elements, and the overall health of plants (Bai *et al*., 2007; Reitz and Trumble, 1996). The use of algae during the growing period is particularly important in extreme weather and soil conditions.

It is believed, though, that the degree of response of plants depends not only on their species and varieties, but also on the dose, frequency of treatment, and their development stage (Khan *et al*., 2009). The frequency of application depends on the individual sensitivity of the species, but the treatment can be performed several times during the growing season. Researchers studying the application of algae extracts to crops showed that during sequential treatments the effects of this substance are cumulative (Reitz and Trumble, 1996; Stirk and Staden, 1996).

Seaweeds are one of the most important marine resources of the world and are used as food, animal feed, and raw material for many industries. They are also used as fertilizer in agriculture and horticulture (Chapman, 1980; Christobel, 2008). Research on plant responses to seaweed extracts has been initiated in the last few years (El-Yazied *et al*., 2012; Kocira *et al*., 2016). Due to the presence of minerals and growth hormones such as IAA, IBA, or cytokinin (Moller and Smith, 1998; 1999), seaweed extracts increase the resistance to plant diseases (Verkleij, 1992). Chemical composition of the extracts shows that they have a very high content of organic carbon, polysaccharides, and microelements and a smaller amount of such macro elements as nitrogen, phosphorus, and potassium (Crouch and Staden, 1993). The beneficial effects on plants are also associated with the content of vitamins and amino acids (Khan *et al*., 2009). Extracts from *Ecklonia maxima* are seen as a new generation of natural growth regulators. They stimulate seed germination and increase the productivity of many crops. Unlike fertilizers, extracts derived from seaweed are biodegradable, non-toxic, environmentally friendly, and safe for humans, animals, and birds (Zodape *et al*., 2009). The use of these substances is gaining in popularity. In most cases Scientific studies confirm the beneficial effect of seaweed extracts on crops (Zodape 2001; Bai *et al*., 2007; Zodape *et al*., 2009), indicating a greater importance of the application time than the dose. The number of flowers and seeds per flower head increased when marigold seedlings were treated with Kelpak immediately after transplanting (Aldworth and Van Staden, 1987; Staden *et al*., 1994). Bai *et al*. (2007) in studies with foliar use of seaweed extracts found that, compared to the control, crops developed shoots about 35% longer, and roots about 22% longer. Contrary to that, there have been reports about no significant influence of phytohormone substances on plants (De Villiers *et al*., 1983; Temple and Bomke, 1989; Nour *et al*., 2010; Zodape *et al*., 2010; Kumar and Sahoo, 2011). In addition, it was stated (Bai *et al*., 2007; Craigie, 2011) that even varieties of the same species can react differently to hormonal treatment, which may explain the lack of effect of the extract on alfalfa in some cases.

Studying the effect of an extract on wheat, Schmidt *et al*. (1999) found no effect of seaweed hormones on the height of the plants, while there was a significant increase of dry matter weight in the first study year characterized by favourable weather conditions. The same authors also observed that algae application had a similar results when, in the later development stages of crops, a biostimulant used one time increased the mass of plants about 17-20%. Numerous scientific reports clearly show that the effect of extracts from algae on the height and weight of plants is determined by the species of algae and the conditions in which the research is conducted. Some researchers (Khan *et al*., 2009; Verkleij, 1992) found no significant effect of algae on plants, while others obtained higher yields, up to 35%, as a result of foliar use of seaweed extracts (Bai *et al*., 2007; Craigie, 2011).

5. Conclusions

The use of *Ecklonia maxima* extract in alfalfa cultivation contributed to an increase in the number of shoots per square meter and the weight of biomass. The extract had no effect on plant density and percentage of leaves in the biomass. The amount of biomass, as shown by the regression analysis, was dependent to a greater degree on the number of shoots than on the number of plants. There were differences between the rates of leaves in each harvest. Most leaves developed in the spring harvest, at the least in the autumn one. The studies showed a significant interaction between the year of the experiment and the number of shoots developed by alfalfa, but with the aging of the crop, the plant density decreased. The shoot number was dependent on meteorological conditions in the growing season and was the highest in years when there were no droughts.

Acknowledgements

The research was carried out as part of the project of the Polish Ministry of Science and Higher Education for the development of young scientists. Topic title: Effect of some biological preparations on soil fertility and productivity of plants alternating grassland; project number 20/MN/11.

References

Aldworth, S., Van Staden, J. 1987. The effect of seaweed concentrate on seedling transplants. S. Afr. J. Bot. 53, 187-89.

Bai, N., Banu, N., Prakash, J., Goldi, S. 2007. Effects of Asparagopsis taxiformis extract on the growth and yield of *Phaseolus aureus*. J. Basic Applied Biology. 1, 6-11.

Benabderrahim, M., Hamza, H., Haddad, M., Ferchichi, A. 2012. Evaluation of yield potential and some agronomic traits variability in mediterranean alfalfa cultivars in the field under tunisian conditions. Electr. J. Plant Breeding. 3, 612-620.

Chapman, D.J. 1980. Seaweeds and their uses. Chapman and Hall Edition, New York, 334p.

Christobel, J. 2008. Effect of seaweed (*Sargassum weightii* L.) on the germination and growth of green gram (*Phaseolus aureus* L.). J. Basic Appl. Biology. 2, 105-108.

Craigie, J. 2011. Seaweed extract stimuli plant science and agriculture. J. Appl. Phycol. 23, 371-393.

Crouch, I., Van Staden, J. 1993. Evidence for the presence of plant growth regulators in commercial seaweed products. J. Plant Growth Regul. 13, 21-29.

De Villiers, J., Kotze, Wag., Joubert, M. 1983. Effect of seaweed foliar spray on fruit quality and mineral nutrition. The Decidous Fruit Grower. 33, 97-101.

El-Yazied, A., El-Gizawy, A.M., Ragab, M., Hamed, E. 2012. Effect of seaweed extract and compost treatments on growth, yield and quality of snap bean. J. American Sci. 8, 1-20.

Hattab, S., Hattab, S., Boussetta, H., Banni, M. 2014. Influence of nitrate fertilization on Cd uptake and oxidative stress parameters in alfalfa plants cultivated in presence of Cd. J. Soil Sci. Plant Nutr. 14, 89-99.

Khan, W., Rayirath, U., Subramanian, S., Jithesh, M., Rayorath, P., Hodges, D., Critchley, A., Craigie, J., Norrie, J., Prithiviraj, B. 2009. Seaweed extracts as biostimulants of plant growth and development. Plant Growth Regul. 28, 386-399.

Kocira, A., Świeca, M., Kocira, S., Złotek, U., Jakubczyk, A. 2016. Enhancement of yield, nutritional and nutraceutical properties of two common bean cultivars following the application of seaweed extract (*Ecklonia maxima*). Saudi J. Biol. Sci. http://dx.doi.org/10.1016/j. sjbs.2016.01.039

Kumar, G., Sahoo, D. 2011. Effect of seaweed liquid extract on growth and yield of *Triticum aestivum* var. Pusa gold. J. Appl. Phycol. 23, 251-255.

Liang, J., Yang, Z., Cao, X., Wu, B., Wu, S. 2011. Preparative isolation of novel antioxidant flavonoids of alfalfa by stop-and-go counter-current chromatography and following online liquid chromatography desalination. J. Chromatogr. A. 12, 6191-6199.

Moller, M., Smith, M. 1998. The significance of the mineral component of seaweed suspension on lettuce (*Lactuca sativa* L.) seedling growth. J. Plant Physiology. 153, 658-663.

Moller, M., Smith, M. 1999. The effect of pruning treatments using seaweed suspensions on the water sensitivity of barley (*Hardeum vulgare* L.) caryopses. Annals Applied Biology. 135, 515-522.

Nour, K.A.M., Mansour, N.T.S., Abd El-Hakim, W.M. 2010. Influence of foliar spray with seaweed extracts on growth, setting and yield of tomato during summer season. J. Plant Production. 1, 961-976.

Regulation (Ec) No 258/97 Of The European Parliament And Of The Council, Oj. Eu L294, 2009.

Reitz, S., Trumble, J. 1996. Effects of cytokinin-containing seaweed extract on *Phaseolus lunatus* L. Influence of nutrient availability and apex removal. Bot. Mar. 39, 33-39.

Sarawaneeyaruk, S., Krajangsang, S., Pringsulaka, O. 2015. The effects of neem extract and azadirachtin on soil microorganisms. J. Soil Sci. Plant Nutr. 15, 1071-1083.

Schmidt, D., Dempsey, J., Kerley, M., Porton, J. 1999. The potential to increase neutral detergent fiber levels in ape diets using reality available produce. Proceedings of the third conference of the American zoo and aquarium association (AZA) nutrition advisory group (NAG) on zoo and wildlife nutrition. Columbus, Ohio, 156-180.

Skowera, B. 2014. Changes of hydrothermal conditions in the Polish area (1971−2010). Fragm. Agron. 31, 74-87.

Sosnowski, J., Jankowski, K., Wiśniewska-Kadżajan, B. 2013a. Effect of Kelp SL on the formation of above-ground biomass of *Festulolium braunii* (K. Richt.) A. Camus. Acta Agrobot. 66, 149-154.

Sosnowski, J., Jankowski, K., Wiśniewska-Kadżajan, B., Jankowska, J. 2013b. Effect of different concentrations of Kelpak bioregulator on the formation of above-ground biomass cocksfoot. J. Ecol. Eng. 14, 48-52.

Staden, J., Upfold, J., Dewes, F. 1994. Effect of seaweed concentrate on growth and development of the marigold Tagetes patula. J. Appl. Phycol. 6, 427-428.

Stirk, W., Staden, J. 1996. Comparison of cytokinin- and auxin- like activity in some commercially used seaweed extracts. J. Appl. Phycol. 8, 503-508.

Sultana, V., Ehteshamul-Haqu, S., Ara, J., Athar, M. 2005. Comparative efficacy of brown, green and red seaweeds in the control of root infecting fungi and okra. Int. J. Environ. Sci. Tech. 2, 129-132.

Temple, D., Bomke, A. 1989. Effects of kelp (*Macrocystis integrifolia* and *Ecklonia maxima*) foliar applications on bean crop growth. Plant Soil. 117, 85-92.

Vasileva, V., Ilieva, A. 2011. Chemical composition, nitrate reductase activity and plastid pigments content in lucerne under the influence of ammonium and nitrate form mineral nitrogen. Agron. Res. 9, 357-364.

Verkleij, F. 1992. Seaweed extracts in agriculture and horticulture: a review. Biol. Agric. Hortic. 8, 309-324.

Zodape, S. 2001. Seaweeds as a biofertilizer. J. Sci. Industrial Res. 60, 378–382.

Zodape, S., Mukherjeea, S., Reddya, M., Chaudharya, D. 2009. Effect of *Kappaphycus alvarezii* (Doty) ex silva. Extract on grain quality, yield and some yield components of wheat (*Triticum aestivum* L.). International J. Plant Product. 3, 97-101.

Zodape, S., Mukhopadhyay, S., Eswaran, K., Reddy, M., Chikara, J. 2010. Enhanced yield and nutritional quality in green gram (*Phaseoulus radiata* L.) treated with seaweed (*Kappaphycus alvarezii*) extract. J. Scientific Industrial Research. 69, 468-471.