NUSANTARA BIOSCIENCE
Vol. 7, No. 1, pp. 33-37
May 2015

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n070106

Influence of foliar application of algae extract and amino acids mixture on fenugreek plants in sandy and clay soils

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Manuscript received: 28 October 2014. Revision accepted: 4 February 2015.

Abstract. *Tarraf SA, Talaat IM, El-Sayed AEB, Balbaa LK. 2015. Influence of foliar application of algae extracts and amino acids mixture on fenugreek plants in sandy and clay soils. Nusantara Bioscience 7: 33-37.* Two pot experiments were conducted to study the effect of foliar application of algae extract and amino acids mixture on the growth and chemical constituents of fenugreek plants (*Trigonella foenum-graecum* L.). Plants were sprayed with different concentrations of algae extract (0.0, 2.5, 5.0 g/L) or equivalent of amino acids mixture (0.0, 0.625, 1.25 g/L). The results indicated that foliar application of amino acids mixture enhanced the vegetative growth of fenugreek plants, especially when plants were sprayed with 1.25 g/L amino acids mixture. Data also show that total nitrogen, essential oil percentage, and yield followed the same trend. These results hold true for plants cultivated in either clay soil or sandy soil. Data also indicated that foliar application of algae extract to fenugreek plants significantly increased plant height, number of leaves, number of branches and fresh and dry weights of plant at vegetative growth stage and flowering stage of fenugreek plant, especially in plants treated with 5 g/L algae extract in sandy and clay soils, respectively. Treatment of fenugreek plants with algae extract markedly increased nitrogen, phosphorous and potassium contents, especially at 5 g/L.

Keywords: Algae, amino acids, fenugreek.

INTRODUCTION

Liquid seaweed extract has been reported as a beneficial treatment for the growth of plants (Metting et al. 1990). It has its usage history of centuries in agriculture (Crouch 1990). *Ascophyllum nodosum* is a prominent seaweed species belonging to the brown algae (Phaeophyceae) in the North Atlantic Ocean (Verkleij 1992). Seaweed extracts have been used as soil conditioners and as a foliar spray to increase growth, yield, and productivity of many crops (Norrie and Keathley 2006). Various responses of plants to seaweed application have been observed. These include vigorous growth, higher yield, increased nutrient uptake, and more resistance to biotic and abiotic stresses (fungal diseases, insect attack, and frost), improved quality and longer shelf life of fruits.

Certain bioactive compounds from algae were early considered with high potential in plant growth stimulation. These compounds affect metabolic processes including photosynthesis, respiration, nucleic acid synthesis, and nutrients uptake. Algal extract mixture contains a wide range of active materials including free amino and organic-acids, phytohormones, vitamins, and enzymes which react as growth promoters (Snedecor and Cochran 1980). In this action, freshwater microalgae extracts appeared to be promising natural fertilizers. They contain high macro and micronutrients concentrations in addition to the natural enzymes and hormones (Shaaban 2001). Moreover, vegetative growth of olive transplants was markedly enhanced as their root zoon was surrounded by pre-digested *Scenedesmus* biomass at the concentration equal to the recommended nitrogen dose. The partial substitution of nitrogen also improved nutrient concentrations and balance by algal bulk (Abdel-Maguid et al. 2004).

The beneficial properties of algae and consequently their extracts for humans, animals, and plants were recognized in the past and are appreciated nowadays, in the development of new biotechnological products. Products with functional properties containing organic compounds derived from natural sources, rather than being a product of heavy organic synthesis are increasingly demanded by consumers. Currently, recipients of products have higher demands, expecting that they will be on one hand "bio" and "organic", but on the other will be a concentrate of compounds with desired properties. This poses a particular challenge for modern biotechnology (Chojnacka et al. 2012).

Primary metabolites such as amino acids, the building blocks in the synthesis of proteins, are involved in plant growth and development (Hounsome et al. 2008). Amino acids are a well-known biostimulant which has positive effects on plant growth, yield and significantly mitigates the injuries caused by abiotic stresses (Kowalczyk and Zielony 2008). Saeed et al. (2005) on soybean found that treatments of amino acids significantly improved growth parameters of shoots and fresh weight as well as pod yield. Liu et al. (2008) revealed that foliar application with the mixture of amino acids to radish plants increased N content of shoots whereas, NO_3 content reduced by 24-38%. El-Zohiri and Asfour (2009) on potato found that spraying of

amino acids at 0.25 ml/L significantly increased vegetative growth expressed as plant height and dry weight of plant. Abo Sedera et al. (2010) revealed that spraying strawberry plants with amino acids (peptone) at 0.5 and 1.0 g/L significantly increased total nitrogen, phosphorus and potassium in plant foliage as well as total yield, weight, TSS, vitamin C and total sugars content of fruits compared to control treatment.

This experiment has been done to study the comparison between the effect (of composite mixture of amino acids as well as blue algae, *Spirulina platensis*, extract which also contains the same proportions of the composite components) on the vegetative growth and seed yield as well as the chemical constituents of fenugreek plant (*Trigonella foenum-graecum* L.) when planted in two different types of soil (clay and sandy soil).

MATERIALS AND METHODS

Plant materials

Two pot experiments were carried out during two successive seasons of (2011/2012-2012/2013) at the screen of National Research Centre, Dokki, Giza, Egypt. Fenugreek seeds were secured from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Seeds were sown in 30 cm diameter earthware pots on 10th October, in the first and second seasons, respectively. Pots were divided into two groups. The first group contained 8 kg loamy clay soil and the second one filled with 8 kg sandy soil. Physical and chemical analyses of the experimental soils are presented in Table (1).

Algal extract and amino acid mixture

The blue alga *Spirulina platensis* was produced at Algal Biotechnology Unit, National Research Centre with a final capacity of 75m^3. Based on the media listed by El-Sayed et al. 2001, the growth media within open pond was enriched by extra amount of potassium carbonate instead of sodium bicarbonate El-Sayed et al. 2008). The comparison was performed based on the initial nitrogen content of both algal extract and ready amino acid mixture to be equal nitrogen content. Chemical analysis of algal extract and amino acids mixture (Table 2) was done by the adopted method of Chapman and Pratt (1978). Amino acid analyzer (Eppendorf LC3000, Germany) proceeded under the conditions of 0.2 ml/minute flow rate: 0 to 50 bar pressure of buffer; 0 to 150 bars pressure of reagent and 123ºC reaction temperature. All used treatments of amino acids mixture were products of Sigma Co. (Germany).

Field experiment

Fenugreek plants (*Trigonella foenum-graecum* L.) were cultivated in two different soils including clay and sandy soils to eliminate the effect of algal bio-extract and amino acid mixture effect on growth. Algal extract was made by algal Biotechnology Unit by fermenting the obtained algal biomass under non-aerobic conditions. Prior fermentation *Spirulina* growth media was enriched by excessive nutritional doses in concern macronutrients (NPK) to increase protein content to reach about 64%. After 45 days, plants were sprayed with different concentrations of algae extract (0.0, 2.5, 5.0 g/L) or equivalent of amino acids mixture (0.0, 0.625, 1.25 g/L) at 1st November in both seasons. Each pot was sprayed with about 80 ml of the prepared treatments. To study the comparison between the effect of each of composite mixture of amino acids, as well as, blue algae extract and which also contains the same proportions of the components of the composite on vegetative growth and seed yield as well as the chemical content of fenugreek plant, when planted in two different types of soil (clay and sandy soils).

Treatments were distributed in complete randomized block design with three replications comprised with three pots for each replicate. Each pot received equal and adequate amounts of water and fertilizers. Phosphorous as calcium superphosphate (15.5% P_2O_5) was mixed with the soil before sowing at the rate of 4.0 g/pot. Three grams of nitrogen as ammonium sulfate (20.5 % N) in three applications (one g for each) with two weeks intervals started 30 days after sowing, also, two grams of potassium sulfate (48% K_2O) were added as soil application. Other agricultural processes were performed according to normal practice. Two plant samples were taken during the growing season, at vegetative growth stage (15 days after treatment) and the second one was at flowering stage (6th December) and yield sample on 5th February.

Table 1. Physical and chemical analyses of the experimental soils.

Variables	Soil types	
	Sand	Clay
Field capacity (%)	13.40	47.60
Wilting point (%)	6.70	23.60
Sand %	96.62	18.50
Silt %	0.67	21.30
Clay %	2.71	60.20
pH	7.20	7.30
EC dS /m	1.40	2.70
$CaCO_3$%	1.50	2.83
Soluble ions mg/100 g soil		
Ca^{++}	9.00	18.00
Mg^{++}	5.00	8.00
Na^{+}	2.80	3.20
K^{+}	2.70	4.00
CO_3^{-}	0.00	0.00
HCO_3^{-}	3.10	4.20
Cl^{-}	4.20	5.10

Table 2. Chemical analysis and amino acid content of algal extract and amino acids mixture.

Analysis %	Algal extract	Amino acids mixture
Amino acids	16	19.58
Nitrogen	10.24	10
Phosphorous	3.4	8
Potassium	1.5	1

Chemical constituents

Fixed oil was extracted from dried seeds according to the procedure stated in the A.O.A.C. (1970). Nitrogen, potassium and phosphorous contents were determined according to the method described by Chapmann and Pratt (1961).

Statistical analysis

The data obtained were subjected to standard analysis of variance procedure according to Snedecor and Cochran (1980). The values of LSD were calculated whenever F values were significant at 5% level.

RESULTS AND DISCUSSION

Plants growth

Data presented in Tables 3 indicate that foliar application of algae extract to fenugreek plants significantly increased plant height, number of leaves, number of branches and fresh and dry weights of plant at vegetative growth stage and flowering stage of fenugreek plant, especially in plants treated with 5 g/L algae extract in sandy and clay soils, respectively. Data also indicate that foliar spray of amino acids mixture on fenugreek plants significantly affected plant height, number of leaves, number of branches as well as fresh and dry weights of plant, especially in plants treated with 1.25 g/L AA. These results hold true for growth parameters in the yield (Table 4).

In this concern, Abd El Moniem and Abd-Allah (2008) found that application of algal extract at 25 to 100% had an announced effect on percentages of bud burst and fruiting buds rather than the check treatment. The effect was obviously appeared till 50% concentration, while the promotion was slight with using concentrations above 50%. Growth characters namely leaf area, shoot length and number of leaves/shoot were greatly stimulated in response to application of Algal extract at 25 to 100% rather than the check treatment These results are in agreement with those obtained by Abdel-Aziz et al (2011) who reported that the application of seaweed had a significant stimulatory effect on growth parameters of *Amaranthus tricolor* plants during two seasons. However, the most effective treatments which had the highest length and diameter of the stem, root length, number of leaves, fresh and dry weight of leaves, stems and roots when spraying with seaweed extract at the concentration of 3.0 cm^3/L compared with untreated plants.

The use of seaweed extracts has been reported to have beneficial effects on plants. Foliar application of seaweed concentrate to seedlings of *Pinus pinea* L. increased shoot length and weight and decreased the root to shoot ratio (Atzmon and Van Staden 1994). Seaweed extracts have been reported to obtain the greater root and shoot development of Kentucky bluegrass (*Poa pratensis* L) (Goatley and Schmidt 1990) Abdel-Maguid et al. (2004) on coratina olive, Gobara (2004) on palms and Hegab et al. (2005) on orange trees. It is evident that, in general, there was a significant positive interaction between saline water and seaweed (*Ascophyllum nodosum*) application on growth parameters recorded in both seasons and verified that combined treatment of water salinity at 1000 ppm and seaweed extract at 3.0 cm^3/L was highly efficient in increasing growth of *Amaranthus tricolor* plants as compared with other treatments, except treated plants with 3.0 cm^3/L seaweed combined with treatment of irrigated with tap water which had non significant.

Rayirath et al. (2009) reported that increasing seaweed application rate from 0 up to 3.0 cm^3/L increased the N, P and K nutrients percentages in leaves, stems and roots of *Amaranthus tricolor* plants. The highest values of Na were obtained when plants untreated with seaweed followed by application of 3.0 cm^3/L. extracts of the brown seaweed (*Ascophyllum nodosum*) enhance the plant tolerance against environmental stresses such as drought, salinity, and frost.

In this concern, amino acids were used to replace NO^{3-} or foliar spray in many plants partially. In most case, the application of amino acids led to decreased nitrate content and increased total nitrogen content in lettuce, Chinese cabbage, onion, pakchoi or other leafy crops (Gunes et al. 1994, 1996; Chen and Gao 2002; Wang et al. 2004). Some authors suggested that plants probably preferred amino acids as sources of reduced nitrogen, and amino acids inhibited nitrate uptake. There was little evidence or data to support the conclusions. It has not been distinguished that increased total nitrogen came of nitrate or amino acids.

Chemical constituents

Data presented in Tables 5 and 6 indicate that treatment of fenugreek plants with algae extract markedly increased nitrogen, phosphorous and potassium contents, especially at 5 g/L. A progressive increase on percentage of N, P and K in the leaves of grapevine plants was observed as a result of increasing concentration of algae extract till 75% than the increase was slightly appeared (Abd El Moniem and Abd-Allah 2008). Table 8. cleared the oil percentage % of seeds as well as the NPK % contents of fenugreek seeds of plants sown in the clay or sandy soil. It could be mentioned that 1.25 g of A.A. as spraying treatment recorded the highest percent of fixed oil content followed by 5 g of blue algae extract treatment. At the same time, the percent of N content showed similar results and recorded 136.9% and 112.6% compared to 100% of control treatment, while the opposite results were obtained with P%. It could be reported that 2.5 g algae show the same trend of P% and as % of control. A slight increase was detected in the content of K and 1.25g of A.A. was the best treatment and recorded 117.2% of control. On the other hand, oil content of seeds grown in sandy soil has been greatly affected when it was sprayed with both see weed extract or amino acids composite mixture, whereas, 5g of blue algae and 1.25g of amino acids mixture recorded 190% and 180% of oil compared to control plants, respectively. Similar results were obtained with the percent of phosphorous content of seeds. These results were different to the same extent with N or K %, while 1.25 of A.A. was the best treatment in increasing both P and K %. The same Table 6 cleared that 5 g liquid algae extract scored the highest proportion of P in the seeds, as well as, the % of control, at the same time

1.25g of A.A. showed the best result in increasing K % of fenugreek seeds.

In conclusion, this research revealed that seaweed extract applied at 30 days after sowing is helpful in obtaining higher yield and quality fenugreek plant. Findings of this work may be helpful to further explore and assess the effect of seaweed extract on other commercial crops.

Table 3. Influence of algae extracts and amino acids mixture on growth of fenugreek plants cultivated in sandy soil and clay soil.

Treatment (g/L)	Vegetative stage					Flowering stage				
	Plant height (cm)	No. leaves	No. of branches	F.W/ plant	D.W./ Plant	Plant height (cm)	No. leaves	No. branches	F.W./ plant	D.W./ plant
Sandy soil										
Control	15.00	14.33	7.67	2.12	1.14	19.67	14.67	8.67	2.93	1.27
2.5 alg	16.67	15.67	5.33	2.27	1.27	20.33	16.67	8.67	2.60	1.39
5 g alg	18.67	19.00	10.00	3.37	1.55	23.33	20.33	10.33	4.99	1.70
0.625 AA	16.67	16.33	8.67	2.75	1.27	20.33	19.00	11.00	3.57	1.57
1.25 AA	22.00	20.67	11.00	3.71	1.51	22.33	29.67	11.00	4.75	1.84
LSD (5%)	2.46	2.87	4.01	0.53	0.19	2.40	1.80	2.09	1.34	0.35
Clay soil										
Control	18.67	14.67	7.33	2.18	1.43	21.00	16.33	8.67	2.60	1.27
2.5 g alg	21.00	19.33	8.67	2.65	1.39	24.33	19.67	8.67	2.93	1.47
5 g alg	21.67	22.67	9.33	2.99	1.52	25.00	23.00	10.00	4.99	1.70
0.625 g AA	19.67	17.67	8.00	2.19	1.51	22.33	19.67	11.00	3.57	1.57
1.25 g AA	25.00	26.33	10.33	3.80	1.71	25.00	19.67	11.00	4.75	1.84
LSD (5%)	2.11	1.11	2.79	0.27	0.09	3.28	1.80	2.09	1.34	0.35

Table 4. Influence of algae extracts and amino acids mixture on yield of fenugreek plants cultivated in sandy soil and clay soil.

	Plant height (cm)	Yield fresh wt/pot	Yield dry wt/pot	No. of pods/plant	Weight of pods x 13	Yield fresh wt/potx13	Yield dry wt/potx13	No. of branches
Sandy soil								
Control	37.00	1.25	0.85	7.00	2.71	16.29	11.05	3.00
2.5 g alg	46.00	2.52	2.41	11.67	4.52	32.76	31.37	3.67
5 g alg	46.33	0.73	0.66	9.00	2.43	9.53	8.54	4.67
0.625 g AA	46.50	1.57	1.07	13.00	5.28	20.37	13.91	5.33
1.25 g AA	61.00	1.95	1.44	16.00	5.69	25.31	18.76	5.33
LSD (5%)	3.27	0.66	0.43	2.71	1.35	8.63	5.63	1.39
Clay soil								
Control	35.67	2.56	0.75	8.33	3.69	33.28	9.79	1.67
2.5 g alg	39.33	4.14	1.46	8.67	5.73	53.82	19.02	3.33
5 g alg	45.33	4.16	1.85	11.00	6.05	54.04	24.09	3.67
0.625 g AA	46.67	4.27	1.67	16.00	7.11	55.47	21.71	4.33
1.25 g AA	47.67	4.36	2.08	16.33	8.47	56.72	27.08	4.33
LSD (5%)	4.70	0.82	0.41	3.84	1.14	10.65	5.36	1.09

Table 5. Influence of algae extracts and amino acids mixture on chemical constituents of fenugreek plants at vegetative growth stage.

Treatment	Clay soil						Sandy soil					
	Nitrogen		Phosphorous		Potassium		Nitrogen		Phosphorous		Potassium	
	%	%of control	%	%of control	%	%of control	%	%of control	%	%of control	%	%of control
Control	2.06	100	0.11	100	1.50	100	3.46	100	0.11	100	1.65	100
2.5 g alg	3.37	163.59	0.29	263.64	1.85	123.33	3.57	103.18	0.20	181.82	1.95	118.18
5 g alg	5.23	253.88	0.30	272.73	1.90	126.67	4.69	135.55	0.30	272.73	2.25	136.36
0.625 g AA	2.73	132.52	0.23	209.09	1.70	113.33	3.78	109.25	0.30	272.73	2.00	121.21
1.25 g AA	3.46	167.96	0.23	209.09	1.95	130.00	5.03	145.38	0.40	363.64	2.30	139.39

Table 6. Influence of algae extracts and amino acids mixture on chemical constituents of fenugreek plant seeds at fruiting stage.

Treatment	Clay soil								Sandy soil							
	Oil		Nitrogen		Phosphorous		Potassium		Oil		Nitrogen		Phosphorous		Potassium	
	%	% of control	%	% of control	%	% of control	%	% of control	%	% of control	%	% of control	%	% of control	%	% of control
Control	0.86	100.0	4.28	100.0	0.07	100.0	1.45	100.0	0.50	100.0	3.88	100.0	0.08	100.0	1.60	100.0
2.5 g alg	1.00	116.3	4.61	107.7	0.27	385.7	1.50	103.5	0.89	178.0	4.00	103.1	0.29	362.0	1.60	100.0
5 g alg	1.36	158.1	4.82	112.6	0.27	385.7	1.55	106.9	0.95	190.0	4.18	107.3	0.30	375.0	1.75	109.4
0.625 g AA	1.12	130.3	4.44	103.7	0.11	157.1	1.50	103.5	0.80	160.0	4.3	110.2	0.2	250.0	1.60	100.0
1.25 g AA	1.73	201.2	5.86	136.9	0.25	357.1	1.70	117.2	0.90	180.0	5.27	135.8	0.29	362.5	1.85	115.6

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