SEAWEED LIQUID FERTILIZER EFFECT ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF BEAN PLANT (*PHAESOLUS VULGARIS* VARIETY PAULISTA) UNDER HYDROPONIC SYSTEM

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Abstract

The seaweed extract application on bean plant (*Phaseolus vulgaris* c. v. Paulista) enhanced the vegetative growth at lower concentrations 25% of *Fucus spiralis* and 25% of *Ulva rigida* that was found to have maximum influencer, on growth parameters as shoot length and root length. When Seaweed liquid extracts (SLE) applied by foliar spray or incorporated in the medium with the Hoagland nutrient solution under hydroponic system, biochemical parameters such as chlorophyll pigment and proteins content were also enhanced when compared to untreated bean plants. High proportions of seaweed extract in medium culture (100% of *Fucus spiralis* extract; 75% and 100% of *Ulva rigida* extract) were found to show negative effect. The examining of nitrate reductase activity showed that foliar application of seaweed extract increased this enzymatic activity. The *Ulva rigida* extract was more effective than *Fucus spirali* extract. (1.48 mol NO_2^- /mg protein/min and 0.653 mol NO_2^-/mg protein/min respectively). However, the incorporation of *Ulva rigida* extract in medium culture showed an important decreasing of nitrate reductase activity. While, the incorporation of *Fucus spiralis* extract in medium culture showed a remarkable increased of nitrate reductase activity particularly at proportion (50% and 75% of *Fucus spiralis* extract). The activation of nitrate reductase enzyme was related to the presence of seaweed extract of *Unlva rigida* and

Fucus spiralis applying on bean plant by foliar spray. Their incorporation in medium culture inhibits this enzymatic activity.

Keywords: Biochemical parameters, chlorophyll, physiological parameters, protein, F*ucus spiralis*, growth parameters, Morocco, Nitrate reductase activity, *Ulva rigida*.

Abbreviations: SLE: Seaweed liquid extracts; N: Nitrogen; NO_2^-: Nitrite, NO_3^-: Nitrate; NH4+: Ammonium; HCl: hydrochloride acid; GB: glycinebetaine; U: *Ulva rigida*; F: *Fucus spiralis*; H: Hoagland's solution

Introduction:

Plant nutrition is one of the most important factors that increase plant production. Nitrogen plays the most recognized role in the plant for its presence in the structure of the protein molecule. In addition, nitrogen is found in such important molecules as purines, pyrimidines, porphyrines and coenzymes. The porphyrin structure is found in metabolically important compounds such as chlorophyll pigments and the cytochromes which are essential in photosynthesis and respiration (Marschner, H., 1995).

Common bean (*Phaseolus vulgaris*) is one of the most important grain food legumes in the world (Van Berkum and al., 1996; Zhang and al., 2008) and Morocco is the main producer of green bean in Africa with 187,497 tonnes per year in 2009 (FAO 2009). These crops are good sources of proteins, vitamins, and minerals such as Fe, Zn, P, Ca, Cu, K, and Mg, and are excellent sources of complex carbohydrates (Camacho Barron and Gonzalez de Mejia, 1998). Minerals accumulation in the seeds have essential role in crop production. Nitrogen (N), a key element, is an essential component of proteins and nucleic acids in plants (Sanchez et al., 2005). The assimilation of nitrogen by plants requires the uptake of NO_3^-, reduction to NO_2^-, the conversion of NO_2^- to NH_4^+, and the incorporation of NH_4^+ into organic compounds (Sivasankar and Oaks, 1996; Stitt, 1999).

Nitrogen availability is often a limiting factor for crop productivity, particularly in developing countries where nitrogen fertilizers are either unavailable or unaffordable (Graham, 1981). Nitrogen fertilization also plays a significant role in crop quality (Sisson et al., 1991). The recommendations in both tropical and temperate areas are that farmers would apply to crops at least minimal doses of nitrogen (Vance, 1998; Fink et al., 1999).

Nitrogen fertilizers are relatively expensive and may contribute to ground and surface water pollution through leaching and soil erosion. Unlike, chemical fertilizers, extracts derived from seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous to humans,

animals and birds. These fertilizers are often found to be more successful than chemical ferlizers (Booth E., 1969).

It has found wide application in modern agriculture for the use of marine macroalgae as fertilizer. Seaweed contain all the trace elements and plant growth hormones required by plant, regulators promoters available to enhance yield attributes (Kingman and Moore, 1982; Crouch and Van staden, 1991; Crouch and Van staden, 1993)

In this study, we have tried to investigate the seaweed liquid extract (SLE) effect on nitrogen metabolism of bean (*Phaesolus vulgaris*), especially the nitrate reductase activity which is the key enzyme of nitrogen nutrition under hydroponic system. In this condition, we have better control of climate and pest factors.

Among factors affecting hydroponic production systems, the nutrient solution is considered to be one of the most important determining factors of crop yield and quality. There are several formulations of nutrient solutions. Nevertheless, most of them are empirically based. In the present study we use Hoagland's nutrient solution.

The main objective of this paper is to evaluate the seaweeds liquid extract effect on the change of some physiological and biochemical parameters in bean plant related to nitrogen metabolism such as the chlorophyll, proteins and nitrate reductase activity. The seaweed extracts obtained from two macroalgae species *Ficus spiralis* and *Ulva rigida* were applied to bean plant cultivated in hydroponic system by foliar spray or incorporated to medium culture. Another objective of this paper is to evaluate the application of different concentration of seaweed extract in enhancing the growth of bean grown in greenhouse.

Materials and Methods:

Collection and Preparation of seaweeds

The seaweed extracts used in the present study were prepared from *Ulva rigida* and *Fucus spiralis* which belongs to Chlorophyceae and Phaeophyceae respectively. They were collected from coastal area of Sidi Bouzid near El jadida city (Morocco) in April 2013.

The alga was brought to the laboratory and washed thoroughly in tap water for 3 or 4 times to remove all epiphytes, sand particles and associated fauna. Fresh material was cut into small pieces and preserved at a temperature of - 20˚C until uses.

One kilogram of fresh seaweed material was ground and boiled separately with a litre of distilled water for an hour and filtered through a double layered muslin cloth to remove debris (Sivasankari et al., 2006). The filtrate was taken as 100% concentration of the seaweed extract and from this, 25% of *Fucus spiralis* and 25% of *Ulva rigida* were prepared by adding

distilled water (Bhosle et al., 1975). In order to preserve organic matter, seaweed extracts were stored at a temperature of - 20˚C until use.

Selection of crop plant and Seeds germination

The crop plant, selected for the present study was (Phaseolus vulgaris L.) c. v. Paulista belonging to the family Fabaceae.

The seeds were collected from National Company of seeds, SONACOS. Seeds were surface sterilized with non ionic detergent 6% for 5 minutes and then rinsed three times with distilled water. Then they are germinated in basins filled with pre-washed sand and placed in an oven at a temperature of 20 C˚.

After 4 days of germination, the young plants were transferred to plastic pots and placed in a greenhouse or in a hydroponics system.

Plant culture under greenhouse

The young plants were transferred to plastic pots containing soil mixture composed of agricultural soil and sand at 1:1 ratio.

All the plants were subjected to water level 100% of field capacity and watered with tap water or sprayed with seaweed extracts during 30 days after sowing.

Plant culture in hydroponic system

The young plants were inoculated and passed carefully through a plastic tube in a pierced rubber stopper. They were fixed with cotton fitted around the hypocotyl.

The culture was conducted hydroponically in controlled culture room conditions at temperature of 25˚C with 16/8 h photoperiod (day/night) (Rodiño et al., 2005).

Treatments

Experiment I: Effect of seaweed liquid on plant growth in greenhouse

The groups of bean plants subjected to water level 100% of field capacity were sprayed with different concentrations (6, 12.5, 25, 50 and 75%) of *Ulva rigida* and *Fucus spiralis* extracts. The plants sprayed with tap water served as control.

The objective of this experiment is to determine the best concentration of seaweed extract that enhanced plant growth.

Experiment II: Seaweed extract application on plants by spray foliar in hydroponic system

- Lot I: The groups of plant grown in 100% Hoagland's solution and sprayed with *Fucus spiralis* extracts (25%).

- Lot II: T The groups of plant grown in 100% Hoagland's solution and sprayed with *Ulva rigida* extract (25%).

Experiment III: Seaweed liquid application on plant by incorporation in medium in hydroponic system

- Lot III: The seedling grown in medium: 50% Hoagland's solution and 50% of *Fucus spiralis* extract (25%)
- Lot IV: The seedling grown in medium: 50% Hoagland's solution and 50% of *Ulva rigida* extract (25%)
- Lot V: The seedling grown in medium: 25% Hoagland's solution and 75% of *Fucus spiralis* extract (25%)
- Lot VI: The seedling grown in medium: 25% Hoagland's solution and 75% of *Ulva rigida* extract (25%)
- Lot VII: The seedling grown in medium: 100% of *Fucus spiralis* extract (25%)
- Lot VIII: The seedling grown in medium: 100% of *Ulva rigida* extract (25%)

The group of plant grown in 100% Hoagland's solution and not treated with seaweed extract served as control for the experiment II and III.

All the experiments were conducted in triplicates.

Growth measurements

Plant growth was measured on the basis of two parameters: Shoot length and root length in cm.

Biochemical and physiological parameters determination

Chlorophyll assay

After pigments extraction from 200 mg of leaf fragments with 4 ml of acetone/ distilled water (80:20 v/v), the chlorophyll content was determined in three independent aliquots and expressed on a dry weight basis (mg. g^{-1}DW) following Arnon (1949). Chlorophyll content was calculated by applying the following formula:

Chlorophylle a = 12.7×DO to 664nm – 2.69×DO to 647nm

Chlorophylle b = 22.9×DO to 647nm – 4.68×DO to 664nm

These coefficients were determined for concentrations expressed in mg/l.

Nitrate reductase activity

The nitrate reductase activity was determined in vivo by Heuer and Plaut method, 1978.

Leaf discs, 0.5 cm in diameter, were removed, weighed and placed in tubes in the presence of 10 ml of 0.05 M phosphate buffer, pH 7.5, containing 0.1 M KNO_3 and 0.1% Triton X-100. The samples were infiltrated under vacuum in order to induce anaerobic conditions in the incubation medium and then transferred to other tubes containing 2.5 ml of the same buffer but without Triton X-100.

The medium was maintained in the dark at 28˚C for 60 minutes. The NO_2^- produced by action of the NR enzyme was determined by drawing a 1 mL aliquot of the incubation medium, and treating this sample with 0.25mL of sulfanilamide 1% in 3M HCl and 0.25mL of N-(1-naphtyl)-ethylenediamine 0.02%. After 20 minutes, the absorbance was measured at 540nm. In order to calculate the amount of NO_2^- contained in the sample, a standard curve was prepared in the same way as the sample, containing from 0 to 2 µg/ml NO_2^-.

The activity of the nitrate reductase is expressed in mol $NO2^-$ produced per mg protein and per minutes

The trials were repeated three times for each treatment.

Protein content

For protein extraction, 50mg of foliar plant were homogenized at 4˚C with a mortar in 2m of phosphate buffer (0.05M, pH 6) and 2.5 % (w/v) polyvinylpolypyrolidone (PVPP) to avoid phenol oxidative effects. The homogenates were centrifuged at 12000g for 30 min two times at 4˚C. Supernatants were kept at -10˚C and used for protein determination.

Protein content was quantified according to Bradford method (Bradford, 1976), using bovine serum albumin (BSA) as standard. Two milliliters of the Bradford reagent and 100 µl of the each protein extract were mixed and then, reaction mixtures were incubated at room temperature for 20 min. The absorbance values were measured by UV-visible spectrophotometer (6305 - UV-visible spectrophotometer (190-1000 nm) – JENWAY) at 595 nm.

Mineral analysis

The material (thalus algae) was kept in the oven at 110˚ C for 12 h, pulverized in the grinder and sieved through a screen with an aperture of 0.5 mm. This powdered material was kept in airtight plastic bottles at room temperature until analysis. Samples were subjected to acid digestion and filtered with Whatman filter paper.

A simple flame photometer (Model 410 Classic, Sherwood Scientific Ltd, UK) with filters for calcium, sodium, and potassium was used. Butane gas and air were supplied as the source of flame. Standard curve with sodium, potassium and calcium concentration between 5 and 30 ppm was established daily. The sodium, potassium and calcium content in sample were determined against the standard curve. Means with standard deviations of triplicate determinations were reported.

Determination of Total Nitrogen

The Thallus of macroalgae was dried at 75˚C for 48 h. Nitrogen content of the samples was determined based on Kjeldahl method (James W. O'Dell, 1993).

Glycinebetaine assay

The thallus of macroalgae was analyzed according to the method of Grieve & Grattan (1983). The extract was prepared in 20 mL test tubes by chopping 0.5 g thallus in 5 mL of toluene-water mixture (0.05% toluene).

All the tubes were mechanically shaken for 24 h at 25˚C. After filtration 0.5 mL of extract was mixed with 1 mL of 2 N HCl solution then and 0.1 mL of potassium tri-iodide solution (containing 7.5 g Iodine and 10 g potassium iodide in 100 mL of 1 N HCl) was added and shaken in an ice cold water bath for 90 min and then 2 mL of ice-cooled water was added after gentle shaking 10 mL of 1,2 dichlorométhane (Chilled at -10˚C) was poured in it. By passing continuous stream of air for 1-2 minutes two layers were separated, upper aqueous layer was discarded and optical density of organic layer was recorded at 365 nm. The concentration of glycinebetaine (GB) was estimated by using standard curve developed with different concentration of GB.

Statistical analysis

All the measurements were made in triplicates. All data presented are means ± standard deviation and were analysed by one way analysis of variance. The statistical significance of differences between means ($P \leq 0.05$) was estimated by test Tukey using Sigma Stat 3.1.

Results

Effect of seaweed liquid extracts on the growth and development

The importance of different seaweed extracts on the growth of bean plants (*Phaseolus vulgaris* L.) was investigated using different concentrations of two species of macroalgae: *Ulva rigida* and *Fucus spiralis*. Table 1 shows diverse results obtained with seaweed liquid extracts on shoot and root length of bean plant. The differences obtained were related to concentration of seaweed liquid extract (SLE) and to algae species. Hence, both treatment 50 % and 25% of each extract of *Ulva rigida* and *Fucus spiralis* provided the significant effects on plant growth and the maximum effect was found with 25% treatment. Therefore, the highest shoot and root length of plants were attained with 25% *Ulva rigida* treatment (23.17cm and 13.78 cm respectively) and with 25% *Fucus spiralis* treatment (26.87 cm and 14.24 cm respectively). The concentrations of SLE (6%, 12.5% and 75%) provide meaningful effect on length steam compared to the control plant.

Table 1. Effect of different concentration of seaweed liquid extracts on Shoot length and root length (cm) of Bean plants cultivated in soil under greenhouse conditions.

Parameter	Treatment type in (%)										
	Control	Ulva rigida					Fucus spiralis				
	0%	6%	12,5 %	25%	50%	75%	6%	12,5 %	25%	50%	75%
Shoot lenght (cm)	16.01 ^{e} ±0.48	16.2 ^{e} ±1.51	17.02 ^{e} ±1.91	23.17 ^{b} ±1.37	20.08 ^{c} ±2.14	18.86 ^{d} ±1.26	18.1 ^{d} ± 0.87	20.4 ^{C} ± 1.15	26.87 ^{a} ±1.4	21.60 ^{b} ±0.40	18.43 ^{d} ±1.12
Root length (cm)	9 ^{d} ±0.72	11.5 ^{b} ±0.61	11.85 ^{b} ±0.67	13.78 ^{a} ±0.74	10.4 ^{c} ±0.78	8.7 ^{d} ±0.76	10.6 ^{c} ±0.58	11.39 ^{b} ±0.7	14.24 ^{a} ±0.59	10.93 ^{c} ±0.68	10.88 ^{c} ±0.74

Results are means ± S.D (n = 3). Different letters show statistically significant differences for P< 0.05 level.

The seaweed extract increased the growth of bean with 25% treatment that is significantly better. Therefore, we use this concentration (25% *Fucus spiralis* and 25% *Ulva rigida* extracts) applied by foliar spray or incorporated in the medium with the Hoagland's nutrient solution under hydroponic system.

Effect of seaweed liquid extracts on biochemical and physiological constituents of bean plant in hydroponic system

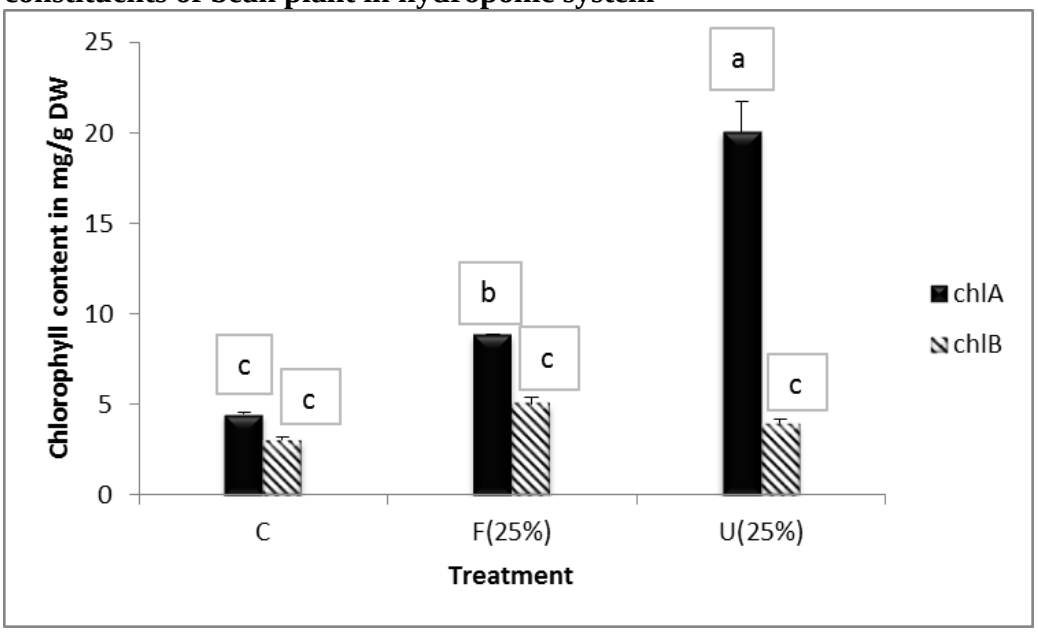

Figure 1: Effect of seaweed extract on chlorophyll content on Bean plant sprayed with 25% of *Ulva rigida* (U 25%) and 25% of *Fucus spiralis* (F 25%).
Chl a: Chlorophyll a; Chl b: Chlorophyll b.
Results are means ± S.D (n = 3). Different letters show statistically significant differences for P< 0.05 level.

Figure 1 showed that foliar application of seaweeds extract enhance chlorophyll content in leaves of plants by both treatments: 25% of *Fucus spiralis* and 25% of *Ulva rigida* when compared to plant control and particularly for chlorophyll a (chl a).

Greatest chlorophyll a quantity was found at 25% of *Ulva rigida* extract (20.08 mg/g DW) compared to 25% of *Fucus spiralis* extracts (8.86 mg/g DW). The lowest value of chlorophyll a amount was recorded in control plant without seaweed liquid extract (4.4 mg/g DW). The level of chlorophyll b in leaves plants was not significantly affected by foliar application of seaweeds extract.

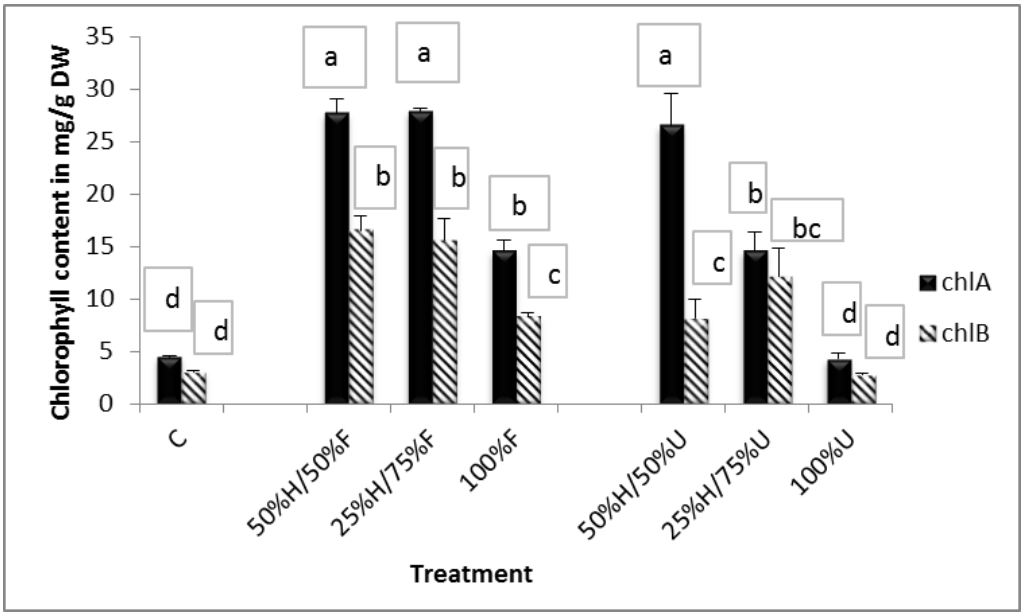

Figure 2: Effect of seaweed extract on chlorophyll content on the bean plants incorporated to Hoagland's medium at different ratio
Chl a, Chlorophyll a; Chl b, chlorophyll b.
C: Control Plants, H: Hoagland's nutrient solution; F: *Fucus spiralis* extract ; U : *Ulva rigida* extract
Results are means ± S.D (n = 3). Different letters show statistically significant differences for P< 0.05 level.

As shown in Figure 2, chlorophyll content (chla a and chl b) in plants treated with seaweeds extract incorporated at different ratio in the medium was significantly increased by both treatments 50%H/50% SLE (27.64 mg/g DW. of chl a; 16.5mg/g DW of chl b for *Fucus spiralis* and 26.4 mg/g DW of chl a; 8.13mg/g DW of chl b for *Ulva rigida*) and 25%H/75% SLE (27.86 mg/g DW. of chl a; 15.57 mg/g DW of chl b for *Fucus spiralis* and 14.60 mg/g DW. of chl a; 12.08 mg/g DW of chl b for *Ulva rigida*) when compared to the control values (4.4 mg/g DW of chl a; 3mg/gDW of chl b). The Highest value of chlorophyll a was recorded by *Fucus* extracts incorporated in medium with proportion 50% and 75% and by *Ulva* extract at proportion 50%. We can also note that treatment with 100% *Fucus spiralis* extract and with 75 % *Ulva rigida* extract have less important effects than the other ratio. While that of 100% *Ulva rigida* extract have no effect in enhancing chlorophyll content and remaine equally effective as that of Hoagland's nutrient solution.

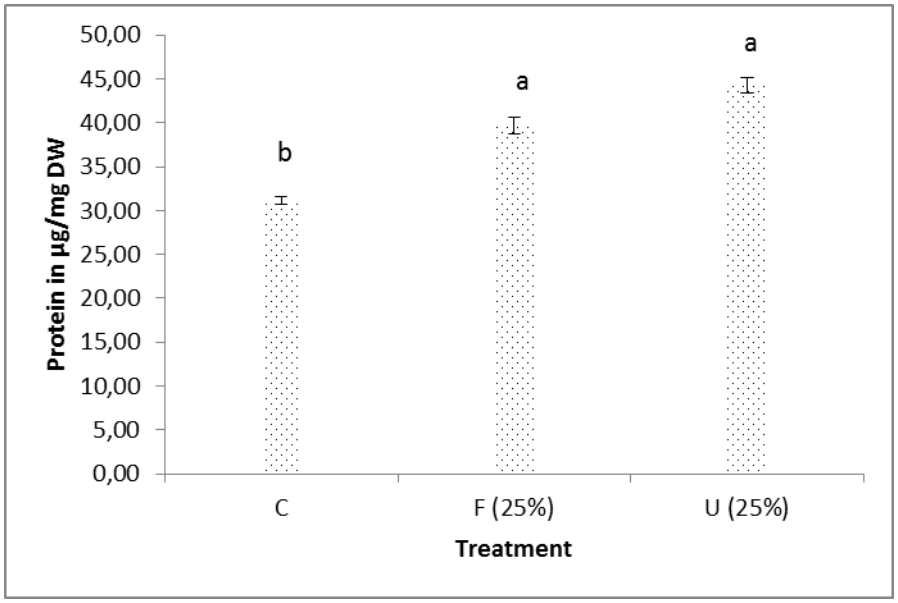

Figure 3: Effect of seaweed extract on protein content in the leaves of Bean plant sprayed with 25% of *Ulva rigida* (U 25%) and 25% of *Fucus spiralis* (F 25%).
C: Plant control without seaweed extract.
Results are means ± S.D (n = 3). Different letters show statistically significant differences for P< 0.05 level.

The protein content in plants was significantly enhanced by both treatments 25% of *Fucus spiralis* extract and 25% of *Ulva rigida* extract (39.67 µg/mg DW and 44.27 µg/mg DW respectively) applyed by foliar spray (Figure 3) when compared to the control plants (31.14 µg/mg DW). The treatment with seaweed extract of *Ulva* and *Fucus* at concentration 25% were found comparable and more effective than exhibited by the Hoagland treatment.

When SLE incorporated in medium culture, the protein content in bean plants increased significantly for all treatment with different proportions: 50%, 75% and 100% of *Ulva rigida* extract and *Fucus spiralis* extract comparatively to the control plants grown in Hoagland nutrient solution. The greatest level was reached with 75% of *Fucus spiralis* extract (107.67 µg/mg DW) followed by 50% of this extract (54.68 µg/mg DW) and by proportions 50% and 75% of *Ulva rigida* extract (63.35 µg/mg DW and 52.48 µg/mg DW respectively). The proportions 100% of *Fucus spiralis* and *Ulva rigida* extract were shown less effective than other treatments.

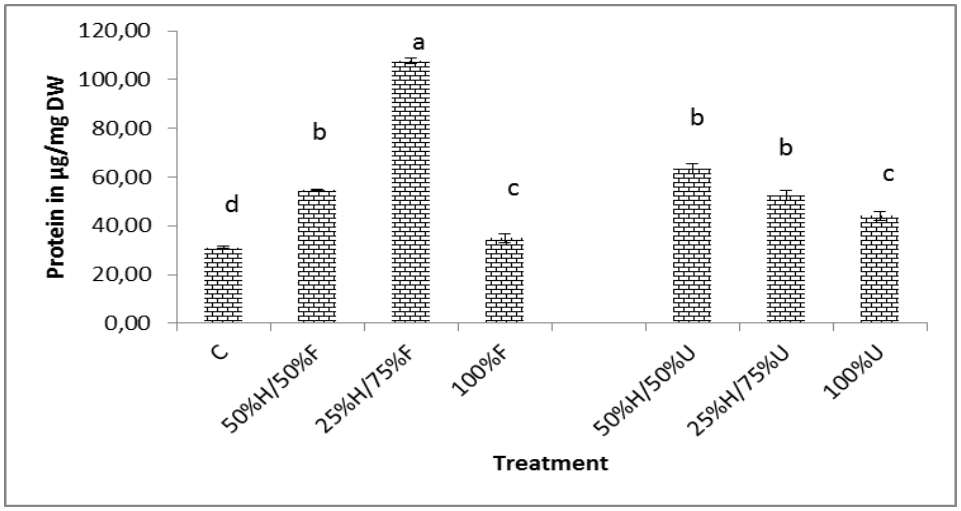

Figure 4: Effect of seaweed extract on protein content in the leaves of Bean plant added to Hoagland's solution (H) at different ratio.
C: Control Plants, H: Hoagland's nutrient solution; F: *Fucus spiralis* extract (25%) ; U : *Ulva rigida* extract (25%)
Results are means ± S.D (n = 3). Different letters show statistically significant differences for P< 0.05 level.

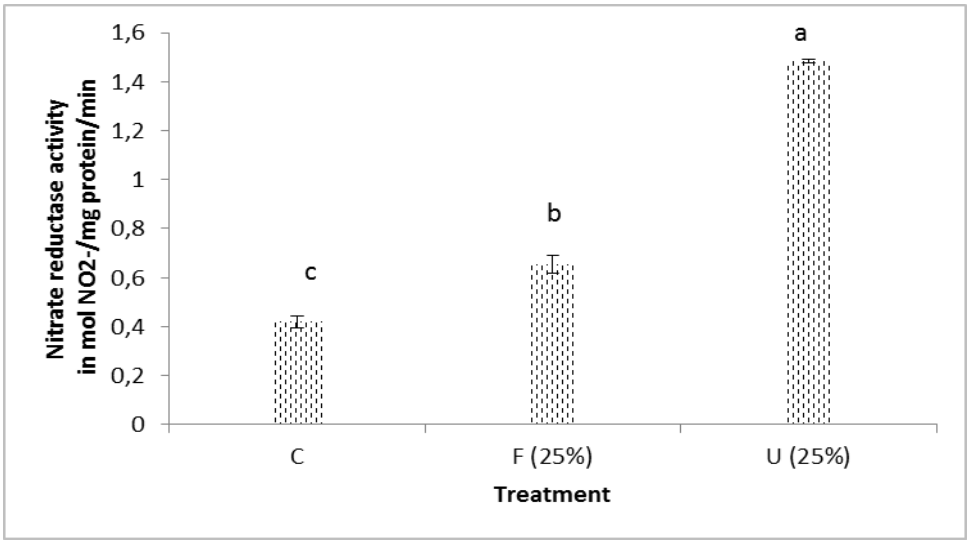

Figure 5: Effect of seaweed extract on leaves Nitrate reductase activity of Bean plant sprayed with 25% of *Fucus spiralis* (F 25%) and 25% of *Ulva rigida* (U 25%)
C: Plant control without seaweed extract.
Results are means ± S.D (n = 3). Different letters show statistically significant differences for P< 0.05 level.

With respect to leaves Nitrate reductase activity (Figure 5), the foliar application of seaweed extract *Fucus spiralis* and *Ulva rigida* have a significant effect in bean leaves (0.653 mol NO_2^-/mg protein/min and 1.48

mol NO $_2^-$/mg protein/min respectively) compared to the control plant (0.42 mol NO_2^-/mg protein/min). The high activity was recorded by treatment *Ulva rigida* (25%).

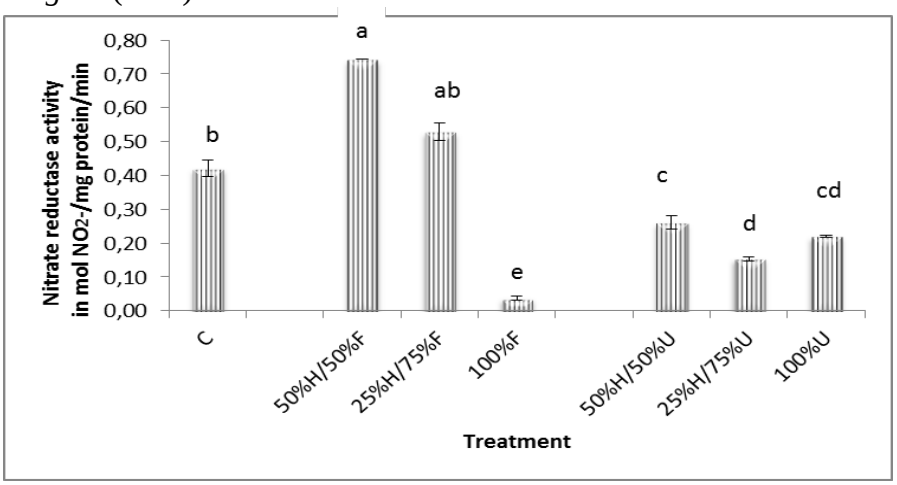

Figure 6: Effect of seaweed extract on Nitrate reductase activity in the leaves of Bean plant treated in the medium with 25% of Ulva rigida (U) and 25% of Fucus spiralis (F) added to Hoagland solution (H) at different ratio.

C: Control Plants, H: Hoagland's nutrient solution; F: *Fucus spiralis* extract ; U: *Ulva rigida* extract

Results are means ± S.D (n = 3). Different letters show statistically significant differences for P< 0.05 level.

As shown in figure 6, when SLE incorporated in medium culture at different ratio, The lower proportions of the *Fucus spiralis* extract (50% and 75%) enhanced significantly the Nitrate reductase activity in bean plants (0.74 NO_2^- mol/ mg protein /min and 0.53 NO_2^-mol/mg protein/min respectively) compared to the control one (0.42 mol NO_2^-/mg protein/min).

In the cases of the other treatment, we found that all proportions of *Ulva rigida* extract (50%; 75% and 100%) and 100% of *Fucus spiralis* extract decreased nitrate reductase activity in leaves plants.

Table 2: Mineral and chemical constituents of *Fucus spiralis* and *Ulva rigida* seaweed extract.

Algal species	Mineral content in ppm			Total nitrogen NTK in (%)	Betain content in mg/g in FW
	Na	Ca	K		
Ulva rigida	42.4 ±0.3	91.9±2.8	55.8±4.4	1.28	0.85±0.02
Fucus spiralis	72.5±0.1	51.2±2.8	142.9±5.4	0.56	0.44±0.07

Na: Sodium; Ca: Calcium; K: Potassium.

Results are means ± S.D (n = 3). Different letters show statistically significant differences for P< 0.05 level.

The Thallus of two macroalgae contained higher levels of sodium (Na), potassium (K), calcium (Ca) (Table2). The *Fucus spiralis* contained more Na and K (72.5 ppm and 142.9 ppm respectively) mineral, while *Ulva rigida* contained more content in Ca (91.9 ppm).

Regarding to the richness of total nitrogen, this green algae (1.288 NTK (%)) contains high level compared to the brown one (0.56 NTK (%)).

The Glycinebetaine analyses showed a significant difference between two algae species studied. The highest accumulation was recorded to *Ulva rigida* (0.85 mg/g FW) compared to *Fucus spiralis* (0.44 mg/g FW).

Discussion

The seaweed extract application in bean plant enhanced the vegetative growth at lower concentration 25% of *Fucus spiralis* and 25% of *Ulva rigida*. Our findings coincide with those of earlier studies carried out on Salvia officinalis (EL KAOUA et al., 2013), on soybean (Rathore, Chaudhary and al., 2009) where there was an increase in vegetative growth by the application of seaweed extract. Similar results were also observed in Cajanus cajan (L.) Millsp. (Mohan et al., 1994) and Vigna sinensis L. (Sivasankari et al., 2006). Our results also agree with those reported by Thirumaran et al. (2009). In fact, lower concentration of seaweed extract prepared from brown algae, *Rosenvingea intricata* induced maximum growth in *Abelmoschus esculentus* plant, while higher concentration of SLE showed a decreasing trend. The negative responses of increased concentration of seaweed extracts on vegetative growth plant can be attributed to presence of regulator hormones or high levels of minerals.

Seaweeds contain all the trace elements and plant growth hormones required by plants. It was also reported that seaweed manure is rich in potassium but poor in nitrogen and phosphorus (Kingman, A.R., and J. Moore, 1982).

The enhancing of vegetative growth can be related to seaweed components such as macro- and microelement nutrients, amino acid, vitamins, cytokinins, auxins, and abscisic acid (ABA)-like growth substances affect cellular metabolism untreated plants leading to enhanced growth and crop yield (Crouch and al., 1992; Crouch and van Staden 1993; Reitz and Trumble 1996; Durand and al., 2003 and Stirk and others 2003). Liquid extract obtained from seaweeds has recently gained much interest as foliar spray for inducing growth and yield in cereal crops, vegatables, fruits, orchards and horticultural plants (Thivy, F., 1961; Metha, V.C. and al., 1967 and Bokil, K.K. and al., 1974).

In our study we evaluated the SLE effect on the change of some biochemicals and physiologicals parameters of bean plant related to nitrogen

metabolism in hydroponic system such as: nitrate reductase activity, chlorophyll content and protein content.

Seaweed application of bean plant by foliar spray or by incorporated in medium culture increases leaf pigment (particulary chl a) and protein content. But at high proportion of seaweed extract in medium culture (100% of *Fucus spiralis* extract; 75% and 100% of *Ulva rigida* extract) decreases these parameters.

Our results coincide with those of studies obtained by Blunden and al., 1977, when seaweed applied at low concentration of *Ascophyllum nodosum* extract to soil or on foliage of tomatoes produced leaves with higher chlorophyll content than those of untreated controls. This increase in chlorophyll content was a result of reduction in chlorophyll degradation, which might be caused in part by betaines in the seaweed extract (Whapham and al., 1993).

We showed that seaweeds extracts were rich of glycinebetaine. This component delays the loss of photosynthetic activity by inhibiting chlorophyll degradation during storage conditions in isolated chloroplasts (Genard and al., 1991).

With respect to nitrate reductase activity, the foliar application of seaweed extract increased this enzymatic activity. *Ulva rigida* extract was found more effective than *Fucus spiralis*. When SLE of *Ulva rigida* extract incorporated in medium culture we noted an important decrease of nitrate reductase activity. However the incorporation of *Fucus spiralis* extract in medium culture showed a remarkable increased of nitrate reductase activity particularly at proportion (50% and 75% of *Fucus spiralis* extract) while a high proportion of this extract (100% of *Fucus spiralis* extract) decrease the nitrate reductase activity.

Our result showed that the activation of nitrate reductase was related to the presence of seaweed extract applied on bean plant by foliar spray. However the incorporation in medium culture inhibits this enzymatic activity.

This decrease in nitrate reductase activity might be due to the consequence of the presence of the high concentration of mineral elements that can inhibit this enzymatic activity. This finding was also reported by Esteban and al., 2003 as the most important enzyme activities within nitrogen metabolism, such as nitrate reductase, nitrite reductase, which were affected negatively by the highest NH_4NO_3 rate applied to *Phaseolus vulgaris* plant at different levels.

The seaweed extract by foliar application provides the assimilation of biostimulant by leaves such as growth hormone or others metabolites that were responsible for enhancement of nitrate reductase activity, synthetic

chlorophyll and protein. We can also note the possible relationship of the nitrate reductase activity with protein content.

Conclusion

The enhancing vegetative growth of bean plant by seaweed extract was related to activation of nitrate reductase, key enzyme of nitrogen metabolism.

The nitrate reductase activity was not dependent on the richness of seaweed extract in mineral element particularly nitrogen. However we can conclude that the biostimulant assimilated by leaves plan by foliar spray can enhance the nitrate reductase activity. Although many of the various chemical components of seaweed extracts and their modes of action remain unknown, it is plausible that these components exhibit synergistic activity.

As perspective, we believe that it would be beneficial to carry out more research including characterization of growth hormones and others metabolites. We can also evaluate the effect of seaweed extract on nitrogen metabolism in root and leaves of bean plant cultivated in medium culture deficiency or richened of minerals elements.

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