



Biofertilizer efficiency of seaweed liquid extracts of marine green and red macro algae on growth and biochemical parameters of Hot Pepper (*Capsicum annuum* L.)

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Abstract

Seaweed liquid extracts are used as nutrient supplements, biostimulants and biofertilizers as an alternative to chemical fertilizers in agriculture. The study was set up to evaluate the biofertilizing efficiency of seaweed liquid extracts (SLE) of green and red marine alga (*Ulva flexuosa*, *Enteromorpha intestinalis* and *Griffithsia teges*) on growth, biochemical and yield of Hot Pepper. To achieve the objectives, Seaweed liquid extracts at concentration 2.0% were prepared and applied as foliar spray on Hot Pepper seedlings raised in experimental pots maintained under natural conditions. After 30 and 70 days, the growth and biochemical parameters and yield attributes were measured, respectively. The results exhibited that the extracts of *Ulva flexuosa* and *Enteromorpha intestinalis* more efficient than *Griffithsia teges* (shoot and root length, total fresh and dry weight, leaf area, the photosynthetic pigments, and phytohormone analysis). The auxins in the (*Ulva flexuosa*, *Enteromorpha intestinalis* and *Griffithsia teges* (7.41 mg/ml, 10.6 mg/ml and 11.58 mg/ml) respectively and gibberelins were (15.00 mg/ml, 15.22 mg/ml and 7.26 mg/ml) respectively. Thus, *Ulva flexuosa* and *Enteromorpha intestinalis* liquid extract could serve as a promising effective organic biostimulant to replace the chemical fertilizers for sustainable agriculture.

Keywords: Liquid biofertilizer, phytohormones, Growth, Biochemical, seaweed liquid extracts (SLE)

Introduction

Chemical fertilizers, pesticides, fungicides, and herbicides give immediate results; however, their continuous use has an adverse impact on the quality of the soil, the beneficial soil microbial communities, the soil's fertility, and on the plants cultivated in these soils. Seaweeds are effectively used as bio-fertilizers because they include

high levels of organic matter, which leads to soil nutrient enrichment (Kumareswari et al., 2015 ; Layek et al., 2018). These extracts are marketed as liquid bio-fertilizers because the chemical analyses of seaweeds and their extracts have revealed the presence of a wide variety of plant growth-promoting substances such as auxins, cytokinins and betaines (Khan et al. 2009) . In addition, they were found to be a better and more suitable alternative to chemical and mineral fertilizers when used in adequate quantities (Mirparsa et al . , 2016).

Many recent studies have discovered wide applications of these marine macro algae (in the form of finely powdered or aqueous extracts) as eco-friendly fertilizers in modern agriculture and horticulture crops improvements (Hernández Herrera et al , . 2018) . In modern agriculture, chemical fertilizers have degraded the fertility of soil making it acidic and rendering it unsuitable for raising crop plants. The intensive use of inputs has led to severe health and environmental hazards viz., soil erosion, water contamination, pesticide poisoning, water logging and depletion of biodiversity. The practice of chemical farming has also put the long-run sustainability of Egypt agriculture and the survival of the farming community at risk. In recent years, the use of natural seaweed as fertilizer has allowed for partial substitution of conventional synthetic fertilizer (Khan et al . , 2009; Zodape et al . , 2010).

In addition, a number of commercial seaweed extract products are available for use in agriculture and horticulture. A number of seaweeds used a liquid fertilizer by applying them as foliar spray, soil drench, or in granular/ powder form as soil conditioners and manure (Thirumaran et al . , 2009).

Seaweeds are one of the important marine bio-resources which are nowadays termed as fantastically promising plants. Seaweeds and their derivatives are used in agriculture as potential plant growth regulators. Moreover, agriculture in many parts of the world (Eyras et al . , 1998) and are an inexpensive local resource in coastal agricultural areas. Seaweed contains all major and minor plant nutrients, trace elements, vitamins, auxins and other bioactive substances. The growth promoting efficiency of fertilizing efficiency of extracts of several marine algae was evaluated in the cereals, pulses and vegetable crops (Kalidass et al . , 2010; Sasikumar et al . , 2011; Zodape et al . , 2011; Bai et al . , 2013; Parthiban et al . , 2013; Kalaivanan et al . , 2012; Hernández-Herrera et al . , 2014).

Hot Pepper (*Capsicum annum*) is an important universal vegetable crop in terms of its commercial value and rural economic significance (Aktas et al . , 2009 and Gonzalez-Diaz et al . , 2009). On the other hand, biostimulants represent precious biostimulants to increase plant growth, yield, and quality in sustainable farming systems, especially the decreased practice of industrial synthetic fertilizers to potentially minimize its harmful impact on the agricultural environment for better future sustainability (Helaly et al . , 2020)

The aim of the study to evaluate the bio-fertilizing potential of liquid extracts of marine green and red algae on growth, biochemical and yield of Hot Pepper under natural environmental condition.

Materials and Methods

Algal Collection

The collected algal samples were selected and harvested by hand during low tide from Baltem, Kafer Elsheikh, Mediterranean coast Fig (1). The collected algal samples washed thoroughly with sea-water and hand brushed to remove macroscopic epiphytes and sand particles. Then, they were washed with tap water to remove adhering salt. The algal samples were blotted and air-dried in room temperature for 6 days to remove excess water. The dried seaweed samples were ground into a fine powder by a mechanic grinder and kept in plastic tubes for further analyses.



Fig (1) collected algal samples

Preparation of liquid extracts of seaweeds

The dried samples of seaweed (1kg) was further made into small pieces and powdered. The powdered samples of seaweed was mixed with water (20 litres) in the proportion of 1:20. Then it was boiled for one hour. After one hour, the mixture was squeezed and filtered through muslin cloth according to (Bhosle et al., 1975). The obtained extracts were designated as stock solution and was used to prepare concentration 2.0 % by mixing appropriate proportions of seaweeds liquid extract (LE) with sterilized distilled water.

Physico-chemical analyses of seaweed extracts

The physical observations such as colour and pH were made using standard methods. The presence of elements such as copper, manganese, zinc, iron, potassium, magnesium, cobalt and sodium were estimated using Atomic Absorption (ICP MS Analyzer - GEBRI/AQ/01/06/00) Environmental and Food Biotechnology Lab, University of Sadat City.

Extraction, Separation, and estimation of growth regulating substances by High-Performance Liquid Chromatography (HPLC)

The extraction procedure was according to the one used by (Shindy and Smith, 1975). Identification and determination of Indol Acetic Acid (IAA), Gibberellic Acid (GA3), and Abscicic acid (ABA), analysis was performed by HPLC- LC1620A.

Preparation of pot study and measurments

Healthy seedling of Hot pepper plants variety " Loggin hybrid " were purchased from Agriculture Research Center , Giza, Egypt .They were sown in plastic pots (9 cm) filled with a sterilized standard soil mix supplemented with sufficient quantity of N P K . Potted plants were treated with 2.0 % concentration of sea weed liquid extracts in the form of foliar spray . In treating plants, growth parameters viz., shoot length, root length, total height, total fresh and dry weight, leaf area , Pho- tosynthetic pigments (Arnon 1949) , were assessed in the leaves of treated plants. Similarly, yield parameters such as number of fruits and fruit weight were also observed. Growth and biochemical parameters were recorded in 30-day-old for treated and control plants. After 70 days, yield characters were observed. Plants irrigated with water alone served as control. All pot experiments were done in three replicates each under natural uniform conditions .

Statistical Analyses

All experiments were run in triplicate. All data were subjected to Analysis of Variance (ANOVA), and the deifferences between means were evaluated by Duncan's Multiple Range Test. SPSS statistic program. statistical analysis software package; version 16.0

Results

Identification of tested algal samples :

In the current study, seaweeds of green algae *Ulva flexusoa* Fig (2), *Enteromorpha intestinalis* (Linnaeus) Nees Fig (3) (family: Ulvaceae), and red algae *Griffithsia teges* (Family: Ceramiaceae)Fig (4) These were identified in accordance with Bhavanath Jha et.al (2009) and confirmed by Prof . Mervat Hosny Prof . algae, Faculty of science – El-Mansoura University.



Fig (2) *Ulva flexusoa*



Fig (3) *Enteromorpha intestinalis*



Fig (4) *Griffithsia teges*

Physio - chemical analysis of liquid extracts of chosen samples

Table .1. Physical analyses of liquid extract of tested samples:

Physical parameters	Ulva flexusoa	Enteromorpha intestinalis	Griffithsia teges
Colour	Green	Green	Red
pH	6.9	6.7	6.3

The mineral analyses of liquid extract of tested algae

All the parameters in chemical analysis of algal extracts (Ulva flexusoa, Enteromorpha intestinalis and Griffithsia teges) given are in ppm were operated in Enviromental and Food Biotechnology Lab, University of Sadat City. The copper (5.734 , 2.230 and 11.086 ppm) manganese (14.365 ,16.231 and 18.163),zinc (11.919, 5.767 and 7.839) iron (6.100 , 5.291 and 12.649), cobalt (1.015, 0.994and 6.562) sodium (1048.126,107.043 and 106.124) nd magnesium (49.752, 20.708 and 28.435) phosphours(7.333, 10.879and 10.640) and potassum (7.785, 9.944and 6.914) were in appreciable level (Table (2)). Among the elements estimated, Sodium , calcium and zinc were found to be abundant in the three extracts.

Table (2) mineral analyses of liquid extract of teted algae :

NO .	Labe l	Ulva flexusoa	Enteromorp ha	Griffithsi a teges	NO .	Labe l	Ulva flexuso	Ent. intestinal	Griffithsi a teges
1	Li	0.367	0.227	0.426	13	Zn	11.919	5.767	7.839
2	B	6.052	3.833	4.295	14	Ga	0.312	0.261	0.633
3	Na	1048.126	107.043	106.124	15	Se	0.105	0.089	0.094
4	Mg	49.752	20.708	28.435	16	Se	0.156	0.127	0.116
5	Al	5.490	5.228	16.158	17	Sr	3.838	3.618	3.739
6	Si	1.990	1.948	3.900	18	Ag	0.044	0.046	0.328
7	P	7.333	10.879	10.640	19	Cd	0.233	0.177	0.573
8	K	7.785	9.944	6.914	20	In	0.073	0.044	0.050
9	Ca	105.296	100.428	60.968	21	Fe	6.100	5.291	12.649
10	Ti	18.297	12.592	19.640	22	Co	1.015	0.994	6.562
11	Cr	0.362	0.277	0.472	23	Ni	0.330	0.247	0.480
12	Mn	14.365	16.231	18.163	24	Cu	5.734	2.230	11.086

Hormone analyses of liquid extract of tested algae -:

The results showed that the standared sample of phytohormone analysis were , the auxins were (5.22 mg/ml) , Absecic acid was (7.36 mg/ml) , gibberellins (11.23 mg/ml) and cytokinins were (4.40 mg/ml) , Table (3) and Fig (5)

Table (3). Hormone analyses of Standard Sample

No	Name	RT Time	Concentration (µg/ml)
1	Gibb	1.0	11.23
2	ABA	1.8	7.36
3	Aux	5.0	5.22
4	Cyto	7.0	4.40

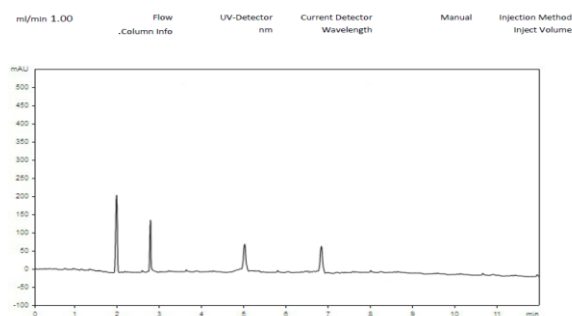


Fig (5). Hormone analyses of Standard Sample

The results showed that phytohormone analysis, the auxins in the *Ulva flexusoa* extract(7.41 mg/ml), Absecic acid was (10.26 mg/ml) and gibberellins (15.00 mg/ml) . The results showed that gibberellins to be more when compared to other hormone Table(4) and Fig (6)

NO.	Name	RT Time	Concentration (Mg/ml)
1	Gibb	2.0	15.00
2	ABA	2.8	10.26
3	Aux	5.0	7.41

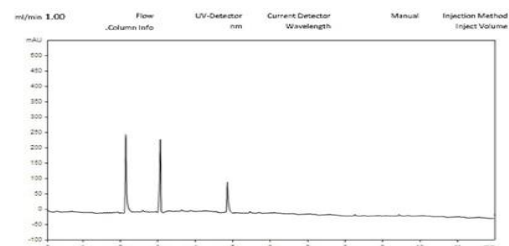


Fig. (6). Hormne analyses of of *Ulva flexusoa*

The results showed that phytohormone analysis, the auxins in the *Enteromorpha intestinalis* extract(10.6 mg/ml) , Absecic acid was (6.33 mg/ml) and gibberellins (15.22 mg/ml) , the results found that, gibberellins to be more when compared to other hormone Table(5) and Fig (7)

NO.	Name	RT Time	Concentration (Mg/ml)
1	Gibb	2.0	15.22
2	ABA	3.0	6.33
3	Aux	5.0	10.6

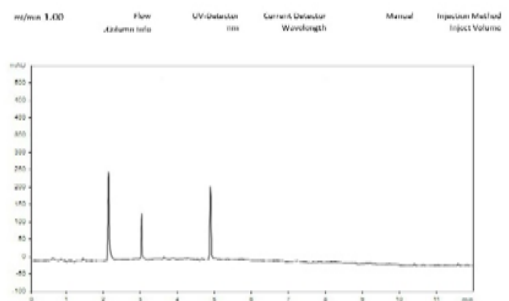


Fig. (7) Hormone analyses of *Enteromorpha* sp.

The results showed that phytohormone analysis, the auxins in the Griffithsia teges extract (11.58 mg/ml) , Absecic acid was (9.15 mg/ml) , gibberellins (7.26 mg/ml) and Cytokinin(5.11 mg/ml) , the results showed that Auxins in higher amounts when compared to other hormones. Table (6) Fig (8)

NO.	Name	RT Time	Concentration (Mg/ml)
1	Gibb	2.0	7.26
2	ABA	3.0	9.15
3	Aux	5.0	11.58
4	Cyto	6.9	5.11

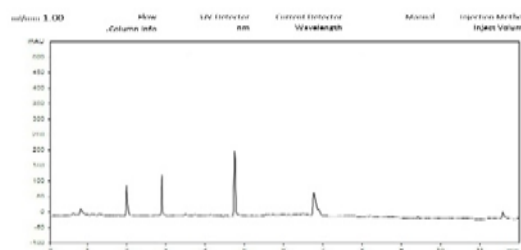


Fig (8) . hormone analyses of Griffithsia teges

Growth parameters and Yield attributes of Hot Pepper plants

Regarding to growth parameters in case of three algal extracts *Ulva flexusoa*, *Enteromorpha intestinalis* and *Griffithsia teges*, treatments were presented in table (7). The total height were (29 , 26 and 17cm) compared with control (20 cm) , The total fresh weight were (5.664,3.570 and 3.110 gm) compared with control (3.690 gm) , the total dry weight were (2.133 , 1.874 and 1.014 gm) compared with control (1.389 gm) Table (7) and Fig. (9).

Table (7) The mean values of the Growth parameters and Yield attributes of Hot

Growth&yield parameters	Co ntr ol	Ulv a Sp.	Ent ero. Sp.	Griffit hsia teges	Growth&yield parameters	Cont rol	Ulva Sp.	Ente ro.S p.	Griffit hsia teges
Shoot length(13	19	18	11	Fresh weight of	3.33	5.11	3.08	2.72
Root length(cm)	7	10	8	6	Fresh weight of root(gm)	0.36	0.55	0.49	0.39
Total height	20	29	26	17	Total Fresh	3.69	5.66	3.57	3.110
Number of branches	3	5	4	2	Dry weight of shoot(gm)	1.18	1.73	1.53	0.768
Number of	12	28	25	18	Dry weight of	0.20	0.40	0.34	0.246
Leaf area	12.	18	12.	9.2	Total Dry weigh	1.38	2.13	1.87	1.014
Number of flowers	2	4	3	2	Number of fruits	4	6	5	2

Pepper plants



Fig (9) : Growth parameters and Yield attributes of Hot Pepper plants

Biochemical characteristics of Hot Pepper plants

In Hot Pepper plants, there was a noticeable increase in bio-chemical parameters when 2.0% of seaweed liquid extracts (SLE) of *Ulva flexusoa* , *Enteromorpha intestinalis* and *Griffithsia teges* applied to Hot Pepper plant. The content of total chlorophyll pigments were enhanced when the Hot Pepper plants were treated with SLE at 2.0% concentrations of (Table 8). However, steep decline was recorded in plants that received 2.0 % of the *Griffithsia tege* extract

The mean values Biochemical characteristics of Hot Pepper plants)(Table

Biochemical parameters	Contr ol	Ulva flexuso a	Enteromorp ha intestinalis.	Griffithsi a teges
Cholrophyll(a)	38.5	57.9	54.00	37.9
Cholrophyll(b)	32.00	48.8	45.9	38.6
Carotine	33.1	46.7	44.3	34.5

Discussion

Growth enhancement by seaweed extracts may be due to components such as macro and micro elements, amino acids, vitamins, cytokinins, auxins and abssisic acid (ABA) like growth substances which affect cellular metabolism in treated plants

leading to enhanced growth and crop yield (Ordog et al., 2004; Durand et al., 2003). Similarly, in our study, the increased growth of Hot Pepper plants could be associated with the occurrence of some growth promoting substances present in the seaweed extract as in other macro algal extract. The growth hormones play role in enhancement of cell size and cell division and together they complement each other as cytokinins are effective (Mooney and Van Staden, 1986 ; Ashour et al.,2021).

In our present study, presence of phytohormones such as auxin, cytokinin and gibberellins in addition macro and micro nutrients such as copper, manganese, iron, zinc, cobalt, potassium, magnesium and sodium were detected in the crude extracts of *Ulva flexuosa*, *Enteromorpha intestinalis* and *Griffithsia tegetes* and the among the three seaweed extracts, *Ulva*, was found to contain maximum amount of phytohormones and nutrient content. Many types of plant growth regulators that have been identified in seaweed extracts, such as auxins, cytokinins, gibberellins and abscisic acid (Khan et al., 2009; Kurepin et al., 2014) .The presence of phytohormones were in agreement with the earlier findings that reported auxins in the extracts of *Ascophyllum nodosum* (Sanderson and Jameson,1986) cytokinins in the extracts of *Ulva* (Sekar, 1995 ; Craft et al., 2007) which stimulates early seedling growth in the plants.

In our study , the foliar application of 2.0% of *Ulva flexuosa* and *Enteromorpha intestinalis* seaweed liquid extracts significantly enhanced the overall growth and physiology of Hot Pepper *Capsicum annum* plant. Similar reports regarding seaweed as biostimulants has been reported in *Calibrachoa* (Elansary et al., 2016) *Lablab purpurens* (Vishnupriya and Flora, 2017), *Capsicum annum* and *Lycopersicum esculentum* (Divya and Niranjana, 2017)

In the present study, The increase in the growth paramerters and photosynthetic pigments at 2.0% concentrations of SLE confirmed the efficiency of foliar spray as it enhanced the absorption of most of the necessary elements by the seedlings. The increase in chlorophyll content could also be a result of reduction in chlorophyll degradation, which might be caused in part by betaines in the seaweed liquid extract (Whapman et al., 1993). In addition, in a study, *Ulva lactuca* extract recommended rate of enhanced the content of photosynthetic pigments in Hot Pepper (Sridhar and Rengasamy, 2010).

Suganthi and Sujatha (2014) reported that foliar application of 5% *Sargasum myricosysutum*, and *Caulerpa racemosa* enhanced the growth attributes viz., plant height, dry matter production, leaf area index, crop growth rate of sunflower hybrid plant.

In our experiment, concentration of 2.0% extracts showed differential responses in the yield characters also. Significant increase in number of flowers and fruits was noticed when 2.0% of green algal extracts was given as foliar spray. However, decrease in number and weight of the fruits was observed in red alga .

Conclusion

Our results show that enhancement of growth, biochemical and yield parameters of Hot Pepper plant may be due to the presence of differential level of micro and macro elements, growth hormones, in the liquid extracts of three algal extracts *Ulva flexuosa*, *Enteromorpha intestinalis* compared with *Griffithsia teges*. However, optimum concentration of seaweed liquid extracts is necessary as the present study exhibited that 2.0 % SLE had better influence on growth and productivity of tested plant and used as eco-friendly fertilizers in modern agriculture under natural environmental condition.

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