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Foliar application of seaweed extracts as a means for enhancing the yield and safety of *Triticum aestivum*

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ABSTRACT:

There is a great contamination of water, soil, air, soil erosion, pest resistance and extensive use of chemical fertilizers cause a turn to induce agriculture for producing safe and clean products. For this purpose, by using natural materials like algae on the beach, organic and environmentally friendly fertilizer was prepared. In this investigation effect of Seaweed Liquid Fertilizer (SLF) of *Ulva fasciata* at concentrations of 0%, 2.5%, 5%, 7.5% and 10% on some parameters of wheat (*Triticum aestivum var. chamran*) has been investigated. Based on the results, above algae can be used as a food supplement solution in the form of foliar spray at 2.5% concentration with a significant increase in morphological and biochemical indices.

Keywords:
Seaweed, *Triticum aestivum, Ulva fasciata,* Fertilizer.

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INTRODUCTION

Iran along with the other countries has expanded its use of chemical fertilizers so that in recent years, it has reached more than 4 million tons per year. Indiscriminate use of chemical fertilizers directly and indirectly created numerous environmental effects, soil erosion and problems of human health. Fertilizers with increasing water demand of land have changed the time of need of nutrients for plant, and this action help rock agglomeration and depletion of soil from humus substances. Chemical fertilizers increase soil and plant needs to more inputs such as fertilizers and pesticides and for this reason, the distribution of chemical fertilizers is also increasing in the country. Based on the obtained results in Iran the use of chemical fertilizers showed growth at the rate of 5.5% per year. The remnants of these substances pollute groundwater and air in addition to, absorbed by the plants and are transmitted to humans through consumption (Serpil, 2012). Considering that the 2.1% of agricultural land of the world is located in Iran and only 3.0 percent of the world's pesticides are used in Iran so, the situation is better than the global average. Sustainable agricultural policy, have prompted experts that the more natural ingredients in the soil to help plants take food needs and thus began the production of organic fertilizers. The purpose of use of organic fertilizers, is to boost soil fertility, harvest more without polluting the environment and providing healthy food and richer. As a result, the final product will be organic products which are free of toxic residues, chemical and preservative (Thirumaran *et al*., 2009). For the first time the use of algae as fertilizer is happened by coastal. From one side algae due to high levels of fiber have an important role in softening the soil and retain moisture on the other side, because of the minerals and trace elements are much more important. Seaweeds have macro and micro nutrients, amino acids, vitamins, cytokinin, auxin and abscisic acid; because of these materials it stimulate growth and plant products, creating resistance to environmental stress, increase the absorption of nutrients from the soil and increases their antioxidant properties (Erulan, 2009). Studies have proved that this product is widely used in science and horticulture industry, so that using of these products is followed by some benefits including increased production, soil nutrients, resistance to certain pests, seed germination and resistance to frost (Rathore *et al*., 2009). Bhosle (1975) observed that the value of seaweeds as fertilizer is not only because of contents like nitrogen, phosphorus and potassium but it is due to its trace elements and metabolites. When aqueous extract of *Sargassum wightii* through the application of foliar spray was applied on *Zizyphus mauritiana* it led to increased yields and fruit quality (Rao, 1991). In a trial that Thirumaran *et al*. (2009) had applied application of seaweed as fertilizer and showed faster growth and a higher rate of development. Today algae extracts are available commercially under names like Cytex, Algifert (marinure), Sea crop 16, Seaspray, Seasol, SM3 and Kelpak (Sivasankari *et al*., 2006) for utilization as fertilizers.

Table 1. Date of the tests and the amount of replication

Experiment stages	Wheat cultivation	First foliar application	First harvest (vegetative stage)	Second foliar application	Second harvest (ear emergence stage)	Third foliar application	Third harvest (ripening stage)
Date	11/03/13	29/12/13	01/02/14	01/02/14	26/02/14	26/02/14	17/04/14
Amount of solution	-	250 ml for 3 repetition	-	250 ml for 3 repetition	-	250 ml for 3 repetition	-

MATERIALS AND METHODS

Collection of seaweeds

The seaweeds utilized as a part of the present investigation was *Ulva fasciata* (class Chlorophyceae). They were gathered from the coastal region of Chabahar, Iran (25° 17_ N and 60° 37_ E) amid November, 2013. The seaweeds were handpicked and washed altogether with seawater to expel all the undesirable impurities.

Preparation of seaweed liquid fertilizer

Freshly gathered seaweeds were shade dried for ten days. Dried material was finely powdered. Fifty gram of finely powdered material was extracted with 500 mL boiling water for 60 min. The hot extract was filtered through a double layered cheese cloth and permitted to cool at room temperature. The subsequent extract was taken as 100% concentration of the SLF (Ramarajan, 2012). As the seaweeds liquid fertilizer contained organic content, they were refrigerated in the vicinity of 0 and 4°C.

Physico-chemical analyses of SLF

The colour, pH, nitrate, phosphorus, potassium, iron, zinc, copper and manganese content were investigated using readily available kits and are depicted in Table 2.

Experimental design and treatments

This investigation had two phases, in the main phase hundred seeds were soaked in 5 petriplates for each treatment. The treatments were 2.5%, 5%, 7.5%, 10% aqueous extracts of seaweeds. Five petriplates of seeds were considered as the control with 10ml of distilled water and the leftovers of them were treated with 10 ml of 2.5%, 5%, 7.5%, 10% of aqueous extract of seaweeds at the first and after three days. All petriplates were taken up against seventh day subsequent to sowing.

Table 2. Physico-chemical properties of SLF of *Ulva fasciata*

General parameters	*Ulva fasciata*
Color	Light green
pH	6.8
Chemical	**parameters**
Nitrate	14
Phosphorus	ND
Potassium	136
Iron	0.32
Zinc	1.1
Copper	0.88
Magnesium	102.4

Second phase was carried out during 2013-2014 years in the Shavoor on the experimental field of Institute of Agricultural Sciences of Khozestan, Ahvaz. The experimental site is located at 31° 50′ N and 48° 28′ E. The experiment was laid out in a randomized block design with three replications and fourteen treatments viz., 2.5, 5.0, 7.5 and 10.0% (v/v) of *Ulva fasciata* saps and a control with water only also, in order to compare seaweed fertilizer and chemical fertilizer a chemical treatment was considered on the technical feasibility. The seeds of wheat were gathered from Chamran University, Khozestan, Ahvaz, Iran. They were surface disinfected with 5% sodium hypochlorite (Shahbazi *et al.*, 2015). The seeds with uniform size, colour and weight were taken for test further. Plots included 30 cm diameter pots with a capacity of 20 kg. Since 150 Kg of seed

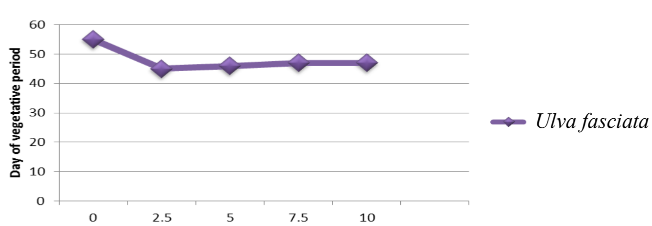

Figure 1. Length of the vegetative period

used per acre and our pots had about 0/07 m^2 area, approximately one gram of seed per pot was given. All used algal fertilizers were applied as foliar spray, three times with an interval of one month, over a period of about 30 days after planting. The plants were harvested at three stages, vegetative, ear emergence (60 days old) and ripening (Table 1).

Growth and biochemical analysis

The growth parameters including shoot length, fresh weight, dry weight, shoot diameter, spike length, spike weight, number of grain per spike, grain weight of a spike, thousand seed weight, seed yield, harvest index of spike, harvest index [general] and leaf area were figured. The biochemical constituents, for example, chlorophyll a, b, and total chlorophyll content, carotenoid, total sugar content, protein, malon dialdehyde and leaf oil content were evaluated in wheat. Also phenology traits such as length of vegetative period and reproductive period and changes in activity of antioxidant enzymes such as peroxidase, catalase and ascorbate peroxidase were studied.

RESULT AND DISCUSSION

The physico-chemical properties of SLF of *Ulva fasciata* were investigated and are exhibited in Table 2. The chemical of the SLF *Ulva fasciata* was light green. The pH of SLF of *Ulva fasciata* was 6.8. The content of potassium was higher than other components and the content of phosphorus was less than others.

The results showed that the highest percentage of germination related to the concentration of 2.5% compared to control (Table 3). A similar experiment on *Ziziphus mauritiana* confirmed this result (Rao, 1991). This increase might be due to the presence of growth hormones such as auxin, gibberellin, cytokinin, and etc. in seaweed extract. Growth hormones increased the starch degradation by recreation of hydrolysis enzymes and therefore increases the germination (Ramarajan, 2012). In the present experiment there was observed a significant rise in growth parameters including shoot and root length, shoot and root dry and fresh weight (Table 3, 4, 5 and 6). Sivasangari (2011) has observed

Table 3. Effect of SLF of *Ulva fasciata* on the growth and biochemical parameters at the germination stage

S. No	Carbohydrate (mg/g.D.w.)	0.019±0.002fe	0.023±0.002dcb	0.022±0.003fe	0.018±0.003dcb	0.020±0.003ba
1	Carotenoid (mg/g.f.w.)	0.263±1.448ba	0.262±4.417cb	0.245±1.703dc	0.240±1.701dc	0.251±2.684cb
2	Chl. a /Chl. b	0.060±0.009e	0.122±0.001ba	0.112±0.000cba	0.101±0.001edcba	0.106±0.005dcba
3	Chl. B (mg/g.f.w.)	0.073±0.001g	0.083±0.001fe	0.077±0.002i	0.072±0.003e	0.098±0.001cb
4	Chl. A (mg/g.f.w.)	0.479±0.005g	0.513±0.003ba	0.484±0.007cb	0.462±0.007edc	0.502±0.004cb
5	Root length (cm)	4.70±0.013hg	6.09±0.014a	5.37±0.024dcba	4.58±0.053edc	5.64±0.015ba
6	D. w. of shoot (g)	0.170±0.05	0.210±0.07	0.191±0.0b	0.182±0.05b	0.208±0.06a
7	F. w. of shoot (g)	1.220±0.034ba	2.636±0.088ba	1.986±0.033ba	1.906±0.065ba	1.974±0.049ba
8	Shoot length (cm)	4.91±0.067edc	9.58±0.214edc	7.56±0.115dcb	7.54±0.020cb	8.17±0.035ba
9	Seed germination (%)	80	86.6	80	73.3	76.6
10	Con.	0	2.5	5	7.5	10

Table 4. Effect of SLF of *Ulva fasciata* on the growth and biochemical parameters at the vegetative stage

S. No	Oil content (mg/g.D.w.)	0.021±0.002fe	0.037±0.002dcb	0.024±0.003fe	0.035±0.003dcb	0.044±0.003ba
1	Carbohydrate (mg/g.D.w.)	15.82±1.448ba	12.24±4.417cb	6.12±1.703dc	5.25±1.701dc	10.96±2.684cb
2	Carotenoid (mg/g.f.w.)	0.060±0.009e	0.122±0.001ba	0.112±0.000cba	0.101±0.001edcb a	0.106±0.005dcba
3	Chl. a /Chl. b	4.102±0.001g	4.032±0.001fe	3.936±0.002i	4.058±0.003e	4.402±0.001cb
4	Chl. b (mg/ g.f.w.)	0.039±0.005g	0.093±0.003ba	0.078±0.007cb	0.069±0.007edc	0.077±0.004cb
5	Chl. a (mg/ g.f.w.)	0.160±0.013hg	0.375±0.014a	0.307±0.024dcba	0.280±0.053edc	0.339±0.015ba
6	Leaf area $(mm)^2$	3366.43±210.5 8h	5485.43±57.08cb	5737.33±87.12b	5790.33±233.73b	6307.27±26.06a
7	D. w. of shoot (g)	0.220±0.034ba	0.288±0.088ba	0.249±0.033ba	0.276±0.065ba	0.349±0.049ba
8	F. w. of shoot (g)	1.42±0.067edc	1.50±0.214edc	1.65±0.115dcb	1.77±0.020cb	1.96±0.035ba
9	Shoot length (cm)	31.75±0.905g	39.23±1.031dcb	39.51±0.290dcb	41.51±1.187ba	43.05±0.863a
10	Con.	0	2.5	5	7.5	10

the same result on *Cyamopsis tetragonoloba*. Increasing in vegetative growth might be due to the presence of phenylacetic acid and similar compounds, growth hormones, macro and micro elements, vitamins and amino acids in algae. The highest content of chlorophyll a, b, total chlorophyll and carotenoids were observed at a concentration of 2.5% compared with the control (Table 3 and 4). The results confirmed the research conducted by Thambiraj *et al*., (2012). Blunden *et al*. (1997) has admitted that if the content of organic and inorganic of algae was separated and a quantity equal to inorganic content applied to plants did not make a great change in leaf chlorophyll contents between control and test plants. This observation strongly suggested that organic portion of algae extract is caused by this increase. The increase in chlorophyll content in addition to being caused by the application of liquid fertilizer of algae is achieved by betaines solution too. These clearly proved that changes on leaf chlorophyll contents caused by the use of seaweed extracts that contained the betaines. It seems probably that presence of betaine in algae extract slowing down the degradation of leaf chlorophyll rather

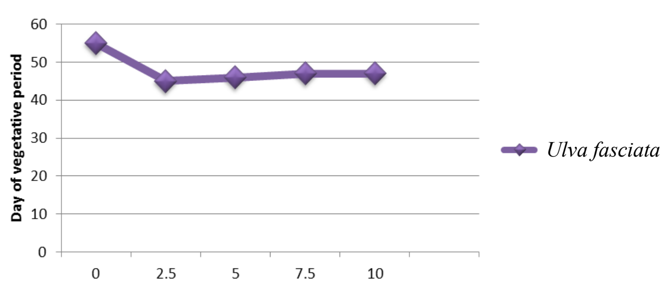

Figure 2. Length of the reproductive period

Table 5. Effect of SLF of *Ulva fasciata* on the growth and biochemical parameters at the ear emergence stage

S. No	Ascorbate peroxidase Activity (min-1/mg protein. ΔOD)	0.298±0.007	0.703±0.027	0.576±0.052	0.473±0.012	0.453±0.034
1	Catalase Activity (min -1/mg protein. ΔOD)	0.286±0.013	0.517±0.020	0.415±0.008	0.254±0.026	0.283±0.014
2	Peroxidase Activity (min-1/mg protein. ΔOD)	4.967±0.073	7.683±0.457	7.786±0.511	7.967±0.467	8.287±0.519
3	MDA (mM^{-1}/g.F.w.)	10.248±0.048	10.860±0.209	10.433±0.186	10.033±0.067	9.267±0.437
4	Total protein (mg/ g.F.W.)	49.37±0.426	54.52±0.700	49.53±0.563	52.42±1.952	53.07±0.852
5	D.W. of shoot (g)	0.597±0.018	1.363±0.017	0.973±0.032	0.753±0.032	1.045±0.037
6	F. w. of shoot (g)	2.68±0.094	4.49±0.025	3.60±0.098	2.87±0.083	3.83±0.112
7	Shoot diameter (mm)	2.73±0.05	3.9±0.03	3.23±0.04	3.3±0.03	3.53±0.05
8	Shoot length (cm)	54.08±0.874	58.73±2.226	49.57±0.745	49.00±0.305	49.80±1.193
9	Con.	0	2.5	5	7.5	10

than increasing its content. As a result, we can confidently say that increasing the amount of pigment resulting from the application of seaweed liquid fertilizer because of the presence of betaine. The total amount of carbohydrates in seeds treated with seaweed at the concentration of 2.5% showed significant increase (Table 3 and 4). This view has also been reported in the seeds of *Trigonella foenum-greacum L* that were treated with algae (Pise and Sabale, 2010). This result may be due to the presence of organic molecules such as organic acids, methionine and even polyamines. This material has formed chelation by connecting the minerals and facilitates absorption of them and thereby increased manufacturing of carbohydrates (Papenfus *et al*., 2013). According to the results plants treated with seaweed has been significantly reduced reproductive and vegetative period especially at 2.5% concentration (Figure 1 and 2). This reduction could be useful for plants which are sensitive to cold or heat weather by shortening the period of vegetative growth, can be harvested before the arrival of cold or heat season. Also, with low growth period, some plants such as vegetables can be planted three times per year. Johnsi (2008) reported that due to algae having hormone GA they can reduce vegetative and reproductive period. Many of the plants until they reach a certain stage of maturity do not produce flowers or cones. Application of GA would be adjustment moving the plant from juvenile to maturity, although the nature of the effect depends on plant species (Taiz and Zeiger, 2010). By applying seaweed liquid fertilizers of *Ulva fasciata*, protein content increased at 2.5% concentration compared to distilled water (Figure 2). Increase in the amount of protein in lower concentrations may be due to the uptake of required elements. Kannan and Tamilselvan (1990) have reported an increase in these indicators that may be due to the presence of Phenyl Acetic Acid (PAA) and similar compounds (P-CH-PAA) as well as some stimulants growth in seaweed liquid fertilizer. In addition, this increase is due to the presence of certain growth-promoting substances such as auxin, abscisic acid, gibberellins, cytokinins and microelements, vitamins and amino acids in the seaweed extracts. The study was conducted by researchers the amount of nitrate reductase was increased after applying seaweed liquid fertilizer. Nitrate reductase enzyme increased ammonium production and thus increase the

Table 6. Effect of SLF of *Ulva fasciata* on the growth and harvest parameters at the ripening stage

S. No	Harvest index [general]	54.68±6.571b	72.90±0.586a	72.38±0.850a	72.46±1.573a	69.25±0.608a
1	Harvest index of spike	59.76±7.206b	73.08±0.479a	73.00±0.900a	71.77±0.900a	69.68±0.312ba
2	Grain weight of a spike (g)	0.619±0.162c	1.172±0.064ba	1.213±0.093ba	1.007±0.022b	1.232±0.119ba
3	Seed yield (g)	11.00±0.599c	19.73±0.452a	19.97±1.290a	17.97±0.172a	19.77±0.880a
4	Thousand seed weight (g)	24.83±6.136b	34.43±0.088ba	34.03±1.478ba	34.11±1.146ba	35.57±1.827a
5	Number of grain per spike	31.53±5.974a	34.13±1.507a	34.20±2.078a	29.80±o.693a	38.60±3.464a
6	spike weight (g)	1.024±0.179c	1.603±0.078ba	1.664±0.139ba	1.461±0.083b	1.766±0.163ba
7	spike length (cm)	13.27±0.371ba	13.38±0.533ba	12.67±0.359b	13.08±0.328ba	14.13±0.792a
8	Total weight (g)	1.250±0.047d	1.513±0.035cba	1.477±0.075dcba	1.361±0.091dc	1.670±0.089ba
9	Shoot length (cm)	62.00±0.577d	77.37±0.961ba	75.57±2.509ba	74.27±1.769b	75.62±1.799ba
10	Con.	0	2.5	5	7.5	10

amino acids and proteins by accessing absorbable nitrogen for plants (Taiz and Zeiger, 2010). Cellular antioxidant defense system played an important role to protect plant cells against oxidative stress caused by reactive oxygen radicals. The MDA dropped by applying seaweed liquid fertilizer of *Ulva fasciata* even lower than the control sample while chemical treatments showed no significant difference compared to the control. By applying seaweed liquid fertilizers of *Ulva fasciata* the amount of peroxidase enzyme was increased but there is a decline in the ascorbate peroxidase and catalase activity. Akila and Jeyados (2010) showed an increase in the peroxidase activity by using seaweed liquid fertilizer of *Sargassum wightii* on the plant of *Helianthus annuus.* L. algae at low concentrations (2.5%) but increasing the concentration of algae has decreased enzyme activity. This result proved algal extract at least in the biosynthesis of Malondialdehyde (MDA) and peroxidase activity, does not cause the production of free radicals and oxidative stress and reduced it to the lowest possible level.

CONCLUSION

The observations revealed that the most growth in parameters is related to 2.5% concentration, but then the most growth is related to highest concentration of 10% This result may be due to a phenomenon called anatonoz at a concentration of 10 percent which includes the demolition of larger molecules like starch into smaller molecules such as glucose and fructose (Taiz and Zeiger, 2010). In the case of oxidative stress that indices antioxidant enzymes and MDA, different results were observed. These results suggested that seaweed *Ulva fasciata* don't make severe stress in plants and doesn't created oxidation problems. The growth in these studied parameters made clear that the algae extracts contains plant nutrients and growth hormones that can increase plant growth without damaging the environment and not disturbing the ecological balance. But since plants need large amounts of nitrogen for advancing their metabolism and algae are not able to provide this amount of nitrogen for plants, 50% conventional chemical fertilizer or even less with the foliar use of algal extract can meet these requirements. According to the above, the algae used in this study can provide micro and part of the macro requirement elements as a nutrient solution supplement so that it can be used as a fertilizer in the form of foliar spray.

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