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Influence of seaweed *Cystosiera crinitophylla* as biostimulants on wheat seedling growth and development.

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Highlights

- **The application of *Cystosiera crinitophylla* as a crude powder mixed with soil or cold aqueous extract foliar spraying caused significant improvements in all the growth parameters and photosynthetic pigments of wheat seedlings.**
- **The preliminary phytochemical screening of *Cystosiera crinitophylla* showed the presence of phenolic compounds, flavonoids, tannins, alkaloids, carbohydrates, and proteins with high relative levels of some minerals.**
- **The practice of application of eco-friendly seaweed extracts is recommended to growers for attaining better germination, growth, and crop yield of wheat.**

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ABSTRACT

The Objective of this study was to explore the effects of the *Cystosiera crinitophylla* extracts on the germination and seedling development of wheat (*Triticum aestavium* L.). We assessed seaweed cold and hot aqueous extract and ethanol extract (1:10 w/v) at different concentrations (2.5, 5, 10, and 20% v/v) on germination percentage and growth parameters of wheat seedlings under laboratory conditions. Additionally, the pot experiment was achieved to assess the effect of the biostimulant action of *Cystosiera crinitophylla* as a crude powder mixed with soil or foliar application on some growth parameters and photosynthetic pigments content of *Triticum aestavium* L. seedlings. The results demonstrated enhancement germination and growth at low extracts concentration, all the three seaweed extracts showed a better rate of germination and growth at optimum extracts concentration ranges between 5–10 % and toxicity at higher extracts concentration. In addition, the results of the pot experiment showed that the application of *C. crinitophylla* as a crude powder mixed with soil or cold aqueous extract, foliar spraying caused significant improvement in all the growth parameters and photosynthetic pigments of wheat seedlings. However, the enhancement was varied and could be application method and concentration-dependent. Furthermore, the preliminary phytochemical screening of seaweed showed the presence of phenolic compounds, flavonoids, tannins, alkaloids, carbohydrates, and proteins with high relative levels of some minerals. The results of the present investigation suggested that the application of biodegradable seaweed extracts is recommended to growers to manage improved germination, growth and of wheat.

1. Introduction

Algae are an important part of the ecosystem and are of the important renewable marine resources, where the seas and oceans is the largest store of algae, which is a good source of food and biofertilizers, as well as used as an antimicrobial, virus, and fungal, and cholesterol and pressure and a source of various chemicals as well as help and stimulate the growth of numerous plant species (Khan *et al.,* 2009). About 15 million metric tons of algal products are produced annually (FAO, 2006) on a whole whereas a major part is used as biofertilizers as it enhances plant growth and yield. They are applied either fresh dried or even used as composts and their products used in agriculture have good tolerance, seed germination, plant growth, yield, tolerance to stress, and resistance against disease infection or pests (Dineshkumar *et al.,* 2018). It has been marketed as an organic fertilizer because it contains many plant growth-stimulating substances such as trace elements, amino acids, betaines, vitamins, ethylene, auxins, gibberellins, and cytokinins (Al-shakankery *et al.,* 2014) and many nutrients for plant growth such as calcium, phosphorus, potassium, iron, copper, and zinc, As well as enhance soil useful microorganisms growth (Hong *et al.,* 2007), improve soil properties and promote seed germination, seedlings development, improved plant growth and yield, increase the efficiency of carrying plants for most environmental stress, increased immune conditions and increase the absorption of nutrients from soils (Kumari *et al.,* 2011). Various forms of seaweed preparation such as liquid seaweed fertilizers and either whole or finally chopped powdered algal fertilizer have been utilized and reported to produce useful effects on cereals and horticultural crops (Ciepiela *et al.,* 2016). Wheat is an important food crop in the world, where most people are on food made from wheat plants depends, despite the contribution of chemical fertilizers to increase agricultural production, but they also contributed to nitrogen and phosphate fertilizers in the soil degradation, air pollution, and water sources and thus harming humans and animals and plants. The use of organic products to improve plant growth has gained much attention and has recently become the new system in agricultural production after chemical fertilizers have proved to have a detrimental effect on the environment and humans (Don and Curry, 2003). Seaweed extracts are a novel Bio-organic fertilizer that is biodegradable, non-toxic, non-polluting, and non-hazardous (Dhargalkar and Pereira, 2005). Several researches on the

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effect of seaweed extracts on the growth of the crops plant were conducted and the results varied depending on the type of algae, the extraction method, the concentration used, method of addition, time, number of times of addition, and the type of plant, is almost applied directly to the soil and/or as a foliar treatment to the plants (Michalak and Chojnacka, 2015). In this work, we evaluated the potential use of aqueous and ethanol extracts of *Cystosiera crinitophylla* as well as crude powder and foliar spraying as a biostimulant.

2. Materials and Methods

2.1. Collection and processing of seaweed

The brown seaweeds *Cystosiera crinitophylla* were collected from the coastal region of eastern Benghazi-Libya during July 2017 and shade dried for 6 days. The dried seaweeds were crushed in an electrical mill until a fine powder was obtained, and stored.

2.2. Preparation of seaweed extracts

There are three simple methods of extracting the *Cystosiera crinitophylla*: (A) cold aqueous extract: 10 g of seaweed powder was mixed with 100 ml distilled water (1:10 w/v) and the mixture was placed in a shaker incubator at 25°C for 24 hours. (B) hot aqueous extract:10 g of seaweed powder was mixed with 100 ml distilled water (1:10 w/v) and The mixture was heated to 60 °C for 24 h in an incubator shaker then the mixtures were filtered through the muslin cloth and filtered with Whatman filter paperNo.1. (C) Ethanol extract: Ethanol 99% has been used for extraction by mixing 10 g of seaweed powder with 100 ml solvent. The mixture was placed in a shaker incubator at 25 °C for 24 hours then have filtered to remove the insoluble materials, the extract was concentrated by evaporating the solvent to dryness, and the solid residue was then dissolved in 2 ml by the same solvent. Now, the extract was made up of 100 ml with distilled water (10%). The filtrate thus obtained is considered as 100% seaweed aqueous extracts and then diluted into different concentrations (2.5, 5, 10, and 20% v/v) with distilled water.

2.3. Germination bioassay

Petri-dish laboratory experiment was carried out to test the biostimulant action of *Cystosiera crinitophylla* extracts (2.5, 5, 10, and 20% v/v) on germination and seedling growth of the recipient species wheat (*Triticum aestivum* L. Local variety). Twenty-five seeds were arranged in 9-cm diameter Petri-dishes on two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from 22-25°C and night temperature from 15-18°C. Five milliliters of each concentration of the seaweed extracts were inserted into each Petri-dishes, the seeds were surface sterilized before sowing by soaking for two minutes in 5% sodium hypochlorite, then, washed several times by distilled water. Treatments were arranged in a completely randomized design with four replications. Seeds were considered germinated when radicle length was 2 mm and the number of germinated seeds was recorded daily, while germination percentage, plumule and radicle length (cm), seedlings dry weight (g), and seedling vigor index (SVI) were recorded after 7 days.
(SVI) = [seedling length (cm) × germination percentage] (Abdul-baki and Anderson, 1973).

2.4. Seedling growth bioassay (Pot experiment)

A pot experiment was applied to test the effect of biostimulant action of *Cystosiera crinitophylla* as a crude powder mixed with soil or cold aqueous extract foliar spraying application on growth parameters and photosynthetic pigments content of *T. aestavium* L. seedlings. To get this, soil samples were collected from the crop fields, air-dried under shade, and sterilized at (125°C for 24h). Seeds of the *T. aestavium* L. were sown in plastic pots (15 cm in diameter) with about 400 g mixed clay and sandy soils (1:1) (w/w) with 5 and 10 gram per one kilogram of crude powder of the seaweed. The experiment was performed under normal laboratory conditions (25°C temperature, 70% relative humidity). Single treatment was run as a control without crude powder. One week after the basal application of seaweed crude powder, the *T. aestavium* L. seeds were planted at the rate of six per pot. After 15 days from the seed planting the foliar spraying (10% and 20% v/v concentration) was done eight times during the experimental period at four days interval. The total spray volume was 10 ml per plant at one time. The experiment consisted of nine treatments as following: (Seaweed crude powder 5 gr (w/w), seaweed crude powder 10 gr (w/w), seaweed foliar spraying 10 % (v/v), seaweed foliar spraying 20% (v/v), seaweed crude powder 5 gr + seaweed foliar spraying 10 %, seaweed crude powder 5 gr + seaweed foliar spraying 20 %, seaweed crude powder 10 gr + seaweed foliar spraying 10%, seaweed crude powder 10 gr + seaweed foliar spraying 20 %, soil without seaweed crude powder + water spray as control treatment. Treatments were set in a Completely Randomized Design (CRD) with three replications. The pots were irrigated every three days with normal tap water. After 45 days the seedling was taken attentively from each treatment. The samples were divided into shoots and roots for the estimation of growth parameters. (Shoot height and root length were measured to the nearest cm. additionally, dry weight of shoots and roots were also estimated). The total chlorophyll was extracted and determined using the spectrophotometric method described by Arnon (1949).

2.5. Preliminary phytochemical screening

Methanol extracts of *Cystosiera crinitophylla* were analyzed to detect the presence of tannins, flavonoids, alkaloids, Proteins, carbohydrate and/or glycosides, and phenolic compounds according to Harborne (1984). The minerals were estimated by used Atomic Absorption (Thermo FS95) according to AOAC (1995).

2.6. Statistical Analysis

The experimental results were subjected to one-way analysis of variance and a Tukey's test was used for the multiple comparisons of means. All statistical analysis was performed with Minitab software version 13.

2. Results and Discussion

The effect of *Cystosiera crinitophylla* different extracts on germination percentage and growth parameters of *T. aestivum* L. are shown in Tables 1, 2 & 3. The results indicated that apply of *C. crinitophylla* extracts significantly promoted the germination and growth parameters of *T. aestivum* L. compared to the control. There was a noticeable increase in germination and growth when 2.5% of seaweed extracts were applied to recipient species. The highest seed germination percentage and growth parameters were recorded with the application of the *C. crinitophylla* extracts at 5% concentration, followed by 10% concentration, the germination percentage, and growth parameters were increased with gradual increase of the *C. crinitophylla* extracts concentrations up to 20% but declined at excessive concentrations. Higher concentrations (20% and above) were found to show an inhibiting effect on all the above parameters regardless of extraction method (Tables 1, 2 & 3).

Data in Table 4 showed that the application of *C. crinitophylla* as a crude powder mixed with soil or cold aqueous extract foliar spraying caused significant enhancement in all the growth parameters of wheat seedlings compared with the control. The maximum shoot and root length was noted under foliar spraying 10 % treatment, while the highest shoot and root dry weight values were recorded under crude powder 10 gr treatment (Table 4). The highest values of total chlorophyll were observed with Crude powder 10 gr +foliar spraying 20% treatment (Table 4). The results demonstrated that the seaweed *C. crinitophylla* contained a high fairly level of iron, potassium, nickel, manganese, cadmium, phosphor, copper, chromium, zinc, sodium, lead, magnesium and nitrogen,

(Table 5). The obtained results from the phytochemical screening of *C. crinitophylla* methanol extract illustrated the presence of phenols, alkaloids, tannins, flavonoids, carbohydrates, proteins, and glycosides. Meanwhile, saponins were rarely detected (Table 6). Seaweed extracts contain growth regulators and mineral nutrients that enhance plant growth (Tarakhovskaya *et al.*, 2007). *T. aestivum* L. seeds treated with low concentrations of seaweed extracts demonstrated higher germination and growth, while at higher concentrations germination and growth were inhibited. Furthermore, the highest improvement in seedling growth was found under cold aqueous extract treatment followed by hot aqueous extract treatment and the lowest seedling growth was noticed with ethanol extract treatment.

Table 1

Effect of different concentrations of *C. crinitophylla* cold aqueous extract on germination and growth parameters of *T. aestivum* L. Data are mean of four replicates (±SE).

Extract conc.%	GP%	PL (cm)	RL (cm)	PDW (g)	RDW (g)	SVI
0.00	NS 90.0±4.08 a	** 9.47±1.38 a	*** 13.26± 2.39 a	** 0.067±0.0026 A	*** 0.218±0.0205 a	** 2045.1±69.9 a
2.50	91.2±6.29 a	9.66±1.66 ab	14.86±2.51 b	0.073±0.0007 Ab	0.288±0.0049 b	2238.2±169.7 ab
5.00	95.0±5.77 a	10.15±1.23 ab	15.11±1.84 b	0.078±0.0053 B	0.285±0.0203 b	2400.9±180.6 b
10.00	98.7±2.50 a	10.27±0.84 b	14.66±2.04 b	0.079±0.0053 B	0.298±0.0112 b	2458.9±85.3 b
20.00	91.2±6.29 a	10.32±1.15 b	13.00±3.30 a	0.079±0.0063 B	0.260±0.0402 ab	2117.1±124.7 ab

GP: Germination percentage, **PL**: Plumule length, **RL**: Radical length, **PDW**: plumule dry weight, **RDW**: radical dry weight, **SVI**: Seedling vigor index, **NS**: Non significant ** = Significant at Ꝑ < 0.01 *** = Significant at Ꝑ < 0.001
Different letters within each column indicate a significant difference at P < 0.05 level.

Table 2

Effect of different concentrations of *C. crinitophylla* hot aqueous extract on germination and growth parameters of *T. aestivum* L. Data are mean of four replicates (±SE).

Extract conc.%	GP%	PL (cm)	RL (cm)	PDW (g)	RDW (g)	SVI
0.00	** 90.0±4.08 a	** 9.47±1.38 a	*** 13.26± 2.39 b	*** 0.067±0.002 a	NS 0.218±0.020	NS 2045.1±69.9
2.50	96.2±2.50 b	10.43±0.83 b	12.68±1.42 b	0.079±0.003 b	0.234±0.032	2225.7±63.1
5.00	100.0±0.00 b	10.12±0.84 ab	12.32±0.95 ab	0.076±0.001 b	0.256±0.014	2245.0±38.5
10.00	97.5±2.89 b	10.03±1.15 ab	12.18±1.87 ab	0.070±0.001 a	0.250±0.021	2167.2±210.8
20.00	96.2±2.50 b	10.03±1.30 ab	11.61±1.43 a	0.070±0.001 a	0.248±0.006	2084.5±106.6

GP: Germination percentage, **PL**: Plumule length, **RL:** Radical length, **PDW**: plumule dry weight, **RDW**: radical dry weight, **SVI:** Seedling vigor index, **NS:** Non significant ** = Significant at Ꝑ < 0.01 *** = Significant at Ꝑ < 0.001
Different letters within each column indicate a significant difference at P < 0.05 level.

Table 3

Effect of different concentrations of *C. crinitophylla* ethanol extract on germination and growth parameters of *T. aestivum* L. Data are mean of four replicates (±SE).

Extract conc.%	GP%	PL (cm)	RL (cm)	PDW (g)	RDW (g)	SVI
0.00	*** 90.0±4.08 bc	*** 9.47±1.38 b	*** 13.26± 2.39 b	*** 0.067±0.002 B	*** 0.218±0.020 b	*** 2045.1±69.9 c
2.50	96.2±2.50 c	10.08±0.49 c	13.41±0.87 b	0.071±0.002 Bc	0.224±0.022 b	2261.6±62.0 d
5.00	93.7±2.50 c	10.02±0.65 c	12.85±0.72 b	0.073±0.001 C	0.243±0.006 b	2144.6±70.0 cd
10.00	85.0±4.08 b	9.11±0.63 ab	11.81±1.49 a	0.069±0.002 Bc	0.205±0.016 ab	1775.3±103.7 b
20.00	77.5±2.88 a	8.81±0.62 a	11.46±1.21 a	0.061±0.001 A	0.175±0.008 a	1571.5±88.0 a

GP: Germination percentage**, PL**: Plumule length, **RL**: Radical length, **PDW**: plumule dry weight, **RDW**: radical dry weight , **SVI**: Seedling vigor index, *** = Significant at Ꝑ < 0.001
Different letters within each column indicate a significant difference at P < 0.05 level.

Table 4

Effect of different levels of *C. crinitophylla* as a crude powder mixed with soil or foliar application on some growth parameters and total chlorophyll of *Triticum aestavium* L. seedlings in a pot experiment. Data are mean of three replicates (±SE).

Treatments	SL (cm)	RL (cm)	SDW (g)	RDW (g)	Total Chlorophyll (mg/g)
Control	*** 26.16±2.30 a	*** 6.66±1.00 A	*** 0.108±0.030 a	* 0.028±0.005 a	*** 1.27±0.004 a
Crude powder 5 gr (w/w)	28.41±2.35 ab	10.25±1.11 Bc	0.189±0.031 b	0.052±0.020 ab	1.54±0.013 bc
Crude powder 10 gr (w/w)	29.83±1.57 b	11.62±0.88 C	0.217±0.015 b	0.066±0.010 b	1.60±0.014 c
Foliar spraying 10 % (v/v)	28.45±1.42 ab	10.29±2.33 Bc	0.173±0.009 b	0.050±0.016 ab	1.82±0.019 e
Foliar spraying 20 %(v/v)	31.29±0.83 b	11.79±2.85 C	0.182±0.008 b	0.040±0.004 ab	1.71±0.011 d
Crude powder 5gr+Foliar spraying10%	29.83±2.02 b	11.29±2.15 Bc	0.172±0.011 b	0.033±0.009 ab	1.76±0.023 de
Crude powder 5gr+Foliar spraying20%	27.20±2.38 ab	9.08±0.76 B	0.156±0.024 ab	0.036±0.002 ab	1.45±0.005 b
Crude powder 10gr+Foliar spraying10%	30.45±1.01 b	10.91±1.63 Bc	0.209±0.012 b	0.045±0.016 ab	1.80±0.023 de
Crude powder 10gr+Foliar spraying20%	28.91±2.49 b	10.25±2.34 Bc	0.176±0.032 b	0.035±0.006 ab	1.87±0.086 e

SL: Shoot length, **RL**: Root length, **SDW**: Shoot dry weight, **RDW**: Root dry weight
*: Significant at $P < 0.05$ ***: Significant at $P < 0.001$
Different letters within each column indicate a significant difference at $P < 0.05$ level.

Table 5

Mineral composition of *Cystosiera crinitophylla.*

Minerals	*C. crinitophylla*
Nitrogen (%)	0.76
Phosphor (mg/g)	0.271
Potassium (mg/g)	15.64
Sodium (mg/g)	20.37
Magnesium (mg/g)	8.11
Iron (mg/g)	1.91
Copper (mg/g)	0.0040
Manganese (mg/g)	0.0208
Nickel (mg/g)	0.0034
Zinc (mg/g)	0.0304
Chromium (mg/g)	0.0061
Cadmium (mg/g)	0.0028
Lead (mg/g)	0.0055

Table 6

Preliminary qualitative phytochemical screening of *C. crinitophylla* methanol extract.

Phytoconstituents	*C. crinitophylla*
Flavonoids	+ + +
Phenolic compounds	+ + +
Tannins	+ + +
Alkaloids	+ + +
Carbohydrates	+ + +
Proteins	+ + +
Glycosides	+ + +
Saponins	+ + -

+ + + abundantly present,
+ + - Moderately present ,
+ - - Present in trace amount.

The enhancement in germination and growth at low concentrations might be due to the presence of growth-promoting substances such as plant hormones, minerals, and vitamins (Mohy El-Din, 2015). Liquid extracts of seaweed as a soil drench to cluster bean plant and noticed maximum influence on growth parameters such as shoot length, root length, total dry weight, and leaf area (Latique *et al.*, 2013). All growth parameters and photosynthetic pigments content of *Triticum aestivum* L. seedling recorded significant differences compared to the control only under *Cystosiera crinitophylla* crude powder at 10-gr treatment. Under foliar spray of *Cystosiera crinitophylla* at 20 %. In general, enhancement in growth parameters was found in concentration and crude powder 10 gr treatments. In this study, the enhancement in all seedling growth parameters and photosynthetic pigment may be due to seaweed extracts being rich in macro and microelements, important plant hormones like Auxins, Gibberellins, and Cytokinin, which induce cell division, and increasing cell enlargement (Khan *et al.*, 2009).

The enhancement in all seedling growth parameters and photosynthetic pigment also might be attributed to the usage of natural components of seaweed extract i.e. polysaccharides, alginates, polyamines, amino acids, nutrients, natural cytokinins, auxins, and auxin-like compounds, and gibberellins. These components affect cellular metabolism processes, thus led to enhance seed germination, seedling growth and stimulate plant vegetative growth (Yusuf *et al.*, 2012). Seaweeds provide an excellent source of compounds such as vitamins, minerals, and growth-promoting substances (Anisimov and Chaikina, 2014). Low concentrations of seaweed extracts had a maximum positive influence on germination and growth as reported in previous studies due to the occurrence of trace elements, plant growth hormones, and vitamins. However, the enhancement was varied and could be extraction method and extract concentration-dependent. Our study concludes that the brown seaweed *Cystosiera crinitophylla* are practically effective and low-cost fertilizers that can be promoted as bio-fertilizers and the perform use of eco-friendly seaweed extracts is recommended in crop production for achieved better yield of wheat after further research.

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