

POTENTIAL OF *ULVA LACTUCA* EXTRACT ON GROWTH, BIOCHEMICAL CONSTITUENTS, AND ACTIVITY OF PEP CARBOXYLASE OF *ZEA MAYS* SEEDLINGS

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Abstract

The effect of applying *Ulva lactuca* aqueous extract (ULAE) with different concentrations on the growth of maize seedlings was studied. The extract was applied as foliar spray and in other experiment was incorporated in the growth nutrient solution. Growth parameters, mineral nutrients, photosynthetic pigments, protein profile, and phosphoenolpyruvate (PEPCase) activity were monitored. Presoaking maize grains in different ULAE was an effective technique to obtain better growth. Seedlings sprayed with 0.5% or 1% ULAE showed a significant increase in growth and biochemical parameters. Higher concentration (5%) showed inhibitory effect. There were variable peptides with different low molecular weights that were synthesized in all treatments except for the control one (100% H). For example, in seedlings sprayed with 5% ULAE proteins as 27, 24, 23, 12 and 10 KDa were synthesized. Similar low molecular weight proteins were synthesized in all other treatments but with different band intensities. Furthermore, treatments with ULAE as a foliar spray or supplemented in the growth medium differentially affected protein expression in *Z. mays* seedlings. New proteins were expressed in the treated seedlings which could be due to the action of components in the extract that are bioactive for growth. PEP carboxylase activity in *Z. mays* seedlings grown in different combinations of Hoagland's solution and ULAE was higher than that of seedlings sprayed with ULAE alone. There was a gradual decrease in enzyme activity in response to increasing ULAE applied as foliar spray and the least activity was recorded in seedlings sprayed with 5% ULAE

Key words: *Ulva lactuca*, Phosphoenolpyruvate, Photosynthetic pigments, SDS-PAGE.

Introduction

Using different chemical fertilizers and their impacts especially on the environment has become an important issue (Dubey, 2010; Eissa *et al.*, 2017). As a result, farmers began to shift from synthetic agricultural fertilizers to organic farming. Extracts of macro algae have currently gained attention as foliar sprays for several plants especially crops due to the presence of growth hormones, trace elements, vitamins and amino acids (Sivasankari, 2006; Abdel Khalik & Osman, 2017).

Application of seaweed extracts stimulated the growth and yield of plants, enhance the tolerance to different stresses, enhance element uptake as well as antioxidant properties (Rathore, 2009; Abdel-Latif & Osman, 2017). The genus *Ulva*, known as sea lettuce, is considered the most commonly grown green macro algae all over the world (Lahaye & Robic, 2007; Alothyqic *et al.*, 2016). Despite its wide distribution, it is sparingly utilized and only a small fraction of biomass is consumed as food or animal feed due to its nutritional ingredients (vitamins, oligo-elements, minerals, and dietary fibers) (Pengzhan *et al.*, 2003; Abouseadaa *et al.*, 2015) organic crop fertilizer (Mulbry *et al.*, 2005), effluent biofilters (Msuya & Neori, 2002) and plant protectant as well (Cluzet *et al.*, 2004). Sridhar & Rengasamy, (2011) observed that the seeds of five different plant species treated with 1.0% (v/v) extract of *Ulva lactuca* and *Sargassum wightii* increased germination and affect protein profile. Another study also recorded that foliar spray of 2% SWE of *U. reticulata* increased the growth, yield, and a number of stomata in *Vigna mungo* (Abdel Khalik *et al.*, 2012; Ganapathy, 2013; Osman *et al.*, 2015).

The C4 pathway of photosynthesis occurs in C4 plants such as maize (*Zea mays* L.). It enables to increase the CO₂ concentration where the enzyme Rubisco (EC 4.1.1.39) is located (Von Caemmerer & Furbank, 2003; Osman *et al.*, 2013). In the bundle sheath cells, the secondary autotrophic carboxylation reaction is catalyzed by Rubisco. The supply of CO₂ in this reaction, however, depends on the first carboxylation reaction of phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) which catalyzes carboxylation of phosphoenolpyruvate (PEP) and bicarbonate in the presence of magnesium ion producing oxaloacetate (OAA) and inorganic phosphate. PEPC is mainly distributed in all photosynthetic organisms including vascular plants, algae, cyanobacteria, and photosynthetic bacteria. In C4 plants, it notably performs the incorporation of atmospheric CO₂ in photosynthesis as well as its plays a role as an anaplerotic enzyme that coordinate both carbon and nitrogen metabolism in all plants (Monreal *et al.*, 2010; Munshi & Osman, 2010). This study aims to evaluate the effect of *U. lactuca* aqueous extract (ULAE) on two *Z. mays* hybrids M10 and M321. Certain physiological and biochemical parameters in maize seedlings such as PEPCase activity and protein profile were analyzed.

Materials and Methods

Preparation of seaweed extract: Marine alga *U. lactuca* was manually collected from Abu-Qir coast, Alexandria, Egypt during October 2015. The fresh seaweed samples were shade -air dried for 2-3 days then oven drying for 12 h at 60°C. The oven dried seaweed was hand crushed and powdered with mixer-grinder. Of the dried material 10 g was extracted with 1000 ml distilled water for 24 h. The

mixture was then filtered through a double-layered muslin cloth. The filtrate thus obtained was considered as 100% seaweed aqueous extract and stored at 4°C. Different concentrations (1, 5, 10, and 40%) were prepared from 100% seaweed extract using distilled water. The extracts thus obtained from *U. lactuca* were applied to maize seedlings cultivated in the hydroponic system by foliar spray or added to the culture medium.

Seed germination bioassay experiment: *Zea mays* grains (M10 and M321) were procured from the Crop Institute, Agricultural Research Center, Giza, Egypt. The grains having the uniform size, shape, color and weight were selected for the study. Ten grains were arranged in 18-cm diameter Petri-dishes on two layers of filter paper (Whatman number 1) under normal laboratory conditions at 20–23°C day/14–16°C night. Afterward, 15 ml of each concentration (or distilled water) were then added. Before sowing, the grains were surface sterilized by soaking for two 2 min. in 4% sodium hypochlorite, then, rinsed four times with double distilled water. In a parallel experiment, the seeds were pre-soaked in different concentrations of ULAE for 24 h before germination. The treatments were arranged in a complete randomized block design with three replicates. The measurements of GP, CL, and RL were recorded daily. Germination percentage was calculated by the following formula:

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Seven-day old seedlings were transferred to the growth units which consisted of 4 polyethylene tubes (5 cm diameter, 51 cm length). Each tube had an out and inlet in order to circulate the nutrient solution (Fig. 1). The capacity of each tube was 1500 mL with 31 pores (8 mm) distributed in two alternation rows, where the seedlings should be settled. The micropipettes tips were used to support the seedlings during growth and when they were harvested. An air pump terminal with a flow rate of (200 ml/min), was used to aerate and circulate the solution. The nutrient solution was completely renewed every three days. The experiment was performed under normal laboratory conditions (20 ± 2°C temperature, 75 ± 2% relative humidity, and 14/10 h light/dark photoperiod).

The growth experiment design: Four treatment groups were designed to test the impact of ULAE application in the growth medium on maize plant cultivated in the hydroponic system. The first group of seedlings was grown in medium containing 50% of half-strength Hoagland's solution and 50% of ULAE (50% U + 50% H). The second group of seedlings was grown in a medium containing 25% of half-strength Hoagland's solution and 75% of ULAE (75% U + 25% H). Whereas, the third group of seedlings was grown in 100% half-strength Hoagland's solution and were not treated with ULAE (control). Seedlings of the fourth group were grown in ULAE alone (10%) which served as a positive control. In a parallel experiment, four foliar applications consisting of three different ULAE concentrations (0.5%, 1%, and 5%) and one control treatment (no spray) were given to seedlings grown

hydroponically within half-strength Hoagland's solution. Fifty ml of different concentrations of the extract (or water for the control treatment) was given at 3 days' intervals up to 14 days. After two weeks, the homogenous seedlings were carefully collected from each treatment and gently blotted with filter paper. Roots were separated from shoots to estimate the different growth and biochemical parameters. Other samples were dried at 65°C till a constant weight was observed to determine the seedling dry weights. All the experiments were conducted in triplicate.



Fig. 1. Polyethylene tubes used in the study as hydroponic growth units.

Determination of minerals: About 100 g of powdered seaweed sample was subjected to acid digestion and analyzed by atomic absorption spectrophotometry for the determination of minerals such as potassium, calcium, magnesium, manganese, iron, zinc, calcium, cadmium, and copper (colorimetric) following the procedures from the Association of Official Analytical Chemists.

Determination of total protein content: The total protein content was estimated according to the method described by Hartree, (1972).

Determination of carbohydrates: A known quantity of the dry matter (root and shoot) was extracted twice with 80% ethanol in a reflux apparatus on a boiling water bath. The two alcoholic extracts and their respective washings were pooled together, evaporated in an air-drying oven at 50°C and the residue was taken in water to make up the final volume. The estimation of total soluble sugars (TSS) was carried out by hydrolyzing an aliquot of the sugar extract with 1.0 N HCl for 30 min and made to a volume before neutralization to phenolphthalein end point. The total soluble sugar fraction was determined by the method described by Dubois *et al.*, (2002). The hydrolysate (2 ml) and 5% phenol (1 ml) were mixed and concentrated H₂SO₄ (5 ml) was added rapidly. The tubes were allowed to stand for 10 min, shaken gently and kept for 10–20 min in a water bath at 30°C. Absorbance was read at 490 nm. A calibrated curve using standard glucose was made and the amount of sugar was calculated as mg/g dry wt. All measurements were conducted in triplicate.

Extraction of PEP carboxylase: Approximately 1.0 g of leaf tissue was ground in a pre-filled mortar with purified sand and 10 ml of ice cold homogenization buffer (0.1M Tris HCl (pH 7.8), 0.5 mM EDTA, 1mM MgSO₄ and 1mM DTE freshly prepared). The method was slightly modified from that described by Abdel-Latif, (2008) to suit the quantities of the enzyme present in the leaf samples investigated. The extract was centrifuged in a Beckman-centrifuge (model 2–21) for 25 min at 16000 rpm. Approximately 300 mg of solid PVP (Polyclar AT, Serva Heidelberg, M.W. 45000) was added to 3 mL of the supernatant and mixed vigorously with a stirrer for 3 s to remove the phenolic compounds, which may interfere with the spectrophotometric determination of the enzyme activity. The mixture was centrifuged at 4°C for 10 min at 8000 rpm and the clear supernatant was used as the source of the enzyme. The activity of PEPCase in this extract (conducted in triplicate) was constant at the room temperature for at least 8 h.

PEPCase assay: The PEPCase activity was determined spectrophotometrically as described by Blanke *et al.*, (1986) at 340 nm by coupling the reaction to the oxidation of NADH in the presence of malate dehydrogenase (MDH). The reaction was started by adding 150 µL enzyme extract followed by immediate mixing. The reaction was linear for at least 4 min and in the proportional to the volume of the extract added to the mixture. The rate of the reaction was measured by observing the decrease in absorbance at 340 nm (oxidation of NADH) in a spectrophotometer (Shimadzu Multipurpose-Recording Spectrophotometer, MPS).

Protein profile: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to distinguish and fragment the total soluble protein from treated maize samples according to the method of Laemmli, (1970).

Statistical Analysis: All the data in the present study were subjected, where appropriate, to standard one-way analysis of variance (ANOVA) and Student's t-test (t-value <0.05 was considered as significant) using the COSTAT 2.00 statistical analysis software from CoHort Software Company (Zar, 1984). In the case when a significant difference was detected by ANOVA, pair-wise comparisons of means were performed using Least Significant Differences (LSD) at 0.05 probability level.

Results

Determination of mineral elements of *U. lactuca*: The essential minerals such Calcium (Ca), Potassium (K), Zinc (Zn), Magnesium (Mg), and Iron (Fe) are present in significant amounts in *U. lactuca* (Table 1). Among these elements, Ca is the most abundant element in the *U. lactuca* seaweed, followed by Potassium. The other elements are Mg, Zn and Fe. The high mineral content of *U. lactuca* seaweed is due to the consumption of the nutritive elements in the medium, where the alga grows.

Biological activity of ULAE on the germination and growth parameters of *Z. mays* M10 and M321: The germination percentage (GP) of *Z. mays* (M10) seeds increased with increasing levels of ULAE (Table 2). The data indicated that ULAE exerted a significant positive effect on the seed germination of the test species and the effect was prominent at the relatively higher concentration (5, 10 and 40%) compared to 1%.

The trend was different in the pre-soaked seeds with different concentrations of ULAE. All the concentrations of the extract reduced the GP except for 5%, where it increased compared to the control. All concentrations of the extract reduced RL except for 10%.

The GP of *Z. mays* M321 was relatively low (4.76%) compared to M10 hybrid. Treatment with different concentrations of ULAE increased the GP in M321 grains. The highest GP was obtained at 5% ULAE (Table 3). Similarly, there was an increase in GP of M321 pre-soaked seeds at the different ULAE concentrations. The data indicated that ULAE exerted a significant positive effect on the germination of M321 seeds and it was prominent at 1, 5, and 40% concentrations.

There was a significant increase in RL of M321 seeds throughout the whole course of the experiment. After two days of sowing, RL was visibly increased from 0.1 cm (control) to 0.93, 1.23, 0.46 and 0.33 cm at concentrations 1, 5, 10 and 40% respectively. At the end of the experiment, it reached 9.93, 15, 10.73 and 3.86 cm at 1, 5, 10 and 40% concentrations respectively compared to the corresponding control (6.1 cm). Regarding coleoptile length (CL), all applied concentrations of extract increased CL except for the highest concentration (40%). There was no coleoptile seen two days after sowing irrespective of the dose or mode of treatment. The dry weight was reduced in all the seedlings irrespective of the dose and mode of application.

Table 1. Minerals composition in *U. lactuca*.

Element	K	Cu	Cd	Ca	Mg	Fe	Mn	Zn	Na
Concentration (ppm)	287.9	5.8	0.8667	3255.86	90.9	71.17	17.63	79.8	27.06

Plant growth bioassay: As shown in (Fig. 2), a mixture of 50% H + 50% U did not affect shoot length significantly, while 25% H + 75% U increased shoot length. Likewise, the root length was also affected by the different treatments; the most pronounced effect was on the seedlings treated with a combination of 25% H + 75% U and 100% ULAE. In a similar way, in the foliar application experiment, the ULAE significantly promoted growth of *Z. mays*. There was a significant increase in growth when the seedlings were sprayed with 0.5% or 1% of ULAE, while the higher concentration (5%) was found inhibitory. Foliar application of 0.5% and 1% ULAE exhibited a significant effect on

seedling length. However, the increase in seedling length was mainly due to the increase in root length rather than shoot length. However, the reduction in the length of seedlings sprayed with 5% ULAE was mainly due to the reduction in root length rather than shoot. Seedlings grown in 100% U as well as 50% H + 50% U had relatively lower fresh weight than the control (Fig. 3). The application of various ULAE combinations did not cause any significant difference in seedlings dry weight. On the other hand, dry weight was significantly enhanced by all treatments of ULAE applied as a foliar spray. The highest leaf area of *Zea mays* was recorded for the seedlings receiving H25%+U75%

treatment, whereas, seedlings sprayed with 5% extract had the lowest leaf area (Fig. 4). As compared to control plants, seedlings grown in 50% U + 50% H, as well as 100% U, showed significant increase in total carbohydrates, while carbohydrate content appeared to be non-significantly affected by 25% H + 75% U treatment (Fig. 5). On the other hand, total carbohydrates were significantly decreased in all foliar spray treatments. The percent decrease, however, was more significant in seedlings sprayed with 0.5% U.

Activity of PEP carboxylase: The rate of reaction was determined by measuring the decrease in absorbance at 340 nm (oxidation of NADH) and the reaction was linear for at least 4 min. The PEP carboxylase activity in maize seedlings grown in different combinations of Hoagland's solution and ULAE was higher than that in seedlings sprayed with ULAE. Seedlings grown in both 100% H or 100% ULAE exhibited a higher PEP carboxylase activity than other combinations. There was a gradual decrease in the enzyme activity in response to increasing ULAE applied as a foliar spray. The lowest PEP carboxylase activity was recorded in seedlings sprayed with 5% ULAE (Fig. 6).

Protein profile: Figure 7 represents the SDS-PAGE protein profile of maize seedlings treated with different combinations of Hoagland's solution and ULAE, which

showed the presence of several peptides, varying in migration position and band intensity. The figure shows that 15,18,17,17,18,15 and 14 protein bands were excised in seedlings treated with 50%+50%, 25%H+75%U, sprayed with 1%, sprayed with 0.5, 100%U, sprayed with 5% and control (100% H) respectively. *Zea mays* seedlings grown in half strength Hoagland's solution (control) showed the presence of 14 peptides with molecular masses ranging from 70 to 31 kDa., while *Zea mays* seedlings grown in 100% U revealed the presence of 15 peptides with molecular masses ranging from 66 to 12 kDa. There were variable protein bands with different low molecular weights that were appeared in all treatments except for the control one (100% H). For example, in seedlings sprayed with 5% ULAE proteins as 27,24,23,12 and 10 KDa were synthesized with their band intensities of 6,3,4,3 and 2 respectively. In seedlings grown in 100% U, low molecular weight proteins ,29,25,22,20 and 12 KDa with their band intensities of 9,3,4,2 and 1 were synthesized. Similar low molecular weight bands were appeared in all other treatments but with different band intensities. Another protein band of molecular weight 80 kDa was appeared in seedlings sprayed with 1% ULAE and in seedlings grown in 25%H + 75% U. Protein band of 66 kDa in the control seedling was not present in all treatments. Another protein band with 70 KDa was also absent in all treatments except for seedlings sprayed with 1% ULAE.

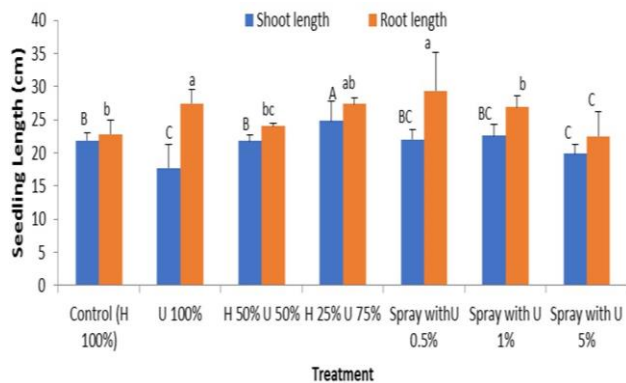


Fig. 2. The effects of applying different concentrations of ULAE added to the growth medium or used as a foliar spray on shoot and root lengths of 14-day-old *Zea mays* seedlings.

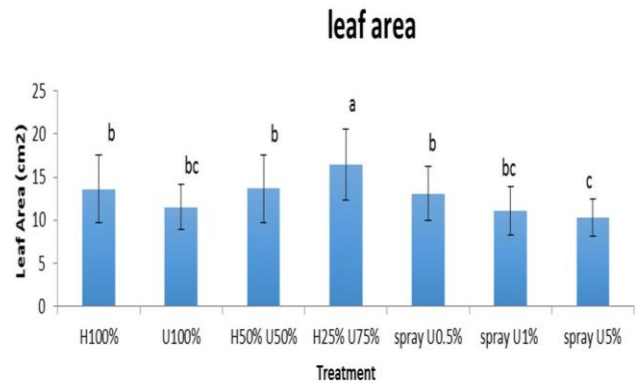


Fig. 4. The effects of applying different concentrations of ULAE added into the growth medium or used as a foliar spray on *Zea mays* leaf area.

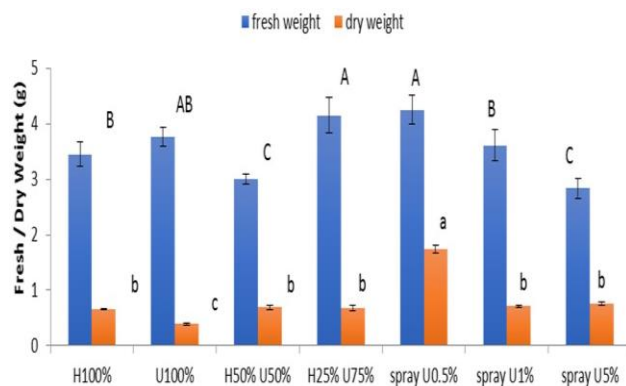


Fig. 3. The effects of applying different concentrations of ULAE added to the growth medium or used as a foliar spray on fresh weight, dry weight of 14-day-old *Zea mays* seedlings.

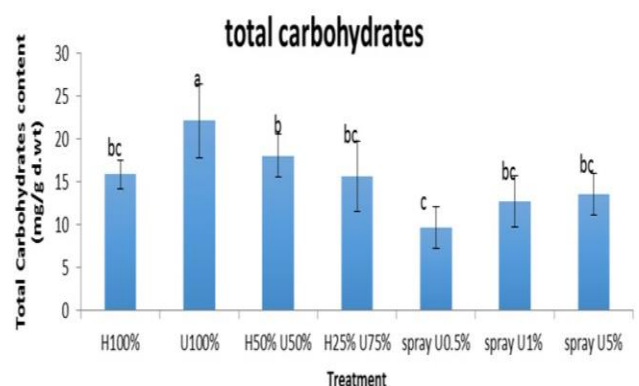


Fig. 5. The effects of application of different concentrations of ULAE added to the growth medium or applied as a foliar spray on the carbohydrate composition (mg/g d. wt) of 14 day-old *Zea mays* seedlings.

Table 2. The effect of different concentrations of *Ulva lactuca* aqueous extract (ULAE) on germination percentage (GP), fresh weight (FW), dry weight (DW), coleoptile length (CL), and radicle length (RL) of *Zea mays* (M10) seeds.

M10		Germination percentage	Fresh weight (g)	Dry weight (g)	Coleoptile and radicle length of second read "Mean \pm SD"	Coleoptile and radicle length of third read "Mean \pm SD"			
Coleoptile		Radicle	Coleoptiles	Radicle	Coleoptile	Radicle			
Irrigated by different concentrations of <i>U. lactuca</i> extract									
C	28.75%	1.95	0.64	-	1.56 \pm 0.04	1.43 \pm 0.37	5.46 \pm 0.34	4.33 \pm 1.24	7.36 \pm 0.98
1%	28.95%	1.51	0.55	-	0.4 \pm 0.2	1.46 \pm 0.46	4.46 \pm 0.81	7.3 \pm 0.52	12.66 \pm 1.43
5%	80.95%	1.31	0.55	-	1.5 \pm 0.07	1.23 \pm 0.51	4.13 \pm 1.24	3.33 \pm 1.61	5.96 \pm 0.44
10%	23.8%	1.96	0.57	-	2.03 \pm 0.23	3.2 \pm 0.13	8.36 \pm 0.49	8.53 \pm 1.44	16.33 \pm 1.37
40%	33.33%	1.58	0.63	-	1.5 \pm 0.54	1.2 \pm 0.85	3.66 \pm 1.27	4.00 \pm 2.43	4.3 \pm 2.01
Pre-soaked in different concentrations of <i>U. lactuca</i> extract									
1%	14.28%	1.76	0.61	-	0.16 \pm 0.04	0.86 \pm 0.21	3.4 \pm 0.34	4.3 \pm 0.89	10.23 \pm 3.03
5%	33.33%	1.87	0.72	-	0.33 \pm 0.17	1.06 \pm 0.62	3.73 \pm 1.07	6.3 \pm 0.85	12.03 \pm 1.28
10%	19.04%	1.86	0.56	-	0.36 \pm 0.21	0.9 \pm 0.27	3.2 \pm 1.36	5.8 \pm 0.57	10.3 \pm 2.00
40%	4.76%	2.39	0.69	-	0.53 \pm 0.69	1.4 \pm 0.87	4 \pm 1.51	6.9 \pm 1.09	8.36 \pm 2.75

Table 3. The effect of different concentrations of *Ulva lactuca* aqueous extract (ULAE) on germination percentage (GP), fresh weight (FW), dry weight (DW), coleoptile length (CL) and radicle length (RL) of *Zea mays* (M321) seeds Plant growth bioassay.

M321		Germination Percentage	Fresh weight (g)	Dry weight (g)	Coleoptile and radicle length of first read "Mean \pm SD"	Coleoptile and radicle length of second read "Mean \pm SD"	Coleoptile and radicle length of third read "Mean \pm SD"		
Coleoptile		Radicle	Coleoptile	Radicle	Coleoptile	Radicle	Radicle		
Irrigated by different concentrations of <i>U. lactuca</i> extract									
C	4.76%	2.27	1.01	-	0.1 \pm 0.13	1.26 \pm 0.51	3.7 \pm 0.98	4.86 \pm 0.61	6.1 \pm 1.78
1%	23.8%	1.73	0.72	-	0.93 \pm 0.50	1.43 \pm 0.37	5.96 \pm 0.84	5.2 \pm 1.55	9.93 \pm 1.79
5%	47.61%	2.40	0.80	-	1.23 \pm 0.67	2.23 \pm 0.55	6.26 \pm 1.35	7.96 \pm 0.34	15 \pm 0.52
10%	4.96%	1.94	0.84	-	0.46 \pm 0.61	1.56 \pm 0.55	4.53 \pm 0.41	6.2 \pm 0.99	10.73 \pm 0.49
40%	28.57%	2.14	0.98	-	0.33 \pm 0.17	0.56 \pm 0.04	2.9 \pm 0.6	3.13 \pm 0.76	3.86 \pm 0.83
Pre-soaked in different concentrations of <i>U. lactuca</i> extract									
1%	23.8	2.00	1.01	-	1.13 \pm 0.61	0.8 \pm 0.2	2.73 \pm 0.57	3.5 \pm 0.34	3.56 \pm 0.98
5%	14.28	2.76	0.99	-	0.2 \pm 1.48	1.00 \pm 0.32	3.56 \pm 1.15	6.6 \pm 1.33	10.16 \pm 2.12
10%	4.76	1.70	0.80	-	0.06 \pm 0.08	1.03 \pm 0.19	3.3 \pm 0.13	4.03 \pm 0.70	8.16 \pm 0.57
40%	14.28	1.63	0.58	-	0.86 \pm 0.08	0.66 \pm 0.04	3.56 \pm 0.34	6.2 \pm 1.30	9.43 \pm 2.54

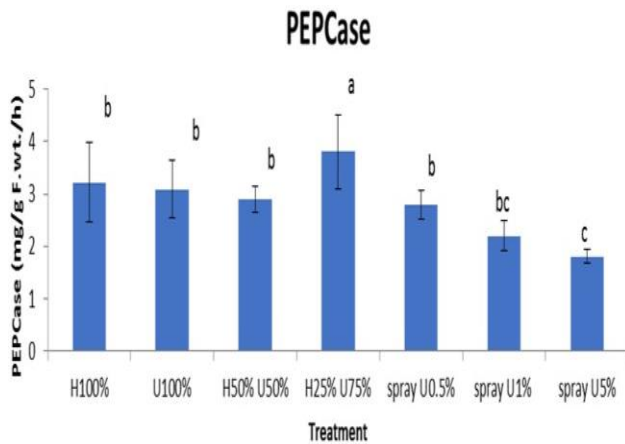


Fig. 6. The effects of application of different concentrations of ULAE added to the growth medium or applied as a foliar spray on the activity of PEP carboxylase (mg/g F.W./h) of 14 day-old *Zea mays* seedlings.

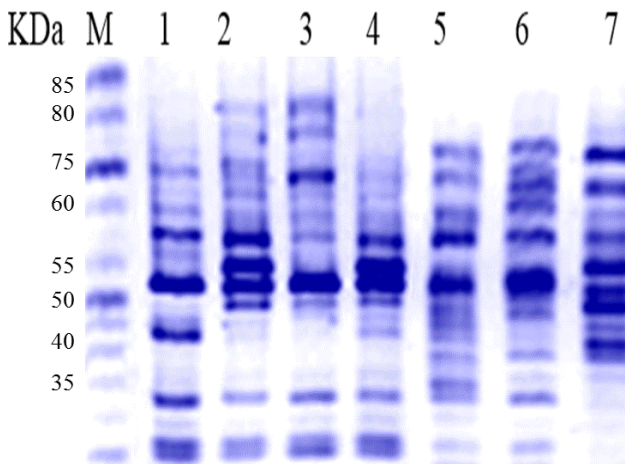


Fig. 7. Protein profile of *Z. mays* seedlings treated with different ULAE concentrations added to Hoagland's solution or used as foliar spray where M: protein marker

- 1- *Z. mays* grown in 50% Hoagland's solution and 50% of *Ulva lactuca* extract.
- 2- *Z. mays* grown in 25% Hoagland's solution and 75% of *Ulva lactuca* extract
- 3- *Z. mays* grown in 100% Hoagland's solution and sprayed with 1% *Ulva lactuca* extract as foliar spray
- 4- *Z. mays* grown in 100% Hoagland's solution and sprayed with 0.5% *Ulva lactuca* extract as foliar spray
- 5- *Z. mays* grown in 100% of *Ulva lactuca* extract
- 6- *Z. mays* grown in 100% Hoagland's solution and sprayed with 5% *Ulva lactuca* extract as foliar spray
- 7- Control treatment: 100% half strength Hoagland's solution

Discussion

The present study investigated the effect of different concentrations of ULAE added to the growth medium or applied as a foliar spray on the seedling of *Zea mays*.

A significant increase in the root hair length and the density is often observed in plants treated with seaweed extracts suggesting that these substances induce a "nutrient acquisition response" that favors nutrient uptake in plants via an increase in the absorptive surface

area (Nardi *et al.*, 2016). A stimulating effect of ULAE on seed germination was demonstrated in this study and the results are in accordance with the reports of (Nedumarum and Perumal, 1991; Osman & Quronfuahl 2015). The present study shows that the germination% of *Z. mays* seeds soaked in ULAE was increased compared to the corresponding control. Similar results were also observed in *Cajanus cajan* (Kalaivanan *et al.*, 2012) and *Lycopersicon* spp. (Alalwani *et al.*, 2012; Bahieldin *et al.*, 2018). This may be attributed to the presence of growth-promoting substances such as IAA and IBA, gibberellins (A&B), and cytokinins (Gupta, 2012; Ramarajan, 2012). There was an increase in the vegetative growth parameters by the application of seaweed extract. This coincides with earlier studies on *Phaseolus vulgaris* L. (Kocira *et al.*, 2013; El-Helow *et al.*, 2-018). The effects of presoaking the wheat grains in a water extract of *U. lactuca* on growth, some enzymatic activities, and protein profile of salinized plants were investigated by Ibrahim *et al.*, (2014). They concluded that presoaking of grains in algal extracts demonstrated a significant enhancement in the percentage of seed germination and other growth parameters. In the present investigation, *Z. mays* (M10 and M321) pre-soaked in different ULAE concentrations showed an increase in RL throughout the experimental course. At the end of the experiment, all applied concentrations showed a positive effect on RL of M321 except for 1% where RL was reduced. Similarly, data indicated that ULAE at 1, 5, and 40% concentrations exerted a significant positive effect on the germination of M321 seeds. Hernández-Herrera *et al.*, (2014) showed that the application of liquid extracts from *U. lactuca* and *P. gymnospora* as a foliar spray increases the shoot and root length of tomato plants. In this study, seedlings grown in 50% U + 50% H, as well as 100% U, induced pronounced increase in total carbohydrates which may be a consequence of increased CO₂ fixation. These results are in good agreement with those obtained by Lozano *et al.*, (1999). The increase in total carbohydrate content has also been seen in *Trigonella foenum-graecum* L. treated with seaweeds (Pise, 2010; Shagufta & Mariyam, 2018). This may be attributed to the increase in chlorophyll which can improve photosynthesis and hence increase the production of carbohydrate. An alternative explanation is that organic molecules such as organic acids, methionine and even PAs in LSF can increase nutrient absorption in plants by chelating the available nutrients, thereby increasing their absorbance and so can increase carbohydrates (Papenfus, 2013). The appearance of low molecular weight protein bands in maize seedlings in the different treatments could be considered as treatment-specific proteins (King, 1991) or indicate a changed pattern of gene expression upon ULAE treatment (Popova *et al.*, 1995). On the other hand, induction of the low molecular weight protein bands of 10, 12 and 22 KDa in all treatments tested could be also triggered by hormones which are present in the extracts and it is tentatively predicted that such proteins might represent phytohormone receptors. Moreover, the lower molecular weight proteins are known to have a role in the stress tolerance process (Waters *et al.*, 1996; Di *et al.*, 2018).

Conclusion

This study showed that a liquid seaweed extract from *U. lactuca* effectively stimulated the growth of maize seedlings, and therefore, it could potentially be a suitable candidate for the production of effective biostimulants. The presence of inorganic minerals in ULAE makes it an excellent choice as an organic fertilizer. The practice of applying eco-friendly seaweed extract can, therefore, be recommended to the farmers to attain better germination and growth of other important crops.

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