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Original Article

Improvement of growth, biomass production and mineral composition of aquaponically grown lettuce by foliar application of two seaweed extracts

Hibat Allah Annabi[1*], Bochra Laribi[1], Anissa Riahi[2], Amira Hachana[3], Hanen Arfaoui[3], Mouna Mechri[4], Yassine Sekma[2], Abdessalem Shili[5], Taoufik Bettaieb[1]
[1]Laboratory of Horticultural Sciences, National Agronomic Institute of Tunisia (INAT), University of Carthage, 43 Av. Charles Nicolle-1082, Tunis, Tunisia
[2]Higher School of Agriculture of Kef (ESA Kef), University of Jendouba-7119, Le Kef, Tunisia
[3]Laboratory of Agricultural Sciences and Techniques, National Institute of Agricultural Research of Tunisia (INRAT), University of Carthage, Tunisia
[4]National Institute of Field Crops (INGC), Boussalem, Jendouba, Tunisia
[5]Research Laboratory Ecosystems & Aquatic Resources, National Agronomic Institute of Tunisia (INAT), University of Carthage, 43 Av. Charles Nicolle-1082, Tunis, Tunisia

**Corresponding author's emails:* hiba.annabi@inat.ucar.tn; hibaannabi5@gmail.com
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Abstract

Seaweed extracts are substances rich in numerous plant growth stimulating molecules such as macro and micronutrients as well as hormones and have been used in agriculture for decades for their renown activity in promoting plant development. This research was conducted to examine the efficiency of two aqueous seaweed extracts as biostimulants in enhancing the growth and mineral composition of lettuce (*Lactuca sativa* var. *longifolia*) grown in aquaponics. Therefore, lettuce plants were grown under a greenhouse in a Nutrient Film Technique aquaponic system. The green algae extracts were prepared from *Chaetomorpha linum* and *Ulva linza* and their application was done by foliar spraying. Results showed that *C. linum* extract positively affected lettuce growth and biomass production. Indeed, this treatment enhanced the majority of the parameters studied: a significant increase in the number of leaves (20.56 leaves developed per plant, $P < 0.05$), the root length (22.30 cm, $P < 0.05$) and the shoot dry weight (13.68 g, $P < 0.05$) was observed. The chlorophyll and carotenoids contents of aquaponically grown lettuce were enhanced by the application of the aforementioned treatment, nevertheless these differences were not statistically significant. Overall results related to the mineral composition highlighted the effectiveness of *U. linza* extract. This latter treatment has significantly improved the Ca (by 8.75%, $P < 0.05$), Fe (by 2.71%, $P < 0.05$), Cu (by 26.88%, $P < 0.05$), Mn (by 7.92%, $P < 0.05$) and Zn (by 45.35%, $P < 0.05$) contents in lettuce leaves. These findings prove the potential of the studied algae extracts as biostimulants, thus encouraging their use in the enhancement of aquaponically grown lettuce productivity.

Keywords: NFT, *Chaetomorpha linum*, *Ulva linza*, Nile Tilapia, Biostimulant

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Introduction

In addition to a substantial surge in the global population, the mid-20th century was marked by the Green Revolution resulting in an increase of crop yields due to the integration of advanced agricultural technologies as well as the use of fertilizers and pesticides. Their excessive utilization deteriorates soil's mineral nutrients availability and the quality of groundwater (Kaur and Purewal, 2019). An environmentally friendly alternative to agrochemicals is biostimulants (Bharti and Suryavanshi, 2021). Indeed, biostimulants are defined as substances obtained from organic material that, when administered in small amounts, exhibit the capacity to promote the growth and development of a plethora of crops under optimal and stressful conditions (Miranda et al., 2024). It is worth mentioning that there are numerous kinds of biostimulants such as beneficial fungi (Arbuscule-Forming Mycorrhiza) and bacteria (Plant Growth-Promoting Rhizobacteria) as well as seaweed extracts (du Jardin, 2015; Khan et al., 2023). These solutions are obtained from marine macroalgae which are aquatic plants found in coastal regions around the globe. In fact, seaweeds are organisms lacking the defining structural attributes typically associated with higher plants, such as roots, stems, and leaves (Raja and Vidya, 2023; Tan et al., 2021). Based on a number of criteria such as photosynthetic pigments, storage compounds, and the composition of cell walls, seaweeds are classified into three main groups: *Phaeophyta* (brown algae), *Rhodophyta* (red algae), and *Chlorophyta* (green algae) (Arora and Sahoo, 2015; Nedumaran, 2017). On one hand, seaweed extracts are characterized by a multifaceted mechanism that entails altering gene expression, metabolic processes as well as phytohormone synthesis (Sujeeth et al., 2022; EBIC, 2023). On the other hand, these biostimulants have been observed to promote the accumulation of compatible solutes and antioxidants thereby mitigating oxidative stress (Zulfiqar et al., 2024). They have multiple functions such as the enhancement of the process of transporting nutrients from roots to shoots (du Jardin, 2015; Bharti and Suryavanshi, 2021). Indeed, these non-chemically synthesized products are known for their biodegradability, their capacity to promote plant growth (mainly due to the existence of phytohormones such as auxins), their tolerance to stress as well as their biological control ability (in which bioactive molecules such as polysaccharides, fatty acids, pigments are applied in biocontrol experiments) have rendered them extremely valuable for sustainable agriculture (Battacharyya et al., 2015; Stirk et al., 2020; Yashavantha Rao et al., 2020; Tan et al., 2021). In this context, countless research has been dedicated to investigating seaweeds as biostimulants in agriculture. It is worth mentioning that the majority of studies have focused on macroalgae belonging to the *Phaeophyta* group including *Ascophyllum nodosum*, *Laminaria spp*., and *Sargassum spp*., a choice largely influenced by their large size and availability (Mooney and van Staden, 1986; Al-Juthery et al., 2020). Conversely, green algae are smaller in size, measuring only a few centimeters to a meter in length. For example, seaweeds belonging to the genus *Ulva* have a sheet-like appearance, while the thallus of *Chaetomorpha linum* resembles entangled mats of hair and can reach diameters of a meter or more (Nedumaran, 2017).

Green seaweeds are notable for their richness in mineral elements (N, Ca, Mg, Zn, Cu, etc.), amino acids, protein (representing 10-26% of their dry matter), vitamins, chlorophylls, carotenoids, phenolic compounds (bromophenols, flavonoids, and phenolic acids) as well as plant growth stimulants (auxins, cytokinins, and betaines). Additionally, these algae contain various fatty acids including palmitic acid, linolenic acid, and arachidonic acid, as well as sulfated polysaccharides, mainly ulvan (Fleurence, 1999; Cotas et al., 2020; García et al., 2020; Hernández-Herrera et al., 2022; Maray et al., 2023; Li et al., 2024). Accordingly, the chemical constituents found in algae are able to modulate plant metabolism and hence, rendering them suitable for use as biofertilizers in agriculture (Stirk et al., 2020). Indeed, biofertilizers are biodegradable and non-toxic to humans and to the environment. They can be employed in a variety of ways, for instance as liquid extracts and thus applied as a foliar spray or soil drench, or in a granular form as a soil amendment. Consequently, seaweed extracts act as biostimulants by enhancing the efficiency and availability of plant nutrients, as well as the quality of the plants themselves and their resilience to external stressors (Chrysargyris et al., 2018; El Boukhari et al., 2020; EBIC, 2023).

Lettuce (*Lactuca sativa* L.) is an annual crop belonging to the *Asteraceae* family. It is one of the most economically important and consumed vegetables globally, due to its notable content of vitamin C, polyphenols, and fiber (Shatilov et al., 2019; Siracausa and Ruberto, 2019). Indeed, among all the different types, romaine lettuce has the highest amount of carotene, every 100 g of uncooked romaine lettuce contains 4 mg of carotene). Typically, this significant crop is cultivated in the soil whereas a growing interest exists in its soilless cultivation including hydroponics and aquaponics (Soares et al., 2015; Kovàscné Madar et al., 2019; Eck et al., 2021). Indeed, aquaponics represent an integrated agricultural technique that combines aquaculture (the rearing of fish) with hydroponics (the cultivation of plants without soil). The waste produced by fish is dissolved in water and converted by nitrifying bacteria into nutrients. These nutriments are assimilated by plants, thereby purifying the water before it is returned to the fish tank (Rharrhour et al., 2022; Madusanka et al., 2023). As a matter of fact, in this mutually beneficial setting, crop cultivation is enhanced due to the plentiful supply of nutrients created by the aquacultural system. This is advantageous since plants assimilate mineral elements, thus sustaining the appropriate maintenance of water quality in the fish tank (Nascimento et al., 2023). This symbiotic system has attracted worldwide attention in recent years due to its high efficiency in terms of water and mineral nutrients (Matysiak et al., 2023). This soilless cultivation technique allows for the production of vegetables and fish at high yields, while simultaneously reducing water wastage and thus making it an environmentally friendly approach (da Silva Cerozi and Fitzsimmons, 2017).

Therefore, the objective of this study was to examine, for the first time, the impact of two green seaweeds liquid extracts, namely *Chaetomorpha linum* and *Ulva linza*, applied by foliar application on the growth and mineral composition of aquaponically cultivated lettuce.

Material and Methods

Macroalgae collection and seaweeds extracts preparation

The collection of the studied green seaweeds, *Chaetomorpha linum* and *Ulva linza,* was done by hand from Guengla beach, Bizerte, Tunisia (Lat 37° 10' N; Long 9° 46'E) in March 2024. Before carrying these macroalgae to the laboratory in plastic bags, where they were identified, they were washed with sea water to remove sand particles. Afterwards, it was washed thoroughly with tap water, shade-dried for several days and then ground into a fine powder. The seaweeds powders were mixed with water in the proportion of 1:20 (w:v) and then boiled for 30 min with constant magnetic stirring (300 rpm). The mixture was filtered through a stainless-steel sieve and then through an ashless filters paper and kept at - 20° C. The obtained extracts were considered as stock solutions and utilized to prepare the seaweed extracts (SEs) at 10% by diluting adequate ratios of stock solutions in distilled water.

Characterization of the experimented seaweeds

Numerous parameters characterizing the physicochemical properties of the experimented SEs were measured. Physical parameters such as pH and electrical conductivity (EC) were measured by standard methods using a multi water quality meter (Lutron WA-2017SD Multi Water Quality Meter, Bangkok, Taiwan). The chemical composition of the dried algae powders was evaluated. The total nitrogen (N) content was determined by the Kjeldahl method (Sher, 1955), while the phosphorus (P_2O_5) content was determined by the Olsen method (Gautheyrou and Gautheyrou, 1966). The estimation of the potassium (K_2O), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) and sodium (Na) contents were performed using the inductively coupled plasma atomic emission spectroscopy method.

Greenhouse growth bioassay

The greenhouse experiment was carried out from February to May 2024. Romaine lettuce seeds were cultivated in seedling trays containing peat under natural light conditions (16 h light: 13 h dark). Seedlings were irrigated with tap water during this period. After 20 days, uniform seedlings were transplanted into pots containing substrates consisting of a mixture of peat, sand and expanded clay aggregates (1:1:1 *w/w/w*) under natural light. The transplantation of the plants into the aquaponic system was done 26 days later and growth was continued for another 8 weeks where the temperature ranged between 19° and 33°C. It is noteworthy to mention that in this experiment; lettuce was grown for 76 days in total.

Aquaponic system design

A coupled aquaponic Nutrient Film Technique (NFT) system was used during this experiment. The system was composed of an 800 L fish rearing tank, a 100 L brush filter used for solid waste removal (mechanical filter) as well as a biofilter that is comprised of a 100 L cylindrical tank equipped of net bags containing bio-balls to provide a high surface area for bacteria. The plant beds used were in triplicates and consisted each of 5 perforated PVC pipes (Length = 300 cm; Width = 10 cm) that had 14 holes / tube (diameter = 7 cm) for inserting the net pots with lettuce plants, potting space between 2 plants was 10 cm. The nutrient rich water was pumped continuously using a water pump from the fish rearing tank into the mechanical filter, biofilter and the grow pipes and then returned by gravity back to the fish tank in a closed water recirculating configuration. An air pump was used to provide oxygen inside the fish culture tank and the biofilter *via* numerous air stones. It is noteworthy to mention that iron (Fe-EDDHA) was added every 3 weeks to prevent plant chlorosis.

Stocking and feeding of fish

Nile tilapia (*Oreochromis niloticus*), a fresh-water fish indigenous to northern Africa, was used for this experiment. Thirty uniformed-sized (350 g) fish were stocked in the fish tank. As a consequence of its broad range of water conditions (pH between 5 and 9; dissolved oxygen < 2 mg L^{-1}) under which it grows, this specie is one of the most commonly reared species in aquaculture (Bonham, 2022). A commercial feed containing an average protein content of 30%, 16% fat, 8% ash and 1.2% phosphorus was offered 3 times per day: at 8:00 AM, at 12:00 PM and at 17:00 PM by automated feeders to the fish.

Physical and chemical water parameters

Several parameters associated with the aquaponic system water quality were assessed: temperature (T), EC, pH and dissolved oxygen (DO_2) were measured using a portable multi water quality meter (Lutron WA-2017SD Multi Water Quality Meter, Bangkok, Taiwan). Dissolved nutrients analyzed were ammonia, nitrite, nitrate and Fe^{2+} using test kits (JBL Proaquatest Lab, JBL, Germany).

Measurements

Morphological characteristics such as number of leaves, leaves length (cm), leaves width (cm), leaf area (cm^2), stem diameter (mm), roots length (cm), and volume of roots (cm^3) were measured. In addition, the fresh weight (g) of shoots (stems and leaves) and roots were assessed by destructive harvests of 9 randomly selected plants. These samples were stored in paper bags and oven-dried for 48 h at 75°C to obtain the dry weight (g). The dry matter contents (DMC) were then determined according to the following equation:

$$\mathbf{DMC}\ (\%) = \frac{\mathbf{DW}}{\mathbf{FW}} \times \mathbf{100}$$

Where FW: fresh weight (g) and DW: dry weight (g).
Afterwards, the dried samples were ground to analyze the total nitrogen (N) content by the Kjeldahl method (Sher, 1955), the phosphorus (P_2O_5) content using the Olsen method (Gautheyrou and Gautheyrou, 1966). Finally, the potassium (K_2O) content was measured by the flame photometric method. The inductively coupled plasma atomic emission spectroscopy method was used to determine calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), and sodium (Na) contents. These measurements were carried out with 3 repetitions per treatment.
Regarding the photosynthetic pigments namely chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, they were determined according to Torrecilas et al. (1984) method and their contents were calculated using Mackinney (1941) and Arnon (1949) equations.
The assessment of the relative water content (RWC) was done according to Schonfeld et al. (1988) while the Lutts et al. (1996) method was used to determine the electrolyte leakage (EL). All measurements were done in triplicates.

Seaweed extract application, experimental design and statistical analysis

The application of both SEs was carried out using a spray bottle, totaling two foliar applications. The first foliar spraying was carried out 4 days after the transplantation of plants into the aquaponic systems, whilst the second one was carried out 2 weeks later. During the first application each plant received 5 mL of the biostimulants while plants received 10 mL during the second application. Control plants were foliar sprayed with distilled water in the same way with the same volumes as previously described. It is noteworthy to mention that the foliar application was chosen because it allows the absorption of biostimulant through the cuticle, epidermal cells and stomata which provides a way to deliver nutrients directly to the leaves thus enhancing nutrient absorption.

The experiment was arranged in a complete random block design with 3 repetitions and 1 factor of variation which is the seaweed extract (SE) tested. The Statistical Analysis System version 9.0 was used in data analysis (SAS Institute, 2002). Statistically significant differences were determined using the one-way analysis of variance (ANOVA) and were presented as mean ± standard deviation. Duncan's multiple range tests were exploited in means comparison at 5% probability.

Results

Characterization of the experimented seaweeds

The results of the determination of the nutrient composition of the dried macroalgae *C. linum* and *U. linza* powders, used substantially for the preparation of SEs, are presented in Table 1.

Table-1. Concentration of macro and micronutrients of the seaweeds.

Constituent	Seaweeds	
	C. linum	***U. linza***
N (%)	$1.20 \pm 0.01^{b*}$	1.93 ± 0.03^{a}
P (mg Kg^{-1} DW)	1.36 ± 0.01^{b}	1.20 ± 0.01^{a}
K (g Kg^{-1} DW)	474.97 ± 11.80^{b}	2863.20 ± 9.60^{a}
Ca (g Kg^{-1} DW)	5.31 ± 0.05^{b}	12.19 ± 0.07^{a}
Mg (g Kg^{-1} DW)	1.87 ± 0.03^{b}	11.66 ± 0.03^{a}
Cu (mg Kg^{-1} DW)	6.88 ± 0.51^{b}	19.62 ± 0.12^{a}
Fe (mg Kg^{-1} DW)	910.52 ± 79.87^{b}	3708.83 ± 15.15^{a}
Mn (mg Kg^{-1} DW)	147.69 ± 13.21^{b}	70.36 ± 0.46^{b}
Zn (mg Kg^{-1} DW)	25.62 ± 2.69^{b}	58.97 ± 0.24^{a}
Na (g Kg^{-1} DW)	9.59 ± 0.14^{b}	17.49 ± 0.16^{a}

*Values followed by different superscripts (a-b) in the same line are significantly different at probability level $P < 0.05$ (Duncan test).

Results obtained from the nutrient composition analysis showed the presence of N, P, K, Cu, Fe, Mn, Zn, Ca, Mg and Na in both experimented macroalgae (Table 1). The concentration of the majority of the tested mineral elements (N, P, K, Cu, Fe, Zn, Ca, Mg and Na) was significantly higher in *U. linza* than those in *C. linum*. However, the Mn concentration was significantly higher in *C. linum* when compared to *U. linza* (Table 1).

The pH values of the studied SEs, namely *C. linum* and *U. linza,* were slightly acidic and practically identical (6.79 and 6.62, respectively). Regarding the EC of the tested SEs, it was observed that *C. linum* pure and diluted liquid extracts EC values (33 mS and 4.34 mS, respectively) were higher than those of the other experimented seaweed extract (10.8 mS and 3.87 mS, respectively). It is noteworthy to mention that the dilution of the liquid extracts induces a decline in the EC.

Effects of the foliar application of SEs on the growth of aquaponically grown lettuce

The foliar application of the SE of *C. linum* was found to be more effective in influencing all of the studied growth parameters in lettuce during the period of its aquaponic cultivation (Figure 1). Indeed, the application of both SEs (*C. linum* and *U. linza*) displayed a significant enhancement of the number of leaves developed per plant by 17.7 % and 10.1 % respectively (P < 0.05) (Figure 1a).

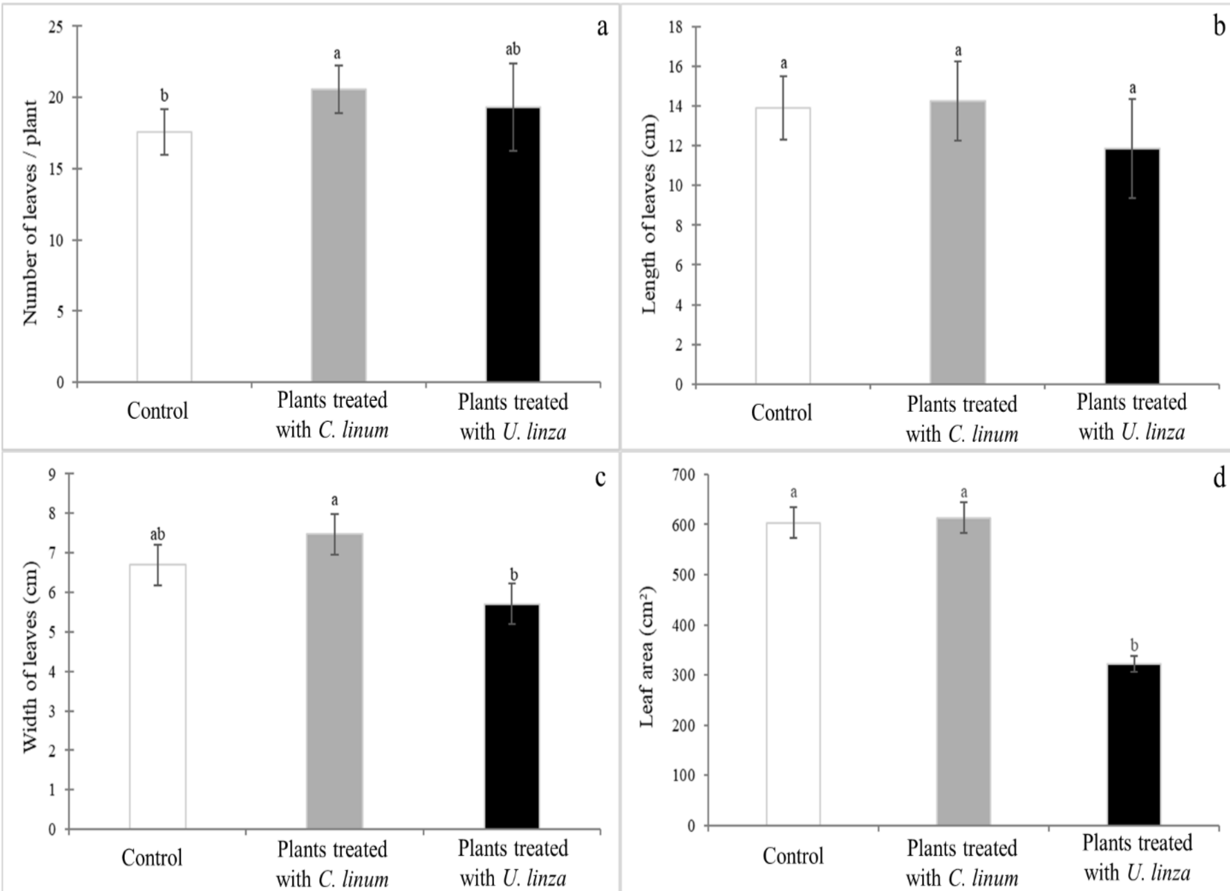

Figure-1. Effect of the SEs application on leaf morphometry: number of leaves (a); length of leaves (b); width of leaves (c) and leaf area (d) of aquaponically grown lettuce (*Lactuca sativa var. longifolia*) plants. Columns denoted by different superscripts (a-b) are significantly different at P < 0.05 (Duncan test); Bars represent standard error.

On the other hand, lettuce plants subjected to these same treatments showed no significant differences in the length of leaves as well as the root volume even though these parameters have showed a slight increase (by 2.59 and 10.92 % respectively, P < 0.05) when *C. linum* SE was applied. However, the foliar application of *U. linza* extract decreased the aforementioned parameters by 14.82 and 12.18 % respectively (P < 0.05) (Figure 1b and Figure 2c). Moreover, *U. linza* extract induced a decline of the root length by 2.89 % when compared to the control whereas the application of C. *linum* SE resulted in a significant increase (by 15.31 %, P < 0.05) of this parameter (Figure 2b).

In addition, although *U. linza* extract had induced a slight decrease in the width of leaves and the stem diameter (from 6.69 to 5.71cm and from 7.64 to 6.97 mm respectively, Figure 1c and Figure 2a) when compared to the control, the differences were not significant.

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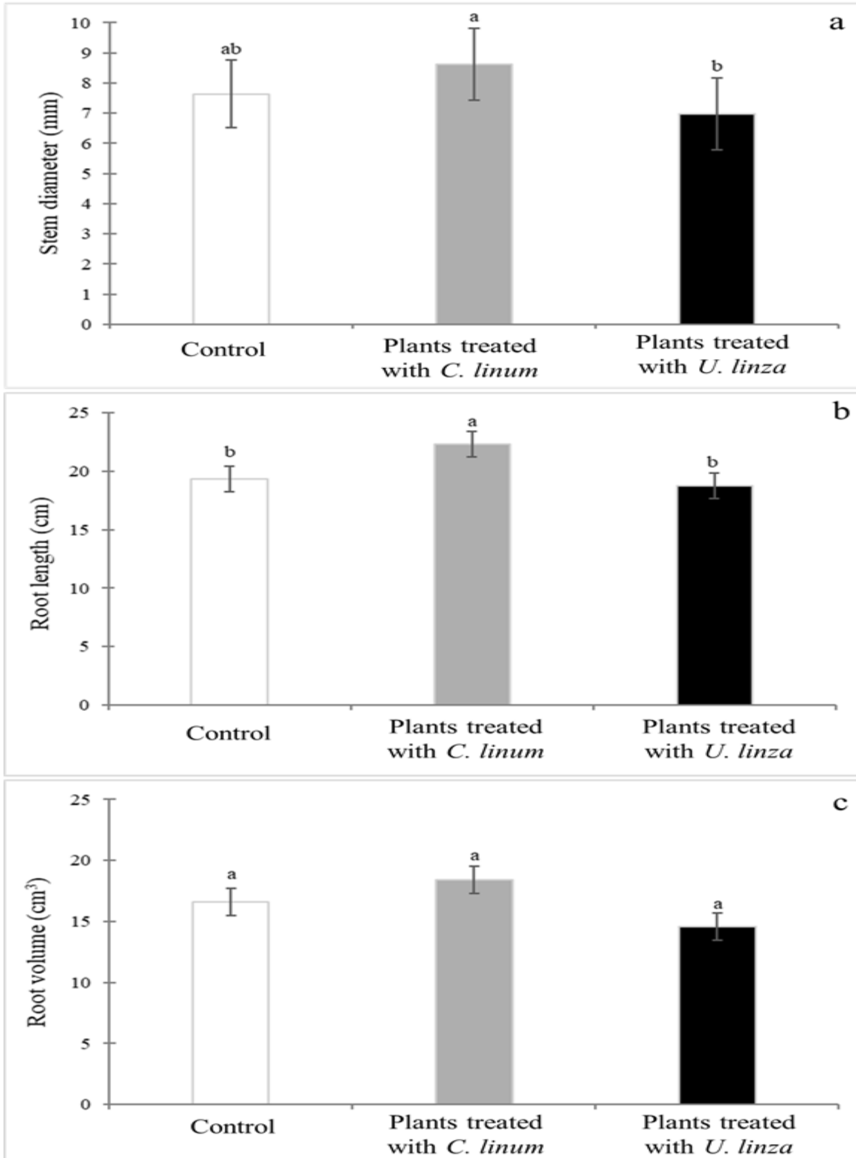

Figure-2. Effect of the SEs application on the stem diameter (a) and root traits: root length (b) and root volume (c) of aquaponically grown lettuce (*Lactuca sativa var. longifolia*) plants. Columns denoted by different superscripts (a-b) are significantly different at $P < 0.05$ (Duncan test); Bars represent standard error.

A slight non-significant increase of 1.55% was observed in the leaf area of lettuce treated with C. *linum* extract in comparison to non-treated plants. In contrast, *U. linza* SE significantly ($P < 0.05$) decreased, by 46.6%, the leaf area when compared to control.

Effects of the foliar application of SEs on the biomass production of aquaponically grown lettuce

The effects of the foliar application of SEs on the biomass production of lettuce grown in aquaponics are presented in Table 2. The application of *C. linum* SE was associated with a stimulatory effect on the biomass production of lettuce plants. Indeed, the fresh and dry weights of shoots were significantly ($P < 0.05$) enhanced by 15.73 and 32.30% respectively while a significant increase (15.43%, $P < 0.05$) of the root dry weight was noted in comparison to the plants treated with *U. linza*. Additionally, *C. linum* was observed to enhance the fresh root weight but without significant differences. Conversely, both *C. linum* and *U. linza* SEs were observed to reduce the root dry matter content (by 7.75 and 12.88 %, respectively, $P < 0.05$) in comparison to the control. Besides, *U. linza* SE exhibited a significant elevation (42.40%, $P < 0.05$) in the shoot dry matter content.

Table-2. Effect of the foliar application of the SEs on the biomass production of aquaponically grown lettuce.

Foliar treatment	Shoots			Roots		
	Fresh weight (g)	Dry weight (g)	Dry matter content (%)	Fresh weight (g)	Dry weight (g)	Dry matter content (%)
Control	127.72 ± 15.88^{a*}	10.34 ± 2.02^{b}	8.16 ± 1.68^{b}	39.89 ± 7.25^{a}	3.37 ± 0.50^{ab}	9.55 ± 1.16^{a}
***C. linum* extract**	147.82 ± 25.43^{a} (+ 15.73 %) **	13.68 ± 1.59^{a} (+ 32.30 %)	9.06 ± 1.36^{b} (+ 11.03 %)	44.85 ± 8.46^{a} (+ 12.43 %)	3.89 ± 0.53^{a} (+ 15.43 %)	8.81 ± 1.20^{a} (- 7.75 %)
***U. linza* extract**	91.23 ± 16.88^{b} (- 36.49 %)	10.86 ± 1.93^{b} (+ 5.03 %)	11.62 ± 2.27^{a} (+ 42.40 %)	38.32 ± 6.48^{a} (- 3.93 %)	3.14 ± 0.51^{b} (- 6.82 %)	8.32 ± 1.40^{a} (- 12.88 %)

*Values followed by different superscripts (a-b) in the same column are significantly different at probability level $P < 0.05$ (Duncan test).
**Values in parentheses represent reduction or increase percentage compared to the control.

Effects of the foliar application of SEs on the mineral composition of aquaponically grown lettuce

The foliar application of both SEs enhanced the levels of the majority of the minerals examined as shown in Figure 3. Indeed, the application of *U. linza* extract has resulted in an increase in the content of essential minerals including Zn, Cu, Ca and Mg. Thus, plants receiving the aforementioned treatment exhibited significantly greater Zn, Cu, and Ca contents (29.10, 10.48 mg Kg^{-1} DW and 8.95 g Kg^{-1} DW, respectively, $P < 0.05$) when compared to the alternative treatment and the control (Figures 3d, g and i). In contrast, *C. linum* extract had a detrimental impact on Cu and Ca amounts, resulting in a significant reduction of 20.7 and 5.71 %, respectively, in comparison to the control (Figures 3d and g).

As for the Mg content, the highest value was noted in lettuce treated with *U. linza* extract, exhibiting a notable enhancement of 14.82% ($P < 0.05$) in comparison to the control (Figure 3e). However, no significant differences were discerned in the Mg content of these plants and that of plants treated with *C. linum* extract (Figure 3e). Indeed, this latter treatment induced a significant improvement (+12.04%, $P < 0.05$) in the Mg content when compared to the control (Figure 2e). In fact, *C. linum* extract significantly ($P < 0.05$) improved N, Mn and Fe contents by 33.33, 12.49 and 12.19%, respectively in comparison to the other treatment and to the control (Figures 3a, f and h).

It has been observed that the two green SEs tested significantly reduced P and K contents. Indeed, *C. linum* significantly reduced these elements by 79.6 and 31.03 %, respectively (Figures 2b and c). As for *U. linza*, the decline was significantly ($P < 0.05$) lower compared to the previous treatment, amounting to 11.26 and 1.2 %, respectively but still significant ($P < 0.05$) compared to the control (Figures 3b and c).

Similarly, the abovementioned treatment decreased the Na content by 3.22%, whilst this same nutrient element was significantly ($P < 0.05$) elevated in lettuce treated with *C. linum* extract.

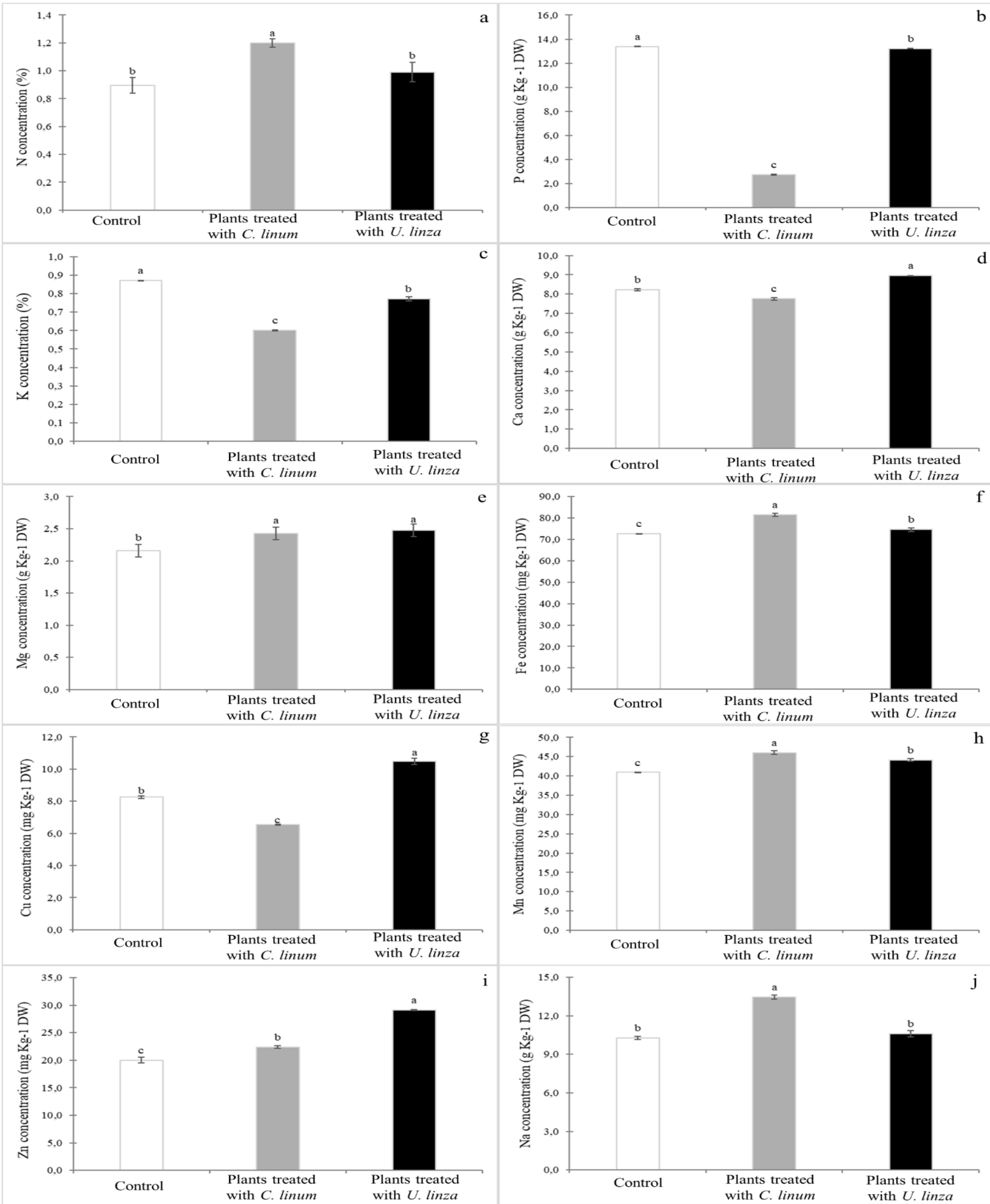

Figure-3. Effect of the application of the SEs on the concentration of Nitrogen (a); Phosphorus (b); Potassium (c); Calcium (d); Magnesium (e); Iron (f); Copper (g); Manganese (h); Zinc (i) and Sodium (j) of aquaponically grown lettuce (*Lactuca sativa var. longifolia*) plants. Columns denoted by different superscripts (a-c) are significantly different at $P<0.05$ (Duncan test); bars represent standard error.

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Effects of the foliar application of SEs on the chlorophyll and carotenoid pigments of aquaponically grown lettuce

The foliar application of the experimented SEs showed no significant differences between the studied treatments when compared to the control regarding the content of photosynthetic pigments (Table 3). The SE of *C. linum* was found to be more effective in enhancing chlorophyll a (+ 6.62%, P < 0.05), b (+ 26.45%, P < 0.05), and total chlorophyll (+ 18.19%, P < 0.05) as well as the carotenoids content (+ 41.72%, P < 0.05). Similarly, the *U. linza* SE has increased the chlorophyll b, total chlorophyll and carotenoids content by 17.25, 9.76, and 23.05 %, respectively (P < 0.05). In contrast, a slight decrease (- 0.74%) in the chlorophyll a content was recorded in plants receiving the aforementioned treatment.

Table-3. Effect of the foliar application of the SEs on the photosynthetic pigments content of aquaponically grown lettuce.

Foliar treatment	Photosynthetic pigments content (μg mg^{-1} FW)			
	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids
Control	157.76 ± 21.23[a]	221.01 ± 59.18[a]	378.64 ± 57.18[a]	77.50 ± 16.60[a]
***C. linum* extract**	168.20 ± 22.48[a] (+ 6.62 %) *	279.46 ± 27.36[a] (+ 26.45 %)	447.51 ± 48.92[a] (+ 18.19 %)	109.83 ± 24.19[a] (+ 41.72 %)
***U. linza* extract**	156.60 ± 27.16[a] (- 0.74 %)	259.14 ± 43.60[a] (+ 17.25 %)	415.59 ± 70.28[a] (+ 9.76 %)	95.36 ± 27.16[a] (+ 23.05 %)

*Values in parentheses represent reduction or increase percentage compared to the control.

Effects of the foliar application of SEs on the RWC and the EL of aquaponically grown lettuce

In terms of RWC, treating plants with *C. linum* resulted in a statistically significant (P < 0.05) increase of 13.91 % in comparison to the control (Figure 4a). Conversely, this same treatment has had a detrimental impact on the EL, leading to a significant (P < 0.05) increase of 2.42 % (Figure 4b). However, no significant differences were recorded in plants treated with *U. linza* SE. Indeed, this treatment resulted in a decline in RWC (-6.61%, P < 0.05) and a slight increase of EL (+0.63%, P < 0.05) as shown in Figure 3a and Figure 3b, respectively.

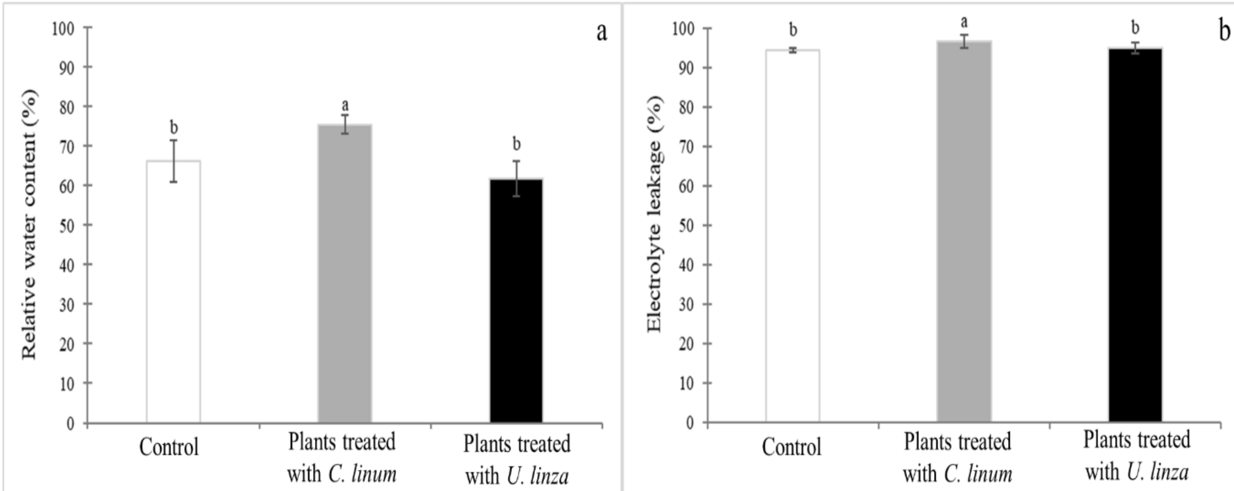

Figure-4. Effect of the application of the SEs on (a) relative water content and (b) electrolyte leakage of aquaponically grown lettuce (*Lactuca sativa* var. *longifolia*) plants. Columns denoted by different superscripts (a-b) are significantly different at $P < 0.05$ (Duncan test); bars represent standard error.

Discussion

Seaweed extracts, defined as plant biostimulants, have been used in the agricultural sector since the 1950s (Stirk et al., 2020). The rising interest in these seaweed-based products is reflected in the increasing number of patents applied for during these last few years, which highlights the growing recognition of their numerous beneficial traits within the agricultural community (Tan et al., 2021; Fatimi, 2022).

In this paper, an experiment was conducted with the aim of assessing and comparing the effects of extracts of two green macroalgae namely *Chaetomorpha linum* and *Ulva linza* on the growth, biomass production, the mineral composition and the chlorophyll pigment contents of aquaponically grown lettuce plants.

Prior studies reported the richness of green algae in mineral elements as well as bioactive compounds characterized by a significant nutritional value (Arora and Sahoo, 2015). Our results related to the N concentration in *C. linum* were lower than that recorded in the same seaweed sampled from the Northern Lake of Tunis (Chebil Ajjabi et al., 2024). Additionally, *C. linum* collected from the coast of the Bulgarian Black Sea contained a lower concentration of K, Na, Cu (15.44 mg g^{-1} DW, 3.09 mg g^{-1} DW, and 0.76 mg per 100g DW) than that observed in this study (Panayotova and Satncheva, 2013). These same authors reported a higher concentration in Ca (7.30 mg g^{-1} DW), Mg (2.50 mg g^{-1} DW), Mn (29.40 mg per 100g DW), Fe (105.97 mg per 100g DW), and Zn (1.87 mg per 100g DW). Besides, *U. linza* collected from the eastern harbor and Abu Qir Bay (Alexandria, Egypt) contained a lower amount of Fe but the Mn, Zn, Cu concentrations were higher in the aforementioned study when compared to our results (Shobier et al., 2023). Nevertheless, it is important to indicate that a fluctuation in the level of algae's multiple compounds is attributable to the analytical methods employed, seasonal variations and its geographical distribution (Hernández-Herrera et al., 2014).

Based on our findings, the application of *C. linum* extract has significantly increased the number of leaves developed by 17.1% when compared to the control. In the same context, Mohammed et al. (2022) reported similar results indicating a significant enhancement of the number of leaves of lettuce (*Lactuca sativa* var. *longifolia* L.) when plants have been treated by foliar spraying of an algal extract. It is noteworthy to mention that our results proved that *U. linza* extract increased this same parameter by 10.1% but not significantly when compared to the control. Furthermore, it has been observed that treating mung bean (*Vigna radiata*) plants by foliar application of *Portieria hornemannii* extract (30%) significantly enhanced the number of leaves developed. However, applying *Turbinaria ornata* (20%) extract by foliar spraying enhanced the number of leaves but not significantly compared to the latter treatment as reported by Karthik and Jayasri (2023). Our findings, related to the number of leaves of plants treated with *U. linza* extract, are similar to those of Chrysargyris et al. (2018) who found that spraying the leaves of lettuce

(*Lactuca sativa* L. cv. Verdede) with *Ascophyllum nodosum* extract has enhanced this parameter, but not significantly in hydroponically grown plants when the nutrient solution contained a low K concentration (125 mg l^{-1}). Furthermore, Hassan et al. (2021) found that cucumber plants treated by foliar application of a mixture of 75% commercial seaweed liquid extract (TAM) which are composed of three algal species namely *Ulva lactuca*, *Jania rubens* and *Pterocladia capillacea,* and 25% NPK mineral fertilizer had the highest number of leaves (32.6 leaves) but this difference was not significant in comparison to the treatments "50% TAM + 25% NPK mineral fertilizer" and "100% TAM" (for the year 2018). As for the slight non-significant diminution in root length observed in plants treated with *U. linza* SE, a similar finding was also reported in the root length of basil plants treated with the extracts of *U. lactuca* and *Padina pavonica*; nevertheless, the differences were not statistically significant (Abudeshesh et al., 2024). Additionally, our findings indicated that aquaponically grown lettuce treated with *C. linum* extract presented significantly longer roots in comparison to non-treated plants (control). These results are in accordance with an earlier study carried out on mung bean and which reported that foliar spraying of *T. ornata* (100%) extract has not significantly affected this parameter in comparison to the aqueous control unlike spraying *Portieria hornemannii* extract (30%) which showed an enhancement of the root length by 120% in treated plants (Karthik and Jayasri, 2023). Also, Bahmani Jafarlou et al. (2023) observed a significant enhancement of the root length of *Calotropis procera* plants (control plants: salt stress = 0 $dS.m^{-1}$) treated with *Sargassum angustifolium* extract (1%). As for the root volume, our findings indicate a slight enhancement of this parameter in lettuce sprayed with *C. linum* SE and a decrease of this parameter in plants treated with *U. linza* extract but these differences were not significant in comparison with control plants. This is in agreement with the results of Bahmani Jafarlou et al. (2023) who observed a non-significant enhancement in the root volume of milkweed seedlings (control plants: salt stress = 0 dS m^{-1}) treated with *S. angustifolium* extract (1.5%), whilst the foliar application with the same seaweed extract (1%) has induced a significant increase of this parameter in the same plants. Regarding leaf length, our findings are in accordance with those found by Chrysargyris et al. (2018) who observed a decrease in this parameter in lettuce grown in hydroponics using nutrient solutions containing a normal (375 mg L^{-1}) and low (125 mg L^{-1}) K concentrations and treated by foliar spraying of *A. nodosum* extract when compared to control plants. Besides, our results showed that the foliar application of *U. linza* SE has negatively affected the stem diameter of aquaponically grown lettuce. Conversely, lettuce treated with *C. linum* extract was marked with slightly bigger stems, but this difference is not statistically significant. Moreover, our findings are in accord with recent studies indicating that the collar diameter of milkweed seedlings decreased but non-significantly in plants receiving 15 $dS.m^{-1}$ and treated by foliar spraying with *S. angustifolium* extract (1.5%) compared to control (salt stress = 15 dS m^{-1}) (Bahmani Jafarlou et al., 2023).

Furthermore, results related to fresh root weight are in accordance with those previously found by Chrysargyris et al. (2018) who found that lettuce plants grown in hydroponics under two K concentrations: normal (375 mg L^{-1}) and low (125 mg L^{-1}) and treated by foliar spraying of *A. nodosum* extract had a slightly higher root fresh weight in comparison with control plants but non-significantly. Moreover, *Padina gymnospora* extract proved its positive effectiveness by significantly enhancing the fresh root weight of tomato plants grown in pots during their vegetative stage (Hernández-Herrera et al., 2022). On the other hand, our results related to the non-significant decrease in this parameter in plants treated with *U. linza* extract are in accordance with the findings of Abudeshesh et al. (2024) who noted that a mixture of the extracts of the algae *Padina pavonica* and *Ulva lactuca* as well as the microalga *Chlorella vulgaris* has induced a slight non-significant decrease of the fresh weight of basil roots.

It is worth mentioning that macroalgae's growth promotion activity is due to numerous active compounds such as phytohormones, mineral elements, polysaccharides, polyphenols, carotenoids, fatty acids and vitamins (Ammar et al., 2022; Mamede et al., 2023). This might explain why even though *U. linza* was richer in macro- and micronutrients than *C. linum*, the former alga was less effective in promoting growth. Indeed, Khan et al. (2009) indicated that mineral nutrients found in seaweed extracts solely are insufficient to explain the growth enhancement induced by these extracts. In addition, the synergy created between these bioactive molecules is what determines the SE efficiency (Krid et al., 2023).

Regarding the fresh shoot weight, our results seem to be consistent with other research which found that treating basil with *P. pavonica* extract induced a non-significant enhancement in this parameter when compared to control (Abudeshesh et al., 2024). Additionally, the application of *P. gymnospora* extract, by drenching the soil, considerably enhanced the fresh shoot weight of tomato plants grown in pots in comparison to control plants (Hernández-Herrera et al., 2022). Moreover, the decrease of the fresh shoot weight observed in lettuce plants treated with *U. linza* extract was also observed by Chrysargyris et al. (2018) who found that treating hydroponically grown lettuce (K = 375 mg L^{-1}) with *A. nodosum* extract resulted in a slight decline of this crop's fresh weight. During this experiment, results related to the shoot dry weight are in accordance with the findings of Abudeshesh et al. (2024) who found that treating basil grown in pots with *U. lactuca* extract has significantly enhanced this parameter in comparison to the control. As for the root dry weight, our results match those observed in an earlier study that showed that the dry root weight of basil plants cultivated in pots and treated with *P. pavonica* extract was enhanced slightly but non-significantly (Abudeshesh et al., 2024). Our findings are also consistent with data obtained by Bahmani Jafarlou et al. (2023) who noted that the application of *S. angustifolium* extract at 1.5% induced a non-significantly inferior effect on dry root weight of milkweed seedlings (seawater stress level = 0) when compared to the control (sprayed with distilled water). Contrary to the expectations, this study did not find a significant difference between the shoot dry matter content of lettuce plants treated with *C. linum* extract and control plants. However, the foliar application of *U. linza* SE induced a significant enhancement of the above-mentioned parameter when compared to control. Indeed, this accords with earlier observations which showed that treating cucumber plants with a commercial seaweed extract (TAM) at a concentration of 50% has induced a non-significant enhancement of the dry matter for the year 2017 (Hassan et al., 2021). Besides, the foliar application of TAM at a concentration of 0.25% significantly enhanced the dry matter of hot pepper plants cultivated during the year 2017 (Ashour et al., 2021). A possible explanation for these results may be the Na present in the SEs. Indeed, Na is a basic nutritional element useful for plants since it enhances plants biomass production (Chanthini et al., 2019). It is noteworthy to mention that large amounts of Na, especially in the presence of chloride, induce damage to crops. In addition, considerable amounts of Na diminish K and Ca absorption (Krastanova et al., 2022). As for the root dry matter content, our findings indicated that liquid extracts of both green seaweeds (*C. linum* and *U. linza*) negatively affected this parameter in aquaponically grown lettuce without significant differences in comparison to the control. This outcome is contrary to that of Chrysargyris et al. (2018) who found that the foliar application of *A. nodosum* extract on lettuce plants cultivated in hydroponics (K = 375 mg L^{-1}) significantly enhanced these plants' root dry matter. Interestingly, these same authors reported that the enhancement of the dry matter was not significant in lettuce roots of plants cultivated in hydroponics using a nutrient solution with a low K concentration (K = 125 mg L^{-1}). Findings of this study showcased that *U. linza* SE (10%) negatively affected the majority of growth parameters as well as the biomass production of lettuce grown in aquaponics. Furthermore, it was observed that the application of fresh and dried seaweeds (*Dictyota dichotoma*, *Gelidium sesquipedale* and *Jania rubens*) at concentrations of 10% and 5%, respectively either by soil drench or by foliar spraying decreased root and shoot lengths as well as the fresh weights of shoots and roots of tomato plants cultivated in salt stress in comparison to lower concentrations of 2% and 5% as well as 0.5%, 2% and 5% using fresh and dried seaweeds, respectively (Krid et al., 2023). Indeed, many researchers proved that applying SEs at lower concentrations improves plants growth whereas higher concentrations induce detrimental effects (Moh et al., 2018; Ashour et al., 2020; Krid et al., 2023; Chebil Ajjabi et al., 2024).

It is important to note that, the analysis of the mineral composition of the studied algae revealed their deficiency in major elements such as N and P. Nevertheless, their richness in other nutrients such as K (responsible for the activation of numerous enzymes that intervene in photosynthesis as well as the metabolism of C and N (Xu et al., 2020)), Ca (enhances plant growth (Chanthini et al., 2019)), Mg (plays a crucial role in the photosynthesis (Chanthini et al., 2019)), Cu (which plays a prominent role in numerous vital processes such as cell wall metabolism (Yruela, 2009)), Fe (vital element since it is responsible for chlorophyll production (Schmidt et al., 2020)), Mn (intervenes in numerous bio-processes such as respiration, protection against diseases as well as photosynthesis (Alejandro et al., 2020)) and Zn (responsible for plant defense against pathogens, photosynthesis, pollen production, boosts antioxidant enzymes' amounts (Rudani et al., 2018; Cabot et al., 2019)).

The determination of the mineral composition of aquaponically grown lettuce leaves revealed various differences due to the application of the tested SEs during this study. Indeed, our results regarding the N concentration in plants sprayed with *C. linum* extract are similar to those reported in a previous study where a non-significant enhancement of this parameter was observed in basil plants treated with *U. lactuca* extract (Abudeshesh et al., 2024). On the other hand, these results are contrary to previous studies which have noted that treating different plant species (hot pepper, strawberry, lettuce and cucumber) with SEs (commercial seaweed extract (TAM) and *A. nodosum* extract) induced a decrease in the N content (Chrysargyris et al., 2018; Ashour et al., 2021; Hassan et al., 2021; Ashour et al., 2022). Our findings revealed that detrimental effects were caused by *C. linum* and *U. linza* extracts on the contents of P and K. Similarly, a significant decrease in P concentration was observed in strawberry leaves plants treated with the commercial seaweed extract TAM at 50% and 100% (Ashour et al., 2022). Alternatively, treating milkweed seedlings with different concentrations (0.5, 1 and 1.5%) of *S. angustifolium* extract has significantly enhanced the K concentration (Bahmani Jafarlou et al., 2023).

It seems possible that these findings are due to the mineral elements (both micro- and macro-) contained in the SEs (Moh et al., 2018). In addition, an enhancement of the assimilation, transportation as well as the accumulation of nutrients might be due to the existence in SEs of phytohormones such as auxin (IAA) who modulate the mineral elements transfer within plants (Yang et al., 2023). Indeed, previous studies reported the presence of phytohormones such as auxin, cytokinin and gibberellin in algae (Stirk and Van Staden, 2014; Rathod et al., 2024). In fact, the existence of the aforementioned hormones (IAA, IBA, cytokinin and gibberellin) was noted in *C. linum* samples collected from the Chilika lagoon located in the Indian east coast (Mohanty and Adhikary, 2018). In addition, the analysis of samples of *U. linza* collected from the coastal region of Dalian (China) revealed the presence of phytohormones (IAA, ABA, jasmonic acid and cytokinin (trans-zeatin and N-isopentenyl adenine)) in their composition (Li et al., 2023).

On one hand, this study revealed that root traits (length and volume) of aquaponically grown romaine lettuce were enhanced by the foliar spraying with *C. linum* SE. On the other hand, the analysis of the mineral composition of these plants indicated their richness in N, Mg, Fe, Mn as well as Zn. These results support and further confirm previous findings reporting the link between root growth and the improvement of nutrient uptake (Wang et al., 2006). Indeed, roots have a pivotal role that surpasses plants' wellness and viability extending to broader physiological and ecological implications. Indeed, they have a crucial role in crop productivity as well as ecosystems preservation. Furthermore, numerous studies have demonstrated that the efficacy with which roots absorb nutritional elements is essential to agricultural production and quality (Cochavi et al., 2020; Anbarasan and Ramesh, 2021). It is noteworthy to mention that root spatial organization is determined by various factors mainly genetic and environmental (Anbarasan and Ramesh, 2021). In fact, plant roots improve nutriment absorption through the synthesize, into the rhizosphere, of organic compounds referred to as root exudates or rhizodeposits (Wang et al., 2006; Senff et al., 2022). Additionally, these substances might be mineral elements, chemotactic substances or allelochemicals which come into contact with fungi, bacteria as well as other crops to establish beneficial circumstances and reduce abiotic and biotic stress on plants (Senff et al., 2022). Indeed, Lee et al. (2006) and Zhou et al. (2020) found that the roots of lettuce grown in a soilless system released several bioactive molecules such as benzoic acid, cinnamic acid, ferulic acid, gallic acid, lauric acid, palmitic acid, phenylacetic acid, phthalic acid, salicylic acid, stearic acid and tannic acid.

Regarding the photosynthetic status, application of both tested SEs did not significantly affect the chlorophyll pigments content. Indeed, *C. linum* extract enhanced all the tested photosynthetic pigments. On the other hand, applying *U. linza* SE positively affected the majority of the chlorophyll pigments except for the chlorophyll a content which underwent a decline. This latter result was also reported by Karthik and Jayasri (2023) who found that the chlorophyll a content in leaves of mung bean plants treated with *T. ornata* extract (100%) was lower, non-significantly, than that of plants treated with the chemical fertilizer control. On the other hand, the foliar application of the brown seaweed *Stoechospermum marginatum* extract at 1% has induced a non-significant enhancement in chlorophyll a content of *Solanum melongena* (Ramya et al., 2015).

Additionally, these same authors found that *S. marginatum* extract at a concentration of 5% enhanced non-significantly the chlorophyll b content in *S. melongena* plants. Our results related to the total chlorophyll content confirm that of Krid et al. (2023) who observed a non-significant increase of the total chlorophyll content in tomato leaves treated with the fresh extract of *Jania rubens* at 5% and when compared to salt control plants. As for the carotenoids content, our findings support the work of Hamouda et al. (2022) who found that seed priming (*Triticum aestivum* L.) using *U. linza* extract at 5% concentration non-significantly enhanced the carotenoids content in comparison to control. These findings may be explained by the fact that algae are rich in amino acids as well as nutrient elements that boost stomatal conductance as well as the photosynthetic capacity, thus promoting the chlorophyll and carotenoids content (Bahmani Jafarlou et al., 2023). Additionally, another reason for the elevation of the chlorophyll pigments is the rise in the number of chloroplasts biosynthesis. Indeed, in a previous study it was found that the application of AZAL5 (a SE prepared from *A. nodosum*) at a concentration of 67 g L^{-1} increased the number of chloroplast/cell (Jannin et al., 2013). Besides, the previously cited authors hypothesized that the aforementioned treatment resulted also in the inhibition of chlorophyll degradation due to an overexpression of certain genes responsible for the coding of protease inhibitors as well as the attenuation of the stay green protein responsible for the degradation in protein leaves (Jannin et al., 2013).

Our findings related to the RWC are similar to those observed by Bahmani Jafarlou et al. (2023), showcasing a significant enhancement of this parameter in milkweed seedling treated with *S. angustifolium* extract at a concentration of 1% and grown in salt stress (7.5 dS m^{-1}) when compared to the control (plants cultivated in salt stress (7.5 dS m^{-1}) without seaweed extract treatment). On the other hand, treating lettuce cultivated in aquaponics with *C. linum* and *U. linza* extracts negatively affected these plant's EL. Contrary to our findings, a decrease in the aforementioned parameter was noted in milkweed seedlings treated with the *S. angustifolium* extract at all tested concentrations (Bahmani Jafarlou et al., 2023).

This experiment adds to a growing corpus of research showing that SE can be used as a biostimulant for plant growth. By applying *C. linum* and *U. linza* extracts we proved the hypothesis that these SEs could be used in the enhancement of the cultivation of lettuce grown in aquaponics. It is noteworthy to mention that the efficiency of these extracts is not caused by one specific component (mineral nutrients, phytohormones or growth regulators) but by their synergism (Krid et al., 2023).

Conclusion

The current study highlighted that the use of *Chaetomorpha linum* and *Ulva linza* as biostimulants has promising effects in the improvement of the aquaponic cultivation of lettuce (*Lactuca sativa* var. *longifolia*). The efficiency of *C. linum* in promoting growth, biomass production as well as the physiological status of lettuce was more evident on the one hand. The foliar application of *U. linza* extract, on the other hand, was proven to positively influence the mineral composition of lettuce leaves. However, the latter treatment's effectiveness, growth-wise, might depend on the applied dose. This may constitute the object of a future study where lower concentrations of *U. linza* SE will be tested. In addition, the composition of these macroalgae in phytohormones, polysaccharides, proteins and other elements responsible for plant growth stimulation remain unanswered at present. A further study with more focus on seaweed extracts' growth promoting molecules as well as their effects on plant bioactive compounds is therefore suggested.

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Contribution of Authors
Annabi HA: Conceived the idea, conducted the research, collected, analyzed, interpreted the results and wrote the original draft
Laribi B: Conceived the idea, supervised the study, was involved in the preparation of the original draft, reviewed, edited and approved the final draft.
Riahi A: Conceived the idea, supervised the study and approved the final draft.

Hachana A & Arfaoui H: Performed the analysis of mineral composition and analyzed the results.
Mechri M: Involved in the methodology.
Sekma Y: Conducted the experiment and collected the data.
Shili A: Identified the seaweed samples.
Bettaieb T: Supervised the study and reviewed the original draft.

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