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Effect of Foliar Spraying of the Alga *Cladophora glomerata* Extract on Some Vegetative Characteristics and Chemical Contents of Hydroponically Grown Lettuce Plants

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ABSTRACT

The experiment was conducted in Al-Diwaniyah Governorate, Iraq, during the 2022-2023 agricultural season (three months) on lettuce (*Lactuca sativa* var. capitata). The study aimed to investigate the effect of spraying lettuce plants with an algal extract from *Cladophora glomerata*, isolated from a stream at the University of Al-Qadisiyah, at concentrations of 2 and 4g/ L. Results indicated an increase in vegetative growth characteristics, including plant height, number of leaves, fresh weight, and total leaf area, with the highest averages being 20.66cm, 24 leaves, 135g, and 5260.26cm², respectively. Additionally, chemical content characteristics such as carbohydrates, vitamin C, total nitrogen, phosphorus, sulfur, and iron showed mean values of 1.6%, 4.12mg/ 100g fresh weight, 3.72%, 0.25%, 72.45mg/ g dry weight, and 1.32%, respectively, when treated with 4g/ L of algal extract. In comparison, control plants showed the lowest values. Treatment with 2g/ L of algal extract resulted in higher mean values for dry weight, total chlorophyll, carotenoids, protein, vitamins A and K, potassium, zinc, calcium, and fiber, with values of 11.64g, 1.89mg/ g, 0.21%, 1.39%, 200.72mg/ 100g fresh weight, 108.47mg/ 100g fresh weight, 1.2%, 2.94mg/ 100g fresh weight, 60.9mg/ 100g, and 1.6%, respectively, compared to the control plants. Statistical analysis revealed significant differences ($P \leq 0.05$) between all parameters, except for plant height and iron, where the differences were not significant among the experimental treatments.

INTRODUCTION

Lactuca sativa Linn., widely known as lettuce, is a leafy green valued globally for its culinary properties. Beyond its use in salads, soups, and stir-fries, lettuce has a rich history of medicinal applications. Grown worldwide, lettuce is a popular raw vegetable, praised for its refreshing flavor and impressive nutritional profile. It is a valuable source of phytonutrients and exhibits remarkable morphological and genetic diversity. Evidence suggests that the Egyptians were the first to cultivate this leafy green. Today, lettuce is produced globally, and its role in folk medicine continues, with documented uses for pain relief, digestive problems, and inflammatory and urogenital infections. Scientific studies have increasingly highlighted the pharmacological potential of lettuce, revealing properties such as anti-bacterial, anti-oxidant, neuroprotective, and even therapeutic eff12ects. The plant's diverse chemical composition includes several groups of intermediate compounds, such as terpenoids, flavonoids, and phenolic compounds, which are likely involved in its observed bioactivity. In addition to its medicinal properties, lettuce offers a wealth of essential nutrients, including vitamins, minerals and organic matter, making it a valuable addition to a healthy diet **(Noumedem *et al*., 2017; Unver & Gurhan, 2024)**. *Lactuca sativa* L. belongs to the Compositae family, in the Arab world, it is called lettuce, from the Latin word (*laci lactics*) meaning milk, or in French (laitue) meaning milk as well; this variety is one of the best food sources of antioxidants and contains high levels of phenols and terpenes and is rich in vitamins A and C **(Kim *et al*., 2016)**. Lettuce is among the best vegetables consumed by humans due to its benefits, including aiding digestion and fighting several diseases **(Ekoungoulou & Mikouendanandi, 2020)**.

Consumers have become more aware of the importance of food in improving health, preventing diseases, and improving product quality **(Nafed Mughrbi & Auzi, 2020)**. This is one of the main reasons farmers are resorting to hydroponics **(Alvarado & Camarillo, 2020)**. Hydroponics is a branch of hydro-culture, a technique for cultivating plants in the absence of soil by dissolving inorganic nutrient solutions in an aqua solvent **(Ekoungoulou & Mikouendanandi, 2020)**. This type of farming has advantages such as rationing the use of nutrients and their types, lower cost and less effort, high productivity of the crop and saving land, especially when resources are scarce, rationing the use of water and fertilizer and reducing the use of agricultural pesticides by reducing the transmission of diseases by hydroponic crops **(Goddek *et al*., 2019; Salman & Hussein, 2023)**.

Algae are autotrophic organisms rich in many active substances such as pigments, chlorophyll, xanthophylls, carotenoids, vitamins, amino acids, fats, and other substances, which would enhance plant growth if their extracts were used as bio-fertilizers for plants by mixing with soil or foliar. Algal extracts are one of the most important sources of nutrients used in agricultural production as a supplement to fertilizers and not as a substitute for them, since they stimulate the plant's physiological functions **(Dmytryk & Chojnacka, 2018)**, and algae are a source of vitamins, antioxidants and minerals **(Nutautaitė *et al*., 2021)**. *Cladophora glomerata* is a unicellular green alga used as animal feed **(Pikosz *et al*., 2019)**. Algae is high in protein and fiber and is used in the foodstuffs manufacturing industries, in addition to several nutrients and vitamins (it contains high levels of pigments, carotenoids, chlorophyll and plant-stimulating hormones **(Michalak & Messyasz, 2021)**. *Cladophora glomerata* has some alkaloid compounds, in addition to many flavonoids, phenols, tannins, and its potential use as a natural source of antifungal agents **(Mohammed *et al*., 2014)**.

An investigation was performed to select the appropriate dose of mineral nitrogen fertilizer (MNF) and optimum concentration of algal extract to minimize the amount of MNF, maximize yield, and improve seed quality for pea plants grown under sandy soil conditions. Twelve treatments were included in this experiment, which were combinations of three rates of MNF (50, 75 and 100%) and four concentrations of algal extract (0. 5, 10 and 15% of the recommended mineral nitrogen rate), compared with other concentrations of seaweed extract; the results showed that spraying pea plants with either 10 or 15% seaweed extract markedly increased plant development characteristics, income yield, seed N and P content, protein content and leaf chlorophyll content **(Nawar & Ibraheim, 2014)**. The impact of two kinds of alga extracts applied topically on the growing, production, nutritional value and chemistry of the plants of the lettuce species grown in gritty soils under trickle watering was also investigated. Relative to the standard treatment, the findings revealed that adding variety of concentrations of algal extracts (Nostoc muscorum and Anabaena oryzae) to lettuce showed a marked improvement in height, leaves number, weight of head, weight and global Income. In addition, significant increases in carotenoids, total chlorophyll, and chlorophyll a were observed at various doses of these extracts. The maximum level of nitrogen, phosphate, potassium and stuffed food content of the plants showed statistically significant differences between all treatments **(Mohsen, 2016)**.

Mohammed *et al.* (2022) noted that the overuse of inorganic fertilizers causes soil degradation, which hurts product quality; as a result, using eco-friendly biologically-derived fertilizers in agriculture has recently elevated. A local area was subject to an experiment conducted to investigate the effects on *Lactuca sativa* L. growth and yield of soil application of organic fertilizer, the coulter spray of algae extract and inorganic fertilizer compared to the control group. The results showed that while leaf area and chlorophyll content were not positively affected, the application of the different fertilizers and their combinations meaningfully increased vegetative growth metrics including length of stem and leaves number. The addition of the compost to the soil and foliar usage of inorganic fertilizer and extract of seaweed resulted in significant increases in head weight, yield and total yield compared to controls. Seaweed, inorganic fertilizer and biofertilizer all had an effect on the N, P and Ca content of lettuce leaves. The use of environmentally friendly seaweed and biofertilizer may result in higher head weight and hence higher yield. Another research study investigated the effects of spraying marine algae extract on the growth, alimentary and nutritional content, osmotic substance levels, different components of the antioxidant response and gene regulation in salt-stressed lettuce plants. The concentration of kaempferol, gallic acid, luteolin, and quinic acid in the algal extract has been applied to detect its phenolic content. As a result of salt stress, proline concentration in leaves was enhanced, whereas mass productivity, fluorescent chlorophyll and elemental content declined. The findings indicated these plants treated using seaweed extract got an improved nutrition status, better resistance to salt stress and a higher capacity to tolerate salt challenges. Moreover, H2O2 and malondialdehyde values were also reduced. Additionally, under mild stress conditions, the activities of antioxidant enzymes increased, indicating a significant improvement in the antioxidant system. Overall, our research suggests that seaweed extract works well as a biocatalyst product during the saline processing of lettuce **(Aloui *et al.*, 2023)**.

This study aimed to assess the impact of the spraying application of *Cladophora glomerata* extract on the vegetative growth and biochemical composition of hydroponically grown lettuce plants.

MATERIALS AND METHODS

Experimental design and procedure

A completely randomized design (CRD) was used to conduct a factorial experiment with three replications. Treatments included control (distilled water) and algal extract at 2 and 4g/ L.

Hydroponics technique

The experiment was conducted in a hydroponic system constructed within a house in Diwaniyah Governorate, Iraq. The system comprised five iron stands, spaced 50cm apart, and vertically reinforced with a roof and base for stability. Each stand supported two, four-meter-long, four-inch diameter pipes arranged 40cm apart. Two water tanks containing deionized (RO) water were connected to the pipes via a water plunger and hose. Nutrient Film Technique (NFT) membrane technology was employed to circulate water through the pipes, providing a continuous nutrient supply to the plants.

Planting seeds and transporting seedlings

Lactuca sativa var. capitata lettuce seeds, procured from Arzuman (Turkey) and suitable for NFT hydroponics, were sown on October 3rd, 2022, in 40 plastic trays (10 seeds per tray) filled with Dutch Patmos growing medium. The trays were placed in Al-Rahman Al-Nursery and Gardens, Al-Arouba district, Diwaniyah Governorate. Seedlings emerged five days later and were transplanted into the hydroponic system after developing 4-5 leaves. Nutrient solutions were introduced ten days post-transplantation, supplemented with calcium sprays applied every 15 days. The initial algal extract treatment was applied on November 23rd, 2022, followed by a second application on December 3rd, 2022, using a one-liter hand sprayer for foliar application at the specified concentrations. Watering was daily performed, and adjusted according to plant needs, especially during the winter months.

Cladophora glomerata extract

The algae extract was obtained from the Environmental Laboratory of the Department of Biology and was isolated from a stream at the University of Al-Qadisiyah.

Characteristics examined

Vegetative growth indicators

Plant height (cm)

A scale has been made to assess the height of each plant from the bottom to the apex. For each treatment, the average plant height was determined by calculating the mean height of all plants within that treatment **(Singh & Stoskopf, 1971)**.

Leaves number (leaves/plant)

The number of leaves on each plant was counted for each replicate in each treatment. The average number of leaves per plant for each treatment was calculated by dividing the total number of leaves by the number of plants **(AOAC, 2019)**.

Fresh and dry weight of total vegetative plant (g/plant)

Three plants were randomly selected from each treatment and weighed using a precise electronic balance. The mean fresh weight per plant was calculated for each treatment by summing the individual plant weights and dividing by three. Subsequently, the plant samples were dried under ambient conditions until their weights were stabilized. The average dry weight per plant was then determined by calculating the mean of the dried plant weights for each treatment.

Total leaf area (Cm2/ Plant)

Leaf area was determined using a method adapted from **Al-Zaidy and Al-Ubaidy (2017)**. Three fully expanded leaves from each replicate were scanned, and their areas were measured using the image analysis software. The total leaf area per plant was then calculated by multiplying the mean leaf area by the mean number of leaves per plant within each replicate.

Chemical content indicators

The method of **Mackinney (1941)** was used to determine the chlorophyll contents. A gram of fresh leaf tissue was homogenized in a glass test tube with ten milliliters of 80% acetone. After 20 hours in the dark at 4°C, the mixture was homogenized again and was kept at 4°C for a one to two hours. The supernatant was acidified with two to three drops of 0.1 N hydrochloric acid and diluted twice with acetone after centrifugation at 3000rpm for ten minutes. Using the appropriate equation, the total chlorophyll concentration was calculated spectrophotometrically at wavelengths of 645 and 663nm.

Total chlorophyll (mg/g tissue) = [20.2(D645) + 8.02(D663] x V/(1000 x W)

Where, D = optical density reading of the chlorophyll extract at the specific

Wavelength, V = final volume of the 80% acetone-chlorophyll extract

W = fresh weight in grams of the tissue extracted

Estimation of carotenoids (µg/g)

Using the technique outlined by **Kirk and Allen (1965)**, the content of carotenoids was measured spectrophotometrically at 480, 663, and 645nm.

Carotenoid (mg/ g)=([A480+0.114A663]-0.638A645)/(A×1000×W)×V

V = Final filter volume (ml), A = Optical density of chlorophyll extracted in the cell (1 cm), W = Leaf fresh weight (g).

Estimation of total carbohydrates (%)

Colorimetric phenol-sulphuric acid procedure prescribed by the **DuBois *et al*. (1956)** was used to assess the amount of carbohydrates in the samples. This method is widely used due to its sensitivity and simplicity. Total carbohydrate concentration was calculated based on a standard curve and the following equation:

Total sugar concentration=A.test /A.STD *Conc.■(of&STD)

Protein estimation

Protein content was quantified using the Bradford technique in both crude and purified extracts **(Bradford, 1976)**. This procedure employs the Coomassie Brilliant Blue G-250 dyestuff dissolved in ethanol and phosphoric solution acid. A calibration curve was constructed using bovine serum albumin, and the absorbency was read at 595nm.

$$Y=aX+b$$

Y= the absorbance, X= protein value, a= slope, b= constant.

Vitamin A

The technique described in **Jadoon *et al.* (2013)** has been used to test for vitamin A concentration. This process forms an iron-ferrozine complex detected at 562nm using spectrophotometry. The complex was prepared in an aqueous solution and stabilized using a pH 6 acetate buffer.

Vitamin K

The technique outlined by **Hassan (1980)** was used to determine the vitamin K concentration. Five grams of fresh plant tissue were homogenized in hexane. Spectrophotometric analysis of vitamin K was based on its reaction with alcoholic sodium hydroxide in alkaline solutions with a concentration below 0.2 M (0.5%).

Estimation of total vitamin C (ascorbic acid) (%)

The 2,4-dinitrophenylhydrazine (DNP) assay reported by **Al-Ani *et al.* (2007)** was used to assess vitamin C concentration. This method uses copper ions to oxidize ascorbic acid to dehydroascorbic acid, followed by conversion to 3-diketo-gulonic acid. The resulting compound reacts with DNP to form a red-colored dihydrazone, which is quantified spectrophotometrically at 520nm.

Estimation of total nitrogen (%) by spectrophotometry

Nitrogen content was determined using the Berthelot reaction as outlined by **Temminghoff and Houba (2004)**. This method involves the formation of a blue-green indophenol dye through the reaction of ammonia with hypochlorite in an alkaline medium. The dye's absorbance at 660nm is directly proportional to the ammonium ion concentration, which serves as an indicator of total nitrogen content in the sample.

Leaves phosphorus content (%)

The ammonium molybdate-ascorbic acid technique was used to evaluate the phosphorus concentration **(Bender & Wood, 2000)**. Phosphorus was detected by the ammoniacal molybdate-ascorbic acid method. A digested plant sample was diluted, and a portion of this diluted sample was reacted with ascorbic acid and ammonium molybdate to form a complex with a blue color. The color intensity, measured spectrophotometrically at 620nm, was correlated to phosphorus concentration using a standard curve. A stock phosphorus solution was prepared from potassium dihydrogen phosphate, and a series of standard solutions were created. The absorbance of these standards was measured, and the phosphorus content of the plant samples was determined using a calibration curve.

Leaf content of potassium (%) and calcium (mg/100 g).

The content of potassium and calcium was determined by flame photometry according to the procedure described by **Tomar and Agarwal (2013)**. Dried plant material was digested in a concentrated acid mixture (sulfuric, nitric, and perchloric acids) and filtered. The resulting solution was diluted and analyzed for K and Ca using a flame photometer equipped with specific filters for each element. The concentrations of K and Ca were calculated using standard curves.

Zinc determination method (µg/g)

Using the full Abnova kit, zinc (Zn) content was established using the procedure prescribed by **Knoell *et al.* (2009)**. This kit allows for direct zinc measurement in biological samples without prior preparation. The assay involves a chromogenic reagent which binds to zinc to form a colored complex. The amount of zinc in the sample is directly related to the intensity of this color, measured at 425nm.

Iron (Fe) (µg/g)

The iron content was estimated using the procedure outlined by **Sandell (1951)**. Plant samples were dried, grounded, and digested with sulfuric acid. The resulting ash was dissolution in HCl and filtering. The filtrate was adjusted to pH 3.5 using sodium citrate, followed by the addition of hydroquinone and orthophenanthroline to reduce iron. The iron-orthophenanthroline complex was detected by spectrophotometry at 508nm. Based on a standard curve prepared from defined iron values, the iron levels of the samples were calculated.

Estimation of crude fiber

The fiber content was assessed according to the procedures of **Song *et al.* (2018)**. The analysis involved a two-step process: initial acid digestion using 12.5% sulfuric acid, followed by base digestion using 12.5% sodium hydroxide. After drying and burning the remaining residue, fiber content was calculated by difference.

Data analysis

SPSS version 26 was chosen for the statistical analysis of the data using two-way analysis of variance (ANOVA). At a significance level of $P \leq 0.05$, mean comparisons were made with the LSD (least significant difference) test.

RESULTS

The results presented in Table (1) demonstrate that spraying the vegetative group of lettuce leaves with *C. glomerata* extract at concentrations of 2 and 4g/ L significantly increased all vegetative characteristics and chemical contents. These two treatments outperformed the control group, which exhibited the lowest values.

Specifically, vegetative growth characteristics, including plant height, number of leaves, fresh weight, and total leaf area, showed the highest means at 20.66cm, 24 leaves, 135g, and 5260.26cm², respectively, when treated with 4g/ L of the algae extract. Similarly, chemical content characteristics, such as carbohydrates, vitamin C, total nitrogen, phosphorus, sulfur, and iron, also increased with mean values of 1.6%, 4.12mg/ 100g fresh weight, 3.72%, 0.25%, 72.45mg/ g dry weight, and 1.32%, respectively, compared to the control plants, which had the lowest mean values.

The treatment with 2g/ L of the algal extract resulted in better mean values for dry weight, total chlorophyll, carotenoids, protein, vitamin A, vitamin K, potassium, zinc, calcium, and fiber, with values of 11.64g, 1.89mg/ g, 0.21%, 1.39%, 200.72mg/ 100g fresh weight, 108.47mg/ 100g fresh weight, 1.2%, 2.94mg/ 100g, 60.9mg/ 100g, and 1.6%, respectively, compared to the reference plants.

Statistical analysis showed that all treatments differed significantly ($P \leq 0.05$), except for plant height and iron content.

Table 1. Effect of foliar spraying with algal extract on vegetative and physiological characteristics of *Lactuca sativa* var. capitata

Plant Parameter	Treatments			LSD
	Control	2g Algal extract	4g Algal extract	
	Mean±SE	Mean±SE	Mean±SE	
Plant height (cm)	18±1.73205	18.66±0.33333	20.66±0.33333	2.84 NS
Number of leaves	20.66±0.88192 b	23.66±1.33333 ab	24±1.73205 a	3.37
Fresh weight (g)	121±0.57735 b	133±0.57735 a	135±0.57735 a	3.87
Dry weight (g)	10.03±0.0928 b	11.46±0.29059 a	11.43±0.46667 a	0.88
Total leaf area Cm^2/plant	4113.09±0.57735 c	4305.22±0.57735 b	5260.26±0.57735 a	43.58
Total chlorophyll mg/gm	1.7±0.02887 c b	1.89±0.06351 a	1.82±0.02667 b	0.122
Carotenoids %	0.13±0.01732 c	0.21±0.01732 a	0.15±0.00577 bc	0.03
Carbohydrates %	1.03±0.01732 b	1.55±0.02887 a	1.6±0.02887 a	0.07
Protein%	0.97±0.01732 c	1.51±0.02887 a	1.39±0.03464 b	0.076

Vitamin A mg/100g.f.wt	133.76±0.88192 c	200.72±1.45297 a	167.37±1.73205 b	3.84
Vitamin C mg/100g.f.wt	2.37±0.17321 c	3.81±0.06009 b	4.12±0.1701 a	0.39
Vitamin K mg/100g.f.wt	65.67±11.46051 b	108.47±5.7735 a	55.51±12.70171 b	28.5
Total Nitrogen %	1.5±0.01732 c	2.52±0.01732 b	3.72±0.01732 a	0.047
Phosphorus %	0.2±0.00577 b	0.24±0.01155 a	0.25±0.01155 a	0.027
Potassium %	0.65±0.02887 c	1.2±0.02887 a	0.76±0.02309 b	0.07
Sulfur mg/g.dry wt	10.59±1.1547 c	23.27±1.1547 b	27.45±1.1547 a	3.16
Zinc mg/100g.f.wt	2.89±0.00577 a	2.94±0.01155 b	2.92±0.00577 b	0.02
Iron%	1.11±0.01732	0.9267±0.31355	1.32±0.06351	0.50 NS
Calcium mg/100g	29.05±0.05 c	60.9±2.88675 a	43.05±1.73205 b	5.33
Fiber %	1.4±0.05774 b	1.6±0.02887 a	0.98±0.04933 c	0.132

- small - --*letters indicated the significant differences between treatments.

DISCUSSION

From the results of the presented research, it was found that the Cladophora extracts increased the vegetative characteristics and chemical content of lettuce plants compared to the control group, and these results were confirmed by statistical analysis at a probability level of 0.05, except for plant height and iron content, which showed no significant differences (Table 1). This superiority can be related to the possibility that algae have a high proportion of macronutrients, micronutrients and amino acids in addition to vitamins, carbohydrates, proteins, natural enzymes and some growth regulators that improve productivity and vegetative growth. Moreover, *C. glomerata* contains nutrients like N, P, K, C, Ca, S, Mg, Mn, Zn, Fe, and Se, and each of these elements has a significant and effective involvement in increasing plant development **(Islam *et al.*, 2019; Wu *et al.*, 2023)**.

Furthermore, the addition of algae extracts significantly increased the nitrogen (N), phosphorus (P), and potassium (K) contents in the leaves. This is likely because algae extracts strengthen the root system, enhancing the uptake and accumulation of nitrogen in the plant. Nitrogen plays a crucial role in chlorophyll formation **(Nutautaitė *et al.*, 2021)**. This finding aligns with the current study, which observed an increase in total chlorophyll levels when plants were treated with seaweed extracts.

Similar results were reported when an algal extract from *Chlorella vulgaris* was applied to broccoli. The treated plants showed superior outcomes compared to the control group in terms of leaf number, stem diameter, plant height, leaf area, total chlorophyll content, flower head diameter, nitrogen and potassium content, and carbohydrates **(Kareem *et al.*, 2022)**.

Chrysargyris *et al.* (2018) also noted that seaweed extracts act as biostimulators. They observed that applying *Ascophyllum* extracts as a foliar fertilizer to hydroponically grown lettuce resulted in an increased relative growth, higher calcium levels, and enhanced antioxidant activity in the plants. The study indicated that the use of algal extract improves foliar metabolism and enhances the efficiency of the aerial parts of the lettuce, allowing more nutrients to reach the roots **(Pacheco *et al.*, 2023)**.

Some research studies suggested that seaweed extract can help make plants more resilient to abiotic stress. Under drought stress, the effect of a seaweed extract called *Sargassum angustifolium* on lettuce development indices was studied. The findings confirmed that treating with the marine algae extract significantly increased plant resistance to drought stress and improved morphological and physiological indices of lettuce ($P< 0.05$). The best results were obtained with a treatment of 1.5g/ L of seaweed extract **(Mohkami, 2022)**. **Ali *et al.* (2021)** noted that due to the bioactive properties of seaweed-derived products, their use in crop cultivation systems is becoming increasingly popular. These products act as phytostimulants, promoting higher plant development and yield in many important crops. They contain components that stimulate plants to develop defense mechanisms against insect infestations, pathogens, and stresses such as drought and salinity. This is often associated with the activation of critical defense genes and pathways within the plant, strengthening its defense mechanisms against potential threats. Seaweed extracts also trigger hormonal activity in plants due to their unique constituents, which interact with the regulation of plant growth. The findings of this study are consistent with those of **Aydın and Demirsoy (2020)**, who observed that seaweed application enhanced root, stem, and leaf dry weight, leading to the highest lettuce seedling quality index as seaweed doses increased.

Rouphael *et al.* (2022) also investigated the effects of plant biostimulants, including seaweed extracts, on lettuce grown under both normal and saline conditions. They found that biostimulants reduced sodium content in saline-grown lettuce and increased the accumulation of stress-related compounds such as glucosinolates, terpenoids, and jasmonates. These results suggest that plant biostimulants can help mitigate the negative effects of salt stress on lettuce.

CONCLUSION

According to the results of the study, the topical application of *Cladophora glomerata* extract to lettuce plants significantly improved their vegetative development and chemical composition compared to the control group. The increased concentration (4g/L) was particularly successful in increasing the amount of carbohydrates, vitamin C and leaves on the plant, as well as their height, number, area, total nitrogen, phosphorus, sulphur and iron contents. Conversely, the lower concentration (2g/L) was superior in enhancing dry weight, chlorophyll, carotenoids, protein, vitamin A, vitamin K, potassium, zinc, calcium and fiber. These results suggest that *Cladophora glomerata* extract is a useful potential foliar fertilizer for improving lettuce yield and nutritional quality. However, the optimum application rate will depend on the desired growth parameters.

REFERENCES

Al-Ani, M.; Opara, L.U.; Al-Bahri, D. and Al-Rahbi, N. (2007). Spectrophotometric quantification of ascorbic acid contents of fruit and vegetables using the 2, 4-dinitrophenylhydrazine method. Journal of Food Agriculture and Environment, 5(3/4): 165.

Al-Zaidy, A. and Al-Ubaidy, R. (2017). Effect of adding wheat peat and spraying with its extract and organic nutrient vegeamino on growth and yield of red cabbage. Iraqi Journal of Agricultural Sciences, 48(2).

Ali, O.; Ramsubhag, A. and Jayaraman, J. (2021). Biostimulant properties of seaweed extracts in plants: Implications towards sustainable crop production. Plants, 10(3): 531.

Aloui, D.; Kalleli, F.; M'Hamdi, M.; Manaa, M.; Abid, G. and Karmous, C. (2023). Marine Algal extract as a biostimulant to improve tolerance to salinity in lettuce plants. Emirates Journal of Food and Agriculture, 12: 1-18.

AOAC. (2019). Official Methods of Analysis of the Association of Official Analytical Chemists: Official Methods of Analysis of AOAC InternationalOfficial methods of analysis (Vol. 12). AOAC. Washington, DC.

Aydın, M.; and Demirsoy, M. (2020). The effects of seaweed extracts and the applications of vermiwash on organic lettuce (Lactuva sativa L.) seedlings. Turkish Journal of Food and Agriculture Sciences, 2(1): 1-4.

Bender, M.; and Wood, C. (2000). Total phosphorus in soil. Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters, 45.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2): 248-254.

Chrysargyris, A.; Xylia, P.; Anastasiou, M.; Pantelides, I.; and Tzortzakis, N. (2018). Effects of Ascophyllum nodosum seaweed extracts on lettuce growth, physiology and fresh-cut salad storage under potassium deficiency. Journal of the Science of Food and Agriculture, 98(15): 5861-5872.

Dmytryk, A.; and Chojnacka, K. (2018). Algae as fertilizers, biostimulants, and regulators of plant growth. Algae biomass: characteristics and applications: towards algae-based products: 115-122.

DuBois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.t.; and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical chemistry, 28(3): 350-356.

Ekoungoulou, R.; and Mikouendanandi, E.B.R.M. (2020). Lettuce (Lactuca sativa L.) production in Republic of Congo using hydroponic system. Open Access Library Journal, 7(5): 1-17.

Goddek, S.; Joyce, A.; Wuertz, S.; Körner, O.; Bläser, I.; Reuter, M.; and Keesman, K.J. (2019). Decoupled aquaponics systems. Aquaponics food production systems, 10: 978-973.

Hassan, S.S. (1980). [16] Spectrophotometric determination of the K vitamins. In Methods in Enzymology (Vol. 67, pp. 125-128). Elsevier.

Islam, M.; Islam, M.H.; Rouf, M.A.; Sultana, M.; and Haque, M.S. (2019). Effects of nitrogen and phosphorus fertilizer on yield and yield attributes of lettuce (Lactuca sativa L.). Journal of Bioscience and Agriculture Research, 23(01): 1872-1884.

Jadoon, S.; Malik, A.; Qazi, M.; and Aziz, M. (2013). Spectrophotometric method for the determination of Vitamin A and E using Ferrozine-Fe (II) complex. Asian Journal of Research in Chemistry, 6(4): 334-340.

Kareem, A.O.; ALGHANMI, H.A.; and JAMEEL, D.A. (2022). Effect of Chlorella vulgaris extract on some vegetative and chemical characteristics of the broccoli plant, Brassica oleracea. Iranian Journal of Ichthyology, 9: 369-376.

Kim, M.J.; Moon, Y.; Tou, J.C.; Mou, B.; and Waterland, N.L. (2016). Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). Journal of Food Composition and Analysis, 49: 19-34.

Kirk, J.; and Allen, R. (1965). Dependence of chloroplast pigment synthesis on protein synthesis: effect of actidione. Biochemical and Biophysical Research Communications, 21(6): 523-530.

Knoell, D.L.; Julian, M.W.; Bao, S.; Besecker, B.; Macre, J.E.; Leikauf, G.D.; DiSilvestro, R.A.; and Crouser, E.D. (2009). Zinc deficiency increases organ damage and mortality in a murine model of polymicrobial sepsis. Critical care medicine, 37(4): 1380-1388.

Mackinney, G. (1941). Absorption of light by chlorophyll solutions. Journal of biological chemistry, 140(2): 315-322.

Michalak, I.; and Messyasz, B. (2021). Concise review of Cladophora spp.: macroalgae of commercial interest. Journal of Applied phycology, 33(1): 133-166.

Mohammed, A.A.; Söylemez, S. and Sarhan, T.Z. (2022). Effect of biofertilizers, seaweed extract and inorganic fertilizer on growth and yield of lettuce (Lactuca sativa var. longifolia L.). Harran Tarım ve Gıda Bilimleri Dergisi, 26(1): 60-71.

Mohammed, D.Y.; Dwaish, A.S. and Jawad, A.-L.M. (2014). Anti–phytopathogenic Activities of *Cladophora glomerata* extract against plant fungi. Baghdad Science Journal, 11(2): 883-887.

Mohkami, A. (2022). Evaluation of the Effect of Sargassum angustifolium C. Agardh Extract on Growth and Yield Indices of Lactuca sativa L. Under Drought Stress Conditions. Journal of Phycological Research, 6(2): 883-894.

Mohsen, A.A.M.A. (2016). The effect of foliar spray with cyanobacterial extracts on growth, yield and quality of lettuce plants (Lactuca sativa L.). Middle East Journal of Agriculture Research.

Nafed Mughrbi, H. and Auzi, A.A. (2020). Lactuca sativa stems as the source of bioactive compounds as well as the leaves. Journal of Pharmacy and Pharmacology, 8: 143-150.

Nawar, D.A. and Ibraheim, S.K.A. (2014). Effect of algae extract and nitrogen fertilizer rates on growth and productivity of peas. Middle East Journal of Agriculture Research, 3(4): 1232-1241.

Noumedem, J.A.K.; Djeussi, D.E.; Hritcu, L.; Mihasan, M. and Kuete, V. (2017). Chapter 20 - Lactuca sativa. In V. Kuete (Ed.), Medicinal Spices and Vegetables from Africa (pp. 437-449). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-809286-6.00020-0

Nutautaitė, M.; Vilienė, V.; Racevičiūtė-Stupelienė, A.; Bliznikas, S.; Karosienė, J. and Koreivienė, J. (2021). Freshwater *Cladophora glomerata* biomass as promising protein and other essential nutrients source for high quality and more sustainable feed production. Agriculture, 11(7): 582.

Pacheco, D.; Cotas, J.; Pereira, L. and Bahcevandziev, K. (2023). A Possible Synergistic Approach: Case Study of Saccharina latissima Extract and Nitrifying Bacteria in Lettuce. Journal of Marine Science and Engineering, 11(9): 1645.

Pikosz, M.; Czerwik-Marcinkowska, J. and Messyasz, B. (2019). The effect of *Cladophora glomerata* exudates on the amino acid composition of Cladophora fracta and Rhizoclonium sp. Open Chemistry, 17(1): 313-324.

Rouphael, Y.; Carillo, P.; Garcia-Perez, P.; Cardarelli, M.; Senizza, B.; Miras-Moreno, B.; Colla, G. and Lucini, L. (2022). Plant biostimulants from seaweeds or vegetal proteins enhance the salinity tolerance in greenhouse lettuce by modulating plant metabolism in a distinctive manner. Scientia Horticulturae, 305: 111368.

Salman, A. and Hussein, W. (2023). Effect of blue and red led light and some plant extract on lettuce growth and yield in NFT technique. IOP Conference Series: Earth and Environmental Science,

Sandell, E.B. (1951). Colorimetric determinations of traces of metals. In (Vol. 71, pp. 245): LWW.

Singh, I. and Stoskopf, N. (1971). Harvest index in cereals 1. Agronomy Journal, 63(2): 224-226.

Song, H.; Wu, H. and Li, X. (2018). Quantitative analysis of chemical components of plant fiber. Applied Chemical Engineering.

Temminghoff, E.E. and Houba, V.J. (2004). Plant analysis procedures. Springer.

Tomar, N.S.; and Agarwal, R. (2013). Influence of treatment of Jatropha curcas L. leachates and potassium on growth and phytochemical constituents of wheat (Triticum aestivum L.).

Unver, T.and Gurhan, I. (2024). Chemical composition and antimicrobial activity of an apolar extract from Lactuca serriola L. leaves. Biochemical Systematics and Ecology, 114: 104832.

Wu, J.Y.; Tso, R.; Teo, H.S. and Haldar, S. (2023). The utility of algae as sources of high value nutritional ingredients, particularly for alternative/complementary proteins to improve human health. Front Nutr, 10: 1277343. https://doi.org/10.3389/fnut.2023.1277343