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Research Article

Effect of *Chlorella vulgaris* extract on some vegetative and chemical characteristics of the broccoli plant, *Brassica oleracea*

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Abstract
This study was aimed to estimate the effect of foliar spraying of the algal extract of *Chlorella vulgaris* on some vegetative and chemical characteristics of broccoli at a concentration of 2 and 4g l^{-1}. The results showed that the highest averages of plant height, stem diameter, number of leaves, leaf area, total chlorophyll content in leaves, inflorescence diameter, nitrogen concentration, potassium concentration, and total carbohydrates as 20.66cm $plant^{-1}$, 11.92mm $plant^{-1}$, 21.66 leaves $plant^{-1}$, 592.9cm^2 $plant^{-1}$, 0.467mg g^{-1}, 11.30cm, 1.45%, 3.11%, 27.83%, respectively at 4g l^{-1} of algal extracts compared with the control treatment. While at the concentration 2g l^{-1} of algal extract, the highest averages in the phosphorous content of the florets disc and iron of 0.694% and 6.61µg g^{-1} were recorded, respectively. The results revealed significant differences between the treatments at $P>0.05$.

Keywords: Algal extract, Broccoli, Chemical characteristics, Inflorescence.

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Introduction

Algae are 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 spraying, through their effects on many vegetables and crop plants (Yoldas et al. 2008). In addition, the algal extract contains many essential elements for plant growth such as nitrogen, phosphorous, potassium, iron, copper, zinc, and boron (Abd El Moniem & Abd-Allah 2008). Algae extracts are among the important organic sources used in agricultural production. They are complement substances, not a substitute for fertilizers, as they stimulate the physiological functions of the plant. They contain many major and micronutrients and protein materials ranging between 45-50% and more than a group of substances growth promoters such as auxins, gibberellins, and cytokinins, and some vitamins, organic acids, and amino acids (El-Motty et al. 2010; Charlie 2003; Al-Musawi 2018). About 31 million tons of algae were used annually in the agricultural field worldwide, which are substances that stimulate plant growth in low concentrations (Stirk et al. 2003). In addition, the algal extracts contain polysaccharides, laminarin, fucoidan and alginate (Rioux et al. 2007). It also contains Betaine, which is a source of nitrogen in low concentrations and a regulator of osmosis in high concentrations, the increased resistance of the plant to salinity and drought may be attributed to the role of these extracts (Naidu et al. 1987).

Chlorella vulgaris belongs to the phylum Chlorophyta and is characterized by a large amount of protein ranging between 50-60%, carbohydrates 15-20%, lipids 12-18%, and its high content chlorophyll. This alga is used in various fields,

including nutritional supplements for humans and animal husbandry and agriculture (Kang & Sim 2004). Among many microalgae, *C. vulgaris* and *C. fusca* have been used as biofertilizers due to their richness in proteins, carbohydrates, fats, and growth hormones (Faheed & Fattah 2008; ÖzdemİR et al. 2016; Dineshkumar et al. 2019). Several studies have indicated that these two algae are highly efficient in improving plant growth and increasing the plant's yield. These two algae were used as bio-fertilizer on different types of plants, including tomato plant, *Solanum lycopersicum* (Garcia-Gonzalez & Sommerfeld 2016), cucumber, *Cucumis sativus*, eggplant, *Solanum melongena*, *Lactuca sativa*, and rice, *Oryza sativa* (Elhafiz et al. 2015), spinach (Cassan et al. 1992; Fan et al. 2013; El-din & Hassan 2016), wheat, *Triticum aestivum* (Shaaban 2001; Renuka et al. 2016), *Zea mays* (Dineshkumar et al. 2019), grapes *Vitis* spp (Abd El Moniem & Abd-Allah 2008), Mangifera indica (El-Sharony et al. 2015), and *Citrus sinensis* (Amro 2015).

This alga is used as a biofertilizer in various forms, including adding it to the soil, increasing soil fertility, soaking the seeds before planting, and spraying as foliar on the plant (Barone et al. 2018). As explained by ÖzdemİR et al. (2016), the use of an algal extract of *Chlorella* on tomato plants grown in greenhouses gave a significant increase in plant growth, yield and some fruit characteristics such as dry weight, total solids, and vitamin C. Puglisi et al. (2020) showed that the algal extract of *C. vulgaris* led to an increase in the germination rate of seeds of sugar beet plant *Beta vulgaris.* While Mutale-Joan et al. (2020) indicated that extracts of different types of *Chlorella* spp. were able to induce an increase in root length, shoots, leaf chlorophyll content, and dry weight in a 40-day old tomato plant. The current study aimed to evaluate the effect of using *Chlorella* algal extract on some vegetative and chemical properties of the broccoli plant, *Brassica oleracea*.

Materials and Methods

Experimental design and procedure: The experiment was conducted in Al-Rahman plantation in Al-Orouba district, Al-Diwaniyah governorate during the agricultural season 2020-2021 to evaluate the effect of foliar spraying of algal extract of *C. vulgaris* fertilizer on some plant and chemical properties on the broccoli plant. On 9/15/2020, broccoli seeds were planted in cork dishes, and on 10/15/2020, after a month of planting, the midwives were transferred to the pots, which are flexible plastic bags with dimensions of 20cm in diameter x 40cm in height and a capacity of 10kg each. Before planting, these bags were filled with sandy soil with pre-determined physical and chemical qualities and Dutch-origin peat moss (Page et al. 1982) with the adding of Dutch-origin peat moss at a 1:2 mixing ratio. The control group was sprayed with distilled water, and the treated plants were sprayed with algal extract fertilizer of 2 and 4g for the first time on 15/11/2020 and the second time on 1/1/2021.

Different concentrations of algal extract fertilizers (2 and 4g) were dissolved in distilled water using an ultrasonic homogenizer. They sprayed on the upper and lower layers of the leaves, as well as the entire vegetative complex of the whole plant, in the early morning to increase the efficiency of the sprayed substance's uptake, taking into account the prior irrigation because drought causes stomata to close (Gruda 2005).

Studied Characteristics: The height of the plant was measured using a tape measure from the soil level in the pot to the top of the plant for all replications, and the average was calculated (Singh & Stoskopf 1971). The average of the leaves number per plant (leaf plant^{-1}) was derived after calculating the number of leaves for each plant and all replicates. The total leaf area (cm^2 plant^{-1}) of all replicates in each treatment was estimated using a Portable Laser Leaf Area Meter Model CI-202 (American Bio-Science Company), by placing the plant leaves on the device platform and dividing the sum of the total leaf area of plants Transaction on the number average.

Total Chlorophyll content in leaves (mg g^{-1} wet weight) was calculated according to Mackinney

(1941). For this, the total chlorophyll content of leaves was determined by taking 1g of fresh plant leaves, cutting them into small pieces, and crushing them in a ceramic mortar in 10ml of acetone as 80% concentration. Then separating, the supernatant from the residue using a centrifuge (Hettich EBA-35, Germany) at 3000rpm for 15min, and repeating the process until the green colour was removed. The following equation was used to calculate the total chlorophyll content of the leaves:

$$\text{Total chlorophyll (mg g}^{-1}) = \frac{[20.2(D_{645}) + 8.02(D_{663})] \times V}{1000 \times W}$$

The stem diameter (cm) of all plants in each treatment was measured with a Digital Vernier Caliper (China), and the average was calculated. The floret disc diameter (cm) average was obtained by dividing the sum of the diameters of the florets in the treatment by the number of their plants. The diameter of the florets disc was measured using a tape measure.

Total nitrogen concentration (%) was determined based on Cresser & Parsons (1979) method, which was as follows: (1) to collect the NH_4 ammonia, it was added 10mL of NaOH (35%) and 10mL of the digested sample solution to a distilling flask containing 50mL of H_3BO_3 (4%) in a nitrogen distillation system (Macro Kjeldahl, Germany), (2) to distill the ammonia, the contents are boiled for 30-40min, (3) the ammonia-containing acid was titrated with sulfuric acid (0.05M), and the volume of acid consumed (during the titration procedure) is determined, and the percentage of total nitrogen in the floret disc is approximated using the following equation:

$$N\% = \frac{1.401[V1M1 - V2M2) - (V3M1 - V4M2)]}{W}$$

Where V1 = milliliters of standard acid put in a flask for the samples, V2 = milliliters of standard NaOH used in titration; V3 = milliliters of standard acid put in a flask for blank, V4 = milliliters of standard NaOH used in titrating blank, M1 = molarity of standard acid, M2 = morality of standard Na OH, W = weight of sample taken (0.2 g) and DF = dilution factor of the sample (if 1g were taken for estimation, the dilution factor would be 100).

The phosphorous content of the samples was estimated using the ammonium molybdate method and ascorbic acid, according to Olsen & Sommers (1982). In this method, 10ml of the digested sample was taken and placed in a volumetric flask of 50ml capacity. The volume was supplemented with distilled water to the mark, and then 10ml of the previous solution was withdrawn and placed in a perforated 100ml flask. The flask was then heated on a hot plate until the color of the solution changed to blue, and the volume was filled with distilled water to the mark. The optical absorption measurements were taken for a succession of concentrations from standard phosphorus solutions to build a phosphorus curve in a UV-visible Spectrophotometer at the wavelength of 620nm. The concentration of phosphorus was calculated using the following equation:

$$P\% = \frac{C \times df \times 100}{1000000} = \frac{C \times 1000 \times 100}{1000000} = \frac{C}{10}$$

Phosphorous concentration ($\mu g.ml^{-1}$) was read from the standard curve. In the formula, df = the dilution factor, which is 100 x 10 = 1000, and is calculated as follows: 0.2g of the sample was completed to 100ml (100 times), 5ml of sample solution was completed to 50ml (10 times), and 1000000: the conversion factor from micrograms to grams.

The potassium concentration of the floret disc in the digested sample was determined using a Flame Photometer (ELICO Model CL 361, Indian) according to Horneck & Hanson (1998).

The iron content of the pink disc (%) was estimated according to Sandell (1951), where samples were taken from the treated florets discs and dried in the oven at a temperature of 70°C until the weight was stable. Then they were ground, and 1ml of sulfuric acid (H_2SO_4) was added to it and left for an hour, then burned by the oven until white. The ash was dissolved in 5mL of 6 N hydrochloric acid (HCL),

filtered through an acid-washed filter paper, and the volume was replenished to 100mL (0.1 N) hydrochloric acid. 25ml of the solution was taken and sodium citrate solution prepared previously by dissolving 250mg of citrate in 1 liter of distilled water was added until the pH became 3.5. Then 1ml of each hydroquinone solution was added to it by dissolving 1g of hydroquinone in 1 liter of water and orthophenanthroline solution was prepared with 0.5g of it in 100ml of distilled water and stored in a dark bottle. The solution was left for an hour at a temperature of 20°C to reduce the amount of iron. Standard iron solutions were prepared at a concentration of 5, 10, 20, 30, 40, 50, 70, and 90mg l^{-1} to draw the standard curve of iron, and using a UV-Vis Spectrophotometer, the optical density was read at the wavelength 508nm.

About total carbohydrates (%), Masuko et al. (2005) sulfuric acid (H_2SO_4)-phenol (C_6H_6O) method is the simplest and most dependable colorimetric method for carbohydrate determination.

Statistical analysis: The results data were analyzed using the analysis of variance test for a two-factor factorial experiment according to the Randomized Complete Block Design (RCBD). The differences between the means of the treatments were compared using the LSD test at $P \leq 0.05$.

Results

The foliar spraying on broccoli plants treated with 2 and 4g l^{-1} of algal extract of *C. vulgaris* showed an increase in the mean plant height as 20.33 and 20.66cm $plant^{-1}$, respectively (Table 1). This increase was not significant compared to the control with the lowest mean of 20.0cm $plant^{-1}$. It was found that 2g l^{-1} of the algal extract decreases the mean stem diameter to 9.73mm $plant^{-1}$. In comparison, the concentration 4g l^{-1} recorded a significant increase in the average stem diameter as 11.92mm $plant^{-1}$ compared with the control group with an average of 9.88mm $plant^{-1}$.

The broccoli treated with 2 and 4g l^{-1} of algal extract recorded a significant increase in the number of leaves at 4g l^{-1} (21.66 leaves $plant^{-1}$) and 2g l^{-1} (18.66 leaves $plant^{-1}$) compared to the control one (16.33 leaves $plant^{-1}$). The broccoli plants treated with the 4g l^{-1} were significantly distinct in the average leaf area (592.9cm^2 $plant^{-1}$) compared to the control group (510.0cm^2 $plant^{-1}$). The concentration of 2g l^{-1} recorded a slight decrease in the average leaf area of 509.4cm^2 $plant^{-1}$.

The concentrations of 2 and 4g l^{-1} were significantly notable in the total chlorophyll content of leaves over the control plants that recorded the lowest average of 0.311mg g^{-1}. The concentration 4g l^{-1} had the highest average of 0.467mg g^{-1}, and 2g l^{-1} an average of 0.410mg g^{-1}. Also, the concentrations 2 and 4g l^{-1} recorded a significant increase in the average diameter of the flowering inflorescence as 11.30 and 9.80cm $plant^{-1}$, respectively, compared with the control plants (7.53cm $plant^{-1}$).

The results indicate that broccoli plants treated with 2 and 4g l^{-1} of algal extract had an increase in the average content of the florets disc of nitrogen as 1.37 and 1.45%, respectively. This increase was not significant compared to the control, with an average of 1.35%. The content of the florets disc of phosphorous in plants treated with concentrations of 2 and 4 g l^{-1} was significantly different, as the 2g l^{-1} treatment was recorded with the highest average of 0.694% than the 4 g l^{-1} with an average of 0.650% compared with the control one (0.578%). While it was found that the concentration of 4g l^{-1} recorded an increase in the mean content of the florets disc of potassium with an average of 3.11%. This increase was not significant compared to the control plants (2.93%), while the concentration of 2g l^{-1} had a slight decrease in the potassium content (2.81%)

The results also showed that the 2g l^{-1} group was significantly different in the flower disc of iron content with an average of 6.61µg g^{-1} compared to the control plants (5.46µg g^{-1}). The concentration 4g l^{-1} recorded a slight increase in the iron content reached 5.67µg g^{-1}. While the concentrations 2 and 4g l^{-1} recorded a significant distinction in the average

Table 1. Effect of the algal extract of *Chlorella vulgaris* on the studied characteristic of broccoli.

Treatments	plant height cm	Leaves number per plant	Leaf area cm^2 $plant^{-1}$	Chlorophyll content mg gm^{-1}	Stem diameter cm	Inflorescence diameter cm	Total nitrogen %	Phosphorous %	potassium %	Iron µg gm-1	Carbohydrate %
Control	20.00	16.33	510.00	0.311	9.88	7.53	1.35	0.578	2.92	5.46	23.04
Algal extract 2g	20.33	18.66	509.4	0.41	9.73	9.8	1.37	0.694	2.81	6.61	25.45
Algal extract 4g	20.66	21.66	592.9	0.467	11.92	11.3	1.45	0.658	3.11	5.67	27.83
LSD	0.935	0.582	62.14	0.058	0.641	0.64	0.57	0.061	0.582	0.57	0.57

percentage of carbohydrates, as the highest average was recorded in plants treated with 4g l^{-1} group (27.83%), followed by that 2g l^{-1} one (25.45%), while the plan control treatment recorded the lowest mean in their total carbohydrate content as 23.04%.

Discussion

Algae contain some minerals, growth regulators, proteins, carbohydrates, vitamins, thiamine, riboflavin and folic acid, and algae extract regulates the physiological and biochemical pathways of plant growth and nutrient uptake. Therefore, they play a vital role in improving agricultural production (Mutale-Joan et al. 2020). Spraying plants with extracts of algae leads to an increase in the efficiency of many physiological processes in the plant and the concentration of auxin, gibberellins, cytokinins, amino acids and macronutrients such as N, P, K, Ca and Mg and microelements such as S, Zn, Co, Mo, Cu, Mn and Fe and the polyamines (Haroun & Hussein 2003; Masojídek & Prášil 2010; Mutale-Joan et al. 2020).

The natural materials provided by algae are relatively small compared to industrial mineral fertilizers. Whereas the foliar spray is the appropriate way to increase the efficiency of biological fertilization, as the plant during the foliar spray process uses more than 90% of these materials, while it was found that the plant absorbs 10% of these materials if it presents in the soil. In this case, foliar spraying can increase in the crop ranging from 12-25% compared to the addition of traditional fertilizer (Ecochem 2022). Algae or its extracts, when sprayed on the leaves in different stages of vegetative growth of plants such as cotton, barley, oats, tomatoes, sugar cane, corn, hot peppers, lettuce, and other various plants, have an important role in increasing plant yields (Howard et al. 2001; Mohammed & Qasim 2021). Algae are bio-fertilizers that support the growth of plants (Ordog 1999; Jackson 1973). Aly et al. (2008) pointed out that activating mechanisms such as vitamins, amino acids, polypeptides, exogenous sugars, antibacterial, and antifungals improve plant growth and crop quality directly or indirectly.

Chlorella vulgaris is the most widely used bio-fertilizer in agriculture or cultivated (Wijffels et al. 2013) because of its high growth rate, simple and widespread cultivation. Its adaptation to soil conditions has become essential for organic farming and maintaining and sustaining soil fertility, ensuring economic feasibility (Kumar et al. 2015). Microalgae like *Chlorella* produce plant hormones similar to cytokinins, isopentylamine, zeatin, ribosides that influence cell division and differentiation and are also important in chloroplast development, the dominance of apical meristems, and delay senescence; these molecules have been identified in this alga through the studies conducted on plants like cucumber, beans, mountain flower, grapes and rice (Shanan & Higazy 2009; Hussain & Hasnain 2012; Romanowska-Duda et al. 2010; Ansari et al. 2022).

Chlorella vulgaris mainly provides large amounts of micro and macronutrients, carbohydrates, proteins and cytokinins (Elarroussia et al. 2016; Kholssi et al. 2019). It was a source of biologically active compounds that activate many physiological processes in the plant including photosynthesis, which regulates the growth and development of the plant (Romanowska-Duda et al. 2010; Bokov et al. 2022). Also, this alga has a positive effect on the absorption of nutrients by the plant, which enhances all physiological processes in it (Ghiloufi et al. 2017; Borchhardt et al. 2017; Huldani et al. 2022). The current study results agree with the findings of Faheed & Fattah (2008) regarding the effect of the algal extract of *C. vulgaris* on *Lactuca sativa*, and Agwa et al. (2017) on the okra *Abelmoschus esculentus*. In conclusion, it was found that foliar spraying at a concentration of 4g l^{-1} of the algal extract of *C. vulgaris* recorded a significant increase in the majority of vegetative and chemical characterization of the broccoli plant.

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