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Scenedesmus quadricauda Cultivate In Media Supplemented With Cheese Whey Act as a Biostimulant For *Lactuca sativa* Vegetative Growth.

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Abstract: Whey is the liquid portion of milk that remains after the curd (milk casein) is separated from the solid portion that results from milk coagulation. Microalgae are known to efficiently remove a range of contaminants from wastewater, combining the high-value biomass production system for commercialization with agroindustry waste treatment is one practical way to lower the costs of microalgae cultivation. This study aims to cultivate *Scenedesmus quadricauda* on standard media supplemented with cheese whey, then the pellet after growth was taken and resuspended in the soaking water to investigate its effect on the growth and development of *Lactuca sativa*. The algal treatments enhanced *Lactuca sativa* growth parameters, as well as an increase in carbohydrate content compared to the control plant during the vegetative stage.

Keywords:: Microalgae, Scenedesmus quadricauda, Whey, Biostimulant, seed priming, Lactuca sativa

Introduction

Microalgae have a lot of biotech promise, and they can be used in various sectors, including the chemical, food, medicinal, and cosmetic industries, as well as for biofuel production [1]. Natural proteins, lipids, nutrients, vitamins, pigments, and enzymes exist in these microorganisms[2, 3].

Microalgae efficiently remove several pollutants in wastewater, such as nitrogen, phosphates, and organic carbons. The high cost of producing microalgae has been a significant barrier to commercialization. Coupling the high-value biomass production mechanism for commercialization with agroindustry waste treatment is a feasible option to minimize microalgae cultivation costs[4]. For biomass production, mixotrophic cultivation is the preferred microalgae cultivation technique [5].

Microalgal biomass or its extracts used for seed germination as microalgae comprise bioactive compounds such as polyamines, natural enzymes, carbohydrates, proteins, and vitamins, all of which are utilized to improve soil characteristics that favor plant nutritional status in terms of vegetative growth and yield. Using microalgae in wastewater treatment limits or eliminates the need for chemicals [6]. The treatment of commercial, urban, and agricultural wastewater has been the subject of several studies.

Whey is a byproduct of the dairy industry that pollutes the environment. It is the residue left over after making different kinds of cheese, yogurt, ice cream, and butter using different methods [7].

Seed pretreatment increases the rate of imbibition, inflow of water-soluble metabolites, softens the testa, and regulates the pre-germinative and biochemical processes to initiate growth and protrusion of the radicle [8].

Studies have shown that the seeds pretreated with microalgal cellular extracts have higher germination and plant growth rates compared to the control along with higher soluble carbohydrate, protein, and free amino acid content[9-11].

Biostimulants are frequently described as substances that increase crop yields by promoting metabolism or optimizing nutrition; however, other benefits have also been noted, including resistance to abiotic processes and minimal environmental impact [12].

Priming is one of the most essential physiological techniques for enhancing seed performance and achieving more rapid and synchronized germination. Primed seeds provide more consistent, earlier, and sometimes higher germination as well as the establishment and growth of seedlings[13].Priming enables some of the metabolic processes required for germination to occur, without germination. Seed priming has been widely utilized to shorten the interval between seed sowing and seedling, and to synchronize emergence [14].

Lactuca commonly known as lettuce, is a genus of flowering plants in the daisy family, Asteraceae. The genus includes at least 50 species, distributed worldwide, but most wild lettuces are xerophytes, adapted to dry habitat types. Lettuce is easily cultivated, although it requires relatively low temperatures to prevent it from flowering quickly. Lettuce is rich in vitamins K and A and a moderate folate and iron source. Lettuce is considered an excellent nutritive source of minerals and vitamins as it is consumed as a fresh green salad [15]. This study aims to cultivate *S. quadricauda* on standard media supplemented with cheese whey to investigate its impact on the growth and development of *Lactuca sativa*

Materials and methods

Isolation and identification of microalgae.

The freshwater sample was collected from the Delta Company for Fertilizers and Chemical Industries. The sample was streaked on Perti dishes and incubated for 10 days. Then, different colonies were recultivated for further purification before cultivation on liquid media utilizing the streak plating technique [16]. Florescent microscopical investigations and Olympus microscopes were used for morphological identification. Isolate was identified according to Akgul, Kizilkaya [17] as *S. quadricauda* is characterized by its oblong cylindrical cells usually in one series.

Growth assessment

S. quadricauda was cultivated in (three replicates) 250ml Erlenmeyer flasks containing 180ml BBM, inoculated by 10% (v/v) with 10 days old activated culture and incubated for seven days at 26^oC, and 16:8h light: dark duration cycle and light intensity was 50 µmol photons m^{-2} s^{-1}. Direct cell count was proceeded using a standard hemocytometer technique to measure the algal growth as a cell count[18].

According to Sarrafzadeh, La [19], the estimation of algal growth can be achieved through spectrophotometric measurement of optical density. Daily measurements of the optical density of algal cultures were made at 440 nm [20].

In addition to the specific growth rate (µ), divisions per day (Dd^{-1}), and division time (Td) were calculated following the subsequent equations according to Guillard [21].

1-Growth rate; $\mu = \ell n\ (N / N_o)\ /dt$

Where N_o is the initial cell density (cell ml^{-1}) and N is the cell density at a given time t.

The doubling per day rate (Dd^{-1}) was calculated as follows:

2-Doubling per day; Dd-1 = $\mu / \ell n2$

3-Division time; $Td = 1/ Dd^{-1}$

Acclimatization of *S. quadricauda* to different whey concentrations

S. quadricauda was treated with different cheese whey concentrations to obtain the ideal concentration at which *L. sativa* recorded the highest growth parameters.

Pot experiment

A pot experiment was designed to investigate the impact of soaking lettuce seeds in the extract of *S. quadricauda* cultivated on standard Bold Basal Medium (BBM) [22] supplemented with different concentrations of whey. Homogeneously sized lettuce seeds were chosen, and the seeds were surface sterilized by immersing them in a 0.01 % HgCl2 solution for three minutes. We properly cleaned the seeds using tap water.

The experiment design was as follows:

T1: Seeds soaked in tap water

T2: Seeds soaked in *S. quadricauda* that cultivated on BBM

T3: Seeds soaked in *S. quadricauda* cultivated on 1.25% cheese whey

T4: Seeds soaked in *S. quadricauda* cultivated on 2.5% cheese whey

T5: Seeds soaked in *S. quadricauda* cultivated on 5% cheese whey

T6: Seeds soaked in *S. quadricauda* cultivated on 10% cheese whey

A week before the experiment, 400 ml of *S. quadricauda* from each concentration were prepared for soaking seeds. The centrifugal process was done, and then the pellet was taken and resuspended in 400 ml of tap water for seeds soaking to minimize the impact of the minerals in the algal growth medium, which could act as a chemical fertilizer, such as nitrates and phosphorous salts.The seeds were soaked in tap water and *S. quadricauda* suspension for 6 hours before sowing in the soil. On 27th November 2022, all groups of lettuce were planted (ten identical seeds in each pot). All sets of seeds were sown in similar earthenware pots (20 cm in diameter and 15 cm in length) filled with equal amounts of garden soil (prepared by mixing clay to sand, 2:1; v/v). The pots were maintained in the greenhouse at regular day/night temperatures and irrigated with tap water when required. After 50 days, samples were taken for growth assessment. The collected samples were used for the determination of growth parameters (shoot length, number of leaves/plants, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight) as well as some metabolic aspects for the shoot only such as total carbohydrates which were estimated by Hedge, Hofreiter [23 and crude protein which was determined by the method of Bradford [24]

Statistical analysis

The data were statistically analyzed using Cochran and Snedecor [25] methodology for randomized complete block design (RCBD). After evaluating the homogeneity using Bartlett's test, a combined analysis of the two growing seasons was conducted. The Duncan test was used to compare means at the 5% probability level.

3. Results and Discussion

Growth curve of *S. quadricauda* on bold's basal medium

The growth curve of *S. quadricauda* was plotted using both optical density and cell count, as illustrated in Fig. (1).

From the beginning until the 6th day of the experiment, both cell count and OD increased. At the end of the experiment, the cell count decreased and OD became constant. The specific growth rate (µ), division per day (Dd^{-1}), and division time (Td) were calculated as mentioned in Table (1).

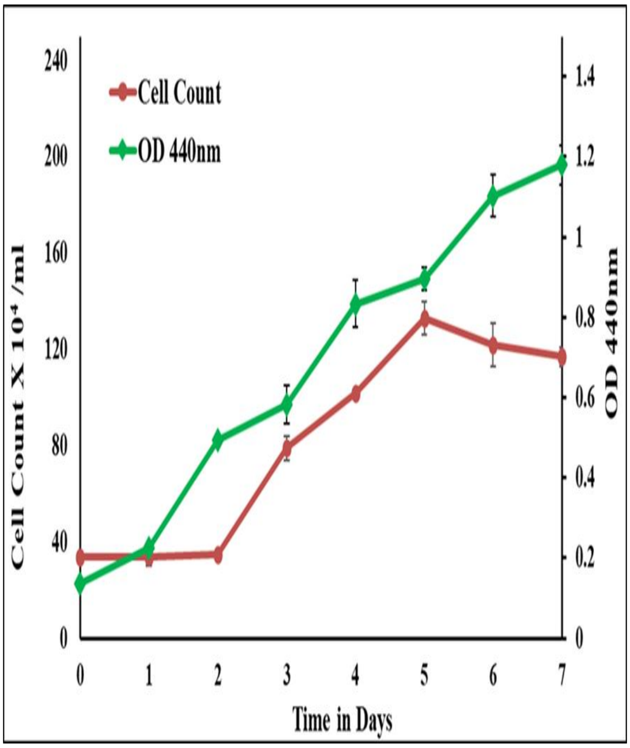

Fig. 1 Growth curves of *S. quadricauda* on bold's basal medium for 7 days.

Table 1 Specific growth rate (µ), division per day (Dd^{-1}), and division time (Td)

Isolate	µ	Dd^{-1}	Td
S. quadricauda	0.1886 ± 0.012	0.2722 ±0.017	3.7033 ± 0.226

Changes in growth parameters

The changes in the estimated growth parameters (shoot length, number of leaves, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight) in response to different treatments, during the vegetative stage, are displayed in Tables (2,3).

Growth parameters of the lettuce shoot.

Except for the significant decrease in lettuce shoot length at T4, the other treatments significantly increased the lettuce plant's shoot length significantly, compared to the control (T1). It was noticed that ((T3) showed the highest shoot length (9.0 ± 0.15) and (T4) gave the lowest value (5.7 ± 0.25). The number of leaves of lettuce plants increased non-significantly by all treatments, compared to the control (T1) and it was noticed that (T6) gave the highest (5 ± 0.2)

A non-significant decrease in *lettuce* shoot fresh weight was detected at (T5) and significantly at T4 but significantly increased by the other treatments. It was noticed that the highest waste concentration (T6), gave the highest value of the shoot fresh weight, and (T4) gave the lowest value (0.22 ± 0.01) compared to the control.

Scenedesmus treatments decreased the shoot dry weight of lettuce plants either non-significantly by treatment with T5 or significantly by T4 but significantly increased by the other treatments. T3 had the highest shoot dry weight (0.146 ± 0.008).

The recent increase in the growth parameters of lettuce shoots was in good agreement with those of Tarraf, Talaat [26] , who noted that applying algal extracts to fenugreek plants significantly enhanced the plants' height, leaf number, branch number, and fresh and dry weights during the vegetative and flowering stages. Additionally, *Chlorella vulgaris* and *Spirulina platensis* fertilizers increased the efficacy of rice plants in terms of plant height, leaf area per plant, number of leaves per plant, and fresh and dry weight per plant [27].

Table 2 Effect of *S. quadricauda* treatments on *L. sativa* shoot growth parameters.

Treatment		Shoot length (cm/plant)	Number of leaves/plants	Shoot fresh weight (g/ plant)	Shoot dry weight (g/ plant)
T1	Control (water)	6.9 ±0.18^{c}	4 ±0.2^{a}	0.328 ±0.007^{c}	0.036 ± 0.001^{c}
T2	*S. quadricauda* on BBM	8.2 ±0.2^{b}	5 ±0.2^{a}	0.44± 0.01^{b}	0.043± 0.001^{b}
T3	*S. quadricauda* on 1.25% CWE	9±0.15^{a}	5 ±0.4 ^{a}	0.47±0.03^{ab}	0.146± 0.0008^{a}
T4	*S. quadricauda* on 2.5% CWE	5.7±0.25^{d}	4 ±0.2 ^{a}	0.22 ±0.01^{d}	0.026 ± 0.001^{d}
T5	*S. quadricauda* on 5% CWE	7.16±0.16^{c}	5 ±0.33^{a}	0.30 ±0.006^{c}	0.035± 0.003^{c}
T6	*S. quadricauda* on 10% CWE	8.3±0.3^{b}	5 ± 0.2^{a}	0.49 ±0.017^{a}	0.042 ± 0.002^{b}

Growth parameters of root

All treatments increased the root length significantly and T3 recorded the highest root length compared to the control.

Only T4 decreased the root fresh weight of *the lettuce* plant significantly and the other treatments increased this parameter; significantly by T2 and non-significantly by T3, T5, and T6.

The root dry weight of the lettuce plant decreased non-significantly at T4 whereas the other *Scenedesmus* treatments increased this parameter; non-significantly by T2 and T6 and significantly by T3 and T5.

The increase in root and shoot lengths of plants may be due to the activity of one or more growth-promoting substances, specifically auxins, gibberellins [28], and cytokinin [29] found in microalgae. It has been found that eukaryotic green algae, or microalgae, can directly promote shoot and root growth [30,31].This may be because algal exopolysaccharides, or EPS, are necessary for nitrogen fixing and the symbiotic relationship between rhizobium and legume. Algal EPS may help attract fungi and microorganisms that benefit plants' rhizosphere[32].

Table 3 Effect of *S. quadricauda* treatments on *L. sativa* root growth parameters.

Treatment		Root length (cm/plant)	Root fresh weight (g/ plant)	Root dry weight (g/ plant)
T1	Control (water)	2.9 ±0.1^{c}	0.04± 0.002^{b}	0.005±0.0004^{cd}
T2	*S. quadricauda* on BBM	4.2± 0.12^{a}	0.056±0.006^{a}	0.007±0.0005^{abc}
T3	*S. quadricauda* on 1.25% CWE	4.4 ±0.24^{a}	0.049 ±0.002^{ab}	0.009±0.001^{a}
T4	*S. quadricauda* on 2.5% CWE	3.5 ±0.15^{b}	0.024±0.002^{c}	0.003± 0.005^{d}
T5	*S. quadricauda* on 5% CWE	4.16± 0.16^{a}	0.041±0.005^{b}	0.008±0.005^{ab}
T6	*S. quadricauda* on 10% CWE	3.5 ±0.15^{b}	0.046±0.0006^{ab}	0.006± 0.0004^{bc}

Changes in Total Carbohydrates and protein content

The content of carbohydrates and protein was determined for only the treatments that have the best responses i.e. T1, T2, and T3. The total carbohydrates and protein content of

lettuce plants decreased significantly by the lowest waste concentration and it was noticed that T1 and T2 recorded the highest content of total protein and carbohydrates as shown in Figs (2,3).

The highest value of total carbohydrates in lettuce plants treated with microalga agreed with Osman, El-Sheekh [33], who found that *Nostoc entophytum* and *Oscillatoria angustissima* increased growth parameters (including root depth, shoot length, dry weights, and leaf area), contents of all pigment fractions (chlorophyll a, chlorophyll b, and carotenoid) in seedlings leaves of pea plant, and carbohydrate content.

In support to the current finding, Faheed and Fattah [34] , stated that in terms of soluble carbohydrate, soluble protein, and total free amino acids, lettuce (Lactuca sativa L.) seed growth was significantly increased when planted in soil with different concentrations of *Chlorella vulgaris* as opposed to the control. This improved the seedlings' fresh and dry weight in addition to their pigment content.

The growth bioregulators present in the microalgal filtrate may have a direct or indirect role in the metabolism of nitrogen and saccharides[35].

The protein content in this study was consistent with those of Elnagar et al.[36]who found that *Corchorus olitorius* treated with both *Chlorella sorkiniana* cultivated on standard BBM and *C.sorokiniana* treated with 50% beet filter cake extract (BFCE) during the flowering stage showed a decline in total proteins compared to untreated control.

These findings disagreed with those of Puglisi, La Bella [37], who demonstrated that the leaves of lettuce seedlings treated with *S. quadricauda SN4* extract had a protein content that was almost 38% higher than that of the control plants. Additionally, inconsistent with those of Fan, and Hodges [38], who found that spinach treated with a commercial algae-based extract showed an increase in total soluble proteins and that this increase was strongly correlated with an increase in the transcription level of regulatory enzymes involved in nitrogen metabolism. According to Haider, and Ayyub [39], tubers sprayed with seaweed extracts showed greater levels of crude protein (up 75%) and total soluble carbohydrates (up 21% from the untreated control).

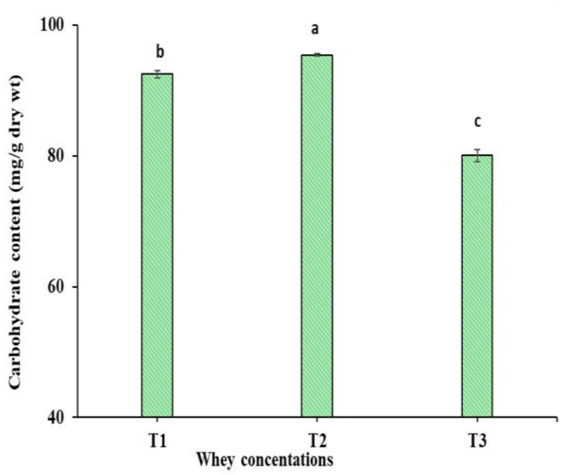

Fig. 2 Effect of *S. quadricauda* on carbohydrate and protein content of *L. sativa* plants ± SE.

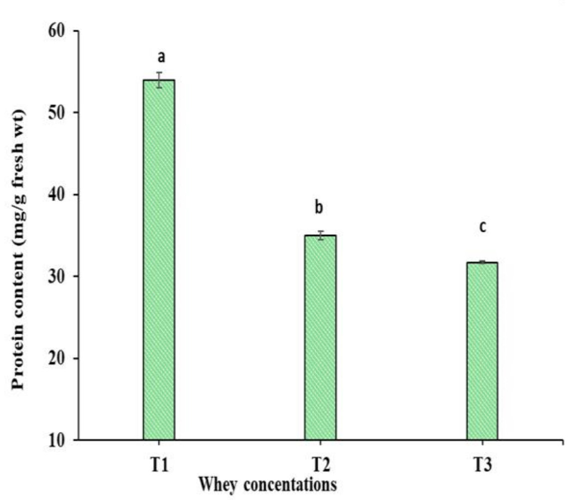

Fig. 3 Effect of *S. quadricauda* on protein content of *L. sativa* plants ± SE.

4. Conclusions

Microalga *S. quadricauda*-based biofertilizers hold great promise as an alternative way to recycle nutrients by enhancing the production of large amounts of biomass that can be upgraded in value and used in various applications. These biofertilizers especially when cultivated on standard BBM supplemented with CWE, which could lower the cost of its production while also consuming waste released into the environment, show a significant potential as biostimulants, due to their phytohormone contents and the presence of other plant-growth promoters, such as amino acids and vitamins that can decrease dependence on chemical fertilizers and can effectively improve the physicochemical properties of the soil of potted lettuce seeds, which would be beneficial to the growth and development of the lettuce plant.

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