SİİRT ÜNİVERSİTESİ Bilimin Işığında

Türkiye Tarımsal Araştırmalar Dergisi
dergipark.org.tr/tutad

Research Article

Turk J Agric Res
2026, 13(1): 1-11
© TÜTAD
ISSN: 2148-2306
e-ISSN: 2528-858X
doi: 10.19159/tutad.1742063

Biostimulant Effects of *Cladophora glomerata* and *Limnospira platensis* on Growth and Rhizosphere Activity of *Mentha spicata*

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Received: 14.07.2025 **Accepted:** 04.03.2026

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Abstract: This study aims to assess the biological properties of rhizosphere soil and the growth performance of *Mentha spicata* through the application of macroalgae and microalgae, promoting sustainable agriculture and environmental conservation. Seedlings were treated various concentrations (0%, 0.2%, 0.4%, and 0.6%) of *C. glomerata* as a macroalgae source and *L. platensis* as a microalgae source. Applications were carried out at 15-day intervals, starting one month after sowing. While the treatments varied in their effects on these growth factors, they significantly enhanced plant metrics compared to the control group. The highest chlorophyll content in leaves was achieved with the combined application of *Cladophora glomerata* (0.6%) and *Limnospira platensis* (0.6%). The findings demonstrated that microalgae and macroalgae treatments not only improved plant growth but also significantly increased β-glucosidase and dehydrogenase enzyme activities in the rhizosphere compared to the control ($p<0.05$). Additionally, soil chlorophyll levels were markedly elevated by algal treatments ($p<0.05$). The results showed that the combined application of *C. glomerata* and *L. platensis* is a promising biostimulant approach for improving mint growth and soil biological activity.

Keywords: Microalgal-based biofertilizers, macroalgae, microalgae, microbial activity, organic agriculture

1. Introduction

Mint (*Mentha spicata*) is a highly valued aromatic plant with significant economic importance, widely used as both a flavouring agent and a medicinal herb. In Türkiye, approximately 60% of mint production takes place in the Nizip district of Gaziantep where local varieties are particularly appreciated for their intense aroma and vibrant color. Nizip mint is distinguished from other varieties because of its distinct aroma and vivid color. Its cultivation is widespread among farmers due to its strong economic profitability. As since mint thrives in water-rich environments, it is extensively grown near the Euphrates River in Türkiye (Gezinç and Hasdemir, 2021).

Organic agriculture, a rapidly growing and environmentally sustainable form of production, emphasizes avoiding pesticides and chemical fertilizers. Chemical fertilizers have been shown to negatively affect soil health and the environment, causing issues such as soil fatigue, water and soil pollution, and toxic residues. The expansion of organic farming is closely tied to increased awareness of these harms and the development of alternative fertilization methods. Among these alternatives, algal fertilizers stand out for their natural composition and ability to support plant growth. Even in small quantities, they significantly contribute to plant nutrition, making them an essential tool in organic farming practices (Gamage et al., 2023).

Microalgae, despite being at the base of the food chain, serve as a crucial food source for fish and other aquatic organisms (Maizatul et al., 2017). As primary producers, they play a foundational role in the ecological hierarchy and are among the most valuable ecological groups. Recent biotechnological research has focused on expanding the applications of microalgae in fields such as agriculture, medicine, food, industry, feed, environmental management, and cosmetics (Khan et al., 2018). Biofertilizers, when applied to plants, seeds, or soil surfaces, allow living microorganisms to colonize the plant and facilitate the delivery of essential nutrients. Microalgal-based biofertilizers, in particular, have been shown to enhance plant growth significantly (Khan et al., 2018; Barone et al., 2019). Cyanobacteria, a key component of soil microflora, play a vital role in improving soil fertility both directly and indirectly (Win et al., 2018). They increase soil nitrogen levels, reduce soil compaction, and produce plant growth promoting substances (Khan et al., 2018; Riba et al., 2020). Cyanobacteria also release high concentrations of amino acids and phytohormones into the soil, enhancing germination rates, plant growth, and the production of secondary metabolites in aromatic plants (Gayathri et al., 2017; Win et al., 2018; Chookalaii et al., 2020; Zarezadeh et al., 2020). These biostimulants have been extensively studied for their impact on various plant parameters (Gayathri et al., 2017; Santini et al., 2021), highlighting their potential as sustainable agricultural tools (Shakeela et al., 2017).

Cladophora glomerata is a filamentous, branched green alga that may be free-floating in shallow waters or attached to substrates in shallow pools such as lakes, canals, shaded coastal areas of lakes, and slow-moving streams (Michalak et al., 2018). The biomass of species such as *Ulva*, *Cladophora*, and *Polysiphonia* has been recognized as a valuable raw material for organic fertilizer production, finding applications in various agricultural practices (Michalak et al., 2017; Küçük et al., 2024). Numerous studies have examined the composting of these algae and their effectiveness as fertilizers (Cavallo et al., 2006; Michalak et al., 2017, 2018; Dziergowska et al., 2021; Sezen and Küçük, 2024). Research has also investigated the biofertilizer potential of five algal species *Cyanothece aeruginosa* (Nägeli) Komárek, 1976; *Limnospira platensis* Gomont, 1892; *Cladophora glomerata*; *Spirogyra jugalis* (Dillwyn) Kütz, 1845; and *Chara globularis* Thuiller, 1799 on the growth and productivity of beans (*Phaseolus radiatus* L.) (Mahadik and Kabnoorkar, 2020). Similarly, studies on tomato seedlings showed that using powdered green algae *Cladophora prolifera* (Roth) Kützing, 1843 as a fertilizer significantly improved yield and development in treated plants (Cavallo et al., 2006). This study aimed to evaluate the biostimulant effects of *Cladophora glomerata* and *Limnospira platensis* on the growth of *M. spicata* and the biological activity of its rhizosphere under greenhouse conditions.

2. Materials and Methods

2.1. Material

Cladophora glomerata was collected from Karkamış Dam in Birecik, Şanlıurfa, Türkiye. The collected algae were cleaned, dried at room temperature, pulverised with a 46.000 rpm blender and stored at -20ºC. The chemical composition analysis of *C. glomerata* was carried out at Akdeniz University Food Safety and Agricultural Research Centre, the major biochemical components are presented in Table 1.

Table 1. Chemical composition of *C. glomerata*

Feature	Value
Moisture (%)	89.50
Ash (%)	8.94
Total protein (%)	19.35
Crude fibre (%)	16.55
Crude fat (%)	2.05
Carbohydrate (%)	60.25
Chlorophyll a (µg g^{-1})	965.30
Chlorophyll b (µg g^{-1})	713.20
Carotene (µg g^{-1})	291.34
Protein (mg g^{-1})	242.50
Carbohydrate (mg g^{-1})	735.15

Limnospira platensis was obtained from Çukurova University, Faculty of Fisheries. *L. platensis* was cultured in BG11 medium in 20 l plastic bottles at a constant temperature of 30 ± 1 ºC, pH 9.7, 16 h light / 8 h dark photoperiod under LED (Light Emitting Diode) illumination and continuous fresh air supply with aquarium pump in Harran University, Faculty of Arts and Sciences, Department of Biology, Hydrobiology-Algology Laboratory (Lopez-Rodriguez et al., 2023). The culture reached maximum density 9-10 days after inoculation and was harvested by filtration through a 20 µm plankton net. After harvesting, *L. platensis* was dried at room temperature, pulverized with a 46.000 rpm blender (Ultra High Speed Blender) and stored at -20 ºC. The composition of *L. platensis* was made at Akdeniz University Food Safety and Agricultural Research Center and is given in Table 2.

Mentha spicata (Nizip mint) cuttings used in the experiment were collected from Kumla and Elifoğlu Villages in the Nizip district. The soil used in the experiment was classified as clay soil, with a composition of 49.79% clay, 27.58% silt and 22.63% sand; characterized by its alkalinity. It is poor in lime and organic matter content. The properties of the used in the experiment are detailed further in Table 3. The soil used in the experiment was sterilized in an autoclave.

Table 2. Approximate composition of *L. platensis* (dry matter basis)

General composition	Composition amount (per 100 g dry weight)
Moisture	2.15 g
Protein	62.40 g
Fat (Lipids)	10.50 g
Ash	8.20 g
N-free extract (NFE)	16.50 g
Minerals	
Phosphorus	935.00 mg
Iron	59.60 mg
Calcium	181.00 mg
Potassium	1.82 g
Sodium	1.11 g
Magnesium	273.00 mg
Colorants	
Phycocyanin	17.30 g
Carotenoids	486.10 mg
Chlorophyll a	1.49 g

NFE: Nitrogen Free Extract, NFE= 100-(Moisture+Crude protein+Crude lipids+Fiber+Ash), Total biomass= Chlorophyll a x 67

Table 3. Characteristics of soil used in the experiment

Properties	Value
EC (dS m^{-1})	0.34
pH	8.3
Lime (%)	20.6
Total nitrogen (N) (%)	0.4
Available phosphorus (P) (kg ha^{-1})	49.7
Exchangeable potassium (K) (kg ha^{-1})	1186
Organic matter (%)	1.71
Clay (%)	49.79
Silt (%)	27.58
Sand (%)	22.63

2.2. Method

2.2.1. Setting up the trial

The experiment was conducted in a greenhouse with natural light in 3 replicates according to factorial experimental design (algae type; *C. glomerata, L. platensis* and mixing, dose were used as factors) and the plants were harvested 5 months after sowing. For each treatment, four healthy plants were selected and planted in 7-liter pots. One month after planting, *C. glomerata* and *L. platensis* were added to the soil separately and in combination [0% (control), 0.2%, 0.4%, 0.6%; w/w]. Applications were carried out 7 times at 15 days intervals. The control (0%) was irrigated with tap water only. Plant health was monitored during the experiment and no disease symptoms were observed.

2.2.2. Basic plant characteristics

Some measurements were taken at harvest. These measurements included the number of leaves per plant, length of the longest branch (cm), and root length (cm), which were measured and recorded using a ruler. Plant dry and wet weight (g $plant^{-1}$), root dry and wet weight (g $plant^{-1}$), and were weighed separately immediately after harvest and recorded as wet weight. The samples were then placed in separate paper bags and dried in an oven at 70 °C until a constant weight was reached. The dry plant portions were weighed on a precision scale recorded (Yeşil and Kara, 2014).

2.2.3. Total chlorophyll of leaves

Leaf samples were crushed in a mortar and soaked in ethyl alcohol in hot water at 80 °C for 25-30 minutes, then centrifuged at 6000 rpm for 10 minutes. The supernatant in the tubes was measured using alcohol as a blank at absorbance values of 652 nm and 665 nm with a spectrophotometer (Thermo Scientific Multiskan GO Microplate Spectrophotometer). The results were calculated according to Girard et al. (2020).

2.2.4. Biochemical analysis of rhizosphere soil

2.2.4.1. β-glucosidase enzyme activity

Toluene, Modified Universal Buffer (MUB) (pH: 6) buffer, p-nitrophenyl-α-d-glucopyranoside (PNG) solution were added to the soil samples taken from the root zone, mixed for 5 minutes and incubated at 37 °C for 1 hour. At the end, 0.5 M $CaCl_2$ and 0.1 M tris hydroxymethyl aminomethane (THAM) buffer (pH:12) were added and shaken. The supernatant was then centrifuged at 10000 x g for 10 minutes and measured spectrophotometrically at 410 nm (Tabatabai, 1982; Küçük and Şinşek, 2019).

2.2.4.2. Dehydrogenase activity

The soil samples from each treatment were separately added to 3% 2,3,5 triphenyltetrazolium chloride. They were incubated for 24 hours at 25 °C. At the end of the incubation period, methanol was added to each sample. The contents were filtered through Whatman filter paper. The filtrates were measured at 485 nm using a spectrophotometer (Tabatabai, 1982).

2.2.4.3. Chlorophyll content of soil

Soil samples (10 g) were transferred into glass bottles and mixed with 30 ml of acetone and dimethyl sulfoxide (DMSO) in a 1:1 ratio. The bottles were capped, incubated at room temperature for 96 hours in the dark and filtered through Whatman No. 1 filter paper. The filtrate obtained was measured by spectrophotometer with wavelengths of 630, 645, 663 nm and the results were calculated as mg g^{-1} chlorophyll soil (Nayak et al., 2004).

2.2.5. Statistical analysis

The data obtained from the experiments were analysed using of variance in JMP 11 statistical programme and the differences between treatments were evaluated by the Least Significant Difference (LSD) multiple comparison test (p<0.05).

3. Results

3.1. Effects of treatments on plant characteristics

As seen in Table 4, the treatments had varying effects on mint plant growth. A combination of 0.6% *C. glomerata* and 0.2% *L. platensis* significantly increased root length (156.5%), root wet weight (441%), and root dry weight (416.7%) compared to the control (p<0.001). Leaf number increased significantly (106.4%) following the application of a mixture conatining 0.2% *C. glomerata* and 0.6% *L. platensis* dried biomasses. In addition, shoot wet and dry weights by 60.5% and 163.6%, respetively, following treatment with 0.6% *C. glomerata* and 0.4% *L. platensis* dose mixtures. Furtermore, a 0.6% application dose of *C. glomerata* and 0.2% of *L. platensis* was found to be effective on branch length compared to other doses (Table 4). *Cladophora glomerata* 0.6% and *L. platensis* 0.4% doses appear to be optimal for plant growth. This treatment combination shows particularly strong effects on root length and root wet weight.

Table 4. Growth parameters of *M. spicata* species treated with different dose mixtures of *C. glomerata* and *L. platensis**

Basic plant characteristics	*C. glomerata* (%)	*L. platensis* (%)			
		0 (Control)	0.2	0.4	0.6
Shoot wet weight (g plant^{-1})	0.0	6.7 g	8.5 fg	8.9 efg	14.6 bcd
	0.2	11.4 def	9.8 efg	8.8 efg	10.5 ef
	0.4	12.0 cde	17.1 ab	9.8 efg	10.4 ef
	0.6	15.0 abc	17.2 ab	18.3 a	14.4 bcd
Shoot dry weight (g plant^{-1})	0.0	1.1 g	2.1 cde	1.5 fg	2.5 abc
	0.2	2.3 bcd	1.4 fg	1.8 fg	1.8 def
	0.4	2.2 cde	2.2 cde	1.3 fg	1.9 def
	0.6	2.4 bcd	2.8 ab	2.9 a	2.3 cde
Root wet weight (g plant^{-1})	0.0	3.9 d	6.3 cd	8.5 bcd	9.9 bcd
	0.2	10.1 bcd	10.8 bcd	8.9 bcd	11.5 bcd
	0.4	17.3 ab	13.1 a-d	13.9 abc	11.5 bcd
	0.6	16.3 ab	21.1 a	10.4 bcd	8.5 bcd
Root dry weight (g plant^{-1})	0.0	1.2 ı	2.8 d-ı	3.2 d-h	3.4 c-g
	0.2	1.9 ghı	2.1 f-ı	2.4 e-ı	4.0 b-e
	0.4	4.0 b-e	3.6 c-g	4.6 a-d	5.1 abc
	0.6	3.8 b-f	6.2 a	5.0 abc	5.4 ab
Root length (cm)	0.0	14.5 e	17.8 b-e	19.8 b-e	15.5 de
	0.2	20.9 b-e	21.8 b-e	25.2 b-e	27.3 abc
	0.4	22.3 b-e	20.2 b-e	26.5 bc	25.6 bcd
	0.6	28.2 ab	37.5 a	20.4 b-e	18.1 b-e
Length of the longest branch (cm)	0.0	7.2 e	7.3 de	8.3 cde	8.8 b-e
	0.2	7.2 de	7.3 de	8.5 b-e	9.8 bc
	0.4	8.5 de	9.2 b-e	9.4 bcd	10.1 bc
	0.6	10.6 b	14.5 a	10.1 bc	9.7 bc
Number of leaves (pcs plant^{-1})	0.0	23.1 f	32.2def	34.5 b-e	35.0 b-e
	0.2	29.7 ef	39.4 a-e	40.9 a-d	47.7 a
	0.4	43.2 abc	44.1 ab	39.4 a-e	37.2 b-e
	0.6	34.6 b-e	34.0 cde	37.1 b-e	37.2 b-e

*: The difference between the averages indicated by the same letter is not statistically significant.

As seen in Figure 1, it was observed that *C. glomerata* and *L. platensis* treatments provided a significant increase in chlorophyll content. Chlorophyll content of leaves; 0.6% application of *C. glomerata* showed an increase of 48.2% compared to the control. The 0.4% application dose of *L. platensis* showed higher chlorophyll content than the other doses. Co-application of 0.2% of *C. glomerata* increased chlorophyll content compared to other treatments (Figure 1).

3.2. Some rhizosphere characteristics of soils

All treatments significantly affected β-glucosidase activity in the rhizosphere. The lowest activity was observed in the control, while the highest activity was recorded with 0.4%

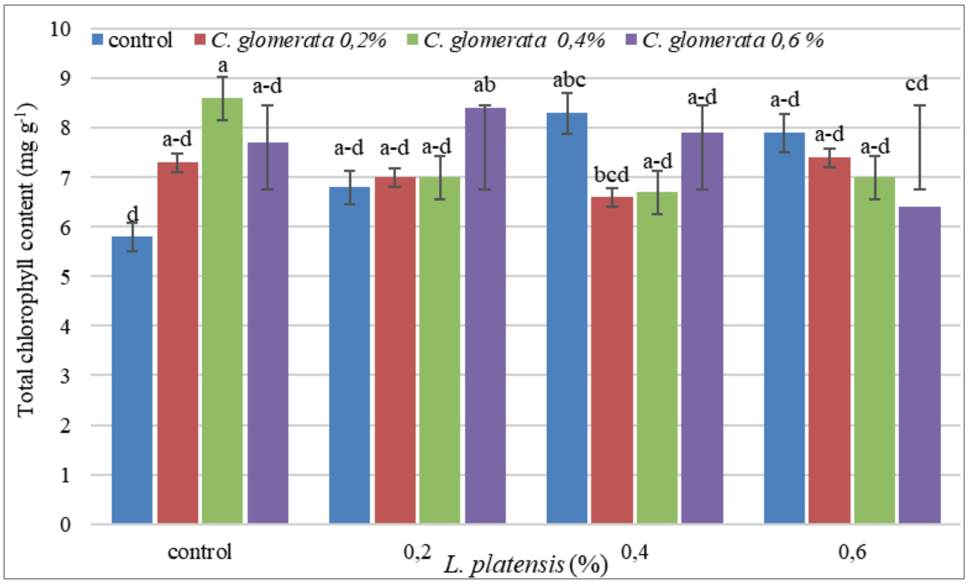

Figure 1. Effects of *C. glomerata* and *L. platensis* treatments on chlorophyll content (mg g^{-1})*

*: The difference between the averages indicated by the same letter is not statistically significant.

C. glomerata and 0.6% *L. platensis* (Figure 2). The effects of treatments on dehydrogenase enzyme activity in the rhizosphere are given in Figure 3. The lowest activity was obtained in the control. A dose-dependent increase in dehydrogenase activity was observed with both *C. glomerata* and *L. platensis* applications, with all treatments showing significantly higher activity than the control ($p<0.05$). The highest activity was obtained with the combined application of *C. glomerata* (0.6%) and *L. platensis* (0.6%). This was followed by the 0.6% *C. glomerata* and 0.4% *L. platensis* and co-application of 0.6% *C. glomerata* and 0.2% *L. platensis* (Figure 3). Soil chlorophyll content was significantly influenced by the treatments. The control group exhiibited the lowest values, while the highest chlorophyll content was observed in the co-application of 0.6% *C. glomerata* and 0.6% *L. platensis*, showing a 99% increase over the control (Figure 4).

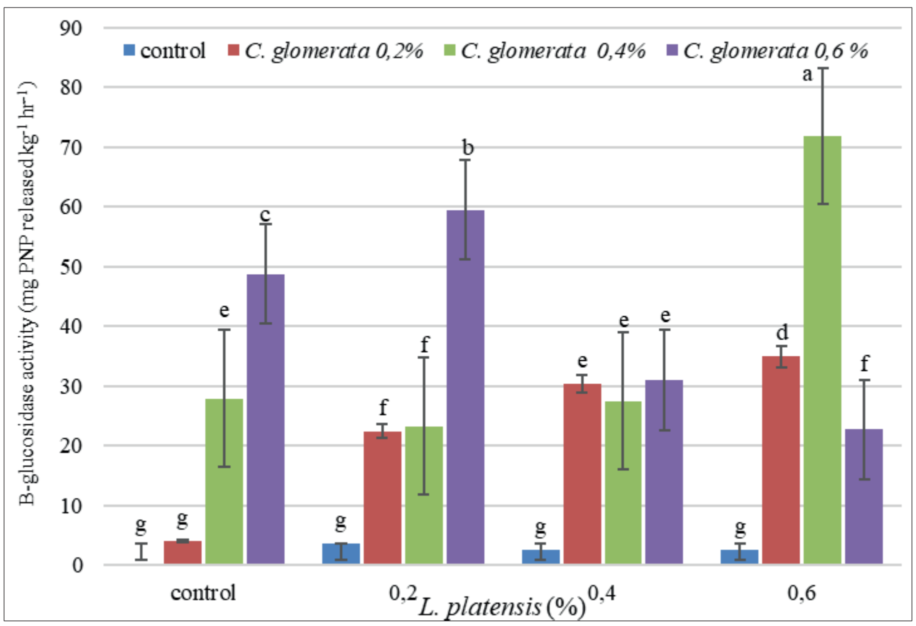

Figure 2. Effects of treatments on β-glucosidase activity in the soil rhizosphere*

*: The difference between the averages indicated by the same letter is not statistically significant.

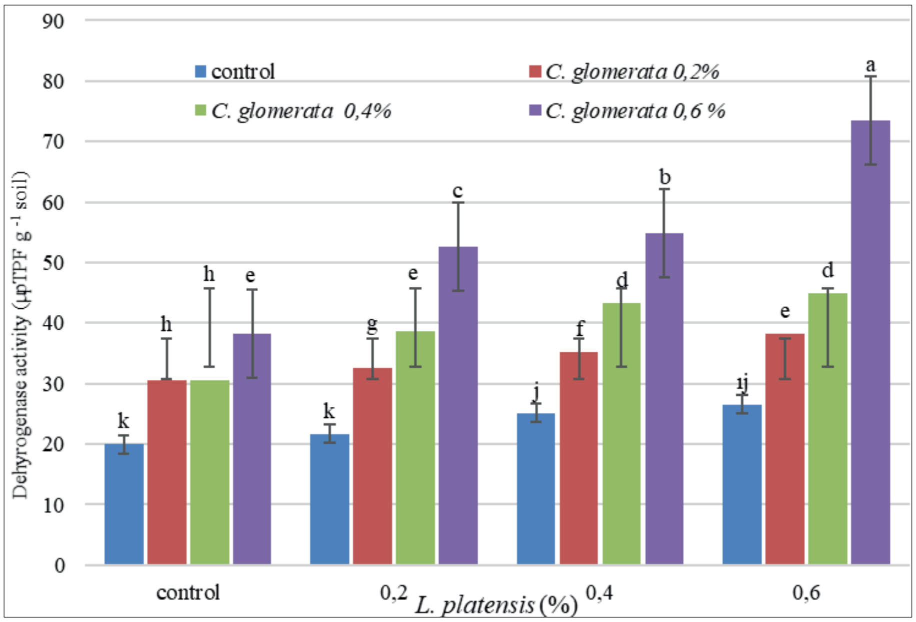

Figure 3. Effects of treatments on dehydrogenase enzyme activity in the soil rhizosphere*

*: The difference between the averages indicated by the same letter is not statistically significant.

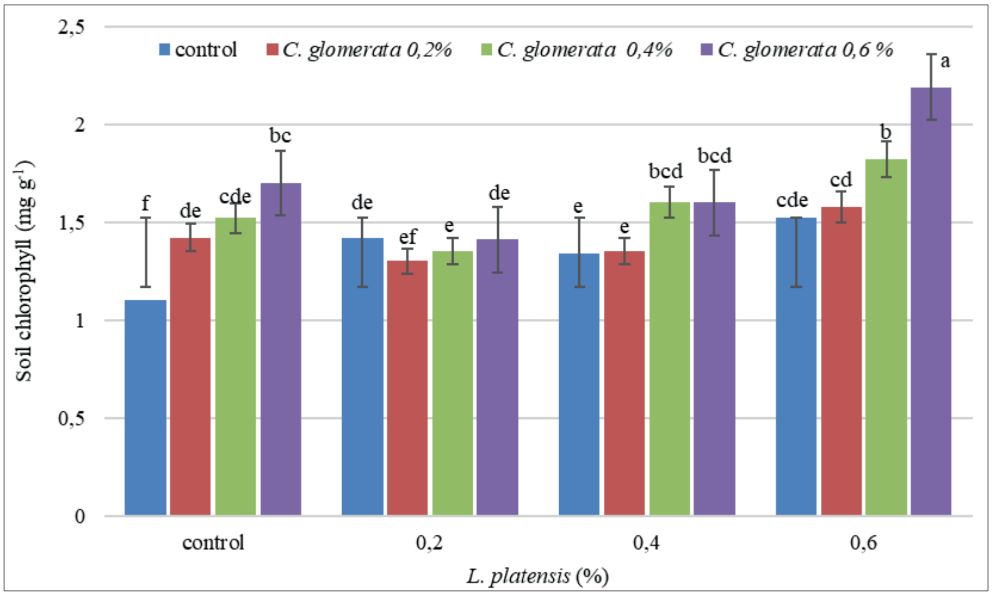

Figure 4. Effects of treatments on soil chlorophyll content*

*: The difference between the averages indicated by the same letter is not statistically significant.

4. Discussion and Conclusion

In the present study, the application of different doses of *C. glomerata* and *L. platensis* significantly enhanced plant biomass compared to the control group. The root length, shoot wet weight of *M. spicata* plants treated with two different algae increased significantly compared to the control. Improvement in basic growth parameters of mint plants, especially plant weight, has been frequently associated with cyanobacterial-based treatments due to their content of phytohormones such as auxins (Supraja et al., 2020; Santini et al., 2021). In a study by Riahi et al. (2023), evaluated the effects of *Wollea* sp. and *Nostoc* sp. treatments on the growth parameters of four *Mentha* species. The researchers reported that *Wollea* sp. and *Nostoc* sp. treatments increased the number of leaves, branching, shoot and root length, while the treatments showed different effects on root and shoot wet weight. All treatments showed a significant improvement in shoot and root wet weight. However, the effects of different doses of the applied algae were different (Table 4). The 0.4% and 0.6% doses of *C. glomerata* were more effective than the other doses, while the 0.6% dose of *L. platensis* was more effective than the other doses (Table 4).

The effect of algal treatments on plant characteristics is attributed to the auxins present in algae, which plant a significant growth. Auxins, the most important group of phytohormones directly involved in increasing plant growth, have been reported to play key role in cell division, plant height, differentiation and root elongation (Mahadik and Kabnoorkar, 2020; Bharti et al., 2021). Auxins produced by algae have been reported to control a range of processes such as cell growth, the formation of vascular tissue, and the development of roots (Santini et al., 2021). It has also been explained that auxins play a role in the biosynthesis of soluble proteins, causing an increase in the content of chlorophyll a and b (Supraja et al., 2020). Auxins have been described to increase nutrient uptake by increasing root length leading to plant growth (Ranjan et al., 2016; Santini et al., 2021). It is also known that phytohormones can affect other soil microorganisms involved in nutrient cycling, thus indirectly providing absorbable forms of nutrients for plants (Shukla et al., 2019; Shah and Smith, 2020; Santini et al., 2021).

In the study by Ceylan and Sezen (2024) on the flavonoid and phenolic compound amounts of *C. glomerata* and *L. platensis* algae used in the study; flavonoid contents of algae were determined as 616.82 and 600.67 µg QEs mg^{-1} ekstrakt (QEs: quercetin equivalents), respectively; phenolic contents were determined as 581.77 and 570.39 µg PEs mg^{-1} ekstrakt (Pes: pyrocatechol equivalents), respectively (Ceylan and Sezen 2024). The high flavonoid and phenolic compounds indicate that *C. glomerata* and *L. platensis* are very useful as biostimulants in plant growth. It has been found that the compounds contained in microalgae improve the root system of the plant and increase mineral nutrition (Ranjan et al., 2016; Gorka et al., 2018; Santini et al., 2021).

In this study, algal treatments significantly enhanced photosynthetic pigment levels in plants compared to the control group ($p<0.05$) (Figure 1). Photosynthetic pigments are crucial indicators for evaluating the quality and efficiency of photosynthesis under different environmental conditions (Chabili et al., 2025). Previous research has demonstrated that growth-promoting substances can increase photosynthetic pigment levels (Anbi et al., 2020). The synthesis of chlorophyll, a key photosynthetic pigment, depends on the availability of macronutrients such as total nitrogen and available phosphorus in the soil (Supraja et al., 2020). Consequently, the application of algal extracts, which can increase the concentration of these nutrients, may stimulate endogenous chlorophyll biosynthesis. Studies have reported that plants treated with algal extracts exhibit higher chlorophyll content, which may result from improved nutrient uptake or the extract's protective effect on chlorophyll. This protective mechanism reduces chlorophyll degradation and delays plant senescence, further supporting plant health and productivity (Supraja et al., 2020; Geries and Elsadany, 2021). In addition to the hormonal effects of algae, it helps plants absorb nutrients more easily and contributes to the photosynthetic activity of the plant.

The enzyme involved in the microbial degradation of cellulose to glucose, an important carbon source for soil microorganisms, has been identified as β-glucosidase (Daunoras et al., 2024). Soils containing easily decomposable organic matter have high β-glucosidase activity (Daunoras et al., 2024). Biosolids, manure, urban sludge and poultry waste added to soils increased the activity of β-glucosidase enzyme in soils (De Almeida et al., 2015). During the growth of certain algal species, the content of intracellular and extracellular polysaccharides increases significantly, which can fix more carbon in the soil and thus increase dissolved organic carbon and total carbon (Jiajun et al., 2019). In this study, the observed increase in β-glucosidase activity in the rhizosphere under algal treatments may be attributed to the addition of more carbon sources to the soils with these treatments. These results are supported by the results of researchers (Jiajun et al., 2019). In studies, it was determined that organic and chemical fertilizers added to the soil increased dehydrogenase enzyme activity and organic fertilizers were more effective in this increase (Daunoras et al., 2024). In this study, the treatments significantly increased β-glucosidase and dehydrogenase activity in the rhizosphere soil compared to the control. The effects of application and different doses of *C. glomerata* and *L. platensis* were statistically significant on β-glucosidase and dehydrogenase activity ($p<0.05$). It has been reported that dehydrogenase activity increases significantly with an increase in the active viable cell population and is directly related to soil microbial biomass (Prasanna et al., 2015). In previous studies, cyanobacterial formulations showed a 10-20% increase in dehydrogenase activity compared to the control (Prasanna et al., 2016). In this study, the increase in enzyme activities of *C. glomerata* and

L. platensis treatments compared to the control may be due to the stimulation of soil enzyme activity (Prasanna et al., 2016; Osorio-Reyes et al., 2023). In addition to improving soil physical and chemical properties, the addition of nutrients to soils increases the microbial population; microorganisms, especially bacteria, participate in nutrient cycles and energy conversion by promoting the decomposition of organic matter and the release of nutrients, and contribute to increased soil fertility (Marks et al., 2019; Bharti et al., 2020). The application of macroalgae and microalgae to soil can promote microbial activity and enzyme production (Shukla et al., 2019). Algae added to soils are a source of carbon, nitrogen and other nutrients for the soil, so their addition affects the activity of soil microbial populations. Therefore, it is thought that the dehydrogenase and β-glucosidase activities of soils increase with the addition of different doses of *C. glomerata* and *L. platensis* compared to the control. Some growth regulators in algae have been reported to improve the soil micro-ecological environment (Chen et al., 2020). Algae, as potential carbon source, are used in various agricultural applications (Gorka et al., 2018). A decrease in the organic carbon content of soils leads to a decrease in soil fertility and degradation of agricultural land. It has been reported that algae can improve soil aggregation and stability by converting carbon in soils to sugars through photosynthesis and by affecting soil microbial community structure. (Marks et al., 2019). In our study, the significant increase in rhizosphere enzyme activities of different doses of *C. glomerata* and *L. platensis* added to the soil compared to the control is thought to be due to the promotion of microbial activity by the applied algae.

When added to soil, algae contribute to soil fertility by improving its biological and physicochemical properties. Extracellular polymeric substances (EPS) produced by algae are released, causing an increase in the carbon content of the soil, thus contributing to soil aggregation and promoting beneficial microbial populations that support nutrient cycling. Algae added to soils can affect plant growth directly by secreting compounds that promote growth and indirectly by facilitating the dissolution of minerals in the environment (Marks et al., 2019). Some algae species secrete extracellular enzymes that decompose organic waste in the soil, and metabolites produced by algae can accumulate in the environment and interact with minerals. This can lead to the formation of organic-mineral complexes that eventually become part of the soil's organic carbon. When microalgae are added to the soil, they become a source of organic carbon. They can also prevent the decrease in soil organic carbon, which leads to the decrease in soil quality and fertility in agricultural lands (De Silva et al., 2024). Microalgae have been reported to incorporate organic carbon into their biomass through photosynthesis and affect the distribution of soil microorganisms (Osorio-Reyes et al., 2023). Since the soil was sterilized, the algal contents applied to the soil showed differences in the chlorophyll content of the soil. These differences were statistically significant compared with the control. Algal treatments also increased soil chlorophyll content compared to the control ($p<0.001$; Figure 4). Soil chlorophyll has been described as an indicator of microalgal productivity and soil health (Bharti et al., 2021). Increasing doses of *C. glomerata* and *L. platensis* increased chlorophyll content. Similar results were also reported by Bharti et al. (2020). The researchers found that *A. laxa* applications caused a significant increase in soil chlorophyll and polysaccharides. Chemical fertilizers added to soils to obtain more plant products also cause the number of beneficial microorganisms in the soil to decrease, thus posing a risk to soil health. In this context, algae used as biostimulants do not cause pollution in the soil, and they increase soil fertility by contributing to both plant health and the increase in the population of beneficial microorganisms in the soil. Considering the economic importance of the mint plant, the findings of this study may be an effective step in increasing the economic value of these valuable plants. In addition, the effectiveness of algae as a biofertilizer in sustainable agriculture can be evaluated for specific plant species. Using algae instead of chemical fertilizers can both reduce fertilizer costs and increase productivity by improving the quality of soils in agricultural areas.

This study showed that *C. glomerata* and *L. platensis* significantly influenced the growth of mint plants, with notable differences observed between application doses. These effects are likely due to the metabolites present in *C. glomerata* and *L. platensis*, which play a role in promoting plant growth. Algal treatments demonstrate potential as effective natural agents for enhancing both the quality and quantity of biomass and valuable metabolites in medicinal plants. Due to their distinct hormone secretion and ion release properties, *C. glomerata* and *L. platensis* have different impacts on key developmental traits of mint, a plant of considerable medicinal and industrial value. Algae have attracted great attention as biofertilizers in environmentally friendly plant production in recent years. However, using algae in combinations may be more effective in increasing soil biological activity, plant disease resistance and productivity of plant products. Therefore, further research to reveal the molecular mechanisms underlying the effect of coculture combination on plant production or plant disease suppression will be necessary for the expansion of sustainable agriculture.

Ethical Statement

The authors declare that ethical approval is not required for this research.

Funding

This study was supported by Harran University Scientific Research Projects (HÜBAP) with Project No: 21254.

Declaration of Author Contributions

Conceptualization, Material, Methodology, Investigation, Data Curation, Formal Analysis, Visualization, Supervision, Funding Acquisition, Writing-Original Draft Preparation, Writing-Review & Editing, *G. SEZEN*; Conceptualization, Material, Methodology, Investigation, Formal Analysis, Writing-Original Draft Preparation, Writing-Review & Editing, *Ç. KÜÇÜK*; Material, Methodology, Formal Analysis, Visualization, Writing-Original Draft Preparation, Writing-Review & Editing, *R. OKUTAN*. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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CITATION: Sezen, G., Küçük, Ç., Okutan, R., 2026. Biostimulant Effects of *Cladophora glomerata* and *Limnospira platensis* on Growth and Rhizosphere Activity of *Mentha spicata*. *Turkish Journal of Agricultural Research*, 13(1): 1-11.