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Research Article

The effects of blue-green algae *Nostoc commune* aqueous extract on the growth parameters, metabolite contents, and antioxidant capacity in seedlings of a local Iraqi rice (Amber) variety

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ABSTRACT- Blue-green algae have significant potential for enhancing the productivity of various agricultural crops. In this study, the effects of *Nostoc commune* extract on the physiological parameters of a local rice variety were evaluated. The extract was prepared at a concentration of 0.5 g per 100 mL of distilled water, and rice seeds were treated with extract dilutions of 0%, 20%, 40%, 60%, 80%, and 100%. Different concentrations of *N. commune* extract produced varied physiological responses in rice. All extract concentrations significantly increased radicle length and seedling weight. Germination percentage was significantly improved by 20% and 60% treatments, while the 40% concentration notably enhanced hypocotyl length. In terms of biochemical parameters, all concentrations significantly increased metabolite content. Most concentrations also led to a significant rise in chlorophyll levels, and carotenoid content was the highest in 100% extract treatment. The 60% concentration resulted in the highest carbohydrate and protein levels. The greatest accumulation of phenols and flavonoids was observed with the 20% extract. Additionally, antioxidant capacity was significantly enhanced by the 20% and 40% concentrations. Overall, based on improvements observed in both growth and biochemical parameters, aqueous extract of *N. commune* at concentrations below 60% can be recommended as an effective biostimulant and biofertilizer for enhancing rice yield.

INTRODUCTION

Fertilizers are essential tools in agricultural production. With the growing global population and increasing demand for food, the use of chemical fertilizers has expanded significantly to boost crop yields. However, the excessive application of chemical fertilizers has resulted in numerous environmental issues, including soil acidification and degradation, water pollution, elevated greenhouse gas emissions, and ozone layer depletion (Bhandari, 2014). In response, green agricultural technologies have emerged as sustainable alternatives that mitigate these environmental risks. Today, biofertilizers and biostimulants play a crucial role in modern green agriculture (Mishra et al., 2013). One of the primary objectives of using these biological inputs is to reduce reliance on chemical fertilizers and provide environmentally friendly alternatives (Kalaivanan et al., 2012). Studies have demonstrated the potential of blue-green algae (BGA) in promoting sustainable agriculture while safeguarding environmental and human health (Chittora et al., 2020). Also known as cyanobacteria, BGAs are photosynthetic microorganisms that thrive in diverse aquatic and terrestrial habitats, including freshwater bodies, oceans, soil, and rocks (Whitton and Potts, 2012). These organisms are valuable sources of natural products, such as secondary metabolites, vitamins, pigments, proteins, and enzymes (Zahra et al., 2020). Their nitrogen-fixing ability makes them particularly beneficial as natural fertilizers for enhancing agricultural productivity (Mishra and Pabbi, 2004). For example, the application of two BGA species, i.e., *Nostoc entophytum* and *Oscillatoria angustissima*, in combination with half the recommended dose of chemical fertilizer in chickpea cultivation led to increase in germination percentage, seed carbohydrate and protein content, and photosynthetic pigments. *N. entophytum* was found to be rich in exopolysaccharides, auxins, and cytokinins, while *O. angustissima* had high gibberellin content (Osman et al., 2010). In addition, several studies have confirmed the effectiveness of BGA extracts in enhancing plant growth and performance. Treatment with *Spirulina platensis* extract improved both vegetative and reproductive growth parameters in chickpea, along with increase in nitrogen, phosphorus, seed protein content, and leaf photosynthetic pigments (Nawar and Ibraheim, 2014). Similarly, *S. platensis* extract stimulated germination, lateral root formation, stem

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elongation, and dry biomass in wheat and barley (Akgül, 2019).

BGA contribute to soil health and plant growth through a variety of mechanisms. By secreting extracellular polysaccharides (Hill et al., 1994), they enhance soil adhesion and promote water retention (Mazor et al., 1996), while increasing soil porosity and improving soil structure (Malam Issa et al., 2007). BGAs also play a role in mitigating soil salinity (Rodríguez et al., 2006) and in suppressing the activity of harmful plant pathogens (Abo-Shady et al., 2007; Kim, 2006). Additionally, their allelopathic properties enable them to inhibit weed growth. Due to their ability to perform photosynthesis and fix atmospheric nitrogen, BGAs can enhance soil carbon and nitrogen levels. They also release organic acids that increase the availability of soluble phosphate in the soil (Saadatnia and Riahi, 2009; Wilson, 2006). Furthermore, by secreting growth-promoting compounds such as plant hormones (including auxins and gibberellins), vitamins, and amino acids, BGAs can significantly stimulate plant development (Vikram and Sikarwar, 2024). Numerous studies have demonstrated the beneficial effects of BGAs on the growth of various crops. These include wheat (Mazhar et al., 2013), corn (Maqubela et al., 2008), tomato, cucumber, squash (Shariatmadari et al., 2013), peppermint (Shariatmadari et al., 2015), lettuce (Puglisi et al., 2020b; Faheed et al., 2008), beet (Puglisi et al., 2020a), and willow (Grzesik et al., 2017). The observed improvements are attributed to the BGAs' roles in enhancing soil structure, enriching soil fertility, and secreting growth-stimulating compounds (Chabili et al., 2024; Obana et al., 2007).

Nostoc is one of the most widespread genera of filamentous, nitrogen-fixing BGA that form microscopic and macroscopic colonies on the Earth's surface (Dodds et al., 1995). The genus *Nostoc* contains high amounts of fiber, amino acids, vitamins, and carbohydrates and has high nutritional value (Gao, 1998; Johnson et al., 2008). The effect of two BGA species *Nostoc carneum* and *Nostoc commune* in combination with chemical fertilizers on rice showed that the combination of BGA with half of the required chemical fertilizers led to an increase in root length, number and weight of seeds in the spike (Chittapun et al., 2018). Treatment of *Gentiana dahurica* with *N. commune* extract also showed that germination and plant growth parameters were improved (Liu et al., 2011).

Rice (*Oryza sativa* L.) is one of the most important agricultural crops and serves as the primary food source for over half of the global population. It is cultivated in more than 100 countries and contributes approximately 21% of the world's energy intake and 15% of the required protein (Delcour and Hoseney, 2010). With global population projections estimated to reach 9–11 billion by 2050 (Pison, 2022), ensuring sufficient food production has become a pressing concern. This anticipated surge in demand presents a significant challenge to the production of high-quality food (Fróna et al., 2019). The germination stage is one of the most critical and vulnerable phases in a plant's life cycle, during which seeds are highly susceptible to environmental stress. Successful germination greatly influences the yield and quality of the final crop (Ling et al., 2014). Based on the prior studies, it was hypothesized that essential mineral elements such as nitrogen, along with bioactive compounds including hormones, polysaccharides, and various metabolites present in the extract of the nitrogen-fixing blue-green alga *Nostoc commune*, could positively influence seed germination and early seedling growth in rice. Accordingly, this study investigated the effects of different concentrations of *N. commune* extract on rice seeds. The findings aim to identify optimal extract levels for use as a biostimulant and biofertilizer in rice cultivation.

MATERIALS AND METHODS

Chemicals and reagents

Methanol, Acetone, and sulfuric acid were purchased from Dr. Mojallali company (Iran). Di-potassium hydrogen phosphate and potassium dihydrogen phosphate (to prepare potassium phosphate buffer), aluminum chloride, sodium carbonate, potassium acetate, phenol, DPPH (2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazin-1-yl), Folin reagent, glucose, Bradford's solution, bovine serum albumin (BSA), gallic acid, routine, and ascorbic acid were obtained from Merck company (India).

Preparation of BGA extract

Colonies of *N. commune* were collected from their natural habitats in the campus of University of Mazandaran (N 52° 40' 53", E 36° 42' 43"). The colonies were immersed in tap water and then washed with distilled water to remove soil. Finally, they were air-dried in the shade in the laboratory at 25 ± 2 °C. The dried colonies were powdered using an electric grinder. To prepare the aqueous extract, 0.5 g of powder was soaked in 100 mL of distilled water and placed on a shaker for 24 hours at a speed of 100 rpm. Then, the extract was subjected to sonication for 30 minutes and finally centrifuged for 5 minutes at a speed of 4000 rpm. The supernatant was used in the next steps after filtering with filter paper (Whatman No. 1).

Germination conditions

Iraqi aromatic rice seeds (Amber) were obtained from Al-Mishkhab region, Iraq. To surface sterilize, seeds were immersed in 3% sodium hypochlorite for 5 minutes and washed with distilled water. Then, 15 seeds were placed on filter paper in Petri dishes. Concentrations of 0%, 20%, 40%, 60%, 80%, and 100% of the aqueous extract were added to the Petri dishes in a volume of 5 mL. Zero concentration (control) contained 5 mL of distilled water. Petri dishes were placed in a culture rack at a temperature of 25 ± 2 °C and irradiated with fluorescent lamps under conditions of 16 hours of light and 8 hours of darkness.

Growth parameters

Seedlings were harvested after 7 days, followed by measurements of growth parameters, including germination percentage, hypocotyl length, radicle length, number of radicle branches, and seedling fresh weight.

Photosynthetic pigments content

First, fresh aerial parts of seedlings (0.05 g) were accurately weighed using a digital balance (DLS100-5, Nano Pajouhan Raga Co., Iran). Then, they were thoroughly homogenized in 3 mL of 80% acetone and placed in an ultrasonic bath (GT Sonic-D3, China) for 10 minutes. After centrifuge at 4000 rpm for 5 minutes, the absorbance of the resulting supernatant was recorded using a spectrophotometer (SU 6100, Philler Scintific, USA) at three wavelengths of 663, 645, and 470 nm. The concentrations of chlorophyll^a, chlorophyll^b and carotenoids were calculated using the following equations (Arnon, 1949):

$$Chlorophyll^a = (12{,}7 \times A663) - (2{,}69 \times A645) \quad \text{Eq. (1)}$$

$$Chlorophyll^b = (22.9 \times A645) - (4.68 \times A663) \quad \text{Eq. (2)}$$

$$Carotenoids = ((1000 \times \mathrm{A470}) - (1.82 \times Chl\ a) - (85.02 \times Chl\ b))/198 \quad \text{Eq. (3)}$$

Soluble carbohydrates content

First, fresh aerial parts of seedlings (0.05 g) were precisely weighed using a digital balance. Then, they were thoroughly homogenized in 3 mL of 100 mM potassium phosphate buffer (pH 7) and placed in an ultrasonic bath for 10 minutes. After centrifugation the mixture at 4000 rpm for 5 minutes, the supernatant was separated and used to measure the soluble carbohydrates content by the phenol-sulfuric acid method. For this purpose, 200 microliters of the supernatant were added to 200 microliters of 5% phenol and vortexed. Then, 1000 microliters of concentrated sulfuric acid were added to the mixture and vortexed again. The mixture was placed in an incubator at 38 °C for 30 minutes. After cooling, the absorbance of the mixture was recorded at a wavelength of 485 nm using a spectrophotometer (DuBois et al., 1956). Finally, the soluble carbohydrates content was reported using the equation obtained from the calibration curve of glucose ($y = 0.0058x + 0.0185; R^2 = 0.9968$).

Proteins content

First, fresh aerial parts of seedlings (0.05 g) were precisely weighed using a digital balance. Then, they were immediately powdered with liquid nitrogen and completely homogenized in 3 mL of 50 mM potassium phosphate buffer (pH 7). After centrifuging the mixture at 12,000 rpm for 10 minutes at 4 °C, the supernatant was separated and used to measure the protein content by the Bradford method. The reaction mixture was prepared by adding 100 microliters of the supernatant to 1000 microliters of Bradford's solution. After 10 minutes, the absorbance of the mixture was recorded at a wavelength of 595 nm using a spectrophotometer (Bradford, 1976). Finally, the total protein content was reported using the equation obtained from the calibration curve of bovine serum albumin (BSA) ($y = 0.004x + 0.0205; R^2 = 0.9968$).

Total phenolic content, total flavonoid content, and total antioxidant capacity

For extraction, fresh aerial parts of seedlings (0.05 g) were precisely weighed using a digital balance. Then, they were completely homogenized in 3 mL of 80% methanol and placed in an ultrasonic bath for 10 minutes. After centrifugation the mixture at 4000 rpm for 5 minutes, the supernatant was separated and used to measure the total phenolic and flavonoid contents, and total antioxidant capacity. Total phenolic content was measured according to the Folin-Ciocalteu method. The volume of 200 microliters of the supernatant was added to 1000 microliters of 10% Folin reagent and vortexed. After 5 minutes, 800 microliters of 7.5% sodium carbonate were added to the mixture and vortexed well. The mixture was placed in the dark for one hour, and then its absorbance was recorded at a wavelength of 765 nm using a spectrophotometer (Ainsworth and Gillespie, 2007). Finally, the total phenolic content was reported using the equation obtained from the calibration curve of gallic acid ($y = 0.0078x + 0.0135; R^2 = 0.9979$).

Total flavonoid content was measured with a colorimeter using aluminum chloride. The volume of 200 microliters of the supernatant was mixed with 40 microliters of 10% aluminum chloride, 40 microliters of 1 M potassium acetate, 600 microliters of 80% methanol, and 1120 microliters of distilled water and vortexed well. The mixture was placed in the dark for 30 minutes, and then its absorbance was recorded at a wavelength of 415 nm using a spectrophotometer (Akkol et al., 2008). Finally, the flavonoid content was reported using the equation obtained from the calibration curve of routine ($y = 0.0059x - 0.0108; R^2 = 0.9991$).

To measure the total antioxidant capacity, one milliliter of the supernatant was mixed with one milliliter of 0.2 mM DPPH (2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazin-1-yl) solution, and then placed in the dark for 30 minutes. The absorbance of the mixture was recorded using a spectrophotometer at a wavelength of 517 nm, and then its inhibition percentage was calculated using the following equation (I: inhibition percentage, Ac: absorbance of control, and Am: absorbance of mixture) (Blois, 1958):

$$I = ((Ac - Am)/Ac) * 100 \quad \text{Eq. (4)}$$

Finally, the total antioxidant capacity was reported using the equation obtained from the calibration curve of ascorbic acid ($y = 2.3253x - 1.9073; R^2 = 0.997$).

Statistical analysis

The experiment was performed in a completely randomized design and each treatment had five replications. The graphs were drawn by Microsoft Office Excel 2013. One-way analysis of variance (ANOVA) was performed on all data using SPSS 20.0, followed by the least significant difference (LSD) method to compare the significance of differences among the treatments ($P < 0.05$).

RESULTS AND DISCUSSION

In this study, rice seeds were treated with different concentrations of aqueous extract of *N. commune* as a biological stimulus and biofertilizer. The results showed improvements in the growth and biochemical parameters of the seedlings.

Growth parameters

Analysis of variance revealed significant differences in the growth parameters of rice seedlings treated with different concentrations of *N. commune* extract ($P < 0.05$) (Table 1). The impact of extract concentration on growth parameters varied among treatments. Specifically, treatments with 20% and 60% extract significantly increased seed germination percentage compared to the control ($P < 0.05$), while other concentrations did not show significant differences ($P \geq 0.05$) (Table 2). Germination percentage increased by 15% and 19% in the 20% and 60% treatments, respectively (Table 3). Hypocotyl length increased significantly in seedlings treated with 40% extract, showing an 8% improvement over the control ($P < 0.05$), while other concentrations had no significant effect on this parameter (Table 2 and Table 3). Radicle length was significantly greater in all treatments compared to the control ($P < 0.05$), with the highest increase (38%) observed in seedlings treated with 40% extract (Table 3). The number of radicle branches did not differ significantly from the control in the 20% and 40% treatments ($P \geq 0.05$), but higher extract concentrations led to a reduction in this parameter (Table 2). While the fresh weight of seedlings across all extract concentrations ranged from 9% to 13% higher than the control, the differences between extract concentrations themselves were not statistically significant. However, all treatments showed a significant increase in fresh weight compared to the control ($P < 0.05$) (Table 2 and Table 3). Overall, the results demonstrated that *N. commune* extract significantly influenced rice seedling growth, particularly in enhancing radicle length, hypocotyl length (at specific concentrations), and seedling fresh weight.

In a study, it was shown that *Ulva lactuca* and *Padina gymnospora* algae extracts can increase the length of shoot and root of tomato (*Solanum lycopersicum*) and improve its growth (Hernández-Herrera et al., 2014). In other studies, the effect of *Scenedesmus* sp. algae extract led to the improvement of growth and increase in root and shoot weight of *Petuniax hybrida* (Plaza et al., 2018) and the effect of *Hypnea musciformis* algae extract increased the shoot length of peanut (*Arachis hypogea*) (Al-Sherif et al., 2015). In this study, the aqueous extract of *N. commune* led to an increase in the length of the hypocotyl and radicle, and the weight of rice seedlings (Table 2). The positive effects of biofertilizers and biostimulants on plant growth indices were primarily attributed to their high content of trace elements and growth regulators (Blunden and Wildgoose, 1977). Plant biomass is closely influenced by nutrient availability, and it has been well established that the presence of growth hormones in algae and BGA extracts enhances nutrient uptake and translocation within the plant, thereby increasing plant weight. Algal extracts contain organic compounds with activity similar to plant hormones such as cytokinins, auxins, and gibberellins, which stimulate cell division and elongation, ultimately promoting overall plant growth (Sunarpi et al., 2011; Crouch and Van Staden, 1993). In addition to the growth regulators and mineral salts, BGA and algal extracts also contain a variety of other beneficial compounds, including vitamins, amino acids, phenylacetic acid, and antioxidants. These substances contribute to improved seed germination and enhanced growth parameters (Thirumaran et al., 2009; Erulan et al., 2009; Sivasankari et al., 2006; Sridhar and Rengasamy, 2011; Zhang and Ervin, 2008). The observed improvements in the growth indices of rice seedlings treated with *N. commune* extract suggest a potentially significant role for this extract in supporting subsequent plant development and increasing crop productivity. Based on these findings, further research on other plant species could support the broader application of *N. commune* extract as an effective biofertilizer and biostimulant in sustainable agriculture.

Table 1. Analysis of variance (ANOVA) for rice seedling growth parameters treated with different concentrations of *Nostoc commune* extract

		Sum of squares	df	Mean square	F	Sig.*
Germination percentage	Extract treatment	611.852	5	122.370	2.581	0.053
	Error	1137.778	24	47.407		
	Total	1749.630	29			
Hypocotyl length	Extract treatment	2.825	5	0.565	2.314	0.053
	Error	16.114	66	0.244		
	Total	18.939	71			
Radicle length	Extract treatment	9.148	5	1.830	23.561	0.000
	Error	5.125	66	0.078		
	Total	14.273	71			
Number of radicle branches	Extract treatment	16.125	5	3.225	13.514	0.000
	Error	15.750	66	.239		
	Total	31.875	71			
Seedling weight	Extract treatment	0.001	5	0.000	3.832	0.004
	Error	0.004	66	0.000		
	Total	0.005	71			

* At a significance level of 0.05

Table 2. Effect of different concentrations of *Nostoc commune* aqueous extract on rice seedling growth parameters

Parameter	Extract concentrations (%)					
	0	20	40	60	80	100
Germination percentage (%)	$70.67^{c} \pm 7.60$	$81.33^{ab} \pm 5.58$	$76.00^{abc} \pm 3.65$	$84.00^{a} \pm 3.65$	$77.33^{abc} \pm 8.94$	$77.33^{abc} \pm 9.43$
Hypocotyl length (mm)	$6.53^{b} \pm 0.42$	$6.35^{b} \pm 0.27$	$6.98^{a} \pm 0.77$	$6.52^{b} \pm 0.51$	$6.51^{b} \pm 0.51$	$6.44^{b} \pm 0.32$
Radicle length (mm)	$2.8^{d} \pm 0.27$	$3.25^{c} \pm 0.26$	$3.86^{a} \pm 0.30$	$3.63^{b} \pm 0.25$	$3.62^{b} \pm 0.27$	$3.73^{ab} \pm 0.29$
Number of radicle branches	$8.42^{a} \pm 0.51$	$8.17^{a} \pm 0.39$	$8.42^{a} \pm 0.51$	$7.50^{b} \pm 0.52$	$7.50^{b} \pm 0.52$	$7.25^{b} \pm 0.45$
Seedling weight (mg)	$0.090^{b} \pm 0.003$	$0.102^{a} \pm 0.009$	$0.101^{a} \pm 0.007$	$0.098^{a} \pm 0.007$	$0.101^{a} \pm 0.011$	$0.101^{a} \pm 0.008$

Values are presented as mean ± standard deviation. Values with different letters in the same row are statistically significant at the 5% probability level in LSD test.

Table 3. Percentage changes in growth parameters of rice seedlings treated with different concentrations of *Nostoc commune* extract compared to the control

Extract concentrations	Germination percentage	Hypocotyl length	Radicle length	Number of radicle branches	Seedling weight
0	0^{c}	0^{b}	0^{d}	0^{a}	0^{b}
20	15^{ab}	-3^{b}	16^{c}	-3^{a}	13^{a}
40	8^{abc}	8^{a}	38^{a}	0^{a}	12^{a}
60	19^{a}	0^{b}	30^{b}	-11^{b}	9^{a}
80	9^{abc}	0^{b}	29^{b}	-11^{b}	12^{a}
100	9^{abc}	-1^{b}	33^{ab}	-10^{b}	12^{a}

Values are in percentage (%) units. Values with different letters in the same column are statistically significant at the 5% probability level in LSD test.

Biochemical parameters

Analysis of variance for biochemical parameters of treated rice seedlings, including metabolite content and total antioxidant capacity, displayed significant differences between different concentrations of *N. commune* extract ($P < 0.05$) (Table 4).

Photosynthetic pigments

The amount of chlorophyll^{a} in the treatment with all concentrations of BGA extract showed a significant increase *vs.* the control ($P < 0.05$) (Fig. 1). Seed treatment with 100% extract concentration resulted in the highest amount of chlorophyll^{a}, which was 67% more than the control (Table 5). The amount of chlorophyll^{b} also showed a significant increase in the treatment with most of the extract concentrations *vs.* the control (Fig. 1). The maximum amount of chlorophyll^{b} was observed in the treatment with 20% extract concentration, which was 30% more than the control (Table 5). The amount of carotenoids increased significantly only in treatment with 100% extract concentration, which was 15% more than the control (Fig. 1 and Table 5).

Chlorophylls play an important role in plant metabolism and their amount affects plant growth. Carotenoids are less efficient in light absorption but play an important role in stress conditions (Alscher and Hess, 1993; Eckhardt et al., 2004; Hou et al., 2007; Sengar et al., 2008). Application of *Ulva polysiphonia* and *Cladophora* sp. algae extracts increased the chlorophyll and carotenoid contents of *Lepidium sativum* and *Triticum aestivum* (Michalak et al., 2016). In addition, extracts of *Anabaena* sp., *Nostoc* sp., and three nitrogen-fixing bacteria (*Azotobacter chroococcum*, *Azospirillum brasilense*, and *Rhizobium* sp.) increased total chlorophyll and carotenoids in *Matthiola Incana* (Shanan and Higazy, 2009). The present findings also revealed that different concentrations of *N. commune* extract had a significant effect on the levels of photosynthetic pigments, including chlorophylls and carotenoids (Fig. 1). Algal extracts are rich in growth regulators such as cytokinins, auxins, and betaines, which are known to enhance chlorophyll content in plants. Cytokinins, in particular, have been reported to increase both the number and size of chloroplasts while also delaying chlorophyll degradation (Thirumaran et al., 2009; Erulan et al., 2009; Sridhar and Rengasamy, 2011). Additionally, betaines contribute to the stabilization and protection of photosynthetic pigments from degradation (Blunden et al., 1996). The presence of essential mineral elements such as magnesium and iron in algal extracts likely supports chlorophyll biosynthesis and enhances photosynthetic activity. Moreover, given nitrogen's central role in the structure of photosynthetic pigments, the presence of nitrogen and amino acids in the nitrogen-fixing *N. commune* extract may further stimulate pigment synthesis (Mutale-Joan et al., 2020; Górka et al., 2018; Pise and Sabale, 2010). The observed increase in photosynthetic pigments in rice seedlings treated with *N. commune* extract likely contributes to the greater photosynthetic output, resulting in elevated levels of carbohydrates and other organic compounds such as proteins. This enhancement in photosynthetic efficiency may ultimately improve plant growth as well as the yield and quality of the rice crop.

Total soluble carbohydrates and proteins

The content of soluble carbohydrates in rice seedlings significantly increased following treatment with 20%, 40%, and 60% concentrations of *N. commune* extract compared to the control ($P < 0.05$) (Fig. 1). Specifically, treatments with 40% and 60% extract concentrations

resulted in 11% and 12% increases in soluble carbohydrates, respectively (Table 5). Similarly, total protein content in seedlings was significantly enhanced by all extract concentrations ($P < 0.05$), with the highest protein level observed in the 60% treatment, 65% higher than the control (Table 5 and Fig. 1). These changes are particularly important given the critical roles that carbohydrates and proteins play in plant physiological processes and growth (Behboudi et al., 2013; Pessarakli, 1999). Supporting evidence from previous studies confirms these findings. For example, *U. lactuca* extract increased carbohydrate content in mung bean (*Vigna radiata*) (Castellanos-Barriga et al., 2017), and *Spirulina platensis* extract improved vegetative and reproductive traits in chickpea (*Cicer arietinum*), including increased nitrogen, phosphorus, and seed protein content (Nawar and Ibraheim, 2014). Similarly, application of *Kappaphycus alvarezii* and *Gracilaria edulis* extracts, in combination with chemical fertilizers, enhanced micronutrient (iron, zinc, copper, and manganese) and protein levels in rice seeds (*O. sativa*) (Layek et al., 2018). In the present study, aqueous extract of *N. commune* significantly increased both soluble carbohydrates and total protein content in the aerial parts of rice seedlings, with the 60% concentration being the most effective (Fig. 1). These improvements may be attributed to the extract's ability to enhance nitrogen availability and nutrient uptake, boost chlorophyll content, and stimulate photosynthesis through the regulation of genes involved in carbon fixation, such as those encoding Rubisco and carbonic anhydrase (Mutale-Joan et al., 2020). Moreover, algal extracts contain a variety of bioactive compounds, including auxins, cytokinins, vitamins, and other organic and inorganic molecules, that contribute to the increased biosynthesis of polysaccharides, pigments, polyphenols, and proteins (Chojnacka et al., 2012; Michalak et al., 2016). Specific components such as aminobutyrate, glycine betaine, and betaine have also been shown to stimulate photosynthesis and carbohydrate accumulation (Sunarpi et al., 2011). The observed increase in protein content may be linked to the enhanced gene expression triggered by hormones present in the extract. Furthermore, nitrogen-fixing BGA such as *N. commune* supply organic nitrogen, further promoting protein synthesis (Osman et al., 2010). Since the photosynthetic apparatus is directly involved in the biosynthesis of plant metabolites, any enhancement of photosynthetic activity can lead to increased carbohydrate and protein levels (Kriedemann, 1986; Khalil et al., 2007; Dewick, 2002). Therefore, the observed elevation in photosynthetic pigment levels (Fig. 1) likely contributed to increased photosynthetic activity, and serves as a plausible mechanism underlying the higher soluble carbohydrate and protein contents in rice seedlings treated with *N. commune* extract.

Total phenolic content, total flavonoid content, and total antioxidant capacity

The total phenolic and flavonoid contents of rice seedlings treated with *N. commune* extract showed significant differences compared to the control ($P < 0.05$) (Fig. 1). Specifically, the total phenolic content increased by 15% in seedlings treated with the 20% extract concentration, while treatments with higher extract concentrations resulted in reduced total phenolic contents relative to the control (Table 5). In contrast, the total flavonoid content significantly increased at all concentrations of the extract, with the 20% concentration exhibiting the highest increase, 39% greater than the control (Table 5 and Fig. 1). Similarly, total antioxidant capacity increased significantly in seedlings treated with 20% and 40% concentrations of *N. commune* extract ($P < 0.05$) (Fig. 1). These treatments enhanced antioxidant capacity by 9% and 11%, respectively, compared to the control (Table 5). Phenolic compounds are key contributors to plant resistance, primarily by improving antioxidant capacity and facilitating the detoxification of reactive oxygen species (ROS) (Allen and Ort, 2001). These results are consistent with previous studies. For instance, red seaweed extract used as a biofertilizer increased total phenolic content in cucumber leaves and fruits (Saedi et al., 2022), while foliar application of *Ascophyllum nodosum* extract significantly improved total phenolic content and antioxidant capacity in *Momordica charantia* L. (Aminifard and Khandan, 2019). In the present study, treatment with 20% aqueous extract of *N. commune* significantly enhanced total phenolic and flavonoid contents, and the antioxidant capacity of the aerial parts of rice seedlings, particularly at 20% and 40% concentrations. The increase in phenolic compounds may be attributed to the presence of bioactive compounds in algal extracts that serve as intermediates or regulators in phenolic biosynthesis. These compounds can stimulate key enzymes such as phenylalanine ammonia-lyase and chalcone synthase, thereby promoting the synthesis of phenolic compounds (Lola-Luz et al., 2014). Additionally, growth hormones and nutrients in the extract may induce carbohydrate production, which serves as a precursor for the biosynthesis of secondary metabolites like phenols and flavonoids (Khandan Deh-Arbab et al., 2020). Algae are well-documented sources of natural antioxidants, including polyphenols, carotenoids, and ascorbic acid that contribute to the enhanced antioxidant properties in treated plants (Cho et al., 2011; Zhang and Ervin, 2004). By activating phenolic biosynthetic pathways, algal extracts can lead to the accumulation of phenolic and flavonoid compounds, enhancing antioxidant capacity, and protecting plant tissues and cell membranes from oxidative damage caused by ROS (Nair et al., 2012). Therefore, the observed increase in total phenolic content in response to *N. commune* extract treatment is likely to contribute to the improved stress resistance and enhanced nutritional quality of the rice.

CONCLUSION

The findings of this study demonstrated that the aqueous extract of *N. commune* has a significant impact on the growth parameters and metabolic profiles of rice (*O. sativa*) seedlings. Application of the extract led to the marked increase in hypocotyl length, radicle length, and seedling fresh weight. However, a progressive decrease in the number of radicle branches was observed with increasing extract concentration. Regarding primary and

secondary metabolites, chlorophyll content increased at most extract concentrations, while flavonoid and protein levels were enhanced across all treatments. Soluble carbohydrate content also rose significantly in seedlings treated with extract concentrations ranging from 20% to 60%, and total phenolic content peaked at the 20% concentration. Moreover, total antioxidant capacity increased significantly in treatments with 20% and 40% extract concentrations. These results suggest that *N. commune* extract functions effectively as a biostimulant and biofertilizer, contributing to both the quantitative and qualitative improvement of this locally cultivated Iraqi rice variety. The observed enhancement in antioxidant capacity may also strengthen plant resilience against biotic and abiotic stress factors, including pests, pathogens, and environmental fluctuations. Given these promising outcomes, further research is recommended to explore the broader applicability of *N. commune* extract in other crops and under diverse field conditions.

Table 4. Analysis of variance (ANOVA) for rice seedling biochemical parameters treated with different concentrations of *Nostoc commune* extract

		Sum of squares	df	Mean square	F	Sig.*
Chlorophyll[a]	Extract Treatment	0.144	5	0.029	126.021	0.000
	Error	0.003	12	0.000		
	Total	0.147	17			
Chlorophyll[b]	Extract Treatment	0.017	5	0.003	232.507	0.000
	Error	0.000	12	0.000		
	Total	0.017	17			
Carotenoid	Extract Treatment	0.002	5	0.000	47.168	0.000
	Error	0.000	12	0.000		
	Total	0.002	17			
Antioxidant capacity	Extract Treatment	0.323	5	0.065	40.950	0.000
	Error	0.019	12	0.002		
	Total	0.342	17			
Flavonoid	Extract Treatment	0.939	5	0.188	50.929	0.000
	Error	0.044	12	0.004		
	Total	0.983	17			
Phenol	Extract Treatment	0.399	5	0.080	29.368	0.000
	Error	0.033	12	0.003		
	Total	0.432	17			
Protein	Extract Treatment	52.692	5	10.538	59.227	0.000
	Error	2.135	12	0.178		
	Total	54.827	17			
Soluble carbohydrate	Extract Treatment	4.273	5	0.855	5.599	0.007
	Error	1.832	12	0.153		
	Total	6.105	17			

* At a significance level of 0.05

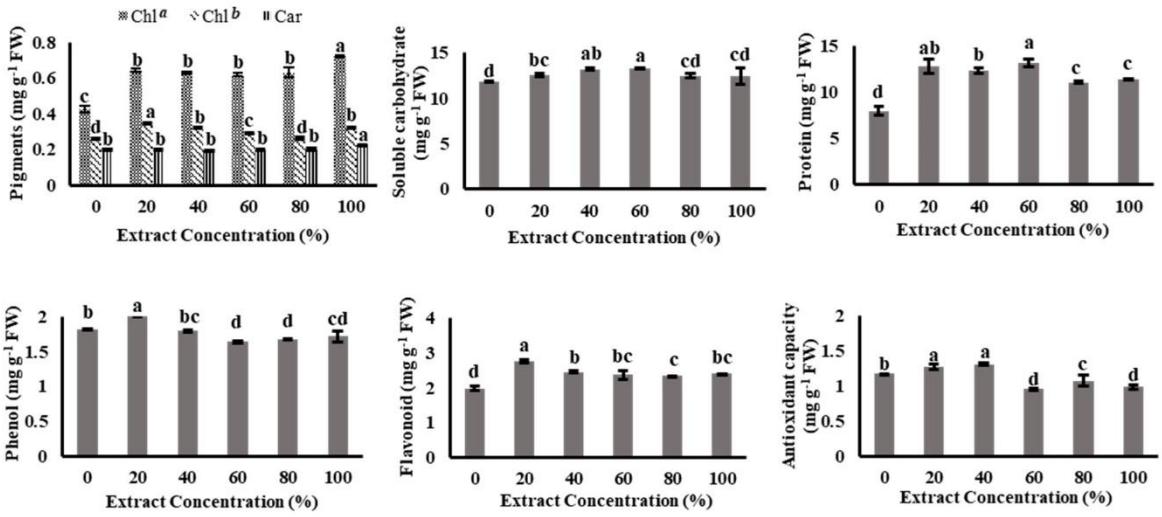

Fig. 1. Amount of plant metabolites and antioxidant capacity of arial parts of rice seedlings treated with *Nostoc commune* extract. The bars and error bars in the charts represent the mean and standard deviation, respectively. Different letters in each graph indicate significant differences among the treatments at the 5% significance level with LSD test. Treatments with at least one same letter are not significantly different.

Table 5. Percentage changes in biochemical parameters of rice seedlings treated with different concentrations of *Nostoc commune* extract compared to the control

Extract concentrations	Chlorophyll^a	Chlorophyll^b	Carotenoid	Soluble carbohydrate	Protein	Phenol	Flavonoid	Antioxidant capacity
0	0^{c}	0^{d}	0^{b}	0^{d}	0^{d}	0^{b}	0^{d}	0^{b}
20	49^{b}	30^{a}	-1^{b}	6^{bc}	61^{ab}	15^{a}	39^{a}	9^{a}
40	47^{b}	19^{b}	-2^{b}	11^{ab}	54^{b}	-1^{bc}	24^{b}	11^{a}
60	44^{b}	7^{c}	-1^{b}	12^{a}	65^{a}	-10^{d}	19^{bc}	-19^{d}
80	47^{b}	0^{d}	0^{b}	5^{cd}	39^{c}	-8^{d}	18^{c}	-9^{c}
100	67^{a}	19^{b}	15^{a}	5^{cd}	43^{c}	-5^{cd}	21^{bc}	-15^{d}

Values are in percentage (%) units. Values with different letters in the same column are statistically significant at the 5% probability level in LSD test.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY

All data analyzed and generated during this study are included in this published article.

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