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# The effect of aqueous extracts of *Nostoc commune* and *Ulva lactuca* algae on quinoa seedlings

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**Abstract**

**Objective**: Biofertilizers and biostimulants improve germination, absorption of nutrients, resistance to stress, growth, performance, and health of plants. Considering the importance of the germination stage, the effect of *Nostoc commune* and *Ulva lactuca* extracts on quinoa was investigated.

**Methods**: In this research, quinoa seeds were treated with different concentrations of green algae *Ulva lactuca* (U) and cyanobacterium *Nostoc commune* (N) extracts, separately and in combination. Then, the growth traits, photosynthetic pigments, and anthocyanins of seedlings were evaluated.

**Results**: The results showed that the length of the hypocotyl and the contents of chlorophyll *a*, chlorophyll *b*, carotenoids, and anthocyanins in the treatments containing *U. lactuca* extract, *N. commune* extract, and combined extract increased significantly, compared to the control. The weight of seedlings increased significantly in the treatments with *U. lactuca* extract and the combined extract, while the length of the radicle increased only in the treatment with the combined extract, compared to the control. The highest hypocotyl length was observed in the treatment with 40% concentration of *U. lactuca* extract, which was 46.70% higher than the control. The highest levels of chlorophyll *a* and chlorophyll *b* were in the treatment with 80% concentration of *U. lactuca* extract, so that the levels of chlorophyll *a* and chlorophyll *b* increased by 87.22% and 52.43%, respectively, compared to the control. The highest radicle length was obtained in the treatment with 50% concentration of the combined extract (4U:1N), with a 20.43% increase over the control. The highest seedling weight was observed in the treatment with 100% concentration of the combined extract (4U:1N), with a 20.90% increase compared to the control. The highest amount of carotenoids occurred in the treatment with 50% concentration of the combined extract (3U:2N), with a 57.74% increase over the control. The highest level of anthocyanins was detected in the treatment with 25% concentration of the combined extract (3U:2N), with a 32.81% increase over the control.

**Conclusion**: These findings indicated that the extracts, especially the *U. lactuca* extract and the combined extract of *U. lactuca* with *N. commune*, can act as biological stimulants for quinoa.

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## Introduction

Germination is always the most critical phase in the growth of plants, and improving germination ability results in better seedling establishment in the soil, expansion of root systems, and increased plant growth (Murungu *et al*. 2003; Gavazzi *et al*. 2008). Germination also influences plant density and performance, preparing them for subsequent growth stages (Sathe *et al*. 1983; Amiri *et al*. 2015).

Biofertilizers and biostimulants can lead to increased germination, absorption of nutrient compounds, increased resistance to biotic and abiotic stresses, and increased growth, performance, and health of plants. In addition, using these green products can increase the quality of the soil by improving its microbial performance, biodiversity, and nutrient composition, and reduce the harmful effects caused by the use of chemical fertilizers and pesticides. It has been reported that biological stimuli induce physiological responses against environmental conditions by sending messages to plants, making plants resist the adverse conditions or improve their growth. Compounds in biostimulants may be precursors of various metabolic pathways. Biostimulants can increase cation exchange capacity, stimulate plasma membrane pumps, activate phenylpropanoid metabolism, or act as sources of energy (Singh 2014; Bulgari *et al*. 2015; Du Jardin 2015; Shanmugam and Seth 2018; Ghasembaghlou *et al*. 2022; Matthews *et al*. 2022).

Many studies have shown that various organisms, including seaweed, blue-green algae (cyanobacteria), and bacteria have been used as biofertilizers and biostimulants. It has been reported that adding the extract or biomass of these organisms to the culture medium of plants promotes the growth (Win *et al*. 2018). Treatment of *Vigna radiata* seeds with aqueous extracts of *Turbinaria ornata*, *Sargassum wightii*, and *Halimeda opuntia* led to an increase in germination percentage, growth traits, photosynthetic pigments, and secondary metabolites (Punitha *et al*. 2024). Also, the priming of *Triticum aestivum* seeds with cyanobacteria led to maintaining the growth rate, relative water content, and the levels of photosynthetic pigments under drought stress conditions, thereby increasing the seedlings' resistance to stress (Sneha *et al*. 2024). Cyanobacteria and algae can be considered as emerging biomass and valuable resources of biostimulants for the development of sustainable agriculture (Di Filippo-Herrera *et al*. 2019; Santini *et al*. 2021).

The genus *Nostoc* is one of the most widespread blue-green filamentous and nitrogen-fixing algae, which has an extraordinary potential to produce a wide range of secondary metabolites (Dodds *et al*. 1995). Furthermore, the *Ulva* genus, a type of marine green algae, contains significant amounts

of Ulvan polysaccharides, which have potential applications as biological preservatives in agriculture. They enhance plant resistance and help combat pathogens, and improve agricultural productivity (Vera *et al*. 2011; Abkhoo and Sabbagh 2016; Sari-Chmayssem *et al*. 2019).

As part of the survival strategy, governments are always looking for alternatives to meet the basic needs of their societies. Therefore, they are looking for products that, in addition to easy access, contain rich and sufficient sources of vital compounds and can be cultivated in harsh environmental conditions (Repo-Carrasco *et al*. 2003). One of these important and alternative products is quinoa (*Chenopodium quinoa* Wild), which has been described as one of the grains of the 21st century (Konishi 2002; Angeli *et al*. 2020). Quinoa belongs to the Chenopodiaceae family (Jancurová *et al*. 2009; Filho *et al*. 2017). Quinoa contains nutritious and valuable compounds such as proteins, carbohydrates, lipids, minerals, phenolic compounds, saponins, and phytoecdysteroids. These components contribute to enhancing its nutritional and medicinal significance (Graf *et al*. 2015; Ng and Wang 2021).

Considering the importance of the germination stage as the basis of vegetative and reproductive growth, in the present study, the effect of *Nostoc commune* and *Ulva lactuca* extracts on quinoa was investigated.

## Materials and Methods

### *Sample preparation*

Quinoa seeds were obtained from the National Salinity Research Center, Yazd, Iran. The cyanobacterium *Nostoc commune* was collected from its natural habitat in the campus of University of Mazandaran (N 52° 40′ 53″, E 36° 42′ 43″), Iran, and the green algae *Ulva lactuca* was collected from the shores of the Persian Gulf (N 56° 13′ 45″, E 27° 09′ 08″). The cyanobacterial and algal samples were transferred to the laboratory and, to remove mud, washed several times with tap water and finally rinsed with distilled water. After drying under shade conditions at the laboratory temperature, they were ground into powder using an electric grinder and stored in a refrigerator.

### *Extraction of cyanobacterial and algal extracts*

To prepare a cyanobacterial aqueous extract, 0.5 grams of *N. commune* powder was mixed with 100 ml of distilled water and placed on a shaker at 100 rpm for 24 hours. To prepare the algal extract, 5 grams of *U. lactuca* powder was mixed with 100 ml of distilled water and placed in a water bath at a temperature of 80 °C for 1 hour. Finally, both extracts were centrifuged for 5 minutes at 4000 rpm, and their supernatant was separated for further experimental procedures (Nazifi *et al*. 2015).

***Germination conditions***

For surface sterilization, quinoa seeds were placed in a 3% sodium hypochlorite solution for 5 minutes and then washed thoroughly with distilled water. Twenty seeds were placed on filter paper in each Petri dish, and 5 ml of the extract was added to them. Treatments consisted of various concentrations of each extract and combinations thereof. The seeds were treated with 20, 40, 60, 80, and 100% dilutions of cyanobacterial and algal extracts, separately. Then, different volume ratios of cyanobacterial (N) and algal (U) extracts including 1U:4N, 2U:3N, 3U:2N, and 4U:1N were combined, and the seeds were treated with 25, 50, 75, and 100% dilutions of them. The control sample in all treatments contained 5 ml of distilled water. Petri dishes were arranged in a completely randomized design, and each treatment had four replications. The Petri dishes were placed in culture racks at 25 °C and under 16 hours of light/8 hours of darkness with fluorescent lamp. After seven days, the seedlings were harvested, and their growth traits and pigments were measured.

***Measurement of growth traits***

The fresh weight of quinoa seedlings was measured immediately after harvesting. Digimizer 5.4 software was used to measure the length of the hypocotyl and radicle.

***Measurement of photosynthetic pigments***

For extraction, 0.05 g of the aerial part of the seedlings was ground in a mortar with 3 ml of 80% acetone in a porcelain mortar. The resulting mixture was placed in an ultrasonic device for 10 minutes and then centrifuged for 5 minutes at 4000 rpm. Finally, the supernatant was separated and its absorbance was measured at wavelengths of 645, 663, and 470 nm using a spectrophotometer. The amount of pigments was calculated in milligrams per gram of fresh weight (mg $g^{-1}$ FW) using the following formulas (Arnon 1949): (Ax: absorption at wavelength x).

$$Chl\ a = (12.7 \times A663) - (2.69 \times A645)$$

$$Chl\ b = (22.9 \times A645) - (4.68 \times A663)$$

$$Carotenoid = \frac{(1000 \times \mathrm{A470}) - (1.82 \times \mathrm{Chl\ a}) - (85.02 \times \mathrm{Chl\ b})}{198}$$

***Measurement of anthocyanin content***

For extraction, 0.05 g of aerial parts of the seedling was ground with 3 ml of acidic methanol (MeOH: HCl, 99: 1, v:v) in a porcelain mortar. The resulting mixture was placed for 24 hours at 5 °C and in

dark conditions. Then, the samples were centrifuged for 5 minutes at 4000 rpm, and the absorbance of the supernatant solution was measured by a spectrophotometer at a wavelength of 550 nm. Finally, the anthocyanins content was determined in millimoles per gram of fresh weight (mmol $g^{-1}$ FW) using the following formula, considering the molar extinction coefficient (ε) of 33000 $mol^{-1}$ $cm^{-1}$ (Hara *et al*. 2003) (A: absorbance, b: cuvette width, c: solution concentration, ε: extinction coefficient).

$$A= \varepsilon bc$$

***Data analysis***

Graphs were drawn using Microsoft Excel 2013 software, and statistical data analysis was done with one-way analysis of variance and Duncan's multiple range test at a significance level of 5% using SPSS 25 software.

## Results and Discussion

***Hypocotyl length***

The treatment of quinoa seeds with different concentrations of *U. lactuca* and *N. commune* extracts showed that the length of the hypocotyl increased significantly in the treatments with all concentrations of *U. lactuca* extract and in the treatments with 80% and 100% concentrations of *N. commune* extract compared to the control ($p \leq 0.05$). In the treatment with *U. lactuca* extract, the highest hypocotyl lengths were recorded in the treatments with 20% and 40% extract concentrations, which were 46.02% and 46.70% higher than the control, respectively. In the treatment with the *N. commune* extract, the 80% and 100% extract concentrations resulted in 9.06% and 12.18% increases in hypocotyl length, respectively, compared to the control (Figure 1).

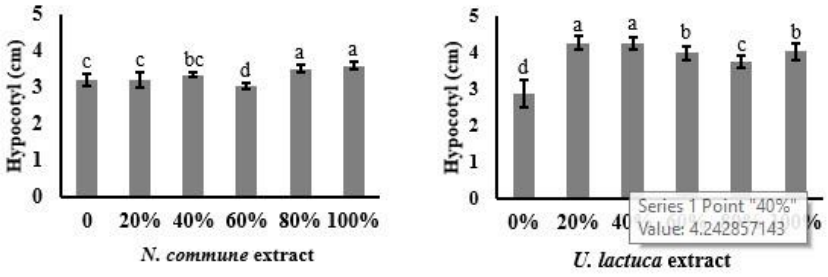


**Figure 1.** Hypocotyl length of quinoa seedlings treated with *Ulva lactuca and Nostoc commune* extracts; Different lowercase letters in the graph indicate significant differences between treatments at the 5% probability level, using Duncan's multiple range test.

The treatment of quinoa seeds with the combined extracts of *U. lactuca* and *N. commune* showed that the length of the hypocotyl increased significantly in all concentrations of the combined extracts

compared to the control ($p \leq 0.05$) (Figure 2). The highest hypocotyl length was observed in the 75% concentration of the 4U:1N extract, with a 31.13% increase compared to the control (Table 1).

**Table 1.** Growth traits of quinoa seedlings in treatment with different concentrations of the combined extracts of *Ulva lactuca* and *Nostoc commune*.

| Treatments | Percentage (%) | Hypocotyl (cm) | Radicle (cm) | Weight (g) |
|---|---|---|---|---|
| Control | 0 | $3.18\pm0.18^{i}$ | $3.72\pm0.46^{bc}$ | $0.067\pm0.006^{cdefgh}$ |
| 1U:4N | 25 | $3.62\pm0.10^{gh}$ | $2.88\pm0.28^{efg}$ | $0.062\pm0.004^{gh}$ |
| | 50 | $3.86\pm0.17^{cde}$ | $3.39\pm0.65^{cd}$ | $0.067\pm0.005^{cdefgh}$ |
| | 75 | $3.88\pm0.12^{cd}$ | $4.05\pm0.80^{b}$ | $0.071\pm0.008^{bcde}$ |
| | 100 | $3.89\pm0.08^{cd}$ | $3.10\pm0.51^{def}$ | $0.071\pm0.011^{bcdef}$ |
| 2U:3N | 25 | $3.68\pm0.11^{fgh}$ | $2.63\pm0.33^{gh}$ | $0.060\pm0.003^{h}$ |
| | 50 | $3.93\pm0.11^{bcd}$ | $3.28\pm0.50^{de}$ | $0.065\pm0.004^{defgh}$ |
| | 75 | $3.96\pm0.13^{bcd}$ | $3.91\pm0.46^{b}$ | $0.073\pm0.006^{abcd}$ |
| | 100 | $3.99\pm0.20^{bcd}$ | $2.74\pm0.17^{fgh}$ | $0.075\pm0.007^{abc}$ |
| 3U:2N | 25 | $3.55\pm0.11^{h}$ | $3.36\pm0.37^{cd}$ | $0.062\pm0.008^{fgh}$ |
| | 50 | $3.71\pm0.20^{efg}$ | $2.35\pm0.26^{h}$ | $0.061\pm0.008^{h}$ |
| | 75 | $4.10\pm0.16^{ab}$ | $4.19\pm0.54^{b}$ | $0.076\pm0.007^{ab}$ |
| | 100 | $4.01\pm0.24^{bc}$ | $2.41\pm0.28^{h}$ | $0.070\pm0.006^{bcdefg}$ |
| 4U:1N | 25 | $3.83\pm0.14^{def}$ | $2.37\pm0.21^{h}$ | $0.064\pm0.004^{efgh}$ |
| | 50 | $3.86\pm0.22^{cde}$ | $4.48\pm0.29^{a}$ | $0.077\pm0.004^{ab}$ |
| | 75 | $4.17\pm0.17^{a}$ | $2.75\pm0.38^{fgh}$ | $0.073\pm0.005^{abcd}$ |
| | 100 | $4.07\pm0.14^{ab}$ | $3.75\pm0.39^{bc}$ | $0.081\pm0.010^{a}$ |

Data are shown as mean ± standard deviation. Different lowercase letters in each column indicate significant differences between treatments at the 5% significance level, using Duncan's multiple range.

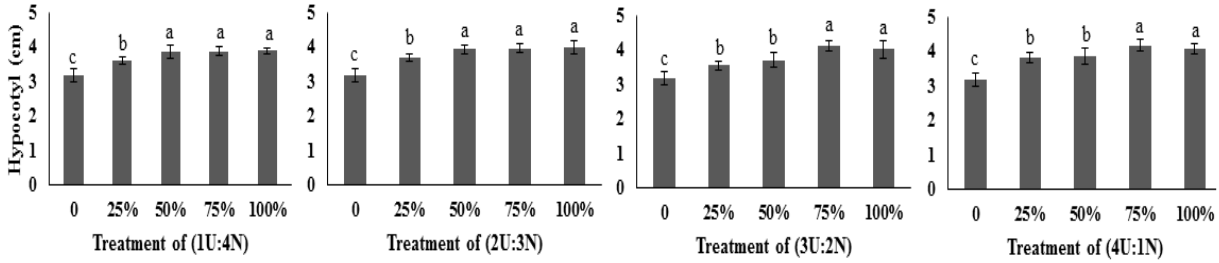


**Figure 2.** Hypocotyl length of quinoa seedlings treated with the combined extracts of *Ulva lactuca and Nostoc commune*; Different lowercase letters in the graph indicate significant differences between treatments at the 5% significance level, using Duncan's multiple range test.

### *Radicle length*

The length of the radicle in the treatment with all concentrations of *U. lactuca* extract was significantly reduced compared to the control. The length of the radicle decreased with increasing the concentration of the extract. Treatment with different concentrations of *N. commune* extract often led

to a significant decrease in the radicle length compared to the control; only the treatment with a concentration of 40% was not significantly different from the control ($p \leq 0.05$) (Figure 3).

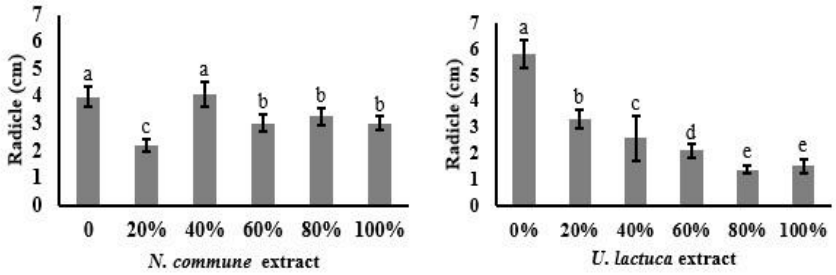


**Figure 3.** Radicle length of quinoa seedlings treated with *Ulva lactuca and Nostoc commune* extracts; Different lowercase letters in the graph indicate significant differences between treatments at the 5% significance level, using Duncan's multiple range test.

The length of the radicle in the 1U:4N and 2U:3N extracts did not increase significantly compared to the control. However, in the treatment with 75% concentration of 3U:2N extract and 50% concentration of 4U:1N extract, a significant increase in the root length was observed compared to the control ($p \leq 0.05$) (Figure 4). The maximum radicle length was observed in 50% concentration of 4U:1N, which increased by 20.43% compared to the control (Table 1).

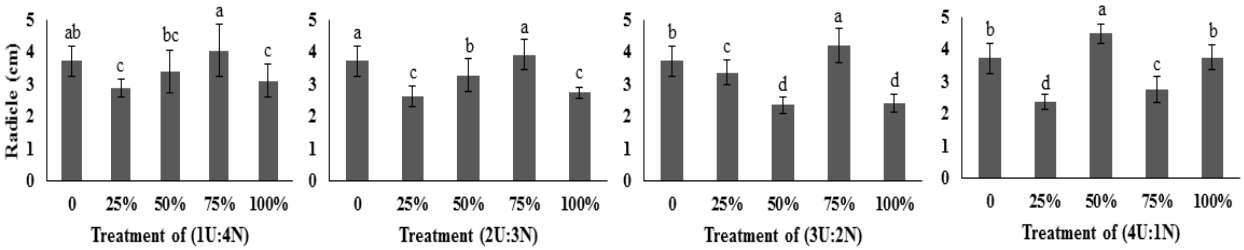


**Figure 4.** Radicle length of quinoa seedlings treated with the combined extracts of *Ulva lactuca and Nostoc commune*; Different lowercase letters in the graph indicate significant differences between treatments at the 5% significance level, using Duncan's multiple range test.

### *Seedling weight*

The fresh weight of seedlings in the treatment with 20% concentration of *U. lactuca* extract significantly increased by 16.39%, compared to the control. Treatment with other concentrations of *U. lactuca* extract showed no significant difference from the control. The fresh weight of seedlings in the treatment with 80% concentration of *N. commune* extract was not significantly different from the control. For the other concentrations of the *N. commune* extract, the fresh weight of seedlings decreased as compared to the control ($p \leq 0.05$) (Figure 5).

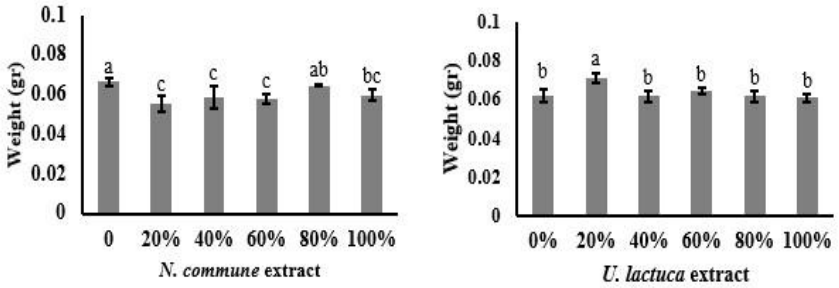


**Figure 5.** Fresh weight of quinoa seedlings treated with *Ulva lactuca and Nostoc commune* extracts; Different lowercase letters in the graph indicate significant differences between treatments at the 5% significance level, using Duncan's multiple range test.

The treatment with 100% concentration of the 2U:3N extract, 75% concentration of the 3U:2N extract, and the 50 and 100% concentrations of the 4U:1N extract showed a significant increase in fresh weight of seedlings compared to the control ($p \leq 0.05$) (Figure 6). The highest seedling fresh weight was observed in 100% concentration of 4U:1N extract, which increased by 20.90%, compared to the control (Table 1).

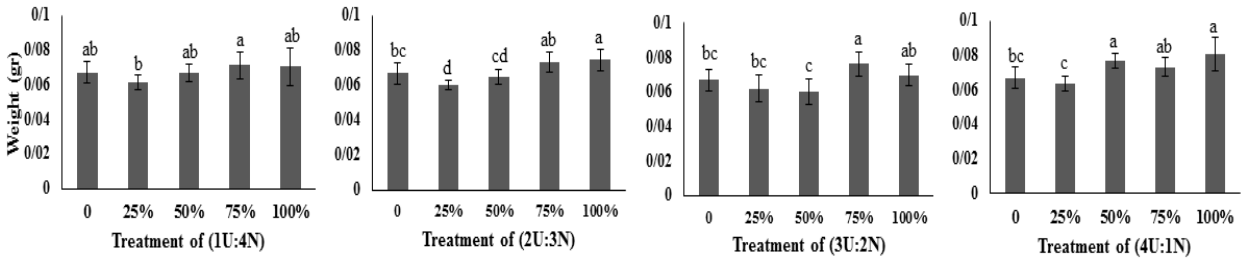


**Figure 6.** Fresh weight of quinoa seedlings treated with the combined extracts of *Ulva lactuca and Nostoc commune*; Different lowercase letters in the graph indicate significant differences between treatments at the 5% significance level, using Duncan's multiple range test.

### *Photosynthetic pigments*

The amount of photosynthetic pigments in the treatment with *U. lactuca* extract enhanced by increasing the concentration of the extract. All concentrations of *U. lactuca* extract resulted in a significant increase in chlorophyll *a*, compared to the control ($p \leq 0.05$), with the 80% and 100% concentrations showing the highest increases of 87.22% and 77.39%, respectively. The amount of chlorophyll *b* and carotenoids also increased significantly in the treatment with most concentrations of *U. lactuca* extract compared to the control ($p \leq 0.05$). The highest amount of chlorophyll *b* was observed in the treatment with 80% and 100% concentrations of *U. lactuca* extract, with an increase of 52.43% and 48.10%, respectively, compared to the control. The highest amount of carotenoids was also in the treatment with 80% and 100% concentrations of *U. lactuca* extract, with an increase of 32.21% and 27.19%, respectively, compared to the control (Figure 7).

Treatment with all concentrations of *N. commune* extract also led to a significant increase in the amount of chlorophyll *a*, and the highest amount of chlorophyll *a* was observed in the treatment with 60% concentration of the cyanobacterial extract, which showed a 38.10% increase over the control ($p \leq 0.05$). The amount of chlorophyll *b* for the concentrations of 40, 60, and 100% of the *N. commune* extract was significantly different ($p \leq 0.05$) from the control, with an increase of 24.77%, 18.01%, and 16.21%, respectively. The amount of carotenoids for most concentrations of the *N. commune* extract increased significantly over the control ($p \leq 0.05$). The treatment with 60% concentration of *N. commune* extract had the highest amount of carotenoids, which led to an increase of 23.07% over the control (Figure 7).

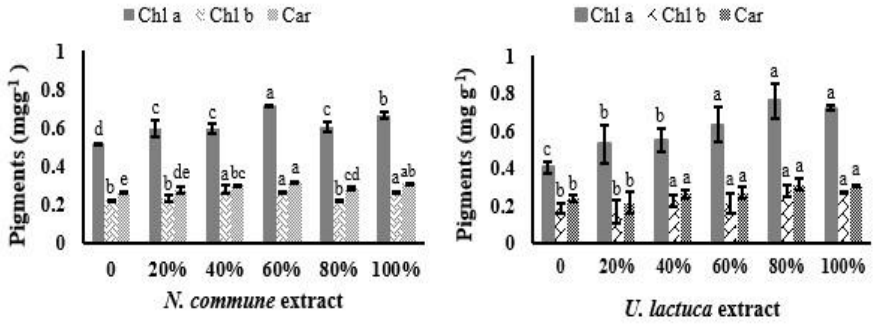


**Figure 7.** The amount of photosynthetic pigments in quinoa seedlings treated with *Ulva lactuca and Nostoc commune* extracts; Different lowercase letters in the graph indicate significant differences between treatments at the 5% significance level, using Duncan's multiple range test; Chl a: Chlorophyll a, Chl b: Chlorophyll b, Car: Carotenoids.

The amount of photosynthetic pigments showed a significant increase over the control in most concentrations of the combined extracts of *U. lactuca* and *N. commune*. ($p \leq 0.05$) (Figure 8). The highest amount of chlorophyll *a* occurred in the 25% concentration of 4U:1N extract and 100% concentration of 2U:3N extract, with a 43.72% increase compared to the control. The highest amounts of chlorophyll *b* were observed in 25% concentration of 4U:1N extract and 100% concentration of 2U:3N extract, which increased by 28.14% and 29.14%, respectively, compared to the control. The highest amount of carotenoids was seen in the 50% concentration of the 3U:2N extract, with a 57.74% increase over the control (Table 2).

### *Anthocyanins*

The amount of anthocyanins in the quinoa seedlings increased significantly in the 20, 40, and 60% concentrations of the *U. lactuca* extract, compared to the control. Increasing the concentration of *U. lactuca* extract above the concentration of 40% led to a decrease in the amount of anthocyanins, so that at the 100% concentration, it was significantly lower than the control. The highest amount of anthocyanins occurred in 20% and 40% extract concentrations, with an increase of 19.2% and

23.19%, respectively, over the control. The amount of anthocyanins in seedlings at the 20% concentration of the *N. commune* extract was significantly increased by 25.86%, compared to the control. Treatment with other concentrations of cyanobacterial extract showed no significant difference from the control ($p \leq 0.05$) (Figure 9).

**Table 2.** The amount of pigments of quinoa seedlings in treatment with different concentrations of the combined extracts of *Ulva lactuca and Nostoc commune*.

| Treatments | Percentage (%) | Chl a (mg $g^{-1}$) | Chl b (mg $g^{-1}$) | Carotenoids (mg $g^{-1}$) | Anthocyanins (mg $g^{-1}$) |
|---|---|---|---|---|---|
| Control | 0 | $0.462\pm0.054^{k}$ | $0.199\pm0.017^{k}$ | $0.239\pm0.014^{h}$ | $0.323\pm0.020^{f}$ |
| 1U:4N | 25 | $0.496\pm0.016^{j}$ | $0.214\pm0.001^{ghi}$ | $0.245\pm0.001^{fgh}$ | $0.414\pm0.008^{ab}$ |
| | 50 | $0.555\pm0.027^{gh}$ | $0.219\pm0.006^{efgh}$ | $0.253\pm0.008^{fgh}$ | $0.318\pm0.009^{f}$ |
| | 75 | $0.508\pm0.004^{ij}$ | $0.207\pm0.001^{jk}$ | $0.242\pm0.001^{gh}$ | $0.323\pm0.001^{f}$ |
| | 100 | $0.627\pm0.006^{bcd}$ | $0.248\pm0.001^{ab}$ | $0.283\pm0.004^{bcd}$ | $0.374\pm0.001^{d}$ |
| 2U:3N | 25 | $0.531\pm0.027^{hi}$ | $0.223\pm0.009^{efg}$ | $0.262\pm0.010^{defgh}$ | $0.372\pm0.007^{d}$ |
| | 50 | $0.636\pm0.004^{abc}$ | $0.244\pm0.002^{bc}$ | $0.286\pm0.001^{bcd}$ | $0.373\pm0.004^{d}$ |
| | 75 | $0.542\pm0.011^{gh}$ | $0.213\pm0.004^{hi}$ | $0.245\pm0.006^{fgh}$ | $0.352\pm0.016^{e}$ |
| | 100 | $0.663\pm0.015^{a}$ | $0.257\pm0.003^{a}$ | $0.289\pm0.001^{bc}$ | $0.345\pm0.001^{e}$ |
| 3U:2N | 25 | $0.552\pm0.011^{gh}$ | $0.239\pm0.001^{bc}$ | $0.280\pm0.001^{bcde}$ | $0.429\pm0.008^{a}$ |
| | 50 | $0.591\pm0.0126^{ef}$ | $0.228\pm0.001^{de}$ | $0.377\pm0.053^{a}$ | $0.389\pm0.007^{cd}$ |
| | 75 | $0.612\pm0.006^{bcde}$ | $0.235\pm0.003^{cd}$ | $0.268\pm0.001^{cdefg}$ | $0.349\pm0.005^{e}$ |
| | 100 | $0.567\pm0.005^{fg}$ | $0.217\pm0.001^{fghi}$ | $0.246\pm0.001^{fgh}$ | $0.336\pm0.001^{ef}$ |
| 4U:1N | 25 | $0.664\pm0.014^{a}$ | $0.255\pm0.003^{a}$ | $0.294\pm0.004^{b}$ | $0.407\pm0.006^{b}$ |
| | 50 | $0.594\pm0.009^{def}$ | $0.228\pm0.001^{de}$ | $0.267\pm0.001^{cdefg}$ | $0.346\pm0.009^{e}$ |
| | 75 | $0.643\pm0.005^{ab}$ | $0.238\pm0.002^{c}$ | $0.270\pm0.003^{bcdef}$ | $0.405\pm0.025^{bc}$ |
| | 100 | $0.605\pm0.009^{cde}$ | $0.225\pm0.004^{ef}$ | $0.256\pm0.004^{efgh}$ | $0.320\pm0.004^{f}$ |

Data are shown as mean ± standard deviation. Different lowercase letters in each column indicate significant differences between treatments at the 5% significance level using Duncan's multiple range.

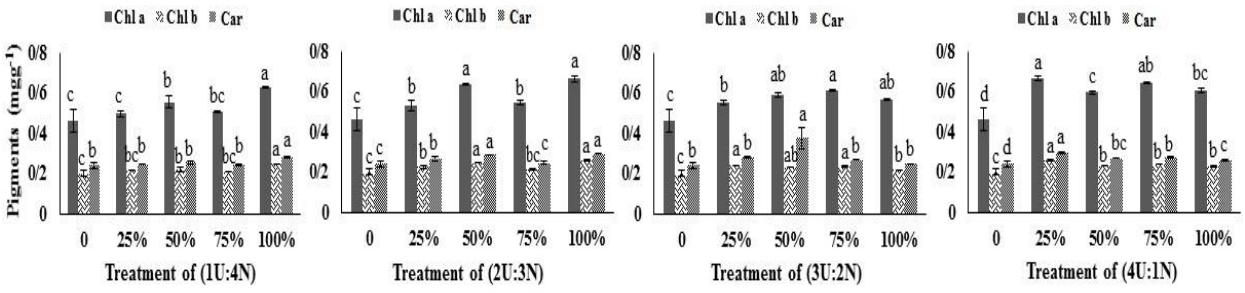


**Figure 8.** The amount of photosynthetic pigments in quinoa seedlings treated with the combined extracts of *Ulva lactuca and Nostoc commune*. Different lowercase letters in the graph indicate significant differences between treatments at the 5% significance level, using Duncan's multiple range test.

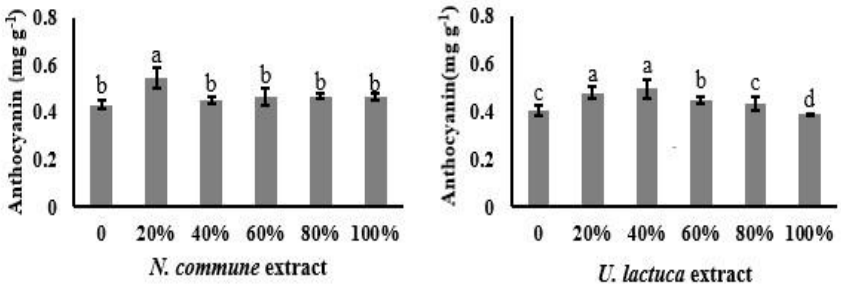


**Figure 9.** The amount of anthocyanins in quinoa seedlings treated with *Ulva lactuca and Nostoc commune* extracts; Different lowercase letters in the graph indicate significant differences between treatments at the 5% significance level, using Duncan's multiple range test.

The amount of anthocyanins in quinoa seedlings increased in the treatment with several concentrations of the combined extract of *U. lactuca* and *N. commune* (Figure 10). Seedlings treated with a concentration of 25% in all combined extracts showed the highest amount of anthocyanins, and the anthocyanins content often decreased with the increase in the concentration of the extract (Figure 10). Among the combined treatments, the highest amount of anthocyanins was seen in the 25% concentration of the 3U:2N extract, which showed an increase of 32.81% over the control ($p \leq 0.05$) (Table 2).

The comparison of the maximum increase in growth characteristics and pigments of quinoa seedlings, when treated with different concentrations of pure and combined extracts of *N. commune* and *U. lactuca*, is shown in Table 3. The greatest increase in the hypocotyl length occurred in the 40% concentration of the *U. lactuca* extract, the 75% concentration of the 4U:1N combination, and 100% concentration of the *N. commune* extract, respectively. The greatest increase in the radicle length was observed in the treatment with 50% concentration of 4U:1N extract, while different concentrations of pure *U. lactuca* and *N. commune* extracts did not increase this trait. The highest increase in the seedling weight was observed in the treatment with 100% concentration of 4U:1N extract and 20% concentration of *U. lactuca* extract, respectively, while different concentrations of *N. commune* extract did not show an increase. The highest amount of chlorophyll *a* was seen in the treatment with 80% concentration of *U. lactuca* extract, 25% concentration of 4U:1N, 100% concentration of 2U:3N, and 60% concentration of *N. commune* extract, respectively. The highest amount of chlorophyll *b* was in the treatment with 80% concentration of *U. lactuca* extract, 25% concentration of 4U:1N, 100% concentration of 2U:3N, and 40% concentration of the *N. commune* extract, respectively. The highest increase in the amount of carotenoids was observed in the treatment with 50% concentration of 3U:2N extract, 80% concentration of *U. lactuca* extract, and 60% concentration of *N. commune* extract, respectively. The highest amount of anthocyanins was observed

in the treatment with 25% concentration of 3U:2N mixed extract, 20% concentration of *N. commune* extract, and 40% concentration of *U. lactuca* extract, respectively.

**Table 3.** Maximum increase of growth characteristics and pigments of quinoa seedlings in treatment with different concentrations of the pure and combined extracts of *Ulva lactuca and Nostoc commune*

| Traits | Combination | *U. lactuca* | *N. commune* |
|---|---|---|---|
| Hypocotyl | 31.13%<br>(at the 75% concentration of the 4U:1N extract) | 46.70%<br>(at the concentration of 40%) | 12.18%<br>(at the concentration of 100%) |
| Radicle | 20.43%<br>(at the 50% concentration of the 4U:1N extract) | - | - |
| Weight | 20.90%<br>(at the 100% concentration of the 4U:1N extract) | 16.39%<br>(at the concentration of 20%) | - |
| Chl a | 43.72%<br>(at the 25% concentration of the 4U:1N and the 100% concentration of 2U:3N extracts) | 87.22%<br>(at the concentration of 80%) | 38.10%<br>(at the 60% concentration) |
| Chl b | 29.14%<br>(at the 25% concentration of the 4U:1N and 100% concentration of 2U:3N extracts) | 52.43%<br>(at the 80% concentration) | 24.77%<br>(at the 40% concentration) |
| Carotenoids | 57.74%<br>(at the 50% concentration of the 3U:2N extract) | 32.21%<br>(at the 80% concentration) | 23.07%<br>(at the 60% concentration) |
| Anthocyanins | 32.81%<br>(at the 25% concentration of the 3U:2N extract) | 23.19%<br>(at the 40% concentration) | 25.86%<br>(at the 20% concentration) |

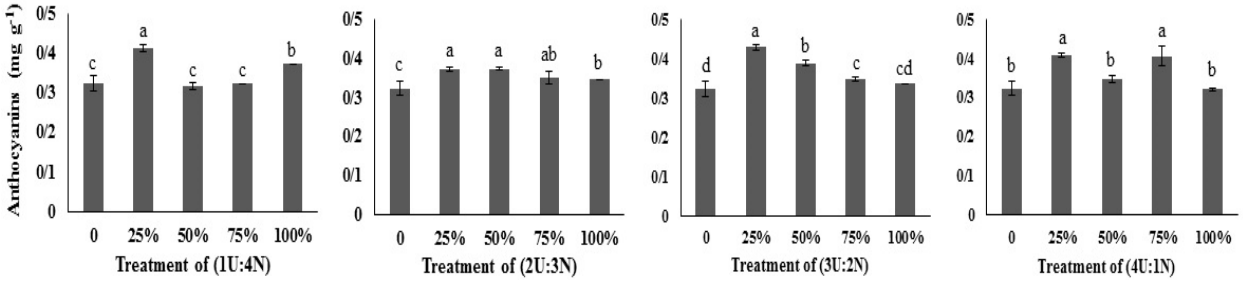


**Figure 10.** The amount of anthocyanins in quinoa seedlings treated with the combined extracts of *Ulva lactuca and Nostoc commune*; Different lowercase letters in the graph indicate significant differences between treatments at the 5% significance level, using Duncan's multiple range test.

## Discussion

Biological stimulants contain biochemical compounds that affect gene expression by activating the signaling pathway and regulating physiological processes, improving growth, increasing resistance, and synthesizing essential metabolites in plants (Singh 2014; Pourbeyrami Hir *et al.* 2021). Several

studies have reported the effect of cyanobacterial and seaweed extracts as fertilizers and biostimulants on plant growth and development (Bai *et al*. 2011; Mai *et al*. 2017; Fleurence *et al*. 2018). Biochemical compounds, phytohormones, amino acids, vitamins, and essential nutrients are part of the biological stimulants in the extracts of cyanobacteria and seaweed, which increase the yield of plants by improving the absorption of nutrients and water, improving the germination process, and increasing stress resistance (Zhang and Ervin 2004, 2008; Aly *et al*. 2008). Some studies have shown the effect of the *Nostoc* cyanobacterium extract and the *Ulva* green algae extract in improving growth and increasing plants' resistance to stress (Liu *et al*. 2011; Shariatmadari *et al*. 2011; Castellanos-Barriga *et al*. 2017; Chittapun *et al*. 2018; Shukla *et al*. 2021). In the present study, treating quinoa seeds with the extract of cyanobacterium *Nostoc commune* and green algae *Ulva lactuca*, separately and in combination, led to the improvement of seedlings' growth characteristics.

The germination stage is one of the most critical stages of the plant's life cycle because it can strengthen the establishment of the plant in the soil by creating an extensive root system and lead to the improvement of the growth of the aerial parts (Murungu *et al*. 2003; Gavazzi *et al*. 2008). In addition, by creating suitable conditions in the germination stage, the resistance of seedlings against stress increases, and the performance of plants also improves (Abedi Firoozjaei *et al*. 2021). The use of *Ulva lactuca* extract led to improvement in seedling length and weight and photosynthetic pigments in beans (Gireesh *et al*. 2011). In addition, using *Ecklonia maxima* seaweed extract could improve the germination of okra seeds by stimulating cell divisions, increasing cell length, and protein synthesis (Makhaye *et al*. 2021). It has also been reported that the *N. commune* cyanobacterial extract at low concentrations improved the germination of *Gentiana daturic* (Liu *et al*. 2011). In the present study, the hypocotyl length in the treatment with pure and combined extracts significantly increased compared to the control, and the treatment with the pure extract of *U. lactuca* showed the greatest increase. While only in the treatment with some concentrations of the combined extract, the radicle length increased significantly over the control. The seedling weight also increased in some concentrations of the treatment with pure *U. lactuca* extract and the combined extract compared to the control (Figures. 1-6, Table 1). It has been reported that seaweed extract contains phytohormones such as auxin and cytokinin. Auxin leads to changes in cell wall flexibility and expansion of plant cells, and cytokinin results in an increase in cell number, stimulation of stem growth, and increased movement of nutrients to the leaves (Dilavarnaik *et al*. 2017; Ruban and Govindasamy 2018). Additionally, seaweed extract enhances the expression of genes related to the synthesis of cytokinin and auxin in plants, leading to an increase in the number of buds, improved growth, increased resistance, and the development of green and healthy leaves in the plant (El-Sayed *et al*. 2015).

Seaweed extract also contains macro- and micro-nutrients, which increase the plants' access to these compounds and improve their growth (Sridhar and Rengasamy 2011; Devi and Mani 2015). Also, cyanobacteria extract contains growth-promoting compounds, including cobalamin, folic acid, nicotinic acid, pantothenic acid, amino acids, sugar, and various phytohormones such as auxin, which stimulate the growth of plants. In addition, cyanobacteria extract can stimulate plant growth by facilitating the absorption of chemical elements in plants (Aly *et al*. 2008; Shariatmadari *et al*. 2011). It has been reported that algae of the genus *Ulva* contain $Al^{3+}$, which, by influencing the $Ca^{2+}$ transport, changes the membrane potential and regulates cell signaling, metabolism, and growth processes, including root growth (Panda *et al*. 2009; Shaaban *et al*. 2017; Farzanah *et al*. 2022). The researchers have suggested that the initiation and development of roots probably required low concentrations of active compounds, hence reduced rooting was observed at higher concentrations of the seaweed extract (Kumari *et al*. 2011). In the present study, increasing the concentration of pure *U. lactuca* extract showed a significant decrease in radicle length (Figure 3). The present results showed that the pure extract of *U. lactuca* is more effective in improving the growth of the hypocotyl and the combined extract is more effective in improving the growth of the radicle and seedling fresh weight (Table 3). These results will be helpful in properly using of cyanobacteria and algae extracts for better utilization of plants.

Photosynthesis is one of the most vital processes of plants, algae, and cyanobacteria in producing biological energy (Sekar and Ramasamy 2015). During photosynthesis, sunlight is received by photosynthetic pigments, and finally, sugar and organic compounds are synthesized. These pigments include chlorophylls and carotenoids, which play a key role in photosynthetic systems and receive sunlight (Roca *et al*. 2024). It has been reported that low concentrations of *Ulva lactuca* extract led to a significant increase in weight, total sugars, total proteins, and photosynthetic pigments content in mung bean seedlings (Castellanos-Barriga *et al*. 2017). In addition, in a study, the *Arthrospira platensis* cyanobacterial extract increased the photosynthetic pigments content in tomato plants (Rachidi *et al*. 2020). Also, in another study, a 0.3 g/liter extract of cyanobacterium *Nostoc piscinale* increased the chlorophyll content of the wheat plant (Takács *et al*. 2019). In the present study, treatment with pure extracts of cyanobacteria *N. commune* and marine green algae *U. lactuca* and the combined extract increased the photosynthetic pigments content in quinoa seedlings (Figures. 7-8, Table 2). The results showed that the highest amount of chlorophyll *a* and *b* was in the treatment with the pure extract of *U. lactuca*, and the highest amount of carotenoids was in the treatment with the combined extract (Table 3). Phytohormones in the seaweed extract can increase chlorophyll synthesis. For example, cytokinins have a protective effect on the photosynthetic system and can

increase the content of photosynthetic pigments. By increasing the rate of chloroplast growth, cytokinins increase the number and size of chloroplasts and expand grana membranes (Chernyad'ev 2009; Cortleven and Schmülling 2015; Hönig *et al*. 2018). Iron and magnesium elements are also part of the structure of chlorophylls, and the presence of these elements in the extract can play a vital role in the organization of chlorophylls (Ruiz *et al*. 2000; Vijayanand *et al*. 2014). In addition, cyanobacteria of the genus *Nostoc* could fix nitrogen and increase the plant's access to nitrogen (Obana *et al*. 2007; Mus *et al*. 2016). There is a correlation between the amount of total nitrogen and the photosynthetic pigments content and the efficiency of plants, including quinoa. Nitrogen is an essential part of chlorophyll structure and increases the synthesis of chlorophyll precursors such as glutamic acid (Amiryousefi *et al*. 2020; Ördög *et al*. 2021; VaziriMehr *et al*. 2024). It has been shown that a series of L-type amino acids in cyanobacteria extract increases the synthesis of photosynthetic pigments due to their signaling role (Mógor *et al*. 2018). Bioactive extracellular compounds present in cyanobacterial extract, through their signaling role lead to increased gene expression and secretion of various phytohormones or other secondary metabolites, which ultimately increase the synthesis of plant pigments (Nowruzi *et al*. 2021). In addition, the extract of cyanobacteria contains phytosterols that play a role in maintaining photosynthetic pigments by increasing the synthesis of brassinosteroids and hormonal activities (Santini *et al*. 2021). The use of pure extracts of *U. lactuca* and *N. commune* and their combined extract is suggested to improve the growth of plants because the treatment with these extracts will lead to an increase in the amount of photosynthetic pigments and subsequently to an increase in the amount of carbohydrates and other organic compounds. Analyzing the extracts of cyanobacteria and algae and identifying effective compounds in plant growth will make their application more targeted.

Anthocyanins are a group of natural pigments that play a role in pathogen defense and fight against fungal infections and insect infestation (Mannino *et al*. 2021). These compounds play a role in camouflage, increasing sunlight absorption, osmotic regulation, temperature, and aging of leaves and organisms (Mannino *et al*. 2021). Color variation in quinoa plant leaves is closely related to the biosynthesis pathway of anthocyanins, so the creation of a wide range of pink, green, yellow, purple, red, and orange colors is attributed to the effective role of anthocyanins (Zhang *et al*. 2024). Various studies have investigated the effects of algal and cyanobacterial extracts on the amount of anthocyanins. The *Sargassum vulgare* seaweed extract increased the amount of phenolic compounds and anthocyanins in red radish leaves (Mahmoud *et al*. 2019). The effects of the cyanobacterium *Spirulina platensis* extract on *Capsicum frutescens* and *Daucus carota* were investigated, which showed a twofold increase in anthocyanin pigments (Rao *et al*. 1996). In the present study, low

concentrations of pure extracts of *N. commune* and *U. lactuca* and their combined extracts led to a significant increase in anthocyanins content in the quinoa seedlings (Figures. 9-10, Table 2). The results showed that the highest amount of anthocyanins was in the treatment with the combined extract (Table 3). Secondary and primary metabolite pathways interact with each other in plants, so the carbohydrates synthesized in the plant can be used as raw materials for developing secondary metabolite pathways. Biostimulants increase the photosynthetic activity in the plant, which leads to more secondary metabolites such as phenolic compounds (Dewick 2002; Giordano *et al*. 2022). Studies have shown that treatment with the seaweed extracts increased the activity of phenylalanine ammonia lyase (PAL), which is the most crucial enzyme responsible for the biosynthesis of polyphenols (Ren and Sun 2014; Rachidi *et al*. 2021; Rasuli *et al*. 2025). Increasing the activity of this enzyme causes the accumulation of flavonoids and increases the content of antioxidants such as anthocyanins (André *et al*. 2009; Nair *et al*. 2012). Also, it has been found that the extract of cyanobacteria can also contain growth stimulants, carbohydrates, proteins, lipids, essential amino acids, vitamins, osmolytes, and polysaccharides that increase the activity of PAL and lead to the formation of anthocyanins and phenolic compounds (Singh *et al*. 2011; Singh 2014; Sarsekeyeva *et al*. 2024).

Considering the antioxidants and protective role of these compounds, it seems that using cyanobacterial and algal extracts as plant growth stimulants will not only protect plants against harsh environmental conditions and plant pests, but will also improve the plant nutritional quality.

## Conclusion

The present results indicated that treatment of quinoa seeds with *Ulva lactuca* and *Nostoc commune* extracts, separately and in combination, were effective in improving seedling growth. The highest chlorophylls content and hypocotyl length were observed in the treatment with the *U. lactuca* extract. The highest carotenoids and anthocyanins content, radicle length, and seedling weight were observed in the treatment with the combined extract. Although treatment with *N. commune* extract improved the growth traits and pigments content of seedlings, it was less effective than the other treatments. However, using these extracts as soil application or foliar application on aerial parts in potted cultivation could probably provide more comprehensive results for the use of these extracts as biostimulants and biofertilizers.

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## Conflict of Interest

The authors declare that they have no conflicts of interest.

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