International Journal of Horticultural Science and Technology
Journal homepage: https://ijhst.ut.ac.ir

Effects of Two Types of Seaweed Extract on Garden Cress Microgreen Characteristics and Essential Oil Compounds

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ARTICLE INFO

Article history:

Received: 12 March 2024,
Received in revised form: 6 May 2024,
Accepted: 22 July 2024

Article type:

Research paper

Keywords:

Ascorbic acid,
Chlorophyll,
Essential oils,
Soluble sugars,
Total phenols

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ABSTRACT

Microgreens are increasingly valued for their rapid growth, nutritional content, and adaptability to small spaces. This study employed a factorial design with a completely randomized setup and three replications to investigate the effects of two types of seaweed extracts (*Ascophyllum nodosum* and *Ecklonia maxima*) at different concentrations (0, 0.5, and 1%) on garden cress microgreens (*Lipidium sativum*). The analysis of variance revealed that the interaction effect between the type of seaweed extract and its concentration significantly influenced all measured traits, except for carotenoid content. Mean comparisons indicated that the treatment with *E. maxima* extract (1%) exhibited the highest values for fresh and dry weight, dry matter percentage, chlorophyll a and b content, total carotenoids, anthocyanins, antioxidants, flavonoids, total phenols, and total protein. Conversely, the treatment with *A. nodosum* extract (1%) caused the highest levels of vitamin C, total sugar content, and essential oil percentage. Moreover, the main effect of seaweed type demonstrated that *E. maxima* extract resulted in the greatest increase in carotenoid content, with a positive correlation observed as the extract concentration increased. Additionally, the analysis identified 25 compounds in the essential oil of garden cress microgreens. Under the influence of the treatment with 1% *E. maxima* extract, the major and main compounds of microgreen essential oil included 1,8-cineole (28%), camphine (7%), limonene (7%), camphor (25.1%), and epi-alpha-bisabolol (8.87%). In general, the application of seaweed extracts enhanced the morphological, physiological, and biochemical traits of garden cress microgreens, highlighting their beneficial influence on plant quality.

Introduction

Microgreens, the young vegetable plants harvested shortly after the emergence of cotyledon leaves, have gained significant popularity in the last decade due to their fresh taste and nutritional benefits. These microgreens offer the potential to enhance dietary value and improve health outcomes, as they are richer in nutrients, intense flavors, phytochemicals, minerals, and vitamins compared to their mature counterparts (Zhang et al., 2021; Rizvi et al., 2023). Harvested at an early growth stage, these seedlings provide multiple advantages, including rapid growth, adaptability to limited spaces, and a concentrated nutrient profile that surpasses mature plants (Xiao et al., 2012).

Garden cress (*Lepidium sativum* L.), a fast-growing plant from the Cruciferae (Brassicaceae) family, is genetically related to cress and mustard,

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sharing their distinct peppery and pungent flavor and aroma. It is known by various names, including "mustard and cress," "garden pepper cress," "pepper grass," and "poor man's pepper." This plant is widely found across Asia in countries such as Iran, Iraq, Palestine, Jordan, Lebanon, Syria, Turkey, Kuwait, Oman, Saudi Arabia, United Arab Emirates, Yemen, Afghanistan, Pakistan, China, Japan, and India (Falana et al., 2014; Vazifeh et al., 2022).

To improve plant growth and nutritional quality, researchers have increasingly turned to organic amendments, with seaweed extract emerging as a promising growth enhancer (Rayorath et al., 2008). Derived from marine algae, seaweed extract is rich in bioactive compounds such as auxins, cytokinins, and gibberellins, which are crucial for promoting plant growth and development (Rayorath et al., 2008; Khan et al., 2009; Craigie, 2011). Studies have shown that the application of seaweed extract stimulates seed germination, enhances root development, and boosts overall plant growth in several crops (Kiraci, 2008; Rayorath et al., 2008; Khan et al., 2009). Additionally, seaweed extract has been reported to increase essential nutrient content and enhance the antioxidant capacity of plants, thereby elevating their nutritional value (Rayorath et al., 2008; Ali et al., 2021). The bioactive compounds in seaweed extract act as growth regulators, influencing physiological processes like photosynthesis, nutrient uptake, and enzyme activity, leading to increased shoot height, leaf area, and overall plant vigor (Khan et al., 2009; Ali et al., 2021).

The application of seaweed extract has demonstrated notable benefits, including improved seed germination, enhanced root development, increased overall plant growth, and better nutrient uptake (Rayorath et al., 2008; Khan et al., 2009; Battacharyya et al., 2015; Ali et al., 2021; Prajapati et al., 2023). Despite extensive research on the impact of organic fertilizers on agricultural products, limited information exists on the effects of seaweed extract on the quality and yield of microgreens. Given the importance of sustainable agriculture and the growing demand for healthy, organic plants, this experiment aimed to investigate the effects of different concentrations of seaweed extract on the morphology, physiological properties, and essential oil composition of garden cress microgreens.

Materials and Methods

Plant materials and growth conditions

Garden cress (*Lepidium sativum* L.) seeds were obtained from the Pakanbazr Company, located in Isfahan Province, Iran. The seeds were cultivated in trays measuring 60 cm in length and 30 cm in width. The cultivation substrate consisted of a mixture of perlite and a super absorbent material, maintaining a relative humidity between 50-60%. The experiment was conducted in a controlled greenhouse environment (32° 68' 36'' N, 51° 51' 74'' E) starting on February 20, 2023. The greenhouse was maintained under specific environmental conditions, with an average temperature of 16–27 °C, a humidity level of 65%, a light intensity of 350 µmol m^{-2} s^{-1}, and a carbon dioxide concentration of 850 ppm.

After two weeks (on March 6, 2023), the microgreens were harvested when they had reached the first true leaf stage, characterized by green and enlarged cotyledons. The aerial parts (shoots) of the microgreens were collected for further physiological and biochemical analyses. Initially, morphological parameters such as fresh weight, dry weight, and dry matter content were measured. Additionally, physiological and biochemical factors, including photosynthetic pigments, total phenolic content, flavonoids, antioxidant capacity, total sugars, proteins, and essential oil content, were evaluated in the microgreens.

Experimental design and treatments

The experiment followed a factorial design with a completely randomized structure, consisting of three replications. The treatments involved two types of seaweed extracts, *Ascophyllum nodosum* and *Ecklonia maxima*, applied at three concentrations: 0%, 0.5%, and 1%. Commercial fertilizers containing the respective seaweed extracts were used to prepare the treatments. Specifically, Armadian fertilizer from Arman Sabz Adineh Company contained *A. nodosum* (Table 1), while Basfoliar fertilizer from Bazargan Kala Company contained *E. maxima* (Table 2). The seaweed extract treatments were applied to the culture trays every two days at a volume of 50 cc.

Plant measurements

Measurement of vegetative traits

At the end of the experiment, the seedlings were removed from the trays, and the stems and roots were weighed separately. The samples were then placed in paper envelopes and dried in an oven at 72 °C for 48 h. After drying, the dry weight was measured. Both fresh and dry weights were recorded using a digital scale with a precision of 0.001 g. The dry matter content was calculated as a percentage by determining the ratio of dry weight to fresh weight.

Table 1. Major components of Armadian fertilizer.

Compounds	Values (%)
A. nodosum extract.	90
Total Nitrogen (N)	2
Available Phosphorus (P_2O_5)	3
Water-Soluble Potassium (K_2O)	22
Free Amino Acids	8
Organic Matter	66
Alginic Acid	14
Mannitol	4

(sourced from Arman Sabz Adineh Company, Tehran Province, Iran).

Table 2. Major components of Basfoliar fertilizer.

Compounds	Values (%)
E. maxima extract	10
Total Nitrogen (N)	3
Available Phosphorus (P_2O_5)	27
Water-Soluble Potassium (K_2O)	18
Water-Soluble Boron	0.01
Water-Soluble Zinc	0.01
Water-Soluble Copper	0.02
Water-Soluble Iron	0.02
Water-Soluble Manganese	0.01
Water-Soluble Molybdenum	0.001

(sourced from Arman Sabz Adineh Company, Tehran Province, Iran).

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content

Photosynthetic pigments were extracted following the method described by Arnon (1949), using 100% acetone as the solvent. A random sample was collected from each experimental unit, and approximately 0.1 g of leaf tissue was placed in a mortar. A total of 10 mL of pure acetone was gradually added to the mortar in two stages. The leaf samples were thoroughly crushed until the green color disappeared, indicating complete pigment extraction. The resultant solution was then centrifuged at 2000 rpm for 10 min.

Following centrifugation, 4 mL of the supernatant from each sample was transferred to a spectrophotometer (UV 160A-Shimadzu Corp., Kyoto, Japan). Absorbance was measured at three wavelengths: 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for carotenoids. The pigment concentrations were calculated using the following equations (Equation 1-4):

$$Chl\ a\ (mg\ g^{-1}) = 11.24 \times A_{661.6} - 2.04 \times A_{644.8}$$ (Equation 1)

$$Chl\ b\ (mg\ g^{-1}) = 20.13 \times A_{644.8} - 4.19 \times A_{661.6}$$ (Equation 2)

$$Total\ Chl = 7.05 \times A_{661.6} + 18.09 \times A_{644.8}$$ (Equation 3)

$$Carotenoids = (100 \times A\_470 - 27.3 \times (mg\ chl\ a) - 104 \times (mg\ chl\ b))/227$$ (Equation 4)

In these equations, Chl a represents the concentration of chlorophyll a, Chl b represents the concentration of chlorophyll b, Car represents the concentration of carotenoids, A_{663} represents the absorbance at 663 nm, A_{645} represents the absorbance at 644.8 nm, and A_{470} represents the absorbance at 470 nm.

Anthocyanin content

Anthocyanin content was determined following the protocol of Islam et al. (2020). A 0.1 g sample of microgreens was combined with 5 mL of an ethanol acid solution (85:15 ratio) in a falcon tube. The tube was wrapped in aluminum foil to prevent light exposure and refrigerated at 4 °C for 24 h. After centrifugation at 10,000 rpm for 6 min, light absorption was measured at 530 nm to calculate the anthocyanin content, expressed in μg g^{-1} FW.

Vitamin C content

Vitamin C content was determined by diluting 10 g of homogenized sample to 100 mL with trichloroacetic acid. Then, 10 mL of the solution was titrated with 2,6-dichloroindophenol reagent. To establish the vitamin C concentration, a standard vitamin C solution of known concentration was also titrated with the same reagent. The vitamin C content of the sample was calculated using a simple ratio (Lisiewska et al., 2006).

Antioxidant capacity

The antioxidant activity of the microgreens was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical inhibition method (Gasemnejad et al., 2011). A DPPH solution (6.25 x 10^{-5} M in 80% methanol) was prepared in a volume of 1000 mL. For extraction, 0.1 g of plant powder was placed in a test tube with 2 mL of 80% methanol, followed by centrifugation and filtration to obtain the leaf extract. Subsequently, 50 µL of microgreen extract was mixed with 950 µL of DPPH solution in small Falcon tubes and vortexed. The mixture was stored in a dark flask at room temperature. After 15 min, the absorbance was measured at 515 nm using a spectrophotometer. Antioxidant activity was expressed as a percentage of DPPH inhibition, calculated from the reduction in absorbance compared to the control.

Total phenolic content

To extract phenolic compounds, 500 mg of dry, ground sample was mixed with 10 mL of 80% aqueous methanol. The mixture was subjected to ultrasonic treatment for 10 min, followed by centrifugation at 1500 rpm for 15 min. The supernatant was separated, and the sediment was re-extracted. Both extracts were combined to form the phenolic extract. Total phenolic content was determined using the Folin-Ciocalteu method. A 0.5 mL aliquot of the phenolic extract was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 10-fold with distilled water) and 2 mL of 7.5% sodium carbonate solution. The mixture was incubated at 45 °C for 15 min, after which the absorbance was measured at 765 nm using a spectrophotometer. A control solution consisting of 80% methanol and reagents was used for comparison purposes (Farooq et al., 2009).

Total flavonoids

The total flavonoid content was measured using water-soluble aluminum chloride, following a method proposed by Ismail et al. (2017). One g of plant sample was extracted with 10 mL of cold methanol. Then, 2 mL of 2.5% aluminum chloride solution was mixed with 2 mL of the extract and vortexed for 30 sec. After a 15-min incubation at room temperature, absorbance was measured at 430 nm using a UV-2100 spectrophotometer (manufactured in the USA). Flavonoid content was calculated based on a standard curve prepared using quercetin.

Total sugar content

The total soluble sugar content was determined using a method described by Albalasmeh et al. (2013). A 500 µL extract sample was combined with 500 µL of distilled water and 3 mL of concentrated sulfuric acid in a test tube, followed by vortexing for 30 sec. The test tube was then placed in an ice bath for 2 min. Once the solution reached room temperature, absorbance was measured at 315 nm using a UV-2100 spectrophotometer (manufactured in the USA). Glucose was used as the standard for the calibration curve.

Total protein content

The Bradford method (Bradford, 1976) was employed to determine total protein content. A 10 µL aliquot of plant extract was placed on a plate, and 500 µL of Bradford reagent was added. After 20 min, absorbance was measured at 595 nm. The protein concentration, expressed in mg g^{-1} DW, was determined by comparison with a standard curve.

Essential oil extraction

Essential oils were extracted from microgreen samples using the water distillation method with a Clevenger apparatus, according to the British Pharmacopoeia guidelines. During the process, the heat increased steam pressure, causing the oil-containing glands to rupture and release essential oils. The volatile compounds, carried by water vapor, passed through the refrigerant into a graduated tube. Due to their lighter density, the essential oils formed a layer above the water phase. The essential oil volume was measured volumetrically in the graduated tube, and the water was carefully drained out by opening the valve. Any remaining water droplets were removed by adding sodium sulfate or using a syringe to ensure a pure oil sample.

The extracted essential oil was transferred to pre-labeled, pre-weighed glass containers and stored in a refrigerator. To ensure the samples were dry, 5 g of each treatment sample was placed in an oven at 50 °C for 24 h. The percentage of moisture content was calculated based on the weight difference between the fresh sample and the

dried sample. Moisture content was determined using the formula provided by Oztekin and Martinov (2007).

$$Amount\ of\ moisture\ based\ on\ fresh\ weight = \frac{(weight\ of\ dry\ material\ +\ weight\ of\ water)}{/\ weight\ of\ water}$$

$$Amount\ of\ moisture\ based\ on\ dry\ weight = \frac{Weight\ of\ dry\ matter}{weight\ of\ water}$$

Determining the composition of microgreen essential oil

The components of essential oils from the microgreen samples were analyzed using a gas chromatography-mass spectrometry (GC/MS) system (Agilent model, University of Isfahan, USA). The system was equipped with an HP-5MS column and a mass detector, with helium as the carrier gas at a flow rate of 1 mL min^{-1}. The column specifications included a length of 30 m, an outer diameter of 0.25 mm, and a film thickness of 0.25 µm. The initial column temperature was set at 60 °C for 4 min and then increased to 260 °C at a rate of 4 °C min^{-1}. The ionization system operated at 70 electron volts, with the ion source temperature maintained at 200 °C. The injector and detector temperatures were set to 290 °C and 300 °C, respectively. Essential oil compounds were identified by comparing their retention indices and mass spectra with standard compounds and the reference library integrated with the GC/MS system (Adams, 2001).

Statistical analysis

Statistical analyses were performed using SAS software (version 9.2). Analysis of variance (ANOVA) and mean comparisons were conducted based on the LSD test at a 5% probability level. Graphs were generated using Microsoft Excel (version 2016).

Results

The analysis of variance (Tables 3 and 4) for the main effects of algae type and concentration, as well as their combined effects on all morphological and physiological traits measured in this study, were found to be significant at the 1% probability level.

Table 3. Variance analysis of some morphological and biochemical traits of garden cress microgreens under fertilizer treatment with different concentrations of two seaweed extracts.

Sources of variation	d.f.	Mean Square							
		Shoot fresh weight	Shoot dry weight	Dry matter content	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll	Carotenoids	Anthocyanins
Seaweed	1	46503.3504**	157.4129**	0.3173*	0.0361**	0.0019**	0.0547**	0.0005*	53.3545**
Concentration	2	296365.0811**	6941.5772**	14.2195**	0.2316**	0.0225**	0.3978**	0.0100**	117.4436**
Seaweed × concentration	2	12840.4920*	78.1175*	0.2328*	0.0107*	0.0005*	0.0152**	0.0003*	14.4121**
error	12	2997.5603	15.9243	0.0549	0.0016	0.0001	0.0018	0.0001	1.4394
Coefficient of variation		5.1071	4.4445	2.8660	4.3821	3.7186	3.5477	4.3017	6.2615

^{ns}, ** and * indicate the non-significant and significant differences at the level of 1% and 5% probability, respectively.

Table 4. Variance analysis of some biochemical traits of garden cress microgreens under fertilizer treatment with different concentrations of two seaweed extracts.

Sources of variation	d.f.	Mean Square						
		Vitamin C	Antioxidant capacity	Total phenol content	Flavonoids	Total sugar	Total proteins	Essential oils
Seaweed	1	18.0801**	4.3022^{ns}	0.5236**	26.4507**	150.6848**	125.8227**	0.5097**
Concentration	2	173.4913**	622.2544**	1.0090**	51.3474**	1503.0130**	726.6651**	1.1167**
Seaweed × concentration	2	4.6113*	13.0188*	0.1315**	6.6060**	43.1195*	33.2001*	0.1846**
error	12	1.1082	2.9944	0.0097	0.4844	10.8181	7.2093	0.0016
Coefficient of variation		3.6715	5.9232	5.8500	6.0354	3.8386	3.6130	4.5405

^{ns}, ** and * indicate the non-significant and significant differences at the level of 1% and 5% probability, respectively.

Fresh and dry weight

The results indicated that as the concentration of algae extract increased in both types of algae, the fresh and dry weight of the microgreen shoots increased. The treatment with 1% concentration of *E. maxima* algae extract showed the highest amount of fresh and dry weights of the microgreens, as illustrated in Figures 1A and B.

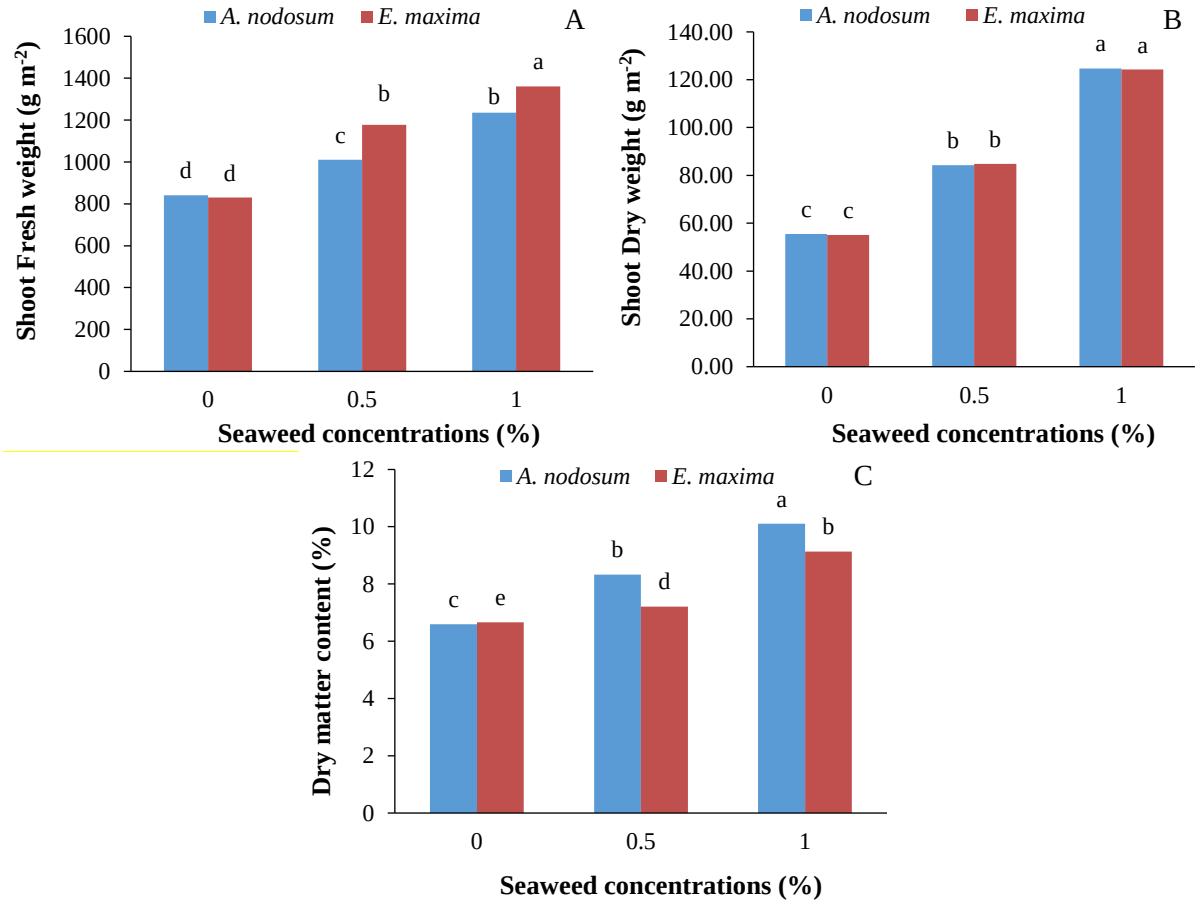

Fig. 1. Interaction effects of applying extracts from two seaweed types, *A. nodosum* and *E. maxima*, at different concentrations on fresh (A) and dry weight (g m^{-2}) (B) and dry matter content (%) (C) of garden cress microgreen. Results are mean values of 3 replicates. Different letters denote significant differences at 0.05 level of LSD test.

Dry matter content

The interaction between the type and concentration of seaweed extract demonstrated that as the concentration of both types of seaweed extract increased, the dry matter content of microgreens increased. The highest dry matter content was observed in microgreens treated with a concentration of 1 mg L^{-1} of *Ascophyllum nodosum* extract (Fig. 1C).

Chlorophyll a, chlorophyll b, and total chlorophyll

The interaction effect of seaweed extract type and concentration showed that increasing the concentration of both types of seaweed extract led to a rise in chlorophyll a, chlorophyll b, and total chlorophyll content. The highest levels of all chlorophyll parameters were recorded in microgreens treated with 1% *Ecklonia maxima* extract concentration (Fig. 2A-C).

Carotenoids and anthocyanins

The findings indicated that in both types of algae, the levels of carotenoids and anthocyanins rose with increasing concentrations of seaweed extract. The treatment with 1% concentration of *E. maxima* algae extract exhibited the highest amounts of carotenoids and anthocyanins, as depicted in Figures 3A and B.

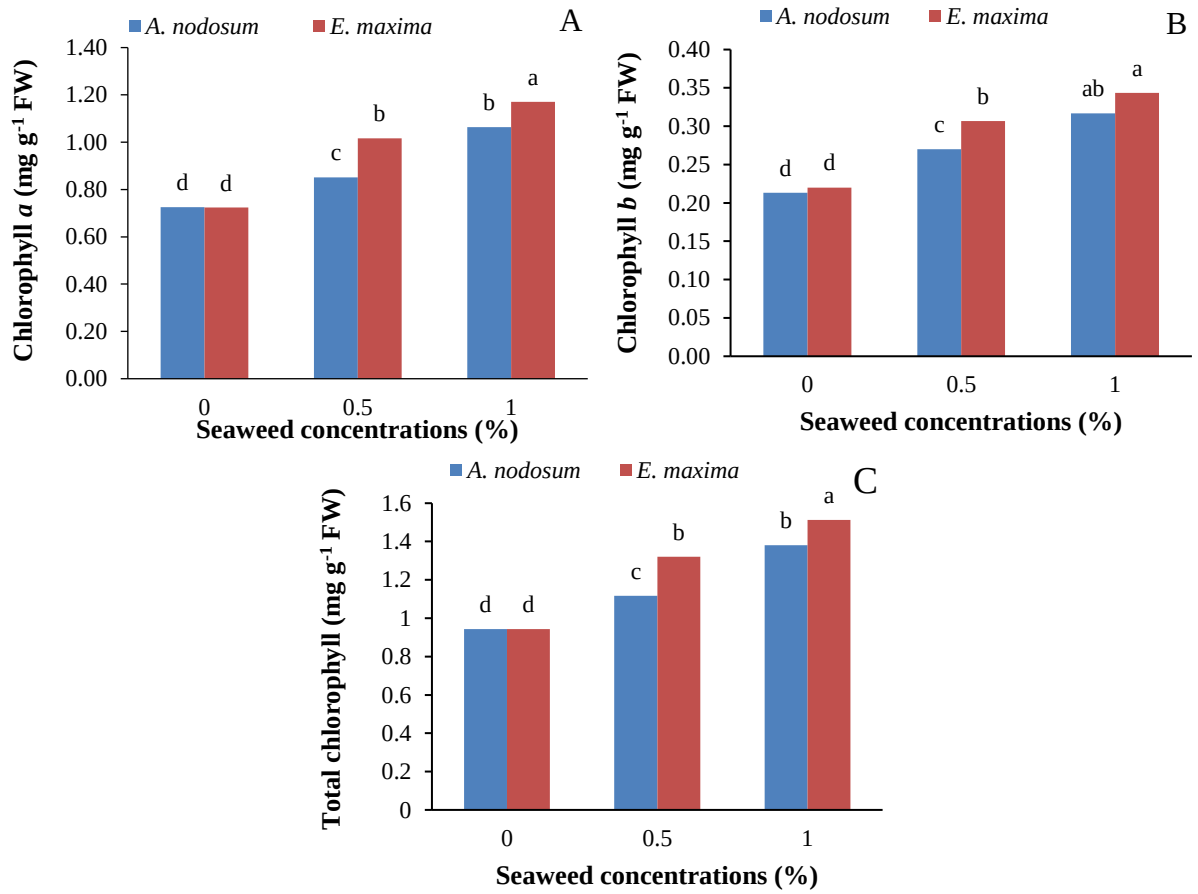

Fig. 2. Interaction effects of applying extracts from two seaweed types, *A. nodosum* and *E. maxima*, at different concentrations on chlorophyll a (A), chlorophyll b (B), and total chlorophyll (mg g^{-1} FW) (C) of garden cress microgreens. Results are mean values of 3 replicates. Different letters denote significant differences at 0.05 level of LSD test.

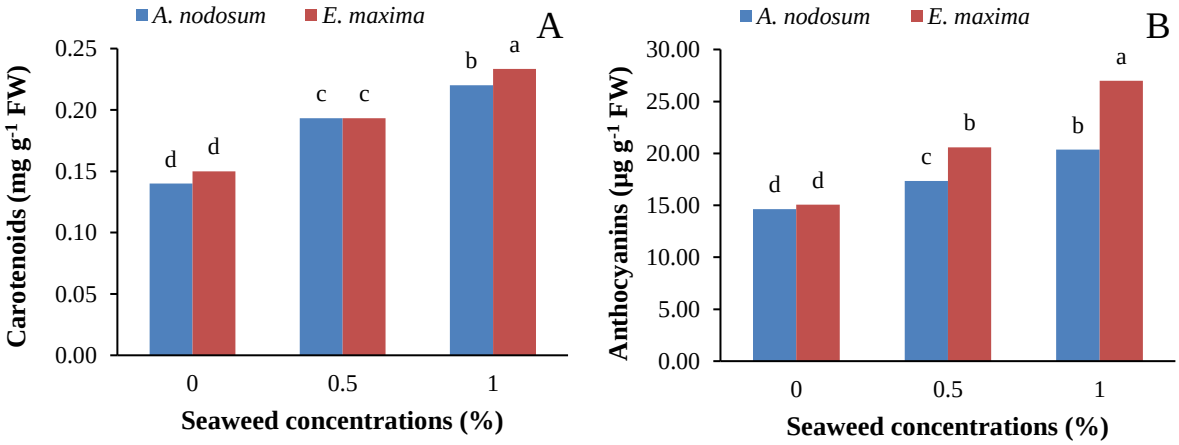

Fig. 3. Interaction effects of applying extracts from two seaweed types, *A. nodosum* and *E. maxima*, at different concentrations on the amount of carotenoids (mg g^{-1} FW) (A) and anthocyanins (µg g^{-1} FW) (B) of garden cress microgreens. Results are mean values of 3 replicates. Different letters denote significant differences at 0.05 level of LSD test.

Vitamin C and antioxidant capacity (DPPH%)

The results indicated that the concentration of seaweed extract positively influenced vitamin C levels in microgreens. As shown in Figure 4A, the highest vitamin C content was recorded in microgreens treated with 1 mg L^{-1} concentration

of *Ascophyllum nodosum* extract. The interactions between seaweed extract type and concentration revealed that antioxidant levels increased with higher concentrations of the extracts. Specifically, the 0.5% and 1% concentrations of seaweed extract resulted in approximately 50% and 100% enhancements in antioxidant capacity, respectively, compared to the control treatment. The highest antioxidant levels were observed in microgreens treated with the 1% concentration of *Ecklonia maxima* extract (Fig. 4B).

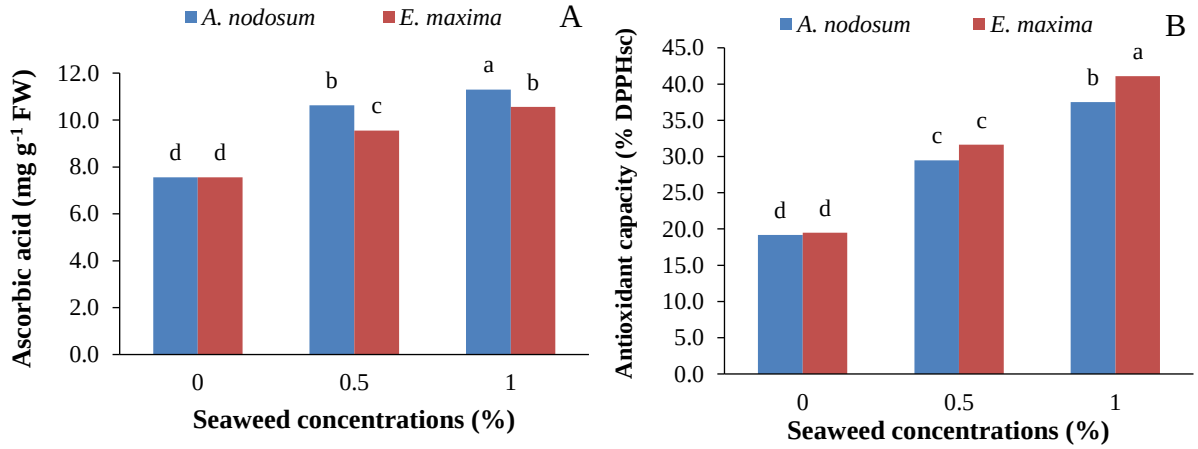

Fig. 4. Interaction effects of applying extracts from two seaweed types, *A. nodosum* and *E. maxima*, at different concentrations on vitamin C (mg g^{-1} FW) (A) and antioxidant capacity (DPPHsc%) (B) of garden cress microgreen. Results are mean values of 3 replicates. Different letters denote significant differences at 0.05 level of LSD test.

Total phenol content and flavonoids

The interaction between the type and concentration of seaweed extract significantly affected the total phenol content in garden cress microgreens. Both *Ascophyllum nodosum* and *Ecklonia maxima* extracts demonstrated an increase in total phenol levels with rising concentrations. Specifically, the 0.5% and 1% concentrations of *A. nodosum* extract resulted in increases of 21% and 42%, respectively. In contrast, the 0.5% and 1% concentrations of *E. maxima* extract yielded increases of 50% and 87%, respectively, compared to the control treatment. The highest total phenol content was observed in microgreens treated with the 1% concentration of *E. maxima* extract (Fig. 5A). The interaction effect of seaweed extract type and concentration also revealed a significant increase in flavonoid levels in both types of algae with higher concentrations of seaweed extract. The 0.5% and 1% concentrations of *A. nodosum* extract led to increases of 22% and 44%, respectively, while the 0.5% and 1% concentrations of *E. maxima* extract resulted in increases of 54% and 92%, respectively, compared to the control treatment (Fig. 5B).

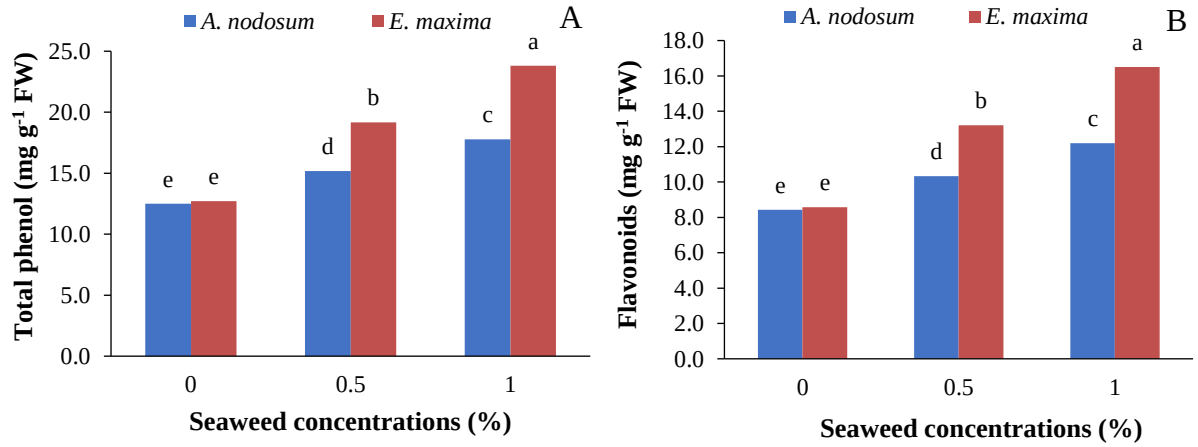

Fig. 5. Interaction effects of applying extracts from two seaweed types, *A. nodosum* and *E. maxima*, at different concentrations on total phenol content (mg g^{-1} FW) (A) and flavonoids (µg g^{-1} FW) (B) of garden cress microgreens. Results are mean values of 3 replicates. Different letters denote significant differences at 0.05 level of LSD test.

Total sugar and total proteins

The comparison of mean values regarding seaweed extract type and concentration revealed that in response to both types of algae, the total sugar and total protein contents increased as the concentration of the algae extract increased. The treatment with 1% *E. maxima* algae extract caused the highest protein levels, while the treatment with 1% *A. nodosum* algae extract caused the highest total sugar levels (Fig. 6A and B).

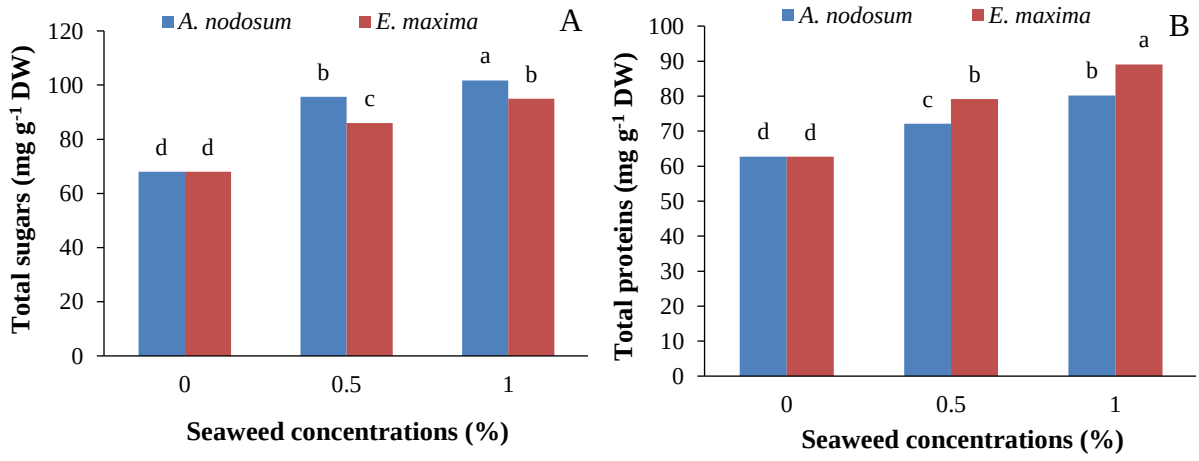

Fig. 6. Interaction effects of applying extracts from two seaweed types, *A. nodosum* and *E. maxima*, at different concentrations on total sugars (mg g^{-1} DW) (A) and total proteins (mg g^{-1} DW) (B) of garden cress microgreens. Results are mean values of 3 replicates. Different letters denote significant differences at 0.05 level of LSD test.

Essential oils (%)

As illustrated in Figure 7, the percentage of essential oil in garden cress microgreens increased with higher concentrations of seaweed extract for both types of algae. The application of 0.5% and 1% concentrations of *Ascophyllum nodosum* extract resulted in increases of 90% and over 100%, respectively. In comparison, the 0.5% and 1% concentrations of *Ecklonia maxima* extract caused increases of 28% and 77%, respectively, compared to the control treatment. Notably, the most significant enhancement in essential oil content was observed in microgreens treated with the 1% concentration of *A. nodosum* extract (Fig. 7).

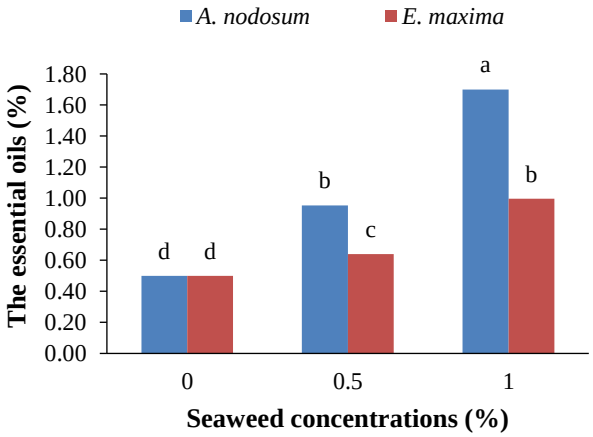

Fig. 7. Interaction effects of applying extracts from two seaweed types, *A. nodosum* and *E. maxima*, at different concentrations on essential oils (%) of garden cress microgreens. Results are mean values of 3 replicates. Different letters denote significant differences at 0.05 level of LSD test.

Essential oils composition

The essential oil composition of garden cress microgreens included 25 identified compounds (Table 5). In the control treatment, the total composition was 84.64%, which increased to 86.88% in response to the 0.5% *Ascophyllum nodosum* concentration, and to 90.22% in response to the 1% *A. nodosum* concentration. Regarding *Ecklonia maxima* extracts, the total compositions were 88.91% in response to the 0.5% concentration and reached 97.55% in response to the 1% concentration. Notably, the application of the 1% concentration of *E. maxima* seaweed extract significantly influenced the composition of essential oils in garden cress microgreens compared to the other treatments. The primary compounds identified in the essential oils of microgreens treated with the 1% *E. maxima* extract included camphine (7%), limonin (7%), 1,8-cineole (28%), camphor (25.1%), and epi-alpha-Bisabolol (8.87%). The identified compounds, their respective percentages, and retention indices are summarized in Table 5.

Table 5. Interaction effects of applying the extracts of two seaweed types, *A. nodosum* and *E. maxima*, at different concentrations on essential oil compounds in garden cress microgreens.

No.	Composition	Retention indices	Control	*A. nodosum* (concentration)		*E. maxima* (concentration)	
				0.5 %	1.0%	0.5%	1.0%
1	α-pinene	920	3.50^{ns}	3.70^{ns}	3.90^{ns}	4.20^{ns}	4.80^{ns}
2	Camphene	938	5.50^{c}	5.53^{c}	5.80^{bc}	6.00^{bc}	7.00^{a}
3	Benzaldehyde	950	0.30^{b}	0.30^{b}	0.40^{a}	0.30^{b}	0.32^{b}
4	Sabinene	962	0.28^{ns}	0.29^{ns}	0.30^{ns}	0.29^{ns}	0.33^{ns}
5	β-pinene	975	1.70^{ns}	1.85^{ns}	2.10^{ns}	2.19^{ns}	2.30^{ns}
6	Myrcene	981	0.10^{ns}	0.20^{ns}	0.20^{ns}	0.20^{ns}	0.20^{ns}
7	*p*-cymene	1010	3.29^{b}	3.47^{b}	3.56^{b}	3.35^{b}	4.12^{a}
8	Limonene	1020	5.70^{ns}	5.90^{ns}	6.24^{ns}	6.50^{ns}	7.00^{ns}
9	1,8-cineole	1041	6.96^{ns}	7.14^{ns}	7.38^{ns}	7.50^{ns}	8.00^{ns}
10	γ-terpinene	1055	2.90^{ns}	3.00^{ns}	3.10^{ns}	3.24^{ns}	3.40^{ns}
11	Linalool	1109	2.94^{ns}	3.12^{ns}	3.35^{ns}	3.24^{ns}	3.47^{ns}
12	Phenylacetonitrile	1120	2.01^{ns}	2.14^{ns}	2.23^{ns}	2.16^{ns}	2.28^{ns}
13	Camphor	1140	24.10^{ns}	24.28^{ns}	24.39^{ns}	24.43^{ns}	25.10^{ns}
14	Borneol	1156	0.67^{ns}	0.70^{ns}	0.80^{ns}	0.73^{ns}	0.84^{ns}
15	α-terpineol	1167	0.10^{ns}	0.20^{ns}	0.20^{ns}	0.20^{ns}	0.20^{ns}
16	Methyl ether thymol	1210	0.68^{ns}	0.72^{ns}	0.79^{ns}	0.80^{ns}	0.87^{ns}
17	Methyl ether	1225	0.20^{ns}	0.30^{ns}	0.30^{ns}	0.30^{ns}	0.30^{ns}
18	Carvacrol Thymol	1276	1.20^{ns}	1.45^{ns}	1.67^{ns}	1.59^{ns}	1.80^{ns}
19	Benzylthiocyanate	1305	2.78^{ns}	2.83^{ns}	2.95^{ns}	3.00^{ns}	3.09^{ns}
20	α-yellangene	1354	0.27^{c}	0.29^{bc}	0.30^{bc}	0.32^{bc}	0.38^{a}
21	Geranyl acetate	1395	1.76^{ns}	1.78^{ns}	1.83^{ns}	1.98^{ns}	2.00^{ns}
22	β-caryophyllene	1432	2.89^{ns}	2.93^{ns}	2.98^{ns}	3.00^{ns}	3.10^{ns}
23	γ-cadinene	1587	1.87^{ns}	1.84^{ns}	1.81^{ns}	1.76^{ns}	1.72^{ns}
24	Caryophyllene oxide	1610	5.05^{ns}	5.14^{ns}	5.26^{ns}	5.48^{ns}	5.90^{ns}
25	Epi- α -bisabolol	1690	8.07^{ns}	8.19^{ns}	8.38^{ns}	8.24^{ns}	8.87^{ns}
	total		84.64	86.88	90.22	91.08	97.55

Discussion

The research findings indicated that the treatment with a 1% concentration of *Ecklonia maxima* seaweed extract resulted in the highest levels of fresh and dry weight, dry matter content, chlorophyll a and b, total carotenoids,

anthocyanins, antioxidants, flavonoids, total phenols, and total proteins. In contrast, the 1% concentration of *Ascophyllum nodosum* seaweed extract produced the highest amounts of total sugars, vitamin C, and essential oil percentage.

The application of seaweed extract via foliar spray has been shown to significantly enhance plant height, photosynthetic pigments, and nutrient levels, such as potassium and phosphorus, ultimately improving yield. Seaweed extracts are recognized for their substantial content of cytokinins, auxins, and betaine, which contribute to elevated chlorophyll levels in leaves (Shehata and El-Khawas, 2003). The presence of betaine in algae extracts is particularly noteworthy for its role in preventing chlorophyll degradation (Shahbazi et al., 2015).

Furthermore, seaweed fertilizers share characteristics with plant growth regulators, not only due to their nitrogen, phosphorus, and potassium content but also because of their trace elements and secondary metabolites (Karthick et al., 2013). The application of seaweed organic fertilizer has demonstrated a notable positive impact on the growth attributes of medicinal plants, leading to enhancements in fresh and dry plant weight, height, chlorophyll and carotenoid levels, and essential oil production.

A study on cucumber plants explored the effects of various seaweed extracts, including two types of red algae and one green algae. The results revealed that the use of green and red seaweed extracts, in conjunction with commercial seaweed extracts and compost, resulted in improved vegetative growth and yield (Ahmed and Shalaby et al., 2012). This aligns with findings from other studies, which reported that the application of seaweed fertilizer positively influenced the fresh and dry weight of seedlings in crops such as Holy basil (Uthirapandi et al., 2018) and Mung bean (Karthik and Jayasri, 2023).

Seaweed extract is known to contain a significant concentration of growth hormones that play a crucial role in plant development. Various researchers have documented the presence of growth-promoting factors, including indoleacetic acid, indolebutyric acid, and gibberellic acid, along with macro- and micronutrients in seaweed fertilizers, all contributing to enhanced plant growth and maturation (Pise and Sabale, 2010). The hormonal compounds, macro- and micronutrients, and growth-regulating substances such as gibberellins, auxins, cytokinins, amino acids, polysaccharides, and betaines present in seaweed extract have been shown to facilitate cell elongation, leaf growth, and internode lengthening while mitigating certain pests and plant diseases.

Consequently, the utilization of seaweed extract, attributed to the presence of growth hormones, enhances nutrient absorption and translocation within plants, leading to increased nutrient concentration in leaves and ultimately resulting in greater plant weight (Sunarpi et al., 2010).

In alignment with the findings of Sunarpi et al. (2010), the application of seaweed extract containing aminobutyrate, glycine betaine, and betaine has been shown to enhance photosynthesis, promote increased sugar and starch production, and elevate chlorophyll levels in leaves and plants, consistent with the outcomes of the present study. Additionally, Spinelli et al. (2010) highlighted the beneficial impact of seaweed extract on chlorophyll levels, which subsequently boosted anthocyanin content in strawberries. Zhang et al. (2011) reported that DPPH is a stable free radical extensively utilized for evaluating antioxidant scavenging activity. Furthermore, Cho et al. (2011) underscored the significant role of antioxidants, particularly polyphenols, found abundantly in marine macroalgae, in antioxidative processes.

Increasing the consumption of algae extract and promoting root growth and development can enhance the plant's utilization of soil nutrients, thereby facilitating the growth of aerial plant parts and increasing the number of meristems that initiate secondary branches. This, in turn, promotes lateral branch formation and positively impacts both fresh and dry weight. The findings of this investigation demonstrate that the application of seaweed extract on microgreens resulted in an increased percentage of essential oil. Notably, the extract from *E. maxima* had a smaller effect on essential oil percentage compared to that from *A. nodosum*, with the 1% concentration showing a more pronounced effect than the 0.5% concentration. The data further revealed that essential oil percentage rises with increasing concentrations of seaweed extract.

Seaweed is known to positively influence the biosynthesis of various compounds, including polysaccharides, proteins, pigments, and polyphenols in plants (Chojnacka et al., 2012). This enhancement of cell metabolism promotes plant growth and increases the number of essential oil glands. As the plant matures, the increase in fresh and dry weight of both roots and shoots directly impacts the quantity and efficacy of essential oil production (Tawfeeq et al., 2016).

The outcomes of this study align with previous research on marjoram (Andrea et al., 2000) and basil (Karagiannidis et al., 2011) regarding the beneficial effects of seaweed fertilizer on essential oil percentage. Furthermore, these results harmonize with the positive impact of

seaweed fertilizer extract on enhancing essential oil content in mint and basil plants (Elansary et al., 2016). An evaluation of seaweed organic fertilizer, humic acid organic fertilizer, and chemical fertilizer on fenugreek revealed that the highest essential oil percentage and yield were achieved with the application of seaweed organic fertilizer. Similar studies on rosemary (Kassem, 2013) and basil (Omer et al., 2016) indicated that the use of seaweed extract led to increased essential oil percentage and enhanced the main compounds within the essential oil, corroborating the present findings.

The enhanced essential oil yield could be attributed to increases in leaf number and surface area, as well as the role of cytokinins in boosting monoterpene synthesis and elevating the number or size of essential oil glands. This study also found that the primary compounds in garden cress microgreens increased with seaweed extract application, consistent with prior research on basil plants. This suggests that the rise in essential oil compounds may be linked to cytokinins enhancing monoterpene biosynthesis, thereby augmenting essential oil components (Gershenzon et al., 2002).

Conclusions

The findings revealed that as the concentration of seaweed extract increased, all measured traits exhibited a consistent upward trend, with the highest levels occurring in response to the 1% concentration. Specifically, the treatment containing 1% *Ecklonia maxima* seaweed extract caused the highest fresh and dry weights, dry matter content, chlorophyll a and b, total carotenoids, anthocyanins, antioxidants, flavonoids, total phenols, and total proteins. Conversely, the treatment that contained 1% *Ascophyllum nodosum* seaweed extract caused the highest amounts of total sugars, vitamin C, and essential oil content. Overall, the application of seaweed extract enhanced the morphological and physiological characteristics of garden cress microgreens.

Acknowledgments

The authors express their gratitude to the Research and Technology Vice-Chancellor of Agricultural Sciences and Natural Resources University of Khuzestan for their support of this research.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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