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Macroalgae contain macro- and microelements, vitamins, amino acids, and phytohormones. These compounds contribute to the development of plants, making macroalgae highly promising as a biofertilizer for different crops. This study aimed to evaluate the potential of three local macroalgae available along the Mediterranean coast, Egypt, two of which are green algae (*Ulva flexusoa* and *Enteromorpha intestinalis*), and one of red algae (*Griffithsia teges*), on nutrition status and fruit quality of "Valencia" orange (*Citrus sinensis*, Osbeck). Aqueous extracts of the three macroalgae were prepared at 0 (control), 5, 10, and 15% concentrations and applied three times as a foliar application throughout the growing period. Results revealed that the macroalgae under investigation contain significant levels of various phytohormones, such as IAA, GA3 ABA, and CK; green algae contain the highest concentrations of GA3, whereas cytokine was observed only in *G. tege.* Macroalgae possess various amounts of essential macro- and microelements, containing Na > Ca >Mg >Mn in addition to Zn, CU, Fe, K, and P. Applying macroalgae extracts significantly affected the mineral composition of orange leaves and the chlorophyll content (presented as SPAD values). Green algae notably impacted fruit quality characteristics, including weight, length and width, juice volume, TSS, and peel color. However, no significant effect was detected on peel thickness or titratable acidity. We concluded that macroalgae extracts could be utilized as an eco-friendly biofertilizer, providing an alternative source for required nutrients in citrus and other fruit orchards.

Keywords: Seaweed, *Ulva flexusoa*, *Enteromorpha intestinalis, Griffithsia teges*, biofertilizer, citrus

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INTRODUCTION

The utilization of chemical fertilizers is increasing steadily because of the growing global population. Continuous usage of chemical fertilizers harms the soil and its beneficial microbial populations. It also leads to accumulating fertilizers in cultivated plants and, consequently, in the food chain and the ecosystem. As a solution, producers are trying to turn towards organic fertilizers such as compost, animal manure, sewage sludge, food processing wastes, *etc*. One of the alternative and sustainable techniques involves the exploitation of macroalgae as biofertilizers.

Macroalgae or seaweed is a sustainable global resource, with annual production exceeding 35.1 million tons (FAO, 2022). It could be used as human food and animal feed as it is a rich source of dietary fiber, essential nutrients, vitamins, omega-3, fatty acids, essential amino acids, and polysaccharides (Rashad and El-Chaghaby, 2020). Also, it is considered a good source of many bioactive compounds used in pharmaceutical applications or other industrial applications (Hamed et al., 2018). Marine algae were classified into three groups based on their pigmentation, including Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae) (Chapman and Chapman, 1980; Rashad and El-Chaghaby, 2020). In addition, macroalgae are a rich source of many different compounds, including plant hormones, antioxidants, antimicrobials, osmoprotectants, essential minerals, and many other organic matters (Zodape, 2001; Godlewska et al., 2016; Nabti et al., 2016).

Many researchers have assessed the impact of powdered or liquid extracts derived from macroalgae as a biofertilizer. The utilization of macroalgae exhibits significant promise for enhancing agriculture since it can improve growth and productivity through various mechanisms, including 1) providing essential macro and micro elements for growth and physiological functions (Mohy El-Din, 2015; Yusuf et al., 2021). 2) Macroalgae contains bioactive compounds that can protect crops from fungal and microbial diseases (Malini et al., 2014; Arioli et al., 2015; El-Sheekh et al., 2021). 3) The addition of macroalgae in the soil can enhance soil structure and augment its water retention capacity (Errati et al., 2022), also improves the ability of crops to alleviate drought and salinity (Zodape, 2001; Hashem et al., 2019). 4) Macroalgae contain various phytohormones, including auxins, gibberellins, cytokinin, and abscisic acid (El Shoubaky and Salem, 2016). These phytohormones play a vital role in cell division, cell elongation, floral induction, fruit set, fruit maturation, *etc*., and thereby affecting

fruit yield, quality, and shelf life (Vijayanand et al., 2014).

On the other hand, the efficacy of liquid macroalgae extracts in influencing plant development under challenging environmental conditions, including higher or lower pH levels, water deficiency, and high temperatures, has been studied previously (Bradacova et al., 2016; Arthur et al., 2013; Nabti et al., 2017). The main effect of macroalgae extracts on alleviating environmental stresses can be attributed to their content of cytokinins, gibberellic acid, and abscisic acid. These phytohormones play an essential role in promoting plant growth in nutrient-stressed conditions and aiding in the recovery of plants after damage (Reitz and Trumble, 1996; Nabti et al., 2016)

Although macroalgae liquid extracts have long been investigated for their agricultural benefits and used on a variety of crops such as beans (Bhosle et al., 1975), wheat (Mohy El-Din, 2015), maize (Bradacova et al., 2016), Hot Pepper (Azzam et al., 2022), the effect of macroalgae application on fruit crops and its quality deserves further investigation and attention.

Citrus is a highly significant fruit crop on a global scale. Citrus fruits are extensively grown in tropical and subtropical regions. Citrus fruits can be eaten fresh, prepared into juice, jams, and marmalades, incorporated into cuisines and beverages, and utilized in the production of pharmaceutical products. Sweet oranges account for over 70% of global citrus production (Elhamady et al., 2009). In Egypt, citrus is the main fruit in the area and production, with over 3,730,685 tonnes (FAOSTAT, 2020). Valencia orange (*Citrus sinensis, Osbeck*) is one of the most important Egyptian sweet orange cultivars exported to several European and Arab markets.

Hence, this study aimed to ascertain the impact of applying liquid extracts derived from three different types of macroalgae, two green algae (*Ulva flexusoa* and *Enteromorpha intestinalis*) and one red algae (*Griffithsia teges*) at various concentrations (0, 5, 10, and 15%) as biofertilizers to Valencia orange trees.

MATERIALS AND METHODS

Algae Collection and preparation of aqueous extract

Algal samples were collected in November 2020 by hand at low tide from the Mediterranean coast in Baltem, Kafer Elsheikh, Egypt (31.59°N, 31.13°E). Collected macroalgae were confirmed by Prof Dr. Mervat Hosny, Professor of Algae, Faculty of Science, Mansoura University, according to Bhavanath Jha *et al.* (2009) and Madkour and El-Shoubaky (2007). The collected samples were washed entirely in seawater to remove sand and other particles. The algae were then transferred to the Botany Lab of the Environmental Studies and Research Institute, Univ. of Sadat City, and washed with tap water to remove any adhered salt. The macroalgae samples were air-dried at room temperature (22±3 ℃) for six days. Dried macroalgae were ground into a fine powder and preserved in plastic tubes in a refrigerator set at 0℃.

One kilogram of dried samples was mixed with 20 liters of sterilized distilled water and boiled for one hour to prepare macroalgae aqueous extracts. The mixture was then squeezed and filtered through muslin cloth in accordance with (Bhosle et al., 1975). The collected extracts were stored as stock solutions and used to make further concentrations (5, 10, and 15%) by mixing them with sterilized distilled water.

Chemical analysis of macroalgae

In dried macroalgae samples, macro and microelements were determined at the Environmental and Food Biotechnology Lab., Genetic Engineering & Biotechnology Research Institute (GEBRI), University of Sadat City, using inductively coupled plasma-atomic emission spectroscopy (ICP-MS; ICAP-Q, Thermofisher, Germany). The phosphorus, potassium, calcium, magnesium, manganese, copper, zinc, iron, and sodium levels were measured following the protocol outlined by Michalak et al. (2015) and Cassap (2016) and expressed as $mg.kg^{-1}$ dry weight.

Hormone analyses of macroalgae extracts

Four phytohormones were analyzed in the collected macroalgae using high-performance liquid chromatography (HPLC- Agilent 1620, Germany). Gibberellic acid (GA3), Abscisic acid (ABA), Indole acidic acid (IAA), and Cytokinin (CK) were separated according to standard methods of Shindy and Smith (1975) by employing a mobile phase of solvents water: acetic acid: ethanol (80: 10: 10 v/v, isocratic mode). The flow rate was set to 1.0 ml/min, and the detector was calibrated to 280 nm using the mobile phase.

Plant materials and experimental conditions

This study was conducted during the two successive seasons, 2021 and 2022, in a private "Valencia"

Orange orchard, budded on Sour Orange (Citrus aurantium L.) rootstock and cultivated at 5x5 meters apart (168 trees/fed.). The trees were grown in clay soil in Menofia Governorate under flood irrigation. Forty trees (15 years old) were chosen to be uniform in vigor, healthy, and free from insects, diseases, and damage. The selected trees were treated with standard and recommended agricultural practices for Valencia orchards in this location, such as fertilizer, irrigation, pruning, and pesticide application (Egyptian Ministry of Agriculture and Land Reclamation, 2003).

Aqueous extraction of three collected macroalgae, including *Ulva flexusoa* (Wulfen) and *Enteromorpha intestinalis* L. (green algae), and *Griffithsia teges* (Harvey) (red algae), were applied three times in February, January and March in both seasons 2021 and 2022. Concentrations of 0, 5, 10, and 15% of the three tested extracts were applied as foliar at a rate of 4-5 liters per tree using a 20-liter Knapsack sprayer with a constant pressure of 2.5 bar. All tested treatments are summarized in Table 1.

Chemical analysis of the soil

To ensure the absence of any deficits in macro and microelements in the selected orchard, soil samples were obtained from eight locations surrounding trees inside the canopy area before applying foliar sprays for the treatments. These samples were taken at three distinct depths: 0-30 cm, 30-60 cm, and 60-90 cm. The electric conductivity (EC) in the soil pastes extract and the soil pH in the 1:5 soil water extract were measured following the method described by Nelson and Sommers (1996). The concentration of macro and microelements, including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), zinc (Zn), iron (Fe) and sodium (Na) were measured at the Environmental and Food Biotechnology Laboratory, GEBRI, using ICP-MS (ICAP-Q, Thermofisher, Germany) in one gram of dry sample after digestion as shown in Table 1.

Chemical analysis of orange leaves

Leaf samples were collected from mature, healthy leaves (second and third) of four representative non-bearing shoots. These leaves were selected in June from spring growth after applying macroalgae extracts. The dried leaves were used to measure macro and micro elements (as previously stated in soil analysis) at the Environmental and Food Biotechnology Laboratory, GEBRI, using ICP-MS (ICAP-Q, Thermofisher, Germany).

Chlorophyll content of orange leaves

The relative chlorophyll content in 30 fully developed and mature leaves per treatment was evaluated using a portable chlorophyll meter SPAD-502 (Konica-Minolta, Osaka, Japan), based on the SPAD value (Soil Plant Analysis Development) that indicates the relative chlorophyll content within the sample leaf.

Physico-chemical characters of orange fruits

At harvest time (two months after the last application), random fruit samples were collected from each replication to assess different fruit quality parameters, including fruit weight (g), fruit length and diameter (cm), peel thickness (mm), and juice volume (cm^2). Total soluble solids (TSS) percentage was measured using a digital refractometer (Milwaukee, model MA871, Milano, Italy), titratable acidity (TA) percentage in the juice was determined using titration with 0.1 N sodium hydroxide, according to (AOAC, 2003).

Peel color was assessed in five randomly chosen fruits at two opposite points using a portable colorimeter (FRU WR18, Shenzhen, China). Subsequently, the average value was calculated for each fruit. The color was measured and recorded using the L*, a*, and b* values, as McGuire (2019) indicated. The L* value represents the brightness, ranging from 0 (representing black) to 100 (representing white). The a* value denotes a green color when negative and a red color when positive, whereas the negative b* value corresponds to a blue color, and the positive b* value corresponds to a yellow one.

Statistical analysis

The study was designed as a Randomized Block Design with four replications (three trees in each replication). The least significant difference value (LSD) was conducted with SPSS software version 16, 2007 (SPSS, Inc., Chicago, IL, USA) to determine the significant differences between mean values at $P \leq 0.05$.

RESULTS

Phytohormone contents of macroalgae extracts

The chromatograms for standard solutions of GA3, IAA, ABA, and CK are shown in Fig 1. Analysis of hormones in *U. flexusoa, E. intestinalis,* and *G. teges* revealed the presence of four different

phytohormones, as summarized in Table 1 and illustrated in Fig 2 (A, B, and C).

Phytohormone analyses of the three examined macroalgae revealed that *E. intestinalis* and *U. flexusoa* had the highest content of GA_3 (15.22 and 15 µg/ml) in comparison to those detected in *G. teges* (7.26 µg/ml). The highest levels of IAA (11.58 and 10.6 µg/ml) were detected in *G. teges* and *E. intestinalis* compared to 7.41 in *U. flexusoa*. The lowest amounts of ABA (6.33 µg/ml) were found in *E. intestinalis*, compared to 9.15 and 10.26 µg/ml in *G. teges* and *U. flexusoa*, respectively. Cytokinin was detected only in *G. teges* at a concentration of 5.11 µg/ml.

Macro and microelements in macroalgae extracts

The data reported in Table 3 illustrates the presence of macro and microelements in the three studied macroalgae. The three studied macroalgae contain high levels of sodium (ranging from 106.1 in *G. teges* to 148.1 $mg.kg^{-1}$ in *U. flexusoa)* and calcium (ranging from 60.9 in *G. teges* to 105.3 in *U. flexusoa).* They also demonstrate moderate magnesium and manganese concentrations, as Mg ranged from 20.7 to 49.8 mg.kg-1 and Mn ranged from 14.4 to 18.2 $mg.kg^{-1}$. In contrast, the P, K, Cu, Zn, and Fe levels are relatively low.

The impact of foliar application using liquid extracts obtained from three macroalgae species (*U. flexusoa, E. intestinalis*, and *G. tegesat*) at rates of 0% (control), 5, 10, and 15% on the levels of macro and micro elements ($mg.kg^{-1}$ dry weight) in the leaves of Valencia orange trees was presented in Table 4. All treatments contain higher concentrations of nitrogen compared to the control. The highest nitrogen content was observed with G10 (3313 $mg.kg^{-1}$) and G5 (2806 $mg.kg^{-1}$), followed by E15 (2731 $mg.kg^{-1}$) and U10 (2635 $mg.kg^{-1}$). The highest phosphorus and potassium were observed with E15, followed by U15. Meanwhile, the highest Ca, Cu, and Mg levels were detected with E10 and U10. The control exhibited a greater sodium content than all the other treatments.

Figure 4 illustrates the effect of the foliar application using extracts of macroalgae (*U. flexusoa, E. intestinalis*, and *G. tegesat*) at different concentrations (0, 5, 10, and 15%) on total chlorophyll content (expressed as SPAD) of Valencia orange leaves. The SPAD values in the leaves of all treatments showed a significant increase compared to the control. The presented data indicated a positive relationship between the extract concentrations of *U. flexusoa and E. intestinalis* and the SPAD values (chlorophyll). On the other hand, there was an inverse relationship between the concentration *of G. tegesat* and the SPAD values. The highest values (74.1, 73.1, and 72.9) were recorded with U15, E15 and G5, respectively.

Table 5 presents the impact of the foliar application of macroalgae extracts at different concentrations on the weight, length, and width of Valencia orange fruits. The fruit exhibited the greatest weight, length, and width values, with U15 extracts reaching 169.4 g, 7.25 cm, and 6.72 cm, respectively. The lowest values of fruit weight and length were recorded with G15, while the minimum values of fruit width (5.89 and 6.14) were recorded with G15 and G10, respectively. There were no significant differences between all other treatments and the control regarding the three measured characters.

The effects of different treatments on Valencia orange juice volume, peel thickness, TSS, and TA percentage are shown in Table 6. The results revealed statistically significant variations in the juice volume and TSS within different treatments. However, no statistically significant differences were observed among the treatments regarding peel thickness and TA. Juice volume measurements ranged from 133.3 to 173.3 cm^2.

All treatments, except for G15, showed no notable variation from the control group. In contrast, G15 exhibited a significant reduction in juice volume compared to the control. Regarding TSS, the readings have varied within a relatively narrow range. The highest TSS value (11.9) was recorded with G15, while the lowest (10.1) was recorded with U15.

The impact of the foliar application of the macroalgae at different concentrations on fruit color was recorded as L*, a*, and b* parameters and presented in Table 7. Data indicated that the impact of various treatments on fruit colors was limited. No statistically significant differences were observed within the treatments regarding the L* and a* values. The control group had the lowest b* values, signifying the least intense yellow color and the poorest coloration. In contrast, the U10 group demonstrated the highest value (69.88), suggesting rapid maturation and excellent coloration.

DISCUSSION

Mineral and phytohormone contents in macroalgae

Our investigation revealed a significant variation in the mineral and phytohormone concentrations among the three studied macroalgae, including green

Table 1. Summarization of the conducted treatments on Valencia Orange trees

Abbreviation	Treatment
U5	Foliar application of *Ulva flexusoa* liquid extract at 5%
U10	Foliar application of *Ulva flexusoa* liquid extract at 10%
U15	Foliar application of *Ulva flexusoa* liquid extract at 15%
E5	Foliar application of *Enteromorpha intestinalis* liquid extract at 5%
E10	Foliar application of *Enteromorpha intestinalis* liquid extract at 10%
E15	Foliar application of *Enteromorpha intestinalis* liquid extract at 15%
G5	Foliar application of *Griffithsia teges* liquid extract at 5%
G10	Foliar application of *Griffithsia teges* liquid extract at 10%
G15	Foliar application of *Griffithsia teges* liquid extract at 15%
Control	Foliar application of tap water

Table 2. Physical and chemical analysis (expressed as $mg.kg^{-1}$) of the soil before macroalgae applications at different depths

Soil depth (cm) Soil parameters	0-30	30-60	60-90
Soil pH	8.23± 0.05	8.20± 0.04	8.13± 0.05
EC ds/m	1.99± 0.02	1.48± 0.02	1.22± 0.03
N	146.2± 17.2	40.60± 23.2	69.30± 14.9
P	9.410± 1.3	11.60± 1.0	14.80± 2.1
K	525.4± 25.6	224.2± 18.2	409.3± 17.3
Ca	46.15± 4.3	21.80± 3.6	32.45± 4.4
Mg	42.89± 4.3	22.77± 1.1	26.26± 2.9
Mn	22.34± 3.2	13.54± 1.5	13.24± 1.7
Cu	2.145± 0.34	0.446± 0.21	1.365± 0.35
Zn	3.698± 1.2	0.879± 0.61	2.784± 0.31
Fe	4.630± 1.3	2.412± 0.96	2.436± 1.01
Na	356.5± 26.2	158.7± 18.8	259.2± 28.1
Ni	0.265± 0.02	0.120± 0.02	0.201± 0.04

Table 3. Macro and microelement contents ($mg.kg^{-1}$ dry weight) in macroalgae of *Ulva flexusoa*, *Enteromorpha intestinalis* and *Griffithsia teges*

Elements ($mg.kg^{-1}$ dry weight)	*U. flexusoa*	*E. intestinalis*	*G. teges*
P	7.33±1.02	10.88±0.63	10.64±0.09
K	7.79±0.91	9.95±1.03	6.93±1.06
Ca	105.3±4.62	100.4±6.35	60.9±2.36
Mg	49.8±5.85	20.7±3.25	28.4±4.15
Mn	14.4±1.98	16.3±2.00	18.2±3.87
Cu	5.74±0.87	2.23±1.39	11.1±3.10
Zn	11.9±2.37	5.77±2.02	7.84±1.99
Fe	6.10±1.09	5.30±1.25	12.7±4.03
Na	148.1±12.6	107.0±22.3	106.1±20.1

Table 4. Macro and microelement contents ($mg.kg^{-1}$ dry weight) in leaves of treated Valencia orange

Treatment	Element ($mg.kg^{-1}$ dry weight)									
	N	P	K	Ca	Fe	Mn	Zn	Cu	Mg	Na
U5	2564	70.5	5119.6	1357	52.0	86.1	23.6	23.6	1364	3155
U10	2635	78.3	6690.2	1570	114	98.2	21.5	28.9	1797	3054
U15	2596	105.5	6873.9	1636	50.3	90.4	27.1	21.9	1580	2389
E5	2100	90.7	8075.1	1800	56.4	90.2	17.2	21.2	2002	1832
E10	2540	72.7	8910.5	1171	114	98.6	21.1	25.9	1738	3118
E15	2731	111.3	10696	1787	56.3	87.03	24.3	25.4	1589	2265
G5	2806	68.8	4221.8	1155	56.9	72.9	20.9	21.2	1392	2706
G10	3313	64.3	4407.8	1123	51.6	90.4	14.1	24.5	1326	2484
G15	2022	71.9	4223.5	1001	45.3	58.9	11.9	15.4	1139	2161
Control	1056	81.1	5568.4	1261	81.2	88.1	12.5	50.5	1196	3663
L.S.D. $_{0.05}$	103.1	8.43	93.1	305.3	3.78	6.37	1.73	2.31	48.7	1139

Ta**ble 5**. Effect of foliar application of three macroalgae at different concentrations on fruit weight, length, and width of Valencia orange.

Treatment	Fruit weight (g)		Fruit length (cm)		Fruit width (cm)	
U5	139.4 ab	±14.1	6.41 ab	±0.28	6.27 ab	±0.24
U10	143.1 ab	±22.5	6.43 ab	±0.48	6.52 ab	±0.33
U15	169.4 a	±18.8	7.25 a	±0.29	6.72 a	±0.25
E5	139.9 ab	±26.3	6.44 ab	±0.36	6.34 ab	±0.43
E10	130.3 ab	±13.4	6.23 ab	±0.18	6.09 ab	±0.03
E15	151.1 ab	±35.2	6.55 ab	±0.66	6.50 ab	±0.64
G5	150.3 ab	±6.7	6.59 ab	±0.12	6.53 ab	±0.05
G10	132.0 ab	±18.7	6.25 ab	±0.37	6.14 b	±0.42
G15	115.6 b	±9.4	6.14 b	±0.12	5.89 b	±0.13
Control	133.2 ab	±32.7	6.36 ab	±0.39	6.20 b	±0.50

*Treatments are presented as U: *Ulva flexusoa; E: Enteromorpha intestinalis;* G: *Griffithsia teges*; at 5, 10 and 15%, control: 0%. The average values followed by the same letter in the same column are not significantly different at $p \leq 0.05$.

Table 6. Effect of foliar application of three macroalgae at different concentrations on juice volume, peel thickness, TSS, and titratable acidity (TA) of Valencia orange fruit.

Treatment	Juice vol. (cm)		Peel thickness (mm)		TSS (%)		TA (%)	
U5	160.0 ab	±27.5	3.7 a	±0.11	10.4 bcd	±1.1	0.37 a	±0.10
U10	147.3 ab	±32.5	3.0 a	±0.15	11.3 abc	±0.4	0.37 a	±0.05
U15	170.0 a	±30.0	4.2 a	±0.10	10.1 d	±0.8	0. 43 a	±0.05
E5	156.7 ab	±36.0	3.3 a	±0.05	11.3 abc	±0.4	0.40 a	±0.11
E10	135.0 bc	±20.5	2.8 a	±0.12	11.4 ab	±0.4	0.33 a	±0.02
E15	170.0 a	±43.0	3.2 a	±0.16	11.0 abcd	±0.2	0.35 a	±0.23
G5	173.3 a	±17.5	4.3 a	±0.05	10.6 bcd	±0.4	0.40 a	±0.05
G10	150.0 ab	±21.7	3.5 a	±0.10	11.5 ab	±0.2	0.35 a	±0.02
G15	133.3 c	±18.6	4.0 a	±0.02	11.9 a	±0.3	0.37 a	±0.05
Control	160.0 ab	±30.0	3.8 a	±0.07	10.6 bcd	±0.2	0.48 a	±0.12

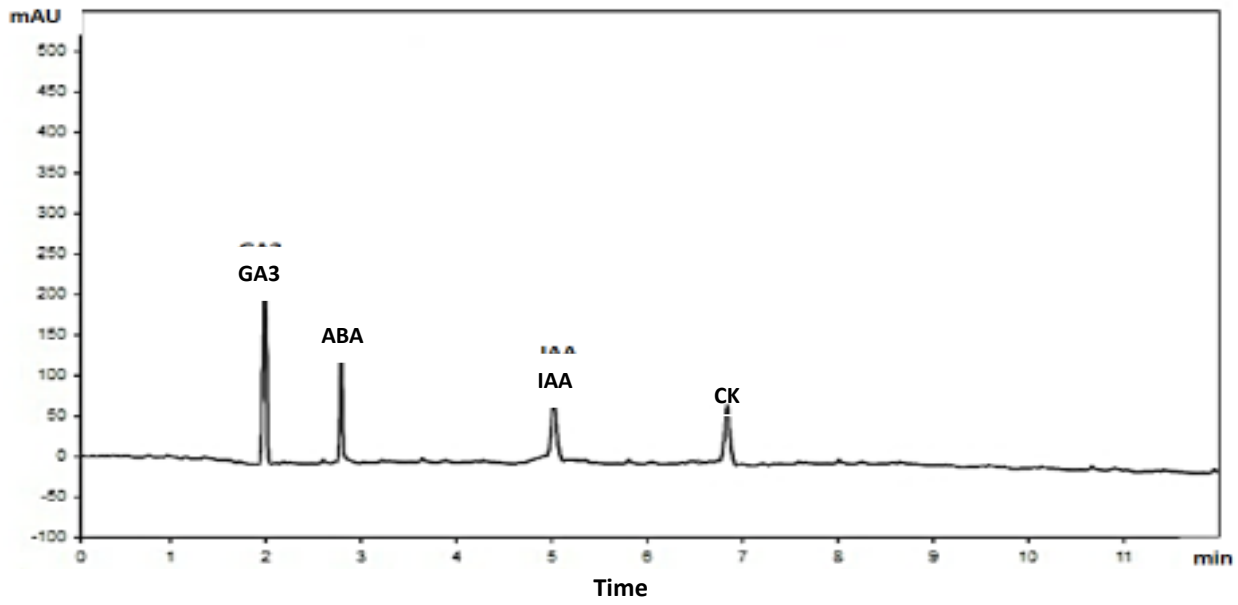
*Treatments are presented as U: *Ulva flexusoa; E: Enteromorpha intestinalis;* G: *Griffithsia teges*; at 5, 10 and 15 %, control: 0%. The average values followed by the same letter in the same column are not significantly different at $p \leq 0.05$.

Table 7. Effect of foliar application of three macroalgae at different concentrations on peel color parameters of Valencia orange fruit.

Treatment	L*		a*		b*	
U5	48.75 a	±4.9	-5.53 a	±5.3	63.03 ab	±4.4
U10	53.12 a	±5.2	-0.80 a	±7.2	69.88 a	±7.4
U15	54. 02 a	±8.8	-3.22 a	±2.9	61.92 abc	±2.4
E5	53.98 a	±7.7	-2.79 a	±4.7	63.60 ab	±8.2
E10	56.07 a	±2.3	-0.30 a	±4.8	67.45 ab	±3.3
E15	57.03 a	±5.2	1.01 a	±6.6	61.62 abc	±9.3
G5	53. 70 a	±1.1	-5.70 a	±3.6	59.92 ab	±4.9
G10	55.80 a	±2.3	0.26 a	±1.7	65.69 ab	±4.2
G15	55.92 a	±2.5	-5.78 a	±4.6	64.35 ab	±3.4
Control	48.90 a	±5.7	-7.37 a	±3.3	52.68 c	±3.0

*Treatments are presented as U: *Ulva flexusoa; E: Enteromorpha intestinalis;* G: *Griffithsia teges*; at 5, 10 and 15 %, control: 0%. The average values followed by the same letter in the same column are not significantly different at $p \leq 0.05$.

algae (*U. flexusoa* and *E. intestinalis*) and red alga (*G. teges*). The highest Ca, Cu, Zn, and Na values were found in *U. flexusoa* macroalgae, while *E. intestinalis* demonstrated the highest recorded values of P and K. On the other hand, *G. teges* showed the greatest level of Mn and Fe. These results were in harmony with many previous works focused on collecting macroalgae from different places and evaluating their chemical composition and other biochemical components. They found that different macroalgae species exhibit valuable content of several minerals, including K, Fe, Mn, Ca, Mg, Na, Zn, Cu, Ni, Co, F, Cr, and Cd (Manivannan et al., 2008; Nabti et al., 2016). Rashad and El-Chaghaby (2020) reported that macroalgae species differed significantly in their mineral contents, phytohormones, antioxidant properties, antibacterial effects, anticancer activities, and bioactive compositions. Ismail et al. (2017) detected that brown species collected from Abu Qir, Alexandrian, Egypt, contain the largest number of elements, followed by red and green macroalgae. Salem et al. (2018) also detected high concentrations of some essential minerals, such as Na, K, Ca, and Mg, in addition to trace elements, such as Fe, Mn, Zn, and



	RT time	Concentration (µg/ml)
GA3	2.0	11.23
ABA	2.8	7.36
IAA	5.0	5.22
CK	7.0	4.40

Figure 1. Chromatogram of GA_3, ABA, IAA, and CK standard solutions.

Cu, in macroalgae. Sodium and potassium levels were highest in brown algae, followed by red and green algae. In contrast, the levels of calcium and magnesium were higher in red algae than in brown and green algae. In general, the chemical composition of macroalgae differs due to the taxonomic type. It can also be affected by seasonal and environmental conditions such as temperature, light, salinity, and nutrient availability in the collecting region (Manivannan et al., 2008; Khairy and El-Shafay, 2013; Nabti et al., 2017).

According to the phytohormonal analysis of the three macroalgae under study, there was a notable variation between the green and red macroalgae. IAA, GA3, and ABA were found in both green and red algae, whereas cytokinin was only found in red algae (*G. teges*). Our findings are consistent with numerous studies that examined the hormonal content of various macroalgae types collected from various locations. Many plant growth regulators, including auxins, cytokinins, gibberellins, and abscisic acid, have been discovered in extracts derived from macroalgae. (Khan et al., 2009; Kurepin et al., 2014). Auxins were reported in the extracts of *Ascophyllum nodosum* (Sanderson and Jameson,1986), and cytokinins in the extracts of *Ulva* (Sekar et al., 1995)) The bioactivity of phytohormones was attributed to extraction techniques, macroalgae types, and environmental stress. Auxins, abscisic acid, and gibberellins were detected in macroalgae collected from the Suez Canal and Timsah Lake, Egypt (El Shoubaky and Salem, 2016). The researchers also noticed the highest concentration of total phytohormones and auxins in green alga (*Ulva rigida*) compared to red alga (*Sarconema filiformae*) and brackish green alga (*Ulva lactuca*). The highest concentration of ABA was found in *Ulva lactuca*, while *Sarconema filiforme* had the highest quantities of gibberellins.

The effect of macroalgae treatments on the nutrient status of Valencia orange trees

Orange leaves treated with E15 and U15 had the highest amount of K, Ca, and Zn. On the other hand, Fe, Mn, Cu, Mg, and Na levels were higher in the E10 and U10 treatments. The increase of macro- and microelements in the leaves of treated Valencia Orange compared to the control could be attributed to the mineral composition of the used macroalgae aqueous extracts. Conversely, the maximum concentration of *G. tegesat* (G15) had lower mineral concentrations than the control and other treatments. These results suggested in previous works that the optimal concentration of macroalgae extract that provides the most beneficial effect

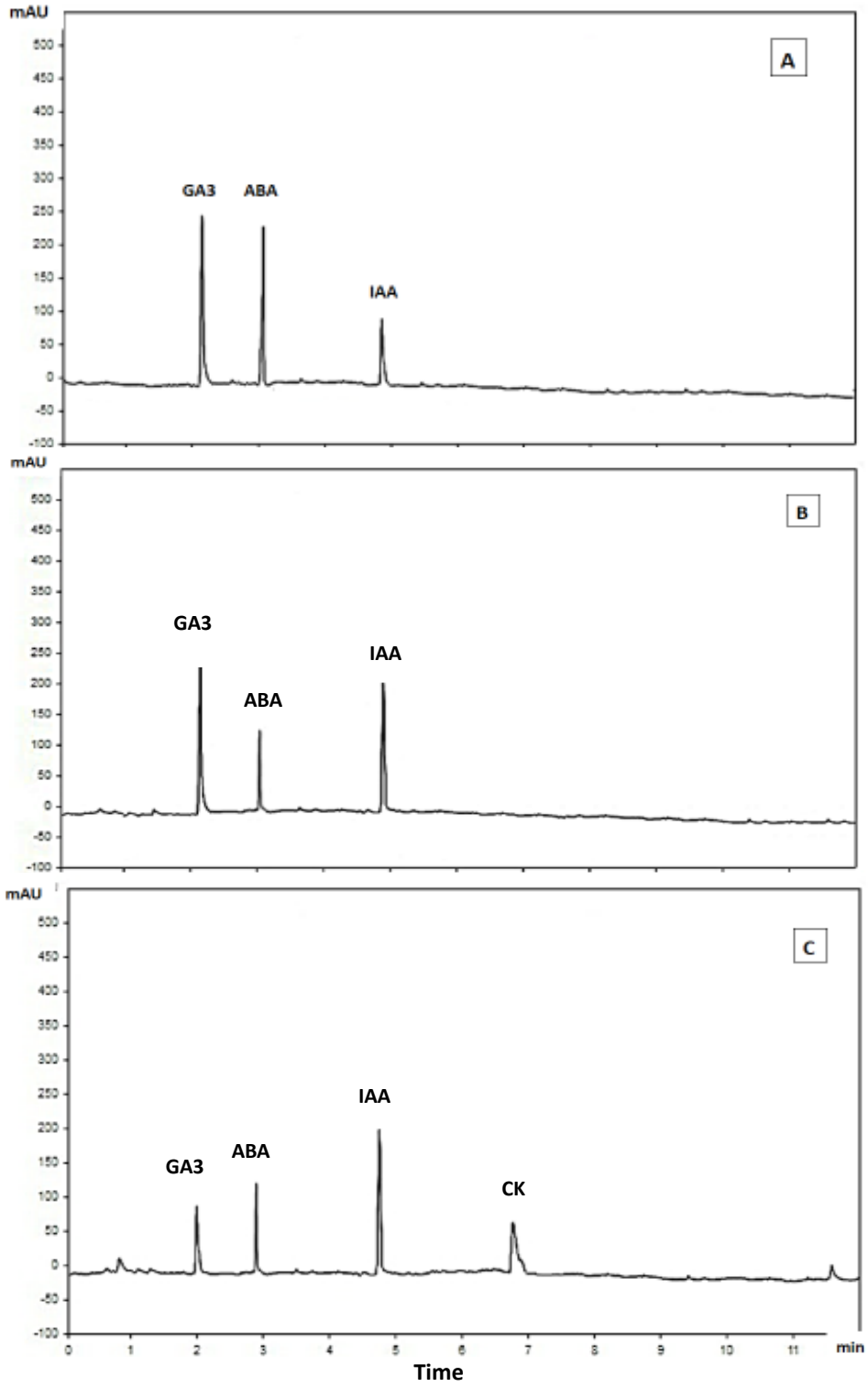


Figure 2. Chromatograms of GA_3, ABA, IAA, and CK in the ~~liquid~~ aqueous extracts of *Ulva flexusoa* (A), *Enteromorpha intestinalis* (B), and *Griffithsia teges* (C)

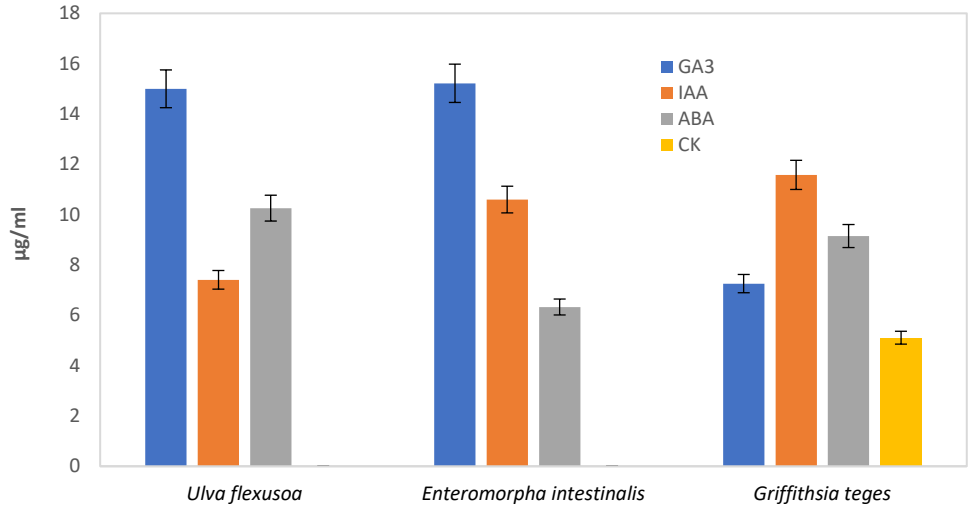


Figure 3. Concentrations of GA3, IAA, ABA, and CK (µg/ml) in macroalgae extracts of *Ulva flexusoa*, *Enteromorpha intestinalis,* and *Griffithsia teges.*

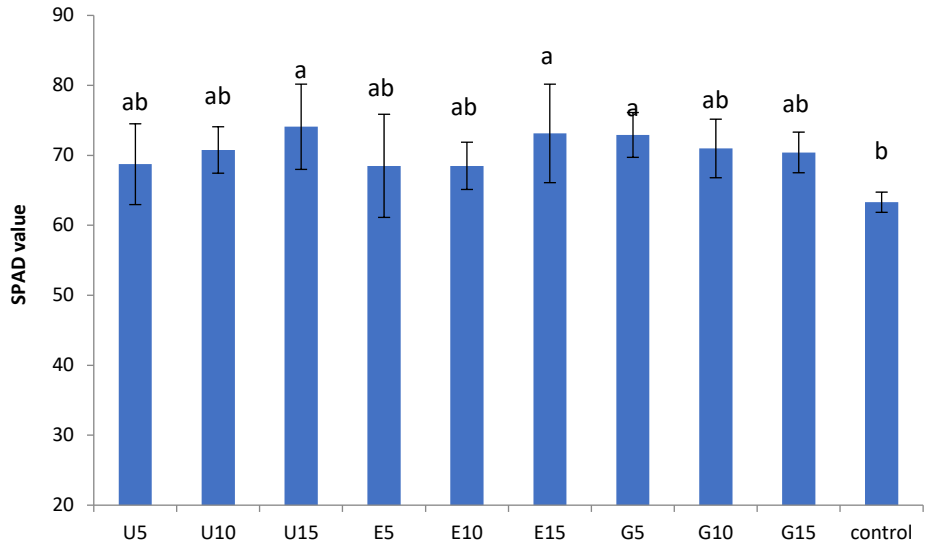


Figure 4. Effect of foliar application of three macroalgae extracts at three different concentrations on relative chlorophyll content in Valencia orange leaves (expressed as SPAD value). Treatments are presented as U: *Ulva flexusoa; E: Enteromorpha intestinalis;* G: *Griffithsia teges*; at 5, 10 and 15 %, control: 0%. Error bars indicate the standard error. Different letters mean significant differences among mean values at $P \leq 0.05$.

depended on the macroalgal type, the kind of treated crops, and their nutritional requirements. Foliar application of macroalgae extracts increased leaf mineral contents of N, P, K, Ca, Mg, Fe, Zn, and Mn in Valencia and Washington Navel orange trees (Hikal, 2015). Silva et al. (2016) assessed the impact of liquid extracts derived from two macroalgae collected from the Portuguese coast (*Sargassum muticum* and *Ascophyllum nodosum)* at 0, 25, 75, and 100% on lettuce. They found that the most favorable effects on plant nutrient content, soil mineral content, and soil pH were observed with the lowest concentration (25%). Hegab et al. (2005) reported that applications of macroalgae extract at 0.5 %, either alone or combined with mono potassium phosphate at 2.0 %, on Balady orange trees were effective in improving the leaves content of N, P, and K compared to control or lower concentrations. Also, Abbas et al. (2008) reported that foliar spray of macroalgae extracts at 3, 6, or 10 ml/L significantly increased N, P, and K content in the leaf of Flame seedless grapevine compared with the control; the best results were recorded with the high concentration of macroalgae (10 ml/L). In addition, applying the solid residue of *Gelidium sesquipedale* (red algae) enhanced organic matter content and increased macro- and microelements in the soil (Errati et al., 2022).

The effect of macroalgae treatments on fruit quality of Valencia oranges

The experimental results demonstrated that applying green algae at various concentrations proved more efficient than red algae (especially in high concentrations of G10 and G15) and significantly increased the mean values of fruit weight, length, and width of the Valencia orange fruits compared to the control. The U15 treatment exhibited the highest fruit weight, length, and width. Conversely, the lowest values were observed in the oranges treated with G15, followed by G10.

Previous studies have demonstrated that using macroalgae can improve metabolic processes and enhance the growth and development of many crops. Safinaz and Ragaa (2013) showed that applying macroalgae as biofertilizers significantly increased maize yield. Azzam et al. (2022) reported that *U. flexusoa* and *E. intestinalis* positively affect shoot and root length, plant fresh and dry weight, photosynthetic pigments, and leaf area of pepper seedlings when compared to *Griffithsia teges*. Macroalgae extracts of *Caulerpa sp.*, *Sargassum sp.*, *Kappaphycus alvarezii,* and *Ulva sp.* enhanced the vegetative parameters of eggplant, including plant height, number of leaves, leaf area, chlorophyll content as well as yield and fruit weight (Yusuf et al., 2021).

The effects of macroalgae extracts were also recorded in citrus and different fruit crops. Foliar application of macroalgae extract on Valencia and Washington Navel orange trees led to considerable enhancements in fruit set %, weight, total soluble solids, TSS/acid ratio, and vitamin C content in both cultivars (Hikal, 2015). The combined application of algae extracts and mono potassium phosphate improved leaf area, yield, fruit weight, TSS %, and vitamin C content and reduced TA % of Balady oranges (Hegab et al., 2005). Also, Abbas et al. (2008) found that foliar spray of seaweed extract at 3, 6, or 10 ml/L had a significant positive effect on the yield, cluster weight, berry size, TSS %, TSS/ acid ratio, and anthocyanin content of Flame seedless grapevines since the application of 6 ml/L increased the yield by 23%. On Barhee date palm, foliar spray with macroalgae extract formulation (1%) in combination with potassium nitrate (2%) improved the yield by about 60.2% compared to the control and improved the physical and chemical characteristics of the date fruits (Alebidi et al., 2021).

Concerning the impact of various treatments on fruit TSS%, findings showed that applying green and red algae increased TSS values at different concentrations. Previous work on Balady orange (Hegab et al., 2005), grapes (Abbas et al., 2008), and Barhee date palms (Alebidi et al., 2021) demonstrated that the application of macroalgae extract led to an increase in fruit TSS. The treatment G15 had the highest TSS value (11.9), which may be attributed to the reduction in juice volume in this treatment, which raised the concentration of soluble solids in the juice.

The lower TSS values observed with U15 and E15 treatments may be related to GA3, whose levels rise with higher macroalgae extract concentrations. The higher concentration of GA3 results in a deceleration of fruit ripening and senescence, leading to a notable reduction in TSS levels. (Zhang et al., 2023).

In general, the changes in the yield and fruit quality of Valencia orange can be attributed to the complex influence of many factors, including endogenous and exogenous factors, such as irrigation, fertilization, biotic and abiotic challenges, *etc*. The differences in fruit weight and quality seen in our research can be attributed to the different compositions found in the macroalgae extracts, such as minerals (K, Fe, Mn, Ca, Mg, Na, Zn, Cu, Ni, Co, F, Cr, and Cd), hormones (IAA, GA3, ABA, and CK), and many other organic matters such as lipids, proteins, carbohydrates, and amino acids (Michalak et al., 2015; Godlewska et al., 2016; Nabti et al., 2016). These components act simultaneously, causing positive effects on plant growth, such as leaf surface area, shoot length, shoot number, root growth, photosynthesis pigments, yield, and fruit quality (Hikal, 2015; Azzam et al., 2022). In addition, phytohormones play a vital role in plant physiological processes, such as growth, cell division and differentiation, and protein synthesis. Gibberellins promote growth and development in citrus and other plants, including vegetative growth, shoot elongation, and fruit set. Exogenous application of IAA and GA3 promotes fruit set, parthenocarpic fruit, and fruit development in citrus and several crops (Gorquet et al., 2005; Mariotti et al., 2011; Bermejo et al., 2018). Cytokinins promote cell division and inhibit plant senescence by inhibiting the decomposition of chlorophyll, proteins, nucleic acids, and other substances and redistributing amino acids, hormones, inorganic salts, and other compounds to other plant parts. In addition, CK can also alleviate the damage to plants caused by some abiotic stresses (Liu et al., 2020). Finally, optimal growth and yield could

be achieved if all nutrients and hormones were balanced; thus, no individual nutrient or essential hormone could be considered a limiting factor in achieving optimal growth and production.

CONCLUSION

Based on the findings obtained from the present study, the application of aqueous extract derived from two green and one red algae macroalgae resulted in an enhancement of chlorophyll and mineral levels in the leaves of Valencia oranges. Green algae (*Ulva flexusoa* and *Enteromorpha intestinalis*) proved more efficient than red algae (*Griffithsia teges*) as an alternative biofertilizer to improve the production and quality of orange fruits. Hence, it could be effective as an eco-friendly biofertilizer for food safety and sustainability. Further research is required to investigate the potential of utilizing macroalgae extracts as replacements for NPK fertilization in citrus and other fruit orchards and to understand the impact of macroalgae and their extracts on yield and plant growth under different stress conditions.

AUTHOR CONTRIBUTIONS

Conceptualization, El-Howeity; experimental work, Azzam, Galal, and Nofal; writing-original draft preparation, Galal; writing-review and editing, Nofal and Galal. All authors have read and agreed to the published version of the manuscript.

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