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Research Article

Plant Growth Bio-stimulants of Seaweed Extract (*Sargasum boveanum*): Implications Towards Sustainable Production of Cucumber

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Abstract: The purpose of this experiment was to compare the growth and quality of cucumber fruits, in response to different concentrations (C_0, $C_{0.75}$, and $C_{1.5}$ g L^{-1}) and different application methods (M_1: foliar sprays, M_2: fertigation, M_3: combined foliar sprays and fertigation) of seaweed extract (SwE). The simultaneous use of the method and different concentration of SwE increased the fresh weight of the leaf, fruit weight, yield, number of leaves, evaporation, fruit length, fruit diameter and firmness, stomatal conductance, and nitrate concentration of fruit. On the other hand, the highest amount of fruit firmness (69.25 and 69.27 N) was observed in $M_2C_{0.75}$ and $M_2C_{1.5}$ compared to other treatments, respectively. The $M_1C_{0.75}$ treatment increased the fruit diameter by 26.52% more than the M_1C_0 treatment. Fruit weight, fruit length, and yield were in the following order in different treatments, $M_1C_{1.5}$> $M_1C_{0.75}$ > $M_3C_{1.5}$. So that only in the $M_1C_{1.5}$ treatment, fruit weight, yield, and fruit length were 25, 52.55, and 25.86% higher than the M_1C_0 treatment, respectively. Generally, the M_1 and M_3 in concentrations of 0.75 and 1.5 created better plant growth, fruit shape, and quality characteristics compared to the second method (M_2) and the C_0 treatment. Therefore, the concentration of 1.5 g L^{-1} and the use of foliar spraying methods, and the combination of foliar spraying and fertigation can be recommended to achieve the maximum yield and quality of cucumber fruits.

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1. Introduction

Recently, the increase in the use of chemical fertilizers has created concerns for the environment and human health. Therefore, considerable research efforts have been made to find new green cultivation technologies and increase the yield and quality of vegetables. In this regard, biostimulant applications are a valuable and environmentally friendly technology for improving the quality traits of vegetables (Consentino et al., 2020; Di Mola et al., 2021; La Bella et al., 2021; Sabatino et al., 2021).

The use of plant biostimulants (BS) or agricultural BS can increase plant growth due to the presence of different compounds and microorganisms, also BS has been considered an effective way to achieve sustainable agricultural production, and to maintain soil health (Rouphael and Colla, 2020; Shabani, 2023). The use of BS motivates natural processes to increase and improve nutrient absorption, nutrient efficiency, product quality, and tolerance to abiotic/biotic stresses (Desoky et al., 2021). Seaweed extract (SwE) contains a complex mixture of polysaccharides, micronutrients, and plant growth hormones and has shown a stimulating effect on plant growth. The use of SwE extract improves the growth and flowering of the plant, as a result of which it increases the yield and quality of products in plants treated with SwE (Rodrigues et al., 2020).

SwE can be used in different ways, such as the application of irrigation fertilizer (near the plant roots), foliar spraying, and their combined application on a variety of flower, vegetable, and tree crops (Haider et al., 2012; Slatnar et al., 2019). The positive effects of SwE on plants under optimal, sub-optimal, or unfavorable conditions are attributed to several biochemical and physiological mechanisms, including stimulating enzymes involved in carbon and nitrogen metabolic pathways, stimulating the synthesis of plant hormones, improving the absorption and accumulation of minerals, and increasing the size of the root system (Consentino et al., 2021). Kocira et al. (2020) also reported that the biostimulants based on SwEs affect the development and resistance of particular parts of plants, such as rhizosphere (roots), phyllosphere (green parts and shoots), and spermosphere (flowers and fruit), and by this way determine the quantity and quality of crop yield produced.

The finding of Ozbay and Demirkiran (2019) on ornamental pepper plants showed that application of SwE improved stem diameter, plant height, number of leaves and leaf area, root and shoot fresh weight, and root and shoot dry weight compared to the control plants. On the other hand, Shafie et al. (2021) demonstrated that the application of different levels of SwE had positive effects on the growth indices, chlorophyll content, carotenoids, and the content of N, P, and K in the leaf of yarrow and the highest shoot dry weight was obtained by the application of SwE by 54% increase compared to the control. In addition, Meng et al. (2022) indicated that the use of SwE in the peanut plant increased the values of photosynthesis, main stem height, lateral branch length, and dry matter accumulation by 13.8%, 45.7%, 225.0%, and 8.7%, respectively. Recent findings showed that SwE in cucumber had a significant effect and increased most studied traits such as plant length, number of leaves, fruit weight, total soluble solids content (TSS), the number of fruits, and total yield (Hassan et al., 2020). Hassan et al. (2021) reported that by increasing the concentration of SwE (0, 1, and 2 g L^{-1}) in cucumber production, plant height, leaf area, leaf dry matter, plant yield, and fruit diameter were increased and leaf nitrogen (N) content was decreased. Also, the application of the SwE had no significant effect on the amount of phosphorus (P) in leaves and fruits. Ashour et al. (2020) findings in a similar study on Jew's Mallow (*Corchorus olitorius* L.) showed a decrease in N concentration and insignificant leaf P content.

Various studies have pointed out the role of SwE extracts on the fruit quality of some vegetables, for example, Colla et al. (2017) reported that foliar applications of SwE improved the total marketable yield of greenhouse tomatoes and Ca^{2+} concentration in the fruit tissue. Also, Mannino et al. (2020) showed that SwE improved tomato fruit development and quality, and by using SwE a two-week reduction of ripening times and a concomitant enhancement of the production percentage, in terms of both fruit yield (110%) and size (85%), were observed. In addition, various studies showed that SwE played an important role in increasing the fresh yield and P, potassium (K), N, and magnesium (Mg) levels of spinach leaves (Rouphael et al., 2018; La Bella et al., 2021). Haidar et al. (2012) showed that the foliar application of SwE concluded a positive response in potato plant growth and yield, moreover, a significant improvement in tuber quality of potato, TSS, N, and protein contents were observed. Similar results were reported in onion (Abbas et al., 2020).

Although there are many studies in the field of using SwE as a foliar application, there is little information about its use as an irrigation fertilizer. Therefore, investigating new methods and their impact on the growth process of plants can be the foundation of new information for researchers and producers. Despite the positive features that have been mentioned about the foliar application of SwE in different plants, disadvantages such as humidity increase in leaf surface and the possibility of fungal disease development in the foliar application method, creating a microscopic effect and the possibility of leaf burns and necrosis, and the difficulty of applying it by the user caused the possibility of using it near the root zone through irrigation fertilizer to be investigated. Therefore, the current research will investigate the growth, physiological, and qualitative characteristics of greenhouse cucumber under hydroponic conditions by studying two different methods of using SwE in different concentrations.

2. Material and Methods

This experiment was carried out in the fall and winter of 2021 in the research greenhouse of Shahid Chamran University of Ahvaz, Ahvaz, Iran, with the geographic location of latitude 31º20´N, and longitude 48º41´E. To conduct the experiment, greenhouse cucumber seeds (var. MRC07, Manier Company, Turkey) were germinated in a glass petri dish in laboratory conditions. In the next step, the germinated seeds were planted in greenhouse conditions under natural light conditions and a day temperature of 25±3 ºC, night temperature of 18±3 °C and humidity of 60±5%, and in disposable glasses. Since the time of seed planting until the emergence of cotyledon leaves, irrigation was done with potable water and after that with a concentration of one quarter of Resh (2013) nutrient solution. Seedlings were transferred to pots containing cocopeat-perlite (70:30 v/v) at the 4 true-leaf stage. Cucumber plants' nutrition during the growing season was carried out by Rash (2013) formula with the following concentrations of N (140), P (50), K (350), Mg (50), Ca (200), S (150), Fe (3), Mn (0.8), B (0.3), Zn (0.1), Cu (0.07), and Mo (0.03) mg L^{-1}. The pH of the nutrient solution was adjusted by 53% nitric acid in the range of 5.8-6. The watering of the plants started with 300 ml per plant and with the growth of the plant, it continued to 500, 700 ml, and finally one liter per plant.

The experiment was conducted based on the split plot randomized complete block design, with 3 replications and two observation plants in each replication. The experimental treatments included different methods of SwE treatment (foliar sprays, fertigation, combined foliar sprays, and fertigation) (as the main factor) and different concentrations (0, 0.75, and 1.5 g L^{-1}) (as the sub-factor) of seaweed extract (*Sargasum boveanum*). To prepare SwE extract, *Sargasum boveanum* seaweed was first collected from the shores of the Persian Gulf in Bushehr city and after the approval of the Persian Gulf Marine Biotechnology Research Center; it was transferred to the laboratory. In order to remove impurities, the washing operation was done several times with distilled water. Seaweed samples were air-dried in laboratory conditions and powdered by a grinder. 15 grams of seaweed powder was mixed with 300 ml of 70% ethanol and stirred on a shaker for 24 hours. After passing the extract through filter paper, the samples were placed in a rotary machine to remove ethanol. The supernatant containing seaweed extract was dried in an oven at 40 °C and the desired extracts were prepared using it (Mohkami and Habibi-Pirkoohi, 2019). Treatments were performed from the second week after transplanting to the tenth week (8 weeks in total). In the foliar application method, the plants were sprayed with 300 ml of the mentioned concentrations, and in the fertigation method, 300 ml of the prepared extract was poured on the pot and next to the cucumber plants, but in the combined method of foliar spraying and fertigation, 150 ml of the prepared concentration was poured on the pot and another 150 ml of the desired concentration was sprayed on the plant. Plant guidance, removal of lateral stems, and adjustment of EC and pH of nutrient solution were carried out regularly.

At the end of the experiment, for measurement of fresh weight (FW) of the root, stem, and leaves of the plants, they were cut from the bottom. The fresh weight of the samples was measured by digital weighing balance. The number of leaves in each experimental treatment was counted and a leaf area meter (LTD, Scientific Instrument, UK) was used to measure the leaf surface. The stem diameter was measured by a digital caliper. The dry weight (DW) of the samples was obtained after drying the roots, stems, leaves, and fruits at 70 °C oven after 48 hours. The dry matter percentage of root, stem, leaf, and fruit was obtained by dividing the dry weight by the fresh weight multiplied by 100. To measure photosynthetic indices such as net photosynthesis rate, stomatal conductance, and transpiration rate, a photosynthesis meter (LCi-SD, UK) was used on complete mature leaves. During the growing season, at each stage of fruit harvesting, the number of fruits was counted by the researcher, the weight of a single fruit was measured by a digital scale, and the length and diameter of the fruit were measured by a digital caliper. The total yield (sum of the weight of single fruits in the entire harvest period) was also recorded and reported at the end of the experiment. The firmness of the fruit was measured by a firmness meter (Santam, STM-1, Iran) and the total dissolved solid of the fruit (TSS) was measured by a refractometer (Atago, A-PAL-1, Japan). In order to evaluate the EC and pH value of the fruit extract, 10 grams of the crushed flesh of cucumber fruits are brought to a volume of 100 ml in a beaker, and after filtering the samples with filter paper, the values of the mentioned traits were recorded and reported (Tabatabai, 2013). Also, in this experiment, the amount of nitrate in the fruit was measured by the method of Cataldo et al. (1975), and nitrate absorbance was measured at 410 nm by a spectrophotometer (UV-1201, Shimadzu, Japan). Dry samples of cucumber leaves were used to determine mineral elements. The plant material was ground with an electric mill and the concentration of P and K was measured by the vanadate-molybdate method using a spectrophotometer (UV-1201, Shimadzu, Japan) at a wavelength of 430 nm and a flame photometer, respectively. The data was evaluated by using SAS 9.1 software (SAS Institute, Cary, NC, USA). Data analysis was performed using the general linear model (GLM) procedure and means were compared using Tukey's test at $p \leq 0.05$ on each of the significant variables measured.

3. Results

3.1. Effect of the application method of seaweed extract

The results of this experiment showed that the application method of SwE caused a highly significant in fresh weight of leaf (FWL), dry weight of leaf (DWL), fruit weight, yield, number of leaves (Num. leaf) leaf area (LA), stomatal conductance, fruit length, fruit diameter, firmness and nitrate concentration of fruit ($p \leq 0.01$) (Tables 1, 2 and 3) (Figure 1). Also, it has been found out a significant increase in dry matter of leaf (DML), fresh weight of root (FWR), fruit dry matter (Fruit DM), and K concentration of leaf ($p \leq 0.05$) (Tables 1, 2, and 3). The highest and lowest FWL were measured in the M_1 (foliar spray) method (264.91 g/plant) and M_2 (fertigation) method (206.73 g plant^{-1}), respectively (Table 1). These results were exactly the same for the DWL with 37.70 g plant^{-1}in the M_1 method and 26.71 g plant^{-1} in the M_2 method, respectively (Table 1). Although the DML in M_2 and M_3 (combined foliar sprays and fertigation) showed no significant difference, but seaweed foliar spray caused an increase of about 9% compared to the other application methods (Table 1). Unlike other measured traits, the highest content of FWR (57.58 g plant^{-1}) and the highest level of cucumber fruit firmness were observed in M_2 (65.58 N) which was significantly higher than the M_1 and M_3 methods (Tables 1 and 3). Compared to the fertigation method, spraying cucumber plants with SwE increased the weight of single fruit by 8.13%, the dry matter of the fruit by 6.60% and the yield of the whole plant by 18.57% (Table 2) (Figure 1). Although the methods of using SwE showed no significant effect on stem diameter, photosynthesis, evaporation, and qualitative characteristics of fruit such as EC, pH, and TSS of the fruit extract, but it significantly affected the quantitative characteristics of leaves and fruits such as the number of leaves, leaf area, K content of leaves, stomatal conductance, length and diameter of fruits (Tables 2 and 3). One of the noticeable results of this experiment was the lower amounts of fruit nitrate in the foliar spray method compared to the M_2 and M_3 methods, so the highest amount of fruit nitrate was 149.54 mg kg^{-1} in the combined method (M_3) and 142.08 mg kg^{-1} in the fertigation method (M_2), respectively and the lowest amount of nitrate was recorded in the spray method with 116.39 mg kg^{-1} fruit fresh weight (Table 3).

3.2. Effects of different concentrations of seaweed

Findings of this experiment indicated that the use of different concentration of SwE had a significant effect on FWL, DWL, DML, fresh weight of stem (FWS), dry weight of stem (DWS), FWR, DWR, DMR, fruit weight, fruit DM, yield, stem diameter, LA, photosynthesis, stomatal conductance, evaporation, fruit length, fruit diameter, firmness, TSS, EC, nitrate and K concentration of cucumber ($p \leq 0.01$), also caused a significant increase in pH of the fruit extract ($p \leq 0.05$) (Tables 1, 2 and 3) (Figure 2). The findings of this study showed that there was a significant difference between different concentrations of SwE in all the quantitative and qualitative traits compared to the control treatment (Tables 1, 2, and 3). In such a way that in traits like FWR, stem and fruit diameter, the $C_{0.75}$ and in traits such as FWL and DWL, fruit weight, yield, number of leaves, stomatal conductance, evaporation, length and firmness of fruit, $C_{1.5}$ took higher values (Tables 1, 2 and 3) (Figure 2). While in other traits such as DML, FWS and DWS, DWR and DMR, fruit DM percentage, LA, photosynthesis rate, TSS, EC, and pH of fruit extract and leaf K percentage, no significant difference was observed between the concentrations of $C_{0.75}$ and $C_{1.5}$ (Tables 1, 2 and 3). No significant difference was observed between the control treatment and different concentrations of SwE in only two traits, the amount of leaf P and stem dry matter percentage (DMS) (Tables 1 and 2).

Table 1. The fresh and dry weight of leaf (FWL and DWL), dry matter of leaf (DML), fresh and dry weight of stem (FWS and DWS), dry matter of stem (DMS), fresh and dry weight of root (FWR and DWR), root dry matter (DMR) of cucumber in response to different concentration (C_0, $C_{0.75}$ and $C_{1.5}$ g L^{-1}) and different application method (M_1: foliar sprays, M_2: fertigation, M_3: combined foliar sprays and fertigation) of seaweed extract (SwE)

Treatments	FWL (g $plant^{-1}$)	DWL (g $plant^{-1}$)	DML (%)	FWS (g $plant^{-1}$)	DWS (g $plant^{-1}$)	DMS (%)	FWR (g $plant^{-1}$)	DWR (g $plant^{-1}$)	DMR (g $plant^{-1}$)
Method (M)	**	**	*	NS	NS	NS	*	NS	NS
Concentration (C)	**	**	**	**	**	NS	**	**	**
M×C	**	NS	NS	NS	NS	NS	NS	NS	NS
Method									
Foliar sprays (M_1)	264.91 ^{a}	37.70 ^{a}	13.77 ^{a}	135.41	10.76	7.91	50.10 ^{ab}	7.81	15.22
Fertigation (M_2)	206.73 ^{b}	26.71 ^{b}	12.73 ^{b}	146.68	12.05	8.15	57.58 ^{a}	8.48	14.33
Combined (M_3)	210.92 ^{b}	27.09 ^{b}	12.63 ^{b}	135.12	10.70	7.88	48.94 ^{b}	7.26	14.41
Concentration(g L^{-1})									
0 (C_0)	152.76 ^{c}	17.94 ^{c}	11.72 ^{b}	106.64 ^{b}	8.28 ^{b}	7.76	39.22 ^{c}	4.63 ^{b}	11.81 ^{b}
0.75 ($C_{0.75}$)	242.42 ^{b}	32.99 ^{b}	13.41 ^{a}	163.99 ^{a}	13.37 ^{a}	8.12	62.12 ^{a}	10.01 ^{a}	16.07 ^{a}
1.5 ($C_{1.5}$)	287.39 ^{a}	40.57 ^{a}	14.00 ^{a}	146.57 ^{a}	11.87 ^{a}	8.06	55.27 ^{b}	8.91 ^{a}	16.09 ^{a}
M×C									
M_1C_0	154.40 ^{d}	18.41	11.91	105.06	8.25	7.85	38.90	4.59	11.79
$M_1C_{0.75}$	312.10 ^{a}	45.49	14.54	157.72	12.70	8.01	59.07	10.33	17.68
$M_1C_{1.5}$	328.24 ^{a}	49.19	14.88	143.45	11.34	7.87	52.33	8.52	16.20
M_2C_0	156.60 ^{d}	18.44	11.76	107.42	8.29	7.71	39.62	4.69	11.83
$M_2C_{0.75}$	198.82 ^{c}	25.79	12.89	168.66	14.04	8.32	68.57	10.80	15.74
$M_2C_{1.5}$	264.77 ^{b}	35.91	13.53	163.95	13.83	8.43	64.55	9.95	15.42
M_3C_0	147.28 ^{d}	16.96	11.51	107.45	8.29	7.72	39.14	4.62	11.80
$M_3C_{0.75}$	216.33 ^{c}	27.69	12.79	165.61	13.39	8.03	58.74	8.91	14.78
$M_3C_{1.5}$	269.16 ^{b}	36.63	13.60	132.32	10.43	7.88	48.93	8.26	16.64

NS= not significant, *= $p \leq 0.05$ and **= $p \leq 0.01$. Means followed by a different lowercase letters in a column were significantly different according to Duncan's multiple-range test ($p \leq 0.05$).

3.3. Combined effects of method and different concentrations of seaweed

The simultaneous use of the method and different concentration of SwE increased FWL, fruit weight, yield, number of leaves, evaporation, fruit length, fruit diameter, and firmness ($p \leq 0.01$) and also caused a significant increase in stomatal conductance and nitrate concentration of fruit ($p \leq 0.05$) (Tables 1, 2 and 3) (Figure 3). In other traits studied, no significant difference was observed in the methods and concentrations of SwE used on cucumber plants. The results of this experiment showed that the simultaneous use of $M_1C_{1.5}$ increased the amount of traits such as FWL, fruit weight, yield, number of leaves, stomatal conductance, and fruit length compared to other treatments. Also, the results showed that there is no significant difference between $M_1C_{0.75}$ and $M_1C_{1.5}$ treatments in traits such as FWL, number of leaves, and stomatal conductance (Tables 1, 2, and 3) (Figure 3). Compared to other treatments $M_2C_{1.5}$ treatment caused a significant increase in evaporation, and nitrate concentration of fruit (Tables 2 and 3). On the other hand, the highest amount of fruit firmness (69.25 and 69.27 N) was observed in $M_2C_{0.75}$ and $M_2C_{1.5}$ compared to other treatments, respectively (Table 3). The $M_1C_{0.75}$ treatment increased the fruit diameter by 26.52% more than the M_1C_0.treatment (Table 3). One of the important indicators of marketability and harvest is the fruit weight, fruit length, and the yield, which were in the following order in different treatments, $M_1C_{1.5}$> $M_1C_{0.75}$ > $M_3C_{1.5}$. So that only in the $M_1C_{1.5}$ treatment, fruit weight, yield, and fruit length were 25, 52.55, and 25.86% higher than the M_1C_0 treatment, respectively (Table 3) (Figure 3). In addition, M_1 and M_3 methods produced even better shaped fruits (Figure 4). Generally, in all three studied methods, the $C_{0.75}$ and $C_{1.5}$ produced better results in terms of plant growth and fruit quality characteristics compared to the C_0 treatment, so in most traits, the M_1 and M_3 in concentrations of 0.75 and 1.5 created better results compared to the second method (M_2) (Tables 1, 2 and 3) (Figures 3 and 4).

Table 2. Stem diameter, number of leaves (Num. leaf), leaf area (LA), photosynthesis, stomatal conductance, evaporation, phosphorus (P), and potassium level in leaves of cucumber in response to different concentrations (C_0, $C_{0.75}$, and $C_{1.5}$ g L^{-1}) and different application method (M_1: foliar sprays, M_2: fertigation, M_3: combined foliar sprays and fertigation) of seaweed extract (SwE)

Treatments	**Stem diameter** (cm)	**Num. leaf**	**LA** (cm^2)	**Photosynthesis** (μmol $m^{-2}s^{-1}$)	**Stomatal conductance** (mmol $m^{-2}s^{-1}$)	**Evaporation** (mmol $m^{-2}s^{-1}$)	**P** (% DW)	**K** (% DW)
Method (M)	NS	**	**	NS	**	NS	NS	*
Concentration (C)	**	**	**	**	**	**	NS	**
M×C	NS	**	NS	NS	*	**	NS	NS
Method								
Foliar sprays (M_1)	0.66	29.11 ^{a}	192.69 ^{a}	8.26	0.11 ^{a}	1.54	0.19	3.94 ^{ab}
Fertigation (M_2)	0.68	25.88 ^{b}	172.00 ^{b}	7.62	0.07 ^{b}	1.20	0.18	3.87 ^{b}
Combined (M_3)	0.65	25.77 ^{b}	174.47 ^{b}	7.55	0.06 ^{b}	1.08	0.22	4.25 ^{a}
Concentration (gL^{-1})								
0 (C_0)	0.57 ^{c}	22.11 ^{c}	142.92 ^{c}	5.22 ^{b}	0.05 ^{b}	0.86 ^{b}	0.19	3.57 ^{b}
0.75 ($C_{0.75}$)	0.72 ^{a}	28.33 ^{b}	193.79 ^{a}	8.78 ^{a}	0.07 ^{b}	1.21 ^{b}	0.20	4.31 ^{a}
1.5 ($C_{1.5}$)	0.69 ^{b}	30.33 ^{a}	202.45 ^{a}	9.43 ^{a}	0.11 ^{a}	1.75 ^{a}	0.21	4.17 ^{a}
M×C								
M_1C_0	0.57	22.33 ^{d}	144.00	5.25	0.07 ^{b}	1.01 ^{ed}	0.19	3.51
$M_1C_{0.75}$	0.71	32.00 ^{a}	212.21	9.20	0.13 ^{a}	1.77 ^{abc}	0.20	4.21
$M_1C_{1.5}$	0.70	33.00 ^{a}	221.87	10.33	0.14 ^{a}	1.83 ^{ab}	0.19	4.11
M_2C_0	0.58	22.66 ^{d}	142.41	5.20	0.05 ^{b}	0.80 ^{ed}	0.19	3.53
$M_2C_{0.75}$	0.74	26.00 ^{c}	182.02	8.73	0.02 ^{b}	0.53 ^{e}	0.17	4.26
$M_2C_{1.5}$	0.72	29.00 ^{b}	191.58	8.93	0.14 ^{a}	2.26 ^{a}	0.19	3.82
M_3C_0	0.56	21.33 ^{d}	142.35	5.20	0.04 ^{b}	0.76 ^{ed}	0.20	3.68
$M_3C_{0.75}$	0.72	27.00 ^{c}	187.14	8.42	0.07 ^{b}	1.32 ^{bcd}	0.23	4.48
$M_3C_{1.5}$	0.65	29.00 ^{b}	193.91	9.04	0.06 ^{b}	1.16 ^{cde}	0.23	4.59

NS= not significant, *= p≤0.05 and **= p≤0.01. Means followed by a different lowercase letters in a column were significantly different according to Duncan's multiple-range test (p≤0.05).

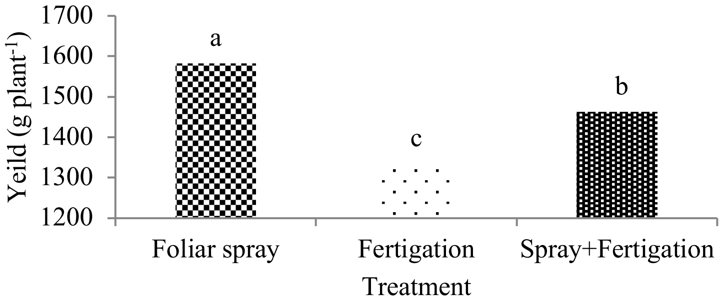

Figure 1. Effect of different application methods of seaweed (*Sargasum boveanum*) extract on cucumber yield.

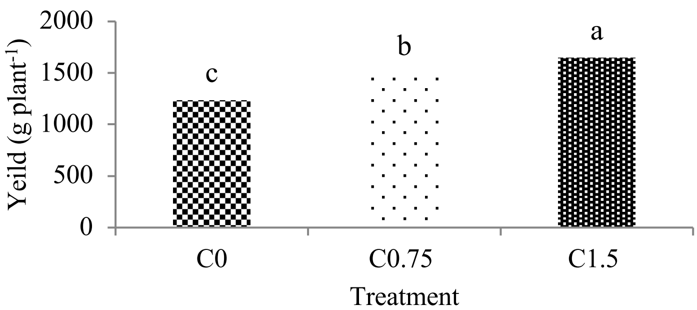

Figure 2. Effect of different concentrations of seaweed (*Sargasum boveanum*) extract on cucumber yield.

Table 3. Quantitative (fruit weight, fruit length, fruit diameter, firmness) and qualitative (fruit dry matter (Fruit DM), total soluble solid (TSS), EC, pH, and nitrate of fruit extract) changes of cucumber fruit in response to different concentration (C0, C0.75, and C1.5 g L-1) and different application method (M1: foliar sprays, M2: fertigation, M3: combined foliar sprays and fertigation) of seaweed extract (SwE)

Treatments	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Firmness (N)	Fruit DM (%)	TSS (%)	EC (dS/m)	pH	Nitrate (mg kg^{-1})
Method (M)	**	**	**	**	*	NS	NS	NS	**
Concentration (C)	**	**	**	**	**	**	**	*	**
M×C	**	**	**	**	NS	NS	NS	NS	*
Method									
Foliar sprays (M_1)	90.11 ^{a}	14.76 ^{a}	2.69 ^{a}	63.80 ^{c}	3.71 ^{a}	3.04	1.61	5.16	116.39 ^{b}
Fertigation (M_2)	83.33 ^{b}	13.96 ^{c}	2.50 ^{c}	65.58 ^{a}	3.48 ^{b}	2.87	1.57	5.01	142.08 ^{a}
Combined (M_3)	85.88 ^{b}	14.39 ^{b}	2.58 ^{b}	65.28 ^{b}	3.56 ^{b}	2.94	1.58	5.23	149.54 ^{a}
Concentration (g L^{-1})									
0 (C_0)	80.33 ^{c}	12.57 ^{c}	2.30 ^{c}	58.01 ^{c}	3.42 ^{b}	2.73^{b}	1.50 ^{b}	4.95 ^{b}	86.59 ^{c}
0.75 ($C_{0.75}$)	87.33 ^{b}	15.18 ^{b}	2.76 ^{a}	67.79 ^{b}	3.59 ^{a}	3.01 ^{a}	1.62 ^{a}	5.27 ^{a}	150.14 ^{b}
1.5 ($C_{1.5}$)	91.66 ^{a}	15.36 ^{a}	2.72 ^{b}	68.87 ^{a}	3.74 ^{a}	3.12 ^{a}	1.64 ^{a}	5.17 ^{a}	171.27 ^{a}
M×C									
M_1C_0	80.00 ^{d}	12.68 ^{g}	2.30 ^{f}	57.66 ^{g}	3.42	2.73	1.49	4.94	81.26 ^{d}
$M_1C_{0.75}$	90.33 ^{b}	15.64 ^{b}	2.91 ^{a}	65.35 ^{e}	3.73	3.13	1.65	5.41	122.42 ^{c}
$M_1C_{1.5}$	100.00 ^{a}	15.96 ^{a}	2.85 ^{b}	68.45 ^{d}	3.99	3.26	1.68	5.12	145.48 ^{c}
M_2C_0	80.00 ^{d}	12.54 ^{h}	2.30 ^{f}	58.22 ^{f}	3.42	2.73	1.51	4.73	88.76 ^{d}
$M_2C_{0.75}$	85.00 ^{c}	14.71 ^{e}	2.61 ^{e}	69.25 ^{a}	3.52	2.93	1.61	5.13	153.30 ^{b}
$M_2C_{1.5}$	85.00 ^{c}	14.64 ^{f}	2.60 ^{e}	69.27 ^{a}	3.51	2.96	1.60	5.16	184.17 ^{a}
M_3C_0	81.00 ^{d}	12.49 ^{h}	2.31 ^{f}	58.22 ^{f}	3.43	2.73	1.52	5.18	89.74 ^{d}
$M_3C_{0.75}$	86.66 ^{bc}	15.20 ^{d}	2.75 ^{c}	68.76 ^{c}	3.54	2.96	1.60	5.27	174.71 ^{a}
$M_3C_{1.5}$	90.00 ^{b}	15.49 ^{c}	2.69 ^{d}	68.87 ^{b}	3.73	3.13	1.63	5.24	184.17 ^{a}

NS= not significant, *= $p \leq 0.05$ and **= $p \leq 0.01$. Means followed by a different lowercase letters in a column were significantly different according to Duncan's multiple-range test ($p \leq 0.05$).

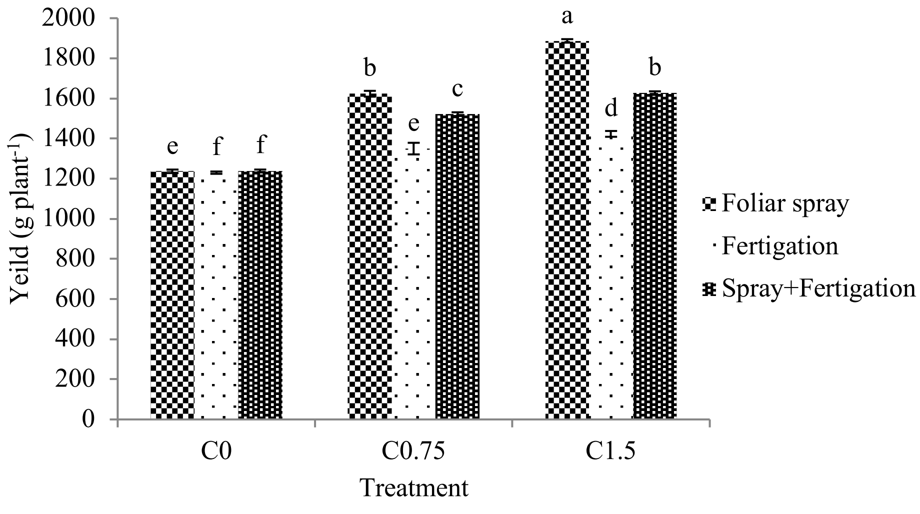

Figure 3. Effect of different application methods and concentration of seaweed (*Sargasum boveanum*) extract on cucumber yield.

Figure 4. Effect of different application methods of seaweed (*Sargasum boveanum*) extract on cucumber shape (Concentration= 1.5 g L^{-1}).

4. Discussion

Native seaweed species should be tested for their industrial and biotechnological potential as a source of bioactive compounds. The use of SwE has several advantages, such as biodegradability, harmlessness and compatibility with the environment, the absence of toxic residues, and resistance to disease due to the existence of a unique and exceptional biomolecular structure compared to chemical fertilizers (Ashour et al., 2020; Hassan et al., 2021). In the present study, the results indicated that the use of SwE obtained from *Sargassum bovianum* increased growth parameters, yield, and photosynthesis. Similar results have been reported by Hamed et al. (2018) and Hassan et al. (2021). These studies showed that SwE with adequate amounts of nutrients, rare-earth elements, vitamins, phytohormones, ascorbic acids, and many other bioactive compounds can increase the growth and yield of cucumber plants. Data are also consistent with La Bella et al. (2021). In this study, the marketable fruit increase prompted by the SwE application was due to a higher fruit mean mass rather than to the higher number of marketable fruits (Data not shown). Therefore, the use of SwE improves the plant's physiological responses, such as stimulating the absorption of nutrients, developing a strong root system, and increasing the leaf area, biomass, photosynthesis, and yield, and is a suitable option to minimize the use of conventional fertilizers (Bajpai, 2016; Abdel-Latif, 2018). Similar results were observed in the improvement of growth rate of *Kappaphycus striatus* plants using two liquid extracts from brown seaweeds, namely *Sargassum cristaefolium* and *Turbinaria conoides*, before out-planting (Tahiluddin et al., 2022). The findings of the researchers showed that the K content in SwE positively increases photosynthesis, meristem growth, and water content in treated plants. Moreover, P content activated root proliferation and increased the ratio of root/stem. Also, Calcium (Ca^{2+}) in SwE contributed to cell elongation, cell stability, and enzyme activation in treated plants (Ahmed et al., 2021).

Xu and Leskovar (2015), reported the application of SwE in spinach plants led to a large leaf area and high photosynthetic rate by improving leaf water relations, maintaining cell turgor pressure, and reducing stomatal limitation. Stimulation of the root system growth by SwE may result from the action of phytohormones like auxins and cytokinins. These compounds are important in the initiation of lateral and adventitious root development, as well as causing increases in total root biomass (Szczepanek et al., 2017). Change in root morphology is one of the major mechanisms by SwE that affect nutrient uptake. Previous findings showed that the increase in K absorption in cucumber plants treated by SwE may be related to several modes of action: (1) the presence of signaling molecules (free amino acids and soluble peptides) in SwE, (2) absorption, movement and accumulation of more nutrients that affect the stimulation of root system architecture, and (3) expression of genes encoding macronutrient transporters in the cell membrane (Lucini et al., 2018; Rouphael et al., 2018; Sestili et al., 2018). According to the results of the researchers, the use of SwE as a foliar spray is currently very useful and advantageous because it can increase vegetative growth and yield in crops such as cereals, leafy vegetables, cucumber, tomato, and pepper (Ali et al., 2016; Rouphael et al., 2018; Trejo Valencia et al., 2018; Ashour et al., 2020). The trend of cucumber production in the future is organic cucumber production because it has high consumer demand. The pricing of these products in the foreign market can be higher than the pricing of normal products and as a result increase value of production and productivity (Trejo Valencia et al., 2018).

In this study, the use of SwE increased the concentration of K in cucumber, which is consistent with the results of Rouphael et al. (2018) and La Bella et al. (2021) in spinach. Results related to the increase of nitrogen, P, K, and Mg uptake in greenhouse cucumbers using SwE have already been reported (Valencia et al., 2018). Abdel-Mawgoud et al. (2010) reported that increasing vegetative growth by SwE was an effect on cucumber fruit growth, in which single fruit weight, fruit diameter, and fruit number were higher due to the absorption of more assimilates into fruits and conversion of fruits to high potential sink (Hassan et al., 2020). To explain these results, we can refer to the extract containing the potassium element, which is more absorbed by the plant than any other element, and is the dominant cation in the plant and its great importance in the process of cell division and regulating permeability. It regulates the membranes in the plant and the transport of sugar and protein, which had a positive effect on increasing the number of fruits, fruits weight, and the yield of cucumber plants (Hassan et al., 2020). Rouphael et al. (2010) study showed that increasing biomass production and crop yield of greenhouse cucumbers treated with SwE can be expected because the plants treated with SwE have a greater capacity to maintain a high rate of net photosynthesis and a better nutritional compounds (high concentration of P, K, magnesium, iron, zinc, and manganese) compared to untreated plants (Rouphael et al., 2010). It seems that in the interaction effects of this experiment, the positive changes in fruit weight, fruit length, and yield of cucumbers are more influenced by the absorbed elements such as K, free amino acids such as lysine, glycine, aspartic acid, etc., beneficial fatty acids and hormones and pseudo-hormones rather than the changes in root architecture and photosynthesis rate which these compounds have been mentioned in the review of the components of *Sargasum boveanum* (Khalifeh et al., 2021).

Mannino et al. (2020) reported that the improved productivity of tomato plants under SwE application can be related to signaling molecules including polysaccharides (alginates, fucoidan, and laminarins), soluble peptides, oligopeptides, and free amino acids, which are made up about 30–40% of the seaweed extract (on dry weight basis) and increase the promoting endogenous phytohormonal biosynthesis (auxin- and/or gibberellin-like activities) thus enhancing crop yield (Ertani et al., 2017; Rouphael et al., 2018).

In this experiment, apart from nitrate content, the biochemical characteristics of the cucumber fruit such as total soluble sugars (TSS), EC, and pH were more influenced by the main effects of the application method and the concentration of the SwE than by the interaction effects of the treatments. Kumari et al. (2011) reported a higher concentration of TSS in tomato crops with soil application and foliar application of *Sargasum johntonii* SwE. The increase in the amount of TSS in cucumber fruit can be directly related to the polysaccharide content of SwE which increases the synthesis of the main compounds of TSS such as organic acids, metabolites, and glucose (Sendur Kumaran, 2016; Mzibra et al., 2021). Trejo Valencia et al. (2018) reported that the use of SwE is very effective for improving fruit formation, fruit shelf life, yield, and increasing fruit quality in cucumbers. In recent studies, it has been shown that if biological stimulants such as SwE are used, made the larger xylem cells and phloem vascular bundles in the stems, and this phenomenon can help to transfer minerals and assimilates to the sink more effectively and increase the concentration of minerals in the fruit. The increased mineral concentration in the fruit of treated plants may also be due to a greater absorption of minerals through stimulation of root growth and the activity of nutrient transporters in cell membranes (Billard et al., 2014; Colla et al., 2017). In agreement with these findings, the results of this experiment clearly indicated the effects of different concentrations of seaweed on root growth, stem diameter, TSS, EC, and pH of fruit extract.

The results of this experiment showed that the SwE application enhanced fruit firmness in the fertigation method more than the foliar spray and combined method. This is consistent with previous research on the influence of plant-based biostimulants (Basile et al., 2021; Consentino et al., 2021). The effect of SwE on fruit firmness is likely related to more absorption and accumulation of Ca in plants treated with SwE compared to the control. The calcium-pectin crosslinks play an important role in the resistance of cell walls and thus the physical and structural characteristics of the fruit. Furthermore, since it is assumed that auxins partake in the transport and uptake of fruit Ca^{2+}, the present study suggested that SwE may have an auxin-like action and improve Ca nutrition in cucumber fruits (Hocking et al., 2016; Basile et al., 2021; Consentino et al., 2021; Cozzolino et al., 2021). It seems that SwE has played a more effective role in fruit firmness by influencing the process of Ca transfer in the fertigation method compared to the foliar spraying and combined methods. In other words, the degree of firmness of the fruit is more influenced by the effective role of SwE in the transfer of Ca in the fertigation method than by the effect of the concentration of Ca in the SwE (foliar spraying method). To prove this claim, more studies are needed in the rhizosphere and at the cellular and molecular levels.

As well as the use of SwE of *Sargasum boveanum* increased the nitrate concentration of cucumber fruit, but the combined use of $M_1C_{0.75}$ controlled the nitrate concentration of cucumber fruit according to the standard of World Health Organization (WHO) (150 mg kg^{-1} of fresh weight). Although, it has been more in the fertigation method than the foliar spraying method, like the firmness of the fruit. It seems that the role of SwE in the fertigation method is more related to the transfer of more ions from the substrate, which has been able to cause more firmness and more nitrates in the fruit. SwE contains amino acids and free peptides to prevent the accumulation of nitrates in the leaf tissue, if used correctly could be associated with an up-regulation of the key nitrogen assimilation genes like nitrate reductase, thus contributing to a higher assimilation of nitrates into amino acids (Tsouvaltzis et al., 2014; Rouphael et al., 2018). Therefore, it seems that the assimilation of nitrate in the foliar spraying method has been done in larger amounts compared to the fertigation and combination methods, which led to a lower concentration of nitrate in cucumber fruits. As a result, long fruits and small diameter in the $M_1C_{1.5}$ compared to the $M_1C_{0.75}$ can be affected by the high level of assimilation of chemical compounds, cell growth and development, and intercellular space in cucumber fruits under seaweed foliar application.

5. Conclusion

The findings of this research showed that although the use of common methods such as foliar spraying of seaweed extract can improve the growth and quality indicators of cucumber fruit in the greenhouse, the use of methods such as the combination of foliar spraying and fertigation can prevent the possible harms of foliar spraying and improve quantitative characteristics such as weight of single fruit, fruit length, fruit firmness and finally total yield. Also, the results of this experiment showed that in most of the traits studied, the concentration of 1.5 g L^{-1} of seaweed extract produced better results than other concentrations in improving indicators such as leaf fresh weight, number of leaves, fruit weight, fruit length, and total yield. It has been suggested for cucumber producers in greenhouses around the world. The fact that the simultaneous use of fertigation and foliar spraying could cause positive changes in the growth and quantitative and qualitative characteristics of cucumber requires more studies in the rhizosphere and the cellular and molecular level of the plant.

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