

Synergism of microorganisms and seaweed extract on vegetative growth, yield and quality of cucumber fruit

Alonso MENDEZ¹, Silvia MARTINEZ¹, Aida LEAL¹,
Armando HERNANDEZ², Juana GARCIA², Miriam SANCHEZ^{3*}

¹Universidad Autónoma Agraria Antonio Narro, Botany Department, 1923 Buenavista, Saltillo, C.P. 25315, Coahuila, México; alonso1977@gmail.com; syma_mx@yahoo.com; aisaler@yahoo.com.mx

²Universidad Autónoma Agraria Antonio Narro, Horticulture Department, 1923 Buenavista, Saltillo, C.P. 25315, Coahuila, México; hernandez865@hotmail.com; jhoana_gs@yahoo.com.mx

³CONAHCYT-Universidad Autónoma Agraria Antonio Narro, Parasitology Department, 1923 Buenavista, Saltillo, C.P. 25315, Coahuila, México; mirisanve@gmail.com (*corresponding author).

Abstract

Natural biostimulants, such as microorganisms and seaweed extracts, are used in agriculture to improve crop yields with a sustainable approach. However, the interaction effects between different biostimulants have not been thoroughly investigated. The objective of this work was to evaluate the synergistic effects of microorganisms (*Glomus intraradices* and *Azospirillum brasilense*) and seaweed extract (*Ascophyllum nodosum* and *Macrocystis pyrifera*) on growth, yield and fruit quality of cucumber plants under soilless cultivation. Two doses of microorganisms (0 and 10 spores mL⁻¹ + 10⁶ CFU) and four concentrations of seaweed extract (0, 250, 500 and 2500 ppm) were evaluated. The experimental design used was a randomized complete block design with a factorial arrangement (2 x 4), with four replications per treatment. The results showed that the equatorial diameter of the fruit, yield and titratable acidity improve when applying microorganisms and seaweed extract in combination; however, when applying the two biostimulants the values of plant height, stem diameter, number of leaves, root length, biomass (fresh and dry), firmness, total soluble solids, vitamin C, chlorophyll (*a* and *total*) and indices of color (L* and b*) did not exceed those obtained when the biostimulants were applied individually. The combined application of microorganisms and seaweed extract improves cucumber yield, but not vegetative growth and, except for titratable acidity, fruit quality attributes.

Keywords: biomass; biostimulants; chlorophyll; titratable acidity; total soluble solids

Introduction

Cucumber (*Cucumis sativus* L.) is a vegetable with great global demand, it is grown for fresh fruits or pickles (González *et al.*, 2020b; Salim *et al.*, 2021). Cucumber production requires moderate to high nutrient rates to achieve high yield and quality (Eifediyi and Remison, 2010; Kumar, 2020). There is currently an urgent need to apply sufficient nutrients to maintain crop growth and yield, thus meeting the food demand of a continuously growing population (Cordell *et al.*, 2009; Ibraheem, 2020). However, the unbalanced use of

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chemical fertilizers has brought problems such as soil deterioration, groundwater contamination and environmental pollution (Pathak *et al.*, 2017; Lin *et al.*, 2019). In this sense, Adesemoyea *et al.* (2010) have indicated that one of the possible solutions to reduce the risk of accumulation of chemical fertilizers in the environment is to combine chemical fertilizers with biostimulants.

Plant biostimulants are any substance (humic and fulvic acids, protein hydrolysates and seaweed extracts) or microorganisms (mycorrhizal fungi and plant growth-promoting rhizobacteria) applied to the plant or the rhizosphere with the aim of improving nutritional efficiency, abiotic stress tolerance, crop quality attributes and/or yield, regardless of their nutrient content (du Jardin, 2015; González *et al.*, 2020a). Microorganisms (arbuscular mycorrhizal fungi and plant growth regulator bacteria) and seaweed extracts are one of the important strategies to ensure sustainable agriculture (Ashour *et al.*, 2020; Ngoroyemoto *et al.*, 2020; Hassan *et al.*, 2021). Arbuscular mycorrhizal fungi increase the absorption of soil minerals (P and N), improve plant tolerance to stress (biotic and abiotic), improve the quality of soil structure and accelerate the biochemical and enzymatic activities of plants (Perner *et al.*, 2007; Altuntas *et al.*, 2015; Sivakumar *et al.*, 2020). The main plant growth regulator bacteria used as biofertilizers include species of the genera *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, *Burkholderia*, *Streptomyces* and *Rhizobia* (Glick, 2012; Pathak *et al.*, 2017). *Azospirillum brasilense* is one of the free-living bacterial species that fixes to atmospheric nitrogen and produces plant hormones (Contreras *et al.*, 2019; Cassán *et al.*, 2020). Seaweed extracts contain macro and microelements, vitamins, amino acids, natural plant growth hormones and sugars (Battacharyya *et al.*, 2015; Ozbay and Demirkiran, 2019; Shukla *et al.*, 2019). Due to the bioactive compounds and specific functions of the microorganisms and seaweed extracts in the growth medium and the plant, the growth and yield of the crops are increased.

González *et al.* (2020a) indicate that the application of microbial and non-microbial biostimulants can efficiently improve yields without increasing the amount of nutrients applied. The efficiency of the combined application of biostimulants based on microorganisms and seaweed extract on the growth and yield of crops has been related to greater availability of nutrients, greater amount of natural hormones and improvement of the microbial population driven by the seaweed extract (Anli *et al.*, 2020; González *et al.*, 2020a). However, the knowledge of the effects of the simultaneous application of microorganisms and seaweed extract on the growth and yield of crops is scarce (González *et al.*, 2020a; Ngoroyemoto *et al.*, 2020; Vafa *et al.*, 2021). In this context, the present work aimed to evaluate the synergistic effects of microorganisms (*Glomus intraradices* and *Azospirillum brasilense*) and seaweed extract (*Ascophyllum nodosum* and *Macrocystis pyrifera*) on the growth, yield and fruit quality of cucumber plants.

Materials and Methods

Location and establishment of the crop

The research work was performed in a greenhouse at Universidad Autónoma Agraria Antonio Narro, located in Saltillo, Coahuila, Mexico. Sowing seeds of cucumber cv. 'Centauro' was performed on June 26, 2020 in black polyethylene containers with a volume of 8 L, using a mixture of acid peat and perlite (1:1, v:v) as growth medium. One seed was placed per container.

Description of the treatments

The treatments consisted of two doses of microorganisms (0 and 10 spores mL⁻¹ + 10⁶ Colony Forming Units or CFU) and four concentrations of seaweed extract (0, 250, 500 and 2,500 ppm). The microorganisms evaluated contained a consortium of *Glomus intraradices* (1,000 spores mL⁻¹) and *Azospirillum brasilense* (1 x 10⁶ CFU mL⁻¹); and a liquid compound of seaweed extract based on *Ascophyllum nodosum* and *Macrocystis*

pyrifera (25%). The microorganisms were applied to the substrate, through three applications during the development of the experiment, the first application was made 15 d after emergence and then every 21 d until completing the three applications. The application of the seaweed extract was carried out by foliar spraying, with three applications during the experiment at intervals of 15 d, the first application was made 15 d after emergence. In all treatments, the nutrient solution proposed by Steiner (1961) (meq L⁻¹: 12 NO₃⁻, 1 H₂PO₄⁻, 7 K⁺, 4 Mg²⁺, 9 Ca²⁺ and 7 SO₄²⁻) was used as the fertilization base. The sources of the macronutrients used for the preparation of the nutrient solution were fertilizer grade ((Ca (NO₃)₂, K NO₃, H NO₃, K₂ SO₄, Mg SO₄ and H₃ PO₄). For the formulation of the base nutrient solution, the chemical properties of the irrigation water were considered. The pH of the solution was adjusted to 6.0 ± 0.1 before each irrigation with H₂ SO₄ at 1 N. The application of the Steiner solution began 15 d after emergence. The irrigations were carried out manually according to the water needs of the plants, applying a sufficient volume of the nutrient solution to maintain a 20% leachate fraction.

Parameters evaluated

The experiment ended on October 4, 2020. The plants were evaluated in terms of internode distance, plant height, number of leaves per plant and stem diameter. In addition, the plant was separated into root, stem and leaves. Plant roots were washed with drinking water to remove excess substrate and root length was evaluated. Subsequently, the fresh weight of each plant organ was determined. The separated organs were placed in a drying oven at 65 °C for 72 h to subsequently record the weight of the dry matter with an analytical balance. Total fresh weight and total dry weight were obtained by adding the weight of each of the evaluated organs.

The harvest of fruits was carried out when they presented the characteristic size of the variety. After harvesting each fruit, the length, equatorial diameter, weight of each fruit and yield per plant were evaluated, the latter being determined by adding the weight of the fruits harvested throughout the crop cycle. The content of total soluble solids, vitamin C, titratable acidity, firmness, chlorophyll (*a*, *b* and *total*) and epidermal color index (L, a* and b*) were determined in the fourth fruit harvested from each plant. Total soluble solids content was determined by placing a drop of fresh juice on the prism of a digital refractometer (ATAGO®, USA Inc., Bellevue, WA, USA). Vitamin C was determined by the 2, 6-dichloroindophenol method by titration. Thielman's reagent was prepared according to the provisions of the AOAC (2000). A 20 g sample of fruit pulp was used, which was added to a flask (50 mL) with 10 mL of 2% HCl and brought to capacity. Subsequently, the content was filtered through cheesecloth into an Erlenmeyer flask. Aliquots of 10 mL were taken and titrated with Thielman's reagent until a pink color appeared without disappearing for 30 seconds. The reading was in spent mL of reagent. To calculate the content of ascorbic acid in the samples, a calibration curve was made with a standard solution of ascorbic acid, according to AOAC (2000). The concentration of vitamin C in the sample was calculated according to the formula:

$$\text{Vitamin C} = \left[\frac{VRT * 0.088 * VT * 100}{VA * P} \right] \quad (1)$$

where: vitamin C = in the sample expressed in mg in 100 g; VRT = volume spent in mL of Thielman's reagent; 0.088 = mg of ascorbic acid equivalent to one mL of Thielman's reagent; VT = total volume in mL of the total filtrate of vitamin C in HCl; VA = volume in mL of the valued aliquot; P = sample weight in grams.

Titratable acidity was determined according to the AOAC method (2000), for which a 10 mL sample of fruit juice was taken and calibrated to 125 mL of distilled water. This solution was titrated with 0.01N NaOH until it reached a pH of 8.3. The results of these measurements were expressed as a percentage of citric acid by applying the following formula:

$$\%acid = \left[\frac{(V_{NaOH} * N_{NaOH} * meq_{acid} * x * 100)}{V} \right] \quad (2)$$

where: V_{NaOH} = volume of NaOH used for the titration; N_{NaOH} = normality of NaOH; $\text{meq}_{\text{acidX}}$ = milliequivalents of acid; V = weight in g or volume in mL of the sample. The base to acid equivalent value for citric acid is 0.064.

Firmness was determined at three points on the fruit using a digital penetrometer (PCE-PTR 200, Grupo PCE, Albacete, Castilla La Mancha, Spain) equipped with a convex tip of 8.0 mm in diameter. The content of chlorophyll *a*, *b* and *total* of the epidermis of the fruit was quantified with the technique described by Witham *et al.* (1971). Lightness coordinates (L^*), color trends from green to red ($-a^*$ and $+a^*$) and from blue to yellow ($-b^*$ and $+b^*$) were measured on two opposite sides of the epidermis of the fruit using a Minolta Chroma Meter CR-400 (MinoltaCorp, Ramsey, NJ, USA).

Design and statistical analysis

The experimental design used was a randomized complete block design with a factorial arrangement (2×4), with four replications per treatment. The data obtained were subjected to an analysis of variance (ANOVA) and the comparison of means with Tukey's test ($\alpha \leq 0.05$) using the SAS program (Statistical Analysis Systems, SAS Institute, 2004) version 9.3.

Results

Plant development

Plant height and leaf number were statistically affected by the doses of microorganisms; while, plant height, stem diameter, internode distance and root length were significantly influenced by the concentrations of seaweed extract; on the other hand, plant height, number of leaves, stem diameter and root length were statistically affected by the interaction of the factors under study (Table 1).

Table 1. Effect of the doses of microorganisms and concentrations of seaweed extract on plant height, number of leaves, stem diameter, internode distance and root length of cucumber plants cv. 'Centaur'

Treatments	Plant height (cm)	Number of leaves (leaf plant ⁻¹)	Stem diameter (cm)	Internode distance (cm)	Root length (cm)
Microorganisms (spores mL ⁻¹ + 10 ⁶ CFU)					
0	576.41b	21.58a	5.66a	15.25a	55.02a
10	605.86a	20.83b	5.62a	15.36a	55.15a
Seaweed extract (ppm)					
0	581.34b	20.66a	5.81a	14.90b	61.26a
250	561.04b	21.00a	5.59ab	15.57a	57.96a
500	641.81a	21.66a	5.47b	15.72a	47.84b
2500	580.34b	21.50a	5.71ab	14.77b	53.29ab
ANOVA					
Microorganisms	0.032	0.036	0.519	0.163	0.953
Seaweed extract	0.001	0.167	0.01	0.001	0.003
Interaction	0.003	0.004	0.022	0.165	0.035

Means with different letters indicate significant effects according to Tukey's multiple comparison test with $p \leq 0.05$; ANOVA = analysis of variance ($P > F$), $\alpha \leq 0.01$ and a reliability of 99% were considered; Interaction = microorganisms * seaweed extract.

The interaction of the factors evaluated (Table 1) showed that when microorganisms were applied to the growth medium, plant height was higher with concentrations of 0 and 500 ppm of seaweed extract; while, when no microorganisms were added, the height of the plant was greater when applying the highest concentrations of seaweed extract (Figure 1A). Stem diameter was greater when microorganisms and seaweed extract were not added; but, when microorganisms were added, the stem diameter was greater when 0 and 2500 ppm of seaweed extract was applied (Figure 1B). On the other hand, when microorganisms were added to the growth medium, the number of leaves was higher when adding 500 ppm of seaweed extract; while, when microorganisms were not added, the number of leaves was higher with concentrations of 0 and 2500 ppm of seaweed extract (Figure 1C). While, when adding microorganisms to the culture medium, the length of the root was greater when no algae extract was applied; but, when no microorganisms were applied, the root length was greater when applying 0 and 250 ppm of algae extract (Figure 1D).

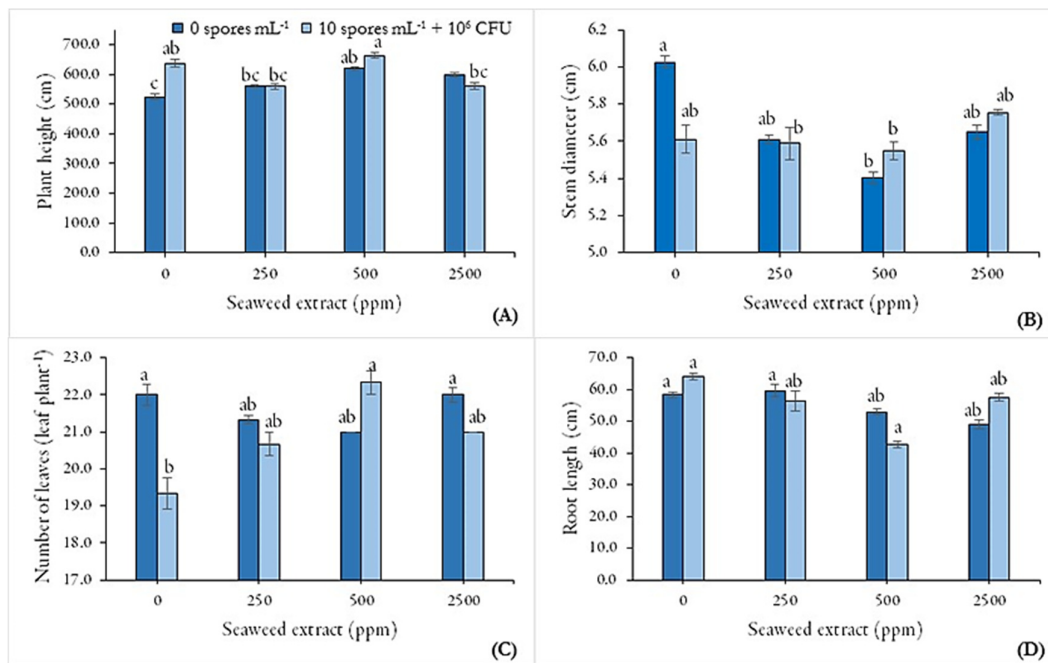


Figure 1. Effect of the interaction of the doses of microorganisms and concentrations of algae extract on the growth of cucumber plants cv. 'Centaur'; (A) plant height; (B) stem diameter; (C) number of leaves; (D) root length

Means with different letters indicate significant effects according to Tukey's multiple comparison test with $p \leq 0.05$. The bars indicate the standard error of the mean ($n = 4$)

Biomass production

Stem fresh weight, root fresh weight, stem dry weight, root dry weight and total dry weight were statistically affected by the dose of microorganisms added to the substrate; while, the stem fresh weight, leaf fresh weight, total fresh weight, stem dry weight, leaf dry weight, root dry weight and total dry weight were statistically influenced by the added concentrations of seaweed extract; likewise, stem fresh weight, leaf fresh weight, total fresh weight, leaf dry weight and total dry weight were affected by the interaction of the factors evaluated (Table 2).

Table 2. Effect of the doses of microorganisms and concentrations of seaweed extract on the fresh and dry weight of stem, leaf, root and total of cucumber plants cv. 'Centaur'

Treatments	Fresh weight (g plant ⁻¹)				Dry weight (g plant ⁻¹)			
	Stem	Leaf	Root	Total	Stem	Leaf	Root	Total
Microorganisms (spores mL ⁻¹ + 10 ⁶ CFU)								
0	178.33b	413.50a	27.83b	619.66a	12.83b	30.04a	1.54b	44.41b
10	192.67a	421.67a	33.38a	647.72a	14.99a	30.33a	1.83a	47.17a
Seaweed extract (ppm)								
0	189.66ab	430.16a	28.00a	647.84a	12.82b	28.50b	1.16b	42.50b
250	185.34b	433.50a	33.59a	652.43a	13.32b	32.83a	2.16a	48.32a
500	210.50a	453.84a	27.84a	692.18a	16.00a	31.66a	1.66ab	49.34a
2500	156.5c	352.84b	33.00a	542.33b	13.50ab	27.75b	1.75a	43.00b
ANOVA								
Microorganisms	0.021	0.572	0.037	0.085	0.004	0.654	0.030	0.022
Seaweed extract	0.001	0.001	0.230	0.001	0.013	0.001	0.001	0.001
Interaction	0.007	0.047	0.247	0.011	0.119	0.001	0.055	0.001

Means with different letters indicate significant effects according to Tukey's multiple comparison test with $p \leq 0.05$; ANOVA = analysis of variance ($Pr > F$), $\alpha \leq 0.01$ and a reliability of 99% were considered; Interaction = microorganisms * seaweed extract.

The interaction of the factors under study (Table 2) indicated that when applying microorganisms to the growth medium the leaf fresh weight, stem fresh weight and total fresh weight were higher when adding 0 and 500 ppm of seaweed extract; but, when these were not added, these parameters were higher with 250 and 500 ppm of seaweed extract (Figure 2A, 2B and 2C). On the other hand, when microorganisms were added to the growth medium the plants reached a higher leaf dry weight and total dry weight when applying a high concentration of seaweed extract; but, when no microorganisms were added the plants reached a higher leaf dry weight and total dry weight when applying 250 ppm of seaweed extracts (Figure 2D and 2E).

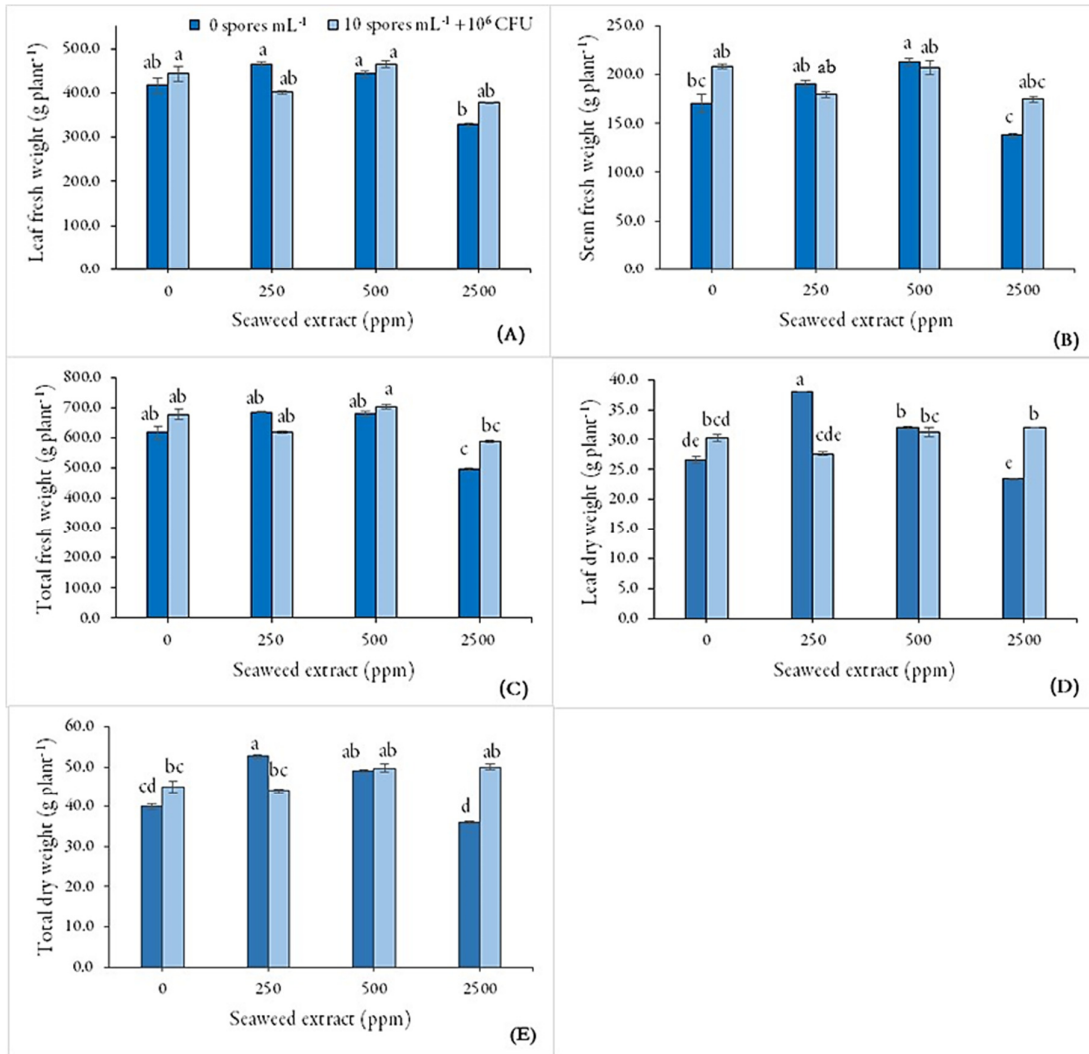


Figure 2. Effect of the interaction of the doses of microorganisms and concentrations of algae extract on the biomass of cucumber plants cv. ‘Centaur’; (A) leaf fresh weight; (B) stem fresh weight; (C) total fresh weight; (D) leaf dry weight; (E) total dry weight. Means with different letters indicate significant effects according to Tukey’s multiple comparison test with $p \leq 0.05$. The bars indicate the standard error of the mean ($n = 4$)

Fruit development and total yield

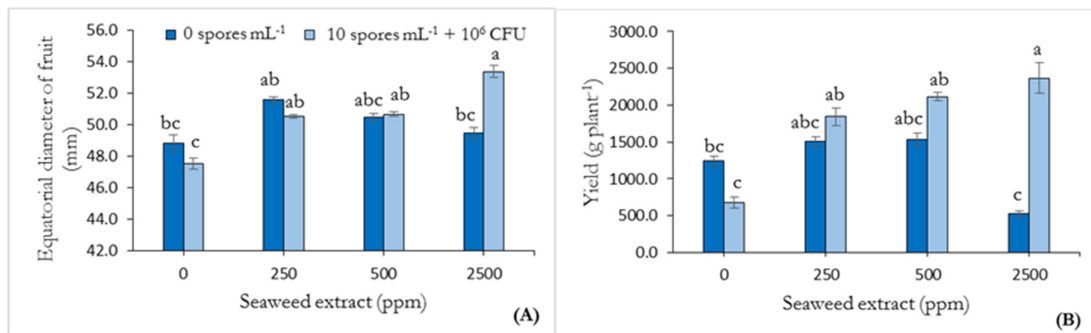
The average fruit weight and yield were statistically affected by the dose of microorganisms added to the substrate; meanwhile, the average fruit weight, fruit length, equatorial fruit diameter and yield were statistically influenced by the concentrations of seaweed extract; likewise, these last two parameters were statistically affected by the interaction of the two factors studied (Table 3).

Table 3. Effect of the doses of microorganisms and concentrations of seaweed extract on the average weight of fruit, longitudinal diameter of fruit, equatorial diameter of fruit and yield of cucumber plants cv. 'Centaur'

Treatments	Average fruit weight (g fruit ⁻¹)	Fruit length (cm)	Equatorial diameter of fruit (mm)	Yield (g plant ⁻¹)
Microorganisms (spores mL ⁻¹ + 10 ⁶ CFU)				
0	345.29a	21.91a	50.54a	1203.8b
10	312.04b	22.14a	50.09a	1752.3a
Seaweed extract (ppm)				
0	309.28b	21.51b	48.20b	959.0b
250	320.74b	21.72b	51.06a	1677.5a
500	301.55b	21.76b	50.57a	1828.2a
2500	383.11a	23.11a	51.42a	1447.5ab
ANOVA				
Microorganisms	0.016	0.321	0.308	0.002
Seaweed extract	0.001	0.001	0.001	0.003
Interaction	0.489	0.084	0.001	0.001

Means with different letters indicate significant effects according to Tukey's multiple comparison test with $p \leq 0.05$; ANOVA = analysis of variance ($Pr > F$), $\alpha \leq 0.01$ and a reliability of 99% were considered; Interaction = microorganisms * seaweed extract.

The interaction between the factors under study (Table 3) indicated that the equatorial diameter of the fruit and total yield were greater when microorganisms were added to the substrate, mainly when it was applied with 2500 ppm of seaweed extract; while, when no microorganisms were added to the substrate, the cucumber fruits presented greater equatorial diameter and total yield when applying 250 and 500 ppm of algae extract (Figure 3A and 3B).

**Figure 3.** Effect of the interaction of the doses of microorganisms and concentrations of algae extract in the production of cucumber fruits cv. 'Centaur'; (A) equatorial diameter of the fruit; (B) yield of cucumber plants

Means with different letters indicate significant effects according to Tukey's multiple comparison test with $p \leq 0.05$. The bars indicate the standard error of the mean ($n = 4$).

Fruit quality

Firmness, total soluble solids and titratable acidity were statistically affected by the dose of microorganisms; while, firmness, total soluble solids, titratable acidity and vitamin C were statistically influenced by the concentrations of seaweed extract, these same variables were statistically influenced by the interaction of the two factors evaluated (Table 4).

Table 4. Effect of the doses of microorganisms and concentrations of seaweed extract on the firmness, total soluble solids, titratable acidity and vitamin C of the fruits of cucumber cv. 'Centaur'

Treatments	Firmness (Newton)	Total soluble solids (^o Brix)	Titratable acidity (% citric acid)	Vitamin C (mg 100 g ⁻¹ fresh weight)
Microorganisms (spores mL ⁻¹ + 10 ⁶ CFU)				
0	56.27b	3.27a	0.09b	36.34a
10	58.16a	2.15b	0.12a	35.62a
Seaweed extract (ppm)				
0	57.88a	2.15c	0.09b	37.48a
250	59.28a	2.64b	0.11ab	36.42a
500	61.34a	2.92a	0.12a	32.83b
2500	50.38b	3.12a	0.10ab	37.19a
ANOVA				
Microorganisms	0.042	0.001	0.001	0.368
Seaweed extract	0.001	0.001	0.004	0.002
Interaction	0.001	0.001	0.001	0.001

Means with different letters indicate significant effects according to Tukey's multiple comparison test with $p \leq 0.05$; ANOVA = analysis of variance ($Pr > F$), $\alpha \leq 0.01$ and a reliability of 99% were considered; Interaction = microorganisms * seaweed extract.

microorganisms were added was greater when no seaweed extract was added; but, when no microorganisms were added, the firmness of the fruit was higher when 250 and 500 ppm of seaweed extract was added (Figure 4A). The content of total soluble solids was higher when microorganisms were not applied to the substrate, with higher growth when the concentrations of seaweed extract was increased; while, when microorganisms were applied, the total soluble solids were similar in the different concentrations of seaweed extract (Figure 4B). In contrast, titratable acidity was higher when microorganisms were added to the substrate, with higher titratable acidity when high concentrations of seaweed extract were added; but, when no microorganisms were added, the titratable acidity of cucumber fruits was reduced by applying seaweed extract (Figure 4C). On the other hand, when microorganisms were added, the vitamin C of the fruits was higher with 0 and 250 ppm of seaweed extract; while, when microorganisms were not added to the substrate, the content of vitamin C in the fruits was higher when adding 2500 ppm of algae extract (Figure 4D).

The content of chlorophyll *a* and the color indices *L**, *a** and *b** were statistically influenced by the doses of microorganisms; while, chlorophyll *a*, chlorophyll *b*, total chlorophyll and the color indices *L**, *a** and *b** were statistically affected by the concentrations of seaweed extract; while, chlorophyll *a*, total chlorophyll and the *L** and *b** color indices were statistically affected by the interaction of the evaluated factors (Table 5).

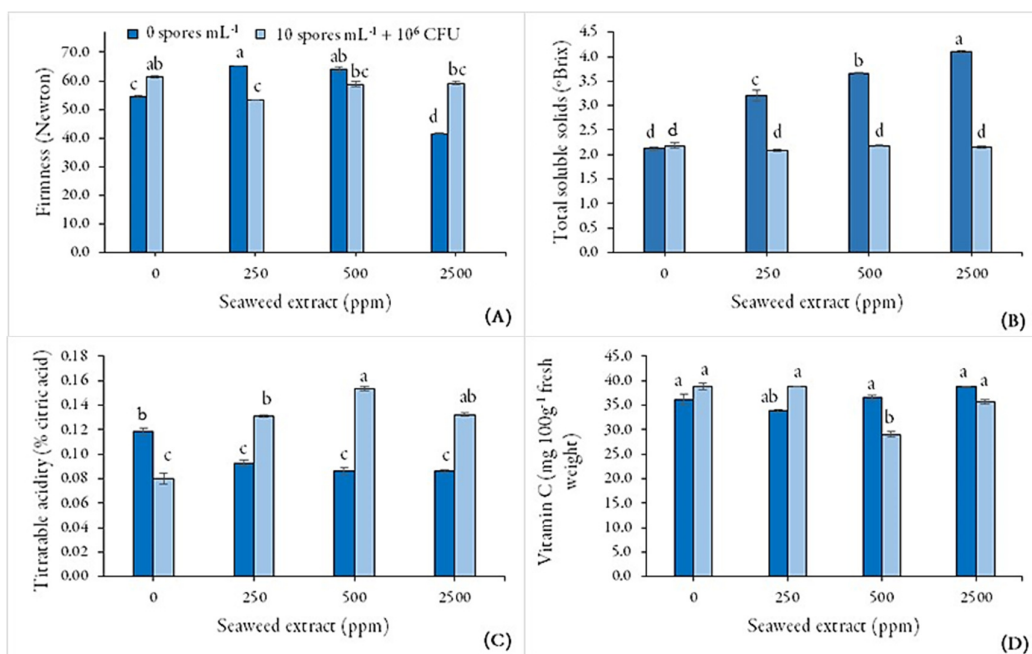


Figure 4. Effect of the interaction of the doses of microorganisms and concentrations of algae extract on the quality of cucumber fruits cv. 'Centaur'; (A) firmness; (B) total soluble solids; (C) titratable acidity; (D) vitamin C

Means with different letters indicate significant effects according to Tukey's multiple comparison test with $p \leq 0.05$. The bars indicate the standard error of the mean ($n = 4$).

Table 5. Effect of the doses of microorganisms and concentrations of seaweed extract on chlorophyll *a*, chlorophyll *b*, total chlorophyll, L^* , a^* and b^* of the epidermis of cucumber fruits cv. 'Centaur'

Treatments	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total Chlorophyll	L^*	a^*	b^*
	(mg g ⁻¹ fresh weight)					
Microorganisms (spores mL ⁻¹ +10 ⁶ CFU)						
0	0.05b	0.05a	0.10a	26.77b	-5.38a	5.59b
10	0.06a	0.05a	0.10a	28.44a	-6.53b	6.40a
Seaweed extract (ppm)						
0	0.06a	0.04b	0.10ab	26.52b	-5.99b	6.13a
250	0.06a	0.05ab	0.17a	29.27a	-6.36b	6.73a
500	0.04b	0.04b	0.08b	27.93ab	-6.57b	6.20a
2500	0.04b	0.06a	0.10ab	26.68b	-4.91a	4.91b
ANOVA						
Microorganisms	0.005	0.228	0.266	0.001	0.001	0.002
Seaweed extract	0.001	0.019	0.005	0.001	0.001	0.001
Interaction	0.001	0.204	0.019	0.001	0.118	0.03

Means with different letters indicate significant effects according to Tukey's multiple comparison test with $p \leq 0.05$; ANOVA = analysis of variance ($Pr > F$), $\alpha \leq 0.01$ and a reliability of 99% were considered; Interaction = microorganisms * seaweed extract.

The interaction of the factors under study (Table 5) showed that when microorganisms were incorporated into the growth medium, the content of chlorophyll *a* and *total* chlorophyll in the fruits were higher with concentrations of 0 and 250 ppm of seaweed extract; while, by not incorporating microorganisms into the substrate, the content of chlorophyll *a* and *total* chlorophyll were higher when applying 250 and 2500 ppm of seaweed extract (Figure 5A and 5B). Likewise, when microorganisms were added to the growth medium, cucumber fruits presented higher *L** and *b** when applying 0 and 250 ppm of seaweed extract; while, when microorganisms were not applied to the growth medium, the value of *L** and *b** were higher when applying seaweed extract at 250 and 500 (Figure 5C and 5D).

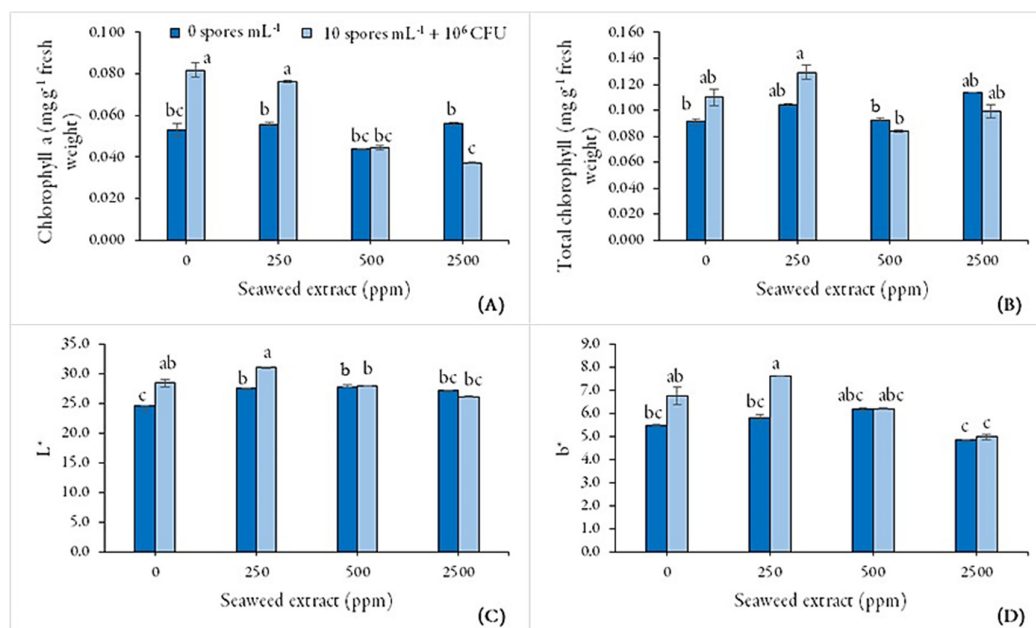


Figure 5. Interaction effect of microorganism doses and algae extract concentrations on chlorophyll content and color index in cucumber fruits cv. 'Centaur'; (A) chlorophyll *a*; (B) *total* chlorophyll; (C) *L**; (D) *b**

Means with different letters indicate significant effects according to Tukey's multiple comparison test with $p \leq 0.05$. The bars indicate the standard error of the mean ($n = 4$).

Discussion

Plant development

Some studies have reported better growth of aonla (Kumar *et al.*, 2014), tomato (Lira *et al.*, 2014) and rice (Ruiz *et al.*, 2011) plants when inoculated with biostimulant based on microorganism (mycorrhizae and *Azospirillum*), attributing this effect to the improvement of the availability of nutrients by microorganisms (Kumar *et al.*, 2014; Lira *et al.*, 2014). In this work, when only the biostimulant based on microorganisms was applied (10 spores mL⁻¹ + 10⁶ CFU and 0 ppm of seaweed extract), an increase in the height of cucumber plants was observed, compared to when no applied this biostimulant (0 spores mL⁻¹ and 0 ppm of seaweed extract); however, the stem diameter, leaf number and root length did not present the same response. On the other hand, it has been reported that seaweed extract stimulates crop development because it contains a wide variety of substances that promote vegetative growth (Craigie, 2011; Battacharyya *et al.*, 2015; Rayorath *et al.*, 2008). Nevertheless, spraying cucumber plants with seaweed extract (without the addition of microorganisms) only favored plant height and internode distance. Likewise, a synergism on plant growth has been reported when

microorganisms and seaweed extract are applied simultaneously (Anli *et al.*, 2020); but, in this study, a positive effect on the growth of cucumber plants was not observed when applying both biostimulants, since the effect on growth obtained when the biostimulants were applied independently was not exceeded.

Biomass production

Biomass accumulation is largely determined by the photosynthetic performance of a plant (Keller *et al.*, 2022). Inoculation of plants with mycorrhizal fungi and growth-promoting bacteria has been reported to improve plant photosynthesis (Ruiz *et al.*, 2011; Chen *et al.*, 2017; Mikiciuk *et al.*, 2019); therefore, they can promote the biomass production of the inoculated plants. Lira *et al.* (2014) observed an increase in the total dry biomass of tomato plants when inoculating the plants with *Azospirillum brasilense* and *Glomus intraradices*. Likewise, Ruiz *et al.* (2011) indicated that when inoculating rice plants with *Glomus intraradices* and *Azospirillum brasilense* they obtained higher shoot fresh weight and root fresh weight. The previous results are in agreement with those obtained in this work, because when applying only the biostimulant based on microorganisms (10^6 spores mL⁻¹ + 10^6 CFU and 0 ppm of seaweed extract) the stem fresh weight, root fresh weight, stem dry weight, leaf dry weight and root dry weight were higher, compared when this biostimulant was not applied. Despite not having obtained greater root length by adding only the biostimulant based on microorganisms a greater accumulation of fresh and dry biomass of the root of the cucumber plants was observed, possibly due to a greater lateral growth of the root stimulated by the microorganisms, this as mentioned in other works (Gutjahr and Paszkowski, 2013; Pozo *et al.*, 2015). On the other hand, seaweed extracts, due to the large amount of bioactive compounds they possess, influence the physiology of plants, being reflected in a higher biomass production (Craigie, 2011; Battacharyya *et al.*, 2015). The foregoing agrees with what was obtained in this work, since the spraying of cucumber plants with seaweed extract (without the addition of microorganisms) improved the leaf fresh weight, stem fresh weight, total fresh weight, stem dry weight, leaf dry weight, root dry weight and total dry weight of cucumber plants, mainly when concentrations of 250 and 500 ppm were applied. The synergism of the biostimulants was not reflected in the accumulation of fresh and dry biomass of the organs of cucumber plants as has been reported in other works (González *et al.*, 2020a; Anli *et al.*, 2020), since when applying them in combination, the biomass reached when the biostimulants were applied individually was not exceeded.

Fruit development and total yield

Microorganisms have been reported to improve plant development and yield (Lira *et al.*, 2014). The positive response in the development and yield of plants when adding microorganisms is mainly due to the fact that they improve the mineralization, mobilization and absorption of nutrients, stimulate the synthesis of plant hormones, fix atmospheric N and accelerate the biochemical and enzymatic activities of plants (Basha and Basha, 2010; Contreras *et al.*, 2019; Sivakumar *et al.*, 2020). In this work, the application single of the biostimulant based on microorganisms (10^6 spores mL⁻¹ + 10^6 CFU and 0 ppm of seaweed extract) did not improve the weight, size and total yield of cucumber fruits, compared when it was not added this biostimulant. The results do not agree with other studies in which it has been reported that inoculating plants with arbuscular mycorrhizal fungi and *Azospirillum brasilense* improves the size and yield of aonla (*Indian Gooseberry*) (Kumar *et al.*, 2014), guava (*Psidium guajava* L.) (Das *et al.*, 2017) and tomato (*Solanum lycopersicon* Mill.) (Lira *et al.*, 2014). Related to the inconsistency in the previous results, it has been indicated that the effects of mycorrhizal fungi on fruit development will depend on the cultivar, the culture conditions, the time of inoculation and the arbuscular mycorrhizal fungus used (Schubert *et al.*, 2020; Mikiciuk *et al.*, 2019). On the other hand, the average fruit weight, fruit length, equatorial fruit diameter and yield were favored when spraying cucumber plants with seaweed extract (without the addition of microorganisms). The favorable behavior of seaweed extracts on the development and yield of the fruits has been reported in works carried out on pepper (Ashour

et al., 2021), cucumber (Hassan *et al.*, 2021), strawberry (Al-Shatri *et al.*, 2020) and tomato (Ali *et al.*, 2016). Although the single application of microorganisms (10^6 spores mL⁻¹ + 10^6 CFU and 0 ppm of seaweed extract) did not improve cucumber fruit development, it was observed that applying the microorganisms in combination with the highest concentration of seaweed extract improved equatorial diameter and yield of cucumber fruits in comparison when none of the biostimulants were added, exceeding it by 9.4% and 91%, respectively. Our results agree with those reported by Vafa *et al.* (2021), who indicated that when applying microorganisms (*Phosphobacteria* sp., *Azotobacter* sp. and *Azospirillum* sp.), mycorrhizal fungi (*Glomus mosseae*) and seaweed extract the yield of wheat grains improved.

Fruit quality

Fruit quality attributes are important factors influencing consumer judgment in the market (Tarantino *et al.*, 2018; Ranjbar *et al.*, 2020). Firmness is an important factor that affects the quality of the fruit, since the useful life of the fruit depends on it (Jahan *et al.*, 2020; Pérez *et al.*, 2020). Several studies have addressed the positive role produced by the inoculation of microorganisms on fruit firmness (Rivera *et al.*, 2012; Pérez *et al.*, 2020). The results obtained in this study are in agreement with previous reports, since the single applying of biostimulant based on microorganisms (10^6 spores mL⁻¹ + 10^6 CFU and 0 ppm of seaweed extract) improved the firmness of cucumber fruits, compared to when this biostimulant was not added. Likewise, when spraying cucumber plants with seaweed extract (without the addition of microorganisms) the firmness of the fruits improved, mainly when concentrations of 250 and 500 ppm were added. The results agree with those reported by Ali *et al.* (2016), who indicated that when spraying tomato plants with *Ascophyllum nodosum* fruits with greater firmness were obtained. The synergism between the microorganisms and the seaweed extract was not reflected in the firmness of the cucumber fruit, since when they were added in combination this parameter decreased. Wang *et al.* (2007) have indicated that the increase in the application of N reduces the firmness of tomato fruits. According to the above, the contribution of N by microorganisms (Azcón *et al.*, 2003; Bashan and Bashan, 2010), seaweed extract (Patel and Mukherjee, 2021) and chemical fertilizers could increase the availability and absorption of N by cucumber plants and, possibly, reduce fruit firmness.

Total soluble solids and titratable acidity determine the quality and flavor of the fruit (González *et al.*, 2020b). The literature shows that the influence of microorganisms on the content of total soluble solids in fruits and their acidity is varied. Cecatto *et al.* (2016) found a decrease in the values of total soluble solids and titratable acidity in some strawberry cultivars inoculated with mycorrhizal fungi. Perez *et al.* (2020) reported that inoculation with *Pseudomonas fluorescens* and *Azospirillum brasilense* did not improve titratable acidity in tomato fruits. In a study by Ansari *et al.* (2018), a marked increase in total soluble solids and titratable acidity was observed in mycorrhizal strawberry fruits. In this work, it was observed that the addition of microorganisms as the only biostimulant (10^6 spores mL⁻¹ + 10^6 CFU and 0 ppm of seaweed extract) did not improve the total soluble solids and the titratable acidity of cucumber fruits, compared when no applied this biostimulant. Whereas, spraying the plants with seaweed extract (without the addition of microorganisms) improved the content of total soluble solids in cucumber fruits, mainly when high concentrations were added; but, this effect did not appear in the titratable acidity. The results agree with those reported in other studies, in which an increase in total soluble solids has been achieved in tomato, grapevine and strawberry fruits by spraying the plants with *Ascophyllum nodosum* (Ali *et al.*, 2016; Frioni *et al.* 2018; Frioni *et al.*, 2018). Likewise, the results coincide with Al-Shatri *et al.* (2020), who indicated a significant decrease in titratable acidity in strawberry fruits when increasing the application of seaweed. The synergism of the combined use of microorganisms and seaweed extract was reflected in the titratable acidity parameter, mainly when high concentrations of seaweed extract was added. Related to the above, it has been indicated that the excessive application of nitrogenous fertilizers can decrease the concentration of soluble sugars and increase the titratable acidity in the fruits (Wang

et al., 2008); therefore, the response of total soluble solids and titratable acidity in cucumber fruits could be related to the greater contribution of N when adding microorganisms, seaweed extracts and chemical fertilizers.

In relation to vitamin C content, microorganisms have been reported to improve vitamin C content in some horticultural crops (Bhantana *et al.*, 2021; El-Hifny and El-Sayed, 2011; Bona *et al.*, 2015). However, in this study the application of microorganisms as the only biostimulant (10 spores mL⁻¹ + 10⁶ CFU and 0 ppm of seaweed extract) did not improve the content of vitamin C, compared to the value reached when this biostimulant was not applied. Likewise, the spraying of algae extract improved the content of vitamin C in cucumber fruits by adding the highest concentration, which is consistent with that reported in other studies (Ali *et al.*, 2016; Trejo *et al.*, 2018). The synergy between the two biostimulants was not reflected in the content of vitamin C in the cucumber fruit. The lack of synergism between the two biostimulants in the content of vitamin C in cucumber fruits may also be related to the high availability and absorption of N by cucumber plants, since it has been indicated that the high application of N can have negative effects on the content of vitamin C in fruits (Simonne *et al.*, 2007).

Cucumber fruit skin color is an important sensory quality characteristic that determines consumer preference (Zhang *et al.*, 2014; Kishor *et al.*, 2021). The green color of cucumber fruits is related to the accumulation of chlorophyll (Hurr *et al.*, 2009). The influence of microorganisms on the accumulation of chlorophyll *a*, chlorophyll *b* and *total* chlorophyll in leaves of different crops has been reported in other studies (Chen *et al.*, 2017; Mikiciuk *et al.*, 2019; Golubkina *et al.* 2020). In this study, although the chlorophyll content in cucumber leaves was not evaluated, it was observed that the reported positive effect of microorganisms on the chlorophyll content in leaves is the same on the chlorophyll content in fruits, since an increase in chlorophyll *a* and *total* chlorophyll content in cucumber fruits when microorganisms were applied as the only biostimulant (10 spores mL⁻¹ + 10⁶ CFU and 0 ppm of seaweed extract), compared when it not applied. Likewise, it has been indicated that seaweed extracts promote chlorophyll biosynthesis and/or minimize its decomposition, resulting in an increase in green color in plant tissues (Sharma *et al.*, 2014). This is in agreement with our results, since a higher content of total chlorophyll was observed in cucumber fruits when a high concentration of seaweed extract was added (without the addition of microorganisms). However, the synergism between the biostimulants was not observed in chlorophyll content. Likewise, the effect of microorganisms, algae extract and interaction of biostimulants on color indices (L*, a* and b*) was the same as that observed on chlorophyll content in cucumber fruits; however, there are currently no reports of the effect of these biostimulants on fruit color indices.

Conclusions

The individual application of microorganisms and seaweed extract improve growth, biomass production and fruit quality of cucumber plants; but, in combination these biostimulants do not present synergism in these parameters. However, the equatorial diameter, yield and titratable acidity of fruits were better with the joint application of microorganisms and seaweed extract, mainly when the microorganisms were applied in combination with high concentrations of seaweed extract.

Authors' Contributions

Conceptualization: AM and MS; Data analysis: SM, AL and AH; Funding acquisition: AM; Investigation: MS and JG; Methodology: SM and AL; Project administration: AM; JS prepared Figures. Writing-review and editing AM, MS. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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