



Article

Use of a Biostimulant Based on Seaweed Extract as a Sustainable Input to Enhance the Quality of Solanaceous Seedlings

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Abstract: Seaweed extracts have several functions in agriculture due to their composition that is rich in nutrients, plant hormones, and bioactive substances. It is a natural product used as a biostimulant especially to promote the growth and development of plants and their tolerance to environmental stresses. The objective of this study was to analyze the response to a biostimulant containing seaweed extract derived from *Ascophyllum nodosum* in the cultivation of tomato and eggplant seedlings, analyzing the growth and physiological parameters in two different regions of Brazil. Cherry tomato and eggplant were cultivated in polyethylene trays for 30 days. In each crop, five treatments were tested, comparing the use of a commercial seaweed extract in application doses and forms, which were the control (without seaweed application); 0.1%, 0.2%, and 0.3% of the seaweed extract applied by irrigation water; and treatment with 0.2% of the seaweed extract by foliar application. This study confirms the efficacy of incorporating seaweed extract from *Ascophyllum nodosum* as a bio-input into the production phase of Solanaceae seedlings. The seedlings which received the seaweed extract significantly increased some morphological parameters, mainly regarding the biomass and length of leaves, stems, and roots. In general, applying both methods through irrigation water and foliar application were effective in providing benefits compared to the control treatment. The intermediate dose (0.2%) was the most effective in promoting improvement in the analyzed parameters. This underscores the importance of obtaining quality seedlings for subsequent planting in the field, potentially leading to better acclimatization and initial adaptation.

Keywords: *Ascophyllum nodosum*; horticulture; eggplant seedling; plant biostimulant; tomato seedling



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1. Introduction

Tomato and eggplant belong to the Solanaceae family and are two species of fruit and vegetables with high economic importance, and seedling production is the first stage in the tomato and eggplant production chain. Obtaining quality seedlings is essential to guarantee the growth and development of several horticultural crops to reach a high productive potential and to have better adaptation to transplanting and adverse climatic conditions whether in a protected environment or in the open field [1,2]. The main factors which influence the formation of quality seedlings are the type of substrate, water supply, environmental conditions, and cultural practices [3,4].

However, there are some strategies that can be used to intensify the seedling production chain, such as the use of phytohormones [5], beneficial elements [6], inoculation

of microorganisms [7–9], and the use of seaweed extracts [10]. Seaweed extracts are considered biostimulants in agriculture, as they promote growth and defense responses to plants. Brown seaweeds is the class of macroalgae which have been used as a biomass source to produce seaweed extracts [11]. They are produced through soft extraction methods, employing low temperatures and pressures and utilizing both physical and chemical techniques for the extraction process [11,12].

Ascophyllum nodosum is one of the seaweed species most used as raw material to produce organic biostimulants in agriculture [13]. Improving the yield in different growing conditions, inducing stress tolerance, and increasing nutrient absorption are important for sustainable agricultural management [10,14,15]. These positive responses are mainly explained by their constitution, consisting of several bioactive compounds, such as polyphenols, polysaccharides, lipids, and amino acids, in addition to the macro- and micronutrients and plant phytohormones [16–19].

Several studies highlight the positive effects of applying seaweed extract in agriculture, but few are focused on the seedling production phase. In the nursery phase, the main management carried out is the supply of organic or chemical fertilizer, according to the requirements of the seedlings, to guarantee adequate nutrition combined with an adequate supply of water, avoiding both an excess and a lack thereof to ensure the healthy development of the seedlings. But the incorporation of seaweed extract has proven efficient in the development, nutrition, and quality of some seedlings [14,20]. At this stage, studies address foliar application and do not focus on studying the benefits of application through irrigation water. Furthermore, it is important to test different application doses and forms to achieve better results.

Most vegetables from the Solanaceae family are planted in the field starting with seedlings. It is important to develop studies that can improve and intensify this process, such as the addition of seaweed extract. This research expected that the application of seaweed extract, regardless of the form of application, can provide an increase in the seedling quality parameters of the Solanaceae species. This study aimed to evaluate the consequences of applying seaweed extract of *Ascophyllum nodosum* in different application doses and forms, analyzing the growth and physiological parameters of tomato and eggplant seedlings in two different regions, in Maringá and Piracicaba, both located in subtropical areas of Brazil.

2. Materials and Methods

2.1. Local and Experimental Conditions

The study was carried out in two experimental sites (Figure 1). The first experiment was conducted at the State University of Maringá, Maringá, Paraná, Brazil (23°40' S, 51°94' W, 514 m of altitude), from 3 November 2021 to 2 December 2021. Climatic conditions during this period were measured by an automatic weather station installed in the center of the protected environment. Temperature, relative humidity, and solar radiation are represented in Figure 2a. Temperature ranged from 21.52 to 28.47 °C, and the relative humidity varied between 39.1% and 83.0%. The solar radiation ranged from 8.16 to 22.58 MJ m⁻² day⁻¹. The second experiment was conducted at São Paulo University in Piracicaba, São Paulo, Brazil (22°71' S, 47°62' W, 546 m of altitude). It was conducted from 8 November 2022 to 7 December 2022. The temperature during this period ranged from 22.24 to 28.08 °C, with the relative humidity varying from 51.7% to 95.9%, and the solar radiation ranged from 5.13 to 20.33 MJ m⁻² day⁻¹ (Figure 2b). Both experiments were conducted in a protected environment, measuring 25 m in length and 14 m in width, with a transparent plastic cover and 4 m of ceiling height, and it was surrounded with a nylon mesh barrier designed to deter aphids.

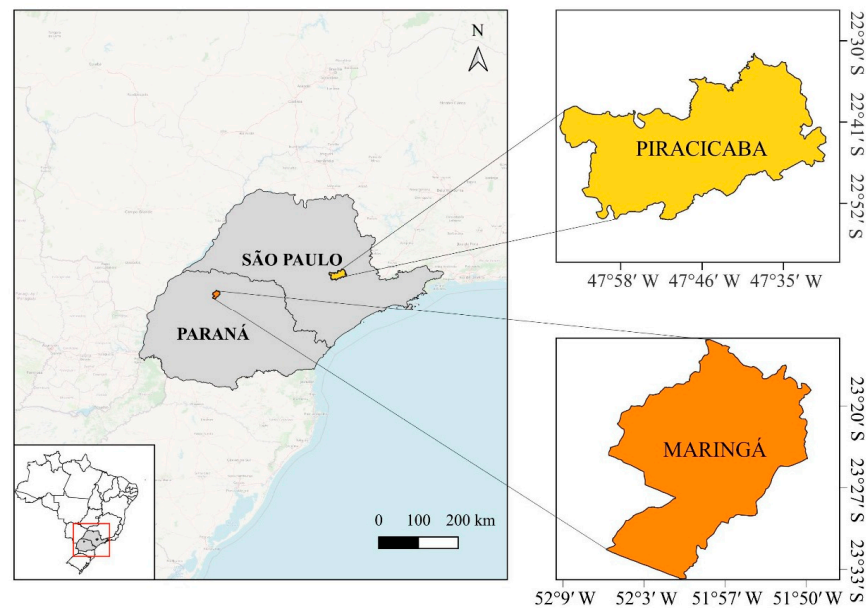


Figure 1. Geographic representation of the experimental sites.

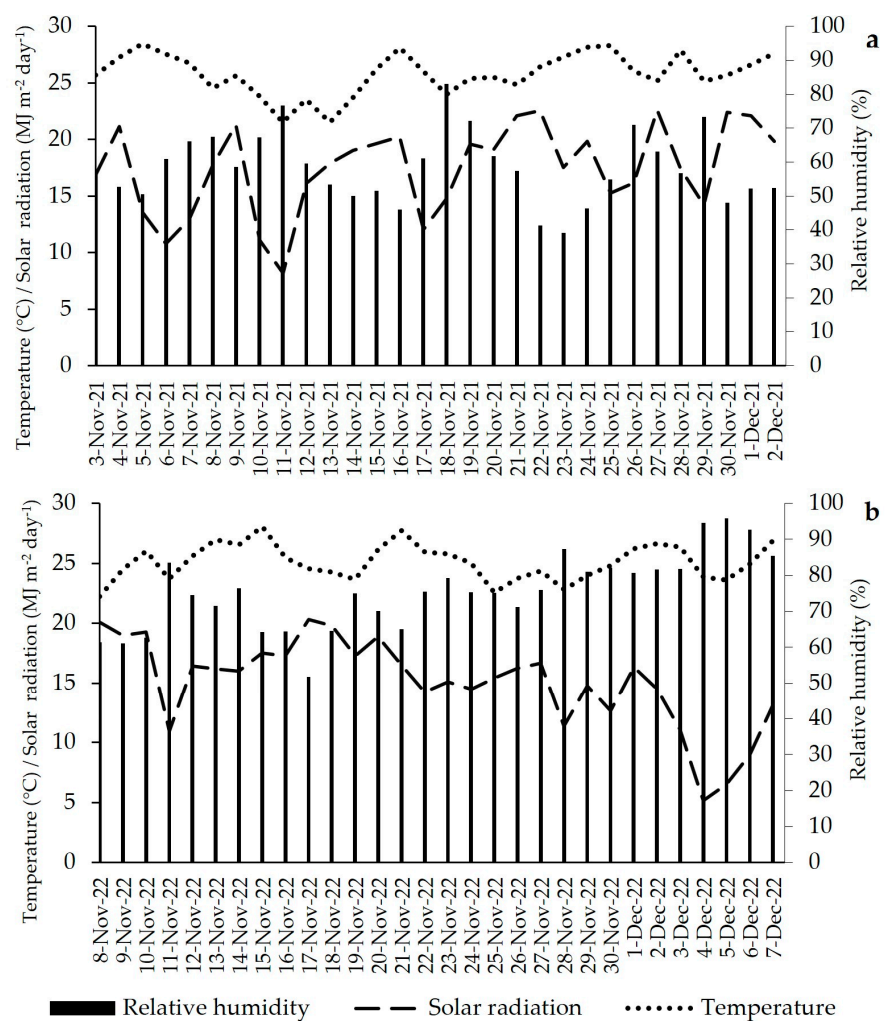


Figure 2. Average temperature (°C), relative air humidity (%), and solar radiation (MJ m⁻²) during the first experiment (a) and second experiment (b).

2.2. Treatments and Experimental Design

Cherry tomato (Sweet heaven[®]) and eggplant (Nápoli[®]) seeds, both produced by Sakata Seed Sudamerica, Bragança Paulista, SP, Brazil, were sown in polyethylene trays containing 128 seeding cells. The cells measuring 35 cm³ were filled using the commercial substrate TN top gold[®] (Agrinobre, Sinimbu, RS, Brazil) and cultivated for 30 days, which is the average time required for complete seedling development used in nurseries. In each crop, five treatments were tested, comparing the use of a commercial seaweed extract in application doses and forms, which were control (without seaweed application); 0.1%, 0.2%, and 0.3% of the seaweed extract applied by irrigation water; and treatment with 0.2% of seaweed extract by foliar application. The commercial product Acadian[®] (Acadian seaplants Ltd., Dartmouth, NS, Canada) was used; it is a product with biostimulant properties that contains seaweed extract of the species *Ascophyllum nodosum*.

The experiments were conducted in a randomized block design, with ten repetitions. Each repetition was a tray with 128 seeding cells. Each tray was divided into 5 equal parts for implantation and division of treatments. For the evaluations, 10 seedlings of each treatment per block (excluding the borders) were chosen randomly for the evaluation of the seedling parameters to obtain the average of each repetition.

2.3. Seaweed Extract Application

The application of the extract was carried out in 6 stages during the productive cycle of the seedlings, on the day of sowing and 5, 10, 15, 20, and 25 days after sowing (DAS) using the biostimulant Acadian[®]. This product is derived solely from *Ascophyllum nodosum* seaweed and harvested in North Atlantic waters. The extraction process involved using an alkali extraction method. Along with fresh seaweed, the product's raw materials include 0.5% citric acid complexing agent, water, and potassium hydroxide (as it was used in the seaweed extraction). The final product is rich in nutrients and bioactive compounds, organic acids, amino acids, betaines, alginates, and polysaccharides. It is available in liquid form with 1.16 g mL⁻¹ of density and an alkali pH of 8.0 [18,21,22].

The seaweed extract applications were conducted in the morning. The application via irrigation water occurred with a manual watering can with a volume of 2 L, and the foliar application was performed with an automatic sprayer. There is no recommended dose of this biostimulant for the seedling production phase. Then, the recommended dose for growing vegetables in the field was taken as a parameter, which is foliar 0.2%, that is, 2 mL of the extract for each 1000 mL of water. At the time of application, only water was applied for the control treatment.

2.4. Seedling Management

Two seeds were sown per tray cell, and after their germination, it was standardized leaving only one seedling per cell, to ensure that all cells had a seedling. The irrigation management of the seedling trays in the protected environment was carried out automatically using a micro-sprinkler system to ensure uniform distribution of water to the growing seedlings, programmed based on the specific requirements of the seedlings and environmental conditions. A schedule was defined to irrigate at regular intervals, and daily irrigations were carried out in two periods of the day (8 a.m. and 4 p.m.), so that each seedling received the appropriate amount for its initial growth and development.

To allocate the trays within the protected environment, 1.0 m high galvanized steel benches were used, so the trays were placed on these benches, with no contact with the local soil surface. At sixteen DAS, foliar fertilization was performed in all treatments using the commercial product Forth Hortalças, which contains macro- and micronutrients, with guarantees of 9% nitrogen, 15% phosphorus, 10% potassium, 2.5% calcium, 2.5% magnesium, 9% sulfur, 0.06% boron, 0.05% copper, 0.22% iron, 0.10% manganese, 0.20% zinc, and 0.005% molybdenum, using a recommended dose for vegetables of 3 g L⁻¹.

2.5. Evaluated Parameters

Morphological and physiological parameters of tomato and eggplant seedlings were evaluated at 30 DAS for both experiments. Regarding the leaves of the seedlings, the length of the largest leaf (cm) was evaluated by using a digital caliper (0.01 mm), and the total fresh mass (g) was obtained in an analytical balance (0.001 g). The total leaf area was obtained using the LI 3100 area meter[®] (LI-COR Biosciences, Lincoln, NE, United States) equipment and expressed in cm². After measuring the fresh mass and the leaf area, the leaves were dried at 70 °C until a constant mass was obtained, and the dry mass (g) was measured on an analytical balance.

Seedling stem height (cm) was evaluated using a graduated tape (1.00 mm); the stem diameter (mm) was evaluated using a digital caliper, and the fresh and dry masses of the stems were obtained in a manner similar to the methodology described for the leaves. The roots were carefully removed from the substrate after washing in running water. The length (cm) of the roots was measured using a graduated tape, as well as the fresh and dry masses (g) of the roots as previously described. Chlorophyll content was estimated on the most expanded leaf of each seedling using a SPAD-502 Plus[®] portable meter (Konica Minolta Sensing, Tokyo, Japan), and the results were expressed in SPAD index.

2.6. Statistical Analysis

The statistical software Sisvar, version 5.6 [23] was used to perform an analysis of variance with F test at 5% probability, and the treatments were compared using Tukey's test in all evaluated parameters after the assumptions of normality and homogeneity of variance by Shapiro–Wilk test were met. From the observed data of all parameters, Pearson's linear correlation coefficient was obtained, regardless of the applied treatment, to verify the positive or negative relationships between the studied parameters.

3. Results

3.1. Foliar Parameters

Analyzing the foliar parameters of the tomato and eggplant seedlings, the seaweed extract application was superior to the control in both experiments (Table 1). In experiment 1, the foliar application of the product was superior to the treatments with soil applications in most parameters for eggplant seedling production.

Table 1. Foliar parameters of tomato and eggplant seedlings subjected to different doses and application methods of *Ascophyllum nodosum* extract in both experiments.

Treatments	Tomato				Eggplant			
	Leaf Length (cm)	Leaf Fresh Mass (g)	Leaf Dry Mass (g)	Leaf Area (cm ²)	Leaf Length (cm)	Leaf Fresh Mass (g)	Leaf Dry Mass (g)	Leaf Area (cm ²)
Experiment 1								
Control	2.00 c	0.16 b	0.020 c	3.15 b	1.62 c	0.13 c	0.026 c	8.09 c
0.1% soil	3.22 a	0.26 a	0.034 b	4.56 a	1.74 c	0.15 c	0.027 c	8.54 bc
0.2% soil	3.08 ab	0.27 a	0.038 a	4.79 a	2.06 bc	0.20 b	0.035 bc	10.65 bc
0.3% soil	3.10 ab	0.28 a	0.039 a	5.10 a	2.38 b	0.26 a	0.041 b	12.00 b
0.2% foliar	2.84 b	0.28 a	0.039 a	4.96 a	3.04 a	0.29 a	0.056 a	15.68 a
CV (%)	5.59	13.26	16.88	12.66	8.26	13.39	9.26	16.38
Experiment 2								
Control	2.45 b	0.21 b	0.032 b	5.14 d	2.73 c	0.27 c	0.026 b	7.94 b
0.1% soil	2.98 a	0.29 a	0.036 b	6.20 cd	3.03 b	0.31 bc	0.028 b	8.71 b
0.2% soil	3.20 a	0.33 a	0.049 a	7.04 bc	3.60 a	0.40 a	0.045 a	12.29 a
0.3% soil	3.38 a	0.36 a	0.049 a	9.04 a	3.50 a	0.38 ab	0.048 a	13.74 a
0.2% foliar	3.23 a	0.35 a	0.046 a	8.27 ab	3.60 a	0.43 a	0.044 a	13.62 a
CV (%)	6.91	10.32	7.70	9.12	9.04	10.49	11.31	9.36

Means ($n = 10$) followed by different letters in the same column and in each experiment realized are significantly different by Tukey's test at $p < 0.05$. CV: coefficient of variation.

However, in experiment 2, the foliar application did not differ statistically from the soil application at the same dose (0.2%). It was observed that for eggplant cultivation, the

lowest dose of seaweed extract was similar to the control or lower than the other doses. Furthermore, the highest dose applied via the soil (0.3%) was similar, in most parameters, to the intermediate dose (0.2%), inferring that the intermediate dose can promote the best productive performance of the seedlings, providing an increase of 87.6% in leaf length, 123.1% in fresh mass, and 93.8% in leaf area compared to the control treatment in experiment 1.

In the tomato seedlings, the lowest dose of the seaweed extract (0.1%), applied via the soil, was sufficient to achieve the best performance in most parameters, resulting in an increase of 61% in leaf length, 62.5% in fresh mass, and 44.8% in leaf area in relation to the control treatment in experiment 1. There was no significant difference between the application methods (foliar and soil) for tomato seedlings.

3.2. Stem Parameters

The application of a biostimulant based on seaweed extract was effective for most of the parameters related to the stem of the two species of the seedlings evaluated (Table 2). The application of the 0.2% dose was statistically the same for both application forms. In experiment 2, the application of 0.2% of the extract via the soil promoted an increase of 26.6% in stem height, 28.1% in diameter, and 58.3% in fresh mass for the production of tomato seedlings. In the eggplant seedlings, these increases were 68.9%, 2.45%, and 83.3%, respectively.

Table 2. Stem parameters of tomato and eggplant seedlings subjected to different doses and application methods of *Ascophyllum nodosum* extract in both experiments.

Treatments	Tomato				Eggplant			
	Stem Height (cm)	Stem Diameter (mm)	Stem Fresh Mass (g)	Stem Dry Mass (g)	Stem Height (cm)	Stem Diameter (mm)	Stem Fresh Mass (g)	Stem Dry Mass (g)
Experiment 1								
Control	3.56 c	1.53 c	0.10 c	0.006 b	2.52 d	1.48 b	0.11 c	0.011 a
0.1% soil	4.78 b	1.82 bc	0.15 b	0.014 a	2.9 cd	1.55 b	0.12 bc	0.012 a
0.2% soil	4.98 ab	2.10 ab	0.15 b	0.016 a	3.34 b	1.57 ab	0.13 b	0.012 a
0.3% soil	5.30 a	2.19 a	0.18 a	0.019 a	2.98 bc	1.53 b	0.13 b	0.012 a
0.2% foliar	4.76 b	1.88 b	0.17 ab	0.017 a	3.5 a	1.71 a	0.17a	0.015 a
CV (%)	5.56	7.95	12.91	19.01	6.51	5.12	12.03	14.63
Experiment 2								
Control	4.13 c	1.53 b	0.12 b	0.013 b	2.25 c	1.18 c	0.12 c	0.008 b
0.1% soil	4.75 b	1.80 ab	0.14 b	0.016 b	3.30 b	1.19 bc	0.14 bc	0.011 b
0.2% soil	5.23 a	1.96 ab	0.19 a	0.023 a	3.80 a	1.21 a	0.22 a	0.021 a
0.3% soil	5.45 a	1.95 ab	0.20 a	0.023 a	3.69 a	1.20 a	0.19 ab	0.019 a
0.2% foliar	5.20 a	2.20 a	0.20 a	0.023 a	3.70 a	1.20 a	0.20 ab	0.019 a
CV (%)	4.77	12.02	12.00	11.98	5.40	4.69	10.39	19.99

Means ($n = 10$) followed by different letters in the same column and in each experiment realized are significantly different by Tukey's test at $p < 0.05$. CV: coefficient of variation.

3.3. Root Parameters

Analyzing the root parameters of the seedlings, the application of seaweed extract was effective in relation to the control seedlings (Table 3). In experiment 1, treatments with seaweed extract were similar; therefore, the lowest dose, applied via the soil (0.1%) was able to provide the best root performance in the production of tomato seedlings. In experiment 2, the intermediate dose (0.2%) was the one that achieved the highest performance in most parameters. In the eggplant seedlings, the foliar application of the seaweed extract showed better results, increasing the fresh mass of the root by 111.7% and 77.8% in the dry mass; however, there was no significant difference in the length of the roots in experiment 1. In experiment 2, the applications of 0.2% via the soil and foliar applications were statistically similar, and both were superior to the control treatment.

Table 3. Root parameters of tomato and eggplant seedlings subjected to different doses and application methods of *Ascophyllum nodosum* extract in both experiments.

Treatments	Tomato			Eggplant		
	Root Fresh Mass (g)	Root Dry Mass (g)	Root Length (cm)	Root Fresh Mass (g)	Root Dry Mass (g)	Root Length (cm)
Experiment 1						
Control	0.12 b	0.007 b	5.98 b	0.17 c	0.018 c	8.38 a
0.1% soil	0.19 a	0.014 a	8.42 a	0.20 bc	0.023 b	9.00 a
0.2% soil	0.20 a	0.016 a	9.22 a	0.22 bc	0.023 b	9.06 a
0.3% soil	0.22 a	0.018 a	10.10 a	0.26 b	0.024 b	9.00 a
0.2% foliar	0.21 a	0.017 a	8.88 a	0.36 a	0.032 a	9.50 a
CV (%)	12.97	18.18	12.29	8.63	13.49	7.73
Experiment 2						
Control	0.16 b	0.017 c	8.63 b	0.10 b	0.010 b	6.10 b
0.1% soil	0.24 a	0.022 b	9.25 b	0.17 b	0.014 b	8.43 a
0.2% soil	0.29 a	0.028 a	11.88 a	0.36 a	0.027 a	9.33 a
0.3% soil	0.26 a	0.021 b	10.75 ab	0.31 a	0.024 a	9.05 a
0.2% foliar	0.27 a	0.024 b	10.30 ab	0.31 a	0.026 a	8.78 a
CV (%)	18.36	17.50	9.70	21.60	14.05	8.12

Means ($n = 10$) followed by different letters in the same column and in each experiment realized are significantly different by Tukey's test at $p < 0.05$. CV: coefficient of variation.

3.4. Chlorophyll Content

Regarding the chlorophyll content of the seedlings, it was observed that the application of the seaweed extract was efficient in increasing the SPAD index of both the tomato and eggplant seedlings (Figure 3).

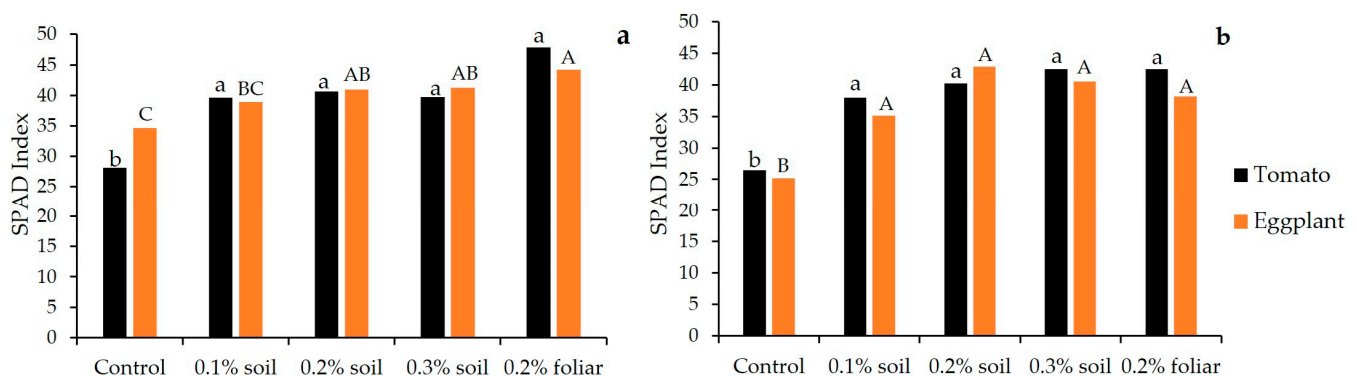


Figure 3. Chlorophyll content of leaf means of tomato and eggplant seedlings subjected to different doses and application methods of *Ascophyllum nodosum* extract for both experiments. Means ($n = 10$) followed by different letters (lowercase letters for tomatoes and capital letters for eggplant) in the same bar and crop in each experiment realized are significantly different by Tukey's test at $p < 0.05$. First experiment (a) and second experiment (b).

In the first and second experiments, the four treatments with the application of the seaweed extract were statistically equal and superior to the control treatment. In the eggplant seedlings, the application of 0.2% of the extract via foliar application showed the best performance in the first experiment, increasing the SPAD index by 27.5% in relation to the control treatment. In the second experiment, there was no difference between the four treatments with the application of the extract; all were superior to the control, highlighting the treatment with 0.2% of the extract applied via irrigation water which provided an increase of 70.1% in chlorophyll content in relation to the control.

3.5. Pearson's Linear Correlation

Pearson's linear correlations were performed to examine the relationships between the parameters in the tomato seedlings (Table 4) and the eggplant seedlings (Table 5), from the first experiment, because the data trend was similar for both experiments.

Table 4. Pearson's correlation of tomato seedling parameters subjected to different doses and application methods of *Ascophyllum nodosum* extract in the first experiment, regardless of the applied treatments.

	Leaf Length	Leaf Fresh Mass	Leaf Dry Mass	Leaf Area	Stem Height	Stem Diameter	Stem Fresh Mass	Stem Dry Mass	Root Fresh Mass	Root Dry Mass	Root Length	Chlorophyll
Leaf length	1.00	-	-	-	-	-	-	-	-	-	-	-
Leaf fresh mass	0.76	1.00	-	-	-	-	-	-	-	-	-	-
Leaf dry mass	0.76	0.74	1.00	-	-	-	-	-	-	-	-	-
Leaf area	0.76	0.72	0.77	1.00	-	-	-	-	-	-	-	-
Stem height	0.83	0.88	0.78	0.79	1.00	-	-	-	-	-	-	-
Stem diameter	0.66	0.67	0.87	0.62	0.77	1.00	-	-	-	-	-	-
Stem fresh mass	0.72	0.75	0.76	0.71	0.83	0.70	1.00	-	-	-	-	-
Stem dry mass	0.68	0.76	0.87	0.66	0.81	0.86	0.83	1.00	-	-	-	-
Root fresh mass	0.62	0.63	0.37	0.54	0.69	0.37	0.55	0.46	1.00	-	-	-
Root dry mass	0.43	0.45	0.41	0.15	0.57	0.55	0.59	0.55	0.57	1.00	-	-
Root length	0.56	0.59	0.62	0.44	0.69	0.66	0.70	0.70	0.61	0.67	1.00	-
Chlorophyll	0.63	0.77	0.55	0.64	0.75	0.41	0.60	0.62	0.62	0.34	0.41	1.00

Table 5. Pearson's correlation of eggplant seedling parameters subjected to different doses and application methods of *Ascophyllum nodosum* extract in the first experiment, regardless of the applied treatments.

	Leaf Length	Leaf Fresh Mass	Leaf Dry Mass	Leaf area	Stem Height	Stem Diameter	Stem Fresh Mass	Stem Dry Mass	Root Fresh Mass	Root Dry Mass	Root Length	Chlorophyll
Leaf length	1.00	-	-	-	-	-	-	-	-	-	-	-
Leaf fresh mass	0.75	1.00	-	-	-	-	-	-	-	-	-	-
Leaf dry mass	0.62	0.72	1.00	-	-	-	-	-	-	-	-	-
Leaf area	0.80	0.76	0.85	1.00	-	-	-	-	-	-	-	-
Stem height	0.72	0.67	0.68	0.67	1.00	-	-	-	-	-	-	-
Stem diameter	0.39	0.53	0.36	0.32	0.69	1.00	-	-	-	-	-	-
Stem fresh mass	0.79	0.73	0.72	0.74	0.76	0.47	1.00	-	-	-	-	-
Stem dry mass	0.85	0.65	0.67	0.75	0.68	0.34	0.72	1.00	-	-	-	-
Root fresh mass	0.69	0.75	0.83	0.75	0.75	0.47	0.82	0.76	1.00	-	-	-
Root dry mass	0.69	0.66	0.75	0.75	0.69	0.39	0.81	0.70	0.92	1.00	-	-
Root length	0.70	0.61	0.70	0.63	0.83	0.41	0.64	0.63	0.75	0.74	1.00	-
Chlorophyll	0.68	0.77	0.67	0.64	0.76	0.66	0.66	0.68	0.73	0.67	0.72	1.00

It was observed that in the tomato seedlings, the leaf length and leaf fresh mass have high correlations with stem height, being (0.83) and (0.88), respectively. Leaf dry mass showed a strong correlation with stem diameter (0.87) and stem dry mass (0.87). Stem height also had a positive correlation with stem fresh mass (0.83) and stem dry mass (0.81), and stem diameter showed a high correlation with stem dry mass (0.86). And both stem mass variables, fresh and dry masses, positively correlated with each other (0.83). The chlorophyll content had the highest correlation with leaf fresh mass (0.77) and stem height (0.75).

Regarding the Pearson's correlation of eggplant seedlings, it can be highlighted that leaf length showed a positive correlation with leaf area (0.80) and stem dry mass (0.85), and leaf dry mass showed a strong correlation with leaf area (0.85) and root fresh mass (0.83). Stem height correlated with root length (0.83). Stem fresh mass had a strong correlation with the root parameters, root fresh mass (0.82), and root dry mass (0.81). And these two root parameters were strongly correlated with each other (0.92). Some parameters showed a strong positive correlation with chlorophyll, such as leaf length (0.77), stem height (0.76), root fresh mass (0.73), and root length (0.72).

4. Discussion

In this study, the effects of applying a biostimulant based on *Ascophyllum nodosum* seaweed in the production phase of Solanaceae seedlings in nurseries were explored. Two forms of application were compared (foliar and soil at a dose of 0.2%), and also three doses of application via the soil (0.1, 0.2, and 0.3%) with the control treatment (without seaweed extract application). The results show the significant potential of seaweed extract in the

production of tomato and eggplant seedlings in the nursery phase. In both experiments, in different regions, the use of seaweed extract was effective in increasing parameters related to the quality of the seedlings, that is, related to the leaves, stems, and roots, as well as in relation to the chlorophyll content. This makes the application of seaweed extract an alternative to obtain seedlings with better morphological characteristics and which can corroborate with the initial adaptation after transplanting in field conditions.

The advantages of seaweed in agricultural crops are well explored to increase productivity, soil fertility, and tolerance against abiotic stresses [13,24,25], and these advantages are mainly due to the rich composition of seaweed, which has several bioactive compounds in its extracts [17,19]. Research involving seaweed extract uses in the initial phase of seedling production is limited, but the data of this study show great potential for this purpose.

Quality seedlings are characterized by a well-developed root system; that is, the greater the root biomass, the better the adaptation of the seedling when planted in the field [1,14,20]. With the application of the seaweed extract, there was an increase in both the biomass and the root length of those seedlings of tomato and eggplant that received the extract. The plant hormone that most directly stimulates seedling root growth in the early stage is indole-3-acetic acid (IAA), which is a form of auxin. Auxins are essential for root development, as they promote the formation of lateral roots and increase the elongation of root cells, facilitating penetration into the soil [26]. Auxin is a phytohormone that is present in the composition of seaweed extracts and may have been responsible for the root increments [27,28]. A more developed root system helps in the greater exploitation of the soil to absorb water and nutrients [29].

Combined with the root increments, it was observed that with the seaweed extract application, there was an increase in some leaf parameters; both seedling species had longer leaves, greater biomass, and greater leaf area per seedling. In addition to auxin also promoting leaf growth by stimulating cell elongation and the formation of new tissues, gibberellins can influence leaf size by promoting cell elongation. This helps to explain that the seedlings that received seaweed extract showed higher values of leaf length, as the presence of these plant phytohormones in the *Ascophyllum nodosum* extract may have promoted these favorable results [30].

In addition to cytokinins regulating leaf growth by stimulating cell division, another important function of this phytohormone is the role of cell differentiation and the formation of chloroplasts, which is important for photosynthesis [27]. Chlorophylls are the green pigments found in chloroplasts, and they are essential for photosynthesis, as they are responsible for absorbing the sunlight necessary for the process [26]. It was observed that the seedlings that received the extract application had a higher chlorophyll content in their leaves, which can be related to the presence of phytohormones in the seaweed extract. It was noted that these seedlings also showed a high correlation with the fresh mass of leaves. In addition to having more chlorophyll, these seedlings had a greater leaf biomass to intercept sunlight. During photosynthesis, the light energy absorbed by chlorophyll is used to convert carbon dioxide and water into sugars and oxygen, causing the seedlings to have greater development, possibly by increasing the photosynthetic rate of plants [17,31,32]. It can also be inferred that seaweed extract contains several organic compounds, mainly polysaccharides, and these compounds may also have played essential roles in the growth and development of the seedlings, including energy storage, structural support, growth regulation, and interactions with the environment [27].

In addition to plant phytohormones and some organic compounds, as components of the *Ascophyllum nodosum* extract, macro- and micronutrients stand out [16–19]. These can also be associated with the better performance of seedlings subjected to the biostimulant, and we can infer that good fertilization can stimulate the development of the root system, allowing the seedlings to absorb water and nutrients more efficiently, thus helping to promote the growth of the seedlings [33]. And the application of the extract via the soil can also improve the availability of nutrients for plants [13,33].

As observed in the correlation results, the increase in chlorophyll is correlated with an increase in some morphological parameters in the seedlings, highlighting that iron and magnesium are essential nutrients to produce chlorophyll, as well as nitrogen, which stimulates the vegetative growth of the seedlings and thus the production of leaves and shoots [26]. At this stage, the seedlings are actively growing and require adequate nutrients for good development. Furthermore, adequate nutrition could help seedlings to better tolerate adverse conditions, such as water and thermal stress, contributing to healthier and more vigorous development of the crops [14,15,20].

In terms of the molecular comprehension of the *Ascophyllum nodosum* extract's action mechanism in plants, the extract has a positive impact on the internal balance of plant hormones by adjusting hormone levels. This adjustment is associated with controlling the induced gene expression involved in secondary metabolism, hormone signal transduction, and enhancement of certain defense enzymes [11,28,34]. The adoption of seaweed extract in the production of seedlings in nurseries may have minimized some possible seedling stress.

Other organic compounds like amino acids and vitamins and also some polysaccharides like fucoidan and laminarin are found in seaweed [16–19], and the use of seaweed extract in this stage of seedling production can help reduce post-transplant stress, favoring the adaptation of plants to the new environment, and can increase plant resistance to biotic stresses (such as pest attacks and diseases) and abiotic stresses (such as drought, salinity, and extreme temperatures), activating plant defense mechanisms [15,17,18,22,35]. In plants, betaine can be produced from amino acids and plays a critical role in protecting against abiotic stresses, serving as an osmoprotectant in plant cells [30]; seedlings that received seaweed extract may also have better performance because of it.

In addition to resulting in higher-quality seedlings, in relation to leaf, stem, and root morphological characteristics, the incorporation of the extract into the seedling production chain in nurseries can promote an advancement at this stage, as observed by the high increments in morphological parameters compared to the control treatment; that is, it will take fewer days to obtain quality seedlings for transplanting in the field. It is possible that the seedlings that received the seaweed extract will show better development after transplanting in the field, as these seedlings will be able to better establish themselves and protect themselves against stressful abiotic situations. This last inference is a suggestion to be analyzed in future work on vegetable cultivation.

5. Conclusions

After two experiments in different locations, this study confirmed the potential of seaweed extract from *Ascophyllum nodosum* as a new bio-input to be incorporated into the production phase of Solanaceae seedlings, especially tomato and eggplant. The addition of the extract significantly increased several morphological parameters of seedling quality. Regarding the forms of application, in general, both the application via irrigation water (directly into the soil) and foliar application of the extract were effective in providing benefits compared to the control treatment; however, foliar application stood out in practically all parameters. The intermediate dose (0.2%) was the one that, on average, best promoted improvement in the analyzed parameters, thus evidencing the importance of obtaining quality seedlings for subsequent planting in the field, possibly promoting better acclimatization and initial adaptation.

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References

- Bantis, F.; Koukounaras, A.; Siomos, A.; Menexes, G.; Dangitsis, C.; Kintzonidis, D. Assessing Quantitative Criteria for Characterization of Quality Categories for Grafted Watermelon Seedlings. *Horticulturae* **2019**, *5*, 16. [\[CrossRef\]](#)
- Qin, K.; Leskovar, D.I. Humic Substances Improve Vegetable Seedling Quality and Post-Transplant Yield Performance under Stress Conditions. *Agriculture* **2020**, *10*, 254. [\[CrossRef\]](#)
- Hsieh, C.Y.; Fang, S.L.; Wu, Y.F.; Chu, Y.C.; Kuo, B.J. Using Sigmoid Growth Curves to Establish Growth Models of Tomato and Eggplant Stems Suitable for Grafting in Subtropical Countries. *Horticulturae* **2021**, *7*, 537. [\[CrossRef\]](#)
- Ramesh, R.; D'Souza, M.; Asolkar, T.; Achari, G.; Gupta, M.J. Rootstocks for the Management of Bacterial Wilt in Eggplant (*Solanum melongena* L.) and Tomato (*Solanum lycopersicum* L.) in the Coastal Regions of India. *Adv. Agric.* **2022**, *2022*, e8594080. [\[CrossRef\]](#)
- Moncada, A.; Vetrano, F.; Esposito, A.; Miceli, A. Fertigation Management and Growth-Promoting Treatments Affect Tomato Transplant Production and Plant Growth after Transplant. *Agronomy* **2020**, *10*, 1504. [\[CrossRef\]](#)
- Puccinelli, M.; Malorgio, F.; Pintimalli, L.; Rosellini, I.; Pezzarossa, B. Biofortification of Lettuce and Basil Seedlings to Produce Selenium Enriched Leafy Vegetables. *Horticulturae* **2022**, *8*, 801. [\[CrossRef\]](#)
- Vetrano, F.; Miceli, C.; Angileri, V.; Frangipane, B.; Moncada, A.; Miceli, A. Effect of Bacterial Inoculum and Fertigation Management on Nursery and Field Production of Lettuce Plants. *Agronomy* **2020**, *10*, 1477. [\[CrossRef\]](#)
- Miceli, A.; Moncada, A.; Vetrano, F. Use of Microbial Biostimulants to Increase the Salinity Tolerance of Vegetable Transplants. *Agronomy* **2021**, *11*, 1143. [\[CrossRef\]](#)
- Mundim, G.S.M.; Maciel, G.M.; Mendes, G.O. *Aspergillus niger* as a Biological Input for Improving Vegetable Seedling Production. *Microorganisms* **2022**, *10*, 674. [\[CrossRef\]](#)
- Carmody, N.; Goñi, O.; Langowski, L.; O'Connell, S. *Ascophyllum nodosum* Extract Biostimulant Processing and Its Impact on Enhancing Heat Stress Tolerance During Tomato Fruit Set. *Front. Plant Sci.* **2020**, *11*, 807. [\[CrossRef\]](#)
- Ali, O.; Ramsubhag, A.; Jayaraman, J. Biostimulant properties of seaweed extracts in plants: Implications towards sustainable crop production. *Plants* **2021**, *10*, 531. [\[CrossRef\]](#)
- Boukhari, M.E.M.E.; Barakate, M.; Bouhia, Y.; Lyamlouli, K. Trends in seaweed extract based biostimulants: Manufacturing process and beneficial effect on soil-plant systems. *Plants* **2020**, *9*, 359. [\[CrossRef\]](#)
- Villa e Vila, V.; Rezende, R.; Marques, P.A.A.; Wenneck, G.S.; Nocchi, R.C.d.F.; Terassi, D.d.S.; Andrean, A.F.B.A.; Matumoto-Pintro, P.T. Seaweed extract of *Ascophyllum nodosum* applied in tomato crop as a biostimulant for improving growth, yield and soil fertility in subtropical condition. *J. Appl. Phycol.* **2023**, *35*, 2531–2541. [\[CrossRef\]](#)
- Langowski, L.; Goñi, O.; Ikuyinminu, E.; Feeney, E.; O'Connell, S. Investigation of the direct effect of a precision *Ascophyllum nodosum* biostimulant on nitrogen use efficiency in wheat seedlings. *Plant Physiol. Biochem.* **2022**, *179*, 44–57. [\[CrossRef\]](#)
- Villa e Vila, V.; Marques, P.A.A.; Rezende, R.; Wenneck, G.S.; Terassi, D.d.S.; Andrean, A.F.B.A.; Nocchi, R.C.d.F.; Matumoto-Pintro, P.T. Deficit Irrigation with *Ascophyllum nodosum* Extract Application as a Strategy to Increase Tomato Yield and Quality. *Agronomy* **2023**, *13*, 1853. [\[CrossRef\]](#)
- Santaniello, A.; Scartazza, A.; Gresta, F.; Loreti, E.; Biasone, A.; Di Tommaso, D.; Piaggese, A.; Perata, P. *Ascophyllum nodosum* seaweed extract alleviates drought stress in Arabidopsis by affecting photosynthetic performance and related gene expression. *Front. Plant Sci.* **2017**, *8*, 1362. [\[CrossRef\]](#) [\[PubMed\]](#)
- Goñi, O.; Quille, P.; O'Connell, S. *Ascophyllum nodosum* extract biostimulants and their role in enhancing tolerance to drought stress in tomato plants. *Plant Physiol. Biochem.* **2018**, *126*, 63–73. [\[CrossRef\]](#) [\[PubMed\]](#)
- Pereira, L.; Morrison, M.; Shukla, P.S.; Critchley, A.T. A concise review of the brown macroalga *Ascophyllum nodosum* (Linnaeus) Le Jolis. *J. Appl. Phycol.* **2020**, *32*, 3561–3584. [\[CrossRef\]](#)
- Rajendran, R.; Jagmohan, S.; Jayaraj, P.; Ali, O.; Ramsubhag, A.; Jayaraman, J. Effects of *Ascophyllum nodosum* extract on sweet pepper plants as an organic biostimulant in grow box home garden conditions. *J. Appl. Phycol.* **2021**, *34*, 647–657. [\[CrossRef\]](#)
- Bernardes, R.S.; Santos, S.C.; Santos, C.C.; Heid, D.M.; Vieira, M.C.; Torales, E.P. *Ascophyllum nodosum* seaweed extract and mineral nitrogen in *Alibertia edulis* seedlings. *Rev. Bras. Eng. Agric. Ambient.* **2023**, *27*, 137–180. [\[CrossRef\]](#)
- Craigie, J. Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.* **2011**, *23*, 371–393. [\[CrossRef\]](#)
- Shukla, P.S.; Mantin, E.; Adil, M.; Bajpai, S.; Critchley, A.; Prithiviraj, B. *Ascophyllum nodosum*-based biostimulants: Sustainable applications in agriculture for the stimulation of plant growth, stress tolerance, and disease management. *Front. Plant Sci.* **2019**, *10*, 655. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ferreira, D.F. SISVAR: A computer analysis system to fixed effects Split plot type designs. *Rev. Bras. Biom.* **2019**, *37*, 529–535. [\[CrossRef\]](#)

24. Agarwal, P.K.; Dangariya, M.; Agarwal, P. Seaweed extracts: Potential biodegradable, environmentally friendly resources for regulating plant defence. *Algal Res.* **2021**, *58*, 102363. [[CrossRef](#)]
25. Cristofano, F.; El-Nakhel, C.; Roupael, Y. Biostimulant substances for sustainable agriculture: Origin, operating mechanisms and effects on cucurbits, leafy greens, and nightshade vegetables species. *Biomolecules* **2021**, *11*, 1103. [[CrossRef](#)] [[PubMed](#)]
26. Taiz, L.; Zeiger, E.; Moller, I.M.; Murphy, A. *Fisiologia Vegetal*, 6th ed.; Artmed Editora: Porto Alegre, Brazil, 2017.
27. Arioli, T.; Mattner, S.; Winberg, P. Applications of seaweed extracts in Australian agriculture: Past, present and future. *J. Appl. Phycol.* **2015**, *27*, 2007–2015. [[CrossRef](#)]
28. De Saeger, J.; Van Praet, S.; Vereecke, D.; Park, J.; Jacques, S.; Han, T.; Depuydt, S. Toward the molecular understanding of the action mechanism of *Ascophyllum nodosum* extracts on plants. *J. Appl. Phycol.* **2020**, *32*, 573–597. [[CrossRef](#)]
29. Mattner, S.; Milinkovic, M.; Arioli, T. Increased growth response of strawberry roots to a commercial extract from *Durvillaea potatorum* and *Ascophyllum nodosum*. *J. Appl. Phycol.* **2018**, *30*, 2943–2951. [[CrossRef](#)] [[PubMed](#)]
30. Ali, O.; Ramsubhag, A.; Jayaraman, J. Biostimulatory activities of *Ascophyllum nodosum* extract in tomato and sweet pepper crops in a tropical environment. *PLoS ONE* **2019**, *14*, e0216710. [[CrossRef](#)]
31. Salvi, L.; Brunetti, C.; Cataldo, E.; Niccolai, A.; Centritto, M.; Ferrini, F.; Mattii, G.B. Effects of *Ascophyllum nodosum* extract on *Vitis vinifera*: Consequences on plant physiology, grape quality and secondary metabolism. *Plant Physiol. Biochem.* **2019**, *139*, 21–32. [[CrossRef](#)]
32. Joshi-Paneri, J.; Chamberland, G.; Donnelly, D. Effects of *Chelidonium majus* and *Ascophyllum nodosum* extracts on growth and photosynthesis of soybean. *Acta Agrobot.* **2020**, *73*, 7313. [[CrossRef](#)]
33. Hussain, H.I.; Kasinadhuni, N.; Arioli, T. The effect of seaweed extract on tomato plant growth, productivity and soil. *J. Appl. Phycol.* **2021**, *33*, 1305–1314. [[CrossRef](#)]
34. Ali, O.; Ramsubhag, A.; Ramnarine, S.D.B.J.; Jayaraman, J. Transcriptomic changes induced by applications of a commercial extract of *Ascophyllum nodosum* on tomato plants. *Sci. Rep.* **2022**, *12*, 8042. [[CrossRef](#)] [[PubMed](#)]
35. Ali, N.; Ramkissoon, A.; Ramsubhag, A.; Jayaraj, J. *Ascophyllum* extract application causes reduction of disease levels in field tomatoes grown in a tropical environment. *Crop Prot.* **2016**, *83*, 67–75. [[CrossRef](#)]

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