

## Article

# Effects of NAA and *Ecklonia maxima* Extracts on Lettuce and Tomato Transplant Production

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**Abstract:** *Ecklonia maxima* and the commercial biostimulants produced from it contain various plant growth regulators that are responsible for the growth stimulation recorded in many crops. Auxins are one of the major plant growth regulators contained in *E. maxima* extracts. The aim of this research was to evaluate the growth-promoting effect of a seaweed extract from *E. maxima* on lettuce and tomato transplant production under nursery conditions, and to compare the effect of this extract with an equal concentration of synthetic auxin. Two doses of natural or synthetic exogenous auxins (50 or 100  $\mu\text{g L}^{-1}$ ) were supplied to the substrate through the irrigation water with an ebb and flow system, 4, 11, and 18 days after sowing. A commercial biostimulant based on *E. maxima* extract was used as a source of natural auxin, while 1-naphthaleneacetic acid (NAA) was used as a synthetic auxin. Seedlings supplied only with water were used as a control. Tomato seedlings treated with 100  $\mu\text{g L}^{-1}$  of natural auxins from *E. maxima* extract produced the tallest plants (+22%), with a higher leaf number (+12%), a wider leaf area (+44%), and a stronger stem (+12%), whereas lettuce seedling growth was promoted by all the treatments, but with a greater effect with increasing auxin supplementation and when using *E. maxima* extract, compared to NAA. The results showed that the supplementation of exogenous synthetic auxin (NAA), or an *E. maxima* extract containing natural auxins, can have a growth-promoting effect on lettuce and tomato seedlings. This effect was more evident on lettuce than tomato. The biostimulant produced from *E. maxima* extracts improved seedling quality and promoted shoot and root growth more than the NAA used as a synthetic source of auxins.

**Keywords:** vegetable nursery; seedling; *Solanum lycopersicum*; *Lactuca sativa*; seaweed extract; synthetic auxins; natural auxins; biostimulants



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## 1. Introduction

One of the main goals of vegetable nurseries is to produce high-quality transplants in a short time. Transplants are commonly used for establishing crops in open fields and under greenhouses in many countries of the world [1], as they may determine many advantages during crop growth and improve vegetable production [2–5]. These benefits can be closely related to the management of the seedlings during early growth (irrigation, fertilization, climatic conditions) [1,6–9] and their quality characteristics (e.g., height, leaf number and area, dry matter content). Nursery growers adopt intensive cultivation systems with daily applications of water and fertilizers for enhancing the seedling growth rate that may affect the sustainability of the vegetable nursery industry. Vegetable seedlings are generally raised in trays filled with a limited volume of substrates with low nutrient content. In these conditions, fertilization allows the shortening of production time and the production of good quality transplants that can guarantee a good performance after transplanting. The use of fertilizers on plants raised in containers with limited volumes of substrates, subjected to daily irrigation, can have a negative impact on the environment [10].

The aim to improve the sustainability of intensive agriculture systems is pulling toward the reduction of synthetic inputs and their substitution with natural resources.

This approach needs a broad knowledge of the mechanisms underlying plant growth and development that are very complex and not yet fully understood. These mechanisms can have a crucial role in the strategies for enhancing crop yield in sustainable ways through the limitation of the use of chemical fertilizers or other synthetic products.

Growth-promoting products or microbes have been used for increasing crop production and sustainability and reducing the need for chemical inputs [11]. Plant growth promoters, also known as biostimulants [12], have different functions (biofertilizers, bio-control, phytostimulation, etc.) [13,14] and are of different types (bacteria, mycorrhizae, humic acid, algae extracts, etc.).

Algae or seaweed extracts are often produced from brown algae (*Phaeophyceae*) [15] and are sold in liquid form as commercial biostimulants. They can increase nutrient uptake, resistance to biotic and abiotic stresses, and improve seed germination and crop yield [16]. Seaweed extracts contain appreciable quantities of the macroelements Ca, Mg, K, Na, P, and S, as well as some trace elements, including Fe [17,18]. Recently, they were reported to also include considerable amounts of polyamines [19], abscisic acid, and brassinosteroids [20,21]. Seaweed extract contains several osmoprotectants, such as the quaternary ammonium compounds betaine and proline, together with mannitol (a common storage sugar-alcohol) [22,23]. Alginates and polysaccharides [23,24] are other components of great biological interest for their reported stimulation of nutrient uptake, translocation, and ability to stimulate root growth [22]. In addition, seaweed extracts also contain many types of hormone-like biologically active molecules [25], such as auxins, cytokinins, ethylene, and gibberellins [26,27]. The content of auxins and cytokinins in seaweed extract was found to be similar to those of turgid leaves of higher plants [17].

*Ecklonia maxima* (Osbeck) Papenfuss is a brown algae seaweed native to the southern oceans, mainly found along the southern Atlantic coast of Africa. It has been harvested over the last years to produce alginate, animal feed, fertilizers, and plant biostimulants [28]. The commercial extracts of this seaweed have been used for growth-promoting effects on adult plants of many crops [29,30], but little is known on their effects at early growth stages. *E. maxima* and the commercial biostimulants produced from it contain various plant growth regulators (auxins, cytokinins, polyamines, gibberellins, abscisic acid, and brassinosteroids) that are responsible for the growth stimulation recorded on many crops [21,31,32]. The auxins are one of the major plant growth regulators contained in some commercial *E. maxima* extracts, such as Basfoliar<sup>®</sup> Kelp SL, which is obtained by gentle mechanical extraction from fresh seaweed and contains 0.2% N, 1.5% P<sub>2</sub>O<sub>5</sub>, 0.03% K<sub>2</sub>O, 11 mg L<sup>-1</sup> of auxins of vegetal origin and amino acids, carbohydrates, proteins, minerals, and trace of cytokinins (0.04%) [33,34].

The mechanisms responsible for the growth-promoting effect of seaweed extract are not yet well known. Understanding their mode of action is highly complicated due to the multiple interactions among the several bioactive compounds within the same extract [35]. Nevertheless, their effects on plant growth, development, and yield are similar to the physiological responses recorded in crop plants treated with plant growth regulators [12,16,36]. Thus, it could be hypothesized that the effects on plants of a seaweed extract containing natural phytohormones could be similar to those determined by the application of an equal concentration of exogenous synthetic plant growth regulators.

A wide range of processes influencing vegetable crop development and growth can be controlled by exogenous auxins [14,37,38]. These effects may vary according to the treated species, plant age, and doses applied [37,39–41].

Among vegetable crops, lettuce and tomato are widely cultivated under different latitudes and conditions (open field or protected cultivation) throughout the year. Lettuce and tomato transplants are produced in very large numbers by nursery industries all year long; thus, there is a growing interest in using biostimulants or plant growth regulators to increase nursery industry sustainability.

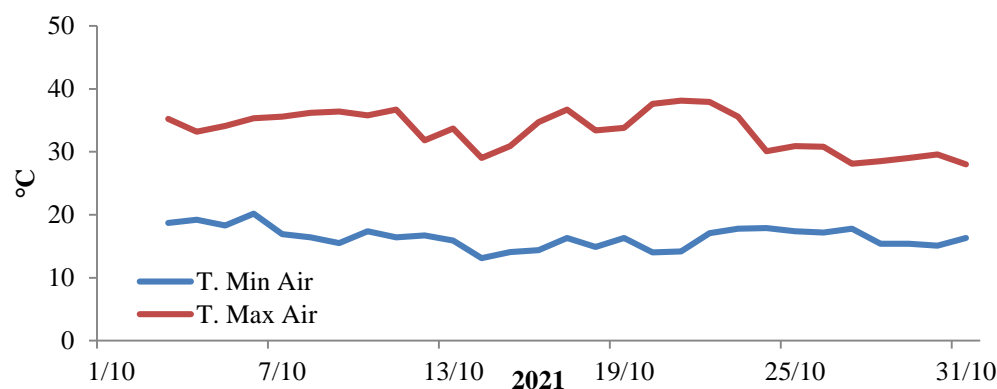
Therefore, the aim of the research was to evaluate the growth-promoting effect of a seaweed extract from *E. maxima* containing natural auxins on lettuce and tomato transplant

production, and to compare the effect of this extract with an equal concentration of a synthetic source of auxins to investigate a possible mode of action of the seaweed extract.

## 2. Materials and Methods

### 2.1. Growth Conditions

The effects of synthetic and natural auxin treatments on transplant production were evaluated in a nursery trial carried out under commercial conditions in a greenhouse situated at the Department of Agricultural, Food, and Forest Sciences (SAAF—University of Palermo, Italy) ( $38^{\circ}6'28''$  N  $13^{\circ}21'3''$  E; altitude 49 m above sea level) during autumn 2021. During the crop cycle, the temperature and the relative humidity of the air inside the greenhouse were monitored using a data logger (mod. 608-H1, Testo s.p.a., Settimo M.se, Italy). A pyranometer (SR30-L, Campbell Scientific, Loughborough, UK) was used for solar radiation measurements. During the experiment, the average temperature outside the greenhouse ranged between  $16.5 \pm 0.4$  °C (night) and  $23.4 \pm 0.5$  °C (day), and the average net solar radiation at noon was  $451 \text{ W}\cdot\text{m}^{-2}$ , with a day length that ranged between 9 and 10 h. Inside the greenhouse, the air temperature was on average  $24.6 \pm 1.2$  °C, and ranged between  $38.1$  °C (day) and  $13.1$  °C (night) (Figure 1), whereas the relative humidity was  $84.8 \pm 1.5\%$  on average, and ranged between 68.2% and 100%; the light intensity at noon was  $41,839 \pm 2451$  lux and ranged from 64,661 to 6097 lux as a function of the cloudiness.



**Figure 1.** Daily average maximum and minimum temperatures of the air inside the greenhouse during seedlings growth.

### 2.2. Transplant Production and Morpho-Physiological Parameter Assessment

Seeds of *Solanum lycopersicum* ‘Marmande’ (Vilmorin, La Méritré, France) and *Lactuca sativa* L. ‘Meraviglia d’inverno’ (Blumen, Piacenza, Italy) were sown into polystyrene trays (104 cells for tomato and 160 for lettuce) filled with a commercial peat substrate (Utilis, GreenView srl, Crocetta del Montello, Italy, containing  $800 \text{ g m}^{-3}$  of a mineral fertilizer NPK 12-11-18). After sowing (1 October 2021), the trays were kept in a dark room at  $25$  °C until seed germination, and were then moved onto fixed benches in the cold greenhouse for seedling growth.

Plantlet emergence occurred 2 and 4 days after sowing for lettuce and tomato, respectively.

Two doses of natural or synthetic exogenous auxins ( $50$  and  $100 \mu\text{g L}^{-1}$ ) were supplied to the substrate through the irrigation water with an ebb and flow system 4, 11, and 18 days after sowing (09, 11, and 13th BBCH lettuce and tomato growth stage, respectively). Seedlings supplied only with water were evaluated as control. 1-naphthaleneacetic acid (NAA; Rigenal P PFnPE VAR 5, Cifo srl, Bologna, Italy) was used as a synthetic auxin, while the source of natural auxins was a commercial extract of *Ecklonia maxima* (Basfoliar® Kelp SL, Compo Expert, Münster, Germany [34]). The two doses of natural auxins corresponded to  $4.55$  or  $9.10 \text{ mL L}^{-1} \text{ H}_2\text{O}$  of the commercial biostimulant ( $11 \text{ mg L}^{-1}$  of auxins is the content of auxins of vegetal origin declared in the product label). During seedling growth, plants were fertilized by sub-fertigating all the trays (including control) with a nutrient solution with  $2 \text{ g L}^{-1}$  of a water-soluble NPK fertilizer (20-20-20) after 14 days from sowing.

The seedlings were sub-irrigated as needed, until they were ready for transplant (on average twice a week).

Plant water use and plant water-use efficiency were calculated from the amount of water consumed by the seedlings for each irrigation and fertigation event by weighing each tray with a digital balance, before refilling the reservoir and after drainage of the exceeding water. Then, plant water use (PWU) was calculated as  $PWU (g\ fw\ L^{-1}\ H_2O) = \text{plant fresh weight (g)}/H_2O (L)$ , and plant water-use efficiency (WUE) was calculated as  $WUE (g\ dw\ L^{-1}\ H_2O) = \text{plant dry weight (g)}/H_2O (L)$  [11]. The total N consumed during the crop cycle was estimated from the volume of nutrient solution supplied to the seedlings, and the nitrogen-use efficiency [42] was calculated as  $NUE (g\ dw\ g^{-1}\ N) = \text{plant total dry weight (g)}/N\ \text{supplied (g)}$ .

Leaf stomatal conductance was measured one week before seedlings were ready for transplanting, using a diffusion porometer (AP4, Delta-T Devices Ltd., Cambridge, England) on two young unshaded leaves of 25 seedlings for each species and each replicate.

Leaf color of each seedling was measured on the upper part of 2 randomly selected leaves, using a colorimeter (CR-400, Minolta corporation, Ltd., Osaka, Japan) that measured  $L^*$  (lightness),  $a^*$  (positive values for reddish colors and negative values for greenish colors) and  $b^*$  (positive values for yellowish colors and negative values for bluish colors). These components were used to calculate hue angle ( $h^\circ$ ) and chroma ( $C^*$ ) as  $h^\circ = 180^\circ + \arctan(b^*/a^*)$  [43] and  $C^* = (a^{*2} + b^{*2})^{1/2}$ .

About one month after sowing (26 and 25 days for lettuce and tomato, respectively) the seedlings were ready to be transplanted (15–16th and 14–15th BBCH growth stage for lettuce and tomato, respectively) and were destructively sampled for the data collecting.

Four replicated samples of 25 seedlings for each species and each treatment were randomly selected and analyzed to evaluate their morphological characteristics (plant height, stem thickness, leaf number). The leaves were detached from the stem and scanned at 300 dpi (Epson Perfection 4180 Photo, Seiko Epson Corp., Suwa, Japan) to obtain digital images that were analyzed with the ImageJ 1.52a software (National Institutes Health, Bethesda, MD, USA) to measure the leaf area.

Leaves, stem, and roots were weighed and dried to a constant weight at 85 °C to determine the fresh and dry biomass and the shoot/root ratio for both fresh and dry weight. The specific leaf area (SLA  $cm^2\ g^{-1}\ dw$ ) was estimated as the leaf area/leaf dry weight.

### 2.3. Statistics and Principal Component Analysis

The experimental design consisted of four replicated samples of 25 seedlings each for every species and every treatment, randomly assigned in four blocks. A one-way ANOVA was performed to evaluate the effects of natural and synthetic auxin treatments on each species. The mean values were compared by the Tukey test at  $p \leq 5\%$  to identify the significant differences among treatments.

All the parameters evaluated in lettuce and tomato seedlings were also used to perform a principal component analysis to investigate the parameters that could effectively discriminate among treatments. The parameters used in the analysis consisted of height, stem diameter, total, leaf, stem, and root fresh and dry weight, shoot/root ratio of fresh and dry weights, dry matter percentage, leaf number, plant and leaf area, SLA, stomatal conductance, PWU, WUE, NUE,  $L^*$ , chroma, and hue angle). The principal components (PCs) were assessed by holding only the factors with eigenvalues higher than 1.0. The plot of the PCs allowed us to study the correlations between the variables of the input data set, and the initial 24 variables were projected into the subspace defined by the first and second PCs, and correlated variables were revealed. The statistical tests, such as homoscedasticity and normality, ANOVA, and PCA, were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

### 3. Results

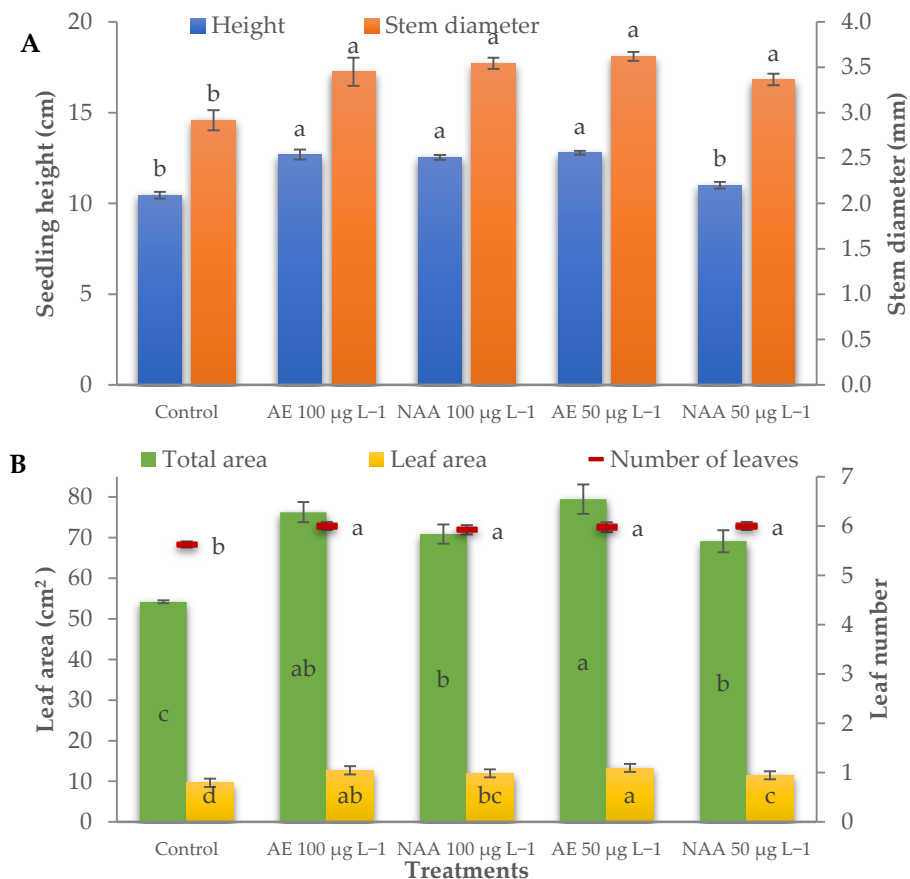
#### 3.1. Morphophysiological Parameters of Lettuce Seedlings

The use of synthetic auxin (NAA) or the algae extract from Basfoliar® Kelp SL (AE) containing natural auxins had significant effects on many morphological and physiological parameters.

The height of the control seedlings was 10.5 cm, and was significantly increased by AE treatments (from seaweed extract) and by 100  $\mu\text{g L}^{-1}$  NAA (12.7 cm on average) (Table 1, Figure 2A). The stem diameter of the treated seedlings was significantly thicker (+19.8%) than the control (2.92 mm). The treated seedlings also had a higher leaf number than the control but differed in leaf size.

The greatest leaf area (79.4  $\text{cm}^2$  seedling $^{-1}$ ; 13.3  $\text{cm}^2$  leaf $^{-1}$ ) was measured in lettuce seedlings treated with 50  $\mu\text{g L}^{-1}$  AE that did not significantly differ from those treated with 100  $\mu\text{g L}^{-1}$  AE; treatments with NAA also increased the leaf area (+29.2% and +21.8% on average for seedling and leaf area, respectively) compared to the control (54.2  $\text{cm}^2$  seedling $^{-1}$ ; 9.6  $\text{cm}^2$  leaf $^{-1}$ ), but to a lower extent than AE (Table 1, Figure 2B). The specific leaf area was negatively affected in the seedlings treated with 50  $\mu\text{g L}^{-1}$  of NAA.

The chromatic characteristics of lettuce leaves ( $L^*$ , chroma and hue) were only slightly influenced by the treatments. The leaf color was found to be significantly lighter than the control in the lettuce seedlings treated with 50  $\mu\text{g L}^{-1}$  AE or NAA, but they showed no significant change in chroma and hue angle (Table 1).



**Figure 2.** Effect of different doses (50  $\mu\text{g L}^{-1}$  or 100  $\mu\text{g L}^{-1}$ ) of natural (AE—algae extract from *Ecklonia maxima*) and synthetic (NAA) exogenous auxins on seedling height and stem diameter (A), and on total seedling area, leaf area, and leaf number (B) of lettuce transplants (bars represent standard errors of the means; points or bars of the same color with different letters are significantly different at  $p < 0.05$  according to the Tukey test).



**Table 1.** Effect of different doses (50 µg L<sup>-1</sup> or 100 µg L<sup>-1</sup>) of natural (AE—algae extract from *Ecklonia maxima*) and synthetic (NAA) exogenous auxins on morphological parameters and chromatic characteristics of lettuce transplants.

Treatments	Height (cm)	Stem Diameter (mm)	Number of Leaves	Leaf Area (cm <sup>2</sup> seedling <sup>-1</sup> )	Leaf Area (cm <sup>2</sup> leaf <sup>-1</sup> )	SLA (cm <sup>2</sup> g dw <sup>-1</sup> )	L*	Chroma	Hue
Control	<sup>z</sup> 10.5 b	2.92 b	5.63 b	54.2 c	9.6 d	1247.6 a	55.9 a	43.3	120.2
AE 100 µg L <sup>-1</sup>	12.7 a	3.45 a	6.00 a	76.2 ab	12.7 ab	1222.4 a	55.4 ab	43.2	120.5
NAA 100 µg L <sup>-1</sup>	12.5 a	3.54 a	5.93 a	70.9 b	12.0 bc	1178.1 a	55.3 ab	43.2	120.3
AE 50 µg L <sup>-1</sup>	12.8 a	3.62 a	5.98 a	79.4 a	13.3 a	1195.9 a	54.6 b	43.5	120.2
NAA 50 µg L <sup>-1</sup>	11.0 b	3.36 a	6.00 a	69.1 b	11.5 c	1044.3 b	54.4 b	44.1	120.7
Significance <sup>x</sup>	***	***	*	***	***	***	*	ns	ns

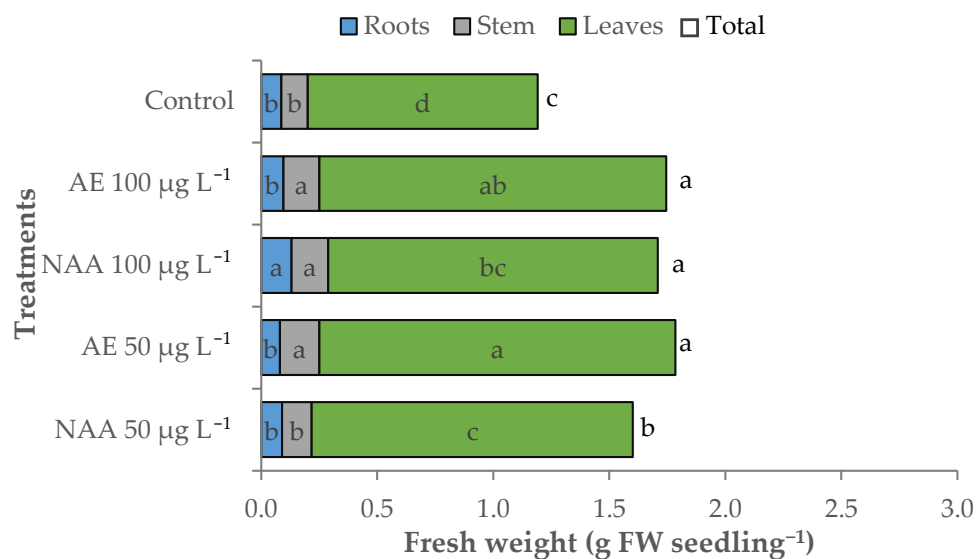
<sup>z</sup> Each value is the mean of 4 replicated samples of 25 seedlings each. Data within a column followed by the same letters do not differ significantly at  $p \leq 0.05$  according to the Tukey test. <sup>x</sup> Significance: ns = not significant; \* significant at  $p < 0.05$ ; \*\*\* significant at  $p < 0.001$ .

The total fresh weight (fw) of the control seedling was 1.19 g fw seedlings<sup>-1</sup> (Table 2, Figure 3). The treatments determined an increase in total fresh weight by 46.6% on average, when *E. maxima* extracts or 100 µg L<sup>-1</sup> of NAA were supplemented to the seedlings, and by 34.4% with 50 µg L<sup>-1</sup> of NAA.

**Table 2.** Effect of different doses (50 µg L<sup>-1</sup> or 100 µg L<sup>-1</sup>) of natural (AE—algae extract from *Ecklonia maxima*) and synthetic (NAA) exogenous auxins on the fresh and dry weight of lettuce transplants.

Treatments	Fresh Weight (g seedling <sup>-1</sup> )					Dry Weight (mg seedling <sup>-1</sup> )					Dry Matter %
	Total	Leaves	Stem	Root	Shoot/Root	Total	Leaves	Stem	Root	Shoot/Root	
Control	<sup>z</sup> 1.19 c	0.99 d	0.12 b	0.09 b	12.9 c	61.3 b	43.5 b	4.5	13.3 a	3.6 c	5.2 a
AE 100 µg L <sup>-1</sup>	1.75 a	1.50 ab	0.16 a	0.10 b	17.5 b	77.7 a	62.5 a	4.5	10.7 b	6.3 b	4.4 b
NAA 100 µg L <sup>-1</sup>	1.71 a	1.42 bc	0.16 a	0.13 a	12.1 c	72.5 a	60.3 a	4.8	7.5 c	8.7 a	4.2 b
AE 50 µg L <sup>-1</sup>	1.78 a	1.53 a	0.17 a	0.08 b	21.4 a	80.2 a	66.4 a	4.5	9.3 bc	7.8 ab	4.5 b
NAA 50 µg L <sup>-1</sup>	1.60 b	1.38 c	0.13 b	0.09 b	17.4 b	80.8 a	66.1 a	4.7	10.0 b	7.3 ab	5.1 a
Significance <sup>x</sup>	***	***	***	***	***	***	***	ns	***	***	***

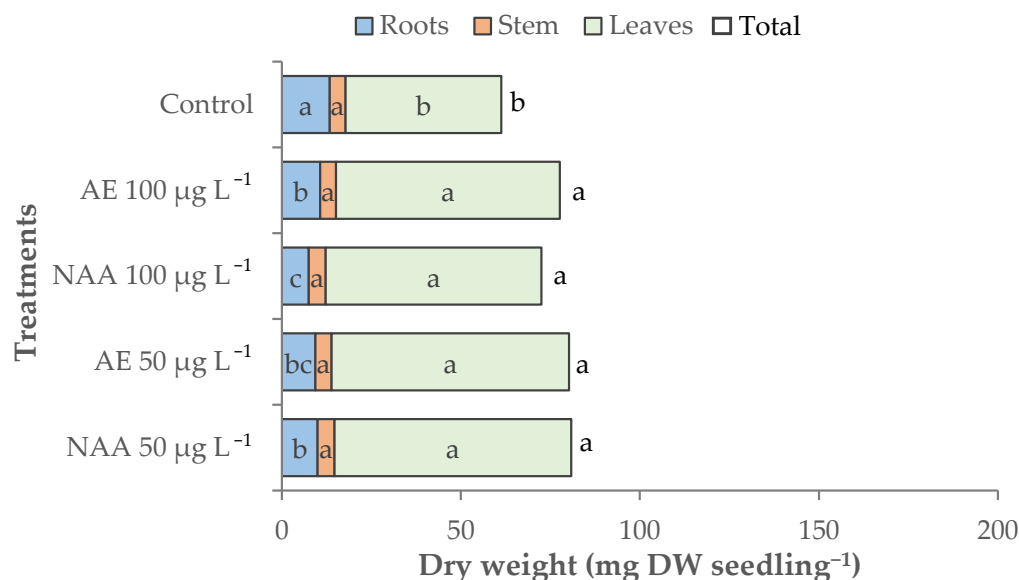
<sup>z</sup> Each value is the mean of 4 replicated samples of 25 seedlings each. Data within a column followed by the same letters do not differ significantly at  $p \leq 0.05$  according to Tukey test. <sup>x</sup> Significance: ns = not significant; \*\*\* significant at  $p < 0.001$ . All percentage data were subjected to angular transformation.



**Figure 3.** Effect of different doses (50 µg L<sup>-1</sup> or 100 µg L<sup>-1</sup>) of natural (AE—algae extract from *Ecklonia maxima*) or synthetic (NAA) exogenous auxins on the total, root, stem, and leaves fresh biomass of lettuce transplants (bars of the same color with different letters are significantly different at  $p < 0.05$  according to the Tukey test).

These differences were mainly due to the leaf weight that recorded greater variations compared to the stem and root fresh weight (Table 2, Figure 3). The seedlings with the highest amount of leaves in terms of fresh weight were those treated with both doses of algae extract (1.53 and 1.50 g fw seedling<sup>-1</sup>, respectively), whereas the lowest leaf fresh biomass was recorded in the control seedlings (0.99 g fw seedling<sup>-1</sup>); intermediate values were observed with NAA treatments. The effect of treatments on fresh biomass partitioning was highlighted by the shoot/root ratio of the fresh weight, which increased up to 21.4 with 50 µg L<sup>-1</sup> of natural auxins (AE) and to 17.5 on average with 100 µg L<sup>-1</sup> AE or 50 µg L<sup>-1</sup> of NAA (Table 2).

The total dry biomass accumulated in control seedlings was 61.3 mg dw seedling<sup>-1</sup> and was significantly increased by all the treatments (+26.9% on average) (Table 2, Figure 4). A similar trend was recorded for leaf dry weight, while root dry weight was significantly reduced by the treatments, especially with 100 µg L<sup>-1</sup> of NAA (−43.5%). The dry matter percentage of lettuce seedlings was significantly lowered by the treatments (4.3% on average), except for the treatment with 50 µg L<sup>-1</sup> of NAA (Table 2).



**Figure 4.** Effect of different doses (50 µg L<sup>-1</sup> or 100 µg L<sup>-1</sup>) of natural (AE—algae extract from *Ecklonia maxima*) or synthetic (NAA) exogenous auxins on the total, root, stem, and leaves dry biomass of lettuce transplants (bars of the same color with different letters are significantly different at  $p < 0.05$  according to the Tukey test).

Only a high concentration (100 µg L<sup>-1</sup>) of natural (AE) and synthetic (NAA) auxins was effective in increasing the stomatal conductance of the leaves (885.3 mmol m<sup>2</sup> s<sup>-1</sup>, on average) while the other treatments did not differ from the control (328.8 mmol m<sup>2</sup> s<sup>-1</sup>) (Table 3).

Control seedlings produced 44.0 g of fresh weight per liter of irrigation water (PWU) and did not differ significantly from the seedlings treated with 50 µg L<sup>-1</sup> of NAA. The use of 100 µg L<sup>-1</sup> AE increased the plant water use by 26.9% compared to control, while 100 µg L<sup>-1</sup> of NAA and 50 µg L<sup>-1</sup> of natural auxins (AE) raised PWU by 14.5% on average (Table 3). No significant effect was recorded on the water use efficiency that was 2.3 g dw L<sup>-1</sup> H<sub>2</sub>O, on average. Small modifications were also found in nitrogen-use efficiency: 11.3 g dw g<sup>-1</sup> N in the control seedlings, which increased significantly (+14.8%) only with 100 µg L<sup>-1</sup> AE.

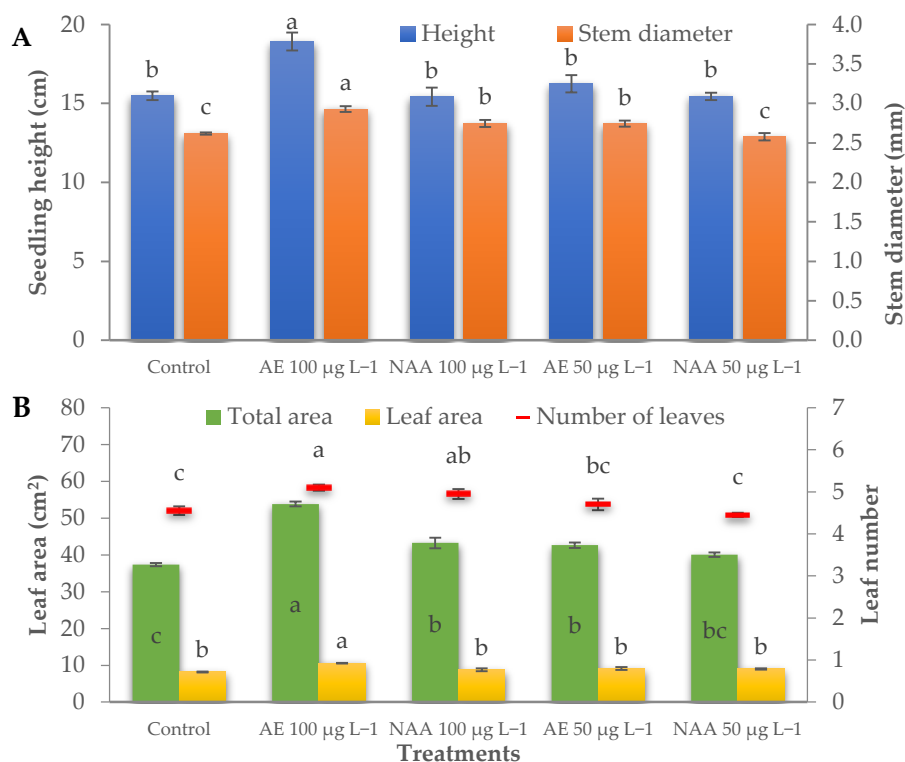
**Table 3.** Effect of different doses ( $50 \mu\text{g L}^{-1}$  or  $100 \mu\text{g L}^{-1}$ ) of natural (AE—algae extract from *Ecklonia maxima*) and synthetic (NAA) exogenous auxins on stomatal conductance, plant water use (PWU), water-use efficiency (WUE), and nitrogen-use efficiency (NUE) of lettuce transplants.

Treatments	Stomatal Conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	PWU ( $\text{g fw L}^{-1} \text{H}_2\text{O}$ )	WUE ( $\text{g dw L}^{-1} \text{H}_2\text{O}$ )	NUE ( $\text{g dw g}^{-1} \text{N}$ )
Control	<sup>z</sup> 328.8 b	44.0 c	2.3	11.3 b
AE $100 \mu\text{g L}^{-1}$	903.0 a	55.8 a	2.5	13.0 a
NAA $100 \mu\text{g L}^{-1}$	867.5 a	51.4 b	2.2	11.7 ab
AE $50 \mu\text{g L}^{-1}$	481.0 b	49.3 b	2.2	12.1 ab
NAA $50 \mu\text{g L}^{-1}$	537.0 b	45.2 c	2.3	12.4 ab
Significance <sup>x</sup>	***	***	ns	*

<sup>z</sup> Each value is the mean of 4 replicated samples of 25 seedlings each. Data within a column followed by the same letters do not differ significantly at  $p \leq 0.05$  according to Tukey test. <sup>x</sup> Significance: ns = not significant; \* significant at  $p < 0.05$ ; \*\*\* significant at  $p < 0.001$ .

### 3.2. Morphophysiological Parameters of Tomato Seedlings

All the morphological parameters were influenced by the treatments. The height of the plants was higher than the control (15.5 cm) only with the use of  $100 \mu\text{g L}^{-1}$  of natural auxins from AE (+22.2%) (Table 4, Figure 5A), which also determined the thickest stem (2.93 mm). A significant increase in stem diameter compared to control (2.62 mm) was also found in the seedling treated with  $100 \mu\text{g L}^{-1}$  of NAA and  $50 \mu\text{g L}^{-1}$  of AE (2.75 mm on average) (Table 4, Figure 5A).



**Figure 5.** Effect of different doses ( $50 \mu\text{g L}^{-1}$  or  $100 \mu\text{g L}^{-1}$ ) of natural (AE—algae extract from *Ecklonia maxima*) and synthetic (NAA) exogenous auxins on seedling height and stem diameter (A), and on total seedling area, leaf area, and leaf number (B) of tomato transplants (bars represent standard errors of the means; points or bars of the same color with different letters are significantly different at  $p < 0.05$  according to the Tukey test).



**Table 4.** Effect of different doses (50  $\mu\text{g L}^{-1}$  or 100  $\mu\text{g L}^{-1}$ ) of natural (AE—algae extract from *Ecklonia maxima*) and synthetic (NAA) exogenous auxins on morphological parameters and chromatic characteristics of tomato transplants.

Treatments	Height (cm)	Stem Diameter (mm)	Number of Leaves	Leaf Area ( $\text{cm}^2$ seedling $^{-1}$ )	Leaf Area ( $\text{cm}^2$ leaf $^{-1}$ )	SLA ( $\text{cm}^2$ g dw $^{-1}$ )	L*	Chroma	Hue
Control	<sup>z</sup> 15.5 b	2.62 c	4.55 c	37.3 c	8.2 b	567.2 b	44.6 b	30.5	126.6 ab
AE 100 $\mu\text{g L}^{-1}$	18.9 a	2.93 a	5.10 a	53.8 a	10.6 a	644.9 a	44.5 b	31.6	126.8 a
NAA 100 $\mu\text{g L}^{-1}$	15.4 b	2.75 b	4.95 ab	43.3 b	8.8 b	678.7 a	45.3 ab	32.2	126.3 ac
AE 50 $\mu\text{g L}^{-1}$	16.3 b	2.74 b	4.70 bc	42.6 b	9.1 b	569.0 b	45.7 ab	31.9	125.7 bc
NAA 50 $\mu\text{g L}^{-1}$	15.4 b	2.58 c	4.45 c	40.1 bc	9.0 b	591.5 b	46.3 a	31.9	125.7 c
Significance <sup>x</sup>	***	***	**	***	***	***	*	ns	*

<sup>z</sup> Each value is the mean of 4 replicated samples of 25 seedlings each. Data within a column followed by the same letters do not differ significantly at  $p \leq 0.05$  according to Tukey test. <sup>x</sup> Significance: ns = not significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; \*\*\* significant at  $p < 0.001$ .

A significantly higher number of leaves than the control (4.55) were found in the seedlings treated with 100  $\mu\text{g L}^{-1}$  of AE or NAA (5.10 and 4.95 leaves seedling $^{-1}$  for natural and synthetic auxins, respectively) (Table 4, Figure 5B). The leaf area of the control seedlings was 8.2  $\text{cm}^2$  leaf $^{-1}$  and 37.3  $\text{cm}^2$  seedling $^{-1}$ . Treatments were effective in increasing the average leaf area only using 100  $\mu\text{g L}^{-1}$  of natural auxins (AE), which determined the greatest leaf area per seedling (53.8  $\text{cm}^2$  seedling $^{-1}$ ). Nevertheless, a significant increase in total leaf area was also recorded with 50  $\mu\text{g L}^{-1}$  of AE and 100  $\mu\text{g L}^{-1}$  of NAA (42.98  $\text{cm}^2$  seedling $^{-1}$  on average) (Table 4, Figure 5B). The treatments with 100  $\mu\text{g L}^{-1}$  of auxins (both AE and NAA) recorded an increase in the specific leaf area by 16.7% on average, compared to the control seedlings (567.2  $\text{cm}^2$  g dw $^{-1}$ ).

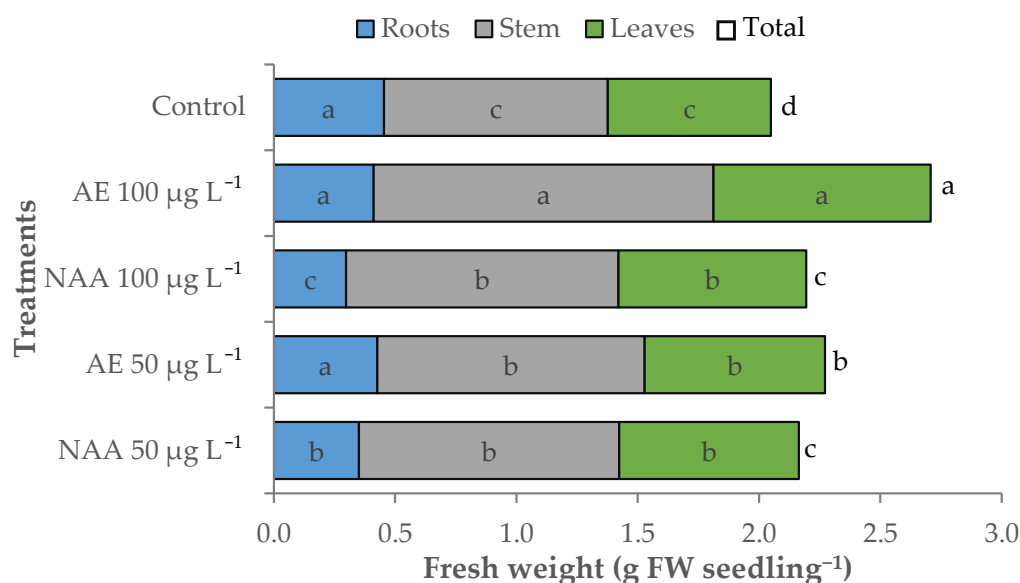
The chromatic characteristics of the tomato leaves (L\*, chroma, and hue) were slightly influenced by the treatments. The leaf color of tomato seedlings treated with 50  $\mu\text{g L}^{-1}$  of NAA was lighter and less greenish (lower hue angle) than the control seedlings. No significant difference was recorded with regards to chroma (Table 4).

Total fresh weight (fw) was 2.05 g fw seedling $^{-1}$  in the control seedlings, which significantly increased by 6.4% in the tomato seedlings treated with NAA (2.18 g fw seedling $^{-1}$  on average). A further increase was recorded when treating seedlings with natural auxins, especially with 100  $\mu\text{g L}^{-1}$  AE (2.71 g fw seedling $^{-1}$ ; +32.1% than control) (Table 5, Figure 6). The main part of the fresh biomass was represented by the stem and the leaves, which resulted in being significantly heavier than the control in the treated seedling, especially those treated with 100  $\mu\text{g L}^{-1}$  AE (+33.0% and +51.7% compared to control for leaf and stem fresh weight, respectively). The seedlings treated with NAA recorded a reduction of root fresh weight compared to control, which was more severe as increasing NAA concentration (−22.5% and −34.4% for 50 and 100  $\mu\text{g L}^{-1}$  NAA, respectively) (Table 5).

**Table 5.** Effect of different doses (50  $\mu\text{g L}^{-1}$  or 100  $\mu\text{g L}^{-1}$ ) of natural (AE—algae extract from *Ecklonia maxima*) and synthetic (NAA) exogenous auxins on the fresh and dry weight of tomato seedlings.

Treatments	Fresh Weight (g seedling $^{-1}$ )					Dry Weight (mg seedling $^{-1}$ )					Dry Matter %
	Total	Leaves	Stem	Root	Shoot/Root	Total	Leaves	Stem	Root	Shoot/Root	
Control	<sup>z</sup> 2.05 d	0.67 c	0.92 c	0.45 a	3.5 d	146.8 c	65.8 c	48.3 d	32.7 a	3.5 c	7.2 a
AE 100 $\mu\text{g L}^{-1}$	2.71 a	0.90 a	1.40 a	0.41 a	5.6 b	185.4 a	83.5 a	71.3 a	30.7 a	5.1 a	6.8 a
NAA 100 $\mu\text{g L}^{-1}$	2.19 c	0.78 b	1.12 b	0.30 c	6.4 a	138.4 d	63.8 c	50.0 cd	24.7 b	4.7 ab	6.3 b
AE 50 $\mu\text{g L}^{-1}$	2.27 b	0.74 b	1.10 b	0.43 a	4.3 c	161.8 b	75.0 b	55.5 b	31.3 a	4.2 b	7.1 a
NAA 50 $\mu\text{g L}^{-1}$	2.16 c	0.74 b	1.07 b	0.35 b	5.2 b	147.8 c	67.8 c	54.0 bc	26.0 b	4.7 ab	6.8 a
Significance <sup>x</sup>	***	***	***	***	***	***	***	***	***	***	***

<sup>z</sup> Each value is the mean of 4 replicated samples of 25 seedlings each. Data within a column followed by the same letters do not differ significantly at  $p \leq 0.05$  according to Tukey test. <sup>x</sup> Significance: \*\*\* significant at  $p < 0.001$ . All percentage data were subjected to angular transformation.



**Figure 6.** Effect of different doses ( $50 \mu\text{g L}^{-1}$  or  $100 \mu\text{g L}^{-1}$ ) of natural (AE—algae extract from *Ecklonia maxima*) or synthetic (NAA) exogenous auxins on the total, root, stem, and leaves fresh biomass of tomato transplants (bars of the same color with different letters are significantly different at  $p < 0.05$  according to the Tukey test).

The modification of fresh biomass partitioning due to the experimental factors was shown by the shoot/root ratio of the fresh weight. The treated seedlings had a higher allocation of biomass in the shoots than control. This effect was higher with  $100 \mu\text{g L}^{-1}$  than  $50 \mu\text{g L}^{-1}$  and using NAA compared to the algae extract (Table 5).

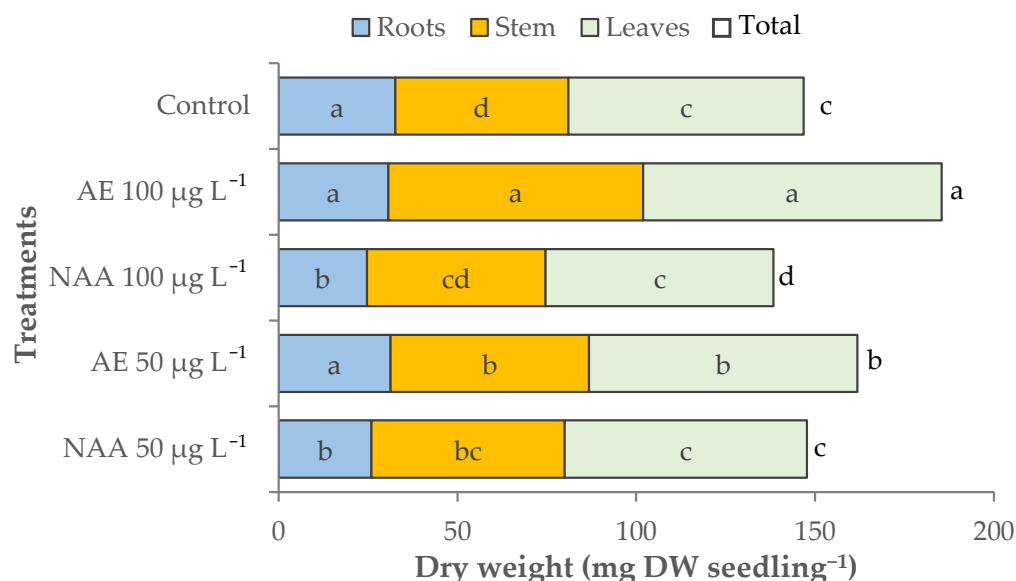
The experimental factors also affected the dry weight (dw) of tomato seedlings that increased significantly when natural auxins from *E. maxima* extracts were applied to the seedlings, while this decreased using  $100 \mu\text{g L}^{-1}$  of NAA (Table 5, Figure 7). The plants treated with  $100 \mu\text{g L}^{-1}$  of natural auxins (AE) had the highest total dry weight ( $185.4 \text{ mg dw seedling}^{-1}$ ) due to the highest dry biomass accumulated in the leaves ( $85.3 \text{ mg seedling}^{-1}$ ) and stem ( $71.3 \text{ mg seedling}^{-1}$ ). The seedlings treated with  $50 \mu\text{g L}^{-1}$  AE had a lower dry biomass accumulation in the leaves and stem, but differed significantly from the control. The seedlings treated with NAA showed small or no significant differences with control seedlings regarding leaf and stem dry weight, but recorded the lowest dry biomass accumulation in the roots. Regarding the dry weight shoot/root ratio, all the treated seedlings showed a higher allocation of dry biomass in the shoots than in the control (3.5). The highest effect on dry biomass partitioning was recorded with  $100 \mu\text{g L}^{-1}$  AE (5.1). A significant variation of dry matter percentage was found only in the seedlings treated with  $100 \mu\text{g L}^{-1}$  of NAA (6.3%) (Table 5).

The leaves with higher stomatal conductance were those of the seedlings treated with  $100 \mu\text{g L}^{-1}$  of natural (AE) or synthetic (NAA) auxins (+87.0% and +54.3% than control, respectively) while the use of  $50 \mu\text{g L}^{-1}$  of AE or NAA had no significant effect (Table 6).

The control seedlings produced  $36.7 \text{ g}$  of fresh biomass per liter of irrigation water (PWU); the treatments with both doses of synthetic auxin and with  $50 \mu\text{g L}^{-1}$  of natural auxins applied through the *Ecklonia maxima* extract increased tomato seedling PWU by 23.6% on average, while the highest PWU value ( $52.2 \text{ g fw L}^{-1} \text{ H}_2\text{O}$ ) was recorded with  $100 \mu\text{g L}^{-1}$  of natural auxins (AE) (+42.3%) (Table 6).

The WUE of tomato seedlings was positively influenced to different extents by the source of auxins and their concentrations. WUE was  $2.6 \text{ g dw L}^{-1} \text{ H}_2\text{O}$  in control seedlings, and increased by 19.6% in the seedlings treated with  $50 \mu\text{g L}^{-1}$  of AE and NAA (3.1, on average). Increasing the concentration of the *Ecklonia maxima* extract further increased WUE up to 3.6, whereas using  $100 \mu\text{g L}^{-1}$  of NAA, WUE was 2.9. The NUE of control seedlings

was 16.9 g dw g<sup>-1</sup> N. This parameter increased by 9.1%, 17.1%, and 31.7%, with 50 µg L<sup>-1</sup> of NAA, 50 and 100 µg L<sup>-1</sup> of natural auxins (AE), respectively (Table 6).



**Figure 7.** Effect of different doses (50 µg L<sup>-1</sup> or 100 µg L<sup>-1</sup>) of natural (AE—algae extract from *Ecklonia maxima*) or synthetic (NAA) exogenous auxins on the total, root, stem, and leaves dry biomass of tomato transplants (bars of the same color with different letters are significantly different at  $p < 0.05$  according to the Tukey test).

**Table 6.** Effect of different doses (50 µg L<sup>-1</sup> or 100 µg L<sup>-1</sup>) of natural (AE—algae extract from *Ecklonia maxima*) and synthetic (NAA) exogenous auxin on stomatal conductance, plant water use (PWU), water-use efficiency (WUE), and nitrogen-use efficiency (NUE) of tomato seedlings.

Treatments	Stomatal Conductance (mmol m <sup>2</sup> s <sup>-1</sup> )	PWU (g fw L <sup>-1</sup> H <sub>2</sub> O)	WUE (g dw L <sup>-1</sup> H <sub>2</sub> O)	NUE (g dw g <sup>-1</sup> N)
Control	<sup>z</sup> 399.3 c	36.7 c	2.6 d	16.9 d
AE 100 µg L <sup>-1</sup>	746.7 a	52.2 a	3.6 a	22.3 a
NAA 100 µg L <sup>-1</sup>	616.0 b	45.9 b	2.9 c	16.1 d
AE 50 µg L <sup>-1</sup>	494.0 c	44.9 b	3.2 b	19.8 b
NAA 50 µg L <sup>-1</sup>	430.0 c	45.3 b	3.1 b	18.6 c
Significance <sup>x</sup>	***	***	***	***

<sup>z</sup> Each value is the mean of 4 replicated samples of 25 seedlings each. Data within a column followed by the same letters do not differ significantly at  $p \leq 0.05$  according to Tukey test. <sup>x</sup> Significance: \*\*\* significant at  $p < 0.001$ .

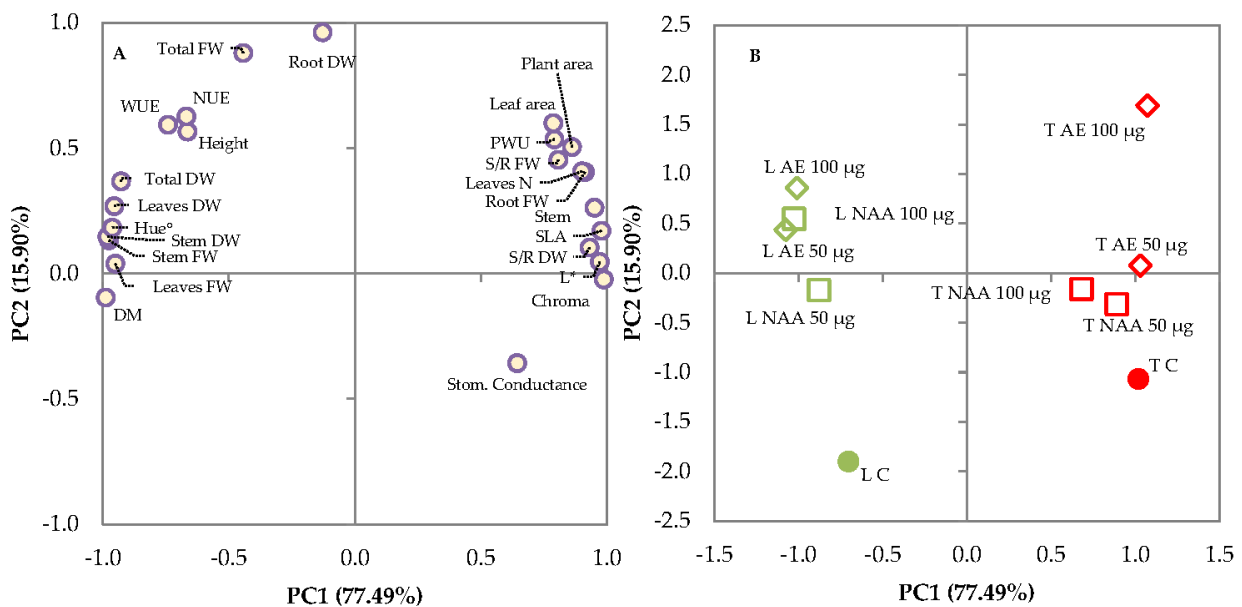
### 3.3. Principal Component Analysis

The principal component analysis revealed two principal components (PCs) with eigenvalues higher than 1.00, representing 77.49% and 15.90% of the total variance, respectively, and combined could represent the initial twenty-four variables, explaining 93.39% of the total variance (Table 7). PC1 was mainly related to stem diameter, leaf, stem, and root fresh weight (fw), shoot/root (S/R) fw, total, leaf and stem dry weight (dw), shoot/root (S/R) dw, dry matter percentage, leaf number, plant and leaf area, specific leaf area (SLA), water-use efficiency (WUE), plant water use (PWU), L\*, chroma, and hue angle; PC2 was related to total fresh weight and root dry weight (Table 7). The projection of the original variables on the plane of the two PCs displayed in the plot of loadings (Figure 8A) illustrates such relationships.

**Table 7.** Correlation of variables to the factors of the principal components analysis (PCA) based on factor loadings.

Variable	PC1	PC2
Height	−0.664	0.567
Stem diameter	<b>0.950</b>	0.263
Total fresh weight	−0.442	<b>0.880</b>
Root fresh weight	<b>0.912</b>	0.405
Stem fresh weight	−0.975	0.133
Leaf fresh weight	−0.950	0.040
Shoot/Root fw	<b>0.807</b>	0.454
Total dry weight	−0.927	0.368
Root dry weight	−0.128	<b>0.963</b>
Stem dry weight	−0.981	0.147
Leaf dry weight	−0.954	0.269
Shoot/Root dw	<b>0.931</b>	0.104
Dry matter %	−0.988	−0.095
Leaf number	<b>0.901</b>	0.408
Plant area	<b>0.861</b>	0.505
Leaf area	<b>0.787</b>	0.600
SLA	<b>0.978</b>	0.171
Stomatal conductance	<b>0.644</b>	−0.357
WUE	−0.741	0.594
PWU	<b>0.791</b>	0.536
NUE	−0.668	0.627
L*	<b>0.971</b>	0.047
Chroma	<b>0.987</b>	−0.023
Hue°	−0.962	0.183

Values in bold within the same factor indicate the variable with the largest correlation.



**Figure 8.** Plots of (A) loadings (morphophysiological characteristics of lettuce and tomato seedlings) and (B) scores (trials) formed by the two principal components from the principal component analysis (PCA). L C: untreated lettuce seedlings; L AE 100 µg and L AE 50 µg lettuce seedlings treated with 100 µg L<sup>−1</sup> and 50 µg L<sup>−1</sup> of natural auxins from *Ecklonia maxima* extract, respectively; L NAA 100 µg and L NAA 50 µg lettuce seedlings treated with 100 µg L<sup>−1</sup> and 50 µg L<sup>−1</sup> of synthetic auxins (1-Naphthaleneacetic acid), respectively; T C: untreated tomato seedlings; T AE 100 µg and T AE 50 µg tomato seedlings treated with 100 µg L<sup>−1</sup> and 50 µg L<sup>−1</sup> of natural auxins from *Ecklonia maxima* extract, respectively; T NAA 100 µg and T NAA 50 µg tomato seedlings treated with 100 µg L<sup>−1</sup> and 50 µg L<sup>−1</sup> of synthetic auxins (1-Naphthaleneacetic acid), respectively.

The differentiation of the treatments with the *E. maxima* biostimulant or NAA to lettuce and tomato seedlings is shown in the plot of scores (Figure 8B), where two main clusters (lettuce and tomato transplants) could be visibly distinguished. The scores of lettuce seedlings were located in the negative part of the PC1 axis, whereas tomato seedlings were located in the positive part of the PC1 axis; the untreated lettuce seedlings were located in the third quadrant ( $-,-$ ) together with those treated with  $50 \mu\text{g L}^{-1}$  of NAA, whereas the untreated tomato seedlings were located in the fourth quadrant ( $+,-$ ) together with those treated with both doses of NAA. The lettuce seedlings treated with natural auxins from *E. maxima* extract (AE) or with  $100 \mu\text{g L}^{-1}$  of NAA were located in the second quadrant ( $-,+$ ) whereas the tomato seedlings treated with *E. maxima* extract (AE) had higher and positive PC2 values, and were located in the first quadrant ( $+,+$ ).

Thus, lettuce and tomato seedlings showed a different response to the *E. maxima* extract or NAA supplemented through the irrigation water. The scores of treated lettuce seedlings increased their PC2 by increasing auxin concentration, but this was seen to a greater extent when using the seaweed extract as a source of natural auxins. Tomato seedlings were less influenced by NAA, but showed a higher sensitivity to the treatments with  $100 \mu\text{g L}^{-1}$  of natural auxins from the *E. maxima* extract. Combining the data from the plot of loadings and scores, it can be concluded that the treatment with natural or synthetic auxins influenced the lettuce and tomato seedlings in different ways.

#### 4. Discussion

In this work, we investigated methods of enhancing the growth of lettuce and tomato seedlings and producing vegetable transplants with good quality characteristics, by supplementing a commercial biostimulant produced from the seaweed *Ecklonia maxima* that can be considered a source of natural auxins, and other plant growth promoters. We also supplied the seedlings with an equal concentration of synthetic auxin (NAA) to elucidate if it can exert similar effects on seedling growth.

It is well known that not all species respond to the same amount and type of auxins, and that the response can be very different even among plants of the same species [44]. The different responses of the two vegetable species to the auxin sources (natural auxins contained in the commercial *Ecklonia maxima* extract, or NAA) can depend on the auxin influx and efflux carriers. Some specialized carriers mediate the auxin's polar transport: incoming carrier proteins of the family AUX1/LAX and outgoing carrier protein of the PIN family. Researchers have proven that the auxin influx carriers (AUX/LAX family) facilitate the uptake of the natural auxin IAA and the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D), but not the synthetic auxin NAA [45–50]. The transport specificities and locations of AUX/LAX family members may contribute to the differences in auxin response. Although all the examined PIN proteins in *Arabidopsis* transport IAA, PIN family members differ in their ability to transport other auxinic compounds [51–53]. All this involves differences in responses to varying auxinic compounds within the same species and among different species [54].

The effects of exogenous auxins on morphological parameters could be contrasting. It has been reported that high concentrations of auxin in maize roots produced shorter plants [40], while Patel et al. [55] revealed that the application of NAA to tomato plants increased their height. In our work, we found the tallest tomato seedlings with the largest stem diameter in the seedlings treated with  $100 \mu\text{g L}^{-1}$  of natural auxins from *Ecklonia maxima* extract. Lettuce seedlings reacted differently to the treatments, with a significant increase in the height and stem diameter with all the treatments with exogenous auxins. According to Immanem et al. [56], auxins and cytokinins show distinct but interconnected stimulation to cambium activity. It cannot be excluded that the presence of traces of cytokinins in the commercial biostimulant used in this experiment may have led to the formation of a more robust stem.

Leaf number is dependent on shoot meristem size and leaf initiation rate [57]. Furthermore, leaf size is determined by the rate and duration of cell division and expansion in the

process of development [58]. The rate of leaf emission and their expansion can influence the time needed for producing transplants in vegetable nurseries and their quality. The exogenous supply of auxins was effective in increasing leaf number, irrespective of auxin source and dose in lettuce seedlings, or only with  $100 \mu\text{g L}^{-1}$  of both auxin sources in tomato seedlings. Even leaf size and total leaf area were affected by the auxin treatments, but auxin source and concentration determined the effects with different strengths on lettuce and tomato seedlings. The plant hormone auxin plays an important role in promoting leaflet formation and blade outgrowth [59], whether it comes from plant biosynthesis or exogenous application [60–63]. This process also involves other hormones. The sites of leaf primordium initiation are determined in the meristem by auxin peaks and by auxin-induced repression of the so-called KNOXI gene [64]; this repression allows gibberellin effects to be enhanced which, in turn, eventually leads to leaflet growth and differentiation. Nevertheless, not every exogenous application acts in the same way: the phenotypic manifestation depends on the origin of the auxin and plant species.

The plants regulate gas exchange and leaf temperature [65,66] through the aperture of stomata which respond to hydraulic and chemical signals [67]. Auxins can determine the stomatal opening and extrusion of  $\text{H}^+$  by activating plasma membrane  $\text{H}^+$ -ATPase and inwardly and outwardly rectifying  $\text{K}^+$  currents [68]. The lettuce and tomato seedlings showed a similar response to the exogenous auxin treatments with regard to stomatal conductance. We recorded that, in lettuce seedlings, the highest concentration of both natural and synthetic auxins determined an increase in stomatal conductance (by 2.7-fold, on average) compared to the control. Similarly, tomato seedlings had a stomatal conductance significantly higher than the control with  $100 \mu\text{g L}^{-1}$  of auxins, but to a greater extent with the use of *Ecklonia maxima* extract that also contained traces of cytokinins. It is reported that stomatal responses to exogenously applied cytokinins are also dependent on plant species and the concentration and type of cytokinin used [67,69]. The increase in stomatal conductance can be related to increases in PUE and WUE [70,71]. This was particularly evident in tomato seedlings, where the treatments with auxins determined the increase in the fresh and dry weight per unit of water consumed compared to the control.

The adaptive responses of the plant to nitrogen involve countless molecules such as peptides, microRNAs, and plant hormones [72–74] that are transported long-distance to mediate organ-to-organ communication. Among those molecules, plant hormones have a decisive role in controlling the uptake and transport of ions and in morphophysiological responses to nutrient cues. According to Dos Santos et al. [75], plant responses to nitrogen supply are dependent on auxin signaling, which can be coming from endogenous biosynthesis or exogenous sources. Zhang et al. [49] have shown that, on rice (*Oryza sativa*), the NUE quantitative trait locus is involved in auxin homeostasis. The promotion of auxin biosynthesis results in improved NUE. In our study, we found that the exogenous supplementation of auxins to tomato seedlings promoted the nitrogen-use efficiency, especially when using *E. maxima* extract containing natural auxins, while lettuce seedlings showed a significant increase in NUE only when using the higher concentration of the *E. maxima* extract.

Cellular auxin levels depend on the rate of anabolism, catabolism, transport, and conjugation at any given time in a tissue [76–78]. *Ecklonia maxima* extract also contains phenolic compounds that influence the metabolism and concentration of active forms of auxins in plants. Particularly, phenolic inhibitors of auxin-oxidase, such as phenolic compounds, chlorogenic acid, and rutin, have been shown to enhance auxin activities [79]. Some of these compounds act as alternative substrates for oxidative enzymes, and thus protect auxins from oxidative breakdown. Aremu et al. [15] stated that plants treated with *Ecklonia maxima* extract had more endogenous auxins. According to Wilson and Van Staden [80], some phenolics may protect auxins from decarboxylation, thereby enhancing the concentration of active forms of this hormone.

It was well documented that most biological processes are not regulated by a single hormone but rather by complex signaling networks controlled by multiple hormones or



other signaling components. Auxins and cytokinins act in consort to control the formation of the embryonic root, root meristem size, root branching, vascular pattern, and shoot phyllotaxy [81–83]. Furthermore, the application of a high quantity of natural auxins induces the plant to increase the production of cytokinins for restoring the natural balance between phytohormones.

In our study, we found a lower effect of NAA compared to natural auxins from *E. maxima* extract, especially for tomato seedlings. The explanations may depend on what was previously mentioned about the protein carriers and on the additional presence of cytokine in the seaweed extract [32,33]. The greater effects of *Ecklonia maxima* treatments on tomato and lettuce seedlings could also be ascribed to the synergistic action of the auxins and all the other components of the biostimulant [18,33]. The biological mechanisms activated by seaweed extracts can be very intricate due to the various number of compounds naturally occurring or commercially added (micronutrients, sugars, and amino acids) that could potentially have a complementary effect on plant growth promotion [84]. Thus, the synergistic nature of the different compounds of a biostimulant solution makes it difficult to isolate the way of action of a single active component [85]. Moreover, the plant-growth regulators contained in the *E. maxima* extract cross-talk among them and could act synergistically or antagonistically on plant growth regulation and plant hormone homeostasis [86].

It is well established that plant roots use inorganic N forms (ammonium, nitrate), but they can also take up organic nitrogen compounds of low molecular mass, such as amino acids [87–89]. Amino acids play a fundamental role in plant protein synthesis and are translocated between different plant organs, both in the xylem and phloem. For most seaweeds, aspartic and glutamic acids together constitute a large part of the amino acid fraction [90]. Additionally, available amino acids in the substrate could promote mineral metabolism in general, such as the redistribution of nitrogen [88,91]. We found that tomato seedlings treated with the biostimulant produced from *E. maxima* (AE) resulted in a greater accumulation of biomass, and a higher content of dry matter, as if the plants had access to a source of organic nitrogen. A non-negligible quantity of ‘organic substances’, equal to 268 mg and 134 mg, was indirectly supplied to every single plant with the supply of 100 and 50  $\mu\text{g L}^{-1}$  of auxins from Basfoliar® Kelp SL. These organic substances also include oligoalginates obtained by depolymerization of alginates that promote the growth of different plants by enhancing nitrogen assimilation and basal metabolism [24,33]. Furthermore, enhanced soil conditions were also found when seaweed extract was applied to substrates, with the alginic acid action targeted at stimulating the bacterial decomposition of organic material [92]. Soluble alginates from seaweed extracts act as an aggregation facilitator among soil particles, thus resulting in increased nutrient uptake [17,92].

The different responses of lettuce and tomato seedlings to the treatments with the biostimulant produced from *E. maxima* (AE) or NAA were well summarized by the principal component analysis. Tomato showed to be mainly influenced by the *E. maxima* treatments, especially when supplementing a high dose, whereas lettuce seedlings were more positively affected by the increasing level of exogenous auxins but with greater growth-promoting effect using natural auxins from *E. maxima* extract (AE). The concentration of seaweed extracts can be a critical factor in determining their effectiveness [93]. Tomato plants showed inhibited root growth under high concentrations of auxins (1:100 seaweed extract:water) whereas a lower concentration (1:600) determined a stimulatory effect [94]. Thus, the differences in plant growth promotion of *E. maxima* extract on the tested species could be ascribed to the different responsiveness of lettuce and tomato seedlings to the doses used in this study.

## 5. Conclusions

The results showed that the supplementation of exogenous synthetic auxin (NAA) or an *Ecklonia maxima* extract containing natural auxins can have a growth-promoting effect on lettuce and tomato seedlings, and improve transplant production under nursery conditions.

This effect was more evident on lettuce than tomato, which reacted differently to the source and concentration of exogenous auxins. The commercial biostimulant produced from *Ecklonia maxima* extracts improved seedling quality and promoted shoot and root growth more than the NAA used as a synthetic source of auxin; thus, the growth-promoting effects of the *Ecklonia maxima* extract could probably also be ascribed to the synergistic effect of the natural auxins and the other organic substances contained in the extract.

Further research may be valuable to elucidate the growth stimulatory effects of *Ecklonia maxima* extract, to explore the effect of this biostimulant on endogenous phytohormones such as gibberellins, and to investigate the potential of this compound alone or with other classical phytohormones under biotic and abiotic stress conditions.

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