



## Article

# Influence of *Ecklonia maxima* Extracts on Growth, Yield, and Postharvest Quality of Hydroponic Leaf Lettuce

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**Abstract:** *Ecklonia maxima* is a brown algae seaweed largely harvested over the last years and used to produce alginate, animal feed, fertilizers, and plant biostimulants. Their extracts are commercially available in various forms and have been applied to many crops for their growth-promoting effects which may vary according to the treated species and doses applied. The aim of the study was to characterize the effect of adding an *Ecklonia maxima* commercial extract (Basfoliar Kelp; 0, 1, 2, and 4 mL L<sup>-1</sup>) to the nutrient solution of a hydroponic floating system on growth, yield, and quality of leaf lettuce at harvest and during cold storage (21 days at 4 °C). The supplementation of the *E. maxima* extract through the mineral nutrient solutions, especially between 2 and 4 mL L<sup>-1</sup>, enhanced plant growth and improved the yield and many morphological and physiological traits (biomass accumulation, leaf expansion, stomatal conductance, water use efficiency, nitrogen use efficiency, etc.). Preharvest treatments with *E. maxima* extract were effective in delaying leaf senescence and extending the shelf-life of fresh-cut leaf lettuce. The delay in leaf decay of treated samples allowed to retain an overall quality over the threshold of marketability for up to 21 d of cold storage, especially using 2 mL L<sup>-1</sup> of extract.

**Keywords:** biostimulants; seaweed extracts; *Lactuca sativa* L. var. *Crispa*; leafy vegetables; hydroponics; floating system



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## 1. Introduction

In the last decades, the rising awareness of consumers on the importance of consuming healthy foods [1] has led to the increase in the demand for vegetable products with the consequent increase of many folds of their production and global trade value [2]. A part of this increase is linked to the increased demand for convenience foods such as fresh-cut vegetables [3]. Ready-to-eat (RTE) products have shown the most rapid expansion among vegetable products, even in developing countries [4]. Consequently, the demand for raw vegetables for minimally processed vegetable production is increasing, and food industries have been urged to utilize new cultivation systems and techniques to produce a sufficient amount of high-quality and safe food without detrimental effects on quality maintenance after minimal processing and during cold storage. Food industries are also interested in increasing crop yield or crop growth rate to reduce raw product cost and be more competitive in pricing.

Moreover, the increasing world population is expected to reach 9 billion by 2050, of which 70% will live in urban centers, and climate change will strain the food supply chain [5]. Thus, there is a growing concern about the importance of supplying vegetables to cities in an environmentally friendly and energy-efficient way [6]. This demand for sustainable urban food could be met by designing and developing vertical farms [7]. This farming system involves large-scale food production in indoor environments, with precisely controlled light, nutrients, and temperatures where plants are stacked in layers that may reach several stories tall. This crop cultivation system is based on hydroponics using cutting-edge

greenhouse technologies [8]. Soilless cultivation systems (or hydroponics) are currently used for many vegetable crops to overcome many biotic or abiotic issues [9–11], improve yield and quality [10,12–14], and extend cultivation area. In the hydroponic cultivation systems, plants are continuously nourished with mineral nutrient solutions prepared by adding mineral fertilizers and chemical elements to the water. The aim to improve the sustainability of intensive agriculture systems is pulling toward the reduction of synthetic inputs and their substitution with natural resources even in innovative cultivation systems such as hydroponics and vertical farming.

The use of growth-promoting products has been suggested for increasing crop production and sustainability and reducing the need for chemical inputs [15]. The plant growth promoters, also known as biostimulants [16], have different functions (biofertilizers, bio-control, phytostimulation, etc.) [17,18] and are of different types (bacteria, mycorrhizae, humic acid, algae extracts, etc.).

Algae or seaweed extracts can be produced from brown algae (*Phaeophyceae*) [19] and are commercially available in liquid form. They can positively affect nutrient uptake, resistance to biotic and abiotic stresses, seed germination, and crop yield [20]. Seaweed extracts contain appreciable quantities of macroelements and some trace elements (Ca, Mg, K, Na, P, S, Fe) [21,22], considerable amounts of polyamines [23], abscisic acid, and brassinosteroids [24,25]. They contain also several osmoprotectants (betaine, proline, mannitol) [26,27] and other components of great biological importance, for their reported promotion of nutrient uptake, translocation, and ability to stimulate root growth [28], such as alginates and polysaccharides [26,27] or many classes of substances with hormone-like activity [29] such as auxins, cytokinins, ethylene, and gibberellins [30,31].

The mode of action of seaweed extracts on plant growth promotion is not well known yet. Understanding the mechanisms responsible for this effect is very complex due to the various interactions among the numerous bioactive compounds contained in the extracts [32]. Nevertheless, similar physiological responses to seaweed extract supplementation have been found in plants treated with plant growth regulators, so the effects on growth, development, and yield have been often related to the hormone-like compounds found in these extracts [16,20,33].

*Ecklonia maxima* (Osbeck) Papenfuss is a brown algae seaweed also known as sea bamboo that grows in the southern oceans and is mainly found along the southern Atlantic coast of Africa. It has been largely collected over the last years and used to produce alginate, animal feed, fertilizers, and plant biostimulants [34]. Their extracts are commercially available in various forms and have been applied to many crops for their growth-promoting effects [35]. The commercial biostimulants produced from *E. maxima* contain various plant growth regulators (auxins, cytokinins, polyamines, gibberellins, abscisic acid, and brassinosteroids) that play a role in the growth stimulation recorded on many crops [25,36,37]. A wide range of processes in plant development and growth can be controlled by exogenous phytohormones [18,38,39]. These effects may vary according to the treated species and doses applied [38,40–42].

Thus, it would be highly worthwhile to test if the supplementation of biostimulants obtained from seaweed extracts through the nutrient solution used in hydroponic cultivation systems could be a cost-effective and easy-to-use technique to increase the productivity and postharvest quality of baby leaf crops, while at the same time reducing the effect on the environment and limiting food waste [12–16].

Therefore, the aim of this study was to characterize the effect of adding an *Ecklonia maxima* commercial extract to the mineral nutrient solution of a hydroponic floating system on the growth, yield, and quality of leaf lettuce at harvest and during cold storage.

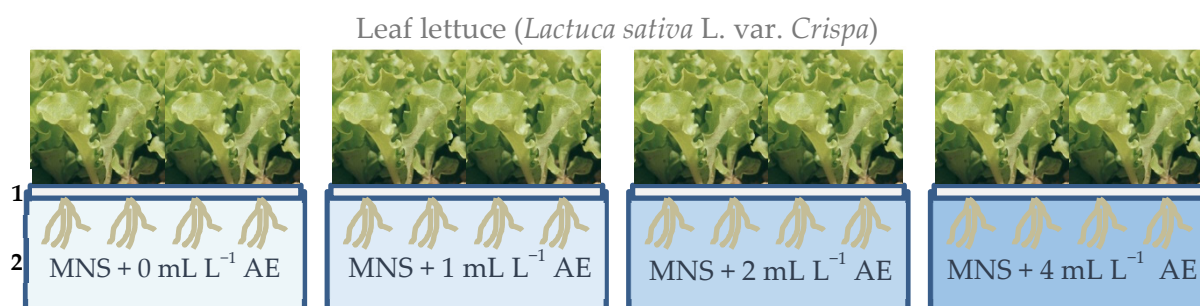
## 2. Materials and Methods

### 2.1. Leaf Lettuce Hydroponic Cultivation

The experiment was carried out in a greenhouse situated at the Department of Agricultural, Food and Forest Sciences (SAAF—University of Palermo, Italy) (38°6′28″ N

13°21'3'' E; altitude 49 m). Leaf lettuce plants (*Lactuca sativa* L. var. *Crispa*) were grown in a hydroponic floating system using nutrient solutions with four concentrations of a commercial *Ecklonia maxima* extract (Basfoliar® Kelp SL Compo Expert, Münster, Germany): 0, 1, 2, and 4 mL L<sup>-1</sup>. The nutrient solutions (MNS) were obtained adding the following nutrients to tap water (pH 7.6; electrical conductivity (EC) 500 µS cm<sup>-1</sup>): 19 mM NO<sub>3</sub><sup>-</sup>, 1.25 mM NH<sub>4</sub><sup>+</sup>, 2 mM H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 11 mM K<sup>+</sup>, 4.5 mM Ca<sup>2+</sup>, 1 mM Mg<sup>2+</sup>, 1.1 mM SO<sub>4</sub><sup>2-</sup>, 40 µM Fe<sup>3+</sup>, 30 µM BO<sub>3</sub><sup>3-</sup>, 5 µM Mn<sup>2+</sup>, 4 µM Zn<sup>2+</sup>, 0.75 µM Cu<sup>2+</sup>, and 0.50 µM Mo [43]. MNS differed only in seaweed extract concentration and had an EC of 2.6 mS cm<sup>-1</sup> and a pH of 6.0. Each nutrient solution was poured into four different tanks (100 cm long × 50 cm wide × 15 cm deep, containing 75 L).

Seeds of leaf lettuce (cv. 'Lattuga da Taglio a Foglia Liscia', Sementi Dotto—SDD SPA, Udine, Italy) were sown on 8 March 2021 into polystyrene panels (300 holes m<sup>-2</sup>) filled with a commercial substrate (Utilis, GreenView Srl, Crocetta del Montello, Italy). The panels were placed in a dark room at 22–24 °C until seed germination and were then transferred into the cold greenhouse. When the seedlings had fully expanded cotyledons (10th BBCH growth stage [44]) the panels were moved to float in the tanks (Figure 1).



**Figure 1.** Graphical representation of the hydroponic floating system made of drilled polystyrene panels floating on mineral nutrient solutions (MNS) with four levels of *Ecklonia maxima* extract (AE) (<sup>1</sup> Drilled polystyrene panels (300 plants m<sup>-2</sup>) floating on MNS; <sup>2</sup> Tanks filled with 75 L of MNS containing increasing concentrations of *Ecklonia maxima* extract).

During the greenhouse experiment, the average outside temperature varied between 6.1 °C (night) and 22.0 °C (day), and the average net solar radiation at noon was 623 W·m<sup>-2</sup>, with a day length that ranged between 9 and 11 h. The air temperature inside the greenhouse was on average 20.0 ± 1.3 °C and varied from 10.0 (night) to 36.4 °C (day), whereas the relative humidity was 78.8 ± 1.9% and varied from 55.0% to 100%; the light intensity at noon was 54,246 ± 3900 lux and varied from 78,801 to 12,970 lux as a function of the cloudiness.

Each treatment was composed of four replicated tanks for each seaweed extract concentration (150 plants for each tank) arranged in a randomized complete block design. The MNS were not aerated during the experiment because leaf lettuce has fast growth and does not necessitate high oxygen concentration in the nutrient solution [45]. Water utilization and the changes in EC and pH of the nutrient solutions were regularly checked and recorded and when the volume dropped by 20% the tanks were refilled with fresh MNS with the same seaweed extract concentration. The polystyrene panels completely covered the tanks so the amount of evaporated water was insignificant and was not considered for assessing the nutrient solutions consumed by the plants of each treatment.

## 2.2. Agronomical and Morpho-Physiological Parameters

The water use efficiency (WUE) was calculated as WUE (g DW L<sup>-1</sup> H<sub>2</sub>O) = plant dry weight (g)/H<sub>2</sub>O (L). At the end of crop cultivation, the nutrient solution that remained in the tanks was examined and the residual N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>3</sub><sup>-</sup> contents were determined reflectometrically by a Merck RQflex10 reflectometer according to the company protocols (Merck, Darmstadt, Germany). This allowed to calculate the uptake of nitro-

gen during the plant growth and to determine Nitrogen Use Efficiency (NUE) [46] as  $\text{NUE (g DW g}^{-1} \text{ N)} = \text{plant total dry weight (g)}/\text{plant N uptake (g)}$ .

The stomatal conductance was evaluated 5 days before harvest on two recently expanded, unshaded leaves of 20 plants for each replicate with a diffusion porometer (AP4, Delta-T Devices Ltd., Cambridge, UK).

At harvest (32 days from sowing), all plants were harvested, and marketable yield was calculated.

The plants were collected 23 days after the beginning of the floating cultivation, and marketable yield was determined after removing decayed or senescent older leaves. Then, 20 plants for each replicate were randomly chosen and destructively sampled to measure plant height, leaf number, main leaf width, and leaf area. Soon after sampling the leaves were weighed, then scanned at 200 dpi (Epson Perfection 1640SU, Seiko Epson Corp., Suwa, Japan) to obtain digital images that were analyzed with the Easy Leaf Area software [47] to calculate the leaf area of each plant. Then, the leaves were dried in an oven at 85 °C until they reach a constant weight and the specific leaf area ( $\text{SLA cm}^2 \text{ g}^{-1}$ ) was calculated as leaf area/leaf dry weight. Another sample of 20 plants randomly selected for each replicate was divided into leaves, stems, and roots. Each fraction was weighed and then dried to constant weight at 85 °C for assessing fresh and dry biomass.

### 2.3. Minimal Processing and Cold Storage

Once harvested, plants were immediately transferred to the laboratory of Vegetable Analysis of the SAAF Department and minimally processed for fresh-cut leaf lettuce production. Leaves were detached from stems, retaining only those free from defects, yellowing, or decay. Then, they were immersed in tap water for 5 min two times and dried by manual centrifugation for 1 min with a handheld salad spinner. At the end of processing, the fresh-cut leaves obtained from each treatment and replicate was weighed and the yield of the minimally processed product was determined as  $(\text{Crop yield weight} - \text{Fresh-cut leaves weight})/\text{Crop yield weight} \times 100$  and the correspondent fresh-cut leaves yield expressed in  $\text{kg m}^{-2}$  was calculated.

Samples of 100 g from each seaweed extract level were directly packed in polyethylene (PE) bags, sealed with a hot bar (Laica VT3112, Vicenza, Italy), and stored at 4 °C for 21 days.

### 2.4. Physico-Chemical and Quality Parameters

The physicochemical characteristics of four randomly selected samples for each seaweed extract treatment were evaluated at the end of processing and at each sampling time (after 7, 14, and 21 days of cold storage). The experimental design was a factorial combination of four seaweed extract levels and four storage times arranged in a randomized complete block design with four replicates.

At packaging and at each sampling time, each sample was weighed to estimate the weight loss ( $\text{g } 100 \text{ g}^{-1}$  of initial fresh weight FW).

Fifteen leaves were randomly selected from each sample to measure upper side leaf color using a Chroma-meter CR-400 (Minolta Corp., Ltd., Osaka, Japan). The CIELAB color components  $L^*$  (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) were recorded and Chroma ( $C^*$ ) and hue angle ( $h^\circ$ ) were calculated as  $C^* = (a^{*2} + b^{*2})^{1/2}$  and  $h^\circ = 180^\circ + \arctan(b^*/a^*)$  [48]. Moreover, total color difference ( $\Delta E$ ) was also determined at each sampling date as  $\Delta E = [(L^* - L^*_0) + (a^* - a^*_0) + (b^* - b^*_0)]^{1/2}$ , where  $L^*_0$ ,  $a^*_0$  and  $b^*_0$  were the control values at the end of processing ( $T_0$ ).

The samples were also analyzed by an informal panel consisting of twelve people (six men and six women, aged 28–57) to evaluate their overall quality (OQ). Each panel member scored the samples from 1 to 5 where 1 = poor/unmarketable (off odors, extensive color changes, and major defects or decay symptoms), 3 = fair/still acceptable and marketable (presence of minor defects or modest color changes), and 5 = excellent (freshly

harvested appearance and full visual and sensory acceptability; free from any off odors, defects, and decay).

Then, twenty grams of each sample were blended with H<sub>2</sub>O (1:5 *w/v*), and the suspensions were centrifuged at 3500 rpm for 10 min. The supernatants were used to assess soluble solids content (SSC), titratable acidity (TA), and nitrate (NO<sub>3</sub><sup>−</sup>) and ascorbic acid content. SSC (°Brix) was measured with a digital refractometer (MTD-045nD, Three-In-One Enterprises Co. Ltd., New Taipei City, Taiwan). TA (mg of citric acid per 100 g of fresh weight) was determined by titrating 10 mL of the supernatants with 0.1 M NaOH up to pH 8.1. The content of NO<sub>3</sub><sup>−</sup> and ascorbic acid (expressed as mg kg<sup>−1</sup> FW and mg 100 g<sup>−1</sup> FW, respectively) were estimated with a reflectometer (Merck RQflex10; Merck, Darmstadt, Germany) [48–50].

### 2.5. Statistics and Principal Component Analyses

The experimental layout consisted of four replicates for each seaweed extract concentration randomly assigned in four blocks. The effects of the *Ecklonia maxima* extract levels on morpho-physiological parameters and yield were determined by performing a one-way ANOVA (four replicated tanks for each biostimulant concentration). Tukey's multiple range test at  $p \leq 5\%$  was used to determine the differences between means. The effects of seaweed extract levels and cold storage on minimally processed leaf lettuce were assessed with a two-way ANOVA (four replicated bags for each biostimulant concentration and each storage time). The least significant differences (LSD) test at  $p \leq 5\%$  was applied to detect significant interactions between factors or significant differences among treatments.

Two different principal components analyses (PCA) were carried out on cultivation and postharvest trials. The first PCA was performed to investigate the relationship between the tested seaweed extract concentrations and the morphological and quality characteristics of leaf lettuce at harvest. The parameters used for the analysis included plant height, stem diameter, whole plant fresh weight (FW), leaf FW, stem FW, roots FW, Shoot/Root FW, whole plant dry weight (DW), leaf DW, stem DW, root DW, S/R DW, epigeal dry matter percentage, root dry matter percentage, yield, leaf yield (% and kg) leaf no., leaf width, leaf area, plant area, SLA, WUE, NUE, stomatal conductance, L\*, chroma, hue angle, SSC, TA, ascorbic acid and NO<sub>3</sub><sup>−</sup>. A second PCA was carried out on morpho-physiological and phytochemical characteristic evolution of fresh-cut leaves during storage to estimate the parameters that more strongly discriminated between preharvest seaweed extract treatments and storage duration. The input matrix for this PCA included weight loss, SSC, TA, ascorbic acid and NO<sub>3</sub><sup>−</sup> content, L\*, chroma, hue angle, color difference ( $\Delta E$ ), and overall quality (OQ). In each PCA, the best level of principal components (PCs) was evaluated by saving factors with eigenvalues higher than 1.0. The plots of the first and second PCs of each PCA enabled the study of the relationships between the variables of each input data set. Moreover, the initial parameters were projected into the subspace identified by the first and second PCs, and correlated variables were revealed. PCA were carried out with SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

## 3. Results

### 3.1. Leaf Lettuce Yield and Quality

At harvest, plant height was significantly affected by the seaweed extract in the nutrient solution especially when the two highest doses were used (2 and 4 mL L<sup>−1</sup>). At these concentrations, plants were 2.4 cm higher on average than control (10.7 cm) but had no significant difference in stem diameter (Table 1).



**Table 1.** Yield and morphological characteristics of green leaf lettuce plants grown in nutrient solutions added with different concentrations of *Ecklonia maxima* extract.

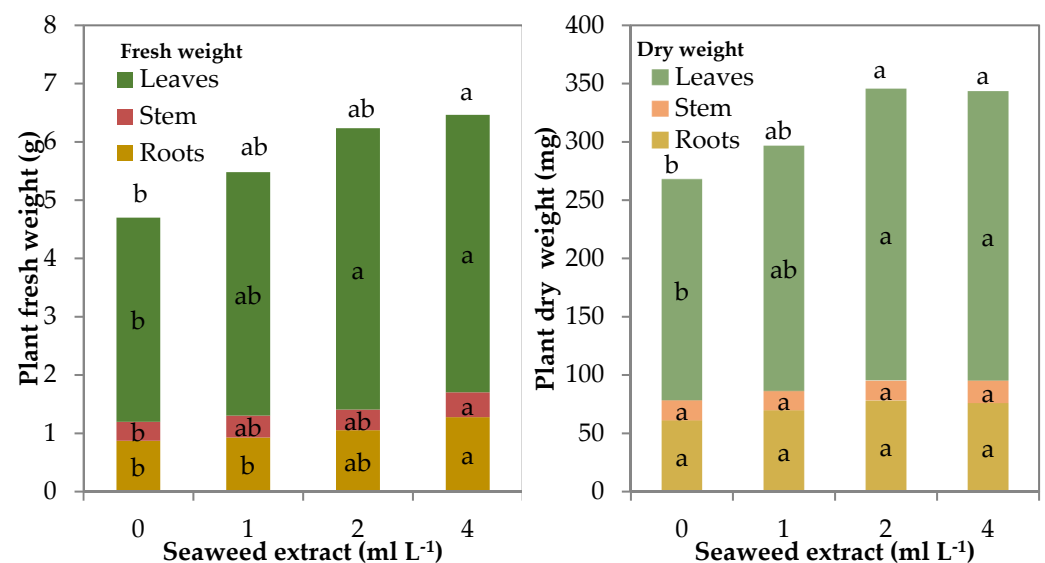
	<i>Ecklonia maxima</i> Extract (mL L <sup>-1</sup> )			
	0	1	2	4
Plant height (cm)	10.7b <sup>1</sup>	12.2ab	13.1a	13.2a
Stem diameter (mm)	5.4a	5.7a	5.8a	5.8a
Plant fresh weight				
Whole plant (g)	4.7b	5.5ab	6.2a	6.5a
Leaves (g)	3.5b	4.2ab	4.8a	4.8a
Stem (g)	0.32b	0.38ab	0.36ab	0.43a
Roots (g)	0.9b	0.9b	1.1ab	1.3a
Ratio shoot/roots	4.4a	5.0a	5.0a	4.1a
Plant dry weight				
Whole plant (mg)	267.8b	296.5ab	345.7a	343.3a
Leaves (mg)	189.6b	210.4ab	250.4a	248.2a
Stem (mg)	17.1a	16.4a	17.1a	19.3a
Roots (mg)	61.1a	69.7a	78.2a	75.8a
Ratio shoot/roots	20.4a	19.5a	20.4a	22.6a
Epigeal dry matter (%)	5.4a	5.0a	5.1a	5.2a
Root dry matter (%)	7.0a	7.6a	7.4a	6.0a
Yield (kg m <sup>-2</sup> )	1.15b	1.37ab	1.55a	1.56a
Fresh cut leaves yield (%)	91.5b	91.8b	93.1a	91.8b
Fresh cut leaves yield (kg m <sup>-2</sup> )	1.05b	1.25ab	1.45a	1.43a
WUE (g DW L <sup>-1</sup> H <sub>2</sub> O)	1.4c	1.6b	1.7ab	1.8a
NUE (g DW g <sup>-1</sup> N)	5.1c	6.0b	6.2ab	6.6a

<sup>1</sup> Results indicate the mean value of four replicates. Data within a row followed by different letters are significantly different at  $p \leq 0.05$  according to Tukey's test.

The total fresh weight (FW) of leaf lettuce plants showed a quadratic trend ( $R^2 = 0.641^{***}$ ) as a function of the seaweed concentration in the mineral nutrient solution (MNS). The total fresh biomass of control plants was 4.7 g plant<sup>-1</sup>, whereas it was significantly higher by 32.6 and 37.5% in the plants grown with 2 and 4 mL L<sup>-1</sup> of biostimulant in the MNS, respectively (Table 1; Figure 2). The main increase in fresh biomass was noted in the leaves with both 2 and 4 mL L<sup>-1</sup> of seaweed extract in the MNS whereas stem and roots had a significantly higher fresh weight than control only with the highest concentration of the biostimulant. Nevertheless, the shoot/root (S/R) ratio did not significantly change by increasing the seaweed extract level in the MNS (Table 1).

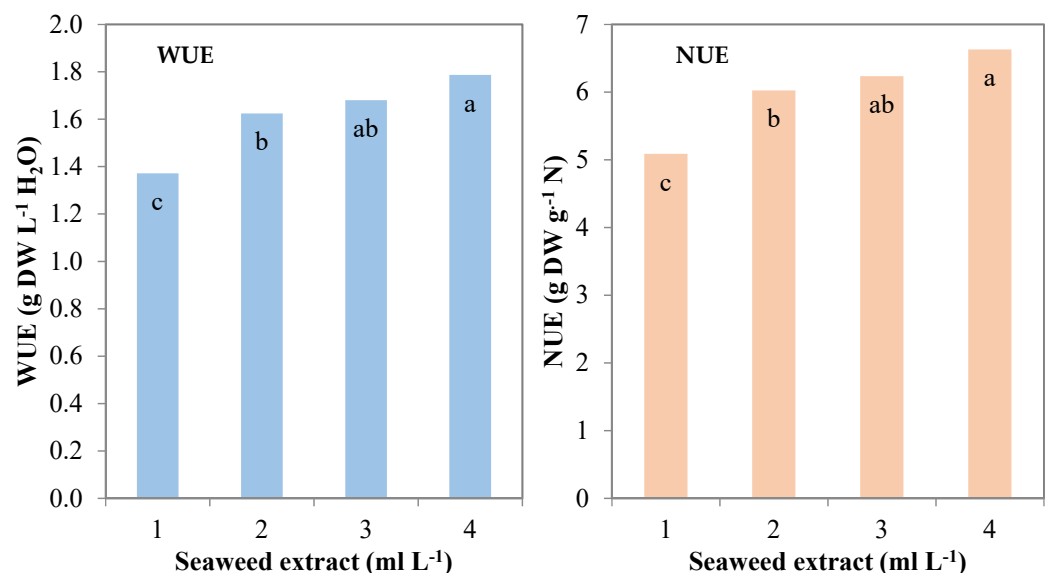
The total dry biomass of leaf lettuce plants showed a quadratic trend ( $R^2 = 0.504^{**}$ ) like that found for fresh biomass. Total dry weight increased significantly only in the plants supplemented with 2 and 4 mL L<sup>-1</sup> of biostimulant in the MNS (Table 1; Figure 2). Stem and root dry weight did not show significant modifications due to the presence of seaweed extract in the MNS, so, the higher total dry weight should be attributed to the rise of the dry biomass of the leaves (+31.5% on average for 2 and 4 mL L<sup>-1</sup>). Even the dry matter partitioning between shoot and roots did not change as found for the fresh shoot/root ratio (Table 1). The dry matter percentage was not affected by the treatments and was 5.2% in the epigeal part and 7.0% in the roots (Table 1).

The plants of leaf lettuce not supplemented with seaweed extract yielded 1.15 kg m<sup>-2</sup>; the addition of the biostimulants to the MNS at the highest concentrations (2 and 4 mL L<sup>-1</sup>) increased the yield by 35.4% on average (about 400 g m<sup>-2</sup> more than control plants) (Table 1). The yield (%) of fresh-cut leaves recorded a significant increase only with 2 mL L<sup>-1</sup> of seaweed extract in the MNS (93.1%) whereas it was 91.7% on average in the other treatments (Table 1). The production of fresh-cut leaves was lowest in control plants (1.04 kg m<sup>-2</sup>) and increased up to 1.45 kg m<sup>-2</sup> with 2 mL L<sup>-1</sup> (Table 1).



**Figure 2.** Fresh and dry biomass of leaf lettuce plants grown in nutrient solutions containing different concentrations of a seaweed extract from *Ecklonia maxima* (bars of the same color with different letters are significantly different at  $p \leq 0.05$  according to Tukey's test).

During the experiment, the consumption of nutrient solution was monitored and recorded to calculate the water use efficiency (WUE) and nitrogen use efficiency (NUE). Control plants recorded  $1.4 \text{ g DW L}^{-1} \text{ H}_2\text{O}$  and  $5.1 \text{ g DW g}^{-1} \text{ N}$  for WUE and NUE, respectively. These parameters followed a quadratic trend ( $R^2 = 0.735^{***}$  and  $R^2 = 0.729^{***}$ , respectively) when increasing the seaweed extract concentration in the MNS and were significantly higher than control with the lowest seaweed extract level ( $1 \text{ mL L}^{-1}$ ;  $1.6 \text{ g DW L}^{-1} \text{ H}_2\text{O}$  and  $6.0 \text{ g DW g}^{-1} \text{ N}$ , respectively), and recorded the highest values at the highest concentration ( $4 \text{ mL L}^{-1}$ ;  $1.8 \text{ g DW L}^{-1} \text{ H}_2\text{O}$  and  $6.6 \text{ g DW g}^{-1} \text{ N}$ , respectively) (Table 1; Figure 3).



**Figure 3.** Water use efficiency (WUE) and nitrogen use efficiency (NUE) of leaf lettuce plants grown in nutrient solutions containing different concentrations of a seaweed extract from *Ecklonia maxima* (Bars of the same color with different letters are significantly different at  $p \leq 0.05$  according to Tukey's test).

The supplementation of the *E. maxima* extract in the MNS also influenced the leaf characteristics of leaf lettuce plants that had 7.9 leaves per plant when grown without the

biostimulant supplementation, while leaf lettuce grown with seaweed extract in the MNS were leafier, particularly with 4 mL L<sup>-1</sup> (8.8 leaves plant<sup>-1</sup>) (Table 2; Figure 4).

**Table 2.** Leaf characteristics of green leaf lettuce plants grown in nutrient solutions added with different concentrations of *Ecklonia maxima* extract.

	<i>Ecklonia maxima</i> Extract (mL L <sup>-1</sup> )			
	0	1	2	4
Number of leaves	7.9b <sup>1</sup>	8.5ab	8.6ab	8.8a
Leaf width (cm)	4.5c	5.1b	5.6a	5.6a
Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )	142.0b	196.5a	199.8a	192.4a
Leaf area (cm <sup>2</sup> leaf <sup>-1</sup> )	18.4b	23.9a	23.9a	22.1ab
SLA (cm <sup>2</sup> g DW <sup>-1</sup> )	763.7b	898.0a	818.5b	790.6b
Stomatal conductance (mmol m <sup>-2</sup> s <sup>-1</sup> )	150.5b	207.1a	198.4a	227.8a
L*	55.2a	55.2a	54.8a	54.7a
Chroma	45.4a	45.4a	45.0a	45.2a
Hue°	121.2a	121.5a	121.5a	121.7a
SST (°Brix)	2.6a	2.7a	2.7a	2.6a
TA <sup>2</sup>	42.3a	38.4a	38.4a	38.4a
Ascorbic acid (mg 100 g <sup>-1</sup> FW)	55.0a	57.3a	57.6a	58.0a
NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> FW)	3120.0a	2540.0b	2891.7ab	2875.3ab

<sup>1</sup> Results indicate the mean value of four replicates. Data within a row followed by different letters are significantly different at  $p \leq 0.05$  according to Tukey's test. <sup>2</sup> Titratable acidity expressed as citric acid.



**Figure 4.** Leaf number and plant and leaf area of leaf lettuce plants grown in nutrient solutions with different concentrations of a seaweed extract from *Ecklonia maxima* (bars of the same color or points with different letters are significantly different at  $p \leq 0.05$  according to Tukey's test).

The leaf morphology was more influenced by the treatments. The effect of biostimulant concentration on leaf width and leaf and plant area followed a quadratic trend ( $R^2 = 0.762^{***}$ ,  $0.727^{**}$ , and  $0.574^*$ , respectively). Leaf width was 4.5 cm in control plants whereas it was significantly wider with the lowest level of biostimulant in the MNS (+12.9%) and recorded the highest width in the plants grown with 2 and 4 mL L<sup>-1</sup> in the MNS (5.6 cm; +23.7% than control plants, on average) (Table 2). The supplementation of seaweed extract was effective in increasing the leaf and plant area to a different extent for each parameter. The leaves were significantly larger than control (18.4 cm<sup>2</sup> leaf<sup>-1</sup>) in the plants supplemented with 1 and 2 mL L<sup>-1</sup> of biostimulant (+30%). Control plants had a total leaf area of 142.0 cm<sup>2</sup> plant<sup>-1</sup>, this parameter increased by 35–40% in all the treated plants (196.2 cm<sup>2</sup> plant<sup>-1</sup> on average).



The *E. maxima* extract influenced the specific leaf area only when supplemented at 1 mL L<sup>-1</sup> (898.0 cm<sup>2</sup> g<sup>-1</sup> DW; 17.6% more than control) (Table 2).

The presence of the *E. maxima* extract in the nutrient solution affected also leaf physiology as shown by the measures of stomatal conductance that was on average 211.1 mmol m<sup>-2</sup> s<sup>-1</sup> in the plants supplied with the biostimulant, 40.3% higher than control plants (150.5 mmol m<sup>-2</sup> s<sup>-1</sup>) (Table 2).

On the contrary, the biostimulant had no effect on leaf color at harvest (Table 2).

To assess the quality of leaf lettuce at harvest, soluble solid content (SSC), titratable acidity (TA), nitrate (NO<sub>3</sub><sup>-</sup>) and ascorbic acid contents of the leaves were determined (Table 2). SSC, TA, and ascorbic acid content showed no significant effect due to the seaweed extract. The nitrate content of leaf lettuce leaves was highest in control plants, which accumulated 3120.0 mg kg<sup>-1</sup> FW and was reduced by biostimulant supplementation, especially when added to the MNS at 1 mg L<sup>-1</sup> (2540.0 mg kg<sup>-1</sup> FW) (Table 2).

### 3.2. Storage of Fresh-Cut Leaf Lettuce

After harvesting, the minimally processed leaf lettuce was stored for 21 days at 4 °C to assess the effect of *E. maxima* extract supplementation and cold storage on postharvest quality changes.

The weight loss of stored samples was not influenced by the treatments during the first week of storage (0.64 g 100 g<sup>-1</sup> FW on average). Afterward, control samples showed a higher weight loss that reached 2.14 g 100 g<sup>-1</sup> FW after 21 days. The treated samples recorded a significant increase of weight loss only at the end of cold storage, but to a lower extent compared to the control (1.41 g 100 g<sup>-1</sup> FW on average) (Table 3).

**Table 3.** Effect of different concentrations of *Ecklonia maxima* extract in the nutrient solution and cold storage on weight loss, soluble solids content (SSC), titratable acidity (TA), ascorbic acid and nitrate (NO<sub>3</sub><sup>-</sup>) content of fresh-cut leaf lettuce.

<i>E. maxima</i> Extract (mL L <sup>-1</sup> )	Storage (d a 4 °C)	Weight Loss (g 100 g <sup>-1</sup> FW)	SSC (°Brix)	TA <sup>3</sup>	Ascorbic Acid (mg 100 g <sup>-1</sup> FW)	NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> FW)
0	0		2.6	42.3c	55.0	3120.0
	7	0.63c <sup>1</sup>	2.7	42.3c	52.5	2545.0
	14	1.00bc	2.6	46.1bc	52.5	2875.0
	21	2.14a	2.9	53.8b	53.0	2787.5
1	0		2.7	38.4c	57.3	2540.0
	7	0.64c	2.7	38.4c	51.2	2485.0
	14	0.72c	3.0	50.0bc	63.2	2720.0
	21	1.15bc	2.5	61.5ab	62.5	2720.0
2	0		2.7	38.4c	57.6	2925.0
	7	0.65c	2.7	46.1bc	59.2	2548.3
	14	0.73c	3.0	50.0bc	61.4	2585.0
	21	1.54b	2.5	59.6ab	59.1	2885.0
4	0		2.6	38.4c	58.0	2875.3
	7	0.64c	2.5	36.5c	51.6	2650.0
	14	0.81c	2.7	48.0bc	61.4	2895.0
	21	1.53b	2.8	65.3a	59.5	2740.0
<i>E. maxima</i> (mL L <sup>-1</sup> )						
	0	1.26	2.7	46.11	53.25	2831.9a
	1	0.84	2.7	47.07	57.00	2616.3b
	2	0.97	2.7	48.51	59.90	2735.8b
	4	0.99	2.7	47.07	57.63	2790.1b
Storage (d a 4 °C)						
	0		2.7	39.4	56.8	2865.1
	7	0.64	2.7	40.8	53.6	2557.1
	14	0.81	2.8	48.5	58.4	2768.8
	21	1.59	2.7	60.0	58.5	2783.1
<i>E. maxima</i>		** <sup>2</sup>	ns	ns	ns	**
Storage		***	ns	***	ns	ns
<i>E. maxima</i> × Storage		*	ns	*	ns	ns

<sup>1</sup> each value is the mean of four replicates. Values in a column followed by different letters are significantly different (LSD test). <sup>2</sup> significance: ns = not significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; \*\*\* significant at  $p < 0.001$ . <sup>3</sup> Titratable acidity expressed as citric acid.

Seaweed extract did not affect the soluble solids and ascorbic acid content that showed no significant change during storage.

The titratable acidity raised during the second part of the storage period in all the samples. It increased by 27.3% in control samples, whereas the treated samples showed a significantly higher level of titratable acidity especially in the samples treated with the highest level of *E. maxima* extract (+70%) (Table 3).

The nitrate content ( $\text{NO}_3^-$ ) of control samples was  $2831.9 \text{ mg kg}^{-1}$  FW on average and was slightly but significantly reduced by the treatments ( $-4.2\%$  on average), whereas no significant change was recorded due to storage time (Table 3).

Color parameters appeared to be variously affected by seaweed extract and storage time. Color lightness ( $L^*$ ) increased from 55.0 to 56.6 during storage on average (Table 4). The color saturation (Chroma) recorded significantly lower values in the treatment with biostimulant in the MNS compared to the control. This parameter recorded a decrease during the initial 14 days of storage followed by a slight increase in the third week. At the beginning of storage, the hue angle was slightly higher than control in the samples treated with the two highest levels of biostimulant. The value of this parameter dropped during storage in control samples down to 118.8 whereas the reduction was lower in the treated samples (119.8 on average). A similar trend was recorded in the changes of color evaluated by calculating the color difference ( $\Delta E$ ) which increased during the storage up to 2.9 in control samples, whereas it ranged from 1.3 to 1.9 in the treated samples at the end of storage time (Table 4).

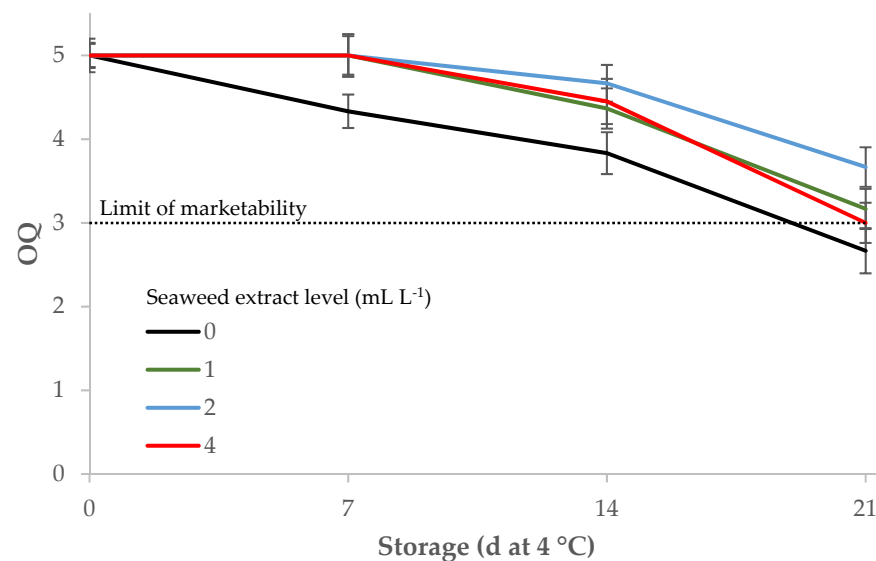
**Table 4.** Effect of different concentrations of *Ecklonia maxima* extract in the nutrient solution and cold storage on leaf appearance of fresh-cut leaf lettuce.

<i>E. maxima</i> Extract (mL L <sup>-1</sup> )	Storage (d a 4 °C)	$L^*$	Chroma	Hue	$\Delta E$	OQ
0	0	55.2	45.4	121.2ab	0.0d	5.00
	7	54.8	44.3	120.9b	1.3bc	4.33
	14	55.7	43.6	120.4bc	1.0c	3.83
	21	57.5	44.9	118.8d	2.9a	2.67
1	0	55.2	45.4	121.5ab	0.9c	5.00
	7	55.0	44.1	120.3bc	1.3bc	5.00
	14	55.2	43.8	120.3bc	0.8c	4.37
	21	55.9	43.9	119.8c	1.3bc	3.17
2	0	54.8	45.0	121.5a	1.1c	5.00
	7	54.1	43.3	121.1ab	1.1c	5.00
	14	54.8	43.2	120.9b	1.5bc	4.67
	21	56.1	43.3	119.9c	1.5bc	3.67
4	0	54.7	45.2	121.7a	0.8c	5.00
	7	54.3	43.7	120.6b	1.1c	5.00
	14	55.0	43.2	120.8b	1.5bc	4.45
	21	56.7	43.8	119.5c	1.9b	3.00
<i>E. maxima</i> (mL L <sup>-1</sup> )						
	0	55.8	44.5a	120.3	1.31	3.96b
	1	55.3	44.3ab	120.5	1.08	4.46a
	2	54.9	43.7c	120.9	1.30	4.58a
	4	55.2	44.0bc	120.7	1.33	4.42a
Storage (d a 4 °C)						
	0	55.0b <sup>1</sup>	45.2a	121.5	0.69	5.00a
	7	54.5b	43.8b	120.7	1.20	4.83ab
	14	55.2b	43.5c	120.6	1.21	4.46b
	21	56.6a	44.0b	119.5	1.91	3.13c
<i>E. maxima</i>		ns <sup>2</sup>	***	***	ns	***
Storage		*	***	***	***	***
<i>E. maxima</i> × Storage		ns	ns	***	***	ns

<sup>1</sup> each value is the mean of four replicates. Values in a column followed by different letters are significantly different (LSD test). <sup>2</sup> significance: ns = not significant; \* significant at  $p < 0.05$ ; \*\*\* significant at  $p < 0.001$ .

The scores for overall quality assessed by an informal panel dropped during storage, but the samples treated with *E. maxima* extract had a significantly better visual quality than the control during the first two weeks of storage, and maintained the marketability score

until the end of the trial, while the overall quality of the control samples was under this threshold at day 21 (Table 4; Figure 5).



**Figure 5.** Influence of *Ecklonia maxima* extract levels in the nutrient solution and storage at 4 °C on the overall quality of fresh-cut leaf lettuce (1: unmarketable; 3: average—limit of marketability; 5: excellent or having a fresh appearance).

### 3.3. Principal Components Analyses

The principal component analysis performed on the agronomic and quality characteristics of leaf lettuce at harvest presented three principal components (PCs) with eigenvalues higher than 1 (Table 5), representing 65.98%, 24.57%, and 9.45% of the total variance, respectively. This suggested that the initial 32 parameters could be represented by three PCs, explaining 100% of the total variance. PC1 was mostly correlated to plant height, stem diameter, whole plant, leaf, stem and root FW, whole plant, leaf, and root DW, yield, leaf yield (kg), WUE, NUE, leaf number, plant and leaf area, stomatal conductance,  $L^*$ , hue angle, TA and ascorbic acid content; PC2 was correlated to S/R FW, stem DW, S/R DW, epigeal and root dry matter percentage, SLA, SSC, and nitrates; PC3 was correlated to leaf yield percentage (Table 5).

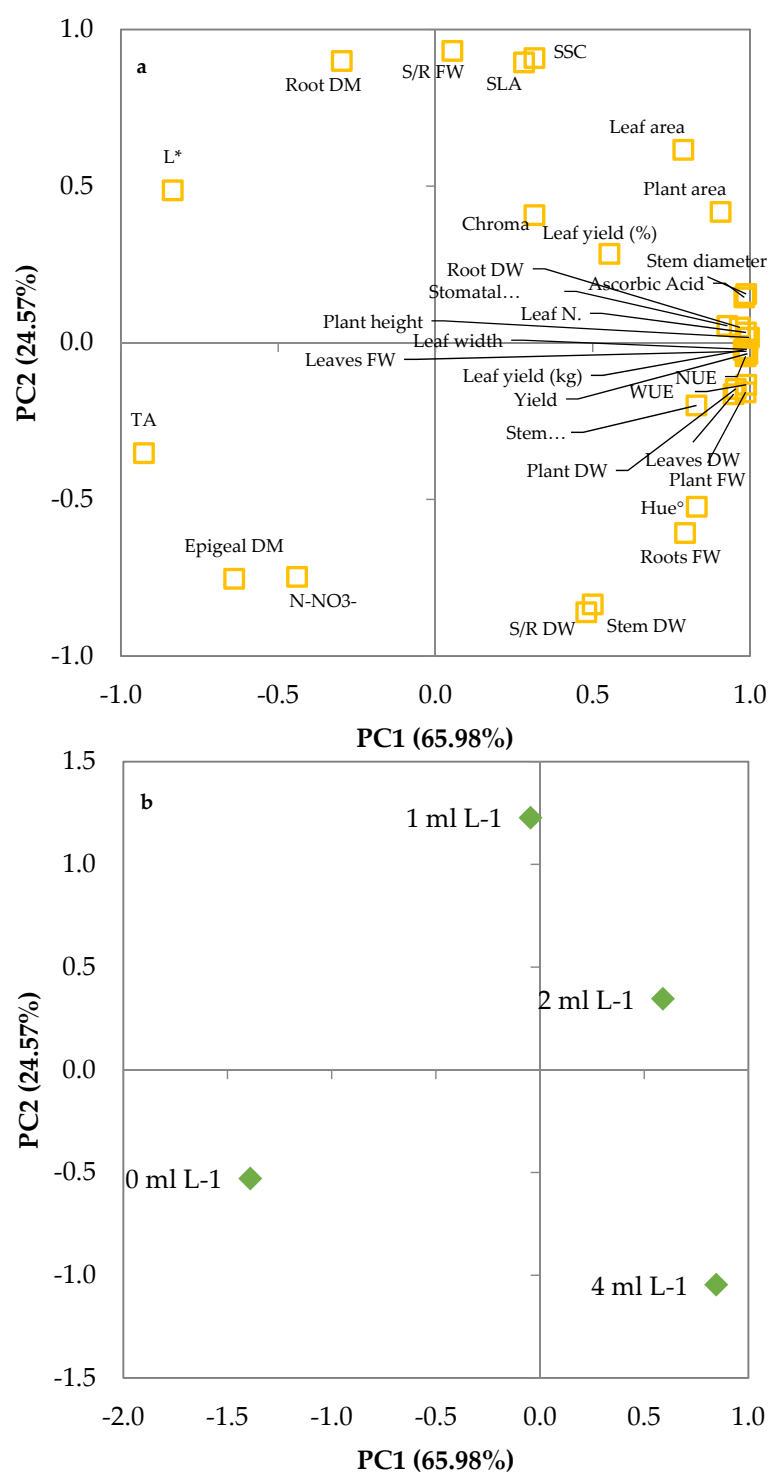
Such a relationship can be clearly evidenced by projecting the original parameters on the plane of the first two PCs, as shown in the plot of loadings (Figure 6a). The different *E. maxima* extract levels can be discriminated in the plot of scores (Figure 6b), where each biostimulant level is clearly separated from the others. The score of control had negative values of PC1 and PC2 (quadrant III; −, −) and was visibly distinguished by the score of the treatments with seaweed extract that were located in the positive part of the PC1 axis (2 and 4 mL L<sup>-1</sup> of *E. maxima* extract) or very close to it (1 mL L<sup>-1</sup> of *E. maxima* extract). Increasing the concentration of seaweed extract in the MNS from 1 to 4 mL L<sup>-1</sup> was negatively related to PC2 and positively related to PC1 so that the score of 1 mL L<sup>-1</sup> was located in the quadrant II (−, +), the score of 2 mL L<sup>-1</sup> was located in the quadrant I (+, +) and the score of 4 mL L<sup>-1</sup> was located in the quadrant IV (+, −). Merging the data from the plot of scores and loadings, it can be concluded that the rise of seaweed extract concentration in the nutrient solution was positively correlated to plant fresh and dry weight, WUE, NUE, stomatal conductance, leaf number, S/R ratios, plant height and yield, and negatively related to  $L^*$ , nitrate content, and dry matter.

**Table 5.** Correlation of variables to the factors of the principal components analysis (PCA) based on the agronomic and quality characteristics of leaf lettuce at harvest.

Variable	PC1	PC2	PC3
Plant height	<b>0.997</b>	0.017	0.072
Stem diameter	<b>0.988</b>	0.156	0.018
Plant FW	<b>0.986</b>	−0.158	0.049
Leaves FW	<b>0.989</b>	−0.027	0.145
Stem FW	<b>0.830</b>	−0.200	−0.520
Roots FW	<b>0.794</b>	−0.607	0.002
S/R FW	0.054	<b>0.932</b>	0.358
Plant DW	<b>0.955</b>	−0.147	0.256
Leaves DW	<b>0.948</b>	−0.164	0.273
Stem DW	0.500	− <b>0.835</b>	−0.227
Root DW	<b>0.968</b>	0.049	0.247
S/R DW	0.480	− <b>0.860</b>	−0.173
Epigeal DM	−0.640	− <b>0.753</b>	0.150
Root DM	−0.298	<b>0.900</b>	0.319
Yield	<b>0.992</b>	−0.035	0.125
Leaf yield (%)	0.553	0.284	<b>0.783</b>
Leaf yield (kg)	<b>0.980</b>	−0.023	0.195
WUE	<b>0.988</b>	−0.134	−0.073
NUE	<b>0.986</b>	−0.043	−0.159
Leaf N.	<b>0.987</b>	0.033	−0.155
Leaf width	<b>0.990</b>	−0.021	0.141
Plant area	<b>0.907</b>	0.418	−0.040
Leaf area	<b>0.788</b>	0.616	0.019
SLA	0.282	<b>0.895</b>	−0.347
Stomatal conductance	<b>0.928</b>	0.054	−0.368
L*	− <b>0.835</b>	0.487	−0.256
Chroma	0.315	0.408	−0.857
Hue°	<b>0.831</b>	−0.523	0.190
SSC	0.315	<b>0.909</b>	0.274
TA	− <b>0.927</b>	−0.352	0.127
Ascorbic acid	<b>0.982</b>	0.145	−0.124
NO <sub>3</sub> <sup>−</sup>	−0.440	− <b>0.747</b>	0.498

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

To have an outlook of the morpho-physiological and biochemical variables characterizing leaf lettuce treated with different biostimulant levels during cold storage, a second PCA was carried out. It showed three PCs with eigenvalues higher than 1.00 (Table 6), representing 53.95%, 14.45%, and 13.41% of the total variance, respectively. Thus, the initial 10 parameters could be expressed by three PCs, explaining 81.81% of the total variance. PC1 was positively correlated to weight loss, titratable acidity, L\* and ΔE and was negatively related to hue angle and overall quality; PC2 was positively correlated to SSC, and PC3 was positively correlated to ascorbic acid and nitrate content (Table 6).



**Figure 6.** Plot of (a) loadings (morpho-physiological and quality characteristics of leaf lettuce plants at harvest) and (b) scores (trials) formed by the two principal components from the PCA analysis on leaf lettuce grown on nutrient solutions with 0, 1, 2 and 4 mL L<sup>-1</sup> of *Ecklonia maxima* extract.

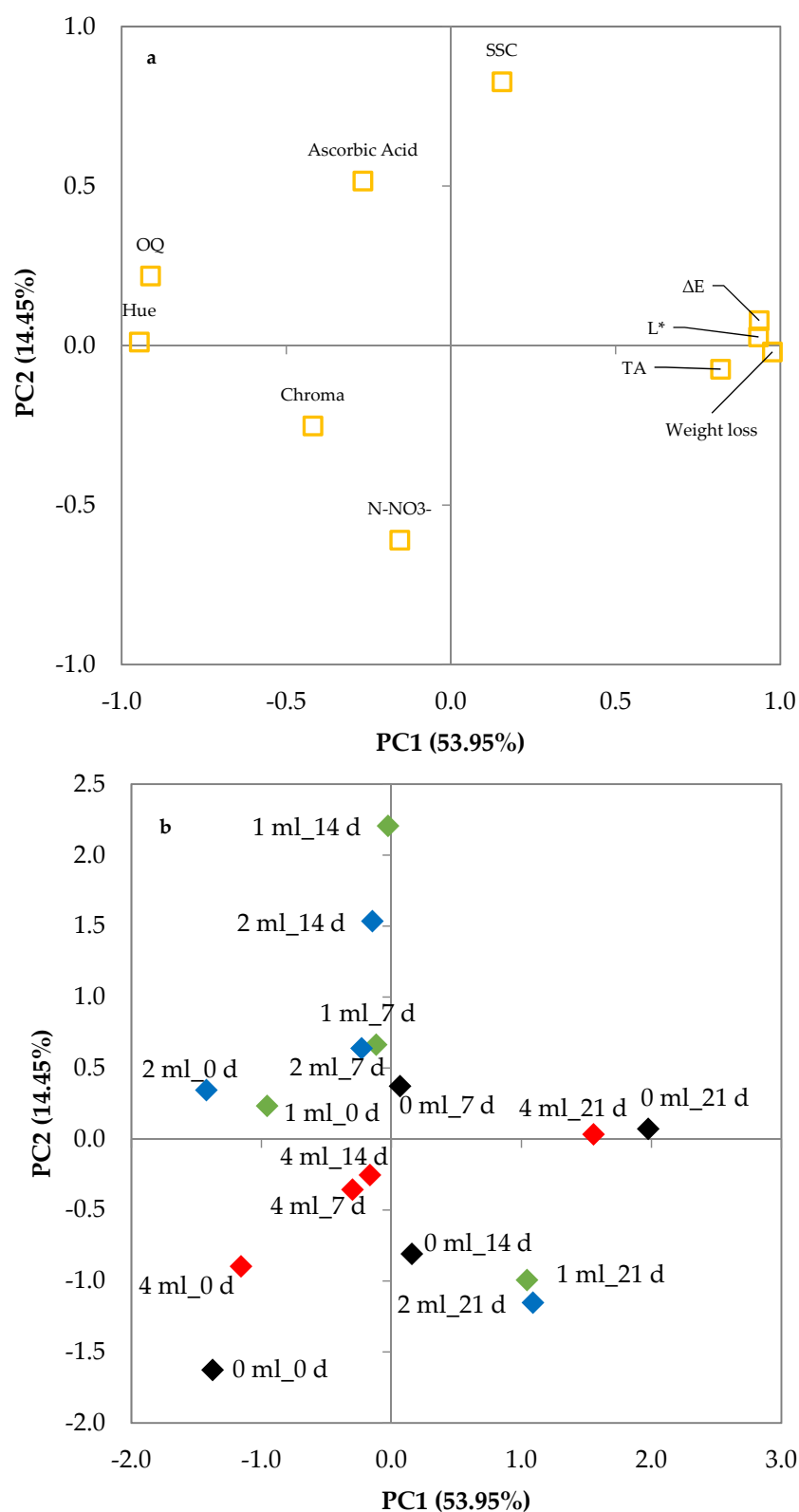
**Table 6.** Correlation of variables to the factors of the principal components analysis (PCA) based on morpho-physiological and biochemical parameters changes during cold storage of leaf lettuce treated with different *Ecklonia maxima* extract levels.

Variable	PC1	PC2	PC3
Weight loss	<b>0.976</b>	−0.020	0.066
SSC	0.155	<b>0.827</b>	0.300
TA	<b>0.819</b>	−0.074	0.283
Ascorbic acid	−0.267	0.516	<b>0.674</b>
NO <sub>3</sub> <sup>−</sup>	−0.155	−0.610	<b>0.724</b>
L*	<b>0.934</b>	0.027	−0.182
Chroma	−0.419	−0.252	0.259
Hue	<b>−0.946</b>	0.011	−0.143
ΔE	<b>0.936</b>	0.078	−0.124
OQ	<b>−0.912</b>	0.218	−0.228

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

The plot of loadings shows the initial 10 variables projected on the plane of the two PCs confirming such a correlation (Figure 7a). The discrimination of the various combinations of preharvest seaweed extract treatments × storage time of fresh-cut leaf lettuce is shown in the plot of scores (Figure 7b). The score of control samples at the beginning of the storage period was located in quadrant III (−, −) whereas it shifted toward the positive part of the PC1 axis during storage ending in quadrant I (+, +) after 21 days of storage. The samples treated with seaweed extract maintained negative values of PC1 but increased PC2 values until 14 days of storage. During this storage period, the scores of the samples treated with 4 mL L<sup>−1</sup> of seaweed extract were all in quadrant III (−, −), whereas the samples treated with 1 or 2 mL L<sup>−1</sup> were located in quadrant II (−, +). At the end of the storage period, the samples treated with 1 or 2 mL L<sup>−1</sup> of seaweed extract had the lowest value of PC1 and PC2 and were closer to the score of control samples at day 14 than those at day 21. Combining the information from the plots, it can be inferred that *E. maxima* extract levels affected leaf lettuce in different ways (Figure 7a,b). Storage time was positively related to PC1 (weight loss, ΔE, L\*, and OQ). During the first 14 days of cold storage, the samples treated with 4 mL L<sup>−1</sup> of seaweed extract showed, generally, lower variations of PC1 and PC2 values than the others, but during the last week of storage, the samples treated with 1 or 2 mL L<sup>−1</sup> showed a lower increase of PC1 values.





**Figure 7.** Plot of (a) loadings (quality characteristics of minimally processed leaf lettuce during 21 d of storage at 4 °C) and (b) scores (trials) formed by the two principal components from the Principal Component Analysis. Leaf lettuce cultivated in nutrient solutions with 0 (black rhombuses), 1 (green rhombuses), 2 (blue rhombuses), and 4 mL L<sup>-1</sup> (red rhombuses) of *Ecklonia maxima* extract; \_0d, \_7d, \_14d, and \_21d: days of storage at 4 °C.

#### 4. Discussion

Seaweed extracts can improve the morpho-physiological and yield characteristics of many vegetable crops [35]. They can be applied in various ways like seed treatment, soil application, and foliar spray. Foliar application is the most common way of supplementation and has been shown to exert biostimulant effects on many crops [51,52] and product quality and shelf life [35]. Nevertheless, this kind of application can leave residues or spots on the leaves that could negatively affect the appearance of leafy vegetables or the processing of fresh-cut leafy vegetables. To overcome this problem, in this experiment we tested the possibility to supply different levels of an *Ecklonia maxima* commercial extract to leaf lettuce grown in a hydroponic floating system through the mineral nutrient solution and estimated their effects on growth and quality at harvest and during cold storage. This is one of the first experiments that has explored this technique for supplying seaweed extract through the mineral nutrient solution of a hydroponic cultivation system under production condition.

The supplementation of the *E. maxima* extract exerted positive effects on leaf lettuce growth even if with various responses to tested concentrations. Leaf lettuce reacted to the increase of *E. maxima* extract concentration in the nutrient solution following a quadratic trend for most of the morphological and growth parameters. Seaweed extracts can improve plant growth at low concentrations or reduce it at high concentrations [53]. Finnie and Van Staden [54] reported that a commercial extract from *E. maxima* promoted the growth of tomato roots when applied at low concentrations while it strongly inhibited root growth at a high concentration.

From the curves of the estimated response to seaweed extract supplementation, it could be found that the concentration with the highest effect on plants falls between 2 and 4 mL L<sup>-1</sup>. In this range, plant growth and fresh and dry matter accumulation increased significantly compared to untreated plants, especially considering leaf biomass. Similarly, Di Mola et al. [55,56] found that weekly foliar application with 3 mL L<sup>-1</sup> of a seaweed extract from *E. maxima* to a baby lettuce crop grown in soil under greenhouse elicited significant increases in plant growth and fresh yield. They recorded an increase of marketable fresh yield that reached 14–17% at most, whereas in our experiment the supplementation of the seaweed extract through the mineral nutrient solution had a similar extent with the lowest dose (1 mL L<sup>-1</sup>), and increased by about 35% leaf fresh and dry weight and yields when the two highest levels were supplied. This could indicate a crop-specific differential response to *E. maxima* treatments and thus they need specific studies to optimize biostimulant use based on crops and application systems.

*E. maxima* extracts have exerted a positive influence on vigor, yield quality and quantity of other crops such as tomato, cucumbers, beans, spinach, zucchini, okra, etc. [36,57–61]. Tomato growth was significantly improved in plants treated with a seaweed extract of *E. maxima* regardless of whether it was supplied as a foliar spray at regular intervals, or as one initial soil drench [62], confirming that this seaweed extract can have a growth-promoting effect even when supplemented at root level as found in our work.

The improvements recorded in terms of biomass accumulation, vegetative growth, and yield could be related to the variations in stomatal conductance measured in the plants supplemented with the seaweed extract from *E. maxima* that recorded increases up to 50% with the highest concentration compared to the untreated plants. Similarly, Rouphael et al. [57] found that the application of *E. maxima* seaweed extract could reduce the stomatal resistance of zucchini leaves even under saline stress conditions.

A higher stomatal conductance may determine the rise of transpiration rates and water consumption, but also stimulates gas exchange and CO<sub>2</sub> assimilation through photosynthesis resulting in increased dry matter accumulation and WUE. A high transpiration rate is not problematic when plants are grown on panels floating on the nutrient solution. Thus, the higher stomatal conductance determined by *E. maxima* seaweed treatments could be beneficial for this cultivation technique.

In our work, we also found an increase in nitrogen use efficiency in seaweed-treated plants (+18.4%, +22.5%, and +30.3%, respectively, with 1, 2, and 4 mL L<sup>-1</sup>). The enhanced growth stimulated by the seaweed extract increased the nitrogen needs of the plants. Seaweed extracts have been shown to influence the regulation of genes that have a significant role in nutrient uptake such as a nitrate transporter gene (NRT1.1) responsible for nitrogen sensing and auxin. The upregulated expression of this gene may result in improved nitrogen assimilation, enhanced growth, and increased yield [35,63,64].

The growth improvements recorded in our experiment in terms of fresh and dry biomass accumulation when plants were grown in a nutrient solution supplemented with the seaweed extract-based biostimulant were associated with modifications of leaf number and leaf morphology. Leafiness and leaf characteristics are very important in leafy vegetables as they can affect yield and marketability.

Leaf number is determined by shoot meristem size and leaf initiation rate [65]. Leaf initiation is thought to be regulated by the positive feedback loop between auxin and its transporter PIN1. Moreover, the increase of auxin level in the vegetative meristem might lead to leaf initiation [66]. The sites of leaf primordium initiation are induced in the meristem by auxin peaks and by auxin-induced repression of the so-called KNOX gene [67]; this repression enhances the effects of gibberellins, which stimulate leaflet growth and differentiation. Thus, auxins play an important role in stimulating leaflet initiation and growth [68] and have been shown to exert this role both if they come from plant biosynthesis or from exogenous applications [69–72]. This process involves also other hormones such as cytokinins. Immanem et al. [73] showed that auxins and cytokinins can exert distinct but interconnected stimulation to cambium activity.

The extracts of brown seaweeds contain auxin, cytokinin, gibberellic acid, and other phytohormones [35]. The growth-promoting effects of seaweed extracts are often linked to the direct or indirect effect of their content in plant growth regulators.

The biostimulant used in our experiment (Basfoliar Kelp SL Compo Expert, Münster, Germany) is produced from *E. maxima* collected on the South African coastline and mechanically cold-processed to obtain a natural solution of phytohormones particularly rich in auxins (11 mg L<sup>-1</sup> of auxins) and containing amino acids, carbohydrates, proteins, vitamins, and traces of cytokinins (0.04%). Thus, the lettuce plants grown in nutrient solution containing the commercial seaweed extract were supplemented with 11–44 µg L<sup>-1</sup> of exogenous auxins according to the biostimulant concentration used.

Exogenous auxins may variously affect the morphological parameters of treated plants. The supplementation of high concentrations of auxin in maize roots produced shorter plants [41], while the application of NAA to tomato plants increased their height [74], confirming that the exogenous application of auxins can determine various phenotypic manifestations according to the origin and concentration of the auxin and plant species. Moreover, the effects of auxin rely on its concentration but also carbohydrate content in the tissues and light conditions may have a role [75].

As reported above, seaweed extracts from *E. maxima* contain cytokinins that can also play an important role in plant development. Cytokinins contained in the seaweed extracts can promote cell multiplication, resulting in increased leaf area [53,76], as also found in the present study.

The growth-promoting effect of seaweed extract produced from *E. maxima* could also be due to other mechanisms of action. The phytohormone-like effect of seaweed extracts could also be determined by other chemical substances in the extract other than phytohormones themselves [35]. *E. maxima* extracts contain several bioactive compounds such as amino acids, polysaccharides, phenolic compounds, osmolytes, etc., that could induce physiological responses reminiscent of phytohormones [35,56]. The phenolic compounds present in *E. maxima* extract could influence the metabolism and level of auxins in plants [77–79]. Some phenolic compounds such as chlorogenic acid and rutin can increase auxin activity as they can act as alternative substrates for oxidative enzymes (auxin-oxidase) and therefore protect auxins from oxidative breakdown [80]. Moreover, some phenolic

compounds may avoid auxin decarboxylation thus increasing the level of active forms of this hormone [81]. This was confirmed by Aremu et al. [19] who found a higher content of endogenous auxin in plants treated with *E. maxima* extract. It has been also suggested that the bioactive compounds in the seaweed extract might trigger a signal transduction pathway throughout elicitation of intrinsic phytohormone synthesis [55].

Another possible mechanism behind the growth-promoting effect of seaweed extract could be found in the stimulation effect on the development of roots system in terms of length, volume, and biomass accumulation that determine an increase of the uptake and translocation of nutrients and their assimilation in the biomass [82]. It was reported that lettuce plants treated with a commercial extract of *E. maxima* grown under optimal conditions increased yield and the content of Ca, K, and Mg in the leaves [83]. *E. maxima*-based biostimulants applied as root application or foliar spray also improved root growth, increased P content, but decreased N content of nutrient-stressed cucumbers, [84]. In our work, we confirmed that lettuce plants recorded an increase in root biomass and yield even when the seaweed extract from *E. maxima* is added to the nutrient solution of a hydroponic floating system.

Seaweed extracts may affect the physiology of treated plants as well, affecting global transcriptome profiles and determining changes in their metabolome [85]. Many metabolic regulatory pathways of plants could be modified by the chemical components of seaweed extracts, thus increasing the transcripts of regulatory enzymes involved in nitrogen and antioxidant metabolisms [83]. A more efficient nitrogen metabolism could explain the lower content of nitrates recorded in our experiment in the leaves of leaf lettuce supplemented with *E. maxima* extract through the nutrient solution. Nevertheless, we did not record any effect on other quality parameters (soluble solid and ascorbic acid content, and titratable acidity).

Preharvest environmental conditions and agronomic practices may influence product characteristics and physiology also after harvesting [86]. To investigate the effects of preharvest treatments with *E. maxima* extract on leaf lettuce shelf life, soon after harvest, the plants were minimally processed and cold-stored as fresh-cut produce for 21 days. Minimally processed leafy vegetables are very perishable products that can suffer fast quality degradation during storage, resulting in loss of commercial value [48]. At harvest, vegetables are detached from the plant losing the source of water, nutrients, and organic molecules including hormones. This can trigger physiological disorders that can be enhanced by preharvest environmental conditions or mineral imbalance arising during growth.

Weight loss is one of the major causes of deterioration occurring during the storage of vegetables as it can strongly affect the appearance and quality of vegetables, especially those minimally processed. It occurs as a consequence of water loss through transpiration or evaporation, but it can be also determined by respiration that degrades carbohydrate reserves [87,88]. Even a little weight loss could adversely affect appearance, salable weight, and texture quality [89]. The weight losses of fresh-cut leaf lettuce leaves were comparable on average during the first two weeks of storage but at the end of storage, the preharvest application of the *E. maxima* extract showed to be effective in limiting the total weight loss compared to control. Processing and leaf tissue deterioration increase the transpiration through the outer periderm or cuticle ending to be the main responsible for water loss [90]. Leaf senescence could be regulated and retarded by cytokinins which can influence different metabolic processes [91]. A decline in cytokinin level has been recorded in senescent leaves, whereas exogenous application of cytokinin can delay leaf senescence in many crops [92]. The effects of exogenous cytokinins on leaf senescence through foliar or root-zone application have been related to increased chlorophyll synthesis, reduced chlorophyll degradation, and decreased lipase, lipoxygenase, and proteolytic activities which are involved in membrane breakdown [92,93]. Membrane integrity has an important role in delaying senescence as it maintains compartmentation and avoids nutrient and enzyme leakage, and water loss [93].

Seaweed extracts contain substances with cytokinin-like activity and may also induce endogenous cytokinin synthesis in the treated plants [92,94,95]. Along with other plant growth regulators such as gibberellins, auxins, brassinosteroids abscisic acid, and polyamines [23,25,37], many cytokinin derivatives have been identified in *E. maxima* extracts [37] confirming their cytokinin-like activity. Moreover, spinach plants treated with *E. maxima* extract recorded a significant increase in the levels of primary endogenous cytokinins compared to the control plants [60]. The higher endogenous concentration was related to a higher number of leaves and higher yield in seaweed-treated spinach plants and could have also affected leaf senescence and membrane integrity of the leaf of lettuce in our experiment explaining the lower weight loss recorded in treated samples at the end of the storage period.

The perceived quality of leafy vegetables is greatly determined by appearance and color as they affect food choice and satisfactoriness and could also impact the consumer's perception of sensory quality. Color characteristics and the way they change during storage can be influenced by preharvest [12,15,48,96,97] or postharvest [98–102] factors. Leafy vegetable color can be altered by browning and yellowing (chlorophyll degradation) phenomena that could negatively affect marketability [48]. In this study, we recorded lower saturation and a slower decrease of hue angle during storage in the samples from plants treated with *E. maxima* extract, which showed a less vivid and more greenish color and consequently a lower chlorophyll degradation [103,104], and smaller variation of  $\Delta E$ . Color variations recorded in treated leaves during cold storage could be also related to an increase in cytokinin leaf content as it is well known that cytokinins delay leaf senescence through the promotion of chlorophyll synthesis or preservation of the chloroplast structure [93]. Moreover, chlorophyll degradation in harvested leaves is ascribed to the shortage of enough endogenous hormones [105,106], thus, the content of various plant growth regulators identified in *E. maxima* extracts could have influenced phytohormones levels and their homeostasis during storage.

The human eye can record even small variations of color changes that can influence the acceptability of leafy vegetable products. The quality perception of the minimally processed leaf lettuce, assessed by evaluating the overall visual quality (OQ) of each sample, appeared to be related to color variations during cold storage. The scores for leaf lettuce OQ assigned by the panel decreased early (after the first week of cold storage) and significantly in the untreated samples whereas they had a significant drop only at the end of the storage period (21 days at 4 °C) in the sample from plants grown with 2 and 4 mL L<sup>-1</sup> of *E. maxima* extract in the nutrient solution. Cold-stored leafy vegetables packed in sealed plastic bags can effectively slow down the occurrence of alterations and decay [86,107,108], but the delayed senescence of the samples treated with the *E. maxima* extract was effective in retaining the overall visual quality of these samples over the threshold of marketability for up to 21 d of cold storage, especially supplementing 2 mL L<sup>-1</sup> of *E. maxima* extract.

Principal component analyses can be effective in unraveling the effects of several experimental factors (genetic materials, pre- and postharvest management) on yield and quality parameters of many vegetable crops, as reported in many experiments carried out on different species and growing and storage conditions [12,86,96,97,109–111]. This was also the case in the present experiment since the different effects determined by the treatments with *E. maxima* extract on leaf lettuce plants were clearly shown by the Principal Component Analyses that outlined the various responses of leaf lettuce during growth and storage to the supplementation of different levels of seaweed extract through the nutrient solution.

The PCA analysis performed on morpho-physiological and quality parameters of leaf lettuce plants at harvest showed that lettuce was positively affected by seaweed extract treatments even if to a different extent for each parameter. Furthermore, plant response to *E. maxima* treatment was shown to be dose-dependent, hence upholding that seaweed extract application needs specific research to optimize biostimulants use based on crops and application systems. Postharvest responses to preharvest *E. maxima* treatments were also



dose-dependent, thus confirming that seaweed extract concentrations should be verified according to yield, quality, and storability goals for different species.

## 5. Conclusions

Growth and biomass accumulation of leaf lettuce plants were affected by *Ecklonia maxima* extract concentration in the nutrient solution of a hydroponic floating system. The presence of the seaweed extract in the mineral nutrient solution was effective as a plant biostimulant and yield enhancer, especially between 2 and 4 mL L<sup>-1</sup>. The growth-promoting effect was evident on various traits such as biomass accumulation, leaf expansion, stomatal conductance, WUE, NUE, etc. Preharvest treatments with *E. maxima* extract was also effective in delaying senescence and enhancing the shelf-life of minimally processed leaf lettuce. The delay in leaf senescence of the samples treated with *E. maxima* extract retained an overall quality over the limit of marketability for up to 21 d of cold storage, especially using 2 mL L<sup>-1</sup> of extract.

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