Growth and Yield Response of Red Leaf Lettuce (*Lactuca sativa* L. cv. Merlot) Substituted with Seaweed Extract as Hydroponic Nutrient Solution

Marielle Jane G. Montecillo[1], Kent Marcial L. Catubis[1*], Reynante A. Cabillo[1] and Pet Roey L. Pascual[2]
College of Agriculture
[1]Cebu Technological University – Tuburan Campus
[2]Cebu Technological University – Barili Campus
Cebu, Philippines
[*]*kentcatubis@gmail.com*

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Abstract

The increasing prices of chemical-based hydroponic nutrient solutions (CHNS) in the market have led to replace a percentage of its recommended rate with available local biostimulants to save hydroponic solution costs. Thus, a study on hydroponics utilizing different concentrations of Eucheuma cottonii seaweed extracts (SWE) as a biostimulant was conducted to determine the growth and yield of red leaf lettuce. The various concentrations of seaweed extract are a percentage substitution of CHNS. The treatments are 100% CHNS, 25% SWE + 75% CHNS, 50% SWE + 50% CHNS, and 75% SWE + 25% CHNS. The plant height of red leaf lettuce under 50% SWE (10.65 cm) is the highest compared to the lettuce grown under 100% CHNS (9.81 cm) and 75% SWE (9.76 cm) one week after treatment application. There was no significant difference in the plant height observed weeks after one week from treatment application. There was no significant difference among treatments on the number of leaves. The leaf width (12.28 cm) and plant weight (28.91 g) of red leaf lettuce under 25% SWE are comparable to the lettuce plant under 100% CHNS (11.47 cm, 33.62 g, respectively). The leaf length, conversely, is the highest at 75% SWE (12.69 cm). Similar growth had been observed among treatments used in the root length and dry weight. Thus, the commercial hydroponic nutrient solution could only be replaced up to 25% with seaweed extract without any significant reduction in growth and yield in lettuce except for plant height and leaf length.

Keywords*:* biostimulant, *Eucheuma cottonii*, hydroponics, lettuce, seaweed

1. Introduction

The advantages of hydroponic farming system have grown in popularity worldwide. New farmers are now engaged in hydroponic farming. It is a soilless method of growing plants through its nutrient-rich water solution as fertilizer. Some advantages of a hydroponic setup are reduced soil-borne pests, diseases, and weeding operations. Since it does not need soil for growing crops, it is best suited in urban areas where soil for planting is very limited. Moreover, hydroponically grown crops yield more than crops grown on soil (Treftz and Omaye, 2015). The yield increase is due to the efficient use of water and nutrients in a hydroponic system (Albaho, 2008). The hydroponic system could also grow herbs, leafy vegetables, and other greenhouse vegetables.

Lettuce (*Lactuca sativa* L.) is the most cultivated crops under a hydroponic system. Its potential to generate income quickly makes it more acceptable to farmers engaging in hydroponics. Nutritionally, lettuce is low in sodium, fat, and calories. It is a good source of fiber, iron, folate, and vitamin C. Lettuce contains several helpful bioactive chemicals for health, especially the red-pigmented lettuce due to its higher phenolic compounds compared to green lettuce. Such phenolic compounds benefit humans as they are anti-aging, anti-inflammatory, antioxidant, and anti-proliferative (Kim *et al*., 2016).

Alternative and low-cost fertilization ought to be considered for growing lettuce in a hydroponic system, as synthetic-based fertilizers, where most of the hydroponic nutrient solution is based, are getting expensive nowadays. Biostimulants of natural origin have been gaining popularity lately as an additive to fertilizers to enhance nutrient uptake, plant growth, and tolerance against biotic and abiotic stresses. Since biostimulants do not directly supply plants with vital nutrients, they are not classified as fertilizers. However, they do include a significant quantity of plant growth hormones, which facilitate the uptake or acquisition of nutrients by means of improved metabolic processes in plants (Drobek *et al*., 2019).

Seaweed extract as a plant biostimulant reported positive effects in some high-value crops. Seaweed species, such as *Ascophyllum nodosum*, *Ecklonia maxima*, and *Sargassum spp*., are widely used for seaweed extract (Xu and Leskovar, 2015; Battacharyya *et al*., 2015). In most cases, seaweed extracts are often applied to plants through foliar or in soil by irrigation (Drobek *et al*., 2019).

Eucheuma cottonii, locally known in the Philippines as '*gusô*,' is among the various species of seaweed still abundantly cultivated for commercial use. It is one of the essential carrageenophytes, a vital source of carrageenan (Pereira and Yarish, 2008). *Eucheuma cottonii*, as a biofertilizer, has been demonstrated to enhance plant growth and development (Yusuf *et al*., 2016; Krishnamoorthy and Abdul Malek, 2022). However, its effect as a biostimulant on plants in a hydroponic system is limited.

Given that *Eucheuma cottonii* contains major and minor minerals, vitamins, cytokinins, auxin, abscisic acid, and other substances that might stimulate plant growth and yield (Rathore *et al*., 2009), this study examined the effects of applying seaweed extract derived from *E. cottonii* on red leaf lettuce in a hydroponic setting. The impact on plant growth and yield responses of substituting seaweed extract at different percentages of the specified dilution rate for a commercial hydroponic solution was evaluated in this study.

2. Methodology

2.1 Planting Material

A 'Merlot' red leaf lettuce cultivar was uniformly sown on a seedling tray with a growing media containing an equal ratio of coconut peat and vermicast. Seedling trays were placed inside the protected screenhouse at Cebu Technological University – Tuburan Campus, College of Agriculture (10° 43' 43.716" N, 123° 49' 28.812" E) for further seed germination. The average temperature and humidity inside the screenhouse were 33.5°C and 55.5%, respectively. Sown seeds were watered every other day. About 90% of the seeds germinated three days after sowing. Grown lettuce seedlings were ready for transplanting two weeks after sowing.

2.2 Preparation of the Growing Boxes

Twelve styrofoam boxes (60.96 cm x 40.64 cm x 15.24 cm) were used as the growing boxes for lettuce following the principle of the Kratky hydroponic system (Kratky, 2009). The lower half of the growing box served as a reservoir for the nutrient solution. A polyethylene plastic film was lined inside the lower half of the growing box to hold the nutrient solution securely. A total of six (6) holes were made per lid or upper half of the growing boxes with the use of an improvised puncher. The puncher was an empty tin can (370 mL capacity) with one lid cut open, sharp enough to bore a hole through the styrofoam boxes.

2.3 Seedling Plug Preparation and Transplanting of Seedlings

Styrocup, with a 237 mL capacity, was utilized as seedling plugs for growing lettuce. A handsaw was used to cut four (4) vertical incisions on the edge of the cup, starting from the bottom and running two-thirds (2/3) along the side of the cup. About one-third (1/3) of the cup was filled with aged coconut coir dust as a growing medium (substitute for the soil). The styrocups were fitted snugly to the holes on the styrofoam box lids (Santos and Ocampo, 2005).

Fourteen (14) days after emergence, the seedlings were transplanted into styrocups.

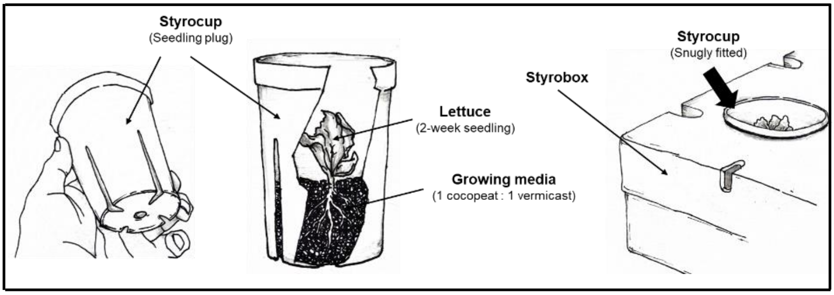


Figure 1. Preparation of seedling plug and transplanting of lettuce seedlings in a Kratky hydroponic system.

2.4 Collection and Preparation of Seaweed Extract

Eucheuma cottonii of seaweed specie was used as raw material for seaweed extract. It was freshly bought at the local market. The bought seaweed was washed with tap water to remove impurities and excess salts. Washed seaweeds were air-dried till completely dry from water. Air-dried seaweeds were chopped into smaller pieces. One kilogram of chopped seaweed was boiled in six (6) liters of distilled water for one (1) hour and was filtered to remove solids using a double-layered cloth. The filtrate was taken as 100% concentration of the seaweed extract (SWE). The extract has a pH of 7.28 and an electrical conductivity (EC) of 1.25 mS/cm based on measurement using the digital pH and EC meter.

2.5 Experimental Design and Treatments

The experiment was conducted under the same protected screenhouse. It was laid out in a Randomized Complete Block Design (RCBD) with three (3) blocks per treatment with a total of four (4) treatments. The treatments were T0 - 100% Commercial hydroponic nutrient solution (CHNS), T1 - 25% SWE + 75% CHNS, T2 – 50% SWE + 50% CHNS, and T3 – 75% SWE + 25% CHNS.

Each box that served as a treatment replicate was assumed to have contributed to certain levels of micro-climate variability, thus serving as a block for the experiment. Each box became inherently blocks of the study, since it separates treatments and replications.

2.6 Data Gathered

Every week, data on plant height and the number of leaves were collected while leaf width, leaf length, root length, root dry weight, and plant weight of lettuce were gathered three (3) weeks after transplanting (termination period).

The plant height of the lettuce plant was measured from its base up to the highest tip. The number of leaves per plant was manually counted and recorded. As to the leaf width and length, the fourth leaf from the apical leaf of each plant was measured using a ruler. Root length was measured with a ruler from the base of the lettuce plant to its longest root tip. Root dry weight and plant weight (per plant) were obtained using a digital weighing scale (capacity: 3,000 g; graduation: 0.1 g).

2.7 Statistical Analysis

All data gathered were tested for normality using the Shapiro-Wilk Test ($n<2000$) and then analyzed through One-Way Analysis of Variance (ANOVA) using the Minitab® statistical software version 17.1.0.0 (Minitab Inc., 2013) to check significant differences among treatment means. Further comparisons were made using Tukey's test ($p < 0.05$) to identify which specific treatment means significantly differ from each other. The bars in the graphs represented the length of confidence intervals (CI) to quantify the margin of error in estimating the population parameter based on a sample.

3. Results and Discussion

3.1 Normality test

All data were normally distributed under the Shapiro-Wilk Normality Test after removing all the outliers from most parameters to achieve normal distribution. Normalized data sets were then analyzed using One-Way ANOVA. The results of the normality test per the parameters are shown in Table 1.

Table 1. The significant value of each treatment per parameter used in the study after removing outliers through the Shapiro-Wilk Normality Test

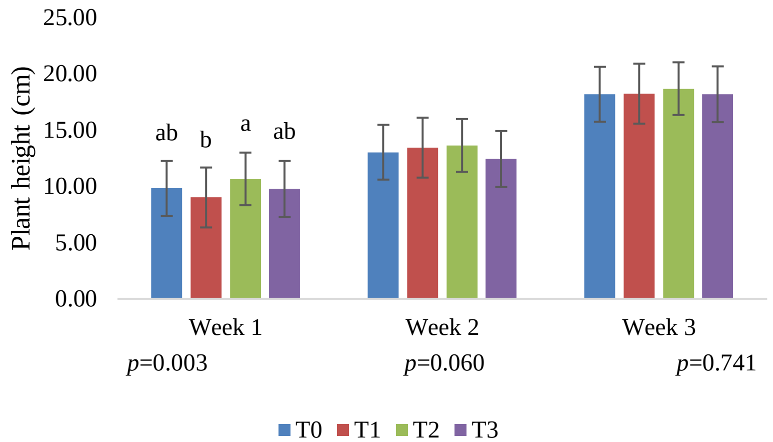
Parameters		Significant value			
		T0	T1	T2	T3
Plant height (cm)	Week 1	.176	.209	.086	.090
	Week 2	.090	.265	.119	.052
	Week 3	.079	.062	.078	.245
Number of leaves	Week 1	.103	.127	.058	.067
	Week 2	.231	.164	.155	.219
	Week 3	.316	.452	.249	.377
Leaf width (cm)		.059	.180	.216	.150
Leaf length (cm)		.053	.439	.181	.169
Root length (cm)		.327	.279	.296	.503
Root dry weight (g)		.055	.087	.191	.157
Plant weight (g)		.506	.569	.781	.693

As a result, all significant values yielded greater than 0.05, suggesting that the data was already normal and, thus, ready for the ANOVA.

3.2 Plant height and Number of leaves

Figure 2 shows the plant height of lettuce in a hydroponic system grown with different levels of seaweed extract and commercial hydroponic nutrient solution. In the first week, the treatment substituted with 50% SWE significantly produced the tallest plants at 10.65 cm compared to all SWE-substituted treatments. From the second week to the termination of the study, results showed that all treatments were comparable.

A study also reported that seaweed extracts support leafy vegetable growth (Chrysargyris *et al*., 2018). Similarly, a 0.5% foliar spray of TAM®, a seaweed extract, increased the plant height of *Capsicum annuum* (88.00 cm) compared to the control group (67.33 cm). In addition, a higher level of TAM® at 1.0% produced a plant height lower than the plants treated with 0.5% of TAM® with a mean value of 72.67 cm (Ashour *et al*., 2021).



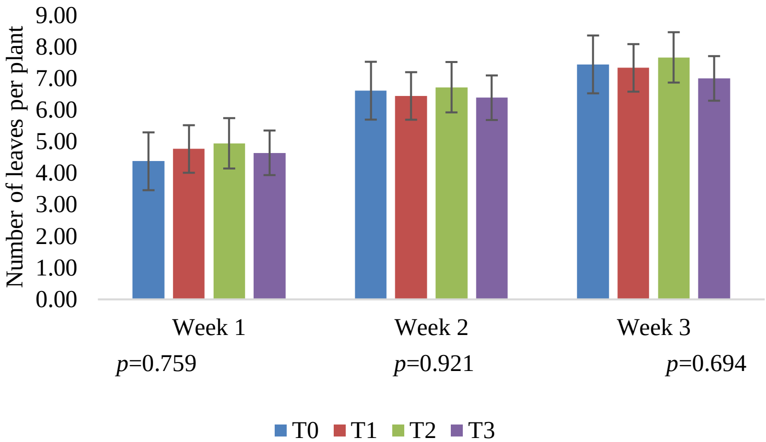
Plant height (cm)	**T0**		**T1**		**T2**		**T3**	
	lower	upper	lower	upper	lower	upper	lower	upper
Week 1	9.23	10.39	8.42	9.58	10.05	11.25	9.17	10.36
Week 2	12.38	13.66	12.70	14.16	12.95	14.31	11.78	13.06
Week 3	17.42	18.92	17.47	18.97	17.92	19.42	17.42	18.92

Treatment means within the same week not sharing letter (s) in common differ significantly from each other at a 5% level of significance based on the Tukey test. Error bars show a population parameter's confidence interval (CI) at 95%.

Legend:

T0 – 100% Control (commercial hydroponic nutrient solution)
T1 – 25% SWE + 75% CHNS
T2 – 50% SWE + 50% CHNS
T3 – 75% SWE + 25% CHNS

Figure 2. Plant height: Weekly plant height (cm) of 'Merlot' red leaf lettuce as affected by the different treatments under the Kratky method of hydroponic system



Number of leaves	**95% Confidence Interval (CI)**							
	T0		**T1**		**T2**		**T3**	
	lower	upper	lower	upper	lower	upper	lower	upper
Week 1	3.59	5.16	4.01	5.52	4.21	5.68	3.81	5.48
Week 2	5.85	7.37	5.69	7.20	5.96	7.48	5.63	7.15
Week 3	6.75	8.14	6.64	8.03	6.97	8.36	6.11	7.89

Treatment means within the same week not sharing letter (s) in common differ significantly from each other at a 5% level of significance based on the Tukey test. Error bars show a population parameter's confidence interval (CI) at 95%.

Legend:

T0 – 100% Control (commercial hydroponic nutrient solution)
T1 – 25% SWE + 75% CHNS
T2 – 50% SWE + 50% CHNS
T3 – 75% SWE + 25% CHNS

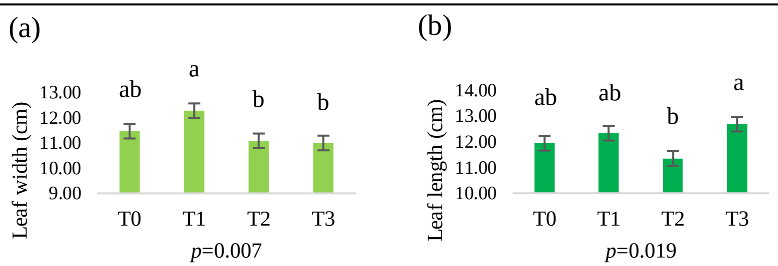
Figure 3. Weekly number of leaves per plant of Merlot red leaf lettuce as affected by the different treatments under the Kratky method of hydroponic system

Figure 3 shows no significant difference in the number of leaves produced from the first treatment application until harvest. The replacement of CHNS, regardless of percent concentration with SWE, has no significant effect on the red lettuce's number of leaves, comparable to 100% CHNS.

Seaweed extract containing plant hormones like cytokinins and auxins stimulated young leaves' rapid growth and shoot tissues (Arthur *et al*., 2003). Mohammed *et al*.'s study (2022) reported that lettuce treated with 4 g/L of seaweed extract significantly increased the number of leaves of the plant (56.00) compared to those under the control group (53.67). Moreover, seaweed extract from *Kappaphycus alvarezii* (also known as *Eucheuma cottonii*) significantly increased the eggplant's number of leaves at week 12 after planting compared to those untreated plants by 54.97% (Yusuf *et al.*, 2021).

3.3 Leaf width and Leaf length

A significant difference in leaf width was observed as influenced by different treatments in the study (Figure 4). Red leaf lettuce grown under 25% SWE and 75% CHNS produced larger leaves with an average leaf width of 12.28 cm compared to those applied with SWE substituted from 50% and 75% at 11.08 cm and 11.00 cm, respectively.



Parameter	95% CI T0		T1		T2		T3	
	lower	upper	lower	upper	lower	upper	lower	upper
Leaf width (cm)	10.90	12.04	11.72	12.84	10.53	11.64	10.44	11.56
Leaf length (cm)	11.36	12.53	11.75	12.92	10.75	11.96	12.07	13.31

Treatment means within the same week not sharing letter (s) in common differ significantly from each other at a 5% level of significance based on the Tukey test. Error bars show a population parameter's confidence interval (CI) at 95%.

Legend:

T0 – 100% Control (commercial hydroponic nutrient solution)
T1 – 25% SWE + 75% CHNS
T2 – 50% SWE + 50% CHNS
T3 – 75% SWE + 25% CHNS

Figure 4. Plant leaf: The leaf width (cm) of 'Merlot' red leaf lettuce at Week 3 after transplanting (a) and the leaf length (cm) of 'Merlot' red leaf lettuce at Week 3 after transplanting (b)

This means that 25% replacement could be allowed only for leaf width before any negative effects could be observed. Such a result could be attributed to the growth-promoting hormones in most seaweed liquid fertilizers, specifically auxins and cytokinins (Panda *et al*., 2012). Generally, cytokinins and auxin plays an integral part in leaf development. It promotes cell division, increasing cell expansion during leaf cell development's proliferation and expansion stages (Xiong and Jiao, 2019; Wu *et al*., 2021). Thus, it helped increase the leaf size of the plant. As reported, the leaf width of green leaf lettuce supplemented with 2 and 4 mL/L of seaweed extract was significantly wider by 23.7% compared to the control plants (Miceli *et al*., 2021). Similarly, eggplant's leaf width was significantly recorded as highest on plants treated with 1.5% v/v of seaweed extract (17.67 cm) compared to plants fertilized only with commercial fertilizer (12.78 cm) and no fertilizer at all (12.33 cm) (Jamili *et al*., 2022). However, it would be important to consider that SWE replacement was made against CHNS, which provided higher nutrients to plants. Thus, only 25% SWE could provide comparable results against 100% CHNS.

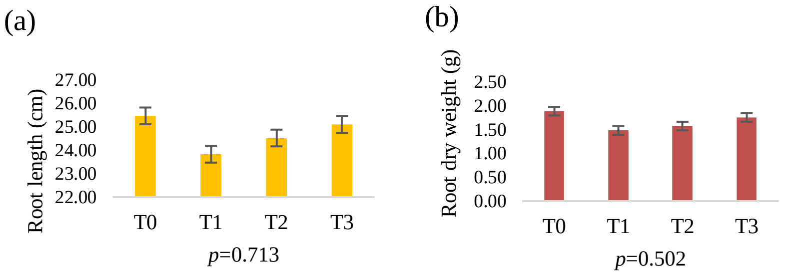
On the other hand, a higher percentage of SWE replacement (50% and 75%) reduced the leaf width of red leaf lettuce. Such a response could be attributed to the micronutrient toxicity (zinc, cadmium, copper, and lead) of higher seaweed extract concentration in plants. As such, the plant's metabolic activity was reduced, negatively affecting both the plant's morphological and physiological parameters (Papenfus *et al*., 2013). This was also likely attributed to the reduced nutrient content of the medium and the reduced CHNS content.

Conversely, a higher concentration of SWE at 75% resulted to a longer leaf length of red lettuce at Week 3 from treatment application, averaging 12.69 cm in length. The result contradicted previous studies, wherein higher seaweed extract levels inhibited plant growth and development. As for the tomatoes, a higher concentration of seaweed extract as foliar fertilizer reduced the leaf area per plant (Sutharsan *et al*., 2014).

However, the plant's response to a higher concentration of seaweed extract could also be credited to the species or variety of crops. In onions, for example, the leaf length of the cultivar '*Phulkara*' was significantly enhanced at 3% SWE and reduced at 0.5-1% SWE. Thus, the study's leaf length result would be the red lettuce cultivar 'Merlot' response to higher SWE concentration (Abbas *et al*., 2020).

3.4 Root length and Root dry weight

Figure 5 shows the average root length and root dry weight per red leaf lettuce plant as affected by different treatments. The data does not provide enough evidence to support the alternative hypothesis. This is due to various factors such as sample size, variability in the data, or the chosen significance level. The nonrejection of the null hypothesis suggests that any observed differences in the data may be due to random chance alone rather than a true underlying difference in the treatments in the population. It indicates that further research or additional evidence may be needed to draw definitive conclusions about the relationship or effect being studied.



Parameter	95% CI							
	T0		T1		T2		T3	
	lower	upper	lower	upper	lower	upper	lower	upper
Root length (cm)	23.36	27.59	21.72	25.95	22.41	26.64	23.00	27.23
Root dry weight (g)	1.47	2.30	1.08	1.88	1.16	1.99	1.37	2.14

Treatment means within the same week not sharing letter (s) in common differ significantly from each other at a 5% level of significance based on the Tukey test. Error bars show a population parameter's confidence interval (CI) at 95%.

Legend:

T0 – 100% Control (commercial hydroponic nutrient solution)
T1 – 25% SWE + 75% CHNS
T2 – 50% SWE + 50% CHNS
T3 – 75% SWE + 25% CHNS

Figure 5. Plant root: The root length (cm) of 'Merlot' red leaf lettuce as affected by the different treatments under the Kratky method of a hydroponic system at Week 3 after transplanting (a) and the root dry weight (g) of red leaf lettuce between treatments at Week 3 after transplanting (b)

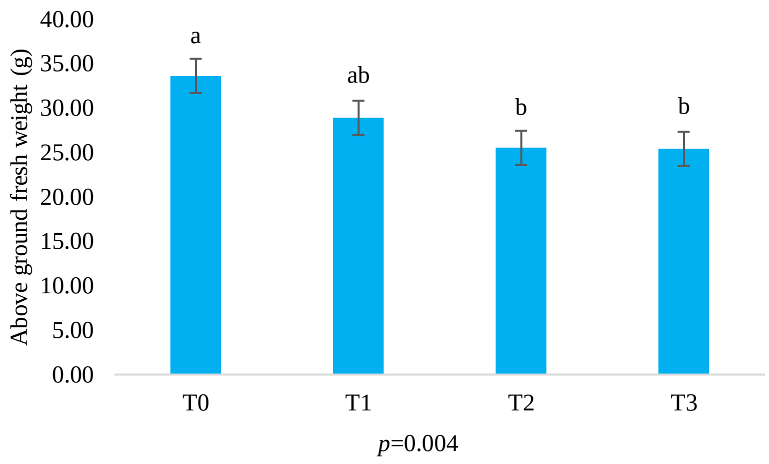
The plant hormone auxin, particularly the Indole-3-Butyric Acid (IBA), present in most seaweed species (Yalçın *et al*., 2019), could maintain the root growth of red leaf lettuce treated with SWE, comparable to those fertilized with 100% CHNS. IBA-derived auxin provided significant roles in the plant's root development, including stimulating and accelerating root growth, lateral root development, and root hair elongation (Frick and Strader, 2018). Similar to the study's result, the root length of eggplant applied with 1.0% v/v of seaweed extract got the longest root with a mean value of 21.61 cm, which was comparable to the plants fertilized with commercial fertilizer with a mean value of 20.28 cm (Jamili *et al*., 2022). Moreover, carob plants have comparable root lengths to those supplemented with 1%, 2%, and 4% liquid seaweed extract (from *Ulva rigida*) after four weeks of culture (Zouari *et al*., 2023).

The study's root dry weight of red leaf lettuce had a similar effect. Root dry weight of lettuce sprayed with seaweed extract was significantly higher compared to lettuce plants without seaweed extract application (Rasouli *et al*., 2022). In addition, *Kappaphycus alvarezii* seaweed extract application on eggplant showed no significant difference in plants fertilized with complete commercial fertilizer (Yusuf *et al*., 2021).

3.5 Plant weight

A significant difference in the fresh weight (g) of lettuce was observed as affected by different treatments in the study (Figure 6). Red leaf lettuce grown in 100% CHNS produced the heaviest crop with an average mean of 33.62 g and was not significantly different only to the treatment substituted with 25% SWE with an average mean of 28.91 g. This means that CHNS could be replaced only with up to 25% SWE before any significant reduction in plant weight could be observed.

The result could be credited to the total nitrogen (N) present and available within the treatments used. Undiluted Simple Nutrient Addition Program (SNAP), a hydroponic fertilizer solution used in the study, had 6.10% wt/vol of nitrogen (UPLB-CAF, n.d.), which was higher than the nitrogen content of *K. alvarezii* seaweed extract (0.45-0.70%), Moringa leaf extract (0.07%), and molasses (0.2-0.8%) (Zodape *et al*., 2009; Kanchani and Harris, 2019; LetCo Swiss S.A., n.d.). As a rule, nitrogen would support all plant metabolic functions such as resource allocation, growth, and development (Yousaf *et al*., 2021). Specifically, nitrogen resulted in higher biomass yields (Blumenthal *et al*., 2008).



Parameter	T0		T1		T2		T3	
	95% CI							
	lower	upper	lower	upper	lower	upper	lower	upper
Plant weight (g)	30.13	37.12	25.41	32.40	22.04	29.03	21.91	28.91

Treatment means within the same week not sharing letter (s) in common differ significantly from each other at a 5% level of significance based on the Tukey test. Error bars show a population parameter's confidence interval (CI) at 95%.

Legend:

T0 – 100% Control (commercial hydroponic nutrient solution)
T1 – 25% SWE + 75% CHNS
T2 – 50% SWE + 50% CHNS
T3 – 75% SWE + 25% CHNS

Figure 6. Plant weight (g) of Merlot red leaf lettuce as affected by the different treatments under the Kratky method of hydroponic system

As to seaweed extract application, a lower concentration of SWE at 25% resulted in a comparable plant weight of red leaf lettuce with 100% CHNS. Plant growth hormones present in seaweed extract positively affected the growth of plants even at low nitrogen content. While not as potent as commercially available hydroponic solutions, seaweed extracts have demonstrated the ability to enhance plant growth. Substituting 25% of the commercial solution with seaweed extract yielded comparable results to using 100% of the commercial solution. Similarly, the average fresh weight of eggplant leaves fertilized with complete synthetic fertilizer at 12 weeks after planting was 25.16 g, which was also comparable to those plants fertilized with *Kappaphycus alvarezii* seaweed extract weighing 34.61 g (Yusuf *et al*., 2021). Moreover, lettuce plants fertilized with NPK fertilizer yielded a fresh plant weight of 21.61 g, which was also significantly similar to the lettuce plants applied with *Sargassum ilicifolium* having an average weight of 19.51 g (Opeña *et al.*, 2022).

Although seaweed extract has growth-promoting hormones that enhance some lettuce growth parameters, a higher concentration of SWE (50% to 75%) reduced the red leaf lettuce plant's weight. As reported, increasing the concentration of seaweed extract led to plant growth inhibition. Beyond 60% of seaweed extract concentration inhibited the germination rate of *Abelmoschus esculentus* and *Solanum lycopersicon* seeds (Arun *et al.*, 2014).

4. Conclusion and Recommendation

Except for plant height in the first week and the leaf length in the third week, the commercial hydroponic nutrient solution could only be replaced up to 25% with seaweed extract without any significant reduction in growth and yield in lettuce. Thus, the 25% seaweed extract replacement from commercial hydroponic nutrient solution is recommended for red lettuce cv. Merlot is recommended.

5. Acknowledgement

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