

Bio-available Fertilizer for Hydroponic Lettuce (*Lactuca sativa L. var. longifolia*) from byproducts of *Chlorella vulgaris*

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Abstract

The byproducts of *Chlorella vulgaris* contain biochemical components, essential nutrients, and biomass, making them viable as bio-available fertilizers for hydroponically grown lettuce (*Lactuca sativa* L. var. *Longifolia*) using the Deep-Water Culture (DWC) system. This study aimed to offer an environmentally friendly alternative to chemical fertilizers, particularly for dry season farming in the Philippines, where chemical inputs contribute to environmental degradation and rising production costs. Exploring alternatives like *C. vulgaris* promotes sustainability, resource efficiency, and supports the UN Sustainable Development Goals. The bio-available fertilizer derived from *C. vulgaris* significantly improved lettuce growth and survival compared to chemical fertilizer and control groups, demonstrating its potential as a sustainable hydroponic input. A six-week experiment using two-way repeated measures ANOVA and descriptive statistics showed that the bio-available fertilizer led to significantly greater shoot length, root length, plant height, leaf area, and number of leaves than the control. It also outperformed chemical fertilizer in shoot and leaf growth. Survival rates were highest in the biofertilizer group, confirming its adaptability under dry-season stress. However, overall growth was still lower than in greenhouse-based studies, such as that of Frasetya et al. (2019), where lettuce reached a height of 22.73 cm under controlled conditions. In contrast, the highest shoot length recorded in this study was 8.6 cm under biofertilizer treatment, highlighting the influence of environmental factors like temperature and humidity. The study concludes that *Chlorella vulgaris* byproducts can serve as an effective and sustainable alternative to chemical fertilizers for hydroponically grown lettuce.

I. INTRODUCTION

The Philippine agricultural industry seeks a technique that can maximize crop production and food quality through sustainable methods, while minimizing the conventional and traditional farming practices. Hydroponics is an innovative sustainable agricultural technique which represents an innovative approach to agricultural farming techniques, minimizing the use of soil as a growth medium and instead using nutrient-rich water for plant cultivation. The hydroponic system has the potential to become a sustainable practice for agriculture because it eliminates the risk of soil-borne plant diseases (Talitag, D., et al. 2022). The hydroponic system offers significant advantages compared to traditional, soil-based agriculture. Additionally, hydroponics provides a solution to environmental problems (Khatri, L., et al. 2023). However, many studies suggest that the use of conventional chemical fertilizers poses health risks and contributes to greenhouse gas emissions major environmental concerns today. The chemical fertilizers have the purpose of providing essential nutrients to plants while compensating for deficiencies in soil composition. However, their widespread use has raised concerns about the effects on plant health, soil fertility, and environmental sustainability (Sarmila, K. C. 2024).

To address the adverse effects of chemical fertilizers on soil and water bodies, a shift toward biofertilizers, organic fertilizers, manure, and biopesticides is encouraged (Kumar, C., et al. 2019). The application of *Chlorella vulgaris* can reduce the use of chemical fertilizers in pepper production (Tian et al. 2022). *C. vulgaris* extract can be considered a bio stimulant, being able to increase lettuce yield by enhancing crop growth and inducing plant metabolism (La Bella, M., et al. 2021). *Chlorella vulgaris* includes a high concentration of nutrients such as vitamins (folic acid, vitamin C, vitamin B complex, biotin, vitamin B1, vitamin D, alpha-tocopherol, and menadione), minerals (Fe, K, Na, Ca, P, and Mg), pigments (lutein, β -carotene, astaxanthin, chlorophyll-a, canthaxanthin, and chlorophyll-b), polysaccharides, and growth factors (Safi, et al., 2024). These nutritional components highlight the potential of *C. vulgaris* in developing bio-available fertilizer for hydroponics through extracting its byproducts.

The integration of *Chlorella vulgaris* in agriculture and biotechnology as a renewable resource can potentially replace harmful practices that negatively affect the environment. With the advancement of innovation, its byproducts may

serve in the development of bio-available fertilizer for hydroponics. This fertilizer is derived from the biomass of organic matter that undergoes biodegradation under optimal conditions. Studies have shown that *C. vulgaris* suspensions can enhance the germination of tomato and cucumber seeds. Algal suspensions of 0.17 and 0.25 g/L improved the root and shoot lengths of tomato and cucumber seeds, respectively (Bumandalai & Tserennadid, 2019). Despite extensive research on *C. vulgaris* for biofuel, bioplastic, and biofertilizer purposes, its potential as a source of bio-available fertilizer for hydroponics remains underexplored.

Recent trends show a shift in research focus from traditional hydroponic solutions, chicken manure, and foliar applications to topics like wastewater management, liquid organic fertilizers, bio stimulants, and eco-enzymes (Syamsia, S., 2024). However, the extraction of byproducts is often costly and typically performed in laboratories with sophisticated equipment. According to Gonçalves et al. (2023), one major limitation is the high cost of producing microalgae biomass and extracting the desired metabolites. This study explores more cost-effective alternatives, such as using heat and flocculation methods to disrupt the cell wall and harness the byproducts of *Chlorella vulgaris*.

In addition, to explore hydroponic experiments, its utility is somewhat constrained by environmental factors. Consequently, it is advisable to consider the exploration of greenhouse and outdoor experimental settings in future investigations to enhance practicality and real-world relevance (A., Wichaphian, et al. 2023). This led the researchers to apply an uncontrolled environment affected by abiotic factors which can affect the growth of the lettuce under DWC hydroponic system treated in bio-available fertilizer, chemical fertilizer and control.

This study aimed to extract byproducts of *Chlorella vulgaris* including the nutritional composition and biomass to convert into valuable bio-available fertilizer, Investigate its effectiveness in the growth of lettuce (*Lactuca sativa L. var Longifolia*) by measuring its morphological parameters of the lettuce plant in the DWC hydroponic system, determine bioavailability of the bio-available fertilizer derived from byproducts of *C. vulgaris* in cultivating lettuce in the hydroponics system, identify which among the fertilizers between 2 treatments (bio-available fertilizer and commercial chemical fertilizer) is effective in stimulating lettuce plants in the hydroponics system rapidly, and determine which treatment (Treatment 1, Treatment 2, or control) can support the lettuce to survive under the uncontrollable environment of the Philippines.

II. METHODS AND MATERIAL

Collection Chlorella spp and Chlorella vulgaris sample

The researchers purchased 1.5 Liters of *Chlorella* spp. with unknown species at the AZA aquatic store located in Gabon, Abucay, Bataan, Central Luzon, Philippines that cultures algae for marine feeds. To strengthen the research validity the researchers purchased 100 mL of *Chlorella vulgaris beijerinck* (Chlorophyta) cultured in BG 11 + nitrogen at the phycology laboratory, Bioscience, University of the Philippines, Los Banos, Laguna, Philippines.

Microalgal comparison (Chlorella spp. Vs Chlorella vulgaris)

The researchers conducted microalgal comparisons at the forensic laboratory at the Pamantasan ng Lungsod ng Muntinlupa under the College of Criminal Justice. This process ensures the researchers that the *Chlorella* spp and *Chlorella vulgaris* share the same morphological attributes. The researchers prepared the sample slides separate from both genus and underwent the comparison. As the researchers observed and compared the 2 microalgae, the researchers found that these two *Chlorella* shared the same characteristic in morphological attributes.

Preparation and synthesizing Organic Fertilizer for Chlorella vulgaris Cultivation.

The *Chlorella vulgaris* was isolated and preparing for cultivation. Firstly, the researchers prepared the materials needed to synthesize organic fertilizer for the *Chlorella vulgaris* cultivation. The banana peel tea was smashed and broken into pieces by the researchers initially extracting the juice of banana peel. The mashed banana peel was exposed at high temperature and let the biochemical component extract through boiling. After 5 mins the researchers settled and cooled down the temperature of the banana peel tea and after it was transferred to a separate container. The researchers also crushed the eggshell and pulverized using mortar and pestle until it became a powdered eggshell. In addition, to collect the ammonium nitrate, the researchers use the urine of the non-infected in any disease donor. The collection of the urine follows the ethical and safety process in which the researchers properly sanitize the bottle and the hand of the donor to avoid cross contamination. The researchers combined the banana peel tea, powdered eggshell and the urine with a small amount in volume to synthesize organic fertilizer necessary for cultivation of *Chlorella vulgaris*.

Cultivation of Chlorella vulgaris in Organic Fertilizer

Chlorella vulgaris underwent the cultivation under the organic fertilizer prepared by the researchers under 25–30 degree Celsius, with 7.0–8.5 pH level and continuous aeration using air pump to oxygenate to ensure maximum biomass yield before extraction within the 3 weeks' time frame. In the study conducted by Josephine, et al. (2022) on cultivation of *Chlorella vulgaris*, the researchers concluded that the ideal condition for increasing the biomass production was a temperature of 25°C, pH 8.0, and blue light (499–465 nm) for the best growth of *C. vulgaris*. These findings helped researchers to cultivate *Chlorella vulgaris* in 3 weeks with the viable application of BPL + urine + CaCO₃ (crash eggshell) at the best growth condition in accordance with the study by Josephine, et al. (2022).

Extraction of Chlorella vulgaris byproducts

The extraction of the *Chlorella vulgaris* byproducts including the biochemical components and the biomass underwent with several process including the initial and final boiling at 90–100 degree Celsius temperature in just an hour followed by the freeze-thaw cycle that underwent 3 repeated cycle under the freezing temperature at the – 11 to –9 degree Celsius within 5–10 hours per cycle and thawing under thawing temperature at the 60–100 degree Celsius in 30 mins in 3 repeated cycle. After the freeze-thaw cycle, the flocculation extraction was processed which was associated with flocculating *Chlorella vulgaris* using the bio flocculant property of moringa oleifera seed powders. As the researchers powdered the seed of moringa, it was mixed with the dechlorinated water and stirred within 60 minutes to extract the bio flocculant property of moringa seed. After an hour, the solution was filtered using a 25 microns filter with 500-mesh. The isolated 20 mL filtrates which contain bio flocculant properties were then mixed with the 500 mL *Chlorella vulgaris* solution (20:500). After 1 hour the settled biomass precipitate was extracted by gradual pouring and filtration to separate the liquid solution from the precipitated biomass. The collected and isolated biomass underwent dehydration and pulverization where the moist biomass was dehydrated by exposure to temperature above the boiling point of water (> 100 degree Celsius) until the biomass dried. After the biomass dehydration, the dried biomass was pulverized during mortar and pestle and then stored in a small sterile container. The sterilization of 600 mL filtrate which served as the bio-available fertilizer was exposed in heat for an hour to ensure that the solution contained zero lived foreign microorganisms. The sterile bio-available fertilizer had the final volume with 500 mL and was transferred to a sterilized container.

Sowing, Germination of Lettuce (Lactuca sativa L. var longifolia) seedling.

The sowing of lettuce seed was sown in foam plugs and the required number of seeds per foam plug were 3 and the required number of foam plugs per group was 9. The timeframe of germination of lettuce seed was 7 days and it was incubated in a rectangular plastic container which serves as an improvised incubator to maintain humidity and

temperature. The guidelines for sowing and germinating romaine seeds came from a study conducted by Lucy Stone in 2024, which outlined the optimal germination conditions, including a temperature range of 18–24°C.

Preparing the DWC hydroponic system set-up.

DWC is a modified hydroponic system with an air stone, reservoir, air pump, tubing, and floating platform (A. A., Stegemeier, et al. 2022). In addition, according to the study conducted by Rajendran, et al (2024) the DWC system included the tank or container that contains the nutrients solution and air pump. The researchers made the improvised DWC using the 9 pieces of 1.5 Liters of plastic containers which served as the nutrient tank. The researchers made 3 holes for every plastic container, enough to hold the net-pot of the foam plug. The 9 plastic containers were divided into 3 for treatment 1, treatment 2 and control group. The researchers used an air pump to oxygenate the lettuce plants required for its growth and development.

*Cultivating Lettuce (*Lactuca sativa* L. var *longifolia*) in the DWC hydroponic system.*

The researchers utilized the DWC hydroponic system method in cultivating lettuce under different treatments, ensuring that the roots directly absorbed the nutrients and water from the treatment of bio-available fertilizer and commercial chemical fertilizer. The control group absorbed water solely from the DWC hydroponic system. The water pH was in the range of 5.5–6.5 under the 22–35 degrees Celsius indirectly absorbing light and cultivated in fully shaded areas. The nutrient solution concentration in treatment 1 and treatment 2 were in the same range of 500–600 ppm to maintain its basic solution for the optimal growth of the lettuce plants per treatment. The researchers prepared the bio-available fertilizer and chemical fertilizer, however both fertilizers had a different concentration. The bio-available fertilizer had the ppm concentration of 3000 ppm while the chemical fertilizer had a 25,395 ppm therefore, the researchers use the dilution formula that equal to $= C_1V_1 + C_2V_2 = C_{Final} (V_1 + V_2)$. The calculated amount in volume of the biofertilizer was 118.0 mL and the chemical fertilizer was 12.0 mL and as the researcher aimed to achieve the 500–600 ppm, the researchers used 1,200 mL of dechlorinated water. The 1,200 mL of dechlorinated water was poured into the 9-nutrient tank and the three (3) 118 mL bio-available fertilizer were poured in the 3-nutrient tank of treatment 1 and the three (3) 12.0 mL of chemical fertilizer were poured in the 3-nutrient tank of chemical fertilizer, and the remaining 3 nutrient tanks were maintained as the solution with non-fertilizer. Treatment 1 and treatment 2 were the same 550 ppm, EC of 1.0 dS/m and 6.0 pH level while the controlled group had the ppm of 310, EC of 0.8 dS/m and 6.0 pH level. The ppm, EC and temperature were measured using the TDS and ECE meter.

The researchers transplanted the 1-week germinated lettuce seed on the improvised DWC hydroponic system, and the researchers used the plastic cups as the net pot however, the researchers were worried about the growth of the lettuce plants thereby it was replaced into a real net pot instead of an alternative one. The growth of the lettuce was observed week by week from week 0 - week 5 by the researchers under the uncontrolled environment.

Morphological Parameters

ensure that the lettuce plant (*Lactuca sativa* L. var. *longifolia*.) treated with the bio-available fertilizer, commercial chemical fertilizer, and controlled from non-fertilizer were being monitored throughout the cultivation timeframe, the researcher observed and measured the following parameters including the shoot length (cm), root length (cm), leaf Area (cm²), number of leaves per plant, and number of surviving plant per group. The researchers measured these parameters every week within 0–5, which the gathered data was utilized to statistically analyze the differences among the treatments.

Statistical Analysis

The researchers employed Two-Way Repeated Measures ANOVA, Tukey Post Hoc Test, and Descriptive Statistics to analyze plant growth parameters (e.g., shoot/root length, leaf area, number of leaves) across treatments and time points. These methods were chosen for their suitability in evaluating repeated measurements and treatment effectiveness, with the Tukey test used when assumptions were met, and descriptive statistics applied if assumptions were violated. All statistical analyses were conducted using Jamovi software (version 2.6.44), a free and user-friendly tool based on the R language.

III. RESULTS AND DISCUSSION

1. Statistical findings morphological parameters of lettuce plant treated in bio-available fertilizer, chemical fertilizer and the non-fertilizer measured repeatedly time to time (0-5 weeks).

A. *The Length of shoots of the lettuce plant(cm).*

The research showed that bio-available fertilizer markedly improved the shoot length of lettuce plants over a six-week period compared to chemical fertilizer and the control group. Repeated measures ANOVA (**table 1**) demonstrated a statistically significant impact of time and treatment on shoot length ($p < .001$), with the biofertilizer treatment consistently producing the highest average shoot lengths throughout all week where the assumption of sphericity was satisfied, Post hoc analysis utilizing Tukey HSD (**Table 2**) revealed that plants treated with biofertilizer exhibited significantly longer shoots compared to those given chemical fertilizer (mean difference = 1.147 cm, $p < .001$) and those without fertilizer (mean difference = 0.711 cm, $p = .003$). Chemical fertilizer resulted in noticeably longer shoots compared to the non-fertilizer group (mean difference = 0.436 cm, $p = .026$). These findings validated that biofertilizer was the most successful method in enhancing shoot growth within the DWC hydroponic system. Microalgae application caused a significant increase in the shoot height values compared to control microalgae application (Turhan and Sensoy 2022). Also, the recent study where the application of *Chlorella vulgaris* offers a contribution to the growth of the shoot length of pepper plant (Tian et al. 2022).

The **Fig. 1** depicts the changes in shoot length over a span of six weeks for the three treatments. Every line signifies a specific treatment, with points displaying the average weekly shoot length and vertical error bars illustrating the 95% confidence intervals. The biofertilizer line climbs sharply, demonstrating the most significant rise in shoot length as time progresses. Chemical Fertilizer exhibits a steadier upward trend, whereas the non-fertilizer treatment displays the slowest growth. The spacing between the lines visually enhances the table's information, showing that the treatments influenced plant growth differently, with biofertilizer resulting in the most notable enhancement.

B. *The Length of roots of the lettuce plant (cm).*

The results of the research indicated that root length markedly grew over the six-week span in all treatment groups, with the biofertilizer group exhibiting the greatest increase. A two-way repeated measures ANOVA (**table 1**) verified a substantial main effect of time on root length ($F = 463.6$, $p < .001$, $\eta^2 = .987$), a notable main effect of treatment ($F = 56.7$, $p < .001$, $\eta^2 = .95$), and a significant interaction between time and treatment ($F = 14.8$, $p < .001$, $\eta^2 = .831$), suggesting that the root growth pattern differed based on the fertilizer type applied. Post hoc Tukey analyses (**table 2**) indicated that both biofertilizer and a lack of fertilizer led to notably longer root lengths compared to chemical fertilizer (mean differences = 1.553 and 1.333 cm, respectively; both $p < .001$), whereas no significant difference was observed between the biofertilizer and no-fertilizer groups ($p = .403$). These findings indicated that chemical fertilizer was the least effective in enhancing root growth, while biofertilizer proved to be more advantageous, although not significantly superior to having no fertilizer at all. In general, the research underscores the restricted effectiveness of chemical fertilizers and the promise of biofertilizers as a more efficient and sustainable option for promoting root

growth. This finding was similar to the trend found by the study of Dai, L., et al (2020) which the root length of the lettuce plant treated under the supernatant *C. vulgaris* were significantly higher as compared to the root length of the lettuce treated in the control group which validated using one-way ANOVA ($p < 0.05$).

The **Fig. 2** illustrated the data in the table and shows how each treatment's root length varies from *Week 0* to *Week 5*. The error bars displayed the confidence intervals for the mean, and each line denoted a treatment group. With the steepest increasing trend, the biofertilizer group (blue line) appeared to have had the largest increase in root length over time. The trend of the non-fertilizer group (orange line) was similar but a little less sharp. Compared to the other two, the Chemical Fertilizer group (gray line) had the least rise and a much flatter slope. With time, the distance between the lines widens, showing clear differences in the effectiveness of the treatments, with biofertilizers being the most effective at encouraging the growth of roots.

C. The total length of plant [roots + shoots] of the lettuce plant (cm).

The research revealed that plant height considerably grew over time and differed by treatment method, with biofertilizer consistently yielding the highest growth. A two-way repeated measures ANOVA (**table 1**) showed a significant main effect of time on overall plant length ($F = 1176.2$, $p < .001$, $\eta^2 = .995$), demonstrating steady plant growth throughout the weeks. A notable interaction between time and treatment was observed ($F = 35.2$, $p < .001$, $\eta^2 = .922$), indicating that the growth pattern varied based on the treatment type used. The primary impact of the treatment was significant as well ($F = 79.3$, $p < .001$, $\eta^2 = .964$), validating that the fertilizer type had a notable influence on plant height. Post hoc Tukey tests (**table 2**) showed that plants receiving biofertilizer were notably taller than those in the chemical fertilizer group (mean difference = 3.04, $p < .001$) and the non-fertilizer group (mean difference = 1.06, $p = .012$). These results confirm the greater efficacy of biofertilizer in enhancing plant growth during the six-week duration.

The **fig. 3** illustrated the growth pattern of plants for each treatment over time. Clearly, when compared to alternative treatments, biofertilizer leads to the quickest and most consistent rise in over several weeks, exhibiting a sharper incline. The chemical fertilizer group showed moderate growth, while the non-fertilizer group experienced slower growth, as suggested by the least steep curve. Despite the variability indicated by the error bars at each point, the consistent separation of the lines from week three onward shows that the effectiveness of the therapies changes over time.

D. Leaf area of the lettuce plant (cm²).

The analysis showed that leaf area grew significantly over time in all treatments, with biofertilizer resulting in the most substantial and steady growth. A two-way repeated measures ANOVA (**table 1**) revealed a significant main effect of time ($F = 1575.9$, $p < .001$, $\eta^2 = .996$), suggesting considerable temporal growth in leaf area. A notable interaction between time and treatment was observed ($F = 17.2$, $p < .001$, $\eta^2 = .851$), indicating that the rate of leaf area growth differed by treatment. Moreover, a notable main effect of treatment was found ($F = 36.2$, $p < .001$, $\eta^2 = .924$), indicating that the type of treatment significantly affected total leaf area. Post hoc comparisons (**Table 2**) indicated that biofertilizer significantly surpassed chemical fertilizer (mean difference = 0.358, $p < .001$), whereas the difference between biofertilizer and non-fertilizer was not statistically meaningful ($p = .105$). Nonetheless, chemical fertilizer led to a notably smaller leaf area compared to no fertilizer (mean difference = -0.251, $p = .003$), suggesting possible adverse effects. These results confirmed that biofertilizer was the most efficient method for improving leaf area and raised doubts regarding the effectiveness of chemical fertilizer. This finding is similar to the present study in which the application of 15% concentration of *C. vulgaris* extract contributed to the lettuce plant production which suggests that the extract may have a benefit on leaf area among growth parameters (Ammaturo, C., et al. 2023).

The trend in **fig. 4** over the course of six weeks for each treatment type is graphically shown by the line graph. The leaf area increased most sharply and steadily with the Biofertilizer treatment (blue line), suggesting a significant beneficial impact. Overall, the increase of the Chemical Fertilizer (gray line) was the slowest. In the early weeks, the biofertilizer performs marginally better than the non-fertilizer treatment (yellow line), which performed better than the chemical fertilizer. Although error bars show more variability in the non-fertilizer group, by week five, both the biofertilizer and non-fertilizer groups converge close to the same leaf area value. Overall, the graph confirmed the table's data and showed that biofertilizer was the best treatment for expanding leaf area.

E. *Number of leaves per lettuce plant.*

The two-way repeated measures ANOVA (**Table 1**) indicated substantial effects of time, treatment, and their interaction on the leaf count per plant (NoL). Time exerted a significant impact ($F \approx 3.27 \times 10^{32}$, $p < .001$, $\eta^2 = 0.95-1$), indicating a notable rise in leaf quantity over a period of six weeks. The interaction effect ($F \approx 5.79 \times 10^{30}$, $p < .001$) indicated that growth patterns differed among treatments, with biofertilizer resulting in the most leaf counts, then the non-fertilizer group, whereas chemical fertilizer exhibited the least growth. Analysis between subjects ($F \approx 2.22 \times 10^{31}$, $p < .001$) indicated a notable treatment effect, although the minimal effect size ($\eta^2 = 0.017$) implies that time was the main factor influencing variation. Nevertheless, assumption evaluations highlight issues: Levene's Test showed variance breaches at Weeks 2 and 4, and multiple tests produced NaN results, indicating possible data problems. The Q-Q plot also indicated departures from normality. Descriptive statistics revealed no variation within the group ($SD = 0$), suggesting extreme consistency that may arise from a limited sample size or rounding, thereby violating ANOVA assumptions. Although the outcomes support biofertilizer use, the results should be viewed carefully because of assumption violations and restricted variability; therefore, additional studies with better design and larger sample sizes are suggested. The findings were opposite to the present study conducted by Vinzon, J. D. C., et al (2023) demonstrating that microalgal treatment using *Chlorella* species *Sorokiniana* has a positive effect on leaf morphometry and leaf counts of *B. rapa chinensis*. *Chlorella* extracts post-treatment, not only the leaf area of the pepper plants increased but also the number of leaves per plant (Tian et al. 2022).

Since assumption tests indicated breaches in variance homogeneity, the lack of sphericity checks, and normality deviations (evident in the Q-Q plot), these results require careful interpretation. Biofertilizer notably improved shoot growth in comparison to chemical fertilizer and no-fertilizer treatments, displaying the greatest average shoot lengths over time. Nonetheless, noted breaches of statistical assumptions like non-normal distribution and unequal variances suggested that the findings need to be regarded carefully. To confirm the strength of these results, it is recommended to perform alternative analyses, like descriptive statistics for overview of results.

TABLE 1 Summary of Two-Way Repeated Measures ANOVA on shoot length, root length, total length of plant, leaf area and no. of leaves per plant in within subjects' effects									
Morphological Parameters		Sum of Squares	df	Mean Square	F	p	η^2_G	η^2	η^2_p
Shoot Length	Week	169.952	5	33.9903	1911.2	<.001	0.992	0.904	0.997
	Week $\times$ Treatment	4.711	10	0.4711	26.5	<.001	0.781	0.025	0.898
	Residual	0.534	30	0.0178					
Root Length	Week	61.496	5	12.2992	463.6	<.001	0.966	0.661	0.987
	Week $\times$ Treatment	3.924	10	0.3924	14.8	<.001	0.647	0.042	0.831
	Residual	0.796	30	0.0265					
Total Length of Plant	Week	400.13	5	80.0258	1176.2	<.001	0.987	0.777	0.995
	Week $\times$ Treatment	23.97	10	2.397	35.2	<.001	0.819	0.047	0.922
	Residual	2.04	30	0.068					
Leaf Area	Week	21.3301	5	4.26602	1575.9	<.001	0.992	0.92	0.996
	Week $\times$ Treatment	0.4654	10	0.04654	17.2	<.001	0.719	0.02	0.851
	Residual	0.0812	30	0.00271					
Number of leaves per plant	Week	75.33	5	15.067	3.27E+32	<.001	1	0.95	1
	Week $\times$ Treatment	2.67	10	0.267	5.79E+30	<.001	1	0.034	1
	Residual	1.38E-30	30	4.61E-32					

Since assumption tests indicated breaches in variance homogeneity, the lack of sphericity checks, and normality deviations (evident in the Q-Q plot), these results require careful interpretation. Biofertilizer notably improved shoot growth in comparison to chemical fertilizer and no-fertilizer treatments, displaying the greatest average shoot lengths over time. Nonetheless, noted breaches of statistical assumptions like non-normal distribution and unequal variances suggested that the findings need to be regarded carefully. To confirm the strength of these results, it is recommended to perform alternative analyses, like descriptive statistics for overview of results.

TABLE 2 Tukey Post Hoc Test for Differences in shoot length, root length, total length of plant, and leaf area Between Treatments								
Comparison								
Morphological Parameters	Treatment		Treatment	Mean Difference	SE	df	t	P _{Tukey}
Shoot Length	Biofertilizer	-	Chemical Fertilizer	1.147	0.121	6	9.51	<.001
		-	Non-fertilizer	0.711	0.121	6	5.9	0.003
	Chemical Fertilizer	-	Non-fertilizer	-0.436	0.121	6	-3.61	0.026
Root Length	Biofertilizer	-	Chemical Fertilizer	1.553	0.158	6	9.84	<.001
		-	Non-fertilizer	0.219	0.158	6	1.39	0.403
	Chemical Fertilizer	-	Non-fertilizer	-1.333	0.158	6	-8.45	<.001
Total Length of Plant	Biofertilizer	-	Chemical Fertilizer	3.04	0.245	6	12.41	<.001
		-	Non-fertilizer	1.06	0.245	6	4.33	0.012
	Chemical Fertilizer	-	Non-fertilizer	-1.98	0.245	6	-8.08	<.001
Leaf Area	Biofertilizer	-	Chemical Fertilizer	0.358	0.0432	6	8.29	<.001
		-	Non-fertilizer	0.107	0.0432	6	2.48	0.105
	Chemical Fertilizer	-	Non-fertilizer	-0.251	0.0432	6	-5.81	0.003

2. Statistical findings in survivability of the lettuce plants under different treatments including bio-available fertilizer, chemical fertilizer and non-fertilizer in DWC hydroponics system during dry season in the Philippines.

The summary of descriptive statistics in **fig. 6** presented showed distinct variations in plant survival among treatment groups from Week 0 to Week 5. All treatments started with complete survival (mean = 9) during Weeks 0 and 1, but variations appeared by Week 2. The bio-available fertilizer group exhibited the most consistent survival, experiencing a minor drop to 7 in Week 2 and leveling off at 6 until Week 5. The non-fertilizer group exhibited a comparable trend but declined more steeply to 6 by Week 2 and stayed at that level. In opposition, the group treated with chemical fertilizer exhibited a sharp and ongoing decrease, dropping to only 1 surviving plant by Week 5. Because of breaches in statistical assumptions (such as singularity and unsuccessful Q-Q plot creation), inferential analysis could not be conducted, rendering descriptive statistics the most trustworthy method. In general, the bio-available fertilizer showed the greatest promise for promoting long-term plant survival in hydroponic environments, whereas chemical fertilizer seemed harmful.

This finding was similar to the present study in cyanobacteria and microalgae used as foliar sprays can enhance plant growth and development, while also offering systemic immune resistance against biotic and abiotic stresses (Parma, P., et al. 2023). Even this recent study is some kind of difference, but this tells that the microalgae offer systemic immune resistance against abiotic and biotic stresses as what the lettuce plant in the hydroponic system survived

under the treatment of bio-available fertilizer from byproducts of *C. vulgaris*. While the study recent study of effect of microalgae *Chlorella vulgaris* on hydroponically grown lettuce focused on yield and quality, it also noted that the use of *C. vulgaris* contributed to healthier plant growth, suggesting potential benefits for plant survivability (Ergun, O., et al. (2020).

In **Fig. 6** the initial weeks (Week 0 and Week 1), all treatments support complete plant survival, averaging 9 plants. Starting from Week 2, variations start to appear. The chemical fertilizer group exhibits a drastic and ongoing decrease in plant survival, reducing to only 1 surviving plant by week 5. In comparison, the con-fertilizer and bio-available fertilizer categories exhibited a slight decrease initially, then maintained a mean of 6 plants from Week 3 to Week 5. This trend indicated that chemical fertilizer could harm plant survival as time passes, whereas bio-available and no-fertilizer options are more beneficial for sustaining long-term plant health.

3. Most Effective Treatment based on the statistical analysis in morphological parameters of lettuce plant growth throughout the 0-5 weeks.

According to the statistical evaluation of morphological factors (shoot length, root length, leaf count per plant, leaf area and plant survival) from Weeks 0 to 5, bio-available fertilizer proved to be the most effective method for promoting lettuce growth. It regularly resulted in the longest and most consistent shoot lengths, the highest leaf count, and the most reliable plant survival over time. Two-way repeated measures ANOVA indicated significant impacts of time and treatment on leaf count, with biofertilizer exceeding both chemical and non-fertilizer groups. Descriptive statistics also reinforced its exceptional capability in enhancing both growth and survival.

In comparison, chemical fertilizer produced the least encouraging outcomes, resulting in slower growth and a significant drop in plant survival. Similar to the present study in which the *Chlorella vulgaris* usage can reduce the application of artificial chemical fertilizers, which deteriorate soil quality (Turhan and Sensoy 2022). Similar study also found in the effectiveness of green microalgae as bio stimulants and biofertilizer through foliar spray and soil drench method for tomato cultivation which the findings was the *C. vulgaris* can serve as an alternative to chemical fertilizers in organic tomato cultivation (Suchithra, M. R., et al. 2021).

4. Bio-availability of *Chlorella vulgaris*' byproducts (nutritional/biochemical composition and dried biomass) as fertilizer in promoting growth for DWC hydroponic system based upon the statistical result.

According to the statistical findings, the bio-available byproducts of *Chlorella vulgaris* (nutritional makeup and dried biomass) proved to be the most efficient fertilizer application in the DWC hydroponic setup. The two-way repeated measures ANOVA indicated that the biofertilizer group exhibited considerably greater shoot length, root length, leaf area, leaf count and plant survival than the chemical and non-fertilizer groups, with robust main effects for treatment and time, along with a significant interaction between the two. Descriptive statistics additionally reinforced these results, as the biofertilizer group recorded the greatest average values in growth parameters and ensured steady plant survival from Weeks 3 to 5. Conversely, the group receiving chemical fertilizer exhibited the least growth and the sharpest decrease in survival, falling from 9 to 1 plant by Week 5.

The data showed that *C. vulgaris* byproducts provide enhanced nutrient availability, fostering both quick vegetative growth and prolonged plant viability, thereby verifying their efficacy as a biofertilizer in hydroponic farming. The similar trend was found to the present study *Chlorella vulgaris* extract acts as a bio stimulant for the growth and development of pepper seedlings. They found that *Chlorella vulgaris* extract can increase growth parameters of the pepper plant, including plant height, leaf area, and stem diameter. (Tian et al. 2022). A similar study also found that the *C. vulgaris* can be considered a biofertilizer that encourages plant growth and nutrient uptake, partially replacing

chemical fertilizers (Turhan and Sensoy. 2022). Also similar to the recent study in which the *Chlorella vulgaris* mixed with cow dung showed increased plant growth parameters, including plant height, number of stem branches, leaves, and root length (Suchithra, M. R., et al. 2021).

IV. CONCLUSION

The *Chlorella vulgaris*' byproduct as bio-available fertilizer greatly improved the growth parameters, including shoot length, root length, leaf area, and survival rates of lettuce plants grown in a Deep-Water Culture hydroponic system during dry hot season in the Philippines. However, the data of number of leaves per plant statistically violated the assumptions of homogeneity in 2-way repeated measures ANOVA with 5% margin of error which suggested a further validation. The result of statistical analysis in morphological parameters of lettuce plant, the bio-available fertilizer surpassed the chemical and non-fertilizer. The evaluations employing repeated measures of ANOVA with 5% margin of error validated significant impacts from both time and treatment, with biofertilizer treatments demonstrating more favorable results. Regarding the plant survivability under the uncontrolled environment, the bio-available fertilizer group sustained the highest survival rates over the six-week duration, whereas the chemical fertilizer group showed a significant decrease. These findings confirmed the efficacy of *Chlorella vulgaris* byproducts as a sustainable and efficient fertilizer choice, fostering robust growth and enhanced resilience in hydroponic lettuce farming during adverse climatic conditions. This result was in favor of the result of the study conducted by Tian et al. (2022), in which the pepper plant was treated with *Chlorella vulgaris* extract, studies conducted by La bella, et al. (2021) and Dineshkumar, R., et al. (2021) which investigated the effect of lettuce and tomato treated in biofertilizer derived from *Chlorella vulgaris* where they all concluded that *Chlorella vulgaris* either the extracts/byproduct or *C. vulgaris* itself has the potential to become sustainable growth stimulant and bio-available fertilizer.

However, despite the result in statistical analysis, the overall growth of the lettuce plant over the 6-week observation and measurement period under the DWC hydroponics system in the uncontrolled environment was still slow as compared to other studies because the highest length of shoot only exhibited 8.6 cm in week 5 (35 days after transplanting) of cultivation. In contrast, in the study conducted by Frasetya et al. (2019), the highest plant height size obtained was 22.73 cm when treated under a controlled environment such as a greenhouse. This made the researchers conclude that the growth of the lettuce plant under a hydroponic system depends not only on the nutrient solution but also on the temperature, humidity, biotic and abiotic factors surrounding the lettuce plants in the DWC hydroponics system.

V. RECOMMENDATIONS

As the researchers face a lot of challenges during the investigation, the researchers provide suggestions and recommendations for future researchers, and these will help the researchers to fill in the research gap. The suggestions and recommendations are the following including the additional investigation of the use of bio-available fertilizers in controlled-environment agriculture, conducting the research study during the transitioning to rainy season, choose appropriate plant subjects that can survive and grow properly under the uncontrolled environment, further evaluation of the effectiveness of the bio-available fertilizer derived from byproducts of *Chlorella vulgaris* by analyzing its nutritional and biochemical composition, utilize the microalgae from Laguna de Bay as a bio-available fertilizer for hydroponic systems, and investigating the additional benefits of *Chlorella vulgaris* byproducts as fertilizer in soil-based cultivation of other vegetable plants under tropical conditions in the Philippines.

Declarations

VI. ACKNOWLEDGEMENT

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VIII. COMPETING INTEREST

The author(s) declare no competing interests.

References

1. Tality, D., Serevo, A. J., Alipio, A. L., & Rosete, M. A. (2022). Cost-Benefit Analysis of Soilless Cultivation System in Tagaytay City, Philippines. *International Journal of Social and Management Studies*, 3(2), 140–156. <https://doi.org/10.5555/ijosmas.v3i2.137>
2. Khatri, L., Kunwar, A., & Bist, D. R. (2024). Hydroponics: Advantages and challenges in soilless farming. *Big Data in Agriculture*, 6(2), 81–88. <https://doi.org/10.26480/bda.02.2024.81.88>
3. Sarmila, K. C. (2024, February 9). *Chemical fertilizers: Impact on plant growth and environmental sustainability*. The Science Notes. <https://thesciencenotes.com/chemical-fertilizers-impacts-plant-growth-environmental-sustainability/>
4. Tian, S. L., Khan, A., Zheng, W. N., Song, L., Liu, J. H., Wang, X. Q., & Li, L. (2022). Effects of Chlorella extracts on growth of Capsicum annuum L. seedlings. *Scientific Reports*, 12, 15455. <https://doi.org/10.1038/s41598-022-19846-6> (Effects of Chlorella extracts on growth of Capsicum annuum L. seedlings - PMC)
5. La Bella, E., Baglieri, A., Rovetto, E. I., Stevanato, P., & Puglisi, I. (2021). Foliar spray application of *Chlorella vulgaris* extract: Effect on the growth of lettuce seedlings. *Agronomy*, 11(2), 308. <https://doi.org/10.3390/agronomy11020308> (Foliar Spray Application of Chlorella vulgaris Extract: Effect on the Growth of Lettuce Seedlings)
6. Safi, C., Zebib, B., Merah, O., Pontalier, P.-Y., & Vaca-Garcia, C. (2024). Chlorella vulgaris is rich in essential nutrients, including vitamins, minerals, and pigments, making it a valuable resource for developing bioavailable fertilizers. *Algal Research*, 68, 102774. <https://doi.org/10.1016/j.algal.2023.102774>
7. Bumandalai, O., & Tserennadid, R. (2019). Effect of Chlorella vulgaris as a biofertilizer on germination of tomato and cucumber seeds. *International Journal of Aquatic Biology*, 7(2), 95–99. <https://doi.org/10.22034/ijab.v7i2.582>
8. Syamsia, S. (2024). The potential of liquid organic fertilizer: A systematic literature review. *Journal of Agriculture*, 3(1). <https://doi.org/10.47709/joa.v3i01.3720>
9. Gonçalves, A., Lopes, D. A., Silva, J. C., & Oliveira, M. B. P. P. (2023). The high cost of microalgae biomass production and metabolite extraction poses challenges; however, alternative methods like heat treatment and

flocculation may offer cost-effective solutions. *Renewable and Sustainable Energy Reviews*, 171, 112957. <https://doi.org/10.1016/j.rser.2023.1129>

10. Wichaphian, A., Sriket, N., Sensupa, S., Pekkoh, J., Pathom-Aree, W., Chromkaew, Y., Suwannarach, N., Kumla, J., Cheirsilp, B., & Srinuanpan, S. (2023). Value-added green biorefinery co-products from ultrasonically assisted DES-pretreated *Chlorella* biomass. *Ultrasonics Sonochemistry*, 100, 106628. <https://doi.org/10.1016/j.ultsonch.2023.106628>
11. Josephine, A., Kumar, T. S., Surendran, B., Rajakumar, S., Kirubakaran, R., & Dharani, G. (2022). Evaluating the effect of various environmental factors on the growth of the marine microalgae *Chlorella vulgaris*. *Frontiers in Marine Science*, 9, 954622. <https://doi.org/10.3389/fmars.2022.954622>
12. Stegelmeier, A. A., Rose, D. M., Joris, B. R., & Glick, B. R. (2022). The use of PGPB to promote plant hydroponic growth. *Plants*, 11(20), 2783. <https://doi.org/10.3390/plants1120278>
13. Rajendran, S., Domalachenpa, T., Arora, H., Li, P., Sharma, A., & Rajauria, G. (2024). Hydroponics: Exploring innovative sustainable technologies and applications across crop production, with Emphasis on potato mini-tuber cultivation. *Heliyon*, 10(5), Article e26823. <https://doi.org/10.1016/j.heliyon.2024.e26823>
14. Turhan, E., & Sensoy, S. (2022). Utilization of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] on plant growth and nutrient uptake of garden cress (*Lepidium sativum* L.) grown in different fertilizer applications. *International Journal of Agriculture, Environment and Food Sciences*, 6(2), 240–245. <https://doi.org/10.31015/jaefs.2022.2.6>
15. Dai, L., Yu, P., Ma, P., Chen, C., Ma, J., Zhang, J., Huang, B., Xin, Z., Zheng, X., & Tang, T. (2024). Effects of the supernatant of *Chlorella vulgaris* cultivated under different culture modes on lettuce (*Lactuca sativa* L.) growth. *Frontiers in Nutrition*, 11, 1437374. <https://doi.org/10.3389/fnut.2024.1437374>
16. Ammaturo, C., Pacheco, D., Cotas, J., Formisano, L., Ciriello, M., Pereira, L., & Bahcevandziev, K. (2023). Use of *Chlorella vulgaris* and *Ulva lactuca* as Biostimulant on Lettuce. *Applied Sciences*, 13(16), 9046. <https://doi.org/10.3390/app13169046>
17. Vinzon, R., Manliclic, A. D. C., Corpuz, M. N. C., & Gigante, E. J. V. (2020). Green microalgae, *Chlorella sorokiniana* promotes the growth of Chinese cabbage (*Brassica rapa chinensis* L. Hanelt). *International Journal of Advanced Research*, 8(1), 3273–3280. https://www.journalijar.com/uploads/334_IJAR-29680.pdf
18. Ergun, O., Dasgan, H. Y., & Isik, O. (2020). Effects of microalgae *Chlorella vulgaris* on hydroponically grown lettuce. In XXX International Horticultural Congress IHC 2018: II International Symposium on Soilless Culture and VIII International Symposium on Seed, Transplant and Stand Establishment of Horticultural Crops (*Acta Horticulturae* No. 1273, pp. 165–170). International Society for Horticultural Science. <https://doi.org/10.17660/ActaHortic.2020.1273.23>
19. Suchithra, M. R., Ramesh, K., & Pradeep, S. (2021). Application of *Chlorella vulgaris* as a biofertilizer and biostimulant enhances tomato cultivation through foliar spray and soil drench methods. *South African Journal of Botany*, 146, 740–750. <https://doi.org/10.1016/j.sajb.2021.12.022>
20. Dineshkumar, R., Sharmila Devi, N., Priya Lakshmi, V., Ahamed Rasheeq, A., Arumugam, A., & Sampathkumar, P. (2021). Biofertilizer and biostimulant properties of the green microalgae *Chlorella vulgaris* on tomato (*Lycopersicon esculentum* Mill L.). *European Journal of Experimental Biology*, 11(5), 1–6. <https://link.springer.com/article/10.1007/s10811-015-0625-2>
21. Frasetya, B., Qurrohman, T., Taofik, A., & Sholehah, M. (2019, December). The evaluation of various nutrient formulation on the growth of lettuce (*Lactuca sativa* var. Arista) in hydroponic raft system at tropic region. *Journal of Physics: Conference Series*, 1402(3), 033025. <https://doi.org/10.1088/1742-6596/1402/3/033025>

22. Gonzaga, N. R., Pepito, S. L. A., Octavio, R. P., Gonzaga, A. B., & Rogers, G. (2017). Growth and yield performance of lettuce (*Lactuca sativa* L.) under protected and conventional cultivation. *Annals of Tropical Research*, 39(Supplement B), 137–143.<https://doi.org/10.32945/atr39sb11.2017>

Figures

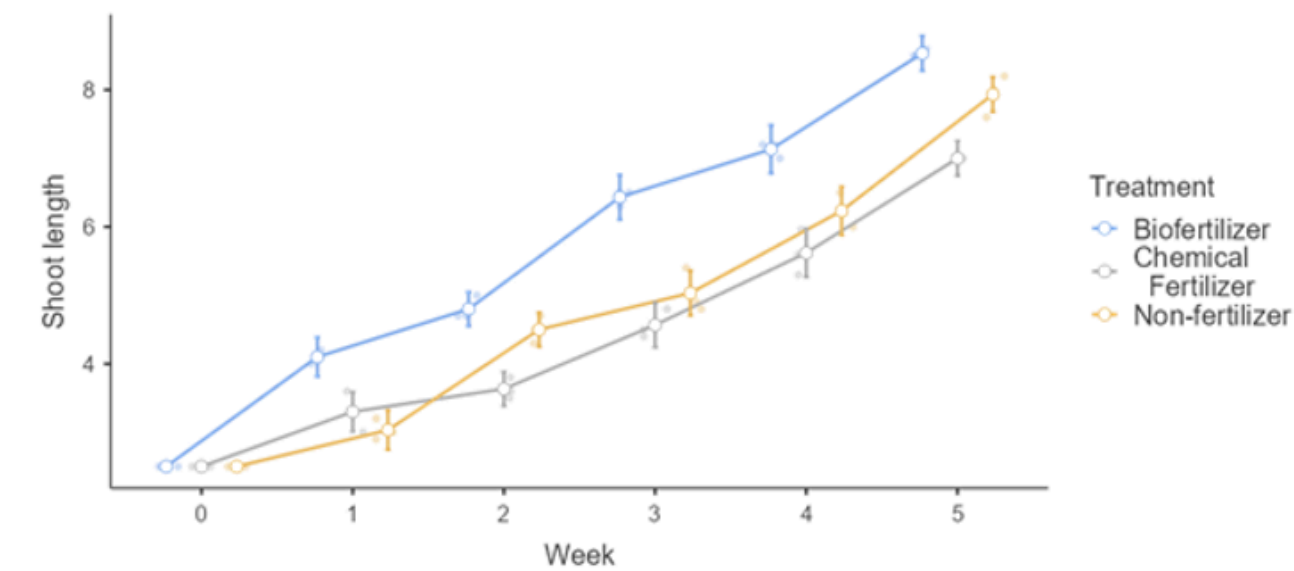


Figure 1
Interaction between Treatment and Time (Week) on Shoot Length

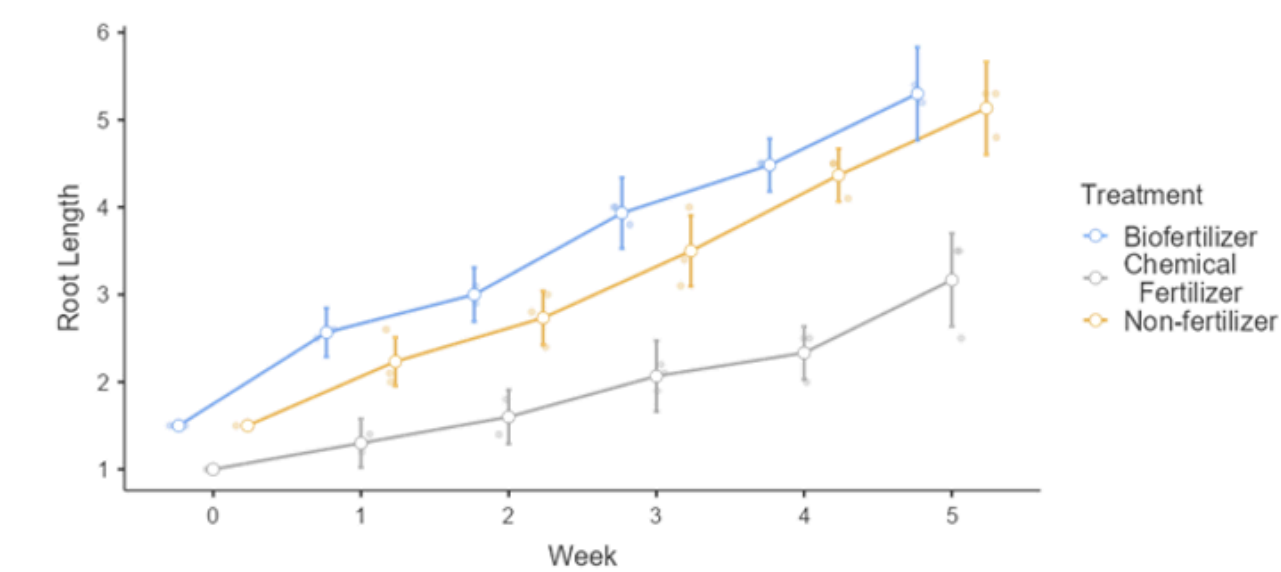


Figure 2
Interaction between Treatment and Time (Week) on root Length

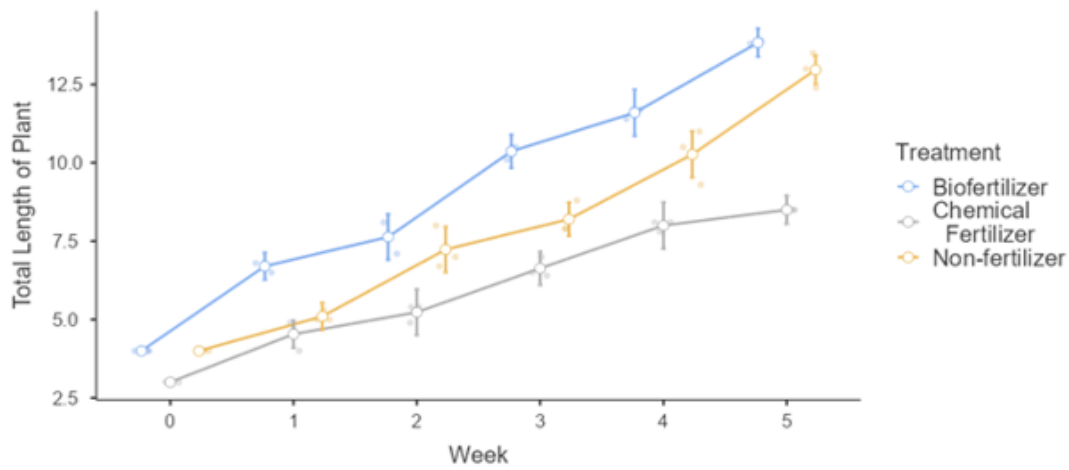


Figure 3

Interaction between Treatment and Time (Week Total Length of plant.

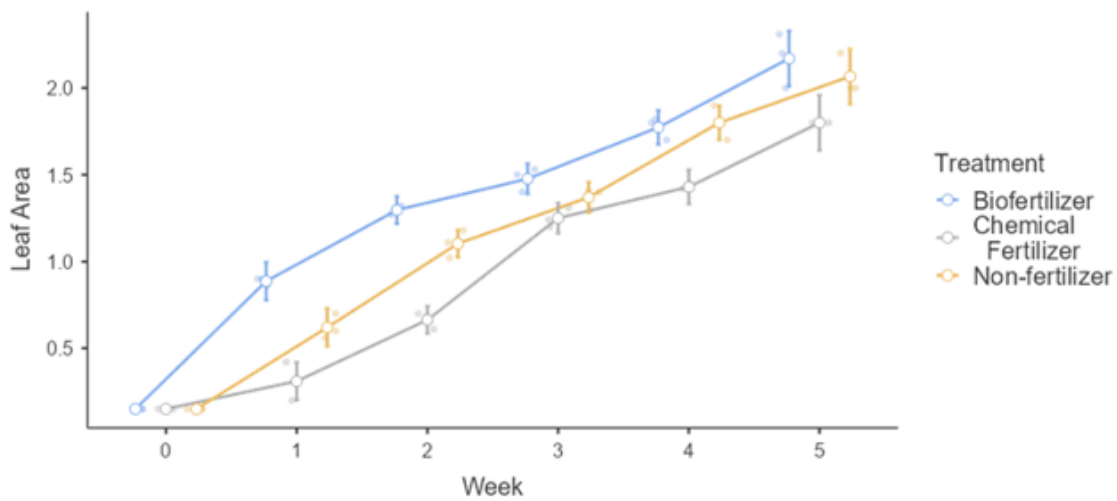


Figure 4

Interaction between Treatment and Time (Week) on leaf area

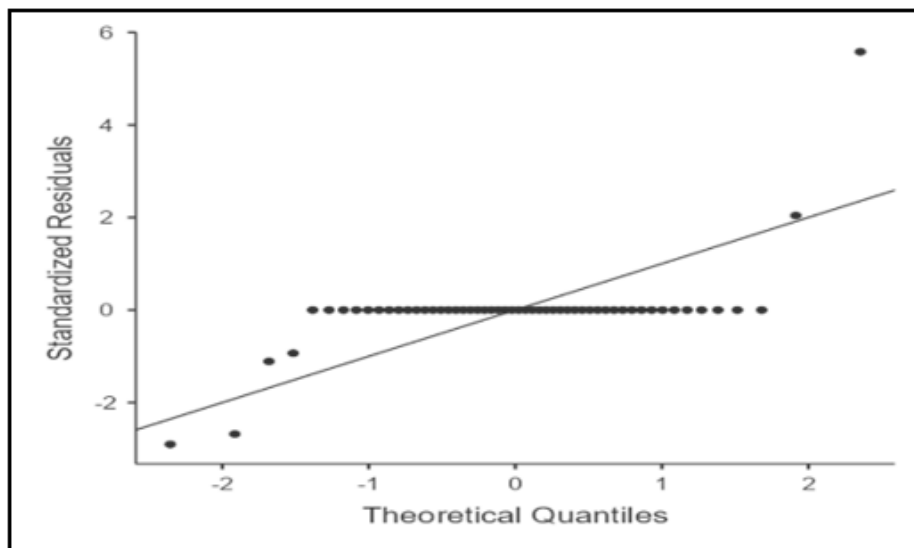


Figure 5

Q-Q Plot of Standardized Residuals for Normality Assessment (Number of Leaves per Plant (NoL))

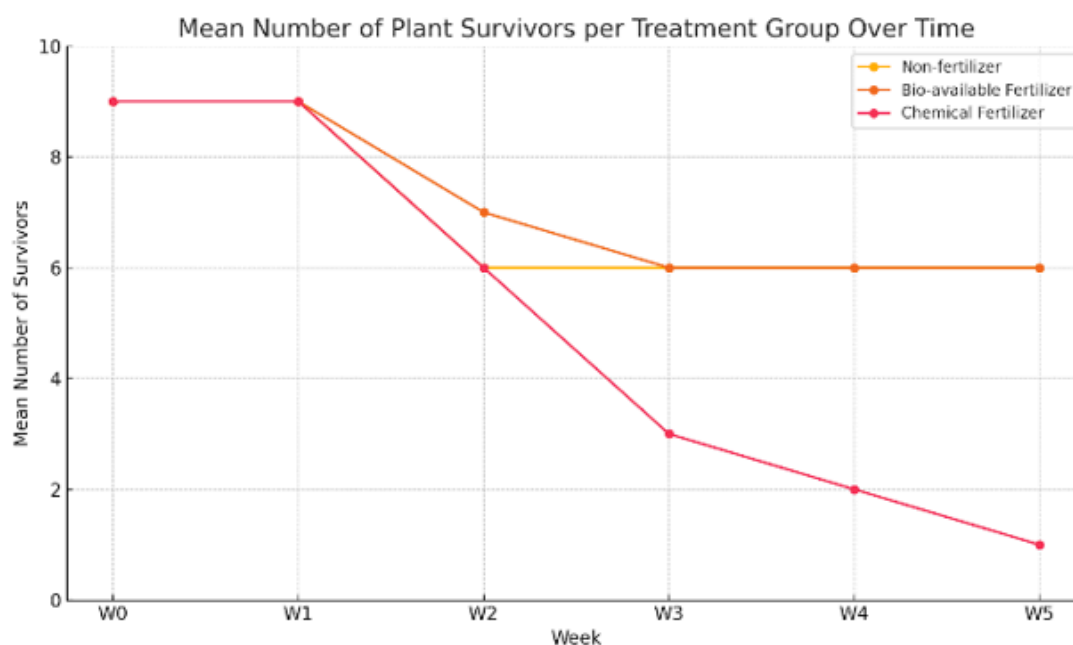


Figure 6

Mean Number of Plant Survivors per Treatment Group from Week 0 to Week 5

Supplementary Files

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