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The Comparative Performance of Plug Preparation Using Different Fertilizer Sources and Concentrations

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Abstract

Plugs are crucial for initiating crop production in greenhouses, soil, and controlled environment agriculture (CEA). Vegetable, fruiting, ornamental, and other horticultural crops that utilize plugs for production have demonstrated superior transplant establishment rate, plant health, and total yield. The APS Laboratory for Sustainable Food at Florida Gulf Coast University investigated the quality of plugs grown based on different concentrations and fertigation sources using synthetic and organic sources. We carried out the growth of "Rex Butterhead" Lettuce (*Latuca sativa*) plugs with five different fertigation treatments, 1) full-strength synthetic starter fertilizer solution; 2) half-strength synthetic starter fertilizer solution; 3) full-strength organic starter fertilizer solution; 4) half-strength organic starter fertilizer solution; 5) no fertilizer for control. Fertilizer treatments were formulated following manufacturer recommendations. The seeds were sown in Oasis® Horticubes and saturated every day with the different fertilizer treatments. The plugs were cultivated for 15 days in a controlled environment until two leaves after the cotyledons had developed. After 15 days, we collected data which included wet weight (g), dry weight (g), leaf area (cm^2), and chlorophyll concentration (mg/cm^2). In addition, we derived data including the Leaf Area Index (LAI, cm^2/cm^2) and Specific Leaf Area (SLA, cm^2/g). Descriptive statistics were used to describe the biomass data. A Tukey's HSD test was carried out to understand the differences between the fertilizer sources. We determined there was a statistically significant difference (P = 7.34E−29) in the measured plug growth

parameters due to the various fertigation sources. We found that all fertilizer treatments produced viable plugs except for the control treatment. Of all the treatments, we concluded the half-strength organic treatment produced the more vigorous plugs with the greatest wet weight (g) and largest total leaf area (cm^2) which was statistically significantly different. Results from this study may inform growers about appropriate fertilizer options for plug production.

Keywords

Controlled Environments, Fertigation, Lettuce, Plugs, Urban Agriculture

1. Introduction

A plug is a plant in the regenerative (immature) stage of the plant life cycle [1]. The growth period between the planted seed and developed plug will determine the overall productivity and health of the matured plant, making the plug development stage critical in plant cultivation [2]. Healthy and optimally sized plugs are needed to produce ornamental, fruiting, or vegetable crops. When compared to direct seeding, vegetable plug transplants are a relatively new phenomenon that first appeared in the 1960s under the name "containerized transplants" [3]. The advantages of plug transplants are that each plant is grown in individual cells, making production and transplantation easier, and the roots are never disturbed in the cultivation process since each plant is containerized [3].

Utilizing plant plugs in crop production offers numerous advantages, which include: 1) a greater quality and consistency of crop as commonly grown in controlled environments; 2) greater control of transplanting dates; 3) mechanical transplanting opportunities; 4) improved water management for transplant establishment in comparison to fresh bare root transplants [4]. Plugs also require less time to grow than the field-produced bare root transplant counterparts and are not exposed to soilborne pathogens in their development phase from planted seeds to established seedlings [4]. Leafy bare-root transplants require 1000 times more water for establishment when compared to plugs, having implications for environmental impacts [5].

Seeds planted directly in the soil, as opposed to grown as plugs and later transplanted, may be exposed to soil fumigant pesticides to combat soilborne pathogens, nematodes, and weeds [4]. In the United States, methyl bromide plus chloropicrin (MBC) was the fumigant of choice used by nurseries, along with the alternatives of dichloro propene 85% + chloropicrin [6] and metam-sodium [7]. Since plugs are usually grown in controlled environments, the use of pesticides is drastically reduced, resulting in more selective pesticide use, reduced worker exposure to pesticides, and lower pesticide residues on crops [4].

Many industries utilize plug transplants, including the floral [3], agricultural

[3], and controlled environment agriculture (CEA) industries, and are heavily reliant on the growth and transport of plant plugs. The plug production enterprise has become a significant industry producing over $2.5 billion worth of plugs annually, meaning that delving into the different techniques for growing the most viable plugs is essential [3]. Besides the United States of America, many other countries utilize plant plugs, including Japan, the Netherlands, China, Mexico, Korea, Israel, Australia, and Canada [3].

Some of the common vegetables grown as plugs include, but are not limited to, tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), cabbage (*Brassica oleracea var. Capitata*), celery (*Apium graveolens*), strawberry (*Fragaria* × *ananassa*), lettuce (*Lactuca sativa*), onion (*Allium cepa*), and various herbs [3]. Ornamental flowering plants can also be grown utilizing plugs which include pansies (*Viola tricolor var. Hortensis*), petunia (*Petunia* × *atkinsiana*), marigold (*Tagetes erecta*), wax begonia (*Begonia* × *semperflorens-cultorum*), salvia (*Salvia officinalis*), and lisianthus (*Eustoma grandiflorum*) [3].

Plugs may be grown in several ways which include directly in the field, or in controlled environments such as greenhouses [8]. In all methods of plug production, fertigation is necessary as plants require nitrogen, phosphorous, and potassium to maintain the normal physiological function of cells [9]. Having the correct fertilizer source and concentration is critical in plug production and ensures ideal growth. According to Mani [10], a lack of nitrogen results in both slow and poor growth, but the excess use of nitrogen results in delayed maturity and low-quality leaves [11].

There are multiple ways to fertilize plugs to provide the necessary nutrient components for growth using either synthetic or organic sources. Synthetically derived fertilizers differ from their organic counterpart because they are synthesized artificially or mined from non-living materials and consist of simple chemical compounds of known composition [9] [12]. Synthetic fertilizers, also known as chemical fertilizers or inorganic fertilizers, offer the advantage of a relatively faster and higher rate of nutrient absorbance by plants compared to organic fertilizers [9]. However, the increased use of synthetic fertilizers has demonstrated numerous flaws including negative environmental effects if managed poorly [13]. These features of synthetic fertilizers cause reduced crop yields due to soil degradation and nutrient imbalances [14]. Excessive and improper use of synthetic fertilizers has an array of adverse environmental impacts, which include increased soil salinity, heavy metal accumulation, water eutrophication, and nitrate accumulation [15].

Organic fertilizers are classified as fertilizers derived from biological or living materials, including manure from livestock, green manure from young plants, especially legumes, and compost from agricultural and food waste [9]. An advantage of organically derived fertilizers is providing the nutritional necessities to sustain plant growth while conjointly suppressing plant pest populations [16]

[17] [18] [19] [20].

With both organic and synthetic fertilizer sources offering varying strengths and weaknesses, this experiment aimed to assess the performance of plug production using different fertigation sources and concentrations. The specific goal was to evaluate plug growth and biomass performance using synthetically and organically derived fertilizers with varying concentrations. Results from this study may provide increased clarity to growers when making a fertilizer selection for plug production.

2. Materials and Methods

2.1. Location

The experiment was carried out at Florida Gulf Coast University (FGCU), located in the city of Fort Myers, Florida, United States of America. The experimental setup was in the Aquarium Room 114 of Academic Building 9 at The Water School. The Florida Gulf Coast University Work Management Center maintained Aquarium Room 114 between 20.5˚C and 22.2˚C for the experiment duration.

The climate of Southwest Florida is characterized by a tropical climate, with a wet summer season and a contrasting dry season [21]. The average annual temperature in Fort Myers was 24.5˚C, with a low temperature of 18.8˚C in January and a high temperature of 28.7˚C in August [22].

2.2. Experimental Setup

The plug production experiment was conducted in a controlled environment, including a thermally insulated grow tent, LED lighting source, and environmental controls that monitored humidity, temperature, and vapor pressure deficit (VPD). These components allowed us to maintain stable growing conditions for plug production with reduced interference from external influences. The experimental setup was similar to the arrangements used by the Yang Laboratory at the University of Connecticut [23] [24] [25] [26] [27].

The thermally insulated grow tent (The Original Gorilla Grow Tent® 5 × 5, Gorilla Inc., Santa Rosa, California) had dimensions of 1.52 × 1.52 × 2.12 m and weighed 33.9 kg. The artificial lighting element consisted of four light fixtures (FREELICHT 4 ft LED Grow Light 60W, Amazon Inc., Seattle, Washington) installed in the tent to provide sufficient light energy for photosynthesis. The lights were attached from the tent ceiling by support ratchets (Heavy-Duty Stainless-Steel Gear Ratchets, AC Infinity, Los Angeles, California). The four linked lights collectively produce 12,000 lumens of light and 14,000 K color temperature. The lights were positioned 0.31 m above the top of the plug trays for the experiment duration.

We utilized a ventilation system using a high-powered fan (CLOUDLINE T6, 6” Inline Duct Fan with Temperature Humidity Controller, AC Infinity, Los Angeles, California) with a duct opening size that is 0.15 m in diameter, an air-

flow capacity of 11.38 cubic meters per minute (CMM), and a power rating of 38 watts. The fan was placed in the top opening of the grow tent in an orientation that directed air outwards. The bottom vent was left open to provide an inflow to the recirculating system of air.

We used light sensors (FUTUREHORTI Light PAR Meter PPFD Tester, Amazon Inc., Seattle, Washington) to collect data on the photosynthetic photon flux density (PPFD) and daily light integral (DLI) in moles per meter square day ($mol/m^2 \cdot day$). To collect air moisture content and temperature data, we utilized the built-in environmental sensor of the fan (CLOUDLINE T6, 6" Inline Duct Fan with Temperature Humidity Controller, AC Infinity, Los Angeles, California). Sensors were placed in the center of the grow tent at the same elevation as the plug trays to represent the environmental conditions the plugs were exposed to. The sensors continuously recorded humidity, temperature, and VPD throughout the growth cycle.

We placed the plugs on a tray stand (SKU number HGC706122, Fast Fit Ltd., Hawthorne Gardening Company, Vancouver, Washington) whose dimensions were 1.22 × 1.22 m. On the stand, we placed five smaller trays (Living Whole Foods Seed Starter Grow Trays, Amazon Inc., Seattle, Washington) with the dimensions of 0.25 × 0.51 m, which housed the growth medium (Horticube XL 104-Cell Sheets, Oasis® Grower Solutions, Kent, Ohio). The trays were sowed with "Rex Butterhead" Lettuce (*Lactuca sativa*) seeds (Johnny's selected seeds, Fairfield, Maine) and were subjected to different fertigation treatments.

The synthetic fertilizer utilized in the experiment was comprised of a combination of (Jack's Nutrients 5-12-26 Part A FeED, JR Peters Inc., Allentown, Pennsylvania) and (Jack's Nutrients 15-0-0 Calcium Nitrate Part B, JR Peters Inc., Allentown, Pennsylvania). The 5-12-26 Part A fertilizer was derived from potassium nitrate, magnesium sulfate, monopotassium phosphate, iron DTPA, iron EDTA, iron EDDHA, copper EDTA, manganese EDTA, zinc ETDA, boric acid, and ammonium molybdate. The specific available nutrients that supported plant growth were 5% total nitrogen (N), 12% available phosphate (P_2O_5), 26% soluble potash (K_2O), 6.3% available magnesium (Mg), and 8.5% available sulfur (S). The Part A fertilizer also contained a micronutrient blend of 0.05% boron (B), 0.015% copper (C), 0.3% iron (Fe), 0.05% manganese (Mn), 0.019% molybdenum (Mo), and 0.015% zinc (Zn). The 15-0-0 Part B (Jack's Nutrients 15-0-0 Calcium Nitrate Part B, JR Peters, Inc., Allentown, Pennsylvania) was derived from calcium nitrate $Ca(NO_3)_2$. The nutrient composition of this fertilizer source was 15% total nitrogen (N) and 18% calcium (Ca). These collective components promote the growth of strong roots and leaves for vegetative plants, which results from successful plug production.

The organic fertilizer that we utilized was (4-6-3 Organic Vegetable Fertilizer (Dr. Earth, Winters, California)). Derived from upcycling food-grade waste, this organic fertilizer included fishbone meal, feather meal, alfalfa meal, potassium sulfate, fish meal, kelp meal, rock phosphate, and kelp flour. The nutrient availa-

bility of this fertilizer was 4% total nitrogen (N), 6% available phosphate (P_2O_5), 3% soluble potash (K_2O), and 7.5% total calcium (Ca). Along with nutrients, the fertilizer mixture contained 6% humic acids derived from Leonardite. Humic acids are molecules that bind to roots to help with the reception of water and nutrients and can dramatically increase the plant's productivity. These collective organic compounds provided the necessary nutrients for plug production.

2.3. Experimental Procedure

The entire plug production process took 15 days during January 2023. We began the experiment by placing five Horticube growth mediums in black 0.25 × 0.51 m starter trays and thoroughly saturated the Horticubes with reverse osmosis (RO) water. We then put a single pelleted "Rex Butterhead" Lettuce (*Lactuca sativa*) seed in each cell of the growth cubes, totaling 104 seeds. We repeated this process four additional times until we had five seed-filled Horticube trays. Next, we placed a layer of newspaper over each of the five trays and placed the covered trays inside the grow tent, where the lighting element was turned off for 48 hours to replicate the natural seed imbibition process. After 48 hours, we used a programmable timer (Mechanical 24-Hour Programmable Dual Outlet Timer, BN Link, Santa Fe, California) to provide 16 hours of continuous light for plug production between 06:00 to 22:00 for the duration of the plug production cycle.

The entire experimental setup allowed us to maintain ideal environmental conditions throughout the growth cycle for plug production. Using light sensors, we determined the DLI ranged from 6.78 to 11.17 mol/m^2·d with an average of 8.76 mol/m^2·d, which fell within the minimum recommended range of 6.5 - 9.7 mol/m^2·d [28]. Utilizing the fan's built-in environmental sensor component, we could monitor the environmental conditions over the plug production cycle. The temperature varied between 21.2˚C and 27.1˚C with an average value of 24.4˚C. The tent's VPD ranged between 0.77 and 2.03 kPa, with an average value of 1.61 kPa. The relative humidity over the growth period varied between 34.5% and 73.71%, averaging 47.1%.

After 48 hours had elapsed, we began the five fertigation treatments of 1) full-strength synthetic fertilizer solution; 2) half-strength synthetic fertilizer solution; 3) full-strength organic fertilizer solution; 4) half-strength organic fertilizer solution; 5) no fertilizer for control. The control tray was treated with plain RO water during the experiment. We formulated various fertigation solutions for plug production following the manufacturers' recommendations for synthetic and organic treatments.

To prepare the full-strength synthetic fertilizer treatment, we mixed 3.6 g of "Jack's Nutrients hydroponic 15-0-0" (calcium nitrate) and 3.8 g of "Jack's Nutrients Part A 5-12-26" in 10 L of RO water. To prepare the half-strength synthetic treatment, we mixed 1.8 g of "Jack's Nutrients 5-12-26 Part A" and 1.9 g of "Jack's Nutrients 15-0-0 Part B" in 10 L of RO water. We mixed 453.6 g of "Dr.

Earth's Organic 4-6-3 Fertilizer" with 9.5 L of RO water to formulate the full-strength organic fertilizer treatment. We mixed 226.8 g of "Dr. Earth's Organic 4-6-3 Fertilizer" with 9.5 L of RO water to prepare the half-organic fertilizer treatment. The control tray was treated with plain RO water during the experiment. To ensure a full mixture of each solution, we utilized a glass stirring rod to maintain an even distribution of the nutrients in the solution.

We fertigated each tray by adding 100 - 400 mL of each fertigation source to their respective trays daily for 13 days, volume added depended on the rate of evaporation and uptake from the plugs. Figure 1 illustrates the experimental setup.

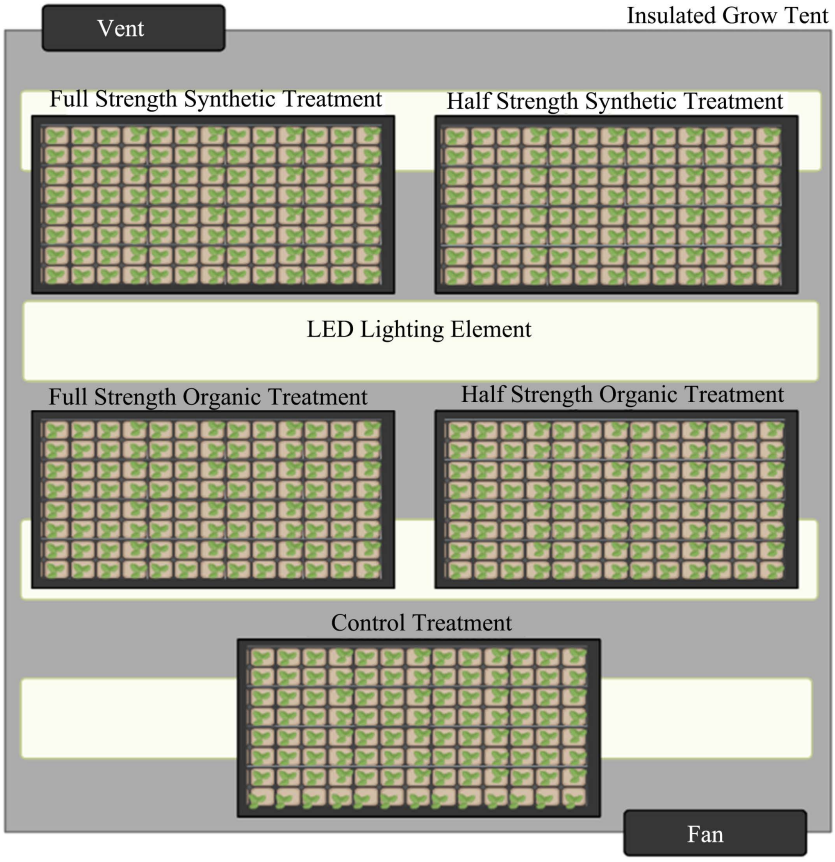

Figure 1. Top view of the experimental setup with five fertigation treatments of 1) Full-strength synthetic starter fertilizer solution; 2) Half-strength synthetic starter fertilizer solution; 3) Full-strength organic starter fertilizer solution; 4) Half-strength organic starter fertilizer solution; 5) No Fertilizer for control for "Rex Butterhead" lettuce (*Lactuca sativa*) plug production.

2.4. Data Acquisition

To find the comparative growth performance of the plugs with different fertigation treatments, we collected biomass data at the end of the growth cycle. On the 15th day of growth, we harvested 20 plugs from each treatment tray in a randomized manner for data collection.

We utilized destructive methods to obtain total leaf area (cm^2), wet weight (g), dry weight (g), and total chlorophyll content (mg/cm^2) for biomass data collection. To obtain the wet weight, we gently pulled each plug out of the Horticube tray, separated the root material from the plug, and weighed the samples. We separated the root mass since it contained fragments of the Horticube material, which, if weighed, would lead to an inconsistent analysis of the biomass. We measured these values first and immediately after harvest to ensure minimum water loss through plant evapotranspiration which would dry out the plugs and lead to inaccurate measurements.

We then utilized a chlorophyll meter (atLEAF CHL Plus Chlorophyll Meter, FT Green LLC, Wilmington, Delaware) to find the total chlorophyll content of each sample. We took three readings per sample and then averaged the values of those readings and converted them to Soil Plant Analysis Development (SPAD) units and subsequently to total chlorophyll content (mg/cm^2). The total chlorophyll content of the crop foliage is calculated by converting the atLEAF CHL values to SPAD and considering the relationship between chlorophyll content and SPAD units [29].

To calculate each plug's total leaf area, we installed the Leafscan app on a mobile device (iPhone 11, Apple Inc., Cupertino, California). We separated and laid out each sample's leaves on a white sheet of paper with four black dots forming a 10.5 × 10.5 cm reference square. The Leafscan application then utilizes the camera on the mobile device to photograph the leaves and, by running an algorithm that measures the green leaf area in comparison to the blank white area, generates the total leaf area value [30]. The Leafscan app calculated the area inside the contour in pixels, and by using the given reference length of 10.5 cm, it converted the leaf pixel area into the surface area [30]. We collected the total leaf area data of 20 samples for each treatment that was later exported in a comma-separated values (csv) format. The Leafscan app measured the leaf area in square centimeters (cm^2) with an accuracy of 0.01 cm^2 [30].

To find the dry weight of the samples collected, we placed all twenty samples in individual brown paper bags and placed them in a drying oven for six days set at a temperature of 65˚C. After six days, we returned to weigh the dried samples and divided the dry weight value obtained by 20 to find the average dry weight of each sample. We averaged the values because the weighing scale we utilized had a precision of 0.001 g and, if individually sampled, would not register on the weighing scale.

2.5. Data Processing and Statistical Analysis

We utilized collected data described in the previous section to derive leaf area index (LAI, cm^2/cm^2) and specific leaf area (SLA, cm^2/g). We also converted SPAD units to total chlorophyll content (mg/cm^2). LAI is defined as the ratio of the total leaf area (cm^2) and the ground area (cm^2). Specific leaf area is defined by the ratio of each plug's total leaf area (cm^2) to dry weight (g). We found the

SLA by dividing the average total leaf area value by the average dry weight value for each treatment. The resulting calculated value is the average SLA for each treatment.

The chlorophyll meter (atLEAF CHL Plus Chlorophyll Meter, FT Green LLC, Wilmington, Delaware) utilized in this experiment records a value based on the transmittance of light through the leaf surface in wavelengths (660 to 940 nm) associated with chlorophyll [29]. From the calculations of Zhu *et al.* [29], it was found that the values computed by the atLEAF chlorophyll meter (r^2 = 0.72) have a strong correlation with SPAD values (r^2 = 0.78). We converted the atLEAF value to the corresponding SPAD unit using this correlation. SPAD units were then converted to total chlorophyll content (mg/cm^2) using the formula (y = 5.52E−04 + 4.04E−04x + 1.25E−05x^2) as described by Richardson *et al.* [31].

We used statistical analysis to understand the effects of fertigation treatments on the biomass output of plugs produced. We processed the data collected and used descriptive statistics to demonstrate our results and implemented an analysis of variance (ANOVA) single factor test and Tukey's Honestly Significant Difference (HSD) procedure, which is a Post-hoc test, to better understand our results using Microsoft Excel.

3. Results

Plug production utilizing different fertigation treatments demonstrated that the biomass output varied significantly based on the fertigation treatment. **Table 1** summarizes the average wet weights, dry weights, total chlorophyll contents, SLA, and LAI from the plug production using the different fertigation treatments. The dry weight, total chlorophyll content, and SLA were not determinable for the control treatment due to the leaves that formed being too small to register in the atLEAF chlorophyll meter, and the dry weight was negligible. None of the control plugs were viable, underscoring the importance of fertigation in plug production.

The wet weights of the plugs produced varied from 0.022 g to 0.280 g over the different treatment methods. Utilizing solely RO water, the control treatment tray produced plugs that had the lowest average wet weight of 0.022 g which was significantly less biomass than all four other treatments, indicating that the supplementation of nutrients, regardless of their source, is necessary for the growth and transplant of viable plugs.

The half-strength organic treatment had the highest average wet weight of 0.280 g, similar to the full-strength synthetic treatment with a wet weight of 0.229 g. There was a difference between the wet weight of the full-strength organic and half-strength organic treatments, with half-strength producing a wet weight 0.128 g greater than the full-strength organic treatment. The difference can be explained by the full-strength solution being more concentrated with nutrients resulting in over fertigation, and thus stunted growth. Half-strength and full-strength synthetic treatments showed slight differences in the wet weight, with full-strength 0.026 g heavier than half-strength for synthetic treatments.

Table 1. Average wet weight (g), dry weight (g), Leaf Area Index (LAI) (cm^2/cm^2), Specific Leaf Area (SLA) (cm^2/g), and total chlorophyll content (mg/cm^2) at harvest, which was the 15th day of plug production.

Fertigation Treatment	Wet Weight	Dry Weight	LAI	SLA	Total Chlorophyll Content
	(g/plug)	(g/plug)	(cm^2/cm^2)	(cm^2/g)	(mg/cm^2)
Full-Strength Synthetic	0.229	0.014	2.83	32.78	0.015
Half-Strength Synthetic	0.203	0.008	2.45	41.48	0.013
Full-Strength Organic	0.152	0.003	1.72	96.47	0.012
Half-Strength Organic	0.280	0.012	3.05	48.56	0.014
Control	0.022	-	0.13	-	-

The dry weight varied between 0.003 and 0.014 g, with the full-strength synthetic treatment having the highest weight value of 0.014 g. The half-strength organic treatment was similar to the full-strength synthetic treatment, with only a slight difference of 0.002 g between the two treatments. The full-strength organic treatment produced plugs with the lowest dry weight value, 0.011 g less than the full-strength synthetic treatment. The full-strength synthetic treatment producing the greatest dry-weight value indicated a higher percentage of non-structural dry-weight components, including glucose, sucrose, and starch, and structural dry-weight components, including cell walls and cytoplasm.

The total chlorophyll content ranged from 0.012 to 0.015 mg/cm^2 across the different treatments, with full-strength synthetic producing the highest value and full-strength organic producing the lowest. Overall, the chlorophyll content had relatively minor variations in the four treatments, with only 0.003 mg/cm^2 variation, indicating that fertigation methods have minuscule effects on the chlorophyll amounts in the leaves.

The LAI ranged from 1.72 to 3.05 cm^2/cm^2, with full-strength organic having the lowest value and half-strength organic having the highest value. The SLA varied from 32.78 to 96.47 cm^2/g, with full-strength organic producing the greatest value and full-strength synthetic producing the lowest.

Significant differences in the biomass of the different plug treatments were observed in terms of wet weight ($F(4,95) = 99$, $P < 0.0001$) and total leaf area ($F(4,95) = 99$, $P < 0.0001$). Tukey's HSD procedure revealed that all treatments resulted in statistically significantly greater wet weight than the control (Mean = 0.022 g). Synthetic treatments produced plugs that weighed statistically more than plugs derived from the full-strength organic treatment (M = 0.152 g), but significantly less than those subjected to the half-strength organic treatment (M = 0.280 g). Regarding the organic treatments, half-strength produced significantly heavier plugs than full-strength concentration.

4. Conclusion

This experiment aimed to determine if there was a variation in the growth performance of plugs when implementing different fertigation treatments using synthetic and organic sources. We found that we could grow plugs ready for transplant by the 15th day for all treatments except for the control, as two leaves after the cotyledons had not developed. Although all treatments except the control produced viable plugs, we can conclude that there is variation in the growth performance of "Rex Butterhead" Lettuce plugs when grown with different fertilizer concentrations and sources. We found the half-strength organic treatment had a plug wet weight that was statistically significantly greater than all other treatments and a leaf area that was greater than all other treatments indicating the half-strength organic treatment produced the most viable plugs with the largest biomass output. This study can help inform commercial and hobby growers about fertilizer choices when producing plugs for their operation.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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