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Evaluation of Lettuce (*Lactuca sativa* L.) Production under Hydroponic System: Nutrient Solution Derived from Fish Waste vs. Inorganic Nutrient Solution

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Abstract: Organic fresh products are appreciated and are gaining a good reputation regarding human health and environmental concerns. Despite the fact that hydroponics are commonly used in vegetable production, growers are looking for sustainable cultivation systems. Therefore, the objective of this study was to investigate the effect of using an organic-based nutrient solution (NS) derived from fish waste in a hydroponic system on the vegetative growth and production of lettuce compared to a conventional inorganic NS. Plant growth, yield, physiological and nutrient content parameters were determined. The results revealed that the overall growth and fresh biomass of the organic NS grown lettuce were relatively lower than those of the inorganic NS. Stomata density was significantly higher in inorganic grown lettuce compared to the organic one. However, the total chlorophyll, carotene, phenolic compounds, and flavonoid contents, as well as antioxidant activity were significantly higher in lettuce grown in organic NS compared to the inorganic one. Leaf nutrient content at harvest was significantly impacted by the type of used fertilizer. Based on these findings, in hydroponic system, organic liquid fertilizer derived from fish waste (as an alternative NS source) requires further improvements to achieve optimal growth and yield comparable to that of conventional inorganic NS.

Keywords: organic nutrient; hydroponic; production; lettuce; antioxidants; phenols



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1. Introduction

Lettuce (*Lactuca sativa* L.) is a member of the *Asteraceae* family and is widely recognized as one of the most important leafy vegetable crops in terms of crop value [1]. It is a delicious vegetable consumed all over the world due to its crispness, pleasant aroma, and high levels of phytonutrients, such as phenolic components and vitamins (C, K and folate) [2,3]. Lettuce is one of the most widely grown hydroponic vegetables. Additionally, several reports have revealed that lettuce has a high yield and good quality when grown in a soilless system [4,5].

Because of increasing environmental and ecological awareness, and potential human health risks caused by the overuse of chemical fertilizers, consumer demand for vegetables and fruits produced organically is growing [6]. As a result, the number of farmers who want to adapt strategies that respect the environment, preserve valuable resources like water, provide healthy and safe products, and use sustainable and efficient agricultural practices is increasing [6].

Recently, greenhouses have started to expand their cultivation techniques to include soilless cultivation technologies such as hydroponics and aeroponics, in addition to conventional soil cultivation [7,8]. In such systems, plants are cultivated in a nutrient solution

(liquid culture) or assisted by means of an inert medium (aggregate culture) [9,10], and all nutritional requirements of the plants are provided through irrigation water enriched with minerals [4]. Furthermore, the system has the potential to reuse nutrients and water, reduce climatic changes, increase production, and reduce the occurrence of pests and soil diseases [10,11]. The main differences between conventional soil cultivation and hydroponic growing systems are water and nutritional supply availability [12]. Such differences can influence the physiological development and biochemistry of plants [12]. However, soilless cultivation can be effectively improved and utilized as an alternative choice for growing healthy food plants such as vegetables and herbs [7,13].

Despite certification issues regarding organic hydroponic products in many countries, hydroponic systems can be more energy and water efficient than soil-based systems, rendering them more sustainable [6]. The use of organic fertilizer solutions has become more common, allowing for organic food production in hydroponic systems [6]. Because of the strong market demand for organic products and the desired flavor of the product, some producers use organic sources of fertilizers in hydroponic culture recirculating [14]. Other reasons include: the need to limit the use of traditional nitrate-based fertilizers in food crops, which has the potential to lower nitrate levels and reduce health-related issues [6,15]. Furthermore, organic-based materials can be utilized as biostimulants, which are biodegradable and can help recover mineral deficiencies (i.e., potassium) to some extent, as observed with the *Ascophyllum nodosum* seaweed application in hydroponically grown lettuce [16]. Previous research also showed that limitation of nutrients restricted the growth of lettuce grown hydroponically through a reduction in stomatal number, size, and conductance [12]. Additionally, the chlorophyll and carotenoid content of lettuce leaves was found to be greatly influenced by the mineral composition of nutrient solution [6].

Liquid fertilizers derived from organic sources are made in a number of ways. They can be made from microbial decomposition of animal by-products such as, fish waste, blood meal, and bone meal, along with mined sulfate of potash [17]. Despite its advantages, the use of organic rather than conventional fertilizers in recirculating cultures creates new challenges for farmers [17,18]. Generally, a difficulty in achieving high plant yield with organic nutrient sources has been reported [14,17]. Organic hydroponics is a complex method that requires more consideration than traditional hydroponics. Organic sources of nutrients may contain too many constituents (for example, micronutrients) or unneeded constituents (for instance, sodium) that require regular ion specific monitoring [14]. It has been suggested that a balanced and stable pH, electrical conductivity (EC) of the nutrient solution, the use of filters, and the presence of adequate microorganisms are the key components of a productive organic hydroponic system [19–21]. Finally, organic fertilizers are often much more expensive relative to inorganic ones, but the benefits (i.e., biomass recycling, lower environmental inputs and constrains etc.) offer can balance this expense over time [17]. Conventional organic producers argue that healthy organic produce can be only produced in soil (living systems), whereas hydroponic cultivation systems can produce high quality crops in more sustainable manners and in places that normally would not be suitable for organic production using soil-based production systems [22]. Moreover, the hydroponic production systems with organic inputs in urban areas may offer the opportunity to produce organic fresh vegetables right where they are most needed without the use of synthetic inputs in locations where traditional organic cultivation is not possible.

Organic production using hydroponic systems is still under investigation, and presents only a small niche of the large organic industry. Because of its complexity and challenges, information on vegetable crop cultivation in hydroponic systems supplemented with organic nutrients, particularly in liquid forms, is limited. Therefore, the objective of the present study was to evaluate the efficacy of using a liquid fertilizer derived from fish waste in hydroponic system on vegetative growth and production, stomata density, chlorophyll, carotene, phenolic compounds, and flavonoid contents, as well as antioxidant activity of leaf lettuce in comparison to a conventional inorganic fertilizer.

2. Materials and Methods

2.1. Growth Conditions

This study took place in a polycarbonate greenhouse at Al Foah experimental farm, United Arab Emirates University, Al Ain, UAE (co-ordinate latitude and longitude of 24.2191° N and 55.7146° E), from October to December 2020, with ambient lighting, average daily temperature 19.2–26.4 °C, and relative humidity 40–60%. A Dutch and Bucket open hydroponic system with auto-pot technology was utilized in this experiment. The system contained several components: tanks of 100 L volume (for nutrient solutions), a small pump to run the system, perlite media to support the plant, pots with depth of 40 cm and 27 L capacity, pipes and micropipes connected to the pots' base. The experiment was set up using the Dutch and Bucket hydroponic system with auto-pot. Eight pots, each with two plants with tanks of nutrient solutions connected with pipes and micropipes to the pots.

The commercial hydroponic inorganic solution based on Hoagland and Arnon solution [23] was obtained from Al Khazna Chem. Fertilizers, Al Ain, UAE. Solution A (1-1-3+T.E+MgO) and solution B (Ca+Fe EDDHA) were mixed and prepared according to the manufacturer instructions. The organic NS was prepared using liquid organic fertilizer derived from fish waste (Zoroa world for liquid fertilizers, Dammam, Saudi Arabia). The full-strength solution contained 27,500, 36,600, and 2300 mg/L for N, P, and K, respectively, micronutrients, and 28.52% organic matter. The recommended dilution by the manufacturer was 1 solution: 100 water (*v/v*). The NS pH was 5.5–5.8 and adjusted as per needed (3 times/week) by adding NaOH or HCl. The electrical conductivity was 2–2.5 ms/cm and corrected every two days by adding appropriate volume of the stock solutions, the solutions were always supplied and the tank were refilled when needed.

2.2. Experimental Design

Seeds of lettuce (*Lactuca sativa* L.) cultivar 'Parris Island cos' (USA), were sown in plastic pots, with peat moss media, placed under greenhouse condition until seedlings emerged. Seedlings (5 cm length, three to four true leaves) were transplanted to the hydroponic system 10 days after germination. Before transplanting in the pots containing horticultural perlite media (1.5–3.0 mm), extensively used in hydroponics in UAE, the seedling's roots were washed to clean the attached peat-based media. Eight pots were used for each NS (organic/inorganic), and two seedlings were transplanted in each pot. The experiment was a complete randomized design with the follow treatments: (i) plants grown in organic-based NS and (ii) plants grown in chemical-based inorganic nutrient solution. Each treatment had eight replicates (pots) and each replicated had two plants/pots.

2.3. Growth Parameters

All lettuce plants grown in the examined treatments were harvested 6 weeks after transplanting. Plant height and leaf number were monitored every other week (0, 2, 4 and 6 weeks) till harvest. At harvest, the following parameters were measured: shoot and root fresh weight, shoot and root dry mass after drying in an oven at 70 °C for 2 days or until a constant weight was achieved. Leaf area, stomata density, chlorophyll and carotene content were determined in the fresh leaf.

2.3.1. Leaf Area

Leaf area was determined by digital image analysis: leaves were detached from each replicate plant and scanned using a flat scanner at 300 dpi (Epson photo 4180, Seiko Epson Corporation, Suwa, Japan). The scanned images were processed with ImageJ software (ver. 1.52a, National Institutes of Health, Bethesda, MD, USA) to measure the leaf area for each treatment [6] and the results were expressed in cm².

2.3.2. Stomata Density

On the lower surface of three lettuce leaves, the area between 2nd-order veins was covered with clear nail varnish to prepare an epidermal impression. The dried layer of nail

varnish was peeled off using sellotape and then adhered onto a slide. From each leaf, 5 disc positions in the intercostal area were randomly chosen, and stomatal density was counted by light microscopy. Image of stomata was observed using 40× magnification with lens area 0.65 mm² [24].

2.3.3. Total Chlorophyll and Carotene Analysis

For the chlorophyll and carotene extraction, 0.25 g of fresh leaves was extracted in 50 mL of 80% methanol using a mortar and a pestle. The chlorophyll content of filtered solution was determined by measuring the absorbance of the supernatant at 663 and 645 nm by spectrophotometer. Carotene content was determined by measuring the absorbance of the supernatant at 470 nm by spectrophotometer [25]. Chlorophyll a and b and carotene were calculated using these formulae:

$$\text{Chlorophyll a (mg/mL)} = 12.7 A_{663} - 2.69 A_{645}$$

$$\text{Chlorophyll b (mg/mL)} = 22.9 A_{645} - 4.68 A_{663}$$

$$\text{Carotene} = (1000 * A_{470}) - 1.82 \text{ Ch a} - 85.02 \text{ Ch b} / 198$$

The contents were expressed as mg/g fresh weight, and total chlorophyll was calculated as the sum of Chlorophyll a and Chlorophyll b.

2.3.4. Total Phenolics, Flavonoids Content and Antioxidant Activity

Extraction of polyphenols and antioxidants was carried out for the dry leaf samples. The obtained extracts were used in the determinations of total phenolic content, total flavonoids content, and antioxidant activity according to Viacava et al. [26] with slight modifications [27].

Total phenolic content was determined using the Folin Ciocalteu reagent. Concentration of total phenolic compounds was calculated using a standard curve of gallic acid and expressed as mg gallic acid equivalents (GAE) per 100 g of DW. Total flavonoids content was determined and expressed as mg of catechin equivalents (CE) per 100 g of DW using a standard curve of quercetin.

Antioxidant activity was determined using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical assay at 517 nm, and the IC₅₀ values (µg/mL extract) were calculated for each sample to find what extract concentration required for a 50 percent reduction of the DPPH radical. Antioxidant activity was also determined by the ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical scavenging activity test. A Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard curve ranging from 50 to 600 µM was generated. The absorbance was then measured at 734 nm. The activity was recorded as milligram of Trolox equivalents (TE) per 100 g dry weight basis (mg TE/100 g DW).

2.4. Nutrient Content of Lettuce Leaf

Dried tissue (0.5 g) from the aerial plant parts from each treatment (8 biological replications; each replication was a pool of 4 individual plants) was ashed and acid-digested (2 N HCl) for nutrient extraction. Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), sulfur (S) and sodium (Na) were determined using an Optical Emission Spectrometry (ICP-OES) [28]. Total nitrogen (N) was measured using the Kjeldahl method (BUCHI, Digest automat K-439 and Distillation Kjelflex K-360, Flawil, Switzerland) [29].

2.5. Statistical Analysis

The obtained data were subjected to statistical analysis with 8 replicates by analysis of variance (ANOVA) using SAS (SAS Institute Inc., 2000, Cary, NC, USA). The means of growth and yield parameters for the two fertilizer treatments (organic and inorganic) were compared using the least significant differences (LSD) test at level $p \leq 0.05$.

3. Results and Discussion

3.1. Plant Height, Number of Leaves, and Leaf Area

The type of fertilizer used had a significant impact ($p \leq 0.05$) on growth parameters of lettuce plants (Figures 1 and 2). This suggests that the source of nutrients in the organic solution somehow caused the differences observed. It is more likely that the poorly balanced nutrient composition in the organic solution led to the observed effects. At week 2, lettuce plants grown in organic NS had a significantly higher number of leaves than those grown in the inorganic nutrient solution. Conversely, at week 6 lettuce plants grown in organic NS had significantly smaller numbers of leaves (~34) than those grown in inorganic NS (~38) (Figure 1). The same trend was noted with plant height and leaf area; plants grown in organic NS had significantly less than those grown inorganically (Figure 2 and Table 1).

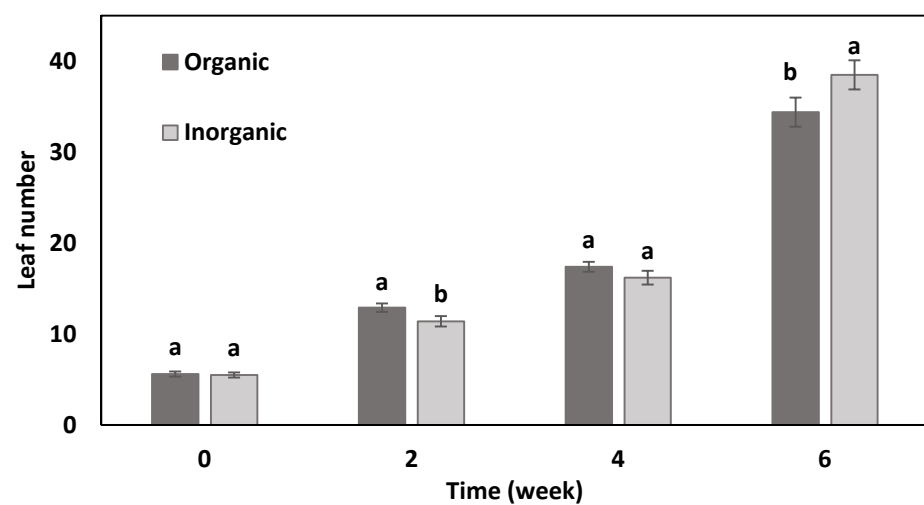


Figure 1. Effect of nutrient solution in a hydroponic system on lettuce leaf number, mean ($n = 8$) \pm SE. Means with different letters on the bar within a time interval are significantly different at $p \leq 0.05$ using the LSD test.

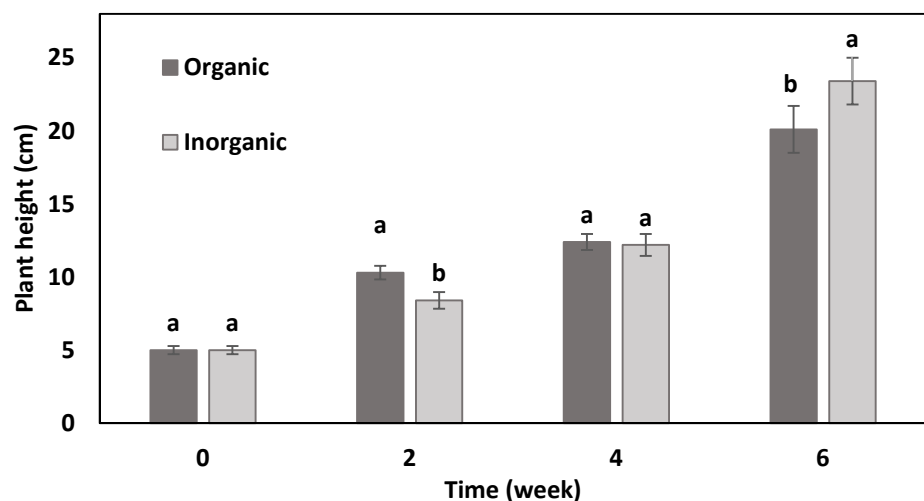


Figure 2. Effect of nutrient solution in a hydroponic system on lettuce plant height, mean ($n = 8$) \pm SE. Means with different letters on the bar within a time interval are significantly different at $p \leq 0.05$ using the LSD test.

Table 1. Effects of using organic and inorganic nutrient solutions in hydroponic system on lettuce fresh shoot and root mass, dry mass, leaf area and stomatal density at harvest.

Treatment	Yield (g/Plant)	Root Fresh Mass (g/Plant)	Shoot Dry Mass (%)	Root Dry Mass (%)	Leaf Area (cm ²)	Stomata Density/mm ² disc
Organic	163.9 ± 2.7 b	36.1 ± 1.7 b	7.01 ± 0.22 a	17.3 ± 0.32 a	172.32 ± 1.25 b	28.6 ± 0.85 b
Inorganic	182.3 ± 2.2 a	37.9 ± 1.9 a	7.4 ± 0.46 a	17.1 ± 0.37 a	181.37 ± 1.36 a	36.3 ± 0.77 a

Values are the mean (n = 8) ± SE. Means with different letters in the same column are significantly different at $p \leq 0.05$ using the LSD test.

Differences in plant height, leaf number and area between the two groups of plants could be attributed to the differences in the composition of nutrient solutions. The reason for the smaller number of leaves, smaller leaf area, and the shorter plants in the lettuce grown in the organic solution could be due to the lower availability of nutrients from organic fertilizer relative to the inorganic one. The nutrient imbalance of nutrient solution derived from fish waste might have increased over time, leading to severe K deficiency. The elemental composition of lettuce leaves, which shows that plants grown in the organic solution had significantly higher N and lower K levels compared to those grown in the inorganic solution. Atkin and Nichols [30] observed that lettuce cultivars grown in a conventional NS had an average of 10% higher growth rates than the organic solution cultivated plants. This is in accordance with the present findings, as the inorganic NS increased the leaf number and plant height at harvest by 11.8% and 16.4%, respectively, compared to the organic NS. Moreover, Atkin and Nichols [30] reported that organically-derived nutrient solutions can be used for the production of lettuce using the nutrient film technique (NFT) system, but with slower growth relative to typical inorganic nutrient solutions. Similar findings have been reported in hydroponically grown strawberries [31]. Likewise, Moncada et al. [6] found that reducing the percentage of mineral NS in favor of the organic one adversely impacted leaf number and area, and stem diameter of basil plants grown hydroponically [6]. Such NS management may prevent any mineral accumulation in roots or leaves that could be phytotoxic and/or have detrimental effects when consumed, for example NO₃ or other micronutrient accumulation.

Organic nutrients from plant and animal remains, unlike inorganic fertilizers, are not readily available to the plant and must be converted to plant-obtainable forms by microbes in the substrate [21]. The rate of microbial-mediated mineralization is considerably variable and depends on many factors, such as the nature of organic nutrient source (for example, composition and particle size), substrate temperature, porosity, and moisture content [32]. The challenge with utilizing organic fertilizers is that the nutrient release rate may not match the nutrients demands of the plant, as this requires continuous adjustment of the hydroponic system and the substrate media to enhance the microbial activity for obtaining the optimum decomposition of the organic materials to be available for the plant to uptake [17,19]. This challenge will limit the grower's ability to properly manage the nutrient supply for the plants and their use may result in the accumulation of salts.

3.2. Fresh and Dry Weights

It was noticed that lettuce plants grown in organic solution had significantly lower ($p \leq 0.05$) fresh shoot and root weights, while no significant effect was noticed for the dry weight (Table 1). Shoot fresh weight decreased by 11.2% in plants grown in organic solution (163.9 ± 2.7 g) compared to those inorganically grown ones (182.3 ± 2.2 g). The lower accumulation of fresh biomass observed in organically nourished plants in relation to the inorganically grown ones could be attributed to the limited availability of mineral nutrients in the organic nutrient solution. These results are consistent with the lower growth parameters reported for the plants grown in organic solutions compared to the inorganically nourished ones (Figures 1 and 2). Comparable findings were reported by Williams and Nelson [14], who found that shoot fresh and dry weights of butterhead lettuce were all smaller in the organically nourished plants compared to inorganic ones. Additionally, the

organically fertilized lettuce cultivars weighed less than their inorganic counterparts [30,33]. Correspondingly, the application of liquid organic fertilizer adversely impacted various basil plant characteristics including fresh and dry biomass, and yield. In addition, these characteristics had lower values when reducing the percentage of inorganic fertilizer in favor of the organic NS [6]. The composition of hydroponic NS (micro- and macronutrients) determines several plant characteristics such as leaf number, leaf area, growth, marketable yield, and crop quality [1,34]. Additionally, the asynchrony of nutrient availability and plant needs can severely hamper organic production. The nutrient imbalance of the nutrient solution produced from fish waste may have increased over time, leading to a severe K shortage. The elemental composition of lettuce leaves demonstrates that plants cultivated in the organic solution had significantly greater N and lower K levels than those grown in the inorganic one. The present results revealed the influence of fertilizer type on the biomass and yield of lettuce grown in a hydroponic system.

3.3. Stomatal Density

The stomatal density was significantly affected by the type of NS used in the hydroponic system (Table 1). The stomatal density was significantly lower ($p \leq 0.05$) in organically nourished lettuce ($\sim 29/\text{mm}^2$) than inorganically grown plants ($36/\text{mm}^2$) (Table 1). These results reflected the lower growth parameters and lower fresh shoot biomass found in lettuce plants grown in organic solution compared to inorganically grown ones (Figures 1 and 2 and Table 1). In plants, stomata play an important role in managing plant use of water and gaining of carbon [24,35,36]. In addition, stomatal density is strongly associated with photosynthetic and growth characteristics, and accordingly biomass accumulation [37]. Stomata can respond directly to N-deprivation-induced signals or in response to carboxylation rates [38]. Previous research showed that limitation of nitrogen restricted the growth of lettuce grown hydroponically through a reduction in stomatal conductance [38]. Additionally, smaller stomatal density caused depression of conductance and/or CO_2 assimilation in *Arabidopsis* [39]. In contrast, Doheny-Adams et al. [40] reported that smaller stomatal density produced greater growth and biomass in *Arabidopsis* under steady light due to suitable temperature and water conditions for metabolic activity and stomatal development with a low metabolic cost. Most recently, in hydroponically grown basil plants, Moncada et al. [6] found that the use of a liquid organic fertilizer in favor of an inorganic one significantly reduced the stomatal conductance. The number, size, distribution, shape, and mobility of stomata are unique characteristics of species and can be changed as a function of environmental adaptation conditions including deficiency of nutrients [12]. The results of this study indicated that the type of fertilizer utilized significantly affected the stomatal density of lettuce plants grown under hydroponic conditions. Hence, for a full understanding of how stomata density changes affect lettuce growth and biomass production under nutrient deficiency conditions, more investigations are needed.

3.4. Chlorophyll and Carotene Contents

The type of used nutrient solutions had a noticeable impact on chlorophyll and carotene contents in lettuce plant (Table 2). While at week 4 there were no significant differences ($p \geq 0.05$) between lettuce plants grown in organic NS and those grown inorganically, in relation to the chlorophyll a and b contents, at week 6 organically grown plants had significantly ($p \leq 0.05$) higher total chlorophyll and chlorophyll a than inorganically grown plants (Table 2). Similar trend was observed with carotene content (Table 2).

Table 2. Effects of NS on chlorophyll (Ch) and carotene contents in leaf lettuce grown in a hydroponic system using organic and inorganic liquid fertilizers.

Time (Week)	Treatment	Leaf Pigment (mg/g FW)			
		Total Ch	Ch a	Ch b	Carotene
Week 4	Organic	1.060 ± 0.03 a	0.811 ± 0.08 b	0.252 ± 0.012 a	0.443 ± 0.04 a
	Inorganic	1.040 ± 0.05 a	0.833 ± 0.06 b	0.211 ± 0.02 b	0.432 ± 0.03 a
Week 6	Organic	1.181 ± 0.04 a	0.891 ± 0.04 a	0.293 ± 0.02 a	0.223 ± 0.02 a
	Inorganic	1.040 ± 0.03 b	0.731 ± 0.02 b	0.313 ± 0.02 a	0.162 ± 0.01 b

Values are the mean (n = 8) ± SE. Means with different letters in the same column within a week (4 and 6) are significantly different at $p \leq 0.05$ using the LSD test.

At week 6, there was no significant difference ($p \geq 0.05$) between the two nourished groups of plants in relation to chlorophyll b content. The differences in the chlorophyll and carotene contents between treatments might be attributable to the composition of the nutrient solutions utilized to nourish the plants that led to stress due to nutrient deficiency. The range of total chlorophyll found in this study (1.04–1.18 mg/g) was lower than that reported by Phibunwatthanawong and Riddech [41] (1.8–22.8 mg/g) and higher than those reported by Fallovo et al. [34] (0.521–0.793 mg/g) and Sapkota et al. [1], in hydroponically grown lettuce. Chlorophyll and carotenoid concentrations are associated with the photosynthetic capability of the plant [42]. Their concentrations provide information on plant vigor, productivity, and the environmental quality [43]. Fallovo et al. [34] observed that the overall chlorophyll content of lettuce leaves was greatly influenced by the composition of the nutrient solution. For instance, N is important for the formation of chlorophyll and lowering the N content in a NS reduces chlorophyll and thereby hampers photosynthesis and crop yield [44]. In the present study, the higher total chlorophyll content noted in organic lettuce relative to the inorganic one was not fully mirrored in the biomass production (Figures 1 and 2, and Table 1). A similar finding was reported by Phibunwatthanawong and Riddech [41]. This may indicate that plant growth and yield are complex characteristics that rely on many factors, including light, other nutrients, CO₂, water, cultivar, and enzymatic activity of plants. Accordingly, chlorophyll is not the only factor that regulates crop growth and yield [1].

3.5. Total Phenolic and Flavonoid Contents

The total phenolic content of organically nourished plants (3168.6 mg GAE/100 g DW) was significantly higher than inorganic grown ones (2715.3 mg GAE/100 g DW) (Table 3). On the other hand, the content of total flavonoids in the lettuces grown with organic solution (714.7) was higher than that for inorganic NS (538.6 mg QE/100 g DW). The biosynthesis of phenolic compounds is mainly mediated by two enzymes, phenylalanine ammonium lyase and tyrosine ammonium lyase, which are participating in the pathway of shikimic acid [45,46]. Based on these findings, the type of NS utilized had a clear impact on total phenolic and flavonoid contents in lettuce leaves, and most likely induced plant stress in some way. However, such observations need further investigation, examining relevant plant stress indicators.

Table 3. Effect of nutrient solution on total phenolics content, total flavonoid content, and antioxidant activity of leaf lettuce grown in a hydroponic system using organic and inorganic liquid fertilizers.

	Organic	Inorganic
Total phenolics (mg/100 g)	3168.6 ± 61.5 a	2715.3 ± 49.6 b
Total flavonoids (mg/100 g)	714.7 ± 42.4 a	538.6 ± 25.6 b
ABTS (mg/100 g)	810.7 ± 10.1 a	723.6 ± 12.7 b
IC ₅₀ (ug/mL)	153.1 ± 1.8 b	157.0 ± 2.1 a

Values are the mean (n = 8) ± SE. Means with different letters in the same row within are significantly different at $p \leq 0.05$ using the LSD test.

3.6. Antioxidant Activity

To evaluate the antioxidant activity of hydroponically grown lettuce plant at harvest, two assays (DPPH and ABTS) were used. The antioxidant activity as determined by ABTS was significantly lower in inorganically growing plants (723.6 mg TE/100 g DW) compared to the organic NS (810.7 mg TE/100 g DW) (Table 3). On the other hand, the lower IC₅₀ numbers in the DPPH measurements indicated a 50 percent reduction in the extract concentration required to scavenge the DPPH radical, signifying an increase in antioxidant activity. Therefore, plants grown in the organic solution had lower IC₅₀ value (153.1 µg/mL) which means they had significantly higher antioxidant activity compared to inorganic plants. Higher levels of phenolic, flavonoids, chlorophyll, and carotenoids in the plant were mostly associated with antioxidant activity, which is consistent with our findings [27,47]. Consequently, the higher antioxidant activity observed in lettuce plants growing in organic NS could be due to the higher content of phenolic compounds, flavonoid, chlorophyll and carotenoids obtained in these plants (Tables 2 and 3). This increase in antioxidant concentration and activity is significant, since it improves the nutritional quality of lettuce plant. As a result, despite the decreased yield, organic treatment can enhance human nutrition.

3.7. Lettuce Nutrient Composition

The elemental composition of lettuce leaves at harvest was significantly ($p \leq 0.05$) affected by the type of fertilizer used (Table 4). For instance, the N content in leaves of organically nourished plants was significantly greater (33,300 mg/kg) than those grown inorganically (28,200 mg/kg) (Table 4). Since N is essential for chlorophyll formation [44], these findings are consistent with chlorophyll levels at harvest, where organically nourished plants showed significantly greater total chlorophyll and chlorophyll a than inorganically grown plants (Table 2). Despite this, organically grown plants had lower growth and fresh weight compared to inorganically ones (Figures 1 and 2 and Table 1). This finding indicates that N is not the only limiting factor for lettuce crop growth [44], and a balanced elemental composition is important for several plant metabolic processes.

Table 4. Effect of using organic and inorganic nutrient solutions in a hydroponic system on the elemental composition of lettuce leaves at harvest.

Element (mg/kg)	Organic	Inorganic
N	33,300.2 ± 87.4 a	28,200.4 ± 93.3 b
K	10,400.3 ± 66.4b	66,500.2 ± 68.1 a
P	6822.4 ± 58.5 a	2631.2 ± 37.9 b
Ca	13,009.4 ± 71.2 b	7630.0 ± 53.2 a
Mg	2852.5 ± 31.1 b	3626.49 ± 21.3 a
Na	23,280.2 ± 47.1 a	11,104.2 ± 55.6 b
S	2129.5 ± 36.7 a	2539.5 ± 47.3 a
Mn	30.90 ± 1.4 b	57.14 ± 3.8 a
Fe	169.8 ± 2.5 a	119.5 ± 6.4 b
Zn	39.38 ± 1.12 b	105.79 ± 5.3 a

Values are the mean ($n = 8$) ± SE. Means with different letters in the same row are significantly different at $p \leq 0.05$ using the LSD test.

In contrast to N, potassium accumulation was higher in inorganically grown plants (66,500 mg/kg) compared to those grown in an organic solution (10,400 mg/kg). This could be attributed to the limited availability of potassium from the organic nutrient solution. Low K levels have been reported in an earlier study in lettuce (cv Verdede) cultivated in a deep flow techniques (DFT) system when high chlorophyll content was measured in the plants, which was also observed in the organically fertilized lettuce in our study [16]. On the other hand, organic fertilizer provided the highest P content (6822 mg/kg), whereas inorganic had 2631 mg/kg. Consistent with our findings, Zandvakili et al. [48] reported high P levels in organically nourished lettuce, however a previous study found no significant difference

in P levels between organic and inorganic hydroponic solutions [49]. Calcium content in leaf tissues was significantly ($p \leq 0.05$) greater in the organically nourished plants as compared to the inorganic ones. In another study, no difference was found in Ca levels for all plants grown in different nutrient solutions [48]. In the present study, inorganic nourished plants had greater Mg accumulation with 3626 mg/kg compared to organic nourished plants (30.9 mg/kg), which could be the result of a concentration effect owing to the restricted growth under this condition. Similarly, in organically amended medium, Warman and Havard [50] reported high Mn content in cabbage (*Brassica oleracea capitata* L.). Nutrient solution derived from fish waste significantly ($p \leq 0.05$) lowered Zn content in lettuce leaves compare to mineral fertilization, which is consistent with a previous report [48]. The larger accumulations of Na and Fe in the leaves were 23,280 and 169.8 mg/kg, respectively, when grown with organic solution compared to inorganic solution (Table 4). There was no significant difference in S content in lettuce leaves between organic and inorganic treatments (Table 4). Based on the above findings, it may be concluded that the nutrient imbalance of the nutrient solution derived from fish waste increased over the time and led to severe K starvation.

4. Conclusions

The present study revealed that lettuce plants grown with organic NS derived from fish waste had lower plant height, leaf number and area, fresh biomass, and stomatal density compared to inorganically grown plants, while the total chlorophyll, chlorophyll a, carotene, phenolic compounds, and flavonoid content as well as antioxidant activity were higher in plants grown in the organic solution compared to the inorganic ones, reflecting the nutritive value of the former. In contrast to N, potassium accumulation was significantly higher in inorganically grown plants compared to those grown in the organic solution. The difference in yield parameters between the two solutions was explained by the availability of mineral nutrients to the plants being lower in the organic solution. This allows the conclusion that even though organic production is sustainable and organic products have more interest in terms of human health and the environment, using organic NSs in hydroponics remains complex. To assess the efficacy and to overcome the limitations of such systems, their contribution to food and nutrition security and overall sustainability, more research is needed. An adaptation of the components used in the hydroponic system such as organic fertilizers and factors influencing nutrient availability is crucial. Additionally, a careful choice of organic fertilizers is recommended, and all the information related to the mineral composition and the availability of the nutrients in the solution should be present as well as how often a solution is refilled.

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