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Organic-based nutrient solutions for sustainable vegetable production in a zero-runoff soilless growing system

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ABSTRACT

As the adoption of soilless production systems escalates to meet the rising demand for safe and healthy fresh produce, the growing environmental awareness and consumer's preference for sustainable production systems are stimulating the reduction of synthetic inputs. A greenhouse study using an auto-pot zero-runoff hydroponic system and lettuce (*Lactuca sativa* L.) as a model vegetable crop was conducted to evaluate the potential of substituting synthetic fertilizer nutrient solutions (NS) with organic-based NS. The use of organic NS resulted in lettuce plants with fewer leaves and a smaller leaf area, plant height, stem diameter, and fresh biomass compared to those grown with inorganic fertilizer. Among the organic NS used, NS B from fish farm waste (159.8 g) and E from plant sources (157.9 g) ensured crop yield performance slightly lower than the inorganic fertilizer NS (175.1 g), but higher than the other humic acid based-organic NS C and D. However, total chlorophyll (0.81 and 0.93 mg/g respectively) and carotene (0.23 and 0.26 mg/g, respectively) levels were higher in organically grown lettuce compared to the control (0.95 0.17 mg/g, respectively). Furthermore, plants grown organically in NS C and D had greater phenolic levels (3.36 and 3.22 g/100 g, respectively) as compared to those nourished with inorganic fertilizer (2.28 g/100g). All organically grown lettuce plants had lower levels of Ca, K, and Mg, and higher P compared to the control. Moreover, all organic NS resulted in lower leaf nitrate levels (ranging from 3.2 to 8.7 mg/kg) compared to the inorganic NS (259.8 mg/kg) based on dry weight. Our findings suggest that organic liquid fertilizers may enable the sustainable production of safe, nutritious, and healthy vegetable crops. However, further study is required to improve and overcome the limitations of such systems.

1. Introduction

Climate change, limited arable land, and water scarcity are among the primary challenges constraining current conventional agriculture systems [1]. In numerous countries, available agricultural land has been repurposed for residential and industrial developments, whereas arid regions have impeded agricultural production in others [2,3]. Concurrently, the demand for high-quality fresh food is increasing with the growing global population. Consequently, scalable and geographically adaptable alternative agricultural systems are necessary to preserve food security without compromising environmental sustainability [1].

Soilless growing systems allow the cultivation of plants without soil. These systems use limited water and nutrient resources efficiently, and thus represent one of the most promising solutions for producing high-quality crops in arid regions and areas with limited availability of fertile agricultural land [4,5]. These systems employ various substrates, including organic and inorganic growing media [6,7], as well as water culture systems with nutrient solutions (NS) prepared from water-soluble fertilizers. As a reliable technology, soilless systems enable the consistent production of a variety of nutritious crops on both large and small scales in the presence of limiting environmental conditions [8,9]. They have the potential to improve the availability of local fresh produce [10] and enhance global food and nutrition security amid climate change [11,12].

Although soilless production systems are highly efficient, they typically depend on synthetic chemical fertilizers. With increasing

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environmental awareness and concerns about the potential human health risks associated with excessive chemical fertilizer use, consumer demand for organic production and consumption is increasing [13–15]. In recent years, there has been significant debate over whether hydroponic farming qualifies as a certified organic farming method in the United States (U.S.) [16], or whether it falls outside the scope of the EU's organic production directive, except for products intended to be sold as potted plants [17]. Employing organic fertilizers can serve as a nutrient-recycling mechanism while reducing the reliance on synthetic mineral fertilizers, particularly in closed-loop hydroponic systems, thereby enhancing sustainability compared to conventional hydroponics.

The continuous demand for organic products has spurred increasing interest in developing organic soilless cultivation systems, or bioponic systems, that employ solely organic fertilizer sources [13–18]. Previous studies have shown that incorporating organic materials into the substrate or using them as liquid fertilizers enhances the efficiency of hydroponic systems. This results in leafy vegetables and fruit of better quality, characterized by higher levels of antioxidant compounds and lower nitrate levels [19–21]. Furthermore, macroalgae can serve as biodegradable biostimulants, which may help replenish potassium minerals to some extent in hydroponic systems [22]. Recent research has also highlighted a limitation: the nutrient content in organic hydroponics.

Identifying reliable organic fertilizer sources that can provide all the required nutrients throughout the crop growth cycle is critical. The choice of organic fertilizer can significantly impact crop affect the yield and quality [23,24]. For instance, *Brassica rapa* L. var. *chinensis* plants grown hydroponically with a diluted liquid biodigestates organic solution exhibited a 47% reduction in yield (fresh weight) compared to those fertilized with an inorganic solution [25]. The availability of specific nutrients can be impeded by the pH and electrical conductivity (EC) of the NS, as well as by a lack of microorganisms essential for the mineralization of organic matter and the release of essential nutrients in soluble inorganic forms that plants can absorb [13,18,26]. This complexity makes bioponic growing systems more challenging than conventional soilless growing systems.

Lettuce (*Lactuca sativa* L.) a highly popular leafy vegetables, thrives in soilless systems and is increasingly valued by consumers for its rich nutritional profile [24]. It is particularly well-suited to hydroponic cultivation, and easily adapts to this method. As a model vegetable crop, lettuce features shallow roots and a relatively short growing season, with high productivity in cyclical cultivation compared to soil-based methods [23].

Given the increasing demand for organic vegetable products, there is a growing interest in the agriculture industry to develop more efficient bioponic systems that employ organic-based fertilizer sources [26]. Therefore, the primary objective of this study was to assess the efficacy liquid organic fertilizers derived from various organic residues such as fish farm waste, plant waste, and humic-based materials, on the growth, yield, and quality of lettuce plants in a zero-runoff auto-pot soilless system, compared to a conventional NS prepared with inorganic fertilizers.

2. Materials and methods

2.1. Growth conditions, treatments, and experimental design

This study was conducted in a polycarbonate greenhouse at the Al Foah experimental farm, United Arab Emirates University, located in Al Ain, UAE (55.7146° E, 24.2191° N), from October to February 2022. Greenhouse temperatures were maintained between 19.2 °C and 26.4 °C, with the relative humidity ranging from between 40 and 60%. Plants received natural sunlight, experiencing day lengths of 10 h: 40 min: 40 s to 11 h: 50 min: 20 s ± 1 h: 20 min:0 s throughout the experimental period.

In this experiment, we utilized a Dutch Bucket hydroponic system integrated with zero-runoff auto-pot technology (Fig. 1). This system operates via a gravity-based mechanism that regulates water flow using a valve. This valve automatically opens when the tray beneath the pot is empty eliminating the need for electricity, pumps, water pressure, or timers. Each unit of the system comprised multiple components: a 100 L tank for NS, pots with a 40 cm depth and 25 L capacity filled with perlite media (Gulf Perlite LLC, UAE), and a network of pipes/micropipes connected to a valve and float at the base of eight pots.

To prepare the organic nutrient solutions, we used four commercial liquid organic fertilizers produced locally by Emirates Biofertilizers Factory, Al Ain, UAE) as follows:

- Nutrient Solution (B): agro-fish (8-2-2), 2% Ca, 30% organic matter, and 50% protein.
- Nutrient Solution (C): nutrihumate (15-10-9), 12% humic acid, and 35% organic matter.
- Nutrient Solution (D): rods-fert (28-14-14), 7% humic acid, 1% Ca, and 2% S plus trace elements.
- Nutrient solution (E): Bio-green (0.3-2-6), 4% alginic acid, 1% amino acid, and 200 ppm cytokinin and GA plus trace elements.

All nutrient solutions were prepared with a fixed EC of 2.0 mS/cm and were checked and corrected every 2 d [24]. The pH of the NS was adjusted to between 5.5 and 5.8) and was checked and readjusted three times per week by adding NaOH or HCl. The tanks were refilled with NS as necessary to maintain the flow.

The 'Parris Island cos' cultivar of lettuce (*Lactuca sativa* L.) (USA) served as the model vegetable crop. Lettuce seedlings were produced in a peat-based growing medium in small plastic pots housed in the same greenhouse. Ten days post-germination, seedlings measuring 5 cm in length and sprouting three to four true leaves were relocated to an auto-pot soilless system. Prior to transplantation into the perlite media, roots of the lettuce seedlings were gently rinsed to remove the peat-based medium. Each pot, within the soilless system, received two seedlings. These were fertigated with either the control hydroponic chemical solution (A) or one of the four organic fertilizer-based NS (B, C, D, and E). The study utilized eight pots, totaling 16 plants (replicates) for each treatment within the soilless system.

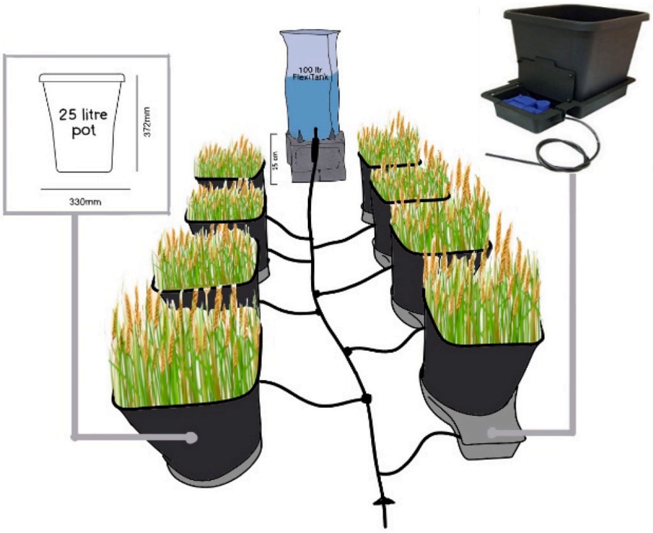

Fig. 1. Example of soilless system with zero-runoff auto-pot technology, each unit including a 100 L tank, eight 25 L pots, main pipe and microtubes connected to a float to control the water flow in a container under each pot.

2.2. Plant growth and physiology assessments

The investigation spanned 60 d after planting (DAP) in the soilless system. Leaf number and plant height measurement were taken at 0, 15, 30, 45, and 60 DAP. Mid-season (30 DAP) and at harvest (60 DAP), stomatal density, leaf area, chlorophyll, and carotene content were measured using fresh leaf samples. At harvest, we measured the fresh weight of the plant shoots and roots, and the dry mass of the leaves and roots after drying in an oven at 70 °C for 48 h. The dry leaf tissue was finely ground and then analyzed for mineral content (N, P, K, Ca, Mg, Cu, Mn, and Fe), nitrate and ammonium levels, and antioxidant compounds and activity. Detailed explanations of all methods are described below:

2.2.1. The leaf area

Samples of three fully developed leaves were randomly collected from each treatment, and their leaf length (mm), width (mm), and area (cm^2) were measured using a leaf area meter (LICOR Photo Electric Area Meter, Model LI-3100, Lincoln, NE, USA) [27].

2.2.2. Stomata density

To prepare an epidermal impression, transparent nail polish was applied to the area on the lower surface of three leaves, specifically between the second-order veins. Once the nail polish dried, Sellotape was used to remove the dry layer containing the leaf impression, which was then fixed to a slide. The number of stomata was counted at five randomly selected disc positions within the intercostal regions of each leaf using light microscopy. Stomatal images were observed at 40× magnification, covering an area of 0.65 mm^2 [28].

2.2.3. Chlorophyll and carotene determination

Chlorophyll *a* and *b*, total chlorophyll, and carotene were extracted from 0.5 g of fresh leaves using a mortar and pestle with 50 mL of 80% methanol. The mixture was filtered, and the resulting supernatant was used to quantify chlorophyll *a* and *b* at absorbances of 663 and 645 nm using a Hitachi U-2001 UV/Vis Spectrometer (OK, USA). The carotenoid content was determined by measuring the absorbance of the supernatant at 470 nm [29]. The following formulas were applied to calculate the total chlorophyll and carotene contents:

Chlorophyll *a* (mg/mL) = 12.7 A6632.69 A645

Chlorophyll *b* (mg/mL) = 22.9 A6454.68 A663

Carotene (mg/mL) = (1000*A470)-1.82 Cha-85.02Chb)/198

The results were reported in milligrams per gram (mg/g) of fresh weight (FW), and the combined amounts of chlorophyll *a* and *b* were used to estimate the total chlorophyll content.

2.3. Antioxidant compounds content and the antioxidant activity

The chemicals 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Folin-Ciocalteu's phenol reagent, catechin, quercetin, and sodium carbonate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals, solvents, and reagents used were of analytical grade.

Polyphenols were extracted from oven-dried leaf samples following the method of Viacava et al. [24], with minor modifications. The resulting extract was used to determine the total phenolic and flavonoid contents. Folin-Ciocalteu's reagent was used to determine the total phenolic content. The sample extract (100 μL) was added, then 2 mL of 6% NaOH was added, and the mixture was allowed to stand for 2 min. Subsequently, 50 μL of Folin-Ciocalteu's reagent was added, and the mixture was incubated for 45 min in the dark at room temperature (22 ± 1 °C). Absorbance at 650 nm was measured with a UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). The total phenolic compound content was calculated and expressed in grams of gallic acid equivalents (GAE) per 100 g of dry weight (DW), using a gallic acid standard curve.

Total flavonoids were quantified following the method of Crozier et al. [30], which involves the use of 5% NaNO2 and 10% AlCl3, followed by the addition of 1 M NaOH. Absorbance was measured at 510 nm using a UV-1601 visible spectrophotometer (Thermo Scientific, Waltham, MA, USA). The total flavonoid content was calculated from a standard curve and expressed in grams of catechin equivalents (CE) per 100 g (DW).

The antioxidant activities were evaluated using DPPH and ABTS radical scavenging assays as described by Chrysargyris et al. [31]. For the DPPH assay, a stock solution of DPPH (50 mg/100 mL) was prepared in absolute methanol. A working solution was then made by diluting a 500 μL aliquot of the stock solution with 4.5 mL of absolute methanol. Then a 500 μL aliquot of the sample was added to the DPPH working solution and kept in the dark for 45 min at room temperature. Subsequently, the absorbance of each sample was measured at 517 nm using a UV-vis spectrophotometer. A working solution without the sample served as the control. For the ABTS assay, stock solutions of ABTS (7 mM) and potassium persulfate (2.6 mM) were prepared separately. The ABTS working solution was created by mixing equal volumes of these stock solutions and allowing them to react in the dark for 12 h. Prior to the assay, this solution was diluted with 80% methanol to achieve an absorbance of approximately 0.710 at 732 nm. The assay was conducted by combining 3 mL of the diluted ABTS solution with 30 μL of the sample, allowing the mixture to stand react in the dark at room temperature for 6 min before measuring the absorbance at 732 nm using a UV-vis spectrophotometer. A diluted ABTS solution without the sample served as the control. The percentage of DPPH and ABTS scavenging was calculated using the following equation:

$$\text{Scavanging } (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.4. Analysis of mineral content in nutrient solutions and plant tissues

Three replicates of ground, dried lettuce leaves were digested using HNO_3 and H_2O_2 at a 1:5 (v/v) ratio in a microwave at 200 °C for 60 min. Concentrations of macro and micro elements (K, P, Ca, Mg, S, Mn, Na, Fe, and Zn) in the prepared leaf material and NS samples were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES Agilent Technologies) following the method described by Hseu [27]. Results were expressed in milligrams per gram (mg/g) or micrograms per gram (μg/g) (DW). Standard reference solutions were purchased from Accu Standard, USA, and a linear regression coefficient (R^2) of 0.9972 was achieved for 1000 mg/L of each metal. Each sample was measured in triplicate. The Kjeldahl method was used to determine the total nitrogen (N) content, which was calculated as a percentage of DW [32]. Nitrate-N (mg/kg) and ammonium-N (%) were quantified in dried and milled lettuce leaves at the national laboratories of the Ministry of Climate Change and Environment, Shrarja, UAE (ISO 17025:2017, UKAS), as described by Cataldi et al. [33]. An ion chromatography instrument IC (Thermo Scientific, Dionex ICS-2100) was used to measure nitrate-N, and a Buchi KjelMaster K-375 was used to measure ammonium-N. A 12.5 mg sample of dried and milled leaves was weighed, and 5 mM hydrochloric acid was used as the extraction solvent. After centrifuging at 3000 rpm for 10 min, the solution was filtered through 0.22-μm nylon filters to remove particulates before injection into the IC.

2.5. Statistical analysis

The research experiment was designed as a completely randomized block. Statistical analysis of the collected data was conducted using one-way analysis of variance (ANOVA) with SAS software (SAS Institute Inc., 2000, Cary, NC., USA). The means of growth, yield, and quality indices for the control group and four organically nourished plants were statistically compared using the least significant differences (LSD) test at $p \leq 0.05$. The experiment was conducted twice, and all analyses were performed in triplicate.

3. Results and discussion

3.1. Elemental composition of nutrient solutions and lettuce leaves

The elemental composition of nutrient solutions and lettuce leaves is presented in Tables 1 and 2. The elemental content of the NS varied significantly ($p \leq 0.05$) (Table 1), with NS D and C exhibiting similar N content with 3.50 and 3.59 mg/g, respectively. These levels of N were higher than those in the control (1.74 mg/g) and other organic NS. Although the NS B solution had a lower N level compared to the other organic NS, its N concentration was not significantly different from that of the control (Table 1). Similarly, the source of fertilizer significantly affected the elemental composition of the leaves at harvest (Table 2). Except for plants nourished with NS B (18.5 mg/g), the N content in leaves of organically produced plants was higher ($p \leq 0.05$) than in the control (26.7 mg/g) (Table 2). Plants grown with NS D and C had the highest N levels, while those supplied with NS B had the lowest. These results align with the N content in the different NS (Table 1). The reduced N concentration in plants grown with NS B may result from the poor availability and slow release of inorganic N from organic NS compared to the inorganic ones. Previous reports suggest that organic fertilizers may not sufficiently support plant growth, as their N content is primarily organic, and thus less beneficial for plant growth and development. In contrast to inorganic fertilizers, which release nutrients immediately, organic fertilizers require mineralization of organic matter to make essential minerals, such as nitrogen, phosphorus, potassium, magnesium and other nutrients, readily available to crops [15].

Table 1
Macro and micromineral content of different nutrient solutions (NS) used in a zero-runoff auto-pot soilless system.

Nutrient solution	Macro elements (mg/g)					
	N	Ca	K	Mg	P	S
A	2.09 ± 0.15^{bc}	80.0 ± 1.91^{a}	57.4 ± 2.45^{ab}	9.02 ± 1.11^{a}	26.1 ± 1.34^{d}	1.35 ± 0.13^{a}
B	1.74 ± 0.12^{c}	34.3 ± 2.76^{b}	65.6 ± 3.31^{a}	5.03 ± 0.61^{b}	95.9 ± 5.72^{c}	1.02 ± 0.05^{b}
C	3.50 ± 0.14^{a}	20.0 ± 1.95^{d}	17.0 ± 0.89^{c}	2.75 ± 0.25^{d}	196.2 ± 6.44^{b}	1.27 ± 0.12^{ab}
D	3.59 ± 0.11^{a}	19.7 ± 1.54^{d}	16.9 ± 1.05^{c}	3.61 ± 0.47^{c}	210.1 ± 7.13^{a}	1.18 ± 0.09^{b}
E	2.42 ± 0.07^{b}	25.7 ± 2.73^{c}	61.7 ± 3.34^{ab}	3.19 ± 0.81^{c}	190.1 ± 5.96^{b}	1.39 ± 0.15^{a}
	Micro elements (µg/g)					
	Fe	Mn	Zn	Cu	Na	Al
A	69.48 ± 3.16^{e}	66.02 ± 2.65^{c}	14.49 ± 0.82^{b}	7.41 ± 0.71b^{c}	8.96 ± 0.61^{b}	0.13 ± 0.03^{d}
B	55.98 ± 4.30^{d}	92.53 ± 7.42^{b}	15.35 ± 1.25^{b}	6.82 ± 0.35^{c}	10.32 ± 0.72^{a}	2.61 ± 0.11^{c}
C	137.9 ± 9.80^{b}	122.31 ± 5.51^{a}	18.09 ± 0.94^{a}	11.93 ± 0.79^{a}	8.52 ± 0.95^{b}	5.24 ± 0.85^{a}
D	174.22 ± 12.34^{a}	90.29 ± 6.30^{b}	18.37 ± 1.41^{a}	12.50 ± 1.05^{a}	8.61 ± 0.65^{b}	4.62 ± 0.76^{a}
E	76.78 ± 5.74^{c}	117.00 ± 7.76^{a}	13.21 ± 0.91^{b}	8.05 ± 0.83^{b}	10.59 ± 0.73^{a}	3.21 ± 0.40^{b}

Mean
SE. Different lowercase letters indicate significant differences at $p \leq 0.05$ as determined by the LSD test.
A = Chemical nutrient solution (control), B= Agro-fish liquid organic fertilizer, C= Nutrihumate liquid organic fertilizer, D = Rods-fert liquid organic fertilizer, E = Bio-green liquid organic fertilizer.

Table 2
Macro and micromineral content of lettuce (cultivar 'Parris Island cos') leaves grown in different NS in a zero-runoff auto-pot soilless system.

	Macro elements (mg/g)					
	N	Ca	K	Mg	P	S
A	26.7 ± 1.3b^{c}	11.90 ± 0.57^{a}	19.85 ± 1.23^{a}	2.88 ± 0.30^{a}	4.33 ± 0.15^{c}	1.43 ± 0.05^{a}
B	18.5 ± 0.97^{c}	6.87 ± 0.31^{b}	10.49 ± 0.47^{b}	1.55 ± 0.04^{c}	4.65 ± 0.25^{c}	1.09 ± 0.07^{c}
C	73.0 ± 1.59^{a}	6.31 ± 0.36^{b}	5.85 ± 0.67^{c}	1.88 ± 0.04^{b}	8.33 ± 0.25^{a}	1.23 ± 0.04^{b}
D	85.4 ± 2.87^{a}	6.31 ± 0.65^{b}	6.38 ± 0.23^{c}	2.01 ± 0.12^{b}	8.68 ± 0.19^{a}	1.18 ± 0.07^{bc}
E	32.2 ± 1.13^{b}	4.58 ± 0.22^{c}	6.64 ± 0.54^{c}	1.43 ± 0.06^{c}	6.71 ± 0.46^{b}	1.39 ± 0.10^{a}
	Micro elements (µg/g)					
	Fe	Mn	Zn	Cu	Na	Al
A	0.43 ± 0.03^{d}	0.155 ± 0.05^{b}	0.143 ± 0.01^{b}	0.261 ± 0.02^{a}	42.5 ± 2.73^{c}	22.6 ± 3.73^{c}
B	1.30 ± 0.89^{a}	0.031 ± 0.00^{c}	0.330 ± 0.02^{a}	0.105 ± 0.03^{a}	90.3 ± 6.18^{a}	20.4 ± 1.55^{c}
C	2.93 ± 0.31^{c}	0.268 ± 0.05^{a}	0.095 ± 0.03^{c}	0.011 ± 0.00^{c}	51.8 ± 2.83^{b}	30.3 ± 2.03^{a}
D	2.53 ± 0.12^{c}	0.262 ± 0.03^{a}	0.134 ± 0.01^{b}	0.025 ± 0.01^{c}	57.4 ± 4.79^{b}	25.0 ± 1.85^{b}
E	4.58 ± 0.75^{b}	0.052 ± 0.01^{c}	0.119 ± 0.01^{c}	0.056 ± 0.01^{c}	89.9 ± 7.33^{a}	28.0 ± 2.93a^{b}

Mean
SE. Different lowercase letters indicate significant differences at $p \leq 0.05$ as determined by the LSD test.
A = Chemical nutrient solution (control), B= Agro-fish liquid organic fertilizer, C= Nutrihumate liquid organic fertilizer, D = Rods-fert liquid organic fertilizer, E = Bio-green liquid organic fertilizer.

The NS exhibited significantly different levels of Ca, K, and Mg contents ($p \leq 0.05$). Organic NS C and D contained lower levels ($p \leq 0.05$) of K, Ca, and Mg than the control (Table 1). Similarly, all organically grown lettuce plants had lower levels of K, Ca, and Mg than the control ($p \leq 0.05$) (Table 2). Additionally, organically grown lettuce from NS C, D, and E had K levels comparable to the control. These results indicate that organic fertilizer feed reduced the Ca, K, and Mg contents in leaf tissues. In contrast, the P content was higher in organic fertilizers (ranging from 95 to 210 mg/g) compared with the inorganic control (26 mg/g) (Table 1), but this did not consistently translate into significant differences in P levels in the leaf tissues of plants grown in the organic versus inorganic solutions (Table 2). Notably, not all organic fertilizers resulted in the highest P levels in lettuce leaves; for example, the P levels in plants grown in NS B were not significantly different from the control. This is consistent with the results of Zandvakili et al. [34], who reported high P levels in lettuce grown with organic NS. Conversely, the Ca content in leaves of organically nourished plants (4.5–6.8 mg/g) was significantly lower than that in the control plants (11.9 mg/g) (Table 2). However, a previous study found no significant difference in Ca levels between organically and inorganically grown lettuce [34]. In this study, the organic NS had lower Mg content (2.7–5 mg/g) ($p \leq 0.05$) than the control (9.2 mg/g) (Table 1). Accordingly, Mg levels in organically grown plants were significantly lower than that in the control plants (Table 2). Organic fertilizers had higher Mn levels (90–122 µg/g) than the inorganic fertilizers (66 µg/g) (Table 1). Plants grown in the control solution had higher Mn content than those grown in NS B and E (Table 2), despite the latter having higher initial Mn levels. Lettuce plants grown with NS C and D accumulated higher levels of Mn (0.268 and 0.262 µg/g, respectively) compared to those supplied with NS B (0.031 µg/g) (Table 2). These results could be due to various factors affecting Mn absorption and accumulation, such as the level of organic matter [35]. A prior study [36] also reported significant Mn levels in *Brassica oleracea* var. *capitata* L. grown in organically fertilized medium. This investigation further revealed that Zn, Cu, Na, S, and Al levels varied significantly between plants fertigated with different fertilizer solutions. Plants grown with NS C, D, and E had lower levels of Zn and

Cu in their leaves than NS B and the control (Table 2), despite having higher initial Zn and Cu levels (except for Zn in NS E, which was slightly lower than the control). This could be due to the lower availability, weaker release, and slower absorption of organic nutrients compared to inorganic NS. Organic nutrients from animal and plant remnants must be transformed by bacteria in the substrate into forms useable by plants [37,38]. Additionally, factors such as the type of organic nutrition supply (e.g., particle size and composition), porosity, substrate moisture, and temperature, impact the rate of microbially-mediated mineralization [38]. We also found revealed that all organically grown plants had higher Na levels than the control. Furthermore, higher accumulations of Fe were observed in organically grown plants (2.5–10 μg/g) compared with the control (0.43 μg/g) (Table 2). The NS B and E treatments accumulated higher levels of Fe than the control and other treatments. Plants grown in NS B had the highest Fe content, despite NS B having the lowest Fe levels among the tested solutions. These findings suggest that nutrient imbalances in organic feeding solutions, due to NS composition, significantly impacted the elemental content of plant leaves at harvest.

3.2. *Effects on plant growth components*

The fertilizer source applied significantly affected the growth components of lettuce plants (Table 3 and Fig. 2). At 15 DAP, the fertilizer source had a significant impact on plant height but did not affect the number of leaves or their size and area; however, by 30 DAP, a significant effect was observed. At 30 and 60 DAP, plants grown with organic NS exhibited lower plant heights and leaf numbers ($p \leq 0.05$) compared to those grown with inorganic fertilizer; yet at 45 DAP, NS B did not differ significantly from the control in terms of plant height (Fig. 2). Among the organic NS, plants fertigated with NS B (fish source) and E (plant source) did not consistently show greater height or a higher leaf count at 30, 45, and 60 DAP compared to other organic NS (Fig. 2). In contrast, NS C (nutrihumate) yielded the lowest plant growth performance. This trend was also observed for leaf area, height, and width (Table 3). These results suggest that organic fertilizer-based NS likely provided fewer nutrients than inorganic fertilizer-based NS. According to Atkin and Nichols [39] and Ahmed et al. [19], organically derived NS can be used to produce lettuce plants using the nutrient film technique (NFT), albeit with slower growth than with standard inorganic NS. Similar findings were reported for hydroponically grown strawberries [20,40]. Moncada et al. [14], found that reducing the mineral NS content in favor of organic NS adversely affected the growth of soilless basil plants, including the stem diameter, leaf number, and area.

Table 3
Effect of NS in a zero-runoff auto-pot soilless system on the morphological characteristics of lettuce leaves 30 and 60 days after planting.

Solution code	Area (cm^2)	Length (cm)	Width (cm)	Stomata density/ mm^2 disc
	30 days			
A	53.80 ± 4.11^{a}	11.34 ± 1.13^{a}	6.79 ± 0.94^{a}	20.0 ± 1.76^{c}
B	34.14 ± 2.43^{b}	9.27 ± 1.21^{b}	6.07 ± 0.66^{b}	27.4 ± 1.38^{b}
C	27.12 ± 3.51^{c}	7.50 ± 0.87^{c}	4.57 ± 0.89^{c}	32.3 ± 2.11^{b}
D	26.67 ± 2.13^{c}	7.04 ± 1.31^{c}	4.64 ± 1.01^{c}	35.0 ± 1.74^{a}
E	30.15 ± 1.72^{b}	9.26 ± 0.84^{b}	5.48 ± 0.71^{bc}	28.4 ± 3.21^{b}
	60 days			
A	73.99 ± 5.10^{a}	15.91 ± 1.33^{a}	8.29 ± 1.01^{a}	29.0 ± 1.16^{b}
B	65.18 ± 3.22^{a}	15.04 ± 1.26^{a}	7.15 ± 0.88^{b}	31.1 ± 1.45^{b}
C	49.55 ± 1.81^{c}	12.90 ± 0.98^{b}	6.50 ± 1.02^{c}	35.9 ± 2.14^{ab}
D	46.63 ± 3.19^{c}	12.24 ± 1.43^{b}	6.52 ± 1.11^{c}	38.4 ± 2.15^{a}
E	58.23 ± 2.25^{b}	14.28 ± 1.12^{b}	7.20 ± 0.64^{b}	31.2 ± 1.78^{b}

Mean
SE. Different lowercase letters within each time interval indicate significant differences at $p \leq 0.05$, as determined by the LSD test.
A = Chemical nutrient solution (control), B= Agro-fish liquid organic fertilizer, C= Nutrihumate liquid organic fertilizer, D = Rods-fert liquid organic fertilizer, E = Biog-reen liquid organic fertilizer.

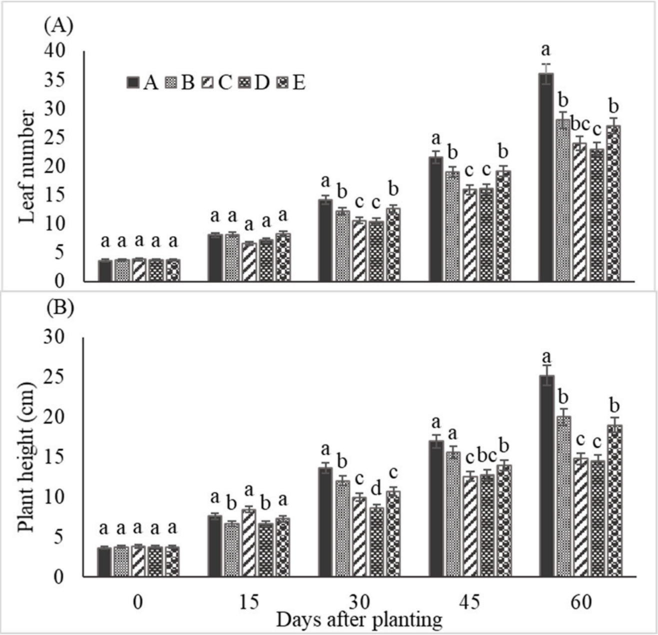

Fig. 2. Effect of nutrient solutions (NS) in a zero-runoff auto-pot soilless system on the leaf number (A) and plant height (B) of lettuce (cultivar 'Parris Island cos'). Mean ± SE with different lowercase letters within a time interval indicate significant differences at $p \leq 0.05$ as determined by the LSD test. A = Chemical nutrient solution (control), B= Agro-fish liquid organic fertilizer, C= Nutrihumate liquid organic fertilizer, D = Rods-fert liquid organic fertilizer, E = Biogreen liquid organic fertilizer.

Unlike water-soluble inorganic fertilizers, organic liquid fertilizers derived from animal and plant waste sources are not readily available to plants. They often require mineralization and transformation into plant-available forms by microorganisms that may be present in the substrate [15,41]. The rate of microbial-mediated mineralization varies significantly and is influenced by various parameters, including the nature of the organic nutrient supply, substrate temperature, moisture, and porosity [15,42]. A major drawback of using organic fertilizers is that the nutrient release rate may not meet the plant's nutrient requirements. To ensure adequate nutrient uptake by plants, continuous adjustment of the soilless system and growing media may be necessary to enhance the microbial activity and achieve an optimal decomposition rate of the organic fertilizer source [37,38].

3.3. *Stomatal density*

The source of NS significantly affected leaf stomatal density (Table 3). At 30 DAP, plants nourished with organic NS exhibited higher stomatal density (27–38/mm^2) than those fertilized inorganically (20–29/mm^2(p<0.05)) (Table 3). Differences were also observed among the organic fertilizer sources ($p \leq 0.05$). Specifically, at 30 DAP, plants treated with NS D solution (35 mm^2) had a higher leaf stomatal density than those receiving other organic NS. A similar trend was observed at harvest, with only plants grown in NS D showing higher stomatal density compared to the control (A), and plants fertigated with NS B and E (Table 3). These findings indicate that the fertilizer source markedly influenced plant physiology, resulting in variations in leaf stomatal density. Such differences may be associated with differences in nutrient availability and relative stress effects. Stomata are critical in modulating plant water use and carbon uptake [21,43], which significantly affects photosynthesis and plant growth [44,45]. Moncada et al. [14] recently reported that soilless-grown basil plants fertilized with organic liquid fertilizer exhibited substantially lower stomatal conductance than those that received chemical fertilizers. The number, distribution, size, form, and mobility of stomata are species-specific traits that can change in response to environmental factors such as nutritional deprivation [22]. Our results indicated that the stomatal density of soilless-grown lettuce leaves was influenced by the type of fertilizer. However, further research is required to fully understand how stomatal density affects lettuce growth and biomass production in soilless systems under nutrient scarcity.

3.4. *Chlorophyll and carotene contents*

Our results revealed significant variations in chlorophyll and carotene contents between plants nourished with different fertilizer sources (Table 4). Generally, at 30 DAP, the total chlorophyll content was higher ($p \leq 0.05$) in organically fertigated lettuce compared to the control, except for plants fertigated with NS C, which had a total chlorophyll content similar to the control (Table 4). Chlorophyll *a* and *b* levels were higher in plants fertigated with NS B and E than in the control plants. Conversely, the carotenoid content was consistently higher in all plants fertigated with organic fertilizer-based NS compared to the control (Table 4). At 60 DAP, the plants fertigated with NS B and C had lower total chlorophyll content than those fertigated with inorganic fertilizer, while the carotenoid content remained higher in organically fertilized plants compared to the control only when using NS C and E. These findings are consistent with the N content of NS (Table 1) and the N uptake by the plants. The amount of chlorophyll and carotenoids in a plant is generally proportional to its photosynthetic capacity, which is related to the N content of their leaves [46].

Table 4
Effect of NS in a zero-runoff auto-pot soilless system on the chlorophyll and carotene contents in lettuce leaves 30 and 60 days after planting.

Solution code	Total Ch (mg/g)	Ch *a* (mg/g)	Ch *b* (mg/g)	Carotene (mg/g)
	30 days after planting			
A	0.95 ± 0.11^{b}	0.69 ± 0.13^{b}	0.26 ± 0.01^{b}	0.17 ± 0.01^{b}
B	1.14 ± 0.12^{a}	0.74 ± 0.17^{a}	0.40 ± 0.02^{a}	0.24 ± 0.00^{a}
C	0.96 ± 0.14^{ab}	0.66 ± 0.05^{b}	0.30 ± 0.00^{b}	0.25 ± 0.02^{a}
D	1.03 ± 0.06^{a}	0.74 ± 0.08^{a}	0.28 ± 0.01^{b}	0.28 ± 0.03^{a}
E	1.16 ± 0.14^{a}	0.78 ± 0.04^{a}	0.38 ± 0.04^{a}	0.25 ± 0.03^{a}
	60 days after planting			
A	0.87 ± 0.06^{a}	0.53 ± 0.05^{a}	0.33 ± 0.02^{ab}	0.18 ± 0.00^{b}
B	0.74 ± 0.16^{b}	0.47 ± 0.03^{b}	0.27 ± 0.01^{b}	0.22 ± 0.03^{ab}
C	0.81 ± 0.16^{b}	0.50 ± 0.06^{ab}	0.30 ± 0.02^{b}	0.26 ± 0.06^{a}
D	0.93 ± 0.08^{a}	0.54 ± 0.03^{a}	0.38 ± 0.02^{a}	0.23 ± 0.03^{ab}
E	0.89 ± 0.07^{a}	0.57 ± 0.03^{a}	0.31 ± 0.01^{b}	0.27 ± 0.02^{a}

Mean
SE. Different lowercase letters within each time interval indicate significant differences at $p \leq 0.05$, as determined by the LSD test.
A = Chemical nutrient solution (control), B= Agro-fish liquid organic fertilizer, C= Nutrihumate liquid organic fertilizer, D = Rods-fert liquid organic fertilizer, E = Bio-green liquid organic fertilizer. Ch = chlorophyll.

Since N is required for chlorophyll synthesis, lower N levels in the NS may result in a reduced chlorophyll content, thereby diminishing photosynthesis and ultimately crop yield [47]. However, in the present study, lettuce fertigated with organic fertilizer exhibited a slightly higher chlorophyll content at 30 DAP compared to those grown with inorganic fertilizer; however, this did not correspond to increased plant growth (Fig. 2, and Table 3). A similar result was reported by Phibunwatthanawong and Riddech [48], who suggested that plant growth and yield are influenced by numerous variables, and factors other than chlorophyll content may have affected plant growth and crop yield.

3.5. *Plant fresh and dry weights*

The NS source significantly influenced lettuce stem diameter, root FW and DW, and total plant FW at harvest ($p \leq 0.05$) (Table 5). Lettuce fertigated with organic NS exhibited lower root FW and DW and total plant FW than those in the control group ($p \leq 0.05$). Among the organic solutions, NS B and E resulted in higher root FW and DW and total plant FW compared to plants fertigated with NS C and D (Table 5). The reduced fresh biomass in organically fertigated plants may be attributed to the differing mineral availability between the two nutrient sources. These findings are consistent with the composition of the tested NS (Table 1), and suggest that the diminished leaf area, stem diameter, and root FW and DW in plants receiving these NS were likely due to a lower availability of macronutrients such as Ca, K, and Mg in the solution (Table 1). Despite higher N and chlorophyll content (Tables 1 and 4), organically grown plants accumulated less biomass than inorganically grown plants (Table 5). This finding indicates that factors other than N availability constrained lettuce plant growth [49] and that a balanced nutrient composition is essential for many metabolic activities in lettuce plants. Williams and Nelson [26] observed that butterhead lettuce shoot FW and DW were smaller in plants grown with organic NS compared to those grown with inorganic NS. Ahmed et al. [19], also found that lettuce fertilized organically accumulated less biomass than those fertilized with inorganic fertilizer. Similarly, the use of liquid organic fertilizer negatively affected several basil plant traits, including their DW and FW [14]. Previous research has demonstrated how the composition of NS in soilless systems influences various plant growth parameters, such as leaf number, leaf area, crop quality, and marketable yield [50,51]. Furthermore, a mismatch between nutrient availability and plant nutrient requirements might limit production in organically fertilized plants compared to those fertilized with water-soluble inorganic NS fertilizers. Our findings reveal the impact of fertilizer source on plant

Table 5
Effect of NS of in a zero-runoff auto-pot soilless system on the stem diameter, fresh weight, and root fresh and dry weight of lettuce plants at harvest.

Solution Code	Stem diameter (mm)	Shoot FW (g)	Shoot DW (g)	Root FW (g)	Root DW (g)
A	16.8 ± 2.06^{a}	175.1 ± 4.28^{a}	7.63 ± 0.41^{a}	45.44 ± 2.57^{a}	8.59 ± 0.72^{a}
B	12.1 ± 1.34^{b}	159.8 ± 3.70^{b}	7.24 ± 0.37^{ab}	41.55 ± 3.16^{b}	8.17 ± 0.43^{a}
C	12.2 ± 1.22^{b}	143.6 ± 1.06^{c}	6.48 ± 0.19^{c}	34.61 ± 2.67^{c}	5.69 ± 0.86^{b}
D	13.1 ± 1.31^{b}	140.2 ± 2.03^{c}	6.35 ± 0.26^{c}	34.96 ± 2.81^{c}	$6.79 \pm$ 0.98b
E	12.3 ± 0.96^{b}	157.9 ± 1.11^{b}	7.10 ± 0.22^{b}	39.88 ± 1.64^{b}	7.52b $\pm 0.46^{a}$

Means
SE. Different lowercase letters indicate significant differences at $p \leq 0.05$, as determined by the LSD test.
A = Chemical nutrient solution (control), B= Agro-fish liquid organic fertilizer, C= Nutrihumate liquid organic fertilizer, D = Rods-fert liquid organic fertilizer, E = Bio-green liquid organic fertilizer.

biomass and lettuce yield in a hydroponic system.

3.6. The nitrate and ammonium content of lettuce leaves

Lettuce is a leafy vegetable that tends to accumulate high levels of nitrates, and since daily nitrate intake affects human health [51,52], its concentration at harvest is an important quality characteristic. Nitrate accumulation occurs when plant nitrate intake surpasses metabolic requirements. Our results showed that the nutrition solution formula significantly influenced the nitrate levels in plant leaves (Fig. 3). Lettuce plants fertilized with organic NS exhibited significantly lower nitrate levels ($p \leq 0.05$), ranging from <3.2 to 8.7 mg/kg DW, compared to the control group at 259.8 mg/kg DW. The use of organic solutions substantially reduced nitrate content in organically grown plants to below the EU's maximum permissible limits (European Commission, 2011) for lettuce (5000 mg/kg FW). Basil plants receiving 100% inorganic mineral NS had significant nitrate levels in their leaves; however, these levels decreased by 88% when the mineral component was reduced to 75% in favor of organic fertilizer in NS [14]. Typically, nitrate accumulation in the petioles and leaves of organic cultivation systems is lower than that in inorganic systems because the N content in organic fertilizers is primarily in the NH_4N form, not NO_3N, which likely accounts for the reduced NO_3N levels in the lettuce [26]. Lettuce is a major source of dietary nitrate for humans [53]. Previous studies have shown a strong correlation between nitrate levels in leafy greens and N application, suggesting that N supply is the main factor driving nitrate accumulation in vegetable crops [54].

Regarding ammonium accumulation in leaves, significant variations were observed among the different plants. The highest NH_4

N accumulation occurred with NS B and E (164.23 and 174.30 mg/kg, respectively), while the lowest occurred with NS C and D (127.2 and 121.5 mg/kg, respectively) (Fig. 3). Although excessive NH_4

N quantities can influence plant growth [34], in this investigation, no detrimental impacts were observed as its concentration was insufficient to affect growth. Therefore, ammonium accumulation may result from the N metabolism in lettuce leaves.

3.7. Total phenolic, flavonoid, and antioxidant activity

Organically grown lettuce exhibited a higher total phenolic content than the control (Fig. 4A). Lettuce treated with the NS E and C demonstrated significantly greater total phenolic content (3.36 and 3.22 g/100 g DW, respectively) ($p \leq 0.05$) compared to the control (2.28 g/100 g DW) and other NS treatments (Fig. 4A). Conversely, NS D solution yielded the highest total flavonoid content (2.68 g/100g DW), while NS B produced the lowest (1.00g/100 g DW) compared with the control and other NS (Fig. 4B). The synthesis of phenolic compounds is primarily regulated by two enzymes of the shikimic acid pathway: tyrosine ammonium lyase and phenylalanine ammonium lyase [55,56]. These findings suggest that the source of NS significantly affects the phenolic and flavonoid contents of lettuce leaves.

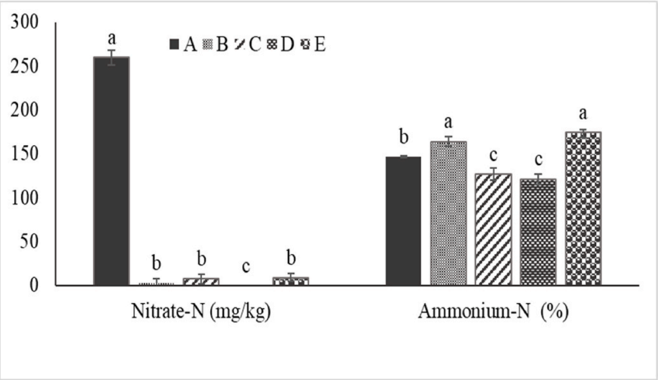

Fig. 3. Effect of NS composition on leaf nitrate and ammonium content of lettuce (cultivar 'Parris Island cos') grown in a zero-runoff auto-pot soilless system. Mean ± SE with different lowercase letters indicate significant differences at $p \leq 0.05$ as determined by the LSD test. A = Chemical nutrient solution (control), B= Agro-fish liquid organic fertilizer, C= Nutrihumate liquid organic fertilizer, D = Rods-fert liquid organic fertilizer, E = Bio-green liquid organic fertilizer.

The results of the antioxidant activity obtained from the DPPH scavenging assay were higher in plants produced from NS C and D than in those treated with inorganic fertilizer (Fig. 4C). Plants from the NS E treatment exhibited slightly lower antioxidant activity (53.01%) (Fig. 4C). The ABTS radical scavenging percentage displayed a similar trend (Fig. 4D). Increased levels of chlorophyll, carotenoids, phenolics, and flavonoids influenced the antioxidant activity in the plant, consistent with previous reports [17,57]. Therefore, the higher levels of phenolics, flavonoids, and total chlorophyll detected in lettuce plants grown with the organic solution may be the cause of the increased antioxidant activity observed in these plants (Table 4 and Fig. 4A and B). The increased level of antioxidant activity is crucial as it enhances the nutritional value of the lettuce. Thus, despite lower production, the application of organic fertilizers may improve human nutrition.

4. Conclusions

The present findings indicate that the performance of organic liquid nutrient solutions (NS) varies greatly depending on the fertilizer source. All organic NS produced lettuce plants with lower yields as compared to those grown with inorganic solutions. Nutrient solutions B (agro-fish) and E (bio-green) performed better than NS C (nutrihumate) and D (rods-fert). Interestingly, organic NS produced lower nitrate levels and higher total chlorophyll, carotene, phenolics, and flavonoid levels in organically grown lettuce, suggesting a higher antioxidant capacity and nutrition value for human consumption. Lettuce plants grown in organic NS exhibited significantly lower levels of Ca, K, and Mg but higher P levels in the lettuce leaves. This suggests that, while organic agriculture is more sustainable, and organic products are increasingly recognized for their environmental and human health benefits, the use of organic NS in hydroponic systems presents challenges. It is critical to modify the components used in hydroponic systems to accommodate organic fertilizers, considering factors that affect nutrient availability. It is recommended that organic fertilizers be carefully selected, and that biofertilizers (microorganisms) be added. Additionally, information on the NS mineral composition and nutrient availability should be provided and their water-soluble composition should be considered. By addressing these factors, a balanced organic NS specifically designed for hydroponic systems can be developed to optimize production. Further research is required to improve the efficacy of such systems and to understand their significance for food security, nutrition, and overall sustainability.

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CRediT authorship contribution statement

K.S.S. Alneyadi: Writing – original draft, Methodology, Investigation, Formal analysis. **M.S.B. Almheiri:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **N. Tzortzakis:** Writing – review & editing, Validation, Supervision, Methodology, Data curation, Conceptualization. **F. Di Gioia:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Z.F.R.**

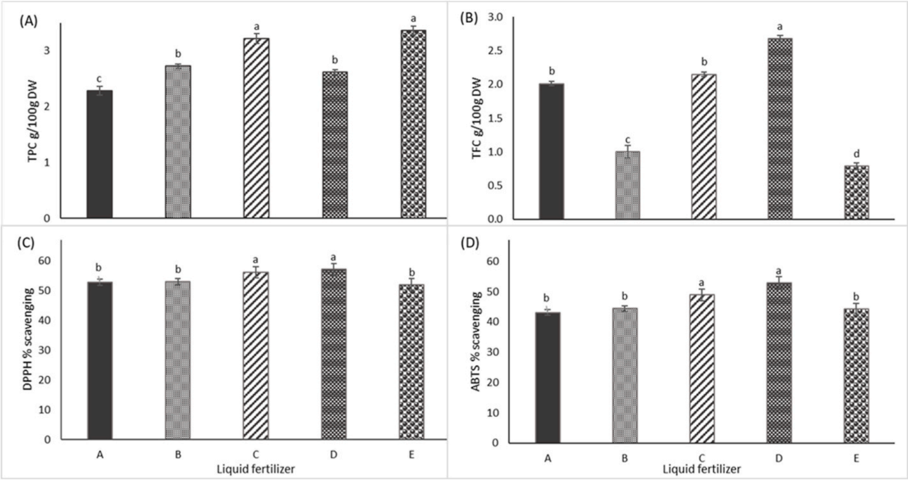

Fig. 4. Effect of NS on total phenolics content (A), total flavonoid content (B), and antioxidant activity (C and D) of lettuce (cultivar 'Parris Island cos') leaves grown in a zero-runoff auto-pot soilless system using organic and inorganic liquid fertilizers. Mean ± SE with different lowercase letters indicate significant differences at $p \leq 0.05$ as determined by the LSD test. A = Chemical nutrient solution (control), B= Agro-fish liquid organic fertilizer, C= Nutrihumate liquid organic fertilizer, D = Rods-fert liquid organic fertilizer, E = Bio-green liquid organic fertilizer.

Ahmed: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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