Comparison of nutrient use efficiency, antioxidant assay, and nutritional quality of butter-head lettuce (*Lactuca sativa* L.) in five cultivation systems

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Abstract: A greenhouse experiment was conducted to evaluate the comparison of nutrient use efficiency, antioxidant assay, and nutritional quality of butter-head lettuce in five cultivation systems. For this experimental study, a split-boxes experimental arrangement in a completely randomized design (CRD) with three aeroponics systems (high pressure (T_1), low pressure (T_2), and ultrasonic nozzle (T_3)), hydroponic system (T_4), and conventional cultivation system (T_5) was designed. The analyses of variance (ANOVA) were employed to assess yield, chlorophyll content, relative water content (RWC), nutrient use efficiency (NUE), 2,2-Diphenyl-1-picrylhydrazyl (DDPH), Hydrogen Peroxide (H_2O_2), Ferric Reducing Antioxidant Power (FRAP), total phenolic (TPC), flavonoid content (TFC) and nutritional quality of butter-head lettuce. The experimental comparative results indicated that the shoot and root (fresh and dry) weight, root-to-shoot ratio, chlorophyll contents, CWC, NUE, DDPH, H_2O_2, FRAP, TPC, TFC, nitrate, vitamin C, and protein level were significantly (p<0.05) higher in T_2 treatment, and the values were significantly (p<0.05) lower in T_1 followed by T_5 treatment. Moreover, as mentioned earlier, the median values of the above-mentioned parameters were measured in aeroponics systems with low-pressure nozzle (T_3). It also found a strong positive correlation (r>0.7) among the Shoot (FW), Chlorophyll, RWC, NUE, among TPC, TFC, DDPH, H_2O_2, and FRAP. This study demonstrates that butter-head lettuce grown in aeroponics systems with high-pressure nozzles may serve as a potential dietary source and is rich in natural antioxidants.

Keywords: aeroponics, high-pressure nozzle, phenolic content, antioxidants, lettuce

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1 Introduction

The COVID-19 pandemic is a global health crisis already having devastating impacts on the world economy, both directly and through necessary measures to contain the spread of the disease. These impacts are also being felt by the food and agriculture sector[1,2]. According to the World Health Organization, the worst impact has yet to come[3,4]. It has also disrupted many agriculture activities, with outbreaks closing numerous facilities worldwide[5]. The lockdown and movement restrictions have reduced the availability of labor, which directly affected outdoor agricultural activities[6]. In addition, given the high degree of uncertainty in the virus and its evolution, the future may pose threats to food security and nutrition, including possible reductions in food productivity and production, depending on the severity and duration of the pandemic and measures to contain the pandemic.

The negative impact of the pandemic on agriculture and food security may be reduced by changing the pattern of cultivation systems. People have recently paid more attention to plant production in closed factories, vertical farms, and indoor planting modules[7]. These production systems can provide a healthy environment for growing food and vegetables full of nutrients because people need healthy eating to support the immune systems. The familiarity with indoor farming and the consumption of leafy vegetables as a nutritional source is a necessity of the present era[8].

Lettuce (*Lactuca sativa L.*) is a leafy green vegetable consumed in large quantities in the world[9], with an annual output of about 27 million tons[10]. It is an excellent source of vitamins and phytonutrients and contains secondary metabolites such as phenolic compounds, flavonoids, phenolic acids, carotenoids, ascorbic acid, and folate, which can enhance health promotion[11]. These phenolic compounds produce antioxidant activity and provide free radical scavenging ability. Polyphenols can prevent cancer and cardiovascular diseases[12]. Nowadays, it has become the interest of many researchers for conventional and organic farming in the open field, soil cultivation in greenhouses, and soilless cultivation in controlled environmental experiments. Different cultivation systems may have distinctive characteristics in production and may affect the characteristics of this leafy green vegetable[13].

Soilless cultivation in a controlled environment has many advantages over other cultural systems that are affected by climatic conditions, soil fertility, and soil-borne diseases; it does not require

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large spaces and intensive labor. Using soilless cultivation for better production, hydroponics, and aeroponics cultivation is the most appropriate modern horticulture soilless techniques[14].

Hydroponics can be defined as the cultivation of crops in water and nutrient solutions with or without the addition of growth media[15,16]. To provide physical support to the plant's roots, artificial and natural media, for example, rock wool, coco peat, clay particles, peat moss, gravel or pots, coconut shells, and sawdust could be used[17,18]. Now a day, hydroponic systems are mainly used for education, research, personal gardening, vegetables (tomatoes, potatoes, spinach, and lettuce), fruits (strawberries and cucumbers), roses, and medical plants. Moreover, aeroponics is a closed air and water/nutrient ecosystem, which can promote the swift growth of plants with virtually no water, soil, or medium[19-21]. This system is economical in using fertilizers and saves water because of the reuse of the nutrient solution. Aeroponics systems save 98% of water, 60% of nutrients, and 100% of pesticides and herbicides[21-23].

According to the aforementioned, this study was intended to compare the nutrient use efficiency, relative water content, chlorophyll content, 2,2-diphenyl-1-picrylhydrazyl, hydrogen peroxide, ferric reducing antioxidant power, phenolic, flavonoid content, and nutritional quality of butter-head lettuce in three various types of aeroponics systems (high pressure, low pressure, and ultrasonic), hydroponics, and conventional (soil) cultivation system.

2 Material and methods

2.1 Experimental site

A greenhouse experiment was conducted during the growing season of January 2020 in the Experimental Station of Jiangsu University, Zhenjiang, China. The site is located at 33°57'54.4536"N and 118°16'15.5568"E. The climate data in the greenhouse was obtained from an automatic weather station (Hobo U12-012, Onset Computer Corp.) placed in the center of the experimental station. The measured maximum average temperature and relative humidity were (21.13±0.32)°C and (63.43±7.13)%, and temperature and minimum relative humidity were (7.65±0.89)°C, and (42.73±3.73)%, respectively, in the greenhouse.

2.2 Experimental design

A split-boxes experimental arrangement in a completely randomized design (CRD) with five treatments (T1: aeroponic system with ultrasonic atomizer, T2: aeroponic system with high-pressure atomizer, T3: aeroponic system with low-pressure atomizer, T4: hydroponics system, and T5: control (soil)) was used with three replications. Previously designed aeroponics systems[24] were utilized for this experiment. The average droplet size of ultrasonic, high-pressure, and low-pressure atomizers was 4.89 μm, 11.24 μm, and 26.35 μm, respectively. In this experimental study, the flooded type of hydroponic system in the blue high-density polyethylene (HDPE) containers with a volumetric capacity of 20 L was used. The control treatments were conducted in plastic pots with a capacity of 5 L. The air-dry clay textural soil (45.7%, silt 37.8%, and sand 16.5%) of 5 kg weight was filled into each pot.

2.3 Plant material and nutrient solution

The Butter-head Lettuce (*Lactuca sativa* L.) seeds were obtained from the Nanjing Ideal Agricultural Science and Technology Co., Ltd. Jiangsu, China. The seeds were planted in polystyrene trays (EPS) with 90 cells containing equal quantities of perlite material. To have good plant growth for the initial seedling, all cultural practices were continued, such as natural sunlight, proper watering, and thinning. Furthermore, Hoagland's full strength modified the chemical composition of the nutrient solution with major and minor nutrients such as Nitrogen (N), Potassium (K), Calcium (Ca), Phosphorous (P), Magnesium (Mg), Boron (B), and Zinc (Zn) as shown in table 1 were used throughout the experiment[16,25].

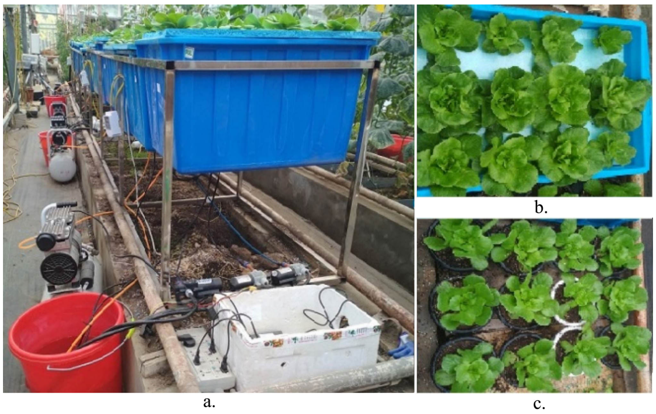


Figure 1 Different aeroponics systems (a), hydroponics system (b), and conventional system (c)

Table 1 Nutrient concentration for lettuce grown in the different cultivation systems

Nutrients	Concentration/mg·L^{-1}	Nutrients	Concentration/mg·L^{-1}
N	210	B	0.4
K	200	Zn	0.35
Ca	235	Mg	45
P	56		

2.4 Physico-chemical properties of soil analysis

To analyze the physicochemical properties of soil, the samples were dried in the open air, grinded, and passed through a 2 mm mesh. Soil pH and ECe were measured as past extract with the help of a digital pH meter and ECe meter (ProfiLine pH 3110, WTW, Weilheim, Germany) with accuracies of 0.01 μS/cm, and 0.1 mS/cm, cation exchange capacity (CEC), and texture (hydrometer method) by hydrometer method[26].

2.5 Chlorophyll Content

The chlorophyll content was measured with the help of a chlorophyll meter (SPAD-502, Konica Minolta Sensing Inc., Osaka, Japan) after 10 d, 20 d, 30 d, and 40 d from a young leaf of butter-head lettuce under each system[27].

2.6 Nutrient use efficiency (NUE)

The nutrient use efficiency was observed after 10 d, 20 d, 30 d, and 40 d with the help of Equation (1)[26,28,29].

$$\mathrm{NUE}=\frac{\mathrm{CY}}{\mathrm{CNU}} \tag{1}$$

where, CY is the crop yield, g/plant; CNU is the crop nutrient uptake, cm^3/plant; Crop nutrient uptake was demonstrated as a purpose of daily radiation (DR) and leaf area index (LAI) intercepted by the crop canopy from Equation (2)[30].

$$\mathrm{CNU}=\frac{\left(\frac{b_1}{24}\times 3600\right)\cdot\left(1-e^{-k_1\cdot LAI}\right)\cdot\left(\frac{DR}{\vartheta\times 10^5}+b_2\right)}{\text{No. of plants per squiremeter}\times 1000} \tag{2}$$

where, LAI is leaf area index, m^2/m^2; *DR* is daily radiation, W/m^2; b_1 and b_2 are the empirical constants; λ is the dormant warmness of water evaporation, MJ/kg; k_1 is the canopy light extinction coefficient; Daily radiation was calculated according to local weather station data, which is located in the center of the greenhouse. The leaf area index is calculated from Equation (3)[31].

$$LAI = \frac{LAI_{max}}{1 + k_2 e^{(-k_3 t)}} \tag{3}$$

where, LAI_{max} is the maximum leaf area index, m²/m²; k_2 and k_3 are the coefficients of the growth functions; t is the plant age, d.

The parameters used in Equations (1)-(3) were obtained from the literature by Massa et al.[30]

2.7 Biomass yield (fresh & dry)

An electronic weighing machine measured the shoot and fresh root biomass of butter-head lettuce in all treatments with an accuracy of 0.1 mg. The fresh weighed samples were inserted carefully into transparent paper envelopes and dried in the oven at 85°C for 72 h, and the same procedure was applied for the measurement of dry weight.

T_1 T_2 T_3 T_4 T_5

Figure 2 Growth of lettuce plants in different cultivation systems

2.8 Measurement of relative water content (RWC)

The relative water content RWC of the lettuce leaves was determined as[32]:

$$RWC = \frac{FW - DW}{TW - DW} \times 100\% \tag{4}$$

where, FW is the fresh weight; DW is the dry weight; TW is the turgid weight of the leaf after equilibration in distilled water for 24 h.

2.9 Preparation of antioxidant extraction

In order to prepare the antioxidant extraction, twenty-seven samples (seven from each system) of butter-head lettuce were collected. A temperature-controlled ultrasonic device (KQ-250DB, Kunshan Ultrasonic Co., Ltd., China) was used to perform a plant ultrasound-assisted extraction (UAE) method for the extraction. Moreover, 10 g of dried lettuce mass was inserted in a glass, and the samples were extracted with 75 mL (95% v/v) ethanol for 10 minutes at 40°C; the process of extraction was repeated five times. After extraction, the mixture was put into a separator for 15 minutes to remove the insoluble, and the remaining extract was collected for further analysis.

2.10 Total phenolic and flavonoids contents determination

A UV-1200 spectrophotometer (SP-75, Shanghai spectrum instruments Co., Ltd, China) was utilized for the modified Folin-Ciocalteu's method to determine the total phenolic content[33] and total flavonoid content[34,35]. The data was expressed as mg/g of garlic acid equivalent to mg/g (mg GE/g) of dry extract.

2.11 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH was evaluated as described by Brand-Williams et al.[36]. Briefly, the extraction solution (100 μg/mL) in 2 mL of methanol was added to 2 mL of DPPH (0.1 mM) solution. Left the mixture in a dark area for 30 min, then measured the absorbance at the maximum λ_{max} 517 nm, with equal amounts of DPPH and methanol as a blank.

The following formula was used to estimate the percentage of DPPH:

$$RSA(\%) = \frac{ADPH - AS}{ADPH} \times 100\% \tag{5}$$

where, ADPH is the control absorbance; AS is the test extracts absorbance.

2.13 Hydrogen peroxide (H_2O_2) scavenging activity

In order to determine the free radical scavenging activity of each extract, the H_2O_2 method was used, as described by Bozin et al.[37] Further, 2 mL methanol extract (100 μg/mL) was added to 4 mL H_2O_2 (20 mM) solution in phosphate buffer with pH value of 7.4. However, After 10 min, measured the absorbance at a maximum of 230 nm against the phosphate buffer blank solution. The following formula was used to calculate the scavenging of H_2O_2 in %:

$$\text{Scavenging of } H_2O_2(\%) = \frac{A_0 - A_1}{A_0} \times 100\%$$

where, A_0 is the control absorbance (phosphate buffer with H_2O_2); A_1 is the test extracts absorbance.

2.14 Ferric reducing antioxidant power (FRAP) assay

The reducing ability was determined by Benzie and Strain[38] and modified method Fe^{3+} to Fe^{2+} by Raza et al.[39] The calibration curve of $FeSO_4$ (y=0.011x−0.01, R^2=0.999) was used to present the results as μmol of Fe (II) dry weight of lettuce per gram.

2.15 Nutritional quality

Three samples from each treatment were lyophilized with liquid nitrogen at –80°C for the determination of Nitrate, Vitamin C, and soluble protein content. The chromatography (ICS 90 DIONEX, US), 2,6-dichloroindophenol dye (AOAC 2000), and Coomassie brilliant blue G-250 dye methods were used to determine the nitrate, vitamin C, and protein level[40-42] respectively in the lettuce leaves.

2.16 Statistical analysis

SPSS Statistics 19.0 and Microsoft Excel 2016 were used to analyze the data. The variance analyses (ANOVA) were employed to assess the yield, nutrient use efficiency, antioxidant properties, and total phenolic and flavonoid content of butter-head lettuce cultivated in aeroponics, hydroponics, and conventional techniques. The correlation of the proposed parameters was tested by regression analyses and Duncan's multiple tests at p≤0.05 significance level.

3 Results

3.1 Soil Physico-chemicals properties

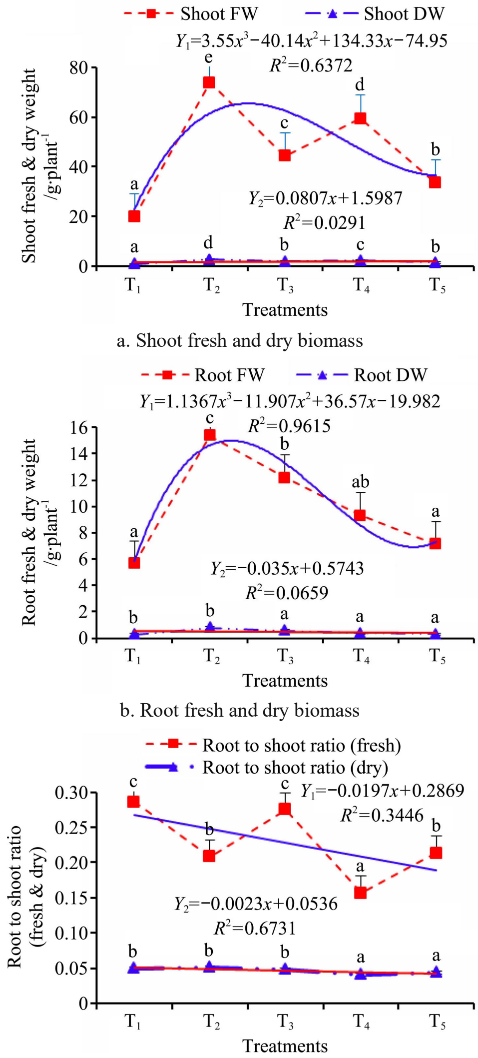
Table 2 lists the results of soil physio-chemical properties of control treatment such as texture, CEC, EC, and pH.

Table 2 Physico-chemical properties of the soil used in the experiment

Texture	CEC/meq·(100 g)$^{-1}$	ECe/dS·m^{-1}	pH
Clay	41.8	1.2	6.8

3.2 Biomass yield (fresh and dry)

The comparative average results of fresh and dry biomass of shoot, root, and root-to-shoot ratio are depicted in Figures 3a-3c. The statistically analyzed results indicated that butter-head lettuce fresh and dry mass of shoot and root were significantly (p<0.05) higher in aeroponics systems with high-pressure nozzle (T_2) over aeroponics system with low pressure (T_1) and ultrasonic nozzle (T_3), hydroponics system (T_4) and conventional farming (T_5). The maximum and minimum shoot fresh and dry weight ((73.74±2.42) g/plant and (2.73±1.17) g/plant) and ((33.40±0.89) g/plant and (0.82±0.57) g/plant) were observed in T_2 and T_1 treatments, respectively. Furthermore, the higher root biomass of (15.37±1.83) g/plant and (5.64±0.56) g/plant were observed in T_2 treatment before and after oven drying. However, the lowest root weight (fresh and dry) was measured in the T_1 treatment. The shoot and root (fresh and dry) weight in all treatments showed a descending trend as T_2, T_4, T_3, T_5, T_1 and T_2, T_3, T_4, T_5, T_1, respectively. The statistical analysis results of the root/shoot ratio revealed a non-significant effect (p>0.05) and mix phenomenon of increasing and decreasing observed in all treatments. The maximum and minimum root/shoot ratio (fresh) of (0.28±0.13) and (0.16±0.09) were achieved in T_1 and T_4 treatments, respectively. However, the maximum and minimum root/shoot dry ratio was observed in T_2 and T_4 treatments. The root/shoot ratios (fresh and dry) in all treatments showed a descending order as T_1, T_3, T_2, T_5, T_4 and T_2, T_1, T_3, T_5, T_4, respectively.



a. Shoot fresh and dry biomass

b. Root fresh and dry biomass

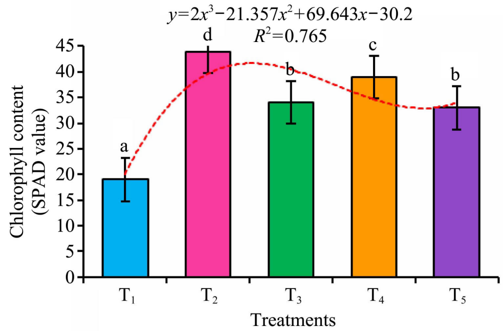
c. Root/shoot radio (fresh and dry)

Note: Different letters denoted significant differences between treatments at p<0.05, and vertical bars indicate the mean value's standard error (SE). Y_1 means fresh and Y_2 means dry.

Figure 3 Shoot fresh and dry biomass, root fresh and dry biomass, and root/shoot ratio (fresh and dry) observed in all treatments

3.3 Chlorophyll content

The statistically analyzed results of chlorophyll content in all treatments are presented in Figure 4. The ANOVA and Duncan's test results showed significant (p<0.05) differences in all treatments. The significantly (p<0.05) higher and lower chlorophyll content values of 44.67±1.34 and 19.43±1.87 were measured in the aeroponics system with high-pressure nozzle (T_2) and aeroponics system with ultrasonic nozzle (T_1). The results of chlorophyll content in all treatments showed a descending trend order as T_2, T_4, T_3, T_5, and T_1.



Note: Different letters denoted significant differences between treatments at p<0.05, and vertical bars indicate the mean value's standard error (SE). The same as below.

Figure 4 Chlorophyll content measured in lettuce leaves in all treatments

3.4 Relative water content (RWC)

The different cultivation systems showed a non-significant (p>0.05) effect except for the aeroponic system with a high-pressure nozzle on relative water content. The results in Figure 5 reveal that the aeroponics system with high-pressure nozzle had higher values (81.46%) than the hydroponics system (75.49%), the aeroponic system with low-pressure nozzle (73.72%), conventional technique (71.09%), and ultrasonic nozzle (67.37%). It was observed that only the T_2 treatment was significantly (p<0.05) different from than T_1, T_3, T_4, and T_5 treatments, respectively.

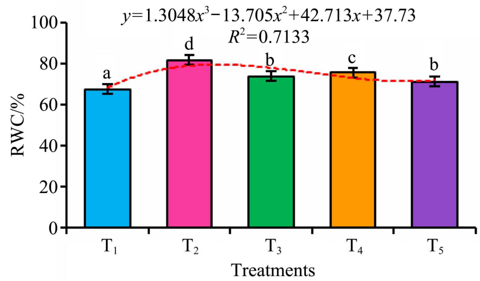


Figure 5 RWC observed in lettuce leaves in all treatments

3.4 Nutrient Use efficiency (NUE)

The statistically analyzed results of nutrient use efficiency are depicted in Figure 6. It was observed that there was a significant (p<0.05) difference in all treatments. The maximum and minimum NUE of 2.95 kg/m^3 and 0.82 kg/m^3 were observed in T_2 and T_1 treatments, respectively. The results also revealed that the NUE significantly increased by 72.10%, 37.49%, 12.53%, and 62.23% in aeroponics systems with high pressure nozzle in T_2 than in T1, T_3, T_4, and T_5 treatments, respectively.

3.5 Correlation between FW (yield), chlorophyll content, RWC, and NUE

The correlation analysis results of fresh weight (yield), chlorophyll content, relative water content, and nutrient used efficiency are listed in Table 3. The results reveal that the parameters had a strong positive correlation with each other. More importantly, the strongest correlation of r=0.99 was observed between chlorophyll and nutrient use efficiency.

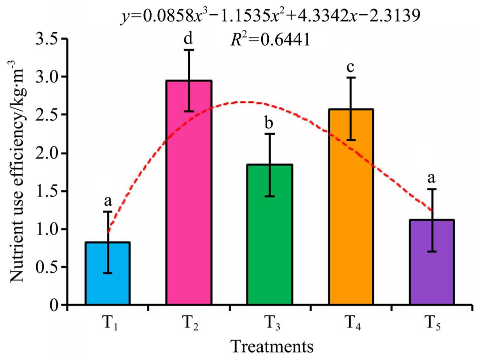


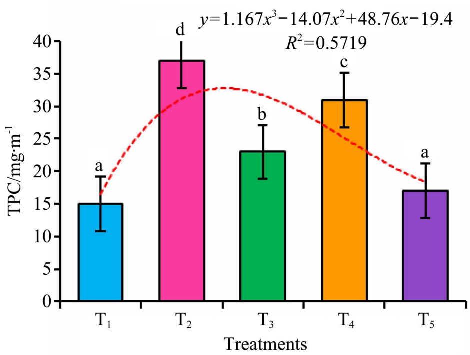
Figure 6 NUE observed 40 d after transplant (DAT) of lettuce in all treatments

Table 3 Correlation between SFW (yield), Chlorophyll content, RWC, and NUE

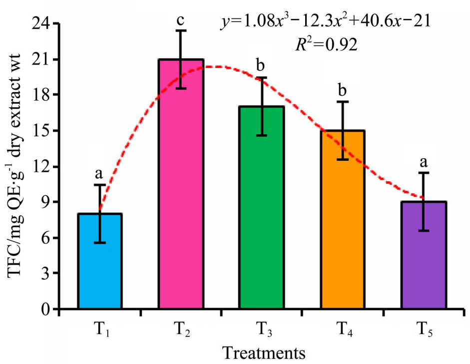
Item	Shoot FW/g	Chlorophyll	RWC
Chlorophyll	0.94		
RWC	0.98	0.93	
NUE	0.87	0.99	0.86

3.6 Total phenolic (TPC) and flavonoid contents (TFC)

The ANOVA and Duncan's test results of TPC and TFC of butter-head lettuce presented significant (p<0.05) differences and greatly varied in all treatments (Figures 7a and 7b). It was observed that the phenolic and flavonoid content was significantly (p<0.05) higher in T_2 and the lowest in T_1 treatment than T_3, T_4, and T_5 treatments. The TPC presented increase of 59.5%, 37.8%, 16.2%, and 54.1% in T_2 than T_1, T_3, T_4, and T_5 treatments respectively. Moreover, the TFC showed increase 61.09%, 19.05%, 28.57%, and 70.59% in T_2 than T_1, T_3, T_4, and T_5 treatments correspondingly.



a. TPC



b. TFC

Figure 7 TPC and TFC values for the dried samples of lettuce in all treatments

3.7 DPPH radical scavenging activity

The statistical analysis results of DPPH radical scavenging activity are presented in Figure 8. The ANOVA and Duncan's test showed significant (p<0.05) differences in all treatments. It can be seen from the figure that the aeroponic system with high pressure nozzle possessed the highest values of 76.8% followed by the hydroponic system (63.4%), and aeroponic system with low pressure nozzle (52.7%). Additionally, with the smallest difference the T_5 (46.7%) and T_1 (45.3%) had the lowest values of DPPH radical scavenging. The trend followed by the treatments was as T_2>T_4>T_3>T_5>T_1, respectively.

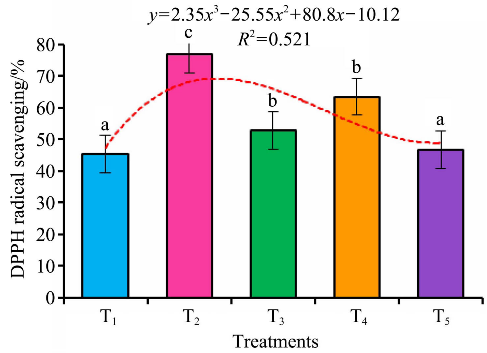


Figure 8 DPPH radical scavenging values for the dried lettuce samples in all treatments

3.8 H_2O_2 scavenging activity

The ANOVA and Duncan's test results of H_2O_2 scavenging activity are depicted in Figure 9. The results reveal that the different systems showed significant (p<0.05) differences in all treatments. The maximum values (61.28%) were observed in aeroponics systems with high-pressure nozzle (T_2) followed by hydroponics systems (53.12%), aeroponic systems with low-pressure nozzle (48.67%), and conventional technique (32.5%). However, the lowest values were measured in an aeroponics system with a low-pressure nozzle.

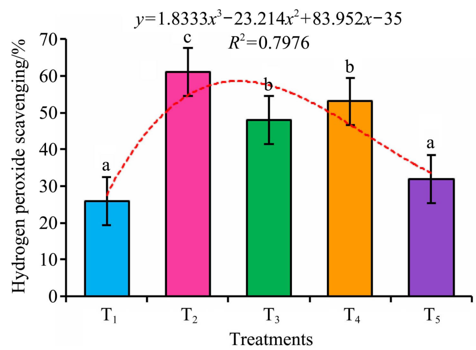


Figure 9 H_2O_2 scavenging values for the dried samples of lettuce in all treatments

3.9 FRAP assay

It can be seen from figure 10 that there is a significant (p<0.05) difference in all treatments of FRAP values of butter-head lettuce. The ANOVA and Duncan's test values presented that the highest values (1.31 μmol/g)) FRAP was observed in the T2 treatment, and the lowest values of 0.89 μmol/g were measured in the T_5 treatment. The FRAP values trend followed by the treatments were T_2>T_4>T_3>T_5>T_1, respectively.

3.10 Correlation between TPC, TFC, and antioxidants assay

The analyzed results of correlations between TCP, TFC, and antioxidants properties are listed in Table 4. It can be seen from the results that a strong positive correlation occurs in TPC, TFC, DDPH, H_2O_2, and FRAP. The maximum r=0.99 was estimated in TPC and DDPH.

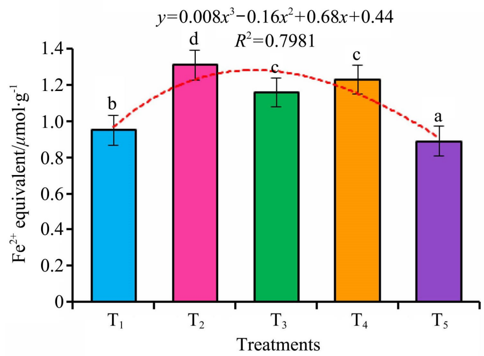


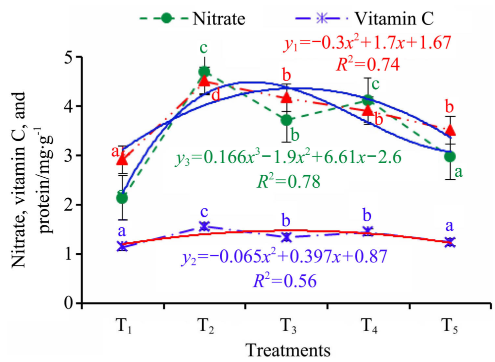
Figure 10 FRAP values for the dried samples of lettuce in all treatments

Table 4 Correlations between TCP, TFC, DDPH, H_2O_2, and FRAP

Item	TPC	TFC	DDPH	H_2O_2
TFC	0.89			
DDPH	0.99	0.87		
H_2O_2	0.96	0.96	0.92	
FRAP	0.95	0.94	0.91	0.96

3.11 Nutritional quality

The statistically analyzed results of nitrate content, vitamin C, and protein level are presented in Figure 11. The ANOVA and Duncan's multiple tests indicated a significant (p<0.05) difference in all treatments. The highest values of nitrate, vitamin C content, and protein level (4.7, 1.56, and 4.5 mg/g) and lowest values were intended in aeroponics systems with an ultrasonic atomizer (T_1) followed by a conventional system (T_5). However, the median values of 3.7, 1.34, and 3.92 mg/g were measured in T_3, T_3, and T_4 treatments, respectively.



Note: Different letters denoted significant differences between treatments at p<0.05, and vertical bars indicate the mean value's standard error (SE)

Figure 11 Nitrate, vitamin C, and protein level of lettuce leave in all treatments

4 Discussion

Leafy vegetables have a significant role in diets and food security, especially during the current COVID-19 pandemic situation around the globe to strengthen the immune system. The familiarity with indoor farming and the consumption of leafy vegetables with great antioxidant properties as well as nutritional sources is a necessity of the present era[8]. The literature review presented a few studies about the different aeroponics systems compared with hydroponics systems and conventional techniques to assess the nutrient use efficiency, crop water content, antioxidant properties, and total phenolic and flavonoid contents of leafy vegetables. The statistically analyzed results indicated that the different cultivation systems had a significant (p<0.05) effect on the shoot and root (fresh & dry) weight of butterhead lettuce. The shoot dry and fresh weight was higher in the aeroponics system with high-pressure nozzle (T_2) followed by a hydroponics system (T_4). The lowest values of shoot fresh and dry weight (g/plant) were found in an aeroponics system with a low-pressure nozzle (T_1) followed by a conventional technique (T_5). Almost, the same situation of root fresh and dry weight was found in all treatments except T_3 and T_4 treatments. The aeroponics systems with low-pressure nozzles (T_3) had significantly higher values of root fresh and dry weight than the hydroponics system (T_4). The root/shoot ratio (fresh and dry) was the interesting and most remarkable difference in the five systems. The significantly higher root-to-shoot ratio was evaluated in the T_3 treatment. The cultivation system and nutrient absorption by the roots significantly can affect crop growth and productivity[43], and the plant nutrient absorption system was better in a good root aeration zone than the no root aeration system (hydroponics & soil)[44]. Another researcher concluded the same kind of results that the root-to-shoot ratio of lettuce cultivated in an aeroponics system was two to three times greater than that cultivated in a hydroponics system and higher than that of subtract culture due to the better circulation of air in the root zone chamber[45].

The results of ANOVA and Duncan's complex difference (p<0.05) showed that the cultivation system had a significant difference in chlorophyll values of butter-head lettuce. The significantly higher chlorophyll values were observed in an aeroponics system with a high-pressure nozzle (T_2). The values of 11.71%, 22.75%, 24.10%, and 36.49 were lower in T_4, T_3, T_5, and T_1 treatments respectively than T_2 treatment. Lakhiar et al.[21] reported similar results that the highest chlorophyll content was observed in air-assisted atomizers than in air-less and ultrasonic nozzle. The difference in chlorophyll values among the same variety can be affected by the factors such as time of harvest, availability of nutrients, environmental conditions, and cultivation systems[46]. Lin et al.[47] reported that chlorophyll content was significantly affected by the cultivation systems and nutrient solution. Additionally, relative water content of lettuce leaves presented significant (p<0.05) effect in aeroponics system with high-pressure nozzle, while other cultivation systems presented non-significant (p>0.05) in other treatments (T_1, T_3, T_4, and T_5). The highest RWC values were calculated in T_2 followed by T_4 treatment and lowest values were found in T_1 followed by T_5 treatment. However, the median values were found observed in T_3 treatment. Hymus et al.[48] reported that root zone with good circulation of CO_2 had higher RWC values. The findings of He et al.[32] also supported our study that bad circulation of root zone CO_2 in the root zone chamber significantly reduced the RWC in plants. NUE is a function of the nutrient input used[49,50], and is the most important indicator for measuring agricultural production[51]. It is an important physiological parameter with high potential yield and low nutrient solution consumption[52]. The ANOVA and Duncan's complex test results of nutrient use efficiency (NUE) showed significant (p<0.05) difference in all cultivation systems. The maximum and minimum NUE values were found in aeroponics system with high-pressure nozzle (T_2) and aeroponics system with ultrasonic nozzle (T_1). The highest, median, and smallest significant (p<0.05) difference of 72.10%, 37.49%, and 12.53 % was found in T_4, T_2, and T_1 treatments respectively. Ali et al.[53] reported that NUE increased in aeroponics system over those of hydroponics system and conventional system. The same researchers also concluded that the NUE of aeroponics system was two and three times greater than hydroponics and conventional cultivation system. Furthermore, total phenolic (TPC) and flavonoid content (TFC) values were significantly (p<0.05) different in all five cultivation systems. The statistically analysed results indicated that maximum and minimum TPC values were observed T_2 and T_1 treatments and the smallest difference of 16.2% was measured between T1 and T4 treatments respectively. The TFC trend was some different from TPC. The values of TFC were higher in T_2 treatment followed by T_3 treatment. The maximum difference of 70.59% was calculated between aeroponics system with high-pressure nozzle and conventional system. The comparative previous studies of aeroponics system, hydroponics system and conventional system demonstrated that cultivation of fruits and vegetables in aeroponics systems had higher values of TPC and TFC than hydroponic and conventional systems[54,55]. It was observed the same results were that aeroponics systems with high-pressure nozzle carried highest TPC and TFC values as compared to other cultivation systems. Lakhiar et al.[21] concluded that the highest TPC values were achieved in air-assisted atomizer. TFC is mainly responsible for the antioxidants of food and leafy vegetables have higher values of it when cultivated in air-circulation system[54,56,57]. The antioxidant activity depends on the species, geographic origin, agricultural practices used, and cultivation systems[58]. In order to measure the antioxidants (DPPH, H_2O_2, and FRAP) values, the ANOVA and Duncan's test results showed significant (p<0.05) difference in all cultivation systems. The highest DPPH, H_2O_2, and FRAP values were observed in T_2 treatment followed by hydroponics system (T_4) treatment. The lowest values of DPPH and H_2O_2 were measured in T_1 and for FRAP the values were significantly lower in conventional technique (T_5). However, the median values of above mentioned parameters were observed in aeroponics system with low pressure nozzle. The conclusion of Lakhiar et al.[21] also, match our results that variation in antioxidants properties can be caused by different atomizers used in aeroponics systems and other cultivation systems. Zambrano-Moreno et al.[55] reported the same kind of results that antioxidants properties values were greater in aeroponics system than hydroponics and conventional technique. The matched findings were found by Rodrigo-García et al.[59] in the hydroponic cultivation system. Additionally, the cultivation systems presented significant (p<0.05) difference in all treatments. The highest nitrate level of 4.7 mg/g was observed in T_2 treatment and 54.5%, 20.9%, 12.3%, and 36.8% higher than T_1, T_3, T_4, and T_5 treatments respectively. The values of vitamin C and protein were also higher in T_2 than in other treatments. The vitamin C and protein levels in all treatments followed a descending trend as T_2, T_4, T_3, T_5, T_1, and T_2, T_3, T_4, T_5, T_1, respectively. The same conclusion was drawn by Chen et al.[60] that the air-assisted atomizer is more suitable atomizer in aeroponics systems for nutritional quality of lettuce. Zhang et al.[33] reported that the aeroponics system with ultrasonic atomizer is not suitable and quality of lettuce was very low.

5 Conclusions

The different cultivation systems had significant (p<0.05) effects on shoot and root (fresh & dry) weight, root/shoot ratio (fresh & dry), RCW, NUE, TPC, TFC, and antioxidants properties. The highest biomass yield, chlorophyll content, total phenolic and flavonoid content values were observed in aeroponics system with high-pressure nozzle (T2) followed by hydroponics system (T4) and the aeroponic system with ultrasonic nozzle (T1) had lower values followed by conventional cultivation system (T5). Further, the antioxidant activity was better in aeroponics system with high-pressure nozzle as compared to other systems. It could be concluded from the scientific study that the lettuce grown in aeroponics system had high nutritional value, used less nutrient solution, and gave higher yield as compared to other cultivation systems. Furthermore, a long-term study is recommended to investigate the effect of different cultivation systems on antioxidants, phenolic and flavonoid properties with respect to harvesting time, growing seasons, temperatures, and different varieties of vegetables and fruits.

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[References]

[1] Carducci B, Keats E, Ruel M, Haddad L, Osendarp S, Bhutta Z. Food systems, diets and nutrition in the wake of COVID-19. Nature Food 2021; 2(2): 68–70.

[2] CDC. Food Safety and Coronavirus Disease 2019 (COVID-19). CDC (2020). https://www.cdc.gov/foodsafety/newsletter/food-safety-and-Coronavirus.html. Accessed on [2020-09-18]

[3] The State of Food Security and Nutrition in the World 2020: Transforming food systems for affordable healthy diets (FAO, IFAD, UNICEF, WFP and WHO, 2020).

[4] Khorsandi P. WFP chief warns of hunger pandemic as Global Food Crises Report launched. World Food Programme Insight 2020; 22. Available:https://www.wfp.org/stories/wfp-chief-warns-hunger-pandemic-global-food-crises-report-launched. Accessed on [2021-02-17].

[5] Nicola M, Alsafi Z, Sohrabi C, Kerwan A, Al-Jabir A, Iosifidis C, et al. The socio-economic implications of the coronavirus and COVID-19 pandemic: A review. Int J Surg, 2020, 78: 185–193.

[6] Organisation for Economic Co-operation and Development. OECD Economic Outlook: June 2020. http://www.oecd.org/economic-outlook/june-2020/. Accessed on [2021-02-18]

[7] Tunio M H, Gao J M. Technological modernization and its influence on agriculture sustainability, aeroponics systems in belt and road countries. Jiangsu Univerity, 2020; pp.1–4.

[8] Aryal S, Baniya M K, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants, 2019; 8(4): 96. doi: 10.3390/plants8040096.

[9] Campos F V, Oliveira J A, Pereira M G, Farnese F S. Nitric oxide and phytohormone interactions in the response of Lactuca sativa to salinity stress. Planta, 2019; 250(5): 1475–1489.

[10] Farrell P, Thow A M, Wate J T, Nonga N, Vatucawaqa P, Brewer T, et al. COVID-19 and Pacific food system resilience: opportunities to build a robust response. Food Security, 2020; 12(4): 783–791.

[11] Sofo A, Lundegårdh B, Mårtensson A, Manfra M, Pepe G, Sommella E, et al. Different agronomic and fertilization systems affect polyphenolic profile, antioxidant capacity and mineral composition of lettuce. Scientia Horticulturae, 2016; 204: 106–115.

[12] Okafor J N, Rautenbauch F, Meyer M, Le Roes-Hill M, Harris T, Jideani V A. Phenolic content, antioxidant, cytotoxic and antiproliferative effects of fractions of *Vigna subterraenea* (L.) verdc from Mpumalanga, South Africa. Heliyon, 2021; 7(11): e08397. doi: 10.1016/j.heliyon.2021.e08397.

[13] de Souza A V, Vieira M R S, Putti F F. Correlações entre compostos fenólicos e atividade antioxidante em casca e polpa de variedades de uva de mesa. Brazilian Journal of Food Technology, 2018; 21: e2017103. doi: 10.1590/1981-6723.10317.

[14] İkiz B, Dasgan H, Dere S. Optimization of root spraying time for fresh onion (*Allium cepa* L.) cultivation in aeroponics. ISHS Acta Horticulturae 1273: VIII International Symposium on Seed, Transplant and Stand Establishment of Horticultural Crops, 2018; pp.101–106. doi: 10.17660/ActaHortic.2020.1273.14.

[15] Spehia R S, Devi M, Singh J, Sharma S, Negi A, Singh S, et al. Lettuce growth and yield in hoagland solution with an organic concoction. International Journal of Vegetable Science, 2018; 24(6): 557–566.

[16] Sapkota S, Sapkota S, Liu Z. Effects of nutrient composition and lettuce cultivar on crop production in hydroponic culture. Horticulturae, 2019; 5(4): 72. doi: 10.3390/horticulturae5040072

[17] Tunio M H, Gao J M, Shaikh S A, Lakhiar I A, Qureshi W A, Solangi K A, et al. Potato production in aeroponics: An emerging food growing system in sustainable agriculture forfood security. Chilean Journal of Agricultural Research, 2020; 80(1): 118–132.

[18] Lakhiar I A, Gao J M, Syed T N, Chandio F A, Tunio M H, Ahmad F, et al. Overview of the aeroponic agriculture–An emerging technology for global food security. Int J Agric & Biol Eng, 2020; 13(1): 1–10.

[19] Lakhiar I A, Gao J M, Syed T N, Chandio F A, Buttar N A. Modern plant cultivation technologies in agriculture under controlled environment: A review on aeroponics. Journal of Plant Interactions, 2018; 13(1): 338–352.

[20] Lakhiar I A, Gao J M, Syed T N, Chandio F A, Buttar N A, Qureshi W A. Monitoring and control systems in agriculture using intelligent sensor techniques: A review of the aeroponic system. Journal of Sensors, 2018; 2018: 8672769. doi: 10.1155/2018/8672769.

[21] Lakhiar I A, Gao J M, Xu X, Syed T N, Chandio F A, Jing Z, et al. Effects of various aeroponic atomizers (droplet sizes) on growth, polyphenol content, and antioxidant activity of leaf lettuce (*Lactuca sativa* L.). Transactions of the ASABE, 2019; 62(6): 1475–1487.

[22] Shabbir A, Mao H, Ullah I, Buttar N A, Ajmal M, Lakhiar I A. Effects of drip irrigation emitter density with various irrigation levels on physiological parameters, root, yield, and quality of cherry tomato. Agronomy, 2020; 10(11): 1685. doi: 10.3390/agronomy10111685.

[23] Gao J, Tunio M H, Chen Y, He R. Design and experiment of low-frequency ultrasonic nozzle integrating air-assistant system and acoustic levitation mechanism. Int J Agric & Biol Eng, 2020; 13(6): 25–33.

[24] Tunio M H, Gao J, Lakhiar I A, Solangi K A, Qureshi W A, Shaikh S A, et al. Influence of atomization nozzles and spraying intervals on growth, biomass yield, and nutrient uptake of butter-head lettuce under aeroponics system. Agronomy, 2021; 11(1): 97. doi: 10.3390/agronomy11010097.

[25] Hoagland D R, Arnon D I. The water culture method for growing plants without soil. Circular California Agricultural Experiment Station, 1950; 347(2). doi: 10.1016/S0140-6736(00)73482-9.

[26] Tunio M H, Gao J M, Talpur M A, Lakhiar I A, Chandio F A, Shaikh SA, et al. Effects of different irrigation frequencies and incorporation of rice straw on yield and water productivity of wheat crop. Int J Agric & Biol Eng, 2020; 13(1): 138–145.

[27] Wang K J, Li W T, Deng L, Lyu Q, Zheng Y Q, Yi S L, et al. Rapid detection of chlorophyll content and distribution in citrus orchards based on low-altitude remote sensing and bio-sensors. Int J Agric & Biol Eng, 2018; 11(2): 164–169.

[28] Djidonou D, Zhao X, Simonne E H, Koch K E, Erickson J E. Yield, water-, and nitrogen-use efficiency in field-grown, grafted tomatoes. HortScience, 2013; 48(4): 485–492.

[29] Soomro A, Mirjat M S, Tunio M, Chandio F A, Tagar A A, Soomro A G. Effect of drip and furrow irrigation methods on water saving, yield and yield components of sunflower crop. Sci Int (Lahore) 2015; 27(3): 2235–2242.

[30] Massa D, Incrocci L, Maggini R, Bibbiani C, Carmassi G, Malorgio F, et al. Simulation of crop water and mineral relations in greenhouse soilless culture. Environmental Modelling & Software, 2011; 26(6): 711–722.

[31] Silberbush M, Ben-Asher J. Simulation study of nutrient uptake by plants from soilless cultures as affected by salinity buildup and transpiration. Plant and Soil, 2001; 233(1): 59–69.

[32] He J, Austin P T, Nichols M A, Lee S K. Elevated root-zone CO_2 protects lettuce plants from midday depression of photosynthesis. Environmental and Experimental Botany, 2007; 61(1): 94–101.

[33] Zhang G W, Hu M M, He L, Fu P, Wang L, Zhou J. Optimization of microwave-assisted enzymatic extraction of polyphenols from waste peanut shells and evaluation of its antioxidant and antibacterial activities in vitro. Food and Bioproducts Processing, 2013; 91(2): 158–168.

[34] Kim D O, Jeong S W, Lee C Y. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry, 2003; 81(3): 321–326.

[35] Blasa M, Candiracci M, Accorsi A, Piacentini M P, Albertini M C, Piatti E. Raw Millefiori honey is packed full of antioxidants. Food Chemistry, 2006; 97(2): 217–22.

[36] Brand-Williams W, Cuvelier M, Berset C. Antioxidative activity of phenolic composition of commercial extracts of sage and rosemary. LWT, 1995; 28: 25–30.

[37] Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Igic R. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). Food Chemistry, 2008; 111(4): 925–929.

[38] Benzie I F F, Strain J J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry, 1996; 239(1): 70–76.

[39] Raza A, Li F, Xu X Q, Tang J. Optimization of ultrasonic-assisted extraction of antioxidant polysaccharides from the stem of *Trapa quadrispinosa* using response surface methodology. International Journal of Biological Macromolecules, 2017; 94: 335–344.

[40] Cataldo D, Maroon M, Schrader L, Youngs V. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Communications in Soil Science and Plant Analysis, 1975; 6(1): 71–80.

[41] Fu Y M, Li L Y, Xie B Z, Dong C, Wang M J, Jia B Y, et al. How to establish a Bioregenerative Life Support System for long-term crewed missions to the Moon or Mars. Astrobiology, 2016; 16(12): 925–936.

[42] Bradford M M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 1976; 72(1–2): 248–254.

[43] Ayi Q, Zeng B, Liu J, Li S, van Bodegom P M, Cornelissen J H. Oxygen absorption by adventitious roots promotes the survival of completely submerged terrestrial plants. Annals of Botany, 2016; 118(4): 675–683.

[44] Faucon M P, Houben D, Lambers H. Plant functional traits: soil and ecosystem services. Trends in Plant Science, 2017; 22(5): 385–394.

[45] Li Q S, Li X Q, Tang B, Gu M M. Growth responses and root characteristics of lettuce grown in aeroponics, hydroponics, and substrate culture. Horticulturae, 2018; 4(4): 35. doi: 10.3390/horticulturae4040035.

[46] Nauš J, Prokopová J, Řebíček J, Špundová M. SPAD chlorophyll meter reading can be pronouncedly affected by chloroplast movement. Photosynthesis Research, 2010; 105(3): 265–271.

[47] Lin K H, Huang M Y, Huang W D, Hsu M H, Yang Z W, Yang CM. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. capitata). Scientia Horticulturae, 2013; 150: 86–91.

[48] Hymus G J, Baker N R, Long S P. Growth in elevated CO_2 can both increase and decrease photochemistry and photoinhibition of photosynthesis in a predictable manner. Dactylis glomerata grown in two levels of nitrogen nutrition. Plant Physiology, 2001; 127(3): 1204–1211.

[49] Coyago-Cruz E, Meléndez-Martínez A J, Moriana A, Girón I F, Martín-Palomo M J, Galindo A, et al. Yield response to regulated deficit irrigation of greenhouse cherry tomatoes. Agricultural water management, 2019; 213: 212–221.

[50] Ullah I, Hanping M, Chuan Z, Javed Q, Azeem A. Optimization of irrigation and nutrient concentration based on economic returns, substrate salt accumulation and water use efficiency for tomato in greenhouse. Archives of Agronomy and Soil Science, 2017; 63(12): 1748–1762.

[51] Hashem M S, El-Abedin T Z, Al-Ghobari H M. Rational water use by applying regulated deficit and partial root-zone drying irrigation techniques in tomato under arid conditions. Chilean journal of agricultural research, 2019; 79(1): 75–88.

[52] Xu C L, Tao H B, Tian B J, Gao Y B, Ren J H, Wang P. Limited irrigation improves water use efficiency and soil reservoir capacity through regulating root and canopy growth of winter wheat. Field Crops Research, 2016; 196: 268–275.

[53] Khater E. Comparison between hydroponic and aeroponic systems for lettuce production. Misr Journal of Agricultural Engineering, 2015; 33: 715-736.

[54] Fontana L, Rossi C A, Hubinger S Z, Ferreira M D, Spoto M H, Sala F C, et al. Physicochemical characterization and sensory evaluation of lettuce cultivated in three growing systems. Horticultura Brasileira, 2018; 36(1): 20–26.

[55] Zambrano-Moreno E L, Chávez-Jáuregui R N, de Lurdes Plaza M, Wessel-Beaver L. Phenolic content and antioxidant capacity in organically and conventionally grown eggplant (*Solanum melongena*) fruits

following thermal processing. Food Science and Technology, 2015; 35(3): 414–420.

[56] de Oliveira J J, Dalmazo G O, Morselli T B G A, de Oliveira V F S, Corrêa L B, Nora L, et al. Composted slaughterhouse sludge as a substitute for chemical fertilizers in the cultures of lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.). Food Science and Technology, 2018; 38(1): 91–97.

[57] Salomão-Oliveira A, Lima E S, Marinho H A, Carvalho R P. Benefits and effectiveness of using *Paullinia cupana*: a review article. Journal of Food and Nutrition Research, 2018; 6(8): 497–503.

[58] Negrao L D, Sousa P V D L, Barradas A M, Brandão A D C AS, Araújo M A D M, Moreira-Araújo R S D R. Bioactive compounds and antioxidant activity of crisphead lettuce (*Lactuca sativa* L.) of three different cultivation systems. Food Science and Technology, 2021; 41(2): 365–370.

[59] Rodrigo-García J, Navarrete-laborde B A, de la Rosa L A, Alvarez-Parrilla E, Núñez-gastélum J A. Effect of Harpin protein as an elicitor on the content of phenolic compounds and antioxidant capacity in two hydroponically grown lettuce (*Lactuca sativa* L.) varieties. Food Science and Technology, 2019; 39(1): 72–77.

[60] Chen X L, Guo W Z, Xue X Z, Wang L C, Qiao X J. Growth and quality responses of 'Green Oak Leaf' lettuce as affected by monochromic or mixed radiation provided by fluorescent lamp (FL) and light-emitting diode (LED). Scientia Horticulturae, 2014; 172: 168–175.