

Effects of droplet size and spray interval on root-to-shoot ratio, photosynthesis efficiency, and nutritional quality of aeroponically grown butterhead lettuce

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Abstract: The proper selection of the atomizer (droplet size) and nutrient solution spray interval is one of the most important factors to be investigated in aeroponics systems for sustainable agriculture. The aim of this study was to research the effects of four aeroponics atomizing nozzles (one air-assisted; A₁, two air-less; A₂ and A₃, and one ultrasonic fogger; A₄) with droplet sizes of 11.24 μm, 26.35 μm, 17.38 μm, and 4.89 μm, respectively, four spray intervals (15 min (I₁), 30 min (I₂), 45 min (I₃) and 60 min (I₄)) at a 5 min of constant spray time by atomizing the Hoagland's nutrient solution on growth, root-to-shoot ratio, photosynthesis characteristics, pigments, and nutritional quality of the aeroponically grown lettuce. The experimental results demonstrated that in A₁ atomizer and I₂ interval, the growth, photosynthesis efficiency, chlorophyll, carotenoids, and nutritional values of the lettuce were significantly higher compared to that grown in A₂ and A₃ atomizers at all spray intervals. The shoot developments were more constrained than root, prominent to the alteration of root-to-shoot ratio (fresh and dry) in the influence of different droplet sizes and spray intervals. Moreover, the plants did not grow well in A₄ atomizer associated with proposed spray intervals. The results disclosed that there was an obvious interaction between droplet sizes (atomizers) and spray intervals for growth, the ratio of root to shoot, photosynthesis efficiency, pigments, and nutritional quality of the aeroponically grown lettuce. This research study increases the awareness of the proper droplet size (atomizer) and the regulation of nutrient solution spray interval for leafy vegetables grown in an aeroponics system.

Keywords: aeroponics system, atomizers, droplet sizes, photosynthesis efficiency, chlorophyll contents, quality of lettuce

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1 Introduction

Food security has become an increasingly important issue worldwide. Demographers predicted that the population will dramatically increase in the coming decades^[1]. At the same time, land specialists such as agronomists, ecologists, and geologists warned that the shortage of arable land is getting worse^[2,3]. It was found that in recent decades additional traditional farmland will be needed to feed a large population. Only 80% of the Earth's arable land is suitable for farming now. Roughly 15% of this land has been rendered unusable for farming due to poor management and unpredicted climate change^[4]. For these reasons, food demand

could exponentially surpass supply, leading to global famine. Another leading issue is that the crops are completely decedent on weather^[4]. To solve the problems, new farming methods have been searched, one of them being soilless culture. Soilless cultivation has many advantages, such as more efficient regulation of nutrients; efficient use of water and fertilizers; high-density cultivation; higher yield per unit area; year-round production; higher quality; easy harvesting process; and negligible contamination from pollutants, pests, and pathogens^[5-8]. Soilless culture, including hydroponics, aquaponics, and aeroponics, is one of the most innovative agricultural strategies that can produce more food from fewer resources^[6,8-12].

With regard to the use of soilless cultivation for vegetable production in developing countries, aeroponics cultivation is the most appropriate technique of soilless cultivation, as it requires less equipment and is inexpensive, locally available, and easy to operate^[7]. International Society for Soilless Cultivation defined aeroponics plant cultivation as “a system in which the root is continuously or discontinuously exposed to the condition of fine drop saturation (a mist) of nutrient solution”^[13]. Plant roots are developed in a two-phase root environment (liquid and air). Aeroponic culture does not involve the antagonism between water and air in the root environment. Continuous contact with oxygen stimulates metabolic processes, which has positive effects on the development of roots and nutrient uptake^[14]. Advantageous effects of the aeroponics culture have been found for tomato,

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cucumber, and strawberry^[15-17], leafy vegetables^[18-20], potato^[11,21,22], herbs and medicinal plants^[23,24], chrysanthemum^[25], anthurium^[26], *Eustoma*, lisianthus and zantedeschia^[23], *Ficus benjamina* L.^[22], *Acacia mangium* Willd.^[27], cranberry^[28], and lettuce^[7,29].

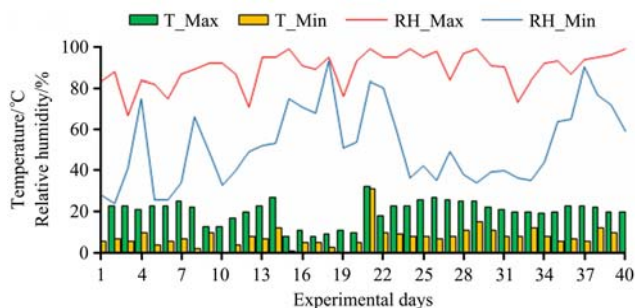
In addition, in an aeroponic system, plant roots receive a direct nutrient solution supply sprayed from the atomizers with different droplet sizes^[30,31]. A study by Tibbitts et al.^[32] reported that with continuous nutrient atomization, plants become dependent on the constant spray, and any interruption of the spray causes loss of plant life. Continuous nutrient atomization can also contribute to fungal and bacterial growth on or near the plants. Moreover, an accurate interval and duration for nutrient atomization scheduling can produce healthier and more natural plant roots than constant atomization. The interval and duration of nutrient atomization help the plants to thrive longer at lower moisture levels and reduce pathogen infection^[33].

Lettuce (*Lactuca sativa* L.) is one of the most world popular vegetables used for fresh consumption as salad^[2,34-37]. Nowadays, it has become the interest of many researchers for indoor experiments due to its high nutrition value and mineral contents^[3]. Therefore, the lettuce was also selected as a test crop. Given the above, the aeroponic atomizer (droplet size), spray time, and interval can be considered as the main parameters influencing the cultivated plant growth^[29,30]. However, in our literature review, we found limited research studies regarding the effects of aeroponic atomizer and spray interval on the photosynthesis characteristics and pigments of any specific leafy plant. It was hypothesized that droplet size and spray intervals may have an impact on growth, root-to-shoot ratio, photosynthesis efficiency, and nutritional quality of lettuce and this data may be helpful for the accuracy of inventories. Thus, the object of this study was to determine the effect of different droplet sizes (atomizers) and spray intervals on root-to-shoot ratio, photosynthesis efficiency, and nutritional quality of aeroponically grown lettuce.

2 Materials and methods

2.1 Experimental site and climate conditions

The experiment was carried out between November-December 2019 at Jiangsu University, China, in a Venlo-type glasshouse equipped with outside and inside shade nets, fans, pad, and spray system. Figure 1 shows the maximum and minimum temperature along with relative humidity during the study period that was recorded daily at 1 min interval with an automatic weather station (Hobo U12-012, Onset Computer Corp.) located in the center of the aeroponic systems.

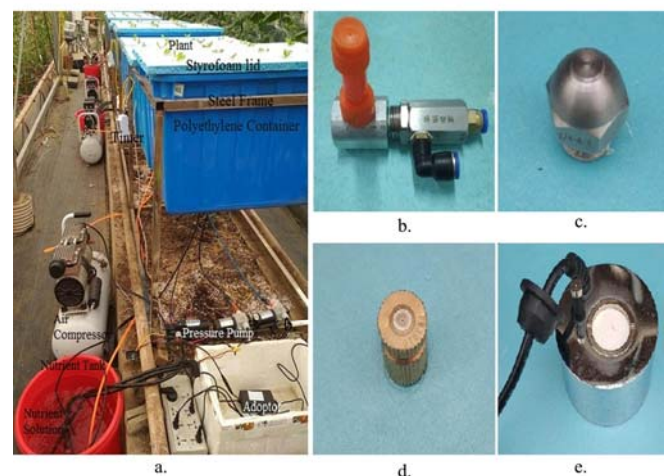


Note: T: Temperature; RH: Relative humidity; Max: Maximum; Min: Minimum
Figure 1 Daily maximum and minimum temperature and relative humidity inside the greenhouse

2.2 Design of the aeroponic system

The experiment was conducted with the existing aeroponic

system previously designed by our research team^[30]. This system consisted of a steel frame and high-density polyethylene (HDPE) containers with styrofoam lids and a volumetric capacity of 140 L (Figure 2a). The systems were designed and manufactured with the four types of spray atomizers (an air-assisted high-pressure atomizer (Figure 2b), two airless atomizers (Figures 2c and 2d), and an ultrasonic fogger (Figure 2e) with different droplet diameters. The air-assisted aeroponic system was designed with three paths: an air inlet path, a water inlet path, and a water outlet path. The systems with airless atomizers were designed with two paths: a water inlet path and water to atomize the liquid. Therefore, in this experiment, the water inlet system was composed of a pressure pump (model PLD-1206, Shijiazhuang City Prandy Electromechanical Equipment Co., Ltd., China), while the air inlet system was composed of an air compressor (OTS-550, Taizhou Outstanding Industry and Trade Co., Ltd, Taizhou, China), as shown in Figure 2a. Furthermore, the ultrasonic aeroponic system consisted of an atomizing chamber, an ultrasonic fogger, a nutrient solution pipeline, a reflux pipeline, a nutrient solution collector pump, a fluid infusion measuring pump, an axial flow fan, and a cultivation box. The fogger is a metal-plated device used to produce ultrasonic vibrations. Normally, the ultrasonic foggers were one to four inches in the nutrient solution within the growth chamber. The flexible polyethylene water and air supply lines were connected to the atomization nozzles through the pressure pump and the air compressor. In addition, the flow of the atomizing nutrient solution was released at different intervals by a digital timer (Figure 2a).



Note: a. Experimental setup b. Air-assisted atomizer c & d. Air-less atomizer e. Piezoelectric ultrasonic fogger

Figure 2 Composition of the experiment system of this study

2.3 Experimental arrangement

The experiments were conducted to study the effects of droplet sizes and spray intervals on growth, root-to-shoot ratio, photosynthesis characteristics, pigments, and nutritional quality of aeroponically grown lettuce. Therefore, four atomizers and four nutrient solution spray intervals were evaluated in a factorial (4×4) randomized complete block design. The air-assisted atomizer (Figure 2b) was designed and manufactured by the authors' research team^[38], and the rest of the atomizers were purchased from the local market near the experimental site.

For the determination of the droplet size of the selected atomizers, a laser particle size analyzer (Winner318B, Jinan Winner Particle Instruments Stock Co., Ltd., Jinan, China), a computer, an air compressor, and a pressure pump were used.

The working pressures of the air compressor, water pump, and water flow rate for the air-assisted atomizer were 0.4 MPa, 0.2 MPa, and 4 L/min, respectively. A pump pressure of 0.2 MPa and a water flow rate of 1 L/min were stable for each airless atomizer, and the flow rate of each ultrasonic fogger was 1 L/min. More importantly, the flow rates and pressures were kept constant for the air compressor and water pump throughout the cultivation period. The detail of the experimental setup is presented in Table 1.

Table 1 Treatments used in the experiment for the lettuce in an aeroponic system

T	Atomizers	S_i /min	S_t /min	D_{av} /μm
A ₁ I ₁	Air-assisted	15	5	11.24
A ₂ I ₁	Airless	15	5	26.35
A ₃ I ₁	Airless	15	5	17.38
A ₄ I ₁	Ultrasonic fogger	15	5	4.89
A ₁ I ₂	Air-assisted	30	5	11.24
A ₂ I ₂	Airless	30	5	26.35
A ₃ I ₂	Airless	30	5	17.38
A ₄ I ₂	Ultrasonic fogger	30	5	4.89
A ₁ I ₃	Air-assisted	45	5	11.24
A ₂ I ₃	Airless	45	5	26.35
A ₃ I ₃	Airless	45	5	17.38
A ₄ I ₃	Ultrasonic fogger	45	5	4.89
A ₁ I ₄	Air-assisted	60	5	11.24
A ₂ I ₄	Airless	60	5	26.35
A ₃ I ₄	Airless	60	5	17.38
A ₄ I ₄	Ultrasonic fogger	60	5	4.89

Note: T: treatments; S_i : Spray interval; S_t : Spray time, D_{av} : Average droplet size.

2.4 Plant material and nutrient solution

Lettuce (*Lactuca sativa* L.) seeds were obtained from Nanjing Ideal Agricultural Science and Technology Co., Ltd. Jiangsu, P.R. China. The seeds were planted in polystyrene trays (EPS) with 72 cells containing equal quantities of perlite material with a chemical composition of SiO, AlO, K₂O, Na₂O, Fe₂O, and H₂O. To have good plant growth for initial seedling, all cultural practices were continued. Moreover, during seeding, natural sunlight was provided with an intensity of approximately 800-900 μmol/m²·s. The seedling transplantation to the aeroponics system was carried out after 15 d of sowing. Each aeroponic box contained 12 plants at a spacing of 14 cm×16 cm. From the two-true-leaf stage, the

seedlings were regularly hand-watered with half-strength Hoagland solution for three days to avoid unnecessary stress on the plants. Additionally, the Hoagland's nutrient solution was sprayed with four different atomizers at 15 min, 30 min, 45 min, and 60 min of spray intervals throughout the experiment. The chemical compositions of micro and macronutrient for leafy vegetables in soilless culture are represented in Table 2. The pH and EC of the nutrient solution were measured every day from each treatment. A fresh nutrient solution with maintained pH (5.8-6.0) and EC (1.6-2.2 dS/m) value was replaced with recycled one on the fifth day during the entire experiment of 40 d after transplant (DAT)^[39,40]. The equipment for measurement of pH and EC were used as reported by our research team Lakhari et al.^[8]

Table 2 Nutrient composition

Composition	Concentration /mg·L ⁻¹	Composition	Concentration /mg·L ⁻¹
Ca(NO ₃) ₂ ·4H ₂ O	945	H ₃ BO ₃	2.86
KNO ₃	607	MnSO ₄ ·4H ₂ O	2.13
NH ₄ H ₂ PO ₄	115	ZnSO ₄ ·7H ₂ O	0.22
MgSO ₄ ·7H ₂ O	493	CuSO ₄ ·5H ₂ O	0.08
FeEDTA	28	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	0.02
pH		5.8-6.0	
EC/dS·m ⁻¹		1.6-2.2	

2.5 Vegetative growth and biomass analysis

After 40 DAT, four plants separately from each treatment were randomly selected for the measurement of average root length (RL), shoot length (SL), leaf area (LA), shoot and root weight of aeroponically cultivated lettuce. The average RL (cm) and SL (cm) were measured using a measuring tape scale. The leaf area (cm²) was measured with a laser leaf area meter (CI-203, CID BioScience, Inc., Camas, Wash.). More importantly, for biomass analysis, the same plants were washed with tap water, and the free surface moisture was immediately removed using a soft paper towel. The fresh weight of roots (RFW), and shoots (SFW) was measured on a scale accurate to 0.0001 g. Furthermore, the root and shoot samples were put into the envelopes and oven-dried at 85°C for 72 h, and the same procedure was applied to measure the dry weight of shoots (SDW), and roots (RDW). The root-to-shoot ratio was calculated as the root weight/shoot weight (fresh and dried)^[2,40-42]. Figure 3 shows the shoot and root growth of aeroponically grown lettuce during the experiment.



Figure 3 Growth for shoot and root of lettuce in aeroponic systems

2.6 Photosynthesis characteristics and pigment content determination

On the 40th day after transplant (DAT) in the aeroponic system, the lettuce finished exponential growth, and we conducted the measurements of net photosynthesis rate (Pn), leaf stomatal conductance (Gs), leaf intercellular CO₂ concentration, (Ci), and transpiration rate (Eleaf) of the fourth leaf using a portable LI-6400XT photosynthesis instrument (Li-Cor 6400-18, Lincoln, NE, USA)^[43]. It is, therefore, randomly selected four plants from each treatment were selected and five repeated measurements were taken from fully expanded leaves of the same four plants for Pn, Gs, Ci, Eleaf, chlorophyll, and carotenoids. The measurements were taken place on the fully shining day from 10:00 a.m. to 1:30 p.m. Moreover, the values of atmospheric pressure 99.9 kPa, and photosynthetic active radiation (PAR) of 800 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ were set in the photosynthesis machine. The optical density was measured with a UV-1200 spectrophotometer (SP-75, Shanghai spectrum instruments Co., Ltd., China) at 663 nm (OD663) for chlorophyll a, 645 nm (OD645) for chlorophyll b and 470 nm (A470)^[44,45]. More importantly, chlorophyll a (Ca), chlorophyll b (Cb), and carotenoids (Cx⁺) were calculated using the following equations^[46]:

$$\text{Ca} = 12.21\text{A}663 - 281\text{A}646$$

$$\text{Cb} = 20.13\text{A}663 - 5.03\text{A}646$$

$$\text{Cx}^+ = \frac{1000\text{A}470 - 3.27\text{Ca} - 104\text{Cb}}{229}$$

2.7 Nutritional quality analysis

For the determination of quality analysis, four samples from each treatment were lyophilized with liquid nitrogen and frozen at -80°C for assay of vitamin C, nitrate, soluble sugar and soluble protein. The 2, 6-dichloroindophenol dye (AOAC 2000) was used to determine vitamin C levels^[47]. Moreover, according to previous studies, a chromatograph (ICS 90 DIONEX, US) was used to detect the nitrate level^[41,48]. The soluble protein content in lettuce leaves was calculated using the Coomassie brilliant blue G-250 dye method^[49]. Finally, anthronesulfuric acid colorimetry was used to examine the soluble sugar content in the lettuce leaves^[50].

2.8 Statistical analysis

The test results are reported as the mean \pm standard error (SE) values. The two-way variance of analyses (ANOVA) with student post hoc comparisons was performed to test for main and interaction of droplet sizes (atomizers) and spray intervals for all variables of growth, photosynthesis efficiency, pigments, and nutritional quality of lettuce. All statistical analyses were performed using SPSS Statistics and Microsoft Excel 2016.

3 Results and discussion

The hypothesis of the present study was confirmed, it was observed that shoot and root growth, fresh and dry weight, root-to-shoot ratio, photosynthesis efficiency, pigments, and nutritional quality of lettuce crop presented a significant influence in air-assisted (A₁), air-less atomizers (A₂ and A₃) at 15 min, 30 min, 45 min, and 60 min of spray intervals with 5 min of constant spray time.

3.1 Droplet size distribution

The percentage of droplets with a cumulative mean diameter for the four different atomizers working at the same pressure is listed in Table 3. Diameters d_{10} , d_{25} , d_{50} , d_{75} , and d_{90} represent the diameters corresponding to cumulative droplet frequencies at 10%,

25%, 50%, 75% and 90%, respectively. The average maximum droplet size diameter (D_{avg}) was calculated in airless atomizer (A₂) followed by A₃, A₁, and A₄ atomizers.

Table 3 Droplet diameters (μm) of different atomizers

Atomizers	Pump pressure/MPa	Flow rate /L·m ⁻¹	d_{10}	d_{25}	d_{50}	d_{75}	d_{90}	D_{avg}
A ₁	0.2	4	3.29	5.73	9.87	15.09	22.2	11.24
A ₂	0.2	1	6.41	13.74	21.72	37.4	52.5	26.35
A ₃	0.2	1	3.32	7.67	15.24	24.98	35.8	17.38
A ₄	0.2	1	0.21	1.49	3.08	6.15	13.14	4.89

3.2 Vegetative growth and biomass analyses

3.2.1 Measurement of root length (RL), shoot length (SL), and leaf area (LA)

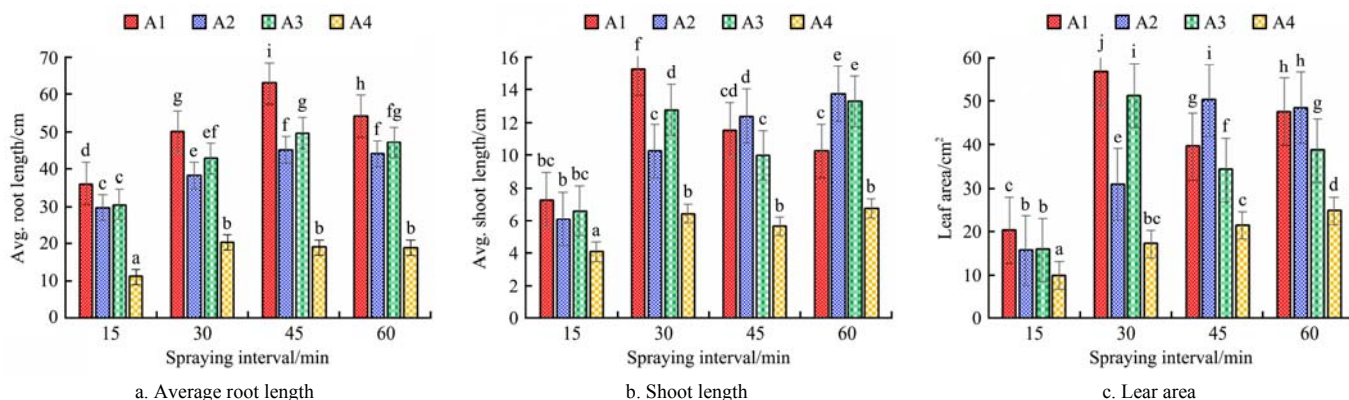
The experimental results of average RL, SL, and LA are presented in Figure 4. It can be seen from the figure, there is noticeable difference between treatments. The ANOVA findings showed significant effects of atomizers and nutrient solution spray intervals on root length, shoot length, and leaf area. It was revealed that the A₁ atomizer and the I₃ spray interval presented the maximum average root length (62.94 cm) in lettuce plants compared to the A₂, A₃, and A₄ atomizers with 15 min, 30 min, 45 min, and 60 min spray intervals. However, the minimum root length (11.25 cm) was observed in ultrasonic fogger (A₄) operated at 15 min spray interval. It was intended from the regression analysis that the A₁ and A₂ atomizers had a significant ($p < 0.05$) effect and A₃ and A₄ atomizers had a nonsignificant effect ($p > 0.05$) at 15 min, 30 min, 45 min, and 60 min spray intervals on root length. The plants sprayed with the A₁ atomizers at different spray intervals appeared stocky compared to those sprayed with the A₂, A₃, and A₄ atomizers. However, the plants sprayed with the A₄ atomizers were slender compared to those sprayed with the A₁, A₂ and A₃ atomizers at different spray intervals. For the average shoot length, an increasing trend was observed for the A₁, A₂, and A₃ atomizers. The highest (15.25 cm) and lowest (4.08 cm) shoot lengths were calculated for A₁I₂ and A₄I₁ treatments. Accordingly, the regression analysis results of average shoot length indicated that the air-assisted atomizer (A₁) and air-less atomizer (A₃) had significant ($p < 0.05$) effects at 15, 30, 45, and 60 min spray intervals. Furthermore, the average leaf area results indicated an increasing trend in A₁, A₂, and A₃ operated at the proposed spray intervals. However, a mixed increasing and decreasing trend was observed for the ultrasonic fogger at four different spray intervals. The highest leaf area of 56.59 cm² was calculated in A₁I₂ treatment, and the lowest leaf area was measured in the ultrasonic fogger at 15 min spray interval (A₄I₁). The regression analysis revealed that the air-assisted atomizer (A₁) and air-less (A₂ and A₃) atomizers had significant ($p < 0.05$) effects and that the ultrasonic fogger had nonsignificant ($p > 0.05$) effects at 15 min, 30 min, 45 min, and 60 min spray intervals on the leaf area. The results of this experimental study contracted with the reported results of Tibbitts et al.^[32], Chiipanthenga et al.^[35], Gao et al.^[51], Buckseth et al.^[18], Lakhier et al.^[6], Lakhier et al.^[52], Tunio et al.^[11] concluded that leafy vegetables treated under droplet sizes were comparably different in morphological parameters with respect to spray interval in aeroponic systems.

3.2.2 Shoot and root fresh and dry weight (SFW, SDW, RFW, RDW)

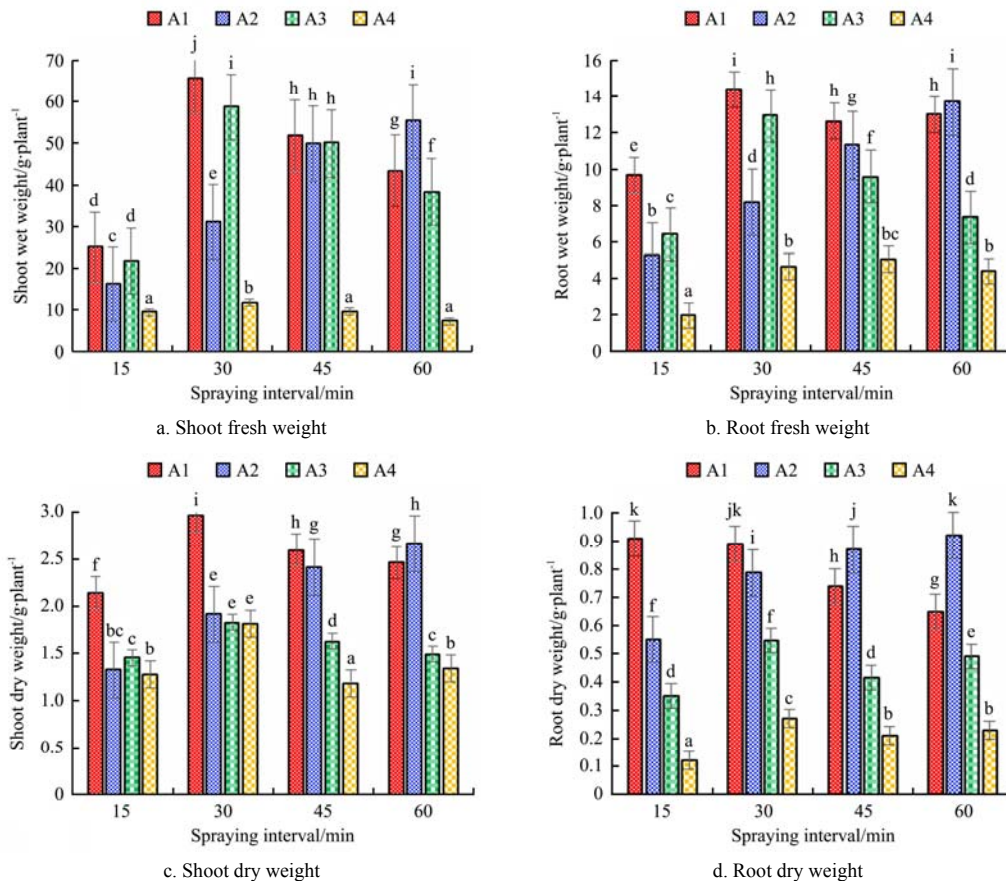
Likewise, there was a significant ($p < 0.05$) difference in shoot and root (fresh & dry weight in all treatments. It is well-known that the plant yield entirely depended on SFW. SFW and RFW

were highest (65.74 g/plant, and 14.37 g/plant) in A₁I₂ treatment and the lowest (7.22 g/plant, and 1.92 g/plant) in A₄I₄, and A₄I₁ treatments, as shown in Figures 5a and 5b. However, the maximum 15.48% reduction in SDW after oven-dried was calculated in A₄I₂ treatment, and the minimum reduction of 3.25% was observed in A₁I₂ treatment (Figure 5b). The descending order for shoot fresh weight in all treatments was A₁, A₃, A₂, A₄ at 30 min, 45 min, 60 min, 15 min of spray intervals. The regression analysis results of shoot and root (fresh and dry) weight indicated that droplet size had a highly significant effect ($p < 0.01$) and that nutrient solution spray interval had a significant ($p < 0.05$) effect on SFW, RFW, SDW, and RDW. More importantly, Ultrasonic foggers had nonsignificant ($p > 0.05$) effects on SFW, RFW, SDW

and RDW in all treatments. The interesting phenomenon of increasing and decreasing root and shoot biomass (fresh & dry) under all atomizers with respect to spray interval was observed^[53]. The greater root growth provided greater shoot biomass (yield) as compared to air-less and ultrasonic foggers misted at different spray intervals^[42]. The present results coincide with the previously stated results that the yield of lettuce plants could be 50-150 g/plant^[2]. Carrasco et al.^[54] reported the same type of results that droplet size affected the fresh weight, dry weight root to shoot ratio of lettuce plants. Another study by Coronel et al.^[55] concluded that interaction between droplet size and spraying interval could significantly affect the biomass but not the root shoot ratio.



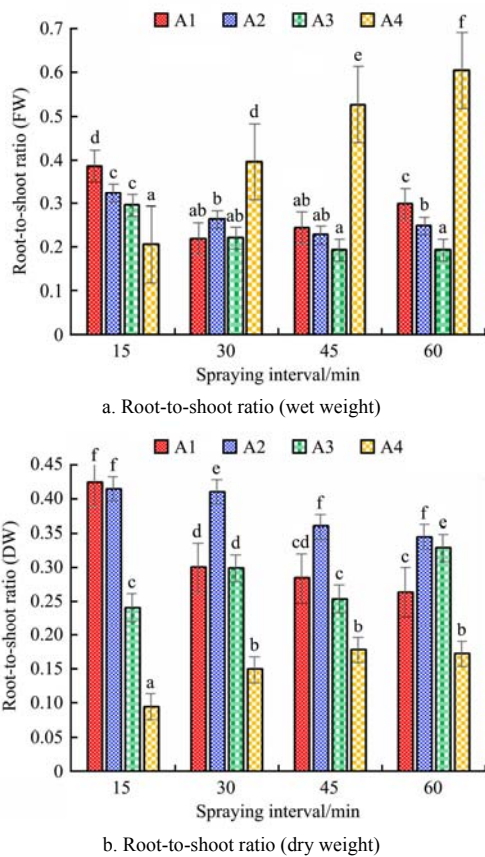
Note: Vertical bars are the mean ± SE; bars labeled with superscript letters are significantly different at $p < 0.05$.
 Figure 4 Average root length, shoot length, and leaf area observed in the lettuce grown in different atomizers operated at different nutrient solution spray intervals



Note: Vertical bars are the mean ± SE; bars labeled with superscript letters are significantly different at $p < 0.05$.
 Figure 5 Shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight observed for the lettuce grown in different atomizers operated at different nutrient solution spray intervals

3.2.3 Root-to-shoot ratio (Fresh & Dry)

The analyzed results of the root-to-shoot ratio on a fresh and dry basis are depicted in Figure 6. The most remarkable result for the root-to-shoot ratio was that the air-assisted atomizer at 15 min and 60 min spray intervals had the maximum root-to-shoot ratio (fresh). The results also revealed that the mixed phenomenon decreasing and increasing trend of root-to-shoot ratio (fresh and dry) occurred in A₁ and A₄ atomizers operated at 15 min, 30 min, 45 min, and 60 min spray intervals. Additionally, the A₂ and A₃ atomizers showed an increasing trend at proposed spray intervals. The regression analysis results for the root-to-shoot ratio (fresh and dry) indicated that only the A₁ had a significant ($p < 0.05$) effect on the root-to-shoot ratio (fresh and dry). The atomizers (A₂, A₃, and A₄) at 15 min, 30 min, 45 min, and 60 min spray intervals had nonsignificant ($p > 0.05$) effects on the root-to-shoot ratio (fresh and dry). The droplet sizes (atomizers) and spray intervals also affected the root-to-shoot ratio. Moreover, the results indicated that the weight of shoot weight was higher than that of root weight under all treatments which significantly differ the values of the root-to-shoot ratio^[46].



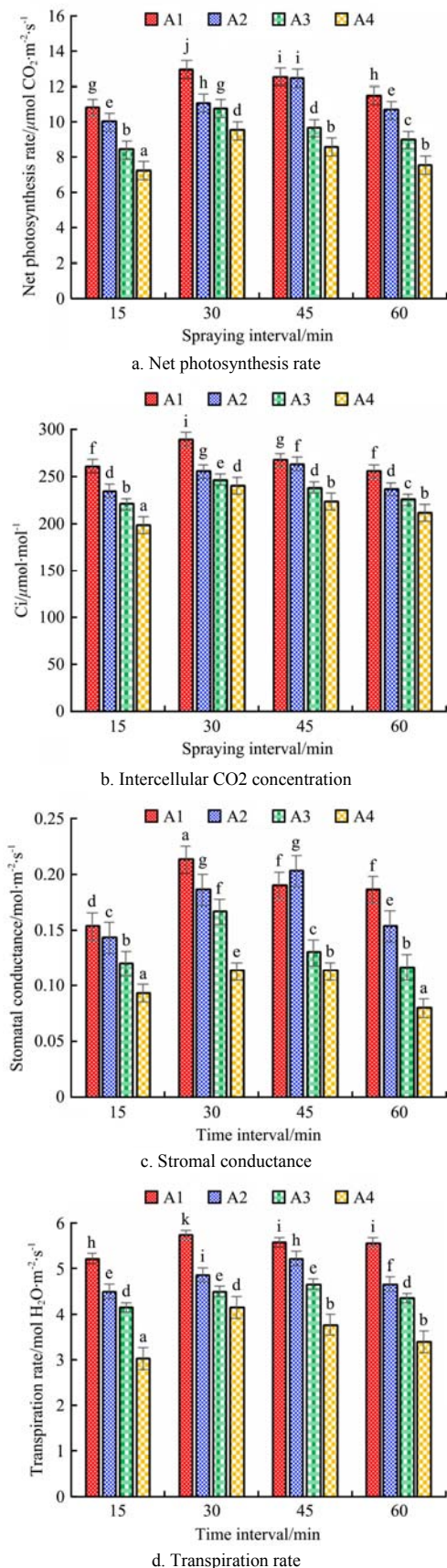
Note: Vertical bars are the mean \pm SE; bars labeled with superscript letters are significantly different at $p < 0.05$.

Figure 6 Root-to-shoot ratio (wet weight) and root-to-shoot ratio (dry weight) observed in the lettuce grown in different atomizers operated at different nutrient solution spray intervals

3.3 The response of photosynthetic characteristics

As shown in Figure 7a, the atomizer type had a significant positive effect and a strong interaction with spray interval on the net photosynthesis rate (Pn) of lettuce. The highest photosynthesis rate ($12.98 \mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$) was observed in A₁I₂ treatment. However, the lowest photosynthesis rate ($7.25 \mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$) was observed in the ultrasonic fogger at 15 min spray interval (A₄I₁). Overall, our data demonstrate that the air-assisted atomizer at different spray intervals presented higher values of Pn

than A₂, A₃, and A₄ atomizers at 15 min, 30 min, 45 min, and 60 min spray intervals.



Note: Vertical bars are the mean \pm SE; bars labeled with superscript letters are significantly different at $p < 0.05$.

Figure 7 Net photosynthesis rate, intercellular CO₂ concentration, stomatal conductance, and transpiration rate in the lettuce grown in different atomizers operated at different time intervals

The experimental results of intercellular CO₂ concentration (Ci) are depicted in Figure 7b. Different atomizers at different spray intervals had significantly ($p < 0.05$) different Ci values. The droplet size (atomizer) without air was directly proportional to the Ci value. In general, the Ci values decreased more in the ultrasonic fogger at 15 min, 30 min, 45 min, and 60 min spray intervals. The minimum reduction was observed in air-assisted atomizers operated at 30 min and 45 min spray intervals. Furthermore, the air-assisted atomizers showed a moderate reduction when spray at 15 min and 60 min spray intervals.

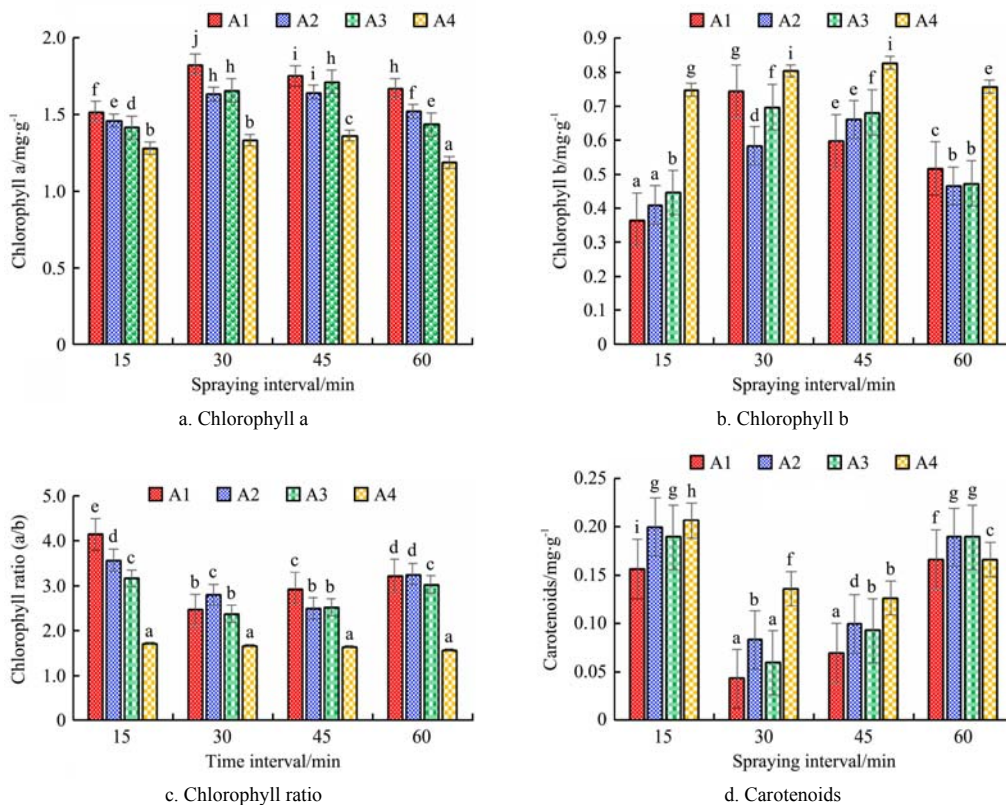
In general, the stomatal conductance (Gs) and transpiration rate (Eleaf) indicated parallel changes with Pn in different combinations of atomizers and spray intervals (Figures 7c and 7d). Similar to Pn, they showed a positive correlation in all treatments. More importantly, in all treatments, the Gs values significantly ($p < 0.05$) increased at the 30 min and 45 min spray intervals. For the Eleaf, the maximum values of 5.72 mol H₂O/m²·s were observed for the A1 atomizer at 30 min spray intervals.

The regression analysis results for Pn, Ci, Gs, and Eleaf indicated that A₁ and A₃ had significant ($p < 0.05$) effects on Pn and that A₁ and A₂ had significant ($p < 0.05$) effects on Ci and Eleaf; however, only A₁ had a significant ($p < 0.05$) effect on Gs at 15 min, 30 min, 45 min, and 60 min spray intervals. The results also reveal that the air-assisted atomizer had a highly significant ($p \leq 0.01$) effect on Pn, Ci, and Gs, while the ultrasonic fogger had a nonsignificant ($p > 0.05$) effect on all parameters at proposed nutrient solution spray intervals. These results revealed that the leaf net photosynthesis rate of lettuce increased with increasing oxygen availability in the root zone chamber^[5]. The benefit of a suitable droplet size (atomizer) with respect to the proper nutrient solution spray interval on plant growth largely results from increased plant photosynthesis^[56,57]. Ougham et al.^[58] reported

similar results, showing that stomatal conductance was correlated with Pn. These results also agreed that droplet size at proper nutrient solution spray intervals increases the Eleaf of plants by increasing the Gs and temperature of the leaf^[59,60].

3.4 Photosynthetic pigments

The interaction of nutrient solution spray intervals and atomizers (droplet size) had a significant ($p < 0.05$) effect on photosynthetic pigments. In figure 8, it can be seen that highest levels of chlorophyll a (1.83 mg/g) and chlorophyll b (0.83 mg/g) were calculated in A₁I₂ and A₄I₃ treatments. The A₁ atomizers at different spray intervals had high advantages from the initial to the final stage, and ultrasonic foggers had a lower level during the entire life cycle. The lowest concentrations of chlorophyll a (1.19 mg/g) and chlorophyll b (0.37 mg/g) were achieved in A₄I₄ and A₁I₁ treatments, respectively. Furthermore, the value added to chlorophyll a was greater than that of chlorophyll b for all treatments; thus, the chlorophyll a/b increased. Moreover, the concentration of carotenoids responded in a highly significant fashion ($p < 0.001$) with different atomizers (droplet sizes), and the four spray intervals had significant ($p < 0.05$) effects. Additionally, the interactions of A₁, A₂ and A₃ had a significant ($p < 0.05$) effect on the concentration of carotenoids at 30 and 45 min spray intervals but not at the 15 min, and 60 min spray intervals. The highest and lowest carotenoid values of 0.21 mg/g and 0.04 mg/g were observed in A₄I₁ and A₁I₂ treatments, respectively. The concentration of carotenoids first increased at the 15 min spray interval, then decreased at the 30 and 45 min intervals, and again increased at the 60 min interval in A₁, A₂, A₃, and A₄. It was observed from the regression analysis results that the A₁ atomizer had a significant ($p < 0.05$) effect on chlorophyll a, b and carotenoids; moreover, A₂ showed significant ($p < 0.05$) effects on chlorophyll a and carotenoids, while A₃ had a significant ($p < 0.05$)



Note: Vertical bars are the mean ± SE; bars labeled with superscript letters are significantly different at $p < 0.05$.

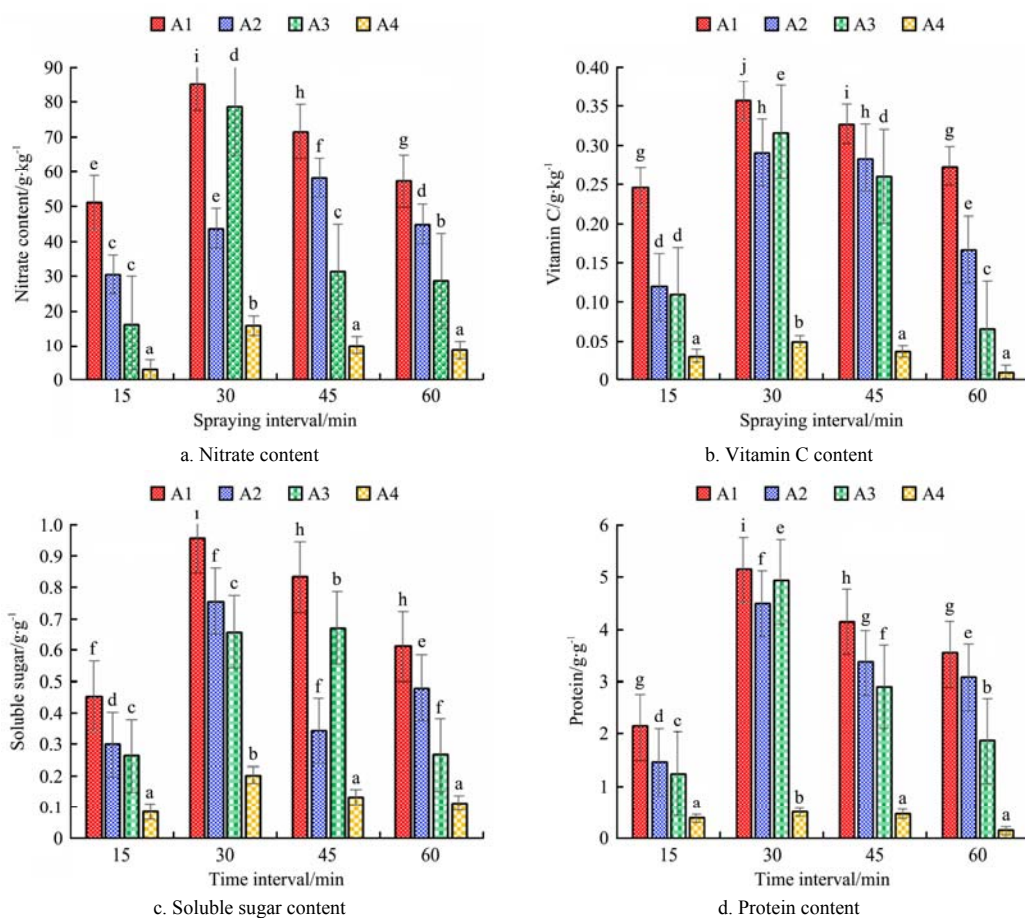
Figure 8 Chlorophyll a, chlorophyll b, chlorophyll ratio, and carotenoids in the lettuce grown in different atomizers at different time intervals

effect only on chlorophyll a. Likewise, the A₄ atomizer had nonsignificant ($p>0.05$) effects on all measured parameters. These research findings support the previous studies that proper droplet size and spray nutrient solutions could increase the chlorophyll content in the leaves^[41]. It was also observed that under A₄ atomizer at four nutrient solution spray intervals, the concentration of chlorophyll was very low; this phenomenon reduced the photosynthesis efficiency and resulted in a very small growth of shoot and root^[61]. Furthermore, it can be seen in Figure 8c that the carotenoids reacted positively to droplet size at spray intervals. The concentration of carotenoids increased firstly at spray intervals of 15 min, then decreased at 30 min and 45 min and again increased at 60 min under A₁, A₂, A₃ and A₄^[43,50]. The carotenoid content was positively associated with the interaction of droplet sizes and spray intervals^[62]. Similar to the results of this study, the positive effects of droplet sizes and spray intervals were reported in kale^[63].

3.5 Nutritional quality of lettuce

As seen in Figure 9, the nitrate concentration of lettuce leaves significantly responded to the atomizer type (droplet size), nutrient solution spray interval and their interaction ($p<0.05$). The maximum and minimum nitrate contents of 85.38 g/kg and 3.08 g/kg were observed for A₁I₂ and A₄I₁ treatment, respectively. Moreover, vitamin C is parabolically correlated with nutrient solution spray intervals. The results revealed that the highest

vitamin C content was observed at the 30 min spray interval for the A₁ atomizer at 0.36 g/kg and that the lowest was observed for A₄I₄. Furthermore, the highly significant ($p<0.001$) effect of droplet size was illustrated for soluble sugar and soluble protein. The A₁ atomizer at 45 min spray intervals had higher values than A₂, and A₃ atomizers at proposed spray intervals. Furthermore, A₄ had nonsignificant ($p<0.05$) effects on nitrate, vitamin C, soluble sugar and protein. The regression analysis results indicated that the air-assisted atomizer operated at 15, 30, 45 and 60 min spray intervals had significant ($p<0.05$) effects on nitrate, vitamin C, soluble sugar and protein. The air-less atomizer (A₂) had a significant ($p<0.05$) effect only on soluble sugar, and the A₃ atomizer had significant ($p<0.05$) effects on nitrate and vitamin C contents. The air-assisted atomizers at 30 min of spray interval were the most suitable atomizer for the nutritional quality of lettuce^[53]. These results coincided with previous results showing that the proper droplet size provides more carbohydrate and photochemical energy to speed up nitrate accumulation^[64]. The results also revealed that the soluble sugar and protein was low at 15 min of spray interval, it was maximum at 30 min of spray interval and then decreased with increasing the spray intervals under all atomizers^[65,66]. The finding of this study concluded that a combination of air-assisted atomizers and 30 min of spray interval could be beneficial to improve the nutritional quality of lettuce.



Note: Vertical bars are the mean \pm SE; bars labeled with superscript letters are significantly different at $p<0.05$.

Figure 9 Nitrate content, vitamin C content, soluble sugar content, and protein in lettuce leaves grown in different atomizers at different time intervals

4 Conclusions

In this study, it was observed that the use of air-assisted

atomizers (A₁) operated at a 30 min nutrient solution spray interval is more suitable than the use of air-less atomizers (A₂ and A₃) for the growth, photosynthesis characteristics, pigments, and

nutritional quality of aeroponically grown lettuce. The results also revealed that ultrasonic foggers (A₄) operated at 15 min, 30 min, 45 min, and 60 min nutrient solution spray intervals are not suitable for lettuce plants cultivated in aeroponics systems. The combination of A₁ and I₂ treatment significantly improved the photosynthesis, and the root characteristics resulted in the increased shoot biomass (yield) and nutritional quality of lettuce leaves when using Hoagland's nutrient solution. However, the reduction in shoot biomass (yield), photosynthesis efficiency, and nutritional quality of lettuce in ultrasonic foggers at spray interval require further investigation in aeroponic systems. Additionally, long-term studies on droplet size and spray intervals of leafy vegetables in aeroponics systems are fully encouraged to conform statements and present data.

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