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Original Article

Hydroponic lettuce production from a biofertilizer compound associated with mineral fertilization

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Abstract

Managing hydroponic lettuce with a nutrient solution enriched with biofertilizer can increase productivity and improve commercial product quality; however, few studies have addressed this topic. This study aimed to evaluate the effects of adding a biofertilizer to the nutrient solution of hydroponic lettuce grown in spring and winter. A randomized block experimental design was used with two nutritional treatments and two growing seasons, each with six replications. For nutrient supply, one treatment did not use biofertilizer, while the other included it. The biofertilizer is a compound based on fulvic acids, an amino acid complex, and alginic acid, applied at a dose of 1 liter per 1,000 liters of nutrient solution. The use of biofertilizer resulted in increases in red and green excitation fluorescence indices (SFR-R and SFR-G), total chlorophyll, flavonoids, and anthocyanins by 32.1%, 41.1%, 30.7%, 10.3%, and 3.5%, respectively, in the spring crop. For nitrogen balance in plants during spring cultivation, the use of biofertilizer promoted increases of 21.7% and 89.9% in red and green excitation nitrogen balance indices (NBI-G and NBI-R), respectively. The use of biofertilizer resulted in average gains, regardless of cultivation period, of 27.6% for root fresh mass, 74.0% for shoot fresh mass, and 11.7% for shoot diameter, as well as increases of 27.8% and 43.4% for stem diameter and number of leaves in spring cultivation. These positive effects indicate that the biofertilizer improves nutrient absorption and stress resistance, resulting in more robust plants with better commercial characteristics.

Keywords: *Lactuca sativa* L., Fulvic acids, Alginic acid, Amino acids, Hydroponics

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Introduction

Lettuce (*Lactuca sativa L.*) is one of the world's most consumed vegetables as it is a good source of fiber, iron, folate, vitamin C and beneficial bioactive compounds (Kim et al., 2016). With a production of 672,000 tons in 2020, it is one of the five most marketed vegetables in Brazil (Conab, 2022) and is an important agricultural crop. In Brazil, 80,000 hectares of lettuce are cultivated, of which 2,000 hectares correspond to hydroponic cultivation (Ibge, 2017; Lima et al., 2018). Lettuce is the most important variety and accounts for 80 % of the vegetables grown in hydroponics in Brazil (Conab, 2022; Sutton et al., 2006).

In hydroponic lettuce cultivation, the plant nutrients are supplied in their inorganic form in a liquid solution (Nguyen et al., 2016). It is a technique in which the plant roots are grown in a static, aerated nutrient solution in a continuous flow. With this technique, studies can be conducted under controlled conditions, allowing better control of characteristics such as temperature, nutrient levels, humidity, radiation, etc., and offering advantages such as the absence of pest infestations in the soil, lower labor costs and greater economy (Jones, 2014). The nutrient solution is the main component of a hydroponic system and its content must meet the nutrient requirements of the plant. Insufficient nutrient doses can lead to nutrient deficiencies in plants, while high doses can lead to physiological damage and thus affect productivity (Setiawati et al., 2019). Therefore, using compounds that can improve plant performance in the nutrient solution may be an alternative to increase the productivity and quality of lettuce (Dasgan et al., 2023a; Demir et al., 2023). Among the various compounds of interest in agricultural production, biofertilizers stand out because of their chemical and biological characteristics and their ability to enhance the sustainability of the production system (Figiel et al., 2025; Gurjar et al., 2023; Kumar et al., 2022).

Biofertilizers have emerged as an ecological alternative to chemical fertilizers (Wichaphian et al., 2025). They are products containing active ingredients or organic agents free of pesticides, acting directly or indirectly on all parts of plants. The use of biofertilizers together with mineral fertilizers at doses up to 50% below the recommended level can maintain or even increase the productivity and quality of lettuce (Dasgan et al., 2023a; Demir et al., 2023; Ikiz et al., 2024). Benefits of using biofertilizers in hydroponics include increased availability and absorption of nutrients, stimulation of root and shoot growth, greater tolerance to abiotic stresses, and reduction in nitrate accumulation, among others (Sen et al., 2025; Wichaphian et al., 2025).

The use of biofertilizers has increased, mainly in soil-based crop management; however, their use in hydroponic systems remains much less common. This is partly due to the limited information available to producers on the subject (Karapetyan, 2024; Singh et al., 2023). The hypothesis of this study is that the combined use of biofertilizers and mineral fertilizers promotes greater productivity and improvements in the growth, physiological, and biochemical characteristics of lettuce. Therefore, the objective of this study was to evaluate the effect of using biofertilizers combined with mineral fertilizers on hydroponic lettuce.

Material and Methods

Area characteristics

The experiment was conducted in a greenhouse at the Federal Institute of Education, Science and Technology of Espírito Santo, Itapina Campus, in Colatina-ES, Brazil, at latitude 19° 29' 54" S, longitude 40° 45' 54" W, and an altitude of 45 meters. Lettuce was grown during the winter (June to September) and spring (September to December) of 2022 in a hydroponic system using the laminar nutrient flow technique (Vought et al., 2024).

The experiment was conducted in a 360 m² greenhouse covered with 150-micrometer-thick plastic film, with side walls protected by black polypropylene screens providing 70% shading. The hydroponic system consisted of two independent subsystems, supplied by a polyethylene tank with a capacity of 500 liters of nutrient solution. The growing benches had six channels, each 6 m long and spaced 0.25 m apart. Solution circulation was automated and operated intermittently from 6 am to 11 am and from 2 pm to 7 pm, every 10 minutes. From 11 am to 2 pm, the system operated for 20 minutes and was off for 10 minutes. At night, the system operated for 10 minutes at midnight and at 3 am. The system provided a flow rate of 1.5 l min^{-1} for each cultivation channel.

In this experiment, lettuce (*Lactuca sativa* L.) of the American Laurel variety was used, with pelleted seeds (TopSeed, Monte Alto, Brazil). The seeds were sown in phenolic foam kept moist with distilled water for three days, then transferred to the growing station.

Transplanting to the production benches occurred when the seedlings had three leaves. Harvesting took place 45 days after sowing.

Experimental design

A randomized block experimental design was used with two nutritional treatments and two growing seasons, with six replications, resulting in 24 plots. Each plot consisted of 30 plants, totaling 720 plants. For nutrient supply, one treatment without biofertilizer (WoB) and another with biofertilizer (WB) were used. In the WoB treatment, considered the control, three nutrient solutions – A, B, and M – were used. Nutrient solution A contained 8.5 kg of potassium nitrate (12% N, 45% K_2O, 1.2% S), 3.75 kg of magnesium sulfate (9% Mg, 12% S), and 1.87 kg of purified monoammonium phosphate (11% N, 60% P_2O_5), all diluted in 50 L of water. Solution B contained 5 kg of calcium nitrate (15% N, 18% Ca) diluted in 50 L of water. Solution M contained 30 g L^{-1} of mineral fertilizer consisting of K_2O (116 g kg^{-1}), S (12.8 g kg^{-1}), Mg (8.6 g kg^{-1}), B (21 g kg^{-1}), Fe (26.6 g kg^{-1}), Cu (3.6 g kg^{-1}), Mn (24.8 g kg^{-1}), Mo (0.36 g kg^{-1}), Zn (33.8 g kg^{-1}), and 60 g L^{-1} of a commercial product containing Fe chelates.

After preparing the nutrient solutions, 500 ml each of solutions A and B and 25 ml of solution M were removed and diluted in 1000 L of water, which was used to nourish the plants. The hydroponic solution was always adjusted to maintain the electrical conductivity at 1.5 mS cm^{-1}. Both the electrical conductivity and the pH of the nutrient solution were kept constant between the two solutions. For the second nutrient management, nutrient solutions A, B, and M were combined with a biofertilizer and diluted in 1000 l of water.

The compound biofertilizer is based on fulvic acids, an amino acid complex, and alginic acid (Ativar® - Litoplant, Linhares, Brazil). Its chemical composition is as follows: organic carbon 20% (260 g L^{-1}); water-soluble potassium 3% (39 g L^{-1}); water-soluble sulfur 3% (39 g L^{-1}); water-soluble nitrogen 4% (52 g L^{-1}); alginic acid 1.5% (15.5 g L^{-1}); free amino acids 14.21% (184.73 g L^{-1}); density 1.30 kg L^{-1}; EC 4.33 mS cm^{-1}; salinity index 32.48%; pH 6.5. For this treatment, 1 liter of biofertilizer was diluted in solution B.

The experiment was conducted in two periods: winter (June to September) and spring (September to December) of 2022. Physiological and yield parameters were evaluated when the plants reached 45 days after sowing, the recommended harvest period for commercial crops (Magalhães et al., 2010).

Physiological parameters

At harvest, non-destructive analyses were performed using the portable multiparameter fluorometer Multiplex 330 (Force-A, Orsay, France). The device illuminated a circular area with a diameter of 8 cm from a distance of 10 cm using six light excitation sources: ultraviolet (UV, 375 nm), blue (B, 450 nm), green (G, 515 nm), and red (R, 635 nm) (Diago et al., 2016). Detection of the emitted fluorescence was used to measure SFR-G, SFR-R, FLAV, ANTH-RG, ANTH-RB, NBI-G, and NBI-R. A complete list of acronyms used is provided in Table 1.

Table-1. List of acronyms used.

Acronyms		Units
SFR-R	Simple Fluorescence Ratio under red excitation	Dimensionless
SFR-G	Simple Fluorescence Ratio under green excitation	Dimensionless
FLAV	Flavonols	Dimensionless
ANTH-RG	Anthocyanins under Red-Green excitation	Dimensionless
ANTH-RB	Anthocyanins under Red-Blue excitation	Dimensionless
NBI-R	Nitrogen Balance Index under red excitation	Dimensionless
NBI-G	Nitrogen Balance Index under green excitation	Dimensionless
NDVI	Normalized difference vegetation index	Dimensionless
FRM	Fresh root mass	g
SPM	Fresh shoot mass	g
SDP	shoot diameter	cm
SD	stem diameter	mm
NL	number of marketable leaves	number

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NDVI was measured with a portable GreenSeeker device (Trimble, Sunnyvale, USA). Values range from -1 to 1, and areas with denser vegetation tend to have NDVI values close to 1 (Cordeiro et al., 2017). The portable ChlorofiLOG (Falker, Porto Alegre, Brazil) was used to assess chlorophyll a, b, and total chlorophyll values. For these analyses, the average of three measurements was taken for all plants in each replicate. All measurements were performed on the 10 central plants of each plot between 8:00 and 9:00 am (GMT, BRS, 3:00 am).

Productivity parameters

All plants in the plot were cut close to the stem and separated into roots and shoots. The fresh root mass (FRM) and shoot mass (SPM) were measured using an analytical balance (Ohaus, Barueri, Brazil). Subsequently, shoot diameter (SDP), stem diameter (SD), and number of marketable leaves per plant (NL) were evaluated. SDP was measured with a ruler graduated in millimeters. SD was measured with a digital caliper (MTX, Guarulhos, Brazil). NL was determined by manually counting the number of marketable leaves per plant.

Statistical analysis

The assumptions of normal distribution and homogeneity of variances were checked. The data were then subjected to analysis of variance, and mean values were compared using the Tukey ($p < 0.05$). Pearson correlation analysis was also performed. All statistical analyses were conducted using R software, with a command developed for the ExpDex.pt data packages (Ferreira et al., 2018).

Results and Discussion

Nutritional management and cultivation period influenced ($p>0.05$) the physiological variables SFR-R, SFR-G, chlorophyll a, chlorophyll b, total chlorophyll, FLAV, ANTH-RB, NBI-G, and NBI-R, but did not affect the NDVI and ANTH-RG indices. The use of biofertilizers combined with the nutrient solution resulted in the highest SFR-R, SFR-G, chlorophyll a, chlorophyll b, and total chlorophyll indices in the spring (Table 2), with increases of 32.1%, 41.1%, 31.0%, 28.5%, and 30.7%, respectively, compared to the use of the nutrient solution alone. During the cultivation period in the spring, increases of 52.2%, 100.0%, 39.4%, and 33.0% were observed compared to winter for the variables SFR-R, SFR-G, chlorophyll a, and total, respectively.
Lower chlorophyll content is associated with a reduced photosynthesis rate in plants. The chlorophyll content of leaves strongly affects their photosynthetic capacity and overall plant yield (Zhou et al., 2022). In winter, plants typically exhibit lower relative growth rates and larger leaf areas due to reduced light intensity and low temperatures (Galieni et al., 2016). In contrast, at higher temperatures, such as in spring, bud formation tends to accelerate and the growing season shortens, which can impact plant productivity (Chen and Zhang, 2023). However, in winter, when temperatures were milder and radiation less intense, no significant differences were observed between plots with and without the addition of biofertilizers (Table 2).

The gains observed in the various chlorophyll indices used in the experiment with biofertilizer (Table 2) may be related to the increased availability of essential nutrients (nitrogen, phosphorus, magnesium, iron) for chlorophyll biosynthesis (Zhang et al., 2023; Jabborova et al., 2025). It is also possible that some biofertilizers favorably regulate genes involved in chlorophyll production, resulting in higher photosynthetic rates (Mthiyane et al., 2024).

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Table-2. Chlorophyll indices (SFR-R, SFR-G, a, b, and total) obtained from hydroponic lettuce leaves grown in a nutrient solution without biofertilizer (WoB) and with biofertilizer (WB) during winter and spring in Colatina, Brazil.

Cultivation period	SFR-R		SFR-G	
	WoB	WB	WoB	WB
Winter	1.42 aA	1.38 aB	1.50 aB	1.46 aB
Spring	1.59 bA	2.10 aA	2.07 bA	2.92 aA
Cultivation period	Chlorophyll a		Chlorophyll b	
	WoB	WB	WoB	WB
Winter	15.64 aB	15.98 aB	2.96 aA	3.10 aA
Spring	17.01 bA	22.28 aA	2.39 bB	3.07 aA
Cultivation period	Chlorophyll total			
	WoB	WB		
Winter	18.62 aA	19.06 aB		
Spring	19.40 bA	25.35 aA		

Average values marked in the column with the same capital letter and in the row with the same lowercase letter are not statistically different according to the Tukey test at a probability level of 5% ($p < 0.05$). SFR-R – Simple fluorescence ratio under red excitation; SFR-G – Simple fluorescence ratio under green excitation.

The FLAV and ANTH-RB indices were higher in winter without the biostimulant, while in spring they increased by 10.3% and 3.5%, respectively, with the biostimulant (Table 3). Under stress, such as winter conditions (Table 3), plants may produce larger quantities of flavonoids and other polyphenols (Rao and Zheng, 2025; Alba et al., 2024). Flavonoids are a specific group of secondary metabolites known as phenolic compounds and play an important role in plant protection, for example, by absorbing shorter wavelength light, sending attractive signals to insects, and regulating hormonal balance and photosynthetic efficiency (Kuljarusnont et al., 2024).

When evaluating the effect of the growing season, our results indicate a significant influence on nutritional management. In winter, the production of flavonoids and ANTH-RB was 18.5% and 35.4% higher, respectively, than the values obtained in spring without the use of biofertilizer, and ANTH-RB production was 25.0% higher with the use of biofertilizer. This behavior indicates that the plant is adapting to more severe environmental conditions. When plants are exposed to cold, they increase the biosynthesis of flavonoids and anthocyanins, which may activate genes involved in flavonoid and anthocyanin pathways (Li et al., 2025; Rao and Zheng, 2025). These compounds also serve as potent antioxidants, eliminating reactive oxygen species and protecting cell membranes (Shomali et al., 2022; Zhuang et al., 2023). Anthocyanins can absorb excess light, protecting photosynthetic tissues from photoinhibition during winter (Agati et al., 2021; Zhang et al., 2019).

Table-3. Indices of flavonoids (FLAV), anthocyanins (ANTH-RB) and nitrogen balance (NBI-G and NBI-R) obtained from hydroponic lettuce leaves grown in a nutrient solution without biofertilizer (WoB) and with biofertilizer (WB) during winter and spring in Colatina, Brazil.

Cultivation period	FLAV		ANTH-RB	
	WoB	WB	WoB	WB
Winter	-0.27 aA	-0.29 bA	-0.65 aA	-0.68 bA
Spring	-0.32 bB	-0.29 aA	-0.88 bB	-0.85 aB
Cultivation period	NBI-G		NBI-R	
	WoB	WB	WoB	WB
Winter	3.13 aA	3.09 aB	2.63 aB	2.17 aB
Spring	2.93 bA	3.76 aA	3.34 bA	4.12 aA

Average values marked in the column with the same capital letter and in the row with the same lowercase letter are not statistically different according to the Tukey test at a probability level of 5% ($p < 0.05$). FLAV – Flavonols; ANTH-RB – Anthocyanins under Red-Blue excitation; NBI-G – Nitrogen balance index under green excitation; NBI-R - Nitrogen balance index under red excitation.

Nitrogen balance values were higher in the spring compared to other growing seasons (Table 3). The indices were 21.7% and 89.9% higher in the spring with the use of biostimulant for NBI-G and NBI-R, respectively. In spring cultivation, the use of biostimulant resulted in increases of 28.3% and 23.4% for NBI-G and NBI-R, respectively, compared to the nutrient solution without the biostimulant.

The use of biofertilizers generally improves nitrogen balance indices by increasing nitrogen absorption by plants and reducing nutrient losses to the environment (Xu et al., 2025; Shikha et al., 2023). This is also supported by the higher chlorophyll values observed in the spring (Table 2). These higher nitrogen balance values are reflected in greener leaves and greater photosynthetic capacity in plants (Navarro-León et al., 2022).

The use of biofertilizer resulted in average gains, regardless of the growing period, of 27.6% for FRM, 74.0% for SPM, and 11.7% for SDP. The SD and NL variables showed gains of 27.8% and 43.4%, respectively, with biofertilizer during spring cultivation. For the growing period, FRM and SDP had average gains of 31.7% and 15.7%, respectively, in spring, regardless of fertilization management, while SPM showed a gain of 54.2% in winter cultivation. SD had a gain of 23.6% in winter without biofertilizer, while NL had a gain of 22.4% in spring with biofertilizer (Table 4).

Table-4. Fresh root mass (FRM) and shoot mass (SPM), Shoot diameter (SDP) and stem diameter (SD) and number of leaves (NL) obtained from hydroponic lettuce leaves grown in a nutrient solution without biofertilizer (WoB) and with biofertilizer (WB) during winter and spring in Colatina, Brazil.

Cultivation period	FRM (g plant^{-1})			SPM (g plant^{-1})		
	WoB	WB	Average	WoB	WB	Average
Winter	11.32	13.28	12.16 B	120.42	213.62	167.02 A
Spring	13.72	18.32	16.02 A	80.54	136.08	108.31 B
Average	12.38 b	15.80 a		100.48 b	174.85 a	
Cultivation period	**SDP (cm)**			**SD (mm)**		
	WoB	WB	Average	WoB	WB	Average
Winter	34.40	39.60	37.00 B	12.08 bA	13.10 aA	12.59
Spring	41.00	44.60	42.80 A	9.77 bB	12.49 aA	11.13
Average	37.70 b	42.10 a		10.93	12.80	
Cultivation period	**NL (unit)**					
	WoB	WB	Average			
Winter	25.00 bA	28.60 aB	26.80			
Spring	24.40 bA	35.00 aA	29.70			
Average	24.70	31.80				

Average values marked in the column with the same capital letter and in the row with the same lowercase letter are not statistically different according to the Tukey test at a probability level of 5% ($p < 0.05$).

The use of biofertilizer can promote greater growth of hydroponic lettuce, increasing the number of leaves and fresh and dry mass, mainly due to the biofertilizer's ability to improve nutrient availability and stimulate plant growth (Dasgan et al., 2023b; Guimarães et al., 2020). In addition, the growth-promoting effect of biofertilizer may be related to increased production of phytohormones that enhance root growth and nutrient absorption (Kumar et al., 2022). When biofertilizer is combined with mineral fertilizers, lettuce productivity and quality may increase (Oliveira et al., 2025), as observed in the study (Table 4).

In general, greater vegetative growth is favored by higher temperatures (Minoli et al., 2022), as observed in spring (Table 4), and the greater the number of leaves the plant reaches, the faster the vegetative development, resulting in plants with greater vigor and precocity (Pereira et al., 2023).

Pearson correlations between physiological and productive parameters were analyzed (Figure 1). A very strong and significant positive correlation was found between chlorophyll indices (SFR-G and SFR-R) and nitrogen balance indices (NBI-G and NBI-R). Nitrogen is the most essential nutrient for plants to maintain photosynthetic processes and ensure the efficient functioning of photosystem II (PII); therefore, its presence in leaves is directly related to the plants' ability to produce chlorophyll (Kai et al., 2025; Fathi, 2022).

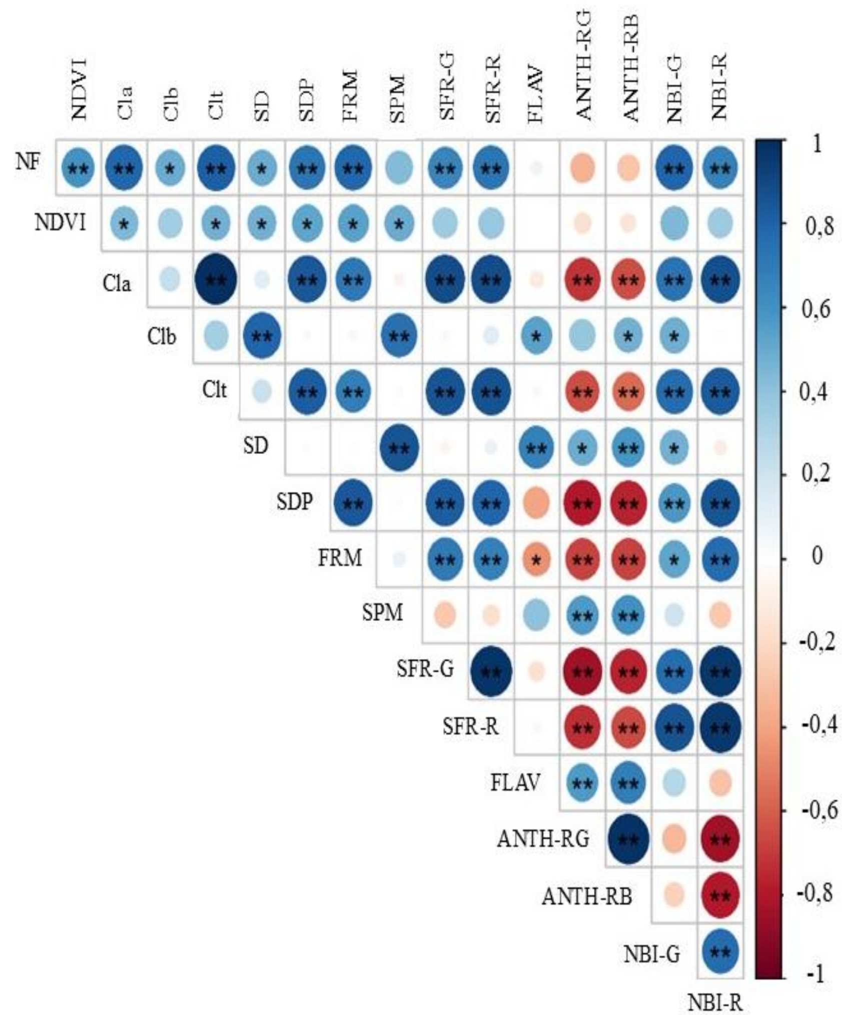

Figure-1. Correlation analysis in hydroponic lettuce leaves grown in nutrient solution without biofertilizer (WoB) and with biofertilizer (WB) during winter and spring in Colatina, Brazil. Significant at $*p < 0.05$; $**p < 0.01$. Positive and negative correlations are shown in blue and red, respectively; the intensity of the color and the size of the circle are proportional to the correlation coefficients.

NL - number of leaves, NDVI - Normalized difference vegetation index, Cla – chlorophyll a, Clb – chlorophyll b, Clt – chlorophyll total, SD - stem diameter, SDP - Shoot diameter, FRM - Fresh root mass, SPM - shoot mass, SFR-G, SFR-R, FLAV - Flavonols, ANTH-RG - Anthocyanins under Red-Green excitation, ANTH-RB - Anthocyanins under Red-Blue excitation, NBI-G - Nitrogen balance index under green excitation, and NBI-R - Nitrogen balance index under red excitation

The anthocyanin index (ANTH-RB) was strongly and negatively affected by nitrogen balance. When plants have higher nitrogen levels, anthocyanin levels decrease because nitrogen reduces the expression of transcription factors that promote anthocyanin production and increases the expression of repressive factors that inhibit anthocyanin synthesis (Zeng et al., 2024; Wang et al., 2024). Additionally, with higher nitrogen levels, plants prioritize primary metabolism, such as growth and protein synthesis, over secondary metabolism, which is responsible for anthocyanin production (Soubeyrand et al., 2018).

In spring, the use of biofertilizer resulted in higher levels of chlorophyll a, chlorophyll b, and total chlorophyll compared to the system without biofertilizer during the same growing season. Correlation analysis showed that chlorophyll a content had a significant positive correlation with the number of leaves, shoot diameter, root fresh mass, and nitrogen balance under green and red light. The greater availability of nitrogen to the plants likely promoted greater aboveground biomass production (Zhou et al., 2022).

Conclusions

Combining biofertilizer use with nutritional management through nutrient solutions proved to be an effective strategy for increasing the productivity of hydroponic lettuce. Biofertilizer use significantly increased the number of leaves, shoot diameter, and chlorophyll index, especially in spring, when environmental conditions were more challenging due to high temperatures. These positive effects indicate that biofertilizer improves nutrient absorption and stress resistance, resulting in more robust plants with better commercial characteristics. Since few studies have addressed the use of biofertilizers in hydroponic systems, further research is essential to evaluate different doses, application times, crops, and sources of biofertilizers mixed with the hydroponic solution.

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Contribution of Authors

Lima SFde & Sales RAde: Analyzed and interpreted data and manuscript write up.

Simon CA, Pedroni AM, Pereira GG, Rangel GC, Jegeski RF & Nunes W: Conducted the experiment and collected data.

Morgado MAD, Souza GSde, Berilli SdaS & Almeida RFde: Assisted in the development of the methodology for physiological studies performed with the Multiplex® fluorometer.

SimonCA & Oliveira EC: Conceptualized the experiment, data analysis and manuscript write up.

All authors read and approved final draft of the manuscript.

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