

## Direct and indirect blue light supplementation on growth, pigments, and gas exchange of young arugula plants

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### Abstract

It is well-established that the blue (430-460 nm) portion of the visible light spectrum beneficially affects plants, primarily by modulating the relationship between photosynthesis and energy metabolism and promoting chlorophyll accumulation. This study aimed to evaluate the effects of direct blue light supplementation (via LEDs operating at 450 nm) and indirect blue light supplementation (via reflective materials) on the growth, pigment content, and gas exchange of young arugula (*Eruca sativa*) plants. Four treatments were tested: control (no supplementation); direct blue light via LED; indirect light via light blue laminate; and indirect light via dark blue laminate. The reflective laminates were placed on the cultivation benches, under the plants. Two hypotheses were tested: 1) The LED, with maximum emission at 450 nm, would have similar performance to the dark blue reflective laminate for plants. 2) The shades of blue in the reflective laminates would influence plant performance. Parameters analyzed included shoot fresh matter, shoot dry matter, contents of chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/b ratio, carotenoids, total chlorophyll/carotenoid ratio, and gas exchange parameters (internal CO<sub>2</sub> concentration (C<sub>i</sub>), net photosynthesis (PN), transpiration (E), stomatal conductance (g<sub>s</sub>), water use efficiency (PN/E), and instantaneous carboxylation efficiency (PN/C<sub>i</sub>)). Results indicated that both blue LED light and dark blue laminate promoted greater accumulation of dry matter and pigments, emerging as the most effective treatments. On the other hand, even though the light blue laminate presented higher light intensity, *Chla*, *Chlb*, Total *Chl* and carotenoids were 19.2%, 18.9%, 19.1%, and 18.89% lower than the control, respectively. For gas exchange, both direct and indirect blue light supplementation led to higher stomatal conductance, transpiration and water use efficiency. However, no statistically significant differences were observed in net A and A/C<sub>i</sub> among treatments, suggesting that combinations of light spectra to optimize plant development in controlled environments, maximizing the benefits of blue light for more efficient production.

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**Keywords:** biomatter; *Eruca sativa*; LED; light supplementation; photosynthetic pigments; photosynthetically active radiation (PAR); protected cultivation; reflective laminate

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## Introduction

The growing demand for fresh, nutritious food has driven an increase in leafy vegetable production, particularly for arugula (*Eruca sativa*). The global cultivation area of arugula (*E. sativa* Mill.) is increasing due to the unique flavor of this species (Zhou and Wu, 2025). Arugula is commonly consumed in salads due to its distinct flavor and high content of vitamins, potassium, sulfur, and iron (Morais *et al.*, 2019). Cultivating arugula as "baby leaf," which involves harvesting whole young leaves between 8-12 cm in length, meets market demand by offering rapid production and a delicate flavor (Piślewska-Bednarek *et al.*, 2018; Santana *et al.*, 2025).

A protected environment in the cultivation of leafy vegetables, such as arugula, provides better growing conditions and plant quality, by regulating parameters such as temperature, humidity, intensity and light quality (Soufi *et al.*, 2023). The spectral balance (relative proportion of different wavelengths) within the growing environment is important for modulating plant growth, development, and gas exchange. Thus, even if two plants have the same light intensity, the biological effect can be completely different depending on the spectral composition in relation to the quality of received photons (the proportion of wavelengths received) (Taiz *et al.*, 2021). However, excessive shading within the greenhouse can reduce the amount of light reaching the plant surface, potentially hindering arugula development (Santana *et al.*, 2025; Dantas *et al.*, 2025), as excessive shading in lettuce has increased the cycle, promoted etiolation, and reduced productivity (Costa Júnior *et al.*, 2019). An alternative to improve light intensity inside the greenhouse is to use benches with reflective materials. Plants can absorb light on the abaxial side of their leaves, which allows for greater light absorption and improves light utilization for photosynthesis (Campos *et al.*, 2023).

The use of blue laminated materials, whether light or dark, shows significant potential in light reflection and optimizing plant growth. The wavelength of blue light (400- 500 nm) can stimulate various physiological processes, including stomatal opening, photosynthesis, and phototropism (Lazzarini *et al.*, 2017). It also influences the biosynthesis of secondary compounds (such as carotenoids and flavonoids) in plants, playing an important role in the crop's antioxidant system, chlorophyll synthesis, and gas exchange (Dou *et al.*, 2020). Some studies confirm that blue reflective materials result in higher photosynthetic pigment content for *Capsicum chinense* seedlings (Bortolheiro *et al.*, 2025), *Brassica oleracea* (Dantas *et al.*, 2025), *Eruca sativa* (Santana *et al.*, 2025), and carotenoid content in *Cichorium intybus* (Araújo *et al.*, 2024).

Another alternative to enhance light intensity is the direct supplementation of blue light via light-emitting diode (LEDs), which aims to improve photosynthetic efficiency by increasing light availability to the leaves, favoring photosynthesis and biomass production (Kong *et al.*, 2019). Artificial lighting plays a fundamental role, with light-emitting diodes (LEDs) establishing themselves as an efficient light source in horticulture. The superior luminous efficiency of LEDs, combined with their low energy consumption, makes them an attractive alternative to traditional fluorescent lamps (Ma *et al.*, 2021). In controlled environment farming systems, blue light-enriched LED technologies have demonstrated strong potential to increase agricultural productivity, nutritional quality, and resilience to environmental stresses, which are essential priorities for sustainable food systems (Su *et al.*, 2025).

Furthermore, the ability of LEDs to emit specific light spectra opens new possibilities for improving plant growth, photosynthetic processes, and plant conservation (Li *et al.*, 2021; Paradiso and Proietti, 2021). Blue light, in particular, has demonstrated effectiveness in promoting the elongation of 52% of arugula seedlings (Johnson *et al.*, 2020; Kong *et al.*, 2023) and improving the quality of microgreens (Ying *et al.*, 2020a).

Blue light plays an important role in regulating plant elongation (Kong and Zheng, 2024). Supplementation at the blue wavelength (450 nm) allows for increased net photosynthesis and greater accumulation of inorganic nutrients in lettuce plants (Soufi *et al.*, 2023).

Given this context, the supplementation of blue light, both direct (using LED on the plant) and indirect (using light and dark blue reflective laminates under the plant), has the potential to optimize the growth, photosynthetic pigment synthesis, and gas exchange in young arugula (*E. sativa*) plants. Specifically, blue light from these sources is expected to contribute to increase photosynthesis, biomass production, and pigment stability, with differentiated effects among the types of supplementations. This study aims to investigate the impact of blue light supplementation, provided both directly (through the use of LED on the plant) and indirect (using light and dark blue reflective laminates under the plant), on the growth, pigment production (chlorophylls and carotenoids), and gas exchange in young arugula plants cultivated in a protected environment.

## Materials and Methods

### *Location and characterization of the experimental area*

Experiments on blue light application in baby arugula cultivation were conducted at the State University of Mato Grosso do Sul (UEMS), Cassilândia University Unit, located at 19°05'46" S and 51°48'50" W, at an altitude of 521 meters. The greenhouse used has dimensions of 18 m in length, 8 m in width, and 4 m in height, totalling 144 m<sup>2</sup>. The roof is covered with a 150-micron low-density polyethylene (LDPE) film, equipped with a light diffuser, anti-drip feature, and a zenithal opening sealed with a 30% white screen. Additionally, the greenhouse features monofilament side and front screens with 50% shading and an ALUMINET<sup>®</sup> 50 aluminized thermos reflective screen under the LDPE film. The screen under the LDPE film was not utilized, i.e., it was retracted.

The greenhouse was not air-conditioned. Ventilation and lighting were natural. The micrometeorological conditions (temperature, humidity, and radiation) inside the environment are described in Table 1. The amount of carbon dioxide inside the environment was not measured. The experiments were fertilized with 2.0 g per pot of Forth Fruits/Vegetables' mineral fertilizer (NPK+9) 10 days after sowing and subsequently irrigated for 15 minutes. Irrigation was done using micro-sprinklers, aiming to maintain substrate moisture close to field capacity.

**Table 1.** Micrometeorological data

Parameters	Cycle 1	Cycle 2	Average
External temperature (°C)	24.9	24.5	24.7
Internal temperature (°C)	27.4	26.1	26.8
External relative umidity (%)	86.0	84.9	85.5
Internal relative umidity (%)	79.8	80.6	80.2
External global radiation (W m <sup>-2</sup> )	454.8	447.4	451.1
Internal global radiation (W m <sup>-2</sup> )	174.7	166.9	170.8
External PAR radiation (μmol m <sup>-2</sup> s <sup>-1</sup> )	1994.3	1974.5	1984.4
Internal PAR radiation (μmol m <sup>-2</sup> s <sup>-1</sup> )	546.0	548.6	547.3
Control reflected PAR radiation (μmol m <sup>-2</sup> s <sup>-1</sup> )	24.6	25.6	25.1
Dark blue reflected PAR light (μmol m <sup>-2</sup> s <sup>-1</sup> )	35.3	33.0	34.2
PAR LED radiation (μmol m <sup>-2</sup> s <sup>-1</sup> )	60.0	60.0	60.0
Reflected PAR light blue radiation (μmol m <sup>-2</sup> s <sup>-1</sup> )	125.8	113.2	119.5

Cycle 1: from 11/12/2023 to 03/01/2024 (23 days); Cycle 2: from 11/01/2024 to 01/02/2024 (21 days)

### *Treatments and experimental design*

The experiments were conducted in a completely randomized design with four treatments, comprising three blue light supplementations and one control, as follows: 1) Control; 2) direct blue light supplementation via LED; 3) indirect blue light supplementation via light blue reflective laminate; and 4) indirect blue light supplementation via dark blue reflective laminate. Four replicates and three experimental units were used, with each experimental unit consisting of a 1.0 L cultivation pot.

The blue light was provided by reflective glossy Formica sheets and an LED light. Light blue and dark blue Formica sheets were utilized. The LED setup consisted of a 100 W Blue LED Floodlight Reflector, which was turned on daily from 7 am to 11 am, totaling four hours of supplementation. The Formica sheets were placed on the cultivation benches, fixed in position for reflection, and measured 1.03 m x 1.25 m (1.29 m<sup>2</sup>). The 1.0 L pots were 10.5 cm high, with a bottom diameter of 9.5 cm and a top diameter of 13.5 cm. They contained Carolina Soil substrate and were sown with Antonella arugula (*E. sativa*) - Super Isla seeds, Lot 165419-000, valid until June 2025. Fifty seeds were distributed at a depth of 0.5 cm, resulting in a sowing density of 0.4 seeds per cm<sup>2</sup>.

### *Experiment implementation, conduction, and data collection*

Two cultivation cycles were conducted. Cycle 1: from December 11, 2023, to January 3, 2024 (23 days); and Cycle 2: from January 11, 2024, to February 1, 2024 (21 days).

The following evaluations were performed: shoot fresh matter (SFM), shoot dry matter (SDM), chlorophyll a (*Chla*) content, chlorophyll b (*Chlb*) content, total chlorophyll (Total *Chl*) content, carotenoids (CRT) content, internal CO<sub>2</sub> concentration (C<sub>i</sub>), transpiration (E), stomatal conductance (g<sub>s</sub>), net photosynthesis (PN), water use efficiency (PN/E), and instantaneous carboxylation efficiency (PN/C<sub>i</sub>).

Determinations of SFM and SDM were performed using an analytical balance with a precision of four decimal places. Dry matter was obtained by drying in a forced-circulation oven at 65 °C for 72 hours.

Extractions of chlorophylls (a and b) and carotenoids followed the methodology of Lichtenthaler (1987). Fresh plant material (0.5 g) was weighed, 5 mL of 80% acetone was added, and samples were stored in 14 mL test tubes for 48 hours in a refrigerator at 25 °C. After this period, the test tubes were centrifuged for 15 minutes at 4000 rpm, and then the supernatant extract was diluted at a ratio of 0.3 mL of extract to 1.7 mL of 80% acetone. Measurements were performed using a spectrophotometer at wavelengths of 470, 647, 653, 663, and 665 nm, with three samples for each replicate.

To determine internal CO<sub>2</sub> concentration (C<sub>i</sub>), transpiration (E), stomatal conductance (g<sub>s</sub>), and CO<sub>2</sub> assimilation rate or net photosynthesis (PN), the leaves in the middle third of the plant were evaluated at 9 a.m., with three readings in each repetition using a portable infrared gas analyzer (LCi, ADC Bioscientific, Hertfordshire, UK). Subsequently, the water use efficiency (PN/E) (ratio between net photosynthesis and transpiration) and the instantaneous carboxylation efficiency (PN/C<sub>i</sub>) (ratio between net photosynthesis and internal CO<sub>2</sub> concentration) were calculated.

Reflected photosynthetically active radiation (PAR) ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) from the cultivation benches was monitored daily at 9:30 am using a portable digital quantum PAR sensor (Apogee MQ-200). Air temperature (°C), relative air humidity (%), and external and internal global radiation (W m<sup>-2</sup>) were collected via an automatic Irriplus station, model E4000, located at the center of the environment and externally, with readings recorded every 60 minutes.

The reflectance spectra of the blue laminated reflective materials (Formica) were obtained using a UV-Vis-NIR spectrophotometer (Lambda 1050 Model, Perkin Elmer), with a 1 nm step at a speed of 100 nm/minute. Small discs of the laminates (f = 1 cm) were inserted into the sample holder of a 150 mm radius integrating sphere.

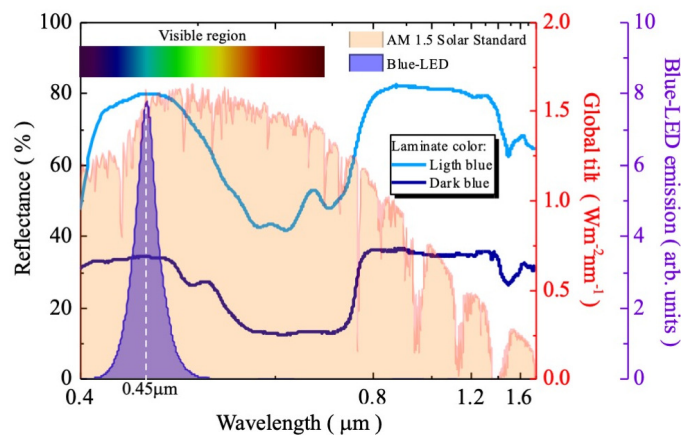
The LED was positioned 55 cm above the plants, and the supplemental PAR intensity applied to the plants is described in Table 1.

### Statistical analysis

Statistical analyses were performed using Sisvar version 5.3 (Ferreira, 2011). Means were subjected to analysis of variance (ANOVA), in a one-way, using the F-test and compared using the LSD test at a 5% significance level. The figures were created using the Excel program.

### Results

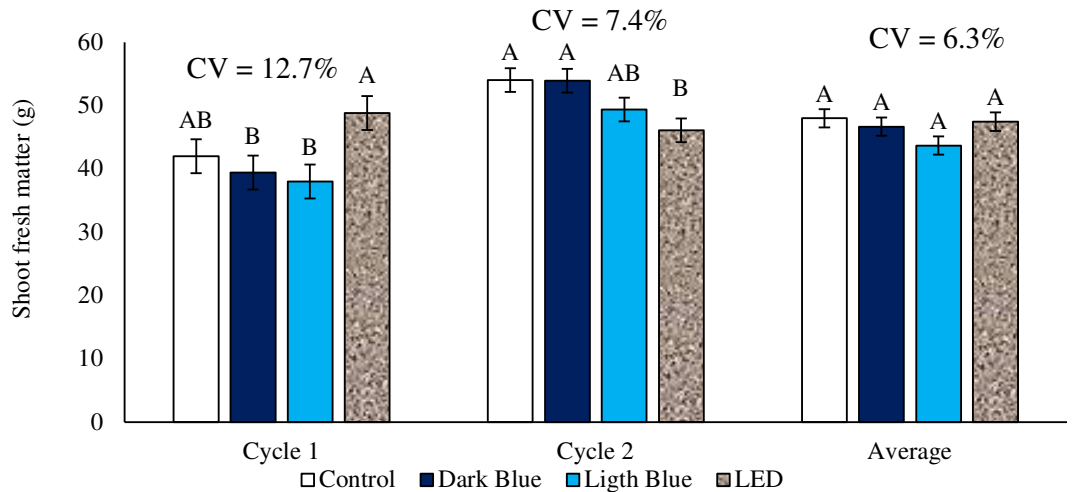
Figure 1 displays the reflectance spectra of the light blue and dark blue Formica<sup>®</sup> sheets, alongside the standard AM 1.5 solar spectrum and the emission spectrum of the blue LED. The blue LED's peak emission is at 450 nm, with a full width at half maximum of approximately 50 nm ( $\sim 2500 \text{ cm}^{-1}$ ). It is evident that while both Formica<sup>®</sup> sheets exhibit similar spectral profiles, greater light reflectance is observed with the light blue Formica<sup>®</sup>, regardless of the analyzed spectral range. The reflectivity of the Formica<sup>®</sup> sheets causes a larger portion of light, particularly for wavelengths shorter than approximately 550 nm, to be reflected and thus directed onto the plant leaves. Additionally, the reflectivity of both Formica<sup>®</sup> sheets in the near-infrared region, at wavelengths greater than 800 nm, is noteworthy, even though solar radiation intensity tends to be lower in this region near-infrared. Despite being less intense, the near-infrared light energy reaches the plant leaves in different proportions when comparing the Formica<sup>®</sup> sheets.



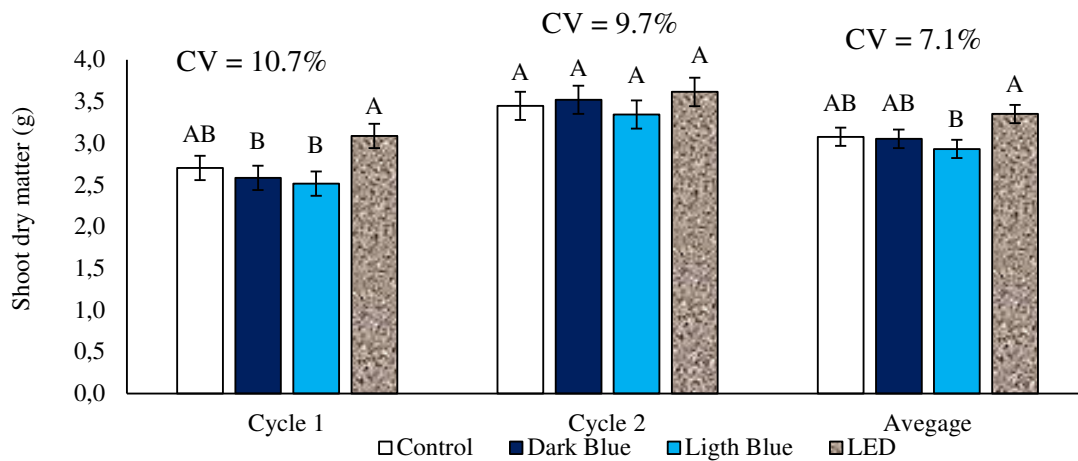
**Figure 1.** Reflectance of blue laminates used for growing baby leaf arugula. The highlighted spectrum corresponds to the AM 1.5 solar standard. The blue-LED emission spectrum is also plotted for comparison.

The average external temperature ( $24.7 \text{ }^{\circ}\text{C}$ ) was lower than the average internal temperature ( $26.8 \text{ }^{\circ}\text{C}$ ), indicating the greenhouse effect created by the polyethylene film; however, this didn't negatively impact the development of the baby leaf arugula. Conversely, the external relative humidity ( $85.5\%$ ) was higher than the internal relative humidity ( $80.2\%$ ). Internal global radiation was significantly reduced compared to external radiation ( $170.8 \text{ W m}^{-2}$  vs.  $451.1 \text{ W m}^{-2}$ ), as was internal PAR, which was approximately 72% lower than external PAR ( $547.3 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  vs.  $1984.4 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ ). This reduction was due to the combined effect of the polyethylene film and the thermo-reflective screen. Among the light supplementation treatments, the LED showed a constant PAR emission ( $60 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ ), while the laminates reflected varying light levels based on colour intensity. The blue laminate reflected the light, on average,  $119.5 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ , and the dark blue laminate reflected  $34.17 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ . The control bench, being grated (open), had a reflected PAR of  $25.1 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  reaching the plants, which was slightly lower than the dark blue laminate. Average temperatures ranging from  $26.1 \text{ }^{\circ}\text{C}$  to  $27.4 \text{ }^{\circ}\text{C}$  were suitable for growing arugula under tropical conditions (Table 1).

In the study of shoot fresh matter (SFM) of arugula plants, results showed significant differences among treatments in both the first and second cultivation cycles. In the first cycle, the SFM of the LED treatment, which did not differ from the control treatment, was 23.8% and 28.5% higher than that of the dark blue laminate and light blue laminate treatments, respectively. In the second cycle, however, the control treatment showed a higher SFM than the LED treatment, but did not differ significantly from the dark blue and light blue laminates treatments. The LED treatment, conversely, showed a 14.7% reduction in relation to control treatment. Averaged across both cycles, the control, dark blue laminate, light blue laminate, and LED treatments did not show statistical differences, indicating that these treatments were equivalent for SFM production over time (Figure 2A).



(A)



(B)

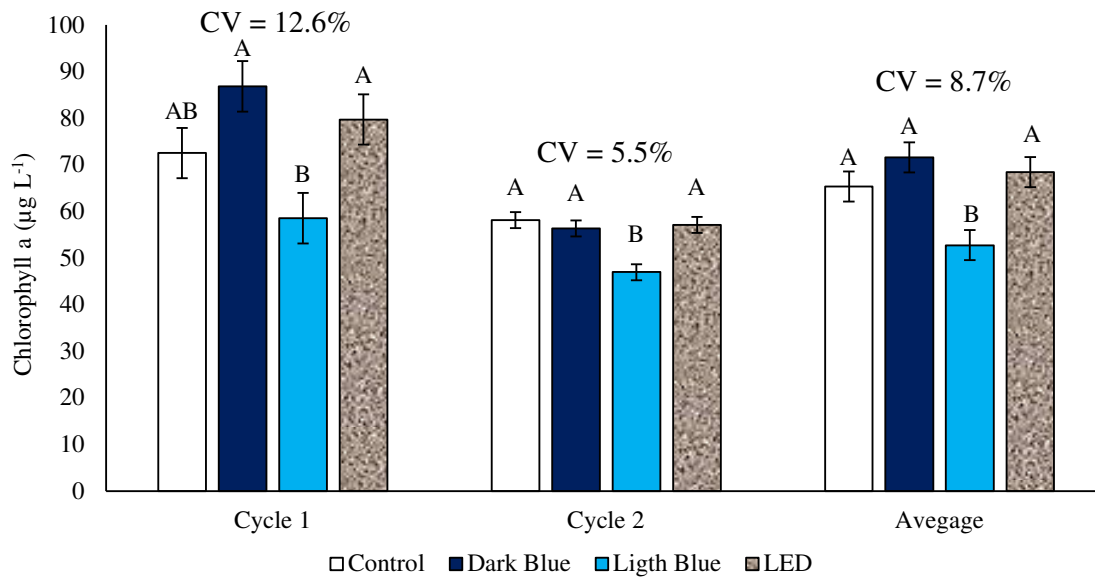
**Figure 2.** Fresh (A) and dry (B) matter of the aerial part of baby arugula under blue light supplementation Cycle 1: from 12/11/2023 to 01/03/2024 (23 days); Cycle 2: from 01/11/2024 to 02/01/2024 (21 days). Capital letters equal do not differ from each other at 5% probability by the LSD test. CV = coefficient of variation. Vertical bars indicate the standard error

Regarding shoot dry matter (SDM) in the first cycle, the SDM of the LED treatment, which did not differ from the control treatment, was 19.4% and 22.6% higher than that of the dark blue laminate and light blue laminate treatments, respectively. In the second cycle, all treatments showed statistically similar values.

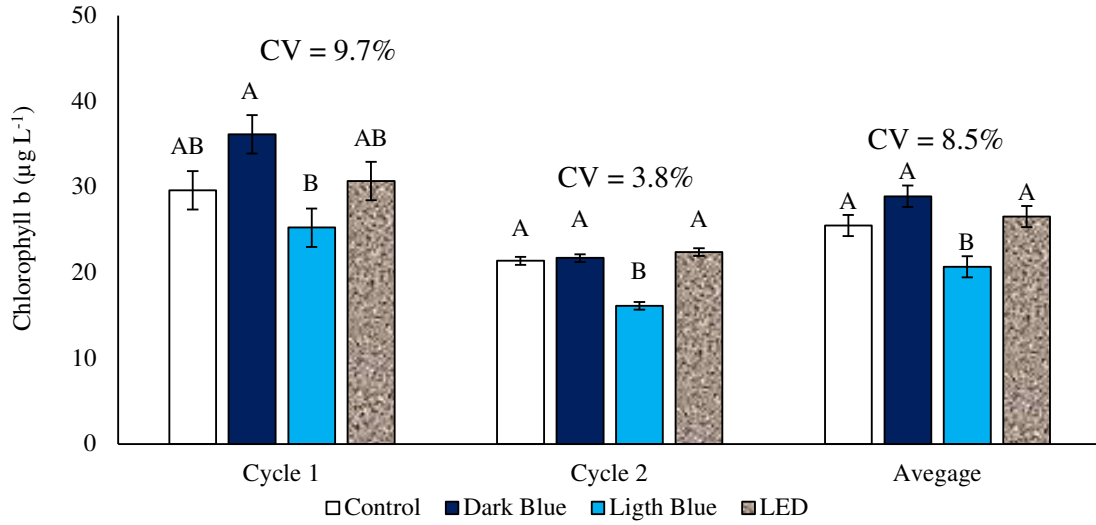
Averaged across both cycles, the LED, control, and dark blue laminate treatments did not show statistical differences. The LED treatment achieved the highest SDM in grams, 12.5% higher than the light blue laminate, which recorded the lowest average across the cycles (Figure 2B).

The analysis of chlorophyll *a* (*Chla*) content revealed differences among treatments, particularly in the first cultivation cycle. Plants grown on the bench with dark blue laminate treatment, which did not differ from the LED and control treatments, showed an increase of 48.3% of the *Chla* content, when compared to the light blue laminate treatment, which had the lowest value among treatments. In the second cycle, the differences between treatments decreased, but the control, dark blue laminate, and LED treatments maintained high values without significant statistical differences, while the light blue laminate continued to show lower values. Averaged across both cycles, the dark blue laminate, LED, and control treatments maintained the highest *Chla* content, with the dark blue laminate being the treatment with the highest percentage content, 35.7% higher than the light blue laminate (Figure 3A).

For chlorophyll *b* (*Chlb*) content, the dark blue laminate treatment in the first cycle, which did not differ from the LED and control treatments, was 43% higher than the light blue laminate treatment, which exhibited the lowest value among treatments. In the second cycle, the control, dark blue laminate, and LED treatments showed no significant differences, maintaining similar values among themselves and superior to the light blue laminate. Averaged across both cycles, the dark blue laminate, LED, and control treatments did not show statistical differences, but the dark blue laminate remained with the highest average percentage *Chlb* content, surpassing the light blue laminate by 39.8% (Figure 3B).



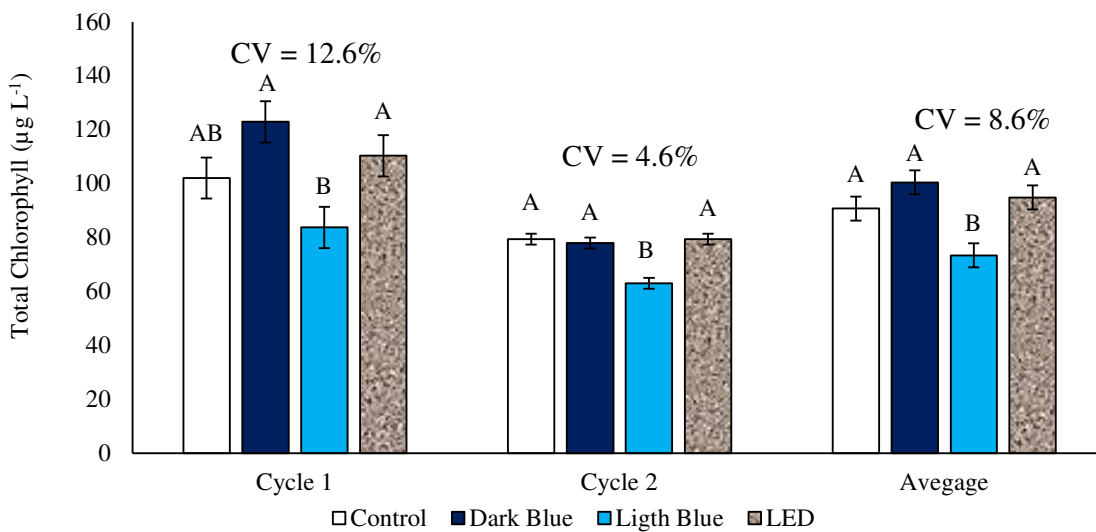
(A)

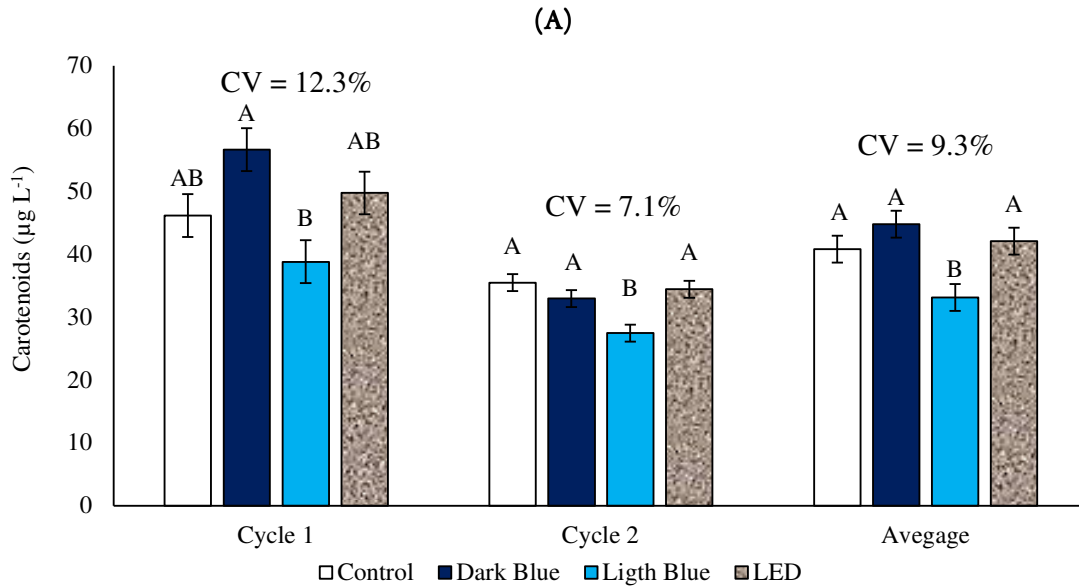


(B)

**Figure 3.** Chlorophyll a (A) and Chlorophyll b (B) of baby arugula under blue light supplementation Cycle 1: from 12/11/2023 to 01/03/2024 (23 days); Cycle 2: from 01/11/2024 to 02/01/2024 (21 days). Capital letters equal do not differ from each other at 5% probability by the LSD test. CV = coefficient of variation. Vertical bars indicate the standard error

For total chlorophyll (Total *Chl*) content, the dark blue laminate treatment in the first cycle, which did not differ from the LED and control treatments, was 46.7% higher than the light blue laminate treatment, which recorded the lowest values. In the second cycle, the control, dark blue laminate, and LED treatments maintained high Total *Chl* values, with no significant differences among them, while the light blue laminate continued to show the lowest values. The average of the two cycles revealed that the dark blue laminate, LED, and control treatments did not differ statistically, but the dark blue laminate maintained the highest percentage content of total chlorophyll, with a 36.8% increase compared to the light blue laminate (Figure 4A). The production of carotenoids in baby arugula across treatments was very similar to Total *Chl* production, with lower contents in the light blue laminate treatment (Figure 4B).

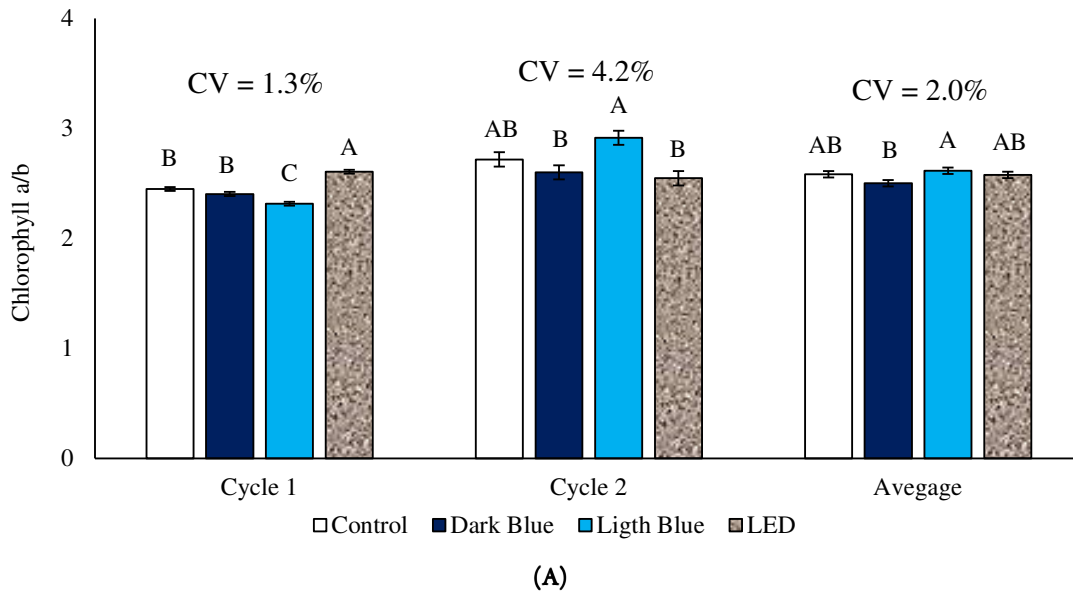


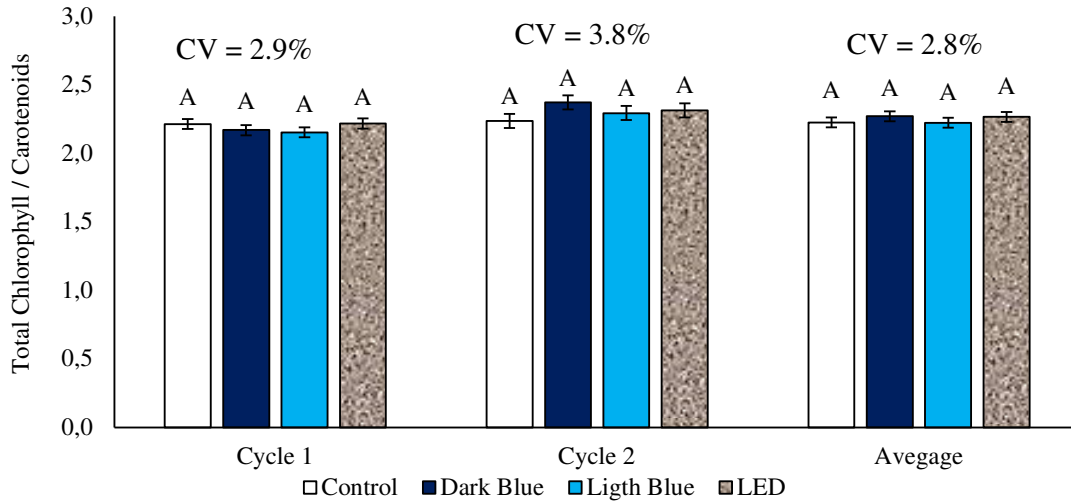


(B)

**Figure 4.** Total chlorophyll (A) and carotenoids (B) of baby arugula under blue light supplementation Cycle 1: from 12/11/2023 to 01/03/2024 (23 days); Cycle 2: from 01/11/2024 to 02/01/2024 (21 days). Capital letters equal do not differ from each other at 5% probability by the LSD test. CV = coefficient of variation. Vertical bars indicate the standard error

For the chlorophyll a to b ratio (*Chla/Chlb*), significant variation among treatments was observed in Cycle 1. Here, the LED treatment showed the highest *Chla/Chlb* ratio, 12.6% higher than the control, while the light blue laminate presented the lowest ratio. In Cycle 2, the light blue treatment obtained a higher value, not differing statistically from the control. Across the average of both cycles, no significant variations were observed between the light blue, control, and LED treatments, with the dark blue treatment showing lowest *Chla/Chlb* ratio that light blue treatment (Figure 5A).





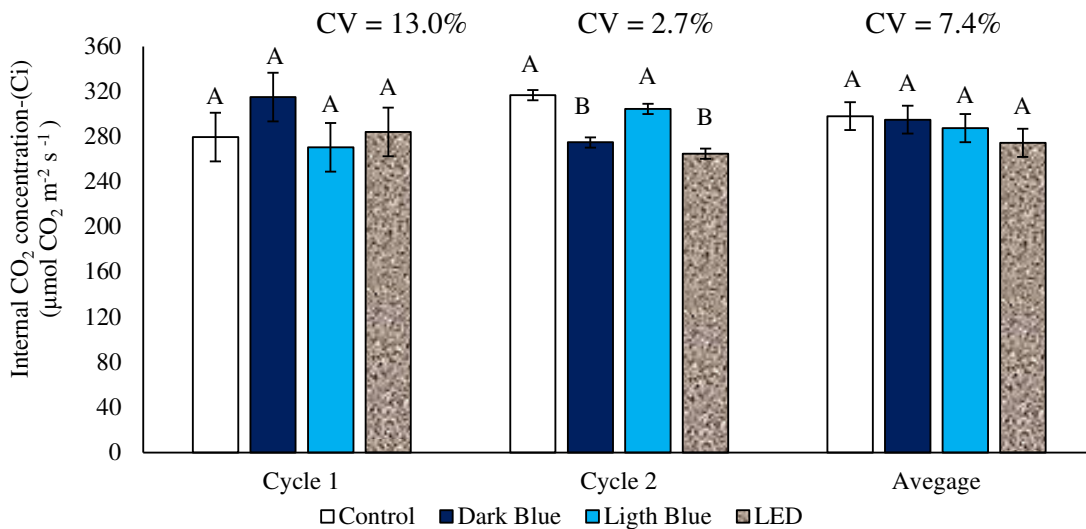
(B)

**Figure 5.** Chlorophyll a/b ratio (A) and total chlorophyll/carotenoids ratio (B) of baby arugula under blue light supplementation

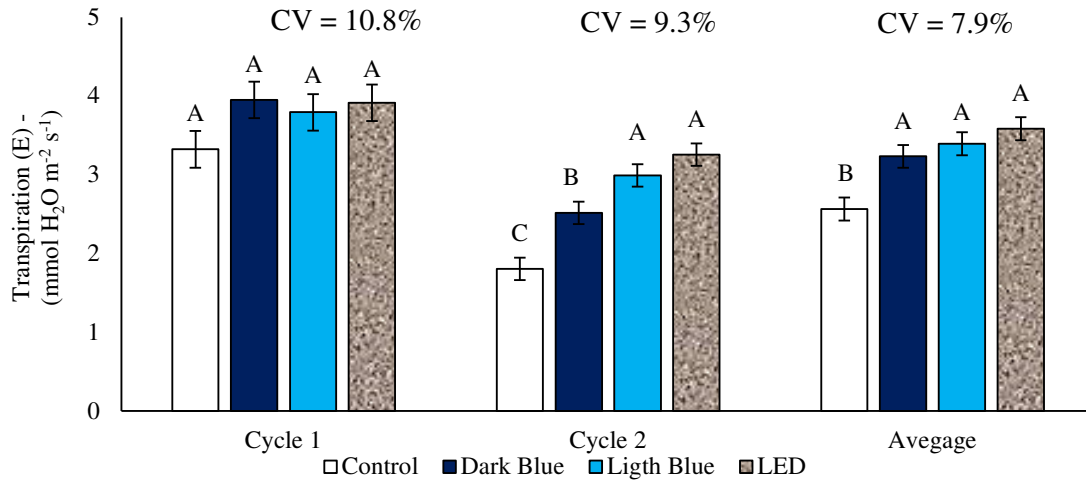
Cycle 1: from 12/11/2023 to 01/03/2024 (23 days); Cycle 2: from 01/11/2024 to 02/01/2024 (21 days). Capital letters equal do not differ from each other at 5% probability by the LSD test. CV = coefficient of variation. Vertical bars indicate the standard error

The analysis of the Total *Chl* to carotenoid ratio (Total *Chl*/CRT) revealed no statistically significant differences among treatments in either cycle, and in average (Figure 5B).

In the analysis of internal CO<sub>2</sub> concentration (Ci), there were no statistical differences between treatments in the first cycle. However, significant differences emerged in the second cycle. The control and light blue laminate treatments did not show statistical differences, but the control exhibited the highest internal CO<sub>2</sub> concentration, which was 19.6% higher than the LED treatment in the second cycle. Averaged across both cycles, the Ci values showed no significant differences (Figure 6A).



(A)



(B)

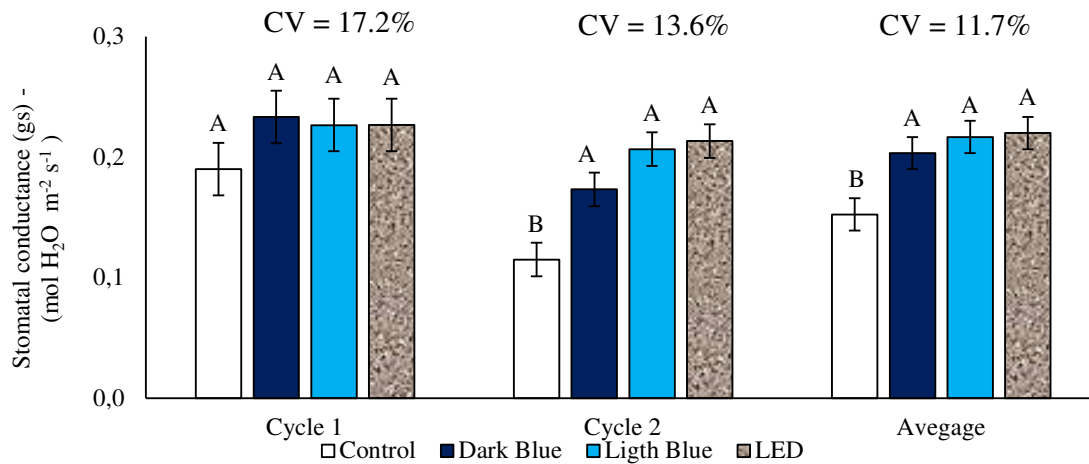
**Figure 6.** Internal CO<sub>2</sub> concentration (A) and transpiration (B) of baby arugula under blue light supplementation

Cycle 1: from 12/11/2023 to 01/03/2024 (23 days); Cycle 2: from 01/11/2024 to 02/01/2024 (21 days).

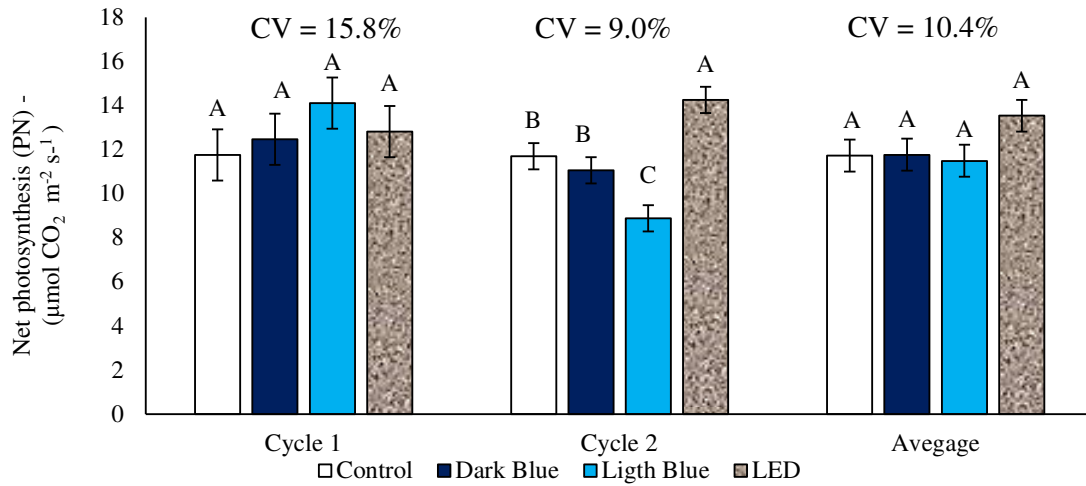
Capital letters equal do not differ from each other at 5% probability by the LSD test. CV = coefficient of variation. Vertical bars indicate the standard error

Regarding transpiration rate (E), no statistical differences were observed among treatments in the first cycle. In the second cycle, the LED and light blue laminate treatments showed the highest average transpiration rates, significantly surpassing the control, which had the lowest average, representing a reduction of approximately 44.5% compared to the LED. Averaged across both cycles, the LED, light blue laminate, and dark blue laminate treatments did not differ statistically, but the LED maintained the highest percentage transpiration rate, with a 40.2% increase compared to the control (Figure 6B).

Regarding stomatal conductance (gs), there was no statistical difference in the first cycle. In the second cycle, the LED, light blue, and dark blue laminate treatments showed the highest averages, while the control exhibited the lowest value, approximately 85.2% lower than the LED. Averaged across both cycles, the LED, light blue, and dark blue laminate treatments did not differ statistically, remaining superior to the control (Figure 7A).



(A)



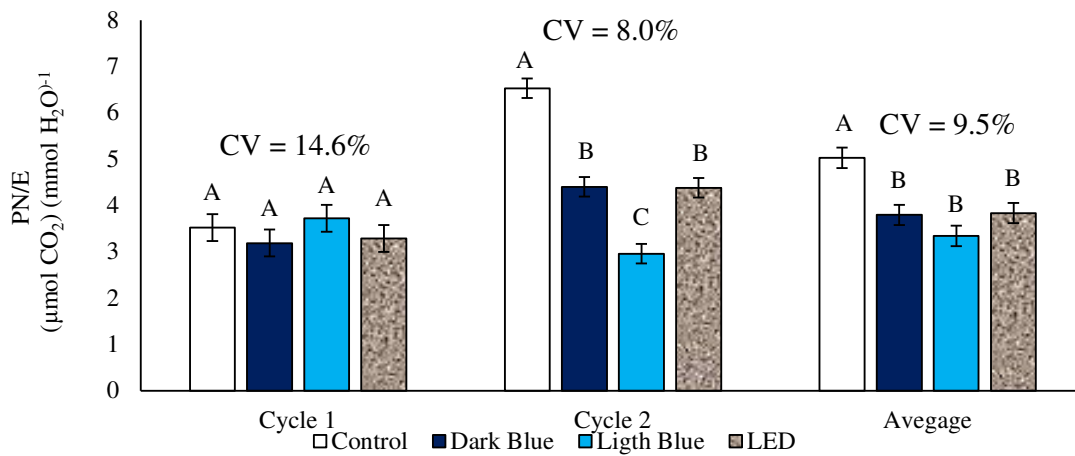
(B)

**Figure 7.** Stomatal conductance (A) and net photosynthesis (B) of baby arugula under blue light supplementation

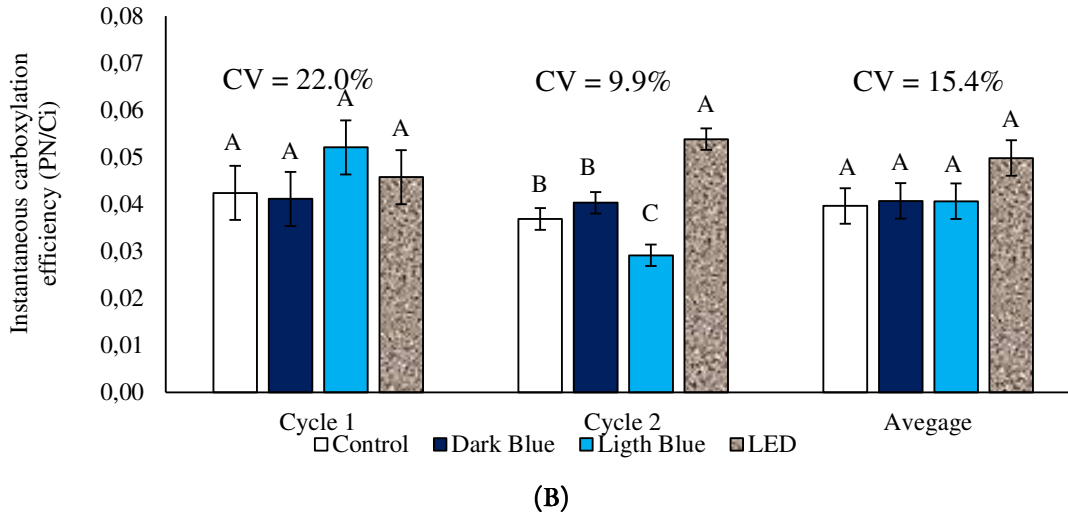
Cycle 1: from 12/11/2023 to 01/03/2024 (23 days); Cycle 2: from 01/11/2024 to 02/01/2024 (21 days). Capital letters equal do not differ from each other at 5% probability by the LSD test. CV = coefficient of variation. Vertical bars indicate the standard error

The net photosynthetic rate (PN) showed no statistical differences among treatments in the first cycle. In the second cycle, it was influenced by the type of illumination, in which the LED, which did not differ from the control and dark blue laminate treatments, showed a higher net photosynthetic rate than the light blue laminate, being 60.5% higher, possibly due to lower transpiration (Figure 6B) and lower stomatal conductance (Figure 7A) observed in the control treatment. Overall, across the average of both cycles, there were no statistical differences between the treatments (Figure 7B).

Regarding water use efficiency (PN/E), there were no statistical differences among treatments in the first cycle. In the second cycle, the control treatment maintained the highest average PN/E, being 50.6% higher than the light blue laminate, which showed the lowest value. On average, the control treatment achieved the best results compared to the dark blue laminate, light blue laminate, and LED illumination, which did not differ statistically and showed the lowest results (Figure 8A).



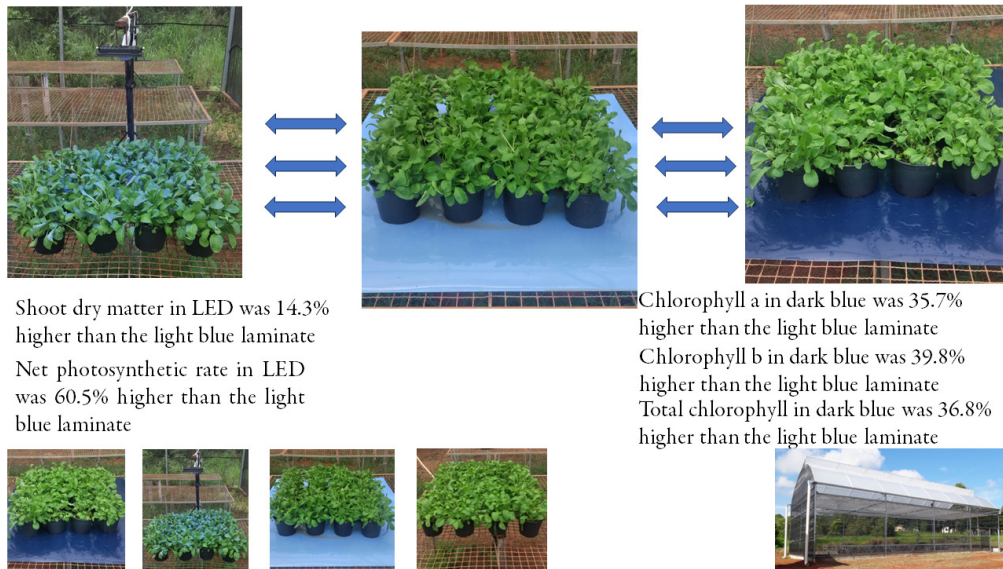
(A)



**Figure 8.** Water use efficiency,  $PN/E$  (A) and instantaneous carboxylation efficiency,  $PN/Ci$  (B) of baby arugula under blue light supplementation  
 Cycle 1: from 12/11/2023 to 01/03/2024 (23 days); Cycle 2: from 01/11/2024 to 02/01/2024 (21 days). Capital letters equal do not differ from each other at 5% probability by the LSD test. CV = coefficient of variation. Vertical bars indicate the standard error

For instantaneous carboxylation efficiency ( $PN/Ci$ ), there were no statistical differences among treatments in the first cycle. The LED illumination stood out in the second cycle, with the highest value, which was 84.6% higher than the light blue laminate, which showed the lowest efficiency. Averaged across both cycles, there were no statistical differences among treatments (Figure 8B).

In summary of the main results, we highlight the following: shoot dry matter in LED was 14.3% higher than the light blue laminate; chlorophyll a in dark blue was 35.7% higher than the light blue laminate; chlorophyll b in dark blue was 39.8% higher than the light blue laminate; total chlorophyll in dark blue was 36.8% higher than the light blue laminate; and net photosynthetic rate in LED was 60.5% higher than the light blue laminate (Figure 9).



**Figure 9.** Summary of the main results

## Discussion

Direct and indirect blue light supplementation for growth, pigmentation, and gas exchange in young arugula plants is a beneficial practice, although its effects may vary with light intensity and application method. Studies show that blue light, at moderate intensities within a narrow spectral range and in color combinations, stimulates photosynthesis and promotes cell elongation, favoring water and nutrient absorption, and thereby increasing the fresh biomass of plants (Johnson *et al.*, 2020; Kong and Zheng, 2020; Ying *et al.*, 2020b).

The absence of significant differences in fresh matter among treatments may indicate that the general growing conditions allowed for uniform basal growth, regardless of the type of supplemental light. However, arugula fresh and dry matter was higher in cultivation cycle 2 (Figure 2A), which had a lower average radiation intensity and temperature (Table 1). Studies such as Shao *et al.* (2020) showed that blue LED supplementation promotes biomass production and secondary metabolite accumulation in hydroponic lettuce but can cause photo-oxidative damage when used for 4 hours. Furthermore, by using blue light to increase the speed of stomatal responses in plant leaves via an engineered ion channel, productivity and water use efficiency can be increased under fluctuating light conditions without affecting carbon fixation (Papanatsiou *et al.*, 2019). In the current study, the applied intensity may not have been sufficient to promote differences in water retention and fresh matter.

Treatments with LED light and dark blue laminate showed higher dry matter compared to the light blue laminate, indicating that the quality and intensity of blue light can directly influence plant growth and biomass accumulation. Moderate and continuous levels of blue light increase photosynthetic efficiency and maintain the normal morphology of *Gerbera jamesonii* plants (Hung *et al.*, 2022). Blue light can increase plant height and dry matter allocation to leaves in some species, but may reduce total dry matter in others, such as basil, depending on the intensity and duration of exposure (Larsen *et al.*, 2020).

Evaluating the two arugula cultivation cycles, it is observed that, under higher levels of radiation and temperature (Table 1), photosynthetic pigments (chlorophylls and carotenoids) in Cycle 1 showed a significant increase with the use of laminates compared to Cycle 2. Nevertheless, the blue LED, dark blue laminate, and control treatments stood out in promoting high levels of chlorophyll *a* (*Chla*), chlorophyll *b* (*Chlb*), and total chlorophyll (Total *Chl*), significantly surpassing the light blue laminate (Figures 3A, 3B and 4A). These results reflect the effectiveness of blue light in stimulating photosynthesis, favoring rubisco activation, and promoting the synthesis of essential photosynthetic pigments, especially when provided directly and stably, as with LED and dark blue laminate (Samuolienė *et al.*, 2017; Izzo *et al.*, 2020; Hung *et al.*, 2022). Blue light promotes *Chla* and *Chlb* accumulation in various vegetable crops, such as *Brassica oleracea* (Dantas *et al.*, 2025), *Eruca sativa* (Santana *et al.*, 2025), *Cucumis sativus* (Wang *et al.*, 2015), *Lactuca sativa* L. (Wang *et al.*, 2016), and *Chlamydomonas reinhardtii* (Li *et al.*, 2021), increasing light capture efficiency and protection against oxidative stress.

The consistent pattern among *Chla*, *Chlb*, and Total *Chl* indicates that blue light contributes in an integrated manner to the balance of pigment metabolism, optimizing light energy capture and photosynthetic efficiency (Duarte and Costa, 2018; Hashimoto *et al.*, 2015; Ma *et al.*, 2020; Kaiser *et al.*, 2019). In contrast, the inferior performance of the light blue laminate can be attributed to the high intensity of the reflected light (Figure 1).

The carotenoid content in arugula plants showed higher levels in the blue LED, dark blue laminate, and control treatments, while the light blue laminate performed the worst (Figure 4B). Blue light is effective in increasing carotenoid synthesis because it acts as an antioxidant agent and protects the plant against oxidative damage, especially in high light intensity environments (Samuolienė *et al.*, 2017). The high intensity of direct blue light from the LED favors carotenoid production by providing a constant energy source that stimulates photosynthesis and the accumulation of these protective pigments. In the case of the light blue laminate, the

amplified intensity of the reflected light may have been too exacerbated to effectively stimulate carotenoid synthesis, resulting in lower concentrations of this pigment compared to the other treatments.

The blue LED, light blue laminate, and control treatments showed the best chlorophyll *a/b* ratios (*Chla/Chlb*), while the dark blue laminate performed worse (Figure 5A). In spinach and other leafy green crops, blue light has been shown to elevate total chlorophyll levels and adjust the chlorophyll ratio to favor a more balanced photon uptake under intense light conditions (Wang *et al.*, 2016). The direct influence of blue light on photosynthetic complexes allows for the adjustment of chlorophyll proportions, which reinforces its applicability in controlled systems, such as greenhouses with supplementary LED lighting (Ouzounis *et al.*, 2015). This blue light supplementation also favors chlorophyll stability in species adapted to protected environments by regulating chlorophyll b synthesis and its proportion with chlorophyll a, adjusting energy capture in photosystems for conditions of lower direct irradiance (Naznin *et al.*, 2019). The inferior performance of the dark blue laminate may have occurred due to the reflection of a more diffuse and lower intensity amount of blue light, resulting in a lower *Chla/Chlb* ratio.

Direct and indirect blue lights yielded better results for transpiration (E) and stomatal conductance (gs), with LED being the best promoter of higher rates. Blue light directly influences stomatal conductance, facilitating stomatal opening and intensifying gas exchange between the plant and the environment (Lin *et al.*, 2018; Vialat-Chabrand *et al.*, 2021). In summary, these results corroborate with Gao *et al.* (2020), where E and gs were highest under blue light in the cultivation of *Allium fistulosum* L.

The control treatment stood out compared to LED and laminates for water use efficiency (PN/E), indicating a transpiration-photosynthesis relationship under natural light. According to Pan *et al.* (2020), increased CO<sub>2</sub> reduces transpiration and water consumption at different light intensity levels, significantly increasing water use efficiency at the leaf and plant level.

The direct and indirect supplementation of blue light significantly impacts the growth and pigmentation in young arugula plants. It proved effective in producing dry biomass and check it photosynthetic pigments, such as chlorophylls and carotenoids, with the LED light and dark blue laminate, and the control treatment showed better performance than the plants in the light blue laminate treatment. In contrast, the light blue laminate showed inferior performance, possibly due to its higher reflected light intensity. These results indicate that the intensity and quality of blue light are crucial for the physiological development of these plants.

Regarding gas exchange, treatments with blue light, both direct and indirect, promoted higher transpiration and stomatal conductance, demonstrating the role of blue light in stomatal opening and gas exchange. This opening encourages the entry of carbon dioxide (CO<sub>2</sub>) into the leaves, increasing the photosynthetic rate and metabolic efficiency. Although increased atmospheric CO<sub>2</sub> can, under certain conditions, induce stomatal closure as a water-saving mechanism, in the present study, the predominance of blue light acts as a physiological stimulus for stomatal opening. Thus, the results reinforce the importance of consistent blue light sources to maximize arugula production.

## Conclusions

Direct blue light (via LED) and indirect blue light (via dark blue laminate) positively impact the growth, photosynthetic pigment production (chlorophylls and carotenoids), and gas exchange (transpiration and stomatal conductance) in young arugula plants in a protected environment. Blue light is a promising tool for managing protected crops. Future studies exploring spectral combinations are recommended to maximize the observed benefits. Therefore, this study highlights blue LED light as a promising tool for controlling growth and photosynthetic performance in arugula cultivation. Future studies are recommended to explore

combinations of light spectra to optimize plant development in controlled environments, maximizing the benefits of blue light for more efficient production.

The dark blue laminate and LED, even though not differing significantly at 5% from the control treatment, increased total chlorophyll by an absolute average of 10.7% and 4.6%, and carotenoids by 9.7% and 3.1%, respectively, compared to the control. On a large production scale, their use may be economically viable, as these pigments are beneficial to human health and add value to production. Thus, we can perform biofortification with bioactive compounds with antioxidant effect (carotenoids) in arugula using dark blue laminate, which is low-cost and does not require electricity, making it an economically viable technology for large and small producers.

### Authors' Contributions

Conceptualization: PHRM, EC, GPVS, FFSB, EPV. Methodology: PHRM, GPVS, EC, FFSB. Investigation: PHRM, GPVS, EPV, FPAPB. Resources: GHCV, EC, FFSB, LHCA, SML. Data curation: PHRM, FPAPB, EC, TD, GHCV. Writing-original draft: PHRM, TD, FPAPB, LHCA, SML, EPV. Writing-review & editing: PHRM, TD, EC, FPAPB, LHCA, SML. Project administration: EC, FFSB, GHCV, EPV. Funding acquisition: EC, FFSB, GHCV, LHCA, SML.

All authors read and approved the final manuscript.

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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