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EFFECT OF BLUE LED LIGHT PHOTOPERIOD ON THE SYNTHESIS OF BIOACTIVE PIGMENTS IN ARUGULA MICROGREENS

Ellen Abreu da Cruz [1]
Carlos Henrique Barbosa Varjão [2]
Ênio Gomes Flôr Souza [3]
Ranniele Luíza Ventura da Silva [4]
Renata Maria Rosas Garcia Almeida [5]
Elaine Christine de Magalhães Cabral Albuquerque [6]

ABSTRACT

Objectives: This study aimed to determine the optimal blue light photoperiod and cultivation time to maximize the synthesis of bioactive pigments (chlorophylls, carotenoids, flavonoids, and anthocyanins) in arugula (*Eruca sativa*) microgreens grown under blue LED lighting.

Theoretical Framework: Microgreens are recognized for their high nutritional value and nutraceutical potential, often containing higher levels of phytonutrients than mature plants. Light quality and quantity, especially the blue spectrum, directly influence plant morphology and chemical composition, stimulating the biosynthesis of key pigments essential for plant metabolism and human health.

Method: Arugula microgreens were cultivated in a growth chamber under five blue LED photoperiods (10, 12, 14, 16, and 18 hours per day), evaluated from the 4th to the 8th day after sowing. The experimental design was completely randomized, with three replicates. Pigment contents were determined by spectrophotometry, and the data were analyzed using variance and regression analyses.

Results and Discussion: Maximum chlorophyll a accumulation was observed on the 8th day with 14 hours of light, while chlorophyll b peaked on the 7th day with approximately 13.7 hours. Total chlorophyll increased over time, with the highest value under 16 hours of light. Carotenoids accumulated most between 12 and 14 hours of exposure but decreased with longer photoperiods. Flavonoids and anthocyanins showed higher levels at 14 and 18 hours and 12 hours, respectively. The 14-hour photoperiod provided the best balance among bioactive pigments.

Research Implications: The results indicate that manipulating the blue LED photoperiod can be an effective strategy to optimize the nutritional and functional quality of arugula microgreens, contributing to more sustainable and technologically advanced production systems.

Originality/Value: This study provides novel data on the influence of blue light photoperiod on the synthesis of bioactive pigments in arugula microgreens, offering insights for artificial lighting management in controlled cultivation and adding value to the final product.

Keywords: Phytonutrients, Antioxidants, *Eruca Sativa*, Light Spectrum, Urban Agriculture, Sustainable Production.

[1] Universidade Federal da Bahia, Instituto Federal de Alagoas, Piranhas, Alagoas, Brasil. E-mail: ellen.cruz@ifal.edu.br
[2] Instituto Federal de Alagoas, Piranhas, Alagoas, Brasil. E-mail: chbv1@aluno.ifal.edu.br
[3] Instituto Federal de Alagoas, Piranhas, Alagoas, Brasil. E-mail: enio.souza@ifal.edu.br
[4] Universidade Federal de Alagoas, Maceió, Alagoas, Brasil. E-mail: agrorluiza@gmail.com
[5] Universidade Federal de Alagoas, Maceió, Alagoas, Brasil. E-mail: rmrga@ctec.ufal.br
[6] Universidade Federal da Bahia, Salvador, Bahia, Brasil. E-mail: elainecmca@ufba.br

EFEITO DO FOTOPERÍODO DE LUZ LED AZUL NA SÍNTESE DE PIGMENTOS BIOATIVOS EM MICROVERDES DE RÚCULA

RESUMO

Objetivos: O presente trabalho teve como objetivo determinar o fotoperíodo ideal de luz LED azul e o tempo de cultivo mais adequado para maximizar a síntese de pigmentos bioativos (clorofilas, carotenoides, flavonoides e antocianinas) em microverdes de rúcula (*Eruca sativa*) cultivados sob iluminação LED azul.

Referencial Teórico: Microverdes destacam-se pelo elevado valor nutricional e potencial nutracêutico, apresentando teores superiores de fitonutrientes em comparação às plantas adultas. A qualidade e quantidade de luz, especialmente o espectro azul, influenciam diretamente a morfologia e a composição química das plantas, estimulando a biossíntese de pigmentos essenciais ao metabolismo vegetal e à saúde humana.

Método: Microverdes de rúcula foram cultivados em câmara de crescimento sob cinco fotoperíodos de luz LED azul (10, 12, 14, 16 e 18 horas diárias), avaliados do 4º ao 8º dia após o plantio. O delineamento experimental foi inteiramente ao acaso, com três repetições. Os teores de pigmentos foram determinados por espectrofotometria, e os dados analisados por variância e regressão.

Resultados e Discussão: O maior acúmulo de clorofila a foi observado no 8º dia com 14 horas de luz, enquanto a clorofila b atingiu o pico no 7º dia com cerca de 13,7 horas. A clorofila total aumentou ao longo dos dias, com valor máximo sob 16 horas de luz. Carotenoides tiveram maior acúmulo entre 12 e 14 horas de exposição, mas decresceram com fotoperíodos mais longos. Flavonoides e antocianinas apresentaram teores mais elevados em fotoperíodos de 14 e 18 horas e 12 horas, respectivamente. O fotoperíodo de 14 horas proporcionou o melhor equilíbrio entre os pigmentos bioativos.

Implicações da Pesquisa: Os resultados indicam que a manipulação do fotoperíodo de luz LED azul pode ser uma estratégia eficiente para otimizar a qualidade nutricional e funcional dos microverdes de rúcula, contribuindo para sistemas de produção mais sustentáveis e tecnificados.

Originalidade/Valor: O estudo contribui com dados inéditos sobre a influência do fotoperíodo de luz LED azul na síntese de pigmentos bioativos em microverdes de rúcula, oferecendo subsídios para o manejo da iluminação artificial em cultivos controlados e agregando valor ao produto final.

Palavras-chave: Fitonutrientes, Antioxidantes, *Eruca Sativa*, Espectro Luminoso, Agricultura Urbana, Produção Sustentável.

EFECTO DEL FOTOPERÍODO DE LUZ LED AZUL EN LA SÍNTESIS DE PIGMENTOS BIOACTIVOS EN MICROBROTES DE RÚCULA

RESUMEN

Objetivos: El presente estudio tuvo como objetivo determinar el fotoperíodo óptimo de luz azul y el tiempo de cultivo más adecuado para maximizar la síntesis de pigmentos bioactivos (clorofilas, carotenoides, flavonoides y antocianinas) en microbrotes de rúcula (*Eruca sativa*) cultivados bajo iluminación LED azul.

Marco Teórico: Los microbrotes se destacan por su alto valor nutricional y potencial nutracéutico, presentando niveles superiores de fitonutrientes en comparación con las plantas adultas. La calidad y cantidad de la luz, especialmente el espectro azul, influyen directamente en la morfología y composición química de las plantas, estimulando la biosíntesis de pigmentos esenciales para el metabolismo vegetal y la salud humana.

Método: Se cultivaron microbrotes de rúcula en cámaras de crecimiento bajo cinco fotoperíodos de luz LED azul (10, 12, 14, 16 y 18 horas diarias), evaluados del 4º al 8º día después de la siembra. El diseño experimental fue completamente al azar, con tres repeticiones. El contenido de pigmentos se determinó por espectrofotometría y los datos se analizaron mediante análisis de varianza y regresión.

Resultados y Discusión: El mayor acúmulo de clorofila a se observó en el octavo día con 14 horas de luz, mientras que la clorofila b alcanzó su punto máximo en el séptimo día con aproximadamente 13,7 horas. La clorofila total aumentó con el tiempo, con el valor más alto bajo 16 horas de luz. Los carotenoides se acumularon más entre 12

y 14 horas de exposición, pero disminuyeron con fotoperíodos más largos. Flavonoides y antocianinas presentaron niveles más altos en fotoperíodos de 14 y 18 horas y 12 horas, respectivamente. El fotoperíodo de 14 horas proporcionó el mejor equilibrio entre los pigmentos bioactivos.

Implicaciones de la Investigación: Los resultados indican que la manipulación del fotoperíodo de luz LED azul puede ser una estrategia eficaz para optimizar la calidad nutricional y funcional de los microbrotes de rúcula, contribuyendo a sistemas de producción más sostenibles y tecnificados.

Originalidad/Valor: El estudio aporta datos inéditos sobre la influencia del fotoperíodo de luz LED azul en la síntesis de pigmentos bioactivos en microbrotes de rúcula, ofreciendo información relevante para el manejo de la iluminación artificial en cultivos controlados y agregando valor al producto final.

Palabras clave: Fitonutrientes, Antioxidantes, *Eruca Sativa*, Espectro Luminoso, Agricultura Urbana, Producción Sostenible.

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1 INTRODUCTION

Microgreens are young plants of plants and herbs grown to the stage when the cotyledons are fully open or the first true leaf emerges (Katsenios *et al.*, 2021). More recently, they are being considered for their high nutritional value and nutraceutical potential, presenting higher values of phytonutrients than their mature equivalents (Katsenios *et al.*, 2021; Zhang *et al.*, 2020; Wright & Holden, 2018; Xiao *et al.*, 2014).

They are consolidated as sustainable and resilient crops, with the potential to integrate more efficient, urban and low environmental impact food systems. They are easily produced due to the need for reduced space, low requirement of nutrients and growth medium and short growth period (Turner *et al.*, 2020). They can be grown outdoors, indoors, in greenhouses or in hydroponic systems (Misra & Gibson, 2021), adapting to different production contexts. This versatility allows its cultivation in urban and controlled environments, including in regions with degraded soils or scarce resources, contributing to food security in crisis scenarios. In addition, its production demands significantly less water than conventional crops, making it an efficient strategy in the face of drought periods associated with climate change (Lone *et al.*, 2024). The use of artificial light, especially high energy efficiency LEDs, also enables the use of renewable sources, reducing the carbon footprint (Dubey *et al.*, 2024; Turner *et al.*, 2020; Xiao *et al.*, 2014).

Light directly influences the growth and chemical composition of plants, as it provides not only the energy source for photosynthesis, but also the signal for a multitude of physiological responses. Light quality (wavelength), amount of light (intensity), photoperiod

(duration) and uniform direction are key components of lighting conditions (Lobiuc *et al.*, 2017; Zhang *et al.*, 2020).

Incandescent and fluorescent lamps are now being replaced by light-emitting diode (LED) lamps, which have recently been applied in controlled environment agriculture for the cultivation of vegetables of various types, including microgreens, and in food processing (Cossu *et al.*, 2021; Alrifai *et al.*, 2019).

Compared to conventional light sources, LED light offers inexpensive, cold and controllable light sources that can provide different spectra in a *selective and quantitative manner (Zhang et al.*, 2020). LED light sources with defined wavelengths and optimised parameters can positively affect the accumulation of bioactive compounds, taste and pigmentation of vegetables (Alrifai *et al.*, 2019).

Among the different light spectra, blue light plays a key role in the development of microgreens, directly influencing their morphology and chemical composition. This wavelength is associated with the regulation of stomatal opening, compact vegetative growth and increased biosynthesis of several bioactive compounds (Alrifai *et al.*, 2019; Lobiuc *et al.*, 2017). Studies show that exposure to blue light can stimulate the production of essential pigments, such as chlorophylls, carotenoids, flavonoids and anthocyanins, which play important roles in both plant metabolism and human health (Zhang *et al.*, 2020).

Chlorophylls are the main pigments involved in photosynthesis, responsible for the absorption of light and conversion into chemical energy. Blue light, together with red light, contributes to the maintenance and increase of chlorophyll content in plants, enhancing their growth and nutritional quality (Xiao *et al.*, 2014). Carotenoids, in addition to acting in photoprotection, have antioxidant properties beneficial to human health, and their synthesis is also stimulated by blue light (Wright & Holden, 2018).

Flavonoids and anthocyanins are phenolic compounds associated with antioxidant and anti-inflammatory effects. Blue light can intensify the biosynthesis of these pigments, promoting a vibrant colour and increasing the nutraceutical value of microgreens (Misra & Gibson, 2021). Thus, the use of LEDs with adjustable spectrum, especially with a significant fraction of blue light, can be an efficient strategy to improve the nutritional and functional quality of these plants.

In view of the above, the objective of this work was to determine the ideal photoperiod of blue LED light and the most appropriate cultivation time to maximise the synthesis of bioactive pigments (chlorophylls, carotenoids, flavonoids and anthocyanins) in arugula microgreens.

2 METHODOLOGY

2.1 PLANT MATERIAL AND GROWING CONDITIONS

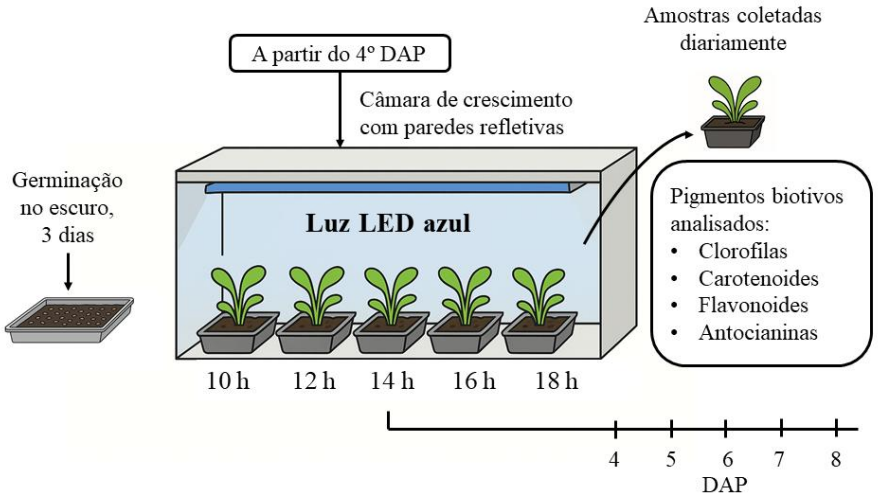
Arugula (*Eruca sativa* cv. "Surya"), from the company ISLA Sementes, were grown on 50 g of commercial substrate (Tropstrato HA Vegetables - Green Life) with nutritional composition: pine bark, vermiculite, Mix 14-16-18, potassium nitrate, simple superphosphate and peat, in plastic containers (trays) of 0.2 L (9.0 cm x 9.0 cm x 4.5 cm) for eight days.

Two grammes of arugula seed were sown per tray, which represented a repetition. The trays, with the seeds without cover, were placed to germinate in the dark (negative photoblastism, in which the germinative behaviour of the seeds is better in the absence than in the presence of light) for three days, to avoid etiolation (abnormal growth of the plant, characterised by thin, elongated and pale stems, due to the lack of adequate light). The trays were arranged randomly and systematically rotated every day to improve the uniformity of light exposure, after the three days in the dark. The plants were watered daily with a slight mist of distilled water.

The experiments were carried out in a growth chamber adapted with temperature and relative humidity monitoring. The day/night temperatures of 25 °C ± 2 °C were established with relative humidity of 60-70%. The adapted chamber remained in a closed room inside the Microbiology Laboratory of the Federal Institute of Alagoas (IFAL), Piranhas Campus.

Figure 1

Representative scheme of the experiment with arugula microgreens under different photoperiods of blue light.



2.2 LIGHTING SYSTEM AND EXPERIMENTAL DESIGN

The microgreens were grown under light culture conditions in a growth chamber adapted and equipped with two LED reflectors (20 W), with predominant wavelength of approximately 450 nm, in blue colour arranged above the trays. The reflectors were activated by a timer (timer). The chamber was lined with aluminium foil for more uniform light scattering.

The experimental design was completely randomised with three replications, with treatments arranged in a factorial scheme (5 x 5), referring to periods of exposure to LED (10, 12, 14, 16 and 18 hours daily), and times of daily evaluation: from the 4th to the 8th day after planting (DAP). The experimental plot consisted of trays with substrate and seeds.

The objective of the study was to evaluate the effect of different photoperiods under blue light specifically, since the literature has already demonstrated that the blue spectrum has a determinant role in the modulation of bioactive pigments. Therefore, no treatments with absence of light or other spectral sources were included in this first exploratory experiment.

We recorded how many days passed from sowing to: breaking of seed coat, root spill, hypocotyl emission, cotyledons formation.

2.3 PIGMENT CONTENT

The pigment content was obtained in triplicate for five consecutive days, from the 4th to the 8th DAP.

2.3.1 Chlorophylls and Carotenoids

The pigments were extracted one hour after harvest. About 200 mg of the microgreen sample was weighed on an analytical scale and then placed in a mortar and added 0.2 g of $CaCO_3$ and 3 mL of 80% acetone. After homogenisation, 2 mL of 80% acetone was added, completing the final volume for 5 mL. This procedure was carried out in a dark environment, and the extract was centrifuged in a refrigerated centrifuge (Universal 320R, Hettich) for ten minutes, with a temperature of 10 °C and rotation at 3,000 rpm. After centrifugation, the supernatant was transferred to a 10 mL test tube and the volume was observed. Aliquots were taken and read in a spectrophotometer (Nova 2000, Femto) at wavelengths of 470 nm, 646 nm and 663 nm. The contents of chlorophylls and carotenoids were calculated using the following

formulas (Lichtenthaler & Wellburn, 1983):

$$CLO_a\ (mg/100\ g) = 20{,}13 \times A_{663} - 2{,}81 \times A_{646} \quad (1)$$

$$CLO_b\ (mg/100\ g) = 20{,}13 \times A_{646} - 5{,}03 \times A_{663} \quad (2)$$

$$CLO_{total}\ (mg/100\ g) = CLO_a + CLO_b \quad (3)$$

$$CAR_{totals}\ (mg/100\ g) = (1000 \times A_{470} - 3.27\ CLO_a - 104 \times CLO_b) / 229 \quad (4)$$

Where, CLO_a refers to the chlorophyll content a; A refers to the absorbance of samples at the corresponding wavelengths (470, 646 and 663 nm); CLO_b refers to the chlorophyll content b; CLO_{total} refers to the total chlorophyll content; and CAR_{totals} refers to the total carotenoid content.

2.3.2 Flavonoids and anthocyanins

1 g of the sample was weighed and 10 mL of the ethanol-HCl mixture (85:15) was added. Soon after, the mixture was macerated for 1 minute and collected in a tube, storing in the refrigerator for 24 hours. After the time elapsed in the refrigerator, the mixture was filtered in cotton and the volume was completed to 10 mL. The reading was done in a spectrophotometer (Nova 2000, Femto) at wavelengths of 374 nm for flavonoid and 535 nm for anthocyanins. The concentrations of flavonoids and anthocyanins were calculated using the following formulas (Francis, 1982):

$$FLA\ (mg/100\ g) = (Fd \times A_{374}) / 76.6 \quad (5)$$

$$TNA\ (mg/100\ g) = (Fd \times A_{535}) / 98.2 \quad (6)$$

Where, FLA refers to the flavonoid content; Fd = 100 / mass (g) / dilution volume (mL); A refers to the absorbance of samples at the corresponding wavelengths (374 and 535 nm); and ANT refers to the anthocyanin content.

2.4 STATISTICAL ANALYSES

The results of the variables were evaluated through analysis of variance (F test), using the Sisvar programme, version 5.6 (Ferreira, 2011), at 5% probability. When there was a significant effect of the treatments, regression analyses were performed.

3 RESULTSS AND DISCUSSÕES

The use of blue LED lighting in the five photoperiods (10, 12, 14, 16 and 18 hours) in a five-day cycle (from 4th to 8th DAP) did not impair the growth of arugula microgreens nor caused visual photodamage in the leaves (Figure 2).

Figure 2

Arugula microgreens after growth using LED light in the 8th DAP and 16 h photoperiod.



3.1 PIGMENT CONTENT

3.1.1 Chlorophyll a

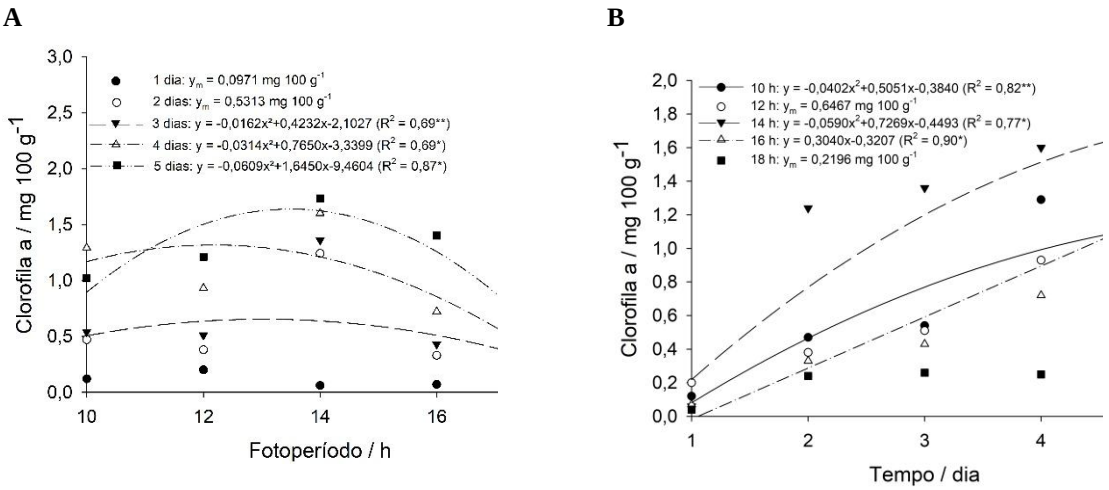
Chlorophylls, pigments produced by plants in response to light, play a key role in photosynthesis and also offer significant benefits to human health, including potent anti-inflammatory properties, supply of essential vitamin precursors and antioxidants (Wu *et al.*, 2007; Zhang *et al.*, 2020).

Only in the 6th, 7th and 8th DAP it was possible to observe a curve that adjusted to the data of chlorophyll a (Figure 3A) when the photoperiods were evaluated in relation to the days. The production of chlorophyll a showed a growth and subsequent reduction in the evaluation

periods (6th to 8th DAP), with the increase of the photoperiod. A similar behaviour was found by Cruz *et al*. (2024), when studying the growth of radish and arugula microgreens under white LED light. These authors also observed a higher production of chlorophyll a in arugula, 4.51 mg/100 g, with a photoperiod of 12.81 h in the 7th DAP. In the present work, it was estimated that a photoperiod of approximately 13.51 h would present the highest chlorophyll a contents, 1.64 mg/100 g (Figure 3A) in the 8th DAP.

Figure 3

Chlorophyll a values of the photoperiod split as a function of the days after planting (DAP) (1 day: 4th DAP; 2 days: 5th DAP; 3 days: 6th DAP; 4 days: 7th DAP; 5 days: 8th DAP) - (A) and DAP x photoperiod - (B).



In general, chlorophyll a showed an increasing behaviour when evaluated the days in relation to the photoperiod (Figure 3B). The highest estimated chlorophyll a content occurred at 8th DAP in the 14-hour photoperiod, with a value of 1.73 mg/100 g (Figure 3B). Higher value was found by Cruz *et al*. (2024), who observed a content of 4.51 mg/100 g of chlorophyll a in arugula grown under white LED with 12.81 h of photoperiod. Studies with combined spectra, as is the case with white LED, suggest that isolated blue light, although efficient, may limit the photosynthetic potential of the crop.

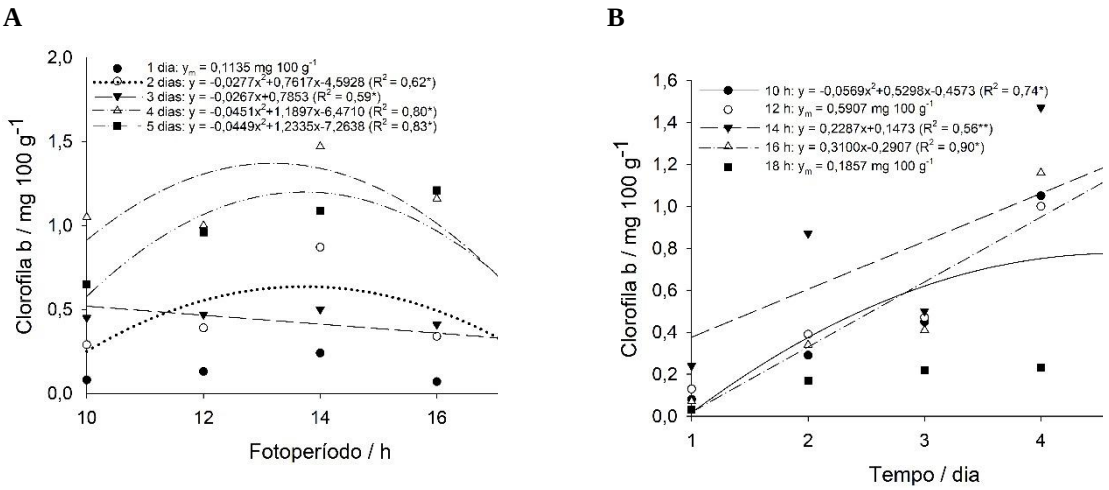
A similar study by Kendirlioglu *et al.* (2015) investigated the impact of photoperiod on *Chlorella vulgaris* pigments. The results showed that, under a photoperiod of 16 hours, the amount of chlorophyll a continued to increase over the days.

3.1.2 Chlorophyll b

The photoperiod splitting in relation to the days of analysis for chlorophyll b only did not show adjustment in the 4th DAP, with lower values observed with 18 h of photoperiod (Figure 4A). On the other days, there was regression adjustment, with the highest production of chlorophyll b, 1.21 mg / 100 g, with 13.74 hours of photoperiod in the 7th DAP (Figure 4A).

Figure 4

Chlorophyll b values of photoperiod splitting as a function of the days after planting (DAP) (1 day: 4th DAP; 2 days: 5th DAP; 3 days: 6th DAP; 4 days: 7th DAP; 5 days: 8th DAP) - (A) and DAP x photoperiod - (B).



There was no regression adjustment for photoperiods of 12 h and 18 h when days were evaluated in relation to photoperiods for arugula chlorophyll b data (Figure 4B).

It can be observed that in the 8th DAP there was a trend of greater accumulation of chlorophyll b in all photoperiods, with a higher value observed in the photoperiod of 16 h, 1.21 mg / 100 g, and lower accumulated value in the photoperiod of 18 h, 0.27 mg / 100 g (Figure 4B). A similar result was found by Liu *et al*. (2022) exploring the effects of different light intensities and photoperiods on growth, nutritional quality and antioxidant properties of two Brassicaceae microgreens (cabbage *Brassica oleracea* L. and Chinese kale *Brassica alboglabra* Bailey) in which the chlorophyll b content in cabbage and Chinese kale microgreens were the highest in the 16 h photoperiod.

The regression adjustment of the days in relation to the 14-hour photoperiod showed a low correlation with the coefficient of 0.56 (Figure 4B). It was not possible to adjust the data

of chlorophyll b of the days in relation to the photoperiods of 12 h and 18 h for arugula, presenting an average value of 0.59 mg / 100 g and 0.18 mg / 100 g, respectively (Figure 4B).

3.1.3 Chlorophyll a:b ratio

The relationship between chlorophyll types (a and b) in microgreens is an indicator of the cultivation conditions, as observed by Wojdyło *et al*. (2020). The presence of chlorophyll b is a sign of adaptation of the plant to shade. In addition, the ratio of *chlorophyll a to b may indicate the level of light exposure: a high proportion of chlorophyll a to chlorophyll b suggests exposure to sun or bright light, while a low proportion indicates exposure to shade (Niroula et al.*, 2019; Wojdyło *et al*., 2020).

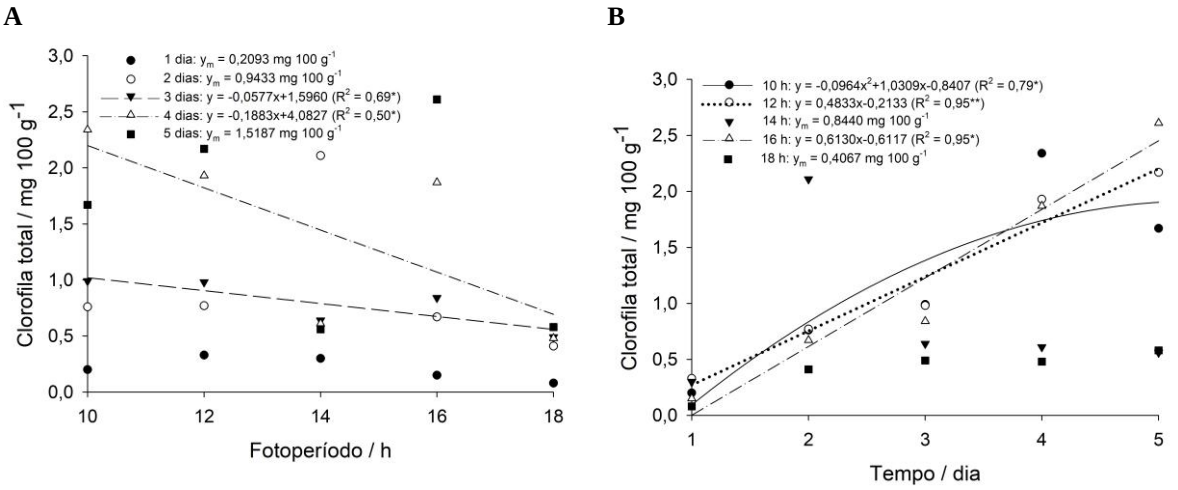
The chlorophyll a:b ratio in this study was lower than the values found by Cruz *et al.* (2024), who observed values from 1.42 to 1.84 for arugula grown in a controlled environment with white LED light. The proportion between the means of chlorophyll a: b according to the photoperiods evaluated in this work ranged from 0.92 to 1.44, demonstrating that the exposure to LED light applied was not excessive and that there was a slight predominance of chlorophyll a.

3.1.4 Total chlorophyll

For the arugula, the photoperiod regression adjustment in relation to the 6th and 7th DAP showed low correlation with the coefficients of 0.69 and 0.50, respectively (Figure 5A). On the other days, there was no regression adjustment, with mean values ranging from 0.21 mg/100 g to 1.52 mg/100 g (Figure 5A).

Figure 5

Total chlorophyll values of the photoperiod split as a function of the days after planting (DAP) (1 day: 4th DAP; 2 days: 5th DAP; 3 days: 6th DAP; 4 days: 7th DAP; 5 days: 8th DAP) - (A) and DAP x photoperiod - (B).



The correlation between time and photoperiod for total chlorophyll generated in arugula microgreen was high, with determination coefficients of 0.79, 0.95 and 0.95 for 10 h, 12 h and 16 h, respectively (Figure 5B). These photoperiods showed a tendency of accumulation of total chlorophyll over the days. In the 8th DAP, the arugula microgreen presented values of 1.67 mg/100 g and 2.17 mg/100 g and 2.61 mg/100 g of total chlorophyll in the photoperiods of 10 h, 12 h and 16 h, respectively (Figure 5B).

3.1.5 Carotenoids

In plants, carotenoids are light-collecting pigments in chloroplasts and are important in protecting plants against photooxidative damage. Since carotenoids are closely related to photosynthesis, the most important factors that impact changes in carotenoid content are the quantity and quality of light (Brazaityte *et al.*, 2015; Zhang *et al.*, 2020).

The carotenoid content was negatively affected with increasing exposure to treatment with blue LED light (Figure 6A). The highest production of carotenoids occurred with the exposure of rocks to a photoperiod between 12 h and 14 h, reaching a value of 1.01 mg/100 g (Figure 6A). A similar result was found by Cruz *et al.* (2024) in which the increase of the photoperiod caused a reduction in the carotenoid content in the arugula microgreen grown under similar conditions. Brazaitytė *et al.* (2015), when using LED spectra in microgreens of the Brassicaceae family, reported levels higher than 2.5 mg/100 g, demonstrating that the

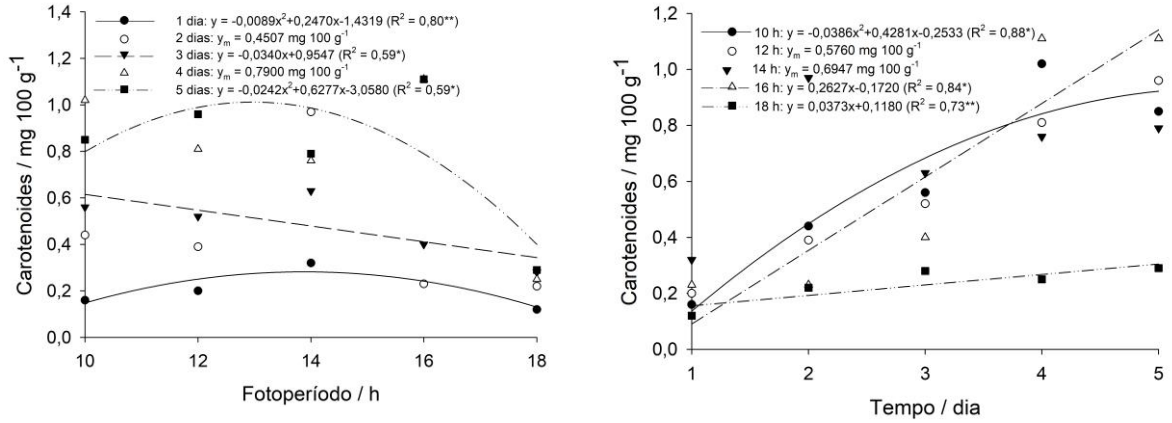
combination of blue light with other wavelengths (such as red) can further optimise the biosynthesis of these pigments.

There was no regression adjustment of the photoperiod in relation to the 5th and 7th DAP for the arugula, and the mean values of 0.45 mg / 100 g and 0.79 mg / 100 g of carotenoids were observed (Figure 6A).

Figure 6

Carotenoid values of photoperiod splitting as a function of the days after planting (DAP) (1 day: 4th DAP; 2 days: 5th DAP; 3 days: 6th DAP; 4 days: 7th DAP; 5 days: 8th DAP) - (A) and DAP x photoperiod - (B).

A **B**



It was observed in the arugula microgreen that the increase of the carotenoids occurred as the days passed (Figure 6B). The highest carotenoid value found for arugula, 1.01 mg/100 g, occurred in the 8th DAP with 16 h of photoperiod. Shibaeva *et al.* (2022) found a carotenoid content of 0.80 mg/100 g in radish after 12 days of sowing, under a photoperiod of 16 hours. These results are consistent with the findings of the present study, which showed that a higher exposure to LED light resulted in lower carotenoid values.

3.1.6 Total chlorophyll: carotenoid ratio (clo:car)

The relationship between chlorophylls and carotenoids is considered an important indicator of the physiological state of plants, reflecting the balance between photosynthetic processes and photoprotection mechanisms (Lichtenthaler & Babani, 2021). Young plants naturally have a high ratio between chlorophyll and carotenoids (clo:car), which gradually

decreases with advancing age. In addition, this relationship can increase significantly when there is a low concentration of carotenoids (Niroula *et al*., 2019).

In the present study, arugula microgreens showed a clo:car ratio ranging from 1.21 to 2.15 (Table 1), with values lower than those reported by Cruz *et al*. (2024), who observed ratios higher than 3.0 in arugula grown under *white LED, while Dube et al*. (2024) observed values ranging from 2.0 to 3.4 in *Brassica juncea* leaves submitted to bacterial inoculation. These data indicate that, although the absolute levels of chlorophylls and carotenoids in the present study were lower, the relationship between them remained within a functionally adequate range for microgreens, reflecting an efficient photosynthetic adaptation to cultivation under blue light. In addition, the fact that these relationships are obtained in only eight days of cultivation and under controlled conditions highlights the feasibility of using microgreens as fast and efficient sources of bioactive pigments, even with restricted light spectra.

Table 1

Mean of the values of the clo:car ratio in relation to the photoperiods for the arugula microgreen.

Photoperiod	Clo:car ratio
10	1.96
12	2.15
14	1.21
16	1.99
18	1.77

3.1.7 Flavonoids

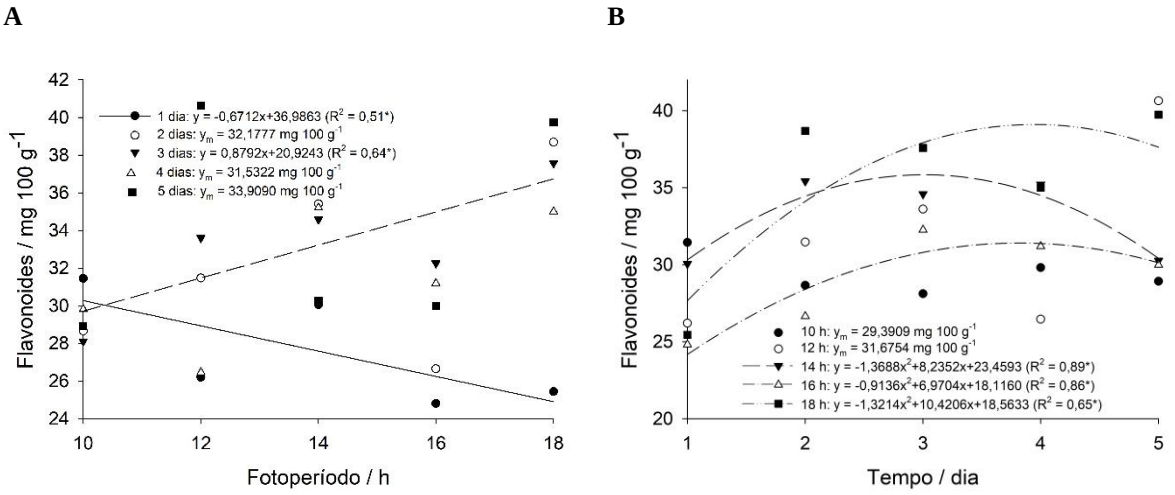
These compounds are produced by plants to protect them against oxidative stress, absorbing free radicals and inhibiting the oxidative reactions promoted by reactive oxygen species (ROS), interrupting the chain of initiation or propagation of oxidative reactions (Podsedek, 2007; Pinnola & Bassi, 2018).

In the 6th DAP, a reduction behaviour of arugula microgreen flavonoids was observed with the increase of photoperiods (Figure 7A). The data showed opposite behaviour in the 4th DAP, in which the flavonoid content showed an increase with the increase of the photoperiod, with a maximum value of 37.58 mg / 100 g (Figure 7A). These results are expressive and are within the range reported in the literature as indicative of foods with high antioxidant potential. Khan *et al*. (2022), for example, observed levels between 20 and 45 mg/100 g in groundnut

leaves under different photoperiods, which positions arugula microgreens under blue LED as promising foods in terms of nutritional functionality.

Figure 7

Flavonoid values of photoperiod splitting as a function of the days after planting (DAP) (1 day: 4th DAP; 2 days: 5th DAP; 3 days: 6th DAP; 4 days: 7th DAP; 5 days: 8th DAP) - (A) and DAP x photoperiod - (B).



Several reports have indicated that flavonoid accumulation occurs in plant tissues in response to a longer exposure to light (Singh *et al.*, 2017). Studies such as Khan *et al.* (2022), which found results that indicated an increase in the amount of flavonoids in the leaves with the increase of the photoperiod in the study of peanuts (8, 12, 16 and 24 h of light) and Cruz *et al.* (2024), which also observed a trend of accumulation of flavonoids over the days for the radish and arugula microgreen, with a higher value in the photoperiod of 12 h and 14 h for radish and 12 h for arugula, corroborate the previous information.

The growth time showed a high correlation with the flavonoid content, with determination coefficients of 0.89 and 0.86 for 14 h and 16 h photoperiod, respectively (Figure 7B). The data indicated a tendency of accumulation of flavonoids, however, a subsequent decrease was noted over the days for the arugula microgreen, with a higher value in the photoperiod of 18 h, 39.11 mg / 100 g, followed by 14 h, 35.84 mg / 100 g (Figure 7B). These values demonstrate the ability of arugula microgreens to synthesise phenolic compounds at levels close to those found in adult plants. Veremeichik *et al.* (2023) found that adult plants of Eruca sativa grown under high light intensities LED showed high levels of flavonoids, evidencing the role of light in stimulating the biosynthesis of these metabolites. The possibility of achieving equivalent levels in a short production cycle and under controlled conditions

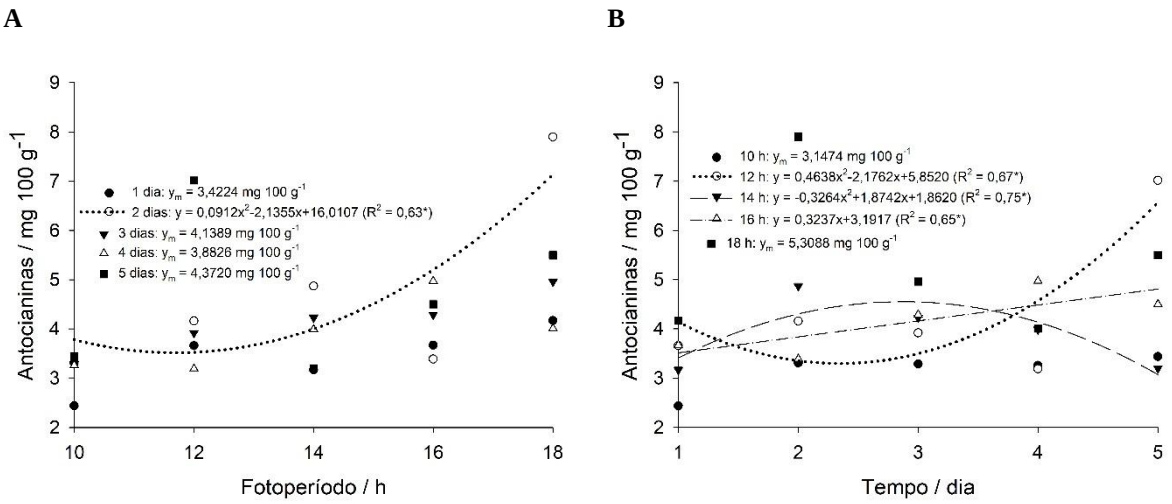
represents an important advantage of microgreens, allowing the supply of foods with high antioxidant and functional potential in urban environments, with lower consumption of resources and time.

3.1.8 Anthocyanins

There was only regression adjustment of the photoperiod splitting in relation to the evaluation days for the arugula in the 5th DAP (Figure 8A), presenting a maximum value of 7.90 mg / 100 g. On the other days, the mean anthocyanins ranged from 3.42 mg/100 g to 4.37 mg/100 g.

Figure 8

Anthocyanin values of photoperiod splitting as a function of the days after planting (DAP) (1 day: 4th DAP; 2 days: 5th DAP; 3 days: 6th DAP; 4 days: 7th DAP; 5 days: 8th DAP) - (A) and DAP x photoperiod - (B).



According to the curves obtained in the unfolding of the days in relation to the photoperiods, an increase was observed with a subsequent fall in the photoperiod of 14 h with the course of the days for the arugula microgreen (Figure 8B). A similar behaviour was observed by Cruz *et al*. (2024) with an increase with subsequent decrease in anthocyanin concentrations in the rocket cultivation using a 16 h photoperiod.

During the days of arugula cultivation using the photoperiods of 12 h and 16 h, a tendency of accumulation of anthocyanins was observed (Figure 8B). Zhang *et al*. (2019) found that radish microgreens grown under blue and ultraviolet-A (UV-A) light showed a significant increase in anthocyanin content when compared to those grown in the dark. The largest

accumulation of anthocyanins in the screen work occurred in the 8th DAP with 7.02 mg / 100 g in the 12-h photoperiod.

It is important to highlight that the accumulation of anthocyanins is highly sensitive to the type of light, cultivation time and plant species. Although the levels obtained under pure blue LED in this study can be considered moderate, they are still within a functionally relevant range, since anthocyanins have antioxidant activity and beneficial health effects even at modest concentrations (Podsedek, 2007).

As observed, the anthocyanin content obtained in the arugula microgreens ranged from 3.42 to 7.90 mg/100 g. Although Veremeichik *et al*. (2023) have reported higher levels in adult plants subjected to intense artificial lighting and combined spectra, the values achieved in this study demonstrate that the cultivation of microgreens under blue light is able to promote the efficient synthesis of this bioactive pigment. The fact that these concentrations are obtained in a short period and under controlled conditions reinforces the potential of microgreens as a practical and sustainable alternative for the production of foods rich in bioactive compounds, with emphasis on their applicability in technified and urban-based agricultural systems.

5 CONCLUSION

Based on the results obtained, it was observed that the photoperiod exerts a significant influence on the content of bioactive pigments in arugula microgreens throughout the cultivation cycle. The highest accumulation of chlorophyll a was recorded in the 8th DAP with 14 hours of photoperiod, while chlorophyll b had a higher content in the 7th DAP with approximately 13.74 hours of photoperiod. The total chlorophyll showed a tendency of accumulation over the days, with the highest value observed with 16 hours of photoperiod.

Regarding carotenoids, it was found that prolonged photoperiods resulted in less accumulation of this pigment, with the highest content found between 12 and 14 hours of exposure. For flavonoids, there was a progressive increase until the 8th DAP, with the highest values obtained in the photoperiods of 14 and 18 hours. The anthocyanins had the highest concentration in the 8th DAP with 12 hours of photoperiod.

Given these findings, considering the balance between pigment contents, the 14-hour photoperiod was the most suitable for the cultivation of arugula microgreens under blue LED lighting. This exposure time favoured the synthesis of chlorophylls and carotenoids, besides promoting the adequate accumulation of flavonoids and anthocyanins, contributing to the nutritional and functional quality of the plant. In addition, the cultivation up to the 8th DAP

was efficient to obtain a balanced profile of bioactive compounds. Further studies may deepen the understanding of the effects of light on other physiological and nutritional parameters, further optimising the management of artificial lighting for the cultivation of microgreens.

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