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Research Article

White light intensities for maximum yield and quality of arugula microgreens[1]

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ABSTRACT

The production of microgreens in plant factories is a great option for urban agriculture, and artificial light is one of the main factors for the success of this cultivation system. This study aimed to evaluate the effect of photosynthetic photon flux density (PPFD) (100, 200, 300, 400, 500 and 600 µmol m^{-2} s^{-1}) of white LED on yield and quality of arugula microgreens. The experiment was conducted in an indoor environment, in a completely randomized experimental design, with six replicates. The highest yield was obtained with 358 µmol m^{-2} s^{-1}, while 90 % of the maximum yield required 234 µmol m^{-2} s^{-1}. The maximum shoot dry mass and cotyledon area were obtained with 439 and 312 µmol m^{-2} s^{-1}, respectively, while the hypocotyl length decreased linearly with increasing PPFD. The highest nitrogen, phosphorus, calcium, sulfur and zinc contents were obtained with 380, 285, 326, 294 and 317 µmol m^{-2} s^{-1}, respectively, while the maximum Fe content was obtained at the lowest light intensity. The contents of chlorophylls, carotenoids, phenolic compounds and antioxidant protection had maximum values with 600 µmol m^{-2} s^{-1}, and the vitamin C with 458 µmol m^{-2} s^{-1}.

KEYWORDS: *Eruca sativa*, urban farm, photosynthetic photon flux density.

RESUMO

Intensidades de luz branca para máxima produtividade e qualidade de rúcula microvegetal

A produção de microvegetais em fábricas de plantas é uma grande opção para a agricultura urbana, e a luz artificial é um dos principais fatores para o sucesso desse sistema de cultivo. Objetivou-se avaliar o efeito da densidade de fluxo de fótons fotossintéticos (DFFF) (100, 200, 300, 400, 500 e 600 µmol m^{-2} s^{-1}) de LED branca na produtividade e qualidade de rúcula microvegetal. O experimento foi conduzido em ambiente interno, em delineamento experimental inteiramente casualizado, com seis repetições. A maior produtividade foi obtida com 358 µmol m^{-2} s^{-1}, enquanto 90 % da máxima produtividade demandou 234 µmol m^{-2} s^{-1}. Massa seca da parte aérea e área cotiledonar máximas foram obtidas com 439 e 312 µmol m^{-2} s^{-1}, respectivamente, enquanto o comprimento do hipocótilo diminuiu linearmente com o aumento da DFFF. Maiores teores de nitrogênio, fósforo, cálcio, enxofre e zinco foram obtidos com 380, 285, 326, 294 e 317 µmol m^{-2} s^{-1}, respectivamente, enquanto o teor máximo de Fe foi obtido com a irradiância mais baixa. Teores de clorofilas, carotenoides, compostos fenólicos e proteção antioxidante tiveram valores máximos com 600 µmol m^{-2} s^{-1}, e a vitamina C com 458 µmol m^{-2} s^{-1}.

PALAVRAS-CHAVE: *Eruca sativa*, agricultura urbana, densidade de fluxo de fótons fotossintéticos.

INTRODUCTION

In the last two decades, the interest in nutritious and pesticide-free foods has increased (Di Gioia et al. 2017). To meet this goal, the production of microgreens in indoor farms has increasingly gained the attention of researchers, producers and consumers (Shibaeva et al. 2022). For researchers, it is a cultivation technology economically and socially applicable to the growing global urbanization. Producers are encouraged by the fact that this is a production system that promotes a considerable increase in food production per unit area (verticalization of production) and greater efficiency of inputs used (sustainability), if compared to crops traditionally grown in the field. As it is a production system that allows controlling the production factors with a view to increasing the contents of nutrients, vitamins and antioxidants, consumers have the possibility to access higher

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quality food (Clifford et al. 2017, Wieth et al. 2019, Yadav et al. 2019).

In this production system, it is worth highlighting the influence of lighting on photosynthesis and photomorphogenesis, which, through the modulation of gene expression made by photoreceptors (Shanker & Venkateswarlu 2011), acts on the growth and accumulation of phytochemicals (Paradiso & Proietti 2022). Thus, the manipulation of the photosynthetic photon flux density (PPFD) has been used in agriculture aiming at higher yield and quality of vegetables (Pocock 2017). In this context, light-emitting diode (LED) has been increasingly used in indoor agriculture, due to the possibility of working with monochromatic or mixed wavelengths, intensities and photoperiods, in addition to their low energy consumption, low heat emission and long lifetime of the light source (Gupta & Agarwal 2017, Bantis et al. 2018).

As for the intensity, Cope & Bugbee (2013) evaluated the effect of PPFD between 100 and 500 µmol m^{-2} s^{-1} (obtained from white LED) on the growth and development of radish (*Raphanus sativus*), soybean (*Glycine max*) and wheat (*Triticum* spp.), and concluded that the response varied according to the species. The maximum fresh mass production was reached with 400 µmol m^{-2} s^{-1} for *R. sativus* and *G. max* and with 500 µmol m^{-2} s^{-1} for *Triticum* spp.

Several studies have evaluated the relationships among PPFD, growth and yield of Brassica microgreens, which are among the most preferred ones. However, few studies have evaluated the effect of white light on microgreen production and none on arugula. Samuolienė et al. (2013) evaluated the effects of four PPFD levels (220, 330, 440 and 545 µmol m^{-2} s^{-1}) on kohlrabi (*Brassica oleracea* var. *gongylodes*), mustard (*Brassica juncea*), red pak-choi (*Brassica rapa* var. *chinensis*) and tatsoi (*Brassica rapa* var. *rosularis*). These authors observed that PPFD levels of 330 and 440 µmol m^{-2} s^{-1} were more adequate, promoting a greater growth and nutritional quality of microgreens. On the other hand, intensities higher than 440 µmol m^{-2} s^{-1} caused light stress to the microgreens, with a negative effect on most of the parameters evaluated. Gerovac et al. (2016) evaluated the effect of light intensities of 105, 210 and 315 µmol m^{-2} s^{-1} on the production of kohlrabi, mizuna (*Brassica rapa* var. *niposinica*) and mustard, and observed that the fresh and dry mass increased by up to 34 and 25 %, respectively, as the intensity increased from 105 to 315 µmol m^{-2} s^{-1}. Jones-Baumgardt et al. (2019) evaluated the effects of pink light intensity on the growth and yield of kale (*Brassica oleracea* var. *acephala*), cabbage (*Brassica oleracea* var. *capitata*), arugula (*Eruca sativa*) and mustard microgreens grown in a growth chamber, and observed that the fresh mass increased by 36, 56, 76 and 82 %, and the dry mass by 65, 69, 122 and 145 %, respectively for kale, cabbage, arugula and mustard, when the intensity increased from 100 to 600 µmol m^{-2} s^{-1}.

Another point to be better understood is the effect of light intensity on the quality of microgreens, as it depends on the concentrations of metabolites produced according to the variations of production factors, especially artificial light in a controlled environment (Zhang et al. 2020). The use of white light increased the total phenolic content in Chinese cabbage (*Brassica oleracea* var. *alboglabra*) microgreens (Qian et al. 2016) and pea (*Pisum sativum*) (Liu et al. 2016) by up to 35 and 45 %, respectively, when the intensity increased from 0 to 30 µmol m^{-2} s^{-1}. In Chinese cabbage microgreens, the antioxidant capacity also responded positively, increasing up to 30 % when the white light intensity increased from 0 to 30 µmol m^{-2} s^{-1} (Qian et al. 2016).

According to the results observed in the previously reported studies, there is clearly a correlation between the species and the ideal light intensity to maximize yield and quality (Pescarini et al. 2023). No studies were found on the effect of white light intensities on the yield and quality of arugula microgreens, what justifies the present study. It is believed that arugula microgreens respond positively to the increase in PPFD with white LED.

Thus, this study aimed to evaluate biometric characteristics, yield, photosynthetic pigments, mineral content and secondary metabolites in arugula microgreens grown under white light intensities.

MATERIAL AND METHODS

The experiment was conducted in an indoor environment, with temperature control of 25 ± 2 ºC, at the Universidade Estadual Paulista, in Jaboticabal, São Paulo state, Brazil (21º15’22”S, 48º18’58”W and 575 m of altitude), in 2022.

The treatments corresponded to six photosynthetic photon flux density (PPFD) levels (100, 200, 300, 400, 500 and 600 µmol m^{-2} s^{-1}), with white light, in a photoperiod of 14 hours. The PPFD was measured with a spectroradiometer (SpectraPen LM 500-PSI) (Figure 1).

The treatments were arranged in a completely randomized experimental design, with six replicates. Each experimental unit was composed of three transparent polypropylene trays (25 x 24 x 5 cm in length, width and height, respectively), which were perforated at their base with 2-mm holes, spaced 5 x 2 cm apart, and filled with Bioplant® organomineral substrate (pH 6 ± 0.3 and electrical conductivity of 0.8 dS m^{-1} ± 0.06), 1-cm-deep layer, previously moistened. Seeds of the 'Folha Larga' arugula cultivar were distributed on the substrate at a density of 102 g of seeds per tray, with a sowing density defined based on Wieth et al. (2019) for purple cabbage microgreens.

After sowing, the trays were irrigated using a microsprayer, stacked and placed in a dark environment at the temperature of 25 ± 2 ºC. After three days, when the cotyledons were already present, the trays with the seedlings were taken to benches under lamps and placed in trays, where nutrient solution was added every 24 hours, so that the seedlings received water and nutrients by capillarity (subirrigation). The nutrient solution used was that recommended by Furlani et al. (1999) for the cultivation of leafy vegetables in nutrient film technique (NFT) hydroponics. In the first three days, 200 mL of nutrient solution were supplied every 24 hours, and, from the fourth day, 250 mL were applied per tray per day.

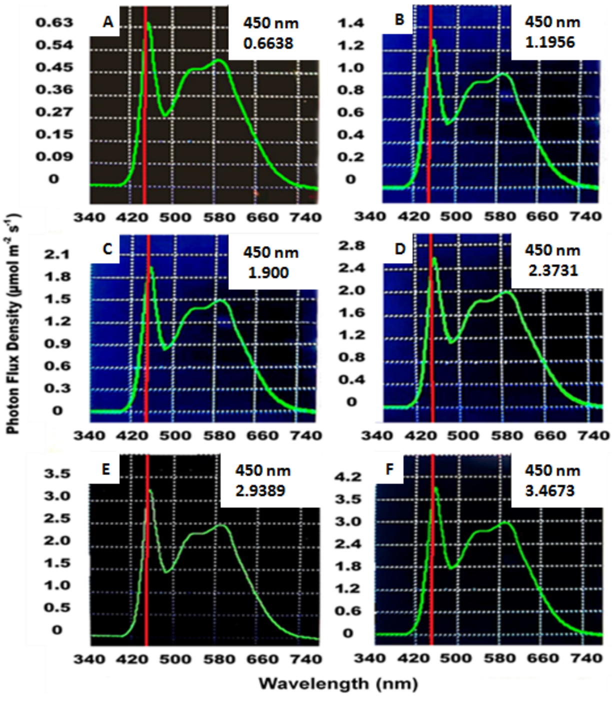

Figure 1. Spectral and photon flux characterization of light-emitting diode (LED) as a function of light intensities of 100 (A), 200 (B), 300 (C), 400 (D), 500 (E) and 600 µmol m^{-2} s^{-1} (F).

The light treatments were kept until harvest, which was carried out at 9 days after the beginning of the lighting period, with full expansion of the cotyledons and preceding the appearance of the first leaf. The harvest was performed manually using scissors, by cutting the hypocotyl of the seedlings at 1 cm above the substrate.

At harvest, the following parameters were evaluated: a) hypocotyl length (cm): determined using a millimeter ruler; b) cotyledon area (cm^2 seedling^{-1}): measured using the ImageJ software (Rasband 2018); c) shoot dry mass (g m^{-2}): obtained after drying in an oven with forced air circulation at 65 ºC; d) yield (g m^{-2}): immediately after removing the light, the seedlings were cut at 1 cm height from the substrate and the shoot fresh mass was weighed on a scale with precision of four decimal places; e) N, P, K, Ca, Mg and S contents (g kg^{-1}) and Fe and Zn contents (mg kg^{-1}): determined in the dry mass of seedlings, according to methodologies described by Miyazawa et al. (2009); f) chlorophyll *a* and *b* and carotenoid contents: obtained by spectrophotometric reading, with results expressed in µg g^{-1} of leaf fresh mass (Lichtenthaler 1987); g) vitamin C determined by titration with DCPIP: 1 g of leaves was ground in 5 mL of 56 mM oxalic acid, using pestle and mortar in a dark environment (Ponting 1943). Subsequently, this mixture was vacuum filtered on Whatman nº 1 filter paper, and the filtrate was collected. Duplicate aliquots of 1 mL of the filtrate were titrated with 3.73 mM of DCPIP (2,6-dichlorophenolindophenol), until the filtrate showed a persistent pink color. Finally, the added volume of DCPIP was compared with a standard curve of ascorbic acid concentrations; h) phenolic content: determined by spectrophotometry of an extract obtained according to Larrauri et al. (1997), using 70 % acetone and 50 % methanol solutions, with subsequent addition of the Folin-Ciocalteu reagent, based on an oxidation-reduction alkaline reaction, where the phenolate ion is oxidized and the Folin-Ciocalteu reagent is reduced. After the reaction, a blue color is produced, and its reading is carried out at an absorbance of 700 nm; i) antioxidant protection (AOP): 1 g of the sample was weighed in a dark environment and placed in a beaker, where 5 mL of

50 % methanol were added, homogenized and left to rest for 60 min. Subsequently, it was centrifuged at 15,000 rpm for 15 min, and the supernatant 1 was placed in a 10 mL volumetric flask. In a second step, 4 mL of 70 % acetone were added to the residue of the first extraction, and it was homogenized, left to rest for 60 min and centrifuged at 15,000 rpm for 15 min, and then the supernatant 2 was collected in the same 10 mL volumetric flask. Subsequently, the volumetric flask was completed with distilled water, resulting in the extract. In a third step, 0.1 mL of the extract and 3.9 mL of the 0.06 mM DPPH radical were added in a test tube and homogenized in a tube shaker. Methyl alcohol was used as blank to calibrate the spectrophotometer, and readings were performed at 515 nm (Larrauri et al. 1997).

The data were subjected to analysis of variance and polynomial regression study, choosing the significant equation with the highest coefficient of determination according to the AgroEstat software (Barbosa & Maldonado Júnior 2015).

RESULTS AND DISCUSSION

The white light intensity affected the biometric characteristics of the arugula seedlings. The hypocotyl length, whose maximum was obtained with 100 μmol m^{-2} s^{-1}, decreased linearly with the increase in PPFD. While there was a 28.3 % reduction in the hypocotyl length between the maximum (10.33 cm) obtained with the lowest light intensity and the minimum (7.41 cm) obtained with the highest intensity evaluated, the cotyledon area, shoot dry mass and yield were described by a second-degree equation, and their maximum values were obtained with 312, 439 and 358 μmol m^{-2} s^{-1}, respectively (Figure 2).

Samuolienė et al. (2013) demonstrated that low intensities of white LED did not allow the normal growth of Brassicaceae microgreens. These authors observed a maximum fresh and dry mass of kohlrabi, mustard, red pak-choi and tatsoi microgreens with 330 to 440 μmol m^{-2} s^{-1}, and the best irradiance varied among the species. Thus, the results obtained in the present study corroborate those reported by Samuolienė et al. (2013), because a low yield was observed at the lowest intensity, with a subsequent increase with maximum at 358 μmol m^{-2} s^{-1}. This PPFD level allowed increments of 76, 109 and 36 % between the minimum and maximum values of yield, shoot dry mass and cotyledon area, respectively. It is worth mentioning that, although the highest yield of arugula microgreens was obtained with 358 μmol m^{-2} s^{-1}, 90 % of the maximum yield were obtained with 234 μmol m^{-2} s^{-1}, which allowed a significant reduction in the PPFD and, consequently, in the energy spent in production. The prices of energy and arugula microgreens in different localities can determine the most economically interesting intensity for the producer.

The highest light intensity evaluated (600 μmol m^{-2} s^{-1}) caused light stress and led to reductions of approximately 28, 49, 12 and 38 % in hypocotyl length, cotyledon area, shoot dry mass and yield, when compared to their maximum values, respectively. Light stress leads to a decrease in carbon fixation rate, resulting in lower growth and biomass gain (Lazzarini et al. 2017).

For the macro and micronutrient contents, the PPFD levels affected the N, P, Ca, S, Fe and Zn, and the highest contents were obtained with 380, 285,

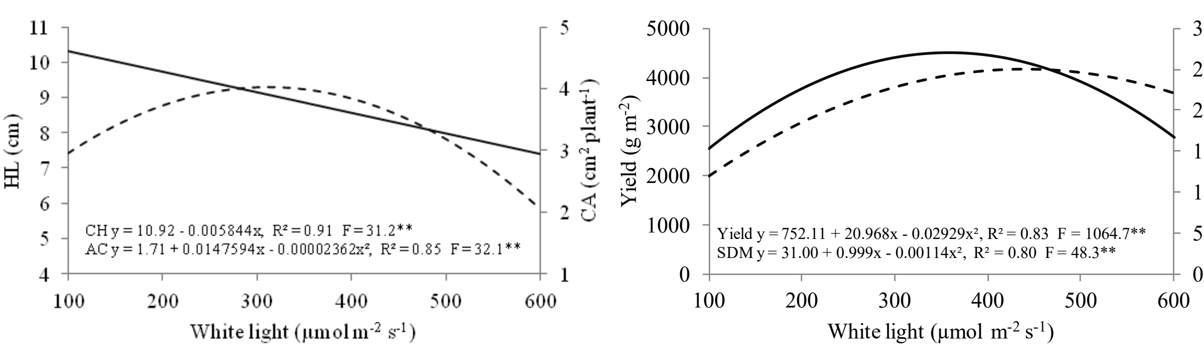

Figure 2. Hypocotyl length (HL), cotyledon area (CA), shoot dry mass (SDM) and yield of arugula microgreens as a function of white light intensity. ** Significant at 1 % of probability by the F test.

326, 294, 100 and 317 μmol m^{-2} s^{-1}, respectively (Figure 3). The K and Mg contents were not described by the polynomial equation and showed mean values of 33.4 and 6.9 g kg^{-1}, respectively.

Except for K (33.4 g kg^{-1}) and Mg (6.9 g kg^{-1}), which were not influenced by light intensity, and Fe, whose maximum content (636 mg kg^{-1}) was obtained with the lowest intensity, the other evaluated nutrients responded with an increase in their contents, and maximum values were obtained with intensities close to the one that promoted the highest yield.

The result observed for Fe content was consistent with that found by Gerovac et al. (2016), who studied the effects of irradiances from 105 to 315 μmol m^{-2} s^{-1} on kohlrabi, mizuna and mustard, and observed a reduction in the Fe concentration as the light intensity increased. These authors attributed their results to the dilution of nutrients in the plant tissue due to the observed increase in dry mass.

The maximum contents of N (45.5 g kg^{-1}), P (21.2 g kg^{-1}), Ca (18.2 g kg^{-1}), S (17.3 g kg^{-1}) and Zn (181.9 mg kg^{-1}) were obtained with 380, 285, 326, 294 and 317 μmol m^{-2} s^{-1}, respectively, which promoted increments of approximately 35, 14, 26, 7 and 37 %, if compared to the contents obtained with the lowest intensity evaluated. One of the qualitative highlights of microgreens, in comparison to traditionally marketed vegetables, is that they are nutritionally richer. In the present study, considering the PPFD that allowed the highest yield (358 μmol m^{-2} s^{-1}), compared to the average contents observed in adult plants (38.9 g kg^{-1} of N, 4.7 g kg^{-1} of P, 17.6 g kg^{-1} of Ca, 7.9 g kg^{-1} of S and 137.3 mg kg^{-1} of Zn) (Rugeles-Reyes et al. 2019, Silva et al. 2020, Nascimento et al. 2022), the contents of N, P, Ca, S and Zn corresponded to increments of approximately 17, 340, 3, 116 and 32 %, respectively, while the Fe value was 27.4 % lower than the average observed in adult plants (442.6 g kg^{-1} of Fe).

The white light PPFD also affected the contents of chlorophylls *a* and *b* and carotenoids, which increased linearly with the increase of the PPFD (Figure 4).

The chlorophyll *a*, chlorophyll *b* and carotenoid contents responded similarly to the increase in PPFD, with minimum and maximum values obtained with the lowest and highest intensities of white light, respectively (Figure 4). The maximum values obtained for pigments resulted in increments of 156.3, 224.0 and 94.3 % for chlorophyll *a*, chlorophyll *b* and carotenoids, respectively, if compared to the lowest irradiance. Tuan et al. (2017), when studying

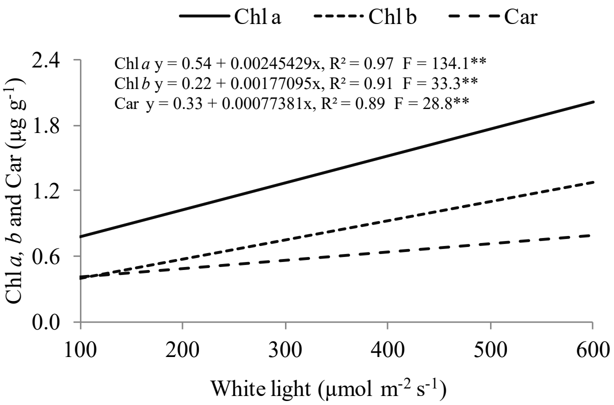

Figure 4. Contents of chlorophyll *a* (Chl *a*), chlorophyll b (Chl *b*) and carotenoids (Car) in the cotyledon fresh mass of arugula microgreens as a function of white light intensity. ** Significant at 1 % of probability by the F test.

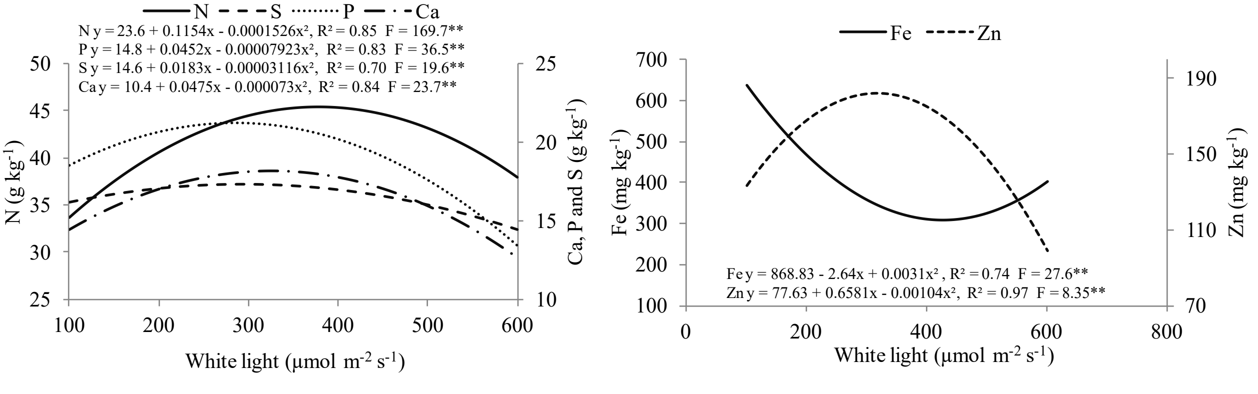

Figure 3. Contents of nitrogen (N), calcium (Ca), phosphorus (P), sulfur (S), iron (Fe) and zinc (Zn) in the shoot dry mass of arugula microgreens as a function of white light intensity. ** Significant at 1 % of probability by the F test.

buckwheat (*Fagopyrum esculentum*) microgreens grown under white LED irradiation, observed a higher expression of genes associated with the biosynthesis of photosynthetic pigments with the increase in PPFD. Zhang et al. (2015) also observed an increased expression of genes associated with biosynthesis of carotenoids, and, consequently, a greater synthesis of these components, indicating a stimulating role of the irradiation in the mechanism that regulates the biosynthesis of photosynthetic pigments.

However, although the chlorophyll and carotenoid contents are considered in the evaluation of the photosynthetic potential of plants (Lazzarini et al. 2017), in the present study, when the PPFD increased from 100 to 600 µmol m^{-2} s^{-1}, the increments in the contents of these pigments were not related to the responses observed for cotyledon area, shoot dry mass and yield of arugula microgreens, which decreased with PPFD greater than 312, 439 and 358 µmol m^{-2} s^{-1}, respectively, what may be indicative of photo-oxidative stress (Lazzarini et al. 2017).

According to Gerovac et al. (2016), the chlorophyll contents of mizuna and mustard microgreens are not influenced by the increase in light intensity, although their dry mass increased by 1 and 2 %, and the yield by 15 and 26.5 %, respectively. However, the authors observed increments of 11 and 13 % in the chlorophyll content of kohlrabi under 315 µmol m^{-2} s^{-1}, when compared to intensities of 105 and 210 µmol m^{-2} s^{-1}, respectively, which promoted a higher yield (Gerovac et al. 2016).

The PPFD levels affected the contents of vitamin C, which showed a 183.5 % increase between the intensity of 458 µmol m^{-2} s^{-1}, which promoted the maximum value (47.61 mg 100 g^{-1} of fresh mass), and the lowest light intensity studied (Figure 5).

Both the phenolic compounds content and antioxidant protection increased as the PPFD increased (Figure 5). The 74.9 % increase in the phenolic compounds of arugula microgreens obtained with 600 µmol m^{-2} s^{-1}, when compared to the lowest PPFD evaluated (100 µmol m^{-2} s^{-1}), resulted in a content of 225 mg of galic acid equivalent [GAE] 100 g^{-1}, a value significantly higher than that of adult plants grown in full sun, with average of 90 mg of GAE 100 g^{-1} (Arbos et al. 2010). Samoulienė et al. (2013) also observed an increase in phenolic compounds in pak choi and tatsoi, when the light intensity increased from 110 to 545 µmol m^{-2} s^{-1}.

An increased synthesis of these phytochemicals in microgreens with LED management has also been observed by Świeca et al. (2012), Lee et al. (2014), Yuan et al. (2015) and Liu et al. (2016), who observed increments in the contents of phenolic compounds of 55 % in lentils (*Lens culinaris*), 465 % in buckwheat, 19 % in soybean and 34 % in Chinese cabbage, respectively.

The antioxidant power reached a maximum of 80 % neutralization of free radicals, which is higher than the 70 % found by Arbos et al. (2010) in adult plants grown in full sun. Huang et al. (2021) concluded that the antioxidant power increased by 73 and 19 %, when comparing choy sum (*Brassica rapa* var. *parachinensis*) shoots grown under 500 µmol m^{-2} s^{-1} to those grown under 50 and 200 µmol m^{-2} s^{-1}, respectively. As the white light intensity is increased, there is an increase in the supply of blue light (Figure 1), a wavelength that stimulates the synthesis of secondary metabolites,

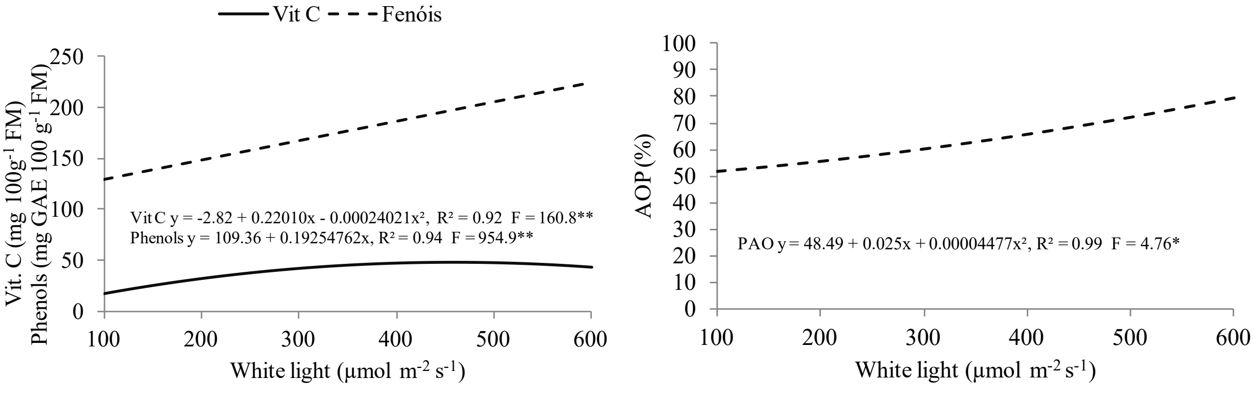

Figure 5. Vitamin C (Vit C), phenols and antioxidant power (AOP) in the shoot fresh mass of arugula microgreens as a function of white light intensity. *, ** Significant at 5 and 1 % of probability by the F test, respectively. FM: fresh mass; GAE: galic acid equivalent.

such as antioxidants (Wang et al. 2016, Wang et al. 2018), which will inhibit or reduce free radical damage (Samoulienė et al. 2013). The positive correlation between antioxidant protection and increase in light intensity was also found in mustard, tatsoi and kohlrabi microgreens by Samoulienė et al. (2013), who studied a mixed LED lighting module with wavelengths of 455, 638, 665 and 731 nm, and observed and increase in the antioxidant content as light intensity increased.

CONCLUSIONS

1. The photosynthetic photon flux density (PPFD) influenced the biometric characteristics, yield, nutritional composition and phytochemical compounds of arugula microgreens;
2. Increasing the PPFD produces smaller microgreens, but a higher leaf area, shoot dry mass and yield, while nutrients are differently influenced or unaffected by increasing PPDF;
3. The maximum yield of microgreens is achieved with 358 μmol m^{-2} s^{-1}, while pigments, vitamin C, phenols and antioxidant power are maximized with 600 μmol m^{-2} s^{-1} and vitamin C with 458 μmol m^{-2} s^{-1}.

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