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The Effect of Light Intensity and Photoperiod on the Yield and Antioxidant Activity of Beet Microgreens Produced in an Indoor System

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Abstract: Microgreens are immature and tender edible vegetables that have become relevant in the market due to their contribution to human health as "functional food". They can be produced in controlled environments, allowing more efficient use of space and resources and facilitating the management of environmental conditions, such as light, temperature, and relative humidity. The study's objective was to evaluate the impact of photoperiod and light intensity on red beet microgreens' yield and the antioxidant compound content. LED growth lamps (spectrum of 75% red, 23% blue, and 2% far-red) under two photoperiods were evaluated: 12 and 16 h, and three intensity levels: 120 (low), 160 (medium), and 220 (high) μmol m^{-2} s^{-1}. The largest photoperiod raised 32, 49, and 25% on phenolic compounds, total betalains, and antioxidant capacity, respectively, but a 23% reduction in microgreens yield was obtained compared with the shortest photoperiod. The low and medium intensities promoted the highest yield, reaching 460 g m^{-2}; yield decreased significantly by 22.1% at high intensity compared to low and medium intensity. Contrastingly, no effect on antioxidant activity was observed with the evaluated range intensities, except for the betalains concentration, which was reduced by 35% under the highest intensity compared to low intensity. On the other hand, resource use efficiency (energy and water) improved under the shortest photoperiod. Thus, an intensity between 120 and 160 μmol m^{-2} s^{-1} and a photoperiod of 12 h favored the microgreen's beet growth and saved electricity; meanwhile, a 16 h photoperiod ameliorated the beet microgreens antioxidant activity under a light spectrum composed of blue:red:far-red = 23:75:2.

Keywords: vertical farming; antioxidant compounds; microgreens; light factors

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1. Introduction

A complex future is projected for humanity concerning health and quality of life due to increased obesity. Chile has the second-highest percentage of adult population obesity in the world after the USA [1]. Consuming fruits and vegetables can significantly reduce the risk of non-communicable diseases, such as cardiovascular diseases, some types of cancer, obesity, and type 2 diabetes [2]. However, according to the National Food Consumption Survey [3], only 5% of the population has a healthy diet, and the remaining 95% require significant dietary modifications. In addition, the consumption of fruits and vegetables is low; only 15.7% of the population (over 15 years of age) comply with the recommendation of consuming five servings daily [1].

Microgreens are a new class of tender and immature vegetables, which generally have two fully developed cotyledons and the incipient appearance of one or two true leaves [4]. Its height varies between 5 and 10 cm, including the stem and cotyledons [5]. Depending on the species, its harvest varies from 7 to 21 days after germination [6,7]. These products have been considered "functional foods" as they present health benefits or have properties that prevent degenerative diseases due to their higher concentrations of pigments, polyphenols,

and antioxidant capacity comparing their mature stage [5,8–11]. Various crops can be produced as microgreens, including vegetables, cereals, and herbs [12].

Beet is among the most widely used species in microgreens production [13]. In addition to their striking appearance, beet microgreens present compounds beneficial to health, such as polyphenols and betalains [13]. Betalains play an essential role in human health, as they eliminate free radicals and, consequently, can prevent the risk of cancer and cardiovascular diseases [14]. For their part, phenolic compounds have been described as presenting an essential contribution to the total antioxidant activity of plants. Therefore, they have a preventive effect on cardiovascular diseases, cancers, diabetes, and diseases associated with oxidative stress [15].

In plant production, factors such as light, air, water, nutrients, appropriate temperature, and relative humidity ranges are required for proper growth and development [16]. Vertical or controlled environment agriculture presents advantages in plant production by minimizing the influence of environmental, seasonal, and geographic conditions and reducing the use of soil and water [17]. These systems provide a reliable and safe food supply throughout the year and consist of rooms with shelves where trays or pots with high-density plants grown under artificial light are distributed. This technology allows it to control light, temperature, and relative humidity, among other productive factors [18].

Light is a fundamental environmental factor influencing plant photosynthesis and morphogenesis [16]. Light parameters include photosynthetic photon flux density (PPFD) or intensity, photoperiod (hours of light), and spectral distribution (wavelength or quality) [19]. Light intensity is critical, as it directly affects CO_2 and H_2O transport through stomata during photosynthesis and transpiration [20]. Therefore, the optimal light intensity can improve the photosynthetic rate and increase productivity. On the other hand, when the light intensity is below a certain compensating intensity, photosynthesis is exceeded by respiration, and plants become net consumers of oxygen [20]. On the other hand, very high light intensities could damage plants due to photoinhibition [21]. According to the review by [22], the most commonly used intensities in microgreens production are 100 to 300 μmol m^{-2} s^{-1} PPFD, considering the range of 100 to 140 μmol m^{-2} s^{-1} as low intensities; 150 to 190 μmol m^{-2} s^{-1} as medium intensities; and intensities above 200 μmol m^{-2} s^{-1} as high intensities.

Photoperiod corresponds to the number of hours of light within a day. Photoperiod variation influences several physiological factors in plants, such as biomass production and changes in secondary compounds in microgreens [23–26]. According to a review by Appolloni et al. [22], the most commonly used indoor photoperiods in plant production are 12 and 16 h, being employed in a large number of studies [7,11,27–29]. It has been established that photoperiods with fewer light hours correspond to short ones, while photoperiods with higher light hours correspond to long ones [30]. Ali et al. [23] evaluated the effect of photoperiods of 6, 12, 18, and 24 h on beet growth and antioxidant activity. They obtained that 18 and 24 h reduced yield, total phenol and betacyanin concentration, and antioxidant capacity, while the 12 h photoperiod got the highest values for the same variables. In *Brassicaceae* microgreens, the literature yields different results for photoperiods between 8 and 24 h. For example, the dry and fresh weight in cabbage and Chinese kale microgreens among 12, 14, 16, 18, and 20 h was promoted by 14 h of light [24], while Filatov and Vetchinnikov [25] observed that the cabbage microgreens fresh weight increased under 8 h photoperiod under 200 μmol m^{-2} s^{-1} versus 16 h photoperiod with 100 μmol m^{-2} s^{-1}. Still, in radish microgreens, the opposite occurred. In mustard microgreens, a 20 h photoperiod raised the fresh weight compared to an 8, 12, 16, and 24 h photoperiod [26]. Likewise, antioxidant capacity can vary among species. Liu et al. [24] indicated no significant difference in DPPH and FRAP for cabbage microgreens under different photoperiod treatments. For Chinese kale microgreens, DPPH was not significantly different among photoperiod treatments, while FRAP was the lowest for the 16 h photoperiod. In red pak choi, tatsoi, and mustard microgreens, the duration of 20 and 24 h evoked an antioxidant response which influenced significantly higher contents of DPPH scavenging activity [31]. On the other

hand, the contents of polyphenols and flavonoids were the lowest for the 20 h photoperiod for cabbage microgreens. For Chinese kale microgreens, the contents of polyphenols and flavonoids were not significantly different among photoperiod treatments [24]. In red pak choi, tatsoi, and mustard, total phenols and flavonols (index) increased under 20 and 24 h photoperiods compared to 8, 12, and 16 h photoperiods [31]. However, as for intensity, it is necessary to evaluate photoperiods for each species and variety because the response to this factor varies.

Red and blue are the most efficient light spectra for plant photosynthesis [32,33]. This is because red and blue light coincides with the maximum absorption of chlorophylls, thus promoting photosynthesis, growth, nutrients, antioxidants, and polyphenol accumulation. Although blue light is less efficient than red light in photosynthesis [33], blue light induces stomatal opening allowing better CO_2 fixation supporting photosynthesis, besides enhanced synthesis of antioxidant compounds [34]. On the other hand, it has been observed that, although far-red would have a limited contribution to photosynthesis due to the low absorption of the plant canopy, it is necessary for efficient photosynthesis [35]. In addition, Ahmed et al. [36] note that adding the far-red spectrum to red and blue illumination substantially improves biomass production and increases light use efficiency.

Studies on beet microgreens regarding their potential human health benefits are limited. Besides, the effect of intensity and photoperiod depends on the species, so the impact of light on microgreens cultivation needs to be evaluated in detail. Therefore, the present study aims to observe the differences in yield and antioxidant activity of beet microgreens under different intensities and indoor photoperiods. The primary purpose is to understand microgreens' growth and phytochemical responses to define the suitable light intensity and photoperiod to increase yield and achieve a better quality of beet microgreens.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

This research consisted of an experimental trial in adapted refrigeration cold rooms of 3.5 × 4.0 × 6 m at the Centro Estudio de Postcosecha (CEPOC) at Universidad de Chile, Santiago, Chile (33°34′ S, 70°38′ W). Three metal shelves measuring 170 × 180 × 45 cm were arranged inside the cold rooms, with three levels per shelf. Three LED growth lamps (Asycar, Santiago, Chile) were mounted on each level, combining 75% red, 23% blue, and 2% far-red. The distance between shelf levels was 60 cm for low intensity (120 ± 2 µmol m^{-2} s^{-1}), 47 cm for medium intensity (160 ± 2.5 µmol m^{-2} s^{-1}), and 33.5 cm for high intensity (220 ± 2.8 µmol m^{-2} s^{-1}). The photoperiods used were programmed for 12 and 16 h with a plug-in analog timer TG-14 (ManHua Electric Co., Ltd., Wenzhou, China). Sowing was carried out on two different cultivation periods to avoid interference between the lamps with different photoperiods. The lighting system began to operate on the day of sowing. Ambient temperature and relative humidity during culture growth were 21 ± 2 °C and 70–80%, respectively. Both variables did not vary significantly during the cultivation of the beet microgreens. The energy released by the heat of the LED lamps was minimal (±2 °C), and there were minor variations in humidity that did not exceed 5%.

Beet (*Beta vulgaris* L. ssp. *vulgaris*) seeds (Rijk Zwaan, De Lier, The Netherlands) were sown at a density of 36 g m^{-2} in a plastic tray (64 × 35 × 6 cm). The substrate used was a mixture of peat DSM2 W R0632 (Kekkilä, Vantaa, Finland) and perlite A6 (Harborlite, Santiago, Chile) in a ratio of 1:1 (*v:v*). The seeded trays were introduced into the cold rooms, and the treatments detailed in Table 1 were applied.

2.2. Physical Evaluations

2.2.1. Yield

On the day of harvest, the fresh weight of all microgreens obtained from each tray was measured in units of grams per square meter (g m^{-2}).

Table 1. Details of the treatments applied to the beet microgreens under the indoor system.

Light Treatment	Intensity	Photoperiod	DLI
	µmol m^{-2} s^{-1}	**h**	**mol m^{-2} d^{-1}**
[1] L12	120	12	5.2
L16	120	16	6.9
M12	160	12	6.9
M16	160	16	9.2
H12	220	12	9.5
H16	220	16	12.7

[1] L: low; M: medium; and H: high intensity.

2.2.2. Dry Matter Percentage

Dry matter was measured from 5 g of harvested microgreens per tray, which were dried in an oven LFO-250F (LabTech, Gyeonggi-do, Republic of Korea) at 60 °C until the sample maintained constant weight. It was then measured through a semi-analytical balance CMN3000-1 (Kern & Sohn GmbH, Balingen, Germany), and the result was presented as a percentage.

2.2.3. Height

The height of the microgreens was measured using a ruler from the point of harvest cut to the cotyledons. For this purpose, 30 microgreens randomly chosen from each repetition were used.

2.2.4. Cotyledon Area

It was obtained through digital images (photographs) of the cotyledons using the ImageJ program (version 1.53k, United States). Twenty microgreens randomly selected from each repetition were used.

2.2.5. Color

Luminosity (L*), Chroma (C*), and Hue (h*) were measured in the cotyledons through the use of a compact tristimulus colorimeter CM-2500d (Konica Minolta Inc., Osaka, Japan) according to Lara et al. [37]. C* corresponds to the purity of a color. A high chroma indicates that the hue has no black, white, or gray. Hue indicates the color itself. Thirty randomly selected microgreens from each replicate were used for measurement. Cotyledons were separated and taped with no gaps, and 30 readings were taken for each replicate.

2.3. Chemical Evaluations

2.3.1. Total Phenolic Content

Total phenolic content was analyzed according to the method proposed by Singleton and Rossi [38] and Lara et al. [37]. In a 2 mL Eppendorf tube, 100 µL of extract (200 mg dry matter plant in 10 mL methanol 70%) and 200 µL of 10% Folin-Ciocalteu reagent were added and allowed to react for 5 min. Subsequently, 800 µL of sodium carbonate (Na_2CO_3) at 700 mM was added and left to react for one hour. After this, 200 µL of the solution was added to a 96-well plate for absorbance measurement at 765 nm in a microplate spectrophotometer ASYS UVM340 (Biochrom Ltd., Cambridge, UK). The total phenolic content was calculated using a calibration curve performed with gallic acid. The results were expressed as mg gallic acid equivalent (GAE) g^{-1} of fresh weight (FW).

2.3.2. Total Betalains Content

Total betalains was determined according to the method by Zin et al. [39]. One mL of the extract (200 mg dry matter plant in 10 mL ethanol 15%) was taken to reduced volume cuvettes. The absorbance was measured in a UV/VIS spectrophotometer Optizen POP (Mecasys Co., Ltd., Daejeon, Korea) at 480 and 535 nm for betaxanthins and betacyanins,

respectively. The quantification of betacyanins and betaxanthins was calculated by the equation: BC = (A × MW × DF × 1000)/(ε × L), where A corresponded to absorbance, MW to molecular weight, DF to dilution factor, ε to molar extinction coefficient, and L to cuvette length. The results were expressed as mg g^{-1} of fresh weight (FW). The values used for the calculations were:

$$\text{For betaxanthins: } \varepsilon = 48{,}000 \text{ L mol}^{-1} \text{ cm}^{-1}, \text{ MW} = 308 \text{ g mol}^{-1}$$

$$\text{For betacyanins: } \varepsilon = 60{,}000 \text{ L mol}^{-1} \text{ cm}^{-1}, \text{ MW} = 550 \text{ g mol}^{-1}$$

The total betalains contents was the sum of betaxanthins plus betacyanins.

2.3.3. Antioxidant Capacity

Antioxidant Capacity by FRAP Method

Antioxidant capacity by FRAP method was carried out according to the method proposed by Benzie and Strain [40]. FRAP reagent was prepared by the addition of acetate buffer 300 mmol L^{-1} (pH 3.6), an aqueous solution of ferric chloride hexahydrate 20 mmol L^{-1} and 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) 10 mmol L^{-1} in HCl 40 mmol L^{-1}, in a 10:1:1 ratio, respectively. It was heated in a thermoregulatory bath at 40 °C for 10 min. Subsequently, 20 µL of the same extract used to measure total phenolic compounds and 600 µL of FRAP reagent were added in a 2 mL Eppendorf tube. After 30 min, 200 µL was extracted and placed in a 96-well plate for measurements every 30 min for five h at 593 nm in a microplate spectrophotometer ASYS UVM340 (Biochrom Ltd., Cambridge, England). Antioxidant capacity by FRAP was calculated through a calibration curve performed with Trolox. The results were expressed as mg trolox equivalent (TE) g^{-1} of fresh weight (FW).

Antioxidant Capacity by DPPH Method

Antioxidant capacity measurement by DPPH was done with slight modifications of the technique employed by Ali et al. [23] and Zheng et al. [41]. In a 2 mL Eppendorf tube, 250 µL of extract (200 mg dry matter plant in 10 mL methanol 70%) and 1 mL of 0.4 mM DPPH reagent were added and allowed to react for 20 min. Then, 200 µL was extracted and transferred to a 96-well plate to measure the absorbance at 517 nm in a microplate spectrophotometer ASYS UVM340 (Biochrom Ltd., Cambridge, England), and 200 µL of the blank solution, prepared with 250 µL of 70% methanol and 1 mL of DPPH reagent, was added. The antioxidant capacity was calculated using the equation:

$$I = ((A_{blank} - A_{sample})/A_{blank}) \times 100\%$$

where A_{sample} corresponded to the absorbance of the reaction between the reagent and the extraction, A_{blank} to the absorbance of the reagent with methanol without the extract, and I to the inhibition of the DPPH free radical expressed as a percentage (%).

2.4. Microbiological Counts

Microbiological determinations were conducted using 10 g of microgreens per replicate. The samples were mixed with 90 mL of 0.1% buffered-peptone water (Merck, Darmstadt, Germany) and homogenized in a sterile bag using a stomacher (model Easy Mix, AES Chemunex, Bruz, France) for 60 s. Serial dilutions were prepared for plating. The total quantities of aerobic mesophiles and psychrophiles were assessed on plate count agar after 2 or 7 days of incubation at 37 and 5 °C, respectively [42,43]. In addition, Enterobacteriaceae counts were performed on violet red bile dextrose agar incubated for 2 days at 37 °C [42]. Besides, microbiological counts were performed on the irrigated substrate before sowing, finding 3.9 ± 0.2 CFU g^{-1}, 5.3 ± 0.0 CFU g^{-1}, and 4.0 ± 0.0 CFU g^{-1} for mesophiles, psychrophiles, and Enterobacteriaceae, respectively.

2.5. Resource Analysis

2.5.1. Energy Use Efficiency (EUE)

Efficiency was calculated by the ratio between the electricity consumption of all the LED lamps and the microgreens production obtained at the end of each culture period and expressed as kg m^{-2} for each intensity-photoperiod combination. The electricity consumption for a photoperiod of 12 h was 97.2 kW (27 lamps × 300 W), whereas for a photoperiod of 16 h, it was 129.6 kW. Therefore, the unit of measurement was g FW kW^{-1} m^{-2}.

2.5.2. Water Use Efficiency (WUE)

Water use efficiency was calculated using the equation: Rn L^{-1}, where Rn corresponded to the yield of microgreens produced at the end of each culture period expressed as kg and liter (L) to the water volume applied during all the same period [44]. The water use efficiency was reported as g FW L^{-1} m^{-2}.

2.6. Experimental Design and Statistical Analysis

The trial was conducted with a completely randomized design with a 3 × 2 factorial structure. The factors considered were: intensity, with three levels (120, 160, and 220 µmol m^{-2} s^{-1} PPFD), and photoperiod, with two levels (12 and 16 h). The data were analyzed using linear mixed models for each evaluated variable. Finally, the differences between the means were compared by LSD Fisher's test with a significance level of 5% ($\alpha = 0.05$) for factor interaction or independent factors when applicable. Statistical analyses were performed with InfoStat software and R programming language, both versions 2020e.

3. Results

3.1. Physical Evaluations

3.1.1. Yield

Significant differences were independently obtained for this variable in the intensity and photoperiod (Table 2). Specifically, the low (459.74 g m^{-2}) and medium (460.5 g m^{-2}) intensities significantly increased yield concerning the high intensity (358.41 g m^{-2}), which was 22% lower than the low and medium intensities. On the other hand, the average yield obtained under 12 and 16 h photoperiods were 482.73 and 369.7 g m^{-2}, which means a decreased beet microgreen yield of 23.4% between both photoperiods.

Table 2. Agronomic characteristics and color (L* = lightness; C* = Chroma, and h = hue) of beet microgreens under different light treatments. Values are the mean of 3 repetitions.

Factor	Yield	Dry Matter	Height	Cotyledon Area	Color		
	g m^{-2}	%	cm	cm^2	L*	C*	h
Intensity (I)	*	ns [1]	ns	ns	*	ns	ns
Low (L)	459.74 a [2]	8.00	3.98	0.52	37.17 a	25.98	83.25
Medium (M)	460.50 a	8.94	3.84	0.51	33.76 ab	24.28	74.40
High (H)	358.41 b	8.96	3.67	0.49	30.21 b	26.34	88.53
Photoperiod (P)	*	*	*	ns	ns	ns	ns
12	482.73 a	6.71 b	4.33 a	0.53	36.54	27.31	84.45
16	369.70 b	10.55 a	3.33 b	0.49	30.88	23.76	79.67
Interaction (IxP)	ns	ns	ns	ns	ns	ns	*
[3] L12	471.24	7.36	4.16	0.53	37.12	26.65	87.89 a
L16	414.72	9.28	3.66	0.51	34.03	24.87	78.60 ab
M12	471.62	7.83	4.09	0.52	35.15	25.80	87.83 a
M16	415.10	9.75	3.59	0.50	32.32	24.02	60.96 b
H12	420.57	7.84	4.00	0.51	33.38	26.83	77.61 ab
H16	364.10	9.76	3.50	0.49	30.55	25.05	99.44 a

[1] Indicates not significant. [2] Different letters on the columns within each factor or interaction indicate significant differences (Fisher's test, * $p < 0.05$). [3] L: low; M: medium; and H: high intensity.

3.1.2. Dry Matter Percentage (DM)

Microgreens' dry matter percentage was significantly affected by the photoperiod. In particular, the dry matter under the 12 h photoperiod (6.7%) was significantly reduced by 36.3% in comparison to the 16 h photoperiod (10.6%) (Table 2).

3.1.3. Height

A significant effect of the photoperiod was recorded for beet microgreens' height (Table 2). Specifically, the height of microgreens grown under 16 h of light was significantly lower by 23% than those produced under a 12 h photoperiod.

3.1.4. Cotyledon Area (CA)

The intensity did not cause significant differences in the leaf area of beet cotyledons, which were at low, medium, and high intensities of 0.52, 0.51, and 0.49 cm^2, respectively (Table 2). The effect of the photoperiod also showed no significant changes in the cotyledon area, which reached 0.53 and 0.49 cm^2 for 12 and 16 h of light, respectively.

3.1.5. Color

Lightness

The lightness under the different intensities showed significant differences (Table 2). Specifically, high intensity caused beet cotyledons to be 18.7% darker than those treated with low intensity. Conversely, medium intensity showed no significant differences between low and high intensity. The effect of the photoperiod did not promote significant differences in the lightness of beet microgreens (Table 2). Notably, the mean values for the 12 and 16 h photoperiods were 36.5 and 30.9, respectively.

Chroma (C*)

The chroma showed similar values under different intensities and photoperiods. The values observed for the 12 and 16 h photoperiods were 27.3 and 23.8, respectively, while low and high intensity values ranged from 24.28 to 26.4 (Table 2).

Hue (h)

Hue was affected by the interaction of intensity x photoperiod. In particular, beet microgreens under M16 (60.96°) showed significantly less yellow than L12 (87.89°), M12 (87.83°), and H16 (99.44°) (Table 2).

3.2. Chemical Evaluations

3.2.1. Total Phenolic Content

Photoperiod was the unique factor that promoted significant differences in the total phenolic content of beet microgreens (Table 3). As the photoperiod increased from 12 to 16 h, the total phenol content increased significantly by 46.4% from 8.99 to 13.16 mg GAE g^{-1} FW. In contrast, intensities showed no significant differences, averaging values between 10.16 and 11.80 mg GAE g^{-1} FW (Table 3).

3.2.2. Total Betalains

The total betalains were significantly affected by both intensity and photoperiod without significant interaction between both factors. Specifically, microgreens grown under low intensity had a substantially higher content than those produced at high intensity by 55.9% (Table 3). On the other hand, the total betalains content doubled as the photoperiod increased from 12 (0.6 mg g^{-1} FW) to 16 h light (0.61 mg g^{-1} FW) (Table 3).

Betaxanthins

The betaxanthins content is described in Table 3. The content of this pigment was independently affected by intensity and photoperiod. As the intensity increased, the betaxanthin content decreased, being significantly higher under low compared to high

intensity by 50%. In contrast, as the photoperiod increased from 12 to 16 h, betaxanthins rose from 0.07 to 0.13 mg g^{-1} FW, i.e., there was an increase of 85.7%.

Table 3. Total phenolic content, total betalain content (betacyanins + betaxanthins), and antioxidant capacity (FRAP and DPPH) of beet microgreens under different light treatments. Values are the mean of 3 replicates.

	Total Phenolic Content	**Total Betalains**	**Betacyanins**	**Betaxantins**	**FRAP**	**DPPH**
Factor	**mg GAE g^{-1} FW**	**mg g^{-1} FW**	**mg g^{-1} FW**	**mg g^{-1} FW**	**mg TE g^{-1} FW**	**%**
Intensity (I)	ns [1]	*	*	ns	ns	ns
Low (L)	10.16	0.53 a [2]	0.12 a	0.41	35.07	35.13
Medium (M)	11.27	0.50 ab	0.11 ab	0.39	38.58	34.05
High (H)	11.80	0.34 b	0.08 b	0.27	39.96	36.94
Photoperiod (P)	*	*	*	*	*	ns
12	8.99 b	0.31 b	0.07 b	0.24 b	32.37 b	37.13
16	13.16 a	0.61 a	0.13 a	0.48 a	43.37 a	33.61
Interaction (IxP)	ns	ns	ns	ns	ns	ns
[3] L12	9.58	0.42	0.10	0.33	33.72	36.13
L16	11.66	0.57	0.13	0.45	39.22	34.37
M12	10.13	0.41	0.09	0.32	35.48	35.59
M16	12.22	0.56	0.12	0.44	40.98	33.83
H12	10.40	0.33	0.08	0.26	36.17	37.04
H16	12.48	0.48	0.11	0.38	41.67	35.28

[1] Indicates not significant. [2] Different letters on the columns within each factor or interaction indicate significant differences (Fisher's test, * $p < 0.05$). [3] L: low; M: medium; and H: high intensity.

Betacyanins

Betacyanins content was impacted by the photoperiod factor (Table 3). In particular, by increasing the photoperiod by 4 h, from 12 to 16 h, the betacyanin content doubled.

3.2.3. Antioxidant Capacity

The photoperiod affected the antioxidant capacity measured by FRAP (Table 3), whereas no significant differences were found with antioxidant capacity by DPPH. Notably, the increase of photoperiod from 12 to 16 h resulted in a considerable increase in the antioxidant activity of the beet microgreens by 25.4% from 32.37 to 43.37 mg TE g^{-1} FW. However, as for intensity, the antioxidant capacity value varied non-significantly between 35.1 and 40 mg TE g^{-1} FW.

3.3. Microbiological Counts

3.3.1. Mesophiles

The intensities caused a significant effect on the mesophiles counts of beet microgreens. In particular, microgreens grown at low intensity showed a significantly lower mesophiles count of 3.67 log CFU g^{-1} than those developed at medium and high intensity, with 4.92 and 5.07 log CFU g^{-1}, respectively (Table 4). Meanwhile, the photoperiod showed no significant differences. The mean microbial counts under 12 and 16 h were 4.41 and 4.69 log CFU g^{-1}, respectively (Table 4).

3.3.2. Psychrophiles

Psychrophiles counts showed no significant differences for both intensity and photoperiod factors or interaction (Table 4). In the case of intensity, counts of 1.45 log CFU g^{-1} or less were found for low to high intensity. Similarly, counts less than 1 and 2.02 log CFU g^{-1} were found at 12 or 16 h, respectively.

Table 4. Microbiological bacterial counts (log CFU g^{-1}) of beet microgreens under different light treatments. Values are the mean of 3 repetitions.

	Mesophiles	**Psychrophiles**	**Enterobacteriaceae**
Factor	**log CFU g^{-1}**	**log CFU g^{-1}**	**log CFU g^{-1}**
Intensity (I)	*	ns [1]	*
Low (L)	3.67 b [2]	1.45	5.45 a
Medium (M)	4.92 a	<1	4.53 b
High (H)	5.07 a	<1	5.08 a
Photoperiod (P)	ns	ns	ns
12	4.41	<1	4.69
16	4.69	2.02	5.35
Interaction (IxP)	ns	ns	*
[3] L12	4.04	<1	5.52 a
L16	4.18	2.90	5.38 ac
M12	4.67	<1	3.94 b
M16	4.81	1.35	5.12 ac
H12	4.74	<1	4.62 bc
H16	4.88	1.81	5.54 a

[1] Indicates not significant. [2] Different letters on the columns within each factor or interaction indicate significant differences (Fisher's test, * $p < 0.05$). [3] L: low; M: medium; and H: high intensity.

3.3.3. Enterobacteriaceae Counts

For these microorganisms, the photoperiod and intensity factors acted dependently (Table 4). In fact, the intensity caused different Enterobacteriaceae counts for the same photoperiod level. Low intensity (L) showed a slight but significant decrease in Enterobacteriaceae counts as the photoperiod increased from 5.52 to 4.62 log CFU g^{-1}. In contrast, increasing the photoperiod from 12 to 16 h caused a significant increase from 3.94 to 5.12 log CFU g^{-1} for medium intensity and from 4.62 to 5.54 log CFU g^{-1} for high intensity.

3.4. Resource

3.4.1. Energy Use Efficiency (EUE)

EUE was affected mainly by the photoperiod factor. Specifically, EUE was increased by under 12 h compared to 16 h periods of light by 31.6% (Table 5).

Table 5. Energy (EUE) and water use (WUE) efficiency of beet microgreens under different light treatments. Values are the mean of 3 repetitions.

	EUE	**WUE**
Factor	**g FW kW^{-1} m^{-2}**	**g FW L^{-1} m^{-2}**
Intensity (I)	ns [1]	*
Low (L)	4.56	33.56 a [2]
Medium (M)	4.56	33.59 a
High (H)	4.04	29.72 b
Photoperiod (P)	*	*
12	4.97	36.60 a
16	3.80	28.00 b
Interaction (IxP)	ns	ns
[3] L12	4.85	35.70
L16	4.27	31.42
M12	4.85	35.73
M16	4.27	31.45
H12	4.33	31.86
H16	3.75	27.58

[1] Indicates not significant. [2] Different letters on the columns within each factor or interaction indicate significant differences (Fisher's test, * $p < 0.05$). [3] L: low; M: medium; and H: high intensity.

3.4.2. Water Use Efficiency (WUE)

WUE was influenced by intensity and photoperiod factors independently. In particular, the low and medium intensity provoked a higher WUE than the higher intensity by 13.1%. On the other hand, a shorter photoperiod (12 h) enhanced WUE compared to a more extended photoperiod (16 h) by 30.7% from 36.6 to 28.0 g FW L^{-1} m^{-2} (Table 5).

4. Discussion

4.1. Environmental Conditions

Among the environmental factors that generate changes in vegetables' organoleptic and functional quality are temperature, light, and to a lesser extent, relative humidity [45]. In this study, neither temperature nor relative humidity significantly varied during beet microgreens cultivation. The energy released from the heat by the LED lamps was minimal. During the growth period of the microgreens, an average increase of 1.8 °C was observed when the cold room lighting system was turned on concerning this. However, this increase would not significantly impact the temperature range between day and night for microgreens produced in confinement could vary by three °C as long as the temperature is in the optimal range for the species [46]. On the other hand, relative humidity also had a slight variation, being 5.1% higher with the lights off compared to the cold room with the lights on. Therefore, neither temperature nor relative humidity varied significantly during the trials, and the responses of the microgreens in the different variables measured must be mainly associated with the variation of the light parameters (photoperiod and intensity).

4.2. Effect of Intensity and Photoperiod on Physical Evaluations

Fresh weight is a vital growth quality parameter of microgreens [11], and it responded differently to light intensity [47–49], as does yield [50]. In this study, the fresh weight was obtained to measure the yield of each treatment. Notably, the high intensity significantly decreased the yield concerning the medium and low intensity. These results followed a similar trend observed for other measured parameters such as L* and total betalains, where significant changes were found at the highest intensity. In the same way, dry matter, height, cotyledon area, total phenolic content, and antioxidant capacity were slightly more extensive at the highest intensity but not significantly. Similarly, in broccoli microgreens, Gao et al. [47] noted that as the intensity increased from 50 to 70 µmol m^{-2} s^{-1} and 70 to 90 µmol m^{-2} s^{-1}, the fresh weight decreased by about 12.6% and 9.7%, respectively. High light intensities could damage photosystem II and promote photoinhibition, reducing the photosynthetic rate [16,45]. The high intensity in this study is associated with the highest daily light integral (DLI) (Table 1). DLI, which represents the total flux of photosynthetic photons irradiated by a light source in a day [51] and combines both the light intensity and photoperiod [52], can cause damage to PSII when it increases. For example, a DLI of 14.4 mol m^{-2} d^{-1} (250 µmol m^{-2} s^{-1}) compared to 8.6 and 4.6 mol m^{-2} d^{-1} (150 and 80 µmol m^{-2} s^{-1}) caused photoinhibition in basil but not lettuce [53]; this means that photoinhibition caused by high DLI is species-specific. Then, in this study, a DLI upper 9.5 mol m^{-2} d^{-1} (220 µmol m^{-2} s^{-1}) could promote photoinhibition in beet microgreens by observing a lower yield. Photoinhibition can result from excess excitation energy directed toward reaction centers, most commonly PSII [54]. On the other hand, it is considered that the extended photoperiod itself may also be a reason for excess light absorbed even if the DLI is not higher than usually required by plants under shorter photoperiods that may cause photoinhibition [55]. At the same time, Bian et al. [56] point out that optimal light intensity could improve photosynthetic activity and the synthesis of phytochemicals in vegetables. Therefore, under these research conditions, the favorable intensity to improve the yield was between 120 to 160 µmol m^{-2} s^{-1} since it increased it by ~28.4% compared to the high intensity of 220 µmol m^{-2} s^{-1} (Table 2).

On the other hand, the literature showed that the photoperiod influenced the growth of various vegetables [57,58]. This research indicates that a higher yield was produced by applying a lower photoperiod. Specifically, beet microgreens grown under the 12 h

photoperiod yielded 30.6% more than those grown under the 16 h photoperiod (Table 2). Coincidently, Ali et al. [23] evaluated photoperiods of 12, 18, and 24 h in beet, red and green amaranth, red spinach, and Swiss chard. The researchers obtained a significant decrease in the fresh weight of all species with increasing photoperiod. These results contradict those obtained by Meas et al. [27], who evaluated photoperiods of 8, 12, 16, and 20 h in microgreens of two varieties of amaranth. They observed a tendency to increase the yield as the hours of light rose, concluding that a more extended lighting period favored a more prolonged photosynthetic activity that translated into a higher crop yield [57]. Likewise, shortening the photoperiod also increased hypocotyl length by 1 cm (Table 2). This result could be associated with the highest yield obtained under 12 h of light, although other variables, like the foliar area, could be related. The cotyledon area increased by 8.2% under a 12 h photoperiod, although the difference was insignificant with 16 h of light. In contrast, the dry matter content diminished significantly when the photoperiod reduced from 16 to 12 h, suggesting a high water content in beet microgreen under the shortest photoperiod.

The light intensity can also influence microgreens' hypocotyl elongation. This research found a tendency to decrease the length with high intensity (Table 2). A similar trend has been observed in different vegetable species. For example, Vetchinnikov et al. [28] noted that increasing the intensity from 100 to 200 μmol m^{-2} s^{-1} diminished the hypocotyl length of radish, cabbage, and basil by 20, 34, and 56%, respectively. Similarly, Jones-Baumgardt et al. [49] observed that when increasing the intensity from 100 to 600 μmol m^{-2} s^{-1}, under the light whose blue: red ratio was 1:5.7 and a 16 h photoperiod, the hypocotyl length of cabbage, rocket, and mustard decreased by 24, 37, and 62%, respectively. Likewise, Gerovac et al. [48] observed that the length of hypocotyls decreased to 30% in kohlrabi, mizuna, and mustard, with an increase in light intensity from 105 to 315 μmol m^{-2} s^{-1}. Similarly, Gao et al. [47] found that broccoli hypocotyl lengths were markedly reduced as light intensity with red:green:blue = 1:1:1 increased (from 30 to 90 μmol m^{-2} s^{-1}). Runkle [59] has pointed out that the decrease in light intensity signals the shade avoidance response. Shade avoidance responses include the elongation of stems and petioles [59]. Additionally, it has been suggested that low light (50 μmol m^{-2} s^{-1}, 16 h light) promoted hypocotyl elongation repressed wall deposition by influencing the accumulation of cellulose, hemicellulose, and pectin [60]. Thus, these signals can be detected by microgreens promoting hypocotyl elongation, increasing their height. On the other hand, the size of the microgreens must be at least 5 cm [7]. However, our results show that none of the applied treatments reached 5 cm in height, so a more extended growth period or a lamp with a spectrum with a higher fraction of red is suggested, which allows a greater elongation of the hypocotyl [61]. In addition, far red can promote hypocotyl elongation. For example, the supplementation of far-red light on the basis of LED red + blue light positively promoted the hypocotyl length in kohlrabi and mustard microgreens [48]. Likewise, LED blue + far-red or far-red monochromatic increased plant height by 6% and 15% for mustard and arugula microgreens [62]. Nevertheless, this action could depend on the amount of far-red photons because a 2% far-red in the spectrum used in this study does not favor beet microgreens hypocotyl elongation.

The quality parameters of microgreens determining the momentary purchase decision are size, shape, and color [45]. The beet's microgreens color is given by pigments named betalains, which are subdivided into two groups based on their structure and chemical composition [63]. Betacyanins are responsible for red–violet pigmentation, and betaxanthins are related to yellow pigmentation [13,64]. Our results showed that the intensity significantly impacted the luminosity (L*). Remarkably, the high intensity promoted a darker (opaquer) color in the cotyledons of beet microgreens, whereas the low intensity caused cotyledons with a lighter (more transparent) color. Furthermore, the enhancement of betaxanthin is associated with higher lightness [65]; hence, the lighter color in beet microgreens would be due to the increase in betaxanthin concentration under the low intensity observed in this study (Table 3). On the other hand, the hue was affected by interaction intensity × photoperiod. Overall, beet microgreens under all treatments were more yellow, except microgreens

under M16, which presented a color more towards red. According to Stintzing et al. [65], total contents and the specific ratios of betacyanin: betaxanthin will determine the resulting hue. Acharya et al. [8] indicated that a higher betacyanin: betaxanthin ratio favored the red–violet color. Then, the color more towards red of beet microgreen under M16 was due to a more significant accumulation of betacyanins or diminished betaxanthins or both, as shown in Table 3. The rest of the treatments would promote the opposite effect.

4.3. Effect of Intensity and Photoperiod on Chemical Evaluations

Phenolic compounds are secondary metabolites that are synthesized throughout the growth and development of plants [8]. They correspond to one of the main contributors to antioxidant activity in beetroot leaves and have been shown to be influenced by photoperiod [8,23]. Similarly, in this study, a longer photoperiod of 16 h favored a higher concentration of phenolic compounds compared to 12 h of light. In contrast, no differences in the phenolic contents were observed among intensities, probably because the range was not wide enough.

The amounts of accumulating betalains responded to the intensity and depended on the irradiation spectrum of the light sources [66,67]. In addition, they can be stimulated by light photoperiods [63]. This research showed that photoperiod and intensity independently affect total betalains, betaxanthins, and betacyanins contents. Specifically, the exposition to 16 h light and the low intensity (120 μmol m^{-2} s^{-1}) significantly and independently raised its concentration in beet microgreens. The research of El-Ashry et al. [68] mentioned that the highest value of betalains (betacyanin and betaxanthin) in reed beet was recorded by exposing the cultures to the red light for 30 days compared to 10 and 20 days. Contrarily, a previous study showed that the maximum production of betacyanin was obtained between 6 to 12 h photoperiodic ranges and decreased after that under 540 μmol m^{-2} s^{-1} using cool white fluorescent and incandescent bulbs [23]. On the other hand, the literature indicates that different intensities impact betalains. For example, Girod and Zryd [69] found that transferring calli red beet from dim light to light with 39 μmol m^{-2} s^{-1} is necessary for induction betalain synthesis. In contrast, an intensity of 500 μmol m^{-2} s^{-1} under white light resulted in a rapid accumulation of betacyanins in *Mesembryanthemum crystallinum* L. (ice plant) (Aizoaceae) compared to 100 and 200 μmol m^{-2} s^{-1} [66]. The literature would indicate that the light effect depends on species and growth stage.

On the other hand, specific genes could be regulated by the light that may affect betalains synthesis. For example, Zhao et al. [70] indicated that the dopa-4,5-dioxygenase gene (DODA), crucial for synthesizing the chromophore betalamic acid involved in betacyanin synthesis, responds to different light quality and quantity. In particular, markedly increased DODA transcript was noted under light treatments (white light, red light, and blue light) compared with dark. Besides, the highest gene expression level from *S. salsa* calli cultured under white light 80 μmol m^{-2} s^{-1} versus blue and red monochromatic lights was observed, indicating that DODA transcription was, at the least in part, responsible for light-regulated changes in betacyanin accumulation. Likewise, Imamura et al. [71] point out that the enzyme CqCYP76AD1-1 is involved in betalain biosynthesis because the accumulation of betalain pigment coincided with its expression under prolonged light exposure (18 h of light) in *Chenopodium quinoa* hypocotyl. According to Zhao et al. [70], the light signal would be sensed by the phytochrome or cryptochrome and passed through multiple intermediates that regulate a transcription factor that control the expression of different enzymes related to the formation of betalains. Hence, the betacyanins or betaxanthins accumulation/production would be held for some genes stimulated mainly by the time light exposition and intensity.

Betalains are pigments with potent antioxidant properties and may confer tolerance to various stress conditions [72]. Moreover, they are one of the main contributors to antioxidant activity in beet leaves [8,23]. The importance of antioxidants is that they can delay or prevent oxidative damage of a substrate when they are in low concentrations [73]. Thus,

antioxidants can reduce the risk of diseases related to oxidative stress, such as neurodegenerative diseases, heart disease, and cancer [7]. In this regard, microgreens have aroused great interest as a functional food since they have higher levels of bioactive compounds and minerals than mature microgreens, which has led to increased consumption [34].

Antioxidants can be altered under different lighting conditions. For example, the antioxidant capacity of DPPH showed considerable differences among several vegetables due to the influence of photoperiodic variations [23]. In contrast, this research showed no significant differences in the DPPH free radical scavenging, while the antioxidant capacity measured by FRAP was significantly affected by photoperiod (Table 3). Mainly, 16 h light exposure increased the antioxidant capacity in beet microgreens compared to the 12 h photoperiod. Similarly, Meas et al. [27] noted a higher antioxidant capacity in amaranth microgreens when applying a 16 h photoperiod compared to a 12 h photoperiod. The higher antioxidant capacity under 16 h light was consistent with the concentration values of betalains, betacyanins, betaxanthins, and phenolic compounds obtained, which were also higher in microgreens grown under greater hours of daily light (Table 3). Phenolic compounds are secondary metabolites that are synthesized throughout the growth and development of plants [8]. They are one of the main contributors to beetroot leaf antioxidant activity and are influenced by photoperiod [8,23]. Similarly, in this study, a longer photoperiod of 16 h favored a higher concentration of phenolic compounds compared to 12 h of light. This result is consistent with Ali et al. [23], who mentioned that the antioxidant capacity of vegetable leaf extracts had a robust positive relationship with betacyanins and total polyphenols under different photoperiods. Thus, the higher antioxidant capacity in beet microgreens is due to a higher accumulation of phenols and betalains.

On the other hand, the intensity did not cause significant differences in beet microgreens' antioxidant capacity and phenolic contents, probably because the range was not wide enough. Samuolienė et al. [74] observed a slight effect on DPPH free radical activity in *Brassica* microgreens between the intensity range of 110 and 545 μmol m^{-2} s^{-1}. In contrast, literature results have shown that intensity impacts the antioxidant capacity of different species. For example, Meas et al. [27] indicated that the amaranth microgreens' antioxidant capacity increased as the intensity rose from 130 to 280 μmol m^{-2} s^{-1}. Likewise, Harakotr et al. [50] found that water convolvulus, red holy basil, dill, and lemon basil microgreens produce greater DPPH free radical scavenging under 330 μmol m^{-2} s^{-1} than 110 and 220 μmol m^{-2} s^{-1}. In other vegetables such as lettuce and endive, the antioxidants compounds were significantly increased by the high intensity (~100 μmol m^{-2} s^{-1}) compared with low intensity (62–78 μmol m^{-2} s^{-1}) [75]. Therefore, it is probable that the lack of differences under this research's low, medium, and high intensities was not significant enough to cause substantial changes in the species evaluated and provoke no environmental stress that induced the accumulation of antioxidant compounds as a defense mechanism [74].

4.4. Effect of Intensity and Photoperiod on Microbiological Counts

According to Verlinden [4], most works on microgreens indicate they are microbiologically safer than sprouts, germinated seeds that usually lack pigmentation. However, it has been posited that microgreens' delicate and soft-textured hypocotyls might favor microbial growth compared to their mature counterparts [12], and they are not exempt from contamination by pathogens. In this way, Priti et al. [76] mentioned that total aerobic bacteria, yeast, and mold, *Escherichia coli*, were recorded well within the limit to cause human illness in mungbean, lentil, and Indian mustard microgreens. Meanwhile, *Salmonella* spp. and *Listeria* spp. were not detected in these microgreens. On the other hand, it should be noted that the extent and quality of light directly or indirectly influence microbial growth [76]. According to D'Souza et al. [77], LEDs provide an alternative to chemical sanitizers in ascertaining microbiological food safety and an additional means of decontamination as microbial resistance becomes a more urgent problem. Specifically, irradiance is used to quantify the amount of monochromatic light in microbial inactivation operations in terms of

light energy. This study showed that mesophiles had significantly higher counts in the high (220 µmol m^{-2} s^{-1}) and medium (160 µmol m^{-2} s^{-1}) than in the low-intensity samples under light with 75% red light, 23% blue light, and 2% far-red light. However, the counts did not exceed the limits of Reglamento Sanitario de los Alimentos chileno (RSA) [78], adopted by the International Commission on Microbiological Specification for Foods. In a study by Chandra et al. [79], mesophilic bacterial counts of 7.1, 7.2, and 7.8 log CFU g^{-1} were obtained for radish, wheat, and unwashed cabbage microgreens, which is considerably higher than that obtained in this study for beet microgreens. According to the RSA [78], the values of microbiological counts of mesophilic bacteria below which ready-to-eat foods would not present a health risk correspond to 6.69 log CFU g^{-1}. Mesophilic bacteria give an estimate of total viable populations; they are indicative of endogenous microflora and contamination of the material. Several studies have quantified them in vegetables, and a high variability related to the vegetable studied has been found, pre-harvest handling and growing conditions, among others [80]. In the photoperiods reviewed, mesophilic bacterial counts were found below the maximum limit established by the RSA [78] in microgreens, and no significant differences between them were found.

According to Kowalska and Szczech [81], assessing the microbiological contamination of vegetables includes determining the levels of Gram-negative bacteria of the Enterobacteriaceae family, with particular relevance to fecal Enterobacteriaceae as general indicators of pollution. The RSA [78] determines that the upper limit value of the microbiological count below which ready-to-eat foods do not represent a health risk corresponds to 5.69 log CFU g^{-1}. In this research, the interaction of the factors photoperiod and intensity was significant, but no particular pattern was observed. However, the values of Enterobacteriaceae counts under treatments M12 and H12 were lower than the rest. Furthermore, all microbiological count values were below the maximum value where ready-to-eat foods would not represent a health risk. Results from Kroupitski et al. [82] indicated that *Salmonella enterica*, which belongs to the same family as Enterobacteriaceae, was observed both on the surface and within the iceberg lettuce leaf tissue when it was illuminated. The highest internalization rate was evident under intense illumination (100 µmol m^{-2} s^{-1}) and was significantly inhibited in the dark. These results imply that the pathogen is attracted to nutrients (mainly sucrose) produced by the photosynthetic process under illumination, especially at high intensity.

Psychrophiles represent a significant group of microorganisms in fresh vegetables, as they can multiply during storage and sale, usually occurring at temperatures between 1 and 5 °C [80]. For psychrophiles, maximum allowable count values are not established. However, the counts of beet microgreens were low compared to their mature counterparts, as shown in the study of Fernandez et al. [80]. They obtained counts of 5.63 ± 0.79 log CFU g^{-1}, while in the present research, the highest value obtained was 1.81 log CFU g^{-1} under H16. In addition, no differences were observed between photoperiods or the intensities evaluated.

The low counts of microorganisms in the beet microgreens reached in this research are probably because the microgreens were harvested one cm above the substrate to avoid contamination. Furthermore, the count of microorganisms in the substrate was always lower than the limit, which did not pose a health risk [78]. According to Verlinden [4], microgreens are mostly harvested without seed, seed coat, or roots; hence, microbial contamination is less of an issue. In addition, the short cultivation cycles of microgreens reduce the contact period with contaminants; meanwhile, controlled conditions and physical barriers of the environment would generate lower contamination of the microgreens. Finally, beet microgreens can be innocuous vegetables that do not risk human health if some factors are appropriately managed, such as using certified seeds, maintaining a sanitized environment, materials and tools, and correct handling.

4.5. Effect of Intensity and Photoperiod on Resources

According to Kozai and Niu [83], electricity is one major component of the production cost in a plant factory with artificial lighting, up to 18–20% of the total production cost.

Likewise, energy intensity is essential for the commercial production of microgreens, as this figure affects the cost of production [26] by having a direct relationship with electricity consumption [84]. However, this research showed that energy use efficiency (EUE) did not present significant differences under the intensities. Still, it is essential to mention that the EUE under high intensity (220 µmol m^{-2} s^{-1}) was 11.49% lower than that of low (120 µmol m^{-2} s^{-1}) and medium (160 µmol m^{-2} s^{-1}) intensity, i.e., there was a higher use of electricity per g FW per m^2, which would increase production costs. Similarly, Vetchinnikov et al. [28] found that electricity cost (kWh kg^{-1}) raised under 200 µmol m^{-2} s^{-1} compared to 50 and 100 µmol m^{-2} s^{-1} in radish, cabbage, and basil microgreens by 157%, 180%, and 155%, respectively. On the other hand, photoperiod can play a fundamental role in plant growth and energy consumption under controlled growth conditions. According to Lanoue et al. [85], 24 h lighting had the highest energy-use efficiency of the lights, indicating that the input energy produced higher biomass in amaranth microgreens than the 16 h of light independently of daily light integral (DLI). Meanwhile, collard green and two cultivars of basil plants grown under the 24 h of light, under a DLI = 14 mol m^{-2} d^{-1}, had the highest energy-use efficiency of the lights, as more biomass was produced with the least amount of input energy versus 24 h light under a DLI = 21 mol m^{-2} d^{-1} and 16 h light under a DLI = 14 or 21 mol m^{-2} d^{-1} [85]. It can be assumed that a longer photoperiod would benefit plant growth, resulting from increased carbon assimilation [24,85], making energy use more efficient. However, this research showed that a more extended exposure period (16 h light) significantly diminished the energy use efficiency for beet microgreen cultivation, generating 23.5% less g FW per kilowatts consumed than 12 h light (Table 5). According to Liu et al. [24], an excessive photoperiod could mediate inhibition of photosynthetic activities, leading to biomass reduction, observed under the 16 h photoperiod of this research, where yield reduction and reduced height of beet microgreens were obtained. Therefore, a shorter photoperiod (12 h of light) allowed better energy use. Likewise, 12 h of light improved the water use efficiency (WUE) in beet microgreen cultivation compared to 16 h photoperiod by 22% (Table 5). In contrast, Pennisi et al. [58] showed that WUE in lettuce, basil, and rocket did not differ significantly among photoperiods. However, the WUE was significantly higher in the chicory under a 16 h photoperiod than under 20 and 24 h light. Thus, shorter photoperiods could generate more fresh mass per water volume. Similarly, lower intensities, such as low (120 µmol m^{-2} s^{-1}) and medium (160 µmol m^{-2} s^{-1}) intensity, used in this study provoked a higher WUE in beet microgreens than did higher intensity (200 µmol m^{-2} s^{-1}). In lettuce and basil, the WUE was progressively increased, as it raised the light intensity from 100 to 200 µmol m^{-2} s^{-1} without any further significant increase for an intensity $\geq$200 µmol m^{-2} s^{-1} and $\geq$250 µmol m^{-2} s^{-1}, respectively [86]. These differences for WUE under different intensities may be due to the phenological stage of the plants; however, the intensity-promoting effect on stomatal conductance, as mentioned by Pennisi et al. [86], could be the common factor that improves yield under the intensities noted. In particular, intensities between 120 to 160 µmol m^{-2} s^{-1} and low light exposition, such as a 12 h photoperiod, are optimum conditions for beet microgreens under indoor cultivation.

5. Conclusions

LED technology improves the performance of red beet microgreens under low and medium intensities and short photoperiods of 12 h of light. The lower light exposure makes the cultivation of this type of microgreen more favorable as it increases energy and water use efficiency and lowers production costs per square meter. On the other hand, longer photoperiods of 16 h of light positively affect phenol content, antioxidant capacity, and concentration of total betalains, betacyanins, and betaxanthins. Therefore, the culture conditions to optimize red beet microgreens' growth and resource utilization under confinement conditions is an intensity between 120 to 160 µmol m^{-2} s^{-1} and a photoperiod of 12 h of light.

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