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Supplementary Light on the Development of Lettuce and Cauliflower Seedlings

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Abstract: The production of seedlings is one of the main activities for implementing agricultural crops. Many factors are involved in producing quality seedlings, including nutrition, health, genetics, and climatic factors such as temperature, humidity, and light. To evaluate the effect of light supplementation, a study was conducted using supplementary artificial light to produce lettuce and cauliflower seedlings. Sowing was carried out in styrofoam trays under a floating irrigation system. Part of the experiment containing the two species, received treatment with LED light for an additional 4 h per day, in addition to solar radiation (10 h·day^{-1}). The remaining seedlings received only solar radiation (without supplementation). After 37 days, the seedlings' biometric (leaf area, root length, aerial dry mass, and root dry mass) and biochemical parameters (phenolic compounds, flavonoids, chlorophyll *a*/*b*, and total chlorophyll) were analyzed. The data showed that the complementary light enhanced the performance in all the biometric parameters evaluated in the experiment for lettuce and cauliflower. The biochemical parameters in lettuce were also higher in seedlings with light supplementation. For cauliflower, supplementary light did not differ from the natural photoperiod for biochemical parameters except for a reduction in the levels of total phenolic compounds. Considering the enhanced biometric and biochemical parameters and greater dry weight and leaf area of the seedlings grown with supplemental light, using such a tool can optimize seedling development, possibly reducing production time in the nursery and providing greater productivity.

Keywords: artificial light; *Brassica oleracea* L.; *Lactuca sativa* L.; light-emitting diode; photoperiod

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1. Introduction

Agricultural activity plays a vital role in the Brazilian economy. The Brazilian vegetable market is highly diversified and segmented. According to Pessoa and Machado Junior [1], crops such as lettuce and cauliflower belong to broadleaf plants that unite around 1.5 million producers. According to data from the National Supply Company (CONAB), 67.97 thousand tons of lettuce and 11.73 thousand tons of cauliflower were marketed in 2022 [2]. These vegetables are a source of vitamin A, β-carotene, calcium, phosphorus, proteins, vitamin C, and fibers, reinforcing these species' nutritional and economic importance [3].

The use of technological resources for farm practices is on the rise so that agricultural supply can keep up with demographic growth without losing quality. Factors such as climate change and water scarcity, the requirement for more resistant species to diseases, and the variation in the incidence of light available for photosynthesis are current problems that demand solutions to support increased crop productivity and quality [4]. Among the technological alternatives for situations with a lack of available photosynthetically active radiation (PAR), the use of artificial light using light-emitting diodes (LEDs) stands out, especially at times of the year that suffer from variations in lighting during the winter period or cloudy/rainy days [5]. Artificial lighting, mainly in closed environments, has been studied to evaluate the incidence and quality of light and the photoperiod, with the aim to optimize the development and increase the quality of plants grown in a controlled environment [6].

Solar radiation intensity (luminosity) is one of the most critical factors in plant production, as it is directly related to the ability of plant organisms to carry out photosynthesis, affecting the entire process of carbon fixation, the production of photoassimilates, and, consequently, the generation of biomass [7]. In addition to providing energy to produce photoassimilates, PAR acts as an element through photoreceptors, such as phytochromes and cryptochromes. Photomorphogenetic responses are stimulated by the action of light on pigments associated with red and blue light and other wavelengths [8].

The range of the electromagnetic spectrum used for photosynthesis is in the wavelength range of 400–750 nm, corresponding to ePAR, and encompasses the visible light region. Within the ePAR, the spectral bands corresponding to blue (400–520 nm) and red (610–720 nm) light are those with the highest energy conversion efficiency by chlorophylls [9]. Therefore, when using artificial light, it is recommended to optimize the system so that most of the artificial light spectrum encompasses these two radiation bands [5].

Several studies have been conducted in recent years on various horticultural species such as lettuce, tomato, and strawberry. The main parameters evaluated were the photoperiod, type of wavelength, proportions between blue and red light, and light intensity, among other parameters [10–12]. It is important to note that the intensity of radiation necessary to carry out photosynthesis and the proportion between colors/wavelengths of the light provided varies according to the needs and physiology of each species [13].

Etae et al. [5] evaluated the effect of different artificial light sources on the growth of green oak lettuce seedlings. The authors reported that the use of 'bar' type LED lighting had a more pronounced effect on the biometric parameters of seedlings without significant changes in chlorophyll and carotenoid contents relative to natural light. In an experiment by Lima et al. [14], using LED lighting in a 1:5 ratio (blue:red) had the most prominent effect on the biometric parameters of curly lettuce seedlings. Paniagua-Pardo et al. [15] reported that exposing kale seeds to red light (600–650 nm) for 12 h accelerated germination, with significant differences also occurring in some biometric parameters (fresh mass and average hypocotyl length) of the seedlings concerning those under natural lighting.

Jones-Baumgardt et al. [16], assessing the use of supplementary lighting in the production of *Brassica* microgreens using different photon flux densities with purple light (85% red and 15% blue), reported that increasing photon flux from 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased the biometric parameters of all species; however, at higher photon densities, there was a reduction in the seedlings' quality. He et al. [17], evaluating the growth and quality parameters of Chinese broccoli (*Brassica alboglabra*) seedlings grown with and without LED light supplementation, commented that the seedlings exposed to supplementary lighting had improved photosynthetic parameters, such as CO_2 assimilation, stomatal conductance, and productivity.

However, the optimal red-to-blue proportion or the need for adding other light colors varies according to each species, even when considering different cultivars or hybrids. Razzak et al. [18] reported that the exposition of lettuce seedlings to green light (520–600 nm) in a proportion of 72% red, 10% green, and 18% blue yielded the best results relative to lettuce growth and average plant weight compared to using only purple (red and blue) lighting. Lee et al. [19] and Legendre and van Iersel [20] commented that lettuce growth was enhanced when plants were exposed to far-red light (700–800 nm). Chung et al. [21] observed that a red:blue proportion of 80:20 was optimal for plant growth and the production of bioactive compounds (phenolic compounds, anthocyanins) in red lettuce seedlings. On the other hand, Li et al. [22] reported that a red/blue ratio of 0.9 was optimal to stimulate the growth of pakchoi (*Brassica chinensis* L.) seedlings.

Artificial lighting is a novel trend in several parts of the world, and few works specifically address the effect of artificial and supplemental light on the growth and development of seedlings of many horticultural species [5,6,12]. Thus, the present work aimed to evaluate the influence of using supplemental purple LED light (12.5% blue light—centroid wavelength of 430 nm; 87.5% red light—centroid wavelength of 670 nm) on the biometric and biochemical parameters of 'crispa' lettuce (*Lactuca sativa* L var. *crispa*) and cauliflower (*Brassica oleracea* L. var. *botrytis*) seedlings.

2. Results and Discussion

For both species, the seedlings emerged approximately nine days after sowing and presented a normal appearance, regardless of the type of lighting received. However, it was noted that seedlings of both species showed a difference in vigor at the time of evaluation after 35 days of sowing between the two treatments. The visual appearance of lettuce and cauliflower seedlings, grown with and without supplementary lighting, is shown in Figure 1.

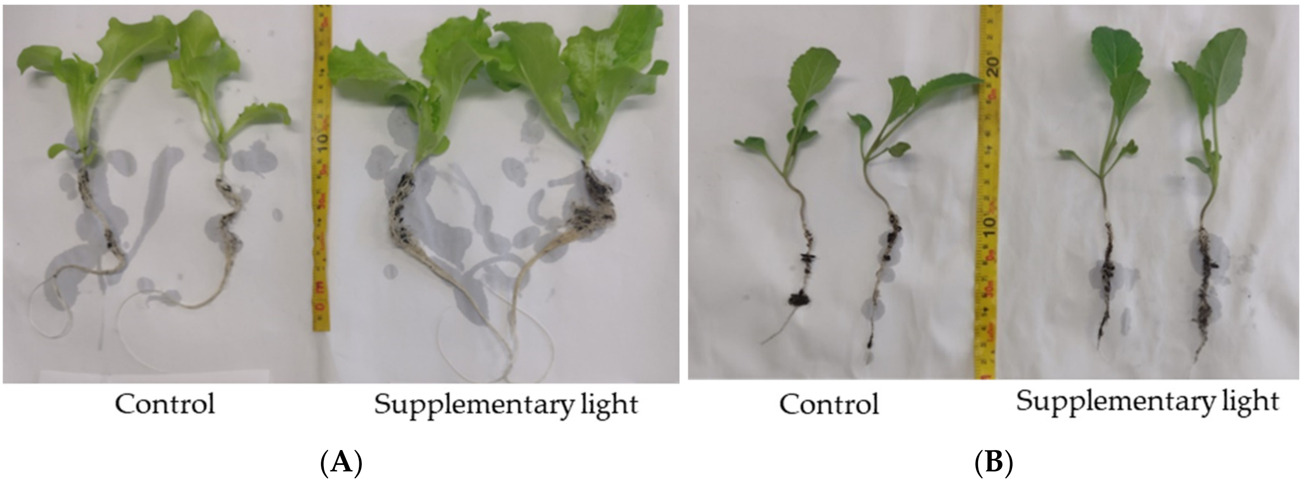

Figure 1. Visual appearance of lettuce (**A**) and cauliflower (**B**) seedlings with and without supplementary light 35 days after sowing.

According to Figure 1, the seedlings grown with supplementary light had a larger apparent size and a more vigorous appearance than those grown without supplemental light. According to Arias et al. [23] and as expected, edaphoclimatic factors, including the presence and intensity of photosynthetically active radiation, influence the vegetative growth and development of seedlings, which may lead to an increase or reduction in vigor depending on the quantities supplied to the developing seedling.

The results regarding the biometric parameters of lettuce seedlings grown with and without supplementary lighting are compiled in Table 1.

Table 1. Biometric parameters evaluated for lettuce seedlings grown in the presence and absence of purple LED light.

Treatment	Root Dry Weight (mg)	Root Length (cm)	Aerial Dry Weight (mg)	Cumulative Leaf Area (cm^2)
Control	20.40 ± 0.44	19.5 ± 2.88	59.7 ± 2.91	3899 ± 742
Supplemental light	69.41 ± 1.57 *	25.3 ± 5.59 *	182.9 ± 1.11 *	9256 ± 1392 *
CV (%)	25.7	19.9	18.2	17.0

Data shown as mean ± SD. *—significant by the *t*-test at a 5% probability of error (n = 15). CV—Coefficient of variation.

Seedlings grown with supplementary lighting had greater dry mass and root length, dry mass area, and leaf area (Table 1). This variation may be related to greater photosynthetic activity, production, and translocation of photoassimilates provided by the longer illuminated period. According to Paik and Huq [8], chlorophyll, phytochromes, and cryptochromes are stimulated, giving a photomorphogenetic response through mechanisms regulated by the photoperiod, which, if extended, can cause growth stimulation in some species.

Artificial light promotes greater growth and increases the photochemical content in vegetable seedlings and plants. According to Etae et al. [5], LED light yields better results than fluorescent light. This occurs because several monochromatic LEDs can be combined, while the fluorescent light has an emission peak in the green light (500–600 nm). These authors, when studying green oak lettuce, observed the most prominent effects with photosynthetic photons in the blue (400–500 nm) and red (600–700 nm) regions, which correspond to the photosynthetically active radiation most absorbed by chlorophylls and other photosynthetic pigments. On the other hand, Hooks et al. [24], evaluating different types of supplementary lighting in the hydroponic cultivation of red-leaf lettuce, did not observe significant differences relative to the plants' dry mass and leaf area.

Amoozgar et al. [25] observed that red light, used alone, is unsuitable for lettuce development until the end of the cycle. Although red light may contribute more to photosynthesis than blue, both wavelengths are necessary for the full development of the species, probably because light with a specific color also has morphological and hormonal regulatory effects. Furthermore, wavelengths other than red and blue are required depending on plant species and characteristics. Lighting systems must provide red and blue wavelengths (and other wavelengths), covering the range necessary for plant photosynthesis [9]. Lima et al. [14], evaluating different color proportions in artificial lighting of curly lettuce, observed greater leaf production with lighting containing five parts of red light for each part of blue light (5:1). Monostori et al. [26], evaluating the effect of different forms of supplementary artificial lighting on wheat growth, observed that LED light containing 65% red and 20% blue (3:1 ratio) promoted anticipation of the cycle and a greater number of shoots and dry aerial weight. Li et al. [27], evaluating two lettuce cultivars, reported that using light with a higher red-light proportion increased plant dry mass (area and root parts). In comparison, light with a greater proportion of blue caused a reduction in dry plant mass.

The results regarding the biometric parameters of cauliflower seedlings grown with and without supplementary lighting are compiled in Table 2.

Table 2. Biometric parameters evaluated for cauliflower seedlings grown in the presence and absence of supplementary purple LED light.

Treatment	Root Dry Weight (mg)	Root Length (cm)	Aerial Dry Weight (mg)	Cumulative Leaf Area (cm^2)
Control	1.53 ± 0.55	8.8 ± 0.92	5.53 ± 0.99	1438 ± 610
Supplemental light	4.45 ± 0.26 *	11.0 ± 2.61 *	12.6 ± 3.69 *	2690 ± 299 *
CV (%)	32.1	13.3	30.0	23.3

Data shown as mean ± SD. *—significant by the *t*-test at a 5% probability of error (n = 15). CV—Coefficient of variation.

Like lettuce, higher values of dry mass and root length, dry aerial mass, and leaf area were also observed in cauliflower seedlings grown using supplementary light. Adequate light intensity and quality [5], together with temperature [28,29], are some edaphoclimatic factors with the greatest impact on the seedling development of different species. However, physical–chemical parameters (Ph, EC) and nutrient contents are also essential for adequate plant development. Paniagua-Pardo et al. [14] evaluated the use of supplementary LED lighting with red, blue, and green colors and photoperiods of 3 h, 6 h, and 12 h on the development of cabbage (*Brassica oleracea* L.). The combination of red and blue LED light with a 12 h photoperiod was the treatment that promoted the highest aerial dry mass.

Zheng et al. [30], evaluating the use of blue LED supplementary light in developing two *Brassica campestris* cultivars, reported that supplementation of up to 50 μmol·m^{-2}·s^{-1} promoted seedling growth. However, blue light supplementation from 100 μmol·m^{-2}·s^{-1} inhibited seedling growth, with lower dry mass. The same authors commented that the root dry mass of green-leafed *B. campestris* seedlings was unaffected. In contrast, the root dry weight of the red-leafed variety was reduced with increasing blue supplementary lighting intensity.

In addition, He et al. [17], assessing the growth of *Brassica alboglabra* seedlings with supplementary LED lighting, commented that providing supplemental light (blue and red) during hazy or cloudy weather has a positive effect on seedling growth, productivity, and nutrient quality, being useful in adverse climate scenarios or poor natural lighting areas.

Some studies in the literature address the effect of red light (600–700 nm) in the stem elongation of plants [31], which may explain the greater seedling length observed (Figure 1). However, Rahman et al. [32] cited that exposure to blue light (400–450 nm), regardless of other wavelengths, may promote the development of leaf area, while red and far-red wavelengths (600–750 nm) promote the reproductive development of the plant to the detriment of the vegetative growth. On the other hand, Ma et al. [33] pointed out that blue light had an inhibitory effect on leaf area. At the same time, the same authors commented that more studies are needed to verify if this is a specific or a general effect.

The observed results show that supplementary and artificial lighting may be an interesting and potential tool to enhance seedling production. The faster growth and bigger size of the seedlings grown under supplemental light indicates that they may be transplanted earlier, reducing nursing time and increasing the turnover, with faster and greater production in a shorter period.

The leaf nutrient contents in lettuce seedlings grown with and without supplementary lighting are presented in Table 3.

Table 3. Nutrient contents in lettuce seedlings grown with and without purple LED light supplementation.

Treatment		Control	Supplemental Lighting	$t_{calculated}$ [1]	CV (%)
N	g·kg^{-1} (d.b.)	44.6 ± 0.2 *	38.4 ± 1.2	7.56	8.93
P		8.6 ± 0.1 *	7.4 ± 0.5	4.80	9.44
K		65.5 ± 1.1 *	49.2 ± 4.2	7.43	16.95
Ca		8.6 ± 0.1 ^{ns}	8.0 ± 0.8	1.54	6.89
Mg		4.9 ± 0.1 *	3.7 ± 0.4	5.35	16.58
S		2.9 ± 0.1 *	2.1 ± 0.1	16.00	19.00
Zn	mg·kg^{-1} (d.b.)	94.4 ± 4.1 *	78.9 ± 3.2	5.99	10.93
Cu		18.7 ± 6.1 ^{ns}	21.2 ± 1.1	0.79	19.24
Mn		128.7 ± 15.7 ^{ns}	160.7 ± 16.1	2.76	15.78
Fe		362.7 ± 7.8 *	209.0 ± 7.8	27.81	31.12
B		17.9 ± 0.8 ^{ns}	16.2 ± 0.1	6.45	6.45

Data shown as mean ± SD. *—significant by the *t*-test at a 5% probability of error (n = 15). [1]—$t_{critical}$ = 4.31. ^{ns}—not significant. CV—Coefficient of variation. d.b.—dry basis.

Seedlings without light supplementation showed significantly higher nutrient values, except Ca, where no statistical difference was observed. In Zn and Fe, statistically higher values were found among micronutrients for seedlings without supplementary lighting. It is important to comment that even with a lower nutrient concentration in the tissue for most nutrients, seedlings grown with supplementary lighting showed higher biomass production and leaf area values, suggesting greater efficiency in using nutrients. Possibly, the supplementary lighting stimulated the production of photoassimilates, even at lower nutrient doses, which can explain the higher biomass accumulation and faster growth. Although there are some statistical differences between treatments, nutrient levels were within the ranges considered normal for lettuce [34].

It Is also Important to observe that, while the contents of several nutrients In the lettuce leaves were smaller in the plants under supplemental lighting than in the control, the seedlings had faster growth, size, and mass. This suggests that supplemental lighting helped seedling growth, even with small nutrient contents in the seedlings' tissues. Such results show that supplemental lighting may help reduce the nutrient demand of lettuce seedlings, saving costs and increasing nursing efficiency.

The nutrient content in the foliar tissue of cauliflower seedlings is compiled in Table 4.

Table 4. Nutrient contents in cauliflower seedlings grown with and without purple LED light supplementation.

Treatment		Control	Supplemental Lighting	$t_{calculated}$ [1]	CV (%)
N	$g \cdot kg^{-1}$ (d.b.)	36.9 ± 2.1	43.3 ± 1.0 *	5.67	9.85
P		5.0 ± 0.1 ns	5.3 ± 0.2	2.68	4.24
K		50.5 ± 0.4 ns	49.5 ± 0.4	4.00	1.29
Ca		12.9 ± 0.1 ns	14.9 ± 0.2	3.32	9.73
Mg		6.9 ± 0.1	9.3 ± 0.2 *	18.43	16.90
S		8.7 ± 0.1 ns	8.7 ± 0.5	0.19	3.44
Zn	$mg \cdot kg^{-1}$ (d.b.)	39.3 ± 2.2	52.6 ± 2.8 *	7.46	17.38
Cu		6.6 ± 0.8 ns	10.3 ± 3.8	1.92	37.06
Mn		78.4 ± 0.8	107.7 ± 7.7 *	7.56	18.81
Fe		105.1 ± 4.9 ns	155.1 ± 30.0	3.29	25.97
B		16.9 ± 0.3	20.3 ± 1.0 *	6.60	11.03

Data shown as mean ± SD. *—significant by the *t*-test at a 5% probability of error (n = 15). [1]—$t_{critical}$ = 4.31. ns—not significant. CV—Coefficient of variation. d.b.—dry basis.

The behavior of the cauliflower seedlings differed from those observed in lettuce. N, Mg, and S contents were statistically higher in the seedlings exposed to supplementary light and Zn, Mn, and B, among micronutrients. Such results suggest that supplementary light may have helped nutrient intake, possibly enhancing or modulating the absorption mechanisms of nutrients [35]. The macronutrient levels were like those observed by Almeida et al. [36].

As Jones-Baumgardt et al. [16] and He et al. [17] commented, providing supplemental lighting to *Brassica* seedlings helps enhance their photosynthetic parameters, which aids plant growth and nutrient intake. Similar behavior was reported by Gómez and Mitchell [37] for the seedlings of several tomato varieties and by Randall and Lopez [38] when testing the effect of high-pressure sodium lamps and LED lighting on the growth and biometric parameters of different horticultural species.

Unlike the behavior shown for lettuce, the contents of some nutrients were higher in the cauliflower seedlings grown under supplemental light. Since the growth and biometric parameters of the cauliflower under supplementary light were greater than in the control, applying supplementary light may have helped nutrient absorption by the seedlings, enhancing their development. In this sense, supplemental light may be a tool to speed up the growth of cauliflower seedlings, reducing the time between sowing and transplantation and saving costs and time.

The levels of phenolic compounds, flavonoids, and chlorophylls-*a* and *b* and total in lettuce seedlings grown with and without supplementary lighting are shown in Table 5.

Seedlings that received supplemental light had higher levels of metabolites than those in the control (without supplementary lighting) treatment. This fact was also observed by Martinazzo et al. [39], commenting that additional lighting can stimulate the synthesis of chlorophylls. Monostori et al. [40] reported that the presence of supplementary lighting increased chlorophyll levels in wheat but that there was no statistical difference for the levels of chlorophyll-*a*, chlorophyll-*b*, total chlorophyll, and carotenoids between white and purple light in a 3:1 ratio (red:blue). Etae et al. [5] and Li et al. [41] also observed no difference between using different colors in the supplementary lighting of two lettuce cultivars with white light, purple light with excess red, and purple light with excess blue.

Table 5. Contents of phenolic compounds, flavonoids, and chlorophylls in lettuce seedlings grown with and without purple LED light supplementation.

Treatment	Chlorophyll-*a* (mg·kg^{-1} d.b.)	Chlorophyll-*b* (mg·kg^{-1} d.b.)	Total Chlorophyll (mg·kg^{-1} d.b.)	*a*/*b* Chlorophyll Ratio	Phenolics (mg·100 g^{-1} d.b.)	Flavonoids (mg·100 g^{-1} d.b.)
Control	175 ± 12	82 ± 18	258 ± 30	2.13 ± 0.17 *	27.2 ± 4.18	29.1 ± 0.89
Suppl. Lighting	203 ± 4 *	136 ± 4 *	340 ± 9 *	1.49 ± 0.07	29.4 ± 1.05 *	37.9 ± 2.67 *
CV (%)	4.7	12.2	7.4	7.1	10.8	5.9

Data shown as mean ± SD. *—significant by the *t*-test at a 5% probability of error (n = 5). d.b.—dry basis. CV—Coefficient of variation.

When grown with supplementary light, lettuce seedlings showed higher levels of phenolic compounds and flavonoids. However, the induction of the production of these substances may be conditioned by the wavelength, as observed by Li et al. [41], where lettuce plants from different cultivars exposed to supplementary light with a higher proportion of blue color showed a higher content of anthocyanins, flavonoids, and carotenoids than plants grown under white light and with a higher proportion of red. The same behavior was observed by Alrajhi et al. [42], whose study evaluated the effect of different lighting compositions on red and green lettuce development. The authors observed that different wavelengths stimulated the production of specific metabolites, such as blue/red light, in a 1:1 ratio relative to the production of phenolic compounds. Hooks et al. [24] reported that applying supplementary lighting affected the levels of phenolic compounds, anthocyanins, carotenoids, and chlorophylls in purple-leaf lettuce plants. However, the authors did not observe specific differences between the colors (wavelengths) tested.

It is important to observe that the ratio of chlorophylls *a*/*b* was significantly lower in the seedlings exposed to supplementary lighting (Table 5), indicating the production of larger amounts of chlorophyll-*b*. This chlorophyll type is associated with photon absorption, helping chlorophyll-*a* capture light to perform photosynthesis [43]. This can explain the observed differences between the contents and ratio of chlorophylls-*a* and -*b*.

The levels of phenolic compounds, flavonoids, and chlorophylls-*a* and *b* and total in cauliflower seedlings grown with and without supplementary purple lighting are shown in Table 6.

Table 6. Contents of phenolic compounds, flavonoids, and chlorophylls in cauliflower seedlings grown with and without purple LED light supplementation.

Treatment	Chlorophyll-*a* (mg·kg^{-1} d.b.)	Chlorophyll-*b* (mg·kg^{-1} d.b.)	Total Chlorophyll (mg·kg^{-1} d.b.)	*a*/*b* Chlorophyll Ratio	Phenolics (mg·100 g^{-1} d.b.)	Flavonoids (mg·100 g^{-1} d.b.)
Control	93.0 ± 34.5 ^{ns}	42.8 ± 17.7 ^{ns}	135.8 ± 52.2 ^{ns}	2.17 ± 0.37 ^{ns}	198.4 ± 28.6 *	28.1 ± 8.6 ^{ns}
Suppl. Lighting	138.7 ± 32.1	69.3 ± 17.5	208.0 ± 49.5	2.00 ± 0.23	136.8 ± 14.0	21.5 ± 1.4
CV (%)	28.8	31.4	29.7	25.8	13.4	25

Data shown as mean ± SD. *—significant by the *t*-test at a 5% probability of error (n = 15). ^{ns}—not significant. d.b.—dry basis. CV—Coefficient of variation.

For cauliflower seedlings, unlike what was observed for lettuce, only the content of phenolic compounds differed statistically regarding the use of supplementary LED light, and the highest content occurred in the control treatment (without the use of supplementary light). Flavonoids, chlorophyll-*a*, chlorophyll-*b*, total chlorophyll, and the *a*/*b* chlorophyll ratio did not differ statistically. These differences are probably the result of the genetic characteristics inherent to each species [44–47].

Lee [48] showed that chlorophyll levels can vary greatly depending on genetic and environmental factors, such as sunlight intensity. According to Engel and Poggiani [49], photosynthetic efficiency is linked to the chlorophyll content of plants, affecting growth and influencing their adaptability to different environments. Martinazzo et al. [39] reported that, in the open field, there were no important differences in the chlorophyll content between shaded plants and plants fully exposed to sunlight.

Rahman et al. [32] observed that while blue light (400–500 nm) stimulates the synthesis of anthocyanins, it has little impact on the production of phenolic compounds. Ma et al. [33] also observed a similar behavior, commenting that red light may inhibit the production of some secondary metabolites, such as phenolics and antioxidant compounds, probably due to primary metabolism (photosynthesis and respiration) stimulation.

Frede et al. [50] observed that using either blue or red light alone did not influence the chlorophyll levels and ratio in *Brassica rapa* ssp. *Chinensis* seedlings; however, when exposed to white LED light, the chlorophyll content was significantly higher than when using blue and red light. On the other hand, Zheng et al. [30] observed an increase in the levels of total phenolic compounds, anthocyanins, and flavonoids in the red and green varieties of *B. campestris* seedlings with exposure to supplemental blue lighting relative to the control (plants without light supplementation). As stated by Rahman et al. [32] and Ma et al. [33], each species has a different response to artificial and supplementary lighting, which also depends on the wavelengths used.

It is also important to highlight that climatic characteristics may directly influence the production of primary and secondary metabolites, biomass production, and growth parameters. The compilation of climate data on temperature, accumulated insolation time, and relative humidity for the experiment period is presented in Table 7.

Table 7. Climate parameters in open air during the experimental period.

Period		Temperature (°C)			Insolation Time (h)	RH (%)
		Average	Minimum	Maximum		
Climatological normal	24–31 May	14.3	11.3	18.9	36.2	82.9
	1–30 June	13.0	9.7	17.5	140.6	62.7
Experiment	24–31 May	13.2	10.4	16.7	18.6	85.7
	1–30 June	11.0	7.9	15.2	119.0	82.5

RH—relative air humidity. Source: INMET (2022).

During the study (24 May 2022 to 30 June 2022), temperatures were lower than the climatological normal, with a lower degree of insolation (137.6 h in the experiment versus 176.8 h in the climatological normal; Table 7). The lower degree of insolation observed may have contributed to the success in the greater production of biomass and leaf area of seedlings, with light supplementation being an important tool in periods of cloudy days. The low insolation during the period may have stimulated the production of pigments in lettuce to increase the degree of absorption of photosynthetically active radiation and phenolic compounds in cauliflower. Furthermore, temperature directly influences the concentration of phenolic compounds, as the higher the temperature or thermal amplitude, the more negative the effect will be on the plant's metabolism, stimulating the production of secondary metabolites to defend against biotic and abiotic stressors [26].

Gobbo Neto and Lopes [51] report that, in general, there is a positive association between the intensity of solar radiation and the production of phenolic compounds, including flavonoids, tannins, and anthocyanins. However, the same authors do not specify the influence of climatic conditions, such as temperature, precipitation, and relative humidity, among other parameters.

3. Materials and Methods

3.1. Experimental Conditions and Species Tested

The experiment was conducted in a greenhouse at the University of Caxias do Sul, municipality of Caxias do Sul, in the state of Rio Grande do Sul (29°10′05″ S, 51°10′06″ W, with an altitude of approx. 800 m, and a Cfa climate according to Köppen classification).

The lettuce cultivar 'crispa' (*Lactuca sativa* var. *crispa*—Isla Sementes®, Porto Alegre, RS, Brazil) was tested, making it possible to sow the whole year, with an average cycle of 35–45 days, showing good tolerance to early bolting, tip burn, and lettuce mosaic virus (LMV-I). A winter variety of cauliflower (*Brassica oleracea* var. *botrytis*—Isla Sementes®, Porto Alegre, RS, Brazil) was used, with an average cycle of 105–120 days.

3.2. Experiment Preparation and Treatments

The sowing of the species was carried out in 128-cell styrofoam trays containing Carolina Soil® substrate (Carolina Soil, Santa Cruz do Sul, RS, Brazil) on 25 May 2022. Substrate properties were Ph 5.5, electrical conductivity of 0.7 $Ds \cdot m^{-1}$, 60 wt.% moisture, dry density of 130 $kg \cdot m^{-3}$, and water retention capacity of 3.0 $g \cdot g^{-1}$. The substrate composition was peat, vermiculite, rice husk, and limestone. Each treatment was composed of one tray, totaling 128 seedlings for each treatment.

The sowed trays were kept in a greenhouse with floating irrigation in Sarruge's [52] nutrient solution (composition: 210 $mg \cdot L^{-1}$ N, 31 $mg \cdot L^{-1}$ P, 234 $mg \cdot L^{-1}$ K, 200 $mg \cdot L^{-1}$ Ca, 48 $mg \cdot L^{-1}$ Mg, 64 $mg \cdot L^{-1}$ S, 0.5 $mg \cdot L^{-1}$ B, 0.5 $mg \cdot L^{-1}$ Mn, 0.05 $mg \cdot L^{-1}$ Zn, 0.02 $mg \cdot L^{-1}$ Cu, 0.01 $mg \cdot L^{-1}$ Mo, 5.0 $mg \cdot L^{-1}$ Fe, and 0.7 $mg \cdot L^{-1}$ Cl), with Ph maintained at 5.5 ± 0.2 and electrical conductivity 2.0 ± 0.3 $Ds \cdot m^{-1}$ for 37 days until the point of seedling transplantation. Sowing was carried out in three trays for each species, with one lettuce seed per cell as they were pelleted seeds and three cauliflower seeds per cell, with manual thinning being carried out, maintaining one seedling per cell after their establishment.

For the treatments with supplemental lighting, a one-meter-long LED luminaire (Luxion®, Caxias do Sul, Brazil) was used, composed of a mixture of two centroid wavelengths, 670 nm (red) and 430 nm (blue), in the proportion of 87.5% and 12.5%, respectively. Such a ratio was chosen based on previous studies [53–55] and the company's luminaire options. The lettuce and cauliflower seedlings were exposed to supplemental light in the same compartment.

The distribution of relative radiation intensity as a function of wavelength for purple light and sunlight was measured using an LMS-6000S spectroradiometer (Lisun Group, Hong Kong, China). The obtained spectra are shown in Figure 2.

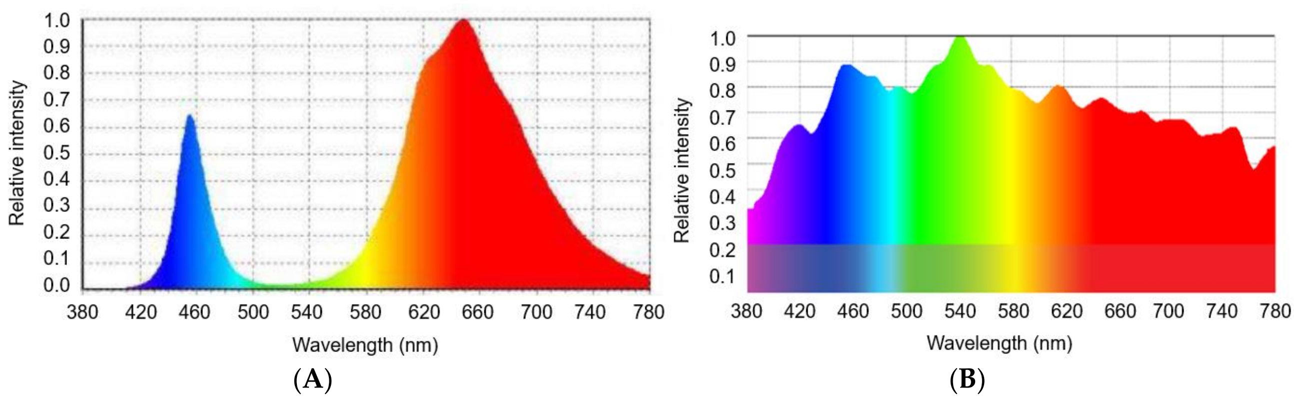

Figure 2. Distribution of relative radiation intensity as a function of wavelength for the purple LED light used in this study (**A**) and sunlight (**B**).

The luminaire was placed 25 cm above the seedlings in the supplementary lighting treatment, generating a PPFD (photosynthetic photon flux density) of 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (measured with the spectroradiometer). In the control treatment, the seedlings received only natural light. The seedlings under the end-of-day supplementary light treatment received an additional four hours per day to the photoperiod (10 h·day^{-1}), switched on 1 h before dawn and 3 h after sunset, with activation carried out with a timer, totaling 14 h.

After 37 days, 30 seedlings from each treatment in each species were selected for evaluation. In the supplemental light treatment, those located below the light cone, with uniform distribution, were considered useful. For control, without supplemental light, the seedlings furthest from the lighting were collected to minimize any interference. The other seedlings were considered borders and not used in the tests. Both treatments received the same amount of fertilizer and were subjected to the same cultural treatments.

3.3. Evaluation of the Biometric and Biochemical Parameters of the Seedlings

The evaluations were carried out on 1 July 2022, where the seedlings of both materials and treatments were removed from the cells, and the root system of the seedlings was washed. The root length and plant height were measured with a measuring tape. Using an AM 350 scanner (ADC BioScientific, London, United Kingdom), the total leaf area of five seedlings from both treatments for both species studied was measured.

Fifteen seedlings were dried in an oven with forced ventilation at 70 ± 5 °C for 72 h. After drying, the dry mass of the shoot and root was determined on an AL500C semi-analytical scale (Marte Científica, São Paulo, SP, Brazil) with an accuracy of 0.001 g. Fifteen seedlings were frozen at −18 ± 3 °C for biochemical/qualitative evaluations.

After weighing, the seedlings were subjected to leaf tissue analysis. The contents of N, P, K, Ca, Mg, S, Zn, Cu, Mn, Fe, and B were determined according to the procedures described by Malavolta et al. [40]. Nitrogen content was determined by the Kjeldahl method, P, S, and B were determined by colorimetry using a B542 spectrophotometer (Micronal, São Paulo, SP, Brazil), K was analyzed by flame photometry using a B462 flame photometer (Micronal, São Paulo, Brazil), and Ca, Mg, Zn, Cu, Mn, and Fe were assessed by atomic absorption spectrometry (AAS) using an AA-55 atomic absorption spectrophotometer (Agilent Technologies, São Paulo, SP, Brazil). The assays were conducted in triplicate for each treatment of each species.

The content of total phenolic compounds was determined by the Folin–Ciocalteau method, according to the procedure proposed by Pereira et al. [41]. Total flavonoid contents were determined by the aluminum chloride colorimetric method, according to the methodology proposed by Matic et al. [56]. Phenolic compounds and flavonoids were extracted from the samples with a hydroalcoholic solution (ethanol 70%, *v*/*v*). The results were expressed in gram milliequivalents of gallic acid per 100 g of plant tissue on a fresh basis for phenolic compounds and gram milliequivalents of quercetin per 100 g of plant tissue on a fresh basis for flavonoids, respectively.

The chlorophyll-*a* and chlorophyll-*b* contents were determined using the method described by Ross [57] after extraction using 80 % *v*/*v* acetone in water [58] and quantification by Arnon equations. Total chlorophyll was calculated as the summation of chlorophyll-*a* and chlorophyll-*b* contents. The chlorophyll contents were presented in milligrams per kilogram of fresh plant material. Phenolic compounds, flavonoids, and chlorophyll contents were determined using a B542 spectrophotometer (Micronal, São Paulo, SP, Brazil).

3.4. Experimental Design and Statistical Analysis

In a completely randomized design, fifteen seedlings per treatment were considered for biometric parameters and three replications of five seedings for biochemical parameters and nutrient content. The results were assessed by the *t*-test at a 5 % error probability using the Microsoft Excel® software, version 2016 (Microsoft, Richmond, VA, USA).

4. Conclusions

Light supplementation was efficient in the production of lettuce and cauliflower seedlings, as all the biometric parameters analyzed were enhanced compared to seedlings grown under natural light only, without harm to the biochemical and nutraceutical parameters. The greater dry mass and leaf area of the seedlings grown under supplemental lighting indicates faster growth. Thus, it may be possible to produce seedlings in a shorter period, increasing the nursery's productivity, especially in adverse weather conditions (e.g., low lighting), and without harmful effects on the seedlings' biochemical and nutraceutical parameters. Moreover, further studies are needed to understand the trends and behaviors observed since each species responds differently to supplemental and artificial lighting, and there is an important influence of the wavelengths used that must be investigated and elucidated.

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