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## Effects of Light Emitting Diode High Intensity on Growth of Lettuce (*Lactuca sativa* L.) and Broccoli (*Brassica oleracea* L.) Seedlings

G. Paniagua Pardo<sup>1\*</sup>, C. Hernández Aguilar<sup>1</sup>, F. Rico Martínez<sup>1</sup>,  
A. Domínguez Pacheco<sup>1</sup>, C. Martínez González<sup>1</sup>  
and M. Martínez Canseco<sup>2</sup>

<sup>1</sup>Department of Systems Engineering, Section of Postgraduate Studies and Research of ESIME "Zacatenco", I.P.N., México D.F., Mexico.

<sup>2</sup>Department of Polymers, Institute of Materials Research, UNAM, México D.F., Mexico.

### Authors' contributions

This research was carried out in collaboration between all authors. Authors GPP, FRM, CHA and ADP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors GPP and FRM managed the analyses of the study. Author CMG managed the literature searches. Author MMC made the absorption spectrums. All authors read and approved the final manuscript.

Original Research Article

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### ABSTRACT

**Aims:** To evaluate the effects of high intensity LED light with different wavelengths (red, blue, green and magenta filter) in physiology and absorption spectrum of lettuce (*Lactuca sativa* L.) and broccoli (*Brassica oleracea* L.) seedlings, with exposure times of 12 hours, in order to improve the growth of vegetables.

**Study Design:** Four treatments of light (red, blue, green, magenta filter) and one control (white light). The experimental design was completely randomized, with two replications of 14 seedlings per experimental unit.

**Place and Duration of Study:** The experiment was conducted at IPN - ESIME Zacatenco University, Mexico DF, between October 2013 and November 2013.

**Methodology:** Seedlings were grown with alternating cycles of light (12hours) and darkness (12 hours). The period of growth of lettuce and broccoli was 29 and 15 days respectively. Physiological variables (fresh and dry weight, average length of hypocotyl,

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\*Corresponding author: Email: [guip70@hotmail.com](mailto:guip70@hotmail.com);

leaf number) and absorption spectrum of these vegetable seedlings were evaluated.

**Results:** Seedlings of broccoli with green light and lettuce with red light exposed to high intensity LED light, showed statistically significant differences in average length of hypocotyl with increments of 33% and 42% compared to control respectively. The highest fresh and dry weight in broccoli seedlings was obtained in red light treatment with increases of 15% and 10% respectively, while in lettuce seedlings was 50 and 41% but with blue light compared to its control.

**Conclusion:** The physiological responses produced by exposure to different wavelengths of high intensity LED light in broccoli and lettuce seedlings, varied according to the wavelength used, where the red LED light treatment had the best results in seedlings of broccoli, and lettuce seedlings was the blue LED light. Suggesting that the use of high intensity LED light is a viable option to improve plant growth in controlled environments.

*Keywords: Light emitting diode; high intensity; lettuce; broccoli; seedling growth.*

## 1. INTRODUCTION

The light plays an important role in photosynthesis, growth and morphogenesis of plants that depends of the wavelength [1] in addition to being one of the variables that affect the concentration of phytochemicals on them [2]. For this reason the artificial lighting systems used for crops in controlled environment are an important factor [3], because they determine the cost and nutritional quality of greenhouse vegetables [4], so that technological advances that may arise in this area are valuable [5]. Traditionally, high pressure sodium lamps, fluorescent and incandescent lamps of different spectral emissions have been used for these purposes [6], but these devices of light have certain limitations, such as its short lifetime, high power consumption and heat emission [7]. The LED (light emitting diode) is a solid state light source, durable and narrow band [8,9] that can be used in a variety of horticultural applications [10], as lighting system in greenhouses, growth chambers and research on plant growth in space [11,12,13,14,15]. These devices can be implemented in dynamic lighting to control growth, development, production and physiological responses of plants [16,17].

At the present time, LED technology has had a great development, with the use of new base materials, such as aluminium indium gallium phosphide (AlIn-GaP) and indium gallium nitride (InGaN), among others [12]. This has allowed the development of high-intensity LEDs with power outputs from 1 to several tens of watts [18]. The benefits that this technology brings are greater light intensity, lower energy consumption (energy cost savings of 40%), increased device longevity compared to other lighting systems, increasing the speed switching, better color control [19] and is a device that can be environmentally friendly because it does not use toxic gases, such as fluorescent lamps and mercury [20], between other.

It is well known that red and blue light are important factors for the plant growth [21]. Blue light suppresses hypocotyl elongation and induces biomass production, and red light induces hypocotyl elongation and expansion in leaf area [22]. However in the last decade has found evidence of the role that the green light has as growth regulator [23]. Green light also affects plant morphology and physiology, including leaf growth, stomatal conductance and early stem elongation [24,23].

Various studies have used LED light for vegetable cultivation among which we can to mention the tomato (*Solanum esculentum*) [25,9], cucumber (*Cucumis sativus*) [26,27], pepper (*Capsicum annuum*) [28,29], spinach (*Spinacia oleracea*) [30], radish (*Raphanus sativus*) [31], strawberries (*Fragaria x ananassa* Duch.) [32], grapes (*Vitis ficifolia*) [33], rice

(*Oryza sativa* L.) [34,35] and lettuce (*Lactuca sativa* L.) [22,36,37,38,39]; depending of quality light found physiological, morphological and concentration of nutrient favorable effects, although responses vary according to the type of plant, so more research is needed with this type of light at different wavelengths to enhance growth and development crops [6].

On the other hand, there are few studies using this type of lighting in broccoli vegetable and few also using high intensity LEDs with greater power than 1 W. Therefore the aim of this study was to evaluate the effects of high-intensity LED light with different wavelengths (red, blue, green and magenta filter) in physiology and absorption spectrum of lettuce (*Lactuca sativa* L.) and broccoli (*Brassica oleracea* L.) seedlings grown for 29 and 15 days respectively, with exposure times of 12hours.

## 2. MATERIALS AND METHODS

### 2.1 Biological Material

Two types of seed were used: lettuce (*Lactuca sativa* L.) White Boston variety and broccoli Waltham 29 variety, ITSCO® brand both, obtained in Mexico City. Seeds were classified according to their size and shape, in order to homogenize them as possible. The experiment was conducted at IPN in ESIME Zacatenco, Mexico City (19°29'56 "N 99°08'06" W).

### 2.2 Lighting System

For growing seeds, a shelf of 145×29.5×41cm was constructed and divided into 10 sections of 14cm. Shelf walls were made of wood and the edge for the support was aluminum. The interior walls of each section of the chamber were covered with aluminum paper. Each section was equipped with 2 high intensity LEDs (SILED®) with a power of 5 W, adjusted to an intensity of 550±5lux, measured with a light meter (Steren®, HER-410 model). Two sections with red LEDs (600-650nm), two with blue LEDs (450-500nm), two with green LEDs (490-540nm), two with white LED covered with a cellophane film magenta (named magenta filter) were used and finally two sections with white LEDs that were used as control (Fig. 1).

### 2.3 Experimental Design

The experiment consisted by four treatments of light (red, blue, green, magenta filter), as well as one control (white light). The experimental design was completely randomized, with two replications of 14 seedlings per experimental unit.

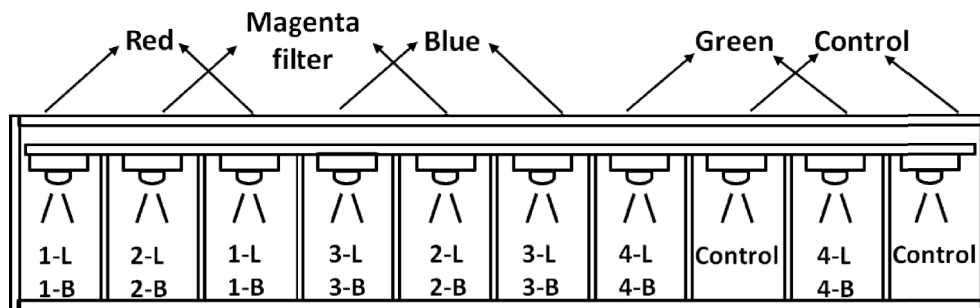


Fig. 1. LEDs panel and treatments distribution

## 2.4 Germination and Seedling Growth

The experiment was conducted in two stages. The first lasted 29 days, where lettuce seedlings were grown; when it was finished, the second stage started, where broccoli seedlings were grown for 15 days, both experiments were developed in the shelf described above with high intensity LED light.

The seeds were germinated in sterile plastic Petri dishes of 5.5cm diameter, using as the substrate a layer of filter paper moistened with 3mL of purified water. Petri dishes with 20 seeds were placed in each section of the shelf, to place them immediately light treatments. Germination took place inside the shelf with alternating cycles of light (12h) and darkness (12h) with an average temperature and humidity of 20.48°C and 31.71%, respectively. After three days, 14 normal seedlings were selected randomly of each section, to be transplanted in germinating trays of black plastic (trays) using as substrate 9.5g of peat moss. Finally 10 trays were obtained with 14 seedlings each one, corresponding to the 10 sections of the shelf. Subsequent to each seedling was added 7ml of purified water to finally place each tray in the corresponding light section. 3ml of purified water were added to seedlings every 48h. After the growth period, the following variables for each type of vegetable were evaluated:

- Absorption spectrum. Two seedlings at random from each replication by treatment were extracted; their leaves were ground in 5ml of ethanol. Subsequently, the absorption spectrum of each solution was measured by UV-VIS spectrophotometer (UNICAM®, model UV-300).
- Average length of hypocotyl (HL). The hypocotyl length (mm) of the 12 seedlings from each replication by treatment were measured, then the average length of the 24 seedlings per treatment was calculated, as a vigor indicator.
- Number of leaves (NL). The counting fresh leaves of seedlings for each replication by light treatment were performed.
- Fresh weight (FW). Having measured the hypocotyl length, the groups of 12 seedlings per replicate were weighed on a bascule (Velab®, VE-1000 model) by treatment.
- Dry weight (DW). Then, the groups were dried in a an electric stove (Riossa®, E-51 model) at a temperature of 65°C for 72h; once this was finished, dry weight (mg) of seedlings groups were determined in a bascule (El Crisol®, AR1140 model).

## 2.5 Statistical Analysis

Data were subjected to analysis of variance, using the GLM procedure of SAS (SAS Institute, 2002), in a completely randomized design with two replications. Comparison of means was performed using low significant differences procedure (LSD), with a significance level of 0.05.

## 3. RESULTS AND DISCUSSION

The result of this investigation confirmed that the growth of lettuce and broccoli can be improved by using light sources with specific wavelength, such as high intensity LED light. Physiological variables (fresh and dry weight, average length of hypocotyl, leaf number) and absorption spectrum of these vegetable seedlings were evaluated. In lettuce seedlings highly significant differences ( $P < 0.01$ ) between treatments of light (red, magenta filter, blue and green) for NL, HL, FW and DW variables were found. Moreover the broccoli seedlings, highly significant differences ( $P < 0.01$ ) between light treatments in HL, FW and DW variables were observed. Table 1 presents the comparison of mean values for the variables with significant differences.

**Table 1. Mean values of physiological variables measured for seedlings of lettuce and broccoli exposed to high-intensity LED light**

Treatment	LED light	HL (mm)	NL	FW (mg)	DW (mg)
<b>Lettuce</b>					
1-L	Red	39.08a	3.48b	836c	37d
2-L	Magenta Filter TA	28.96b	3.68b	706d	37.1c
3-L	Blue	25.8c	4.12a	1470a	64.5a
4-L	Green	0d	0c	0e	0e
Control	White	27.36bc	4.28a	980b	45.7b
<b>Broccoli</b>					
1-B	Red	101.55b	2 <sup>a</sup>	735 <sup>a</sup>	38.75a
2-B	Magenta Filter	86.15cd	2 <sup>a</sup>	655b	33.3c
3-B	Blue	92.9c	2 <sup>a</sup>	615c	33.7c
4-B	Green	111.15a	2 <sup>a</sup>	580d	26.45d
Control	White	83.2d	2.05a	635bc	35.2b

Means with the same letter in a column are statistically equal (LSD, 0.05). HL = average length of hypocotyl, NL = number of leaves, FW = fresh weight, DW = dry weight

### 3.1 Average length of hypocotyls

Seedling growth was evaluated through average length of hypocotyl (HL) variable, where treatments 4-B (green light) and 1-B (red light) resulted in an increase of 33% and 22% in broccoli seedlings height, while lettuce seedlings obtained greatest height with treatment 1-L (red light) with 42% of increase, all of them regarding their control. McCoshum and Kiss [40] and Johkan et al. [22] have reported that the green light promotes plant growth, results that are in agreement with this investigation. McCoshum and Kiss [40] indicate that this could be the effect of phytochrome A. Other authors have mentioned the effects of green light on the growth and development of plants [41,42]. In other crops such as red cabbage, similar results were found with the use of green super bright LEDs with an intensity of 1200 lx [43]. This type of light causes excessive stem elongation [22], which also happened in this research bringing as result a thinning, due to it is recommended to use it in combination with other wavelengths, as reported by [15,23] to enhance the growth of lettuce.

Also reported by other authors using red LED light to achieve greater plant height, as found by [37] in seedlings of red lettuce (*Lactuca sativa* L. cv. Banchu Red Fire) grown for 17 days, who reported greater height compared to the blue LED light. This is because the phytochromes act monitoring the balance of red and infrared light and when they detect changes respond through photomorphogenesis in the plant [10]. Other plants such as grapes [33], strawberry [32] and radish [31] in environments with red LED light showed similar results, which is according to the findings of this investigation using high intensity LED light.

### 3.2 Number of Leaves, Fresh and Dry Weight

In lettuce seedlings NL variable showed the highest value in the control, however treatment 3-L (blue light) is in the same statistical group. In broccoli seedlings NL variable, did not show significant differences between treatments.

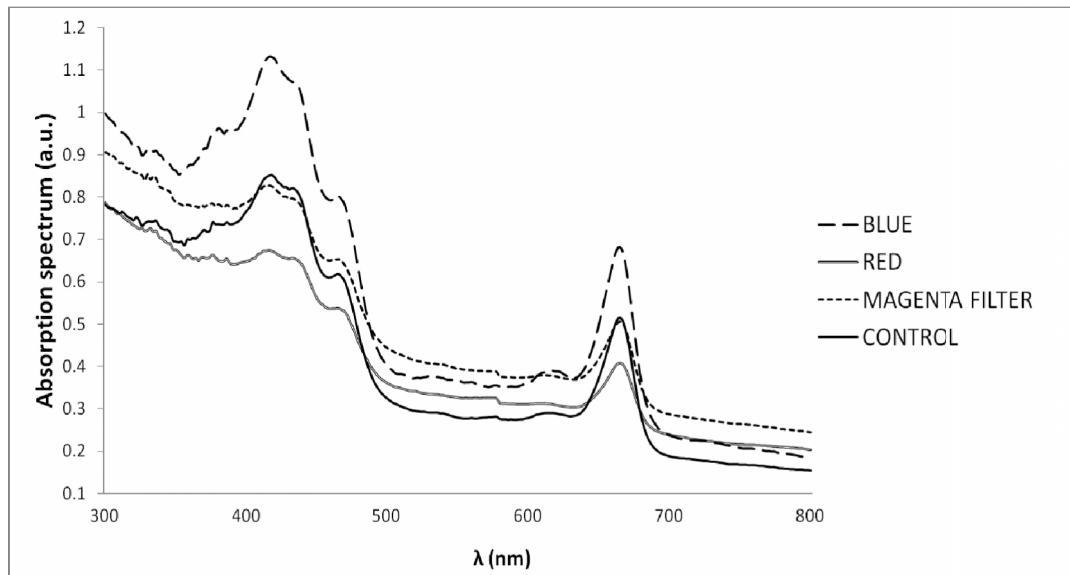
On the other hand FW and DW variables showed statistical significant differences against control with increments of 15% and 10% in treatment 1-B (red light) for broccoli seedlings grown for 15 days and treatment 4-B (green light) had the lowest weight. In contrast for lettuce seedlings grown for 29 days, the best treatment was 3-L (blue light) with increases of

50 and 41% in FW and DW against the control respectively, while the remaining treatments 1-L (red light) and 2-L (magenta filter) showed decreases of 14 and 27% for FW variable and 19 and 18% for DW variable compared to control respectively.

Several studies have mentioned that the use of red light causes a reduction in biomass, so it is necessary to complement them with blue light [44,45,1], a result that differs from observed in this study for seedlings of broccoli. However, other authors have reported similar findings to this research with other species of plants grown in enriched environments by red light [46,47,48,49]. Hogewoning et al. [26] mention that blue light irradiation promotes the production of biomass, which is consistent with that found for lettuce seedlings. These differences in results may be explained by the fact that the light responses differ according plant species [6], and the red light increases starch accumulation in leaves [50,49]. Besides growth time of seedlings in this experiment was different for broccoli and lettuce, and it is reported that light requirements for each phase of plant development might be different [8]. These results suggest that the red light is required in early stages of growth for a good development in cultivating broccoli.

### 3.3 Absorption Spectrums

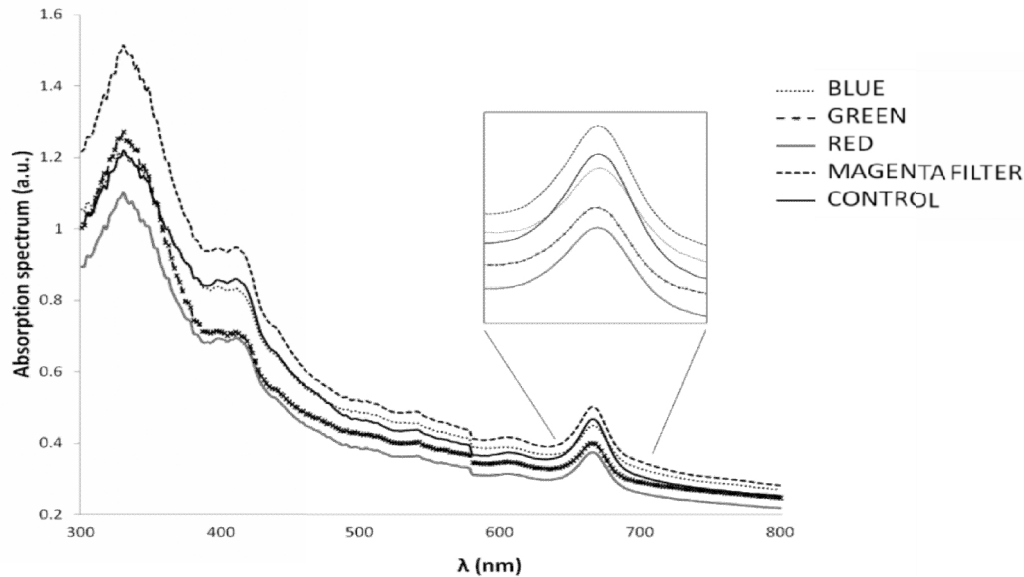
Plant pigments have absorption patterns of specific wavelength known as absorption spectrum [39]. In this research, the amplitudes of the absorption spectrum obtained by UV-VIS spectrophotometry for the different light treatments in seedlings of lettuce and broccoli, reported no statistically significant differences between treatments of high intensity LED light in the 300-700nm range, although their spectrums show certain trends as seen in Fig. 2 and 3.



**Fig. 2. Absorption spectrums for lettuce seedlings exposed to high-intensity LED light**

In the range of 300-700nm, it is possible to observe that exposure regimes of high intensity LED light affected the amplitude of the spectral signal. For lettuce is possible to observe that treatment with the highest amplitude corresponds to the blue LED light, followed by treatment with the magenta filter and control with similar values between them, leaving below all the red LED light treatment (Fig. 2).

Regarding broccoli seedlings in the range of 300 to 700nm is observed that treatment with magenta filter has the highest amplitude compared to all other treatments, while treatment with blue, green and control had similar amplitude. The lowest amplitude was in the red LED light treatment as happened in lettuce seedlings. While treatments with green and red light in the range of 350 to 700nm for broccoli had the same behavior (Fig. 3).



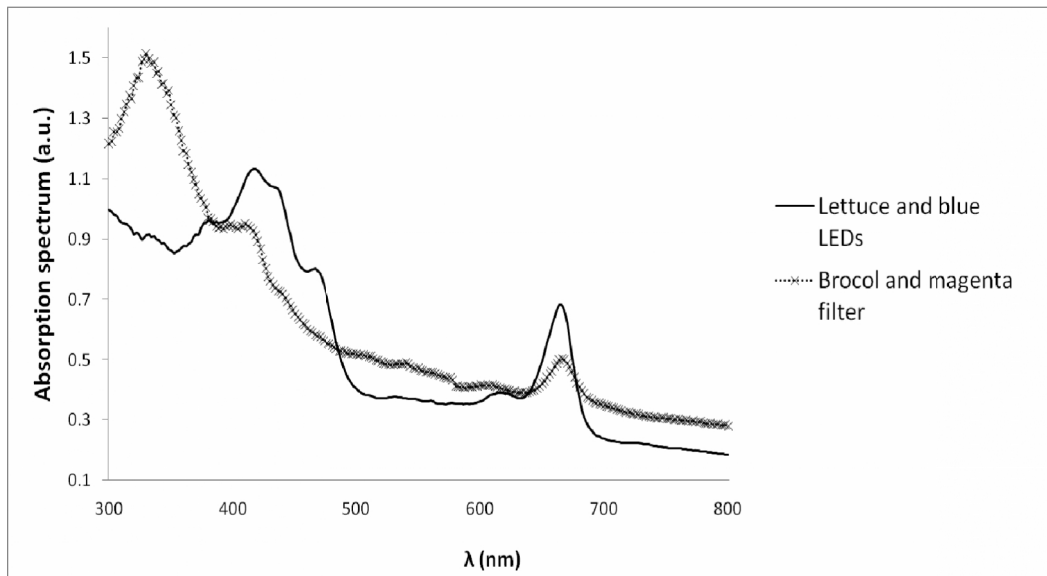
**Fig. 3. Absorption spectrums for broccoli seedlings exposed to high-intensity LED light**

Can be observed in the spectrums that broccoli seedlings developed higher amplitudes of absorption in 300-470 nm, compared to lettuce seedlings. In contrast, the spectrum of the lettuce seedlings developed higher amplitudes of absorption in 600-700nm, compared to broccoli seedlings (Fig. 4).

The results of the mean values of the spectrums show that the best treatments were made up of white LED light and magenta filter for seedlings of broccoli and blue LED light in lettuce seedlings. The filter works by removing the component of green light, so the treatment was under the influence of components of blue, red and other light; this result could be explained as some authors have argued that chlorophyll production is induced with the use of blue LEDs [33,51] and which is consistent with what was found in lettuce. On the other hand treatments with red light, showed lower values of the absorption spectrum which represents a minor amount of chlorophyll, however the treatment had the highest values for FW and DW in broccoli seedlings. Saebo et al. [50] and Li et al. [51] reported that seedlings with low chlorophyll content seem to use it more efficiently under red LED light, which could explain the high values of FW and DW in this investigation.

The use of technological advances to confront socially relevant issues such as food production is an imminent need. It is estimated that by 2050 year to increase 50% food production to avoid disaster in this area [52]. For this reason, plant production in controlled environments is a possibility that has begun to grow rapidly worldwide [45]. The primary variables to be monitored in the production of plants and vegetables in a controlled environment are water, CO<sub>2</sub>, temperature and light for achieve optimal growth and development. Using appropriate illumination systems allows control of light variable as factor

controlling growth and morphogenesis in plants and vegetables [53,8,22]. The light is manipulated to improve the quality and production of plants in greenhouses [54] and high intensity LED light could be an alternative to improve performance in this type of agriculture.



**Fig. 4. Absorption spectrums for lettuce and broccoli seedlings exposed to high-intensity LED light**

The color light treatments in this study presented results above control for physiological variables evaluated, however treatment with specific light is not optimal for the improvement of all variables because light requirements for each phase of plant development may be different [8]. In this research is evident with the use of green light, which achieves longest hypocotyl length and in contrast the lowest fresh and dry weight. This suggests the need to continue investigating the effects of high intensity LED light in order to create new lighting systems based on this type of light for the production of vegetables and other plants in a controlled environment.

#### 4. CONCLUSION

Seedlings of broccoli (*Brassica oleracea* L.) with green light and lettuce (*Lactuca sativa* L.) with red light exposed to high intensity LED light developed for 15 and 29 days respectively, showed statistically significant differences in average length of hypocotyl with increments of 33% and 42% compared to control respectively. The highest fresh and dry weight in broccoli seedlings was obtained in red light treatment with increases of 15% and 10% respectively, while in lettuce seedlings was 50 and 41% but with blue light compared to its control. These results allow to infer that the physiological responses produced by exposure to different wavelengths of high intensity LED light in broccoli and lettuce seedlings, varied according to the wavelength used, where the red LED light treatment had the best results in seedlings of broccoli, and lettuce seedlings was the blue LED light. The results suggest that the use of high intensity LED light is a viable option to improve plant growth in controlled environments.



## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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