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Original Research Article

Effect of Interchanging Red and Blue LED Irradiation Sequence on Growth of Lettuce

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Abstract: Light-emitting diode (LED) is commonly employed as supplementary light sources to enhance plant growth and quality. Nowadays, a combination of red (R) and blue (B) LED sources is mainly used since it can boost plant photosynthesis rate and biomass. Generally, both light sources are continuously irradiated for 16–18 hours for plant photoperiod, which may result in high electricity costs. Therefore, interchanging use of red and blue LED irradiation is introduced to reduce the use of electricity, thus minimising the operating cost. In this study, a growth chamber with red and blue LED was constructed and its intensity and power distribution were assessed. The effect of blue and red LED irradiation sequence on plant growth parameters and chlorophyll content was examined. Three light treatments over a period of 16 hours (RB16 - 16 hours constant simultaneous R- and B-light, R4/B4 - 4 hours interval interchanging R- and B-light and R8/B8 - 8 hours interval interchanging R- and B-light) were exposed to lettuce under controlled room temperature. Results revealed that the different light treatments influence lettuce plant morphology such as plant height, leaf width and leaf number. However, the results are similar for fresh and dry mass, and chlorophyll concentration. It is found that, both plant height and leaf width were the highest under R4/B4 and RB16, respectively. Such findings would be extremely valuable information on the use of interchanging LED irradiation in plant development for commercial production, resulting in optimized power consumption.

Keywords: Interchanging red and blue light; LED; photoperiod; lettuce

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1. Introduction

Supplementary light sources that are used for plant cultivation are such as fluorescent, metal-halide, high-pressure sodium, and incandescent lamps. In a light spectrum, plants respond well to specific wavelengths which LEDs can produce specific light wavelengths that is a huge advantage of LEDs in the growing indoor market. Thus, growers can now optimise lights for plant growth by using LEDs. The changes in the light spectrum have a strong influence on the parameters or variables of the leaf's physiology, anatomy and morphology (Hogewoning *et al*., 2010). Moreover, some research has stated that photoperiod, light quality, and periodic temperature variation have brought some impact on the growth of lettuce and other plants like radishes (Lin *et al*., 2013).

Furthermore, the impacts of blue (B) light and red (R) light on plant and plant growth in terms of light quality has grabbed a lot of attention since these wavelengths are mostly absorbed by photosynthetic pigments and have a significant influence on plant architecture and development (Metallo *et al*., 2018). R-light for normal plant growth acts as an integral part of the light spectrum as red light works on plant morphogenesis by generating photochromic transformations and regulates the synthesis of phytochemicals like phenolic and oxalate (Chen *et al*., 2016). Furthermore, simultaneous R- and B-light irradiation exceeded R or B-light irradiation in terms of lettuce fresh mass accumulation (Shimokawa *et al*., 2014).

Generally, both light sources are continuously irradiated for 16–18 hours for plant photoperiod, which may result in high electricity costs, especially for commercial farming. Therefore, interchanging use of R- and B-LED irradiation is introduced to reduce the use of electricity, thus minimising the operating cost. Dissimilarities in light irradiation patterns have been proven to impact plant development in recent studies (Chen *et al*., 2017; Kuno *et al*., 2017; Masuda *et al*., 2021; Shimokawa *et al*., 2014). Shimokawa *et al*. (2014) looked at the effects of single, simultaneous and alternating B- and R-LED light irradiations on leaf lettuce growth rates in which these lights showed that alternating B- and R-light irradiation was a better pattern of irradiation to boost the growth rate of leaf lettuce varieties. However, the effect of low photosynthetic photon flux density (PPFD) of R- and B-LED irradiation on plant growth has not been previously explored. Thus, the objective of this study is to evaluate the effect of interchanging low PPFD R and B LED irradiation sequences on the growth of lettuce.

In this study, a growth chamber with R and B LED was constructed, and its intensity and power distribution were assessed. Plant growth parameters such as plant height, leaf width, leaf number, fresh mass (FM), dry mass (DM) and chlorophyll were investigated to determine the effects of interchanging R and B LED irradiation on the growth of lettuce (Chen *et al*., 2016; Jing *et al*., 2021; Metallo *et al*., 2018). These parameters (plant height, leaf width, leaf number, FM and DM) can be measured in a simple non-destructive technique using a Vernier calliper or optical imaging techniques. The chlorophyll content was measured by the destructive method to evaluate the effect of interchanging R- and B-LED light on photosynthesis.

2. Materials and Methods

2.1 Experimental Setup and Growth Condition

In this study, leaf lettuce seeds (Lettuce Looseleaf 4, Green World, Kuala Lumpur, Malaysia) were utilised in the experiments. The seeds were germinated in a mini germination box (CitiFarm, Selangor, Malaysia) with perlite (CitiFarm, Selangor, Malaysia) size of 3–6 mm for 7 days and grown under fluorescent light. The water was irrigated daily to ensure that the substrate was completely soaked. For each treatment, the nine germinated lettuce seedlings (seedlings with two to three true leaves) were transplanted into a Styrofoam plate with nine holes and placed in a container (38 × 30 × 11 cm) filled with 8 L nutrients solution (mixture of A and B fertiliser) and hydroponically cultured in a controlled room. Electrical conductivity (EC) and pH of the nutrient solutions were adjusted approximately at 500–600 ppm and 5.5–6.5, respectively and both parameters were monitored daily using EC and pH meter, respectively. Air temperature and relative humidity in the room were maintained approximately at 25 ℃ and 60% respectively using 2 units of air conditioner which were alternately turned on and off at 12 h intervals. The transplanted lettuces were placed under growth chambers, irradiated with three treatments with different R- and B-LED irradiance sequences described below and harvested at 31 days after sowing (DAS).

2.2 Red and Blue LED Irradiation Sequence

R and B LED irradiation treatments were performed in a growth chamber (45 × 40 × 26 cm) installed with RGB LED strips (12V SMD 5050, China) at the ceiling of the chamber. The irradiance patterns of the RGB LED strips for each treatment were controlled automatically using an Arduino microcontroller (Mega 2560 Rev3, Arduino, Italy). The three irradiance patterns for light treatments utilised in this study: (1) constant simultaneous R- and B-light for 16 h (RB16) at 22 $\mu mol \cdot m^{-2} \cdot s^{-1}$; (2) interchanging R-light at 9.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 4 h and B-light at 21.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 4 hours (R4/B4); (3) interchanging R-light at 9.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 8 h and B-light at 21.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 8 h (R8/B8) as shown in Figure 1.

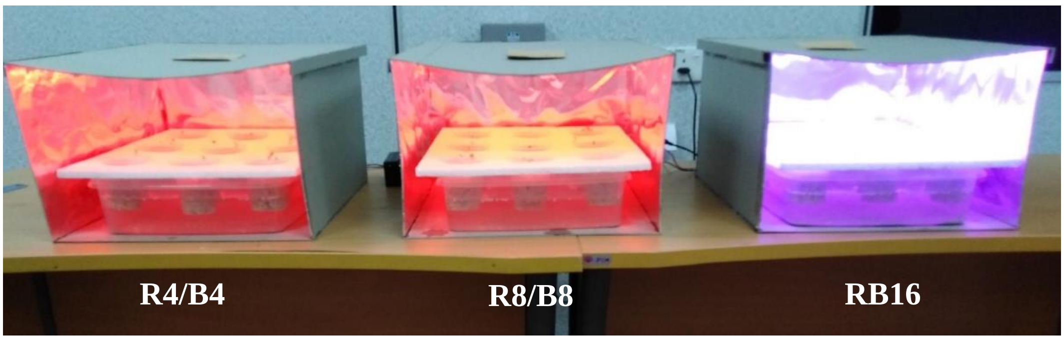

Figure 1. Irradiation sequence used for three light treatments

The photosynthetic photon flux density (PPFD) was measured with a light quantum meter (LI-250A, LI-COR, Nebraska, USA) equipped with a quantum sensor (LI-190R, LI-COR, Nebraska, USA), set at 20 cm below the LED. The PPFD is the amount (also referred to as intensity) of photosynthetically active radiation (PAR) light of wavelength between 400 nm to 700 nm that lands on a square meter each second. These wavelengths correspond to light used by most plants to drive photosynthesis. The measured PPFD was the maximum power from the RGB LED strips. The details of the irradiance sequences are shown in Figure 2.

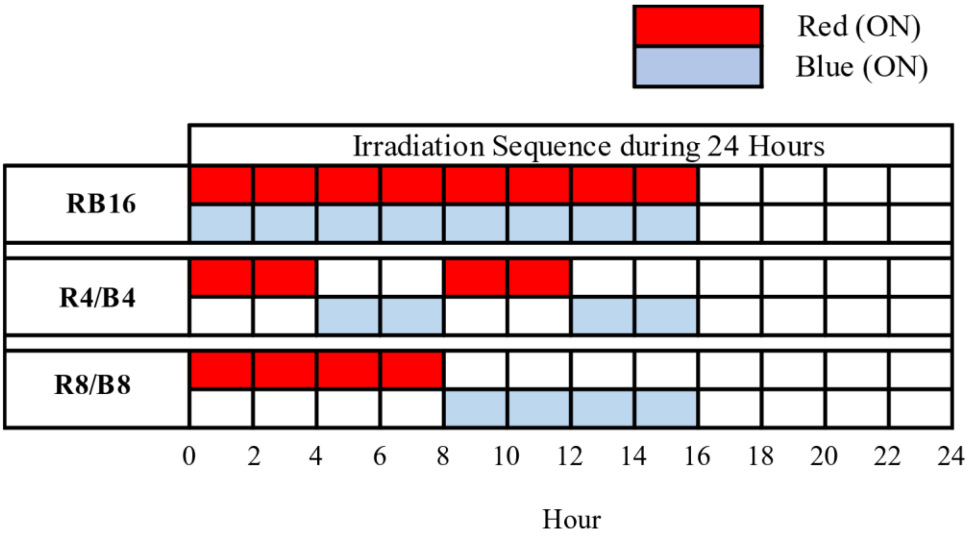

Figure 2. Irradiation sequence used for three light treatments

2.3 Measurement of Plant Morphology and Growth Characteristic

After 31 DAS, the lettuces were harvested and measured for plant height, leaf width, leaf number, FM and chlorophyll. Lettuce leaves were dried in the oven at 60 °C for 24 h to determine the dDM.

2.4 Determination of Chlorophyll Content

A total of 0.20 g of FM lettuce leaves was collected. The chlorophyll was extracted by grinding the leaf disc in 1 mL of 100% acetone (C_3H_6O) with 0.1 g of calcium carbonate ($CaCO_3$) in a mortar. The extract was poured into a test tube to bring the final extract to 5 mL. Then, the extract was filtered by using filter paper and the liquid was collected. The mixture was centrifuged at 10,000 rpm for 5 mins. The solution was transferred into a cuvette, and then the absorbance was measured using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 645 nm and 663 nm wavelength simultaneously. The absorbance values were recorded. Chlorophyll content was measured using the Equations 1, 2 and 3 described by Ritchie (2008):

$$\text{Chlorophyll a } (\mu g\ ml^{-1}) = -1.93\ A_{645} + 11.93\ A_{663} \quad (1)$$

$$\text{Chlorophyll b } (\mu g\ ml^{-1}) = 20.36\ A_{645} - 5.50\ A_{663} \quad (2)$$

$$\text{Total Chlorophyll } (\mu g\ ml^{-1}) = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (3)$$

where A_{λ} is the absorbance coefficient.

3. Results

3.1. Distribution of measured PPFD in the growth chamber

Figure 3(A) and Figure 3(B) show the distribution of measured PPFD set at 20 cm below the LED for red and blue light respectively.

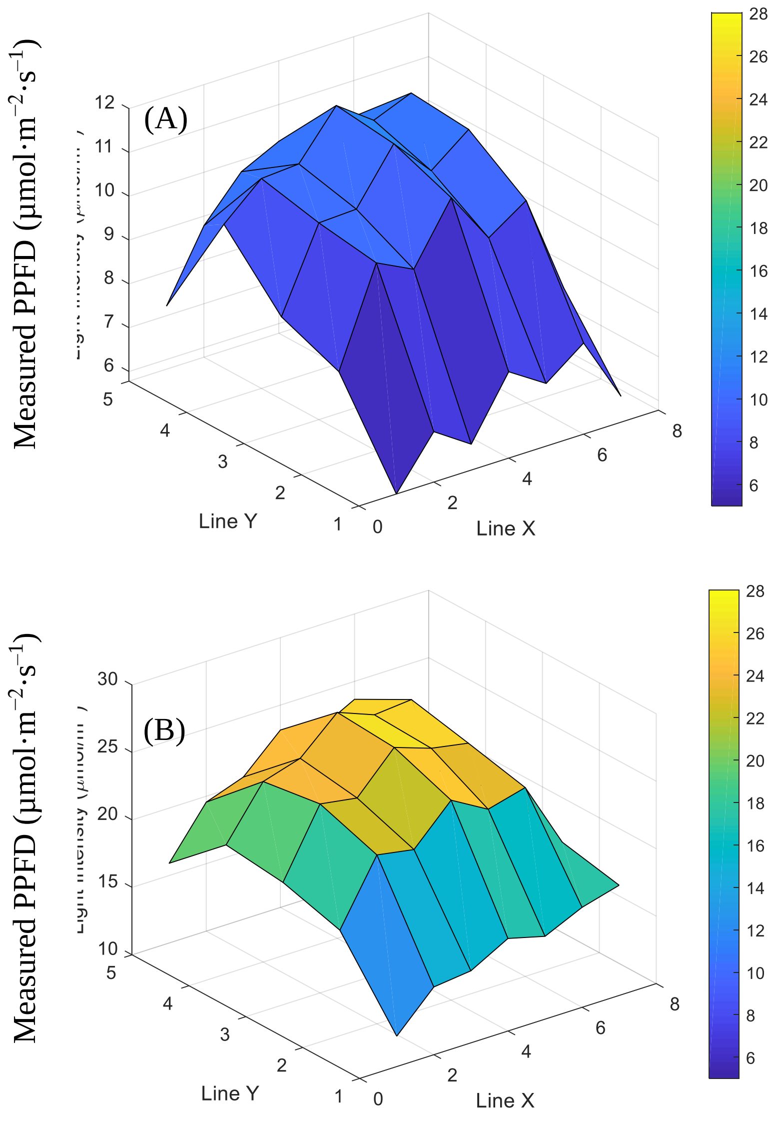

Figure 3. Distribution of measured PPFD for red (A) and blue (B) light in the growth chamber set at 20 cm below the LED

3.2 Plant Morphology and Growth Characteristics

Figure 4(A) shows the measured plant height, leaf width, and leaf number, while Figure 4(B) demonstrates the FM and DM of the harvested lettuce plants under different light treatments. The plant height was highest under R4/B4 with a 40% increase in contrast to RB16 and R8/B8, but no significant difference was observed with other treatments. The leaf width was the highest under RB16 but not significantly different among other treatments. The leaf number, FM and DM were similar among treatments.

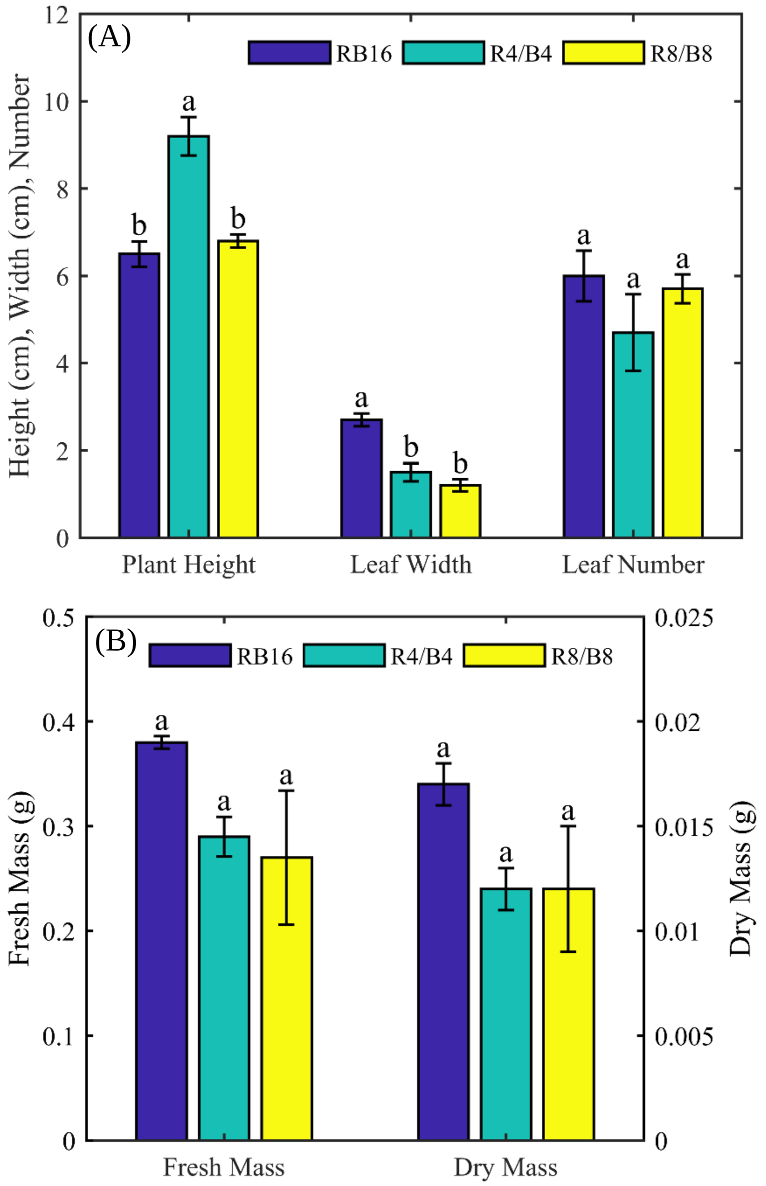

Figure 4. (A) Plant height, leaf width, leaf number, (B) fresh mass, and dry mass of the harvested lettuce plant under different light treatments. Data are averages (n = 3) – standard error (SE). Bars labelled with the different letters are significantly different among the treatments by Tukey-Kramer honest significant difference test at $P < 0.05$

3.3 Chlorophyll Contents

Figure 5 shows the chlorophyll contents of lettuce plants with various light treatments. The highest chlorophyll a, chlorophyll b and total chlorophyll were observed in RB16 plants, followed by R8/B8, while the lowest was observed in R4/B4 plants.

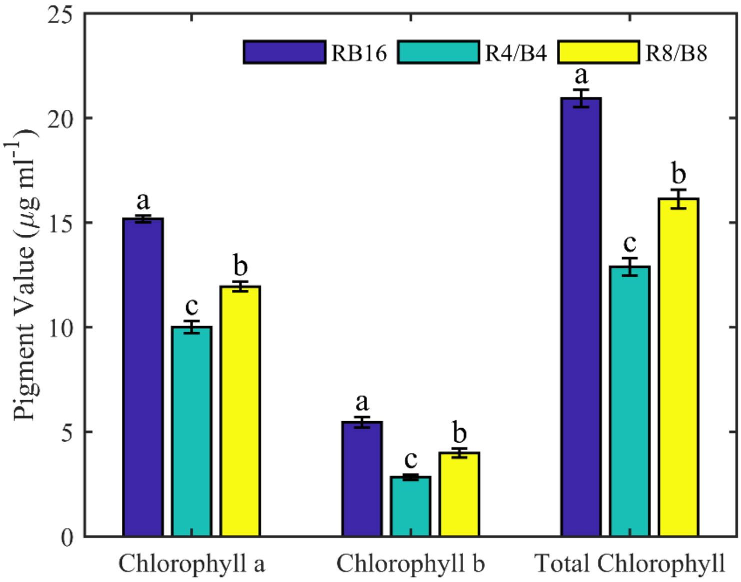

Figure 5. Chlorophyll a, chlorophyll b and total chlorophyll of the harvested lettuce plant under different light treatments. Data are averages (n = 3) – standard error (SE). Bars labelled with the different letters are significantly different among the treatments by Tukey-Kramer honest significant difference test at $P < 0.05$

4. Discussion

In our study, we found that the highest measured PPFD for R and B LED irradiation were 11.7 μmol·m^{-2}·s^{-1} and 26.8 μmol·m^{-2}·s^{-1}, respectively measured at the centre of the position growth chamber set at 20 cm below LED. The average measured PPFD (35 points measured data) for R- and B-LED irradiation were 9.3 ± 1.6 μmol·m^{-2}·s^{-1} (standard deviation) and 21.1 ± 3.7 μmol·m^{-2}·s^{-1} (standard deviation), respectively. We also found that the measured PPFD both at R- and B-light (Figure 3) showed around 26% lower PPFD value at the front region (Line Y = 1) if compared to the back region (Line Y = 5). This occurs because of light dispersing out from the front portion of the growth chamber (refer to Figure 1). In contrast, the combined R- and B-light PPFD used in other similar studies (Huang *et al*., 2021; Ohtake *et al*., 2018; Shimokawa *et al*., 2014) were around more than 100 μmol·m^{-2}·s^{-1}. The limitation of PPFD in our experimental setup may be restricted to the RGB LED strips installed in our growth chamber.

Despite low PPFD light in our experiment, we discovered that interchanging irradiation R4/B4 promoted higher plant height in contrast to simultaneous R- and B-LED light irradiation (RB16), but a similar trend was not observed for R8/B8. Simultaneous irradiation RB16 induced larger leaf width compared with interchanging irradiation R4/B4 and R8/B8. Our findings were not consistent with results reported by Shimokawa *et al*. (2014), Ohtake *et al*. (2018) and Huang *et al*. (2021), in which, interchanging irradiation R- and B-LED irradiation induced plant growth compared to simultaneous RB16. The discrepancies might be attributed to inadequate light intensity under RB and R/B. In addition, the R/B light intensity ratio was fixed to (R: B = 1: 2 – R is less than B). In contrast, Shimokawa *et al*. (2014) and Ohtake *et al*. (2018) used the R/B light intensity ratio of R: B = 5: 3 and R: B = 3: 1, respectively (R is more than B). The R-light influenced the growth of stem diameter and plant height compared to B-light as discovered by Chen *et al*. (2014). In our study, the R/B light intensity ratio was fixed by the LED strip manufacturer.

The leaf number, FM and DM showed no significant differences between the treatments. Thus, interchanging R- and B-light R4/B4 and R8/B8 with approximately 50% reduction of electricity usage can be employed to produce a similar yield with RB16 (with the assumption of R- and B-light having similar electrical power consumption).

Ohtake *et al*. (2018) reported that the total chlorophyll content was not significantly different between simultaneous RB16 and interchanging R/B light, particularly at 22 DAS and 31 DAS. In contrast to the findings, the study shows that the highest chlorophyll a, chlorophyll b and total chlorophyll were detected under RB16 treatment compared to others. The content orders of chlorophyll a, chlorophyll b and total chlorophyll were RB16 > R8/B8 > R4/B4. The content of chlorophyll a was about three times as much as that of Chlorophyll b irrespective of the various light treatment.

5. Conclusions

In this study, a growth chamber installed with R- and B-LED was constructed. The measured average PPFD of R- and B-light were 9.3 ± 1.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 21.1 ± 3.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Significant effects of plant height and leaf width, chlorophyll a, chlorophyll b and total chlorophyll were observed under R4/B4 and RB16 respectively. The leaf number, FM and DM showed no significant differences between light treatments. The interchanging R- and B-light on plant development for commercial production can be considered to optimise power consumption thus reducing electricity costs.

Author Contributions: Conceptualization, Muhammad Firdaus Abdul Muttalib.; methodology, Nur Fathin Syahirah Romli.; validation and formal analysis, Muhammad Firdaus Abdul Muttalib, Nur Fathin Syahirah Romli and Mohd Fauzie Jusoh.; resources, Muhammad Firdaus Abdul Muttalib.; writing—original draft preparation, Nur Fathin Syahirah Romli.; writing—review and editing, Muhammad Firdaus Abdul Muttalib and Mohd Fauzie Jusoh.

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Conflicts of Interest: The authors declare no conflict of interest.

References

Chen, X. L., Guo, W. Z., Xue, X. Z., *et al*. (2014). Growth and quality responses of 'Green Oak Leaf' lettuce as affected by monochromic or mixed radiation provided by fluorescent lamp (FL) and light-emitting diode (LED). *Scientia Horticulturae*, 172, 168–175. https://doi.org/10.1016/j.scienta.2014.04.009

Chen, X. L., Xue, X. Z., Guo, W. Z., *et al*. (2016). Growth and nutritional properties of lettuce affected by mixed irradiation of white and supplemental light provided by light-emitting diode. *Scientia Horticulturae*, 200, 111–118. https://doi.org/10.1016/j.scienta.2016.01.007

Chen, X. L., Yang, Q. C., Song, W. P., *et al*. (2017). Growth and nutritional properties of lettuce affected by different alternating intervals of red and blue LED irradiation. *Scientia Horticulturae*, 223, 44–52. https://doi.org/10.1016/j.scienta.2017.04.037

Hogewoning, S. W., Trouwborst, G., Maljaars, H., *et al*. (2010). Blue light dose–responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *Journal of Experimental Botany*, 61(11), 3107–3117. https://doi.org/10.1093/jxb/erq132

Huang, J., Xu, Y-l., Duan, F-m., *et al*. (2021). Improvement of the growth and nutritional quality of two-leaf-color Pak Choi by supplemental alternating red and blue light. *HortScience*, 56, 118–125. https://doi.org/10.21273/HORTSCI15180-20

Kuno, Y., Shimizu, H., Nakashima, H., *et al*. (2017). Effects of irradiation patterns and light quality of red and blue light-emitting diodes on growth of leaf lettuce (Lactuca sativa L.Greenwave). *Environmental Control in Biology*, 55(3), 129–135. https://doi.org/10.2525/ecb.55.129

Lin, K. H., Huang, M. Y., Huang, W. D., *et al*. (2013). The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. *capitata*). *Scientia Horticulturae*, *150*, 86–91. https://doi.org/10.1016/j.scienta.2012.10.002

Masuda, K., Nakashima, H., Miyasaka, J., *et al*. (2021). Quantification of the effects of alternating and simultaneous red and blue irradiations on plant morphology and shoot fresh weight in leaf lettuce 'Green wave'. *Environmental Control in Biology*, 59(4), 181–190. https://doi.org/10.2525/ecb.59.181

Metallo, R. M., Kopsell, D. A., Sams, C. E., *et al*. (2018). Influence of blue/red *vs*. white LED light treatments on biomass, shoot morphology, and quality parameters of hydroponically grown kale. *Scientia Horticulturae*, 235, 189–197. https://doi.org/10.1016/j.scienta.2018.02.061

Ohtake, N., Ishikura, M., Suzuki, H., *et al*. (2018). Continuous irradiation with alternating red and blue light enhances plant growth while keeping nutritional quality in lettuce. *HortScience*, 53(12), 1804–1809. https://doi.org/10.21273/HORTSCI13469-18

Ritchie, R. J. (2008). Universal chlorophyll equations for estimating chlorophylls a, b, c, and d and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. *Photosynthetica*, 46(1), 115-126. https://doi.org/10.1007/s11099-008-0019-7

Shimokawa, A., Tonooka, Y., Matsumoto, M., *et al*. (2014). Effect of alternating red and blue light irradiation generated by light emitting diodes on the growth of leaf lettuce. *BioRxiv*, 003103. https://doi.org/10.1101/003103