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Effect of Varying Light Spectrum on Antioxidant Production of Red Leaf Lettuces Cultivated using Fish Wastewater

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Wavelengths of blue (B) and red (R) light can influence plants growth and development, specifically in the production of antioxidants. This research aims to determine the optimum B and R light percentage to enhance overall growth and antioxidant levels in red leaf lettuces cultivated from fish wastewater (Omnicare Farm, Malaysia). Lettuces were grown under various combinations of light-emitting diode (LED) light treatment (33 % B + 67 % R; 40 % B + 60 % R and 50 % B + 50 % R), in which the light percentages were quantified using light spectrum analyser for 27 days. The photosynthetic photon flux density (PPFD), photoperiod and temperature were maintained at 250 μmol/m^2.s, 16 h and 30 ± 3 ºC. Under varying light spectra, the plant growth parameters were studied in terms of length and weight while the leaf surface color was measured for lightness (L*), chroma (C*), hue degree (h°), redness (a*) and yellowness (b*). After the LED treatment, the antioxidant levels were analyzed based on the anthocyanin concentration after the LED treatment. The increased B light percentage from 33 to 50% caused a 91.5 and 71.3% reduction in the overall weight and leaf length. However, it increased the anthocyanin concentration by 6.5-fold. This shows that a higher B light percentage resulted in undesirable red leaf lettuce growth reduction but promoted the production of antioxidant levels (anthocyanin concentration) in the lettuces.

1. Introduction

Antioxidants are biologically active compounds that can protect organisms from getting infected by diseases. Examples of plant antioxidant compounds include tocopherols, carotenoids, phenolic acid, flavonoids and dipropenes. Furthermore, secondary plant-derived metabolites such as phenolic compounds have the potential to clear free radicals throughout the whole plant, such as fruits, leaves, seeds, roots and skin (Mathew and Abraham, 2006). These antioxidant substances are beneficial to the human body, as epidemiological evidence has shown that antioxidant flavonoids helps to protect against lung cancer, stroke, and coronary heart disease (Brandt et al., 2004). This leads to increased interest in the research of antioxidant levels evaluation in plants.

Many studies have shown that leafy vegetables such as lettuce, red spinach and red pak choi grown in hydroponic systems contain high amounts of antioxidant compounds (Vaštakaitė et al., 2015). This is because hydroponically plants are cultivated in a nutrient solution which contains sufficient nutrients, particularly potassium for the plant's physiological and chemical components development (Ahanger et al., 2015). Recent developments in hydroponics agricultural methods had led to the development of aquaponics system which is an integration between hydroponics and aquaculture (cultivation of aquatic animals). This system functions by exchanging the waste by-product from the fishes as food for the microbes to undergo microbial activity, then is converted into fertilizer for the hydroponic cultivated plants to absorb the nutrients (Maucieri et al., 2017). While this agricultural method is considered to be more sustainable, the production of fish wastewater may not contain sufficient nutrients for the development of antioxidants in plants.

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The concentration of antioxidant phytochemicals could be increased during plant growth in response to the surrounding environment, such as environmental conditions, the chemical composition in a nutrient solution, or lighting parameters (light spectrum) (Naznin et al., 2019). This is because plants undergo oxidative stress under extreme conditions. Hence, the antioxidant enzymes present in the plants will produce more antioxidant compounds to protect the plants from these oxidative stresses (Soengas et al., 2018). Out of these parameters, the light spectrum would significantly affect the accumulation of plant antioxidant pigments (Bayat et al., 2018). The type of light spectrum can affect the growth and development of plants due to the ability to stimulate plant metabolite synthesis. White light consisting of all color spectrums can promote morphology and increase the plant's photosynthetic rate. In specific, yellow/orange light is responsible for the synthesis of chlorophyll (Lanoue et al., 2018); green light is able to boost the photosynthetic capacity in deeper leaf layers; red light is responsible for the control of chloroplast function, stomatal opening, petiole growth and reproductive system whereas blue light promotes the accumulation of plant pigments and photosynthesis (Ramalho et al., 2002).

The blue (B) and red (R) light radiation can be adsorbed strongly by the chloroplast at the upper layer of the leaves. This is because the chlorophyll a and chlorophyll b, the main photosynthetic pigments in the plants mainly absorb blue light with maximum absorption at 430 and 453 nm, and red light with maximum absorption at 663 and 642 nm of the light spectrum (Terashima et al., 2009). Hence, red and blue lights are widely used in the cultivation of plants. However, monochromatic light can result in photoinhibition which could cause damage to all the components in the photosynthetic machinery of a plant (Bayat et al., 2018). Therefore, a mixture of blue and red LEDs has been used widely as an artificial light source for plant cultivation. Previous studies also showed that a mixture of blue and red light could increase the accumulation of antioxidant compounds in plants. Naznin et al. (2019) studied the influence of various percentages of R and B light on the growth performance and antioxidant levels in lettuce, spinach, kale, basil and sweet pepper. In their study, four different spectral treatments, 100 % R; 5 % B + 95 % R; 9 % B + 91 % R and 17 % B + 83 % R, were used. They concluded that a higher B light percentage at 17 % B can not only increase the fresh plant mass, but also increase chlorophyll concentration, carotenoid concentration and antioxidant levels by up to 78 % in plant cultivation. However, these results were not supported by Son and Oh (2013) who showed that the fresh mass of red and green lettuces decreased with the increasing B light percentage. The authors showed that the B and R light percentage with the highest antioxidant capacity was 47 % B + 5 3% R. They also concluded that the antioxidant levels in lettuces increase with the increasing B light percentage.

Multiple research works published in literature had reported on the positive effects of light spectrum on hydroponic plants cultivated in a controlled nutrient solution. However to date, there had been minimal studies on the influence of B and R light on the physical growth of plants cultivated in fish wastewater. Due to the dynamic nature of fish wastewater generation, it is crucial to understand how red leaf lettuce plants cultivated in fish wastewater respond to the change in B and R light percentages. Therefore, this study aimed to study the responses of red leaf lettuce plants cultivated in fish wastewater in terms of physical plant parameters such as length, aerial weight and leaf surface color, and chemical properties based on anthocyanin concentration after LED treatment.

2. Materials and methodology

2.1 Materials

Seeds of *Lactuca sativa* red leaf lettuce (Evergreen, Malaysia) were used to conduct this experiment. Tilapia fish wastewater obtained from Omnicare Farm Malaysia was used as the nutrient solution for this study. Methanol (Bumi Pharma, Malaysia), acetone (Fulltime Asia, Malaysia), 1 % formic acid (R&M Chemicals, UK), ethanol (Fulltime Asia, Malaysia), petroleum ether (R&M Chemicals, UK), ethyl acetate (Carlo Erba, Italy) and distilled water were used in the extraction of anthocyanin extract. Two buffer solutions: 0.4 M sodium acetate and 0.025 M potassium chloride buffer (R&M Chemicals, UK) were prepared for the anthocyanin analysis.

2.2 Methodology

2.2.1 LED light treatment

A deep water culture (DWC) hydroponics system was constructed using a 75 L container (590 mm length × 430 mm width × 430 mm height). Polystyrene with dimensions of 540 mm length × 380 mm width × 13 mm height, was used to dangle the net pots so that the lettuce roots could submerge into the fish wastewater to obtain the nutrients needed. Each DWC hydroponic system was used to grow six germinated lettuces. Hence, six small holes, which are 8 cm apart were made on the polystyrene to dangle the net pots. The fish wastewater was oxygenated via microbubbles using a submersible air pump. A light-emitting diode (LED) light was hung on a polyvinyl chloride (PVC) framework above the plants to emit a constant photosynthetic photon flux density (PPFD) of 250 $\mu mol/m^2.s$. The whole DWC hydroponics system was then covered with a reflective aluminum

bubble foil so that the lettuce would not be able to absorb the natural light outside. A black cloth was used to cover the container to prevent the growth of algae in the fish wastewater.
Subsequently, *L. sativa* red leaf lettuce seeds were germinated into a growth foam and placed on top of a water surface in a container. The container was then placed in darkness at a temperature of 30 ± 3 ºC for germination. After germinating for four days, the lettuce seedlings were transplanted to the growth chamber filled with fish wastewater which acts as the plant's nutrients. The lettuce plants were illuminated by 300W LED light (JT-GL-TP300) with various percentages of blue (B, 452 nm) and red (R, 660 nm) light. In this study, three spectral treatments were used, namely, 33 % B + 67 % R; 40 % B + 60 % R, and 50 % B + 50 % R. The photoperiod was set at 16/8 h (day/night). The ambient temperature of the fish wastewater was kept at 30 ± 3 ºC, while the pH and dissolved oxygen concentration were maintained at 6.8 and 5 ppm.

2.2.2 Physical plant growth and color analysis

All lettuce plants were harvested after 27 days of LED light treatment. The average overall weight of the six red leaf lettuces was determined immediately upon harvesting using a weighing scale. The roots of red leaf lettuce were then separated from the shoots, and the average shoot weight and root weight were determined. The average root length, leaf width, and leaf length were then measured.
The largest leaf from every plant grown within the data set (6 leaves per LED light treatment) were utilized to determine the leaf surface's color variation parameters such as lightness (L*), a* and b* values were obtained using a chromameter (CR-410, Konica Minolta, Japan). The hue degree, h° was determined using Eq(1), where 0° = red-purple; 90° = yellow; 180° = bluish green; and 270° = blue. The chroma, C*, which indicates the color saturation or intensity, was calculated using Eq(2).

$$h^{\circ} = tan^{-1}\frac{b^*}{a^*} \quad (1)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

2.2.3 Anthocyanins extraction and analysis

The extraction process was conducted by homogenizing 5 mL of methanol/acetone/distilled water with the ratio of 3.5:3.5:3 containing 1 % formic acid into every 1 g of crushed leaves. The extraction temperature was set at 30 °C in a shaking incubator (DH-WIS00220, Daihan Scientific, South Korea) for 30 min. The extraction process was carried out twice using the same crushed fresh red lettuce leaves. First, all the collected extracts were combined and filtered using two layers of cheesecloth. Subsequently, the filtrate obtained was centrifuged at 6,000 rpm for 15 min using a centrifuge (EBA 200, Hettich, Germany). In order to remove methanol and acetone, the supernatant obtained was evaporated at a temperature of 75 °C using a rotary evaporator (WEV-1001V, Daihan Scientific, South Korea) connected to a vacuum pump (A1000S, Eyela, Japan). After evaporation, two successive extractions were conducted using a twofold volume of petroleum ether in a separatory funnel to eliminate the lipophilic pigments from the aqueous phase. Then, three successive extractions were done on the aqueous phase obtained using ethyl acetate in a separatory funnel whereby the ratio of ethyl acetate to the aqueous phase is 1:1. The non-ethyl acetate phase (aqueous phase) obtained was evaporated at 95 °C. The evaporated sample was then used as an anthocyanin sample (Sun et al., 2009).
The anthocyanin extracts were dissolved in 0.4 M sodium acetate (pH 4.5) and 0.025 M potassium chloride buffer (pH 1.0) with a dilution factor of 50. The diluted sample's absorbance was measured using a UV-Visible spectrophotometer (UV-1900, Shimadzu, Japan) at 520 and 700 nm against a blank cell containing distilled water. The results were conveyed as the amount of anthocyanin per gram of fresh weight sample (μg/g FW).

3. Results and discussion

3.1 Plant growth measurement

Table 1 shows the physical results of the overall growth of red leaf lettuces concerning varying B and R light percentages. Based on the data obtained in Table 1, the overall plant growth was the highest under 33 % B + 67 % R, followed by 40 % B + 60 % R and 50 % B + 50 % R LED light treatment. The overall weight, shoot weight, root weight, root length, leaf width, and leaf length decreased by 91.49, 93.33, 88.24, 61.51, 66.67, and 71.34 %, when the B light percentage increased from 33 to 50 %. This is due to the low percentage of R light, which reduces red leaf lettuce's growth. R light promotes photophosphorylation in the guard cells of the stomata, which then stimulates the opening of the stomata to absorb more carbon dioxide from the surrounding air. This phenomenon leads to the increment of glucose conversion during photosynthesis (Liu and Liu, 2022).

Table 1: Effect of various B and R light percentages on the plant growth

B and R light percentage	33 % B + 67 % R	40 % B + 60 % R	50 % B + 50 % R
Overall weight (g)	9.40 ± 5.98	2.62 ± 1.65	0.80 ± 0.59
Shoot weight (g)	6.00 ± 4.06	1.03 ± 0.68	0.40 ± 0.30
Root weight (g)	3.40 ± 1.95	1.58 ± 0.98	0.40 ± 0.30
Root length (cm)	11.82 ± 5.63	4.05 ± 0.88	4.55 ± 1.64
Leaf width (cm)	6.66 ± 1.44	3.92 ± 1.39	2.22 ± 1.12
Leaf length (cm)	10.92 ± 1.75	4.68 ± 1.22	3.13 ± 1.46

The results obtained in this experiment were similar to those of Pennisi et al. (2019). Their reported findings showed that the plant height of lettuces and fresh weight of sweet basil increased with the increasing B light percentage until they reached the maximum point at 25 % B + 75 % R and decreased with the further increase of B light percentage. The growth response of red leaf lettuces was observed and the plant size obtained under 33 % B + 67 % R LED light treatment was the most desirable. Hence, decreasing the R light percentage influences plant growth negatively. Figure 1 demonstrates the physical growth after 27 days of LED treatment.

(a) (b) (c)

Figure 1: Physical plant growth at varying light spectrum of (a) 33 % B + 67 % R, (b) 40 % B + 60 % R and (c) 50 % B + 50 % R

3.2 Leaf surface color measurement

Table 2 summarizes the effect of different B and R light percentages on the changes in color parameters, including L*, a*, b*, C*)and h°. Results demonstrated that the L* increases with increasing B light, which indicated that the red leaf lettuces cultivated under 50 % B + 50 % R LED light treatment had the lightest color in comparison with 40 % B + 60 % R and 33 % B + 67 % R LED light treatment. The results are tallied with the c* values that quantitatively determines the color's chromatic scale. The highest c* value was recorded at 9.85 ± 4.00 under 33 % B + 67 % R LED light treatment, which indicates that the color is bright-toned. On the other hand, the c* value obtained under 40 % B + 60 % R and 50 % B + 50 % R LED light treatment was 1.87 and 2.48, indicating that the color is light-toned.

Table 2: Effect of various B and R light percentages on the leaf surface color

B and R light percentage	L*	a*	b*	c*	h°
33 % B + 67 % R	61.83 ± 9.34	2.79 ± 1.11	9.27 ± 4.36	9.85 ± 4.00	69.24 ± 12.55
40 % B + 60 % R	65.27 ± 0.93	1.79 ± 0.11	-0.50 ± 0.26	1.87 ± 0.07	344.36 ± 8.15
50 % B + 50 % R	73.04 ± 0.46	2.47 ± 0.07	-0.21 ± 0.12	2.48 ± 0.08	355.24 ± 2.59

The a* value represents the redness of the plants cultivated. Results from the color analysis showed that there is no clear indicative trend on the effect of varying B and R light percentages on the a* value, as there is no significant difference in the a* value of red leaf lettuces (1.8–2.8) cultivated under all LED light treatments. Nevertheless, all the lettuces cultivated under all light spectral treatments had a positive a* value, which suggests that the red leaf lettuces cultivated were red.

In contrast, the b* value demonstrated a significant difference in the values with increase B light. The b* value obtained under 33 % B + 67 % R LED light treatment was 9.27 ± 4.36, which is a positive value. Positive b* value means that the red leaf lettuces obtained was yellowish. In contrast, the b* value obtained under 40 % B + 60 % R and 50 % B + 50 % R LED light treatment were -0.50 ± 0.26 and -0.21 ± 0.12. Both the b* values obtained under 40 % B + 60 % R and 50 % B + 50 % R LED light treatment were a negative value. A greater negative b* value indicates that the red leaf lettuces obtained was a bluish and less yellow color, which is more desirable (Zheng et al., 2003). This also means that increasing B light percentage is able to decrease the degree of yellowness and increase the degree of blueness in red leaf lettuces. The results were in good agreement with the h° measured, which indicates the color space of a chromameter. The h° obtained under 40 % B + 60 % R and 50 % B + 50 % R LED light treatment were 344.36 ± 8.15 and 355.24 ± 2.59 , which are close to 0° or 360°. This suggested that the red leaf lettuces obtained under 40 % B + 60 % R and 50 % B + 50 % R LED light treatment were between red and blue color, which was in good agreement with the trend from the b* values. However, the h° value obtained under 33 % B + 67 % R LED light treatment was 69.24 ± 12.55, which suggested that the red leaf lettuces obtained were between red and yellow color. Hence, it can be concluded that increasing B light percentage is able to obtain red leaf lettuces that are in the red-blue zone.

3.3 Anthocyanin analysis

The effect of different B and R light percentages on the antioxidant properties of red leaf lettuces was analyzed in terms of anthocyanin concentration as demonstrated in Figure 2. Figure 2 shows that the anthocyanin concentration was the highest under 50 % B + 50 % R at 21.63 µg/g FW, followed by 40 % B + 60 % R at 19.90 µg/g FW and 33 % B + 67 % R at 3.35 µg/g FW. The anthocyanin concentration in red leaf lettuce under 50 % B + 50 % R LED light treatment was 6.46 times greater compared to the anthocyanin concentration in red leaf lettuce under 33% B + 67% R LED light treatment, which suggests that the anthocyanin concentration increased with increasing B light percentage. This is because B light is responsible for accumulating plant antioxidant compounds due to the photostress induced on plants (Mao et al., 2022).

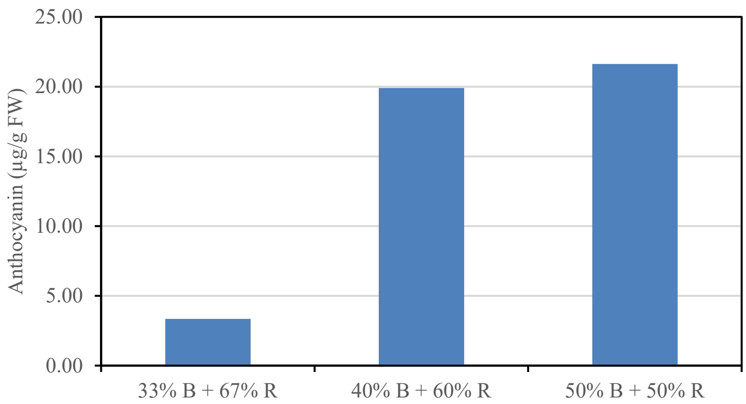

Figure 2: Effect of various B and R light percentages on anthocyanin concentration

The anthocyanin concentration of red leaf lettuce increased by 5.94 times when the B light percentage increased from 33 to 40 % and by 8.69 %, with a further increase of B light percentage to 50 %. This shows that the rate of increase in the anthocyanin concentration decreases with a further increase in the B light percentage, suggesting that a further increase in the B light percentage would not increase the anthocyanin concentration by a significant amount. Hence, there is an optimum B, and R light percentage as a further increase in the B light percentage will lead to a reduction in the growth parameters, including overall weight, shoot weight, root weight, root length, leaf width, and leaf length, without a significant increase in the anthocyanin concentration. It is important to note that the optimum light spectrum for anthocyanin concentration is plant-dependent. Reported findings from Vaštakaitė et al. (2015) demonstrated that the highest total anthocyanin concentration was obtained in tatsoi under 25 % B + 75 % R, in red pak choi under 100 % R and 8 % B + 92 % R, and in basil under 33 % B + 67 % R LED light treatment. Thus, it can be concluded that the effect of increasing B light percentage on the anthocyanin concentration varies among plant species.

4. Conclusions

The physical growth parameters of fish wastewater cultivated red leaf lettuces were significantly affected by varying the light spectrum. When the B light percentage increases from 33 to 50 %, the overall weight, shoot weight, root weight, root length, leaf width and leaf length were reduced by 91.49, 93.33, 88.24, 61.51, 66.67 and 71.34 % respectively. In terms of antioxidant levels, increasing B light percentage from 33 to 50% increases the anthocyanin concentration value by 6.46 times. This concludes that increasing B light percentage (reducing R light percentage) will detrimentally affect the red leaf lettuce physical growth but promotes the production of anthocyanin and thus, increases the antioxidant activity of red leaf lettuces. This is because R light generally influences plant growth by increasing the dry and fresh plant weight whereas B light promotes the accumulation of plant antioxidant pigments, photosynthetic function, chloroplast development, and chlorophyll formation.

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