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Photoperiod and light spectrum effects on growth, pigment and ascorbic acid content of *Lactuca sativa* cv. Fire Red under controlled growth environment

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Abstract

The application of Light Emitting Diodes (LEDs) in agricultural sector has become popular in recent years. LEDs can even be used in total absence of natural sunlight in raising indoor vegetables and ornamental plants. Appropriate light spectral regions must be studied according to crop species or varieties, especially for plants with high commercial values. The present experiment was conducted to determine the effects of monochromic LED of Red (R) and Blue (B), and their combinations at 1:1, 1:2 and 2:1,at 18 W and 240 V, on production of *Lactuca sativa* cv. Fire Red in controlled laboratory environment in total absence of natural light. Each LED irradiation treatment was assigned as sub plot with photoperiods (PPs) ranging from 12 h to 18 has main plots in a split plot design to study their combined effects on growth, pigment and ascorbic acid (AA) contents of this attractive red colour vegetable. Seedlings in moist jiffy blocks at one week after sowing were transferred to polyvinyl chloride (PVC) troughs of 5 cm diameter containing quarter strength Hoagland solution at electrical conductivity ranging from 1,100 to 1,300 µS/cm and pH 6-7. Plants were grown with nutrient film technique at a distance of approximately 8 cm below LED. Results obtained showed that R was not appropriate for growing this vegetable while analysis of variance indicated that plants irradiated with B gained the greatest plant height at five weeks at harvest, but these plants had the lowest number of leaves. Highest fresh weight (FW) as marketing attribute of this vegetable was obtained with 18 h PP. When combined 18 h PP with BR or 2BR, it was the best for production of *L. sativa* cv. Fire Red with the highest anthocyanin content.

Introduction

Plant needs light as one of the common ecological sources to grow. Light Emitting Diodes(LEDs) have great achievement as alternative light supplies for indoor plant cultivation under controlled environment (Folta *et al.*, 2005; Ilieva *et al.*, 2010; Yang *et al.*, 2012; Sirtautas *et al.*, 2014). LED innovation gave several benefits including low electricity consumption, long life, flexible light spectrum and intensity, low heat emission and high energy-conversion productivity (Schuerger *et al.*, 1997; Massa *et al.*, 2008; Morrow 2008; Yeh and Chung, 2009). Some commercial crops, for example, *Melissa officinalis* and *Ocimum basilicum* (Fraszczak *et al.*, 2014), *Capsicum annuum* (Schuerger *et al.*,1997), *Pisum sativum* (Wu *et al.*, 2007), *Lycopersicume sculentum* hybrid 'Raissa F1' (Brazaityte *et al.*, 2010) and *Lactuca sativa* (Kim, Goins, Wheeler *et al.*, 2004; Li and Kubota, 2009; Lin *et al.*, 2013; Ouzounis *et al.*, 2015) have already been proven under LEDswith promising results. Plant physiological processes of photosynthesis, photomorphogenesis, inflorescence induction, seed germination, phytocompound and biomass enhancement can be achieved by adjusting light wavelengths reaching the plants (Hahn *et al.*, 2000; Dewir *et al.*, 2006; Massa *et al.*, 2008; Pinho, 2008; Yeh and Chung, 2009; Vanninen *et al.*, 2010; Hogewoninget al., 2012; Gupta and Jatothu, 2013; Aasamaaand Aphalo, 2016; Dueck *et al.*, 2016).

Monochromatic Blue light (B) has been reported to enhance epidermal flavonoids (Hoffmann *et al.*, 2014). B was vital in the chloroplast advancement, chlorophyll synthesis, photomorphogenesis, stomatal opening and red pigment of anthocyanin biosynthesis (Bukhov *et al.*, 1995; Giliberto *et al.*, 2005; Xu *et al.*, 2012; Gorai *et al.*, 2014; Shi *et al.*, 2014; Ranade and Gil, 2016). However, the level of phenolics, antioxidant activities, vitamins, tannins and other secondary metabolites may be further increased by combining B with other light wavelengths (Tegelberg *et al.*, 2004; Wu *et al.*, 2007; Pallozzi *et al.*, 2013; Taulavuoria *et al.*, 2016). For example, Folta and Childers (2008) reported that the development of *Fragaria x ananassa* grown for 40 days in a plant growth chamber was higher when irradiated with

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combined B and Red light LED (R) contrasted with B or R alone. Yorio *et al*. (2001) reported that combination of Red Green Blue light spectrum (RGB) increased plant biomass and leaf area of *Lactuca sativa* cv. Waldmann's Green, while B did not affect biomass or photosynthesis of *Spinacea oleracea* cv. Nordic IV and *Raphanus sativus* cv. Cherriette; light requirements seem to be species specific.

Photoperiod (PP), on the other hand, indicates the length of day or presence of light for plants grown under LED at total absence of natural light. Plants generally use photoreceptor proteins of phytochromes or cryptochromes to detect length of light, as well as absence of light or darkness (Chia and Kubota, 2010; Craig and Runkle, 2016). Changes in relative length of night or darkness are naturally affected by geographical locality, distance from Equator and time of the year. Such changes have been reported to affect seed germination, plant growth and yield, while flowering of some temperate plant species depends on critical length of night (Miralles and Richard, 2000; Lefsrud *et al.*, 2006; Ali *et al.*, 2009; Xu *et al.*, 2016). For indoor plants, adequate amount of supplemental light supplied to plants, like that from LED, must be achieved through both the intensity and length of the light to fulfil photosynthesis and other plant physiological functions.

Although *L. sativa* has been reported successful with LED, the efficiencies of monochromic light spectral regions on certain variety of lettuce was rarely reported. In this study, a local lowland attractive red lettuce of *L. sativa* cv. Fire Red was grown with nutrient film technique using LED in laboratory in total absence of natural light. The effects of B and R, applied singly or in different ratios, combined with varying PPs towards growth, pigment and ascorbic acid (AA) contents of this vegetable under controlled laboratory environment were presented.

Materials and Methods

Location of study

The experiment was conducted in air-conditioned laboratory of Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Puncak Alam, Selangor (N 3°11.84', E 101°26.93'). The average temperature and relative humidity of the laboratory were 25±2°C and 55±5%, respectively. This red colour vegetable was also planted simultaneously in greenhouse of Faculty of Plantation and Agrotechnology within the same campus for comparison purpose.

Test material

Lactuca sativa cv. Fire Red seeds purchased from a local vegetable seed supplier were germinated on paper towel moistened with tap water in enclosed clear plastic containers. The plastic containers were placed at about 5 cm below Warm White LED (WW) at 14 h PP in the laboratory. On the next day, two germinated seeds were transferred to each jiffy block pre-moistened with tap water and allowed to grow for a week, also at about 5 cm below WW, to two-leaf stage with height of approximately 3-3.5 cm. These seedlings were then transferred to polyvinyl chloride (PVC) troughs for experimentation under varying PPs and light spectrums. At the same time, seedlings were also planted similarly in trough in the greenhouse. Thinning of seedlings was carried out after three days to have only one seedling to grow in each jiffy block.

Experimental procedure

All seedlings were grown for four more weeks using nutrient film technique at 15 cm apart in PVC troughs of 110 cm in length and 5 cm in diameter on wooden racks in laboratory, and on a bench in the greenhouse, respectively. Each trough was filled with 1.2 litres of quarter strength Hoagland solution to provide solution depth of approximately 4 cm in the trough. Nutrient solution was at electrical conductivity (EC) in the range of 1,100 to 1,300 µS/cm and pH of 6 to 7. EC and pH of the solution in troughs were checked twice weekly and adjusted accordingly when necessary. Troughs were also added with quarter strength Hoagland solution accordingly when the solution level dropped to below 3 cm. There was no other fertilizer applied throughout the experiment. There was also no solution circulation or aeration using pump throughout this vegetable production period.

Seedlings in laboratory were subjected to different PPs under B(450 nm) and R (660 nm), applied singly or at ratios of 1:1 (BR), 2:1 (2BR) or 1:2 (2RB), in the laboratory. In this study, 18W 240V LED lightings providing B, R, BR, 2BR and 2RB, respectively, were specially manufactured for this experiment on growing *L. sativa* cv. Fire Red. This PP in combination with light spectrum experimentation was carried out based on a split plot design. Different PPs of 12 h, 15 h and 18 h were main plots using three four-tier wooden racks. Within each main plot (rack), B, R, BR, 2BR and 2RB, respectively, were sub plots, each occupying a level (tier) of each rack at random. Every level of each rack was covered with blackout cloth at all sides to ensure that the plants in each level received only the assigned light spectrum. Seedlings were irradiated at a direct distance of

approximately 8 cm below LED lightings and the positions of LED lightings were adjusted higher accordingly as the plants grew taller and bigger. Each treatment was replicated six times, each with a single plant. Seedlings in the greenhouse, on the other hand, were all dependent on availability of natural sunlight and weather condition throughout the same period. Based on Hobo light/temperature datalogger placed in the greenhouse, 80% of the study period of four weeks was found with sunny days before noon time, providing sufficient light for this vegetable. The temperature in the greenhouse as recorded by the datalogger, however, could be as high as 34°C at noon during hot days.

Growth measurements

Height and number of leaves of each plant were recorded weekly. Height was measured from root collar to the highest level of the plant in its own form and appearance as affected by PP and light spectrum. Leaves with length of ≥ 2 cm, on the other hand, were taken note for the growth parameter on number of leaves. At five weeks after sowing, all plants were harvested by cutting them at their root collars. Fresh weight (FW) of each plant was recorded. FW of each plant without root system was weighed using an electrical balance.

Pigment content analysis

Analysis of chlorophyll and carotenoid contents of the leaves was carried out according to Lichtenthaler and Wellburn (1983). Fresh leaves were weighed to approximately 0.2 g, cut into 2 mm strips, and added with 20 ml of 99% aceton in test tubes. Pigment extraction using acetone was carried out in a refrigerator in the dark for 24 h. The leaf strips turned whitish when the pigments were extracted into acetone after 24 h. The chlorophyll and carotenoid concentrations in acetone were read using UV-VIS spectrophotometer (Sastec/ST-UV8000) at 470 nm, 662 nm and 645 nm. The amount of chlorophylls and carotenoids of the plants was calculated using the formulas below.

$$\text{Chlorophyll } a \text{ (Chl. } a\text{) (mg/100g FW)} = \frac{[11.75\ A_{662} - 2.35\ A_{645}] \times \text{dilution factor} \times \text{volume of product (ml)} \times 100}{1000 \times \text{weight of sample (g)}}$$

$$\text{Chlorophyll } b \text{ (Chl. } b\text{) (mg/100g FW)} = \frac{[18.61\ A_{645} - 3.96\ A_{662}] \times \text{dilution factor} \times \text{volume of product (ml)} \times 100}{1000 \times \text{weight of sample (g)}}$$

$$\text{Carotenoids (mg/100g FW)} = \frac{[1000\ A_{470} - 2.27\ \text{Chl. } a - 81.4\ \text{Chl. } b]/227 \times \text{dilution factor} \times \text{volume of product (ml)} \times 100}{1000 \times \text{weight of sample (g)}}$$

Anthocyanin was extracted from leaf strips of approximately 2 g in FW with 20 ml mixture of 95% ethanol and 1.5 *N* hydrochloric acid (HCl) (85:15, v/v). Extraction of anthocyanin was carried out in the dark for 24 h in a refrigerator. The anthocyanin content was then determined at 535 nm using the UV-VIS spectrophotometer. The amount of anthocyanin was calculated according to the formula below.

$$\text{Anthocyanin content (µg/g FW)} = \frac{A_{535} \times \text{volume of product (ml)} \times \text{dilution factor} \times 1000}{E^{1\%}_{1cm,\ 535} \times \text{weight of sample (g)}}$$

where,

$E^{1\%}_{1cm,\ 535}$ value for the acid – ethanol solvent measured in a 1 cm cell at 535nm is 98.2.

Ascorbic acid (AA) assay

AA content of the leaves was determined using indophenol titration method (Association of Vitamin Chemists, 1966; Favell, 1998). Solution of 3% meta-phosphoric acid (HPO_3) was prepared by dissolving 3g HPO_3 into 100 ml distilled water. The solution was kept cool under dark condition in the refrigerator to avoid oxidation. Standard solution of AA was prepared for calculation of dye factor by adding 100 mg AA into 100 ml 3% HPO_3 followed by dilution of 10 ml of this solution with 3% $HPO_3$to 100 ml. This gave AA standard solution of 0.1 mg/ml or 100 mg/l. To determine the dye factor, 5 ml standard solution of AA was added with 5 ml 3% HPO_3. Then, the mixture was titrated with 2,6-dichlorophenol indophenols until pale pink colour persisted in the mixture. The dye was prepared by dissolving 21 mg sodium bicarbonate into 75 ml distilled water followed by boiling until dissolved before dissolving 25 mg sodium 2,6-dichlorophenol-indophenols into the solution. Then, the solution was topped up to 100 ml using distilled water, cooled and put aside for use. Dye factor was calculated according to the formula:

$$\text{Dye Factor} = \frac{\text{amount of AA (mg)}}{\text{volume of titrate (ml)}} = \frac{0.5\ \text{mg}}{\text{volume of titrate (ml)}}$$

To determine AA content of the leaves, approximately 1.5 g leaves were weighed using an analytical balance. Then, the leaves were blended with 10 ml of cooled 3% HPO_3 followed by filtration using a funnel and filter paper. An amount of 3 ml filtrate was immediately titrated with 2,6-dichlorophenol indophenols until pale pink colour persisted in the mixture. AA content of the leaves was calculated as:

$$\text{AA content (mg/100 g FW)} = \frac{\text{volume of dye (ml)} \times \text{dye factor} \times \text{volume of product (ml)} \times 100}{\text{weight of sample (g)} \times \text{volume of sample for titration (ml)}}$$

Table 1. Mean plant height in cm

	Period (weeks)				
PP (h)	1	2	3	4	5
12	3.27[a]	6.23[b]	8.75[b]	14.08[a]	19.63[a]
15	3.23[a]	6.56[b]	9.23[b]	14.31[a]	22.92[a]
18	3.21[a]	7.44[a]	10.42[a]	15.50[a]	22.96[a]
Means with the same letter within PP are not significantly different at 5% probability level.					
LED					
B	3.25[a]	7.72[a]	11.44[a]	18.33[a]	26.86[a]
BR	3.22[a]	6.47[bc]	8.67[b]	13.81[b]	20.47[b]
2BR	3.31[a]	5.89[c]	8.33[b]	12.97[b]	19.28[b]
2RB	3.17[a]	6.89[ab]	9.42[b]	13.42[b]	20.72[b]
Means with the same letter within LED are not significantly different at 5% probability level.					
PP x LED F-test significance	ns	ns	ns	ns	ns

ns indicates P≥0.05.
Greenhouse plants grown simultaneously had mean height of 4.3, 6.4, 8.8, 14.8 and 18.3 cm at 1, 2, 3, 4 and 5 weeks after sowing, respectively.

Statistical analysis

Data of the laboratory plants were subjected to analysis of variance (ANOVA) and treatment means were compared using Tukey's Honestly Significant Difference (HSD) test at 5% level of significance. Minitab Version 17 was used for statistical analysis. Means of measurements of plants grown in the greenhouse, on the other hand, were calculated and used for reference purposes.

Results

There was no significant interaction between PP and LED treatment in plant height of *L. sativa* cv. Fire Red grown in laboratory (Table 1). Monochromic 660 nm (R) failed to grow this vegetable as it resulted in etiolated plants with narrowand long pale green to yellowish leaves for about three weeks and the plants died at four weeks after sowing. With plants raised under B, BR, 2BR and 2RB, PP significantly affected the height of plant on week two and three after sowing (Table 1). However, plant height became comparable later by four and five weeks, irrespective of 12 to 18 h PP. There was more than 35% increment in height from fourth to fifth week. In addition to PP, different light spectrums of B, BR, 2BR and 2RB also significantly affected plant height from second week to end of the experiment (Table 1). B had always resulted in greatest plant height throughout this indoor experimentation. When B combined with R, plant height was lower than that with B alone, measuring at approximately 20 cm, but did not differ significantly despite the difference in ratio of B to R at 1:1, 2:1 or 1:2 as studied. These plants were slightly taller than those grown simultaneously under natural light in the greenhouse for the same period (Table 1).

There was generally no significant difference in number of leaves among plants subjected to different PPs and light spectrums in laboratory at second and third week (Table 2). PP, however, interacted significantly with LED treatment in leaf development at fourth and fifth week. Plants receiving longer PPs of 15 h or 18 h in combination with BR, 2BR or 2RB had significantly more than 10 leaves as compared to those raised under B, which had less than 10 leaves, at fifth week. Monochromic B was hence, unsuitable for indoor vegetable production. Although B allowed the greatest plant height growth,it was the least efficient in production of leaves.When compared to greenhouse plants, *L. sativa* cv. Fire Red gained higher productivity indoor under LED, despite good weather with almost sufficient sunshine for this vegetable in the greenhouse during the same period (Table 2).

Plant FW at five weeks as marketing attribute of *L. sativa* cv Fire Red, on the other hand, was only affected by PP indicating that this vegetable is a light demanding species and therefore, PP was an important determinant for its indoor growth (Table 3). 18 h PP was the best for this leafy vegetable production, resulting in average single plant FW of more than 30 g while those subjected to 12 h and 15 h PP were 17.93 g and 25.54 g, respectively (Table 4). It was clear at this point that 12 h PP was insufficient for growing *L. sativa* cv. Fire Red in total absence of natural light. Plants in the greenhouse, on the other hand, could only gain mean FW of 14.9g by five weeks after sowing (Table 4).

Leaf chlorophylls and carotenoids were not affected significantly by PP and LED treatment as studied (Table 3). Plants grown with LED, however, had relatively lower mean chlorophyll

Table 2. Mean number of leaves

PP (h)	LED	Period (weeks) 2	3	4	5
12	B	5.00^{a}	5.83^{a}	5.33^{d}	7.33^{c}
	BR	5.00^{a}	6.00^{a}	6.67^{abc}	10.17^{abc}
	2BR	5.00^{a}	6.00^{a}	6.83^{abc}	9.83^{abc}
	2RB	5.00^{a}	6.00^{a}	6.83^{abc}	8.00^{bc}
15	B	5.00^{a}	6.00^{a}	5.33^{d}	7.50^{bc}
	BR	5.00^{a}	6.00^{a}	6.33^{bcd}	10.33^{ab}
	2BR	5.00^{a}	6.00^{a}	7.50^{a}	11.33^{a}
	2RB	5.00^{a}	6.00^{a}	7.00^{ab}	12.00^{a}
18	B	4.50^{b}	5.83^{a}	5.83^{d}	8.33^{bc}
	BR	5.00^{a}	6.00^{a}	7.50^{a}	12.17^{a}
	2BR	5.00^{a}	6.00^{a}	7.00^{ab}	11.33^{a}
	2RB	5.00^{a}	6.00^{a}	7.33^{ab}	10.00^{abc}

Means with the same letter within column are not significantly different at 5% probability level.
Greenhouse plant grown simultaneously had mean number of leaves of 4, 5, 5.3 and 6.7 at 2, 3, 4 and 5 weeks after sowing, respectively.

Table 3. F-values for variables affecting FW, pigment and AA contents at harvest

Source	FW	Chlorophyll content	Chl. *a*: Chl. *b*	Carotenoid content	Anthocyanin content	AA content
Block	12.94*	0.55^{ns}	4.63^{ns}	0.28^{ns}	0.88^{ns}	1.77^{ns}
PP	67.74***	0.93^{ns}	0.99^{ns}	0.52^{ns}	13.6*	1.69^{ns}
Block x PP	0.38^{ns}	2.09^{ns}	0.75^{ns}	2.59^{ns}	2.31^{ns}	0.57^{ns}
LED	1.98^{ns}	0.94^{ns}	1.73^{ns}	2.47^{ns}	12.72***	1.60^{ns}
PP x LED	1.80^{ns}	1.62^{ns}	0.52^{ns}	0.74^{ns}	2.07^{ns}	1.92^{ns}

ns, * and *** indicate no significant difference at 5%, significant difference at 5%and 0.1% level of significance, respectively.

and carotenoid contents of 74.57 mg/100 g FW and 12.02 mg/100g FW, respectively, as compared to those in the greenhouse with chlorophyll and carotenoid contents of 95.4 mg/100 g FW and 15.5 mg/100 g FW, respectively. In terms of Chl. *a*:Chl. *b*, the plants had ratio of 2.5-2.7, irrespective of the growth environment in laboratory under LED or in greenhouse.

Anthocyanin content of the leaves, on the other hand, was significantly affected by PP and LED spectral region, respectively (Table 3). 18 h PP and light spectral quality of BR or 2BR produced *L. sativa* cv. Fire Red with the highest anthocyanin content of more than 110.0 µg/g FW (Table 5). The amount of red pigments synthesized with these treatments was almost double of that obtained by plants subjected to only 12 h PP or B treatment. B alone seemed to inhibit biosynthesis of anthocyanins in this red colour vegetable besides resulting in less sturdy plants with lower leaf number. However, combination of B with R as BR or 2BR as mentioned was beneficial for expression of the red pigments of this colour vegetable. The expression of anthocyanin content in laboratory plants under BR or 2BR was also higher than that exhibited by the greenhouse plants with mean anthocyanin content of 107.6 µg/g FW (Table 5). Despite the importance of both B and R in biosynthesis of anthocyanins, R seemed to be needed in lower quantity for *L. sativa* cv. Fire Red to synthesis the red pigments as 2RB was found to reduce anthocyanin content in this vegetable as compared to BR or 2BR.

As for AA content of the leaves, PP and LED treatments as studied had no significant effect on this antioxidant content in *L. sativa* cv. Fire Red. The laboratory plants raised under LED had mean AA content of 64.55 mg/100g FW, which was relatively higher than that gained by the greenhouse plants with AA of 60.8 mg/100g FW.

Discussion

Growing vegetables or plants under LED or artificial light sources is generally different from that under natural sunlight with wide range of light spectrum. The varying wavelengths and photon energies from natural sunlight stimulate photosynthesis that subsequently affects all other related or distinctly related physiological processes and energy transport in plants in different degrees (Takahashi *et al.,* 2010; Hildner *et al.*, 2013). Plant's photosynthetic efficiency varies with the frequency and conversion rate of light, via certain light wavelengths, combined with the light intensity and PP, temperature, carbon dioxide and water availability and many other biotic and abiotic factors in a very complex manner (McCree, 1972; Lavergne and Trissl, 1995; Heo *et al.*, 2006; Kurilcik

Table 4.Mean plant FW at harvest as affected by PP

PP (h)	Mean FW (g)
12	17.93[c]
15	25.54[b]
18	30.29[a]

Means with the same letter are not significantly different at 5% probability level.
Greenhouse plant grown simultaneously had mean FW of 14.9 g at harvest.

et al., 2008; Trouwborst *et al*., 2016). As opposed to monochromic light source or combination of just two or a few light wavelengths provided by artificial light sources, for example LED, certain biological functions, pigment synthesis, secondary metabolite and antioxidant abundance in plants can hence be different, or certain physiological processes in plants can even fail when only certain light wavelengths are available (Chow *et al*., 2005; Hogewoning *et al.*, 2010; Schreiber *et al*., 2012).

In the current study, PP of less than 18 h provided by LED was not sufficient for optimum FW gain in *L. sativa* cv. Fire Red. Despite morphologically acceptable in terms of plant height and number of leaves when grown under combined B and R for PP of 12 h and 15 h, shorter PPs were not sufficient for photosynthesis and biomass production of this vegetable at cellular level resulting in plants of lower FW with lower market value. It is indeed a light demanding vegetable. Shen *et al.* (2014) also showed that longer PP application gave greater plant height and leaf number of *L. sativa* var. Dasusheng. Miralles and Richard (2000) described that constant longer PP reduced the production duration of *Hordeum vulgare* 'Arapiles', and semi-dwarf *Triticum aestivum* 'UQ 189' from sowing to double ridge. Xu *et al.* (2016) also supported that longer PP exposure to cultures of *Dunaliella salina* CCAP 19/30 was necessary for faster growth rates and higher cell densities.

In terms of light spectral requirements, B greatly affected the height of *L. sativa* cv. Fire Red to harvest the light more efficiently. Most of other researchers also reported that B alone contributed more in the height growth of plant. Wu *et al.* (2007) demonstrated that B LED was effective in increasing stem length of *Pisum sativum* seedlings, as compared with R or combined B with R. Another study carried out by Hernandez and Kubota (2016) also mentioned that B increased stem elongation in *Cucumis sativus* cv. Cumlaude.

Despite greatest plant height gain, B alone was inappropriate for production of *L. sativa* cv. Fire Red. This red lettuce needed both B and R for more leaf development and sturdier plant form while such combination was also essential for

Table 5.Mean anthocyanin content of leaves at harvest as affected by PP and light spectrum

PP (h)	Mean anthocyanin content (µg/g FW)
12	49.18[c]
15	86.93[b]
18	138.75[a]
Means with the same letter within PP are not significantly different at 5% probability level.	
LED	
B	57.27[b]
BR	114.62[a]
2BR	124.64[a]
2RB	69.96[b]

Means with the same letter within LED are not significantly different at 5% probability level.
Greenhouse plant grown simultaneously had mean anthocyanin content of 107.6 µg/g FW at harvest.

biosynthesis and expression of anthocyanins as one of the important attributes of this colour vegetable. Rather similar findings on BR requirements for plant growth and metabolite expressions were agreed by other researchers. Earlier study by Lian *et al.* (2002) described that BR enhanced the growth of *Lilium oriental* hybrid 'Pesaro'. Their results also demonstrated that manipulating quality of light changed the growth of this Lilium hybrid during the enlargement stage. Domurath *et al*. (2012) later suggested that combination of B and R was necessary to enhance the plant growth because B alone gave lower yield of photosynthesis.

In line with higher anthocyanin content synthesis in *L. sativa* cv. Fire Red in the current study, past research also found 4.3 times higher anthocyanin content on *Perilla frutescens* var. acuta Kudo raised under BR as compared to that in the green light (G) (Nishimura *et al*., 2009). In a later study carried out by Heo *et al.* (2012), anthocyanin accumulation was enhanced by BR and inhibited by B in *L. sativa* 'Ttuksum' and 'Jaju'. Anthocyanin content in leaves of *Oryza sativa* cultivar indica, IR1552, was the highest in RB but less in R and B alone, where that of the latter two was even lower than the red pigments gained by the plant grown in G (Chen *et al*., 2014). In terms of chlorophylls, some previous reports showed that the absorption spectrum of Chl. *a* and Chl. *b* was in the range of B and R, but different proportions of B and R lights could significantly affect plant growth and development (Brown *et al*., 1995; Kinoshita *et al*., 2001; Kim, Hahn, Heo *et al.*, 2004; Johkan *et al.*, 2010).

More recent study carried out by Ranade and Gil (2016) also mentioned that combined B and R increased biomass as well as the fibre dimensions, which rendered structural stability to *Pinus sylvestris* seedlings. In contrast, Wojciechowska *et al.* (2015) demonstrated that supplemental LED of higher

R as 9RB was suitable to increase the yield and simultaneously nutritional value of *Valerianella locusta* 'Nordhollandse' in greenhouse conditions. Light spectral requirements are indeed species or even cultivar specific. Such mechanisms of plant growth regulation by monochromic artificial irradiance, singly or in combination of two or a few limited wavelengths, are still poorly understood. Plant development and production have always been recognized as outcome of combined environmental attributes interacting with multiple plant internal factors simultaneously.

In the present study on *L. sativa* cv. Fire Red, monochromic B and R LED in combinations of 1:1 or 2:1 seemed to be a promising tool to replace natural light for production of this vegetable based on the advantages of greater FW and anthocyanin biosynthesis in this vegetable when referred to greenhouse plants. BR and 2BR irradiance was also acceptable for indoor production of this colour lettuce as such light spectrum did not affect its AA content, which is an important antioxidant for humans. Such findings are encouraging indoor production of this vegetable as a pesticide free food option in Malaysia under controlled environment despite her tropical climate with sufficient sunshine generally. Besides guaranteed shorter period of production, the application of low energy but long lasting LEDs also enables more efficient land use by means of vertical vegetable production using nutrient film techniques, especially in air-conditioned premises like supermarkets and hypermarkets. It also avoids opening of more farm lands and keeps the forests better for ecological services.

Conclusion

In total absence of natural light, combination of B and R in 1:1 or 2:1 at 18 h PP was effective in producing *L. sativa* cv. Fire Red of higher FW and anthocyanin content when referred to greenhouse plant. Such irradiance also gave appropriate plant morphology and AA content for pesticide free indoor production of this vegetable.

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