Mal J Nutr 30(2): 257-270, 2024

Effects of LED treatments on the growth and nutritional content of lettuce (*Lactuca sativa*) in a hydroponic vertical farming system

Mei Xin Koh & Ajit Singh*

School of Biosciences, University of Nottingham Malaysia Campus, Selangor, Malaysia

ABSTRACT

Introduction: LED-integrated hydroponic vertical farming system (HVFS) may address food security challenges arising from urbanisation, while antioxidants in vegetables may prevent cardiovascular disease. This study aimed to explore the LED effects on yield, antioxidant properties, and vitamin C content in Butterhead lettuce (BL) and Italian lettuce (IL). **Methods:** Three LED combinations were used: 77% red, 14% green, and 9% blue lights (77R:14G:9B); 7% red, 41% green, and 52% blue lights (7R:41G:52B); and 25% red, 67% green, and 8% blue lights (25R:67G:8B). The concentration and pH of nutrient solution in HVFS ranged from 0.878-2.061ppm and 6.0-6.6, respectively, with a 12:12 light-dark cycle. Chlorophyll and anthocyanin contents were measured using chlorophyll and anthocyanin meters, respectively. Total phenolic and vitamin C content were measured using Folin-Ciocalteu colorimetric assay. Total flavonoid and antioxidant activity were determined through aluminium chloride colorimetric assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) colorimetric assay, respectively. Completely randomised design was adapted with three replications. **Results:** BL exhibited greater yield, anthocyanin and chlorophyll contents, while IL had higher vitamin C content. Significantly (p<0.001) higher yields were produced under 77R:14G:9B and 25R:67G:8B. Significantly higher phenolic (p<0.001) and flavonoid (p=0.003) contents were produced under 25R:67G:8B. Vitamin C was significantly (p=0.037) higher under 25R:67G:8B than 7R:41G:52B. **Conclusion:** LED treatment with higher proportion of red light generated greater yield, whereas higher proportion of green light gave higher phenolic, flavonoids, and vitamin C accumulation. This study provided preliminary data on boosting crop yield and phytochemical contents, which may improve food self-production and population health.

Keywords: antioxidant, butterhead, food security, phytochemicals, yield

INTRODUCTION

Urbanisation is a complicated socio-economic process that modifies the built environment, changing previously rural towns into urban settlements while simultaneously shifting the spatial distribution of a population from rural to urban areas (United Nations, Department of Economic and Social Affairs and Population Division, 2019). The global urban population has increased from 0.75 billion in 1950 to 4.22 billion in 2018, and it is predicted to continue growing until 6.68 billion in 2050 (United Nations, Department of Economic and Social Affairs and Population Division,

*Corresponding author: Dr Ajit Singh
School of Biosciences, University of Nottingham Malaysia, Selangor, Malaysia
Tel: (6)012-2396105; Fax: (6)(03)89248167; E-mail: ajit.singh@nottingham.edu.my
doi: https://doi.org/10.31246/mjn-2023-0107

2019). With the increasing rate of urbanisation, more lands are diverted from agricultural production and used for urban development, resulting in less food production. At the same time, increasing population leads to higher demands for food and subsequently resulting in food insecurity. Therefore, light-emitting diode (LED)-integrated hydroponic vertical farming system (HVFS) can be considered as a way to solve this problem because it can be used to grow foods in nutrient solution on vertically stacked layers, producing higher yields than traditional farming. It has also been shown that greater yield could be produced under LED treatment than under natural sunlight (Chua *et al.*, 2020).

Besides, it was reported that about 32% of deaths worldwide in 2019 were attributable to cardiovascular disease (CVD) (World Health Organization, 2021). Plant-based foods are excellent sustainable food sources and they are rich and safe sources of natural antioxidants. Bioactive substances, such as polyphenols, flavonoids, anthocyanins, and chlorophylls, in plant-based foods are crucial as they may help to reduce the prevalence of CVD. Antioxidants could help control the oxidation of low-density lipoprotein, hence improving lipid profile and significantly control the emergence and progression of CVD (Ciumărnean *et al.*, 2020).

Whether growing plants indoors or outdoors, light is a crucial environmental component influencing plant growth and development. Therefore, LED technology can be used to improve the effectiveness of indoor plant growth where sunlight may be deficient. LED lights are more environmentally friendly than other artificial light sources because of their great light efficiency, low heat emission, and low power consumption. Also, combinations of various LED lights can deliver high flux and specialised wavelengths for plant culture, which trigger plant development (Monostori *et al.*, 2018). While light intensity affects photosynthesis the most, it was found that spectral composition plays a role as well in modifying carbon dioxide assimilation and electron transport processes for photosynthesis (Monostori *et al.*, 2018). However, the precise lighting condition, light intensity, and spectrum makeup necessary for the optimal growth and nutritional value of various plant species are still not well understood.

Most research done so far has focused on the importance of red (600-700nm) and blue (400-500nm) lights when used separately or in combination due to the high absorption by photosynthetic pigments at these wavelengths. For instance, a study conducted by Spalholz, Perkins-Veazie & Hernández (2020) has discovered higher total phenolic, anthocyanin, and chlorophyll contents in lettuce under combinations of red and blue lights than under sunlight. Green light (500-600nm) has recently been given a more important function to play. It is essential for fostering biomass formation in deeper leaf and lower canopy regions where other lights are deficient (Smith, McAusland & Murchie, 2017). Photosynthesis rate and fresh weight were found to be significantly greater when supplementing red-blue light with appropriate quantities of green light (Smith, McAusland & Murchie, 2017).

Lettuce is a popular greenhouse vegetable widely consumed and it serves as a model crop for studies on how plants react to light quality (Li & Kubota, 2009). According to Brazaitytė *et al.* (2022), it is one of the highly valuable crops grown in controlled environment agriculture, which is rich in fibre, minerals, various vitamins (vitamin C, vitamin E), bioactive compounds such as folate, and phenolic compounds like flavonoids and anthocyanins. In addition to its rich content and wide consumption, cultivating lettuce in a closed production system, such as HVFS,

may also contribute to the efforts to meet the world's rising food demand (Rehman *et al.*, 2017). Thus, implementing LED-integrated HVFS can help in achieving easier and faster food self-production, as well as promoting antioxidant intake, which is beneficial for a wide variety of non-communicable diseases (e.g., CVD) at the same time. The only barrier that limits the widespread implementation of LED-integrated agriculture is its high initial installation cost (Rehman *et al.*, 2017). Nonetheless, this cost may be justified if we take into account its long lifetime (Rehman *et al.*, 2017). A study has also estimated that the power consumption for eight hours a day of LED lights is just about 32.12 kWh/year (Khorasanizadeh *et al.*, 2015).

In this study, we hypothesised that growing crops on the LED-integrated HVFS would positively affect the fresh yields and antioxidant content of lettuce cultivars. Thus, the objectives of this experiment were to investigate the effects of different LED treatments on the yield, phytochemicals, and vitamin C contents of lettuce cultivars, namely Butterhead lettuce (BL) and Italian lettuce (IL).

MATERIALS AND METHODS

Experimental site

This research project was carried out at the LED-integrated HVFS located in Block C at University of Nottingham Malaysia. The average room temperature was about 27°C. From sowing until data collection, this experiment took place from 27 October 2022 until 21 February 2023.

Experimental materials

Seeds of BL (*Lactuca sativa var. capitata*) and IL microgreens (*Lactuca sativa L. var. ramose*) were obtained from Baba™ Smart Grow and New Trio Products, respectively. Fertilisers used were manufactured by SQM Holland BV and distributed by Agroniche Sdn Bhd. EC meter (TDS&EC, China) and pH meter (Danoplus Ltd., Hong Kong) used for monitoring the electrical conductivity (EC) and pH of nutrient solution in HVFS were obtained from the Biosciences Lab at University of Nottingham Malaysia.

Germination test

Ten-day germination test was done to determine the quality of seeds. Seeds of each type were placed onto moistened filter papers separately in different agar plates and observed for germination in darkness for two days, followed by eight days in the presence of light.

Nursery management

Nursery management was done to ensure that the seedlings could survive and be able to grow well when transplanted onto the HVFS. First, seeds of the two cultivars were soaked separately in two agar plates with water and kept in the chiller at 5°C for 24 hours. After 24 hours, the seeds were removed from the chiller and prepared for sowing. Each type of seed was sown in 70 germination sponge cubes with 2-4 seeds per cube. The seeds in sponge cubes were then allowed to germinate in a plastic tray indoors away from sunlight for the first two days, followed by outdoor germination under the exposure of sunlight. Watering and monitoring were carried out daily to prevent seedlings from drying out. At nine days after sowing (DAS), thinning was done to ensure only one seedling germinated in each cube. At 12DAS, 27 seedlings of each type were selected and transplantation onto the HVFS was carried out. Nine seedlings of each lettuce cultivar were put into individual net pots and transplanted onto each row of the three LED treatments at 12DAS.

Treatments

The LED light combinations and light intensity were measured using a spectral irradiance colorimeter (OHSP-350C, Hangzhou HopooLight and Color Technology Co. Ltd., China). Three

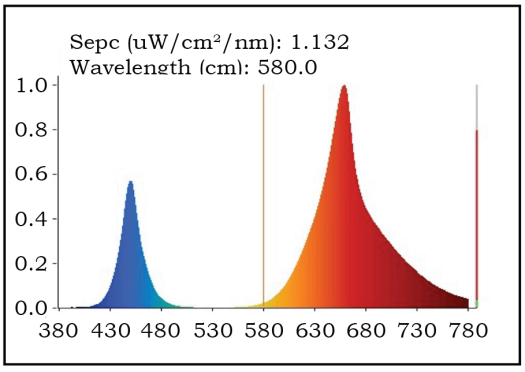
different LED treatments were set up, with combinations of red, blue, and green lights: 77% red, 14% green, and 9% blue lights (77R:14G:9B) with a light intensity of 250fc; 7% red, 41% green, and 52% blue lights (7R:41G:52B) with a light intensity of 145fc; 25% red, 67% green, and 8% blue lights (25R:67G:8B) with a light intensity of 635fc. 25R:67G:8B served as the control treatment. The emission spectra of each light treatment are shown in Figure 1.

Setup of HVFS

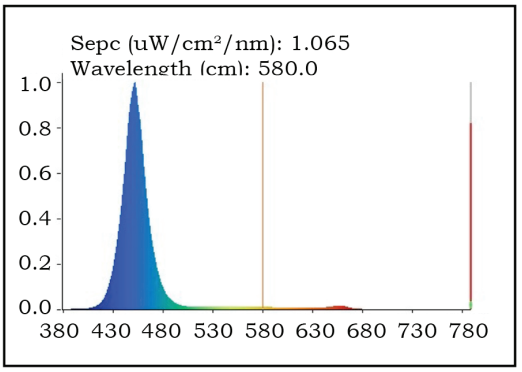
The HVFS was mainly made up of polyvinyl chloride (PVC) pipes, stainless steel rack, digital timers, LED light tubes, and water pump, with a reservoir tank at the bottom. All tubes were interlinked and eventually linked to the water reservoir tank to ensure that the water flowing down would be pumped up by the water pump again for nutrient solution recirculation. Fertilisers were added to supply nutrients for plant growth. About 6250 grams of solid Fertiliser A (Ultrasol® Calcium, SQM Holland BV, Westdorpe, Netherlands) and 6250 grams of solid Fertiliser B (Ultrasol® Lettuce Hydroponica, SQM Holland BV, Westdorpe, Netherlands) were dissolved in 25 litres of water, respectively, in two individual HDPE jerry cans as stock solutions. The compositions of fertilisers are shown in Table 1. 150mL of Fertiliser A (EC=5.434ppm) and 150mL of Fertiliser B (EC=9.747ppm) were added and mixed with about 50 litres of water in the reservoir tank, producing an electrical conductivity (EC) of 0.920ppm.

Maintenance of conditions in HVFS

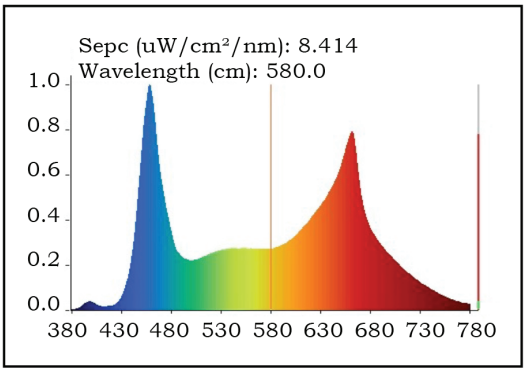
The HVFS was run indoors with an average temperature of 27±1°C. Digital timers were used to switch on the LED lights from 6 a.m. to 6 p.m., providing a 12-hour-light/12-hour-dark photoperiod cycle as study has shown that such photoperiod cycle promotes better lettuce growth (Hang *et al.*, 2019). The water pump was switched on for 24



a. 77R:14G:9B



b. 7R:41G:52B



c. 25R:67G:8B

Figure 1. Spectra distribution of three LED treatments on HVFS.
a. 77R:14G:9B, **b.** 7R:41G:52B, and **c.** 25R:67G:8B. 77R:14G:9b=77% red, 14% green, and 9% blue lights; 7R:41G:52B=7% red, 41% green, and 52% blue light; 25R:67G:8B= 25% red, 67% green, and 8% blue light.

Table 1. Content of fertiliser A (Ultrasol® Calcium) and fertiliser B (Ultrasol® Lettuce Hydroponica)

Content	*Fertiliser A*	*Fertiliser B*
% Total nitrogen (N)	15.5	7.8
% Nitric nitrogen	14.3	7.8
% Ammoniacal nitrogen	1.2	
% Calcium oxide (CaO) water-soluble	26.3	
% Water-soluble phosphorus pentoxide (P_2O_5)		9.6
% Phosphorus pentoxide (P_2O_5) soluble in neutral ammonium citrate and in water		9.6
% Water-soluble potassium oxide (K_2O)		40.9
% Water-soluble sulphur trioxide (SO_3)		11.3
% Water-soluble boron (B)		0.04
% Water-soluble copper (Cu)		0.006
% Copper (Cu) 100% chelated by EDTA		0.006
% Water-soluble iron (Fe)		0.207
% Iron (Fe) 100% chelated by EDTA		0.207
% Water-soluble manganese (Mn)		0.035
% Manganese (Mn) 100% chelated by EDTA		0.035
% Water-soluble molybdenum (Mo)		0.009
% Water-soluble zinc (Zn)		0.042
% Zinc (Zn) 100% chelated by EDTA		0.042

EDTA: Ethylenediamine tetraacetic acid

hours for continuous recirculation of the nutrient solution. The pH of nutrient solution was maintained between 6.0 to 6.6. The EC of nutrient solution was measured daily and increased according to plant growth from about 1ppm to 2ppm. Modification of EC was done by adding dissolved fertilisers or tap water to the nutrient solution. Increment of pH from acidic condition was done by adding 1M NaOH.

Chlorophyll and anthocyanin contents

Chlorophyll and anthocyanin contents were measured using a handheld chlorophyll meter (SPAD-502, Konica Minolta Inc., Tokyo, Japan) and a portable anthocyanin meter (ACM-200, Opti-Science Inc., NH, USA), respectively, at 2 weeks after transplanting (WAT), 3WAT, and 4WAT by clamping the sensor compartment of the meter onto three different leaves on each plant. Then, the average measurement was calculated.

Fresh weight

At 4WAT, plants were harvested. The roots of lettuce were removed using scissors, leaving only the shoot part. Fresh weight was determined immediately using an electronic weighing balance (GF-61000, A&D Ltd, Japan).

Sample extraction

BL and IL under each treatment were separated into individual paper bags and dried in an oven (Memmert GmbH + Co. KG, Schwabach, Germany) set at 60°C for five days and two days, respectively, until a constant weight was achieved. The dried BL samples were ground into powders using a blender (HR2096/01, Koninklijke Phillips N.V., Amsterdam, Netherlands), while dried IL samples were ground into powders using a set of mortar and pestle. The powders were stored in individual zip bags, with three bags of powders for each type of lettuce under each treatment. The zip bags were

stored in a freezer at -20°C until needed for the preparation of plant extracts. Preparation of plant extracts was done by dissolving the sample powder in 30% v/v of ethanol in 1:10 ratio (powder: solvent) (Hefnawy & Ramadan, 2013). In this case, 0.2 grams of powder was mixed with 2ml solvent and stirred using a spatula for even dissolution. 6 ml of distilled water was then added to dilute the homogenates. The mixture was poured into individual 50ml falcon tubes and incubated in hot water bath at 80°C for an hour, followed by centrifugation at 27°C and 10 000rpm for 10 minutes using a centrifuge (Eppendorf AG 22331, Eppendorf SE, Hamburg, Germany). After incubation, filtration was done using Filtres Fioroni filter papers grade 601 (150mm) to obtain the liquid extracts.

Total phenolic content

Total phenolic content was determined using Folin-Ciocalteu colorimetric assay modified from Javanmardi *et al.* (2003). 75µl of extracts was mixed with 75µl of 0.2M Folic-Ciocalteu reagent and left still for three minutes. Then, 100µl of 7.5% w/v aqueous sodium bicarbonate was added and incubated at room temperature for an hour. 250µl of each mixture was pipetted into a 96-well plate (SPL Life Sciences Co., Ltd, Korea) with flat bottom. Absorbance was measured using a microplate reader (Epoch, BioTek Instruments Inc, Vermont, USA) at 760nm, with distilled water as the blank and ethanol as the negative control. The standard calibration curve was generated using 50-300mg/L gallic acid solutions. Total phenolic content was expressed as microgram of Gallic Acid Equivalents (mg GAE)/g of extract.

Total flavonoid content

Total flavonoid content of sample extracts was determined using a modified colorimetric method by Heimler *et al.* (2005). 0.25ml of sample extract was added into a mixture of 0.75ml of 5% sodium nitrate, 0.15ml of 10% aluminium chloride, and 0.5ml of 1M sodium hydroxide solutions in a 5ml Eppendorf tube, followed by the addition of distilled water until the volume reached 2.5ml. Incubation of the mixture was done at room temperature for 5 minutes. The absorbance was measured at 520nm using UV spectrophotometer (Libra S12, Biochrom Ltd, Cambridge, England) against distilled water as the blank. Total flavonoid content (mg QE/0.25ml extract) was determined by comparing the absorbance rate of the sample with the standard curve of quercetin hydrate at different concentrations (0 to 10000µM).

Total vitamin C content

Modified from Jagota & Dani (1982), 0.5ml of extract was mixed with 0.8ml of 10% trichloroacetic acid, shaken vigorously and kept on ice for 5 minutes. Centrifugation was done at 4°C and 10,000rpm for five minutes. Next, 0.2ml of the mixture was diluted using 2ml of distilled water. 0.2ml of 0.2M Folin-Ciocalteu reagent was added and shaken vigorously. The mixture was left at room temperature for ten minutes and the absorbance was read at 760nm using a UV spectrophotometer (Libra S12, Biochrom Ltd, Cambridge, England) against distilled water as the blank. Vitamin C content was estimated using an ascorbic acid standard curve (0-0.03g/ml).

Antioxidant activity

Modified DPPH radical scavenging method from Zhang & Hamauzu (2004) was used to measure antioxidant activity. 0.2ml of sample extract was added into 1ml of 0.1mM DPPH. Absorbance was measured immediately at 521nm using a UV spectrophotometer (Ultrospec 8000, Biochrom Ltd, Cambridge, England) against distilled water as the blank, and after three minutes at room temperature.

The sample extract was replaced by distilled water to act as negative control.

Antioxidant activity was estimated using the following equation:

$$\text{Percentage Inhibition DPPH} = \frac{\text{Initial absorbance} - \text{Absorbance after 3 min}}{\text{Initial absorbance}} \times 100$$

Experimental design and statistical analysis

This experiment adapted a completely randomised design and triplicate measurements. Data were analysed using IBM SPSS Statistics for Windows version 28.0 (IBM Corp, Armonk, New York), with normally distributed data subjected to two-way ANOVA and Tukey's test at 5% significance level. Data of vitamin C content was subjected to $\log^{10}$ transformation before two-way ANOVA analysis. Chlorophyll and anthocyanin contents were not normally distributed and were subjected to Independent-Samples Kruskal-Wallis Test. Table 2 provides an overview of all the data collected.

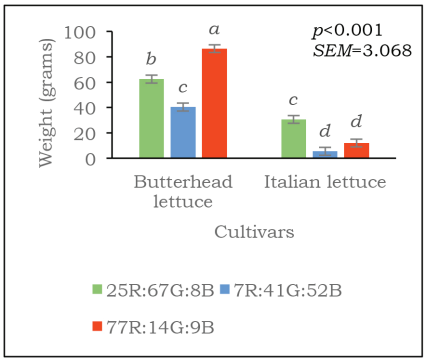
RESULTS

Fresh yield

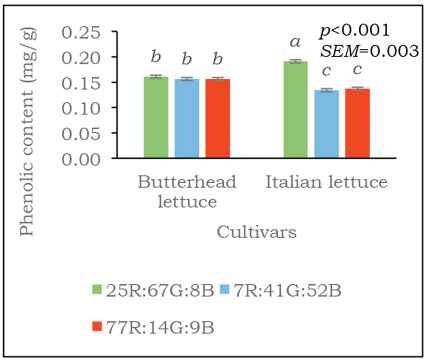
BL produced significantly (p<0.001) higher fresh yields than IL. Overall, 77R:14G:9B and 25R:67G:8B treatments generated significantly (p<0.001) greater fresh yields than 7R:41G:52B treatment. The interactions between cultivars and LED treatments were significant (p<0.001). As shown in Figure 2a, BL and IL responded differently to LED treatments, in which the highest fresh weight of BL was produced under the 77R:14G:9B treatment, while the highest fresh weight of IL was produced under the 25R:67G:8B treatment.

Chlorophyll content

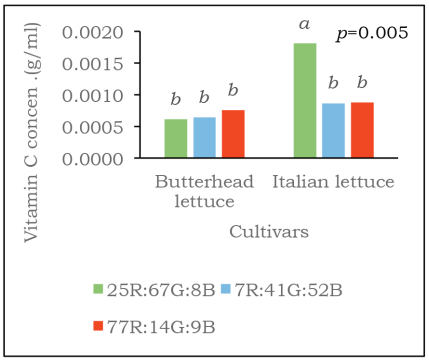
The chlorophyll content of BL was significantly (p<0.001) higher than IL. No significant effect of LED treatments on chlorophyll content was observed.



a. Fresh yield



b. Phenolic content



c. Vitamin C concentration

Figure 2. Effect of interaction between cultivar and LED treatment on **a.** fresh yield, **b.** phenolic content, and **c.** vitamin C concentration (n=3). Bars with the same letter are not significantly different using Tukey's test at 5% significance level. Error bars are SEM values. 25R:67G:8B= 25% red, 67% green and 8% blue light; 7R:41G:52B=7% red, 41% green, and 52% blue light; 77R:14G:9B= 77% red, 14% green, and 9% blue light. SEM=standard error of mean.

Table 2. Effect of cultivars, LED treatments, and their interactions on the fresh weight, chlorophyll content, anthocyanin content, phenolic content, antioxidant activity, vitamin C concentration and flavonoid content of lettuce after harvest (n=3)

Treatment	*Fresh weight (grams)*	*Chlorophyll content (SPAD)*			*Anthocyanin content (ACI)*			*Phenolic content (mg GAE)/g*	*Flavonoid content (mg QE/0.25ml extract)*	*Vitamin C concentration (g/ml)*	*Antioxidant activity (% inhibition DPPH)*
		2WAT	*3WAT*	*4WAT*	*2WAT*	*3WAT*	*4WAT*				
Cultivars (C)											
Butterhead lettuce (BL)	63.0[a]	37.4[a]	39.8[a]	39.2[a]	5.00[a]	5.20[a]	6.20[a]	0.158	4.26	0.000667[b]	15.2
Italian lettuce (IL)	16.0[b]	15.7[b]	16.7[b]	20.7[b]	1.90[b]	1.90[b]	2.50[b]	0.154	4.14	0.001109[a]	17.6
SEM	1.77	-	-	-	-	-	-	0.002	0.140	-	1.77
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.200	0.558	<0.001	0.369
LEDs											
77R:14G:9B	49.2[a]	24.4	25.4	31.7	3.35	3.35	4.25	0.147[b]	3.87[b]	0.000813[ab]	12.7
7R:41G:52B	22.8[b]	27.7	28.0	29.5	3.45	3.70	3.90	0.145[b]	3.92[b]	0.000745[b]	18.2
25R:67G:8B (Control)	46.4[a]	30.6	29.5	29.0	3.70	3.95	4.45	0.176[a]	4.81[a]	0.00105[a]	18.3
SEM	2.17	-	-	-	-	-	-	0.002	0.171	-	2.18
p-value	<0.001	0.431	0.717	0.522	0.696	0.764	0.699	<0.001	0.003	0.037	0.162
Interaction (*p*-value)											
C x LEDs	<0.001	-	-	-	-	-	-	<0.001	0.060	0.005	0.975

WAT: Week after transplanting; GAE: Gallic acid equivalent; QE: Quercetin equivalent; *SEM*: Standard error of mean.

Means in a column indicated by the same letter are not significantly different using Tukey's test at 5% significance level. Vitamin C content was not normally distributed and the data shown were back-transformed from $\log^{10}$ transformation method. Medians for chlorophyll and anthocyanin contents indicated by the same letter are not significantly different using the Independent-Samples Kruskal-Wallis test at 5% significance level. 77R:14G:9B= 77% red, 14% green, and 9% blue light; 7R:41G:52B=7% red, 41% green, and 52% blue light; 25R:67G:8B= 25% red, 67% green, and 8% blue light.

Anthocyanin content
Overall, BL contained significantly (p<0.001) higher concentration of anthocyanin than IL. However, the distribution of anthocyanin content was the same across all three LED treatments.

Phenolic content
The phenolic contents of BL and IL were not significantly different. The effects of different LED treatments on phenolic contents were significantly (p<0.001) different, with 25R:67G:8B treatment resulting in significantly higher concentration than both 77R:14G:9B and 7R:41G:52B treatments, which were not significantly different from each other. The interaction between types of cultivars and LED treatments was significant too. Referring to Figure 2b, BL was found to respond similarly under all three LED treatments, while IL under the 25R:67G:8B treatment was found to produce significantly higher phenolic concentration than 77R:14G:9B and 7R:41G:52B treatments.

Flavonoid content
The flavonoid contents of BL and IL were not significantly different. LED treatments had significant (p=0.003) effects on the average flavonoid content, with 25R:67G:8B treatment resulting in the highest content, followed by 7R:41G:52B and 77R:14G:9B treatments, which had no significant difference between them. Also, BL and IL responded similarly to different LED treatments.

Vitamin C concentration
IL had significantly (p<0.001) higher vitamin C than BL. LED treatments also had significant (p=0.037) effects on vitamin C accumulation in lettuce, with 25R:67G:8B treatment resulting in the highest content and 7R:41G:52B treatment resulting in the lowest content, while the effect of 77R:14G:9B treatment was not significantly different from them. The two cultivars were also found to respond significantly (p=0.005) differently to the treatments. As shown in Figure 2c, BL responded similarly to all three treatments, whereas IL under the 25R:67G:8B treatment produced significantly higher vitamin C than 77R:14G:9B and 7R:41G:52B treatments.

Antioxidant activity
Neither types of cultivars nor LED treatments showed significant effects on antioxidant activity and their interactions. Therefore, antioxidant activity was not influenced by all these factors.

DISCUSSION

Fresh yield
The high proportion of red light (R) (600-700nm) in the 77R:14G:9B treatment could promote the conversion of inactive phytochrome (red light-absorbing form) to active phytochrome (far-red light-absorbing form), which regulates the downstream components of the light signalling pathway and plays a role in initiating germination, de-etiolation, chlorophyll biosynthesis, and leaf expansion (Tripathi *et al.*, 2019). For 25R:67G:8B treatment, the high proportion of green light (G) could penetrate deep into the mesophyll layer of the leaf and increase carbon dioxide fixation rate, where R and blue lights (B) are deficient to drive photosynthesis (Smith *et al.*, 2017). The lower result of 7R:41G:52B treatment might be related to overstimulation of cryptochrome (CRY1) by B, hence inhibiting gibberellin biosynthesis, which is essential for plant growth (Matsuo *et al.*, 2019).

Chlorophyll and anthocyanin contents
Chlorophyll and anthocyanin contents were not affected by LED treatments. These results might be due to some limitations in this experiment such as light intensity and genetic variation. A

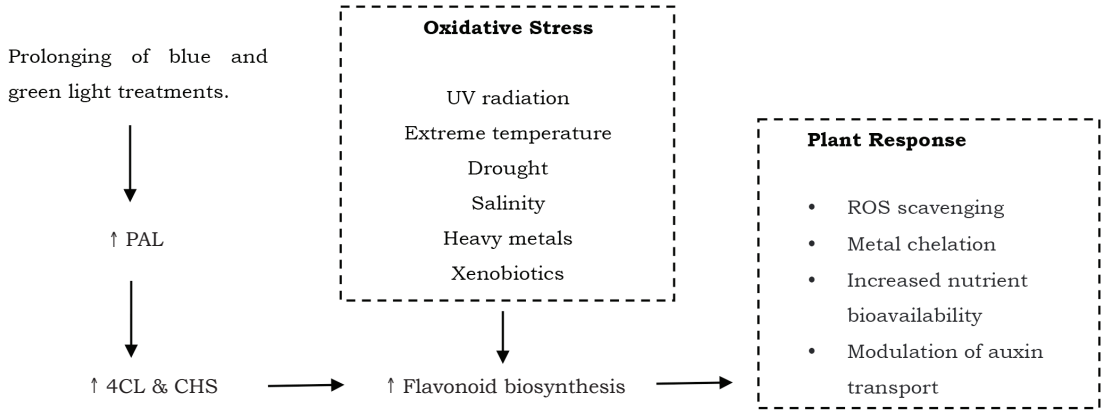


Figure 3. The impacts of light and oxidative stress on the production of flavonoids and plant response. Adapted from Liu *et al.* (2018) and Ghitti *et al.* (2022).
PAL=phenylalanine ammonia lyase; 4CL=4-coumaroyl CoA-ligase; CHS=chalcone synthase; ROS=Reactive Oxygen Species

study has shown that the accumulation of chlorophyll in lettuce was promoted by a light intensity of 350µmol/m^2/s to 600µmol/m^2/s at 23°C to 30°C, so the accumulation in our study might have been influenced by the low light intensity provided for the growth of lettuce (Zhou, Li & Wang, 2022). Meanwhile, the low, insignificant difference of anthocyanin present in both lettuce cultivars might be due to the lack of anthocyanin pigments, which are responsible for the purple or blue colour in green leaves.

Phenolic content

Higher phenolic content was observed under the 25R:67G:8B treatment. Our result was consistent with a study done by Fazal *et al.* (2016), which showed enhanced total phenolic production by G in a medicinal plant known as self-heal. According to Son *et al.* (2012), phenolic concentration and antioxidant capacity are effectively promoted by monochromatic B due to the activation of the phenylalanine ammonium lyase (PAL) gateway enzyme during the phenolic compounds' biosynthesis. Meanwhile, a study has shown that supplementation of G to B could help phenolic compound accumulation, as shorter G wavelengths of less than 530nm are also recognised as a part of the canonical cryptochrome and phototropin B response, while longer G wavelengths of about 570nm could counteract B cryptochrome activation and hence phenolic compound accumulation (Brazaitytė *et al.*, 2022). Therefore, higher phenolic content might have been induced as a G wavelength of less than 530nm was provided to the lettuce in this experiment. Shading by other plants on the HVFS might be a reason for the insignificant difference in BL.

Flavonoid concentration

Higher flavonoid concentration was found under the 25R:67G:8B treatment. B and G were shown to increase flavonoid biosynthesis through the mechanisms shown in Figure 3 (Liu *et al.*, 2018; Ghitti *et al.*, 2022). In this experiment, higher production of flavonoids was found under the 25R:67G:8B treatment than the 7R:41G:52B treatment, which might be due to the higher light intensity of the former treatment. Also, studies have shown that the production of flavonoids is induced by certain factors to protect against harsh environments (Figure 3) like drought, cold, heat, UV radiation, and

pathogens, acting as detoxifying agents, allelopathic compounds, and signalling molecules (Liu *et al.*, 2018; Ghitti *et al.*, 2022). The potential stress factor in our study might be the temporarily insufficient nutrients provided during 2WAT. Therefore, the additive effects of LED treatment with different oxidative stress factors might have resulted in different levels of flavonoid production, as obtained by other studies. A similar result was obtained by Klimek-Szczykutowicz *et al.* (2022) that a combination of 50% G, 35% R, and 15% B, which was similar to the 25R:67G:8B treatment in this experiment, had resulted in a significantly higher flavonoid concentration in watercress.

Vitamin C concentration

Higher vitamin C concentrations were found under the 25R:67G:8B and 77R:14G:9B treatments. Many studies have shown different results under different LED treatments, which might be due to different stress factors that have occurred. The stress factors that might have occurred in our study were suboptimal temperature (27±1°C) and light intensity. A study has shown higher vitamin C accumulation in sweet corn seedlings at a cultivation temperature of 40°C (Xiang *et al.*, 2020). Another study has also found higher vitamin C accumulation in tomatoes at higher light levels (Ntagkas *et al.*, 2019). In our study, 25R:67G:8B and 77R:14G:9B treatments provided stronger light intensities than the 7R:41G:52B treatment, which resulted in higher vitamin C accumulation.

Antioxidant activity

Antioxidant activity was not affected by LED treatments. Several research studies have also revealed the negligible impact of various lighting conditions on antioxidant properties. For example, the antioxidant potential of purple basil callus under different lighting conditions was not much different as compared to other similar studies conducted using DPPH assay (Nazir *et al.*, 2020). This might be due to certain limitations of the DPPH assay, in which the yellowish-green pigments or colours of the extract might have affected the light absorption in the spectrophotometer, hence interfering with the radical scavenging potential determination.

It might also be due to the development of both non-enzymatic and enzymatic antioxidant systems in plants to combat excessive reactive oxygen species. It has been established that LED light affects not only antioxidant contents, but also antioxidative enzymes, of which mechanisms are yet to be discovered (Adil, Haider Abbasi & Ul Haq, 2019). Since no test was conducted to measure the antioxidative enzymes in this study, the phenolic compounds, flavonoids, and vitamin C concentrations measured might not reflect the actual total antioxidant activity. When processed with heat and consumed, both non-enzymatic and enzymatic antioxidant compounds might be affected or reduced.

Strengths and limitations

This research has implemented an innovative approach to grow crops indoors in a vertical way, which has proven the practicality of the HVFS, which is both energy-efficient and space-saving. It has been carried out under a well-controlled, chemical-free, and pest-free environment, which significantly reduced the effects of various external factors that may be encountered using the normal farming method.

Despite the significant findings, several limitations should still be addressed. Firstly, the small sample size used might not represent the general growth or phytochemical contents of lettuce cultivars. We have adapted the sample size of n=3 due to practical constraints, such as resource availability and time constraint, though the experiment was conducted under controlled, homogeneous conditions.

Secondly, shading by self-leaves due to overgrowth might have affected photosynthesis and hence nutrient accumulation. Thirdly, tip burn on lettuce was observed due to insufficient calcium supplied, but calcium fertiliser was added once this was realised. Next, the temperature for plant growth might not be optimal as the experiment was conducted indoors with an average temperature of 27±1°C. A study conducted by Choi, Paek & Lee (2000) found that the optimum day growing temperatures for butterhead and leaf lettuces were 22 to 26°C and 20 to 24°C during the early and middle growth stages, respectively, while the optimum night temperature was 15 to 20°C. Also, the long storage time of samples after harvest might have caused some degree of phytochemical degradation.

CONCLUSION

In conclusion, this study observed that BL outperformed IL in terms of yield, chlorophyll and anthocyanin contents, while for vitamin C content, it was the opposite. Also, a higher R proportion (77R:14G:9B) positively affected the lettuce's fresh yield, while a higher G proportion (25R:67G:8B) positively affected phytochemical (phenolic, vitamin C, and flavonoid contents) accumulation. No significant difference was observed for chlorophyll content, anthocyanin content, and total antioxidant activity under different LED treatments.

To address the issue of food insecurity related to rising urbanisation rate, cultivating lettuce cultivars under the 77R:14G:9B or 25R:67G:8B treatment might aid in producing the greatest yield of crops. To address the issue of rising CVD, cultivating lettuce under the 25R:67G:8B treatment could aid in producing the greatest phenolic, vitamin C, and flavonoid contents. Thus, this study provided preliminary data for optimising LED treatment combinations for optimal plant growth and phytochemical content, which are essential for addressing food security and health issues. This study also added to the understanding of LED-integrated HVFS, and the inclusion of variable indicators has provided a more comprehensive knowledge of how LED treatments affect the quality of crops. Aside from LED-integrated HVFS, future research can also be done to look into the potential use of solar photovoltaic hydroponic system in order to achieve a more sustainable form of agricultural production.

Acknowledgement

This research was fully funded by School of Biosciences, University of Nottingham Malaysia. I would like to express my sincere gratitude to Dr. Ajit Singh for the great support and contributions throughout the study, which has been pivotal in the completion of this work.

Authors' contributions

Koh MX, the main investigator, conceptualised, designed, and conducted the study. Koh MX also led the data collection, data analysis and interpretation, drafted and reviewed the manuscript. Singh A contributed to the design of the study, data analysis and interpretation, drafting and review of the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

References

Adil M, Haider Abbasi BH & ul Haq I (2019). Red light controlled callus morphogenetic patterns and secondary metabolites production in Withania somnifera L. *Biotechnol Rep* 24:p. e00380.

Brazaitytė A, Vaštakaitė-Kairienė V, Sutulienė R, Rasiukevičiūtė N, Viršilė A, Miliauskienė J, Laužikė K, Valiuškaitė A, Dėnė L, Chrapačienė S, Kupčinskienė A & Samuolienė G (2022). Phenolic compounds content evaluation of lettuce grown under short-term preharvest daytime or nighttime supplemental LEDs. *Plants* 11(9):1123.

Choi KY, Paek KY & Lee YB (2000). Effect of air temperature on tipburn incidence of butterhead and leaf lettuce in a plant factory. In: Kubota C & Chun C (eds) Transplant Production in the 21st Century. Springer, Dordrecht. pp166–171.

Chua HS, Lai SW, Paramasivam S, Goh TT & Chen GC (2020). Effect of artificial night lighting on the growth of loose head lettuce in hydroponic system. *Sains Malays* 49(12):2891–2900.

Ciumărnean L, Milaciu MV, Runcan O, Vesa Ştefan C, Răchişan AL, Negrean V, Perné MG, Donca VI, Alexescu TG, Para I & Dogaru G (2020). The Effects of Flavonoids in Cardiovascular Diseases. *Molecules* 25(18):4320.

Fazal H, Abbasi BH, Ahmad N, Ali SS, Akbar F & Kanwal F (2016). Correlation of different spectral lights with biomass accumulation and production of antioxidant secondary metabolites in callus cultures of medicinally important Prunella vulgaris L. *J Photochem Photobiol B* 159:1–7.

Ghitti E, Rolli E, Crotti E & Borin S (2022). Flavonoids are intra- and inter-kingdom modulator signals. *Microorganisms* 10(12):2479.

Hang T, Lu N, Takagaki M & Mao H (2019). Leaf area model based on thermal effectiveness and photosynthetically active radiation in lettuce grown in mini-plant factories under different light cycles. *Sci Hortic* 252:113–120.

Hefnawy HTM & Ramadan MF (2013). Protective effects of Lactuca sativa ethanolic extract on carbon tetrachloride induced oxidative damage in rats. *Asian Pac J Trop Dis* 3(4):277–285.

Heimler D, Vignolini P, Dini MG, Vincieri FF & Romani A (2005). Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. *Food Chem* 99(3):464–469.

Jagota SK & Dani HM (1982). A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. *Anal Biochem* 127(1):178–182.

Javanmardi J, Stushnoff C, Locke E & Vivanco JM (2003). Antioxidant activity and total phenolic content of Iranian Ocimum accessions. *Food Chem* 83(4):547–550.

Khorasanizadeh H, Parkkinen J, Parthiban R & David Moore J (2015). Energy and economic benefits of LED adoption in Malaysia. *Renew Sustain Energy Rev* 49:629–637.

Klimek-Szczykutowicz M, Prokopiuk B, Dziurka K, Pawłowska B, Ekiert H & Szopa A (2022). The influence of different wavelengths of LED light on the production of glucosinolates and phenolic compounds and the antioxidant potential in vitro cultures of Nasturtium officinale (watercress). *Plant Cell Tiss Organ Cult* 149:113–122.

Li Q & Kubota C (2009). Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environ Exp Bot* 67(1):59–64.

Liu Y, Fang S, Yang W, Shang X & Fu X (2018). Light quality affects flavonoid production and related gene expression in Cyclocarya paliurus. *J Photochem Photobiol B* 179:66–73.

Matsuo S, Nanya K, Imanishi S, Honda I & Goto E (2019). Effects of blue and red lights on gibberellin metabolism in tomato seedlings. *Hort J* 88(1):76–82.

Monostori I, Heilmann M, Kocsy G, Rakszegi M, Ahres M, Altenbach SB, Szalai G, Pál M, Toldi D, Simon-Sarkadi L, Harnos N, Galiba G & Darko É (2018). LED Lighting – Modification of Growth, Metabolism, Yield and Flour Composition in Wheat by Spectral Quality and Intensity. *Front Plant Sci* 9:605

Nazir M, Ullah MA, Younas M, Siddiquah A, Shah M, Giglioli-Guivarc'h N, Hano C & Abbasi BH (2020). Light-mediated biosynthesis of phenylpropanoid metabolites and antioxidant potential in callus cultures of purple basil (Ocimum basilicum L. var purpurascens)'. *Plant Cell Tissue Organ Cult* 142(1):107–120.

Ntagkas N, Woltering E, Bouras S, de Vos RC, Dieleman JA, Nicole CC, Labrie C & Marcelis LF (2019). Light-induced vitamin C accumulation in tomato fruits is independent of carbohydrate availability. *Plants* 8(4):86.

Rehman M, Ullah S, Bao Y, Wang B, Peng D & Liu L (2017). Light-emitting diodes: whether an efficient source of light for indoor plants? *Environ Sci Pollut Res Int* 24(32):24743–24752.

Smith HL, McAusland L & Murchie EH (2017). Don't ignore the green light: exploring diverse roles in plant processes. *J Exp Bot* 68(9):2099–2110.

Son KH, Park JH, Kim D & Oh MM (2012). Leaf shape index, growth, and phytochemicals in two leaf lettuce cultivars grown under monochromatic light-emitting diodes. *Korean J Hortic Sci Technol* 30(6):664–672.

Spalholz H, Perkins-Veazie P & Hernández R (2020). Impact of sun-simulated white light and varied blue:red spectrums on the growth, morphology, development, and phytochemical content of green- and red-leaf lettuce at different growth stages. *Sci Hortic* 264:109195.

Tripathi S, Hoang QTN, Han YJ & Kim JI (2019). Regulation of photomorphogenic development by plant phytochromes. *Int J Mol Sci* 20(24):6165.

United Nations, Department of Economic and Social Affairs and Population Division (2019). *In: World Urbanization Prospects: The 2018 Revision (ST/ESA/SER.A/420).* From https://population.un.org/wup/publications/Files/WUP2018-Report.pdf [Retrieved March 11 2023].

WHO (2021). *In: Cardiovascular diseases (CVDs).* From https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) [Retrieved March 11 2023].

Xiang N, Hu J, Wen T, Brennan MA, Brennan CS & Guo X (2020). Effects of temperature stress on the accumulation of ascorbic acid and folates in sweet corn (*Zea mays* L.) seedlings. *J Sci Food Agric* 100(4):1694–1701.

Zhang D & Hamauzu Y (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chem* 88(4):503–509.

Zhou J, Li P & Wang J (2022). Effects of light intensity and temperature on the photosynthesis characteristics and yield of lettuce. *Hortic* 8(2):178.