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Effects of Light-Emitting Diode Light Irradiance Levels on Yield, Antioxidants and Antioxidant Capacities of Indigenous Vegetable Microgreens

Bhornchai Harakotr [1,*], Sophit Srijunteuk[1], Panumart Rithichai[1] and Sompong Tabunhan[2]

[1] *Department of Agricultural Technology, Faculty of Science and Technology, Thammasat University, Pathum Thani 12120, Thailand*
[2] *Chulabhorn International College of Medicine, Thammasat University, Pathum Thani 12120, Thailand*

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ABSTRACT

Light-Emitting Diode (LED) lights allow specific wavelengths to be selected. These may be associated with microgreen growth, nitrate accumulation, and synthesis of bioactive compounds. The objective of this study was to assess the effect of LED irradiance on the yield, antioxidant production, and antioxidant capacity of microgreens from five traditional vegetables: rat-tailed radish (*Raphanus sativus* Linn var. *caudatus* Ale.), water convolvulus (*Ipomoea aguatica* Forsk), red holy basil (*Ocimum sanctum* L.), dill (*Anethum graveolens* L.), and lemon basil (*O. africanum* Lour). Samples were grown on a peat substrate in a controlled environment (8-12 days from sowing to harvesting, 25±2 ºC and 16-h photoperiod, 0.05% CO_2 concentration, and 60±2% RHs). Light irradiance levels of 330, 220, and 110 µmol.m^{-2}.s^{-1} photosynthetically active flux density (PPFD) were compared, with fluorescence lighting as control. The different species showed different responses to irradiance levels. Irradiance at 330 µmol.m^{-2}.s^{-1} PPFD was found to be optimal for growth and accumulation of bioactive compounds by water convolvulus, red holy basil, dill, and lemon basil microgreens, producing the greatest dry weight, total phenolic and flavonoid content, and ABTS and DPPH free radical scavenging. Rat-tailed radish microgreen was not significantly responsive to the irradiance level. We recommend the use of LED lighting to enhance productivity and promote higher production of bioactive compounds in indigenous vegetable microgreen cultivation.

Keywords: Artificial lights; PPFD; Phenolic compounds; Antioxidant activity; Microgreens

***Corresponding author:** harakotr@tu.ac.th **doi:** 10.14456/scitechasia.2019.21

1. Introduction

Interest in functional foods is increasing, driven by changing consumer tastes and growing awareness of the health and nutritional benefits of such foods and their potential for disease prevention [1]. Microgreens are a new class of specialty crop, defined as the tender, immature shoots from the seeds of vegetables, herbs, flowers, and cereals. Microgreens are generally harvested above the soil, at the base of the hypocotyls, as soon as the first pair of true leaves appear and when the cotyledons are fully expanded but still turgid. This is usually within 7-12 days of seed germination, with the exact time depending on the species and growing conditions [2]. Microgreens have become popular with health-aware consumers and are particularly suited to urban areas, given their short production cycle and small growing space were required. Many studies have identified microgreens as rich sources of bioactive compounds including minerals, phylloquinone, β-carotene, α-tocopherols, and phenolic compounds. Their nitrate and ammonium content is lower than that of mature plants [2-4].

Growing conditions play a significant role in the growth, yield, quality, and bioactive compound accumulation of microgreens. Environmental factors such as light levels, watering, temperature, and supply of elicitors and nutrients are important in the production of high-quality crops [5, 6]. In particular, microgreens may be biofortified by raising the spectra and irradiance levels of lighting, as this influences physiological changes and secondary metabolite production [7, 8]. Microgreens are cultivated in fields, greenhouses, and controlled environments or houses, the latter requiring an artificial light source. Light emitting diodes (LEDs) are a rapidly developing energy efficient technology for agriculture. Combining LEDs with red, blue, and far red wavelengths have been reported to enhance seed and microgreen production [2, 7]. LED light supplementation promotes growth, yield, and the production of bioactive compounds in a range of microgreens [8, 9]. Samuoliene et al. [8] reported that 330 $\mu mol.m^{-2}.s^{-1}$ PPFD irradiance is optimal for growth and bioactive compound formation in *Brassica* microgreens. They demonstrated increases in anthocyanin and phenolic production, enhanced free radical scavenging, and reduced nitrate content. In contrast, high light levels induced mild photooxidative stress that had negative impacts. Indigenous vegetables constitute a repository of materials for microgreen production [4, 10, 11]. However, little information is available on the optimal conditions and irradiance levels for growth and bioactive compound production by different traditional vegetable species. Therefore, the objective of the current study was to assess the effect of LED irradiance levels on the yield, antioxidant production, and antioxidant activity of some traditional vegetable microgreens.

2. Materials and Methods

2.1 Plant material and culture

Five Thai indigenous vegetables were investigated: rat-tailed radish (*Raphanus sativus* Linn var. *caudatus* Ale.), water convolvulus (*Ipomoea aguatica* Forsk), red holy basil (*Ocimum sanctum* L.), dill (*Anethum graveolens* L.), and lemon basil (*O. africanum* Lour.) were selected based on their antioxidant and antioxidant capacity levels (unpublished). Samples were grown on a sterilized peat substrate of pH 5.5-6 (Klasmann-TS3, Germany) in plastic vessels (10x15x6 cm) for 8-12 days from sowing to harvesting. Depending on seed size and weight, 3-5 g were seeded per box. The microgreens were watered daily using a small hand sprayer.

2.2 Lighting conditions

Experiments were performed in a controlled environment room with inner

dimensions 4x3x3 m in the Faculty of Science and Technology, Thammasat University, Pathum Thani, Thailand. Trays were placed on stainless steel growing racks with a single vertical layer. The entire rack covered an area of approximately 1.2 x 0.8 m and stood 1.8 m high. Each light treatment used four replicate batches per species. Growing conditions were as follows: a constant temperature of 25±2 ºC, 16-h photoperiod, 0.05% CO_2 concentration, and relative air humidity (RHs) of 60±2%. Average room temperatures and RHs were logged every 15 min by a HOBO data logger (Onset Computer Corporation, USA).

LED units were designed following Samuoliene et al. [8]. They comprised LEDs with emission wavelengths in the blue (I=445 nm), red (I=638 and 665 nm), and far-red (I=735 nm) regions (1154*15.8*1.0-LM-20S1B, Grows Laboratory, Thailand). The irradiated area was approximately 0.6 m^2. A range of irradiance levels, expressed as photosynthetic photon flux densities (PPFD), were set for each lighting unit (110±1, 220±1, and 330±1 $\mu mol.m^{-2}.s^{-1}$, Table 1). Fluorescent bulbs (45±2 $\mu mol.m^{-2}.s^{-1}$) were used as the control. The PPFD irradiance level was measured and regulated using a Line Quantum Sensor (LI-191R, LI-COR, USA).

Table 1. Combinations of light-emitting diodes and photosynthetic photon flux densities (PPFD).

PPFD ($\mu mol.m^{-2}.s^{-1}$)	Blue (445nm)	Red (638nm)	Red (665nm)	Far-red (735nm)
330±1	25	136	166	3
220±1	17	90	111	2
110±1	8	45	55	1

Note: Based on Samuoliene et al. [8].

2.3 Sampling

At harvesting time (8-12 days after seeding, depending on the species), microgreen stems with cotyledons were gently cut. The plant specimens were weighed and immediately deep-frozen in liquid nitrogen to stop enzymatic activity, then lyophilized until dry. Dried samples were finely ground in a sample mill (Mill CM 290 Cemotec™, Switzerland), sieved through a 60-mesh screen, thoroughly mixed, and stored at -20 ˚C until analysis. Dry weight was recorded by freeze-drying, following Xia et al. [11].

2.4 Antioxidant and antioxidant capacity Analysis

Antioxidants were extracted from the microgreen powders using a modified method of Harakotr et al. [12] . Approximately 2 g of sample were added to a flask containing 25 mL of 70% aqueous acetone acidified by the addition of 0.01% HCl extracts. The flask was shaken on a platform shaker at room temperature for 2 h. After centrifugation at 4,000 rpm and 4 °C for 10 min, the supernatant was collected. Extraction was then repeated two further times. Supernatants were pooled and evaporated at 40 °C under vacuum. The residue was re-dissolved in 25 mL of 80% ethanol. Extracts were stored at -20 °C until chemical analysis.

The total phenolic content was determined using the Folin–Ciocalteu method, as used by Harakotr et al. [12]. Aliquot of extract was mixed with 100 µL of Folin–Ciocalteu reagent and allowed to stand at room temperature for 5 min, followed by the addition of 30 µL of 20% Na_2CO_3 solution. The mixture was allowed to stand in the dark for a further 2 h, and absorbance was measured at 765 nm (Shimadzu mod. UV-128, Japan). The total phenolic content of the extracts was calculated and expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/ g DW) , based on the gallic acid standard curve.

The total flavonoid content was determined using the colorimetric method of Kubola and Siriamornpun [13], with slight modification. Briefly, aliquot of the extract was made up to 2.25 ml with distilled water, mixed with 0.15 mL of 5% $NaNO_2$ solution,

then 0.3 mL of 10% $AlCl_3 \cdot 6H_2O$ solution was added and the mixture was incubated for a further 5 min before 1.0 mL of 1 M NaOH was added. The solution was mixed thoroughly using a vortex mixer. Absorbance was measured immediately at 510 nm (Shimadzu mod. UV-128, Japan). Results were expressed as µg catechin equivalent per 1 g of dried weight (µg CE/g DW).

The two most common radical scavenging assays were conducted using 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, following Harakotr et al. [12]. The ABTS and DPPH free radical scavenging capacities were expressed as % inhibition of activity, and were calculated as follows:

$$\frac{(A_o - A_e)\ x100}{A_o}$$

where A_o = absorbance without extract
A_e = absorbance with extract

2.5 Statistical analysis

The data were analyzed by the means of the four replicates ± standard deviation. The data were subjected to one-way analysis of variance (ANOVA) and means within each species were compared with LSD at $p \leq 0.05$ using the STATISTIX 9.0 software.

3. Results and Discussion

3.1 Effect of LED light irradiance levels on yield

The microgreen yield varied from species to species, but was also affected by the LED irradiance level (Fig. 1). No significant difference was found in the dry weights of rat-tailed radish and dill. Significant differences were found with other species. The 330 µmol.m^{-2}.s^{-1} PPFD irradiance level was most efficient in increasing the dry mass of microgreens from water convolvulus (1.66 times control), red holy basil (1.90 times), and lemon basil (1.95 times). Growth and photosynthetic performance can be improved through stimulating morphological and physiological responses by increasing the PPFD irradiance level [15]. Optimization of light irradiance intensity contributes to CO_2 fixation and controls stomatal opening [16]. This weak response of photosynthetic pigments to PPFD irradiance levels may be related to the development stages of growth, as microgreens are very young vegetables with cotyledons and the first true leaves [14].

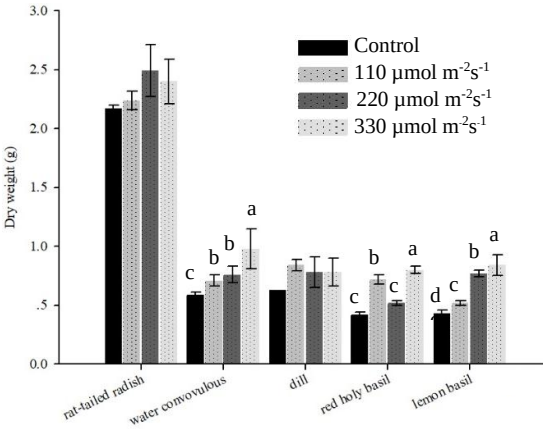

Fig. 1. Effect of LED light intensity on dry weight of indigenous vegetable microgreens

Note: Within each vegetable species, means with different letters are significantly different (P<0.05).

3.2 Effect of LED light irradiance levels on total phenolic and flavonoid contents

Different irradiance levels were associated with different total phenolic contents (Fig. 2). Total phenolic content accumulation in the rat-tailed radish microgreen was not significantly correlated with PPFD irradiance levels**.** In water convolvulus microgreen, total phenolic content under 220 µmol.m^{-2}. s^{-1} was lower than control, though no statistically significant difference was found under 110 or 330 µmol.m^{-2}.s^{-1}. The increased bioactive compound concentrations under low- and high-light irradiance levels may reflect rapid adaptation to suboptimal lighting conditions [7]. However, microgreens from three

species (dill, red holy basil, and lemon basil) showed greater total phenolic accumulation at 110-330 µmol $m^{-2}.s^{-1}$ PPFD irradiance than control**.** At the highest PPFD irradiance level (330 µmol.$m^{-2}.s^{-1}$) total phenolic accumulation was significantly increased in dill (2.51 times control) and red holy basil microgreens (10**.**20 times). This finding was in agreement with a previous study, which reported that a 330 µmol.$m^{-2}.s^{-1}$ light irradiance level increased the total phenolic contents of *Brassica* microgreens [8] . The irradiance level is involved in both regulations of photosynthetic processes and production of bioactive compounds [7**]**. Lobiuc et al. [16] reported that the effect of light intensity on synthesis of compounds by *O. basilicum* L**.** microgreens differed among species. In our study, the phenolic content of lemon basil microgreens grown under LED lighting was 3.09-3.48 times greater than control, though no significant difference was found between different light intensities**.** An increased light irradiance level was associated with an increase in the phenolic compound content of these microgreens**.**

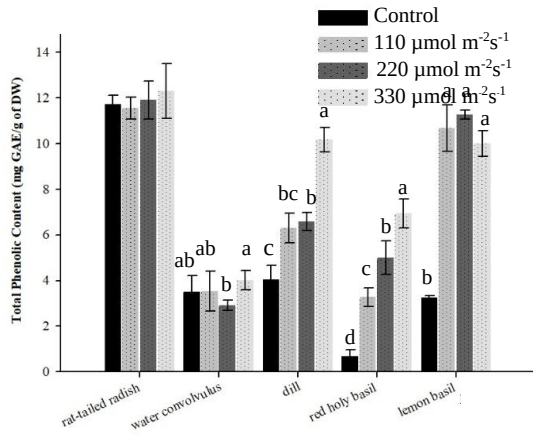

Fig. 2. Effect of LED light intensity on total phenolic content of indigenous vegetable microgreens.

Note: Within each vegetable species, means with different letters are significantly different (P<0.05).

Total flavonoid content was also associated with the irradiance level (Fig**.** 3). Rat-tailed radish microgreen grown under LED lighting had a reduced flavonoid content, though when the level was below 220 µmol.$m^{-2}.s^{-1}$ PPFD irradiance, no significant difference was found with control. Water convolvulus and dill microgreens produced the highest total flavonoid content at an irradiance level of 330 µmol $m^{-2}.s^{-1}$. At lower irradiance levels, no difference was found with control**.** The total flavonoid content of red holy basil microgreen grown under LEDs was 1.23-1.37 times greater than the control, though no significant difference was found between different intensities. This was attributed to anthocyanin accumulation (data not shown).

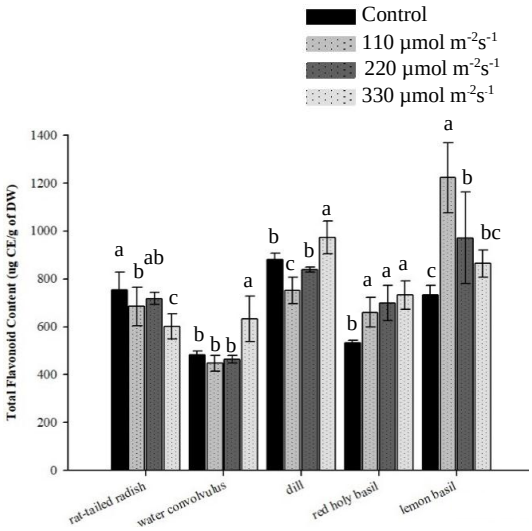

Fig. 3. Effect of LED light intensity on total flavonoid content of indigenous vegetable microgreens.

Note: Within each vegetable species, means with different letters are significantly different (P<0.05).

The PPFD irradiance level has been found to be involved in the regulation of anthocyanin biosynthesis chalcone synthase **(***CHS***)** and dihydroflavonol-4-reductase **(***DFRA***)** gene expression **[**5**]**. For production of bioactive compounds, high light intensities have been reported as optimal when growing vegetable sprouts in

controlled environments such as indoor cultivation [17]. Lemon basil microgreen was found to have higher total flavonoid content than control when cultivated under LED light, though higher PPFD irradiance levels tended to decrease the total flavonoid content. Our results are consistent with those of Fu et al. [18], who suggested that light efficiency is highest at low irradiance levels.

Table 2. Effect of LED light intensity on antioxidant capacity (%inhibition) of indigenous vegetable micro greens.

Microgreens	PPFD ($\mu mol.m^{-2}.s^{-1}$)	Antioxidant capacities	
		ABTS	DPPH
rat-tailed radish	Control	41.00±1.66	42.80±2.00^{b}
	110	39.75±1.80	42.74±1.63^{b}
	220	42.29±2.02	46.16±0.056^{a}
	330	42.29±1.63	45.96±1.83^{a}
	F-test	ns	**
water- convolvulus	Control	11.21±0.55^{b}	22.86±0.44^{b}
	110	12.21±1.90^{b}	23.11±0.67^{b}
	220	13.07±1.44^{b}	24.16±1.65^{a}
	330	18.89±0.84^{a}	31.74±0.80^{a}
	F-test	**	*
dill	Control	9.86±1.17^{d}	15.34±1.52^{c}
	110	20.04±2.73^{c}	24.91±4.97^{b}
	220	24.36±1.58^{b}	29.10±2.01^{ab}
	330	29.00±1.35^{a}	33.14±1.28^{a}
	F-test	**	**
red holy basil	Control	19.50±0.83^{d}	25.8±1.52^{c}
	110	30.21±4.29^{c}	38.42±5.07^{b}
	220	39.52±0.44^{b}	49.44±4.86^{b}
	330	50.95±3.44^{a}	68.26±14.18^{a}
	F-test	**	*
lemon basil	Control	19.11±1.46^{b}	27.80±1.08^{c}
	110	37.83±7.54^{a}	51.68±7.73^{b}
	220	39.14±7.28^{a}	60.93±9.82^{a}
	330	43.68±5.41^{a}	62.55±5.50^{a}
	F-test	*	**

Note: *,** significant difference at $p < 0.05$ and 0.01 level, respectively.
Within the vegetable species, means in the same columns with different letters are significant at $p < 0.05$ level.

3.3 Effect of LED light irradiance levels on antioxidant capacity

The irradiance level was associated with the antioxidant characteristics of the Thai traditional microgreens (Table 2). For ABTS free radical scavenging in rat-tailed radish microgreen, no significant differences were found between the PPFD irradiance level and control. However, cultivation under 220 and 330 $\mu mol.m^{-2}.s^{-1}$ PPFD irradiance promoted DDPH free radical scavenging. The highest PPFD irradiance level was associated with increased ABTS and DPPH free radical scavenging in water convolvulus microgreen. However, no significant difference in DPPH free radical scavenging was found between 220 and 330 $\mu mol.m^{-2}.\ s^{-1}$ PPFD irradiance. The

antioxidant capacities of dill and red holy basil microgreen were significantly increased when grown under LEDs, though to different extents. The greatest antioxidant capacities were found at 330 $\mu mol.m^{-2}.s^{-1}$ PPFD irradiance. Lemon basil grown under LED light showed greater antioxidant capacity than control. No significant differences in DPPH free radical scavenging were found among LED treatments, though the highest PPFD irradiance level was associated with enhanced scavenging. This may be because, when exposed to high light irradiance, photostress activates photoprotective mechanisms, which in turn affect secondary metabolites and free radical scavenging [8]. In the case of Chinese kale sprouts, the use of LED lighting has been recommended for improvement of nutritional and phytochemical properties, as well as enhancing antioxidant capacities [19].

4. Conclusions

The highest (30 $\mu mol.m^{-2}.s^{-1}$) PPFD irradiance level is recommended when growing water convolvulus, red holy basil, dill, or lemon basil microgreens, as this maximized dry weight, total phenolic content, total flavonoid content, and ABTS and DPPH free radical scavenging**.** At the lowest (110 $\mu mol.m^{-2}.s^{-1}$ PPFD) irradiance level, dry weight, antioxidant accumulation, and antioxidant capacity were suppressed. Rat-tailed radish microgreen showed no significant response to the light irradiance level.

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