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Volatile oil concentration and growth of thyme (*Thymus vulgaris* L.) plants responded to red to blue light ratios

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Abstract

The objective of this study was to evaluate the growth and volatile oil concentrations of thyme (*Thymus vulgaris* L.) plants under different red to blue light ratios of 4:1 (R4B1), 2:1 (R2B1), 1:1 (R1B1), and 1:2 (R1B2) with light-emitting diodes (LEDs) and fluorescent light (FL) in a plant factory. Thyme plants were sampled at three intervals of 12, 24, and 36 d after treatment. The results showed that the growth and medicinal components accumulation of thyme plants were significantly affected by different light qualities. The significant higher biomass, leaf area, and volatile oil concentrations of thyme plants were obtained under treatment R4B1 compared with treatment FL, regardless of the cultivation period. When analyzing the volatile oil constituents of thyme plants, thymol, γ-terpinene, p-cymene and α-terpinene were detected as the main constituents. However, the response of these different constituents varied with different light qualities. The above results indicated that the targeted constituent concentrations could be manipulated by employing different light qualities according to various purposes. Based on the above results, R4B1 can be considered as the optimal light treatment for thyme plants growth and volatile oil production in a closed production system with LEDs.

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INTRODUCTION

Many herbs have been widely used for socio-cultural, spiritual, and medicinal purposes in many countries[1]. Daily diet with fresh or processed medicinal plants became popular due to rising awareness of their health benefits[2]. Thyme (*Thymus vulgaris* L.), a commonly used herb and medicinal plant, is popularly used in daily diets due to its unique aromatic flavor and relatively high concentration of phytonutrients or bioactive secondary metabolites[3]. The main essential oil constituents of thyme are thymol and carvacrol[4]. Thymol has antifungal activity against a number of species including *Cryptococcus neoformans*, *Aspergillus*, *Sapralegnia*, and *Zygorohynchus* sp.[5,6], and is reported to have strong antioxidant, antispasmodic, antiseptic, antitussive, expectorant, and carminative properties due to a wide range of phenolic type compounds such as flavonoids and rosmarinic acid[7–9].

Thyme, as a valuable medicinal plant, is widely cultivated in many regions in the world, although it has originated from the Western Mediterranean region and southern Italy[10]. However, a stable and reliable supply of thyme has become a big issue due to unsuitable and/or changeable microclimates in the open field or a semi-closed greenhouse. To guarantee quality and quantity of medicinal plants such as thyme, an enclosed plant production system with artificial light (aka plant factory) is gradually used for medicinal plant production, especially in Asian countries[2,11,12].

In a plant factory, environmental factors such as light, air temperature, and CO_2 concentration, can be controlled to desired levels due to airtightness and thermal insulation of the structure and by using artificial light as the sole light source for plant production. Light, as a signal and energy source, is one of the most important environmental factors for plant growth and development in a plant factory[13]. Light can regulate plant behavior depending on different light environmental factors. Compared to light intensity and photoperiod, light wavelength showed a more complex effects on plant morphogenesis and physiology, especially on accumulation of medicinal components[14]. Plants can sense and respond to a broad range of light spectrum, from UV-C (260 nm) to far-red (720–780 nm). Compared with other wavelengths, red and blue lights absorbed by photosynthetic pigments are reported to be more effective for plant production[15,16]. Light-emitting diode (LED) is the preferred light source in a plant factory because of its advantages, such as customizable wavelength, high light use efficiency and lower lamp surface temperature and longevity, compared to fluorescent lamps (FL)[17].

Many studies reported the enhancement of growth and medicinal component content through manipulation of the light quality under indoor controlled environments[2,11,18]. For most herb species, red combined with blue light significantly increased plant yield than monochromatic red or blue light. The yield of herb plants decreased as blue light proportion increased, but this trend varied among species[19]. Some studies also showed that the concentrations of essential oils and phenolic compounds in various herbs were enhanced, and the antioxidant capacities were improved by employing red, blue, and/or ultraviolet light compared with white light or sunlight[2]. Studies conducted by Dong et al.[20] showed that the total essential oil concentrations of *Mentha piperita*, *M. spicata*, and

M. longifolia were the highest when cultivated under red light with a photosynthetic photon flux density (PPFD) of 500 μmol·m^{-2}·s^{-1} and a photoperiod of 16 h for 60 d, and were 39% and 86% higher than those grown under blue and white light, respectively, while the total essential oil concentration was the lowest under sunlight. The total essential oil concentration in sweet basil was the lowest when cultivated under red light with the PPFD of 50 μmol·m^{-2}·s^{-1} and photoperiod of 16 h for 70 d, and 1.2–4.4 times higher when cultivated under blue light than that under white light[21]. Specific light wavelength combinations increased volatile compound concentrations, yield, and antioxidant capacity of basil plants. For instance, in basil plants, higher levels of monoterpenoid volatiles were observed under blue/red/yellow or blue/red/green wavelengths, while higher levels of sesquiterpenoid volatile molecules were induced under blue/red/far-red treatment[11]. The aforementioned reports strongly indicated that the medicinal secondary metabolites could be manipulated by different light qualities. However, the effects of single or mixed light quality may vary for different plant species or cultivars. Suitable light quality for improving growth and medicinal secondary metabolites in plants should be carefully investigated, especially in a plant factory.

In this study, we investigated different red to blue light (R/B) ratios and FL regimens on thyme plants, to enhance thyme plant production and nutritional quality in a plant factory. Effects of light quality on thyme plant growth and essential oil accumulation were analyzed by analyzing growth parameters, chlorophyll fluorescence, and content of medicinal components. The results of this study would be useful as a guide when designing a light environment for thyme cultivation under controlled environment.

MATERIALS AND METHODS

Plant factory and cultivation conditions

The plant factory used in this experiment was 2.2 m wide, 4 m long and 2.5 m high. Four basic modules (0.7 m wide, 1.5 m long and 2.4 m high each) were placed in the plant factory. Each module consisted of three shelves with two vertically adjustable LED panels (0.7 m long and 0.7 m wide each) per shelf and a culture bed (0.68 m wide, 1.5 m long and 0.1 m deep). The culture bed was covered with styrofoam board for holding the plants, the nutrient solution was circulated one hour per day. CO_2 was supplied from a CO_2 gas cylinder for CO_2 enrichment in the plant factory. A heat pump (cooling capacity: 14 kW, HFW-75-2, Beijing, China) was used to control air temperature and relative humidity inside the plant factory.

The cultivation conditions of the plant factory were maintained at PPFD (emitted from FLs and LEDs) of 160 μmol·m^{-2}·s^{-1} at the plant canopy level and a photoperiod of 16 h, the air temperature of 20 ± 0.5 °C and 25 ± 0.5 °C during dark and light period, respectively, with a relative humidity of 65% ± 5%, CO_2 concentration of 1,000 ± 50 μmol·mol^{-1}. LED panels (Dongguan Bio-lighting Sciences and Technology Co. Ltd, China) equipped with red and blue LEDs with the peak wavelength of 660 nm and 450 nm, respectively, were employed in this experiment.

Plant materials

Thyme seeds (*Thymus vulgaris* L.) were supplied by the Department of Medicinal and Aromatic plants, Agriculture Research Center (A.R.C.), Ministry of Agriculture, Egypt. Thyme seeds were germinated under dark for 10 d using an incubator (GLED-250PY; Luxi technology Co., Ltd., Beijing, China) with an air temperature of 20 ± 0.5 °C. Then, seedlings were moved to the plant factory with the PPFD of 100 μmol·m^{-2}·s^{-1} provided by FL (TL-D56W, Osram, Fluora, Munich, Germany). The seedlings with two fully expanded leaves were selected and cultured at a plant density of 50 plant m^{-2} in each treatment.

Experimental design

Five treatments with different R/B ratios of 4:1 (R4B1), 2:1 (R2B1), 1:1 (R1B1), 1:2 (R1B2) and white FL (TL-D56W, Osram, Fluora, Munich, Germany) were set up in this experiment. The FL treatment was used as the control. Distance between the light panels and/or lamps and cultivation bed was set at 0.4 m in all treatments. The deep flow technique cultivation system with 0.05 m depth of Yamasaki nutrient solution (pH: 5.8 and EC: 1.4 dS·m^{-1}) was applied in this experiment for plant growth. Cultivation period of this experiment was 36 d. The experiment was repeated three times.

Morphological and growth measurements

During the cultivation period, the morphological and growth parameters was measured on day 12, 24 and 36 after treatment. Five plants were selected randomly for samples on day 12, 24 and 36 after treatment. Morphological and growth parameters, such as leaf number, leaf area, shoot height, shoot fresh and dry weights, root length, and root fresh and dry weights were measured. The leaf area of each plant was measured by leaf area meter LI-3000C (LI-COR biosciences, Lincoln, USA). Dry weights of the samples were weighed after drying at 70 °C in a drying oven for 48 h.

Essential oil measurements

On day 36 after treatment, essential oil in dry thyme plants was isolated using hydrodistillation with a drying system (the Polish version of the Clevenger apparatus) according to El-Zaeddi et al.[22]. In a 500 mL round bottom flask, 15.0 g freshly chopped herb shoots (including stems and leaves) were mixed with 150 mL distilled water, 1.0 g sodium chloride and 50 μL of benzyl acetate as an internal standard (987 mg·L^{-1}). After the above mixture was left to boil for 1 h, the vapors were condensed by a cold refrigerant. The drying apparatus was added by 1 mL of cyclohexane at the beginning of the hydrodistillation process to retain the essential oil distilled from the samples of herbs shoots. After extracting for 60 min, the solvent enriched with the volatile compounds was transferred into a 2.5 mL vial. Before the gas chromatography-mass spectrometry (GC-MS) analyses were conducted, the vial was dried over anhydrous sodium sulfate and kept at 15 °C. The extractions were repeated three times.

Chromatographic analyses

The GC-MS analysis were used for analyzing and identifying the volatile compounds. According to Abdel-Hamid et al.[23], GC-MS system (Agilent Technologies) was equipped with mass spectrometer (5977A) and gas chromatograph (7890B) detectors. Samples were diluted with hexane (1:19, v/v). The GC was equipped with HP-5MS column with an internal diameter of 30 m × 0.25 mm and film thickness of 0.25 μm. Helium as the carrier gas (a flow rate of 1.0 mL/min and a split ratio of 1:30) with the injection volume of 1 μl was applied for analyses, and the following temperature program: 40 °C for 1 min; rising at 4 °C /min to 150 °C and held for 6 min; rising at 4 °C/min to

210 °C and held for 1 min. The injector and detector were held at 280 and 220 °C, respectively. Mass spectra were obtained by electron ionization at 70 eV using a spectral range of m/z 50-550 and solvent delay 3 min. By comparing the spectrum fragmentation pattern with those in Wiley and NIST Mass Spectral Library data, the identification of different constituents was determined.

Chlorophyll concentrations

Chlorophyll concentrations were measured by excising samples at a similar position of thyme leaves in each treatment. The 0.1−0.2 g fresh weight of leaves were added to 20 ml of 95% ethyl alcohol until leaves turned white color. The optical density was measured using UV-1800 spectrophotometer (Shimadzu, Japan) at 663 nm (OD 633) and 645 nm (OD 645) for determining chlorophyll a (Chl a) and chlorophyll b (Chl b). The total chlorophyll concentrations (Chl) were calculated according to Wang et al.[24].

Statistical analysis

The data were evaluated by analysis of variance (ANOVA). Significant differences between treatments were determined using Duncan's test with a confidence level of 95% ($P < 0.05$), according to the method of Gomez & Gomez[25]. The data analysis was carried out three times.

RESULTS

Thyme plant morphology and growth

The morphology and growth parameters of thyme plant were significantly affected by different light qualities (Table 1). Based on the measurements on day 12, 24, and 36, significantly higher morphology and growth parameters were observed under treatment R4B1 compared with those under treatment FL, regardless of the cultivation period. However, morphology and growth parameters were affected by different R/B ratios and varied under different sampling times. On day 12, the highest shoot fresh/dry weight and root fresh weight were observed under treatment R4B1. No significant differences in root fresh/dry weight, root length, shoot fresh weight and plant height were observed in treatments R2B1, R1B1, and R1B2. On day 24, no significant differences in morphology and growth parameters of thyme plant were observed between treatments R4B1 and R2B1, except for root length. On day 36, no significant differences in all morphology and growth parameters were observed between treatments R4B1 and R2B1. However, significantly higher root fresh weight, root length, leaf area, plant height, and branch number were observed in treatment R4B1 than those under R1B1 and R1B2.

Chlorophyll concentration

Changes in chlorophyll a and b concentrations of thyme plants as influenced by light qualities varied on day 12, 24 and 36 after treatment (Table 2). On day 12, no significant differences in chlorophyll a were evident among the different R/B ratios, but were all significantly higher than the control treatment FL. For chlorophyll b, higher values were found under treatment R4B1 and R2B1 than R1B1, R1B2, and FL. On day 24, no significant differences in chlorophyll a and b of thyme plants were observed among all treatments. On day 36, the highest chlorophyll a and b levels were obtained under treatment R2B1. No significant differences in chlorophyll a and b of thyme plants were observed among treatment R4B1, R1B1, and R1B2. A significantly higher chlorophyll a and b levels were obtained under treatment R2B1 and R4B1 than under treatment FL.

Table 1. Thyme plant morphology and growth as affected by different light qualities.

Days after treatment	Light quality	Plant height (cm)	Branch numbers	shoots fresh weight (g)	shoots dry weight (g)	Leaf area (cm^2)	Root length (cm)	Roots fresh weight (g)	Roots dry weight (g)
12 days	R4B1	15.63 A	15.33 A	2.56 A	0.57 A	27.47 A	17.37 A	0.76 A	0.06 A
	R2B1	14.63AB	14.00 AB	1.86 B	0.51 B	25.30 A	15.60 A	0.59 B	0.06 A
	R1B1	13.73 B	12.00 BC	1.55 BC	0.41 C	20.20 B	14.80 A	0.57 B	0.05 A
	R1B2	13.97 B	14.33 AB	1.57 BC	0.33 D	18.04 BC	15.53 A	0.60 B	0.05 A
	FL.	13.37 B	11.00 C	1.32 C	0.27 D	15.68 C	11.30 B	0.34 C	0.03 B
24 days	R4B1	26.00 A	28.00 A	20.28 A	4.69 A	156.80 A	18.33 A	3.20 A	0.26 A
	R2B1	25.00 AB	26.33 AB	19.46 AB	4.22 A	150.30 A	16.00 B	2.66 AB	0.20 AB
	R1B1	23.00 AB	23.00 BC	17.42 AB	2.96 B	134.40 B	15.00 B	2.27 AB	0.16 BC
	R1B2	23.83 B	24.0 ABC	18.02 AB	3.00 B	131.60 B	14.67 B	1.98 B	0.15 BC
	FL.	20.00 C	21.00 C	17.13 B	2.79 B	127.90 B	14.00 B	2.04 B	0.11 C
36 days	R4B1	29.67 A	40.67 A	32.24 A	5.60 A	199.80 A	21.33 A	3.73 A	0.27 A
	R2B1	27.33 AB	38.00 AB	29.81 A	5.15 A	191.30 A	20.00 AB	2.89 AB	0.24 AB
	R1B1	26.00 B	35.33 BC	29.21 A	4.88 AB	159.80 B	18.67 B	2.56 B	0.22 AB
	R1B2	26.67 B	32.00 CD	23.31 B	3.81 B	154.20 B	19.00 AB	2.44 B	0.20 B
	FL.	25.00 B	28.67 D	22.13 B	3.88 B	137.90 C	18.00 B	2.19 B	0.14 C

FL = fluorescent lamp; R = Red; B = Blue. Means followed by different letters within a column are significantly different at $P < 0.05$ by Duncan's test.

Table 2. Chlorophyll a and b concentration of thyme leaves as affected by different light qualities.

Days after treatment	12 days					24 days					36 days				
Light quality	R4B1	R2B1	R1B1	R1B2	FL	R4B1	R2B1	R1B1	R1B2	FL	R4B1	R2B1	R1B1	R1B2	FL
Chl(a)(mg/l)	16.75 AB	18.64 A	16.38 AB	16.49 AB	14.43 B	15.71 A	18.91 A	17.77 A	16.92 A	15.26 A	19.90 B	23.10 A	19.36 B	18.30 BC	16.45 C
Chl(b)(mg/l)	6.29 AB	6.82 A	4.83 B	4.96 B	4.98 B	5.11 A	6.30A	5.56 A	4.80 A	4.73 A	6.63 B	7.73 A	5.96 BC	5.90 BC	5.37 C

FL = fluorescent lamp; R = Red; B = Blue. Means followed by different letters within a column are significantly different at $P < 0.05$ by Duncan's test.

Volatile oil concentration

Volatile oil percentage and total volatile oil yield of thyme plants were significantly increased by treatment R4B1, R2B1, and R1B1 than treatments R1B2 and FL. However, no significant differences in volatile oil percentage and total volatile oil yield of thyme plants were observed under treatments R4B1, R2B1, and R1B1. A similar volatile oil percentage and total volatile oil yield of thyme plants were found under treatments R1B2 and FL. The average volatile oil percentage and the total volatile oil yield under treatment R4B1 increased by 27.6% and 77.8%, respectively, than those under treatment FL (Table 3).

Table 3. Volatile oil of thyme plants as affected by different light qualities.

Light quality	Volatile oil (%)	Volatile oil yield (mL plant^{-1})
R4B1	2.82A	0.16 A
R2B1	2.77 A	0.14 A
R1B1	2.70 A	0.13 A
R1B2	2.25 B	0.09 B
FL	2.21 B	0.09 B

FL = fluorescent lamp; R = Red; B = Blue. Means followed by different letters within a column are significantly different at $P < 0.05$ by Duncan's test.

Volatile oil constituents

The volatile oil components of thyme plants under different light treatments were identified by GC/MS. About 31 components were identified which represented nearly from 99.46% to 99.83% (Table 4). The volatile oils of thyme plants were characterized as high levels of thymol (48.22%–49.83%), γ-terpinene (17.89%–19.11%), p-cymene (7.48%–8.85%) and α-terpinene (3.64%–4.25%). Thymol, γ-terpinene and α-terpinene reached their maximum relative percentage under treatment R4B1 and R1B1, while the maximum relative percentage of p-cymene was obtained under treatment R1B2. The monoterpenes hydrocarbons (NH) group ranged between 37.71% and

Table 4. Volatile oil constituents of thyme plants as affected by different light qualities.

Compound	KI*	R4B1	R2B1	R1B1	R1B2	FL
[I]: Monoterpenes Hydrocarbons [MH]						
α-Thujene	913	2.18	1.98	2.18	2.18	1.91
α-Pinene, (–)-	938	0.96	0.99	0.96	0.96	0.94
Camphene	953	0.22	0.30	0.22	0.22	0.23
Sabinene	965	0.06	0.05	0.06	0.06	0.05
(–)-β-Pinene	980	0.27	0.28	0.27	0.27	0.30
β-Myrcene	986	2.25	2.27	2.25	2.25	2.42
α--Phellandrene	996	0.43	0.43	0.43	0.43	0.48
DELTA3-Carene	1004	0.12	0.12	0.12	0.12	0.14
p-Cymene	1025	7.48	7.70	7.48	7.48	7.54
D-Limonene	1030	0.65	0.67	0.65	0.65	1.10
Eucalyptol	1046	0.17	0.19	0.17	0.17	0.32
α-Terpinene	1057	4.25	4.03	4.25	4.25	4.23
γ-Terpinene	1057	19.11	18.72	19.11	19.11	17.89
α-Terpinolene	1087	0.15	0.14	0.15	0.15	0.16
Total MH		38.30	37.87	38.30	38.30	37.71
[II] Oxygenated Monoterpenes Hydrocarbons [OM]						
cis-Sabinene hydrate	1063	1.13	1.15	1.13	1.13	1.29
Linalool	1098	1.91	1.87	1.91	1.91	1.98
Terpinen-4-ol	1176	0.69	1.06	0.69	0.69	0.63
alpha-Terpineol	1189	0.13	0.13	0.13	0.13	0.15
Thymol methyl ether	1217	0.07	0.29	0.07	0.07	0.65
Carvacrol methyl ether	1247	0.16	0.34	0.16	0.16	0.78
Thymol	1293	49.83	49.06	49.83	49.83	48.22
Phenol, 2-methyl-5-(1-methylethyl)-	1307	3.71	3.26	3.71	3.71	3.76
Total OM		57.63	57.16	57.63	57.63	57.46
[III] Sesquterpenes Hydrocarbons [SH]						
Caryophyllene	1415	2.03	2.29	2.03	2.03	2.33
α-Humulene	1455	0.06	0.08	0.06	0.06	0.08
γ-Muurolene	1478	–	0.30	0.27	–	0.30
α-Muurolene	1486	0.27	–	0.14	0.27	0.06
γ-Cadinene	1515	0.15	0.23	0.15	0.15	0.24
delta-Cadinene	1526	0.25	0.32	0.25	0.25	0.34
γ-eudesmol	1608	–	0.13	–	–	0.10
Total SH		2.76	3.35	2.90	2.76	3.45
IV: Oxygenated Sesquterpenes [OS]						
tau-Cadinol	1640	0.15	0.28	0.15	0.15	0.31
Total OS		0.15	0.28	0.15	0.15	0.31
[V]: Various compounds [VC]						
1-Octen-3-ol	977	0.85	0.80	0.85	0.85	0.59
Total VC		0.85	0.80	0.85	0.85	0.59
Total Identified Compounds		99.69	99.46	99.83	99.69	99.52

FL = fluorescent lamp; R = Red; B = Blue.

Table 5. Correlation coefficient between the parameters.

Parameters	Height (cm) 1	Fresh weight (g) 2	Dry weight (g) 3	Branches number/plant 4	Leaf area (cm^2) 5	Root length (cm) 6	Root fresh weight (g) 7	Root dry weight (g) 8	Chl(a) ($mg\,L^{-1}$) 9	Chl (b) ($mg\,L^{-1}$) 10	V. oil (%) 11	V. oil yield ($L\,Plant^{-1}$) 12
1	1	0.882*	0.774	0.771**	0.959**	0.935**	0.919**	0.823**	0.604*	0.674**	0.680**	0.773**
2		1	0.958**	0.933**	0.898**	0.900**	0.961**	0.930**	0.807**	0.790**	0.925**	0.958**
3			1	0.907**	0.788**	0.815**	0.891**	0.884**	0.755**	0.724**	0.978**	0.961**
4				1	0.832**	0.883**	0.852**	0.811**	0.735**	0.712**	0.899**	0.983**
5					1	0.950**	0.891**	0.821**	0.683**	0.733**	0.697**	0.820**
6						1	0.872**	0.817**	0.601*	0.640*	0.739**	0.871**
7							1	0.858**	0.744**	0.736**	0.841**	0.871**
8								1	0.796**	0.807**	0.868**	0.863**
9									1	0.956**	0.790**	0.773**
10										1	0.748**	0.750**
11											1	0.956**
12												1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

38.30%. Volatile oil of thyme plants was characterized by high amounts of oxygenated monoterpene hydrocarbons (OM). The highest percentage of this group was recorded at 57.63% under treatment R4B1, R1B1 and R1B2.

Correlation coefficient analysis between all the parameters

The majority of agronomic traits are complex in their genetic behavior and the result of the interaction of numerous component factors. It is of paramount importance to figure out the relationship between yield and its component traits to correctly select promising plant varieties. Correlation studies provide information on correlated response of important plant traits and therefore leads to a directional model for yield response. In our study, herb yield and essential oil yield were very important economic traits for thyme plant. According to our results (Table 5), all vegetative characters had a positive and significant correlation coefficient with fresh and dry weights of shoots especially branch number. Also, all traits in this study exhibited a significant and positive correlation with essential oil percentage and essential oil yield especially fresh and dry weights of shoots.

DISCUSSION

In this experiment, thyme plant growth, such as fresh weight, dry weight and leaf area, were significantly affected by different light qualities. Similar results were observed in several other species, such as lettuce[24], 'Wala' basil[26], green basil, and purple basil 'Red Rubin'[2]. These results indicate that the growth of thyme plants under combined red and blue lights, can be improved with higher red light portion. However, the red light portion threshold was not observed in this experiment. The lowest growth rate of thyme plants was observed under treatment FL in this experiment. The above results indicate that the combined red and blue LED lights had a pronounced effect on thyme growth compared with FL with a wide range of spectrum since the absorption peak of photosynthetic pigments was observed under red and blue lights rather than other wavelengths.

The effects of light environment, especially light quality, on plant growth is complicated because light is absorbed by multiple plant photoreceptors including phytochromes, cryptochromes, and phototropins, and generates a wide range of specific physiological responses[27,28]. It is known that red light is important for shoot/stem elongation, phytochrome responses, and changes in plant anatomy[29]. However, blue light is important in chlorophyll biosynthesis, stomatal opening, enzyme synthesis, and maturation of chloroplasts and photosynthesis[30]. This may be one reason why chlorophyll concentrations of thyme plants growing under R/B ratios of 4:1, 1:1, and 1:2 did not have significant differences but had the highest values under R/B ratio of 2:1.

Although blue light is very effective for chlorophyll biosynthesis and stomata opening, many studies showed that blue light was less effective than red light for driving photosynthesis[31–33]. The reason is that blue light can be absorbed by lower-efficiency pigments such as carotenoids or inactive pigments such as anthocyanins leading to a reduction in blue light energy that absorbed by the chlorophyll pigments[34]. Shimizu et al.[31] reported that plant biomass and photosynthesis rate had similar response to

monochromatic blue and red light. Piovene et al.[35] reported that basil shoot fresh weight increased by 214% when blue light portion increased from 15% to 59% under combined red and blue lights at PPFD of 200 μmol·m^{-2}·s^{-1} with a 16 h photoperiod. The above studies demonstrated that significant effects of blue light on photosynthesis and growth of plants is species-dependent.

Light is not only an energy source for photosynthesis but also an environmental signal to regulate plant growth and medicinal component accumulation. In this experiment, volatile oil concentrations and yield of thyme plant were improved when grown under different R/B ratios than under FL, but the level of enhancement varied according to R/B ratios treatments. Volatile oil constituents were also analyzed, and the results showed that, thymol, γ-terpinene, p-cymene and α-terpinene were the main constituents of volatile oil which suggested that the essential oil analyzed belonged to the thymol chemotype in agreement with previous studies[36]. Similar studies reported that the major compounds in essential oil of *T. vulgaris* were p-cymene, γ-terpinene, and thymol[37–40]. In this respect, the total essential oil concentration of *Mentha piperita*, *M. spicata*, and *M. longifolia* were highest when cultivated under red light compared with those cultivated under blue or white light, and were the lowest under sunlight[41]. Similarly, l-menthol, in *M. arvensis*, the main component of essential oil, was 1.4 times higher under red light than under blue or green light[42]. The concentration of glycyrrhizic acid, 50 times sweeter than sugar, in root tissues of Chinese liquorice was reported to be the highest under red light, followed by white light, and the lowest under blue light[43]. However, the total essential oil of sweet basil was the lowest under red light and was 1.2–4.4 times higher grown under blue light than that under white light[21]. Except for enhancement of essential oil concentration, light wavelength composition may also affect the composition of essential oils in herbs. For instance, myrcene and linalool were the second and third major components of essential oils in sweet basil when cultivated under blue light, while α-pinene and β-pinene were highest under green and red lights, and plants under white light showed an intermediate response[21]. Mexican mint (*Plectranthus amboinicus*) produced greater amounts of sesquiterpenes under blue light, while plants produced greater amounts of monoterpenes under red light[44]. Therefore, targeted compound concentrations of medicinal plants could be enhanced by adjusting light quality according to various purposes.

CONCLUSIONS

In this experiment, response of thyme (*Thymus vulgaris* L.) plant growth and nutritional quality to different light qualities were investigated in a plant factory. Compared with FL, the combined red and blue lights were more efficient in improving plant growth and volatile oil concentrations. Significantly higher fresh/dry weights, leaf area, and volatile oil concentrations of thyme plants were obtained when grown under treatment R4B1 than under treatment FL. Thymol, γ-terpinene, p-cymene, and α-terpinene were the main constituents of volatile oils of thyme plants grown in a controlled environment system. Volatile oil constituents and their related concentrations varied according to light qualities. The above results indicated that treatment R4B1 can be considered as the optimal light regimen for thyme plants growth and volatile oil production in a plant factory with LEDs.

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Conflict of interest

The authors declare that they have no conflict of interest. Yuxin Tong is the Editorial Board member of the journal *Technology in Horticulture*. He was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer-review handled independently of this Editorial Board member and her research groups.

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