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ARTICLE

The effects of different color lights on the growth, glandular trichome development and essential oil content of *Mentha arvensis L*

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Abstract: *Mentha arvensis L.* is well known for producing the monoterpenes menthol; its essential oil (EO) is widely used in pharmaceutics, cosmetics, food and other industries. The peltate glandular trichome (PGT) is the site responsible for the production and storage of EO in mint. Light is one of the most important environmental factors that contributes to plant growth and development, and secondary metabolism. In this report, we have investigated the influence of additional red light (White + Red, W+R) and blue light (White + Blue, W+B) on the parameters of growth, EO, and PGT in M. arvensis. Interestingly, the plant growth parameters, such as the number of branches, plant height, fresh weight, leaf area and number of leaves were significantly greater both under W+R and W+B treatments compared to the control group. Red light proved more beneficial for increasing plant height (by 15.26%) and fresh weight (by 58.42%), whereas blue light was more effective in boosting the number of branches (by 83.68%) and leaves (by 65.07%). The highest increases in essential oil (EO) content (by 36.36%), yield (by 101.72%), and peltate glandular trichome (PGT) density (by 35.64%) were observed under W+B treatment. However, both W+R and W+B had a slightly negative effect on the quality of mint EO, where menthol content decreased by 4.32 per cent and 11.88 per cent respectively. These results proved that red light is more conducive to biomass accumulation, while blue light is more conducive to EO synthesis and PGT development in M. arvensis.

Keywords: *Mentha arvensis, essential oil, light quality, glandular trichome;*

INTRODUCTION

Essential oil (EO) of *Mentha arvensis L.* (Japanese mint, Asian mint, menthol mint) is the source of commercial natural menthol, which is valued for its aromatic and medicinal properties, therefore, it is widely used in pharmaceutics, cosmetics, food and many other industries [1, 2]. Given its high demand, menthol mint EO production has been the second largest production worldwide [1]. A dominant component of menthol mint EO is monoterpenes, and the synthesis of terpenoids in mint relies on the MEP (methylerythritol phosphate) pathway (Barros et al., 2015). Mint EO is synthesized and stored in the secretory cells from peltate glandular trichome (PGT) [3].

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Previous studies revealed that stimulation of biosynthesis of secondary metabolites and the development of GPT are two effective ways to increase the content of secondary metabolites in many medicinal plants [4-7]. Light is one of the most important environmental factors affecting plant growth and development, and secondary metabolism [8, 9]. Using light rather than chemicals to control plant architecture can reduce negative environmental impacts [10]. Light signals affect the growth, biosynthesis of terpenoids and the development of glandular trichomes mainly in two ways: light intensity and light quality. High-intensity or long-term light exposure usually promotes the synthesis of terpenoids and the development of glandular trichomes. For example, in *Mentha arvensis* L., *Mentha canadensis* L., and *Artemisia annua* L., high-intensity of light significantly increased the content of EO and the density of PGT, and effectively activated the expression of genes related to terpenoids synthesis and glandular trichome development. While weak-intensity of light reduced these effects [5, 11, 12]. Among different light qualities, ultraviolet B and C irradiation have been reported to improve the terpenoids content in plant, but they are harmful to plant growth and development, red light (R) and blue (B) light have particularly significant effects on terpenoids synthesis and glandular trichome development without affecting plant growth and development [7, 8, 13]. For example, light treatment, rich in R or B, significantly enhanced the growth, increased the content of secondary products of *Ocimum basilicum* L.and *Digitalis purpurea* L., and significantly increased the density and size of *D. purpurea* glandular trichomes [14, 15]. However, optimal development of plants cannot be achieved using R light alone, but it needs Blight as well, to regulate processes at variance with photosynthesis [16-18]. B light has been documented to influence vegetative growth, photo-morphogenesis, and secondary metabolite production in plants [19, 20].

The aim of the study is to develop an efficient plant growth system with the addition of R and B LEDs, and to examine the influence of LEDs on plant growth, EO and PGT parameters. Therefore, we screened the light sources to examine if they could promote mint EO content and production without affecting plant growth and development.

MATERIALS AND METHODS

Plant material and climate conditions

Root segments of mint plants were cut, each segment containing one growth point, and planted in the substrate (peat soil: perlite, 3:1). Eight plants were planted in each pot, the pot size was 49x21x15 cm, and 3 independent replicates were performed. Plants were cultured in a tissue culture room, the cultured environment condition were 25℃, light intensity 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, humidity 75 per cent, and full light.

Light treatments

All the LED lights were bought from Chunying Optoelectronics Technology Co., Ltd., Guangdong, China. LED light source was placed at the top of the plants. White light (W) was used to simulate natural light. Based on white light, 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ red light and blue light were added, named W+R and W+B respectively (Figure 1). *M. arvensis* was harvested and used for detecting the growth, PGT and EO parameters respectively after 10 weeks, at which time flower buds appeared indicating the maturity of mint [21].

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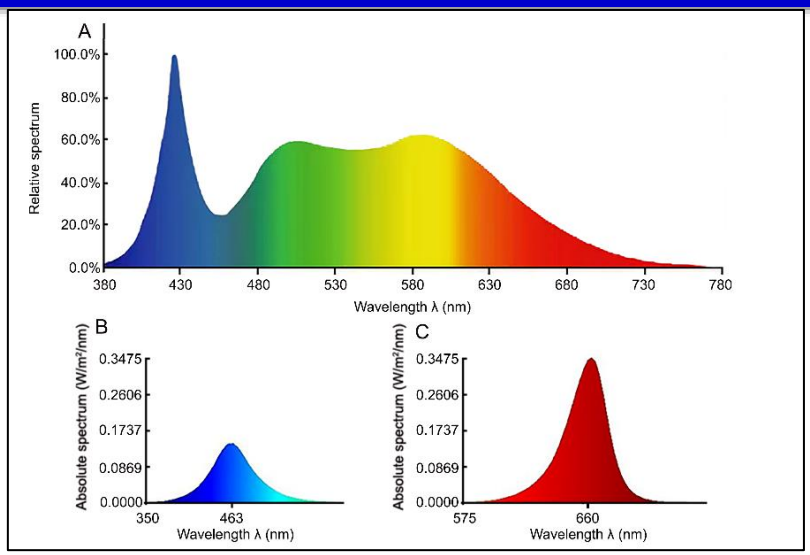

Figure 1. Spectra of lighting treatments used. (A) Simulate natural light (W), (B) Blue light, (C) Red light.

Growth parameters analysis

Plant height - use a measuring tape to determine the height from the substrate surface to the top of the plant. Number of branches - count the number of branches (length over 4 cm, leaves over 4 pairs), and randomly select 10 plants in each group to measure various indicators. Fresh weight - the total fresh weight of the aboveground parts of each plant was weighed with an electronic balance. The number of leaves, the number of pair leaves were counted on the entire plant. The third pair of fully expanded mature leaves from the top was used to measure the leaf area. The images of leaves were scanned by printer, and then ImageJ (http://rsb.info.nih.gov/ij) was used to calculate the area of the leaves. All of the experiments were performed for more than 3 replicates.

The density and size of peltate glandular trichome analysis

The third pair of mature leaves from the top of mint was collected for determine the density and size of peltate glandular trichomes. Images of the top, middle, and bottom of the paraxial face of each leaf were obtained using fluorescence microscopy (Olympus, Tokyo, Japan). A×10 objective was used for imaging.

Fluorescence was imaged by UV light. Counting the number of peltate glandular trichomes and measuring the leaf area were performed using the ImageJ program [22].

Essential oil extraction and analysis

Essential mint oil was extracted by way of hydro-distillation in a Clevenger apparatus from 200 g fresh plant material for 40 min. EO content (expressed as % w/w) was evaluated, the EO products were quantified (in grams per gram tissue fresh weight), and the composition was analyzed by gas chromatography mass spectrometry (GC-MS). Agilent 7890B-5977A gas chromatograph-mass spectrometer was used for GC-MS detection. Detection conditions included: chromatographic column was DB-WAX, carrier gas was helium, quadrupole temperature was 150°C, ionization mode was EI+, and carrier gas flow rate was 1 mL/min [23].

Data analysis

Values presented are the mean ± standard

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deviation (SD) of the three replicates. One-way variance analysis (ANOVA) was employed to analyze the data performed by GraphPad Prism (version 8.0, USA), and statistical significance was set at **P*<0.05, ***P*<0.01 and ****P*<0.001[24].

RESULTS AND DISCUSSION

Effects of red light and blue light on the growth of *Mentha arvensis* L.

Among different light qualities, ultraviolet B and C irradiation has been reported to improve the terpenoid content in plants, but they are harmful to plant growth and development. However, red and blue light could enhance terpenoid accumulation by promoting the expression of the genes involved in terpenoid biosynthesis [13]. Therefore, we used red and blue light as the light sources to examine whether they could promote EO content in *M. arvensis* without affecting plant growth and development (Figure 2). In *M. arvensis*, the results showed that plants growing under different light treatments exhibited distinct growth responses and biomass production. Generally, the plants grew healthier both under W+R and W+B treatments than those in the control group W. Growth parameters, such as the plant height, number of branches, and fresh weight, increased significantly under W+R and W+B treatments compared to the control group. Under W+R treatment, the plant height of *M. arvensis* increased by 15.3 per cent, the number of branches increased by 75.0 per cent, the fresh weight increased by 58.4 per cent, and the number of leaves increased by 39.6 per cent, compared to the control group. Under W+B treatment, the plant height increased by 10.9 per cent, the number of branches increased by 83.7 per cent, the fresh weight increased by 48.0 per cent, and the number of leaves increased by 65.0 per cent, compared to the control group. However, the leaf area decreased by 15.4 per cent under W+B treatment as compared to the control group.

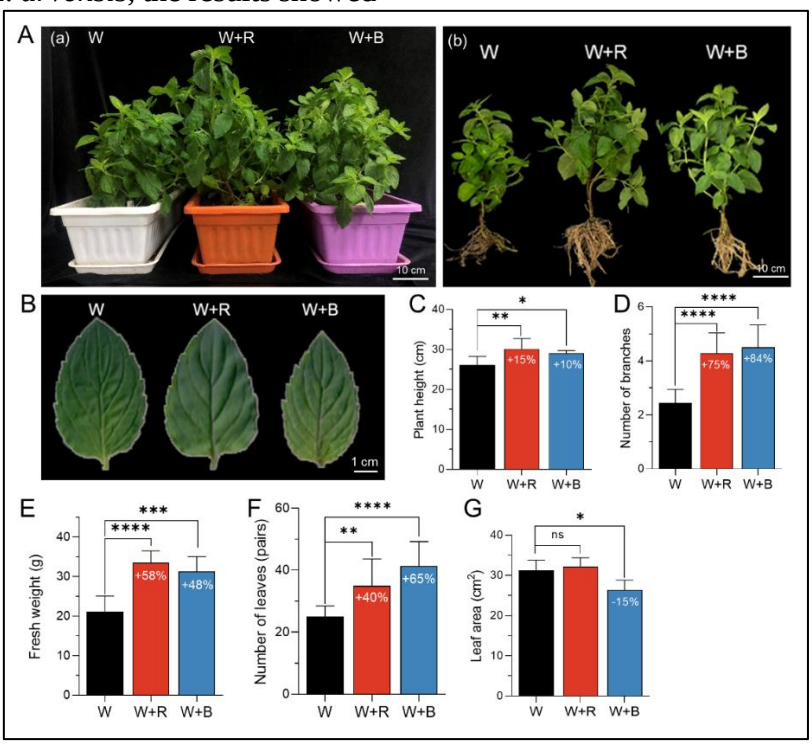

Figure 2. Different light treatments affect the growth of *Mentha arvensis*. (A) Morphology of the whole plants; (B) Morphology of leaf; (C) Plant height; (D) Number of branches; (E) Fresh weight; (F) Number of leaves; (G) leaf area. W: Simulate natural light; W+R: Simulate natural light plus red light; (W+B): Simulate natural light plus blue light. *P<0.05.

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A number of research have been done to study the influence of R and B light on plant anatomy, photosynthesis, and morphology [25]. For instance, Rihan et al. (2022) found that treatments with R-rich light significantly increased leaf area compared to other light spectra [26]. In *Digitalis purpurea*, the highest number of leaves and leaf area were observed under R-rich LED treatment [15]. Lin et al. (2021) reported a significant positive impact of red-rich light on lemon balm growth parameters, including biomass and secondary metabolite content [27]. Similarly, wheat seedlings showed enhanced growth under W+R light [28]. B light was reported to enhance plant growth and development in lettuce [29]; however, Kong and Nemali (2021) observed a reduction in leaf area under B-rich LED treatment [30]. In contrast, Wang et al. found that cucumber's leaf area increased under weak B light [31].

Our study showed that both R light and B light contribute positively to biomass accumulation. R light appears to play a more significant role in the photomorphogenic development of *M. arvensis*; moreover, B light narrowed the leaf area. The differences between plants may be due to the varied responses of different species to light and the differences in experimental methods.

Effects of red and blue light on essential oil content and yield of *Mentha arvensis*

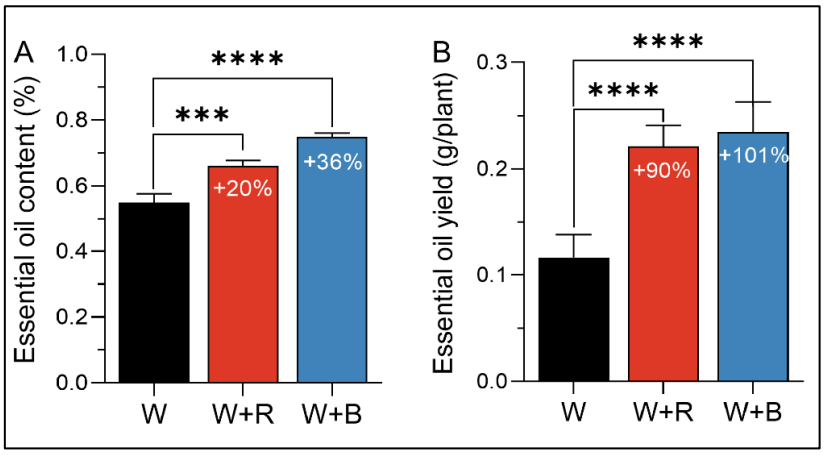

Figure 3. Different light treatments affect essential oil content and yield of *Mentha arvensis*. *P<0.05.

The results showed that both R light and B light significantly increased the content and yield of EO in *M. arvensis* (Figure 3). The EO content increased by 20.0 and 36.4 per cent respectively, and the yield increased by 90.0 and 101.7 v respectively under W+R and W+B treatments, compared to the control group. These findings suggest that B light may have a greater influence on EO biosynthesis.

Both R light and Blight are extensively acknowledged as effective elicitors that regulate the accumulation of bioactive compounds in medicinal plants [8]. For instance, exposure to R-rich light resulted in a 34.7 per cent increase in artemisinin levels in *A. annua* [13], an 83 per cent rise in salidroside concentration in Rhodiola rosea L. [32]. Additionally, there was a significant enhancement in secondary metabolism, and glandular trichomes in *Digitalis purpurea* were notably larger as compared to those under fluorescent lighting [15]. Under B-rich light conditions, a notable increase in bioactive compound content was observed across various plant species. Specifically, *A. annua* showed an 82.6 per cent increase in artemisinin content [13], Camptotheca acuminata Decne L.exhibited an approximately 30.0 per cent rise in

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camptothecin levels [33], and *Ocimum basilicum* L. experienced an approximate 140.0 per cent enhancement in EO content when compared to the control group [34].

Consequently, plant responses to light signals in terms of secondary metabolism vary. Our study revealed that both B light and R light significantly enhanced the EO content and yield in *M. arvensis*. Notably, the influence of blue light on these parameters appears to be more pronounced.

Effects of red and blue light on glandular trichome density and size of *Mentha arvensis*

Peltate glandular trichome (PGT) is the site of EO synthesis and storage in mint plants [35]. Therefore, we assessed the density and size of PGT using fluorescence microscopy. The results demonstrated that both R light and B light significantly increased the PGT density in mint (Figure 4). PGT density increased by 28.7 per cent under W+R treatment, and by and 35.6 per cent under W+B treatment, compared to the control group. Importantly, the data suggest that B light may have a more substantial role in promoting PGT development. Our results are similar to previous studies such as in *Digitalis purpurea* [15].

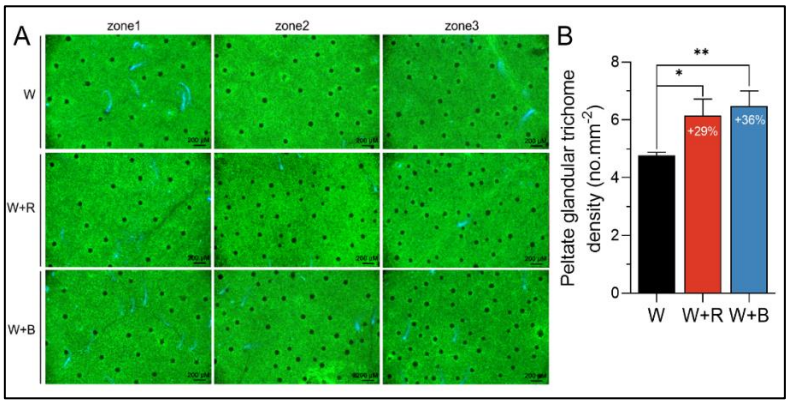

Figure 4. Different light treatments affect glandular trichome density and size in *Mentha arvensis*. *P<0.05.

Effects of red and blue light on essential oil composition of *Mentha arvensis*

A total of 27 volatile substances were identified from *M. arvensis* EO treated with different LED lights, accounting for more than 97.92 per cent of the total volatile substances, with monoterpenes as the dominant component (Table 1). No significant differences were observed in the types and contents of chemicals in mint EO between the W+R treatment and the control group. However, the W+B group showed significant differences when compared to both W+R and the control groups. Under W treatment, the top five volatile compounds were L-menthol (77.10%), L-menthone (11.46%), pulegone (3.73%), isomenthol (2.11%) and isomenthone (1.44%), collectively accounting for 95.85 per cent of the total volatiles. Under W+R treatment, the top five volatile compounds were L-menthol (73.77%), L-menthone (13.40%), pulegone (4.71%), isomenthol (2.22%) and germacrene D (0.87%), accounting for 94.97 per cent of the total volatiles. Under W+B treatment, the top five volatile compounds were L-menthol (67.94%), L-menthone (13.01%), pulegone (9.64%), isomenthol (2.21%) and isomenthone (1.50%), accounting for 94.30 per cent of the total volatiles.

L-menthol is the primary component of *M. arvensis* and is also the most important

indicator for evaluating the quality of *M. arvensis* EO [36, 37]. Souza et al. (2014) identified menthol (70.00%) as the main component of menthol mint EO [38]. Padalia et al. (2013) analyzed oil from 9 cultivars of *M.arvensis* and reported menthol content ranging from 73.70 to 85.80 per cent [39]. A few studies have revealed that light signals affect the composition of secondary metabolites in many medicinal plants. For example, Marco et al. (2020) observed that the EO of *Mentha arvensis* had a higher L-menthol concentration under low-light conditions (137 µmol photons m^{-2} s^{-1}) than that produced under high-light treatments (406 and 543 µmol photons m^{-2} s^{-1})[1]. Our study revealed that L-menthol content exceeded 67.94 per cent across all treatments. Specifically, the W group had the highest L-menthol content, accounting for 77.10 per cent of the total volatiles, while it decreased by 4.33 and 11.89 per cent in the W+R and W+B group, respectively. The findings of our study showed that the content of menthol was similar to that of previous studies. Moreover, our results indicated that supplementing R land B light, slightly reduced the menthol content and had a slight negative impact on the *M. arvensis* EO.

Table 1. Effect of different LED lighting treatments on the essential oil composition of *Mentha arvensis.*

		Component		Relative content (%)		
NO.	RT	Name	CAS	W	W+R	W+B
1	34.34	L-menthol	002216-51-5	77.10	73.77	67.94
2	20.21	L-menthone	014073-97-3	11.46	13.40	13.01
3	33.13	Pulegone	000089-82-7	3.73	4.71	9.64
4	31.12	Isomenthol	000490-99-3	2.11	2.22	2.21
5	22.31	Isomenthone	000491-07-6	1.44	/	1.50
6	37.10	Germacrene D	023986-74-5	0.86	0.87	1.20
7	38.09	Piperitone	000089-81-6	0.50	0.60	0.50
8	16.43	3-Octanol	000589-98-0	0.40	0.41	0.56
9	28.22	Isopulegone	029606-79-9	0.28	0.34	0.35
10	28.82	Isopulegol	000089-79-2	0.28	0.31	0.39
11	29.90	β-Caryophyllene	000087-44-5	0.28	0.27	0.42
12	37.50	α-Terpineol	000098-55-5	0.16	0.21	0.23
13	38.59	Bicyclogermacrene	024703-35-3	0.15	0.15	0.21
14	8.38	Limonene	000138-86-3	0.10	0.12	0.20
15	50.28	Jasmone	000488-10-8	0.08	0.08	0.09
16	23.13	Cis-3-Hexenyl Isovalerate	035154-45-1	0.07	0.09	0.09
17	36.57	lavandulol	000498-16-8	0.06	0.07	0.18
18	59.29	α-Cadinol	000481-34-5	0.05	0.05	0.08
19	8.72	Eucalyptol	000470-82-6	0.05	0.06	0.07
20	5.90	β-pinene	000127-91-3	0.05	0.05	0.06
21	4.14	α-pinene	000080-56-8	0.05	0.04	0.04
22	15.64	3-Hexen-1-ol, (Z)	000928-96-1	0.03	0.04	0.03
23	7.44	β-Myrcene	000123-35-3	0.03	0.04	0.05
24	48.78	Piperitenone	000491-09-8	0.03	/	0.09
25	58.61	tau-Muurolol	019912-62-0	0.03	0.01	0.02
26	6.25	Sabinene	003387-41-5	0.02	0.02	0.03
27	58.37	tau-Cadinol	005937-11-1	0.01	/	0.01
				99.41	**97.92**	**99.21**

Note: RT, Retention time; CAS: Chemical abstracts service; "/", Not detected or a component with a relative peak area less than 0.01%.

CONCLUSIONS

In conclusion, our study revealed that both red and blue light enhance the growth of the plant, essential oil content, yield, as well as the peltate glandular trichome density of *Mentha arvensis* L. Red light contributes more significantly to biomass accumulation, while blue light contributes more to essential oil biosynthesis and peltate glandular trichome development. Therefore, a combination of red and blue light may offer more favorable conditions for mint growth and essential oil biosynthesis.

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