



Manipulating Light Conditions for Enhanced Growth and Improved Phytochemical Composition in Peppermint: Uncovering the Nuances

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ABSTRACT

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This study examined the effects of supplemental blue and UV-A light on the growth, phytochemical composition, and essential oil (EO) production of peppermint (*Mentha × piperita* L.) under greenhouse conditions. Plants were subjected to four lighting treatments using LEDs: natural light (control), UV-A, blue light, and a combination of UV-A and blue light. Blue light significantly increased leaf area (391.78 mm²) and shoot fresh weight (48.78 g), while the combined UV-A and blue light treatment enhanced total chlorophyll (2.03 mg g⁻¹ FW) and carotenoid content (0.46 mg g⁻¹ FW). UV-A light alone improved antioxidant capacity (38.34% inhibition) and flavonoid content (32.78 mg g⁻¹ DW), but decreased both leaf area (253.42 mm²) and shoot fresh weight (33.35 g). Phytochemical analysis showed that blue light produced the highest EO yield (1.84% w/w), whereas the combined UV-A and blue light treatment resulted in the lowest (1.13% w/w). Gas chromatography-mass spectrometry (GC-MS) analysis revealed that blue light increased the concentrations of menthone (18.62–24.30%) and menthofuran (2.32–2.63%) but reduced menthol content. The highest menthol levels were found in the control (41.20%) and the combined UV-A + blue light treatment (37.45%). Significant positive correlations ($r^2 > 0.50$) were observed among antioxidant activity, menthone, total carotenoids, and flavonoids, underscoring the interconnected roles of these compounds in stress response and secondary metabolism. Overall, the findings demonstrate that light spectrum manipulation can be an effective strategy for optimizing peppermint growth, phytochemical profile, and EO production. Blue light improves biomass and EO yield, while UV-A enhances antioxidant capacity and secondary metabolite accumulation. However, combining UV-A and blue light may compromise EO yield, suggesting the importance of fine-tuning light regimes to achieve desired trade-offs between yield and quality in peppermint cultivation.

Abbreviations: 2-diphenyl-1-picrylhydrazyl (DPPH), Controlled environment agriculture (CEA), Chlorophyll (chl), Dry weight (DW), Dry matter (DM), Essential oils (EOs), Fresh weight (FW), Gas chromatography (GC), Gas chromatography-mass spectrometry (GC-MS), Reactive oxygen species (ROS), Retention indices (RI), Ultraviolet (UV)

Introduction

Light is the primary source of energy for photosynthesis and a key environmental cue regulating plant growth, photomorphogenesis, and

the biosynthesis of both primary and secondary metabolites (Hogewoning et al., 2010; Johkan et al., 2010; Islam et al., 2012). Among the various spectral

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components of light, ultraviolet (UV) and blue light are particularly influential in modulating plant physiology and metabolism. Over recent decades, climate change, largely driven by anthropogenic activities, has altered atmospheric composition, including the depletion of the ozone layer, resulting in elevated levels of UV radiation reaching the Earth's surface (Bernhard et al., 2020). UV radiation comprises three bands: UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (100–280 nm). These wavelengths affect plant systems through mechanisms such as DNA damage, the production of reactive oxygen species (ROS), and interference with photosynthesis (Hollósy, 2002; Ahmadi et al., 2019; Duan et al., 2022). Although high levels of UV radiation can be detrimental to plant growth and productivity, moderate UV exposure often induces beneficial stress responses, including the enhanced production of secondary metabolites (Verdaguer et al., 2017). These compounds, particularly flavonoids and phenolic acids, function as antioxidants, helping to mitigate oxidative stress and strengthen plant defense mechanisms (Takshak and Agrawal, 2019; Khare et al., 2020). In addition to UV light, blue light (400–500 nm) has garnered increasing interest for its regulatory role in plant growth and secondary metabolite biosynthesis. Blue light is perceived by specific photoreceptors such as cryptochromes and phototropins, which trigger signaling cascades that influence gene expression, chloroplast development, stomatal conductance, and metabolic activity (Cashmore et al., 1999; Folta and Carvalho, 2015). Numerous studies have shown that blue light enhances the accumulation of phenolic compounds, flavonoids, and essential oils in various plant species (Galvão and Fankhauser, 2015; Taulavuori et al., 2016). However, these effects are highly species-specific and influenced by factors such as genotype, light intensity, and exposure duration, underscoring the need for further research to optimize its use (Johkan et al., 2010; Islam et al., 2012). Peppermint (*Mentha × piperita* L.), a widely cultivated aromatic and medicinal herb in the Lamiaceae family, is prized for its essential oil (EO), which has broad applications in the pharmaceutical, food, and cosmetic industries (McKay and Blumberg, 2006). The EO profile of peppermint, mainly composed of menthol, menthone, and menthofuran, is known to be sensitive to environmental conditions, particularly light quality (Telci et al., 2011). Previous studies have reported that UV-A supplementation can improve leaf area, chlorophyll content, and total EO yield, whereas UV-B exposure increases overall EO content but alters its composition—typically decreasing menthol while increasing menthone and menthofuran (Maffei and Scannerini, 1999, 2000).

Similarly, blue light has been shown to enhance phenolic content, antioxidant activity, and EO biosynthesis in related species such as *Perilla frutescens* (Nguyen and Oh, 2022). Despite growing interest in spectral manipulation, research exploring the combined effects of UV and blue light on peppermint remains limited. Understanding these interactive effects is critical for developing optimized light regimes that enhance both yield and phytochemical quality in controlled environment agriculture.

This study investigated the effects of supplemental UV-A and blue light on the growth, physiological responses, and secondary metabolite production of peppermint (*Mentha × piperita* L.). While the individual effects of UV and blue light have been previously studied, their combined influence on peppermint's biochemical profile remains poorly understood. Emerging evidence suggests that blue light may mitigate UV-induced stress by modulating antioxidant defense pathways; however, the specific mechanisms involved in peppermint have yet to be elucidated (Hoffmann et al., 2015). By addressing this knowledge gap, the present research aims to inform strategies for optimizing light quality in controlled environments, thereby enhancing both the agronomic performance and commercial value of peppermint.

Material and methods

Plant materials and growth conditions

The experiment was conducted from 2021 to 2022 in the Research Greenhouse and Laboratory of Horticultural Science at Bu-Ali Sina University. Peppermint (*Mentha × piperita* L.) transplants were propagated from herbaceous cuttings rooted in September 2021. Each 3 L pot was filled with a growing medium consisting of equal parts garden soil, leaf compost, and greenhouse sand. The substrate had a pH of 6.8, an electrical conductivity (EC) of 1.2 dS m⁻¹, and an organic matter content of 3.5%. One rhizome was planted per pot. Plants were grown under controlled greenhouse conditions and received standard horticultural care. Soil moisture was maintained at field capacity through twice-weekly drip irrigation. A balanced NPK fertilizer (20–20–20) was applied at a concentration of 200 mg L⁻¹ every two weeks to ensure adequate nutrient availability. Environmental conditions, including daily minimum and maximum temperatures and the natural sunlight intensity inside the greenhouse, are shown in Figure 1. Plants were harvested 30 d after the initiation of light treatments for subsequent physiological and biochemical analyses.

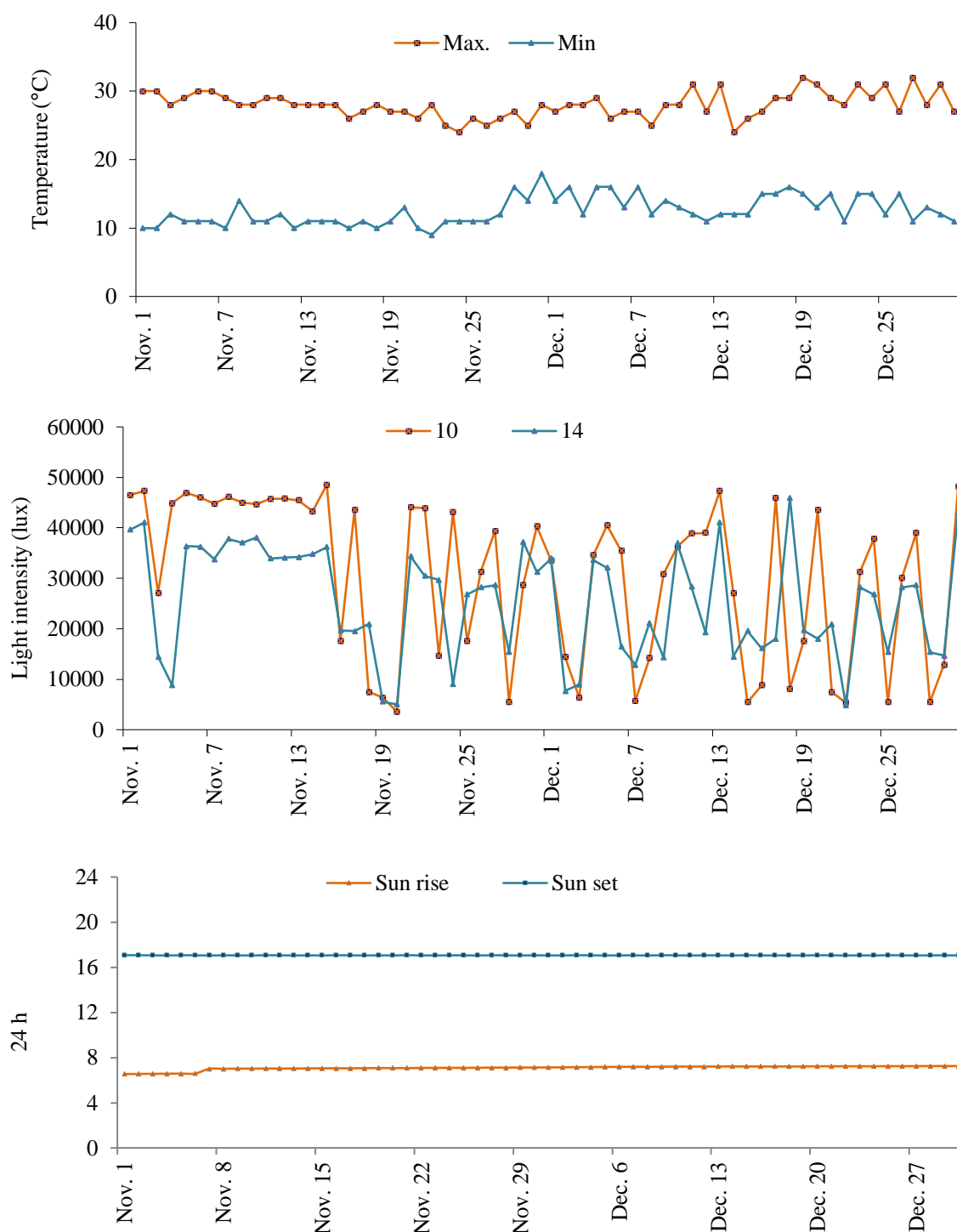


Fig. 1. Daily temperatures (a), light intensity (b) and natural sunlight duration (c) inside the greenhouse at the research site during the experiment.

Light treatments

The research was conducted using a completely randomized design with three replications, each consisting of three experimental units. Treatments included a control (natural light without supplemental lighting), UV-A (365 nm at $35 \mu\text{mol m}^{-2} \text{s}^{-1}$), blue light (440 nm at $80 \mu\text{mol m}^{-2} \text{s}^{-1}$), and a

combination of UV-A and blue light (UV-A+Blue, 50% UV-A and 50% blue LEDs). The LED strands were powered by a 12V supply and installed 60 cm above the pot surface, with aluminum film layers used to prevent light interference. To ensure consistency, the total duration of supplemental lighting was four h d^{-1} , provided at specific time

intervals (9:00, 11:00, 13:00, and 15:00, each for 1 h). The experiment lasted for three months (April 20 to July 20), after which plants were harvested, and their agromorphological and phytochemical traits were assessed. While light intensity varied between treatments, the study focused on the qualitative effects of different spectral compositions rather than achieving identical daily light integrals (Fig. S1).

Agro-morphological traits assessment

Shoot length and plant height were measured with a ruler (1 mm accuracy), and the average shoot and internode lengths were then calculated. A digital caliper (Mitutoyo Corporation, Japan; 0.05 mm accuracy) was used to measure stem diameter. The leaf area was measured using ImageJ, a Java-based image processing software developed by the National Institute of Health (available at <http://rsbweb.nih.gov/ij/>) (Collins, 2007). To assess biomass and total yield, plants were carefully removed from their pots, and the roots were washed to remove soil residues. Fresh weight (FW) was measured using a precision digital scale (LW303i, Bel Engineering, Italy; 0.001 g accuracy). All leaves were separated and weighed (LFW). Subsequently, leaves, branches, and roots were individually placed in an oven at 70 °C for 48 h for drying, after which their dry weight (DW) was recorded.

Chlorophyll and carotenoid concentrations estimation

The chlorophyll concentration was assessed using the procedure outlined by Porra et al. (1989). Initially, 100 mg of fresh leaf tissue was weighed and ground in a Chinese mortar with 5 mL of 80% acetone. The homogenate was centrifuged at $5000 \times g$ for 5 min, and the supernatant was carefully collected. The residue was re-extracted by adding 5 mL of 80% acetone, grinding again, and centrifuging. This process was repeated until the supernatant became colorless. The final volume was adjusted to 20 mL with 80% acetone. A spectrophotometer (Cary 100, Varian, USA) was used to measure absorbance (A) at 664 nm and 645 nm for chlorophylls and 470 nm for carotenoids. The concentrations of chlorophylls and carotenoids (mg g^{-1} fresh weight) were determined using the following equations:

$$\text{Chl } a = (12.25 \times A_{664}) - (2.55 \times A_{645})$$

$$\text{Chl } b = (30.13 \times A_{645}) - (4.91 \times A_{664})$$

$$\begin{aligned} \text{Total chlorophyll } (a + b) \\ = (17.76 \times A_{645}) \\ + (7.34 \times A_{664}) \end{aligned}$$

Carotenoids

$$= \frac{(1000 \times A_{470}) - (1.82 \times \text{Chl } a) - (85.02 \times \text{Chl } b)}{198}$$

Where A represents the absorbance at the specified wavelength.

Soluble carbohydrate concentration estimation

Soluble protein content was determined using the Bradford (1976) method. Fresh leaf tissue (0.5 g) was homogenized in 6.25 mL of extraction buffer and stored at 4 °C for 24 h. The mixture was then ground in a Chinese mortar and centrifuged at $6000 \times g$ for 20 min. After centrifugation, 0.1 mL of the supernatant was mixed with 5 mL of Biuret reagent, stirred briefly, and its absorbance (A) was measured at 595 nm using a spectrophotometer (Varian Cary 100, USA). The protein concentration (mg g^{-1} fresh weight) was calculated using the following equation:

$$\text{Protein } (\text{mg g}^{-1} \text{ FW}) = \frac{A \times 6.25}{1000} \times 0.5$$

Where A represents the absorbance at 595 nm.

Soluble carbohydrate concentration estimation

Soluble carbohydrate content was determined following the method of Paquin and Lechasseur (1979). Fresh leaf tissue (0.5 g) was finely chopped and homogenized in 5 mL of 96% ethanol using a porcelain mortar. The homogenate was centrifuged at 6,000 rpm for 15 min, and the supernatant was collected. The remaining residue was re-extracted twice with 5 mL of 70% ethanol, using the same centrifugation conditions each time. All supernatants were pooled to obtain the final extract. To quantify soluble carbohydrates, 0.1 mL of the combined extract was mixed with 3 mL of anthrone reagent (prepared by dissolving 0.2 g anthrone in 100 mL of 95% sulfuric acid). The mixture was incubated in a water bath at 95 °C for 10 min. After cooling to room temperature, the absorbance was measured at 625 nm using a spectrophotometer. Soluble carbohydrate concentrations were calculated based on a standard curve prepared with known concentrations of glucose.

Assessment of phenolics and antioxidant activities

Preparing extracts

To acquire the compounds, a 500 mg sample of air-dried leaf was crushed using a mortar. The crushed leaf samples were then mixed with 5 mL of 85% methanol. The mixture was stirred at a speed of 60 rpm for 60 min and subsequently centrifuged at 4000 rpm for 15 min. After centrifugation, the resulting supernatant was separated. This process was repeated for the lower phase (residual phase). This supernatant was added to the supernatant from the previous step and used as an extract.

Total phenol concentration

Determination of the concentration of total phenols was carried out utilizing the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). To perform this, 1500 μL of a 10% Folin-Ciocalteu reagent was added to 300 μL of the plant extract and allowed to incubate at room temperature for 5 min. Subsequently, 1200 μL of 7.5% sodium carbonate was added, and the mixture was transferred to a shaker, where it was kept at room temperature in the dark for 90 min. Finally, the absorbance of the solution was measured at a wavelength of 765 nm (Carry 100, Varian, USA) using a spectrophotometer. The quantification of total phenols was determined by employing a standard gallic acid curve, and the results were expressed as milligrams of gallic acid per gram of extract dry weight.

Flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric method described by Chang et al. (2002). The assay was performed by preparing a reaction mixture consisting of 0.1 mL of 10% aluminum chloride, 0.1 mL of potassium acetate, and 2.8 mL of distilled water. To this mixture, 0.5 mL of the plant extract was added. The samples were then incubated at room temperature for 30 min to allow for color development. Following incubation, the absorbance of the reaction mixture was measured at 415 nm using a spectrophotometer (Varian Cary 100, USA). Total flavonoid content was quantified using a standard curve generated with known concentrations of quercetin. Results were expressed as milligrams of quercetin equivalent per gram of extract weight (mg QE g^{-1}).

Anthocyanin content assessment

The measurement of anthocyanin concentration was conducted following the methodology outlined by Rapisarda et al. (2000). First, 95% methanol and 35% hydrochloric acid solution with volume ratios of 80 to 20 were prepared. A volume of 10 mL was extracted from the aforementioned solution and substituted with 2.5 mL of the herbal extract. Then, the absorption (A) of the samples at 532 nm was read by spectrophotometer (Carry 100, Varian, USA). The concentration of anthocyanins was calculated using the formula $[C \text{ mg L}^{-1} = \frac{A}{402.3 \times 10,000} \times DF]$, where the anthocyanin concentration is represented by C, the dilution factor is represented by DF, and the absorption value is represented by A.

Tannin content assessment

The measurement of tannin content was conducted using the Folin-Denis reagent. In this procedure, 250 μL of plant extract was mixed with 1375 μL of distilled water. To this solution, 125 μL of Folin-Denis reagent was added. After a 3 min interval, the

initial solution was mixed with an additional 250 μL of sodium carbonate solution and 8 mL of distilled water. The resulting mixture was shaken for 60 min, and the spectrophotometer was used to measure the absorbance at a wavelength of 725 nm (Carry 100, Varian, USA). The total tannin content was determined by converting it to an equivalent amount of tannic acid, measured in mg g^{-1} DW.

Antioxidant capacity

The evaluation of antioxidant activity was performed using the method described by Brand-Williams et al. (1995), which employs 2,2-diphenyl-1-picrylhydrazyl (DPPH). To initiate the process, a DPPH solution was prepared with a concentration of 0.05 mM in 85% methanol. Then, 500 μL of the plant extract and an equal volume of distilled water were added to the solution, and the resulting mixture underwent centrifugation at 10,000 rpm for 5 min. From this solution, a volume of 75 μL was extracted and combined with 2925 μL of DPPH. A spectrophotometer was used to measure the absorbance of the samples at 515 nm (Carry 100, Varian, USA). Subsequently, the samples were stored in a dark environment at room temperature for a duration of 30 min, and the absorbance was measured again.

The percent of inhibition was calculated using the [Activity (%) = $\frac{(At0 - At30) \times 100}{At0}$] equation

Essential oil extraction

The hydrodistillation of 40 g of air-dried leaves was carried out using a Clevenger-type apparatus. The distillation process involved 700 mL of distilled water and lasted for 3 h. The resulting EO was collected and dried using anhydrous sodium sulfate. Subsequently, the EO was stored in opaque vials and stored in a refrigerator at 4-5 °C until further analysis. The EO content was determined by considering the weight of the sample after drying.

Gas chromatography–mass spectrometry (GC–MS)

A Thermo Fischer capillary gas chromatograph was utilized to directly connect to a mass spectrometer system for the analysis of volatile constituents (TRACE GC, ThermoQuest-Finnigan). The analysis was conducted using an HP-5MS capillary column measuring 30 m in length and 0.25 mm in diameter, with a film thickness of 0.25 μm . The thermal program used a gradual increase in temperature, starting at 40 °C and reaching 240 °C with a rate of 4 °C min^{-1} . The injection chamber and linear transfer temperature were consistently maintained at 260 °C. The components were identified by determining their retention indices (RI) relative to a series of n-alkanes (C8-C20) acquired from Fluka in Buchs/sg,

Switzerland. Additionally, the recorded mass spectra of the components were compared to those stored in the spectrometer database (NIST MS Library v. 2.0) and referenced literature to confirm their identities.

Gas chromatography analysis (GC)

The EO that was extracted underwent dilution with hexane at a ratio of 10:100. From this mixture, a 0.1 mL sample was extracted for gas chromatographic analysis. The analysis was conducted using a ThermoQuest-Finnigan Trace gas chromatograph

(GC) equipped with an HP-5MS non-polar fused silica capillary column. The following operating conditions were applied: the temperature program started at 60 °C and remained constant for 2 min, then increased to 250 °C at a rate of 10 °C min⁻¹, with a final temperature retention of 5 min. The injector was set to "split mode" at a ratio of 1:100, and helium served as the carrier gas at a flow rate of 1 mL min⁻¹. The injector temperature was maintained at 250 °C, while the detector (flame ionization detector) operated at a temperature of 200 °C (Fig. 2).

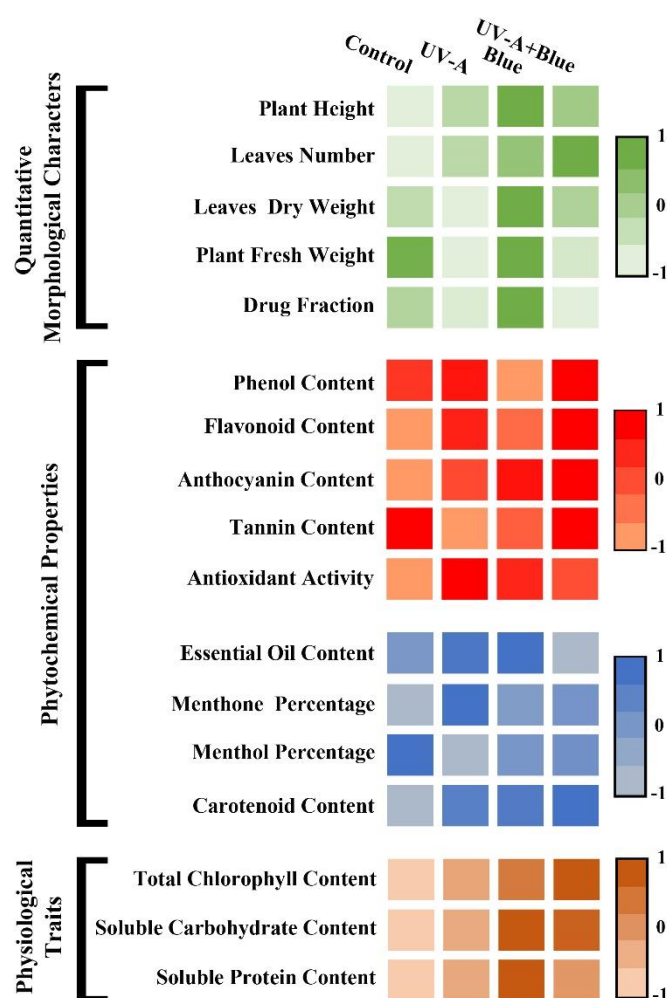


Fig. 2. Heatmap of the effect of different light spectrums on quantitative morphological characters, phytochemical properties and physiological traits of peppermint.

Statistical analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA) with the PROC GLM procedure in SAS software version 9.1. Prior to ANOVA, the normality of the data distribution was assessed using the Shapiro-Wilk test. To compare means, Duncan's multiple range test (DMRT) was

applied at a significance level of $P < 0.05$. Additionally, charts were created using Microsoft Excel, and a heatmap was generated to illustrate the effects of light treatments on the studied traits, following the approach used in a previous study on marjoram (Azizi et al., 2012).

Results

Quantitative morphological traits

Based on the results, the supplementary exposure had a significant effect ($P \leq 0.05$) on the number of

branches (NB), the number of nodes (NN) and leaf area (LA). However, it did not affect the stem length (SL), internode length (IL), shoot diameter (SD), and the number of leaves (NL) (Table 1).

Table 1. Effects of supplemental blue light and UV-A on some agro-morphological traits of peppermint.

Traits	Unit	Treatment			
		Control	UV-A	UV-A+Blue	Blue
NB**	-	24.33±1.15 ^a	18.33±1.53 ^b	14±1 ^c	14.66±1.53 ^c
SL ^{ns}	cm	13.99±1.35 ^a	15.79±1.36 ^a	16.90±1.73 ^a	19.06±5.99 ^a
SD ^{ns}	mm	0.16±0.01 ^a	0.16±0.02 ^a	0.16±0.02 ^a	0.18±0.02 ^a
NN*	-	14.08±0.36 ^b	17.1±0.98 ^a	14.23±1.25 ^b	16.55±1.15 ^a
IL ^{ns}	cm	1.03±0.1 ^a	0.99±0.09 ^a	1.27±0.2 ^a	1.20±0.29 ^a
NL ^{ns}	-	16.57±0.62 ^a	18.53±5.47 ^a	22.23±2.12 ^a	20.4±2.54 ^a
LA*	mm ²	325.28±41.33 ^{ab}	253.42±45.85 ^b	369.7±11.19 ^a	391.78±70.39 ^a
LFW*	g	19.09±3.07 ^{ab}	13.31±4.3 ^b	18.67±3.67 ^{ab}	23.88±0.69 ^a
SFW**	g	43.95±4.19 ^a	33.35±1.21 ^b	47.39±2.42 ^a	48.78±2.34 ^a
PFW ^{ns}	g	167.75±22.13 ^a	134.62±7.64 ^a	138.40±21.92 ^a	168.8±17.37 ^a
RFW**	g	104.71±17.03 ^a	93.29±1.15 ^a	62.2±5.71 ^b	103.85±8.07 ^a
LDW*	g	4.05±0.38 ^b	3.27±1.11 ^b	4.46±1.22 ^{ab}	5.91±0.38 ^a
SDW ^{ns}	g	9.83±0.93 ^a	7.32±1.85 ^a	8.87±1.35 ^a	9.05±2.05 ^a
PDW**	g	30.75±2.85 ^{ab}	26.68±3.41 ^b	25.88±0.99 ^b	34.69±2.13 ^a
RDW**	g	16.87±2.47 ^{ab}	16.09±0.74 ^b	12.54±1.75 ^c	19.72±0.98 ^a

NB (No. of branches), SL (Stem length), SD (Stem diameter), NN (No. of nodes), IL (Internode length), NL (No. of leaves), LA (Leaf area), LFW (Leaf fresh weight), SFW (Shoot fresh weight), PFW (Plant fresh weight), RFW (Root fresh weight), LDW (Leaf dry weight), SDW (Shoot dry weight), PDW (Plant dry weight), RDW (Root dry weight).

Different lowercase letters (a, b, c) indicate statistically significant differences between treatments. The error bars represent standard deviation (SD). The sample size (N) for each treatment was 3. P-value thresholds: * = $P \leq 0.05$, ** = $P \leq 0.01$, ^{ns} = not significant.

Thirty days after treatment initiation, control plants exhibited the highest number of branches (NB), averaging 24.3 per plant, whereas the UV-A + blue light treatment resulted in the lowest NB (14.0 per plant), which was not significantly different from the blue light treatment. UV-A treatment increased NB compared to the blue and UV-A + blue treatments, with no significant difference between UV-A and blue light. The average NB values were 17.1, 16.55, 14.23, and 14.08 for UV-A, blue, control, and UV-A + blue treatments, respectively. Leaf area (LA) was highest under blue light, averaging 391.78 mm², though not significantly different from the UV-A + blue and control treatments. The lowest LA (253.42 mm²) was observed under UV-A light (Table 1). Significant differences ($P \leq 0.05$) were found among treatments in leaf fresh weight (LFW), leaf dry weight (LDW), shoot fresh weight (SFW), root fresh weight (RFW), and root dry weight (RDW) (Table 1). The highest LFW (23.88 g plant⁻¹) was recorded under blue light, with no significant difference from the control and UV-A + blue treatments. The lowest LFW occurred under UV-A treatment. Blue light also produced the highest LDW (5.91 g), while UV-A treatment resulted in the lowest (3.27 g). Shoot fresh weight was highest under blue light (48.78 g) and lowest under UV-A light (33.35 g). However,

SFW under blue light was not significantly different from the control and UV-A + blue treatments. Shoot dry weight (SDW) showed no significant variation among treatments, ranging from 7.32 to 9.83 g. Root fresh weight was highest in control plants (103.85 g), with no significant difference from blue and UV-A treatments, whereas the UV-A + blue treatment yielded the lowest RFW (62.5 g). Root dry weight followed a similar trend, with the highest value under blue light (19.72 g) and the lowest under UV-A + blue light (12.54 g). As shown in Figure 2, blue light had the most pronounced positive effect on morphological traits such as stem length (SL), SDW, and plant fresh weight (PFW). In contrast, the combination of UV-A and blue light resulted in the highest number of leaves (NL).

Chlorophyll and carotenoid content

Supplementary exposure had a substantial and statistically significant effect ($P \leq 0.01$) on the synthesis of chlorophyll *a* (Chl*a*), chlorophyll *b* (Chl*b*), total chlorophyll (TChl), and total carotenoid (TC) in the leaves. The UV-A+Blue light treatment demonstrated the highest TChl, measuring 2.03 mg g⁻¹ FW, whereas the control plant exhibited the lowest TChl 1.2 mg g⁻¹ FW (Table 2). Similarly, the UV-A+Blue light treatment yielded the highest Chl*a*

content with a value of 1.41 mg g⁻¹ FW, while the control plants displayed the lowest (0.74 mg g⁻¹ FW). Regarding Chl_b, the UV-A+Blue light treatment exhibited the highest content, which did not show a significant difference compared to the levels observed in the blue light and UV-A treatments, measuring at 0.61, 0.54, and 0.49 mg g⁻¹ FW, respectively. Conversely, the control treatment exhibited the lowest Chl_b content, measuring at 0.37

mg g⁻¹ FW. The UV-A+Blue light treatment also showed the highest TC value with an average of 0.46 mg g⁻¹ FW, followed by plants exposed to blue light and UV-A treatments (0.44 and 0.43 mg g⁻¹ FW), while the control group had the lowest TC content. These findings, as presented in Table 2, revealed that the UV-A+Blue light treatment had a substantial effect on the pigment concentrations, as compared to the control, blue, or UV-A light.

Table 2. Effects of supplemental blue light and UV-A on chlorophyll and carotenoid content of peppermint.

Traits	Unit	Treatment			
		Control	UV-A	UV-A+Blue	Blue
Total chlorophyll**	mg g ⁻¹ FW	1.12±0.08 ^d	1.43±0.11 ^c	2.03±0.08 ^a	1.77±0.07 ^b
Chlorophyll <i>a</i> **	mg g ⁻¹ FW	0.74±0.05 ^c	0.93±0.05 ^c	1.41±0.1 ^a	1.22±0.05 ^b
Chlorophyll <i>b</i> **	mg g ⁻¹ FW	0.37±0.04 ^b	0.49±0.01 ^{ab}	0.61±0.03 ^a	0.54±0.035 ^a
Total Carotenoid**	mg g ⁻¹ FW	0.30±0.03 ^b	0.43±0.04 ^a	0.46±0.11 ^a	0.44±0.05 ^a

Different lowercase letters (a, b, c) indicate statistically significant differences between treatments. The error bars represent standard deviation (SD). The sample size (N) for each treatment was 3. P-value thresholds: * = P ≤ 0.05, ** = P ≤ 0.01, ^{ns} = not significant.

Soluble protein and carbohydrate variations

The concentrations of protein and soluble carbohydrates were significantly affected by supplemental light treatments (P < 0.01). Among all treatments, blue light resulted in the highest protein content, reaching 21.71 mg g⁻¹ fresh weight (FW) (Table 3). In contrast, the control treatment exhibited the lowest protein level (20.98 mg g⁻¹ FW), which

was not significantly different from those observed under UV-A and combined UV-A + blue light treatments. Similarly, blue light produced the highest concentration of soluble carbohydrates (5.82 mg g⁻¹ FW), although this value was not significantly different from that of the combined UV-A + blue light treatment. The control group showed the lowest carbohydrate content, averaging 2.37 mg g⁻¹ FW (Table 3).

Table 3. Effects of supplemental blue light and UV-A on soluble carbohydrate and total protein content of peppermint.

Traits	Unit	Treatment			
		Control	UV-A	UV-A+Blue	Blue
Carbohydrate content **	mg g ⁻¹ FW	2.37±0.08 ^c	3.37±0.17 ^b	5.55±0.07 ^a	5.82±0.08 ^a
Total protein**	mg g ⁻¹ FW	20.98±0.8 ^b	21.20±0.65 ^b	21.31±0.69 ^b	21.71±0.55 ^a

Different lowercase letters (a, b, c) indicate statistically significant differences between treatments. The error bars represent standard deviation (SD). The sample size (N) for each treatment was 3. P-value thresholds: * = P ≤ 0.05, ** = P ≤ 0.01, ^{ns} = not significant.

Phytochemical properties

Supplemental light treatments had a significant impact on several biochemical parameters, including phenol, flavonoid, and tannin content, antioxidant activity (P≤0.01), and anthocyanin concentration (P ≤ 0.05). The combined UV-A + blue light treatment produced the highest phenol content (39.64 mg g⁻¹ DW), though it was not significantly different from the UV-A and control treatments. The lowest phenol content was observed under blue light (38.19 mg g⁻¹ DW). Flavonoid content was also highest in the UV-A + blue light treatment (32.78 mg g⁻¹ DW), followed closely by the UV-A treatment, with no significant difference between them. The control group exhibited the lowest flavonoid concentration (27.70 mg g⁻¹ DW) (Table 4). Anthocyanin content peaked under the UV-A + blue light treatment (7.23 mg g⁻¹ DW), with no significant differences

compared to the UV-A and blue light treatments. The control group showed the lowest anthocyanin level (5.61 mg g⁻¹ DW), which was statistically similar to the UV-A treatment. Tannin content was highest under both the UV-A + blue light and control treatments (1.58 mg g⁻¹ DW), while the UV-A treatment recorded the lowest tannin content (1.45 mg g⁻¹ DW). The highest antioxidant activity was observed under UV-A light (38.34% inhibition), whereas the control treatment exhibited the lowest activity (11.83% inhibition) (Table 4).

Essential oil content and composition

A total of 41 to 43 compounds were identified in peppermint essential oil (EO) across all treatments (Table 5, Fig. S2). Oxygenated monoterpenes were the most abundant class of compounds, accounting for 87.13-88.64% of the total EO, with the highest

proportion observed in the control treatment (88.64%). Monoterpene hydrocarbons were present in smaller amounts (5.32-7.14%), while

sesquiterpene hydrocarbons and oxygenated sesquiterpenes constituted 3.62-4.83% and 0.63-1.05% of the EO, respectively.

Table 4. Effects of supplemental blue light and UV-A on phenolic properties and antioxidant power of peppermint.

Traits	Unit	Treatment			
		Control	UV-A	UV-A+Blue	Blue
Phenol content**	mg g ⁻¹ DW	39.14±0.85 ^a	39.45±0.35 ^a	39.64±0.55 ^a	38.19±0.56 ^b
Flavonoid content **	mg g ⁻¹ DW	27.70±0.35 ^c	31.7±0.49 ^a	32.78±0.64 ^a	29.29±0.77 ^b
Anthocyanin content *	mg g ⁻¹ DW	5.61±0.65 ^b	6.46±0.44 ^{ab}	7.23±0.63 ^a	7.05±0.15 ^a
Tannin content **	mg g ⁻¹ DW	1.58±0.02 ^a	1.45±0.02 ^c	1.58±0.03 ^a	1.50±0.01 ^b
Antioxidant activity **	(% of inhibition)	11.83±2.09 ^d	38.34±1.34 ^a	24.87±2.92 ^c	31.92±1.87 ^b

Different lowercase letters (a, b, c) indicate statistically significant differences between treatments. The error bars represent standard deviation (SD). The sample size (N) for each treatment was 3. P-value thresholds: * = $P \leq 0.05$, ** = $P \leq 0.01$, ^{ns} = not significant.

Table 5. Effects of supplemental blue light and UV-A on essential oil content and oil composition of peppermint.

NO.	Compounds	RI	Treatment			
			Control	UV-A	UV-A+Blue	Blue
1	α -thujene	926	0.02	0.03	0.03	0.04
2	α -Pinene	934	0.58	0.74	0.63	0.97
3	Sabinene	973	0.48	0.57	0.50	0.69
4	β -Pinene	978	0.96	1.15	1.02	1.46
5	β -Myrcene	990	0.21	0.26	0.23	0.35
6	α -Terpinene	1017	0.25	0.21	0.22	0.17
7	<i>p</i> -Cymene	1025	0.04	0.07	0.06	0.07
8	Limonene	1031	2.54	2.8	2.40	3.05
9	1,8-Cineol	1034	6.17	6.9	6.27	7.66
10	Z- β -Ocimene	1036	0.03	0.04	0.03	0.11
11	E- β -Ocimene	1046	0.11	0.19	0.16	0.14
12	γ -Terpinene	1058	0.02	0.03	0.02	0.02
13	cis-Sabinene hydrate	1068	1.35	1.38	1.20	1.05
14	α -Terpinolene	1090	0.08	0.07	0.08	0.07
15	Linalool	1101	0.12	0.10	0.10	0.08
16	Isopulegol	1151	0.09	0.05	0.07	0.07
17	menthone	1159	14.68 ^c	24.30 ^a	19.71 ^b	18.62 ^b
18	Isomenthone	1161	6.55 ^{ab}	7.09 ^a	5.43 ^b	7.27 ^a
19	menthofuran	1169	1.81 ^c	2.63 ^a	2.31 ^b	2.32 ^b
20	neoMenthol	1172	2.67	2.10	2.46	2.33
21	Menthol	1190	41.20 ^a	32.28 ^b	37.45 ^a	36.8 ^a
22	neoisoMenthol	1194	0.49	0.24	0.59	0.34
23	3-p-Menthol	1198	0.41	0.12	0.15	0.15
24	α -Terpineol	1200	0.19	0.12	0.24	0.14
25	Methyl salicylate	1203	0.02	0.05	0.04	-
26	Pulegone	1245	4.43	4.56	4.37	3.61
27	Piperitone	1258	0.46	0.31	0.37	0.31
28	Neomenthyl acetate	1277	0.36	0.01	0.18	0.03
29	Menthyl acetate	1298	7.37	5.46	6.45	6.12
30	iso-Menthyl acetate	1310	0.27	0.24	0.21	0.23
31	β -Bourbonene	1387	0.07	0.07	0.07	0.06
32	trans-Caryophyllene	1423	1.57	1.54	1.86	1.41
33	β -Farnesene (E)	1458	0.35	0.32	0.37	0.30
34	Germacrene D	1486	1.75	1.84	2.17	1.59
35	Bicyclogermacrene	1500	0.21	0.22	0.27	0.18
36	Germacrene A	1508	0.06	0.05	0.06	0.05
37	δ -Cadinene	1526	0.06	0.03	0.03	0.03
38	Germacrene D-4-ol	1579	0.02	0.01	0.01	0.01
39	Spathulenol	1582	0.06	0.04	0.05	0.05
40	Caryophyllene oxide	1586	0.28	0.11	0.18	0.09
41	Viridiflorol	1597	0.57	0.49	0.61	0.45
42	epi- α -Murolool	1646	0.09	ND	0.01	-
43	α -Cadinol	1659	0.03	0.02	0.03	0.03
Monoterpene hydrocarbons			5.32	6.16	7.14	5.38
Oxygenated monoterpenes			88.64	87.94	87.13	87.6

NO.	Compounds	RI	Treatment			
			Control	UV-A	UV-A+Blue	Blue
	Sesquiterpene hydrocarbons		4.07	4.07	3.62	4.83
	Oxygenated sesquiterpenes		1.05	0.67	0.63	0.89
	Total identified		99.09	98.84	98.52	98.70
	Essential oil content [% (w/w)]		1.47 ^b	1.79 ^a	1.84 ^a	1.13 ^c

Different lowercase letters (a, b, c) indicate statistically significant differences between treatments.

The essential oil (EO) of peppermint was primarily composed of menthol (32.28–41.20%), menthone (14.68–24.30%), 1,8-cineole (6.17–7.66%), menthyl acetate (5.46–7.37%), and isomenthone (5.43–7.27%). Among these constituents, menthol was the most abundant, with the highest concentration observed in the control treatment (41.20%) and the lowest under UV-A light (32.28%). In contrast, menthone content was significantly elevated under UV-A light (24.30%) compared to other treatments. The highest level of 1,8-cineole (7.66%) was recorded in plants exposed to blue light. Supplementary light treatments significantly affected EO yield ($P \leq 0.01$). The highest EO content was obtained under blue light (1.84% w/w), followed by UV-A light (1.79% w/w), whereas the lowest yield was recorded under the combined UV-A + blue light treatment (1.13% w/w). This indicates a potential antagonistic effect of combined UV-A and blue light on EO production, despite the positive effects of each when applied individually. Light treatments also significantly influenced the composition of EO constituents ($P \leq 0.05$). For example, menthofuran, a compound considered undesirable for EO quality, was highest under UV-A light (2.63%) and lowest in the control treatment (1.81%). Notably, UV-A treatment increased menthone levels but reduced menthol content, underscoring the role of specific light spectra in modulating the biosynthesis of key EO components. These findings highlight the sensitivity of peppermint EO yield and composition to light quality and the need for precise light management in controlled cultivation systems.

Pearson's correlation among important traits

To investigate the interrelationships among key morphological traits, phytochemical properties, and physiological responses under supplemental LED lighting, a comprehensive Pearson correlation analysis was performed (Fig. 3). Several strong positive correlations ($r^2 > 0.50$) were observed among antioxidant activity, menthone content, total carotenoids, chlorophyll *b*, total protein, total flavonoids, and total anthocyanins. Notably, menthone ($r^2 = 0.91$) and carotenoids ($r^2 = 0.78$) exhibited the strongest correlations with antioxidant activity, suggesting their central roles as major contributors to the plant's antioxidative capacity under different light treatments.

Discussion

This study contributed to a deeper understanding of how specific LED light spectra, particularly blue and UV-A wavelengths, regulate the growth, morphology, and biochemical composition of peppermint. Our findings reaffirm that light quality significantly influences plant development, aligning with previous studies on peppermint and other medicinal species (Macedo et al., 2011; Li et al., 2020). Light quality plays a critical role in plant morphogenesis by modulating cell division and elongation. Blue light, in particular, is well known for suppressing stem elongation while promoting compact growth and leaf expansion (Maffei and Scannerini, 1999; Wang et al., 2016). In the present study, blue light significantly increased leaf area (LA), supporting the hypothesis that it enhances lateral expansion, possibly through mechanisms such as improved stomatal conductance and increased photosynthetic efficiency. In contrast, exposure to UV-A light reduced both leaf area and shoot fresh weight, likely due to its inhibitory effects on cell division and elongation, as previously reported in peppermint and other species (Maffei and Scannerini, 2000; Hopkins et al., 2002). Interestingly, internode length (IL) and stem length (SL) remained unaffected by spectral treatments, a trend consistent with findings in basil and tomato, suggesting species-specific morphological responses to light spectrum variations (Huché-Thélier et al., 2016; Tabbert et al., 2022). Given that leaf size is a major determinant of biomass accumulation—owing to its role in light interception and photosynthesis (Lin et al., 2018)—the observed increase in LA under blue light may explain the maintenance of overall biomass despite UV-A exposure. This suggests a potential compensatory mechanism when both spectra are applied. Although red light is traditionally associated with increased biomass accumulation in *Melissa officinalis* (Aghakarim et al., 2023), blue light has also been linked to enhanced dry matter production in *Rosmarinus officinalis* (El Haddaji et al., 2023; Rahimi-Rizi et al., 2023). These results are consistent with those of Matsuda et al. (2008) and Brelsford et al. (2019), who reported that blue light enhances photomorphogenic responses and promotes resource allocation toward leaf expansion and structural development.

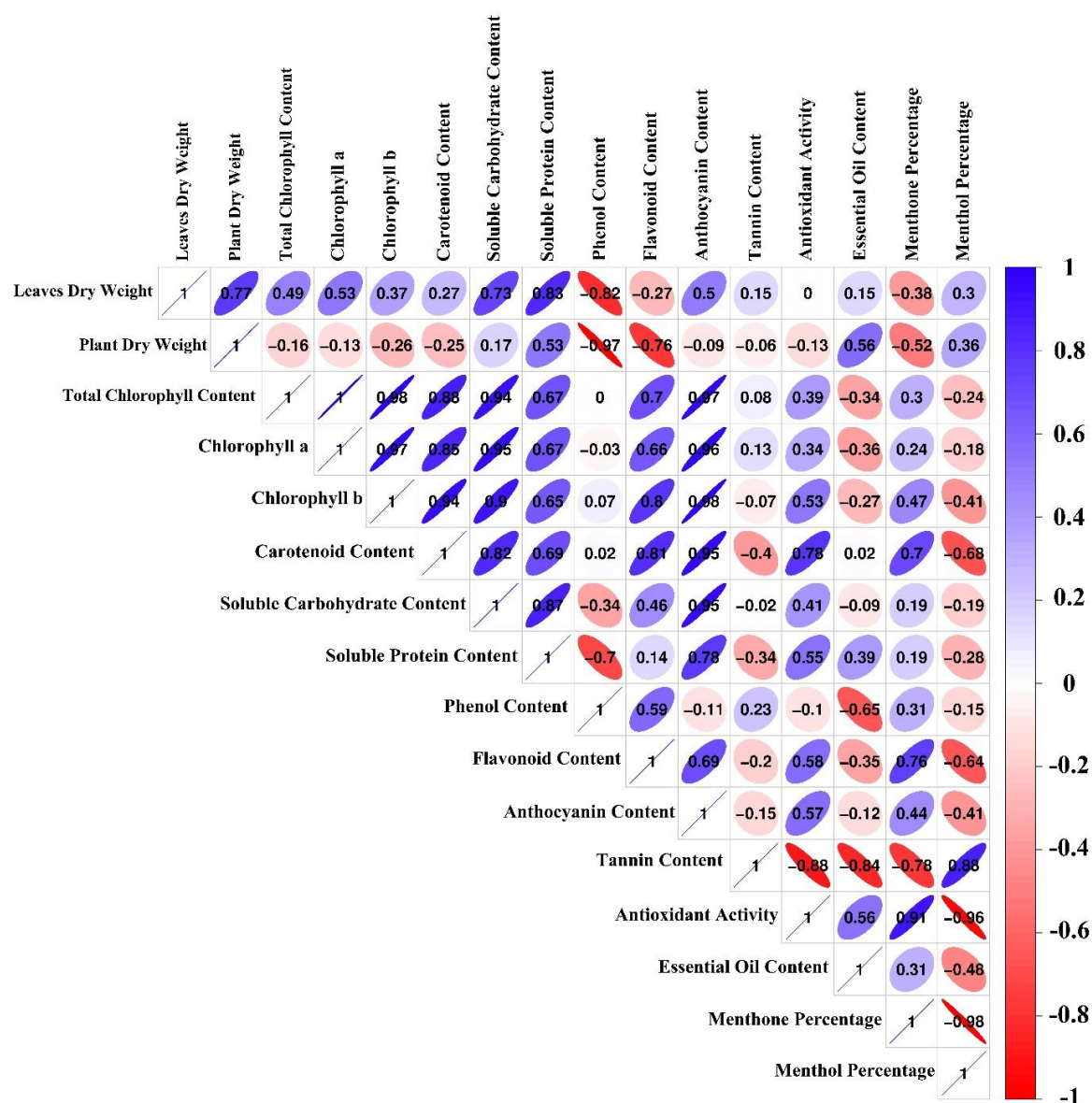


Fig. 3. Pearson's correlation coefficients between the parameters of peppermint after supplemental LED treatments.

The relationship between blue light and chlorophyll biosynthesis is well established, particularly during the early stages of plant development (Senger and Bauer, 1987; Quian-Ulloa and Stange, 2021). In our study, the combination of UV-A and blue light significantly enhanced total chlorophyll content, consistent with previous reports in *Cannabis sativa*, *Ocimum basilicum*, and *Brassica oleracea* (Johkan et al., 2010; Cheng et al., 2022). This combined spectral treatment also promoted anthocyanin accumulation, a response frequently observed in *Arabidopsis thaliana* and other UV-sensitive species (Brelsford et al., 2019). These findings suggest that UV-A and blue light act synergistically to regulate pigment biosynthesis, thereby enhancing both photosynthetic efficiency and stress tolerance. Light

quality is a key determinant of metabolic pathways, modulating both primary and secondary metabolism. Blue and red wavelengths, in particular, are known to enhance carbohydrate accumulation by improving photosynthetic performance, while blue light specifically promotes protein synthesis and stabilizes protein structures (Barro et al., 1989). In line with these observations, our study showed that blue light led to the highest levels of soluble carbohydrates and proteins, consistent with findings in *Brassica oleracea* (Chen et al., 2014). The biosynthesis of phenolic compounds and flavonoids is closely linked to light-induced activation of chalcone synthase, an enzyme highly responsive to UV-A, UV-B, and blue light (Zhou et al., 2013; Yan et al., 2019). Low doses of UV radiation, particularly UV-A, have been

shown to induce defense responses that elevate flavonoid content, as reported in *Silene littorea* and *Ocimum basilicum* (Kang et al., 2022). Our results corroborate these findings, with UV-A and blue light treatments resulting in the highest levels of total phenolics and flavonoids, mirroring outcomes in *Lactuca sativa* (Lee et al., 2019) and *Lepidium sativum* (Ajdanian et al., 2019). Moreover, peppermint essential oil (EO) composition is strongly influenced by light-induced shifts in secondary metabolism. Blue light has been shown to increase menthone, isomenthone, and menthofuran concentrations while reducing menthol levels, a key quality indicator of peppermint EO, as previously noted by Maffei and Scannerini (1999). Our findings support this pattern: blue light enhanced EO yield but was associated with reduced menthol content. Similarly, UV-A light increased the concentration of menthofuran, a less desirable EO component, echoing observations in *Ocimum basilicum*, where blue light reduced estragole levels (Carvalho et al., 2016). These results underscore the pivotal role of light spectra in modulating EO quality through targeted regulation of secondary metabolite biosynthesis.

Interestingly, monoterpene transformations in *Mentha × piperita* have been shown to accelerate under UV-B exposure, particularly under conditions of low solar radiation (Behn et al., 2010). This highlights the potential of spectral manipulation as a precise tool for fine-tuning essential oil (EO) composition, optimizing yield while preserving or enhancing desirable aromatic and therapeutic qualities. While antioxidant capacity in many plant species is commonly attributed to phenolic and flavonoid accumulation, evidence suggests that in peppermint, chlorophyll and anthocyanin levels may play a more prominent role (Mahmud et al., 2019; Mohd Yusof et al., 2021). In the present study, the UV-A treatment yielded the highest antioxidant capacity (38.34% inhibition), whereas the control treatment showed the lowest value (11.83%). These results are consistent with previous findings in *Ocimum basilicum* and *Perilla frutescens* (Carvalho et al., 2016; Nguyen and Oh, 2022), supporting the hypothesis that specific light wavelengths, particularly in the UV spectrum, activate stress-response pathways that enhance antioxidant potential. These findings underscore the complex but predictable relationships between light spectrum, secondary metabolite biosynthesis, and functional quality traits in medicinal and aromatic plants.

Conclusion

This study specified the pivotal role of blue and UV-A light in modulating peppermint growth, morphology, and biochemical traits. Blue light significantly promoted leaf expansion and biomass

accumulation, whereas UV-A enhanced antioxidant activity and increased phenolic compound concentrations. The combined application of UV-A and blue light synergistically elevated chlorophyll and anthocyanin levels, suggesting improved stress resilience. While blue light led to the highest essential oil (EO) yield, it also shifted EO composition, thereby reducing menthol while increasing menthone and menthofuran levels. These findings highlight the potential of targeted spectral manipulation as a strategy to optimize peppermint cultivation for improved phytochemical profiles. Future studies should investigate the long-term effects of spectral treatments and their interactions with other environmental variables to refine and personalize light-based cultivation protocols for medicinal and aromatic plants.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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Supplementary Materials

The Effects of Supplementary Lights on Peppermint

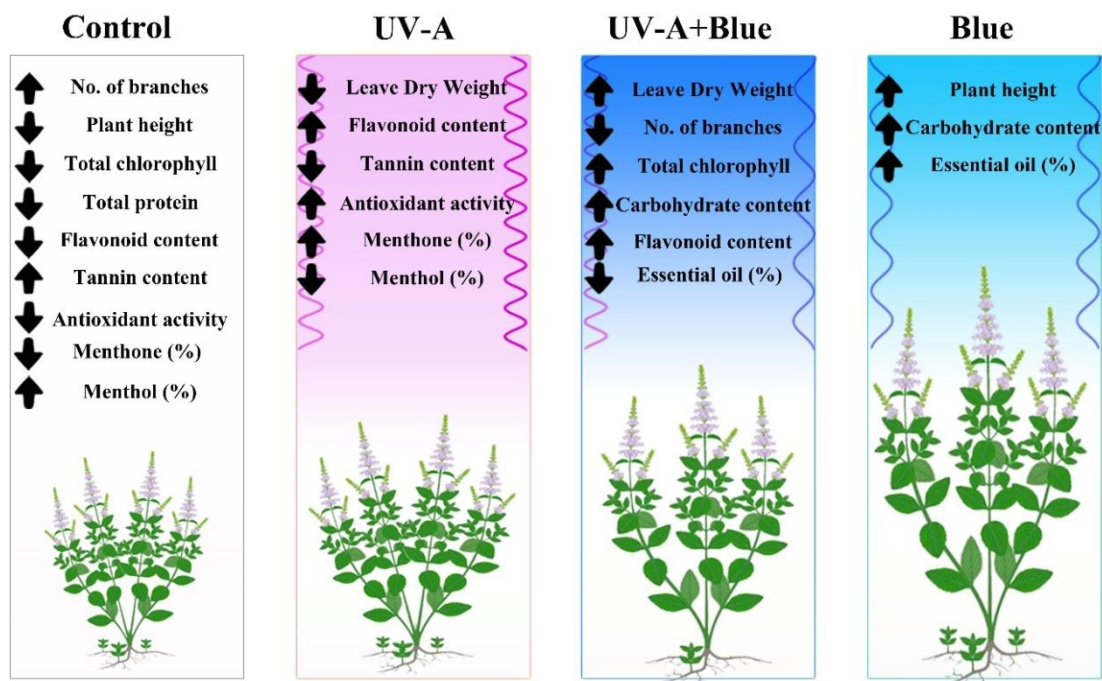
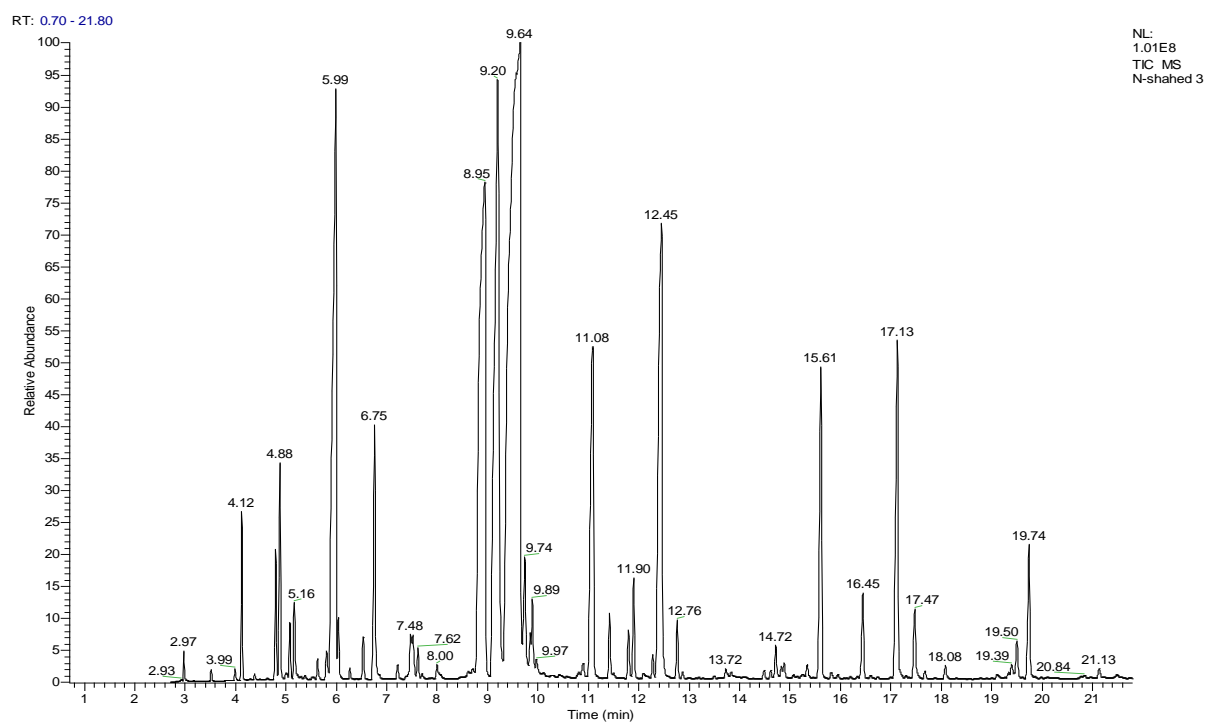


Fig. S1. The effect of supplementary lights on peppermint.

Fig. S2. GC-MS chromatogram of peppermint essential oil (*Mentha piperita*) in the control treatment.