

The Impact of LED Spectra on *Cannabis sativa* Production

Victorio Morello

Department of Bioresource Engineering

McGill University

Montreal, Quebec, Canada

A thesis submitted to McGill University in partial fulfillment of the
requirements of the degree of Master of Science

April 2021

©Victorio Morello, 2021

Abstract

Cannabis sativa is a highly responsive plant; of all the important environmental factors impacting *C. sativa*'s growth, lighting remains critical as it highly influences growth, secondary metabolite production and operational costs. The objective of this study was to investigate and evaluate the impact of six light spectra, including Blue (430 nm), Red (630 nm), Rose (430+630 nm, ratio 1:10), Purple (430+630 nm, ratio 2:1), and Amber (595 nm) lights, in addition to a double-ended high-pressure sodium (HPS) light (control), on *C. sativa* (intermediate chemotype) growth traits and secondary metabolite (cannabinoid and terpene) profiles. An HPS spectrum resulted in the highest inflorescence mass ($133.59 \text{ g} \pm 9.17$), and monochromatic blue light yielded has significantly less inflorescence mass ($76.39 \text{ g} \pm 3.21$). Different metrics used changes the views of the impact of light spectrum on tetrahydrocannabinol (THC) contents. Blue light resulted in the highest THC content ($10.17 \text{ \% w/w} \pm 0.13$) per gram dried inflorescence, yet the lowest THC content per plant ($1.44 \text{ g plant}^{-1} \pm 0.11$). The highest THC content per plant corresponded to *C. sativa* plants cultivated under the HPS spectrum ($2.54 \text{ g plant}^{-1} \pm 0.29$). Similar to THC, blue light influenced cannabigerol (CBG) and terpene biosynthesis, as THC content increased with increased blue light fraction, whereas blue light has less impact on cannabidiol (CBD) biosynthesis in the intermediate chemotype *C. sativa*. Overall, HPS spectrum significantly increased inflorescence mass, consequently leading to the highest production of values secondary metabolites per plant. As the combined effects of light spectrum on both growth traits and secondary metabolites have important ramifications for industry, inappropriate spectral design (high blue light or common blue/red LED light spectrum) for *C. sativa* growth would lead to a great reduction

in cannabinoid production (20-40 %). These findings show promise in helping producers choose spectral designs that meet specific *C. sativa* production goals.

Résumé

La *Cannabis sativa* est une plante hautement réactive à son milieu. De tous les facteurs environnementaux contribuant à sa croissance, la luminosité de bout-à-bout en est un des plus critiques à raison de son influence sur sa poussée, sa production de métabolites et sur son coût opérationnel en industrie. L'objectif de cette étude était d'évaluer l'impact de six différents spectres de lumière sur la croissance et le profil métabolique secondaire (cannabinoïde et terpène) de la *C. sativa* de chimiotype intermédiaire: 430 nm, *bleu*; 630 nm, *rouge*; 430+630 nm, ratio 1:10, *rose*; 430+630 nm, ratio 2:1, *violet*; 595 nm, *ambre*; et un contrôle avec une lumière de sodium à haute pression (HPS) et à double fonction. Le spectre HPS a produit la masse d'inflorescence la plus élevée de $133,59 \pm 9,17$ g, et la lumière monochromatique bleu a produit la masse d'inflorescence la plus basse de $76,39 \pm 3,21$ g. Les différents paramètres utilisées font varier les interprétations de l'impact du spectre de lumière sur la teneur en tétrahydrocannabinol (THC) : la lumière bleue a produit la teneur en THC la plus élevée de $10,17 \pm 0,13$ % w/w par gramme d'inflorescence sèche; mais encore la lumière bleue a aussi produit la teneur en THC par plante la plus basse de $1,44 \pm 0,11$ g par plante. La teneur en THC par plante la plus élevée de $2,54 \pm 0,29$ g par plante a été produite sous le spectre HPS. La lumière bleue a influencé la biosynthèse de cannabigerol (CBG) et de terpène, de concert avec l'augmentation de la teneur en THC

induite par l'augmentation de la fraction de lumière bleue. La lumière bleue a eu moins d'effet sur la biosynthèse de cannabidiol (CBD). Somme toute, le spectre HPS a significativement augmenté la masse d'influorescence générant ainsi la production la plus élevée de métabolites secondaires par plante. Puisque l'effet combiné du spectre de lumière sur la croissance et le profil métabolique secondaire peut potentiellement entraîner des ramifications importantes sur l'industrie du cannabis, un design spectral inadéquat, consistant en une lumière bleu élevé ou bleu/rouge LED au cours de la croissance de la *C. sativa* réduirait la production de cannabinoïde par une marge de 20 à 40 %. Les conclusions de cette thèse sont prometteuses dans leur potentiel à aider les producteurs de cannabis dans l'élaboration d'un spectre lumineux permettant d'atteindre des objectifs spécifiques de production de *C. sativa*.

Acknowledgements

I would like to thank Martin Robitaille who managed the plants every day, with his help all plants grow uniformly and properly. I gained a mentor in many aspects thanks to him. I would also like to thank my supervisor, Dr. Mark Lefsrud. Working with Dr. Mark led to many opportunities that would not be possible without him, it was a pleasure to see how far we pushed our vision. I am thankful for my mother who let me follow this path of passion as well as the rest of my family who supported me through the writing process. All the friends I made during my time at school will be remembered with fondness especially Natalie Wu who weathered COVID with me. Her support helped me move forward. I am thankful to my committee members, Dr. Valérie Orsat for her time as someone who was at the helm of the COVID response. Lastly, I am grateful for Exka for funding this study.

Contribution of Authors

Dr. Mark Lefsrud conceived and designed the experiment. Victorio Morello performed the experiments, analysis and wrote each chapter. Natalie Wu, Bo-Sen Wu, and Sarah MacPherson are the major editors that helped focus the writing. Martin Robitaille managed the plants day to day on an agreed plan created with Victorio. Pierre-Quan Francoeur translated the abstract.

Table of Content

Abstract	2
Résumé	3
Acknowledgements	5
Contribution of Authors	5
List of Figures	8
List of Tables	9
List of Abbreviation	10
1. Introduction	11
2. Literature review	14
3. Materials and methods	17
3.1 Plant materials and cultivation environment	17
3.2 Light spectra and plant cultivation	19
3.3 Plant measurement and harvest	24
3.4 Statistical analysis	25
4. Results	26
4.1 Morphology	26
4.2 Biomass	28
4.3 Nutrient uptake	30
4.4 Phytochemicals	33
4.4.1 THC and CBD	33
4.4.2 CBG and CBDV	37

4.4.3 Total terpene production	39
5. Discussion	43
5.1 Inflorescence mass	43
5.2 Nutrient uptake	44
5.3 Cannabinoids	45
5.4 Terpenes	47
5.5 Limitations (difference between replicates)	48
6. Conclusion	49
7. References	51
Appendix	55

List of Figures

Figure 1. Flowchart of plants for both replicates	18
Figure 2. Relative spectra of lights	19
Figure 3. Mean plant height graph	27
Figure 4. Representative images of each treatment	28
Figure 5. Inflorescence fresh and dry mass graph	30
Figure 6. Average THC and CBD graph	34
Figure 7. Average CBG and CBDV graph	39
Figure 8. Total terpenes produced graph	40
Figure 9: Photosynthetically active radiation (PAR) over the plant life cycle per light treatment of both replicates.	45

List of Tables

Table 1. Watering and nutrient solution schedule	22
Table 2. Leaf tissue nutrient analysis	32
Table 3. Concentrations of six most abundant terpene compounds	42
Table 4: Concentrations (mg/g) of 80 terpene compounds in <i>C. sativa</i>	56

List of Abbreviation

THC	tetrahydrocannabinol
THCA	tetrahydrocannabinolic acid
CBD	cannabidiol
CBDA	cannabidiolic acid
CBG	cannabigerol
CBGA	cannabigerolic acid
CBDV	cannabidivarin
HPS	high pressure sodium
LED	light-emitting diodes
PPFD	photosynthetic photon flux density
SE	standard error
PS	photosystem
Chl	chlorophyll

1. Introduction

Cannabis sativa has been exploited as a medicinal plant for over two millennia (Petrovska 2012). Considerable effort has aimed at investigating this plant's secondary metabolites, including cannabinoids and terpenes (Turner, Elsohly et al. 1980, Jin, Dai et al. 2020, Livingston, Quilichini et al. 2020). Major cannabinoids, including Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD), aid in reducing chronic pain, chemotherapy-induced nausea, vomiting, and improving multiple sclerosis spasticity symptoms (Van Klingerren and Ten Ham 1976, White, Munson et al. 1976, Mao, Price et al. 2000, Gonçalves, Rosado et al. 2019). Established evidence suggests that the combined action of cannabinoids and terpenes generates an "entourage effect" and postulated synergy between these two cannabis-derived psychoactive compounds (Johnson, Burnell-Nugent et al. 2010). Apart from these major secondary metabolites, *C. sativa* synthesizes and accumulates more than 500 known secondary metabolites (ElSohly, Radwan et al. 2017). With global movement on cannabis decriminalization and legalization, *C. sativa* production has become one of the most rapidly expanding markets (Bahji and Stephenson 2019, Eichhorn Bilodeau, Wu et al. 2019).

Legal commercial *C. sativa* production is legislated by a regulatory framework that controls production, distribution, and sale. Cannabis production commonly occurs indoors as licensed producers must adhere to regulations for production that can be difficult to meet in outdoor growing environments (Cox 2018). Enclosed growing allows optimization of crop production and secondary metabolite biosynthesis by fine-tuning environmental conditions, including light, CO₂ concentrations and temperature (Eichhorn Bilodeau, Wu et al. 2019, Jin, Jin et al. 2019). For example, elevating CO₂ levels result in higher

photosynthetic carbon assimilation, thus accelerating plant growth and improving productivity (Zelitch 1975). The impact of CO₂ concentration and temperature on *C. sativa*'s photosynthetic activity has been evaluated (Chandra, Lata et al. 2008, Chandra, Lata et al. 2011). Elevated CO₂ concentrations (250 to 750 μmol mol⁻¹) substantially increases net photosynthetic rates in *C. sativa* (Chandra, Lata et al. 2008) and the optimum temperature for cultivating medical drug type *C. sativa* ranges between 25-35 °C; however, temperatures above 30 °C can lead to adverse effects on photosynthesis and *C. sativa* growth (Chandra, Lata et al. 2008).

Light is another crucial parameter for enclosed *C. sativa* production, as it greatly impacts the growth and development of all plants through light intensity and spectra (Hawley, Graham et al. 2018, Magagnini, Grassi et al. 2018). Lighting represents one of the highest operation costs for *C. sativa* production (Mills 2012). In the greenhouse industry, light-emitting diodes (LEDs) have been widely used for plant cultivation as they are more energy-efficient over conventional light sources, including high-pressure sodium (HPS) lamps (Singh, Basu et al. 2015, Wu, Hitti et al. 2019). The impact of light spectrum on *C. sativa* cultivation has been reported with different lighting systems, including HPS (Vanhove, Van Damme et al. 2011, Potter and Duncombe 2012) and LEDs (Namdar, Charuvi et al. 2019, Amrein, Rinner et al. 2020, Danziger and Bernstein 2021). These studies concluded that blue light led to increased cannabinoid content, while supplemental green light induced both cannabinoid and terpene accumulation (Hawley, Graham et al. 2018, Namdar, Charuvi et al. 2019). Light spectrum also influenced *C. sativa* height and inflorescence dry mass (Magagnini, Grassi et al. 2018). However, these studies were conducted under various mixed-light spectra (i.e. mixture of blue and red

light with other supplementing light). It is difficult to dissect the impact of wavelength on *C. sativa*'s morphological traits and secondary metabolite production with these varied spectra. It makes comparison among studies difficult since it is unknown if such beneficial responses were triggered by the synergistic impact of combined wavelengths. This proves challenging when determining what light wavelengths are essential for growth traits and secondary metabolite production in *C. sativa*.

The objective of this study was to investigate and evaluate the impact of light spectra, including monochromatic light (blue, amber, and red wavelengths) and mixed light spectra with different ratios (blue and red wavelengths), on *C. sativa* inflorescence yield and its secondary metabolite (cannabinoid and terpene) profile. Intermediate chemotype *C. sativa* plants with both THC and CBD were cultivated under six light treatments, including five different LED spectra and an HPS spectrum in a controlled environment. Results from this study provide detailed information on how light spectrum impacts *C. sativa* production and accumulation of secondary metabolites. As LEDs are gaining momentum as a standard lighting system for grow facilities in this nascent legal industry, these data may help when constructing an optimal spectrum for *C. sativa* growth.

2. Literature Review

Light is a necessary environmental element required for plant growth. The main light source for most plants on Earth is sunlight which is composed of visible light, ultraviolet and infrared radiation (Moan 2001). Due to our atmosphere only 60% of the visible light and infrared radiation reaches Earth (Moan 2001). Of that energy 40% is visible light which has wavelengths of 380 and 740 nm (Moan 2001)

During the plant's life cycle, the amount and quality of light a plant receives causes a variety of effects such as growth, photomorphogenesis and production. The light energy produced by the sun is converted to chemical energy in plants through a process known as photosynthesis (Reese 2008). Photosynthesis is an anabolic process in which energy rich organic molecules/compounds are synthesized from CO₂ and H₂O using solar energy (Kochhar, 2020).

As simple as a leaf is, there is multileveled structure to harness the sun's energy. This reaction takes place in the mesophyll, the ground tissue of a leaf which is between the upper and lower epidermis or skin on the leaf (Campbell, 2008). Inside the mesophyll cell there are about 30 to 50 chloroplasts which are organelles inside the cell (Campbell, 2008) (Kochhar, 2020). The fluid within the chloroplast is called the stroma and it is enclosed by two membranes (Campbell, 2008). The thylakoids are a system of interconnected membranous sacs surrounded by the stroma (Campbell, 2008). The thylakoid sacs are normally stacked into columns called grana (Campbell, 2008). The thylakoid membrane contains photosynthetic pigments which are organized into networks called photosystems. These photosystems included pigments including chlorophyll (Chl) (Campbell, 2008, Kochhar, 2020).

In green plants, pigments can be categorized into two groups, vital pigments and accessory pigments (Kochhar, 2020). Vital pigments are Chl a while accessory pigments are composed of Chl b, carotenoids and phycobilin (Kochhar, 2020). Each of these pigments has a specific absorption spectrum which indicates which specific sets of wavelengths it can absorb (Croft and Chen 2017). Chlorophyll a has a peak absorption at 449 nm and 660 nm, Chl b has its peak absorption at 453 nm and 642 nm while carotenoids such as carotene and xanthophyll pigments have their absorption peaks are 440 nm and 470 nm (Kochhar, 2020). Chlorophyll a and Chl b have similar peak absorptions since both pigments have a similar structure. Chlorophyll molecules have a tennis racket like shape with a porphyrin ring above a phytol tail (S. L. Kochhar 2020). The main difference between Chl a and Chl b is one functional bond on the top of the racket head which allows each to absorb slightly different wavelengths (Reese 2008, S. L. Kochhar 2020).

The thylakoid takes in light energy and water to produce ATP and NADPH for the Calvin cycle. There are two different photosystems (PS) in the thylakoid membrane (Kochhar, 2020). PS II is found in the appressed regions of the granal thylakoids while PS I is found in the non-appressed region (Kochhar, 2020). These photosystems have similar multi-subunit protein complexes (Fromme, Jordan et al. 2001). The central structure of these complexes is a reaction center which acts as an energy sink allowing chlorophyll to absorb the longest wavelength (either P680 & P700 depending on the photosystem). Surrounding the reaction center is the light harvesting complex which contains photosynthetic pigments. PSII is the preliminary reaction where H₂O splitting occurs (Rögner, Boekema et al. 1996). In the presence of light, 2H₂O + CO₂ is converted

to O₂ as well as energy in the form of an electron (Rögner, Boekema et al. 1996). The electron is then delivered via the electron transport chain to PSI (Kochhar, 2020). As the electron is moved to PSI, it drops in energy which allows chemiosmosis to form ATP. PSI accepts light and electrons to further produce electrons which are used in the formation of NADPH.(Reese 2008)

Studies examining which precise wavelength is used in photosynthesis started as early as 1883 (Engelmann 1883). These studies took light divided with a prism and passed through a filament of algae (Engelmann 1883). Oxygen consuming bacteria was placed around the algae to grow where CO₂ was consumed by the photosynthesis algae which grew similar to the action spectrum of photosynthesis (Engelmann 1883). Current understanding of quantifying light for plants begins with McCree's research published in 1971 (McCree 1972). Light with wavelengths of 400-700 nm were shown to be absorbed and used for photosynthesis at different degrees of efficiency (McCree 1972). To measure the amount of light a plant uses, photosynthetically active radiation or PAR was introduced. PAR is defined as light between 400-700 nm and is measured as an irradiance (number of photons over a period of time on an area) recorded as $\mu\text{mol m}^{-2} \text{s}^{-1}$. McCree's experiment set-up the current action spectrum of photosynthesis by measuring the CO₂ consumed by 22 different variety of crop plants under different light wavelengths, covering the range of 350-750 nm in 25 nm intervals (McCree 1972). Plant's leaves use of CO₂ was determined using an infrared gas analyzer. Preliminary PAR curves were recorded which showed which wavelengths of light optimally photosynthesized per plant. Two broad max peaks in CO₂ consumption common across

the plants were 440 nm and 620 nm with a secondary maxima at 670 nm (McCree 1972).

3. Materials and methods

3.1 Plant materials and cultivation environment

Figure 1 summarizes cannabis propagation and cultivation conditions used in this study, including plant number, photosynthetic photon flux density (PPFD) level, and photoperiod used at each growing stage. One hundred *C. sativa* 'Babbas Erkle Cookies' (intermediate chemotype) cuttings were obtained from WeedMD Inc. (Aylmer, ON, Canada). Out of the initial 100 cuttings, 72 plants (uniform in size) were transplanted and placed in a growth chamber for the first crop cycle (Replicate 1), while remaining plants were used as mother plants. Cuttings for the second crop cycle (Replicate 2) came from these mother plants. The growing area for each light treatment was 1.2 by 1.2 m and divided into a 3 by 4 grid. Mother plants were maintained under fluorescent light (RAZR2, Fluence, Austin, TX, US). Cuttings for Replicate 2 were rooted using indole-3-butyric acid gel (Technaflora, Mission, BC, Canada) and rapid rooter plugs (General Hydroponics, Santa Rosa, CA, US), with an 18 h d⁻¹ photoperiod and a PPFD level of 125 $\mu\text{mol m}^{-2} \text{s}^{-1}$ under high humidity (> 90 %), using a propagating tray with transparent dome cover. PPFD was determined with a LI-250A Light Meter and a LI-193 Spherical Underwater Quantum Sensor (LI-COR, Lincoln, NE). Successfully propagated cuttings showing adventitious roots were transplanted into 750 mL square pots with Canna-coco coconut husk mixture (Canna, Toronto, ON, Canada) and 10 mL Myke Tree & Shrub Mycorrhizae

was added to the pots (Premier Tech, Rivière-du-Loup, QC, Canada). For each crop growth cycle, plants were divided into six experimental groups differentiated by six light treatments (12 plants/light treatment then 9 plant/light once flowered).

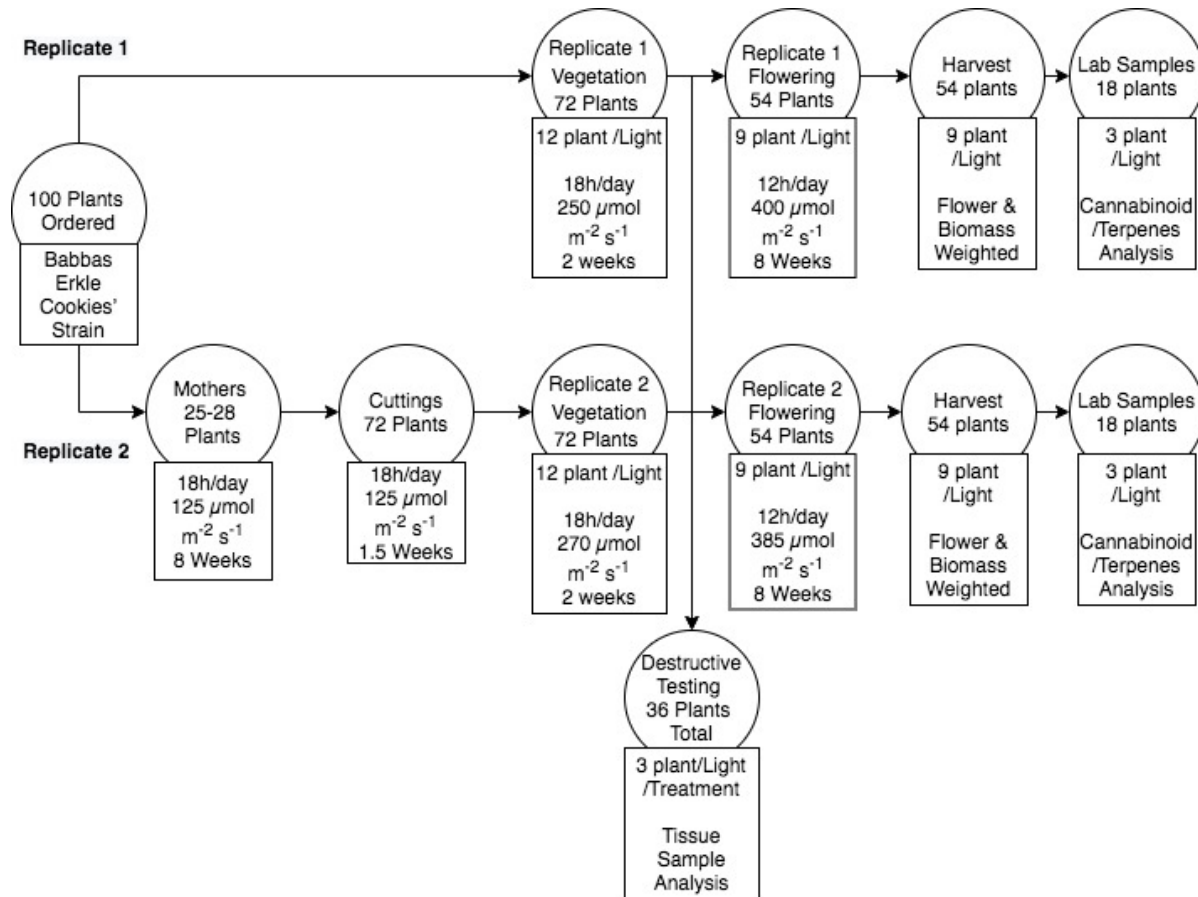


Figure 1. Flowchart of plants for both replicates, including photoperiod and light intensity for growing plants.

Each treatment was covered by 80% black shade cloths that were doubled layered to reduce stray light (~96% reduction). Shade curtains were used on three walls with the front side open. Stray light testing was done with all lights on, except the treatment zone being tested. Measurements were made at the center base of the table and stray light

bleeding was negligible ($< 2\%$). A 50 cm void space was left at the base under each shade curtain to allow air circulation. An exhaust fan cycled air between the growth room and outside environment, and four fans were placed in the room to allow for constant air movement. Nets were installed horizontally 15 cm over the plants to serve as support. Strings were added to support and maintain vertical orientation of the plants. During the day, the temperature of the grow room was kept between $28 \pm 2\text{ }^{\circ}\text{C}$, and relative humidity was between 40–55 %. Night-time temperature started at 25–27 $^{\circ}\text{C}$ and steadily decreased to 19–21 $^{\circ}\text{C}$ as outside temperature lowered. The night-time temperature of Replicate 2 was $\sim 4\text{ }^{\circ}\text{C}$ cooler than Replicate 1, while maintaining relative humidity between 50–65%.

3.2 Light spectra and plant cultivation

Light treatments comprised five different LED lights (Vanq Technology, Shenzhen, China) and a 750-W double-ended HPS lamp, which served as the control (Gavita, Aalsmeer, Netherlands) (Figure 2), for which all spectral composition data were confirmed with a spectroradiometer (ALP00051300010731, Asensetek, Gatineau, QC, Canada). The six different light treatments included: (1) a double-ended HPS lamp (main peak at 605 nm, a secondary peak at 575 nm, and a valley at 590 nm; Figure 1A); (2) a sole 590-nm spectrum ('Amber'); (3) a sole 630-nm spectrum ('Red'); (4) a combined 430-nm and 630-nm spectrum with a 1:10 ratio ('Rose'); (5) a combined 430-nm and 630-nm spectrum with a 2:1 ratio ('Purple'); and (6) a sole 430-nm spectrum ('Blue'). The HPS lamp, a traditional light fixture commonly used in commercial production, was used as a control. All LED spectra emitted 600 W from four 150 W LED chips equipped with glass circular

optic lenses (90° viewing angle). The HPS lamp was a larger single cylinder bulb with an aluminum reflection hood, which was powered at 750 W.

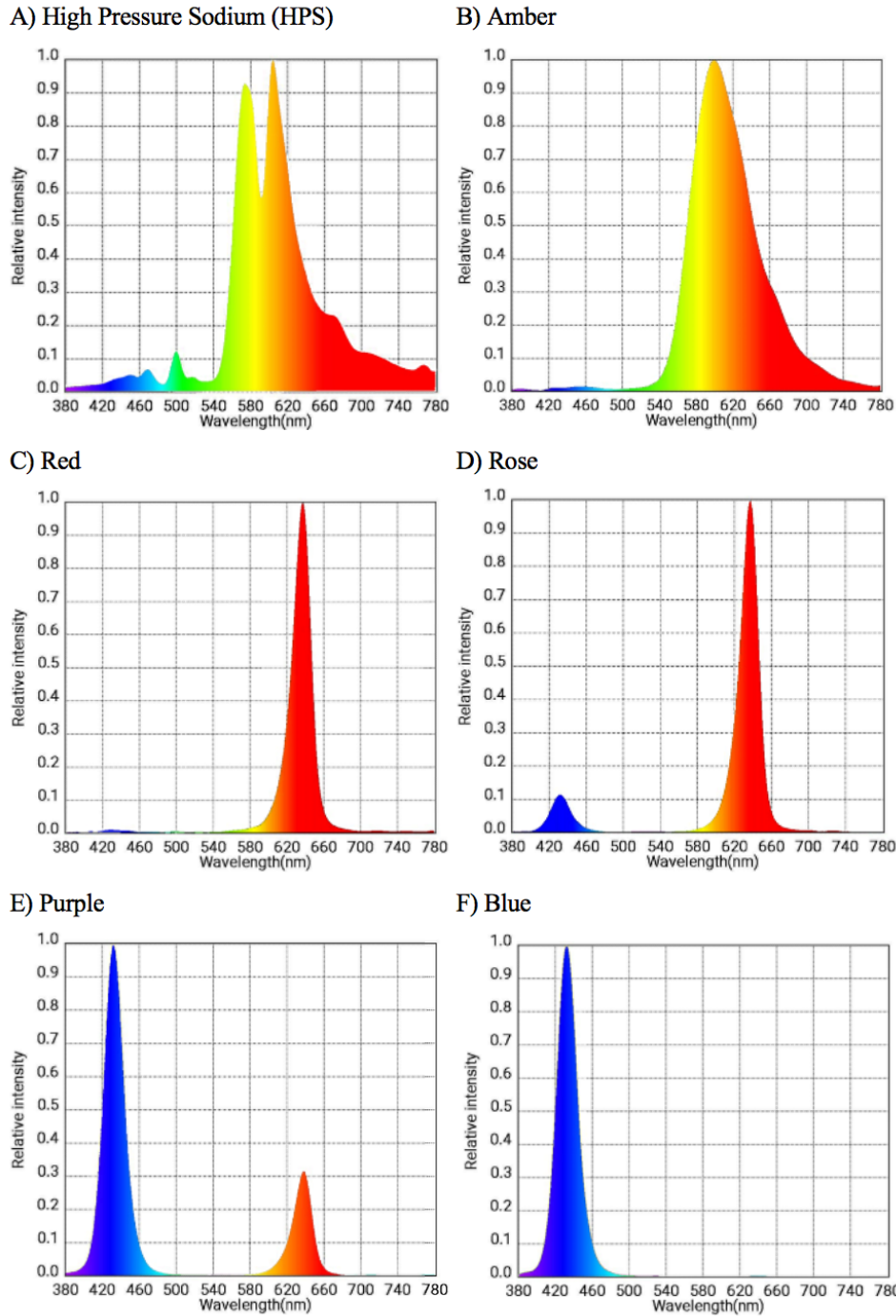


Figure 2. Relative spectra of a double-ended HPS lamp (control) and five experimental LED light treatments for cannabis plant cultivation: (A) double-ended HPS, (B) Amber

(590 nm), (C) Red (630 nm), (D) Rose (430 nm and 630 nm with a 1:10 ratio), (E) Purple (430 and 630 nm spectrum with a 2:1 ratio), and (F) Blue (430 nm) treatments. HPS: High Pressure Sodium.

All lights were suspended above the center of each treatment table, and experimental groups (12 potted plants/treatment) were placed in plastic flood tables under each light treatment. The PPFD level and photoperiod were adjusted based on plant growth stages. During the first two weeks (day 0–13, vegetation stage), plants were grown with an 18-h d⁻¹ photoperiod at 250–270 $\mu\text{mol m}^{-2} \text{s}^{-1}$. At the end of the vegetation stage (day 13), 3 plants were removed and the plant materials (i.e. leaves and stem) were sent for nutrient uptake analysis. The remaining 9 plants were transplanted into 2-L pots with additional Canna-coco coconut husk mixture and 20 mL Myke Tree & Shrub Mycorrhizae and switched to inductive photoperiod (12 h d⁻¹) for 8 weeks (day 14–70) until harvest. Once being switched to inductive photoperiod, PPFD levels were increased by $\sim 20 \mu\text{mol m}^{-2} \text{s}^{-1}$ every week until plants stopped growing vertically (day 35, 3rd week of flowering), which was then maintained at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Plant densities were 8.3 plant m⁻² during vegetative stage, and 6.25 plant m⁻² during flowering period. During the cultivation, plants were randomly reorganized every 3 days, which avoids inconsistent PPFD levels caused by light uniformity. In the meantime, PPFDs under each light treatment were confirmed and adjusted to the set point by adjusting the height of the lights.

Plants were trimmed on day 35 (3rd week of flowering) to avoid mildew using the following guidelines: (1) All fan leaves on the bottom two thirds of the primary stem were removed; (2) branches that grew from the bottom one third of the main-stem were stripped

of all fan leaves except for the top two fan leaves while all inflorescence -leaves were kept.; (3) Leaves in contact with inflorescence s were removed. Removed biomass trimmings were analysed.

Nutrient solutions consisted of tap water (Montréal, QC, Canada), coco A&B nutrient solutions (Canna, Toronto, ON, Canada) with nutrient content, monopotassium phosphate (KH_2PO_4) powder, potassium sulfate (K_2SO_4) and a pH down solution comprised of phosphoric acid (HGDi Technologies, Montreal, QC, Canada). This nutrient mix was increased from 500 ppm to 975 ppm over eight weeks (Table 1). The first watering was done by hand to compact the coco grow medium, and sequential watering was provided by two submersible pumps (728305, EcoPlus, Austin, TX, US) every 2–4 days. The amount of water provided increased during the study and tripled once plants were transplanted into their final pots. A nutrient flush was started on day 59 (no nutrients were added to the irrigation water) and lasted until harvest.

Table 1. Watering and nutrient solution schedule. Watering 1 for Replicate 2 occurred before plants were in the growth chamber. Watering 25 did not occur in Replicate 2 as flush started earlier than in Replicate 1.

Waterin g Number	Stage	Days after transplant (Replicate 1)	Days after transplant (Replicate 2)	Vol. provided (mL)	PPM	A&B (mL)	KH₂PO₄ (g)	K₂SO₄ (g)
1	Vegetative	0	-	150	500	210	0	0
2		4	0	200	600	270	0	0
3		6	5	200	650	300	0	0
4		8	9	200	650	300	0	0
5		10	11	250	700	325	0	0
6		12	12	250	700	325	0	0
7	Flowering	13	13	750	700	325	0	0
8		16	17	850	700	325	0	0
9		19	20	800	700	325	0	0
10		22	23	900	700	325	0	0
11		24	26	900	700	325	0	0
12		26	28	1080	750	350	0	0
13		29	31	900	750	350	0	0
14		31	33	900	750	250	100	0
15		33	36	1080	800	300	100	0
16		35	38	900	850	325	100	0

17	37	40	900	868	350	125	0	
18	40	42	1080	868	350	125	0	
19	43	45	1000	900	325	200	0	
20	45	48	775	900	325	200	0	
21	47	50	775	925	400	0	50	
22	49	52	775	925	400	0	50	
23	51	54	1080	975	400	0	75	
24	53	57	1080	975	400	0	75	
25	56	-	400	975	400	0	75	
26	58	59	1200	120	0	0	0	
27	61	61	600	120	0	0	0	
28	62	63	600	120	0	0	0	
29	64	64	1200	120	0	0	0	
30	66	66	600	120	0	0	0	
31	68	68	600	120	0	0	0	
32	70	70	HARVEST					

3.3 Plant measurement and harvest

During light treatments, plant height and the images were recorded. Plant height was measured once a week, and heights were recorded from the base of the plant to the highest new stem growth (excluding leaves). Images of plants growing under each light treatment were captured weekly. While images were taken, lighting from each treatment was switched off and flash from a digital camera was used. The camera was placed above the plant canopy, and the distance from the camera to the plant canopy was consistent (46 cm. Images were analyzed with a color histogram using ImageJ 1.48v software (Bethesda, MD, US), to determine the effect of light treatments on inflorescence and leaf colorations.

The plants removed on day 14, prior to the flowering stage, were cut at the base of the stem and growing medium. Harvested plant tissues were weighed with a kitchen scale (POC-P221-CA1, NEXT-SHINE, N/A, China) and biomass was dried in a Hamilton-Beach 32100C Food Dehydrator (Glen Allen, VI, USA) for 11 h at 50 °C. Dried plant material (stem and leaves) was sent for nutrient analyses to a third-party laboratory (A&L Labs, London, ON, Canada).

Remaining plants (9 per experimental group) were harvested eight weeks after initiating the flowering photoperiod. Inflorescence, stems, and leaves of the plants were weighed and placed on a screen for drying separated by plant. The inflorescences were placed in a dark room with a dehumidifier (<20 % RH) and dried for 3 days. Dried inflorescences were placed into a plastic bag for curing. Over a one week curing period, bags were opened once a day for 10 minutes.

Cannabinoid and terpene analyses were performed on dried plant material from each treatment using three separate 4-g inflorescence samples taken from three plants per light treatment per replicate. Each 4-g sample comprised a portion of the biggest inflorescence as well as equal parts of medium and small sized inflorescences. Cannabinoid and terpene were performed by Laboratoire PhytoChemia (Saguenay, QC, Canada). Measured cannabinoids included THC, tetrahydrocannabinolic acid (THCA), CBD, cannabidiolic acid (CBDA), cannabigerol (CBG), cannabigerolic acid (CBGA), and Cannabidivarin (CBDV). According to the Canadian Access to Cannabis for Medical Purposes Regulation, total THC is defined as $THCA * 0.877 + THC$. This equation was also applied to CBD and CBG by substituting the preferred cannabinoid (Hawley, Graham et al. 2018).

3.4 Statistical analysis

Statistical analyses were performed using JASP software (University of Amsterdam, Netherlands). Two-way ANOVAs were calculated by categorizing light and replication. Tukey post-hoc tests were performed on all data to find significant differences; if the data did not pass equality of variance by failing the Levene's test ($p < 0.05$), a Games Howell post hoc test was used to validate any results.

4. Results

C. sativa 'Babbas Erkle Cookies' plants were cultivated under six different light spectra for 10 weeks (2 weeks vegetative + 8 weeks flowering) to compare morphology, yield, nutrient uptake and phytochemical profiles of major cannabinoids and terpenes. Light spectra included a double-ended HPS (control), Blue (430 nm), Red (630 nm), Rose (430+630 nm, ratio 1:10), Purple (430+630 nm, ratio 2:1), and Amber (595 nm) light. The experiment was repeated to investigate differences between replicates.

4.1 Morphology

Plant height was measured after 21 days of flowering (Figure 3). Plants cultivated under Amber light resulted in the tallest plants, with a mean height of 79.61 cm \pm 1.53, followed by Red light (74.84 cm \pm 0.85), Rose light (74.65 cm \pm 1.09), HPS light (73.09 cm \pm 1.73), Purple light (70.65 cm \pm 1.22) and Blue light (66.75 cm \pm 1.23). Several significant differences were observed between light treatments ($p \leq 0.001$). Plants cultivated with Amber light were significantly taller than plants cultivated under Red, Rose, HPS, Purple and Blue lights ($p = 0.028$, $p = 0.020$, $p \leq 0.001$, $p \leq 0.001$, $p \leq 0.001$, respectively). Plants cultivated with blue light were significantly shorter than those cultivated under HPS, Rose and Red lights (all $p \leq 0.001$).

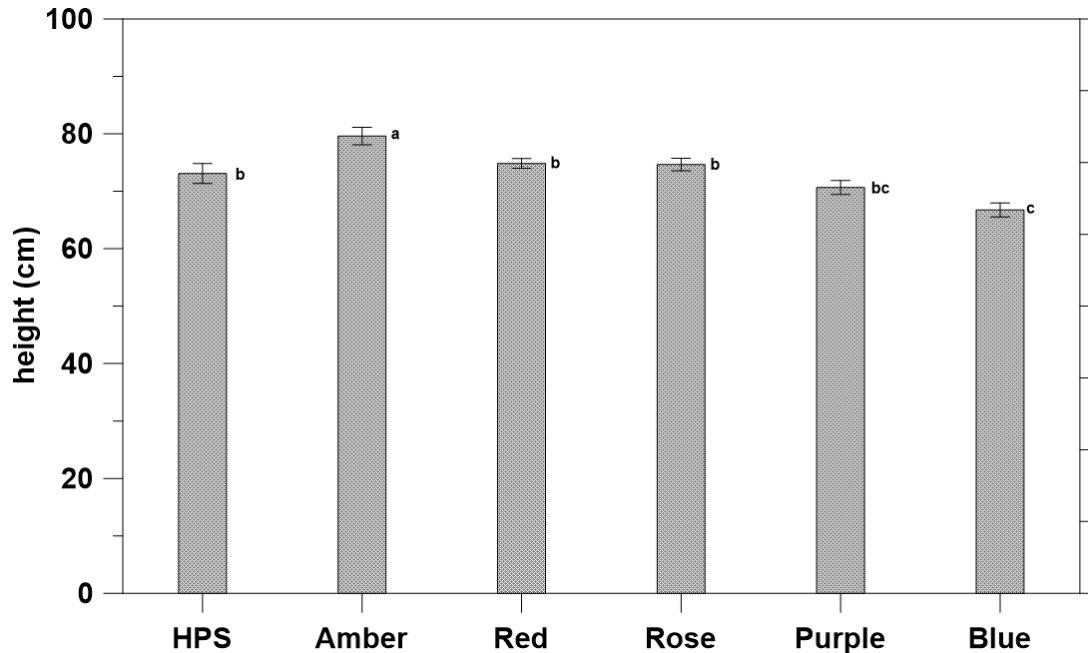


Figure 3. Mean plant height \pm SE (n=18, total of 108 plants) at day 21 of the flowering stage of both replicates under each light treatment. Data are presented in mean. Letters represent significance differences using Tukey's post hoc.

Leaf and inflorescence coloration for *C. sativa* plants cultivated under different light treatments were compared one week before harvest (Figure 4). Color histogram results (RGB values) showed that different lights did not influence inflorescence coloration. Rather, leaf coloration was affected and similar RGB profiles were observed for plants cultivated under HPS and Rose lights. A higher fraction of green color was observed for plants cultivated under Amber and Red lights, and leaves grown under these two light treatments were visibly brighter green than plants cultivated under the other treatments. Both Purple and Blue lights resulted in the lower RGB values, and Blue light resulted in the lowest fraction of blue color.



Figure 4. Representative images of leaf and inflorescence coloration under each light treatment one week before harvest.

4.2 Biomass

Mean inflorescence fresh and dry mass were measured after harvest (Figure 5). HPS light resulted in the highest inflorescence fresh mass ($133.59 \text{ g} \pm 9.17$), followed by Rose ($128.95 \text{ g} \pm 3.76$), Red ($122.98 \text{ g} \pm 4.17$), Amber ($103.11 \text{ g} \pm 4.81$), Purple (97.10 g

± 3.43), and Blue light in last ($76.39 \text{ g} \pm 3.21$). Statistically significant differences were observed for fresh inflorescence mass in three light treatments. Blue LED light resulted in significantly less fresh inflorescence mass than all other light treatments (all $p \leq 0.001$). HPS, Rose, and Red light yielded significantly greater fresh inflorescence mass than Purple and Amber light (all Purple $p \leq 0.001$, Amber: HPS $p = 0.013$, Rose $p = 0.002$, Red $p = 0.040$). The ranking of fresh inflorescence mass was consistent for both replicates. Significance differences were similar for the second crop cycle, with a few exceptions. In replication 1, Amber treatment was not significant to Red or Rose treatment, and Blue treatment was not significant to Purple treatment. In replication 2, Purple treatment was not significant different to Red treatment, and Blue treatment was not significantly different than Purple or Amber treatment. Drying reduced inflorescence mass by approximately 80% for each light treatment, and the moisture content was between 11–14 %. HPS yielded the greatest inflorescence dry mass ($26.26 \text{ g} \pm 1.84$), followed by Rose ($25.30 \text{ g} \pm 1.03$), Red ($23.83 \text{ g} \pm 0.88$), Purple ($18.74 \text{ g} \pm 0.82$), Amber (18.33 ± 0.82) and Blue ($15.49 \text{ g} \pm 0.80$) light. Dry mass under Blue light did not significant differ from dry mass measured for Amber and Purple light.

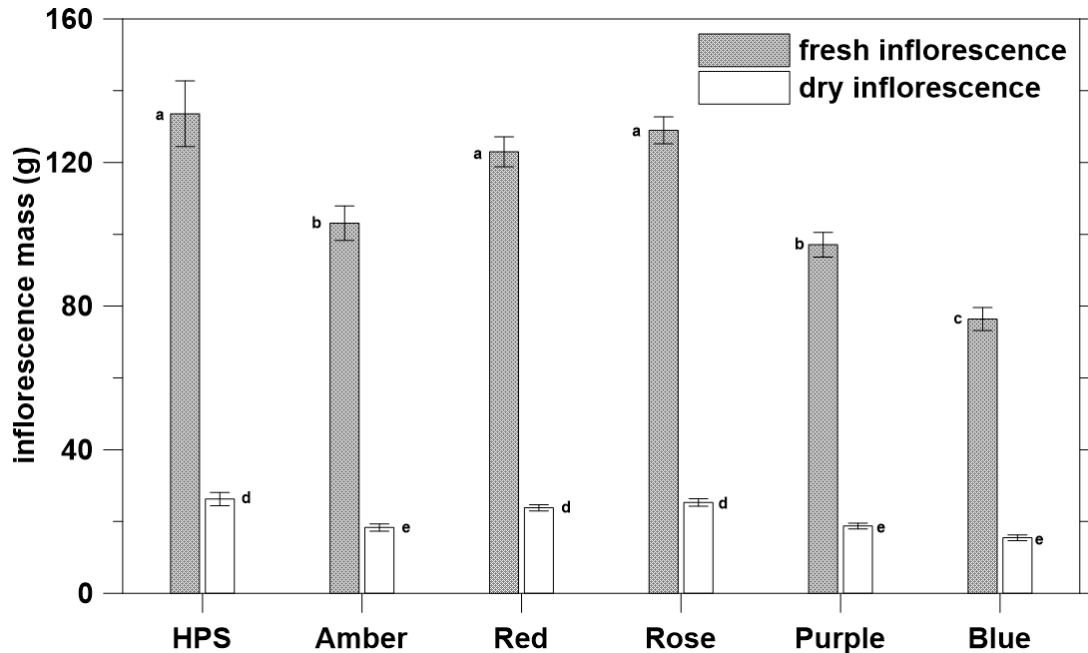


Figure 5. Mean \pm SE (n=18) inflorescence fresh and dry mass of both replicates under each light treatment. Letters represent significance differences found using Games Howell's post hoc.

4.3 Nutrient uptake

The plants removed at the end of vegetative stages had their leaves and stems sampled for nutrient uptake analysis (destructive testing) (Table 2). Significant differences were noted for nitrogen uptake between light treatments ($p= 0.016$). More nitrogen was present in dried tissue from *C. sativa* plants cultivated under Blue ($4.45 \% \pm 0.18$) and Purple ($4.34 \% \pm 0.14$) light when compared to other treatments, followed by HPS ($4.04 \% \pm 0.17$), Red ($3.95 \% \pm 0.17$), Rose ($3.93 \% \pm 0.10$) and Amber ($3.78 \% \pm 0.25$) light. Blue was significantly greater than Amber ($p= 0.024$) seen from post-hoc test. Dried tissue from plants cultivated under Rose light ($0.84 \% \pm 0.03$) contained more phosphorus and

potassium than tissue from plants cultivated under the other light treatments, followed by Purple (0.81 % \pm 0.04), Red (0.79 % \pm 0.02), Amber (0.76 % \pm 0.04), HPS (0.84 % \pm 0.03) and Blue (0.74 % \pm 0.03) light treatments. Significant differences in nutrient uptake of phosphorus between replicates were observed ($p= 0.002$). The potassium concentration measured in tissue from plants grown under Rose light was 4.16 % \pm 0.03, followed by Red (3.93 % \pm 0.28), Purple (3.84 % \pm 0.17), Amber (3.73 % \pm 0.31), Blue (3.67 % \pm 0.23), and HPS (3.57 % \pm 0.25). Significant differences in potassium content between replicates were observed ($p\leq 0.001$).

Other nutrient analyses of interest include magnesium, boron, and zinc. Magnesium was highest in Blue (0.67 % \pm 0.03) then Purple (0.62 % \pm 0.03), Red (0.57 % \pm 0.02), HPS (0.56 % \pm 0.04), Rose (0.55 % \pm 0.03) and Amber (0.50 % \pm 0.04). There was significant difference between lights for magnesium and Blue was found to be significantly greater than Amber. Blue had more boron with 32.5 ppm \pm 2.4 followed by Purple (27.2 ppm \pm 1.3), HPS (26.8 ppm \pm 2.2), Red (25.8 ppm \pm 2.0), Rose (24.7 ppm \pm 0.6), and Amber (23.2 ppm \pm 2.1). There was significant difference between the lights ($p\leq 0.001$) as well as the replicate ($p\leq 0.001$). Blue had significant greater boron than HPS ($p= 0.041$), Red ($p= 0.011$), Rose ($p= 0.002$), and Amber ($p\leq 0.001$). Zinc was found the highest in Blue (61.5 ppm \pm 3.2) followed by Purple (58.0 ppm \pm 2.9), Red (57.5 ppm \pm 3.0), HPS (57.2 ppm \pm 5.6), Amber (54.5 ppm \pm 5.3), and Rose (51.3 ppm \pm 2.4). Blue had significant more zinc ppm than rose ($p= 0.024$)

Table 2. Leaf tissue nutrient analysis for *C. sativa* plants cultivated under six different light treatments (mean \pm SE). The data presented are average of six plants from both replicates, and the highest among treatments are in bold. Letters represent significant difference using Tukey's post hoc test.

Elements	HPS	Amber	Red	Rose	Purple	Blue
Nitrogen (%)	4.04 \pm 0.17 ^{ab}	3.78 \pm 0.25 ^b	3.95 \pm 0.17 ^{ab}	3.93 \pm 0.10 ^{ab}	4.34 \pm 0.14 ^{ab}	4.45\pm 0.18^a
Phosphorus (%)	0.76 \pm 0.03	0.76 \pm 0.04	0.79 \pm 0.02	0.84\pm 0.03	0.81 \pm 0.04	0.74 \pm 0.03
Potassium (%)	3.57 \pm 0.25	3.73 \pm 0.31	3.97 \pm 0.28	4.16\pm 0.03	3.84 \pm 0.17	3.67 \pm 0.23
Sulfur (%)	0.29 \pm 0.02	0.27 \pm 0.02	0.30 \pm 0.02	0.30 \pm 0.01	0.32\pm 0.01	0.32\pm 0.02
Magnesium (%)	0.56 \pm 0.04 ^{ab}	0.50 \pm 0.04 ^b	0.57 \pm 0.02 ^{ab}	0.55 \pm 0.03 ^{ab}	0.62 \pm 0.03 ^{ab}	0.67\pm 0.03^a
Calcium (%)	4.67 \pm 0.44	4.08 \pm 0.50	4.51 \pm 0.38	4.41 \pm 0.19	4.63 \pm 0.26	5.28\pm 0.55
Sodium (%)	0.06 \pm 0.006,	0.07\pm 0.005	0.06 \pm 0.004,	0.06 \pm 0.004,	0.06 \pm 0.002	0.06 \pm 0.003
Nitrate nitrogen (ppm)	2108.0 \pm 203.1	1945.0 \pm 195.4	2220.0\pm 152.6	1973.3 \pm 152.9	1983.3 \pm 187.6	2123.3 \pm 230.0
Boron (ppm)	26.8 \pm 2.2 ^b	23.2 \pm 2.1 ^b	25.8 \pm 2.0 ^b	24.7 \pm 0.6 ^b	27.2 \pm 1.3 ^{ab}	32.5\pm 2.4^a
Zinc (ppm)	57.2 \pm 5.6 ^{ab}	54.5 \pm 5.3 ^{ab}	57.5 \pm 3.0 ^{ab}	51.3 \pm 2.4 ^b	58.0 \pm 2.9 ^{ab}	61.5\pm 3.2^a
Manganese (ppm)	75.8 \pm 10.9	63.7 \pm 11.4	67.8 \pm 9.4	69.3 \pm 5.6	75.7 \pm 6.6	87.2\pm 14.1
Iron (ppm)	135.5 \pm 10.3	128.7 \pm 12.3	147.8 \pm 8.3	126.8 \pm 8.1	157.5\pm 7.9	145.2 \pm 15.0
Copper (ppm)	7.0 \pm 0.4	7.0 \pm 0.4	7.0 \pm 0.4	6.2 \pm 0.2	7.5 \pm 0.2	8.0\pm 0.4
Aluminum (ppm)	13.7 \pm 2.4	12.7 \pm 1.6	10.7 \pm 1.9	12.0 \pm 2.1	15.7\pm 1.7	13.2 \pm 1.6

4.4 Phytochemicals

4.4.1 THC and CBD

Figure 6 summarizes the effect of light treatments on THC and CBD contents in *C. sativa* crops cultivated in two identical and controlled environments. The effect of wavelength on total THC content was statistically significant ($p \leq 0.001$). The highest total THC content was observed in plants grown under Blue ($10.17 \% \pm 0.13$), and Purple ($8.98 \% \pm 0.49$) light. HPS and Rose light had intermediate total THC content ($8.50 \% \pm 0.47$, $8.19 \% \pm 0.59$, respectively), while Red and Amber light had the least total THC content in the dry inflorescence ($6.66 \% \pm 0.8$, $6.36 \% \pm 0.74$). Total THC content in plants cultivated under Blue light was significantly higher than that of plants cultivated under HPS, Amber, Red and Rose light ($p = 0.013$, $p \leq 0.001$, $p \leq 0.001$, $p = 0.003$ respectively). The effect of Amber and Red light on total THC content was significantly lower than HPS, Rose and Purple light (Amber treatment: $p = 0.001$, $p = 0.005$, $p \leq 0.001$, Red treatment: $p = 0.005$, $p = 0.026$, $p \leq 0.001$, respectively).

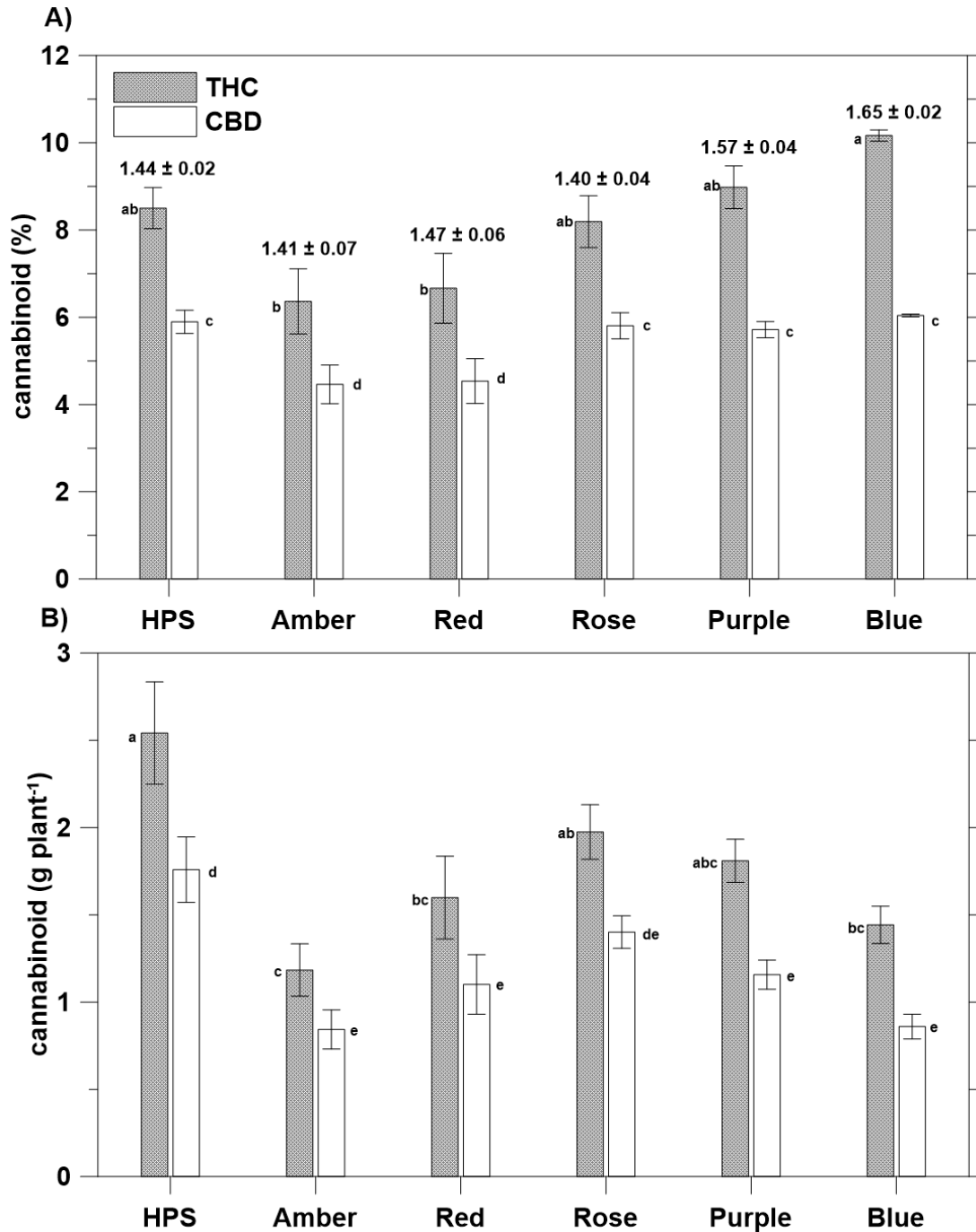


Figure 6. Average total THC and CBD concentrations in *C. sativa* inflorescence under each light treatment. (A) THC and CBD concentrations in percentage (%), and (B) THC and CBD concentrations per plant (g plant⁻¹). Values presented in mean ± SE

(n=3/treatment/replicate). Letters represent significance differences found using Tukey's post hoc. Values in Figure 7A refer to THC and CBD ratio (mean \pm SE). Total of 36 plants.

Differences in cannabinoid content between replicate were analysed to identify alterations in two seemingly identical and environmentally controlled crop cycles. A higher THC percentage was observed in the second crop cycle (Replicate 2) when compared to the first (Replicate 1) for each light treatment. The hierarchical order of total THC content was the same between replicates overall, with the exception that the effects of HPS light matched those of Rose light in the second crop cycle (Replicate 2; 9.35 % \pm 0.46 versus 9.38 % \pm 0.13), while for Replicate 1 (7.65 % \pm 0.42 versus 7.00 % \pm 0.57). In Replicate 1, Amber and Red treatments were significantly less than HPS, Purple and Blue treatments (Amber treatment: $p=0.048$, $p=0.023$, $p\leq 0.001$, Red treatment: $p=0.041$, $p=0.020$, $p\leq 0.001$). Rose treatment was significantly less from Blue treatment with $p=0.023$. In Replicate 2, Amber treatment was significantly less than HPS, Rose, Purple and Blue treatments ($p=0.038$, $p=0.034$, $p=0.004$, $p<0.001$), and Red treatment was significantly less than Purple and Blue treatments ($p=0.046$, $p=0.011$). The treatments without blue light resulted in lower THC content than plants given a small amount of blue light. Increasing the proportion of blue light shows an increase in total THC concentration. When considering total inflorescence dry mass, plants cultivated under HPS had the highest THC content per plant (2.54 g \pm 0.29), followed by Rose (1.98 g \pm 0.16), purple (1.81 g \pm 0.12), Red (1.60 g \pm 0.24), Blue (1.44 g \pm 0.11) and Amber (1.18 g \pm 0.15) light. Post hoc analyses showed that HPS light was significantly different from Amber, Blue and

Red light treatments for this parameter ($p \leq 0.001$, $p = 0.003$, $p = 0.013$), and Rose light was significantly different from Amber light ($p = 0.049$).

The effect of wavelength on CBD content from both replicates was statistically significant ($p \leq 0.001$) (Figure 6B). The highest CBD content was observed for inflorescence cultivated under the Blue light treatment ($6.04 \% \pm 0.03$), followed by HPS, Rose and Purple light treatments ($5.89 \% \pm 0.26$, $5.8 \% \pm 0.29$, and $5.72 \% \pm 0.18$, respectively). Inflorescence for plants cultivated under Red and Amber light treatments had the lowest CBD content ($4.54 \% \pm 0.51$, $4.46 \% \pm 0.44$).

A significant difference of CBD concentration between replicates was observed ($p \leq 0.001$) but no significant difference for the interaction effect of the light and replication. Using the post hoc test, inflorescence cultivated under Red and Amber light treatments have significantly less CBD concentration than HPS, Rose, Purple and Blue light treatments (Red: $p = 0.010$, $p = 0.019$, $p = 0.032$, $p = 0.004$; Amber: $p = 0.006$, $p = 0.012$, $p = 0.020$, $p = 0.002$). Data did not pass the test for equality of variance and validating with a more conservative post hoc test did not prove significance. Inflorescence in Replicate 2 had more CBD in each light treatment and did not follow the same ranking as the first replication. Inflorescence analysed from Replicate 1 showed that Blue light treatment resulted in the greatest CBD content ($6.02 \% \pm 0.04$), followed by HPS, Purple and Rose light treatments ($5.5 \% \pm 0.28$, $5.41 \% \pm 0.26$, $5.23 \% \pm 0.23$). Amber and Red light treatments had the least CBD content ($3.96 \% \pm 0.79$, $3.59 \% \pm 0.41$). An ANOVA shows that there is a significant difference between lights ($p = 0.008$). Tukey's post hoc test shows that Blue light treatment is significantly different from Red and Amber treatments ($p = 0.012$, $p \leq 0.036$). Data from Replicate 1 were not of equal variance and could not be

validated. Replicate 2 data had Rose and HPS light with the most CBD content ($6.38 \% \pm 0.24$, $6.29 \% \pm 0.35$) followed by Blue, Purple, Red and Amber light treatments ($6.06 \% \pm 0.05$, $6.03 \% \pm 0.08$, $5.48 \% \pm 0.51$, $4.96 \% \pm 0.31$). An ANOVA showed significance difference ($p= 0.044$) but a post hoc test did not find any specific significant differences between any lights.

Total CBD concentration per plant showed that HPS treatment yielded the most total CBD per plant ($1.76 \text{ g} \pm 0.19$) in both replicates, followed by Rose ($1.40 \text{ g} \pm 0.09$), Purple ($1.16 \text{ g} \pm 0.08$), Red ($1.10 \text{ g} \pm 0.17$), Blue ($0.86 \text{ g} \pm 0.07$) and Amber light treatments ($0.84 \text{ g} \pm 0.11$). There were statistical significant differences between the light treatments ($p \leq 0.001$). Post hoc shows HPS treatment is significantly different than Amber, Blue, Red and Purple treatments ($p \leq 0.001$, $p \leq 0.001$, $p= 0.014$, and $p= 0.03$, respectively). The THC to CBD ratio was impacted by light treatments. Blue treatment had the highest THC to CBD ratio with 1.68 ± 0.019 . There is a significant difference between light treatments ($p \leq 0.001$). Initial post hoc showed that Blue light treatment was significantly higher than HPS, Amber, Red and Rose treatments (all $p \leq 0.001$). Purple treatment was significant greater to Rose and Amber treatments ($p= 0.016$, $p= 0.029$). Validation was needed and showed Blue had a significantly higher ratio than HPS ($p \leq 0.001$) and Rose treatments ($p= 0.004$).

4.4.2 CBG and CBDV

Percentages of CBG and CBDV determined in dry inflorescence samples (Figure 7). Inflorescence with the most CBG content was cultivated under Blue ($0.18 \% \pm 0.011$) and Purple ($0.15 \% \pm 0.014$) light, followed by HPS and Rose light treatments ($0.09 \% \pm$

0.011 and 0.09 % \pm 0.013). Comparable amounts were determined for inflorescence cultivated under Amber and Red light treatments (0.05 % \pm 0.008, 0.05 % \pm 0.001).

Statistical analysis showed statistical difference ($p \leq 0.001$) between replicates ($p \leq 0.001$) but not on the interaction effect of light treatment and replicates. Overall Tukey post hoc test showed that Blue and Purple treatments were significantly greater than HPS, Amber, Red, and Rose treatments ($p \leq 0.001$ for all). Rose and HPS treatments were significantly different than Red and Amber treatments (Rose treatment: $p = 0.014$ both, HPS treatment: $p = 0.019$ both). Replication 1 kept the same ranking as the overall results including equivalences. Replicate 2 did not have equivalent values for treatments; Rose treatment had more total CBG than HPS treatment (0.12 % \pm 0.013 to 0.11 % \pm 0.009), and Red treatment had more than Amber treatment (0.06 % \pm 0.009 to 0.05 % \pm 0.013).

CBDV was measured without an equivalent CBDVa measurement. No amount was found in Replication 1. Replication 2 had the most CBDV in Rose treatment (0.09 % \pm 0.006) with equal amounts between Purple and HPS treatments (0.08 % \pm 0.003, 0.08 % \pm 0.006). Blue treatment (0.07 % \pm 0.002) followed by Red (0.06 % \pm 0.006) and Amber treatments (0.06 % \pm 0.006). No significance can be claimed with this data.

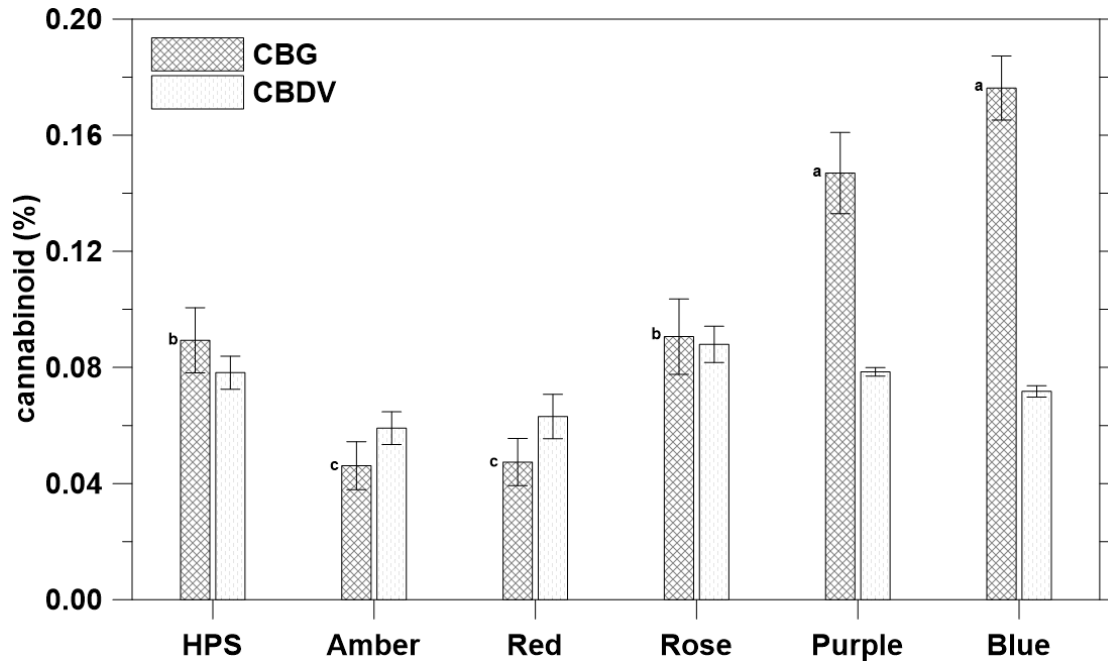


Figure 7. Average CBG (%), $CBGA \times 0.877 + CBG$ and CBDV content in *C. sativa* inflorescence under each light treatment. Data is presented in mean \pm SE (n=6). For CBDV, data represent mean from Replicate 2 (n=3), no trace was found in Replicate 1. Letters represent significance differences found using Tukey's post hoc. Total of 36 plants.

4.4.3 Total terpene production

Terpene production varied depending on light treatment (Figure 8). Purple light treatment resulted in the largest amount of terpenes produced ($29.43 \text{ mg g}^{-1} \pm 1.15$), followed by Blue ($28.48 \text{ mg g}^{-1} \pm 1.14$), HPS ($25.83 \text{ mg g}^{-1} \pm 1.00$), Rose ($24.51 \text{ mg g}^{-1} \pm 1.38$), Red ($20.30 \text{ mg g}^{-1} \pm 2.16$), and Amber ($18.10 \text{ mg g}^{-1} \pm 1.30$) light treatments. There was a significant difference between light treatments ($p \leq 0.001$), and replicates ($p = 0.015$), as well as a significant interaction effect of the light*replicate ($p = 0.014$). Post hoc analysis showed that total terpenes produced with Purple light treatment were

significantly greater than the amount of terpenes produced with Rose, Red, and Amber light treatments ($p= 0.043$, $p\leq 0.001$, $p\leq 0.001$ respectively). Total terpenes produced with Amber light were significantly less than the amount produced HPS, Blue and Rose light treatments ($p\leq 0.001$, $p\leq 0.001$, $p= 0.005$ respectively). Red light treatment resulted in significantly less total terpenes than the amount of Blue and HPS light treatments ($p\leq 0.001$, $p= 0.018$). Total terpenes produced per plant was greatest in plants cultivated under HPS light ($0.78 \text{ g} \pm 0.08 \text{ g}$). Purple ($0.6 \text{ g} \pm 0.06$) and Rose ($0.6 \text{ g} \pm 0.05$) produced equivalent amounts of terpenes, followed by Red ($0.49 \text{ g} \pm 0.07$), Blue ($0.41 \text{ g} \pm 0.05$) and Amber light treatments ($0.34 \text{ g} \pm 0.04$). There was statistical difference between lights ($p\leq 0.001$). Post hoc shows that HPS treatment was significantly different than Amber ($p\leq 0.001$), Blue ($p= 0.004$) and Red treatments ($p= 0.037$).

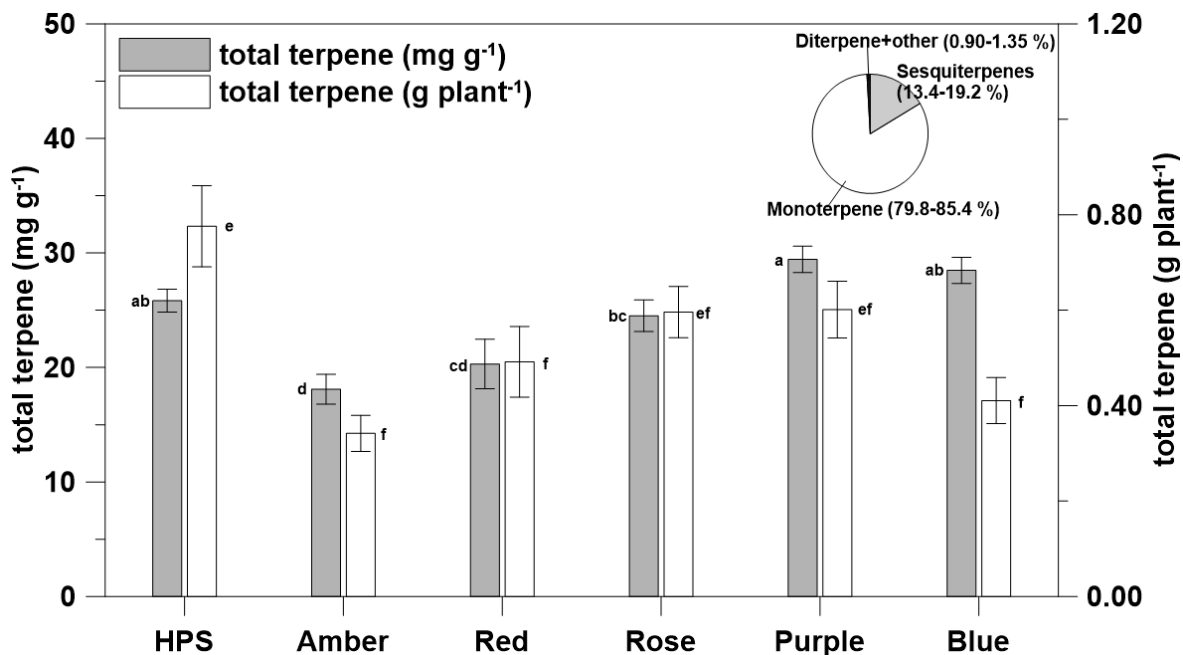


Figure 8. Total terpenes produced (mg g^{-1} and g plant^{-1}) under each light treatment. Data presented as mean values \pm SE ($n=6$). Letters represent significance differences found

using Tukey's post hoc. Total of 36 plants. The pie chart on the left top corner shows the percentages of monoterpene, sesquiterpene, and diterpenes between light treatments.

A total of 87 terpenes compounds were quantified, and the six most abundant ($> 500 \mu\text{g mg}^{-1}$ in at least one treatment) are presented in Table 3. Myrcene was produced more than any other terpene for all light treatments. Purple light produced the most myrcene ($8.91 \text{ mg g}^{-1} \pm 0.53$), and followed by Blue, HPS, Rose, Red, and Amber light. Amber was significantly less than Purple ($p \leq 0.001$), Blue ($p = 0.008$), Rose ($p = 0.042$) and HPS ($p = 0.027$). Red was also significantly less than Purple ($p = 0.005$). α -Pinene was the second most abundant produced terpene for inflorescence cultivated under all light treatments. Blue light produced the most α -Pinene ($5.73 \text{ mg g}^{-1} \pm 0.38$), and followed by Purple, HPS, Rose, Red, and Amber light. Limonene was the third overall most produced terpene and inflorescence cultivated under Purple light produced the most limonene ($3.05 \text{ mg g}^{-1} \pm 0.12$), followed by HPS, Blue, Rose, Red, and Amber light. Amber has significantly less limonene than Purple ($p \leq 0.001$), HPS ($p = 0.041$) and Blue ($p = 0.044$). Red has significantly less limonene than Purple ($p = 0.023$). Purple light produced the most β -pinene ($2.70 \text{ mg g}^{-1} \pm 0.12$), followed by Blue, HPS, Rose, Red and Amber light treatments. Purple and Blue were significantly greater in β -pinene than Amber (both $p = 0.003$) and Red (Purple $p = 0.011$, Blue $p = 0.014$). Inflorescence cultivated under Blue light produced more linalool than other light treatments ($0.847 \text{ mg g}^{-1} \pm 0.04$), followed by Purple, HPS, Rose, Red, and Amber light. Amber was significantly less than Purple and Blue (both $p \leq 0.001$). (E)- β -Ocimene was produced most in the Purple ($0.75 \text{ mg g}^{-1} \pm 0.07$), followed by Blue, Rose, HPS, Red, and Amber light. Amber has

significantly less (E)- β -ocimene than Purple ($p \leq 0.001$), Blue ($p = 0.022$), and Rose ($p = 0.026$). Purple had significantly greater (E)- β -ocimene than Red ($p = 0.009$).

Table 3. Concentrations (mg g⁻¹) of six most abundant terpene compounds in *C. sativa* plants cultivated in both replicates under different light treatments. Bolded numbers are the highest terpene for each light treatment.

Terpene Compound	Light treatment					
	HPS	Amber	Red	Rose	Purple	Blue
Myrcene	7.50 $\pm 0.43^{ab}$	4.80 $\pm 0.44^c$	5.66 $\pm 0.75^{bc}$	7.34 $\pm 0.57^{ab}$	8.91 $\pm 0.53^a$	7.89 $\pm 0.65^a$
α -Pinene	5.33 ± 0.45	4.48 ± 0.25	4.73 ± 0.12	5.13 ± 0.31	5.71 ± 0.36	5.73 ± 0.38
Limonene	2.66 $\pm 0.12^{ab}$	1.85 $\pm 0.16^c$	2.17 $\pm 0.24^{bc}$	2.59 $\pm 0.17^{abc}$	3.05 $\pm 0.11^a$	2.65 $\pm 0.23^{ab}$
β -Pinene	2.47 $\pm 0.16^{ab}$	1.92 $\pm 0.10^b$	2.03 $\pm 0.11^b$	2.30 $\pm 0.08^{ab}$	2.70 $\pm 0.13^a$	2.68 $\pm 0.16^a$
Linalool	0.717 $\pm 0.06^{ab}$	0.428 $\pm 0.06^b$	0.467 $\pm 0.09^{ab}$	0.59 $\pm 0.06^{ab}$	0.84 $\pm 0.04^a$	0.845 $\pm 0.02^a$
(E)- β -Ocimene	0.596 $\pm 0.056^{abc}$	0.347 $\pm 0.039^c$	0.437 $\pm 0.066^{bc}$	0.625 $\pm 0.069^{ab}$	0.75 $\pm 0.066^a$	0.631 $\pm 0.053^{ab}$

5. Discussion

In this work we explored the effects of six light spectra on *C. sativa* morphology, nutrient uptake yield and phytochemistry. We investigated how changes in light spectrum influences plant morphology and secondary metabolite production, including cannabinoids and terpenes. Markedly, the alterations in light spectrum highly impacted growth traits and secondary metabolite profiles, predominately under HPS and Blue light.

5.1 Inflorescence mass

Inflorescence mass is a key factor in determining cannabis yield for growers. HPS had the greatest amount of flower growth followed closely by Rose and Red. A study recently conducted by Magagnini, Grassi et al. (2018) supports these results, reporting that that *C. sativa* plants (drug chemotype “G-170”) grown under HPS have heavier inflorescence than plants grown under LEDs. Although spectral compositions were nearly identical between HPS and Amber light, the latter yielded 30 % less fresh inflorescence mass. Differences between these two spectra had HPS light with a lower percentage of 595-nm light, with small peaks from 420 nm to 460 nm and 500 nm, when compared to the Amber LED spectrum. These differences in spectral composition, particularly for 500-nm light, may be why there is considerable difference in inflorescence mass between HPS and Amber. When comparing Red and Rose (high red and low blue ratio) light treatments, supplementing a small fraction of blue light does not impact fresh inflorescence mass. Hawley, Graham et al. (2018) examined the impact of subcanopy light with 440+530+660 nm light under 440+660 nm background light on *C. sativa* ‘WP:Med (Wappa)’, and reported higher dry inflorescence mass (~27 %) with the subcanopy light. Therefore, we

conclude that a small fraction of green light (500 nm to 530 nm) leads to greater inflorescence mass, and that supplementing with a small fraction of green light can benefit *C. sativa* inflorescence mass accumulation.

A high fraction of blue light, or blue-dominant light, resulted in lower fresh inflorescence mass and the lowest plant height. This was expected, as dwarfing of plants grown under blue-dominant light has been observed for other greenhouse crops (Son and Oh 2013, Wollaeger and Runkle 2015). Not all plants experience shortening with blue light (Kong, Stasiak et al. 2018). After three weeks of flowering, there were slight differences in plant height among treatments, excluding blue light. Amber light led to the tallest plants. Although the impact of amber LED light on *C. sativa*'s morphological traits has not been fully determined, amber light resulted in higher *C. sativa* plant height (Magagnini, Grassi et al. 2018) and plant elongation of greenhouse crops (Grimstad 1987, Britz and Sager 1990).

5.2 Nutrient uptake

Blue light resulted in higher nitrogen, magnesium, boron, and zinc accumulation in *C. sativa* leaf tissues. The impact of light spectrum on *C. sativa* nutrient uptake has not been well examined. Recent studies have attempted to determine the effects of different nutrient solutions on *C. sativa* growth and cannabinoid accumulation (Bernstein, Gorelick et al. 2019, Saloner and Bernstein 2020). Bernstein et al. (2019) reported that enhanced NPK (nitrogen, phosphorus and potassium) treatments led to increased biomass production (total shoot fresh mass) and increased nitrogen in plant tissue. Saloner and Bernstein (2020) attempted to determine the optimal nitrogen supply (30–320 mg L⁻¹ N)

by monitoring *C. sativa* growth including morphology, biomass yield, nutrient uptake in plant tissues. The authors reported that nitrogen availability could influence *C. sativa* growth, as the leaf fresh mass was the highest at 180 mg L⁻¹ N and it decreased as increasing nitrogen supply (Saloner and Bernstein, 2020). However, enhanced NPK treatments also led to approximately a reduction in THC level (~20 %) and an increase in CBG level in inflorescence leaves (Bernstein et al., 2019). These responses to enhanced NPK treatments are similar to what we observed under Blue light treatment in this work. Further identified effects of light spectrum and nutrition management can potentially developed for control in cannabis growth and secondary metabolite accumulation.

5.3 Cannabinoids

In this work, plants grown under blue-dominant light (Blue and Purple) yielded the highest THC content (%) (Figure 6A). Blue light induces secondary metabolite biosynthesis (Warner, Wu et al. 2021), and supplemental blue light results in high THC content (Magagnini, Grassi et al. 2018, Namdar, Charuvi et al. 2019). We investigated how blue light impacted THC biosynthesis by using spectra with different fractions of blue light (HPS, Blue, Purple, and Rose light treatments). Our data support these previous studies and indicate that THC content increases with increasing blue light (Magagnini, Grassi et al. 2018, Namdar, Charuvi et al. 2019). Noted that HPS light resulted in comparable THC percentage over Rose and Purple treatments, which suggests that amber-rich HPS spectrum is as efficient at driving THC biosynthesis as commonly LED light recipes used (mixture of blue and red light) in controlled environment agriculture.

Unlike THC, different fractions of blue light did not impact CBD biosynthesis. Comparable CBD percentages were found in the inflorescence of plants cultivated among Blue, Purple, Rose, and HPS light treatments (5.72 to 6.04 %), while lower CBD percentages were observed in Amber and Red treatment (~ 4.5 %). Conflict results have been reported regarding the effect of blue light on CBD biosynthesis in drug chemotype *C. sativa* (Hawley, Graham et al. 2018, Magagnini, Grassi et al. 2018, Namdar, Charuvi et al. 2019). Magagnini, Grassi et al. (2018) and Namdar, Charuvi et al. (2019) reported that blue light induced CBD biosynthesis, whereas Hawley, Graham et al. (2018) reported no impact of light spectrum on CBD biosynthesis. The impact of blue light on CBD biosynthesis in drug chemotype cannabis plants varies among studies, yet our data suggest that blue light has a lesser effect on CBD biosynthesis for intermediate-type cannabis plants.

While considering the full plant, HPS treatment produced the most cannabinoid (THC and CBD) contents per plant. The light treatments containing blue light resulted in 22 to 43 % less THC per plant. Total CBD content per plant has not been reported yet, as most studies were conducted with drug chemotype *C. sativa* (Hawley, Graham et al. 2018, Magagnini, Grassi et al. 2018, Namdar, Charuvi et al. 2019). It is important to note that although blue light may induce high THC and CBD percentages, it appears to suppress inflorescence growth, resulting in less secondary metabolite produced per plant. This could be of concern to growers as blue light can lead to greater reduction in overall cannabinoid content. Of further industrial relevance, we suggest that reporting THC content per plant (g plant^{-1}) may be more relevant from a grower's perspective, since it shows how much THC was produced in total per growing cycle, rather than presenting

THC content percentage-wise (% mass or % w/w) (Magagnini, Grassi et al. 2018, Eichhorn Bilodeau, Wu et al. 2019). Examining the plant for the total amount of THC in the full plant would also be useful when trying to extract oils. Light spectrum highly impact inflorescence mass formation, and it consequently impact the overall THC content per plant.

There was a consistent decrease in CBG content as the fraction of blue light decreased among Rose, Purple, and Blue treatments. This finding agrees with a recent report (Magagnini, Grassi et al. 2018), whereby LED light containing blue light resulted in higher CBG content than HPS, and that the CBG percentages increased as the fraction of blue light increased. CBDV, another important non-psychoactive cannabinoid, was impacted by light treatment in a way that was similar to CBD, except the highest was not induced by blue-dominant light. Instead, Rose light, which had the highest fraction of red light, produced the most CBDV. To our knowledge, the impact of light spectrum on CBDV biosynthesis has not yet been reported, and here we show that not all cannabinoids accumulate when *C. sativa* is cultivated under blue light.

5.4 Terpenes

Terpenes are responsible for inflorescence odour and flavour profiles (Booth, Page et al. 2017). Light treatments with blue-dominant spectra led to higher total terpene production, monoterpenes, and sesquiterpenes. The Purple light treatment resulted in the highest total terpene concentrations (mg g^{-1}), followed by Blue light treatment. Higher concentrations of monoterpenes and sesquiterpenes were observed under these two light treatments, with a higher fraction of blue light, and these data agree with a precedent

study (Hawley, Graham et al. 2018), in which higher concentrations of monoterpenes such as α -pinene and limonene were reported under supplemental blue, green and red light. Although there is a relatively low fraction of blue light in HPS treatment, it induced comparable total terpene concentrations to Blue light treatment. Although different cultivation approaches were applied, this finding on HPS treatment agrees with Namdar, Charuvi et al. (2019). The authors reported that a higher total terpene concentration was observed when plants were grown under a mixture of light with high blue and low red and flowered under HPS light. In this work, the same spectral treatment was applied throughout the vegetative and flowering stages, and higher total terpene concentrations were observed under both HPS and Purple (with a higher fraction of blue light) treatments.

5.5 Limitations (difference between replicates)

Slightly differences in inflorescence mass, THC, and CBD appeared between different replicates, and plants with different origins may have affected how the plants grew and developed. The plants used to conduct Replicate 1 were sent in a closed box with little air exchange for two days, before being taken out and transplanted in its first pot and put in a growth chamber. The plants used for Replicate 2 were cloned from the original plants at various times and were under fluorescent light before being potted and placed in the growth chamber. Plants were given a week of standardized lighting before being placed in a treatment to get their growth rate as similar as possible. Plant height could have another metric to follow since Replicate 2 were smaller when being put in the growth chamber. Further to this, there was a small difference in PPF levels (15–20 %) and nighttime temperatures (4 °C) between replicates. The plants from replicate 2

produced less inflorescence, but more cannabinoids and terpenes than replicate 1. The plants were much smaller in replicate 2 while transplanting into the growth chamber (56% size of R1), at the starting of flowering (76% size of R1), and final height (92% size of R1). The size of the plants may be why they produced less flower (90% of R1). Another limitation for this study was how PPF were adjusted; LED lights needed to be adjusted by hand. This cause issues for the safety of the plants and the people moving the lights as a mistake here would kill plants and/or hurt the operators. Dimmable LED lights would have given a higher level of control on the light input.

6. Conclusion

The light spectrum considerably affects growth and secondary metabolite derivation in *C. sativa*. Monochromatic blue lights exemplify how secondary metabolite production may be manipulated yet this may prove disadvantageous for growth traits such as inflorescence size. Dichromatic LED light can balance out these deleterious effects on growth traits while maintaining secondary metabolite levels that are comparable to conventional HPS light. Inflorescence yield is linked to the HPS spectrum, which is amber-rich light and contains a low fraction of blue light. When considering whole plants as a means to quantify cannabinoid levels of a given crop, HPS light resulted in the highest cannabinoid content (g plant^{-1}), whereas LED light with different blue-red light ratios lowered cannabinoid content. It is important for growers to consider the impact of individual light wavelengths on both growth traits and secondary metabolite production since inappropriate spectral design could lead to a greater reduction in overall THC production. Data highlight the importance of optimizing plant growth conditions for

maximizing cannabis production. Future studies could expand on this research by deploying light qualities richer in the amber region of the spectrum and modifying the fraction of blue light with a proper metric to determine secondary metabolite production in *C. sativa*.

7. References

- Amrein, P., et al. (2020). "Influence of Light Spectra on the Production of Cannabinoids." Medical Cannabis and Cannabinoids **3**(2): 103-110.
- Bahji, A. and C. Stephenson (2019). "International perspectives on the implications of cannabis legalization: a systematic review & thematic analysis." International Journal of Environmental Research and Public Health Reports **16**(17): 3095.
- Bernstein, N., et al. (2019). "Impact of N, P, K, and Humic Acid Supplementation on the Chemical Profile of Medical Cannabis (*Cannabis sativa* L)." Frontiers in Plant Science **10**.
- Booth, J. K., et al. (2017). "Terpene synthases from *Cannabis sativa*." PloS one **12**(3): e0173911.
- Britz, S. J. and J. C. Sager (1990). "Photomorphogenesis and photoassimilation in soybean and sorghum grown under broad spectrum or blue-deficient light sources." Plant physiology **94**(2): 448-454.
- Chandra, S., et al. (2008). "Photosynthetic response of *Cannabis sativa* L. to variations in photosynthetic photon flux densities, temperature and CO₂ conditions." Physiology and Molecular Biology of Plants **14**(4): 299-306.
- Chandra, S., et al. (2011). "Photosynthetic response of *Cannabis sativa* L., an important medicinal plant, to elevated levels of CO₂." Physiology and Molecular Biology of Plants **17**(3): 291-295.
- Cox, C. (2018). "The Canadian Cannabis Act legalizes and regulates recreational cannabis use in 2018." Health Policy **122**(3): 205-209.
- Croft, H. and J. Chen (2017). "Leaf pigment content." Reference Module in Earth Systems and Environmental Sciences. Oxford: Elsevier Inc: 1-22.
- Danziger, N. and N. Bernstein (2021). "Light matters: Effect of light spectra on cannabinoid profile and plant development of medical cannabis (*Cannabis sativa* L.)." Industrial Crops and Products **164**: 113351.
- Eichhorn Bilodeau, S., et al. (2019). "An update on plant photobiology and implications for cannabis production." Frontiers in plant science **10**: 296.
- ElSohly, M. A., et al. (2017). Phytochemistry of *Cannabis sativa* L. Phytocannabinoids. A. D. Kinghorn, H. Falk, S. Gibbons, J. i. Kobayashi and W. Herz. Cham, Switzerland, Springer: 1-36.
- Engelmann, T. W. (1883). "Bacterium photometricum." Ein Beitrag zur vergleichenden Physiologie des Licht- und Farbensinnes **30**.

Fromme, P., et al. (2001). "Structure of photosystem I." Biochimica et Biophysica Acta (BBA) - Bioenergetics **1507**(1-3): 5-31.

Gonçalves, J., et al. (2019). "Cannabis and its secondary metabolites: Their use as therapeutic drugs, toxicological aspects, and analytical determination." Medicines **6**(1): 31.

Grimstad, S. (1987). "The effect of supplemental irradiation with different light sources on growth and flowering of gloxinia (*Sinningia speciosa* (Lodd.) Hiern)." Scientia Horticulturae **32**(3-4): 297-305.

Hawley, D., et al. (2018). "Improving Cannabis Bud Quality and Yield with Subcanopy Lighting." HortScience **53**(11): 1593-1599.

Jin, D., et al. (2020). "Secondary Metabolites Profiled in Cannabis Inflorescences, Leaves, Stem Barks, and Roots for Medicinal Purposes." Scientific reports **10**(1): 1-14.

Jin, D., et al. (2019). "Cannabis Indoor Growing Conditions, Management Practices, and Post-Harvest Treatment: A Review." American Journal of Plant Sciences **10**(6): 925-946.

Johnson, J. R., et al. (2010). "Multicenter, double-blind, randomized, placebo-controlled, parallel-group study of the efficacy, safety, and tolerability of THC: CBD extract and THC extract in patients with intractable cancer-related pain." Journal of Pain Symptom Management **39**(2): 167-179.

Kong, Y., et al. (2018). "Blue light associated with low phytochrome activity can promote elongation growth as shade-avoidance response: A comparison with red light in four bedding plant species." Environmental and Experimental Botany **155**: 345-359.

Livingston, S. J., et al. (2020). "Cannabis glandular trichomes alter morphology and metabolite content during flower maturation." The Plant Journal **101**(1): 37-56.

Magagnini, G., et al. (2018). "The effect of light spectrum on the morphology and cannabinoid content of *Cannabis sativa* L." Medical Cannabis and Cannabinoids **1**(1): 19-27.

Mao, J., et al. (2000). "Two distinctive antinociceptive systems in rats with pathological pain." Neuroscience Letters **280**(1): 13-16.

McCree, K. (1972). "The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agric. Meteorol. 9, 191–216."

Mills, E. (2012). "The carbon footprint of indoor Cannabis production." Energy Policy **46**: 58-67.

Moan, J. (2001). "Visible light and UV radiation." Radiation at Home, Outdoors and in the Workplace: 69-85.

Namdar, D., et al. (2019). "LED lighting affects the composition and biological activity of *Cannabis sativa* secondary metabolites." Industrial Crops and Products **132**: 177-185.

Petrovska, B. B. (2012). "Historical review of medicinal plants' usage." Pharmacognosy Reviews **6**(11): 1.

Potter, D. J. and P. Duncombe (2012). "The effect of electrical lighting power and irradiance on indoor-grown cannabis potency and yield." Journal of forensic sciences **57**(3): 618-622.

Reese, C. (2008). "Biology 8th edition."

Rögner, M., et al. (1996). "How does photosystem 2 split water? The structural basis of efficient energy conversion." Trends in Biochemical Sciences **21**(2): 44-49.

S. L. Kochhar, S. K. G. (2020). Plant Physiology - Theory and Applications, 2nd Edition.

Saloner, A. and N. Bernstein (2020). "Response of Medical Cannabis (*Cannabis sativa* L.) to Nitrogen Supply Under Long Photoperiod." Frontiers in Plant Science **11**.

Singh, D., et al. (2015). "LEDs for energy efficient greenhouse lighting." Renewable and Sustainable Energy Reviews **49**: 139-147.

Son, K.-H. and M.-M. Oh (2013). "Leaf shape, growth, and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes." HortScience **48**(8): 988-995.

Turner, C. E., et al. (1980). "Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents." Journal of Natural Products **43**(2): 169-234.

Van Klingerren, B. and M. Ten Ham (1976). "Antibacterial activity of Δ^9 -tetrahydrocannabinol and cannabidiol." Antonie van Leeuwenhoek **42**(1-2): 9-12.

Vanhove, W., et al. (2011). "Factors determining yield and quality of illicit indoor cannabis (*Cannabis* spp.) production." Forensic Science International **212**(1-3): 158-163.

Warner, R., et al. (2021). "A review of strawberry photobiology and fruit flavonoids in controlled environments." Frontiers in plant science **12**: 611893.

White, A., et al. (1976). "Effects of Δ^9 -tetrahydrocannabinol in Lewis lung adenocarcinoma cells in tissue culture." Journal of the National Cancer Institute **56**(3): 655-658.

Wollaeger, H. M. and E. S. Runkle (2015). "Growth and acclimation of impatiens, salvia, petunia, and tomato seedlings to blue and red light." HortScience **50**(4): 522-529.

Wu, B.-S., et al. (2019). "Comparison and perspective of conventional and LED lighting for photobiology and industry applications." Environmental and Experimental Botany: 103953.

Zelitch, I. (1975). "Improving the efficiency of photosynthesis." Science **188**(4188): 626-633.

Appendix

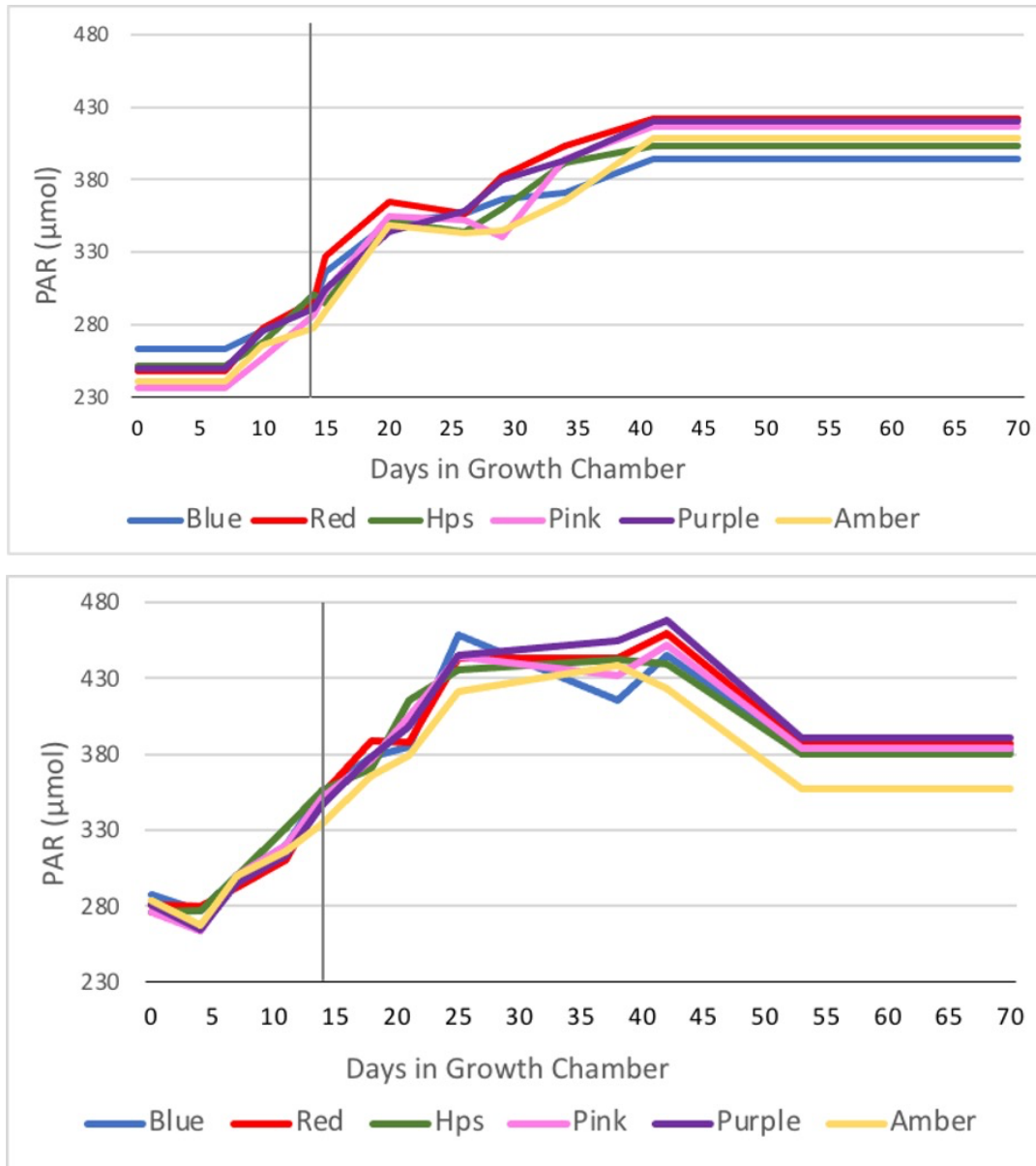


Figure 9: Photosynthetically active radiation (PAR) over the plant life cycle per light treatment of both replicates. As plant grew lights were moved to keep PAR within 10% of each other. The vertical black line indicates when flowering started.

Terpene Compound	HPS	Amber	Red	Rose	Purple	Blue
Myrcene	7.504 ±0.43 ^{ab}	4.809 ±0.44 ^c	5.669 ±0.757 ^{bc}	7.347 ±0.573 ^{ab}	8.914 ±0.53^a	7.898 ±0.653 ^a
α-Pinene	5.339 ±0.458	4.488 ±0.256	4.738 ±0.129	5.138 ±0.317	5.715 ±0.367	5.733 ±0.385
Limonene	2.666 ±0.122 ^{ab}	1.857 ±0.162 ^c	2.178 ±0.246 ^{bc}	2.594 ±0.177 ^{abc}	3.051 ±0.119^a	2.656 ±0.23 ^{ab}
β-Pinene	2.474 ±0.162 ^{ab}	1.929 ±0.106 ^b	2.03 ±0.116 ^b	2.3 ±0.082 ^{ab}	2.708 ±0.136^a	2.687 ±0.16 ^a
Linalool	0.717 ±0.065 ^{ab}	0.428 ±0.067 ^b	0.467 ±0.097 ^{ab}	0.59 ±0.068 ^{ab}	0.847 ±0.045^a	0.845 ±0.02 ^a
(E)-β-Ocimene	0.596 ±0.056 ^{abc}	0.347 ±0.039 ^c	0.437 ±0.066 ^{bc}	0.625 ±0.069 ^{ab}	0.75 ±0.066^a	0.631 ±0.053 ^{ab}
endo-Fenchol	0.402 ±0.026	0.298 ±0.046	0.298 ±0.052	0.373 ±0.041	0.458 ±0.025	0.46 ±0.012
β-Caryophyllene	0.396 ±0.031	0.233 ±0.028	0.275 ±0.046	0.371 ±0.041	0.413 ±0.039	0.377 ±0.076
Selina-4(15),7(11)-diene	0.363 ±0.031 ^{abc}	0.217 ±0.024 ^c	0.26 ±0.042 ^{bc}	0.323 ±0.04 ^{abc}	0.414 ±0.052 ^{ab}	0.466 ±0.021^a
Selina-3,7(11)-diene	0.487 ±0.038 ^{ab}	0.284 ±0.032 ^c	0.336 ±0.053 ^{bc}	0.43 ±0.053 ^{abc}	0.556 ±0.058 ^a	0.614 ±0.026^a
Guaiol	0.344 ±0.04 ^{ab}	0.189 ±0.029 ^b	0.21 ±0.043 ^b	0.293 ±0.043 ^{ab}	0.39 ±0.056 ^a	0.454 ±0.031^a
10-epi-γ-Eudesmol	0.327 ±0.066 ^{ab}	0.193 ±0.032 ^b	0.217 ±0.048 ^b	0.3 ±0.05 ^{ab}	0.397 ±0.064 ^{ab}	0.456 ±0.027^a
Bulnesol	0.375 ±0.05 ^{ab}	0.213 ±0.039 ^b	0.24 ±0.055 ^b	0.32 ±0.057 ^{ab}	0.408 ±0.056 ^{ab}	0.492 ±0.048^a
trans-Pinene hydrate	0.292 ±0.024	0.208 ±0.036	0.211 ±0.04	0.266 ±0.032	0.324 ±0.022	0.329 ±0.012
α-Terpineol	0.38 ±0.032	0.275 ±0.046	0.283 ±0.054	0.343 ±0.041	0.426 ±0.03	0.434 ±0.014
Germacrene B	0.232 ±0.032 ^{abc}	0.105 ±0.019 ^c	0.121 ±0.03 ^c	0.162 ±0.028 ^{bc}	0.258 ±0.04 ^{ab}	0.322 ±0.032^a
Citronellol	0.174 ±0.014 ^{ab}	0.124 ±0.022 ^b	0.129 ±0.027 ^{ab}	0.151 ±0.014 ^b	0.219 ±0.012 ^a	0.233 ±0.01^a
α-Eudesmol	0.209 ±0.027 ^{ab}	0.118 ±0.021 ^b	0.118 ±0.034 ^b	0.172 ±0.03 ^{ab}	0.237 ±0.037 ^{ab}	0.284 ±0.017^a
β-Eudesmol	0.178 ±0.022 ^{abc}	0.095 ±0.016 ^c	0.107 ±0.023 ^{bc}	0.144 ±0.024 ^{abc}	0.201 ±0.03 ^{ab}	0.237 ±0.015^a
γ-Eudesmol	0.067 ±0.007 ^a	0.023 ±0.007 ^b	0.035 ±0.007 ^{ab}	0.046 ±0.008 ^{ab}	0.074 ±0.009^a	0.066 ±0.01 ^a
α-Bisabolol	0.12 ±0.01	0.069 ±0.008	0.093 ±0.017	0.109 ±0.013	0.116 ±0.017	0.117 ±0.011
Borneol	0.136 ±0.006	0.1 ±0.012	0.106 ±0.015	0.121 ±0.008	0.148 ±0.004	0.147 ±0.005
Hexanol	0.017 ±0.005	0.01 ±0.001	0.013 ±0.003	0.022 ±0.007	0.008 ±0.001	0.01 ±0.001
α-Thujene	0.009 ±0.001 ^{ab}	0.008 ±0 ^b	0.008 ±0 ^b	0.009 ±0.001 ^{ab}	0.01 ±0.001^a	0.01 ±0^a
α-Fenchene	0.006 ±0	0.006 ±0	0.006 ±0	0.006 ±0	0.006 ±0	0.007 ±0.001
α-Humulene	0.152 ±0.013 ^{abc}	0.09 ±0.011 ^c	0.103 ±0.019 ^{bc}	0.142 ±0.017 ^{abc}	0.16 ±0.017 ^a	0.17 ±0.01^{ab}
Camphene	0.15 ±0.008 ^{ab}	0.126 ±0.007 ^b	0.129 ±0.007 ^b	0.147 ±0.004 ^{ab}	0.166 ±0.005^a	0.162 ±0.011 ^a
γ-Elemene	0.092 ±0.009 ^{bc}	0.041 ±0.006 ^d	0.046 ±0.01 ^d	0.062 ±0.006 ^{cd}	0.1 ±0.009 ^{ab}	0.129 ±0.006^a

Sabinene	0.011 ±0.003	0.011 ±0.001	0.02 ±0.005	0.024 ±0.006	0.012 ±0.004	0.017 ±0.002
α-Phellandrene	0.011 ±0.001	0.011 ±0.002	0.009 ±0.001	0.015 ±0.002	0.013 ±0.002	0.015 ±0.001
β-Phellandrene	0.075 ±0.016	0.073 ±0.007	0.079 ±0.006	0.097 ±0.009	0.106 ±0.019	0.105 ±0.015
1,8-Cineole	0.053 ±0.022	0.014 ±0.003	0.051 ±0.016	0.021 ±0.002	0.088 ±0.032	0.088 ±0.025
(Z)-β-Ocimene	0.014 ±0.001 ^{ab}	0.01 ±0.001 ^b	0.011 ±0.001 ^{ab}	0.013 ±0.001 ^{ab}	0.017 ±0.001^a	0.015 ±0.002 ^{ab}
γ-Terpinene	0.013 ±0.001 ^{ab}	0.01 ±0.001 ^c	0.011 ±0.001 ^{bc}	0.013 ±0 ^{ab}	0.015 ±0.001^a	0.014 ±0.001 ^{ab}
cis-Sabinene hydrate	0.033 ±0.004	0.026 ±0.006	0.026 ±0.005	0.03 ±0.002	0.032 ±0.005	0.033 ±0.007
Fenchone	0.066 ±0.004	0.054 ±0.006	0.055 ±0.008	0.064 ±0.005	0.074 ±0.003	0.067 ±0.002
Terpinolene	0.039 ±0.003 ^{ab}	0.031 ±0.003 ^b	0.035 ±0.004 ^{ab}	0.04 ±0.002 ^{ab}	0.046 ±0.002^a	0.037 ±0.004 ^{ab}
trans-Sabinene hydrate	0.01 ±0.001	0.009 ±0.001	0.01 ±0.001	0.01 ±0.001	0.012 ±0.001	0.012 ±0.001
cis-Pinene hydrate	0.07 ±0.006	0.049 ±0.008	0.055 ±0.009	0.063 ±0.007	0.076 ±0.005	0.077 ±0.004
Camphene hydrate	0.027 ±0.002	0.02 ±0.002	0.023 ±0.003	0.025 ±0.002	0.03 ±0.002	0.033 ±0.008
lpsdienol	0.039 ±0.002	0.035 ±0.001	0.04 ±0.004	0.032 ±0.006	0.04 ±0.001	0.035 ±0.002
Terpinen-4-ol	0.015 ±0.001 ^b	0.013 ±0.001 ^b	0.014 ±0.002 ^b	0.016 ±0.001 ^{ab}	0.021 ±0.003^{ab}	0.021 ±0.001^a
Geraniol	0.015 ±0.002	0.012 ±0.002	0.012 ±0.002	0.014 ±0.001	0.018 ±0.001	0.015 ±0.001
α-Cubebene	0.008 ±0	0.007 ±0	0.008 ±0.001	0.008 ±0.001	0.009 ±0.001	0.01 ±0.001
α-Ylangene	0.008 ±0.001	0.006 ±0	0.006 ±0.001	0.009 ±0.001	0.009 ±0.001	0.009 ±0
Hexyl hexanoate	0.06 ±0.008	0.058 ±0.005	0.062 ±0.011	0.038 ±0.009	0.045 ±0.003	0.054 ±0.003
trans-α-Bergamotene	0.053 ±0.013	0.022 ±0.002	0.028 ±0.004	0.03 ±0.002	0.034 ±0.005	0.026 ±0.004
allo-Aromadendrene	0.006 ±0.001	0.005 ±0	0.006 ±0.001	0.006 ±0	0.006 ±0	0.005 ±0.001
(E)-β-Farnesene	0.017 ±0.002	0.011 ±0.001	0.013 ±0.002	0.015 ±0.002	0.017 ±0.003	0.014 ±0.003
β-Selinene	0.078 ±0.005 ^a	0.044 ±0.003 ^b	0.06 ±0.007 ^{ab}	0.046 ±0.012 ^{ab}	0.083 ±0.009 ^a	0.093 ±0.006^a
α-Selinene	0.068 ±0.005	0.056 ±0.01	0.057 ±0.008	0.07 ±0.005	0.096 ±0.016	0.066 ±0.022
β-Bisabolene	0.014 ±0.002	0.012 ±0.002	0.016 ±0.003	0.014 ±0.002	0.014 ±0.004	0.018 ±0.003
(3E,6E)-α-Farnesene	0.078 ±0.013 ^{ab}	0.048 ±0.007 ^b	0.052 ±0.01 ^{ab}	0.055 ±0.017 ^{ab}	0.083 ±0.02 ^{ab}	0.116 ±0.02^a
Spirovetiva-1(10),7(11)-diene	0.054 ±0.007	0.037 ±0.005	0.034 ±0.008	0.048 ±0.011	0.045 ±0.004	0.065 ±0.009
(E)-α-Bisabolene	0.087 ±0.005^a	0.057 ±0.004 ^b	0.076 ±0.01 ^{ab}	0.082 ±0.006 ^{ab}	0.085 ±0.008 ^{ab}	0.08 ±0.005 ^{ab}
Eudesma-5,7(11)-diene	0.039 ±0.003 ^{ab}	0.028 ±0.002 ^b	0.03 ±0.003 ^b	0.037 ±0.003 ^{ab}	0.045 ±0.005^a	0.045 ±0.002^a
(E)-Nerolidol	0.058 ±0.006 ^{ab}	0.041 ±0.004 ^b	0.045 ±0.006 ^{ab}	0.055 ±0.006 ^{ab}	0.066 ±0.008 ^{ab}	0.072 ±0.006^a

Caryophyllene oxide	0.021 ±0.003	0.019 ±0.008	0.013 ±0.004	0.045 ±0.017	0.07 ±0.027	0.087 ±0.027
Humulene epoxide II	0.041 ±0.005 ^{ab}	0.02 ±0.002 ^c	0.022 ±0.005 ^{bc}	0.031 ±0.005 ^{abc}	0.047 ±0.007^a	0.045 ±0.003 ^a
Juniper camphor	0.044 ±0.005 ^{abc}	0.025 ±0.003 ^c	0.031 ±0.006 ^{bc}	0.038 ±0.005 ^{abc}	0.049 ±0.007 ^{ab}	0.056 ±0.003^a
Aromadendrane-4,10-diol	0.018 ±0.002	0.013 ±0.002	0.021 ±0.002	0.02 ±0.002	0.021 ±0.002	0.017 ±0.003
meta-Camphorene	0.021 ±0.003 ^{ab}	0.013 ±0.002 ^b	0.013 ±0.003 ^{ab}	0.017 ±0.003 ^{ab}	0.024 ±0.003 ^{ab}	0.025 ±0.001^a
Phytol	0.127 ±0.017	0.138 ±0.029	0.193 ±0.031	0.18 ±0.035	0.259 ±0.061	0.185 ±0.031
δ-Guaiene	0.018 ±0.002	0.034 ±0	0.019 ±0.003	0.024 ±0.005	0.029 ±0.006	0.026 ±0.002
Eremophila-1(10),7(11)-diene	0.041 ±0.003	0.035 ±0.002	0.03 ±0.005	0.046 ±0.005	0.053 ±0.008	0.058 ±0.012
Cryptomeridiol	0.028 ±0.009	0.007 ±0.001	0.036 ±0.003	0.035 ±0.005	0.031 ±0.012	0.039 ±0.009
para-Cymene	0.006 ±0.001	0.005 ±0.001	0.006 ±0	0.007 ±0.001	0.006 ±0.001	0.006 ±0.001
α-Terpinene	0.007 ±0.001	0.009 ±0.002	0.007 ±0.001	0.009 ±0.002	0.008 ±0.002	0.007 ±0.001
Valencene	0.018 ±0.003	*	0.014 ±0.002	0.017 ±0.006	0.034 ±0.014	0.097 ±0.002
α-Santalene	0.012 ±0.002	0.007 ±0.003	0.012 ±0.001	0.014 ±0	0.01 ±0.003	0.082 ±0.062
(4Z)-Decenol	0.016 ±0.003	0.013 ±0.002	*	0.023 ±0.001	0.019 ±0.001	0.019 ±0.001
Selin-6-en-4α-ol	0.019 ±0.006	0.005 ±0.001	0.01 ±0.002	0.011 ±0.001	0.011 ±0.002	0.014 ±0.002
α-Guaiene	0.003 ±0.001	0.002 ±0	0.016 ±0.002	*	0.018 ±0.008	0.019 ±0.006
Hashishene	0.002 ±0.001	0.002 ±0.001	0.003 ±0	*	0.006 ±0.001	0.004 ±0
Decanol	0.005 ±0.001 ^{ab}	0.004 ±0 ^b	0.007 ±0^a	*	0.004 ±0.001 ^b	0.003 ±0 ^b
Epoxyterpinolene	*	*	*	*	*	0.007 ±0.001
Δ3-Carene	0.004 ±0	*	0.003 ±0	0.004 ±0	*	0.003 ±0.001
para-Cymenene	0.006 ±0.001	*	*	*	0.004 ±0.001	0.001 ±0
Selin-6-en-4α-ol isomer	*	*	*	*	*	0.043 ±0.004
Caryophylla-3,8(13)-dien-5β-ol	0.017 ±0.003	0.004 ±0	0.012 ±0.002	0.01 ±0.001	0.012 ±0.002	0.018 ±0.007
(2E,6E)-Farnesol	0.041 ±0.006 ^{ab}	0.022 ±0.007 ^{ab}	0.047 ±0.004^{ab}	0.015 ±0 ^b	0.024 ±0.001 ^{ab}	0.045 ±0 ^a
Hexyl butyrate	*	*	*	*	*	0.004 ±0.001
para-Cymen-8-ol	*	*	0.003 ±0	*	*	*

Table 4: Concentrations (mg/g) of 80 terpene compounds in *C. sativa* plants cultivated under different light treatments.