The effect of different light emitting diodes spectra on vegetative cannabis plant growth parameters and antioxidant activity

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

December 2023

Acknowledgements

I would like to thank Dr. Mark Lefsrud, my supervisor, for providing generous support and invaluable guidance. Working with Dr. Mark Lefsrud allowed for many opportunities that would not be possible without him, it was a pleasure to see how far we pushed our vision. I would also like to thank Dr. Sarah MacPherson who helped me a lot during my project and always was patient. I would like to thank my spouse, Amin, who without his love and support for this project would also not have been possible. Last, I am grateful for my friends that helped me in every step, specifically Vincent Desaulniers Brousseau.

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Abstract

Cannabis sativa stands as one of the world's oldest plant sources for food and textile fiber. This plant can tolerate a high photosynthetic photon flux density (PPFD) for effective photosynthesis and growth, with light being a pivotal environmental factor influencing cannabis growth and development. The aim of this study was to examine and assess the impact of four different light emitting diode (LED) spectra, including wide amber (595 nm), narrow amber (595 nm) + violet (430 nm), narrow amber (595 nm) + violet (430 nm) + blue (485 nm), and a white LED (blue; 446 nm + amber; 595 nm + red; 653 nm) on cannabis vegetative growth parameters (plant height, stem diameter, SPAD value, fresh mass, and dry mass), as well as antioxidant activity using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and ferric reducing antioxidant power (FRAP) assays. Additionally, a comparison was made between two propagation methods (seedlings and cuttings). Significant differences were observed between the treatments, with wide amber yielding the highest growth parameters; SPAD value (61.3), fresh mass (14.4 g), dry mass (3.9 g), plant height (22.5 cm), and stem diameter (4.4 mm). White LEDs exhibited the least favorable outcomes for all growth parameters including plant height (37 %), stem diameter (17 %), SPAD value (2.7 %) fresh mass (35 %), and dry mass (33 %) than other treatment. Antioxidant activity results show that narrow amber + 430 nm + 485 nm demonstrated the highest antioxidant activity in both FRAP (0.0074 %) and TEAC (88.2 %) among all treatments, with wide amber showing the lowest levels (27.5 % for TEAC and 0.0055 % for FRAP). This research revealed a slight variance between the two propagation methods based on morphological parameters. Cuttings exhibited higher plant height (7.0 %), stem diameter (2.7 %), fresh mass (8 %), dry mass (9.0 %), and SPAD (3.0 %) compared to seedlings. These findings indicate that cannabis leaves could potentially serve as a source of antioxidants for other downstream products. Furthermore, the results confirmed that different LED treatments had a significant effect on growth and antioxidant activity, offering promising guidance for cannabis growers to select spectral designs aligned with specific *C. sativa* production goals.

Résumé

Cannabis sativa figure parmi les sources végétales les plus anciennes au monde pour la nourriture et les fibres textiles. La lumière étant un facteur environnemental essentiel influençant la croissance et le développement du cannabis, cette plante peut tolérer une densité de flux de photons photosynthétiques (PPFD) élevée pour une photosynthèse et une croissance efficace. L'objectif de cette étude était d'examiner et d'évaluer l'impact de quatre spectres différents de diodes électroluminescentes (LED), comprenant l'ambre large (595 nm), l'ambre étroit (595 nm) + le violet (430 nm), l'ambre étroit (595 nm) + le violet (430 nm) + le bleu (485 nm), et une LED blanche (bleu; 446 nm + ambre; 595 nm + rouge; 653 nm) sur les paramètres de croissance végétative du cannabis (hauteur de la plante, diamètre de la tige, valeur SPAD, masse fraîche et masse sèche), ainsi que l'activité antioxydante en utilisant les tests DPPH et FRAP. De plus, une comparaison a été faite entre deux méthodes de propagation (plants issus de graines et boutures). Des différences significatives ont été observées entre les traitements, l'ambre large produisant les paramètres de croissance les plus élevés ; valeur SPAD (61,3), masse fraîche (14,4 g), masse sèche (3,9 g), hauteur de la plante (22,5 cm) et diamètre de la tige (4,4 mm). Le blanc a montré les résultats les moins favorables pour tous les paramètres de croissance, y compris la hauteur de la plante (37 %), le diamètre de la tige (17 %), la valeur SPAD (2,7 %), la masse fraîche (35 %) et la masse sèche (33 %). Les résultats de l'activité antioxydante ont montré que l'ambre étroit + 430 nm + 485 nm présentait la plus forte activité antioxydante à la fois dans le test FRAP (0,0074 %) et le test TEAC (88,2 %) parmi tous les traitements, l'ambre large montrant les niveaux les plus bas (27,5 % pour le TEAC et 0,0055 % pour le FRAP). Cette recherche a révélé une légère variation entre les deux méthodes de propagation basées sur les paramètres morphologiques. Les boutures ont montré des niveaux plus élevés en hauteur de la plante (7,0 %), en diamètre de la tige (2,7 %), en masse fraîche (8 %), en masse sèche (9,0 %) et en valeur SPAD (3,0 %) par rapport aux plants issus de graines. Ces résultats indiquent que les feuilles de cannabis pourraient potentiellement servir de source d'antioxydants pour d'autres produits dérivés. De plus, les résultats confirment que différents traitements LED peuvent soit améliorer, soit diminuer de manière significative les paramètres de croissance et l'activité antioxydante, offrant des orientations prometteuses aux cultivateurs de cannabis pour choisir des conceptions spectrales alignées avec des objectifs de production spécifiques de *C. sativa*.

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List of abbreviations

$^1O_2$= singlet-oxygen

AA = ascorbic acid

ABTS= 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)

APX = ascorbate peroxidase

CAT = catalase

CBD= cannabidiol

CBG = cannabigerol

CBGA = cannabigerolic acid

Chl a and b= Chlorophylls a and b

CUPRAC= Cupric Reducing Antioxidant Capacity

delta-9-Tetrahydrocannabinol

DPPH= 2,2-diphenyl-1-picryl-hydrazyl-hydrate

EGCG = (−)-epigallocatechin-3-gallate.

EOD-FR = end of day far- red (EOD-FR)

FRAP= Ferric Reducing Antioxidant Power

GA = gibberellin

GR = glutathione reductase

GSHPx = glutathione peroxidase

H_2O_2 = hydrogen peroxide

HO = hydroxyl radical

HPLC= high-performance liquid chromatography

HPS = high pressure sodium

IAA = Indole-3-acetic acid

IBA= Indole-3-butyric acid

LED= Light-emitting diode

M= mass

NADPH= nicotinamide adenine dinucleotide phosphate

O_2^- = superoxide

PAL= phenylalanine ammonia-lyase

PGE_2 = Prostaglandin E_2

phyA= phytochrome A

PPFD= photon flux density

PSI and PSII= photosystem I and II complexes

Resv = resveratrol

RH= relative humidity

ROS = reactive oxygen species

SDP = short-day plants

SET= single electron transfer

SLW= specific leaf weight

SOD = superoxide dismutase

SPLET= sequential proton loss electron transfer

TFC= total flavonoids

THC= tetrahydrocannabinol

THCVA = tetrahydrocannabivarin acid

TPC= total polyphenols

TSC = total soluble carbohydrates

1 Introduction

Cannabis (*Cannabis sativa*) plants have been cultivated around the world (Raman, 1998) for three major products: hemp fibre, cannabis seeds, and narcotic or therapeutic medicines (Andre et al., 2016; Bonini et al., 2018; Zuk-Golaszewska & Golaszewski, 2018). Cannabis stems are used to obtain hemp fibre, which has been used for generations to make textiles, rope, and sacking (Bouloc et al., 2013). It has a 70 % cellulose content, is tough and resilient, and can grow up to 15 feet. The fibre has been used to create paper, and it has been suggested that it could take the place of wood pulp paper manufacturing (Correia, 2004). The "seeds" (fruit or achene) can be roasted and consumed by humans, used as birdfeed or fishermen's bait, or pressed to produce a greenish yellow oil that has been used in food as well as varnishes, paints, and soap (Fairbairn et al., 1976). Cannabis flowers, leaves and the resulting extracts have a variety of pharmacological effects on humans and is the most well-known application of cannabis (Raman, 1998).

There are two plant forms of cannabis: one that produces seeds and the other that is barren (without seeds) (Small & Cronquist, 1976). These terms reflect that the plant is dioecious, meaning that it produces male and female blooms on different plants (Field et al., 2013). Staminate flowers are produced by the male plant, while pistillate blooms are produced by the female plant, both of which eventually produce fruit and seeds (Small & Cronquist, 1976). The male inflorescence is composed of many individual flowers borne on flowering branches up to 18 cm long and stands out from the leaves (Clarke, 1999). Female cannabis flowers appear in pairs of florets positioned within the leaf axils and these pairs aligned with the smaller branchlets of the secondary axillary branch, which emerge between them (Small & Naraine, 2016). Essentially, each inflorescence represents compressed higher-order branchlets, maintaining a phytomer structure identical to the larger phytomers developed during long-day photoperiods and these condensed branchlets contained single leaflet leaves, an axillary shoot, one or two individual flowers, and bracts (Hesami et al., 2023). Virtually every aerial part of the cannabis plant is covered in minute hairs or trichomes. There are either simple trichomes or glandular trichomes containing a resin (Raman, 1998). Clarke (1981) described that there are five main types of trichomes: (a) long, unicellular, smooth, curved, covering trichomes; (b) more squat, unicellular, cystolith covering trichomes, containing calcium carbonate; (c) bulbous, glandular trichomes; (d) capitate-sessile (i.e. without a stalk), glandular trichomes, and (e) capitate-stalked, glandular trichomes.

Capitate-stalked, glandular trichomes are found on the bracts and floral leaves only (Raman et al., 2017). The capitate glandular trichomes contain cannabinoids, the unique phytochemicals found in cannabis, some of which are responsible for the psychedelic properties of the plant. (Turner et al., 1978). This is present in secretory sacs which consist of a distended area bounded by a sheath, formed between secretory cells of the trichome (Hammond, 1977). Cannabinoids are not found in the non-glandular (covering) trichomes (Schnetzler & Teixeira, 2017).

Indoor cultivation of *C. sativa* under controlled environmental conditions allows for a complete control of the plant life cycle, resulting in management of the quality and quantity of biomass (Chandra et al., 2017). This is important when producing *C. sativa* with a specific chemical profile for pharmaceutical use (ElSohly et al., 2017). The environmental parameters such as light level, photoperiod, humidity, temperature, CO_2 concentration, and circulation of air play a critical role in the indoor production of this plant (Hoogenboom, 2022; Rodriguez Morrison, 2021). Light is one of the most important factors that effected on cannabis growth (Trancoso et al., 2022). Cannabis plant necessitates high PPFD for effective photosynthesis and growth, with light being a pivotal environmental factor influencing cannabis growth and development (ElSohly et al., 2017). Studies showed that cannabis can perform much better if grown at 1500 μmol m^{-2} s^{-1} PPFD (Chandra et al., 2008; Chandra et al., 2015) and a study by Rodriguez-Morrison et al. (2021b) reported that dry inflorescence yield enhances linearly with increasing canopy-level PPFD up to 1,800 μmol m^{-2} s^{-1} .

Light-emitting diode (LED) technology is often utilised in indoor farming systems. One of the significant advantages with LEDs is the potential for energy savings (Dahlberg & Lindén, 2019). According to Jessup et al. (2012) and Martineau et al. (2012), LEDs save between 50 -70 percent in energy consumption and carbon emissions compared to other conventional technologies, which makes them appropriate for indoor horticultural cultivation. Other advantages of using LEDs are the superior control over color, intensity, and directions as well as the lifespan of 50,000 – 100,000 hours (two to five times longer than advanced fluorescent light). The most efficient LEDs on the market are producing 148 μmol J^{-1} (Hjort & Sandberg, 2013).

This study investigated the effect of different LED spectra on the cannabis production through the vegetative stage when propagated as cuttings or seedlings. Plant parameters and the antioxidant activity of cannabis leaves were compared between spectra and propagation method.

2 Literature review

Cannabis sativa, referred to as marijuana, is an annual and dioecious plant originating from eastern and central Asia that is part of the Cannabaceae family (Mirzamohammad et al., 2021). The Cannabis genus is commonly reported as only constituting a single species. However, *C. sativa* may be divided into three sub-species: *C. sativa* ssp. *sativa*, *C. sativa* ssp. *indica*, and *C. sativa* ssp. *ruderalis*. The first two species, often referred to as "*Sativa*" and "*Indica*", are the main cannabis plant species of recreational and medicinal interest (McPartland, 2017). They have distinct yet opposing tetrahydrocannabinol (THC) and cannabidiol (CBD) ratios; *C. sativa* ssp. *indica* typically possesses a high CBD to THC ratio, whereas the reverse is known for *C. sativa* ssp. *Sativa* (Fischedick et al., 2010). In today's marketplace, however, these distinctions are almost meaningless as new accessions have been created from crossbreeding. *C. ruderalis* is the least known subspecies, and it is not commercially produced because of low plant yields (Small, 2017).

Some cannabis biotypes with high THC content have been widely utilized for both therapeutic and recreational purposes in South Asian countries, creating a close relationship to social and religious rites (Adhikary et al., 2021). For hundreds of years, it has been considered by many as a complex herbal medicine (Jin et al., 2020a) used to treat a variety of conditions, including pain, spasms, asthma, sleeplessness, depression, and appetite loss (Fattore, 2015). Modern research suggests that cannabis and cannabinoids have therapeutic potential for numerous other conditions, many of which have not been previously reported in traditional use, including multiple sclerosis, Huntington's disease, Parkinson's disease, glaucoma, hypertension, stress and psychiatric disorders, Alzheimer's disease and dementia, and anti-neoplasia (Jin et al., 2020a).

The most economically important component for the cannabis industry are the chemical compounds produced by the plant (ElSohly et al., 2017). Among the 545 known components in cannabis, there are at least 104 phytocannabinoids, 120 terpenoids (including 61 monoterpenes, 52 sesquiterpenoids, and 5 triterpenoids), 26 flavonoids, and 11 steroids (Jin et al., 2020b). The phytocannabinoids are a class of C21 terpenophenolic chemicals generated exclusively by cannabis (Gonçalves et al., 2019). They have an alkylresorcinol-rich lipid structure that is categorized as neutral cannabinoids (devoid of a carboxyl group) and cannabinoid acids (with carboxyl group) (Jin et al., 2020b). The neutral forms of cannabinoids are decarboxylated from the accumulated cannabinoid acids (Raja et al., 2020). Cannabis leaves, which contain flavonoid and

terpenoids, have been used in traditional medicine for anti-inflammatory, anti-rheumatic, analgesic, anticonvulsant, antioxidant and neuroprotective, larvicidal, gastroprotective properties (Ashaari et al., 2018). One recent analysis indicates that antioxidants are predominantly found in the leaves (Jin et al., 2020b).

2.1 Light

Light is one of the most crucial environmental factors influencing plant growth and development (Aleric & Katherine Kirkman, 2005). It exerts a wide array of effects on photosynthetic activity and photomorphogenic responses throughout a plant's life (Pocock, 2015; Naznin et al., 2016; Ouzounis et al., 2016). Nearly half of the sun's total radiation reaching the Earth's surface consists of visible light, which spans from wavelengths of 380 to 740 nm (Eichhorn Bilodeau et al., 2019; Frouin et al., 1989). This visible light range is flanked by shorter wavelengths, including ultraviolet (UV) radiation (10 – 400 nm) and infrared radiation (IR; 750 – 1 mm). Together, these account for approximately half of the solar radiation that reaches the Earth's surface (Cooper & Adams, 2023; Parrish, 2012). Within the visible light spectrum, color segments were found distinct, such as violet (~400 – 450 nm), blue (~450 – 500 nm), green (~520 – 565 nm), yellow (~565 – 590 nm), orange (~600 – 625 nm), red (~625 – 700 nm), and far-red (700 – 1440 nm) (Austin et al., 2021). The most critical part of the light spectrum for plants, known as photosynthetically active radiation (PAR), falls within the 400 – 700 nm range (McCree, 1972; McCree, 1971).

2.2 Photosynthesis

Photosynthesis plays a pivotal role in the growth of plants, as there exists a strong connection between a plant's productivity and its photosynthetic rates within a particular environment (Lawlor, 1995). Photosynthesis represents the intricate series of reactions through which plant and phototrophic cells capture, transfer, and convert light energy into chemical potential stored within the carbon bonds of carbohydrates (Janssen et al., 2014). This essential process takes place within the chloroplast, an organelle containing chlorophyll that is dedicated to energy production (Jensen & Leister, 2014). Chloroplasts are primarily located in the cytoplasm

of palisade and spongy mesophyll cells situated between the outer epidermal layers of leaves (Bolhar-Nordenkampf & Draxler, 1993). The energy-producing photooxidation-reduction reactions of photosynthesis occur within the innermost thylakoid membrane system of the chloroplast, which forms networks of flattened thylakoid disks, often arranged in stacks known as grana (Cooper & Hausman, 2004).

Embedded within the thylakoid membrane are protein complexes consisting of five membranes, which play roles in electron transport and the simultaneous generation of the energy-carrying molecules nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP), crucial for fueling carbohydrate synthesis. Between these complexes are the photosystem I and II complexes (PSI and PSII), membrane proteins named in the order of their discovery, albeit contrary to their evolutionary sequence in nature (Cooper & Hausman, 2004). These photosystems contain arrays of associated chlorophyll and carotenoid antenna pigments, molecules that are responsible for capturing light energy for photosynthesis (Nelson & Junge, 2015). These pigments are organized in a manner that maximizes the capture and transfer of light energy (Ramus, 1981). Plant pigments exhibit specific patterns of absorbance wavelengths known as the absorbance spectrum. Chlorophylls a and b (Chl a and b) have strong light absorption in the red and blue regions, with less absorption occurring in the green wavelengths. When dissolved in acetone, Chl a shows peak absorbance at 430 nm and 663 nm, while Chl b peaks at 453 and 642 nm. In acetone, the pigments β-carotene and lutein display strong absorption in the blue region of light, with maximum peaks at 454 and 448 nm, respectively (Taiz & Zeiger, 2002).

Carotenoids are auxiliary pigments in photosynthesis with absorbance spectra falling within the 400–550 nm range. Carotenoids prevent photo-oxidative damage caused by the photosynthetic light harvesting apparatus by dissipating the excess energy of the single excited chlorophyll (^{1}Chl*) and possibly a triplet excited chlorophyll (^{3}Chl*) within light reaction centers, as well as scavenging any evolved singlet-oxygen ($^1O_2$) (Hashimoto et al., 2016; Maoka, 2020). β-carotene has an absorbance peak at 461 nm (Karnjanawipagul et al., 2010), while lutein presents two separate absorbance peaks at 422 nm and 474 nm (Dugo et al., 2006).

2.3 The effect of lights on photosynthesis and plants growth

2.3.1 Red (~625 – 700 nm) and far-red (> 700 nm) light

Light-dependent changes in plant growth and development are regulated by plant hormones (De Wit et al., 2016). Red light plays a role in regulating flowering quality, quantity, and duration (Demotes-Mainard et al., 2016). The inhibition of flowering with red light is mediated by red light receptors, including phytochromes (Higuchi et al., 2012). For instance, in marigold plants, the number of visible flower buds was approximately five times higher when grown under fluorescent light supplemented with red LEDs or under fluorescent light alone, compared to when exposed to monochromatic blue or red light. However, salvia plants did not form flower buds when grown under monochromatic blue or red light, or when fluorescent light was supplemented with FR light for marigold (*Tagetes minuta*) plants (Mah et al., 2018).

Internode elongation is influenced by the R:FR ratio, primarily through the modulation of gibberellin (GA) and auxin (Li et al., 2020) and (Yang et al., 2018). In cowpea (*Vigna sinensis*), increased elongation in response to end of day far- red (EOD-FR) light is likely due to reduced inactivation of GA1, which result from the removal of the active phytochrome form Pfr (Martínez-García et al., 2000). In hybrid aspen (*Populus tremula* × *tremuloides*), overexpression of phytochrome A (phyA) led to decreased shoot elongation, accompanied by reduced cell numbers and cell lengths, and this was associated with decreased GA and IAA (Indole-3-acetic acid) levels (Olsen et al., 1997). However, after overexpressing phyA lines and wild-type plants were exposed to EOD-FR light, there were no significant differences in plant length, cell lengths, cell numbers, or GA levels, indicating that phyA is not involved in the response to EOD-FR (Olsen & Junttila, 2002).

Plants grown under canopy shade conditions or in proximity to other plants exhibit various responses to changes in the R:FR ratios of ambient light (Possart et al., 2014). This phenomenon, known as shade avoidance or the near-neighbor detection response, is characterized by accelerated flowering time (i.e., visible within the expanded floral bud) and rapid stem and leaf elongation (Franklin, 2008). Demotes-Mainard et al. (2016) and Van Ieperen (2012) found that FR light reflected from neighboring seedlings increased the R:FR ratio received by plants, leading to a density-dependent increase in stem length, chloroplast content, chlorophyll a/b ratio, and CO_2 fixation rate, while reducing leaf thickness. The effects of FR light or a low R:FR ratio have been

extensively studied in various plant species and developmental stages (Cao et al., 2018; Demotes-Mainard et al., 2016; Mathews, 2006). Rondanini et al. (2014) conducted grain yield under varying levels of irradiance and R:FR ratio and has shown that photomorphogenic signals are integrated early during vegetative growth, and irradiance has a more significant impact than R/FR signals on rapeseed (*Brassica napus*).

The response of cannabis to narrow bandwidth UV and combined blue: red light was examined by Jenkins (2021); it was reported that the plants were grown under UV and red: blue LEDs produced higher THC than plants under white light (3.6 % and 2.9 % respectively) and terpene concentration of *β*-caryophyllene was significantly decreased in the UV light treatment versus white LED.

Kusuma et al. (2021) investigated the impact of far-red lights (700 – 800 nm) on cannabis flowering and found that photons with longer wavelengths emitted by far-red LEDs can activate the conversion of phytochromes from P_{fr} to P_r, subsequently influencing plant development. According to their findings, applying far-red LEDs continuously for 24 h a day to cannabis plants can both deactivate P_{fr} to P_r, leading to stem elongation, and activate P_{fr} to P_r, causing a delay in flowering for short-day plants (SDP). This results are consistent with Carter (2022) study that examined the effect of far-red light on yield and growth of cannabis. They reported that with an increase in far-red light resulted in a decrease in yield and an increase in height.

Hawley et al. (2018) explored the impact of combined LED lights (red: blue (RB) and red: blue: green (RBG)) on the quality and yield of cannabis buds. The findings indicated that both combinations led to an increase in yield and the concentration of total delta-9-Tetrahydrocannabinol (Δ9-THC) in the bud tissue. Additionally, RGB significantly raised the concentrations of α-pinene and borneol. In contrast, both RB and RGB increased the concentrations of cis-nerolidol compared to the control treatment. Notably, RGB had the most significant influence on altering terpene content, while RB resulted in a more uniform profile of cannabinoids and terpenes in the buds.

2.3.2 Blue (~450–520 nm) and UV (< 400 nm) light

Blue and UV light exert diverse effects on plants (Verdaguer et al., 2017). High intensity blue-LEDs promote plant growth by regulating stomatal movement (Wang et al., 2016) and preserving the integrity of chloroplast proteins (Muneer et al., 2014). Blue light controls phototropism by inhibiting stem elongation. In instances where one side of a stem receives less blue light than the other, it elongates at a faster rate, causing the stem to curve towards the light source (Liscum & Briggs, 1995). Plants are grown under blue and UV lights synthesize more carotenoids and anthocyanins (Carvalho et al., 2016). Research indicates that high intensities of UV-B radiation induce stress in plants by causing DNA damage, photoinhibition, lipid peroxidation, and finally, growth retardation (Hideg et al., 2013). Blue light enhances the total soluble carbohydrates (TSC) and starch accumulation efficiency, while red: blue treatment enhances the fresh mass and dry mass of the plant (Fan et al., 2013).

The effect of blue light (430 nm and 465 nm) and UV-A (380 nm and 400 nm) on Chinese kale (*Brassica oleracea var. alboglabra*) and pak-choi (*Brassica rapa subsp. chinensis*) was recently examined by Li et al. (2020). Results showed that blue and UV-A light played a predominant role in increasing plant biomass and influencing morphology, as well as antioxidant compound content (Vitamin C, vitamin E, phenolics, and individual flavonols), antioxidant activity, and total glucosinolates accumulation. 400 nm UV-A light and 430 nm blue light were efficient in increasing secondary metabolites of pak-choi.

Plants synthesize phenolic compounds in response to certain environmental signals or stresses (Naikoo et al., 2019). Siipola et al. (2015) investigated the effect of five different filters on pea (*Pisum sativum*) including: attenuate UV-B; attenuate UV-B + UV-A (< 370 nm), attenuate UV-B + UV-A; attenuate UV-B, UV-A and blue light, with a polythene filter as a control and results reported that flavonoid content in the leaf adaxial epidermis significantly reduced by blue light and the whole-leaf concentrations of quercetin derivatives relative to kaempferol derivatives are decreased. In contrast, UV-B responses were not significant. The results additionally showed that pea plants regulate epidermal UV-A absorbance and accumulation of individual flavonoids by perceiving complex radiation signals that extend into the visible region of the solar spectrum. Furthermore, solar blue light instead of solar UV-B radiation can be the main regulator of phenolic compound accumulation in plants that germinate and develop outdoors.

Magagnini et al. (2018) reported that UV-B light elicits THC accumulation in both leaves and buds of cannabis. Kotiranta et al. (2024) investigated morphology, inflorescence yield and secondary metabolite of cannabis under different ratios of red/far red and blue/UV light. The findings revealed that altering the R: FR ratio or the amount of short-wavelength radiation in the spectrum can influence the morphology, yield, and secondary metabolite accumulation in cannabis. Additionally, they reported that concentrations of CBD, tetrahydrocannabivarin acid (THCVA), cannabigerolic acid (CBGA), and the total measured terpene concentrations increased with a higher red/far-red light intensity ratio. Blue and UV treatments resulted in an increase in cannabinoid and THCVA concentrations. However, they reported that blue/UV treatments did not have any effect on morphology, the total measured terpene concentration, and the total measured cannabinoid concentrations.

Islam et al. (2021) applied various light ratios, including red, blue, UV, green, far red, and white light to identify compounds directly involved in a light-stress environment, serving as stress markers. The results indicated that acidic form of THC (THCA) played a significant role as a stress marker, with CBDA following closely. Conversely, THC and CBD exhibited a minimal response as stress-indicating compounds under such conditions. Plants cultivated under UV-A mediated spectral combinations demonstrated higher concentrations of THCA, CBDA, and THC.

Rodriguez-Morrison et al. (2021a) revealed that UV LEDs as a production tool did not result in any commercially significant advantages in cannabis yield or the composition of secondary metabolites in inflorescences. They observed that UV LED usage led to an escalation in the severity of UV-induced morphological effects, including reductions in whole-plant size, leaf size, leaf malformations, and stigma browning, as well as physiological impacts such as a decrease in leaf photosynthetic rate. Results showed that with an increase the level of UV exposure, there was a decrease in the proportion of the total dry inflorescence yield derived from apical tissues, and the total terpene content in inflorescences also decreased. However, the relative concentrations of individual terpenes varied by cultivar.

2.3.3 Green (~520–560 nm) light

While most studies have concentrated on the impacts of red and blue light, there have been several studies that have explored the effects of green light with contradictory findings with respect

to plant morphology (Macedo et al., 2011; Olle & Viršile, 2013). Use of various experimental setups may be the reason of this inconsistency. A common factor in many studies is that there is a constant level of total PPFD across the different treatments and this means the intensities of blue and red wavelengths decreased as the intensity of green light increased (Schenkels et al., 2020). Green light, characterized by its high transmittance and reflectance, has the potential to penetrate deeper into the plant canopy compared to red and blue light (Terashima et al., 2009). This is because red and blue light are mainly absorbed by the upper leaves (Meng et al., 2004; Zhang et al., 2011). This property may potentially enhance light interception and whole-canopy photosynthesis. With this perspective, the impact of substituting a portion of red and/or blue light with green light on plant grown and development in basil (*Ocimum basilicum*) was examined by Dou et al. (2019) and finding demonstrated that the combined treatment of red, blue, and green light induced stem elongation in green basil plants. However, green light treatments had no discernible effects on petiole elongation, leaf expansion, leaf thickness, or plant yield.

Kang et al. (2016) investigated the addition of green light to various ratios of red and blue light on leaf photosynthetic rate, growth, and morphology of lettuce (*Lactuca sativa*) plants. Results revealed that leaf photosynthetic rate was highest under a combination of 80 % red and 20 % blue light, and it decreased significantly with use of green light instead of blue light. Furthermore, as the fraction of blue light increased, leaf size and overall plant growth decreased significantly. However, the addition of green light, while significantly reducing the leaf photosynthetic rate, did not hamper plant growth. Consequently, using 10 % (15 μmol m^{-2} s^{-1}) green light did not have a positive impact on lettuce growth.

Mahlberg and Hemphill (1983) concluded that in different light environments it was possible to manipulate the cannabinoid content of cannabis measured in young leaves. The authors used colored filters to alter the light spectrum and concluded that the THC content of leaves from plants grown under shaded daylight and filtered red and blue light did not differ significantly from the THC content in daylight controls, while leaves from plants grown under filtered green light and darkness contained significantly lower levels of THC than those from plants grown in sunlight. Magagnini et al. (2018) reported that cannabis plants grown under high green light irradiation had less amount of THC in flower than plants grown under blue and UV lights. In a study by Islam et al. (2021), green light reportedly has a significant role in CBDA synthesis and its conversion to

CBD. Notably, FR light influences CBDA and CBD accumulation along with green light, whereas white and UV-A play a negative role in this process.

2.4 LED history

Over the past few decades, LED technology has thrived, primarily due to its notable attributes, including high efficiency, reliability, robust construction, low power consumption, and long-lasting durability. These factors have played a pivotal role in driving the rapid progress of solid-state lighting, which relies on high-brightness visible LEDs (Pattison et al., 2018). For controlled environment plant cultivation, LEDs present a promising lighting technology compared to traditional lighting sources (Massa et al., 2008; Morrow, 2008). They offer various advantages, such as a controllable light output, minimal heat generation, cool emitting surfaces, and a range of available wavelengths (Massa et al., 2015; Morrow, 2008; Yeh and Chung, 2009).

LEDs are constructed from semiconductor materials that convert electricity into light with 80-90 % more efficient than traditional lighting (Yam & Hassan, 2005). For decades, researchers in the field of semiconductor devices have aspired to create blue LEDs. The groundbreaking technological achievements by Nakamura in the early 1990s (Nakamura et al., 1993), which led to the production of GaN-based blue and green LEDs, have had a profound impact on LED technology (Manasreh, 2000). Following the development of high-brightness blue LEDs, the LED market has experienced significant growth. By combining the three primary colors red, yellow, and blue full-color displays, white light sources were achieved (Pearton et al., 1999) . LEDs now stand as a superior tool for investigating plant responses to different spectra (Wu, 2019). Nonetheless, LEDs are semiconductor devices that require precise hardware controls to maintain their junction temperature and ensure stable spectral properties (Van Driel et al., 2017).

White LED arrays are the largest market share for LEDs, producing white light through the combinations of phosphors and blue LEDs (Cho et al., 2017). This approach is likely to remain the primary driver of the market for some time, despite the potential advantage of individual red, green, blue LED sources due to their higher overall power efficiency (Pust et al., 2015). Ongoing advancements are being made in phosphor-based LEDs, enhancing both their light output and

efficiency. These improvements are a result of higher LED efficiency and the development of phosphors that are better suited for use with blue or near-UV LED light (Pust et al., 2015).

2.5 LEDs benefits

2.5.1 Production bioactive compounds in crops

Light quality has a significant impact on the accumulation of various metabolites in plants; compared to white light, the presence of single-spectral red or blue LEDs results in increased accumulation of plant metabolites, both primary and secondary, including soluble sugars, starch, vitamin C, soluble proteins, and polyphenols (Qi & Sembok, 2019; Yavari, 2020). Additionally, red: blue combination LED further leads to higher accumulation of anthocyanin, total polyphenols, and flavonoids (Kokalj et al., 2019; Lobiuc et al., 2017). The results of research showed that red: blue and blue LEDs increased the accumulation of flavonoids and anthocyanin in Chinese cabbage (Lobiuc et al., 2017; Sng et al., 2021; Zhang et al., 2020). Notably, red LEDs have a more pronounced impact on anthocyanin accumulation and enhanced expression of anthocyanin biosynthesis genes, such as MdMYB10 and MdUFGT compared to blue LEDs (Hasan et al., 2017). The supplementation of ambient light with red, blue, green, red: far-red, or red: blue LEDs further increases the accumulation of organic acids, phenolic compounds, vitamin C, α-tocopherol, carotenoids, and glucosinolates in various crops (Alrifai et al., 2019; Hasan et al., 2017; Hashim et al., 2021; Zhang et al., 2021),

Ginsenosides, significant plant secondary metabolites produced through the isoprenoid pathway in ginseng plants (*Panax ginseng*) and known for their medicinal value, exhibit a notable increase in concentration (from 2 % to 74 %) in ginseng roots when exposed to blue LEDs at 450 nm and 470 nm, in comparison to ginseng roots grown in dark conditions. This suggests that LEDs could potentially act as elicitors, triggering the expression of key enzymes like squalene synthase in the isoprenoid pathway or inducing the production of reactive oxygen species (ROS), subsequently enhancing the activity of defense-related genes, thus leading to increased synthesis of ginsenosides. In red ginseng, blue LED exposure has been observed to stimulate the production of high levels of pharmacologically significant components (Park et al., 2012). Additional evidence indicates that red lights, enhances carbohydrate accumulation, whereas blue LED light treatment promotes protein formation (Ghate et al., 2013).

Increased accumulation of secondary metabolites in response to light, including UV light, can be a stress response or a sun-screening effect to protect plants from ionizing radiation (Park et al., 2012). Light affects signal transduction pathways, which include enzymes, metabolites, and secondary messengers (Tisch & Schmoll, 2010). The evidence strongly suggests that light could be used to produce medicinally important secondary metabolites in plants (Mitchell et al., 2012; Morrow, 2008). However, the effect of different single- or mixed-spectral light ratios may vary according to the plant species or cultivars (Kozai, 2016). To enhance the nutritional traits of crops, use of blue LEDs and/or combined red: blue LEDs might be the best choice, under controlled cultivation practices (Hasan et al., 2017).

2.5.1.1 Cannabis secondary metabolites and LEDs

Single-spectrum blue and red LEDs have a significant impact on the accumulation of cannabinoids, terpenes, and alkanes in cannabis when compared to white fluorescent and high pressure sodium (HPS) light (Namdar et al., 2019). In a study, blue LEDs resulted in increased THC concentration, as well as enhanced levels of cannabigerol (CBG) and various terpenes (Morello et al., 2022). However, recent findings in hemp have provided conflicting results regarding the interaction between blue light and cannabinoid content. In this case, the light spectrum did not affect the accumulation of CBD or THC (Westmoreland et al., 2021). In addition to blue light, it appears that supplementary green light may stimulate the accumulation of secondary metabolites in cannabis plants, including Δ9-THC and terpenes such as limonene, linalool, and myrcene (Booth & Bohlmann, 2019; Hawley et al., 2018). This is likely due to additional light, particularly a spectrum with a notable green light component, which is typically absorbed by certain terpenes, enabled the plants to increase terpene production in response to this environmental condition (Hawley, 2018). Islam et al. (2021) reported that green light had an impact on the synthesis of CBD and CBDA. Lydon et al. (1987) examined the effect of UVB on photosynthesis, growth and cannabinoids; there were no significant physiological or morphological differences between UV-B treatments in either drug or fiber-type plants, yet THC concentrations (not other cannabinoids) increased in leaves of drug types. It was suggested that cannabinoids may play a role in UV protection.

2.5.1.1.1 Terpenes

Terpenes in cannabis plants, including monoterpenes and sesquiterpenes, are present in smaller quantities compared to cannabinoids, they are primarily concentrated in the glandular trichomes and serve various essential functions and are functionally diverse (Booth et al., 2017). Terpenes are volatile aromatic compounds that play a significant role in influencing the taste and aroma of plants (Booth & Bohlmann, 2019). Additionally, they act as a defense mechanism against biotic stresses and function as plant hormones that regulate growth (Booth & Bohlmann, 2019; Brenneisen, 2007). Researchers have additionally observed that increased solar UV radiation is associated with higher levels of CBDA, terpenes, and cannaflavins in the cannabis hemp and UV-B radiation leads to an increase in the number of trichomes (Giupponi et al., 2020) and while several studies suggest that UV-B radiation has a positive effect on the content of monoterpenes in plants with glandular trichomes (Behn et al., 2010; Ioannidis et al., 2002; Tang et al., 2020).

2.5.1.1.2 Flavonoids

Flavonoids are highly responsive to the quality of light they are exposed to, and when plants are grown under UV, blue, and FR light treatments, flavonoid concentrations tend to be higher (Ferreyra et al., 2021; Idris et al., 2018). Flavonoids encompass several classes, including flavonols, flavones, flavanones, anthocyanins, and isoflavonoids, each characterized by different accessory groups attached to a central 15-carbon skeleton (Bhatla & Lal, 2023). The three-carbon unit connecting the phenyl groups typically forms a third ring through cyclization with oxygen, resulting in a core structure known as 2-phenylbenzopyranone. Flavonoids are often found in conjugation with sugar, either as O-glycosides or C-glycosides, but they can exist as aglycones (Ramesh et al., 2021). Jin et al. (2020b) found that cannabis leaves are a rich source of flavonoids compared to roots, stems, and inflorescences. They identified a total of twenty-six flavonoids in cannabis plants, which exist as methylated and prenylated aglycones, or conjugated O-glycosides or C-glycosides of compounds like orientin, vitexin, isovitexin, quercetin, luteolin, kaempferol, and apigenin. Interestingly, unlike cannabinoid accumulation, the content of individual and total flavonoids decrease as plants age. This variation in reported values may be attributed to differences in plant age and chemovar variety. Islam et al. (2021) revealed that light has a significant impact on flavonoid accumulation in cannabis. Total flavonoid content increases under UV light combined with a 60 % red light source. Electrical blue LED and far-red light sources have shown effects on flavonoid accumulation.

2.5.2 LEDs enhance antioxidant activity

The quality of light has a significant impact on the photo-oxidative properties of plants as it influences the antioxidant defense system, leading to an increase in the activity of antioxidative enzymes. The role of LEDs in inducing the production of secondary plant metabolites appears to be linked with the enzyme phenylalanine ammonia-lyase (PAL), which plays a crucial role in the initial step of the phenyl propanoid pathway and synthesis of anthocyanins and phenols (Xie et al., 2020).

Notably, three prenylated/geranylated flavones, known as cannflavins A, B, and C, have been isolated in *C. sativa*. Although cannflavins are often considered unique to *C. sativa*, cannflavin A has been found in (*Mimulus bigelovii)*, a plant in the Phrymaceae family (Bautista et al., 2021). Cannflavin A and B display anti-inflammatory effects by inhibiting the activities of

microsomal prostaglandin E_2 (PGE_2) synthase-1 and 5-lipoxygenase, resulting in reduced PGE_2 and leukotriene production, respectively (Werz et al., 2014) and their pathway starts with p-coumaroyl-CoA derived from phenylalanine and involves phenylalanine ammonia-lyase (PAL) (Flores-Sanchez & Verpoorte, 2008). Photoreceptors are responsible for activating various signal transduction pathways that regulate light-dependent responses through transcription factors and related gene expression. Shorter wavelengths, particularly in the blue and UV light range, have proven to be the most effective in enhancing the accumulation of anthocyanins and flavonoids. This often occurs by upregulating the expression of genes in the flavonoid pathway or relevant transcription factors. For example, strawberries exposed to blue light exhibited a significant increase in anthocyanin content and higher transcript levels of FaCHS, a crucial enzyme in the biosynthesis of flavonoids and anthocyanins (Magagnini et al., 2018)

It is suggested that the up-regulation of PAL in the presence of red: blue LEDs may be responsible for the increased production of plant secondary metabolites (Giliberto et al., 2005). Some studies showed that vegetables, such as peas, lettuce, barley (*Hordeum vulgare*), and tomatoes (*Solanum lycopersicum*), exhibit improved antioxidant properties in response to the use of single-spectral or combined red (625 – 630 nm) and blue lights (465 – 470 nm) compared to traditional white light sources (Kim et al., 2013; Lin et al., 2022; Samuolienė et al., 2011; Wu et al., 2023). Furthermore, green (510 nm), yellow (595 nm) LEDs enhance both antioxidant properties and anthocyanin accumulation (Dong et al., 2014; Samuolienė et al., 2011).

2.5.3 LEDs improve nutritional traits of produce postharvest

LEDs have been utilized in growth chambers and greenhouses to enhance plant biomass and nutrient levels. Their energy-efficient structure, compact size, long lifespan, and relatively cool surfaces have also made LEDs valuable in the postharvest treatment of agricultural produce. The aim of postharvest processing is to maintain the desired visual attributes of the harvested crops, along with their texture, nutritional value, and flavor (Perera et al., 2022). LEDs with narrow bandwidths at various wavelengths can impact the accumulation of volatile compounds and improve the characteristics of diverse postharvest fresh produce. These include lettuce (Kitazaki et al., 2018), pak-choi (Zhou et al., 2020) , cucumber (*Cucumis sativus*) (Łaźny et al., 2023), and

blueberries (Routray et al., 2018), peaches (*Prunus persica*) (Gong et al., 2015), strawberries (*Fragaria ananassa*) (Chong et al., 2022; Zhou et al., 2020) and tomatoes (Nájera et al., 2018).

2.5.4 LEDs provide protection against food spoilage and crop loss

In the realm of the agri-food industry, electrical light treatments have emerged as a valuable tool. These treatments are used for disinfecting water and food, as well as for enhancing the health and growth of plants by harnessing light energy across different wavelengths (Prasad et al., 2020) For instance, single-spectral blue LEDs have proven effective in reducing postharvest decay in citrus fruits caused by penicillium species, especially when compared to dark conditions (Muthukumar, 2019). Moreover, the infection of fruits has been observed to decrease due to the light-triggered stimulation of lipid signaling, leading to the accumulation of phospholipase A2, ethylene, and octanal (Muthukumar, 2019).

Several critical factors must be considered in LEDs applications, including light wavelength, treatment duration, dosage, illumination temperature, relative humidity, and microbiological conditions (Prasad et al., 2020). When fresh-cut papaya (*Carica papaya*) contaminated with salmonella was exposed to blue light LEDs with a 405 nm wavelength for 48 h, it resulted in a noticeable reduction in the bacterial population (Kim et al., 2017; Poonia et al., 2022). Kim et al., (2017) reported that antibacterial efficacy of freshly cut mangoes (*Mangifera indica*) under 405 nm LEDs was more effective when compared to no LED treatment. It found that cell counts of a three-strain cocktail (*Escherichia coli* O157:H7, three serotypes of *L. monocytogenes*, and five serotypes of *Salmonella* spp.) all decreased under the LED treatment.

2.6 Antioxidants

Antioxidants are bioactive compounds, even in small amounts, slow or stop oxidation processes influenced by ROS or ambient oxygen enzymes (Racchi, 2013). Plants produce antioxidants as defensive mechanisms to counteract oxidative stress caused by imbalances of ROS including the singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO) (Scandalios, 2005). ROS result in direct or indirect damages to nucleic acids and

lipids in plant cells acting as signaling molecules involved in plant growth as well as development, and pathogen defense responses (Gill & Tuteja, 2010).

Various studies have reported diverse naturally occurring antioxidants are found in medicinal plants with different concentrations, physical and chemical properties (André et al., 2020; Chang et al., 2006; Cömert et al., 2020; Krawitzky et al., 2014; Mullen et al., 2002; Padhi et al., 2017; Pandey & Rizvi, 2009; Pisoschi et al., 2009). Polyphenols such as phenolic acids and flavonoids are powerful antioxidants with high scavenging properties (Pandey & Rizvi, 2009). In cannabis, cannabinoids are antioxidant agents as they can scavenge free radicals and reduce metal ions (Dawidowicz et al., 2021b).

Hacke et al. (2019) compared the antioxidant activity of CBD and THC and result showed that antioxidant activity of CBD and THC was compared against that of the well-defined antioxidants such as ascorbic acid (AA), resveratrol (Resv) and (−)-epigallocatechin-3-gallate (EGCG). Clear evidence of the synergistic and antagonistic effects between CBD and THC regarding to their antioxidant activities was observed.

Hampson et al. (2000) suggested that these two cannabinoids may be viewed as more powerful neuroprotective substances compared to ascorbate and tocopherol. Additionally, their research illustrated that CBD and THC could prevent oxidative damage induced by hydroperoxide in neuronal cell cultures from rats. In a separate study, Chen and Buck (2000) documented that CBD and THC could function as antioxidants at concentrations below one micromolar, effectively averting oxidative cell death.

As mentioned above, cannabis contains flavonoids known for their antioxidant activity (Wedman-St Louis, 2019). Ahmed et al. (2019) compared the amount of flavonoids in cannabis leaves in compare with bitter apple *(Citrullus colocynthis)* leaves. The results showed that the total flavonoid content in cannabis leaves is higher than that in bitter apple leaves, indicating that the antioxidant activity of cannabis is greater than that of bitter apple.

2.6.1 Antioxidant classification

Antioxidants encompass a diverse group of molecules that are challenging to categorize based on shared structural properties (Packer & Valacchi, 2002). It is additionally important to

consider other compounds that do not directly act as antioxidants but that influence the process indirectly. They can do this by modulating the actions of direct antioxidants or by regulating the production of antioxidant proteins, thereby promoting their synthesis and availability. These substances maybe referred to "pro-antioxidants" (Vertuani et al., 2004). In the past, several classification methods have been attempted, considering various factors such as origin (natural or synthetic) (Augustyniak et al., 2010), nature (enzymatic or non-enzymatic) (Panda, 2012), chemical-physical properties (hydrophilic or lipophilic) (Jimenez-Alvarez et al., 2008), structure (e.g., flavonoids, polyphenols) (Van Acker et al., 1996), and mechanisms of action (preventive, chain-breaking, etc.) (Scott, 1988). Since the functions of antioxidants are expressed through a complex network, it might be more informative to categorize them based on their function-structure relationships. Based on this there can be several classes of antioxidant and pro-antioxidant agents. Given the whit range of molecules that contribute to antioxidant effects, both directly and indirectly, we can consider the following classes: vitamins, fats and lipids, amino acids, peptides and proteins, antioxidants derived from plants, minerals, and enzymes (Vertuani et al., 2004). For a comprehensive overview, Figure 1 illustrates the principal categories of antioxidant and pro-antioxidant molecules classified based on their structure and function.

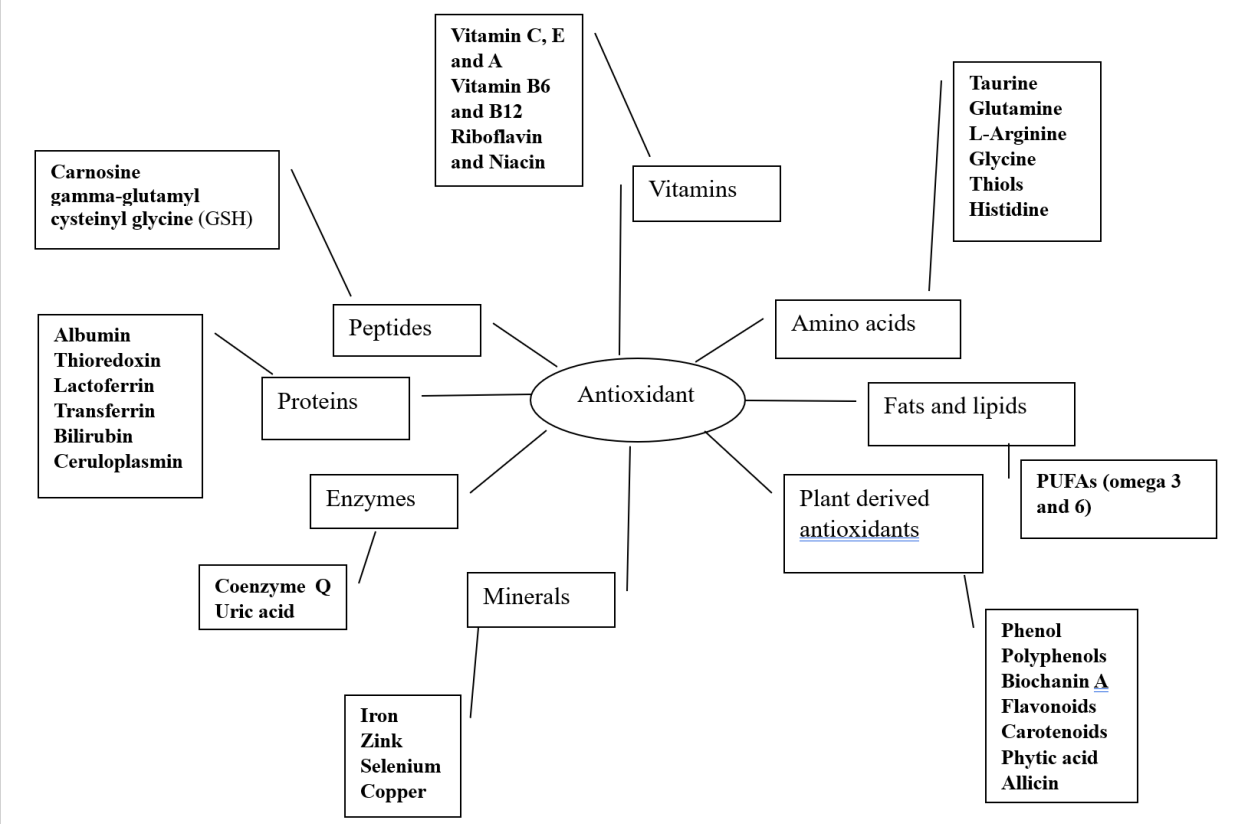

Figure 1. Antioxidant classification based on structure and function.

2.6.1.1 Primary or secondary antioxidants

Antioxidants may be classified as primary or secondary antioxidant agents, according to their direct or indirect antioxidant defence mechanism (Pisoschi et al., 2009; Pisoschi & Pop, 2015). Primary antioxidants such as catalase act as chain-breaking antioxidants by reacting directly with free radicals. Primary antioxidants transform free radicals to more stable, nonradical products by hydrogen or electron donation (Agati et al., 2020). Secondary antioxidants including glutathione-s-transferase work indirectly as singlet oxygen quenchers, peroxide decomposers, metal chelators, oxidative enzyme inhibitors and UV radiation absorbers (Pisoschi & Pop, 2015). The main aim of secondary antioxidants is to prevent lipid oxidation. They work synergistically by regenerating primary antioxidants and thereby restore the antioxidant activity of primary antioxidants to ensure their continuous antioxidant activity (Amorati & Valgimigli, 2015).

2.6.1.2 Hydrophilic or lipophilic antioxidants

Although antioxidants are classified as either lipid-soluble (hydrophobic) and water-soluble (hydrophilic), plant-based antioxidants such as phenolic compounds and vitamin C are mostly hydrophilic (Dias et al., 2011; Haida & Hakiman, 2019a). Phenolic compounds in plants act as structural polymers (lignin), ultraviolet protectors (flavonoids), signal compounds (salicylic acid and flavonoids), and defence response chemicals (tannins and phytoalexins) (Lin et al., 2016). Hydrophobic antioxidants such as carotenoids and vitamin E protect cell membranes from lipid peroxidation (Pulido et al., 2003).

2.6.1.3 Enzymatic or non-enzymatic antioxidants

Antioxidants may further be classified as enzymatic or non-enzymatic based on the catalytic action (Haida & Hakiman, 2019b). Enzymatic antioxidants (e.g. superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GSHPx) convert harmful oxidative products in a multi-step enzymatic process in presence of cofactors such as copper, zinc, manganese, and iron to stable hydrogen peroxide (H_2O_2) and then to water. Non-enzymatic antioxidants (e.g. vitamins A and E, plant polyphenols, and carotenoids) function by preventing the spread of free radicals (Haida & Hakiman, 2019a).

2.6.1.4 Natural or synthetic antioxidants

The primary method in which antioxidants are formed is through their synthesis by various microorganisms, fungi, and even animals, but most commonly by plants. These are referred to as natural antioxidants. However, this is a misconception, as plants produce antioxidants primarily for their own protection against various threats (Anwar et al., 2018).

Another way to produce antioxidant production is through an industrial process. These antioxidants are called synthetic antioxidants. Contrary to natural antioxidants, they have been quality- and safety-tested to protect consumers (Flieger et al., 2021). Natural antioxidants mostly contain a pyrocatechol or a pyrogallol group, they are ortho-disubstituted phenolic compounds, which are less common among synthetic antioxidants because of their higher toxicity. Higher toxicity can be advantageous for plants in the protection against pests. Most synthetic antioxidants

are para-disubstituted phenolic compounds as they are less toxic than ortho-disubstituted compounds (Pokorný, 2007).

2.6.2 Effect of light on antioxidants activity in plants

Changes in light quality due to the spectral properties of tissue pigments strongly affect plant anatomical, physiological, morphological, and biochemical parameters of leaves. Activities of antioxidant enzymes in plants induced by light spectrum are more complex and often reported with contrasting results (Falcioni et al., 2017). For example, activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in *Camptotheca acuminata* seedlings were affected by differential light quality. SOD, POD and CAT activities increased by 16.6, 370.0 and 489.3 % under blue light, respectively, and by 9.8, 99.9 and 156.4 % under yellow light respectively, but decreased by 8.5, 44.8 and 11.9 % under red light respectively, compared with the control. Thus, blue light and yellow light induced higher antioxidant enzyme activities whereas red light decreased them (Yu et al., 2017). A study reported that supplementation of blue light improved the antioxidant activity of *Kalanchoe pinnata* (Nascimento et al., 2013).

Dawidowicz et. al., (2021) showed that cannabinoids CBG, CBD, Δ9-THC, CBN, CBGA CBDA and Δ9-THCA exhibit antioxidant activity manifesting itself in their ability to scavenge free radicals, to protect oxidation process and to reduce metal ions.

Various studies have investigated the effect of LEDs on cannabis antioxidant activity (Eaves et al., 2020; Lalge et al., 2017; Lydon et al., 1987; Morello, 2021). For instance, a study comprising 10 combinations of light on the antioxidant activity of cannabis executed by Islam et al. (2021) revealed that the levels of total polyphenols (TPC) and total flavonoids (TFC) varied with changes in light spectrum. Higher TPC was observed in treatment with light combination with 60 % red light and 20 % blue light. Furthermore, the presence of red light was found to boost cytokinin levels, stimulating the synthesis of phenolic compounds, with far-red light aiding in enhancing the plants' antioxidant capacity. Results additionally showed that intensity of red light and its ratio to other light sources seemed to influence the production of secondary metabolites. Both TPC and TFC decreased when red light sources exceeded 70 % but increased at 50-60 % and dropped when below 40 % compared to natural light. Supplementary UV radiation increased flavanols and other secondary metabolites, acting as a stress response to shield plants from

radiation. The combination of UV radiation with 60 % red light was particularly noteworthy. Moreover, blue LEDs and far-red light enhanced secondary metabolites and improve the nutritional quality of cannabis crops, including ascorbate, total phenolic compounds, total flavonoid contents, and antioxidant activity. Moreover, an increase in the intensity of red light in comparison to blue light was noted to boost plant flavonoid levels.

Islam et al. (2022) examined the effects of light-spectral quality on growth-related morpho-physiological traits of cannabis and the results reported that in treatments with more red-light compared with less red and blue light a significant increase in H_2O_2 concentration occurred, leading to lipid peroxidation during the later stages of growth. Moreover, treatment with red: blue 85:2, consistently showed higher accumulation of cannabinoids. It is evident that elevated levels of ROS caused cellular stress in the plant, as indicated by increased osmolyte synthesis and enzyme activity. This stress accelerated plant maturation and contributed to the higher accumulation of cannabinoids in cannabis plants. Finally, it was concluded that ROS metabolism plays a crucial role in the morpho-physiological adaptation and cannabinoid accumulation in hemp plants. These findings offer valuable insights into the use of LED lighting to optimize cannabinoid production.

The effects of different ratios of red to blue light quality on the growth and cannabinoid synthesis of hemp were reported by Wei et al. (2021) and results showed that increasing the red-light ratio with constant blue light effectively supported hemp growth (in terms of plant height, stem diameter), number of leaves and notably increased aboveground biomass, hemp flowers biomass and the CBD content in both leaves and flowers compared to HPS and other treatments with less red-light. The red: blue 16: 1 ratio was the best choice for indoor hemp cultivation aimed at achieving higher CBD yields compared to HPS.

2.7 Antioxidant assays

The DPPH method is an antioxidant assay based on electron transfer, whereby DPPH persists as a free radical resulting from the spare electron's delocalization throughout the entire molecule. Unlike the majority of free radicals, DPPH does not dimerize and an absorption band with a maximum at 520 nm, demonstrated by a purple colour, indicates delocalization on the DPPH molecule (Vertuani et al., 2004). The reduced (molecular) form of DPPH is produced when DPPH

interacts with a hydrogen donor, and the violet colour vanishes. As a result, the reduction in absorbance is linearly related to the antioxidant content (Kedare & Singh, 2011; Thaipong et al., 2006).

The FRAP assay offers a relatively straightforward, rapid, and cost-effective approach to directly measure the overall antioxidant activity of substances that can donate electrons (reductive antioxidants) in each sample (Benzie & Devaki, 2018). In this assay, the reduction of ferric ions (Fe_3^+) to ferrous ions (Fe_2^+) serves as the indicator reaction, which is linked to a noticeable color change (Lamuela-Raventós, 2018). The FRAP assay can be applied to a wide variety of sample types and is adaptable for different setups, including a basic manual version that requires minimal specialized equipment, a semi-automated version using a microplate reader, and even a fully automated mode through a customized program on a biochemical analyzer. The reagents used are stable and pose minimal toxicity risks (Danet, 2021). The method boasts high sensitivity and precision, with consistent stoichiometric factors for the reacting antioxidants across a broad range of concentrations. It is robust, meaning that minor variations in reaction conditions do not significantly affect the results obtained (Lamuela-Raventós, 2018).

Different groups have researched antioxidant activity in cannabis extracts using DPPH and FRAP assays (Aazza, 2021; Kalinowska et al., 2022; Muscarà et al., 2021; Smeriglio et al., 2016; Stasiłowicz-Krzemień et al., 2023). The results of a study by Hacke et al. (2019) examining several cannabis extracts with varying ratios of CBD and THC revealed that pure CBD is a more effective DPPH scavenger than pure THC, as the former displayed a lower half maximal inhibitory concentration (IC_{50}) value than the latter. Furthermore, they believed, although both substances are isomers, CBD has two hydroxyls (phenolic groups) and THC only has one. This distinction may account for the increased antioxidant activity of CBD over THC observed. Dawidowicz et al. (2021a) compared 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Cupric reducing antioxidant capacity (CUPRAC), FRAP and DPPH assays for measuring cannabis antioxidants and reported that neutral cannabinoids CBG, CBD, Δ9-THC and CBN have more antioxidant activity than Trolox when radicals and metal ions are reduced by electron transfer from phenolic groups using the Single electron transfer (SET) mechanism. It was dditionally concluded that the DPPH assay's determination of the mutual relationships between the antioxidant activities

of specific cannabinoids is less clear-cut than the ABTS assay results, which makes their scientific interpretation significantly more challenging for a number of reasons.

When comparing the antioxidant activity of hemp seed oil and milk thistle (*Silybum marianum*) seed oil using DPPH, FRAP, CUPRAC, and ABTS assays in a study by Kalinowska et al. (2022), it was determined that hemp seed oil was rich in (-) epicatechin, catechin, vanillic and ferulic acids, which were not detected in the extract obtained from milk thistle seed oil. Interestingly, the higher content of phenolic compounds in milk thistle seed oil, as determined by HPLC, did not necessarily correlate with the antioxidant potential determined by ABTS and DPPH assays. Additionally, hemp seed oil displayed a higher antioxidant potential when evaluated using these two spectrophotometric methods, possibly due to the involvement of unsaturated acids in quenching DPPH radicals and ABTS cation radicals (hemp seed oil contained a greater number of unsaturated acids compared to milk thistle seed oil). Furthermore, both hemp and milk thistle seed oils were rich in phenolic antioxidants and unsaturated fatty acids, making them sensitive to oxidation.

Mirzamohammad et al. (2021) used salicylic acid as a stress inducer at different concentrations on cannabis, and the antioxidant activity was measured using the FRAP and DPPH assays. The results indicated that the antioxidant capacity, as determined by the DPPH and FRAP assays, was affected by various concentrations of salicylic acid ($p < 0.01$). When different concentrations of salicylic acid were applied as foliar treatment, the antioxidant capacity in cannabis plants increased from 12.80 to 21.58 μmol $Fe^{++}g^{-1}$ dry mass (FRAP) and from 52.8 % to 76.6 % (DPPH).

2.8 Effect of growth stage on cannabis secondary metabolites

Cannabis secondary metabolites are different during its growth stages (vegetative and flowering stage) and in various parts of the plant (Jin et al., 2020b). Abdollahi et al. (2020) compared cannabis secondary metabolites between different varieties and growth stages, and it found significant differences between treatments. The results reported that one of the cultivars (Yazd) had the highest essential oil yield during the vegetative stage, while for another cultivar (Fed 17), it was observed during the flowering stage. Moreover, the content of sesquiterpenes

decreased during plant growth, while monoterpenes exhibited the opposite trend, with their quantity increasing as the plant grew.

According to Jin et al. (2020b), a total of twenty-six flavonoids have been recognized in cannabis plants. Results indicated that no flavonoids were detected in the roots and stem bark and in inflorescence, flavonoids were detected in lower amounts (0.07 – 0.14 %), while the highest concentrations were found in leaves (0.34 – 0.44 %). Additionally, the study revealed that leaves contain cannabinoids and various terpenoids. A study by Stasiłowicz-Krzemień et al. (2023) investigated the antioxidant potential of cannabis leaves across various cultivars and noted that all cultivars served as sources of both cannabinoids and antioxidant activity.

Another study reported variations in the essential oil composition of cannabis across different months and seasons. This research revealed that terpenoids undergo changes during growth, with the majority exhibiting higher concentrations during the vegetative stage (Verma et al., 2014). The diversity and proportion of secondary metabolites in the plant vary based on several factors, including the cannabis variety, plant part, environmental conditions, and the maturity of the plant (Ahmed & Hijri, 2021; Fischedick et al., 2010).

Moher et al. (2022) investigated the impact of light intensity on cannabis growth and results revealed that PPFD levels ranging between 600 and 900 μmol m^{-2} s^{-1} seemed to strike an optimal balance for key morphological parameters in vegetative cannabis while minimizing energy consumption associated with excessively high light intensities, considering various production strategies such as enhance plant structure and shorten the length. They additionally reported that an increase in light intensity resulted in smaller leaflets, generally displaying smaller and more numerous serrations along the leaflet margins. Individual leaf size showed a linear decrease, while individual leaf biomass exhibited a linear increase, resulting in an 87 % increase in specific leaf weight (SLW) at the maximum versus minimum average PPFD (APPFD).

Rodriguez-Morrison et al. (2021b) explored the impact of light intensity on leaves and photosynthesis during the flowering stage. Results noted that leaf light response curves varied both with localized PPFD and temporally, throughout the flowering cycle and the leaf light response cannot reliably predict whole-plant responses to light intensity, especially in terms of crop yield. Additionally, dry inflorescence yield exhibited a linear increase with canopy-level PPFD up to 1800 μmol m^{-2} s^{-1}, while leaf-level photosynthesis saturated well below this threshold in 1800

μmol m^{-2} s^{-1}. This study found that the density of the apical inflorescence and harvest index also increased linearly with rising light intensity.

In conclusion, there is limited research about the vegetative stage for cannabis plants and how environment can affect this stage of plant growth in cannabis. Research has focused mainly on cannabinoids, Δ9-THC, and CBD in particular, as such, female flower tops are harvested while other parts are often discarded by growers. This is a potentially unnecessary waste (Jin et al., 2020b). Research on the cannabis vegetative stage and antioxidant activity of cannabis leaves may elucidate an additional opportunity for industrial producers by using conventionally discarded cannabis leaves for medical or other industries.

3 Material and methods

3.1 Plant materials and cultivation environment

Two cannabis propagation methods (seeds and cuttings) were used to compare the effect of different LED spectra on vegetative cannabis plant growth. Cannabis seeds (OG Kush) were sown in biodegradable pots (Jiffy Group International BV, Lindtsedijk 20a, NL-3336 LE Zwijndrecht) filled with COCO soil (CANNA Canada Corp., Toronto, ON, Canada). Samples were placed in a climate-controlled growth chamber (Model: PHCBI, MLR-325, PHC Corporation, North America, Wood dale, IL, US) under different light treatments and a vegetative light cycle (16 h photoperiod, 55 - 60 % relative humidity at 25 °C) for 4 weeks. During this stage, seedlings were watered every two days and supplemented with half strength fertilizer COCO A and B (5 % NO_3^-, 0.1 % NH_4^+, 4 % P_2O_5, 3 % K_2O, 7 % CaO, 3 % MgO, 2 % SO_3, 0.007 % B, 0.001 % Cu, 0.02 % Fe DTPA, 0.0003 % Fe EDTA, 0.01 % Mn, 0.002 % Mo, 0.007 % Zn, 0.5 % fulic and humic acid (CANNA Canada Corp., Ontario, Canada).

A total of 10 seedlings were cultivated from a single mother plant in a (1.30 m × 2.50 m) growth tent area and the plants were maintained under a wide PC amber (595 nm) LED (UT CustomGrow, U Technology, Calgary, Alberta, Canada) as mother plants. Cuttings were rooted using indole-3-butyric acid gel (Technaflora, Mission, BC, Canada), with an 18 h photoperiod and a PPFD level of 500 µmol m^{-2} s^{-1} under high humidity (> 90 %), using a propagating tray with a transparent dome cover. PPFD was determined with a light meter (MQ-500, Apogee instruments, Logan, UT, US). Successfully propagated cuttings showing adventitious roots were transplanted into 250 mL square pots with coco soil (CANNA Canada Corp.) and transplanted to the growth chamber under the different LED treatments. The cuttings were similarly grown under a vegetative light cycle (16 h photoperiod, 55 – 60 % RH, and air temperature of 25 °C) for 2 weeks. During this stage, plants were watered every two days and supplemented with the fertilizer COCO A and B.

3.2 Light spectra and plant cultivation

Light treatments for the cannabis seedlings and cuttings comprised four different LED arrays: wide amber (595 nm), narrow amber (595 nm) + violet (430 nm), narrow amber (595 nm) + violet (430 nm) + blue (485 nm) (U Technology), and white LED (blue; 446 nm + amber; 595 nm + red; 653 nm) (Shenzhen SOSEN Electronics Co, SS-200VP-56BH, China). Spectra for these

LED light treatments are reported in Figure 2 to 5. The growing area for each LED light treatment was 45 ×45 cm and the areas were separated by growing tray. PPFD was determined by a light meter and provided 350 – 400 µmol m^{-2} s^{-1}. Six plants grew under each LED light treatment in 3 replications, using 72 plants total.

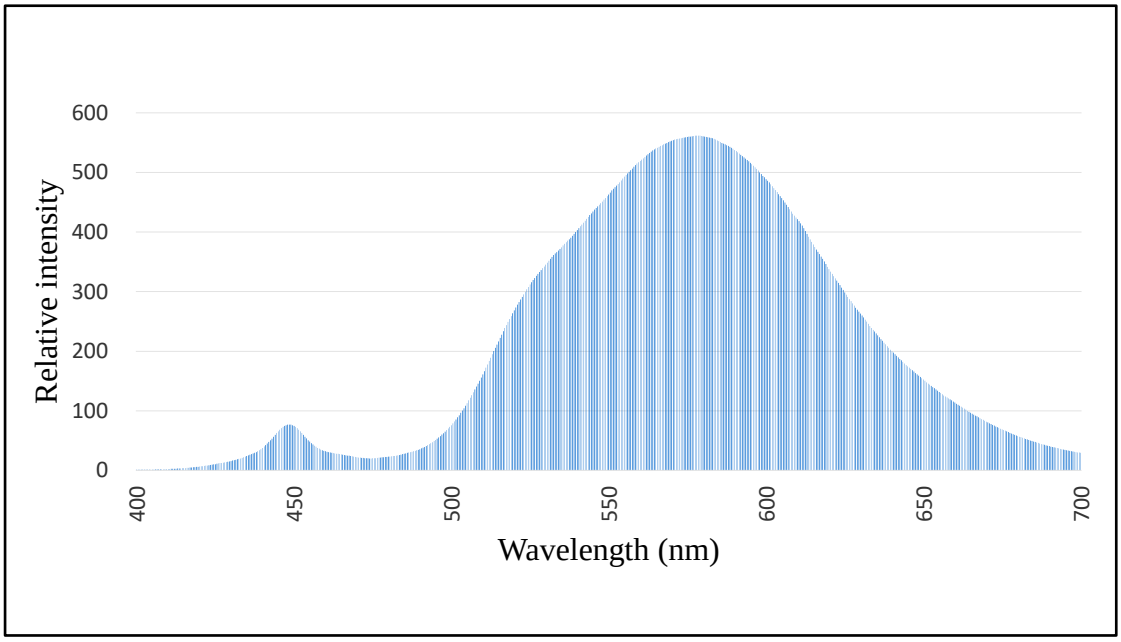

Figure 2. Wide PC amber (595 nm) LED light treatment

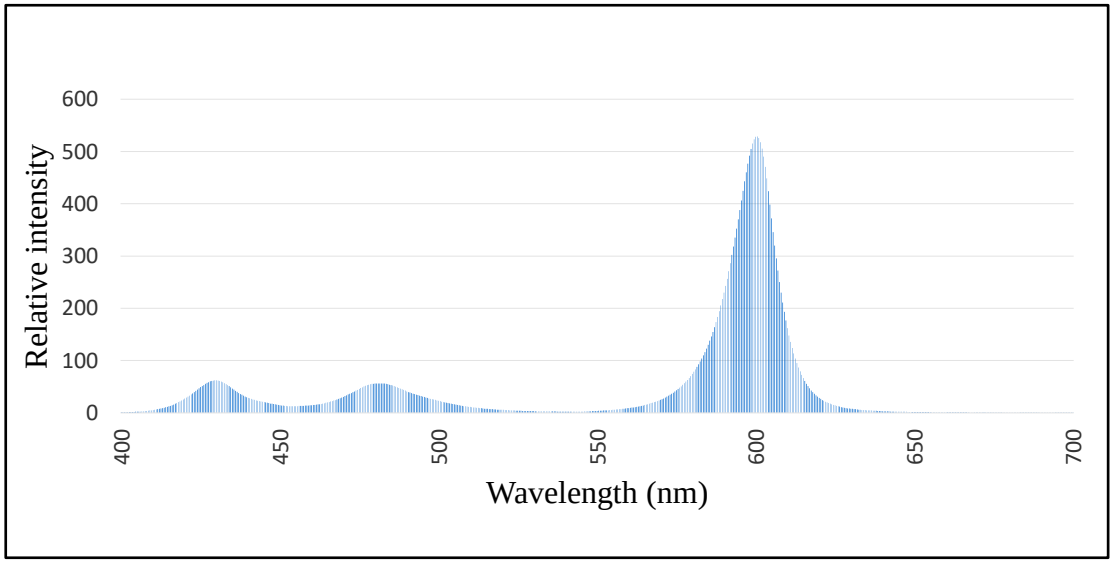

Figure 3. Narrow amber (595 nm) + 430 nm + 485 nm LED light treatment

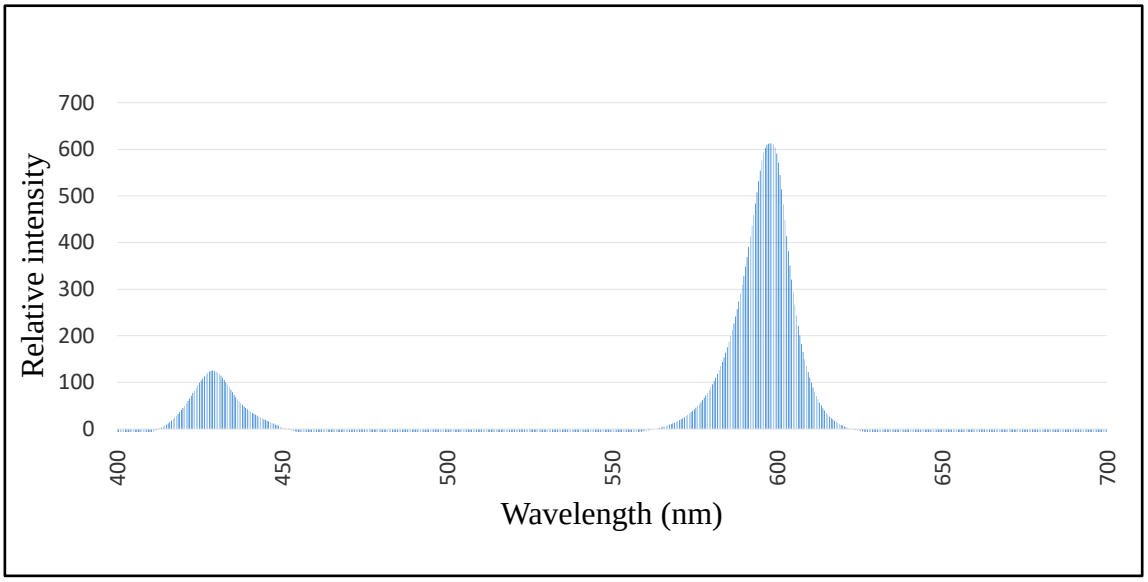

Figure 4. Narrow amber (595 nm) + 430 nm LED light treatment

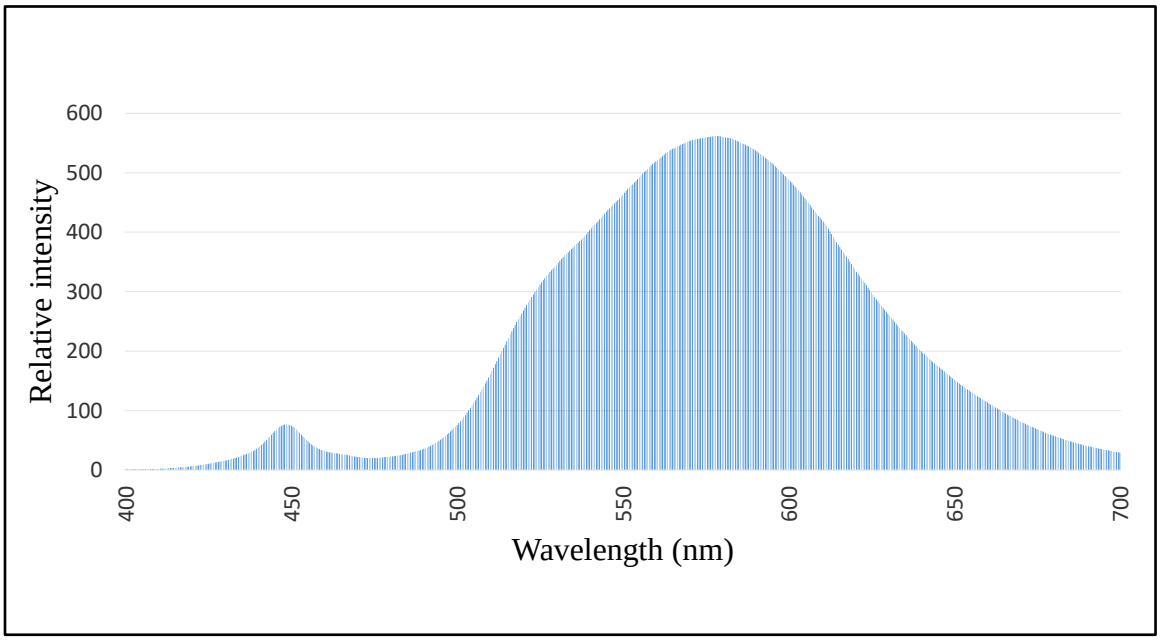

Figure 5. White LED light treatment

3.3 Plant growth parameters and harvest

After harvest, plant height was measured from the base of the plant to the highest new stem growth (excluding leaves). The stem diameter was measured with Vernier calipers 10 cm from the plant base.

Fresh mass was measured by scale (Mettler AE50 analytical balance, Columbus, OH, US) directly after harvest. Prior to drying and analysis, harvested samples were pre-frozen at -5 ºC for at least 24 h to prepare for freeze drying.

SPAD values were measured with a SPAD chlorophyll meter (ISPAD- 502plus, Zhejiang, China) directly after harvest.

3.4 Cannabis freeze drying

The cannabis biomass was freeze-dried using optimal conditions (Addo et al., 2023). In brief, samples were placed in plastic trays and transferred to a laboratory-scale vacuum freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH Gamma 1–16 LSCplus, Osterode, Lower Saxony, Germany) with a condenser temperature of -55 °C. The sample was freeze-dried at 20 °C for 24 h at 0.85 mbar until the dry basis moisture content was 12 %. Dried samples were stored in a food-safe plastic bag and kept in a refrigerator at 5 °C. Dried mass was measured directly after freeze-drying (AE50 analytical balance, Mettler).

3.5 Secondary metabolites extraction

A total of 0.75 g each representative biomass sample from cannabis cuttings and seedlings was weighed in a 50 mL Falcon tube. Samples were ground by hand with a mortar and pestle, then reweighted to ensure sample mass prior to extraction. Each Falcon tube was filled with 20 mL high-pressure liquid chromatography HPLC-grade methanol (Thermo Fisher Scientific, Waltham, MA, US) and vortexed for 20 min at 500 rpm (Thermo Scientific). Each sample was filtered for 20 min using Whatman^{TM} filter paper (Thermo Fisher Scientific in Waltham, Massachusetts, USA). To guarantee that 99.5 % of the secondary metabolites were recovered, residual cannabis material was transferred into fresh 50 mL Falcon tubes and placed to a second extraction process.

A 40 × dilution total extract was produced by combining the second extract with the same quantity of the first extract.

3.6 Measuring antioxidant activity with the DPPH assay

The DPPH test was adapted for cannabis (Addo et al., 2023) and, was used to measure the antioxidant activity in the cannabis leaves. Different serial dilutions of a 10 mM Trolox® standard (Sigma-Aldrich, Saint Louis, MI, US) in HPLC-grade methanol (Thermo Fisher Scientific) were used to create a calibration curve. A stock solution of 0.1 mM DPPH (Sigma-Aldrich, Saint Louis, MA, US) in HPLC-grade methanol was prepared fresh daily. In 15 mL Falcon tubes, aliquots (100 µL) of extracted samples or standards were added together with 2900 µL of DPPH ion stock solution. After vigorous vortexing (Thermo Fisher Scientific) for 30 s, the mixture was incubated for 30 min in the dark at room temperature. The Ultropec 2100 pro ultraviolet/visible spectrophotometer (Biochrom Limited, Cambridge, England) was used to detect absorbances at 517 nm. The spectrophotometer was calibrated using HPLC-grade methanol, and a DPPH solution was employed as a control. The sample average radical scavenging activity was calculated and the DPPH inhibition (%) was analyzed using Equation (1). Equation (2) was used to determine the concentration (M) of Trolox equivalent antioxidant activity (TEAC) using the calibration curve. Using Equation (3), the results are expressed as the amount (M) of Trolox equivalent antioxidant activity (TEAC) per gram of dry matter sample.

$$\%\ \text{DPPH inhibition} = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \quad (1)$$

$$TEAC\ (M) = \frac{\frac{(\%DPPH\ Iinhibision - 1.2737)}{0.3637}}{1000} \quad (2)$$

$$TEAC\ (M) Dry\ matter\ (g)^{-1} = \frac{extraction\ volume\ (0.04L) \times TEAC\ (M)}{Analysis\ volume (0.0001L) \times (Sample\ mass - (\%MC \times sample\ mass))} \quad (3)$$

3.7 Measuring antioxidant activity with the FRAP assay

FRAP assay used was based on a previously adapted method for cannabis (Addo et al., 2023). Trolox (Sigma-Aldrich) was serially diluted at different concentrations (10 to 0.004 mM) to create the standard curve. The FRAP reagent was made using a 300 mM sodium acetate buffer (pH 3.6), 20 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) (Sigma Aldrich) solution in 40 M hydrochloric acid (Thermo Fisher Scientific), and 20 mM ferric chloride ($FeCl_3$) (Sigma-Aldrich) solution in the ratios of 10:1:1 (v/v), respectively.

Daily fresh preparations of the FRAP solution were made and it was warmed at 37 °C for 10 min in a water bath before use. In 15 ml Falcon tubes (Thermo Fisher Scientific), an aliquot (100 µL) of the extracted sample or standard was added to 2900 µL FRAP stock solution. The mixture was incubated for 60 min at room temperature and in complete darkness after being vigorously vortexed (Thermo Fisher Scientific) for 30 s. The Ultropec 2100 pro ultraviolet/visible spectrophotometer (Biochrom Limited, Cambridge, England) was used to detect absorbances at 593 nm. The spectrophotometer was zeroed with HPLC-grade methanol and the FRAP solution was used as a control. Equation (4) was used to compute FRAP inhibition. The calibration curve and Equation (5) were used to determine the FRAP value (antioxidant activity). Equation (6) is used to calculate the FRAP value (M) per gram of dry matter sample.

$$FRAP\ Inhibision(AU) = Absorbance_{\ sample} - Absorbance_{Control} \quad (4)$$

$$\text{FRAP Value (M)} = \frac{\frac{(FRAP\ Inhibition - 0.0588)}{0.2101}}{1000} \quad (5)$$

$$FRAP\ value\ (M)\ dry\ matter\ (g)^{-1} = \frac{Extraction\ volume\ (0.04\ L) \times FRAP\ value\ (M)}{Analysis\ volume\ (0.0001L) \times (Sample\ mass - (\%MC \times sample\ mass)} \quad (6)$$

3.8 Statistical analysis

All experimental conditions were performed in triplicate. Averages and standard deviation were determined with MS Excel. With a 95 % confidence level ($P \leq 0.05$), statistical analyses were carried out using JMP software (JMP 4.3 SAS Institute Inc., Cary, NC, USA). The Student's t-test was used to compare means. JMP software was used to determined how the independent variables (different LED and antioxidant assay) affected the dependent variables (antioxidants activity, plant height, stem diameter, SPAD, fresh mass, and dry mass). To assess the association between the independent and dependent variables, the least-square multiple regression technique was employed. The results of an analysis of variance (ANOVA) were used to determine whether there were any samples that differed significantly ($P \leq 0.05$).

4 Results

4.1 Plant height

Plant height was measured at harvest (Figure 6). Plants cultivated under wide amber LED resulted in the tallest plants, with a mean height of 20.1 cm ± 1.1 for seedlings and 22.4 cm ± 1 for cuttings. This was followed by 8.9 cm ± 0.5 for seedlings and 11.6 cm ± 1 for cuttings grown under narrow amber + 430 light, 7.8 cm ± 0.5 for seedlings and 8.4 cm ± 0.6 for cuttings under narrow amber + 430 + 485 light and 7.2 cm ± 0.1 and 7.4 cm ± 0.2 under white light. Several significant differences were observed between light treatments ($p \leq 0.001$) based on variance analysis. Plants cultivated under wide amber light were significantly taller than plants cultivated under narrow amber + 430 nm, narrow amber + 430 nm + 485 nm and the white light. Results for cuttings were the same as for seedlings (Tables 1 and 2).

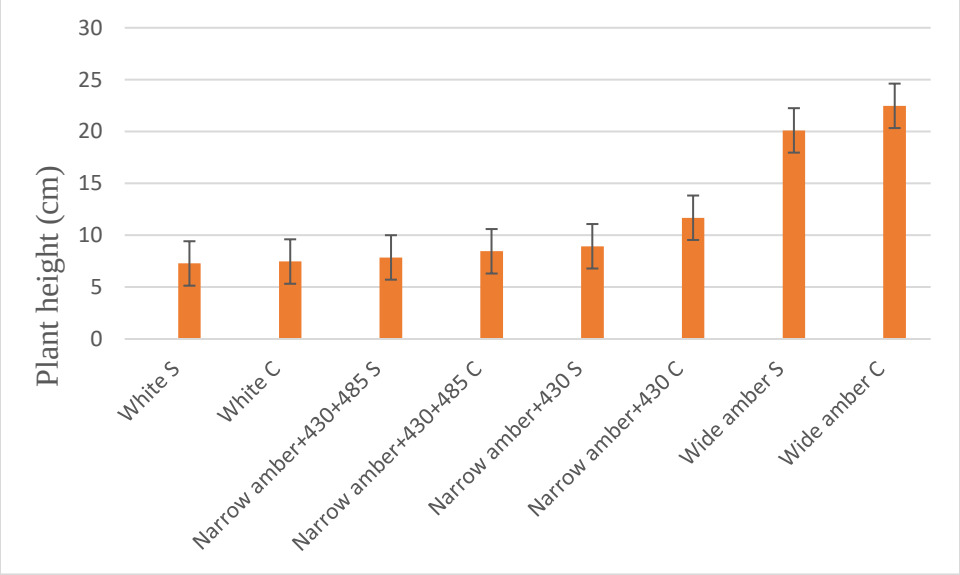

Figure 6. Mean plant height (cm) (S: seedlings and C: cuttings) ± SE (Total of 144 plants) of all replicates under each light treatment. Wide and narrow amber had peak irradiance at 595 nm.

4.2 Fresh and dry mass

The fresh and dry mass of vegetative cannabis plants grown from cuttings and seed were measured after harvest (Figures 7 and 8). Wide amber LED yielded the highest fresh mass (13.3 g ± 0.3 for seedlings and 14.3 g ± 0.2 for cuttings), followed by narrow amber + 430 (8.2 g ± 0.6 for seedlings and 9.2 g ± 0.1 for cuttings), narrow amber +430 + 485 (7 g ± 0.1 for seedlings and 7.9 g ± 0.08 for cuttings) and white (4.9 g ± 0.8 for seedlings and 6.4 g ± 0.9 for cuttings) light. Wide amber LED resulted in the highest dry mass (3.2 g ± 0.6 for seedlings and 3.8 g ± 0.05 for cuttings), followed by narrow amber + 430 (2 g ± 0.1 for seedlings and 2.8 g 0.1 for cuttings), narrow amber + 430 + 485 (1.8 g ± 0.1 for seedlings and 2.3 g ± 0.04 for cuttings) and white (1.4 g ± 0.2 for seedlings and 1.8 g ± 0.4 for cuttings) light. Statistically significant differences were observed for fresh leaf mass between light treatments. White LED light resulted in significantly less fresh mass than all other light treatments ($p \leq 0.001$). Narrow amber + 430 nm and narrow amber + 430 nm + 485 nm light were comparable and yielded significantly greater fresh leaf mass than white. Finally, wide amber showed the highest leaf fresh mass for both seedlings and cuttings. Results were similar for dry mass between treatments and there was a significant different between light treatments ($p \leq 0.001$). Wide amber LED with maximum dry mass and white with minimum in both seedlings and cuttings, displayed the most significant difference with each other (Tables 3, 4, 5 and 6). Comparing between two cultivated methods showed that cuttings had more fresh and dry mass than seedlings samples.

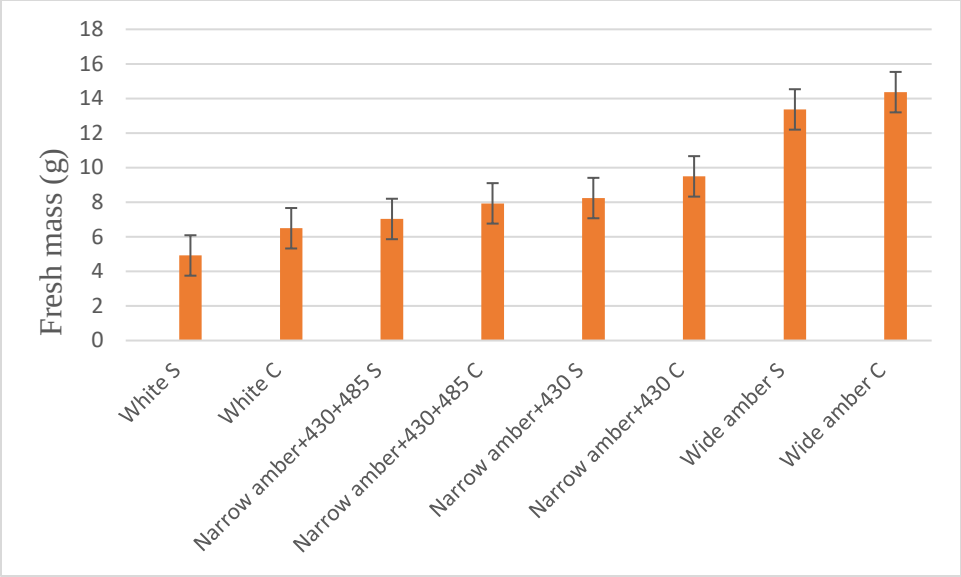

Figure 7. Mean fresh mass (grams) of cannabis seedlings (S) and cuttings (C) cultivated under each light treatment. Error bars represent ± SE (n=18 per group).

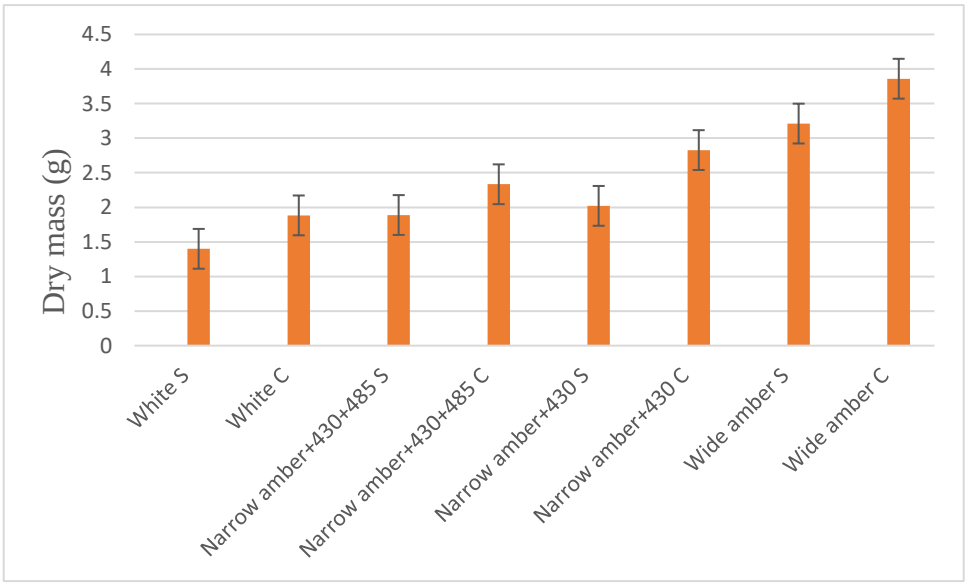

Figure 8. Mean dry mass (grams) of cannabis seedlings (S) and cuttings (C) cultivated under each light treatment for both cultivated methods. Error bars represent +/- SE (n=18 per group).

4.3 Stem diameter

The average stem diameter was assessed from the plant base (Figure 9). The wide amber LED yielded the most substantial stem diameter (4.2 cm ± 0.01 for seedlings and 4.4 cm ± 0.4 for cuttings), with subsequent rankings held by the narrow amber + 430 nm light (4.01 cm ± 0.04 for seedlings and 3.4 cm ± 0.2 for cuttings), and last, the white treatment (2.9 cm ± 0.15 for seedlings and 3.2 cm ± 0.2 for cuttings). Statistically significant differences were evident in stem diameter between the various light treatments. The white LED light treatment exhibited notably smaller stem diameters compared to all other light treatments ($p \leq 0.001$). The narrow amber + 430 and narrow amber + 430 nm + 485 nm treatments were closely aligned and produced significantly larger stem diameters than the white treatment. The wide amber treatment displayed the highest stem diameter for both seedlings and cuttings (Tables 7 and 8). When comparing results between the two cultivation methods, stem diameters were similar, yet cuttings had the maximum stem diameter in all treatments except narrow amber + 430 and narrow amber +430 + 485.

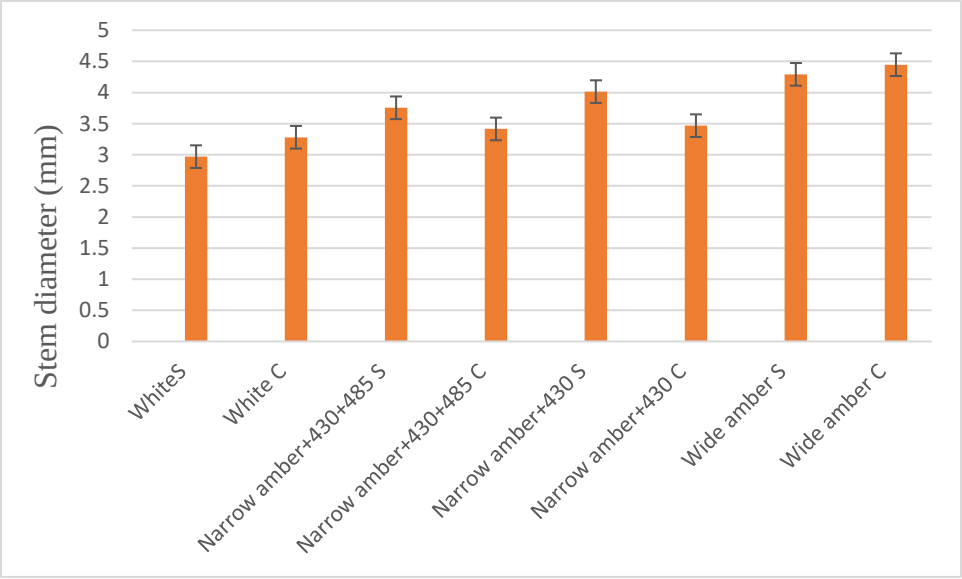

Figure 9. Mean stem diameter (mm) +/- SE of cannabis seedlings (S) and cuttings (C) cultivated under each light treatment (n= 18 per treatment)

4.4 SPAD value

SPAD values of leaves from cannabis seedlings and cuttings grown under each experimental light treatment were evaluated (Figure 10). The wide amber LED resulted in the most SPAD values (61.2 ± 1.2 for seedlings and 64.7 ± 1.6 for cuttings). Subsequent rankings were occupied by the narrow amber + 430 light treatment (57.5 ± 1.6 for seedlings and 60.2 ± 2.7 for cuttings), succeeded by the narrow amber + 430 + 485 light treatment (53.6 ± 1.2 for seedlings and 56.7 ± 0.4 for cuttings), and finally, the white light treatment (56.8 ± 5.1 for seedlings and 56.8 ± 1.7 for cuttings). Significant differences in SPAD between the light treatments were evident ($p \leq 0.001$). The wide amber LED showcased the highest SPAD value in comparison to all other light treatments. The white and narrow amber + 430 + 485 treatments closely resembled each other and led to significantly smaller SPAD values than the narrow amber + 430 light treatment (Tables 9 and 10). Between cannabis seedlings and cuttings, cuttings had greater SPAD values than seedlings, with the exception of the white light, which was the same.

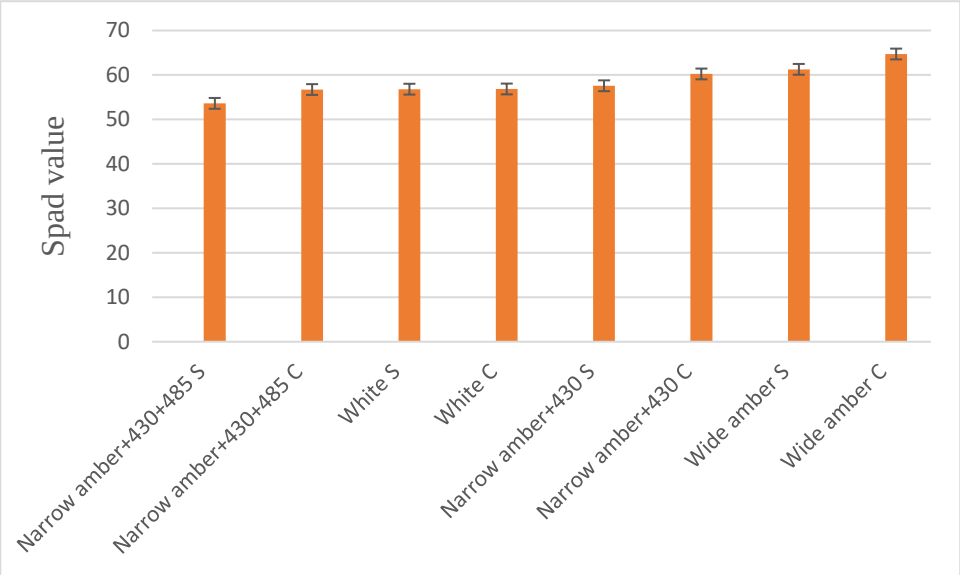

Figure 10. Mean SPAD value +/- SE of leaves from cannabis seedlings (S) and cuttings (C) cultivated under each light treatment (n= 18 per treatment)

4.5 Antioxidant activity

Trolox equivalent antioxidant capacity (TEAC) data for all cannabis seedlings and cuttings grown under different light treatment are presented in Figure 11. Between the treatments, the narrow amber + 430 + 485 light exhibited notably higher antioxidant potential than the other treatments (mean of 86.6 % ± 2.3 for seedlings and 88.1 % ± 3.6 for cuttings). Conversely, the wide amber LED displayed the lowest antioxidant potential between the treatments (20.2 % ± 0.6 for seedlings and 27.5 % ± 2.6 for cuttings), followed by the white treatment (28.8 % ± 1.1 for seedlings and 31.30 % ± 1.08 for cuttings), and the narrow amber + 430 treatment (48.5 % ± 2.5 for seedlings and 52.9 % ± 1.5 for cuttings). Statistical analysis revealed significant differences between treatments for both seedlings and cuttings ($p \leq 0.001$) (Tables 9 and 10). Moreover, distinctions were observed between the antioxidant activities of cuttings and seedlings. Across all light treatments, the antioxidant activity was same for cuttings and seedlings.

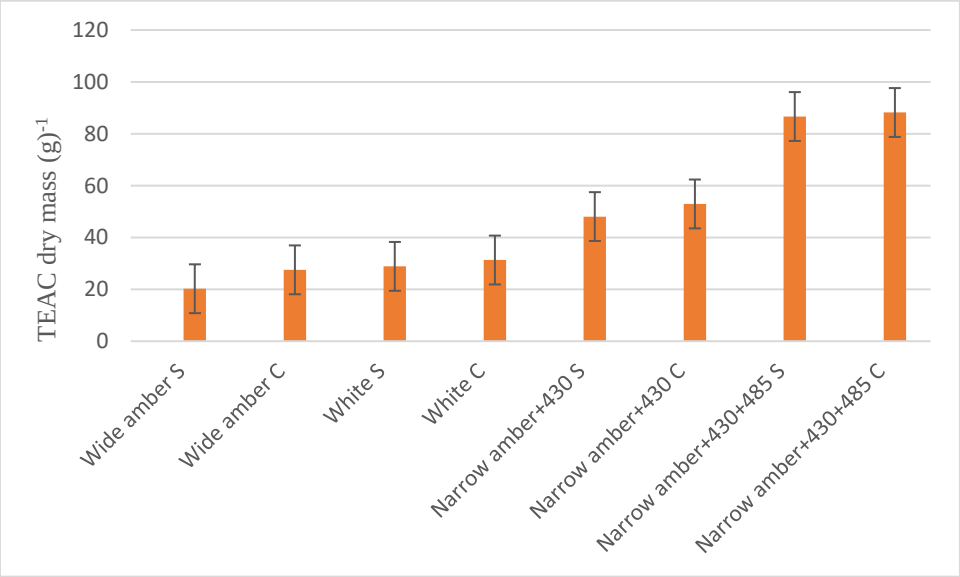

Figure 11: Mean TEAC (M) dry matter $(g)^{-1}$ +/-SE for cannabis seedlings and cuttings grown under each light treatment (n=18 per group)

4.6 FRAP

The antioxidant potential of cannabis extracts obtained from dried cannabis leaves of plants cultivated from seeds or cuttings was assessed using the FRAP dry mass $(g)^{-1}$ method. Samples exposed to wide amber LED lighting exhibited lower antioxidant activity, with values of 0.0046 ± 0.001 for seedlings and 0.0055 ± 0.0001 for cuttings (Figure 12). The highest antioxidant activity was observed in plants subjected to narrow amber + 430 nm + 485 nm lighting, with values of 0.0072 ± 0.002 for seedlings and 0.0074 ± 0.002 for cuttings. This was followed by plants exposed to narrow amber + 430 nm light, which displayed values of 0.0062 ± 0.0006 for seedlings and 0.0066 ± 0.0001 for cuttings, as well as those exposed to white light, with values of 0.0057 ± 0.0002 for seedlings and 0.0060 ± 0.0001 for cuttings. Statistical analysis revealed significant differences between these treatments ($p \leq 0.001$) (Tables 13 and 14). These results indicate that both seedlings and cuttings exhibited antioxidant activity as determined by the FRAP assay, and there were no significant different between cuttings and seedlings.

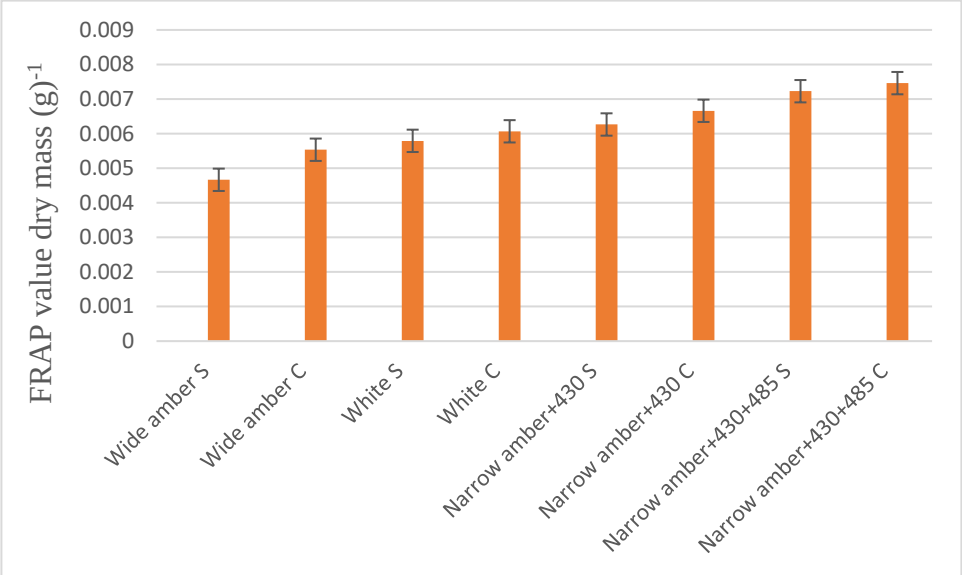

Figure 12. Mean +/- SE FRAP value dry mass $(g)^{-1}$ of cannabis seedlings (S) or cuttings (C) cultivated under each light treatment.

5 Discussion

The effect of four different light spectra on vegetative *C. sativa* plant growth parameters and antioxidant activity were examined. We explored how variations in light spectrum affect the way the plants grow and their ability to produce antioxidants, as measured by two assays (DPPH and FRAP). It was found that changes in the light spectrum had a significant influence on the plant growth parameters during the vegetative stage and on antioxidant activity in leaf tissue.

5.1 Plant height

The experiment revealed significant variations in plant height under different light conditions during the vegetative stage. Specifically, plants under wide amber light were 80 % taller than those subjected to other treatments, while plants under white lighting were 37 % shorter than other treatments. Amber plants were 65 % taller compared to white LED, and 61 % and 51 % taller than those exposed to narrow amber + 430 and narrow amber + 485, respectively. Narrow amber + 430 nm and narrow amber + 430 + 485 yielded similar results in terms of plant height. Reichel et al. (2021) and Xu et al. (2021) indicated that blue light prevents the elongation of internodes in the stem due to low R/FR and inhibit cell division. Lalge et al. (2017) corroborated these findings, demonstrating that the combination of blue and red light prevents stem elongation and results in shorter plants.

Morello et al, (2021) examined the impact of different LEDs on flowering cannabis, plant growth and chemical composition. Results reported that plants cultivated under amber light were significantly taller (79.6 cm) than those cultivated under red, rose, HPS, purple, and blue lights. Conversely, plants cultivated under blue light were notably shorter (66.7 cm) than those subjected to other treatments.

These data are consistent with research conducted by Magagnini et al. (2018) which reported that cannabis plant grown under sole HPS light may suffer from unbalanced morphology expressed by excessive leaf and stem elongation; this is due to the low R:FR ratio (i.e., the ratio between red and far-red light) and low blue light emission of the HPS lamp (Magagnini et al., 2018) .

5.2 Fresh and dry mass

The fresh and dry mass of cannabis cuttings and seedling leaves exhibited notable variations among the various light treatments. Exposure to wide amber light resulted in the highest fresh mass (54 %) and dry mass (45 %) compared to other treatments and white light plants were 35 % fresh mass and 33 % dry mass less than other treatments. Specifically, amber surpassed white light, narrow amber +430 +485, and narrow amber +430 +485 lights by 58 %, 45 %, and 40 % in fresh mass, and 54 %, 40 %, and 30 % in dry mass, respectively. A study conducted by Morello et al. (2022) reported that both fresh and dry inflorescence mass in cannabis samples exposed to amber light (103.11 g and 18.33 g respectively) exceeded those under blue light (66.7 g and 15.4 g respectively).

Further research observed that plants grown under LEDs emitting light at blue: red (1:1) had most inflorescence biomass compared to those under HPS and white LED lighting respectively. They additionally found that CBGA, a precursor for most cannabinoids, was affected by the white LED treatment. Additionally results showed that CBGA accumulation was stimulated by blue-rich light as compared to red rich HPS light and the major cannabinoids CBDA, THCA and CBCA were affected by light quality and the amount increased by blue LEDs (Danziger & Bernstein, 2021).

5.3 Stem diameter and SPAD value

The stem diameter of cannabis seedlings and cuttings was measured after harvest. The results revealed significant differences between the treatments. The wide amber had the highest stem diameter (16 %) and SPAD (5 %) value than other treatments, while the narrowest stem diameter and SPAD was associated with white lighting 17 % and 2.7 % respectively than other treatments. The study reported wide amber light had 27 %, 18 % and 13 % stem diameter more than white, narrow amber +430 +485 and narrow amber + 430 + 485 lights, respectively. In terms of SPAD value the different percentages for wide amber was 12 %, 9 % and 6 % compared to white, narrow amber + 430 + 485 and narrow amber + 430 + 485 lights, respectively. Cheng et al. (2022) reported that cannabis samples grown under blue light exhibited a greater stem diameter (10.2 %) and chlorophyll content (7.4 %) than those grown under white, red, and red-blue lights. In addition, Wei et al. (2021) found that cannabis plants grown under LED treatment with a red-

to-blue ratio of 9.3:1 and a PPFD of 191 µmol m^{-2} s^{-1}) displayed 50 % stem diameter more than, plants under a combined wavelength LED (red-to-blue ratio of 6.5:1 and a PPFD of 28.2 µmol m^{-2} s^{-1}) and LED (with a red-to-blue ratio of 7.2:1 and a PPFD of 41.7 µmol m^{-2} s^{-1}).

Another study reported that cannabis plants grown under LEDs with peak wavelengths at 650 nm and 450 nm displayed higher levels of chlorophyll a and b compared to those grown under HPS and white LED lighting. Vegetative cannabis plants exposed to wide amber LED lighting exhibited 24 % chlorophyll a and 33 % chlorophyll b content (measured using SPAD) more than those subjected to narrow amber combined with 430 nm, narrow amber combined with 430 nm and 485 nm, and white light (Danziger & Bernstein, 2021).

5.4 Antioxidant activity

Plant-based antioxidants work by neutralizing free radicals through the donation of electrons or hydrogen atoms, thus preventing damage to tissue cells (Haida & Hakiman, 2019b). In this study, we compared the antioxidant activity of cannabis seedlings and cuttings grown under different LED lighting conditions using two distinct assays. The outcomes from the DPPH and FRAP assays indicated that plants grown under narrow amber + 430 + 485 LEDs demonstrated the highest antioxidant activity, registering 17.7 % for FRAP and 82 % for DPPH, surpassing other treatments, while wide amber lighting yielded the 15 % by FRAP and 50 % by DPPH antioxidant activity. In comparison to wide amber, white, and narrow amber + 430 lights, narrow amber + 430 + 485 plants exhibited 30 %, 19 %, and 12 % higher FRAP values, and 72 %, 65 %, and 42 % higher DPPH values, respectively.

These findings are consistent with Lin et al. (2022), which found that coriander (*Coriandrum sativum*) plants grown under blue and red light displayed 86.2 % and 71.5 % antioxidant activity respectively compared to those exposed to green light and mixed LED lighting.

Cheng et al. (2022) compared different LEDs, including white, blue, red, and a mix of 50 % red with 50 % blue light, on hemp plants. They measured antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD) and found that blue reduced POD activity (16.6 %). Furthermore, SOD and POD activities were increased under both RL and RBL compared with WL. These changes were believed to be linked to modifications in protein functions in various

plant tissues, and their study suggests that SOD and POD play roles in protecting protoplasm and cell integrity.

According to Magagnini et al. (2018), LED combination with high blue light has been shown to reduce oxidative stress in industrial hemp plants and was associated with concentrations of CBD (35 %) and CBG (207 %) compared HPS, and total cannabinoids recorded 4.3 g plant^{-1} for this treatment.

In our study, we compared the FRAP and DPPH assays and found that both cannabis seedlings and cuttings exhibited antioxidant activity. These results suggest that both the FRAP and DPPH assays are suitable methods for assessing the antioxidant activity of cannabis leaves. However, it is important to note that the data from the FRAP assay were more consistent with each other than the results obtained from the DPPH assay. Additionally, differences between treatments were more pronounced in the DPPH assay than with the FRAP assay. These findings align with the research of Dawidowicz et al. (2021), who studied the antioxidant activity of cannabinoids using different assays. Their DPPH results showed that only CBG and Δ9-THC exhibited stronger antioxidant activity than Trolox, while in the FRAP assay, CBN and Δ9-THC displayed the highest antioxidant activity compared to CBG and CBD. The authors attributed these differences to the presence of COOH groups in cannabinoids, which impacted their antioxidant potency to varying degrees (Dawidowicz et al., 2021a). The existence of a carboxyl group linked to the aromatic ring of the phenolic group results in charge distribution and decreases electron density, promoting the creation of the phenolic radical. It is important to note that the electron transfer from the antioxidant molecule to the ABTS cation radical coincides with the detachment of a proton from the phenolic –OH group. In the case of acidic forms of cannabinoids, the active phenolic –OH group and the –COOH group are in the ortho position. Consequently, they participate in the formation of intramolecular hydrogen bonding. This impedes the release of the proton from the phenolic group, as evidenced by the observed lack of influence of the –COOH group on the antioxidant properties of acidic cannabinoids compared to neutral ones (McMurry, 2016).

5.5 Seedling or cutting propation

The objective of any improvement program is to deploy the best genetically improved plants efficiently and extensively, whether through seed or vegetative propagation. This study aimed to examine the effect of different light spectra on cannabis plants during the vegetative stage to further optimize seedling and clonal propagation. Morphological parameters and antioxidant activity were assessed post-harvest. Minimal differences were observed between the two propagation methods, with closely aligned measurements for all parameters.

Plant height exhibited variations of 2 %, 7 %, 10 %, and 9 % between cutting and seedling samples under white, narrow amber + 430 + 485, narrow amber + 430, and wide amber lights, respectively, resulting in an overall difference of 7 %. Stem diameter showed a total difference of 2.7 % between cuttings and seedlings. Cuttings under white and wide amber lights had larger stem diameters than seedlings by 9 % and 4 %, respectively. In contrast, seedling samples grown under narrow amber + 430 + 485 and narrow amber + 430 exhibited larger stem diameters by 8 % and 12%, respectively.

Regarding fresh mass, cuttings exceeded seedlings by a total of 8 %, with specific differences of 12 %, 7 %, 3 %, and 7 % under white, narrow amber + 430 + 485, narrow amber + 430, and wide amber lights, respectively. Dry mass exhibited a total difference of 9 %, with specific variations of 10 %, 10 %, 11 %, and 8 % under white, narrow amber + 430 + 485, narrow amber + 430, and wide amber lights, respectively.

Furthermore, the SPAD value was consistently 3 % higher in cuttings compared to seedlings, with specific differences of 4 %, 0 %, 4 %, and 4 % for narrow amber + 430 + 485, white, narrow amber + 430, and wide amber lights, respectively. It seems that this is due to cuttings being more robust than seedlings in terms of growth. Additionally, there was no significant difference between the two methods in terms of antioxidant activity. A study examining the effect of propagation method (seedlings and cuttings) on growth and wood quality of *Eucalyptus globulus*, as it was concluded that there were no significant differences between the two types of plant material cuttings versus seedlings (Gaspar et al., 2005).

Caplan et al. (2018) concentrated on the vegetative propagation of cannabis using stem cuttings. The results revealed that cuttings possessing three leaves displayed root quality 15 %

higher than those with two leaves. However, the quantity of leaves did not affect the success rate of rooting. The placement of the cutting had a minimal impact on both the success of rooting and its quality. To achieve optimal levels of rooting success and root quality, the recommendation was for cuttings from either apical or basal positions to have a minimum of three fully expanded, uncut leaves. Furthermore, the study proposed that the indole-3-butyric acid (IBA) rooting hormone tested in the experiment is preferable to the willow-based product.

6 Conclusion

The growth and antioxidant activity of *Cannabis sativa* are significantly influenced by the light spectrum. In this study, vegetative cannabis plants exposed to wide amber light (595 nm) displayed the highest morphological parameters, surpassing other treatments. This superiority included plant height (80 %), stem diameter (16 %), SPAD value (5 %), fresh mass (54 %), and dry mass (45 %). Conversely, cannabis plants under white LED lighting exhibited the lowest values for morphological parameters, with plant height (37 %), stem diameter (17 %), SPAD value (2.7 %) fresh mass (35 %), and dry mass (33 %) being lower than all other treatments.

Samples cultivated under narrow amber + 430 + 485 exhibited notable antioxidant activity, registering 17.7 % by FRAP and 82 % by DPPH. This effect is attributed to the blue wavelengths (430 nm and 485 nm), which induce a more pronounced stress response. In contrast, samples under wide amber LED showed the least antioxidant activity.

Furthermore, when comparing the two propagation methods (cuttings and seedlings), there were slight differences in morphological parameters. Cuttings displayed a maximum difference of 7 % in plant height, 2.7 % in stem diameter, 8 % in fresh mass, 9 % in dry mass, and 3 % in SPAD compared to seedlings. However, there was no significant difference between the two cultivation methods in terms of antioxidant activity.

Finally, the vegetative growth of cannabis is greatly influenced by different wavelengths of light. Growers can consider the impact of light wavelengths on cannabis vegetative growth and by the results of this study cannabis leaves may signify potential as utilized for medical purposes or in other industries, rather than being solely discarded as waste by cannabis producers.

7 General summary

7.1 Major findings

- Blue light reduced the height, fresh mass, and dry mass of cannabis plants but increased their antioxidant activity.
- Amber light increased the height, fresh mass, and dry mass of cannabis plants, while also boosting their antioxidant activity.
- Leaves emerged as a significant reservoir of antioxidant activity.

There have been numerous studies examining cannabis growth and secondary metabolites under various LED lighting conditions, but there is limited data regarding the impact of narrow amber light on cannabis. Additionally, while cannabis inflorescence is the final product in many industries, most studies have centered around cannabis inflorescence, with fewer focusing on cannabis leaves and their secondary metabolites. The objective of selecting antioxidant activity of leaves is to demonstrate potential utilization of leaves.

7.2 Limitation

- Like any controlled laboratory study, the results obtained from model cannabis plants may not entirely reflect the responses of all cannabis varieties. Variability among different cultivars can yield different outcomes.
- This study only investigated four different LED wavelengths, whereas industries employ various lights and wavelengths.
- The combinations of light were fixed during cannabis plant growth in this study, but using different lights based on cannabis growth stage may enhance efficiency.

Despite these limitations, this study lays the groundwork for further research and potential applications in cannabis growth and antioxidant activity. Future studies could build upon these findings by considering more complex LED wavelengths and different parts of the plant to enhance the practical applicability of the research.

7.3 Future suggestion work

- Investigate the effects of different LED treatments on cannabis antioxidants and secondary metabolites.

- To deepen our understanding of the effects of LEDs on visual and nutritional quality, it is recommended to broaden the investigation by exploring a wider range of wavelengths.
- Explore different parts of plants as potential sources of antioxidants.
- Evaluate different variations of cannabis under the same conditions to determine which variety serves as the best antioxidant source.
- Examine secondary metabolites and nutritional elements of cannabis leaves.
- Examine different antioxidant assays on cannabis leaves.

By exploring the effects of LED lighting on cannabis growth and antioxidant activity, this thesis not only contributes to understanding the impact of LEDs on cannabis growth but also demonstrates that cannabis leaves are not waste; rather, they represent a viable source of antioxidants. Ultimately, this research has the potential to positively influence cannabis waste reduction in industries.

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9 Appendix

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant height	24	2255.302	93.9709	9.3553	<.0001
Error	47	472.0983	10.0446		
C. Total	71	2727.4			

Table 1. Analysis of variance for cannabis cutting plant height.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant height	23	2152.611	93.5918	44.5639	<.0001
Error	48	100.8083	2.1002		
C. Total	71	2253.419			

Table 2. Analysis of variance for cannabis seedling plant height.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Fresh mass	23	706.4165	30.7138	25.9644	<.0001
Error	48	56.78	1.1829		
C. Total	71	763.1965			

Table 3. Analysis of variance for fresh mass of cannabis seedlings.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Fresh mass	23	645.1911	28.0518	15.4852	<.0001
Error	48	86.95333	1.8115		
C. Total	71	732.1444			

Table 4. Analysis of variance for fresh mass of cannabis cuttings

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Dried mass	23	35.40778	1.53947	9.7017	<.0001
Error	48	7.616667	0.15868		
C. Total	71	43.02444			

Table 5. Analysis of variance for dried mass of cannabis seedlings.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Dried mass	24	43.67523	1.8198	6.4484	<.0001
Error	47	13.2638	0.28221		
C. Total	71	56.93903			

Table 6. Analysis of variance for dried mass of cannabis cuttings.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Stem diameter	23	20.67215	0.898789	8.4936	<.0001
Error	48	5.079333	0.105819		
C. Total	71	25.75149			

Table 7. Analysis of variance for stem diameter of cannabis seedlings

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Stem diameter	24	19.1083	0.796179	4.524	<.0001
Error	47	8.2716	0.175991		
C. Total	71	27.3799			

Table 8. Analysis of variance for stem diameter of cannabis cuttings

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
SPAD	23	822.2481	35.7499	1.9446	0.0262
Error	48	882.4385	18.3841		
C. Total	71	1704.687			

Table 9. Analysis of variance for cannabis seedling SPAD values

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
SPAD	24	929.0521	38.7105	4.2596	<.0001
Error	47	427.1306	9.0879		
C. Total	71	1356.183			

Table 10. Analysis of variance for cannabis cutting SPAD values

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
DPPH	23	53096.38	2308.54	7.0304	<.0001
Error	48	15761.49	328.36		
C. Total	71	68857.87			

Table 11. Aanalysis of variance for antioxidant activity of cannabis seedlings by DPPH assay.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
DPPH	24	71007.65	2958.65	8.9905	<.0001
Error	47	15467.11	329.09		
C. Total	71	86474.76			

Table 12. Aanalysis of variance for antioxidant activity of cannabis cuttings by DPPH assay.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
FRAP	23	8.72E-05	3.79E-06	4.0986	<.0001
Error	48	4.44E-05	9.25E-07		
C. Total	71	0.000132			

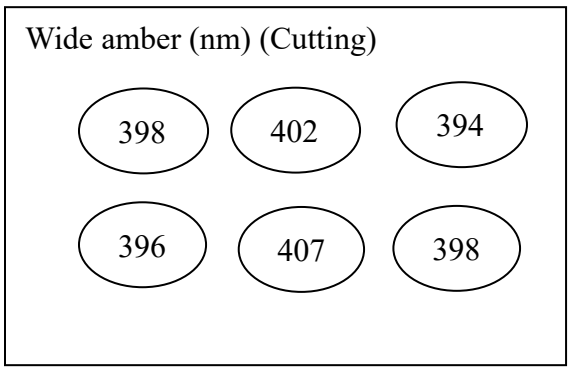
Table 13. Aanalysis of variance for antioxidant activity of cannabis seedlings by FRAP assay.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
FRAP	24	6.34E-05	2.64E-06	4.5695	<.0001
Error	47	2.72E-05	5.78E-07		
C. Total	71	9.06E-05			

Table 14. Aanalysis of variance for antioxidant activity of cannabis cuttings by FRAP assay.

10 Light mapping

Figure 13. Light mapping for cutting and seedlings samples under wide amber (nm) LED



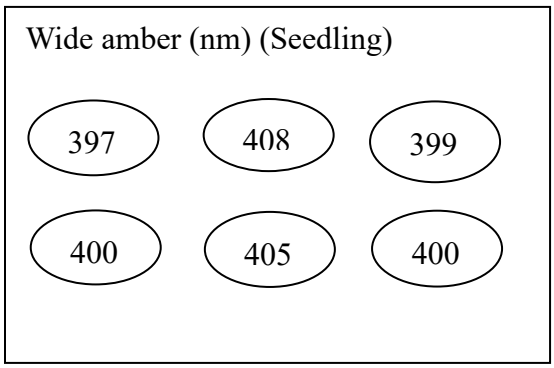
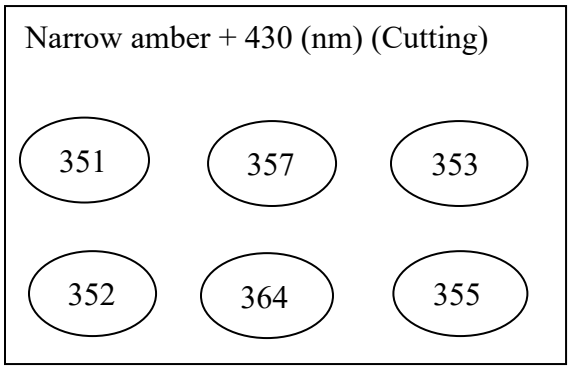


Figure 14. Light mapping for cutting and seedlings samples under narrow amber + 430 (nm) LED



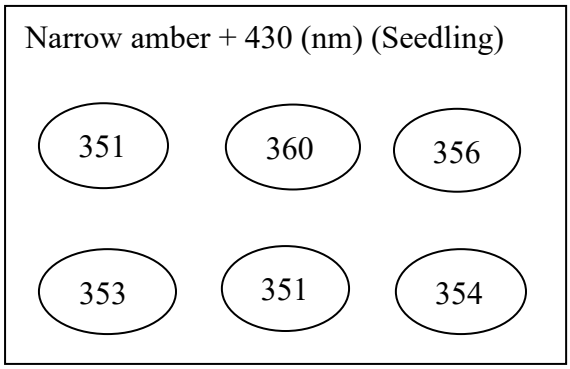
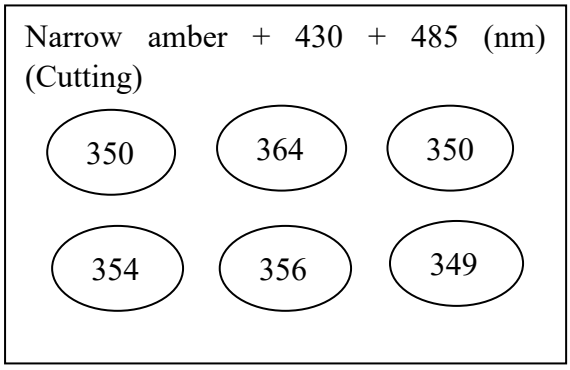


Figure 15. Light mapping for cutting and seedlings samples under narrow amber + 430 (nm) + 485 (nm) LED



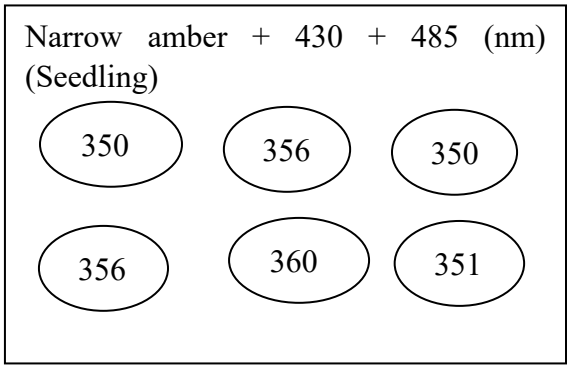


Figure 16. Light mapping for cutting and seedlings samples under white (nm) LED