Influence of light intensity and supplemental UV on biomass production, morphology and specialized metabolites of medicinal cannabis (*Cannabis sativa* L.)

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Cover photograph. 'Medicinal cannabis plants (Original Blitz cultivar) from one experimental plot

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Abstract

The use of cannabis as a medical treatment has propelled interest and research within the rapidly expanding cannabis industry. However, the medical cannabis industry currently faces a great challenge in ensuring an adequate quantity and quality of cannabis plants. This study delves into the effects of ultraviolet (UV) radiation and photosynthetic photon flux density (PPFD) on *Cannabis sativa* L., focusing on how these factors influence cannabinoid and terpenoid production during the flowering phase. The medical cannabis industry, driven by the therapeutic value of Δ-9-THC and terpenoids, seeks to optimize cultivation techniques for enhancing these compounds. UV radiation, known to augment secondary metabolites including terpenoids and phenolic compounds, presents a promising avenue for indoor cultivation.

In this study plants (cv. Original Blitz) was grown in climate controlled rooms without solar light, where two PPFD levels (600 and 1000 µmol m^{-2} s^{-1}) were applied with or without the addition of UVA-B (3.5 µmol m^{-2} s^{-1}). Low PPFD and UV initially (after 6 weeks in short-days) increased cannabinoid and terpenoid accumulation. The breeder, (Perfect Plants), recommends harvesting Original Blitz 6-7 weeks into the generative phase. For this study, we extended the generative phase beyond this recommended harvesting point, to 8 weeks, to gain a comprehensive understanding of cannabinoid and terpenoid accumulation throughout the entire (extended) generative phase. In week 8 of the short-day phase, no treatment effects were observed, suggesting that UV might accelerate cannabinoid production, yet it doesn't influence the final concentration. High PPFD treatments, regardless of UV, yielded lower cannabinoid and terpenoid concentrations. Specific monoterpenoids like limonene, α- and β-pinene were more abundant under low PPFD without UV, while myrcene showed an increase under high PPFD without UV. Additionally, sesquiterpenoids concentrations peaked under low PPFD devoid of UV, indicating UV's possible inhibitory effect on terpenoid synthesis in certain scenarios. The study also noted differential responses of various cannabinoids to PPFD and UV. Δ9-THC, for instance, exhibited a slight increase under high PPFD with UV, contrasting with Δ8-THC and CBG, which favored low PPFD without UV. These findings underscore the nuanced relationship between light intensity, UV supplementation, and cannabis phytochemical production, highlighting the need for tailored lighting strategies in cannabis cultivation to optimize medicinal properties.

Introduction

UV has been applied in horticultural production as a postharvest treatment for potential protection against insects and pathogens through the production of phenolic compounds and regulation of genes required for plant defense (Neugart & Schreiner, 2018; Pate, 1983; Schreiner et al., 2012). However, increasing evidence shows that pre-harvest UV applications have the potential to improve specialized metabolites content which have led to more research trying to understand the effects on the chemical profiles in plant tissues (Schreiner et al., 2012). For example, UV application on cannabis has been entirely focused on the flowering phase (two months) aiming to promote the induction of cannabinoids in the flowers (Lydon et al., 1987; Rodriguez-Morrison et al., 2021). However, UV effects have been described to be beneficial or detrimental in the production of specialized metabolites. For example, UV-A treatment applied during the last two weeks of the flowering phase, increased cannabinoids content; while terpenoids were either increased or decreased upon UV-A depending on the cultivar (Jenkins, 2021). Furthermore, UV-A applications resulted less detrimental to the leaf photosynthesis, floral yield, and specialized metabolites accumulation compared to UV-B light treatments (Jenkins, 2021; Rodriguez-Morrison et al., 2021). However, aside from the duration of application, it is important to consider the interaction with the background photosynthetic photon flux density. In a study by Dou et al., 2019, low PPFD levels (160 µmol $m^{-2}s^{-1}$) with low UV-B led to a higher accumulation of phenolic compounds compared to high PPFD (224 µmol $m^{-2}s^{-1}$) with low UV-B, highlighting the importance of UV doses combined with adequate PPFD levels. It is important to explore the effect of different duration of UV application during the production cycle and at different PPFD levels aiming to identify which light intensity (background light)/UV light is mostly beneficial in cannabis cultivation. The goal of this study is to accomplish a suitable UV supplementation allowing the mitigation of the negative effects on growth while boosting the production of specialized metabolites

Materials and Methods

The study was structured to assess the impact UV light combined with two PPFD levels, resulting in four distinct treatments. These were implemented across two blocks within the same climate-controlled chamber. This chamber, spanning an area of 12m^2, was subdivided into eight sections. This arrangement facilitated two replicates of the four treatments, with each section encompassing 1m^2 (Fig. 1). Initially, each treatment started with sixteen plants, from which the nine most uniform in size and structure were selected for the flowering phase. This resulted in a total of 128 plants during the vegetative stage, reduced to 72 during the flowering stage.

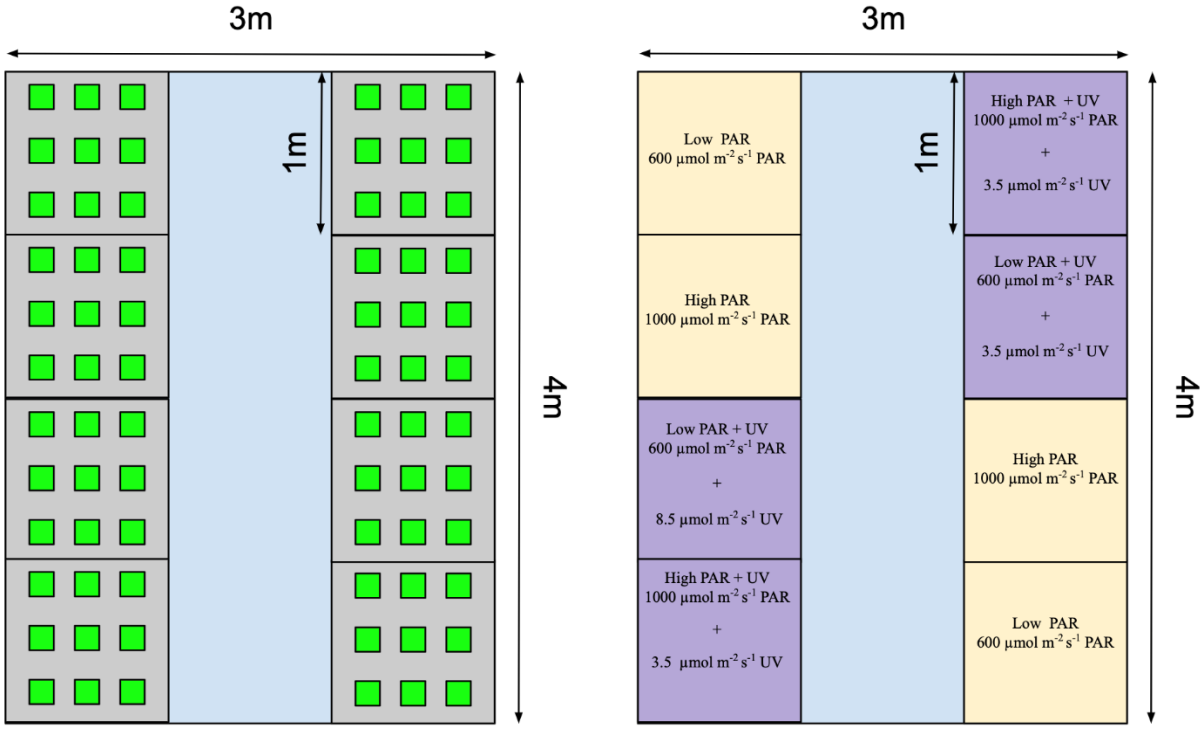

Figure 1 Schematic representation of the experimental layout detailing spatial arrangement and treatment distribution. The left panel illustrates the room's configuration and plant positioning, while the right panel delineates treatment and block distribution, highlighting variations in PAR and UV intensities. Treatments were composed of either 600 or 1000 µmol m^{-2} s^{-1} PAR. Compartments shaded in beige indicate PAR treatments without UV exposure, whereas those in purple indicate PAR treatments supplemented with 3.5 µmol m^{-2} s^{-1} of UV radiation.

Plant Material and Propagation

Cuttings from the medical cannabis variety "Original Blitz" (Perfect Plants, Honselersdijk, the Netherlands) were propagated in controlled indoor conditions using LED lighting. A total of 200 cuttings were prepared, each trimmed to retain a single apical meristem and two fully expanded leaves near the apex. The basal 2 cm of each cutting's stem was treated with a rooting gel containing 0.3% indole butyric acid (Clonex, Growth Technology Ltd, Somerset, United Kingdom) before being inserted into 3.6×3.6×4 cm stone wool plugs (Grodan, Roermond, the Netherlands). A photoperiod of 18 hours of light and 6 hours of darkness was maintained, with the rooting chamber temperature controlled at 28/28 ± 1˚C (day/night) and relative humidity at 100%. This environment was sustained until at least 80% of the plugs exhibited a minimum of two roots emerging from sides of the plugs, typically between 10 and 12 days. The rooting phase concluded on day 12, with 128 successfully rooted cuttings being transferred to larger 15×15×15 cm stonewool blocks (Grodan).

Experimental light set-up

During the rooting phase (days 1-11), the light composition from the LEDs was set at 70% red, 17 green, and 13% blue. The PAR intensity gradually increased from 100 µmol m^{-2} s^{-1} to 400 µmol m^{-2} s^{-1} in daily increments of 50 µmol m^{-2} s^{-1} over six days (days 6-11). Once transferred to the climate chamber, the plants were exposed to more intense LEDs (DLI APEX 800 FS-DC, Dutch Lighting Innovations, The Netherlands) with a PAR emission spectrum of 12% blue, 32% green, and 56% red. The height of PAR light fixtures were adjusted based on plant height growth to maintain the set light intensities, with PAR intensities measured thrice weekly at canopy level using a PAR sensor (LI-250A, LI-COR Biosciences, USA). The UV fixtures emitted a spectrum comprising 20% UV-A, 1% UV-B, with the remaining 79% spanning 400-800 nm wavelengths, as detailed in Fig 2.2B. These fixtures were consistently positioned 100 cm above the canopy, in accordance with manufacturer recommendations, achieving an intensity of 3.5 µmol m^{-2} s^{-1}, as verified by a spectrophotometer (Ocean Optics HDX, Ocean Insight, United States). The daily UV radiation dose was regulated by modifying exposure duration.

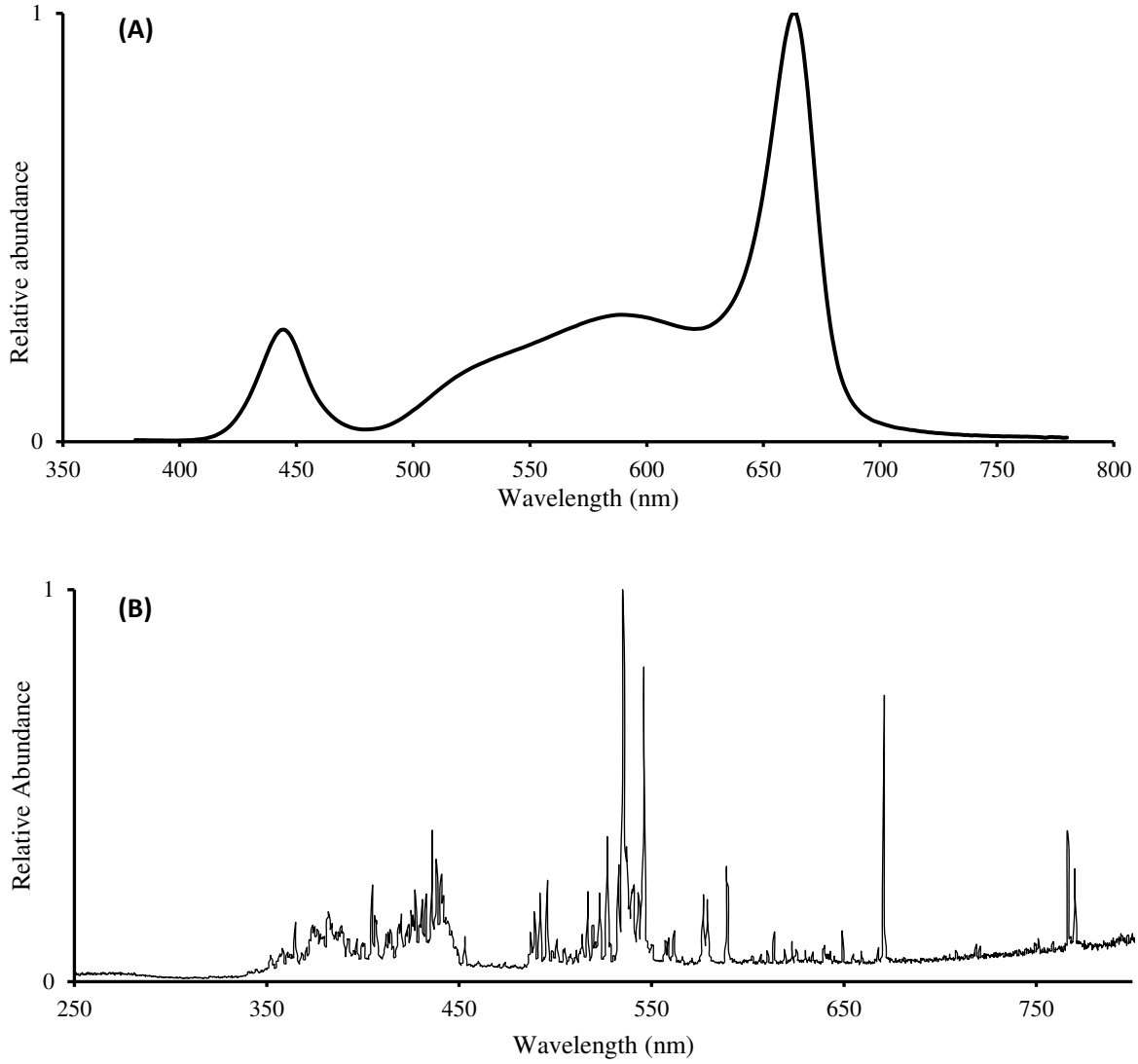

Figure 2 Spectral distribution of the PAR light fixtures, (A) and UV light fixtures (B).

Vegetative Phase

During the vegetative stage, the plants were subjected to an 18-hour photoperiod for 12 days post-rooting (day12-day23). The temperature was set to 26/22 ± 1 °C (day/night), relative humidity (RH) at 80%, and CO_2 concentration maintained at 800/400 ppm (day/night). Nine days post-transplant (day21), the apical meristems were pruned, and all lateral shoots, except the youngest four, were removed. PAR intensity was kept constant at 400 µmol m^{-2} s^{-1}, adjusting lamp height as necessary to accommodate plant growth. From day24, after transplanting, plants in UV treatments began receiving gradually increasing UV radiation doses at day's end. This increment involved maintaining a constant intensity of 3.5 µmol m^{-2} s^{-1} while lengthening exposure time by 30 minutes daily, resulting in daily increments of 6300 µmol m^{-2} until reaching a total dose of 50,400 µmol m^{-2} after eight days (day32).

Generative Phase

The short-day phase commenced on day 35 after transplanting, and lasted 56 days, with nine plants per treatment (planting density = 9 plants m^{-2}) selected based on uniformity in shoot length, number of expanded leaves, and leaf condition across the four main shoots. The cultivar 'Original Blitz' is suggested (by Perfect Plants) to be harvested 42-49 days into the generative phase. For this trial we decided to extend the generative phase by 7 days to get a full overview on cannabinoid and terpenoid accumulation during the generative phase. The cultivar "Original Blitz" requires a critical night length of 12 hours. RH was reduced to 65% during the day and 60% at night; CO_2 concentration was initially kept at 800 ppm during the day and 400 ppm at night. At transition into the short-day phase, daytime CO_2 concentration was increased to 1000 ppm. By day 53, the final PAR intensities were established: 600 µmol m^{-2} s^{-1} for the low-PAR treatment and 1000 µmol m^{-2} s^{-1} for the high-PAR intensity treatments. Plants receiving UV radiation had already been exposed to the full dose (3.5 µmol m^{-2} s^{-1} for 4 hours) at the start of the short-day phase (day 35).

Fertigation

Irrigation was implemented via a drip system, delivering 100ml of nutrient solution per cycle, for a total of 3-12 cycles per day, adjusted according to plant size and irrigation demand. The electrical conductivity (EC) of the solution was initially 2.2 dS m^{-1} during the rooting phase, increasing to 2.5 dS m^{-1} by day 15 in the vegetative phase. The pH of the nutrient solution was maintained between 5.6 and 5.8 throughout the vegetative and generative phases, although the macro-nutrient ratio was modified. The fertigation recipe consisted out of Veg A&B for the vegetive phase, and Bloom A&B for the generative phase (King Solomon, Ventura, CA, The United States).

Assessment of Plant Morphology

Dry weights of the flowers, leaves that had been trimmed from the flowers, regular leaves, and stems were quantified. Flower weights were determined after trimming flower leaves with an industrial trimmer (MT Tumbler 200; Master Products, Girona, Spain). Leaf area was determined based on the total number of regular leaves, using a LI-3100C area meter (LI-COR Inc., Lincoln, Nebraska, USA). Dry weight was determined using a ventilated oven (24h at 70 °C, followed by 48h at 105 °C).

Specialized metabolites analysis

Cannabinoids and Terpenoids extraction

Flower sampling took place at three different developmental stages, namely week four, six, eight, after flowering induction started (WAF). Flower samples were collected from the top inflorescence of the four main shoots in every plant and cryopreserved at – 80 ° C. For the extraction of cannabinoids and terpenoids, 200 mg of fresh flower was combined with 2 mL of n-Hexane, with 1 mg mL^{-1} squalene as an internal standard, and then sonicated for 15 min. The extract was then filtered in a 188 Pasteur's pipette layered with glass-wool and sodium sulphate. The filtered extract was diluted 5 times with n-Hexane and contained in glass vial suitable for Gas-Chromatography and Mass-spectrometry (GC-MS).

GC-MS analysis

The extracts were analyzed on an Agilent GC-MS (7890) equipped with a 30-m x 0.25-mm i.d., 0.25 µm fil thickness column (Zebron, 5 MS) and a mass-selective detector (model 5972A, Hewlett-Packard). The GC-MS operational method was based on the **Manual for specialized metabolites analyses of cannabis flower heads** (Kappers & Verstappen, 2020).

Statistical analysis

The statical analysis for specialized metabolites was conducted in RStudio version 4.3.0 (R Development Core Team, © 2009-2023 RStudio, PBC). The shown data represents the result from two replicate experiments, hence the averages and standard errors of the mean were calculated based on two replicates per treatment (n = 2); each replicate was based on 9 plants per treatment. A two-way analysis of variance (ANOVA) with blocking was performed. When the blocking was not significant, the two-way ANOVA was performed without blocking effect. The assumption of normal distribution was tested by Shapiro-Wilk's test, while homogeneity of variances was assumed because of the limited number of replication. The mean separation was done with Tukey's Honestly Significant Difference (HSD) post-hoc test at $p = 0.05$.

Results and Discussion

Plant growth and development

The inclusion of UV radiation in our study revealed intriguing effects on flower weight, which varied with the photosynthetic photon flux density (PPFD). At higher PPFD, UV addition led to an increase in flower weight, whereas at lower PPFD, a reduction in flower weight was observed (Fig. 4A). This observation does not align with the findings of (Llewellyn et al., 2021), who reported no significant impacts of UV on morphological parameters in their study. One potential explanation for this phenomenon could be that plants acclimated to higher light intensities possess enhanced mechanisms to mitigate UV-induced photodamage.

Interestingly, the application of UV also resulted in increased plant height at lower PPFD (Fig. 3, 4B), suggesting a possible reallocation of dry matter from inflorescences to stem growth. This response to UVA varies among species; for instance, wheat, cotton, and sorghum have shown inhibited growth under UVA exposure (Kataria et al., 2013), whereas positive growth responses were noted in Chinese kale baby leaf (Li et al., 2020), kale (Lee, Kwon, et al., 2019; Lee, Oh, et al., 2019), and tomato seedlings (Kang et al., 2018).

Moreover, our study found that leaf area, specific leaf area (SLA), and the harvest index (the ratio of flower dry weight to total dry weight) were not significantly affected by UV treatment, regardless of PPFD levels (Fig. 4C,D,E). This finding is consistent with outcomes reported in other crop studies under similar conditions.

Figure 3 Photographic representation of the plants architecture at harvest at 600 µmol m^{-2} s^{-1} without UV (left) and with UV (right)

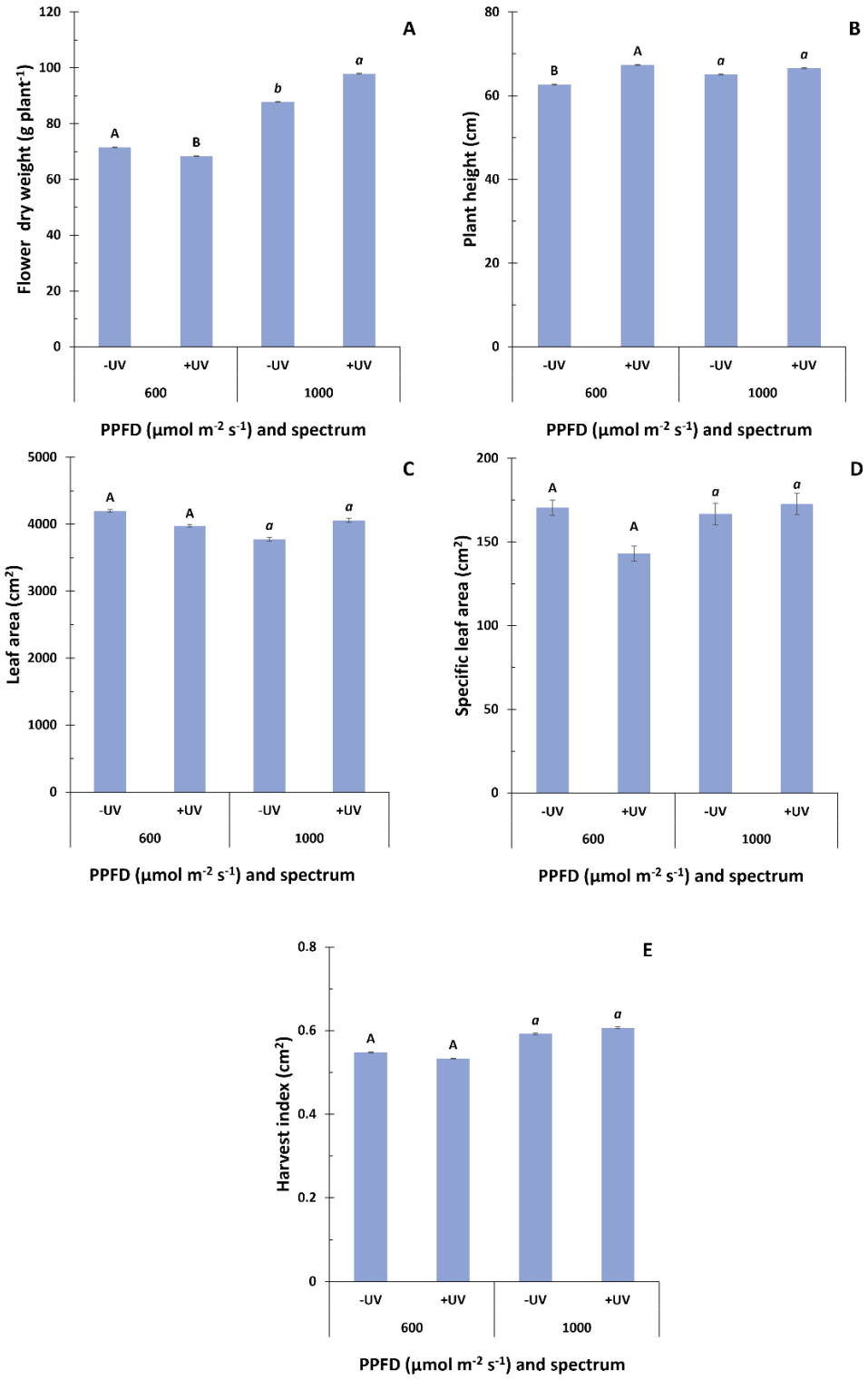

Figure 4 The effect of PPFD (600 and 1000 µmol m^{-2} s^{-1}) and spectrum (with or without UV) on flower dry weight (A), plant height (B), leaf area (C), specific leaf area (D), and harvest index (E). Bars indicate means of both two blocks (n = 2) each consisting of 9 replicate plants. Error bars represent standard error of means (SEM). Different letters (within lowercase, uppercase, and italics) indicate significant differences between treatments with and without UV (Fisher's Unprotected LSD test*, p-value = 0.1)*

Plant specialized metabolites

The effect of supplemental UV and PPFD differs between developmental stages. At four and eight (harvest) weeks after the flowering phase started there were no significant differences in the total cannabinoid and terpenoid concentration across light treatments (**Fig. 5**).

At six weeks after flowering phase started, a low PPFD level significantly increased the total cannabinoid and terpenoid concentration compared to a high PPFD level (**Fig. 5**). Also, UV supplementation significantly increased total cannabinoid concentration compared to no UV supplementation. Similarly, total terpenoid concentration tended to increase (*p-value = 0.155*) under UV supplementation compared to no UV supplementation (**Fig. 5**). This suggests that while UV light may enhance the rate at which cannabinoids and terpenoids accumulate, it does not affect their maximum concentrations achieved.

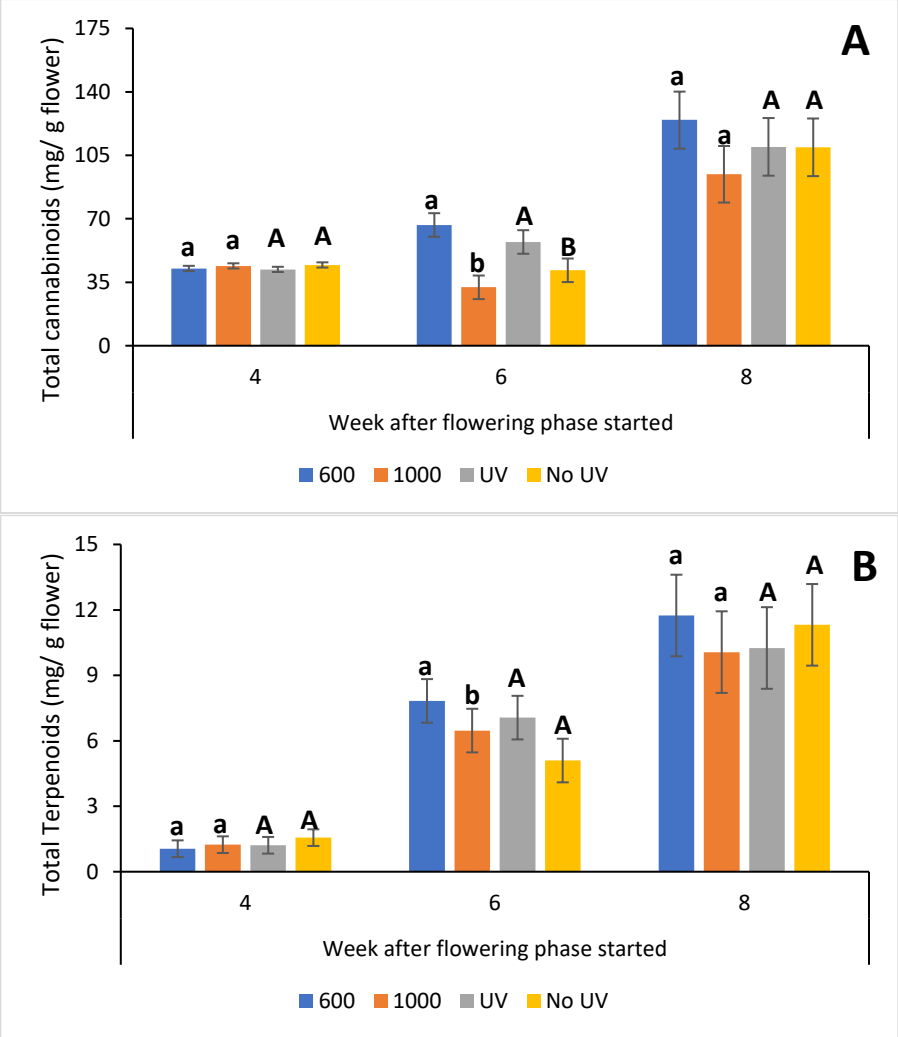

Figure 5. Effect of two PPFD levels (600 and 1000 µmol $m^{-2}s^{-1}$) or UV supplementation on total cannabinoids (A) and terpenoids (B) concentration of C. Sativa cv. Original Blitz plants at six weeks after flowering phase started. Data represent mean ± standard error of the mean (SEM) of two blocks (each mean value is based in 5 plants n = 5). Different lower case letters above the mean values represent significant differences between PPFD levels, while upper case letters represent significant differences between UV supplementation *(*Fischer's Unprotected LSD test*, p-value = 0.1).*

Conclusion

Our research uncovers the interaction between supplemental UV radiation and PPFD in influencing cannabis growth, development, and chemical profile, particularly highlighting stage-specific optimal conditions for cannabinoid and terpenoid production. During the flowering phase, low PPFD combined with UV initially (6 weeks after the flowering phase started) increased cannabinoid and terpenoid concentrations, possibly allowing for earlier harvest times. 8 weeks into the flowering phase no effect of light treatments were found. The observation suggests that UV light's role in enhancing cannabinoids and terpenoids' accumulation rates does not extend to increasing their peak concentrations. Perfect Plants (breeder) recommends harvesting the original blitz 6-7 weeks days into the generative phase. For our study, we extended this phase with 7 days to thoroughly examine cannabinoid and terpenoid accumulation throughout the generative phase. This study highlights the complex relationship between light intensity, UV supplementation, and cannabis phytochemical production, underlining the importance of tailored light management in cannabis cultivation. These insights are vital for optimizing cultivation techniques to enhance medicinal properties, providing guidance for environmental condition adjustments to achieve desired chemical profiles in cannabis. Applying UV light resulted in higher cannabinoid and terpenoid levels by the sixth week of the flowering phase, suggesting the potential of earlier harvests, which could reduce operational costs. Further studies are required to determine the effect of earlier harvests on cannabinoids, terpenoids, and inflorescence yield.

References

Dou, H., Niu, G., & Gu, M. (2019). Pre-Harvest UV-B Radiation and Photosynthetic Photon Flux Density Interactively Affect Plant Photosynthesis, Growth, and Secondary Metabolites Accumulation in Basil (Ocimum Basilicum) Plants. *Agronomy*, *9*(8), 434. https://doi.org/10.3390/agronomy9080434

Jenkins, M. W. (2021). Cannabis sativa L. Response to Narrow Bandwidth UV and the Combination of Blue and Red Light during the Final Stages of Flowering on Leaf Level Gas-Exchange Parameters, Secondary Metabolite Production, and Yield. *Agricultural Sciences*, *12*(12), 1414–1432. https://doi.org/10.4236/as.2021.1212090

Kang, S., Zhang, Y., Zhang, Y., Zou, J., Yang, Q., & Li, T. (2018). Ultraviolet-A Radiation Stimulates Growth of Indoor Cultivated Tomato (Solanum lycopersicum) Seedlings. *HortScience*, *53*(10), 1429–1433. https://doi.org/10.21273/HORTSCI13347-18

Kataria, S., Guruprasad, K. N., Ahuja, S., & Singh, B. (2013). Enhancement of growth, photosynthetic performance and yield by exclusion of ambient UV components in C3 and C4 plants. *Journal of Photochemistry and Photobiology B: Biology*, *127*, 140–152. https://doi.org/10.1016/j.jphotobiol.2013.08.013

Lee, J.-H., Kwon, M. C., Jung, E. S., Lee, C. H., & Oh, M.-M. (2019). Physiological and Metabolomic Responses of Kale to Combined Chilling and UV-A Treatment. *International Journal of Molecular Sciences*, *20*(19), 4950. https://doi.org/10.3390/ijms20194950

Lee, J.-H., Oh, M.-M., & Son, K.-H. (2019). Short-Term Ultraviolet (UV)-A Light-Emitting Diode (LED) Radiation Improves Biomass and Bioactive Compounds of Kale. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.01042

Li, Y., Zheng, Y., Zheng, D., Zhang, Y., Song, S., Su, W., & Liu, H. (2020). Effects of Supplementary Blue and UV-A LED Lights on Morphology and Phytochemicals of Brassicaceae Baby-Leaves. *Molecules*, *25*(23), 5678. https://doi.org/10.3390/molecules25235678

Llewellyn, D., Golem, S., Foley, E., Dinka, S., Jones, A. M. P., & Zheng, Y. (2021). Cannabis yield increased proportionally with light intensity, but additional ultraviolet radiation did not affect yield or cannabinoid content. *Preprints*, *2021030327*, 1–19. https://doi.org/10.20944/PREPRINTS202103.0327.V1

Lydon, J., Teramura, A. H., & Coffman, C. B. (1987). UV-B RADIATION EFFECTS ON PHOTOSYNTHESIS, GROWTH and CANNABINOID PRODUCTION OF TWO Cannabis sativa CHEMOTYPES. *Photochemistry and Photobiology*, *46*(2), 201–206. https://doi.org/10.1111/j.1751-1097.1987.tb04757.x

Neugart, S., & Schreiner, M. (2018). UVB and UVA as eustressors in horticultural and agricultural crops. *Scientia Horticulturae*, *234*, 370–381. https://doi.org/10.1016/j.scienta.2018.02.021

Pate, D. W. (1983). Possible role of ultraviolet radiation in evolution ofCannabis chemotypes. *Economic Botany*, *37*(4), 396–405. https://doi.org/10.1007/BF02904200

Rodriguez-Morrison, V., Llewellyn, D., & Zheng, Y. (2021). Cannabis Yield, Potency, and Leaf Photosynthesis Respond Differently to Increasing Light Levels in an Indoor Environment. *Frontiers in Plant Science*, *12*, 456. https://doi.org/10.3389/fpls.2021.646020

Schreiner, M., Mewis, I., Huyskens-Keil, S., Jansen, M. A. K., Zrenner, R., Winkler, J. B., O'Brien, N., & Krumbein, A. (2012). UV-B-Induced Secondary Plant Metabolites - Potential Benefits for Plant and Human Health. *Critical Reviews in Plant Sciences*, *31*(3), 229–240. https://doi.org/10.1080/07352689.2012.664979