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Influence of Light Spectra on the Production of Cannabinoids

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Keywords
LED · Light spectrum · Cannabinoid content · Indoor growth · Photosynthetic photon flux density

Abstract
In recent years, more attention has been paid to cannabis from both medical and political points of view. This study investigates the influence of 5 different light spectra on the active substance content in THC-poor hemp of the Alessia chemotype II variety. The focus is on comparing conventional growing under metal halide lamps with growing under high-pressure sodium (HPS) vapor lamps with regard to different spectra of LED lighting modules. Growing was carried out in 10 growing boxes under controlled and mostly identical conditions for all boxes. The photoperiod during the vegetative phase was 18 h light and photosynthetic photon flux density ~520 µmol·m^{-2} s^{-1}. The flowering phase was 12 h light and ~540 µmol·m^{-2} s^{-1}. During the experiment, CO_2, temperature, and humidity were measured and logged. Additionally, weekly measurements of chlorophyll, electric conductivity of the fertilizer, activity measurement (salt content) of the soil, and pH value of the soil were checked. The content of cannabinoids was measured by high-performance liquid chromatography (HPLC). Plant height and growth were monitored during the whole experiment by cameras taking pictures every 30 min and loading them onto a cloud storage platform. Cannabinoid content was measured using HPLC. Plant wet weight was determined at the end of the experiment and showed that plants under the high pressure lamp treatment had less flower weight than those under the LED treatment. In conclusion, it could be shown that certain LED spectra can considerably increase the amount of cannabinoids with respect to conventional illumination (HPS).

Introduction

The reaction of plants to light strongly depends on the amount and ratio of different wavelengths in the illumination spectrum. Unlike the cultivation of most other plants, cultivating cannabis is not primarily focused on the crop yield, size, and/or weight but on the content of several chemical compounds, such as specific cannabinoids and terpenes.

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Growing plants indoors has the great advantage of having better control of various ambient conditions, such as CO_2, humidity, or temperature. With the right environment, the plants can grow with less stress and produce more flowers. Moreover, 4–6 harvests per year become possible.

One significant negative factor is the high energy consumption in darkrooms. One percent of the energy consumption in the USA is used for cannabis cultivation. The average cultivator needs 37% of the energy just for conventional lighting (metal halide lamps [MHLs] and high-pressure sodium [HPS] lamps). For average US conditions, producing 1 kg of processed cannabis results in 4,600 kg of CO_2 emissions to the atmosphere (and 50% more when off-grid diesel power generation is used) [1].

Concerning the growing area, photosynthetic photon flux density (PPFD) is an important parameter with respect to yield. The PPFD value depends on distance, radiation characteristics, and light source. For accounting purposes, it is better to compare cost/PPFD rather than cost/Watt since solid-state lighting with LEDs will use less energy for the same number of photons [2].

Already in 1883 Engelmann was able to demonstrate experimentally which algal segments were releasing the most O_2 and thus photosynthesizing the most using aerobic bacteria. Light in the violet-blue and red portions of the spectrum is most effective in driving photosynthesis [3].

Additional experiments showed the process of photosynthesis. Photosynthesis is the only process in nature to generate chemical energy from light. Chloroplasts, as the key organelles for this reaction, work in 2 steps. The first step is photosystem II, which needs a wavelength peak of ~680 nm, and the second step is photosystem I, which needs a wavelength peak of around 700 nm [4]. Furthermore, it could be shown that mixing 680 and 700 nm wavelengths is more effective than just 1 (Emerson effect) [5].

A recent study from 2018 showed an increase of the cannabinoids cannabidiol (CBD) and THC in the flowers with LEDs. The PPFD for all setups was ~450 $\mu mol \cdot m^{-2}\ s^{-1}$[6].

Nowadays, LED technology allows for specific illumination spectra and higher lighting efficiency. The physical limit is 683 lm/W @ 555 nm. For white light, it is about 350 lm/W. Also, the PPFD/W of LED technology depends on the ballast, optics, and wavelength. LEDs are more robust and have a longer lifetime than conventional lights.

To date, 90% of Canadian cultivators are HPS cultivators, since they are proven and are cheaper to purchase. The development of LEDs is fast and prices are decreasing [7]. This study investigates the impact of various light spectra with respect to the yield of cannabinoids.

Materials and Methods

In order to examine the impact of the spectral composition of illuminating light, 10 growing boxes were equipped with several light sources (2 HPS lamps and 4 different LED light spectra in 2 boxes each).

Unrooted *C. sativa* L. cuttings from multiple motherplants of "Alessia" chemotype II (Ai Fame, Schönengrund, Switzerland) were dipped into root hormone powder (indole-3-butyric acid), inserted into easy plugs CT104C (Eazy Plug in DS Goirle, the Netherlands), and watered. The cuttings were kept in a greenhouse (57 × 38 × 22 cm) in 90% relative humidity at ~25°C (± 2°C) and were lit for 18 h (16:00 until 10:00) per day by 2 LED lights (Philips CoreLine Batten 4,000 K) with ~195 $\mu mol \cdot m^{-2}\ s^{-1}$. After 12 days, all the cuttings had taken root, and the greenhouse windows were opened for acclimatization. After 14 days in the greenhouse, the best rooted cuttings were transplanted into 1-L trays with earth substrate 144 from Ricoter (Aarberg, Switzerland) and placed in the growing boxes (115 × 65 × 115 cm). After 3 days in the growing chambers, they were fertilized for the first time.

Ten plants were placed in each of the 10 boxes. Each box was equipped with a Dosatron Compact D07RE125 and a Raspberry Pi 3 (RPI) for measurement. The vegetation fertilizer was "Plantaaktiv 18 + 12 + 18 Type A" (Hauert, Grossaffoltern, Switzerland) with an electric conductivity between 1 and 1.2 mS/cm and for the flowering, "Plantaaktiv 10 + 20 + 30 Type B" (Hauert, Grossaffoltern, Switzerland) with an electric conductivity between 1.3 and 1.5 mS/cm without pH correction. The multifunctional device "COMBI 5000" (STEP Systems, Nuremberg, Germany) measured activity measurement and pH directly in the soil. The activity measurement value was between 0.25 and 0.5 g/L for all the plants during the test, which is a good value for cannabis. The pH was ~5 (±1), with recommended pH values being in the range of 5.8–6.5 with soil.

The MHL "Philips MASTER HPI-T Plus 400 W–4,500 K" used as the standard lighting for the Ai Fame in the vegetation phase operated with 400 W was used for the flowering phase for 18 h (16:00 until 10:00) and the sodium vapor lamp "Sylvania SHP-TS GroLux 400 W–2,050 K" operated with 275 W for 12 h (22:00 until 10:00). The ballast was the Gavita DigiStar 400 W dimmable.

The LED light source was the model Q6W (SANlight, Bludenz, Austria) with different LED chips from Osram. All are dimmable and had a maximum consumption of 215 W/230 V AC (see Fig. 1). As mentioned above, 4 different spectra were used in the experiment.

For the flowering phase, the PPFD measurements took place inside the closed growing box with the plants present. The measurement was carried out in the middle of the box at a distance of 52 cm for the LED and 48 cm for the HPS from the light source (47 ± 1 cm from the bottom of the box). For the vegetation phase, a PPFD value of ~520 $\mu mol \cdot m^{-2}\ s^{-1}$ was measured by the MHL as reference for the LEDs (without plants in the box). After 22 days, the plants were transplanted into 2-L pots, and the PPFD measure-

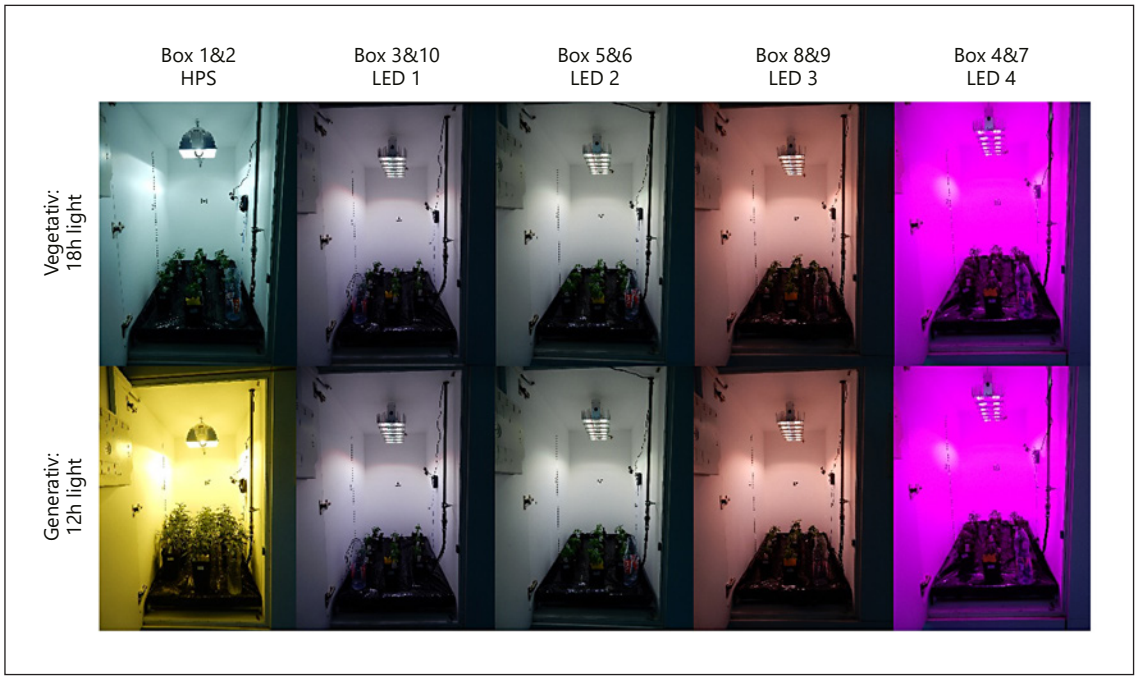


Fig. 1. Box setup. HPS, high-pressure sodium.

ment of the HPS showed ~540 μmol·m^{-2} s^{-1} (with plants present in the box). The PPFD values were set after measurement by the LEDs.

The RPI was connected with a CO_2, temperature, and humidity sensor "SCD30" (Sensirion, Steaffa ZH, Switzerland) at pot height (11 cm from bottom) and a pressure, temperature, and humidity sensor "BME280" (Bosch, Gerlingen, Germany) at flower height (45 cm from bottom). The sensors took measurements every 30 s. Five different spectra were tested (see Fig. 2, 3). Two boxes had the same light spectrum. Additionally, the boxes with the same spectrum were monitored using 2 different types of camera. One box had the camera "Raspberry Pi Modul V2" and the other had the camera "Raspberry Pi NoIR V2" on the RPI (without IR filter) for motion detection and for capturing pictures every 30 min for documentation. The climate was temperature controlled by 1 exhaust fan and 1 inlet fan for all boxes. The temperature during the test was mostly at 25°C (±5°C). Humidity was 50 %Rh (±30%Rh). The CO_2 level was mostly in the range between 400 and 800 ppm during the test (see further details online: https://my.pcloud.com/publink/show?code=kZMP6CkZv6CG1q4SJp4oaLp8vfXhmX5l3wE7).

The weekly height measurements were carried out with a meter rule from the pot rim. The measurements for chlorophyll were done with a SPAD205 (Konica Minolta, Tokyo, Japan), whereas the measurements for the spectrogram and PPFD were done with a MSC15 (Gigahertz Optik). Also, measurements were done with the Raman spectrometer Mira M3 (Metrohm, Herisau, Switzerland) for the detection of the cannabinoid level.

During the last 2 weeks of the experiment, the hyperspectral camera "MV1-D2048 × 1088-HS03-96-G2" (Photonfocus, Lachen SZ, Switzerland) with a bandwidth between 460 and 630 nm was used. During 2–3 days, each spectrum was tested. Hereby, the focus was to determine using the image the existence of cannabinoids in the flowers (trichomes).

The cannabinoid content (±0.3%) of the leaves and flowers for each box was measured with the high-performance liquid chromatograph "Agilent 1100 Series" (Agilent). Here, the measuring process is divided into the following steps: for preparation, the test material was dried at 60°C in the oven. After that, the test material was crushed in a hammer mill. Then, 100 ± 10 mg of the material was weighed (XPE205-Mettler Toledo [QAK: 0100]) in a 100-mL volumetric flask. The rest of the material (more than 500 mg) was used for moisture measurement (HB43-S-Mettler Toledo [QAK: 0014]). The volumetric flask was filled with methanol-chloroform solution (9:1) to the 100-mL mark. Then, it was extracted in an ultrasonic cleaning bath (Sondrex Digitec-Bandelin [QAK: 101]) for 30 min. Finally, small vials were filled and put in the HPLC system.

Cannabinoid content was expressed as the content of the acid and neutral pieces (general formula) [8].

% of THC Total = % of THC + (% of THCA × 0.877),
% of CBD Total = % of CBD + (% of CBDA × 0.877)
% of CBG Total = % of CBG + (% of CBGA × 0.878).

Results

The cannabinoid content of CBD and wet weight of the flowers showed the best results with LED lamps. Most CBD [g] was grown under spectrum 4, which had

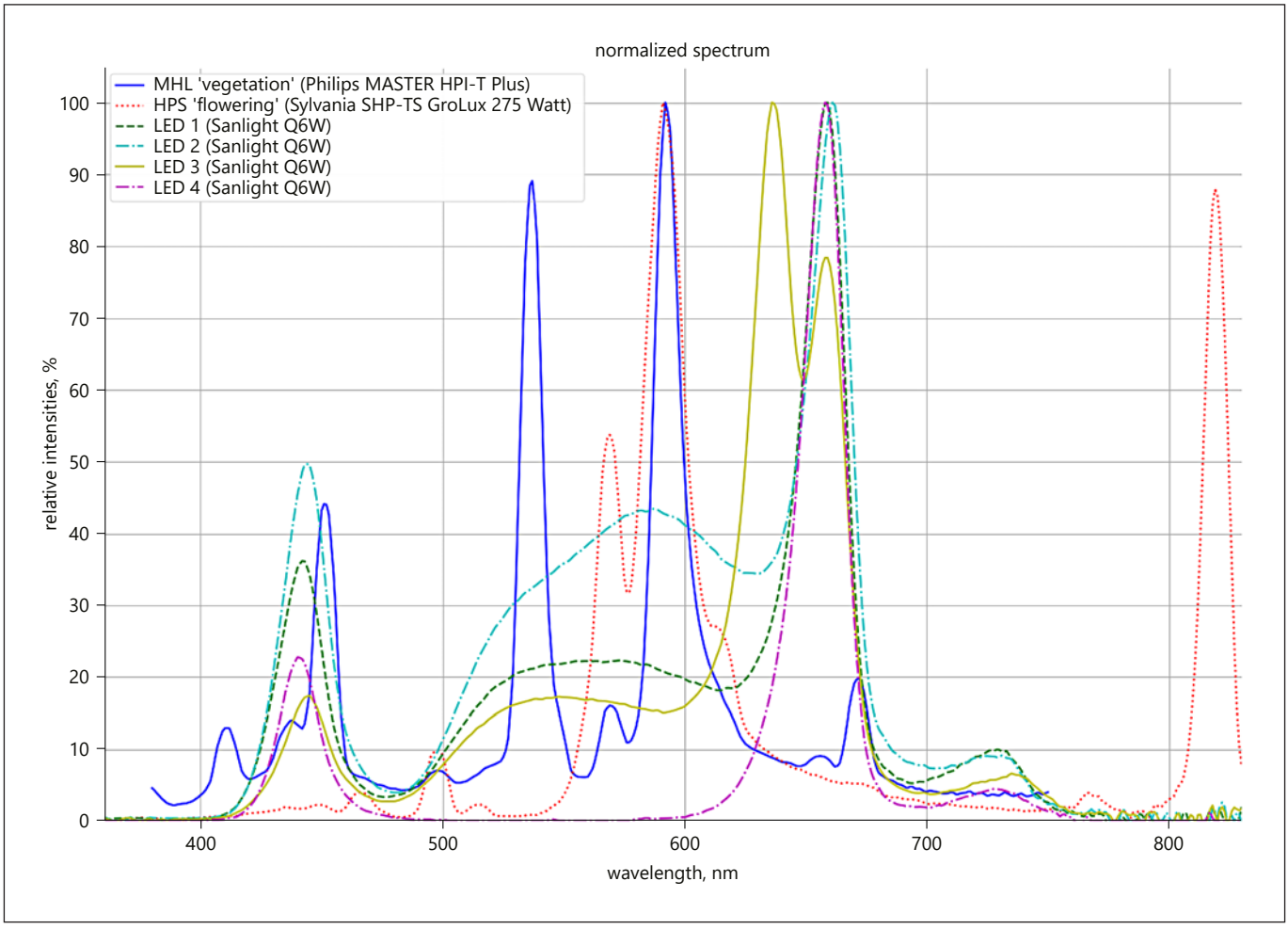


Fig. 2. Light spectrum of different light sources (measured with MSC15). MHL, metal halide lamp; HPS, high-pressure sodium.

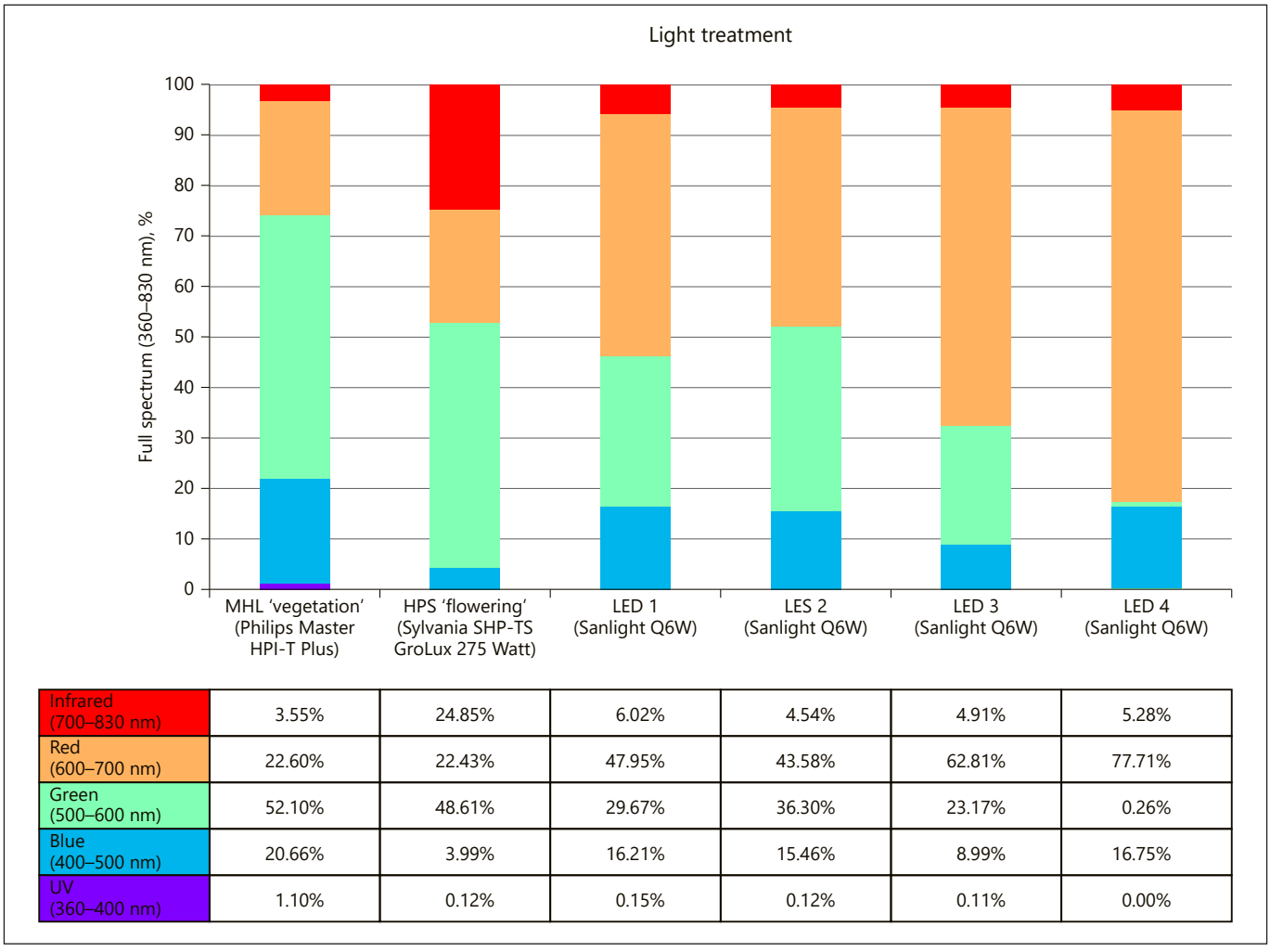
3 times more CBD than the reference boxes with HPS (see Fig. 4). Light distribution (PPFD) for the LEDs was more homogeneous than in the case of the HPS. The difference between maximum and minimum measurements of an area in the vegetation phase was for the LEDs ± 110 µmol·m^{-2} s^{-1} and MHL ± 200 µmol·m^{-2} s^{-1}. In the flowering phase, the difference for the LEDs was ± 200 µmol·m^{-2} s^{-1} and for the HPS ± 250 µmol·m^{-2} s^{-1} (see Fig. 5, 6).

Simulation programs for simulating artificial light, for example, Relux, could show this effect in the beginning of the experiment [9]. In an industrial production environment, lights are applied at a higher distance to the plants in order to avoid heat burn on the leaves and to have a better working area. With the above-mentioned simulation program, it is possible to demonstrate the same PPFD values due to multiple light systems with an overlapping light level even in a bigger growth area.

In contrast to an earlier study [6], this experiment could not confirm significantly higher plants with HPS lamps. The plants from each box had a height between 24 and 32 cm on average. In addition to that, the number of leaves from each box was approximately 25 ± 5 before the flowering phase. In the flowering phase, the number of leaves was stable.

The investigations using Raman spectroscopy could not demonstrate a clear correlation between peaks and CBD in comparison to pure CBD crystals (>99%). In this case, further studies are necessary.

As a matter of fact, the use of the hyperspectral camera with a bandwidth between 460 and 630 nm proved unsuitable for the detection of cannabinoids. The information



Infrared (700–830 nm)	3.55%	24.85%	6.02%	4.54%	4.91%	5.28%
Red (600–700 nm)	22.60%	22.43%	47.95%	43.58%	62.81%	77.71%
Green (500–600 nm)	52.10%	48.61%	29.67%	36.30%	23.17%	0.26%
Blue (400–500 nm)	20.66%	3.99%	16.21%	15.46%	8.99%	16.75%
UV (360–400 nm)	1.10%	0.12%	0.15%	0.12%	0.11%	0.00%

Fig. 3. Color spectrum comparison of the light sources. MHL, metal halide lamp; HPS, high-pressure sodium.

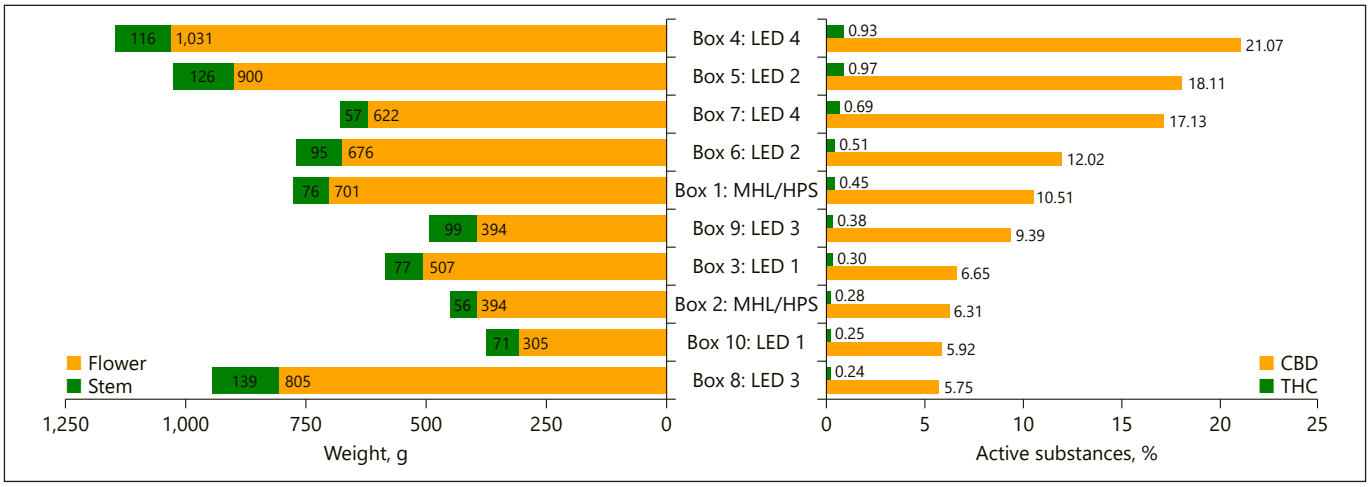


Fig. 4. Weight and cannabinoid content of the last measurement with HPLC. MHL, metal halide lamp; HPS, high-pressure sodium; HPLC, high-performance liquid chromatography; CBD, cannabidiol.

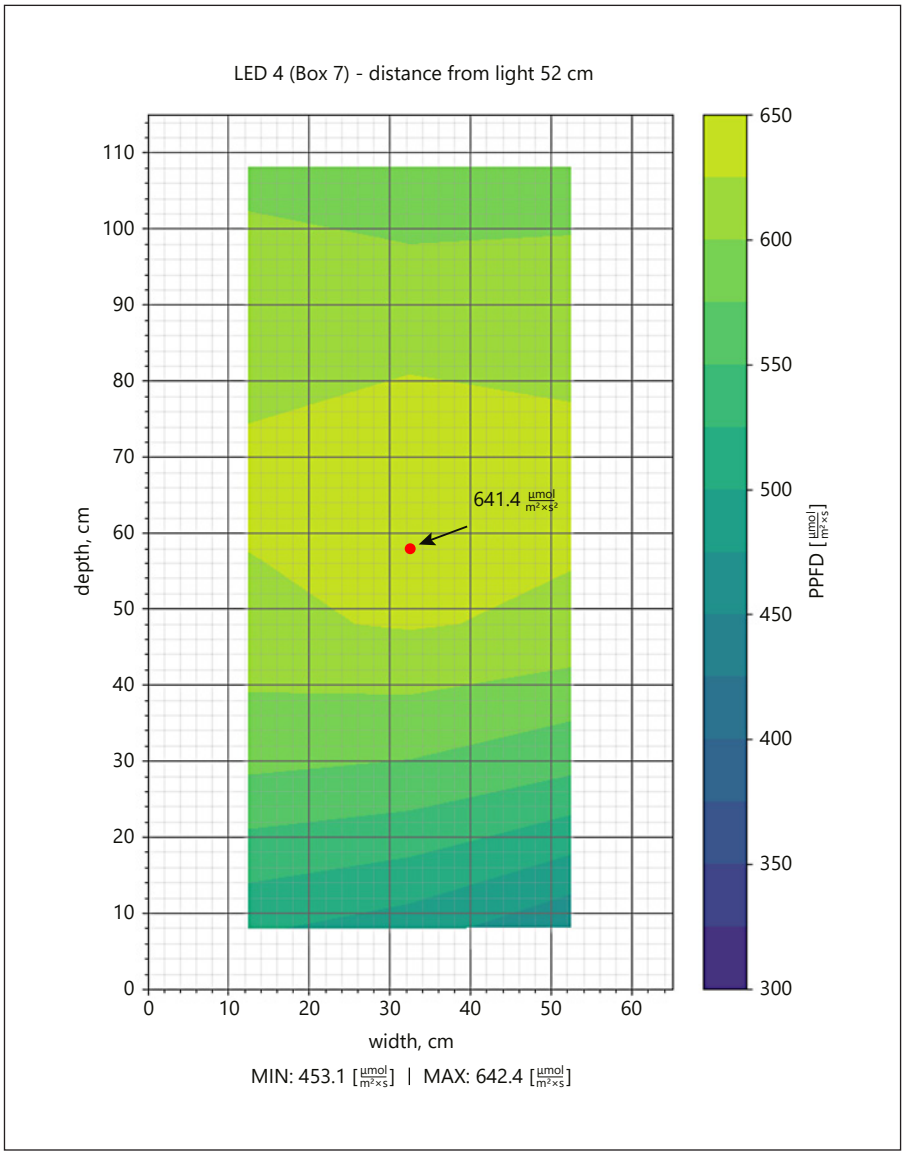


Fig. 5. PPFD distribution of LED 4 (box 7). PPFD, photosynthetic photon flux density.

from the pictures taken using the hyperspectral camera was the same as those taken by a conventional photographic camera. Moreover, requiring wavelengths in the infrared range, the NDVI value could not be calculated. This value serves as an indicator for the health of the plant [10].

The timelapse of the plant delivered a lot of information about the plant, for example, vermin or growing status. Furthermore, the height of the plant may be monitored with a calibrated monocamera [11].

The efficiency of the LED solid-state lighting sources is increasing fast. Through the application of modular LED systems, the latest and most efficient LEDs on the market may be used with decreasing costs.

Discussion/Conclusion

The experiment has shown that the LEDs are better than HPS lamps with regard to harvest weight and active ingredient content. In accordance with earlier investigations [2], this experiment has shown that the blue/red LED spectrum achieved the best results with respect to weight and the active ingredient content CBDA. Most importantly, under spectrum 4, which gives a pink color impression, the yield of the cannabinoid content was higher than that for all the other boxes. The plants under the "white" and "warm white" LED spectra also achieved better results in terms of weight than the plants under the

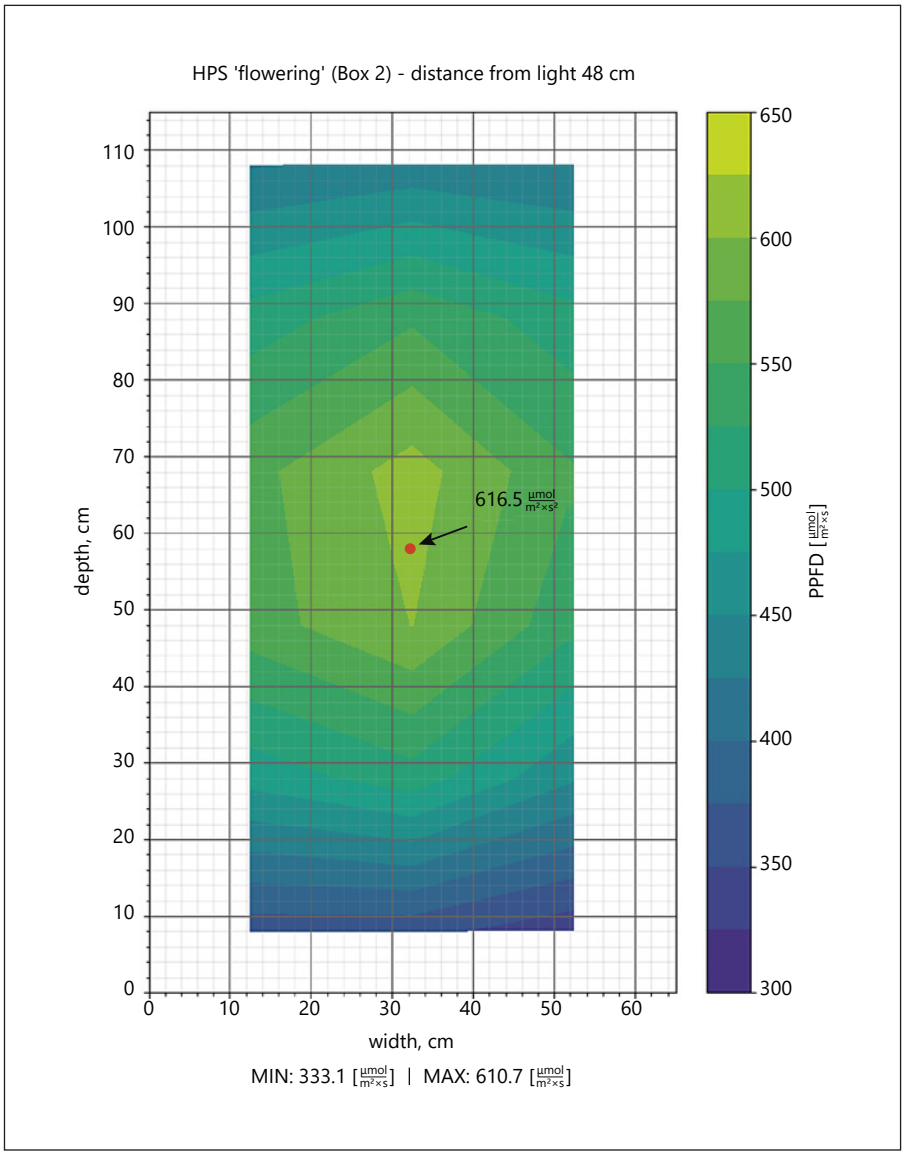


Fig. 6. PPFD distribution of HPS (box 2). PPFD, photosynthetic photon flux density; HPS, high-pressure sodium.

gas discharge lamps, whereas the "cold white" LEDs had the lowest yield.

During the first run of the experiment, several problems were encountered. For instance, the water supply via the dosing units was not sufficient, resulting in different fertilizer mixtures in different boxes. This problem could be solved in the last 4 weeks of flowering. Apart from that, due to a malfunction of the dosing unit, plants in box 7 were fertilized with undiluted fertilizer. This had a negative impact on the plants, and they were replaced by other plants which nonetheless still showed a rather high content of CBDA.

The results could be better with a more stable Dosatron system. For this, the test needs to be repeated.

In the future, it would be interesting to test the impact of infrared lighting (Emerson effect) [5] and UV light. Also, for wake up and good night, a higher red intensity could increase the flower weight. The Raman spectrometer showed some interesting peaks but have to be tested again. The hyperspectral camera was not interesting in this case. A normal camera could see as much as this hyperspectral camera. More interesting would be the NDVI value, which needs the infrared wavelength. Because of the spectrum from the light source of the camera, this camera also would not work.

Acknowledgements

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Statement of Ethics

No subjects (test persons) were involved in the full experiment.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Ai Fame: Laboratory for cannabinoid analyses, growing boxes, cutting box, and interesting facts about hemp breeding. SANlight Research: 8× LED lamps (Q6W) specifically chosen by them. NTB: Raspberry Pi measurement system and human resource (test leader).

Author Contributions

Prof. Dr. Stefan Rinner (NTB Buchs): Professor of physics and photonics. Prof. Dr. Tindaro Pittorino (NTB Buchs): Professor of electronics. Joan Espel (Ai Fame): Head of laboratory at Ai Lab, Schönengrund. David Schmidmayr (SANlight): SANlight Research GmbH, Bludenz. Pascal Amrein (NTB Buchs): Bachelor student of photonics.

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