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Original Article

The Effects of Varying Dark Cycles and Pulse Width Modulation on Fresh Weight in Helianthus Annuus Microgreens

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Abstract *- To aid in the growth of microgreens, lighting factors like light quality, photoperiod, and light intensity are modified to achieve desired results for the plants. This research established the consequence of different dark cycles and pulse width modulation on the fresh weight of Helianthus annuus microgreens. A ratio of 75% red and 25% blue LED light was used to deliver five seconds of light cycles at 100 μmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD) to the tested plants. Central composite design (CCD) using the response surface methodology (RSM) was applied to derive thirteen lighting treatments. The result of the experiments showed that dark cycles of 11 – 15 seconds and PWM frequencies of 300-500 Hz at a 75% duty ratio achieved the maximum outcome. These values can be used on LED lighting systems to accomplish optimum production in the fresh weight of Helianthus annuus microgreens*

Keywords *- Dark cycles, Fresh weight, Light treatments, Microgreens, Pulse width modulation.*

1. Introduction

Microgreens are nutrient-rich young versions of vegetable crops derived from plant seeds [1] with unique tastes and textures. They are popularly used as a garnish for soups, salads, and other dishes [2] because of their distinctive color and high nutritional content [3]. Seeds are planted in a growing medium inside a plastic tray and grown for a week to several weeks, depending on the crop type. Microgreens are harvested without the roots once the first set of true leaves grows out [4]. Crops are typically grown in controlled environments where lighting, climate, and other environmental factors can be automatically regulated [5].

Lighting plays a vital part in growing microgreens. One of the popular lighting types used today is light–emitting diodes (LEDs) because of their high efficiency and low power consumption [6]. LEDs can be used as supplemental light [7] or as a single light source for crops [8]. Most studies involving illuminating microgreens focus on varying the light conditions like the light quality, photoperiod, light intensity, or a combination of each. Light quality means the type of wavelength subjected to the plant. For example, subjecting a cabbage microgreen to 15% blue and 85% red light produces this crop's optimum yield [9]. Research on light quality varies depending on the type of crop and the expected effect on the plant. Photoperiod on the other hand, is the duration of time when a plant is exposed to the presence and absence of light. Depending on the desired results, researchers configure the time a crop is subjected to light and dark, which can vary from hours to seconds. Commonly used photoperiods are 16 hours of the light cycle and 8 hours of the dark cycle (16/8) [10], 12 hours of light and 12 hours of dark (12/12) [11], or even 24 hours of continuous light exposure (24/0) [12]. Light intensity focus on a particular wavelength (between 400 nm to 700 nm), known as PAR (photosynthetically active radiation) that falls to the surface area of the crop or the planting area [13]. The amount of PAR that reaches a square meter of crop canopy per second is measured in photosynthetic photon flux density (PPFD) [14]. To conserve electricity consumption, lighting systems propose using pulse width modulation (PWM) [15,16]. PWM reduces the total power consumption of lighting systems by switching on and off the supply voltage at a rapid rate [17]. Similarly, a single PWM driver can be used to drive several LEDs, thus saving on electronic components when developing a lighting system [18].

Research on microgreens shows that lighting experiment results vary depending on the plant type or crop variety. Microgreens are harvested by weight. They are considered highly perishable because of their short shelf life [41]. It would be advantageous for growers to have the means to increase the weight of their crop to achieve optimum profit. With the growing interest in microgreens research, there is still a lack of published papers involving the influence of light, specifically the impact of seconds-long exposure combined with different dark exposure times and variable PWM frequencies on the plants' mass. Similar studies on fresh weight contrast mainly because of the dissimilar results when experimenting on different microgreen specie or varieties [20].

The objective of this study was to discover the influence of varied dark cycles and different PWM frequencies of *Helianthus annuus* microgreens and derive the optimal combination from achieving targeted fresh crop weight.

2. Related Work

2.1. Factors Affecting Microgreen Growth

Photoperiod directly affects plants' biological clock, which regulates their physiological development [21]. One promising research [22] involving photoperiod is the rapid change in light exposure of plants (measured in seconds) instead of the usual hourly duration. The study showed that exposing plants to alternating five seconds of light and darkness produces the same results compared to the usual 12/12h photoperiod while significantly lowering power consumption by at least 30% to 50%. The research suggests further experimenting on other microgreen varieties by extending their dark cycle exposure. In [23], a comparison was made between the energy consumption of continuous and pulsed lighting (100 Hz, 500 Hz, and 1000 Hz). Assessments were done on chilli peppers using several combinations of red and blue light in dissimilar ratios. Each combination was also tested for different duty cycles (40%, 50%, 60%, 70%, 80%, and 90%). The results showed that the best frequency to use is 500 Hz at 50% duty cycle achieving an average of 11% energy savings for all the tested light treatments.

PPFD values that can affect the fresh weight of crops vary depending on the light quality used as well as the duration on which the plants are exposed to light. A study [24] has tested the effects of light quality treatments (mainly blue light mixed with low levels of supplemental lights) on the growth of several flowering plants using either 50 or 100 μmol m^{-2} s^{-1}. Outputs showed an increase in biomass and leafstalk occurred under a PPFD of 100 μmol m^{-2} s^{-1} using pure blue light compared to the other light treatments, mainly resulting from a shade-avoidance response on tested bedding plants. In [25], tests using different PPFD were made with a constant ratio of red and blue lights over a 16/8h photoperiod. The treatments were tested on selected microgreens. The presented output showed that PPFD is directly proportional to fresh weight, with 600 μmol m^{-2} s^{-1} being the threshold for the tested crops.

Another study [26] conducted three light treatment experiments on two amaranth cultivars. The first experiment tested the effect of several light qualities (red, blue, white, and red with blue) with a sustained PPFD value of 130 μmol m^{-2} s^{-1}. The succeeding experimentations assessed the effects on the plants using varying values of PPFD (130, 180, 230, 280 μmol m^{-2} s^{-1}) with a constant red and blue ratio of 70:30 (70R:30B). Finally, the crops were illuminated with different photoperiods (8/16h, 12/12h, 16/8h,20/4h) having a constant PPFD of 280 μmol m^{-2} s^{-1} and 70R:30B ratio. Results showed that the 70R:30B ratio with a photoperiod of 16/8h and a PPFD of 280 μmol m^{-2} s^{-1} provided the optimum yield and micronutrient enhancement for the tested crops.

2.2. LED Lighting Systems using Internet-of-Things (IoT)

LED lighting use pulse width modulation to control the light intensity of LEDs. It can be done by controlling the frequency and the duty cycle of the PWM signal [27]. Frequency is the speed by which the signals change per second, usually measured in hertz. The duty cycle is the percentage of a signal active in a given period. For example, a 25% duty signal means that the voltage is supplied on only a quarter of the total time. In [28], researchers used PWM to control the brightness of RGB LEDs in a display panel. Individual LEDs were controlled independently, leading to increased stability and efficiency of the LED panel. A similar study [29] also used PWM to remotely control the LED brightness by using a web application on LabVIEW. Movement is detected by a passive infrared (PIR) sensor which sends a signal to a microcontroller that generates the programmed PWM output to the LEDs.

The inexpensiveness of microcontrollers makes generating PWM easier, and integration with currently available LEDs is highly possible [30]. The study [31] developed a lighting system using a microcontroller to generate PWM pulses and control the illumination of a LED light. The system used a web-based remote dashboard to control the frequency and duty cycle of the PWM signal. In the case of the developed system, the frequency can be set starting from 1 Hz to 20 kHz. The system has the option to change the signal duty cycle from 25% to 100%. The research demonstrated that a PWM signal can be controlled by a web-based interface and offer variable frequency and duty cycle inputs for users.

Single-board computers like Raspberry Pi afford the flexibility and robustness needed in data interchange and processing [32]. A study [33] introduced light, temperature, humidity, and soil moisture sensors to feed real-time data to an Arduino board. The collected data is then sent to Raspberry Pi running node-red, which displays the environmental conditions in a graphical interface. The study showed that it is possible to access sensor-collected information using the proposed IoT network remotely. A similar study [34] also used sensors sending data to a Raspberry Pi to monitor the water level inside a storage tank.

Reaching the minimum water threshold activates the pump, while the sprinkler automatically operates when the soil is dry. Water depth, temperature, and other parameters are monitored through a web server running on the same network accessible by a smart phone. The system was capable of sending email notifications to the user.

The gathered information proves that light intensity, photoperiod, and light quality significantly affect the fresh weight of microgreens. Although, light quality treatments to be used vary depending on the type of crop, the testing environment, and the tools used during the experiments. With the cited studies serving as the basis for the experiments, the researchers used PPFD values of 100 μmol m^{-2} s^{-1} under a varying photoperiod of 75R:25B LED light ratio. Five seconds of a light cycle on all experiments were used together with varying dark cycle values. PWM signals going to the LEDs were regulated by an Arduino microcontroller linked to a Raspberry Pi single-board computer running a web-based program for command inputs and monitoring.

3. Materials and Methods

3.1. Plant Growth Conditions

A soilless mix composed of a mixture of pumice, cocopeat, composted/carbonized rice hull, and vermicast was used as a growing medium and placed in a 32cm x 24cm x 5cm (L x W x H) plastic tray. Thirty grams of black oil *Helianthus Annuus* seeds were sown in the medium (as shown in Figure 1) and subjected to a blackout period of 120 hours. During this period, the trays were stacked on top of each other, and the seeds were sprayed every 12 hours with distilled water to aid germination. The temperature was maintained below 30° while the humidity was kept at an average of 60%. On the fifth day, each tray of seeds was placed inside a growth chamber and subjected to a specific lighting treatment. Each lighting treatment lasted three days, with the plants being bottom-watered every 12 hours.

Steel shelves measuring 100 cm x 40 cm x 200 cm (L x W x H) were divided into three aluminum foil-covered paperboards for the developed growth chamber. Each chamber measured 40 cm x 36 cm x 48 cm (L x W x H) inside, which had two 12-volt fans placed on opposite sides for airflow. Humidity and temperature sensors were also placed inside the chamber for monitoring. Gathered data is then fed into an Arduino microcontroller that provides the LEDs' needed PWM frequency and On/Off signal. A Raspberry Pi running Node-Red is connected to the microcontroller to control the light exposure and PWM. A LED lighting panel having a 75R:25B light ratio is placed on the upper portion of the chamber that generates a PPFD value of 100 ± 10 μmol m^{-2} s^{-1} (measured using a spectrometer, Hopoocolor HPL200P) and a 75% duty cycle (measured using an oscilloscope, Tektronix TBS 1102B-EDU).

Fig. 1 Helianthus Annuus seeds sown in a soilless growing medium inside the controlled chamber.

Fig. 2 Helianthus Annuus microgreens grown from a single experiment run.

3.2. Light Treatments

Response surface methodology (RSM) was used to determine the effect of the varied dark cycles and pulse width modulation on the fresh weight of *Helianthus Annuus* microgreens. RSM is a statistical tool to discover the connection between several quantitative parameters and their response.[32–34] Specifically, central composite design (CCD) was utilized to derive the number of experiments performed.

Fig. 3 Sample of ten Helianthus Annuus microgreens collected randomly from a single tray.

Two factors were used with two levels, requiring thirteen tests with four cube points, five center points, and four axial points. The dark cycle (ranging from 5 seconds to 20 seconds) and the PWM frequency (ranging from 500 Hz to 2000 Hz) served as the two factors for the CCD. Light exposure was maintained at five seconds throughout each of the entire experiment runs. After 72 hours of specific lighting treatment, sample plants are collected from the growth tray for weighing (as shown in Figure 2).

3.3. Weight Measurements

The fresh weight of ten samples per experiment was collected from the growth tray (see Figure 3). Each plant was washed with running water to eliminate excess materials and growing medium in the roots. The plants were then pat-dried and individually weighed using a calibrated balance (Kern KB 360-3N), and the average weight value for all ten samples for each experiment was recorded. Minitab 21 (64-bit) program was used for the CCD fresh weight response, while data processing was handled in Microsoft Excel 2019.

Table 1. Variable response based on process values

Runs	**Dark Cycle (s)**	**PWM Frequency (Hz)**	**Fresh Weight (gms)**
1	5	500	0.818
2	13	189	0.678
3	13	1250	0.695
4	13	1250	0.649
5	13	2310	0.735
6	2	1250	0.623
7	13	1250	0.543
8	5	2000	0.628
9	13	1250	0.911
10	20	2000	0.687
11	20	500	0.922
12	23	1250	0.630
13	13	1250	0.579

4. Results and Discussion

Fresh weight is influenced by intensity, photoperiod, and light quality. By having the light intensity (100 ± 10 µmol m-2 s-1) at a constant rate and the light quality (75R:25B) at a constant ratio, the influence of PWM frequency together and varying dark cycles under photoperiod were tested. Table I shows the fresh weight variable response based on the derived process variable combinations. The experiments show that dark cycles influence fresh weight. Analysis of the results indicates that the long dark cycles lead to an increase in the average mass of the microgreens. The longer time between light exposures tends to produce cotyledons with longer and wider surface areas as a response. An increase in the leaf size, in turn, adds to the overall weight of the plant. The same effects were also presented in [38], with the tested lettuce crops being heavier on longer photoperiods.

Pulse width modulation frequencies also significantly affect the overall fresh weight during harvest. Lower PWM frequencies used during the experiments resulted in higher values for the fresh weight. This also aligned with the research that determined that a frequency of 500 Hz (as compared to 1 kHz) produced higher biomass for baby lettuce even when compared to continuous lighting treatments [39]. Although, this effect on weight may be different for a seedling than for a mature plant. A study [40] on the same lettuce plants discovered that the fresh weight increased when higher frequencies (above 1.5 kHz) were used as compared to lower values (500 Hz and below) when the plants were grown for thirty days, even when the PPFD values have maintained an average of 50 µmol m^{-2} s^{-1}.

Similarly, the contour and response surface plots established as a function of the dark cycles and PWM frequency support the analysis of the results. As revealed from the surface plot shown in Figure 4, there is an apparent increase in the fresh weight whenever there is an increase in the dark cycles. The highest values were achieved with dark cycles starting at ten seconds. This is supported by the study that dark cycles longer than ten seconds under a constant five-second light exposure affect plant development [27]. In contrast, fresh weight tends to decrease as the PWM increases in value, especially in frequencies larger than 500 Hz.

The contour plot (Figure 5) further illustrates that the fresh weight is at its heaviest when the dark cycle is in the range between 15- 18 seconds and the PWM frequency does not exceed 500 Hz. Dark cycles shorter than 10 seconds produced plants with smaller cotyledons with the lightest weight seen in the lowest values. The Minitab software's response optimizer was then used to calculate the suggested optimal values of the two parameters that would achieve maximum fresh weight. The results coincided with the observed range of numbers suggesting a frequency of 189 Hz, with the dark intervals being 19 seconds long.

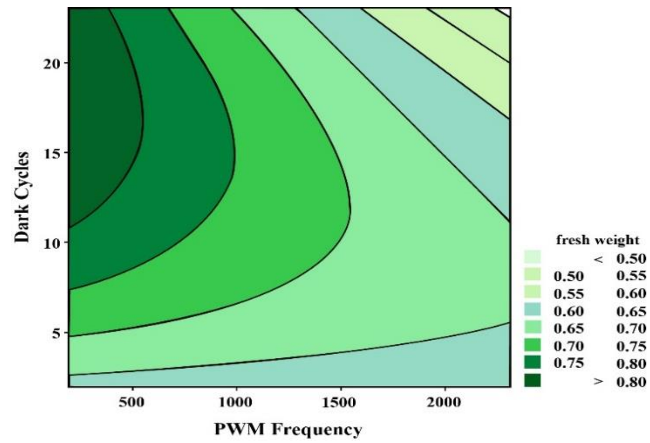

Fig. 4 Response surface plot results

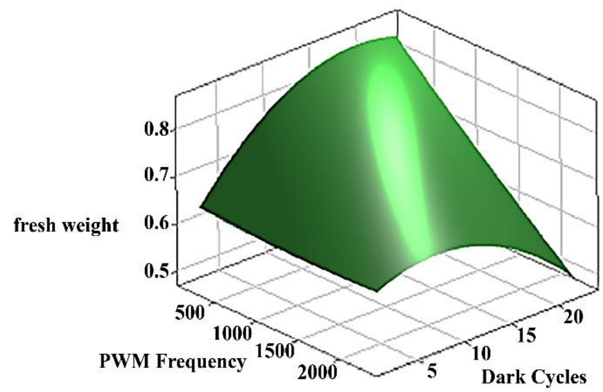

Fig. 5 Contour plot results

5. Conclusion

The paper highlights the results of exposing *Helianthus annuus* plants to rapid switching between light and dark cycles together with pulse width modulation to affect the overall fresh weight of the plants. Each growth tray was run under specific light treatments derived from the RSM methodology.

PWM frequencies of 300-500 Hz and 75% duty ratio at five seconds of light and 11 – 15 seconds of dark cycles had the most positive development in the overall fresh weight of *Helianthus Annuus* microgreens under a controlled environment. Recognizing the optimal PWM frequencies and dark cycles has enormous potential in research and the industry of microgreens.

For future studies, supplementary light sources using different supplementary colors can be used to determine other possible effects on the weight of the plant. The same experiments can also be tested on other microgreens species to contrast responses to their growth parameters. Research can also be done on the consequences of the experimental lighting treatment on other external morphology and the production of micronutrients in the crops.

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