Advanced and Sustainable Technologies (ASET) eISSN 2976-2294
Volume 3 (Special Issue), March 2024 [71-77]

Effects of Blue and Green Light-Emitting Diode (LED) on the Vegetative and Reproductive Growth of Black Jelly Mushroom *(Auricularia auricula-judae)*

Roshita Ibrahim[1*] and Lim Yi Mao[2]

[1]Centre of Excellence in Biomass Utilization, Faculty of Chemical Engineering & Technology, Universiti Malaysia Perlis (UniMAP), Uniciti Alam Campus, Sg Chuchuh, 02100 Padang Besar, Perlis, Malaysia.
[2]Faculty of Chemical Engineering & Technology, Universiti Malaysia Perlis (UniMAP), Uniciti Alam Campus, Sg Chuchuh, 02100 Padang Besar, Perlis, Malaysia.

Received 27 September 2023, Revised 14 October 2023, Accepted 18 October 2023

ABSTRACT

Light is an essential source during the cultivation of mushrooms, which significantly affects the productivity of black jelly mushrooms (Auricularia auricula-judae) in both vegetative and reproductive growth. The application of a light-emitting diode (LED) is proven to be beneficial in several plants and mushroom cultivation. This study focused on the effects of blue and green LED treatments on the vegetative and reproductive growth of black jelly mushroom. During vegetative and reproductive stages, mushroom bags were exposed to three conditions, Control (without LED treatment), blue LED and green LED. The LED treated mushroom bags showed a slower vegetative growth rate compared to the control. However, exposure to Blue LED during the reproductive stage tends to be the most effective treatment among all the LED treatments as it took the least time for pinhead emergence (26.2 to 32.6 days), and green LED shortened the time for fruiting bodies formation (17.0 to 19.4 days). Based on the yield of fruiting bodies, exposure to blue LED during the reproductive stage also resulted in a maximum yield (302.4 g) with a larger mushroom size (13.56 cm). In conclusion, the application of LED during the vegetative stage is unnecessary, but the application of blue and green LEDs during the reproductive stage could improve the growth and yield of black jelly mushrooms.

Keywords: Black jelly mushroom (*Auricularia auricula-judae*), blue and green light-emitting diode (LED), vegetative and reproductive growth, yield

1. INTRODUCTION

Black jelly mushroom (*Auricularia auricula-judae*) belongs to the Auriculariaceae family, and it is known as wood ear or cloud ear mushroom. The black jelly mushroom grows on both dead and live woods in mild climate conditions throughout the globe annually. It is also known for its jelly-like consistency and distinct chewiness, with a dark brown color. It is sharply rounded, usually reminiscent of a floppy ear, although the fruit bodies can also be formed like cups [1] (Figure 1). This life cycle of mushrooms is divided into two phases: vegetative and reproductive growth. The vegetative growth phase indicates the linear growth of fungal mycelia, dissolving complex substrate components into simpler molecules and absorbing them as nutrients. When low temperature, high humidity, sufficient oxygen, and sometimes light are offered, the mycelia cease the vegetative growth and begin reproductive growth when it develops a fruiting body, an ear-like body that is also known as a mature black jelly mushroom. Mushroom cultivation is basically the practice of obtaining a fruiting body by artificially repeating these two growing stages [2].

*Corresponding author: roshita@unimap.edu.my

Figure 1: Black jelly mushroom (Auricularia auricula-judae).

Black jelly mushroom is generally cultivated in an outdoor netted mushroom house, in which the fruiting development depends on sunlight as a light source. In such circumstances, the amount of light acquired by oyster mushrooms for forming fruiting bodies may be inconsistent as it depends on the condition of climate or weather, which greatly influences productivity performances. One of the constraints in mushroom industries is the limited application of advanced technological development in agricultural fields to regulate the growth development of mushrooms. Growers normally use fertilizers and pesticides to control the mushroom's growth and reduce the risk of contamination in mushroom house, respectively. From the aspect of bio-ethics, the use of artificial stimulation of light is an alternative way considered a safer approach in regulating the growth development and morphogenesis of the mushrooms [3].

Light is a crucial influence on the formation of fruiting bodies. Under the effect of light conditions, the differentiation of mycelium will be even apparent in modifying the associated secondary metabolite responses to vegetative mycelium or in fruiting bodies [4]. Light-emitting diodes (LEDs), are electronic semiconductor devices that have evolved tremendously in recent years. These devices emit electromagnetic radiation from the near-infrared range up to the ultraviolet, as is widely known. When their junctions are biased forward, the current passing through the junction causes the emission of photons due to the passage of electrons from the conduction band [5]. This light potential resource prior exploited by the agro-food sector by developing farm structures and greenhouses installed with LEDs to optimize crop cultivation [6].

LEDs have been demonstrated to be artificial flexible lighting sources that can influence biological processes [7]. They are compact and easy to install desirable lighting on each shelf of mushroom cultivations. LED requires low electricity, has a longer life span, and causes less heat ray emission [8]. Blue and green light photoreceptors receive photons and convert photon energy into cells to control fungal photo-responses by expressing differential genes, such as mushroom differentiation [9]. The light was directly exposed to the surface of fruiting mushrooms and significantly overwhelmed their quality and production quantity [10]. Based on other studies, *Pleurotus namko* and *P. eryngii* fruiting bodies under the illumination of blue light produce higher productivity and quantity of the fruiting bodies [10, 11].

2. MATERIAL AND METHODS

2.1 Mushroom Bags Preparation

Black jelly mushroom cultivation began with media preparation with well-mixed substrate consisting of $CaCO_3$, rice bran, and rubber tree sawdust, which were mixed based on the ratio of 1:10:100 accordingly. A small volume of water was added to the media to maintain the moisture content of the substrate at 67% to 69% to prevent it from falling apart. Each polypropylene bag was filled with approximately 850g of the media and sterilized in an autoclave at 121°C for 30 minutes. The sterilized media bags were cooled in laminar flow for at least 16 hours, and 10 g of black jelly mushroom culture on wheat grains was used to inoculate the bags. The inoculated bags were arranged on the rack vertically for the vegetative stage, which is also called a spawning period.

2.2 Vegetative Stage Analyses: Mycelium Growth Rate and Number of Days to Fill up the Mushroom Bags

During the spawning process, three treatments were involved, which were Control (without any LED treatment), Blue LED, and Green LED treatments. At this stage, linear mycelium growth was measured at every 5 days interval using a ruler for each bag until it completely filled up the bag. The number of days taken for the mycelium to fill up the bag was also recorded for each bag. Fifteen bags were used to represent each treatment. All the bags were randomly arranged for the exposure to blue or green LED, and the remaining bags that were not exposed to either blue or green LED served as control. Exposure of blue and green LED used were at 1 watt and exposed for 12 hours every day. All the mushroom bags were stored in the mushroom house with minimum light exposure of only 10%, a temperature of 28-31°C, and 80-90% RH. The mushroom house was watered daily to maintain the required humidity.

2.3 Reproductive Stage Analyses

After the mycelium filled up the bags, three slits of 10 cm length were made vertically on the mushroom bags to allow for pinhead emergence and fruiting body formation. Throughout this reproductive stage, 7 treatments were involved, represented by 5 bags each and was exposed to the following LED treatments for 12 hours every day: Treatment 1: Veg. no LED / Rep. no LED (Control); Treatment 2: Veg. No LED / Rep. Blue LED; Treatment 3: Veg. No LED / Rep. Green LED; Treatment 4: Veg. Blue LED / Rep. Blue LED; Treatment 5: Veg. Blue LED / Rep. Green LED; Treatment 6: Veg. Green LED / Rep. Blue LED and Treatment 7: Veg. Green LED / Rep. Green LED.

2.3.1 Number of Days for Pinhead Emergence and Fruiting Bodies Formation

The mushroom bags were monitored regularly to determine the exact number of days taken for the pinhead emergence and fruiting body formation starting from the day the slits were made.

2.3.2 Yield (Weight and Size of Mushroom Fruiting Bodies)

The fruiting bodies of mushrooms were harvested from substrate bags when matured. The mushroom yields were analyzed in terms of the total fresh weight of fruiting bodies and their size from 4 harvestings. The fresh weight of all fruiting bodies was weighed using a weighing balance and recorded. The average size of the harvested mushrooms fruiting bodies for each treatment were measured in terms of diameter from each end side of the pileus using a ruler and recorded.

3. RESULTS AND DISCUSSION

3.1 Vegetative Stage: Mycelium Growth Rate and Number of Days to Fill up the Mushroom Bags

There were significant differences (P<0.05) in the mycelium growth rate among the treatments (Table 1). Control bags, without receiving any LED, showed a faster growth rate of 0.362 cm/day, whereas bags exposed to blue and green LED had a slower growth rate (0.322 and o.318 cm/day, respectively). Since the composition and condition of the substrate were standardized in this study, it is evident that nutritional contents, along with some other factors (including exposure to LEDs), influence the differences in mycelium growth observed. Therefore, LED treatments using blue and green lights did not positively affect the mycelium growth, most probably because any LED is not required during the vegetative stage of black jelly mushroom, which causes the mycelium to reduce the growth rate. In a study on *Pleurotus spp*., exposure to green light (515-530 nm) during the vegetative stage reduced the mycelial biomass growth but increased the cellulolytic and xylanolytic activities [12].

Based on Table 1, all the LED-treated bags took significantly (P<0.05) longer time for the mycelium to fill-up the bag (73-74 days) compared to control (65 days). This result was in line with the results for mycelium growth rate.

Table 1: The means of mycelium growth rate (cm/day) and number of days taken to filled-up the bag of black jelly mushrooms subjected to blue and green LED treatments.

Different LED treatment	Mycelium Growth Rate (cm/day)	Number of Days to Fill up the Mushroom Bag (day)*
Control	0.362 ± 0.015^{a}	65.000 ± 10.574^{c}
Blue LED	0.322 ± 0.015^{bc}	$73.000 + 11.497^{ab}$
Green LED	0.318 ± 0.010^{c}	$74.000 + 10.249^{a}$

Note : Values are means of 15 replicates. Means (n=15) ± standard deviation
a-c : Values bearing the different superscripts within the same column are significantly different at 5% level (P<0.05).
* : values were used to calculate the total time taken until first harvesting (Table 2)

3.2 Reproductive Stage

3.2.1 Number of Days for Pinhead Emergence and Fruiting Bodies Formation

Table 2 shows the number of days taken for pinhead emergence, fruiting bodies formation, and the total time taken until first harvesting. For pinhead emergence, treatments with blue and green LEDs took a shorter time (26.2-32.6 days), where the fastest was from bags exposed to blue LED during vegetative and reproductive stages, and the longest was control (36.4 days). Pinhead emergence was initiated by abrupt changes in the environment like light intensity, exposure to high concentrations of carbon dioxide, and increased humidity and temperature [13]. [14] reported that small pinheads-like were formed one to two weeks after the bags were fully colonized.

Fruiting bodies formation is another reproductive stage and final stage during mushroom cultivation [15]. The time taken for fruiting body formation was recorded by counting from the day of pinhead emergence until the day developed fruiting bodies can be harvested. The results obtained from Table 2 show that there were significant differences (P<0.05) in the number of days for fruiting body formation in all the treatments, where LED-treated bags had a shorter time (17 – 22 days) compared to the control (29 days). Bags exposed to green LED during vegetative and reproductive stages took the shortest time of only 17 days.

Among all the treatments, the least total time taken for the first harvesting of black jelly mushrooms was led by treatment with no LED during the vegetative stage and blue LED during the reproductive stage (116 days). In general, treatment involving LEDs took a shorter time (116 – 125 days) compared to control, which took the longest time of 130 days. The normal expansion of pileus requires light, and the formation of spores requires phototropism [16]. A study on several species of mushrooms reported that exposure to blue LED during the reproductive stage gave rise to effective fruiting body formation due to the presence of a blue-light photoreceptor as a resident protein, which consists of a photoreactive domain responding to light stimuli required for fruiting development [17].

Table 2: The number of days for pinhead emergence and fruiting bodies formation of black jelly mushroom subjected to blue and green LED treatments.

Different LED treatment	Number of days		
	Pinhead emergence	Fruiting bodies formation	Total Time Taken Until First Harvesting[#]
Control	36.400± 6.007[a]	29.000± 4.997[a]	130[a]
Veg. No LED / Rep. Blue LED	29.400± 9.560[bc]	22.000± 4.504[b]	116[d]
Veg. No LED / Rep. Green LED	32.600± 8.374[ab]	21.400± 3.994[b]	119[c]
Veg. Blue LED / Rep. Blue LED	26.200± 6.574[c]	19.200± 4.028[c]	119[c]
Veg. Blue LED / Rep. Green LED	28.000± 7.009[bc]	19.400± 5.004[c]	120[bc]
Veg. Green LED / Rep. Blue LED	30.200± 7.494[bc]	20.600± 4.889[bc]	125[b]
Veg. Green LED / Rep. Green LED	30.000± 8.904[bc]	17.000± 5.099[d]	121[bc]

Note : Veg. – vegetative stage; Rep. – reproductive stage. Values are means of 5 replicates. Means (n=5) ± standard deviation

a-d : Values bearing the different superscript within the same column are significantly different at 5% level (P<0.05).

: values are summation of number of days taken for mycelium to fill up the bag (Table 1), pinhead emergence and fruiting bodies formation.

3.2.2 Yield (Weight and Size of Mushroom Fruiting Bodies)

A study reported that cultivation of mushrooms under light ranges between 340-520 nm increased the yield of the fruiting body due to the activation of ATP synthase, which is present in the fruiting body [18]. Table 3 shows that blue light exposure with a wavelength of 475nm gave the maximum amount of total fresh weight for black jelly mushroom (270.8 – 307.4 g), followed by green LED (248.68 – 262.86 g), and the least yield was obtained from control (245.5 g).

The size of mushrooms was also influenced by the LEDs treatments during the reproductive stage, where treatments with blue LED had significantly (P<0.05) larger size (11.70 – 13.56 cm diameter) followed by green LED (9.38 – 10.80 cm diameter) and the least was from control bag which mean diameter only 8.85 cm.

Table 3: The total weight and size of black jelly mushrooms subjected to blue and green LED treatments.

Different LED treatment	Total weight of fruiting bodies (g)	mushroom diameter (cm)
Control	245.50± 31.017[b]	8.85± 2.443[c]
Veg. No LED / Rep. Blue LED	307.40± 31.574[a]	13.56± 2.804[a]
Veg. No LED / Rep. Green LED	262.86± 29.880[ab]	10.80± 2.330[b]
Veg. Blue LED / Rep. Blue LED	270.80± 29.281[a]	11.76± 2.880[ab]
Veg. Blue LED / Rep. Green LED	248.68± 30.007[b]	9.38± 1.991[bc]
Veg. Green LED / Rep. Blue LED	292.40± 30.925[a]	11.70± 2.729[ab]
Veg. Green LED / Rep. Green LED	249.70± 31.098[b]	10.24± 2.903[b]

Note : Veg. – vegetative stage; Rep. – reproductive stage. Values are means of 5 replicates. Means (n=5) ± standard deviation

a-c : Values bearing the different superscripts within the same column are significantly different at 5% level (P<0.05).

4. CONCLUSION

LED-treated bags showed a positive effect on reproductive growth performance, such as the number of days for pinhead emergence and fruiting bodies formation. However, there was a negative effect during the vegetative stage. Generally, blue LED exposure during the reproductive stage contributed to maximum yield and resulted in a larger pileus size. This research has proven that Blue and Green LED treatments showed improvement in reproductive growth and yield. However, mycelium growth preferred no LED treatment during vegetative growth. Blue light can be considered the best color LED to enhance the reproductive growth and production of black jelly mushrooms. In short, the treatment of No LED during the vegetative stage and Blue LED during the reproductive stage is the best treatment that resulted in the fastest growth and highest yield of fruiting bodies for black jelly mushrooms.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the financial support provided by the Ministry of Higher Education Malaysia through Fundamental Research Grant Scheme (FRGS/1/2016/WAB01/UNIMAP/03/5) and all the staffs in Faculty of Chemical Engineering & Technology and Institute of Sustainable Agrotechnology (INSAT) of Universiti Malaysia Perlis (UniMAP).

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