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Effects of Color Light Emitting Diode (LED) on the Mycelial Growth, Fruiting Body Production, and Antioxidant Activity of *Lentinus tigrinus*

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ABSTRACT

Lentinus tigrinus is an edible and medicinal mushroom and is being cultivated in fruiting bags consisting of rice straw and sawdust-based substrate. In the present work, we evaluated the influence of light emitting diode (LED) on the growth of mycelia, fruiting body production and antioxidant activities of *L. tigrinus*. The culture plates and fruiting bags were incubated and grown in an improvised LED chamber, and the DPPH radical scavenging activity and the total phenolic content of the harvested fruiting bodies were determined. Mycelia in blue LED recorded the widest mycelial diameter (88.83 mm) while fluorescent light showed the lowest mycelial growth (78.33 mm). However, *L. tigrinus* grown on the 8 parts of rice-straw and 2 parts of sawdust exposed under blue LED registered the shortest incubation period (12.33 days) and primordial initiation (17.67 days), highest number of fruiting bodies (27.67), yield (37.59 g), and biological efficiency (12.53%). Fruiting bodies harvested from the dark condition recorded the wides t diameter of pileus and longest stipe. Moreover, among color LED, the highest phenolic content (25.04 mg gallic acid equivalent per g sample) and radical scavenging activity (61.29%) were noted in those grown under blue and red LEDs, respectively.

Keywords: color LED, *Lentinus tigrinus*, radical scavenging activity, total phenolic content

INTRODUCTION

Lentinus tigrinus, Family Polyporaceae, is a wood-rotting basiodiomycete that is normally found growing on fallen logs. This mushroom has been successfully rescued from the wild, cultured in optimum mycelial growth conditions and in sawdust-rice straw-based formulation as substrate for fruiting body production (Dulay et al. 2012). It contains carbohydrates, proteins, fibers, minerals, and the extract exhibits hypoglycemic effect in alloxan-induced mice, antibacterial, antioxidant, and teratogenic activities (Dulay et al. 2014a; Dulay et al. 2014b; Dulay et al. 2017a). In addition, we

reported in our previous works that the different indigenous liquid culture media and select tropical fruit juices significantly influenced the mycelial biomass production and antioxidant of *L. tigrinus* (Dulay et al. 2015; Dulay et al. 2017b). Indeed, nutritional composition of the medium is very vital in mushroom production.

Light is an essential energy source on earth. This environmental factor may stimulate or inhibit the growth and development of many fungi. However, illumination is one of the important requirements in mushroom production particularly during fruiting stage. The effects of light emitting diode (LED) on the growth of mushrooms have been studied with an intention to improve the yield and quality of cultured mushrooms. Some of these include *Hypsizygus marmoreus, Pholiota nameko, Neolentinus lepideus*, and *Pleurotus eryngii* (Namba et al. 2002; Kaori et al. 2005; Jang et al. 2011; Kim et al. 2012, Jang et al. 2013, Jang et al. 2015). However, the mentioned mushrooms showed varied responses when exposed to LED, indicating their unique characteristics.

Herein, we evaluated the effects of color LED on the mycelia growth, fruiting body production, and antioxidant activity of *L. tigrinus* in our intention to develop techniques for efficient production of mushroom with antioxidant properties.

MATERIALS AND METHODS

Strain source and culture inoculant. Pure culture of *L. tigrinus* was obtained from the culture collection of the Center for Tropical Mushroom Research and Development. An agar block mycelia from the pure culture of *L. tigrinus* was aseptically inoculated onto a sterilized potato dextrose agar (PDA) plate and incubated at room temperature to allow the mycelial growth. After 7 days of incubation, mycelial discs were prepared using a flame sterile 10 mm-diameter cork borer and these were used as inoculant in the growth evaluation.

Evaluation of mycelial growth. The growth performance of secondary mycelia of *L. tigrinus* was evaluated under the three color LED (red, blue, and green), fluorescent light, and dark conditions which served as the control. Coconut water agar was adjusted to pH 6, sterilized at 121°C, 15 psi for 20 min, poured into sterile petri plates, and allowed to solidify. Each treatment was replicated 3 times. A 10 mm-diameter mycelial disc was aseptically inoculated onto the plated medium. The inoculated plates were sealed with cling wrap and incubated in the improvised LED chamber at 30°C. The diameter of mycelial growth was measured using a vernier caliper and the mycelial density was determined as very thin (+), thin (++), thick (+++), and very thick (++++). The treatment that showed the widest mycelial diameter and had most luxuriant growth was identified as the best illumination condition.

Evaluation of fruiting body production. The preparation of grain spawn and formulation rice straw and sawdust-based substrate for fruiting body production of *L. tigrinus* were the same as our previous report (Dulay et al. 2012). The inoculated bags were incubated in the improvised LED chamber at 30°C until the fruiting bags were fully ramified by mycelia. Each treatment was replicated 10 times. The number of days of incubation and primordial initiation were recorded. Fruiting bags were opened and watered to allow the emergence of fruiting bodies. The number and weight of fruiting bodies were determined and the length of stipe and diameter of pileus were measured prior to air-drying. The percentage biological efficiency was also calculated.

Ethanol extraction. A total of 10 g of powdered air-dried fruiting bodies from each treatment were extracted in 500 ml of 80% ethanol for 48 hours. Extracts were filtered using Whatman filter No. 2 and concentrated to dryness using rotary evaporator. The extract yield was determined.

DPPH radical scavenging activity assay. The concentrated extract was used to make a stock solution and aliquot was taken to make 1000 µg/ml dilution and 1000 µg/ml cathechin as control. One ml of the prepared stock solution was mixed with 4 ml of 0.1 mM DPPH solution in separate plastic cuvette. Reaction was done in triplicate. The prepared mixtures were incubated in the dark at 37°C for 30 min. The absorbance readings were monitored at 517 nm using a UV VIS spectrophotometer. The ability to scavenge the DPPH radical was calculated using the formula: % Radical Scavenging Effect = [(Acontrol – Asample) / Acontrol] x 100.

Estimation of total phenolic content. The amount of total phenolics in the extract was determined using Folin-Ciocalteu method (Sunita and Dhananjay 2010). Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). The different concentrations of gallic acid and 1 mg/ml concentration of extract were prepared in methanol. Each sample (0.5 ml) was introduced into test tubes and mixed with 2.5 ml of a 10-fold dilute Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 min at room temperature prior to absorbance reading at 760 nm spectrophotometrically. All tests were performed in triplicate.

Statistical analysis. Treatments were laid out following Completely Randomized Design (CRD). Data were analyzed using Analysis of Variance (ANOVA) and means were compared using Least Significant Difference (LSD) at 5% level of significance.

RESULTS AND DISCUSSION

Effects of color LED on the mycelia growth of *L. tigrinus*. All mushrooms emerge from their vegetative structures called mycelia. Aside from substrate, the emergence of fruiting bodies is also influenced by many factors such as light. In this study, the effects of red, blue, and green LED on the growth performance of secondary mycelia of *L. tigrinus* was evaluated. Table 1 shows the mean diameter of mycelial growth and mycelial density of *L. tigrinus* grown on coconut water agar incubated under the different illumination conditions. Apparently, among treatment, mycelia incubated under blue LED significantly produced the highest mean mycelial diameter of 88.83 mm on the fourth day of observation, whereas those under red LED registered the lowest mean mycelial diameter of 81.00 mm. Very thick mycelial growth was observed in all cultures (Figure 1). The results of the present study indicate that blue LED was the most suitable illumination condition that favored the mycelial growth of *L. tigrinus*. The same was also observed in other basidiomycetes like *Pleurotus eryngii* (Wu et al. 2013). This favorable response to blue LED could be due to its characteristic that is being one of the cool lights in the spectrum compared to red and other LED colors which belong to warm light (Börner et al. 2001).

Table 1. Mycelial growth diameter and mycelial density of *L. tigrinus* grown on coconut water agar incubated under the different illumination conditions.

Illumination	Diameter of Mycelial Growth (mm)			Mycelial Density
	Day 2	Day 3	Day 4	
Red LED	33.17 ± 1.76^{a}	54.83 ± 2.02^{b}	81.00 ± 0.50^{cd}	++++
Green LED	32.33 ± 0.76^{a}	54.17 ± 1.04^{bc}	83.83 ± 1.44^{b}	++++
Blue LED	34.00 ± 1.32^{a}	60.00 ± 4.86^{a}	88.83 ± 3.46^{a}	++++
Fluorescent light	32.67 ± 0.76^{a}	51.50 ± 3.46^{c}	78.33 ± 1.15^{e}	++++
Dark	32.33 ± 1.04^{a}	52.50 ± 0.50^{bc}	80.17 ± 0.76^{d}	++++

Values are mean ± SD of three replicates. Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance.
In a column mycelial density: very thin (+), thin (++), thick (+++), very thick (++++).

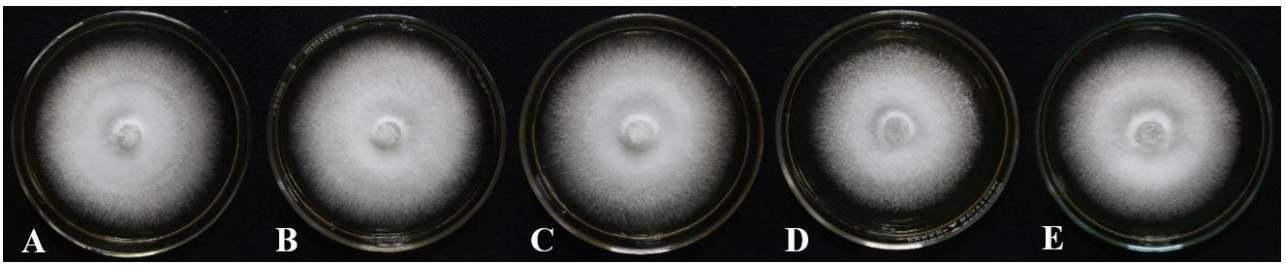

Figure 1. Growth performance of *L. tigrinus* mycelia incubated under the different illumination conditions: (A) red LED, (B) green LED, (C) blue LED, (D) fluorescent light, and (E) dark.

Effects of color LED on the fruiting body production of *L. tigrinus.* Incubation is the phase of mushroom production where the mycelia from the spawn permeate into the substrates until full ramification and primordial initiation. In this present work, the effect of color LED on the vegetative phase and fruiting body production was investigated. The mean number of days of incubation for mycelial ramification and primordial initiation of *L. tigrinus* were presented in Table 2. Among treatments, blue LED recorded the shortest period of incubation for mycelial ramification (12.33 days) and primordial initiation (17.67 days) whereas the most extensive periods were noted in those under fluorescent light. However, the effects of blue and red LED were not significantly different to each other in terms of period of incubation. The fully ramified substrates with *L. tigrinus* mycelia as influenced by the different illumination conditions are shown in Figure 2. This positive response to blue LED does not conform with the observation of Saadatmand et al. (2014), in which the shortest period of incubation (6 days) of *Pleurotus florida* was acquired in those incubated under dark condition. However, the orange and red LEDs prolonged the cultivation period of *Neolentinus lepideus* (Jang et al. 2015).

Table 2. Number of days of incubation for mycelial ramification and primordial initiation of *L. tigrinus* incubated under the different illumination conditions.

Illumination	Incubation Period (day)	Days to Primordial Initiation
Red LED	14.00^{ab}	22.33^{bc}
Green LED	14.33^{b}	21.67^{b}
Blue LED	12.33^{a}	17.67^{a}
Fluorescent light	16.67^{c}	25.33^{d}
Dark	14.67^{b}	24.67^{cd}

Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance.

Figure 2. Fully ramified mycelia of *L. tigrinus* on rice straw-sawdust based substrate formulation incubated under the different illumination conditions: (A) red LED, (B) green LED, (C) blue LED, (D) fluorescent light, and (E) dark.

The fully ramified substrates were opened, watered, and further exposed to the different illumination conditions in order to evaluate the fruiting body performance of *L. tigrinus*. There were significant differences found in the yield of fruiting bodies within two flushes among the different conditions (Table 3). The fruiting bags exposed under blue LED produced the highest yield of 37.59 g (12.53% biological efficiency) while those under red LED recorded the lowest yield. The fruiting bodies grown on the substrate as affected by the different illumination conditions are shown in Figure 3. These results strongly indicate that blue LED was the most favorable illumination condition for fruiting body production of *L. tigrinus*.

The number of fruiting bodies, lenght of stipe, and diameter of pileus were also determined and the data are shown in Table 3. Apparently, fruiting bags exposed to blue LED significantly recorded the highest number of fruiting bodies (27.67) but registered lowest in terms of the diameter of pileus and length of stipe. However, those under dark condition produced the highest diameter of pileus and length of stipe but with low number of fruiting bodies. Thus, the quantity and size of fruiting bodies were greatly affected by the color LED.

Some cultivated mushrooms are also reported to have significant response to LED. Jang et al. (2015) reported the higher yields of *Neolentinus lepideus* under the white, blue, and green LEDs, wider diameter of pileus under the white, blue, and green LEDs, longer and thicker stipes under the orange and red LEDs and lights-out, and the high quality of fruiting bodies was attained when lights-out. Kim et al. (2012) demonstrated that *P. eryngii* cultivated under red, green, and mixed light (R*G), affects the intensity color of the pileus and the length of the stipe but were similar in those of the fluorescent light,

and those under UV-A, blue, and mixed light (B*R, B*G, B*R*G*U) had a dark pileus color and had a short stipe. These findings clearly indicate that mushrooms have different responses to LED lights.

Table 3. Yield, biological efficiency, and size of fruiting bodies of fruiting bodies of *L. tigrinus* grown on rice straw-sawdust based substrate under the different illumination conditions.

Illumination	Yield per bag (g)	Biological Efficiency (%)	Number of Fruiting bodies	Diameter of Pileus (mm)	Length of Stipe (mm)
Red LED	17.79^{d}	5.93^{d}	3.00^{b}	79.57^{a}	30.17^{bc}
Green LED	26.23^{b}	8.74^{b}	6.00^{b}	79.92^{a}	34.19^{b}
Blue LED	37.59^{a}	12.53^{a}	27.67^{a}	59.37^{b}	27.00^{c}
Fluorescent light	23.60^{bcd}	7.87^{bcd}	6.33^{b}	67.54^{ab}	27.20^{c}
Dark	24.21^{bc}	8.07^{bc}	5.33^{b}	80.40^{a}	47.61^{a}

Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance.

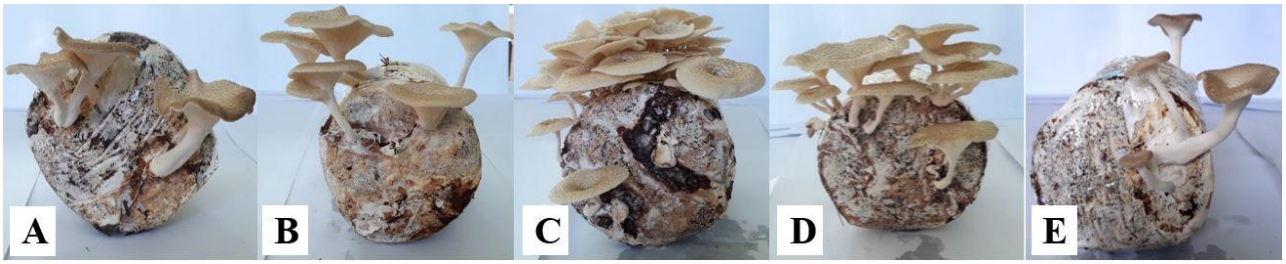

Figure 3. Fruiting bodies of *L. tigrinus* grown on rice straw-sawdust based substrate formulation incubated under the different illumination conditions: (A) red LED, (B) green LED, (C) blue LED, (D) fluorescent light, and (E) dark.

Influence of color LED on the antioxidant property of *L. tigrinus.* Mushrooms possess different antioxidant properties, depending on their contents of antioxidant molecules, which are, in turn, strongly affected by specific lighting conditions (Jang et al. 2013; Wu et al. 2016). In order to determine the effect of LED on the antioxidant activity of *L. tigrinus*, the DPPH radical scavenging activity (RSA) and total phenolic content (TPC) of the extracts were analyzed. Table 4 presented the results of the analyses. It can be seen that, among treatment, extract of mushroom from the dark condition recorded the highest RSA of 75.00%, followed by those under red LED (61.29%). The lowest activity was noted in green LED. However, in terms of the TPC, extract of mushroom from the blue LED registered the highest among all treatments. This was followed by those under red LED. Apparently, the antioxidant activity of fruiting bodies of *L. tigrinus* may vary depending on the type of light conditions.

Table 4. Radical scavenging activity and total phenolic content of ethanolic extracts of fruiting bodies of *L. tigrinus* exposed to different illumination conditions.

Illumination	Radical Scavenging Activity (%)	Total Phenolic Content (mg GAE / g sample)
Red LED	61.29	20.46
Green LED	41.94	5.86
Blue LED	43.55	25.04
Fluorescent light	57.26	16.29
Dark	75.00	6.50
Catechin (control)	83.06	-

On the other hand, in the previous work of Jang et al. (2013), they found out that the DPPH radical scavenging activity and reducing power of *H. marmoreus* were higher in response to all LED treatments than fluorescent lamp and darkness treatment. They also added that the ergosterol content and polyphenol content of the fruiting bodies were higher when cultivated under blue and green LEDs, respectively. Moreover, the DPPH radical scavenging activity of the fruiting bodies of *P. eryngii* was found higher when cultivated under blue, green, and yellow light (except red light), the polyphenol was high under the four LEDs, and the ergosterol was higher under the green light (Jang et al. 2011).

In conclusion, light is an essential factor for the growth of mushroom. LED greatly affects the mycelial growth performance, fruiting body production and antioxidant activity of *L. tigrinus*. Cultivation of *L. tigrinus* under blue LED is a useful technique for producing quality mycelia and fruiting bodies, as well as high phenolic content. The effect of different light intensities must be explored in the next studies.

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