

## Light emitting diode enhances the biomass yield and antioxidant activity of Philippine wild mushroom *Lentinus swartzii*

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### Abstract

*Lentinus swartzii* is a basidiomycetous fungus that usually grows on fallen logs during rainy season. In this study, the effects of color light-emitting diode (LED) on the mycelial biomass, fruiting body production, and antioxidant properties of *L. swartzii* were evaluated. The inoculated culture bottles and fruiting bags were incubated under the three-color LEDs (red, green, and blue), and dark condition (control) in a chamber. Phenolic content and scavenging activity mycelia and fruiting bodies ethanol extracts were also analyzed. In liquid culture, red LED cultures produced the highest mycelial dry weight (0.464 g), while green LED cultures registered the highest number of primordia (6.0). The initiation of primordia was not stimulated under red LED and dark condition. However, in fruiting body production, fruiting bags exposed under red LED showed the shortest period of incubation for mycelial ramification (21.20 days), produced the maximum yield of fruiting body (35.73 g) and biological efficiency (7.14%). All LED mycelial extracts exhibited higher radical scavenging activities (RSA) than catechin, and green LED mycelial extract registered the highest phenolic content (PC) (34.21 mg GAE g<sup>-1</sup> sample). The red LED fruiting body extract recorded the highest RSA (28.06%) and PC (26.08 mg GAE g<sup>-1</sup> sample). Therefore, cultivation of *L. swartzii* in red LED chamber is a practical technique for enhancing biomass production and antioxidant properties.

**Keywords:** *Lentinus swartzii*, Light-emitting diode, Mycelial and fruiting body biomass, Radical scavenging activity, Phenolic content

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## Introduction

The Philippines is known to have very diverse mycological resources, particularly mushrooms that are naturally found growing on leaf litters, soil,

stumps, tree trunks, fallen logs, termite mound, lawns, meadows and gardens. The period of May to September is believed to be the best time to collect mushrooms in the wild, which are basically for food. Mushrooms have been valued as nutritious food and



are effective natural alternative for various diseases. They are ideal source of proteins, carbohydrates, crude fibers, fats, vitamins, minerals, and mycochemicals such as saponins, alkaloids, flavonoids, anthraquinones, atherones, phenols, steroids, coumarins, and fatty acids (Ghorai et al., 2009; Beluhan and Ranogajec, 2011; Sogan et al., 2018; Dulay and Pamiloza, 2018; Aquino et al., 2018). Mushroom extracts and bioactive components have been shown to exhibit antiviral, antiparasitic, antitumor, anticancer, antioxidant, antifungal, antibacterial, immunomodulatory, anti-inflammatory, antidiabetic, nephroprotective, neuroprotective and hepatoprotective properties (Wasser, 2002; Wasser and Weis, 1999). Moreover, mushrooms are also being used in agro-industrial wastes management (Philippoussis et al., 2011; Dulay et al., 2012a).

With the increasing demand for mushrooms, practical and economical production technologies have been developed by the Center for Tropical Mushroom Research and Development in Central Luzon, Philippines with the intention to improve their biological efficiency, thereby, increasing their production. These technologies include the zero-rice waste technology, tilapia and mushroom growth chamber system, aseptic cultivation technology, submerged culture using liquid media, and agro-industrial wastes utilization in mushroom production. However, in Malaysia, electrical shock, bright light, cold temperature (5°C) and high intensity of sound were found effective physical treatments in promoting spawn running and pinhead formation, and in increasing the productivity of *Pleurotus sajor-caju* (Ibrahim et al., 2015). Further, it was demonstrated that the different acoustic sound treatments at 75 dB enhanced the productivity and growth of mycelia of the same mushroom (Ibrahim et al., 2017).

Light is an important energy source on earth. It can regulate growth, growth direction, reproduction, and formation of pigment which is necessary for the survival and distribution of fungi (Idnurm and Heitman, 2005). Illumination is also one of the important environmental requirements in the growth and development of mushrooms. The presence of light stimulates the mycelial growth of *Schizophyllum commune*, *Volvariella volvacea*, *Lentinus sajor-caju* and *Lentinus tigrinus* (Kalaw et al., 2016; Dulay et al. 2012b; Reyes et al. 1998). Light-emitting diode (LED) has high energy efficiency and it does not emit heat rays, which is more advantageous than fluorescent lamps (Jang et al., 2013). Literature proves that blue

LED improves the growth and production of mycelial and basidiocarp, and antioxidant activity of *L. tigrinus* (Damaso et al., 2018), while red LED enhances the mycelial biomass yield and phenolic content of *Ganoderma lucidum* (Alcazar et al., 2018), which indicate that mushrooms respond differently to LED. *Lentinus swartzii*, which belongs to the Family Polyporaceae, is a newly cultivated mushroom in the Philippines. Fruiting bodies of this mushroom have generally light-coloured pileus with dense brown to black smooth scales and are characterized to have leathery stipe. The wild strain is found growing gregariously on dead wood of mango (*Mangifera indica*) in Central Luzon region. Unlike other *Lentinus*, reports about this species are very limited. Studies on the culture conditions and functionality profile of *L. swartzii* are currently under investigation in our laboratory. Recently, we reported the nutritional requirements for mycelial growth and the basidiospore germination and morphogenesis of *L. swartzii* (Dulay et al., 2020a; 2020b). The optimum culture conditions, biomass production, nutritional compositions, biological activities of its relative, *L. tigrinus*, have been reported in various studies (Dulay et al., 2012b; 2012c; 2014; 2015; 2017a; 2017b; Ragasa et al., 2018). On this note, *L. swartzii* potentially demonstrates the same properties, hence further study is necessary.

Herein, we aimed to investigate the effects of color LED on the mycelial biomass production, fruiting body production, antioxidant property of *L. swartzii* in our desire to generate effective technique for efficient biomass production of this mushroom with antioxidant properties.

## Material and Methods

### Mushroom culture

Mycelial culture of *L. swartzii* from the collections of Bioassay Laboratory, Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines was used in this study. The mushroom was cultured in potato dextrose agar plates for five days, which served as source of mycelial inoculant in the growth performance assays.

### Evaluation of mycelia biomass production

In this study, the effect of color LEDs on the production of mycelia of *L. swartzii* in submerged culture condition



was assessed. Coconut water was used as liquid medium. Fifty ml of coconut water was dispensed into each transparent glass bottle with cotton plug. A total of 20 bottled media was prepared. These were sterilized at 15 psi, 121°C for 20 min in an autoclave. When cooled, each bottled medium was inoculated with 10 mm-diameter discs of mycelia from the 5-day old culture. The inoculated bottled media were incubated in the LED chamber for 15 days. Five bottles were placed in each treatment. After incubation, mycelia mats were harvested, air-dried and weighed. The number of initiated primordia was recorded. The final volume of the spent media was also recorded. The air-dried mycelia were subjected to ethanol extraction for antioxidant and total phenolic analyses.

### Evaluation of fruiting body production

The preparation of grain spawn and substrate formulation using sawdust and rice straw for fruiting body production of *L. swartzii* were the same as the previous report on *L. tigrinus* (Dulay et al., 2012b). A total of 20 bags were prepared. The bags were sterilized at 15 psi, 121°C for one hour. Forty grams of the grain spawn were inoculated into each bag and placed in the LED chamber for incubation until the full mycelial ramification. Five bags were placed in each treatment. The period of incubation was recorded. Fruiting bags were opened and watered to allow primordial development and eventually develop into mature fruiting bodies. The yield and biological efficiency were determined. The fruiting bodies were air-dried, pulverized, and were subjected to ethanol extraction for the assays.

### Ethanol extraction

Five grams of pulverized mycelia and fruiting bodies of *L. swartzii* were soaked in 250 ml of 95% ethanol. After 48 hours, filtration was done using Whatman filter No. 2, and the ethanol was separated from the extract through rotary evaporation. The yield extracts were placed separately in vials and were refrigerated until needed for the assay.

### Antioxidant activity assay

The standard method of Kolak et al. (2006) on scavenging activity assay using the stable 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) was used to determine the antioxidant activities of the extracts. The DPPH radical scavenging activity (RSA) of the extracts was calculated based on the absorbance reading at 517 nm using a UV VIS spectrophotometer

(Spectrumlab 752S, Hinotek Instrument Co., LTD, China). On the other hand, phenolic content (PC), in gallic acid equivalents (GAE), of the extracts was determined using Folin-Ciocalteu method (Sunita and Dhananjay, 2010) and was calculated based on the absorbance reading at 760 nm using a UV VIS spectrophotometer.

### Statistical analysis

Analysis of Variance (ANOVA) and Least Significant Difference (LSD) at 5% significance level in SAS System Version 9.0 were used to analyze the data.

## Results

### Effect of color LED on mycelial biomass production

The effects of color LED on the mycelial biomass production of *L. swartzii* were evaluated. The mycelial dry weight, volume loss of culture spent, and number of primordia are presented in Table 1. Among treatments, red LED recorded the highest mycelial dry weight (0.464 g). However, the other two color LEDs registered lower mycelial dry weight, which was comparable to dark condition. Surprisingly, color LED also stimulated the initiation of primordia in liquid culture. It can be noted that green LED recorded the highest number of primordia, followed by the blue LED. However, no primordium was observed in those incubated under red LED and dark condition. Figure 1 shows the liquid cultures of *L. swartzii* as affected by the color LEDs and dark condition. In terms of the volume loss of culture spent, no significant difference was observed among treatments, indicating that the utilization of liquid media by mushroom was not affected by LED.

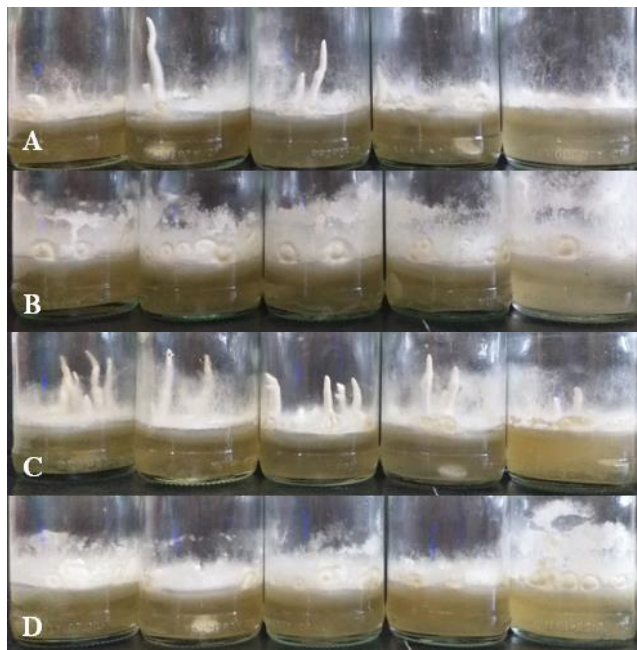
**Table-1. Mean mycelial weight, number of primordia, and volume loss of culture spent of *Lentinus swartzii* grown in liquid culture using coconut water after 15 days of incubation in color LEDs.**

Treatment	Mycelial Weight (g dry wt.)	Number of primordia	Volume loss (ml)
Blue LED	0.412 ± 0.013 <sup>b</sup>	3.20 ± 0.45 <sup>b</sup>	18.50 ± 0.61 <sup>a</sup>
Red LED	0.464 ± 0.011 <sup>a</sup>	0.00 ± 0.00 <sup>c</sup>	19.00 ± 1.00 <sup>a</sup>
Green LED	0.416 ± 0.013 <sup>b</sup>	6.00 ± 2.83 <sup>a</sup>	17.90 ± 1.39 <sup>a</sup>
Dark condition	0.422 ± 0.008 <sup>b</sup>	0.00 ± 0.00 <sup>c</sup>	18.10 ± 0.74 <sup>a</sup>

Values are mean ± SD of five replicates. Means having the same letter of superscript in the same column are



not significantly different from each other at 5% level of significance.



**Figure-1.** Liquid cultures of *Lentinus swartzii* exposed in color LEDs (A): blue LED, (B): red LED, (C): green LED and (D): dark after 15 days of incubation.

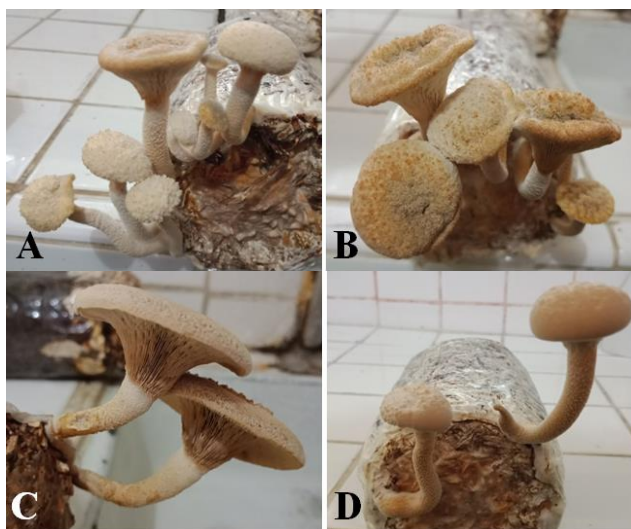
**Effect of color LED on fruiting body production**

The effects of color LEDs on the ramification of mycelia and production of basidiocarp of *L. swartzii* were also investigated (Table 2). Apparently, the shortest period of incubation for mycelial ramification was observed in those incubated under red LED (21.20 days), but statistically comparable to those under blue LED and dark condition. In contrast, the extensive period for mycelial ramification was noted in green LED. The mean weight of fruiting bodies of *L. swartzii* is also presented in Table 2. Noticeably, the fruiting bags exposed under red LED produced that highest weight of fruiting bodies (35.73 g), which corresponds to a biological efficiency of 7.14%. However, no significant difference was found on the yields of red and blue LEDs. The lowest yield, on the other hand, was recorded in those grown under dark condition, which was statistically comparable to those under green LED. Figure 2 depicts the fruiting bodies grown on the substrate as affected by the different treatments.

**Table-2.** Mean number of day of mycelial ramification, weight of fruiting body, and percentage biological efficiency of *Lentinus swartzii* grown on rice straw-sawdust based substrate in color LEDs.

Treatment	Mycelial ramification (day)	Weight of fruiting bodies per bag (g)	Biological Efficiency (%)
Blue LED	22.40 ± 0.55 <sup>ab</sup>	32.54 ± 1.84 <sup>a</sup>	6.51
Red LED	21.20 ± 0.45 <sup>b</sup>	35.73 ± 1.58 <sup>a</sup>	7.14
Green LED	23.00 ± 0.71 <sup>a</sup>	23.58 ± 1.79 <sup>b</sup>	4.72
Dark condition	22.40 ± 0.89 <sup>ab</sup>	21.35 ± 3.66 <sup>b</sup>	4.27

Values are mean ± SD of five replicates. 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.** Fruiting bodies of *Lentinus swartzii* grown on rice straw-sawdust based substrate incubated in color LEDs (A): blue LED, (B): red LED, (C): green LED and (D): dark.

**Antioxidant activity of mycelia and fruiting body extracts**

The RSA and PC of mycelial and fruiting body extracts of *L. swartzii* are presented in Table 3. Apparently, extract of mycelia grown under red LED had the highest RSA of 73.95%, but statistically comparable to the mycelial extracts from blue and green LEDs. In terms of PC, mycelial extract from green LED had the highest PC of 34.21 mg GAE g<sup>-1</sup> sample. Mycelial extract from dark condition significantly recorded the lowest RSA and PC. However, among ethanolic extracts of fruiting bodies, red LED recorded the highest RSA (28.06%) and PC (26.08 mg GAE g<sup>-1</sup> sample). Blue and green LEDs showed the minimum RSA and PC, respectively.

**Table-3. Radical scavenging activity and total phenolic content of ethanolic extracts of mycelia and fruiting body of *Lentinus swartzii* exposed in color LEDs.**

Extract	Radical Scavenging Activity (%)		Total Phenolic Content (mg GAE / g sample)	
	Mycelia	Fruiting body	Mycelia	Fruiting body
Blue LED	72.26 ± 1.28 <sup>a</sup>	25.18 ± 0.42 <sup>c</sup>	26.71 ± 0.52 <sup>b</sup>	14.83 ± 0.64 <sup>b</sup>
Red LED	73.95 ± 0.49 <sup>a</sup>	28.06 ± 0.83 <sup>b</sup>	26.08 ± 0.64 <sup>b</sup>	26.08 ± 0.84 <sup>a</sup>
Green LED	73.11 ± 2.22 <sup>a</sup>	27.34 ± 0.42 <sup>bc</sup>	34.21 ± 0.32 <sup>a</sup>	9.63 ± 0.63 <sup>c</sup>
Dark condition	59.66 ± 0.84 <sup>b</sup>	26.62 ± 1.10 <sup>bc</sup>	24.21 ± 0.55 <sup>c</sup>	9.83 ± 0.42 <sup>c</sup>
Catechin	60.50 ± 2.95 <sup>b</sup>	69.06 ± 1.10 <sup>a</sup>	-	-

Values are mean ± SD of triplicate tests. Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance.

## Discussion

The mycelial growth of mushroom is not only influenced by the nutritional factors but also by physical conditions such as light. According to Ambra et al. (2004), morphogenesis of organisms is stimulated by light. It has been reported also that light is very vital in the accumulation and formation of food reserves necessary for the development of mycelia into basidiocarp (Chang and Miles, 2004). The present study demonstrated that, among color LEDs used, red LED was found to be the most favourable light condition for the efficient mycelial biomass production. However, green and blue LEDs stimulated the formation of primordia of *L. swartzii* in the liquid culture condition. Likewise, the most efficient growth and production of mycelial biomass of *Ganoderma lucidum* was found in red LED (Alcazar et al., 2018). *G. lucidum* mycelia grew faster in red LED, blue LED and dark condition than under other light qualities (Wang et al., 2011). Moreover, red LED induced the conidiation of *Beauveria bassiana*, in which the produced conidia were highly tolerant to ultraviolet radiation and induced sporulation in *Physarum polycephalum* ((Pittarate et al., 2018; Starostzik and Marwan, 1995). The suitability of red LED to the different above-mentioned fungal species is probably because red light is the basal component in lighting spectra, in which sole red light is sufficient for normal

plant growth and photosynthesis (Olle and Virsille, 2013). The difference in the mycelial growth response of *L. swartzii* to color LED could be due to the quality of light requirement of mycelia of mushroom.

Light promotes the development of basidiocarp of mushrooms. The results of the present study indicate that red LED could promote mycelial ramification of *L. swartzii* in the substrate. However, Saadatmand et al. (2014) reported that the shortest period of incubation of *Pleurotus florida* is acquired when incubated under dark condition. In addition, *L. tigrinus* mycelia showed favourable response to blue LED (Damaso et al., 2018). Red LED was found to be the most favourable LED for fruiting body production of *L. swartzii*. Some cultivated mushrooms are also reported to show positive response to LED. For instance, Kim et al. (2012) posited that the pileus color intensity and stipe length of *P. eryngii* were affected grown under red, green, and mixed light (R\*G). In contrast to the present results, Damaso et al. (2018) reported that blue LED is the most favourable illumination condition for fruiting body production of *L. tigrinus*. It is safe to mention therefore that mushrooms, even belonging to same genus, have different responses to color LED.

Mushrooms exhibit different antioxidant properties, depending on the bioactive compounds and antioxidant molecules present during development, which are strongly influenced by light (Jang et al., 2013; Wu et al., 2016). The antioxidant activities of the extracts of mycelia and fruiting bodies of *L. swartzii* produced under color LEDs and dark condition were determined. Interestingly, mycelial extracts from all color LEDs had higher radical scavenging activities when compared to the positive control, catechin, indicating the very promising potential of LED-grown mycelia of *L. swartzii* as source of antioxidant agents. Fundamentally, the highest scavenging activity and phenolic content of fruiting body could be acquired when grown under red LED. This positive response to red LED could probably be due to the ability of red-light absorbing photoreceptors to receive photons and transduce the photon energy into cells to regulate fungal photoresponses through differential gene expression which can regulate biochemical processes (Kurtzman and Martínez-Carrera, 2013; Correa et al., 2003). Previous works reported a significant influence of LEDs on the antioxidant property of fruiting bodies of mushrooms and of plants. For instance, *Pleurotus eryngii* and *Hericium marmoreus* recorded high



radical scavenging activity when grown in blue LED (Jang et al., 2011; 2013). However, in plants, red LED wavelength increases the antioxidant properties and promotes accumulation of anthocyanin, which can be accounted to the increased gene expression for biosynthesis of anthocyanin (Lekkham et al., 2016). The antioxidants reduce the risk for neurodegenerative, cardiovascular diseases and cancer (Prakash et al., 2001). Noteworthy, the present work showed that mycelial extracts have higher scavenging activity and phenolic content than any of the fruiting body extracts. Moreover, the mycelia and fruiting body have varied responses to different color LEDs.

## Conclusion

The present work has shown that red LED enhances the efficient mycelial biomass production in liquid culture and basidiocarp production of *L. swartzii* in the rice straw and sawdust-based substrate. Mycelial extracts from the three-color LEDs and the fruiting body extract from red LED exhibited higher radical scavenging activity. The maximum phenolic content of mycelia and fruiting body could be acquired when incubated under green and red LEDs, respectively. Therefore, cultivation of *L. swartzii* cum exposure to red LED is a useful technique in improving biomass production as well as the antioxidant properties of *L. swartzii*. In order to fully maximize the utilization of this technique, extensive studies on the effects of color LEDs to commercially cultivated mushrooms must be carried out.

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### Contribution of Authors

Tiniola RC: Conceptualization of the study, literature review, experimental design and data collection, manuscript writing  
Pambid RC & Bautista AS: Research design and methodology assessment, manuscript critiquing, final reading and approval  
Dulay RMR: Conceptualization of the study, statistical analysis and data interpretation, manuscript writing

