



Effect of Light-Emitting Diodes on the Mycelial Biomass Production and Antioxidant Activity of *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst.

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Abstract

Light is an important factor for the growth of many forms of life, including mushrooms. This paper highlights the effects of the different wavelengths (red, 650 nm; blue, 450 nm; green, 525 nm) of light-emitting diodes (LEDs) on the growth, biomass production, and antioxidant activities of *Ganoderma lucidum*. *G. lucidum* is a wood-degrading fungus in the Basidiomycota that typically grows on logs. Mycelia were grown on coconut water agar (CWA) solid media for analysis of mycelial colony diameter as well as coconut water (CW) submerged culture for analysis of mycelial biomass weight. Both set-ups were incubated in variously-colored LED chambers. The DPPH radical scavenging activity (RSA) and total phenolic content (TPC) of the harvested mycelia were also determined. After three days of incubation, *G. lucidum* mycelia under red LED recorded the highest mean mycelial colony diameter of 72.50 mm. In terms of mycelia biomass production, *G. lucidum* mycelia exposed in red LED and dark produced the heaviest weight. On the other hand, mycelia grown under green LED had the highest RSA of 66.49%, while those harvested from red LED showed the highest TPC of 81.29 mg GAE / g of sample. Our results demonstrate that LED color influences the growth and production of mycelia, as well as the antioxidant activities of *G. lucidum*.

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

Introduction

Ganoderma lucidum (Leys.:Fr.) Karst, Family Ganodermaceae, is a red shiny to chocolate brown, soft to firm, corky, and flat basidiomycetous fungus that belongs to the order Polyporales. It is locally recognized as *kabuteng kahoy* and commonly found growing on trunks of trees and fallen logs. The optimal growth conditions of the Philippine strain of this mushroom have been established (Magday et

al., 2014) and the mycelia efficiently cultured on coconut water gelatin with a pH 6.0, in illuminated, sealed, and room temperature (32°C) conditions. Corn grit and 70% rice straw and 30% sawdust formulation serve as the best spawning material and substrate for *G. lucidum* fructification, respectively. In addition, we reported in our previous works that the liquid culture media significantly influenced the growth and antioxidant activities of *G. lucidum* (Bustillos et al., 2018).

G. lucidum is considered as a wonder mushroom due to numerous biological activities. This mushroom contains β -glucan and ganoderic acid, which are capable of lowering the risks of diabetes, asthma, arthritis, hepatitis, bronchitis, insomnia, ulcer, anorexia, leucopenia and even malignant cancer (Stamets, 2000; Benzie et al., 2004). *G. lucidum* also displayed antioxidant, hypoglycemic, anti-tumor, and immune-stimulating effects (Wasser, 2002; Yang et al., 2002; Han et al., 2006; Liu et al., 2010). Moreover, *G. lucidum* hot water extract demonstrated aphrodisiac and diuretic activities in male mice (Dulay et al., 2016), exhibited anticoagulative effect in intrinsic pathway (Dulay et al., 2016), and showed toxic and teratogenic effects in zebrafish embryos (Dulay et al., 2012).

Fungi sense not only the different qualities of light but also the different intensities of light and are capable of sensing light over a broad spectrum, from ultraviolet (UV) to far-red light (Idnurm & Heitman, 2005). For instance, *Aspergillus nidulans* performed asexual reproduction when treated with red light, while sexual reproduction occurred when exposed to far-red light (Blumenstein et al., 2005). *Neurospora crassa* was affected by light due to a blue light photoreceptor that adjusts the protein syntheses in different synthetic pathways with or without light (Borkovich et al., 2004). In addition, there are studies reported about the effect of light-emitting diode (LED) on growth and yield of some basidiomycetes including *Pholiota nameko* and *Pleurotus eryngii* (Kaori et al., 2005; Jang et al., 2011).

Herein, we demonstrated the influence of colored LED on the mycelial growth in both solid and submerged cultures and on the antioxidant activity of *G. lucidum* with the intention to develop techniques for efficient cultivation of mushroom mycelia with enhanced bioactivity.

Materials and Methods

Strain Source and Culture Inoculant

Pure culture of wild *G. lucidum* was obtained from the culture collection of the Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. Agar block mycelia from the pure culture was aseptically inoculated onto a sterilized potato dextrose agar (PDA) plate and incubated at 30°C 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 on Solid Media

The effects of colored LEDs on the mycelial growth of *G. lucidum* were evaluated. Coconut water agar adjusted to pH 6 was sterilized at 121°C, 15 psi for 20 min, poured into sterile petri plates, and allowed to solidify. Each treatment was replicated three times. A 10 mm-diameter mycelial disc was aseptically inoculated onto the plated medium. The culture plates were incubated in the colored LED chambers at 30°C. The mycelial colony diameter was measured using a vernier caliper. The treatment that showed the widest mycelial diameter and had the most luxuriant growth was identified as the best illumination condition.

Mycelial Biomass Production

Coconut water was used as the basal medium in mycelial production. Medium (50 ml) was dispensed in each 250 ml-capacity glass culture bottle, plugged with cotton, and covered with paper. Five replicates per treatment were done. Culture media were sterilized in an autoclave at 121°C, 15 psi for 20 minutes. After cooling, 10 mm-diameter mycelial disc was inoculated into each medium. Cultures were incubated in the colored LED chambers at 30°C, static condition for 15 days to allow mycelia growth. The mycelia were harvested, air-dried, and weighed.

Ethanol Extraction

Five grams of mycelial samples 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.

Radical Scavenging Activity and Total Phenolic Content Analysis

The standard method of Kolak et al. (2006) on free radical scavenging activity of the sample using the stable 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical was followed with minor modifications. The ability to scavenge the DPPH radical was calculated using the formula: % Radical Scavenging Effect = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$. However, the total phenolics of extract was determined using Folin-Ciocalteu method of Sunita and Dhananjay (2010) with modifications. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). Each test was replicated three times.

Statistical Analysis

Completely Randomized Design (CRD) was followed to lay-out the treatment. Analysis of Variance (ANOVA) and Least Significant Difference (LSD) at 5% level of significance in The SAS System Version 9.0 (SAS Institute Inc. Cary, NC, USA) were used to analyze and compare the data.

Results and Discussion

Influence of Colored LEDs on the Mycelial Growth and Biomass Production

The growth of mycelia is influenced not only by the nutritional composition of the media but also by physical factors such as illumination (Magday et al., 2014). Accordingly, the effect of different colored LEDs on *G. lucidum* mycelial growth was evaluated in the present work. Table 1 presents the mycelial colony diameter when grown on coconut water agar incubated under the different colored LEDs. Among the different conditions, red LED produced the largest mycelial colony diameter of 72.50 mm after 3 days of incubation. Blue LED, however, recorded the lowest mycelial colony diameter of 62.33 mm. Analysis of variance revealed that different colored LEDs significantly influenced the mycelial growth on solid culture medium. The mycelial cultures exposed at the different colored LEDs are shown in Figure 1. The results strongly indicate that red LED, among LEDs evaluated, was found preferable for the efficient *G. lucidum* mycelial growth.

Table 1

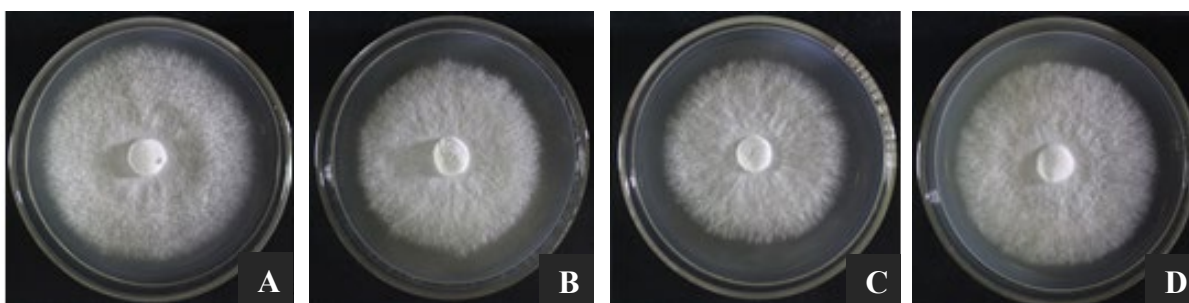
Mycelial Colony Diameter of G. lucidum Grown on Coconut Water Agar Incubated Under Colored LEDs

Treatment	Diameter of Mycelial Colony (mm)		
	Day 1	Day 2	Day 3
Red LED	23.00 ± 0.50 ^a	49.17 ± 1.04 ^a	72.50 ± 0.00 ^a
Green LED	20.17 ± 0.29 ^b	42.67 ± 0.29 ^b	66.17 ± 0.29 ^b
Blue LED	18.17 ± 0.29 ^c	40.33 ± 0.76 ^c	62.33 ± 0.76 ^c
Dark condition	20.33 ± 0.29 ^b	42.83 ± 0.29 ^b	66.33 ± 1.89 ^b

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.

Figure 1

Mycelial Growth of G. lucidum on Coconut Water Agar Under Colored LEDs: (A) Red LED, (B) Green LED, (C) Blue LED, And In (D) Dark Condition After 3 Days of Incubation



The effect of colored LEDs on the mycelial biomass production of *G. lucidum* is shown in Table 2. Apparently, the highest yield of mycelial biomass was recorded in those incubated under red LED and dark conditions with both mean of 0.5 g d.w. However, mycelia from both green and blue LED registered the lowest biomass yield of 0.40 g d.w. The liquid cultures of *G. lucidum* under the different colored LEDs are shown in Figure 2. These results suggest that the different colored LEDs significantly influenced the mycelial biomass production of *G. lucidum*.

Table 2

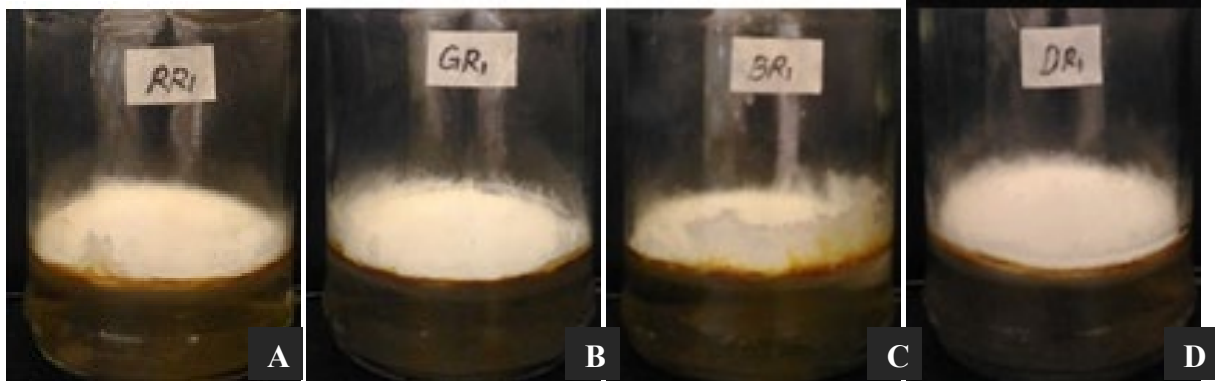
Yield of Mycelial of G. lucidum Grown in Liquid Culture Using Coconut Water Medium After 15 Days of Incubation Under Colored LEDs

Treatment	Fresh Weight (g)	Dry Weight (g)
Red LED	7.20 ^a	0.50 ^a
Green LED	5.24 ^b	0.40 ^b
Blue LED	5.94 ^b	0.40 ^b
Dark condition	7.18 ^a	0.50 ^a

Values are mean 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.

Figure 2

Liquid Cultures of *G. lucidum* Using Coconut Water Under Colored LEDs: (A) Red LED, (B) Green LED, (C) Blue LED, and in (D) Dark Condition After 15 Days of Incubation



The effects of light to mycelial growth, fruiting body production, conidiation, spore germination, metabolites and bioactivities, and quality characteristics of fungi are dependent on the wavelength of light and fungal species (Ellis et al., 1999; Poyedinok et al., 2003; Rangel et al., 2011; Kim et al., 2012; Cheng et al., 2012; Röhrig et al., 2013; Damaso et al., 2018). In the present work, red LED was found to be the most effective treatment for efficient growth and production of *G. lucidum* mycelia. However, in the study of Wang et al. (2011), *G. lucidum* mycelia grew faster in red LED, blue LED, and dark condition than under other light qualities. Zapata et al. (2009) reported that the optimum production of *G. lucidum* was obtained at wavelengths between 425 and 475 nm, which correspond to the blue light, followed in order by white light, dark condition, red light, and yellow light. In case of *P. eryngii*, red LED was found to be the preferred condition for cultivation with better quality (Kim et al., 2012) while dark was found favorable for mycelial production (Yan et al., 2013). Moreover, red LED induced the conidiation of *Beauveria bassiana* and these produced conidia were highly tolerant to ultraviolet radiation (Pittarate et al., 2018) and induced sporulation in *Physarum polycephalum* (Starostzik & Marwan, 1995). The relatively slow mycelial growth in blue LED obtained in this work is conforms with the observation of Röhrig et al. (2013) who reported that blue light has the ability to reduce mycelial growth of *Apergillus nidulans*.

Although many fungi respond to blue light, some species responds to the wavelengths from red to far-red light. Red light is recognized by the photoreceptor phytochromes (PHYs) (Schumacher, 2017). The phytochrome, FphA and PHY-2, of *A. nidulans* and *Neurospora*, respectively, binds to biliverdin chromophore and display a Pr absorption peak at ~700 nm, corresponding to red light (Brandt et al., 2008; Froehlich et al., 2005). This mechanism of positive response to red light of the two model fungi is probably similar to the mechanism in *G. lucidum*, which needs to be elucidated in the future study.

Effect of Colored LEDs on the Antioxidant Activity

The DPPH RSA and TPC of the mycelia of *G. lucidum* grown under different light conditions were also analyzed in order to determine whether colored LEDs could improve bioactivity of mycelia. The RSA and TPC of mycelia grown under LEDs are presented in Table 3. Among light conditions, mycelia cultured under green LED significantly showed the highest RSA with a mean of 66.49%. This was followed in order by those under red LED, dark, and blue LED. On the other hand, mycelia from red LED contained the highest amount of TPC with a mean of 81.29 mg GAE / g of sample. However, mycelia from green and blue LED had lower amounts of TPC when compared to those incubated in the dark condition. It can be noted that mycelia from green LED, which showed the highest RSA, contained the

lowest TPC, among light conditions. This could probably be attributable to other compounds in the mycelia that also acts as antioxidants.

Table 3

Radical Scavenging Activity and Total Phenolic Content of G. lucidum Mycelia Grown Under the Colored LEDs

Treatment	Radical Scavenging Activity (%)	Total Phenolic (mg GAE / g of sample)
Red LED	55.73 ^c	81.29 ^a
Green LED	66.49 ^b	67.13 ^c
Blue LED	23.02 ^e	67.33 ^c
Dark condition	36.80 ^d	71.71 ^b
Cathechin	84.80 ^a	-

Values are mean 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.

Previous works also reported the significant influence of LEDs on the antioxidant property of fruiting bodies of mushrooms. 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 has a more pronounced effect on the anthocyanin accumulation, which can be attributed to the increased expression of genes for biosynthesis of anthocyanin (Lekkham et al., 2016). Moreover, Park et al. (2012) mentioned that exposure to 450 nm and 470 nm light from LED can induce the production of high amounts of ginsenosides, thereby enhancing the pharmacological properties of ginseng. The findings of the aforementioned previous works and the present study strongly suggest that LED light can be used for the production of medicinally important secondary metabolites of both mushrooms and plants. LED-treated mushrooms could have rich antioxidants and other secondary metabolites that can provide health benefits to humankind.

In conclusion, the different LED wavelengths affect the growth and production of *G. lucidum* mycelia. Among LED, red LED was the most favorable treatment for the luxuriant mycelial growth, efficient mycelial production, and total phenolics. Mycelia under green LED had the highest radical scavenging activity. This technique of using LED in mushroom production is very useful in providing innovative and practical way to produce higher yield of mushroom biomass with effective antioxidant activity. Furthermore, the influence of the varying LED wavelengths on the fructification, bioactive secondary metabolites, and functional activities of *G. lucidum* must be studied.

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