Effect of Color of Light Emitting Diode on Development of Fruit Body in *Hypsizygus marmoreus*

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**Abstract** This study was conducted to identify a suitable color of light for development of the fruit body in *Hypsizygus marmoreus*. To accomplish this, samples were irradiated with blue (475 nm), green (525 nm), yellow (590 nm), or red (660 nm) light emitting diodes (LEDs) to induce the formation of fruiting bodies after mycelia growth. The diameter and thickness of the pileus and length of stipes in samples subjected to blue LED treatment were similar to those of subjected to fluorescent light (control), and the lengths of the stipes were highest in response to treatment with the red LED and darkness. The commercial yields of plants subjected to blue and green LED treatment were similar to those of the control. In conclusion, cultivation of *H. marmoreus* coupled with exposure to blue LED is useful for inducing high quality fruit bodies as well as higher levels of ergosterol, DPPH radical scavenging activity, total polyphenol content and reducing power.

**Keywords** Ergosterol, Light, Light emitting diodes, Mushroom

Light emitting diodes (LEDs) have longer life and greater energy efficiency than fluorescent lamps, and, unlike commonly used artificial lighting systems, LEDs do not emit heat rays. Moreover, estimations of the electrical energy conversion efficiency of a LED system for plant irradiation suggest that it may be as much as twice that of fluorescent systems [1]. An important advantage of LED radiation systems is that the peak spectral output of the LEDs coincides closely with the red absorption peak of chlorophyll and the reported wavelengths of maximum photosynthetic efficiency [2]. Thus, a greater photosynthetic efficiency can be expected from radiation emitted by these LEDs than from lamps generating radiation at 400 to 700 nm [1]. The occurrence of fruiting bodies of Basidiomycetes is facilitated by light. In addition, the primordia of oak mushroom and oyster mushroom and the pileus of winter mushroom and scale lentinus is promoted by light [3]. The use of blue light for oak mushroom has been shown to induce pigmentation of the primordial stage [4]. Furthermore, primordia formation and basidiocarp maturation in *Coprinus stercorarius* have separate requirements for light, with both showing maximum activity in the 440~470 nm range but with slightly different peak values [5]. *H. marmoreus* have excellent storage properties, so this mushroom was good export agriculture products in Korea. In Korea, there has been very little research conducted to investigate the effects of light on mushroom bottle cultivation; however, most mushrooms, including *H. marmoreus*, require light to develop properly. Therefore, we investigated the application of LED light to the cultivation of edible mushrooms in this study.

In this study, *H. marmoreus* Mangak No. 2 were investigated. Mycelia isolates were grown on potato dextrose agar. And the substrate for Mangak No. 2 consisted of Douglas fir sawdust, corncobs, soybean hulls, and wheat bran at a 40:30:15:15 ratio (v/v).

Samples were grown for 80 days at 20 ± 1°C, with samples being moved to an LED growth chamber after primordia formed. The size of the LED growth chamber was 950 (W) × 400 (H) × 1,000 (D) mm, and this chamber was divided into two zones, and we put in the LED chamber for the mushroom 2 box (32 bottles). In addition, the growth chambers contained two LED bars per zone (Fig. 1). The environmental conditions of the growth chamber...
were 15 ± 1°C, relative humidity 90 ± 5%, and CO₂ 1,500 ± 100 ppm. And we control the quantity of light below 150 lux by regulators. The experiment control was fluorescent lamp and darkness treatment. Following the growth period, samples were harvested and various growth properties were investigated.

For analysis of ergosterol, freeze dried mushroom sample powder (0.3 g) was accurately weighed into 250 mL round bottom flasks and mixed with 10 mL of sodium ascorbate solution (17.5 g of sodium ascorbate in 100 mL of 2 M NaOH), 50 mL of ethanol (95% pure, Riverbank Chemicals, Singapore), and 10 mL of 50% potassium hydroxide (85% pure, Merck Chemicals, Darmstadt, Germany). The mixture was then saponified under reflux at 80°C for 1 hr, after which it was immediately cooled to room temperature and transferred into a separating funnel. Each tube was cooled and 3 mL of deionized water plus 2 mL of n-hexane were added. Next, the mixture was vortexed for a few seconds, after which the sample was allowed to settle for 2 min, and approximately 1.5 mL of the supernatant (n-hexane + ergosterol) were removed. The sample was then placed in a new glass tube and evaporated under N₂ gas. The dried sample solution was stored in a dark, −20°C refrigerator until further analysis. The extraction sample solution (20 µL) was injected into a Shimadzu Prominence high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) with a C18 column (C18 100A 5 µm column, 4.6 mm × 150 mm). The column was eluted with HPLC grade 99.9% methanol at 1.0 mL/min and 40°C and the ergosterol peak was detected at 280 nm.

To measure the DPPH (1,1-diphenyl-2-pycrylhydrazyl) scavenging activity, aliquots of 0.9 mL of 4.1 × 10⁻⁵ M DPPH ethanolic solution were mixed with 0.1 mL of the extracts. The mixture was then vigorously shaken and allowed to stand for 10 min under subdued light, after which the absorbance was measured at 517 nm. The DPPH radical scavenging activity (%) was calculated by the following equation: radical scavenging activity (%) = (1 − A_{sample}/A_{control}) × 100.

To determine the total phenolic content, the concentrations of phenolic compounds in the water extracts of mushrooms, expressed as gallic acid equivalents, were measured. The sample was mixed with 0.2 mL of 50% Folin and Ciocalteu's phenol reagent (Sigma, St. Louis, MO, USA). After 3 min, 1 mL of saturated 2% Na₂CO₃ was added to the mixture and it was diluted to 10 mL by adding distilled water. The reaction was kept in the dark for 30 min, after which its absorbance was read at 750 nm. A calibration curve was constructed using different concentrations of gallic acid as a standard.

To determine the reducing power, various concentrations of mushroom extract in 1 mL of methyl alcohol were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 min, after which 2.5 mL of trichloroacetic acid (10%) were added and it was centrifuged for 10 min at 12,000 rpm, and the absorbance was measured at 700 nm. Ascorbic acid was used as a standard. The EC₅₀ value, which was considered to be the effective concentration at which the absorbance was 0.5 for reducing power, was obtained by interpolation from linear regression analysis.

The primordia formed by *H. marmoreus* cultivated under LEDs are shown in Fig. 2. Fluorescent lamps and green LEDs resulted in rapid formation of the pileus, while darkness and red LEDs resulted in slow formation. Under blue LEDs, the primordia did not form.

The lightness value of the pileus was lowest under the fluorescent lamp, blue LEDs and green LEDs, and highest under the red LEDs and in the dark (Table 1). The fruit body grown under long-wavelength light tends to be white [6], while those grown under short-wavelength light tend to be black. Moreover, the pileus of *Pholiota nameko* grown under fluorescent lamps and blue LEDs were black [7]. Taken together, these findings were consistent with the results of our study.

The configuration of the pileus grown under blue LEDs

![Fig. 1. Light emitting diode (LED) growth chamber. A, Chamber photography; B, Mushroom in the chamber.](image-url)

![Fig. 2. Photographs of primordia of *Hypsizygus marmoreus* grown under different light emitting diodes (LEDs).](image-url)
was similar to those cultivated under fluorescent light, and was longer than those grown under other treatments. In addition, the length of stipes of oyster mushrooms cultivated under red LEDs was longer than that of mushrooms cultivated under other conditions (Table 2). The commercial yields of mushrooms cultivated under blue LEDs, green LEDs and fluorescent lights were similar, with the highest value of 88% being observed in response to the blue LED treatment (Table 3). The longest diameter of pileus of *H. marmoreus* was observed following treatment with blue LEDs [8], and an increase in the yield of fruit body was reported in mushrooms cultivated under 340–520 nm light because of activation of ATP synthase [6]. Overall, the results presented herein were similar to those of prior studies, and blue LEDs produced fruit body with better features than the other treatments (Fig. 3).

The ergosterol content of the fruit body of mushrooms cultivated under blue LEDs was highest, while that of mushrooms grown under red LEDs was lowest (Fig. 4). DPPH radical scavenging activity of *H. marmoreus* was higher in response to all LED treatments than fluorescent

### Table 1. Effects of light emitting diode (LED) source on pileus color

| Light source  | Lightness (L) | Redness (a) | Yellowness (b) |
|---------------|---------------|-------------|----------------|
| Fluorescent lamp | 37 c         | 6.8 ab      | 15.9 c        |
| Darkness      | 66 a          | 5.6 b       | 23.7 a        |
| Blue LED      | 36 c          | 6.6 ab      | 15.6 c        |
| Green LED     | 39 c          | 7.3 a       | 18.3 b        |
| Yellow LED    | 59 b          | 7.2 a       | 24.4 a        |
| Red LED       | 72 a          | 4.5 c       | 23.8 a        |

*Values followed by the same letter do not differ significantly at \( p > 0.05 \) according to Duncan’s multiple range test.

### Table 2. Effect of light emitting diode (LED) sources on properties of fruit body in *Hypsizygus marmoreus*

| Light source | Diameter of pileus (mm) | Thickness of stipe (mm) | Length of stipe (mm) | Diameter of pileus/Length of stipe |
|--------------|-------------------------|-------------------------|----------------------|-----------------------------------|
| Fluorescent lamp | 24 a                   | 12 a                    | 79 c                 | 0.30                              |
| Darkness     | 16 c                    | 10 b                    | 89 c                 | 0.21                              |
| Blue LED     | 22 a                    | 12 a                    | 68 d                 | 0.15                              |
| Green LED    | 19 b                    | 10 b                    | 98 b                 | 0.14                              |
| Yellow LED   | 14 c                    | 10 b                    | 103 a                | 0.16                              |

*Values followed by the same letter do not differ significantly at \( p > 0.05 \) according to Duncan’s multiple range test.

### Table 3. Effect of light emitting diode (LED) source on yield of *Hypsizygus marmoreus*

| Light source | No. of available stipes (No./850 mL) | Yield (g/850 mL) | Commercial yields (g/850 mL) | Commercial yields index (%) |
|--------------|--------------------------------------|------------------|-------------------------------|----------------------------|
| Fluorescent lamp | 28 ab                  | 148 b            | 122 ab                        | 82                          |
| Darkness     | 31 a                   | 156 a            | 114 b                         | 73                          |
| Blue LED     | 29 ab                  | 159 a            | 135 a                         | 85                          |
| Green LED    | 35 a                   | 158 a            | 130 a                         | 82                          |
| Yellow LED   | 28 ab                  | 129 c            | 96 c                          | 74                          |
| Red LED      | 27 b                   | 126 c            | 77 d                          | 61                          |

*Values followed by the same letter do not differ significantly at \( p > 0.05 \) according to Duncan’s multiple range test.

*Commercial yields/yield × 100.*
lamp and darkness treatment (Fig. 5). The total polyphenol content of \textit{H. marmoreus} was higher under green LEDs than the other treatments (Fig. 6). The reducing power of \textit{H. marmoreus} according to light sources was higher under all LED treatments than under fluorescent lights and darkness (Fig. 7).

In conclusion, cultivation of \textit{H. marmoreus} under blue LEDs is useful for generating fruit body with good features, as well as high ergosterol levels, DPPH radical scavenging activity, total polyphenol content and reducing power.

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