

## Enhanced Production of Fruiting Body and Bioactive Ingredients of *Cordyceps militaris* with LED Light Illumination Optimization

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### ABSTRACT

This study aimed to study the effect of different color combinations of LED light on the quantity of primordia, the formation of the fruiting body, and the yield of bioactive ingredients produced in solid culture *C. militaris*. The results show that appropriate color combinations and light intensities of Light-Emitting Diodes (LEDs) can significantly increase the quantity of primordia, promote the formation of fruiting bodies, and increase the yield of bioactive ingredients (cordycepin, adenosine, mannitol, and polysaccharides) in solid culture of *Cordyceps militaris*. LEDs have several advantages such as small size, long lifespan, fast response time, high efficiency and stability, and the ability to emit a narrow wavelength range of monochromatic light from a cold source. Our results show that both red and green-LEDs can shorten the period of primordia appearance. Red-LED increased the number of primordia and biological efficiency and red and blue LED combinations increased the biomass of the fruiting body. A light intensity of 1,000 Lux resulted in the fastest appearance of primordia, and the highest average fruiting body biomass and biological efficiency. Under different red and blue LED combinations, the highest amounts of cordycepin, adenosine, mannitol, and polysaccharide in the fruiting body of *C. militaris* were  $15.63 \pm 0.72$  (100 Lux),  $23.17 \pm 1.22$  (1,000 Lux),  $19.84 \pm 0.84$  (300 Lux), and  $28.69 \pm 1.52$  mg g<sup>-1</sup> (1,000 Lux), respectively. Appropriate color combinations and intensities of LED light can significantly increase the quantity of the primordium, promote the formation of the fruiting body, and increase the yield of bioactive ingredients produced in solid culture *C. militaris*.

**Keywords:** Biological efficiency, Cordycepin, LED color combinations, Primordium.

### INTRODUCTION

*Cordyceps militaris* is a parasitic fungus of the pupa and larva of Lepidoptera insects, and has similar chemical properties as the fungus used in traditional Tibetan and Chinese medicine, *Ophiocordyceps sinensis*. Its fruiting body is easier to cultivate by artificial cultivation and has been used as a

substitute for *O. sinensis* (Shrestha *et al.*, 2012b).

*C. militaris* has diverse biological activities, including pro-sexual, anti-inflammatory, anti-oxidant, anti-aging, anti-tumor, immunomodulatory, anti-microbial, insecticidal, anti-fibrotic, steroidogenic, hypolipidemic, anti-angiogenic, anti-diabetic, anti-fatigue, neuroprotective, liver-protective, renoprotective, as well as

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pneumoprotective activities (Das *et al.*, 2010). *C. militaris* contains several bioactive ingredients, such as cordycepin (Olatunji *et al.*, 2016; Yuan *et al.*, 2016), adenosine (Guo *et al.*, 2016; Liang *et al.*, 2014), mannitol (Guo *et al.*, 2016; Liang *et al.*, 2014), and polysaccharides (Liu *et al.*, 2016; Luo *et al.*, 2017). Besides being a popular health food, *C. militaris* has a high potential to be developed as a raw material for new drugs.

Light-Emitting Diodes (LEDs) have many advantages such as small size, long lifespan, fast response time, high efficiency and stability, and the ability to emit a narrow wavelength range of monochromatic light from a cold source (Fung *et al.*, 2016; Li *et al.*, 2016; Wu and Ma, 2016). Monochromatic LED has already been used for the production of exopolysaccharides and mycelium of *C. militaris* solid culture. (Kho *et al.*, 2016). Green or yellow light gave the best growth rates, while blue, green, yellow, red, and white light all promoted exopolysaccharide synthesis, compared with dark culture. A previous study has also reported that the highest contents of adenosine were obtained with red light and pink light was the most optimal for cordycepin accumulation in the fruiting body of *C. militaris* (Dong *et al.*, 2012). The cordycepin content was significantly higher under white (Red:Blue:Far-red= 6:3:1) light treatment, with a 62% increase with a combination of different colored LED lights in comparison to fluorescent light (Yi *et al.*, 2014).

As stated above, the appearance time and number of primordia, transformation rate, maturation time, biological efficiency of the fruiting body, and the contents of the bioactive ingredients of *C. militaris* are all significant factors that affect production costs and product competitiveness. The effects of LED light illumination on mycelium and bioactive ingredients, such as exopolysaccharides, adenosine, and cordycepin in the fruiting body of *C. militaris* have been described previously. However, there are no studies on the appearance time and numbers of primordia, transformation rate and maturation time of the fruiting body, or biological efficiency in

relation to bioactive ingredients. Furthermore, the effects of LED light illumination on the formation of the primordium and fruiting body, and contents of bioactive ingredients, such as cordycepin, adenosine, mannitol, and polysaccharides, in the fruiting body of *C. militaris* are currently unknown. This study aimed to study the effect of different color combinations of LED light on the quantity of primordia, the formation of the fruiting body, and the yield of bioactive ingredients produced in solid culture *C. militaris*.

## MATERIALS AND METHODS

### Fungal Strain

*Cordyceps militaris* strain THH (BCRC 930167) was obtained from Culture Collection and Research Centre, Food Industry Research and Development Institute, Hsinchu, Taiwan.

### Liquid Culture Medium

The liquid culture medium consisted of glucose 10 g L<sup>-1</sup>, malt extract 3 g L<sup>-1</sup>, peptone 5 g L<sup>-1</sup>, and yeast extract 3 g L<sup>-1</sup>, pH 6.0. Two hundred milliliters of liquid culture medium was added to a 500 mL flask and autoclaved at 121°C for 20 minutes.

### Liquid Culture Preparation

Ten cultures (diameter around 0.4–0.5 cm) from Potato Dextrose Agar (PDA) slants containing pre-cultured *C. militaris* were subcultured into liquid culture medium and grown for 10 days at 25°C with shaking at 140 rpm.

### Brown Rice Culture Medium

Twenty-five grams of brown rice and 40 mL of liquid culture medium were added into a 500 mL bottle and sealed with two layers of

polypropylene film before sterilization at 121°C for 1 hour.

### Solid Culture of Fruiting Body

After sterilization and cooling, 6 mL of liquid culture was added to the 500 mL culture bottles under aseptic conditions. Each treatment was carried out in triplicate. Every culture bottle was sealed with a breathable lid and wrapped with Kraft paper. The culture bottles were placed in 20°C and 60% relative humidity and cultured for 7–10 days in the dark with constant temperature and humidity. After the brown rice culture was filled with mycelium, the bottles were transferred to an incubation room at 60% humidity for LED illumination. Light cycles were controlled using timers and the incubation temperature was fixed at 22°C. LED light sources were attached to the top of stainless steel shelves and different light colors and light intensities were used for experiments.

### Light System Conditions

Three different wavelengths of light were used for single LED experiments: blue light (465–475 nm), green light (520–530 nm), and red light (625–630 nm), with fluorescent light as a control. For experiments using LED combinations, four combinations of LED lights were used: red+green (6:3), red+blue (8:1), green+blue (3:6), and red+green+blue (6:2:1), and fluorescent light was used as a control group. A fixed voltage of 24V was used together with a variable resistor added to the circuit for the adjustment of current. LED light intensity was controlled by the current and supplemented by height adjustment. A photometer was used to directly measure the average intensity light, in order to provide variable light intensity for *C. militaris* culture experiments. In LED color experiments, the light intensity was fixed at 500 Lux ( $\pm 15\%$ ). In light intensity

experiments, four different light intensities were used: 100 ( $\pm 15\%$ ), 300 ( $\pm 15\%$ ), 500 ( $\pm 15\%$ ), and 1,000 Lux ( $\pm 15\%$ ) with photoperiods (light/dark) of 16/8 hours. Each experimental group in the above-mentioned experiments consisted of 10 bottles that were observed once every day.

### Sample Preparations for Analysis

The dried fruiting bodies of *C. militaris* were ground into powder and mixed with distilled water. The extraction was carried out using an ultrasonic water bath for 10 min. The solution was placed in boiling deionized water and extracted for 3 hours. The supernatant was centrifuged at 14,000×g for 10 minutes and filtered through 0.22  $\mu\text{m}$  membrane filter. The filtrate was used for the determination of cordycepin, adenosine, and mannitol by HPLC analysis. Anhydrous ethanol was added to the supernatant after extraction to a final concentration of 80% (v/v), and the mixture was kept in a beaker overnight at 4°C. The precipitate was collected after centrifugation at 5,000×g for 15 minutes to obtain crude polysaccharides for the determination of polysaccharide content by phenol–sulfuric acid method.

Previously described methods with slight modifications were used for the analysis of cordycepin (Meena *et al.*, 2010), adenosine (Ikeda *et al.*, 2008), mannitol (Hu *et al.*, 2015) and polysaccharides (Dong *et al.*, 1996).

### HPLC Analysis for Cordycepin, Adenosine, and Mannitol

The concentrations of cordycepin and adenosine were determined by HPLC equipped with a UV detector (L-4250, HITACHI L-6200) and Cosmosil C-18 column (4.6×250 mm, 5  $\mu\text{m}$ ). The chromatogram was monitored by UV absorbance at 260 nm. The standards for cordycepin and adenosine were purchased



from Sigma Chemical Corporation and injected at five sample volumes of 7.8125–500  $\mu\text{g mL}^{-1}$  to create the calibration curve. The mobile phase consisted of methanol and water (15/85, v/v). The flow rate was 1.0  $\text{mL min}^{-1}$  and the column temperature was 30°C. The concentration of mannitol was determined by HPLC with a refractive index detection system and Cosmosil NH2 column (4.6×250 mm, 5  $\mu\text{m}$ ). The mobile phase was 0.05  $\text{g L}^{-1}$  EDTA calcium disodium solution with a flow rate of 0.5  $\text{mL min}^{-1}$  and the column temperature of 85°C.

### Determination of Polysaccharide Content

The determination of polysaccharide content was performed by phenol-sulfuric acid method (Dong *et al.*, 1996) after ethanol precipitation of the sample. Briefly, 1 mL of crude polysaccharide solution was mixed with 3 mL concentrated sulfuric acid to initiate the reaction. Phenol (0.6 mL; 5%) was then added and the mixture was kept at 100°C for 15 minutes. After cooling to the room temperature, the absorbance of the reaction mixture was measured at 490 nm using a spectrophotometer (polysaccharide content was determined with d-glucose as standard).

### Statistical Analysis

Data from each test were presented as the mean±standard deviation. The statistical significance of the difference between the means of individual groups was assessed using 1-way analysis of variance with Duncan's multiple range test.

## RESULTS

### Effects of Different Monochromatic Light

The effects of different monochromatic light on the formation of the primordium and fruiting body, and biological efficiency of *C. militaris* are shown in Table 1. Compared with the control group, the red and green lights only shorten the primordium formation time and do not affect the maturation of the fruiting body. Red light was also observed to increase the number of primordia and biological efficiency. No significant effects on the biological efficiency or period of fruiting body maturation were observed for any of the other colored LEDs. Red and blue lights were found to increase the biomass of fruiting bodies. When red light was used for illumination, the period of primordia appearance was the shortest at 5.35 days, the number of primordia

**Table 1.** Effects of illumination of monochromatic light LED on primordia, fruiting body and biological efficiency of *Cordyceps militaris*.<sup>a</sup>

| LED color                   | Period of primordia appearance (Day) <sup>b</sup> | Number of primordia per bottle <sup>c</sup> | Biomass of fruiting body (g) per bottle <sup>d</sup> | Period of fruiting body maturation (Day) <sup>e</sup> | Biological efficiency (%) <sup>f</sup> |
|-----------------------------|---|---|--|---|--|
| Red                         | 5.35 ± 0.42 <sup>b</sup>                          | 24.30 ± 2.06 <sup>a</sup>                   | 2.11 ± 0.15 <sup>a</sup>                             | 34.10 ± 1.91 <sup>a</sup>                             | 52.25 ± 9.04 <sup>a</sup>              |
| Green                       | 5.48 ± 0.39 <sup>b</sup>                          | 21.70 ± 2.36 <sup>b</sup>                   | 1.72 ± 0.13 <sup>b</sup>                             | 33.30 ± 2.16 <sup>a</sup>                             | 39.82 ± 2.80 <sup>b</sup>              |
| Blue                        | 6.40 ± 0.73 <sup>a</sup>                          | 17.30 ± 3.16 <sup>c</sup>                   | 1.96 ± 0.21 <sup>a</sup>                             | 32.30 ± 3.86 <sup>a</sup>                             | 42.52 ± 6.30 <sup>b</sup>              |
| Fluorescent light (Control) | 6.77 ± 0.51 <sup>a</sup>                          | 18.70 ± 2.83 <sup>c</sup>                   | 2.01 ± 0.18 <sup>a</sup>                             | 33.90 ± 2.08 <sup>a</sup>                             | 45.97 ± 6.17 <sup>b</sup>              |

<sup>a</sup> Means with different letters in the same column are significantly different ( $P < 0.05$ ). Light distance: 27 cm, Light/Dark cycle: 16/8 hours. <sup>b</sup> Time from LED light illumination to the first primordium appearance in bottle.

<sup>c</sup> The number of primordia produced in bottle on the 14<sup>th</sup> day. <sup>d</sup> The total dry weight of fruiting bodies collected in every bottle (500 mL jar) after two months of cultivation. <sup>e</sup> Time from secretion of yellow pigments by aerial mycelia in bottle walls to 80% of fruiting bodies with height greater than 2 cm. <sup>f</sup> (Fresh weight of fruiting bodies/Dry weight of culture medium)×100%.

was the most at 24.30, the biomass of fruiting body was the highest at 2.11 g bottle<sup>-1</sup>, the period of fruiting body maturation was the shortest at 34.10 days, and biological efficiency was the highest at 52.25%.

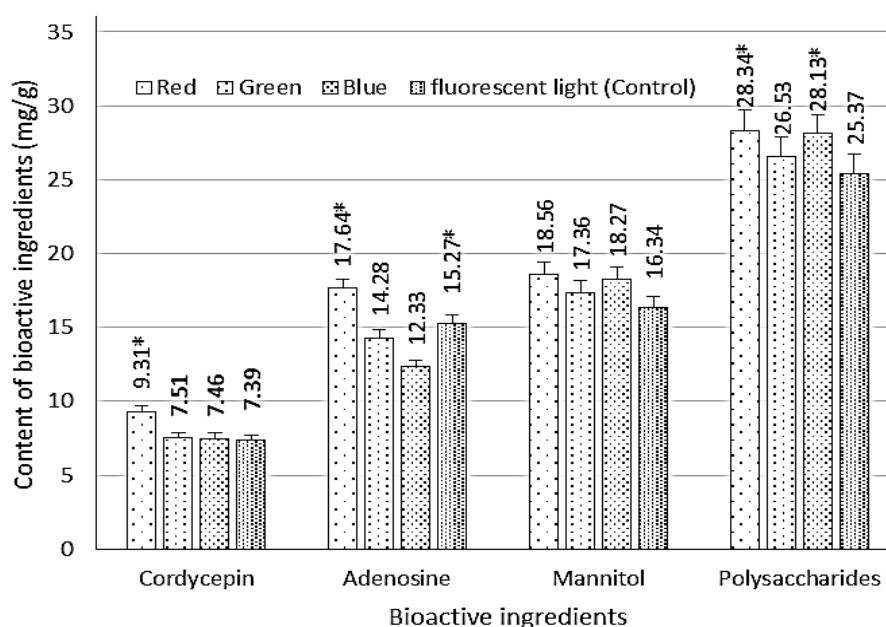
In this study, the biological efficiency was 39.82–52.25% when monochromatic LED light was used to induce the formation of fruiting bodies in *C. militaris*, with red light exhibiting the highest biological efficiency. There were no significant differences detected between green, blue, and fluorescent lights. The biological efficiency in this study was lower than that observed by Qin *et al.* (2003) (66.8–86.6%), who used different grains (rice, brown rice, millet, corn flour, wheat grain, and sorghum). These differences are likely attributed to the different media or cultivars used in the previous study. Shrestha *et al.* (2012a) showed that the biological efficiency was only 14–27% when brown rice was used as the culture medium for cultivation of different isolates and F<sub>1</sub> progeny strains from multi-ascospores, which was significantly lower than the biological efficiency in this

study.

Kho *et al.* (2016) found that illumination by blue light or white light significantly decreases the growth rate of *C. militaris* mycelium in plate cultures. The results of our study demonstrated that illumination by blue light or fluorescent light significantly delayed the period of primordia appearance, and decreased primordia numbers. In addition, green light significantly decreased the biomass of the fruiting body and the biological efficiency in comparison with other colored lights. There were no significant differences observed in the period of fruiting body maturation among fluorescent light and LED color light illumination.

#### Effects of Different Monochromatic Light on Bioactive Ingredients in Fruiting Body of *C. militaris*

The effects of different monochromatic light on bioactive ingredients in *C. militaris* fruiting body are shown in Figure 1. Red



**Figure 1.** Effects of illumination of monochromatic light LED set at 27 cm above the top of the bottle and light cycle of 16:8 hours (light:dark) on production of bioactive ingredients from *Cordyceps militaris* fruiting body. [Values are means, with standard errors represented by error bars. Mean values were significantly different from the control (fluorescent light): \*  $P < 0.05$ ;  $n = 10$ ].



light significantly increased cordycepin and adenosine production. There were no significant differences among different colored LEDs in the synthesis of mannitol and polysaccharides. When illuminated by red light, the production of cordycepin, adenosine, mannitol, and polysaccharide in the fruiting body were the highest at 9.31, 17.64, 18.56, and 28.34 mg g<sup>-1</sup>, respectively.

Red light showed higher production of cordycepin compared with fluorescent light, while there were no significant differences between green, blue, and fluorescent lights. Yi *et al.* (2014) reported that red light significantly increased cordycepin content in *C. militaris* fruiting body, but there were no significant differences when far-red light, blue light or multiple color (red+blue+far-red) light combinations were compared with fluorescent light. However, Dong *et al.* (2012) found that the LED color light combination of red+blue (2:1) was more favorable for cordycepin production in the *C. militaris* fruiting body, and red light showed no significant differences from normal sunlight.

While red light promoted adenosine synthesis, both green and blue lights had inhibitory effects. Dong *et al.* (2012) also found that red light could promote adenosine synthesis in the *C. militaris* fruiting body, while blue light resembled sunlight and showed no effect on adenosine synthesis.

There are previous studies on the effects of LED color on the mannitol content in *C.*

*militaris* fruiting body. The results of our study demonstrated that red light, green-red and blue light could promote mannitol synthesis compared with fluorescent light, and there were no significant differences between these three colors.

Red light, green light, and blue light all significantly increased polysaccharide content compared with fluorescent light. Although there were no significant differences between these three colors, both blue light and red light showed slightly increased polysaccharide production. Kho *et al.* (2016) demonstrated that amongst *C. militaris* cultivated under five different LED colors (blue, green, yellow, red, and white), a 10-day plate culture of *C. militaris* mycelium grown in blue light had the highest exopolysaccharide content (2,404.2 mg L<sup>-1</sup>) compared with dark cultivation, although there were no significant differences among those lights.

#### Effects on Formation of Primordia, Fruiting Body, and Biological Efficiency of *C. militaris*

Table 2 shows the effects of illumination by multiple color/light combinations of LED on the formation and development of the primordium and fruiting body. Compared with the control group, the color light combinations red+green, red+blue and red+green+blue all shortened the period of primordia appearance. The average number

**Table 2.** Effects of illumination of multiple color light combinations on primordia, fruiting body, and biological efficiency of *Cordyceps militaris*.<sup>a</sup>

| LED color                   | Period of primordia appearance (Day) | Number of primordia per bottle | Biomass of fruiting body (mg) per bottle | Period of fruiting body maturation (Day) | Biological efficiency (%)  |
|-----------------------------|--------------------------------------|--------------------------------|--|--|----------------------------|
| Red+Green                   | 5.88 ± 0.13 <sup>b</sup>             | 15.30 ± 2.51 <sup>b</sup>      | 2.02 ± 0.23 <sup>ab</sup>                | 39.30 ± 2.89 <sup>a</sup>                | 47.73 ± 4.18 <sup>ab</sup> |
| Red+Blue                    | 5.58 ± 0.29 <sup>b</sup>             | 21.10 ± 3.15 <sup>a</sup>      | 2.40 ± 0.18 <sup>a</sup>                 | 40.20 ± 3.61 <sup>a</sup>                | 56.65 ± 6.25 <sup>a</sup>  |
| Green+Blue                  | 6.88 ± 0.38 <sup>a</sup>             | 21.00 ± 2.65 <sup>a</sup>      | 1.77 ± 0.22 <sup>b</sup>                 | 40.50 ± 2.52 <sup>a</sup>                | 39.66 ± 5.92 <sup>b</sup>  |
| Red+Green+Blue              | 5.93 ± 0.23 <sup>b</sup>             | 17.60 ± 2.23 <sup>ab</sup>     | 2.23 ± 0.26 <sup>a</sup>                 | 37.10 ± 2.08 <sup>a</sup>                | 52.96 ± 7.88 <sup>a</sup>  |
| Fluorescent light (Control) | 6.20 ± 0.51 <sup>b</sup>             | 16.30 ± 2.15 <sup>b</sup>      | 2.16 ± 0.29 <sup>ab</sup>                | 40.10 ± 3.46 <sup>a</sup>                | 51.40 ± 8.84 <sup>ab</sup> |

<sup>a</sup> Means with different letters in the same column are significantly different (P < 0.05). Light distance: 27 cm, Light/Dark cycle: 16/8 hours.

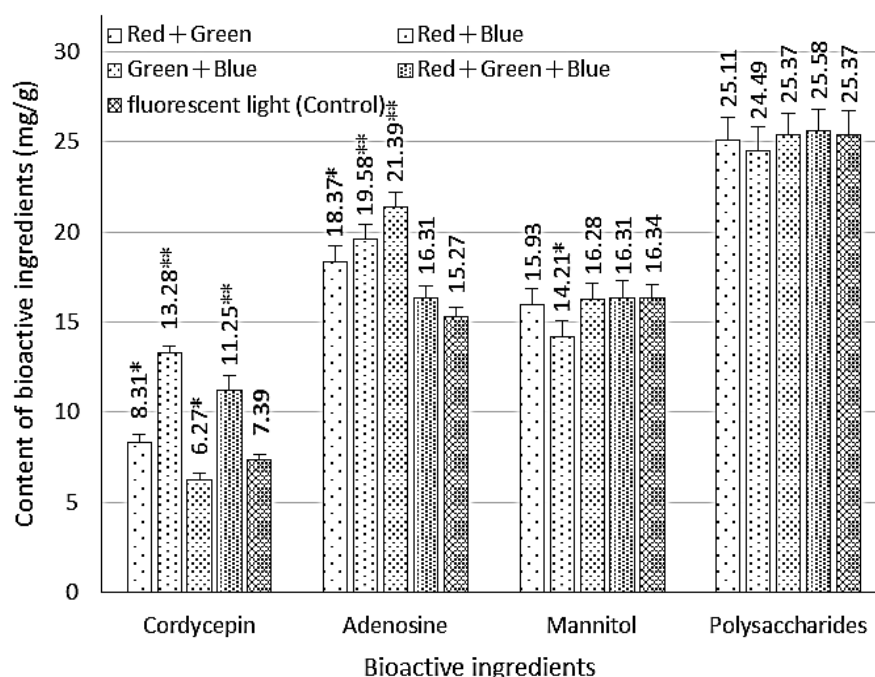
of primordia formed was significantly higher with the color/light combinations red+blue and red+green+blue. Also, red+blue and red+green+blue significantly promoted the biomass and biological efficiency of fruiting body. Illumination by color/light combinations showed no significant effects on fruiting body maturation period.

Yi *et al.* (2014) found that color/light combinations could affect that quantity of *C. militaris* primordia formed and fruiting body biomass. In addition, using different ratios of red, far-red, and blue light, they found that the combination of red+far-red+blue LED in ratios of 6:1:3 and 4:4:2 produced the highest quantity of *C. militaris* primordia and highest fruiting body biomass, respectively. These results demonstrate that the light combinations for inducing primordia formation and fruiting body biomass are not the same. Dong *et al.* (2012) found that pink light (Red:Blue= 2:1) produced the best results for fruiting body

biomass and biological efficiency of *C. militaris*.

### Effects on the Bioactive Ingredients

Figure 2 shows the effects of multiple light combinations on the bioactive ingredients in *C. militaris* fruiting body. Compared with the control group, the color/light combinations of both red+blue and red+green+blue could significantly promote cordycepin synthesis. The cordycepin yield ( $13.28 \pm 0.76 \text{ mg g}^{-1}$ ) with the combination red+blue was 1.79 times that of the control group ( $7.39 \pm 0.46 \text{ mg g}^{-1}$ ), and further studies on light intensity were carried out using this light combination. Compared with the control group, the combinations red+green, red+blue, and green+blue promoted adenosine synthesis, and the combination red+green+blue showed significant inhibition of adenosine synthesis. No significant effects on mannitol and



**Figure 2.** Effects of illumination of multiple light LED set at 27 cm above the top of bottle and light cycle 16/8 hours (light:dark) on production of bioactive ingredients from *Cordyceps militaris* fruiting body. [Values are means, with standard errors represented by error bars. Mean values were significantly different from the control (fluorescent light): \*  $P < 0.05$  and \*\*  $P < 0.01$ ;  $n = 10$ ].



polysaccharide production were observed with different combinations of LED lights.

Yi *et al.* (2014) showed that illumination by red light significantly increased cordycepin content in the *C. militaris* fruiting body, however, the combination red+blue+far-red showed no significant differences compared with fluorescent light. Cordycepin yield under illumination by red light reached 13.4 mg g<sup>-1</sup>, which was close to the cordycepin yield (13.28 mg g<sup>-1</sup>) when illuminated under the combination red+blue light in this study. Dong *et al.* (2012) also found that different colored lights are required for different bioactive ingredients in *C. militaris*. The best colored lights for promoting cordycepin synthesis in decreasing order were pink, blue, sunlight, and red color; the best colored lights for promoting adenosine synthesis in decreasing order were red, pink, blue, sunlight, and pink light (Red:Blue= 2:1) had the highest cordycepin yield of 0.67 mg g<sup>-1</sup>. In our study, the combination red+blue light also contributed to the highest cordycepin yield of 13.28 mg g<sup>-1</sup> in the *C. militaris* fruiting body among colored light combinations.

### Effects of Light Intensity on Formation of Primordia, Fruiting Body, and Biological Efficiency of *C. militaris*

The color/light combination red+blue (8:1) promoted cordycepin synthesis in *C. militaris* fruiting body. Table 3 shows the effects of different light intensities using the combination red+blue on the formation of

primordia, fruiting body and biological efficiency. Results showed that a light intensity of 1,000 Lux could shorten the period of primordia appearance by about 1 day and increase the average fruiting body biomass per bottle. As light intensity increases, the average number of primordia in the bottle also increases. The biological efficiency of *C. militaris* fruiting body under light intensities of 500 and 1,000 Lux was significantly higher than that under light intensities of 100 and 300 Lux. As light intensity increased, the fruiting body biomass and biological efficiency increased, showing that fruiting body biomass and biological efficiency are correlated with light-intensity.

Wu *et al.* (2016) investigated the effects of light intensity on *C. militaris* cultivation and carried out experiments under three different ranges of light intensity of fluorescent light, namely, 1,250±250, 1,750±250, and 2,500±500 Lux. Their results showed that the highest fruiting body yield and biological efficiency were 4.06 g dry weight/bottle and 86.83%, respectively, under 1,750±250 Lux during the primordial initiation stage and the fruiting stage. Our study found that the biomass of fruiting body and biological efficiency were the highest at 1,000 Lux, followed by at 500 Lux, which could be attributed to differences in light sources (fluorescent light vs. LED light) and intensity ranges.

Compared with high intensity LED, low intensity LED was more favorable for cordycepin production of the fungus. Similar

**Table 3.** Effects of illumination of different light intensities of LED on primordia, fruiting body and biological efficiency of *Cordyceps militaris*.<sup>a</sup>

| Light intensity (Lux) | Period of primordia appearance (Day) | Number of primordia per bottle | Biomass of fruiting body (g) per bottle | Period of fruiting body maturation (Day) | Biological efficiency (%) |
|-----------------------|--------------------------------------|--------------------------------|---|--|---------------------------|
| 100                   | 5.96 ± 0.31 <sup>a</sup>             | 17.10 ± 1.73 <sup>b</sup>      | 1.95 ± 0.18 <sup>b</sup>                | 38.30 ± 2.08 <sup>a</sup>                | 47.51 ± 6.49 <sup>b</sup> |
| 300                   | 5.75 ± 0.38 <sup>a</sup>             | 17.40 ± 2.52 <sup>b</sup>      | 2.08 ± 0.13 <sup>b</sup>                | 37.60 ± 2.65 <sup>a</sup>                | 51.75 ± 2.70 <sup>b</sup> |
| 500                   | 5.67 ± 0.31 <sup>a</sup>             | 23.20 ± 2.65 <sup>a</sup>      | 2.39 ± 0.11 <sup>b</sup>                | 32.80 ± 1.00 <sup>b</sup>                | 60.87 ± 2.65 <sup>a</sup> |
| 1000                  | 4.67 ± 0.26 <sup>b</sup>             | 23.60 ± 2.52 <sup>a</sup>      | 2.84 ± 0.13 <sup>a</sup>                | 36.30 ± 2.08 <sup>a</sup>                | 66.87 ± 5.06 <sup>a</sup> |

<sup>a</sup> Means with different letters in the same column are significantly different (P< 0.05). Light cycle (Light/Dark): 16/8 hours.



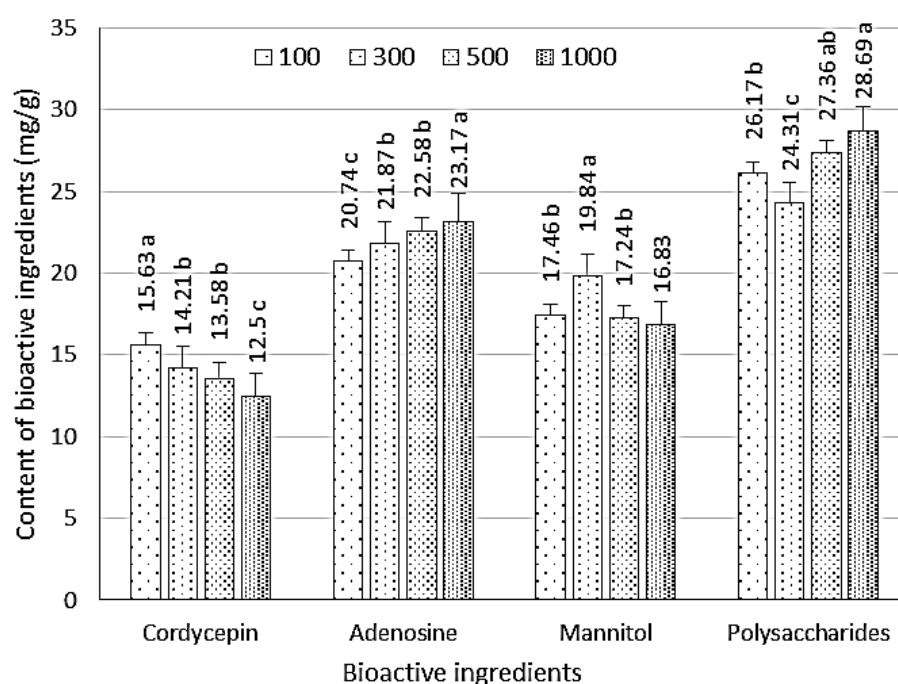
observations were found in a recent study of *Monascus ruber* M7 (Wang *et al.*, 2016), where a low intensity (500 Lux) of blue light decreased biomass yield and increased red pigment accumulation, but did not stimulate citrinin synthesis. Additionally, a high intensity (1,500 Lux) of blue light decreased citrinin synthesis without affecting biomass or red pigment accumulation.

### Effects of Light Intensity on Bioactive Ingredients in *C. militaris* Fruiting Body

Figure 3 shows the effects of different light intensities of the color/light combination red+blue on bioactive ingredients in *C. militaris* fruiting body. As light intensity increases, cordycepin yield gradually decreases, while adenosine yield gradually increases. The light intensities of 100 and 1,000 Lux were the most favorable for the

yield of cordycepin ( $15.63 \pm 0.72$  mg g<sup>-1</sup>) and adenosine ( $23.17 \pm 1.22$  mg g<sup>-1</sup>), respectively. The highest yields of mannitol ( $19.84 \pm 0.84$  mg g<sup>-1</sup>) and polysaccharide ( $28.69 \pm 1.52$  mg g<sup>-1</sup>) were obtained with light intensities of 300 and 1,000 Lux, respectively.

Wu *et al.* (2016) also found that different intensities of fluorescent light were required for the formation of primordia, production of the fruiting body, and synthesis of cordycepin, mannitol, and polysaccharides in *C. militaris*. A light intensity of  $1,250 \pm 250$  Lux was favorable for the synthesis of cordycepin and polysaccharide, and mannitol synthesis was more optimal at  $1,750 \pm 250$  Lux. Fluorescent light intensity had no effect on adenosine synthesis at the stages of primordial initiation and fruiting body. However, our study showed that LED light intensity did affect adenosine content in the fruiting body of *C. militaris*.



**Figure 3.** Effects of LED light intensity by the color light combination set at light/dark cycle 16:8 hours on productions of bioactive ingredients from *C. militaris* fruiting body. (The means not sharing a common letter are significantly different among the same bioactive ingredient at  $P < 0.05$ ,  $n = 10$ ).



## DISCUSSION

Illumination by red light and green light significantly shortened the period of *C. militaris* primordia appearance and increased the numbers of primordia. Red light significantly increased cordycepin and adenosine production. There were no significant differences between different colored LEDs in the synthesis of mannitol and polysaccharides. The color/light combinations red+green, red+blue and red+green+blue shortened the period of primordia appearance, but green+blue was not favorable for primordia formation. The combinations red+blue and red+green+blue were the most favorable for cordycepin synthesis, and the red+green+blue inhibited adenosine synthesis. As light intensity increased, the average number of primordia in bottle also increased, along with a decrease in fruiting body maturation period. The biological efficiency of the *C. militaris* fruiting body under the light intensities of 500 and 1,000 Lux were significantly higher than that under the light intensities of 100 and 300 Lux. As light intensity increased, the fruiting body biomass and biological efficiency also increased, showing that fruiting body biomass and biological efficiency are correlated with light-intensity. Under illumination with the combination red+blue light, light intensity increased and cordycepin yield gradually decreased, while adenosine yield gradually increased. Illumination with 100 and 1,000 Lux were the most favorable for cordycepin and adenosine yield, respectively. The highest mannitol and polysaccharide yields were obtained under light intensities of 300 and 1,000 Lux, respectively. Appropriate color combinations and light intensities of LED light can significantly increase the quantity of primordia produced, promote the formation of the fruiting body, and increase the yield of bioactive ingredients.

In conclusion, the color and intensity of illumination in the cultivation of *C. militaris* can greatly affect the production of the

fruiting body and bioactive ingredients. These results are of great significance for the bioengineering of new products.

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## افزایش تولید اندام باردهی و اجزای زیست فعال *Cordyceps militaris* با بهینه سازی نور چراغ دیودهای نوری (ال ای دی)

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### چکیده

هدف این آزمایش مطالعه اثر ترکیب های مختلف نور دیودهای نوری روی مقدار بافت ابتدایی (primordia)، تشکیل اندام باردهی، و اجزای زیست فعال تولید شده در کشت جامد *C. militaris* بود. نتایج نشان داد که ترکیب مناسب رنگ و شدت نور دیودهای نوری به افزایش مقدار بافت ابتدایی، افزایش تشکیل اندام های باردهی، و تولید اجزای زیست فعال ( شامل cordycepin، آدنوزین، مانیتول، و پلی ساکارید ها) در کشت جامد *Cordyceps militaris* منجر شد. دیودهای نوری فواید مختلفی دارند مانند کوچک بودن، دوام طولانی، سرعت واکنش سریع، کارایی بالا و پایداری، و توانایی انتشار یک محدوده باریک از طول موج نور تک رنگ از یک منبع سرد. نتایج ما نشان داد که هر دو دیودهای قرمز و سبز رنگ می توانند دوره ظهور بافت ابتدایی را کوتاه کنند. دیودهای نوری قرمز تعداد بافت ابتدایی و کارایی بیولوژیکی را زیاد کرد و ترکیب دیودهای نوری قرمز و آبی موجب افزایش زیست توده اندام باردهی شد. شدت نوری برابر ۱۰۰۰ لوکس موجب سریعترین ظهور بافت ابتدایی و بالاترین میانگین زیست توده اندام باردهی و کارایی بیولوژیکی شد. تحت ترکیب های مختلف دیودهای نوری قرمز و آبی، بیشترین مقدار cordycepin، آدنوزین، مانیتول، و پلی ساکارید ها در اندام باردهی *C. militaris* به ترتیب برابر بود با  $15/63 \pm 0/72$  میلیگرم در گرم برای ۱۰۰ لوکس،  $23/17 \pm 1/22$  میلیگرم در گرم برای ۱۰۰۰ لوکس،  $19/84 \pm 0/84$  میلیگرم در گرم برای ۳۰۰ لوکس، و  $28/69 \pm 1/52$  میلیگرم در گرم برای ۱۰۰۰ لوکس. ترکیب مناسب رنگ و شدت نور دیودهای نوری می تواند به طور معناداری باعث افزایش مقدار بافت های ابتدایی، ارتقای تشکیل اندام باردهی، و افزایش اجزای زیست فعال تولید شده در کشت جامد *C. militaris* شود.