

## LIGHT-STIMULATIVE EFFECTS ON THE CULTIVATION OF EDIBLE MUSHROOMS BY USING BLUE LED

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### ABSTRACT

Fruiting body formation of mushrooms is closely involved with “light”, which seriously affects their productivities in both quality and quantity. Primordium formation in several cultivable mushrooms requires light and seldom occurs under continuous darkness. Light also induces the development of fruiting bodies including stipe elongation and cap formation. LED (light emitting diode) has many advantages over current lightings and has been gradually replaced everywhere in recent years. These prompted us to develop more effective lightings in the cultivation of mushrooms, by using blue LED. The cultivations of popular mushrooms eaten in Japan (*Lentinula edodes*, *Flammulina velutipes*, *Hypsizygus marmoreus*, *Grifola frondosa*, *Pholiota nameko* and *Pleurotus eryngii*) were carried out with mushroom blocks (sawdust substrate media) under several lighting conditions. The exposure to continuous light by blue LED during vegetatively growing mycelial stage in *L. edodes* brought higher productivity and quantity of fruiting bodies than using usual white fluorescent lamps. The characteristic fruiting bodies of *P. nameko* and *P. eryngii* were efficiently obtained by the irradiation with blue LED during fruiting body formation, and high intensity at the primordial stage in *G. frondosa* caused high productivity of fruiting bodies, which were derived from highly induced primordia. Moreover, the surface exfoliation of cultivated mushroom-blocks, a serious issue especially found in *F. velutipes*, was successfully avoided by the exposure to blue LED at primordium formation. We present that several conditions of light environment artificially controlled by LED, a new valuable device, provide us more efficient production of cultivable mushrooms.

**Keywords:** Light; Blue LED; Fruiting body formation; Cultivation; Edible mushrooms

## INTRODUCTION

“Light” is closely involved with fruiting body formation of mushrooms. In particular, the productivities of several cultivable mushrooms have been found to depend on light environment in both quality and quantity. Primordium formation in several cultivable mushrooms such as *Lentinula edodes* (black mushroom, *shiitake*), *Flammulina velutipes* (winter mushroom, *enoki* mushroom), *Hypsizigus marmoreus* (*buna-shimeji*), *Grifola frondosa* (*maitake* mushroom) and *Pleurotus ostreatus* (oyster mushroom) requires light, and seldom occurs under continuous darkness [1-5]. Light also induces the development of fruiting bodies including stipe elongation and cap formation. These phenomena have been also acquired through the continuous processes of trial and error in ordinary mushroom cultivations. An attentive control of the light environment during mushroom cultivation is effective in the improvement of availabilities of valuable products of fruiting bodies.

To perceive environmental light stimuli essential for the initiation of fruiting development in mushrooms, sensory factors and/or machinery such as photoreceptors are believed to be necessary. Our group has analyzed several photoreceptors and photoresponsive factors, which are involved with fruiting body formation in mushrooms. In the basidiomycetous mushroom *Coprinopsis cinerea*, *dst1* and *dst2*, which were evidenced to be involved with photomorphogenesis, were genetically analyzed in detail [6-8]. The PHRA protein homologous to the *C. cinerea dst1* product, a blue-light photoreceptor in *L. edodes*, was identified as a resident protein containing a photo-reactive domain responding to light stimuli essential for fruiting development [9]. The photoreceptor complex of PHRA and PHRB likely regulates the transcription of the tyrosinase gene, whose product makes fruiting bodies turn brown [10]. Two developmental regulators in *L. edodes*, PRIB [11] and Le.CDC5 [12], are photoresponsive transcription factors and those abundant expressions completely coordinate with fruiting body formation in response to blue light [13]. PRIB and Le.CDC5 are suggested to be involved with fruiting development because of their binding activities to specific DNA sequences and the phosphorylation of those by protein kinase A [12,14].

In several cultivation houses for mushrooms, there is a variety of brightness because required artificial lightings are usually set up on the ceiling. Synchronized cultivation through fruiting development, which directly influences productive performances, is inhibited by the uneven luminous intensities under such a condition. To ensure the cultural synchronizations of fruiting development stages (mycelium, primordium, fruiting body, etc.), many cultivators usually give a great care to lighting environment in their cultivation houses.

LED (light emitting diode) has many advantages over current lightings and has been gradually replaced everywhere in recent years. Generally, lighting equipments using LED are compact and it is easy to install desirable lightings on each shelf of mushroom cultivations. The detailed advantages of LED are follows:

- (i) Saving electricity: The required electricity emitting the same intensity as usual fluorescent lamps illuminate is low. It can be expected to reduce a waste of electricity.
- (ii) Long life: The frequency of replacing bulbs is extremely lower than fluorescent lamps.
- (iii) Small and lightweight: As described above, installing lightings on each shelf can diminish places with uneven luminous intensities.
- (iv) Low generation of heat: High light-emitting efficiency causes less heat than usual fluorescent lamps. The problems, drying products and media by heat, are also settled.
- (v) Single wavelength & its selectivity: Desirable wavelength of light can be selected because LED has a sharp wavelength peak.

(vi) Strong structure: LED bulbs are stronger than fluorescent lamps.

The attempt to adopt LED lightings as the equipment in mushroom cultivation has already progressed. However, there are few analytical reports on optimal or favorable LED usages: improvement of light conditions (light wavelength, strength, timing of irradiations), designs of LED devices, installing LED on shelves, etc.

In this study, we describe the experiments for edible mushrooms cultivated in Japan, *L. edodes*, *F. velutipes*, *H. marmoreus*, *G. frondosa*, *Pholiota nameko* and *Pleurotus eryngii*, using blue LED, whose techniques provide us several valuable merits including further efficient production, change of fruiting body shapes, low electricity in cultivation houses.

## MATERIALS AND METHODS

**Mushroom strains.** The following commercial strains harboring favorable characteristics were used: (i) *L. edodes*: 607 (Hokken Co., Ltd.), XR1 (Mori & Company, Ltd.); (ii) *F. velutipes*: G-5 (Nagano-Nokoken); (iii) *H. marmoreus*: NN-12 (Nagano-Nokoken); (iv) *P. nameko*: KX-N007, KX-N008, KX-N009 (Kinokkusu Corporation); (v) *G. frondosa*: 51 (Mori & Company, Ltd.); (vi) *P. eryngii*: Nara PE2 (Nara prefecture).

**Lighting devices.** The LEDs used in the experiments are as follows: Red, light emission 580-660 nm, maximum at 631 nm; yellow, light emission 550-630 nm, maximum at 597 nm; Blue, light emission 420-550 nm, maximum at 463 nm; Green, light emission 470-600 nm, maximum at 517 nm. Those LEDs were suitably designed for mushroom cultivation, and the lighting devices were specially constructed with waterproof treatment (Panasonic Corporation). The usual white fluorescent lamp (FL15N, Panasonic Corporation) was also used as the ordinary current lightings. Contained UV wavelength was omitted from white fluorescent lamp by the specified exclusive filters (RuRu Corporation). The intensities of those illuminations were measured with both the LI-190 quantum sensor and the LI-200SA pyranometer, which were connected to the LI-250 light meter (LI-COR, Inc.), and were adjusted by regulating an electrical current in combination with the light diffusion sheet (KIRYU Corporation).

**Media and cultivations.** The cultivation experiments under the following conditions for individual cultivable mushroom strains were carried out. (i) *L. edodes*: Medium blocks containing sawdust, rice- and wheat-bran were 1kg in weight with appropriate moisture content (62%). After autoclaved, the inoculated blocks were incubated at 21 °C for vegetative mycelium growth and then at 17 °C for fruiting under several lightings conditions; (ii) *F. velutipes*: The inoculated medium bottles were incubated at 16 °C and were irradiated with several lightings (blue LED, white fluorescent lamp or continuous darkness) under various durations of light. The fruiting body formation was carried out at 5 °C with 80 % humidity; (iii) *H. marmoreus*: The durations of light were divided into four terms (0-20, 20-40, 40-64, 60-80 days after inoculation). Cultivations were carried out under several lightings such as blue LED, fluorescent lamp or continuous darkness. The used medium was constituted of the sawdust of Japanese cedar, rice-bran and several nutrients with appropriate moisture content (65%). The treatment for the inducing fruiting body formation was carried out at 15 °C with 100% humidity under various light conditions; (iv) *P. nameko*: The medium blocks constituting of sawdust and several nutrients were bottled and were inoculated after the sterilization by autoclaving. The cultivation was carried out at 20 °C

with appropriate moisture content (65%), under continuous darkness for 30 days after inoculation, and then the medium blocks in bottles were irradiated with blue LED or white fluorescent lamp. After continuous irradiation for 20 days, when the forming of primordia was confirmed, the surfaces of those bottles were divided into two types, with and without scratching (a physical treatment for primordium formation). The formation of fruiting bodies were carried out at 14 °C with at least 90% humidity; (v) *G. frondosa*: Inoculated medium blocks constituting of sawdust and several nutrients were incubated at 22 °C with 65% humidity for 4 weeks under continuous darkness, and then the treatment for fruiting body formation was carried out at 17 °C with 90% humidity under several light conditions. When pores on the bottom side of caps had spread to 2-3 mm, fruiting bodies were harvested. (vi) *P. eryngii*: From 4 days later after inoculation, those medium bottles were incubated at 27 °C under the irradiation with red, yellow, green and blue LED for 1day. The growth rates (mycelial extensions) on agar media under various light conditions were also measured. To test effective light conditions for fruiting body formation, vegetative mycelia in test tubes were irradiated with blue LED or white fluorescent lamp for 15 days after 4weeks of inoculation, and then the treatment for fruiting body formation was carried out. Phenotypes of produced fruiting bodies under several light conditions were compared.

## RESULTS AND DISCUSSION

***L. edodes***. In the cultivation of *L. edodes* using medium blocks, half-opened houses with the combination lighting, both sun and artificial light, are widely used. These cultivation houses, however, usually waste quite a few electricity to keep an optimal air condition due to low insulation, and would be replaced by completely sheltered ones in the near future. Since it is an important issue for such closed rooms to regulate lighting and air conditions, LED, which has the advantages of waterproofing, high durability and low electricity over current lightings, has the potential for improving lighting environment in mushroom cultivation. Our group has been developing the practical application of lighting techniques using by LED in *L. edodes* cultivation [15].

At first, effective lightings by blue LED at vegetative mycelium stage were investigated (Table 1). The higher the intensity of light was, the better the yield of fruiting bodies became in both strains. The irradiation with blue LED was more efficient in the yield of fruiting bodies than white fluorescent lamp. Consequently, the blue LED irradiation during vegetative mycelium growth appears to produce fruiting bodies owing to high promotion of the formation of primordia.

**Table 1:** Produced fruiting bodies of *L. edodes* cultivated under various light conditions

Type of lighting Intensity of light ( $\mu\text{mol}/\text{m}^2\cdot\text{s}$ )	Fluorescent 5.4	Blue LED		
		1.2	3.6	7.8
Weight (g)	328.6 ± 69.3	331.7 ± 38.7	336.0 ± 24.7	340.2 ± 28.7
The number of fruiting bodies	26.9 ± 9.1	32.7 ± 8.8	34.1 ± 10.2	30.8 ± 11.9
The number of valuable fruiting bodies	9.7 ± 2.3	10.1 ± 2.8	10.3 ± 3.1	11.7 ± 2.0

The irradiation with blue LED 470 nm (peak wavelength) at vegetative mycelium stage increased the productivity of fruiting bodies, whereas the continuous exposure of medium blocks to the same LED during fruiting body formation brought about a slight diminution in the yield of fruiting bodies. The alternative irradiations by 625 nm red or 520 nm green LED had no apparent

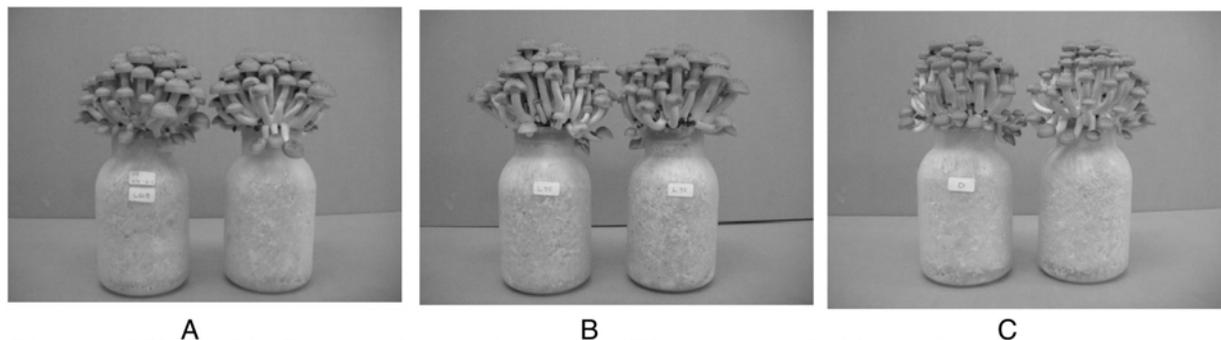
effect on the vegetative mycelium growth. The combined irradiation of blue LED at vegetative mycelia stage and usual fluorescent lamp during fruiting body formation succeeded in increasing the yield of valuable fruiting bodies, which are generally favored by the consuming public. In conclusion, the optimal intensity of the irradiation by blue LED during vegetative mycelium growth is estimated at  $10.5 \mu\text{mol}/\text{m}^2\cdot\text{s}$ .

In the case of the cultivation accompanied with the treatment by high temperature during vegetative mycelium growth, the blue LED irradiation brought about high yield of fruiting bodies.

To investigate the influence by UV, the cultivation under the irradiation with fluorescent lamps, from which UV wavelength was omitted by using the exclusive filters, was carried out. Nevertheless, there was no difference between with and without UV, suggesting that the wavelength around UV has no effect on fruiting body formation of *L. edodes* and that blue LED is sufficiently effective in use for the cultivation of *L. edodes*, even in the absence of UV.

***H. marmoreus***. For the production of highly valued fruiting bodies of *H. marmoreus*, it is important to adjust the size of cap diameters with hemispheric shape. In this study, we attempted to produce favorable fine arrays of the caps of *H. marmoreus* fruiting bodies by regulation of lightings in cultivation houses.

Generally, the adequate intensity of illumination in cultivation of *H. marmoreus* is estimated at 300-700 lx [16]. As experimental results, the growth of stipes was suppressed by blue light but was facilitated by yellow light. Obvious pigmentations of caps were observed under the irradiation of continuous light for at least 12 hr. The favorable colored caps were obtained effectively under blue light. Although there was no remarkable difference in the yield of fruiting bodies between blue LED and white fluorescent lamp, the duration of exposure to light was related with several characteristics of fruiting bodies such as cap diameters, stem lengths, etc. (Fig. 1).



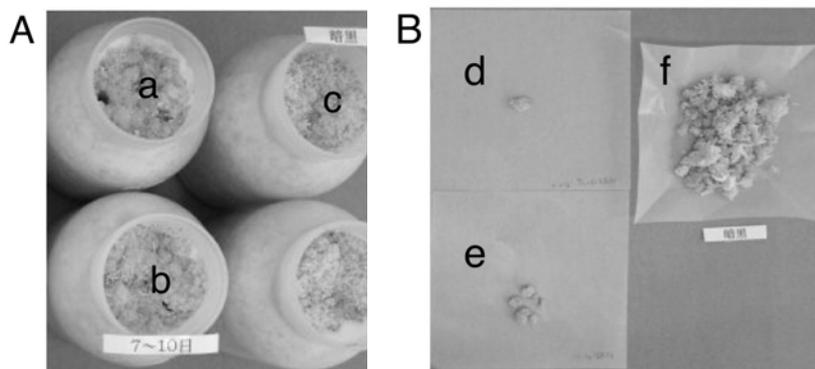
**Figure 1:** Effects of the light durations on the shapes of *H. marmoreus* fruiting bodies.

The irradiation with light was done at 40-60 days (A) and 40-70 days (B) after inoculation. The cultivation without light during vegetative mycelium growth was also carried out (C).

By means of the regulation of lightings in this study, the duration till the initiation of fruiting body formation was successfully shortened. The numbers of fruiting bodies were also increased by the devised irradiations with either blue LED or fluorescent lamp; however, the irradiations at early stages of vegetative mycelium growth (at 0 or 20 days after inoculation) showed the tendency to contain unfavorable phenotypes of fruiting bodies (small diameters of caps, bad synchronization of fruiting). Unexpectedly, the yield of fruiting bodies by the irradiation with blue LED at 20 days after inoculation was significantly lower than with either fluorescent lamp or continuous darkness.

The irradiation with light at 40 days after inoculation shortened the duration of overall cultivation. Furthermore, the irradiation with blue LED at the last period of mycelial growth (60 days after inoculation) gave an optimal result in the yield of fruiting bodies. The optimal intensity of blue LED was  $12 \mu\text{mol}/\text{m}^2\cdot\text{s}$ , as well as the observation in the ordinary cultivation under white fluorescent lamps. UV irradiation had no remarkable effect on the productivity of *H. marmoreus* fruiting bodies.

***F. velutipes***. For effective cultivation of *F. velutipes* fruiting bodies, the fine-tunings of cap diameter and stipe length of fruiting bodies are generally required. The growth of caps in *F. velutipes* is known to be affected especially by blue light. In this study, the cultivations under various lightings were examined. The optimal intensity of illumination through cultivation was known as 70-150 lx [17]. Indeed, this optimal condition of environmental light gave similar productivities also in our study, even if the durations of light were arbitrarily interrupted. The surface exfoliation of cultivated mushroom-blocks, a serious issue especially found in *F. velutipes*, was successfully avoided by the exposure to blue LED at the primordium formation stage (Fig. 2).



**Figure 2:** The surface exfoliations of the cultivation bottles of *F. velutipes*.

A: Medium bottles cultivated under blue LED (a), with fluorescent lamp (b) and continuous darkness (c).

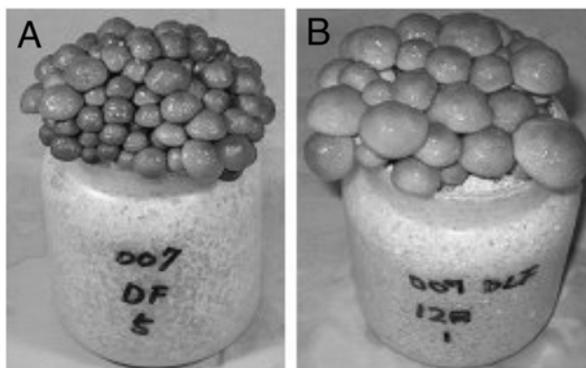
B: The exfoliated medium blocks (d), (e), (f) were derived from the bottles (a), (b), (c), respectively.

The adequate irradiations with either blue LED or white fluorescent lamp during vegetative mycelium growth provided good productions and the yield of fruiting bodies. No obvious difference in several favorable characteristics, such as stem length, stem diameter, stem abnormality, cap diameter, and cap thickness, was observed in the harvested fruiting bodies under the examined conditions using various lightings. Consequently, the lighting of blue LED brought approximately the same productivity as usual fluorescent lamp. The longer medium bottles were irradiated with blue LED or white fluorescent lamp in particular during the late stage of vegetative mycelial growth, the higher the yield of *F. velutipes* fruiting bodies became. Our results suggest that light irradiation during the initiation of fruiting body formation has the remarkable effect on avoiding surface exfoliations of medium blocks; however, such irradiations also bring about slightly lower yield of fruiting bodies. UV had no remarkable effect on fruiting body formation of *F. velutipes*, as well as other examined mushrooms.

***P. nameko***. Most of the cultivations of *P. nameko*, whose glutinous character is appreciated in Japan, are carried out in controlled cultivation houses using medium blocks in bottles. Our group

has been developing effective lightings for *P. nameko* cultivations [18]. Under the slight intensity of light (0.01 lx), the shape of *P. nameko* fruiting bodies revealed an aberrant phenotype similar to the etiolated seedlings of soybean, suggesting that a certain intensity of light is essential for the *P. nameko* cultivation. The increase of light intensity gave dark brownish caps of fruiting bodies, suggesting the relationship between the intensity of environmental light during cultivation and the pigmentation of *P. nameko* fruiting bodies. The optimal intensity of light through cultivation was estimated at 0.2-10 lx, which gave efficient production of favorable and valuable fruiting bodies.

The irradiation with blue LED at the early stage of vegetative mycelium growth brought about higher production of primordia, however, most of those primordia could not grow up to mature fruiting bodies, implying that the timing of the irradiation of light is likely to affect the characteristics of fruiting body development of *P. nameko*. The irradiations with either blue LED or fluorescent lamp during the late stage of vegetative mycelium growth succeeded in the effective induction of primordial formation, and were capable of producing large fruiting bodies for short term (Fig. 3). Our results suggest that blue LED has the potential to control the morphogenesis of fruiting bodies of *P. nameko*. Although the yield of fruiting bodies under the irradiation with blue LED during vegetative mycelium growth was generally higher than usual white fluorescent lamp, the irradiation with blue LED during fruiting body formation had the opposite effect and decreased the yield of fruiting bodies. UV had also no obvious effect on fruiting body formation of *P. nameko*.



**Figure 3:** Effects by blue LED on fruiting bodies of *P. nameko*. A: Fruiting bodies without light irradiation during mycelial growth. B: Produced fruiting bodies after the irradiation with blue LED for 12 days at late stage of mycelial growth.

***G. frondosa*.** There are various public demands for colors and shapes of *G. frondosa* fruiting bodies. Light environment through cultivation has been understood to be capable of controlling favorable characteristics of generated fruiting bodies in *G. frondosa*. In this study, several periods of time for the beginning of cultivation, primordial formation, initiation of fruiting body formation and harvest of fruiting bodies, were investigated under various light conditions using blue LED and usual fluorescent lamp. The suitable intensity of light (approximately 13  $\mu\text{mol}/\text{m}^2\cdot\text{s}$ ) brought about both the fastest primordium formation and the highest yield of fruiting bodies among all examined conditions. The exceedingly aberrant phenotype of primordia was observed under continuous darkness (Fig. 4). Although those media could generate relatively small primordia subsequently to the cold treatment for initiation of fruiting body formation,

grown-up fruiting bodies derived from those small primordia still retained obviously defect phenotypes with fragile tissues. The light irradiation till primordium formation had no obvious effect to the pigmentation of the upper surface of fruiting bodies. Consequently, light is likely to be indispensable to the primordium formation in *G. frondosa*.



**Figure 4:** Fruiting bodies of *G. frondosa* developed under various light conditions. A: Using white fluorescent lamp. B: Using blue LED. C: Without light.

The culture of vegetative mycelia under continuous darkness was effective for fruiting body formation, whereas slight intensity of light at the initiation of primordial development was observed to be essential for good production of fruiting bodies. The irradiation with blue LED during fruiting body formation gave higher production of fruiting bodies, however, the timing of harvest of fruiting bodies was later than using white fluorescent lamp. The irradiation with blue LED during vegetative mycelium growth had no effect on the pigmentation of fruiting bodies, while the surface of those fruiting bodies seemed to become fluffy. The irradiation with white fluorescent lamp omitting UV wavelength had no obvious effect on phenotype of fruiting bodies; however, the duration till harvest was longer and the yield of fruiting bodies was higher than using usual fluorescent lamp. It remains to analyze these phenomena.

***P. eryngii*.** Most of cultivated fruiting bodies of *P. eryngii* have small caps and large stipes, because the consumers prefer those unique taste and food texture. To cultivate such fruiting bodies, only a few fruiting bodies per bottle are generally produced by means of thinning out small fruiting bodies [19]. In this study, several light conditions for mycelial growth, primordium formation and fattening up fruiting bodies were investigated, in order to contribute effective cultivation and production of favorable *P. eryngii* fruiting bodies.

Vegetative mycelium growth on agar media was inhibited by irradiation with light, especially with blue light. Moreover, the higher the intensity of light became, the slower mycelia grew, remarkably under either blue or white light. The strong intensity of light ( $30 \mu\text{mol}/\text{m}^2 \cdot \text{s}$ ) highly inhibited mycelial growth (approximately 40% lower than the mycelial growth rate under usual growth condition). The light wavelength during fruiting body formation significantly affected morphology of fruiting bodies (Fig. 5). Although the spherical primordia under light grew up to mature fruiting bodies with usual stipes and caps, the primordia formed under continuous darkness developed characteristic long stipes. Consequently, both the extension of stipes and the inhibition of cap development were observed under continuous darkness, whereas light irradiation generally appeared to induce an appropriate extension of stipes and the progress of cap development. Light also facilitated the pigmentation of caps.



Darkness Red Yellow Green Blue White  
**Figure 5:** The phenotypes of *P. eryngii* fruiting bodies developed under various light qualities. The details of used LEDs are described in the Materials and methods. The usual fluorescent lamp (white) was also used.

The light irradiation at vegetative mycelia in bottles had no obvious effect on mycelial growth. However, at the initiation of fruiting body formation, the irradiation with light brought about remarkable differences in fruiting body production. After the light irradiation at primordium formation, the subsequent cultivation under continuous darkness could provide the highest yield of fruiting bodies among all experimental conditions. The later the light irradiation started, the longer the overall duration of cultivation became, under either blue LED or white fluorescent lamp. UV has no obvious effect on fruiting body formation of *P. eryngii*. The low intensity of light produced fruiting bodies with small caps and long stipes. After the formation of primordia, the longer the duration under continuous darkness was, the larger stipes became. The intensity of blue LED significantly influenced the phenotypes of produced fruiting bodies. The stronger the intensity of light was, the larger the obtained fruiting bodies became. The stronger the intensity of light was, the more darkened the pigmentation of fruiting bodies became. In conclusion, phenotypes of produced fruiting bodies of *P. eryngii* are likely able to be modified by means of the regulation of light environment through cultivation.

## CONCLUSIONS

For cultivation of edible mushrooms, LED has many useful characters such as low electricity, ecological, etc., and is more effective than the ordinary current lightings such as white fluorescent lamp. The appropriate irradiation with blue LED is effective to increase the yield of fruiting bodies and to improve the productivity of high valued phenotypes, which are favorable to the consuming public. Consequently, UV irradiation has no apparent effect on fruiting body formation of the examined mushrooms. The attentive control of light environment for mushroom cultivation has the potential of further effective productions of fruiting bodies in cultivation houses in the near future.

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