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Effects of light irradiation on spore germination, spawn run, protein content and fruiting body formation in *Pleurotus florida* Singer

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Abstract

In this study, the effects of cool and warm white fluorescent light and combination of these lights on the spawn run, fruiting body formation, dry weight and fresh weight of fruit bodies, spore germination and protein content in *Pleurotus florida* Singer were examined. For investigating fruiting body formation, polyethylene bags were employed to contain the sterilized wheat straw substrate and been inoculated with 30 g of spawn. On the third day after spawn inoculation, all cultures were divided into six sections and irradiated with warm white and cool white fluorescent lamps and combination of these two for four and eight hours, separately. Control group was kept in darkness throughout the experimental period. To investigation of irradiation on spore germination, spores were firstly separated and germinated by using sucrose gradient and water agar medium, respectively. Results showed that in the control group, pin heads formed faster than other groups. Meanwhile, the number of germinated spores was the highest. On the other hand, under warm light fluorescent, the highest wet weight of fruiting body was observed. Protein content increased after 4-hour warm-white-light treatment. The dry weight of fruiting bodies was increased under irradiated treatments.

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Introduction

Mushrooms are nutritionally functional food and a source of physiologically beneficial and nontoxic medicine (Wasser and Weis, 1999). The significant medicinal effect of mushrooms and their metabolites have attracted attention of the public (Jose and Janardhanan, 2000). The production of mushrooms is regarded as the second most important commercial microbial technology besides to yeast (Pathak *et al*., 2009). Some polysaccharides or polysaccharide–protein complexes from mushrooms are able to stimulate the non-specific immune system and to exert antitumor activity through the stimulation of the host's defense mechanism (Chihara *et al*., 1969; Mizuno, 1999; Wasser and Weis, 1999; Reshetnikov *et al*., 2001). In one study, it was found that *Ganoderma lucidum* (Curtis) P. Karst., *Phellinus rimosus* (Berk.) Piat, *P. florida* and *Pleurotus pulmonarius* (Fr.) Quél. possessed profound antioxidant and antitumor activities (Thekkuttuparambil *et al*., 2007). *P. florida* belongs to basidiomycetes, agaricales and tricholomataceae (Alexopolous *et al*., 1996; Zoberi, 1972), and has a great nutritional and medicinal value. The genus *Pleurotus* (Fr.) P. Kumm includes various edible mushroom species and has important medical and biotechnological properties as well as environmental applications (Cohen *et al*., 2002). Many study results showed that light prevented spawn run, enhanced primordia formation and was necessary for the fruiting-body formation of *Pleurotus* species. The requirements for light are different during various stages of growth (Okwujiako, 2001; Datta and Chakraborty, 2002; Marino *et al*., 2003). Vegetative mycelia of *P. ostreatus* could differentiate to primordia which subsequently turn into fruiting bodies on synthetic sucrose-asparagine medium when exposed to light at low temperature (Lee *et al*., 2011). Light promotes the accumulation of beta-carotene and the phototropism of the fruit body of *Mucor* Fresen. (Silva *et al*., 2006).

Effects of light on development and identification of blind mutants of *Coprinopsis* P. Karst. were studied by Kamada *et al* (2010). In *Phycomyces*, one wc-1 gene(mad A) and one wc-2 gene(mad B), are necessary for all the responses of this fungus to light (Idnurm *et al*., 2006; Sanz *et al*., 2009).

In the present research, the effects of light and exposure time on the fruit body formation and yields, spore germination, spawn spreading and protein contents in *P. florida* were studied.

Materials and methods

Fungi cultivation

P. florida mycelium was obtained by tissue-culture method (Jonathan and Fasidi, 2003). Pure mycelium was used for spawn preparing. Polyethylene bags prepared with 16×35 diameters were filled with sterilized wheat straw. Each bag inoculated with 30 g of spawn. The bags were placed at 25-30°C and 60-90% relative humidity under darkness condition. After three days of incubation, the bags were irradiated under following conditions and then were kept in the dark until the end of experiments.

Light-irradiation conditions

In this study, various irradiation conditions were used as:

(i) Two cool-white-Fluorescent lamps (12W+12W), for 4 hour (4h-cwFl).

(ii) Two cool-white-Fluorescent lamps (12W+12W), for 8 hour (8h-cwFl).

(iii) Two warm-white-Fluorescent lamps (12W+12W), for 4 hour (4h-wwFl).

(iv) Two warm-white-Fluorescent lamps (12W+12W), for 8 hour (8h-wwFl).

(v) One cool-white-Fluorescent lamp + one warm-white-Fluorescent lamp, (12W+12W), for 4 hour (4h- (w+c) wFl).

(vi) One cool-white-Fluorescent lamp + one warm-white-Fluorescent lamp, (12W+12W), for 8 hour (8h- (w+c) wFl).

(vii) Continuous darkness (control).

The light intensity was measured using a light Guarda Fx-101 luxmeter. Light intensities of all treatments were 1000 lux.

Spore germination

Spore suspension was obtained by floating of gills

powder in 50% (*w/w*) NaCl and then filtered by a 38-micron mesh, followed by sterilized-water washing. Afterwards, spore suspension was centrifuged at 900 g^{-1} for 2 minutes at 20°C. Supernatant was used for spore culturing on water agar medium. Spores were incubated for three days in darkness conditions at 28°C. After three days of incubation, the spores were irradiated according to above conditions, and then were returned to dark cultivation. After 30 days, study of spore germination was conducted with a light microscope.

Statistical analysis

There were four replication for each treatment. The means were quoted with their standard error (S.E.). Statistical analyses were carried out using analysis of variance and the Duncan multiple range test (Sokal and Rohlf, 1969).

Results

Fig. 1 shows that spawning rate of the inoculation in control group was higher compared to the groups processed by light irradiation. Darkness benefits the growing of mycelia with the shortest spawning period which was 6 days. Moreover, pin head formation in the control group was faster than that of other groups. The 4h-wwFl treatment gave the longest period for fruiting body formation, which was 9 days. The differences among these period times were significant ($p \leq 0.01$).

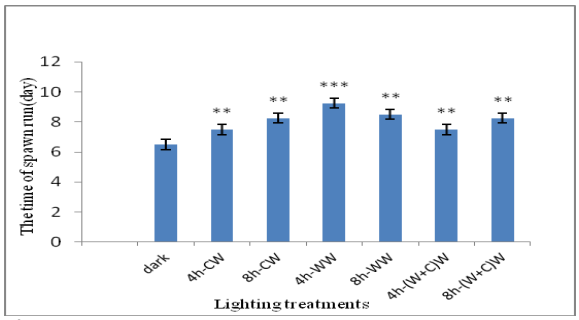

Fig. 1. The time of spawn run in different light treatments and control sample (dark).

The maximum fruiting-body number was formed after treatment of 4h-cwFl whereas the minimum numbers of fruiting body was derived after treatment of 8h-(w+c)wFl (Fig. 2).

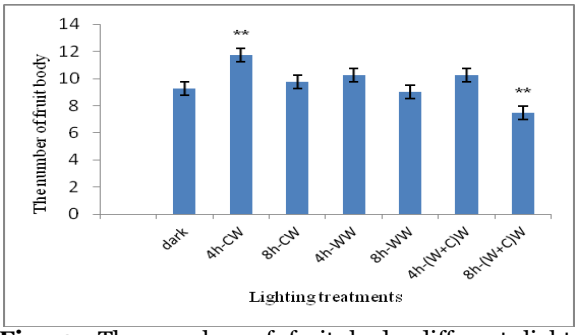

Fig. 2. The number of fruit body different light treatments and control sample (dark).

Fig. 3 shows that the means of wet weight of *Pleurotus* fruit bodies were increased significantly after warm-white-light treatments and were extremely decreased cool-white-light treatments or integration of these two.

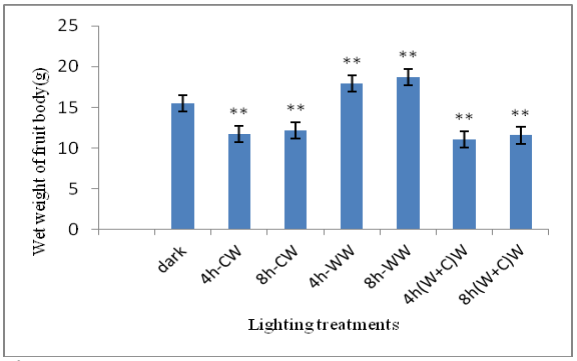

Fig. 3. The wet weight of fruit body in different light treatments and control sample (dark).

Fig. 4 showed that dry weights of fruiting bodies after light irradiated treatments were increased than that of control.

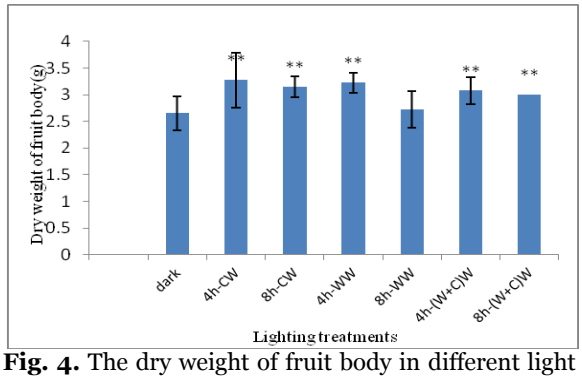

Fig. 4. The dry weight of fruit body in different light treatments and control sample (dark).

Protein content of samples in the control group or treated by cool-white light was lower than that in other treating groups (Fig. 5).

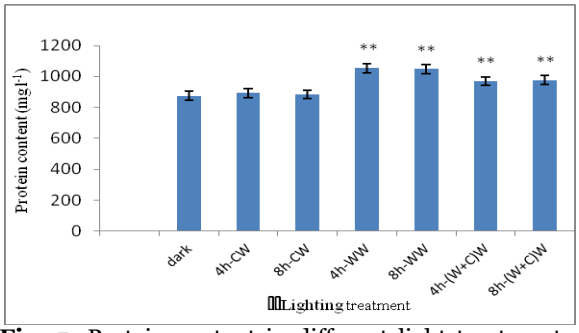

Fig. 5. Protein content in different light treatments and control sample (dark).

The warm-white light increased protein content and raised the wet or dry weights of fruiting bodies (Fig. 4). Compared to the sample growing under continues darkness, warm-white light did not only increased the dry weight of fruiting body but also enhanced its formation, thus this kind of light is more suitable for being employed in fruiting body formation (Fig. 6). However, it prolonged mushroom initiation and the spawn running time.

Fig. 6. The mushroom bags were grown in different light treatments and control sample (dark).

The number of germinated spores in control group was more than that in the lighting-irradiated groups within which exposure times were increased and germinations of spores were reduced (Fig. 7).

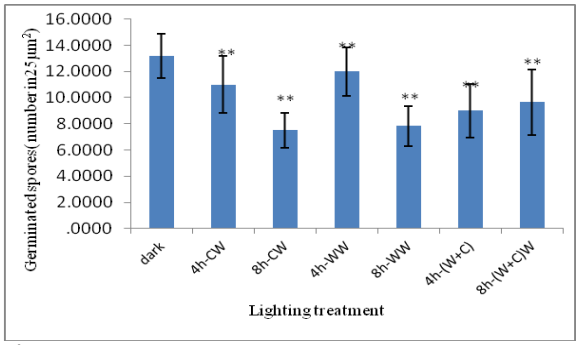

Fig. 7. The number of germinated spores in different light treatments and control sample (dark).

Discussion

The growth of mycelium in the control group observed in this study which was incubated in darkness all thorough the experimental period was consiederably faster than that of the group treated in irradiated conditions. Consequently in this controlled group spawn running time was reduced.

According to Khandakar *et al* (2008) the best mycelial growth of *Pleuroyus citrinopilearus* was found in absence of lighted conditions and the least mycelial growth was found in 40-60 lux condition.

Zandrazil (1982) reported that absence of light yielded the most mycelial growth.On the other hand, the results indicated that the use of light increased the number of fruit bodies, wet weight, dry weight and protein content.

Another study conducted by Stamet and Chilton (1983) demonstrated that lighting is required for *Agaricus bitorquis* and *A. brunnensence*, while *Pleurotus ostreatus* is known to be phototropic and more responsive to an exposure of 2000 lux hr-1 for 12 days.

In some group of mushroom lighted conditions is required to trigger fruiting, although, unlike green plants (Kuforiji and Fasidi, 2005), fungi do not require light to produce carbohydrate.

Okwujiako (2001) investigated the effect of light on the vegetative growth and fruiting body formation of *Pleurotus sajor-caju* , and found that light inhibited the vegetative growth of *P. sajor-caju* but was necessary for the production of fruiting bodies in vitro and cultivation on rice straws. It was also observed that there was no stimulative effect of light on fruiting body inintiation for mycelium less than 5 days old.

Light stimuli in mushroom are rquired to activate photoreceptors in, for example, *Coprinopsis cinerea*, dst1 and dst 2 which were evidenced to be involved in photomorphogenesis. The photoreceptors where genetically analyzed in detail in Tarashima *et al*

(2005) and Kuratani *et al* (2010).

In the present research we indicated that 4h-cool white light increased the fruit body number but the compaction of mycelium and mushroom productivity was raised in both cool and warm white light for 4-h and 8-h.

Lee and *et al* (2011) speculated that the reductones produced during the developmental stages may play a significant regulatory role in cellular response of *P. ostreatus* to light. It can be suggested that the reductones produced in the vegetative mycelia prior to the initiation of fruiting is an important factor in meditating the effect of light on the development of the fruit body in *P. ostreatus*.

As it was illustrated in this study there were differences between the mushroom responses to cool and warm light fluorescent lamps and these differences are generated by the color and temperature of the two lamps. Warm color temperatures tend to enhance red and orange colors, where as cool color temperatures enhance blue colors. Fungi responses are produced commonly because of the variation in wavelengths between the ultraviolet and blue regions of the spectrum. Blue light is the type lost associated with fungal photomorphogenesis. In addition, blue light can activate fungal metabolic pathways or direct growth of fungal structures (Idnurm *et al*., 2010).

Fewer cases are known in which longer wavelength in yellow and red regions affects fungi (Ingold and Peach, 1970; Newman, 1968; Page, 1962). In the ascomycete *Neurospora crassa* blue light is perceived by the white collar (WC) complex, a protein complex formed by WC-1 and WC-2. WC-1 is a protein with a flavin-binding and a zinc-finger domain which interacts with WC-2, another zinc-finger domain protein. The WC complex operates as a photoreceptor and a transcription factor for blue-light responses in *Neurospora*. Proteins similar to WC-1 and WC-2 have been described in other fungi, suggesting a general role for WC complex as a fungal receptor for blue light (Corrochano, 2007).

Despite the stimulatory effects of light on the fruiting body formation, light significantly decreased the spore germination. In addition to that, an encrease in exposure time considerably reduced the spore germination percentage. In cool and warm light combination, the inhibitory effects were also decreased. Our finding was inconsistent with Matinuddin Khan (1977), that reported white light with high intensity activated germination of conidiospores of *Aspergillus niger*. Jeffery *et al* (1990) reported wild-type *A. nidulans* requires light to conidiate and cinsequently demonstrated that light-dependent conidiation was determined by the allelic state of velvet (ve.A) gene. They proposed that the initiation of late gene expression is regulated by velvet and controlled by a red light photoreceptor, whose properties are reminiscent of phytochrome-mediated responses observed in higher plants. On the other hand, in some fungi, for instance, *Puccinia graminis* f.sp.*tritici*, spore germination is inhibited by continuous irradiation (Lucas et.al, 1975).

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