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Effects of Photoperiods on the Growth of the Entomopathogenic Fungi, *Paecilomyces japonica*, During the Production of the Silkworm-dongchunghacho, Silkworm Vegetable Wasp and Plant Worm

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Effects of photoperiods, 24L or 24D, on the growth of the silkworm-dongchunghacho, the silkworm vegetable wasp and plant worm, were investigated. Exposure of the fungi under the photoperiod of 24L for at least 3 days during the cultivation of the fungi after the completion of endosclerotium in the host accelerated the spore formation, but the growth of the fruiting bodies was inhibited. On the contrary, the photoperiod of 24D inhibited the spore formation, but accelerated the growth of fruiting bodies without spores. Accordingly, to produce silkworm vegetable wasp and plant worm of large-size fruiting bodies with over 3 cm in length, it is indicated that recommendable light condition is a photoperiod of 24D during the cultivation until the length of the fruiting body arrives at over 3 cm.

Key words : Photoperiod, Silkworm vegetable wasp and plant worm, Silkworm-dongchunghacho

Introduction

Paecilomyces species is a common fungus which attacks lepidopteran larvae, pupae and adults, and can be found easily from many mountainous areas in Korea (Sung *et al.*, 1997). A method for artificial mass-production of the silkworm vegetable wasp and plant worm with *P. japonica*, silkworm-dongchunghacho, was established, and its many pharmacological activities were reported in recent years (Cho, 1999; Choi, 1999; Shin, 1999). In China, originally, vegetable wasp and plant worm with *Cordyceps* indicates the fruiting body formed on the larval integument of its insect host, which is insect-born mushroom showing special appearance produced from the larvae attacked by entomopathogenic fungi, *C. sinensis* (Lin, 1999).

The silkworm-dongchunghacho is a new type of *Cordyceps*, being very different from the above traditional Chinese *C. sinensis* in many regards, and also is being mass-produced artificially and successfully in industrial scale by the silkworm farmers in Korea (Cho, 1999). Therefore, the silkworm-dongchunghacho is very important for the improvement of the insect-industry and new sericulture. Now, the silkworm-dongchunghacho is being used as health foods since elucidation of its immuno-stimulating, anti-tumor, anti-fatigue and anti-aging effects, and also is very expensive (Cho, 1999; Choi, 1999; Shin, 1999). Accordingly, the farmers want to produce the highly expensive and the best quality of the silkworm-dongchunghacho for the economic benefit. The silkworm-dongchunghacho that the length of the fruiting body is more over 3 cm long can be evaluated as a best one, but sometimes the farmers fail to produce such a best dongchunghacho due to shorter fruiting body in the light of length.

Infection ratios of *P. japonica* varied on the pupae or adult of the corresponding developmental stages in the silkworm, *Bombyx mori* (Lee and Park, 1998) and the content of the amino acids in a fruiting body varied according to the nutritional sources (Lee *et al.*, 1998). The addition of sugars and powder of dried silkworm pupae to *in vitro* culture medium influenced the growth of the fruiting body (Choi *et al.*, 1999). The production of silkworm larval type of dongchunghacho was able to be established

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by injection of the *P. japonica* into the larval body (Lee *et al.*, 1999) and the incubation time after inoculation of the fungi influenced to the productivity of the above insect mushroom (Lee *et al.*, 1999).

There are many environmental factors *in vitro* or *in vivo* culture system on the growth and productivity of the entomopathogenic fungi such as oxygen, carbon dioxide, carbon source, nitrogen source, growth factors, vitamins, water availability, pH, temperature and light etc (Carlile and Watkinson, 1996), but we expected that photoperiod during the cultivation of the silkworm-dongchunghacho will be the key factor on the growth of the fruiting body in the light of the present *in vivo* cultivation of the fungi in the silkworm, *B. mori*. In the present *in vivo* cultivation system of the silkworm-dongchunghacho, temperature, humidity and photoperiod are considered as the important environmental factors on the growth of the fungi because the above three can be controlled artificially in the practical sericultural farm. Among them, the optimum criterions for temperature and humidity are elucidated previously (Cho, 1999), but not for photoperiod. Therefore, this study was carried out to find the effects of photoperiods on the growth of the fruiting body and the spore formation of the silkworm-dongchunghacho.

Materials and Methods

Insect and rearing

Bombyx mori, Baegokjam, F_1 hybrid between the Japanese parental line Jam123 and the Chinese parental line Jam124 was used in this study, and reared with fresh mulberry leaves as described on the guide book of the silkworm rearing of National Institute of Agricultural Science and Technology, RDA, Korea.

Inoculation of the entomopathogenic fungi

The 4th day-old pupae were immersed for about 2 min in 70% ethanol solution for disinfection in the clean bench, and then the pupae were taken out from the fluid and dried in the same clean bench. The species of entomopathogenic fungi used in the study was *Paecilomyces japonica* (Cho, 1999). The conidia in PD (potato dextrose) medium were inoculated by immersion of the pupae into the medium for about 1 min and the inoculation concentration of the fungi was 10^8 spores/ml.

Induction of endosclerotium and photoperiods

To induce the formation of the endosclerotium, the pupae that *P. japonic*a was inoculated by immersion were cultivated at 28°C, 95% R.H. for 10 days. After the fungus finished to produce the endosclerotium (hyphal bodies) in the host, the host pupae were continuously cultivated at 19-20°C, 90% R.H. under the photoperiod of 24D until the completion of fruiting bodies. During the cultivation under the photoperiod of 24D, we checked the growth of the fruiting body emerging from the host's integument every day. The experimental plots were designed according to the length of the fruiting bodies under the following conditions: (T1), 1 cm; (T2), 2 cm; (T3), 3 cm; (T4), 4 cm; (T5), 5 cm in length of the fruiting bodies. The tested number of each experimental plot was 40 host pupae growing fruiting bodies with a same length. These experimental plots were also divided into two photoperiods of 24L and 24D, and cultured separately under two photoperiods during the subsequently given 3 days for testing in the every experimental plot. After 3 days of exposure of the host pupae under the photoperiods of 24L or 24D, the length of fruiting body and the degree of spore formation were checked out.

Source of illumination

The common fluorescent light being used in the current farm house was applied in the present study. The pupae inoculated with *P. japonica* were continuously cultivated under the given photoperiods in the experimental bioincubator equipped with illuminating apparatus of fluorescent light.

Results and Discussion

The 5 days-old silkworm pupae were inoculated with *P. japonica,* a new type *Cordyceps,* developed for an industrial purpose in Korea, and the endosclerotium was induced at 28 90% R.H. under the photoperiod of 24D for 10 days. After the completion of the endosclerotium in the host pupae tested, the temperature and humidity during subsequent cultivation period for inducing the fruiting body formation until the end of this test were 1920 and 90% R.H., respectively, and the photoperiod was 24D condition. During this period, two photoperiods of 24L and 24D were newly applied to the each experimental plot designed according to the length of the fruiting bodies. The result for the length of fruiting bodies under two photoperiods was described in Table 1. In the all treatments (T_1 to T_5), the photoperiod of 24D accelerated the growth of the fruiting bodies without spores. The length of the fruiting bodies emerging from the host pupae in the all treatments became longer when they were kept in dark condition throughout (24D). When the cultivation of the silkworm-dongchunghacho was carried out under the photoperiod of 24D, the growth of the entomopathogenic fungi decreased more significantly than when they were

Table 1. Length of fruiting bodies of the silkworm-dongchunghacho under the photoperiods of 24L and 24D

Treatments*	Photoperiods	Length of fruiting bodies before application of photoperiod (cm)	Length of fruiting bodies after 3 days under the photoperiods of 24L and 24D (cm)	Ratio for the length of fruiting bodies between photoperiods (24D/24L× 100 in length) (%)
T1	24L	1.00	1.46	161
	24D	1.00	2.39	
T2	24L	2.00	2.33	122
	24D	2.00	2.84	
T3	24L	3.00	3.25	109
	24D	3.00	3.53	
T4	24L	4.00	4.18	113
	24D	4.00	4.73	
T5	24L	5.00	5.23	105
	24D	5.00	5.48	
Mean	24L	3.00	3.29	122
	24D	3.00	3.79	

*Treatment plots, T1, T2, T3, T4 and T5 indicate respectively 1, 2, 3, 4 and 5 cm in the length of fruiting bodies before the photoperiods of 24L or 24D are applied.

Table 2. The spore formation of the silkworm-dongchunghacho under the photoperiods of 24L and 24D

Treatments	Spore formation*	
	Photoperiod of 24L	Photoperiod of 24D
T1	+++	-
T2	+++	-
T3	+++	-
T4	+++	±
T5	+++	±

*+++, Spores are covered nearly all of the surface of the fruiting bodies; +, Spores are observed a little bit of the surface of the fruiting bodies; –, Spores are not observed. See Table 1 for further legend.

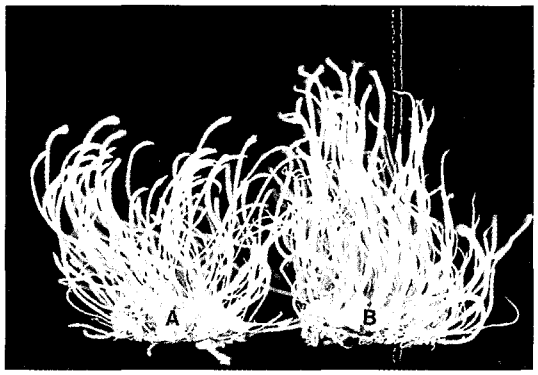

Fig. 1. Comparison of the silkworm-dongchunhacho produced from the *B. mori* pupae under the photoperiods of 24L (A) and 24D (B).

cultivated under the photoperiod of 24D. The comparative growth rates (24D/24L 100 in length) ranged from 161% to 105%, and average of the comparative growth rates was 122%(Table 1).

The degree of spore formation according to the photoperiods was shown in Table 2 and Fig. 1. The brightness for 3 days in the all treatments accelerated the spore formation in the fungi. From these results, the photoperiod of 24D during the cultivation of the silkworm-dongchunghacho with *P. japonica* after the completion of endosclerotium inhibited significantly the formation of spores, and also maintained the continuous growth of the fruiting body without spores. On the contrary, the photoperiod of 24L gave rise to a fruiting body containing spores by only 3 days under the light condition (Carlile and Watkinson, 1996).

There is the related report that illumination will increase or more commonly reduce the rate at which fungi spread across an agar surface *in vitro* culture system (Carlile and Watkinson, 1996), but it is very difficult to find out any report on the effect of illumination during the period of the *in vivo* cultivation of the silkworm-dongchunghacho with *P. japonica* as an entomopathogenic fungus. Such effects by illumination can sometimes be different according to the species of the fungi and the cultivation systems, *in vitro* or *in vivo*, and the different species can also require different conditions for optimal growth. In the present study, the stimulating or inhibiting effects of photoperiods on the growth of fruiting bodies were basically tested: stimulation of the growth of fruiting bodies without spores under the photoperiod of 24D, and the inhibition of the

growth of fruiting bodies and the stimulation of spore formation under the photoperiod of 24L. The condition of the common fluorescent light for all day long gave rise to a fruit body containing spores in this study, but the darkness condition for all day long delayed the spore fomation and showed the continuous growth of the fruit body without spore formation. It indicates that the spore formation and the growth of fruit body of the silkworm-dongchunghacho are primarily dependent on photoperiod in case of the same temperature and humidity conditions.

In conclusion, to harvest a better silkworm-dongchunghacho of longer fruiting bodies with over 3 cm in length, the photoperiod of 24D will be more useful for practical application in the sericultural farming. In future, the effects of more detailed photoperiods will be investigated.

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