Factors Affecting Growth of *Trichoderma* spp. with Special Reference to Control of Green Mildew in *Agaricus bisporus.*

Won Mok Park*. Dong Soo Kim*,
Yong Hwan Park*. Hoo Sup Chung.**

양송이 푸른곰팡이병균(*Trichoderma* spp.)의 생장에 미치는 요인과 방제법

박원목 · 김동수 · 박용환 · 정후섭

Abstract

Four species of *Trichoderma* causing green mildew of *Agaricus bisporus* were isolated from 38 spots of mushroom growing areas in Korea. These are *T. koningi*, *T. lignorum*, *T. glaucum* and an unidentified species, and their frequencies of occurrence are 50%, 32%, 13% and 5% respectively. All of these species grew well in potato dextrose, Waksman's and Richard's solution, and preferred acid (pH. 4) to neutral.

The temperature in mushroom house should be kept at 15°C during cropping period, not only for the high yield of mushroom but also for the prevention of green mildew of mushroom caused by *Trichoderma* spp.

T. lignorum was killed in soil on an exposure of 60 minutes at 70°C and when exposed for 30 minutes at 80°C. Peak heat procedure of compost eliminated *T. lignorum* and *T. koningi.*

Introduction

It is a well established fact that many *Trichoderma* spp. produce a toxic substance which kills many soil borne micro-organisms[5,13]. *Trichoderma* spp. grow rapidly in mushroom compost and casing soil, when the conditions are favorable. The mushroom growers often suffer heavy damage from these fungi.

Much research have been done with *Trichoderma* spp. as mushroom pathogens. Kneebone & Merek[6] reported that there were at least 3 species of *Trichoderma* which attack *Agaricus bisporus*, a cultivated mushroom. Weindling & Emerson[14] obtained the highest yields of a toxic substance from the culture of *T. lignorum* grown in a highly acid medium (pH. 3 to 4.). Park, et al[9] indicated that the highest yield of mushroom was produced in casing soil at pH 7.5. Flegg[3] showed that the temperatures in the range of 14° to 19°C produced high yield and good quality of mushroom. Park & Park[10] reported that beginning 15 days after casing, the keeping of a temperature of 15°C during the cropping period resulted in a high yield of mushroom. Wuest, et al[15] indicated *T. viride* could be eradicated in the soil by an exposure of 30 minutes at 54.4°C. Lambert & Ayers[7] reported that *T. koningi* was killed in 4 hours at 60°C. Rasmussen[11] reported that the one of the effects of peak heat of

* Department of Applied Mycology and Mushroom, Institute of Plant Environment, Office of Rural Development, Suwon, Korea.
** Department of Agricultural Biology, College of Agriculture, Seoul, National University, Suwon, Korea.

compost was pasteurization of compost.

The present experiments were conducted to determine the frequencies of important *Trichoderma* spp. causing green mildew of *A. bisporus* in Korea and the effect of environmental factors on the control of the green mildew.

Materials and Methods

Mycelial growth of *Trichoderma spp.*

Eight different liquid media were used to grow the two species, *T. lignorum* and *T. koningi*. Ingredients of each medium per 1000ml of distilled water were as follows:

a. Potato Dextrose: Potato 200g, Dextrose 20g.
b. Malt Extract: Malt extract 20g.
c. Corn Meal: Corn meal 20g, Pepton 20g, Dextrose 20g.
d. Yeast Starch: Yeast extract 4g, Soluble starch 15g, K_2HPO_4 1g, $MgSO_47H_2O$ 0.01g.
e. Waksman's Solution: Glucose 10g, Pepton 5g, KH_2PO_4 1g, $MgSO_47H_2O$ 0.5g.
f. Czapek's Solution: $NaNO_3$ 3g, K_2HPO_4 1g, KCl 0.5g, $MgSO_47H_2O$ 0.5g $FeSO_4.7H_2O$ 0.01g, Sucrose 30g.
g. Petri Mineral Solution: Ca $(NO_3)_2$ 0.4g, $MgSO_47H_2O$ 0.15g, KH_2PO_4 0.15g, KCl 0.06g.
h. Richard's Solution: KNO_3 10g, KH_2PO_4 5g, $MgSO_4.7H_2O$ 2.5g, FeCl 0.02g, Sucrose 50g.

Conidial suspensions in the amount of 0.2ml of each of the two species of *Trichoderma* were used separately to inoculate into the each of eight media. Each test tube contained 25ml of the medium with four replicates. After inoculation, the test tubes were held for 20 days in an incubator at 25°C. During this period the cultures were agitated 3 times daily. The mycelial mats in the test tubes were placed on filter papers (Toyo Roshi Kaisha, No. 5). The filter papers and mycelial mats were dried for 30 minutes at 105°C in a drying oven. The dried material was then weighed to determine yield of the fungi from the different culture media.

The effect of pH of medium on mycelial growth.

The pH of the media was adjusted between the range from 4 to 8 by the use of a buffer solution (citrate-phosphate)[2] into Richard's solution. The 0.2ml of conidial suspension of *T. lignorum* was inoculated to 100ml of the medium in 250 ml Erlenmeyer flask with four replications. They were grown for 10 days in an incubator at 25°C. During this period the cultures were agitated 3 times daily. Dry weight of the fungus was obtained as described previously.

The effect of temperature on symptom appearance.

A. bisporus were grown in 850 cm^2 box, in a mushroom house with the temperature at about 25°C during spawn growth. The conidial suspension of *T. lignorum* mixed with the casing soil, just before casing. To induce symptoms the temperature was maintained at about 15°C during first flush and then it was raised to about 22°C for 2 days at beginning of second flush. The temperature was dropped again to 18°C after the 2 days at 22°C. Each test-plot consisted of four randomized replicates. The numbers of the healthy and diseased mushrooms were counted to determine the relationship of the symptoms to temperature.

Thermal death time of *T. lignorum*.

Test tubes were filled with a mixture of soil and conidial suspension. Four test tubes were then dipped in a water bath for 30 minutes and the other four for 60 minutes. The temperature in the water baths varied at 10°C intervals through the range of 40°C to 90°C. The timing of the period commenced when the soil heated to the treatment temperature. Following treatment, the inoculated and treated soil was placed on potato sucrose agar (PSA), and incubated for 5 days, to obtain the thermal death point of the fungus.

The effect of peak heat of compost on control of *Trichoderma spp.*

The room and compost temperature was kept initially at 60°C for 12 hr. after which the temperature was kept at 40°C for 5 days. The heat source was steam. Test tubes were filled to about 80% of their capacity with fragments of compost infested with *T.*

lignorum and *T. koningi*, The test tubes were inserted into the compost during peak heat procedure. After that exposure, the fragments of compost in the test tubes were placed on the PSA. and incubated for 5 days at 25°C to determine the effect of such treatment on the growth of pathogens.

Results and Discussions

Frequencies of *Trichoderma* spp. invading *A. bisporus*.

To investigate the frequencies of *Trichoderma spp.* causing green mildew of *A. bisporus*, *Trichoderma spp.* were isolated from diseased fruit bodies and casing soils at 38 mushroom growing areas through Korea. *Trichoderma* spp. were isolated on the acidified PSA. The identification was done by colony types and color[4,8]. on Czapek's agar. The results indicated that there are 4 species of *Trichoderma* from *A. bisporus* in Korea. They are *T. koningi*, *T. lignorum*, *T. glaucum* and one unidentified species, and their frequencies are 50%, 32%, 13% and 5% respectively. Early studies on *Trichoderma* spp. showed that there were at least 3 species of *Trichoderma* which damaged mushrooms[6]. It is considered that *T.koningi* and *T. lignorum* are the most important species in Korea, because of their high frequencies of occurrence.

Mycelial growth of *Trichoderma spp.*

Trichoderma grows well in most media tested. Among the natural media, potato dextrose was the best and among the synthetic media, Richard's and Waksman's solution were very satisfactory for growth, but the growth in Petri mineral solution was meager. (Table 1).

Table 1. Dry weight of mycelia grown in various liquid media for 20 days at 52°C.

Media	*T. koningi*	*T. lignorum*
Potato Dextrose	76(mg)	92(mg)
Malt Extract	25	22
Corn Meal	51	55
Yeast Starch	31	62
Waksman	52	45
Czapek	22	29
Petri Mineral	2	2
Richard	48	59
L.S.D.$_{0.05}$	12	8.9

The poor growth in Petri mineral solution might be due to the lack of carbon sources. Therefore decreasing the organic matters from casing soil might be helpful in reducing the damage from *Trichoderma*.

The effect of pH of medium on mycelial growth.

Trichoderma grew rapidly in acidic solution. The optimum pH level for *T. lignorum* whereas mushroom (*A. bisporus*) was a reverse relationship. (Fig. 1). *T. lignorum* preferred acid(pH 4) to neutral, while

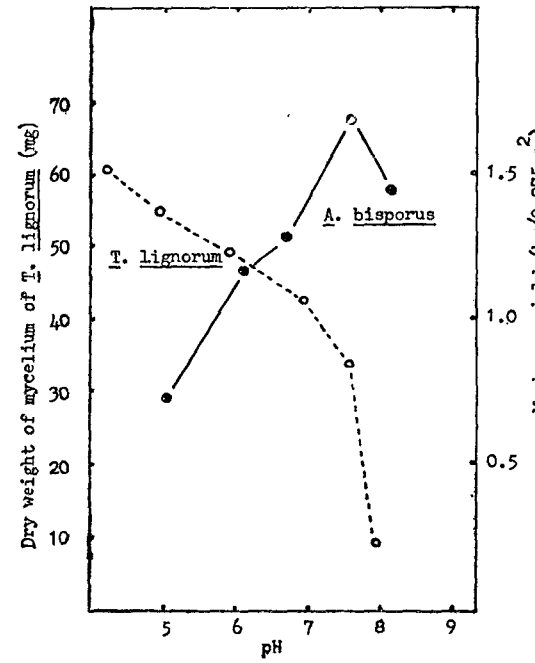

Fig. 1. The effect of pH on yield of *A. bisporus* (Kor. J. Pl. Prot. 10:59-62) and mycelial growth of *T. lignorum*.

mushroom preferred near neutral acidity (pH 7.5). Thomas[12] reported that the addition of lime to casing soil aided in preventing development of green mold which reduces mushroom yields. Weindling & Emerson[14] reported that the highest yields of an antibiotic substance were obtained from a culture of *T. lignorum* grown in a highly acid medium(pH 3 to 4). It has been reported that the highest yield of mushroom can

be obtained at neutral casing soil (pH 7 to 8)[9]. The results of these studies indicate that the neutralization of casing soil is very important, not only for mushroom yield, but also for the prevention of *Trichoderma*.

The effect of temperature on symptom appearance.

The present experiments showed that when mushroom grew at 15°C, the appearance of symptoms of *Trichoderma* was 4.8%, while the room temperature was raised to 22°C for 2 days in beginning of second flush, the symptom appearance increased to 53.1% during second flush. This showed that the effect of temperature on disease occurance was very important and during cropping the temperature should be kept at 15°C for high yield of mushroom as well as preventing the disease. For spring cropping, the cropping should be finished before the air temperature rise to 20°C. Park & Park[10] indicated the optimum temperature during cropping period was 15°C, also Flegg[3] showed that the temperature in the range 14°C to 19°C produced mushrooms which grew faster and larger.

Thermal death time of *T. lignorum*.

Trichoderma lignorum was killed when exposed for 60 minutes at 70°C and when exposed for 30 minutes at 80°C. (Fig. 2). Beck & Rasmussen[1] stated that

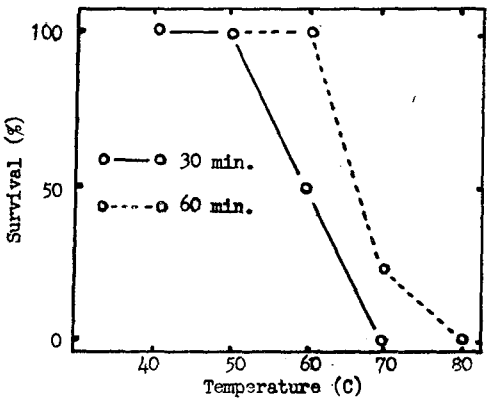

Fig. 2. The survival percentage of *T. lignorum* at various temperatures.

Trichoderma and some other mushoom pathogens in soil could be disinfected by exposing the soil to 60°C for 5 hours. Lambert & Ayers[7] reported that *Trichoderma koningi* was killed when exposed 4 hours at 60°C. The reasons for the differences of thermal death points and times among the reports be assumed the difference of testing methods, soil conditions and isolates used. It is considered that for satisfactory disinfection, the soil should be exposed for 30 minutes at a temperature of 80°C to steam sterilization.

The effect of peak heat of compost on control of *Trichoderma spp.*

Exposures to the peak heat procedure eliminated *T. lignorum* and *T. koningi*. Rasmussen[11] also reported the one of the effects of peak heat was pasteurization of compost. The present experiment showed complete eradication of *Trichoderma* spp. in compost by peak heat. It is considered the peak heat is very satisfactory process to sterilize compost.

적 요

본 시험은 우리나라에서 발생하는 푸른곰팡이병균의 종(種)의 빈도와 발병환경 및 방제법을 구명코저 실시하였다. 시험결과 *Trichoderma koningi*, T. *lignorum*, T. *glaucum* 과 미동정의 1종등 4종의 병원균이 분리되었고 이들의 빈도는 각각 50%, 32%, 13%와 5%이었다. 푸른곰팡이병균은 감자배양액, 왁스만배양액과 리차드배양액에서 생육이 잘 되었으며 중성~염기성배지에서는 생육이 불량한 반면 산성에서 생육이 왕성하였으며 최적산도는 pH4 였다. 양송이 수확기간중 재배사내의 온도는 15°C 내외 일 때 본병의 발생이 적었고 수량이 많으며 20°C 이상에서는 본병의 발생이 격심하였다. 푸른곰팡이병균은 복토흙 소독시 70°C 에서 60분, 혹은 80°C 에서 30분간 열처리하므로서 완전히 사멸하였고 퇴비 후발효 과정에서도 사멸되었다.

Literature cited.

1. Bech, K. and C.R. Rasmussen. 1965. Experiments with soil disinfectants for casing material and their effect on yield. Mushroom Science 6:515-521.
2. Colowick, S.P. and N.O. Kaplan. 1955. Methods in Enzymology. Vol. 1. 835p. Academic Press. N.Y.
3. Flegg, P.B. 1968. Effect of temperature on the growth, water content and condition on mushroom

sporophores. A.R. Glasshouse Crops Res. Inst. 1968. 159-163.
4. Gilman, J.C. 1957. A manual of soil fungi. Iowa State College Press. Ames, Iowa, U.S.A. 212-214.
5. Hino, I. 1935. Antagonistic action of soil microbes with special reference to plant hygiene. Trans. Third Int. Congr. Soil Sci. 173-174.
6. Kneebone, L.R. and E.L. Merek, 1961. Brief outline of and controls for mushroom pathogens, indicator molds, and weed molds and competitors. 9-11.
7. Lambert E.B. and T.T. Ayers. 1957. Thermal death times for some pests of cultivated mushroom (*Agaricus campestris* L.). Plant Dis. Reptr. 41: 348-353.
8. LeClerg, E.L. 1930. Cultural studies of some soil fungi. Mycologia 22:186-210.
9. Park, W.M., D.S., Kim, Y.H. Park, and B.H. Kwack. 1971. Effect of pH of casing soil and calcium on mycelial growth and yield of *Agaricus bisporus*(Lge.) Sing. Kor. J. Pl. Prot. (10)1:59-62.
10. Park, Y.H. and W.M. Park. 1969. The effect of casing time and temperature on mushroom yield and quality. Annual Research Report of Institute of Plant Environment, O.R.D., 7:175-185.
11. Rasmussen, C.R. 1959. Peak-heating and its effect on cropping yields. Mushroom Science 4:349-361.
12. Thomas Violet K. 1963. Cultivated mushrooms a specialty crop. America Mushroom Institute.
13. Weindling, R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology 22 (10):837-845.
14. Weindling, R. and O.H. Emerson. 1936. The isolation of a toxic substance from the culture filtrate of *Trichoderma*. Phytopathology 26(11): 1068-1070.
15. Wuest, P.J., K.F. Baker, and W.S. Conway. 1970. Sensitivity of selected mushroom pathogens to aerated steam. Phytopathology 60:1274-1275.