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Some Biological Studies on *Mycogone perniciosa* Magn. Causing Wet Bubble in Cultivated Mushroom, *Agaricus bisporus* (Lange) Sing.

(I) Antagonistic Relationships between *M. perniciosa* and Microfloral Organisms in the Casing Soil. (II) Interactions between *M. perniciosa* and *A. bisporus*.

Young Sik Han, Kwan Chul Shin, Dong Soo Kim

Institute of Agricultural Sciences, O.R.D., Suweon, Korea

양송이〔*Agaricus bisporus*(Lange) Sing.〕에 마이코곤病을 誘發하는 *Mycogone perniciosa* Magn.에 관한 生物學的 硏究 (Ⅰ) *M. perniciosa*와 양송이 覆土中 微生物사이의 拮抗的 關係 (Ⅱ) *M. perniciosa*와 *A. bisporus*사이의 相互關係

韓龍植・申寬澈・金東秀
農村振興廳 農業技術硏究所

Abstract : These experiments were conducted to learn the antibiosis of microfloral organisms in the casing soil to *Mycogone perniciosa*, and interactions between *M. perniciosa* and *Agaricus bisporus*.

The results obtained were as follows:

1. In vitro tests, the development of *M. perniciosa* was suppressed from the unidentified microfloral organisms in the casing soil.

All the infected sporophores of *A. bisporus* occurred within an area applied with spore suspension of *M. perniciosa* as spot treatment and no infected ones in the area around the spot treated under cropping conditions.

2. In vitro tests, although the antagonistic relationship between *M. perniciosa* and *A. bisporus* was somewhat different in varying the kind of media, *A. bisporus* ultimately overgrew the colony of *M. perniciosa*.

When spore inoculation of *M. perniciosa* was applied on the surface of grain spawn of *A. bisporus* and the mid layer of casing soil under cropping conditions, no infected sporophores were produced, whereas the infected sporophores were only produced on casing soil inoculated on the surface of casing soil with spore suspension.

Introduction

The interactions between pathogenic and nonpathogenic microorganisms in the soil exert a positive or negative effect on the incidence of soil-borne diseases. It has been known for many years that soil microorganisms may limit the growth of soil-borne fungal pathogens.

Mycogone perniciosa Magn. is a pathogen which causes wet bubble disease in the cultivated mushroom, *Agaricus bisporus* (Lange) Sing.

The antagonistic relationships between *M. perniciosa* and microfloral organism in the casing soil have received little attention though this fungus is well known to be soil-borne pathogen.

Smith (1924) stated that spread of the disease by the growth of the mycelium of *M. perniciosa* in the casing soil was usual.

On the other hand, Fletcher and Ganney (1968) reported that spore inoculation of the fungus on the surface of compost before casing resulted in no disease and spread through the casing soil was negligible. In their report, however, the investigation of the reason for these phenomena was not undertaken.

The parasitic or antagonistic relationships between *M. perniciosa* and *A. bisporus* were observed by serveral workers (Smith, 1924; Labrousse, 1936; Chage and Sarazin, 1936; Garibova, 1968; Fletcher and Ganney, 1968).

Accordingly, the purpose of this study was to learn the antibiosis of microfloral organisms in the casing soil to *M. perniciosa* and to record further observations on the interaction between *M. perniciosa* and *A. bisporus*.

Materials and Methods

Antibiosis of certain microorganisms in the casing soil to *M. perniciosa*.

a) Isolation of microorganisms from the mixture of loam and sand loam prepared for casing.——The soil samples were taken from the mixture of loam and sand loam prepared and stockpiled for casing. The two types of soil were generally collected from the B horizontal layer at different locations. The soil dilution plate technique was employed for the isolation of microorganisms and soil samples were diluted 100,000 times with distilled water. The media used for the isolation of soil microorganisms are as follows and the soil extract was prepared by autoclaving 1kg of mixture of loam and sand loam in 1 liter of water for 20 minuted and filtering the suspension.

(1) Peptone dextrose agar: agar 20.0g, KH_2PO_4 1.0g $MgSO_4 \cdot 7H_2O$ 0.5g, peptone 5.0g, dextrose 10.0g, distilled water 1,000ml; add 1 : 30,000 rose bengal after dissolving above, and 30g/ml streptomycin to cooled agar before pouring.

(2) Modified soil extract agar: agar 15.0g, K_2HPO_4 0.4g, $(NH_4)_2HPO_4$ 0.5g, $MgSO_4 \cdot 7H_2O$ 0.05g, $MgCl_2$ 0.1g, $FeCl_3$ 0.01g, $CaCl_2$ 0.1g, peptone 1.0g, yeast extract 1.0g, soil extract solution 250ml, tap water 750ml.

(3) Soil extract agar: agar 15.0g, glucose 1.0g, K_2HPO_4 0.5g, KNO_3 0.1g, soil extract 100ml, tap water 900ml.

(4) Nutrient agar: agar 20.0g, beef extract 3.0g, peptone 10.0g, distilled water 1,000ml.

(5) Potato dextrose agar: peeled and sliced potatoes 250g, agar 20.0g, dextrose 20.0g, distilled water 1,000 ml.

Potato dextrose agar was added to plate to which two drops of 50% lactic acid either was supplied or not. All cultures were incubated in the laboratory at 23~25°C for 5 days.

The resulting colonies were purely cultured and maintained in tubes of potato dextrose agar for further study.

b) Screening test for investigation antifungal activity of isolates against *M. perniciosa*.——The agar medium, consisting of 20g agar, 10g glucose, 2g yeast extract, 100ml soil extract and 900ml distilled water, was used in the screening test with all soil isolates. A 6mm agar disc of *M. perniciosa* was put in the center of petri dish with three agar discs containing one of the isolates placed at points equ-

idistant from it. All cultures were incubated at about 25°C. The growth of the soil isolates in the vicinity of *M. perniciosa* was examined 7 and 14 days after incubation. Types of inhibition by soil isolates were rated as follow outlined by Porter (1924).

(1) Mutual inhibition at considerable distance.

(2) Slight inhibition. Both organisms are inhibited but approach each other until almost in contact, when growth ceases.

(3) Growth around the contending organism.

(4) Mutually intermingling.

(5) Growth superficial over the contending organism.

To check the above observations further, the 2 isolates of bacteria and 2 isolates of fungi most antagonistic to *M. perniciosa* were selected on the basis of these inhibition types.

A mixture of loam and sand loam was placed on one half of the Petri plate and after autoclaving at 121°C for 60 minutes, potato sucrose agar was carefully poured into the second half of the plate. Inoculation was made at the edge of the half of the plate containing soil with a 0.2 ml spore suspension of *M. perniciosa*. In one set, soil was moistened with a 0.5ml broth culture of bacteria or spore suspension of fungi. Another set was moistened with 0.5ml broth without bacteria or with sterile water as a check. Throughout this experiment, the growth of the organism on the surface of the soil and the half of the plate containing potato sucrose agar was observed.

To determine whether the bacteria cause lysis of *M. perniciosa* in soil, a part of the soil was taken from the plate with spore of *M. perniciosa* and the broth culture of the bacteria, and a test was done using the soil dilution plate technique on Martin's peptone dextrose agar. The broth culture of bacteria used was made from a culture of bacteria in nutrient dextrose broth kept at 25°C for 2 days.

c) The effect of soil pasteurization on the antifungal activity of soil microfloral orgarism to *M. perniciosa*.— A test-tube was packed with a mixture of loam and sand loam and then inoculated with 0.5ml spore suspension of *M. perniciosa* near the top of the soil. The soil used was subjected to the following treatments; (1) exposed to 80°C for 60 minutes in the water bath. (2) non-treated. (3) autoclaved at 121°C for 60 minutes as a check.

Following treatment and inoculation with 10 replicates, the test-tubes were incubated at about 25° C. About 2cm depth of soil was taken from the portion below the 2cm and 3cm depths from the top of the soil in test-tube at 11 and 21 days after inoculation. Transfers were placed on Martin's peptone dextrose agar plates and incubated at about 25°C for 7 days to examine the growth of *M. perniciosa* from the transfers. In addition, the above three kinds of treated soil were diluted to 10^{-4} and 10^{-5} respectively, and 1ml of soil suspension was place in a Petri dish and covered with warm potato sucrose agar. The plates were inoculated with an inoculum disc of *M. perniciosa* and incubated at about 25°C, after which growth was recorded.

d) Test under cropping conditions——To confirm the results obtained from laboratory experiments, an experiment was done using a plastic box (33×24×12.5cm) in the mushroom house. Mushrooms were grown on rice straw compost cased with approximately 3 cm of steam sterilized sand loam and loam mixture. Inoculation treatments were done with a spore suspension, the spore suspension was made with distilled water and applied as spot treatment of 0.5ml per spot on the surface of casing soil 5 days after casing. Five equidistant spots were plotted on each box and 10 boxes were used.

Throughout the experiment, the incidence of healthy and infected sporophorse in the area around spots treated were checked.

Interactions between *A. bisporus* and *M. perniciosa*.

To investigate the antagonistic relationship between growing mycelium of the white colored strain of *A. bisporus* and *M. perniciosa*, the following tests were undertaken employing four different agar media.

The ingredients of each medium were as follows and the soil extract was prepared by autoclaving 1kg of mixture of loam and sand loam in 1 liter

of water for 20 minutes and filtering the suspension:

(1) Soil extract agar (SEA)(I): agar 20g, soil extract solution 1,000 ml.

(2) Soil extract agar (SEA)(II):agar 15g, glucose 1.0g, K_2HPO_4 0.5g, KNO_3 0.1g, soil extract 100ml, distilled water 900ml.

(3) Modified soil extract agar (MSEA): agar 15g, K_2HPO_4 0.4g, $(NH_4)_2HPO_4$ 0.5g, $MgSO_4 \cdot 7H_2O$ 0.05g, $MgCl_2$ 0.1g, $FeCl_3$ 0.01g, $CaCl_2$ 0.1g, peptone 1.0g, yeast extract 1.0g, soil extract 250ml, distilled water 750ml.

(4) Potato sucrose agar (FSA): potato 250g, sucrose 20g, agar 20g, tap water 1,000ml.

These experiments were done employing the following methods.

Method A(biculture method)— Grain spawns of *A. bisporus* were placed on the edge of a Petri dish and a disc inoculum of *M. perniciosa* was spaced on the opposite side of same plate. Plates were incubated at 25°C with 10 replicates.

Method B—1 ml of spore suspension of *M. perniciosa* was placed in the bottom of Petri dish and covered with 20 ml of warm media. The plates were inoculated with *A. bisporus*, 3 grain spawns each, and incubated at 25°C with 10 replicates. In both tests the growth of *A. bisporus* in the vicinity of *M. perniciosa* was observed.

A study was made to ascertain whether growth inhibiting substances are produced by both test organisms. A technique was used for killing young fungus colonies without disturbing the culture medium. Petri dishes with fungus colonies grown on potato sucrose agar and soil extract agar(II) for a period at about 25°C were placed in the desicators. Chloroform-soaked cotton was placed at the bottom of the desicators. Dishes containing test organisms and dishes with medium only as controls were left for 20 hours. The dishes were then removed and kept on a laboratory bench for 3 days to allow evaporation of the chloroform. The agar media with a colony of *A. bisporus* killed by chloroform were then seeded with a 2 ml spore suspension and disc inoculum of *M. perniciosa*. Likewise, the agar media with a killed colony of *M. perniciosa* were seeded with mycelial fragments of *A. bisporus*. The influence of the test organisms killed by chloroform on the growth of each organism was noted.

To investigate the parasitic relationship between *A. bisporus* and *M. perniciosa*, an experiment was done using 300ml glass beakers in a growth chamber. Beakers containing 70gr. of grain spawn of the white colored strain of *A. bisporus* were cased with a 4 cm layer of steam sterilized soil (1hr. at 80°C) A mixture of casing soil was made of 3 parts of loam and 1 part of sand loam. Inoculation was made with a spore suspension of *M. perniciosa* and four different series of inoculation treatments, randomized with 4 replicates, were applied as follows: (1) spawn surface immediately before casing. (2) mid layer of casing soil. (3) surface of casing soil immediately after casing. (4) no inoculation as a check. The temperature was kept at the range of 23°C to 25°C while the spawn was running through the casing soil and at about 16°C during the formation of sporophores. Throughout the experiment, the incidence of healthy and diseased sporophores were checked in each treatment.

Results and Discussions

Antibiosis of certain soil microorganisms to *M. perniciosa*

a) Isolation and screening of soil isolates—A total of 36 soil isolates were obtained from the casing soil, representing 16 isolates of bacteria and 20 isolates of fungi.

Large zones of inhibition, more than 10mm wide, were observed around the two isolates of the bacterial colonies on the agar medium. In the early stages, two other isolates of bacteria produced inhibition zones of more than 5mm, but hyphae of *M. perniciosa* later penetrated sparsely into the inhibition zone areas, whereas the others among the 16 isolates of bacteria were overgrown by colonies of *M. perniciosa*.

In contrast, all isolates of fungi were inhibitory to *M. perniciosa*. Among the 20 isolates of fungi, 13 isolates overgrew the colonies of *M. perniciosa* and the others were inhibitory in immediate contact

Table 1. Isolation and antifungal activity of soil isolates against *Mycogone perniciosa*

Isolates a)		Remarks
16 isolates of bacteria	2 isolates	Wide zones of inhibition more than 10mm.
	2 isolates	Inhibition zone more than 5mm, but later penetrated sparsely by *M. perniciosa*.
	12 isolates	Overgrown by colonies of *M. pernicicsa*.
20 isolates of fungi	12 isolates	Overgrew the colonies of *M. perniciosa*
	8 isolates	Both organisms were inhibited but approached each other unitl in immediated contact.

a. Identification was not undertaken.

with the advancing margin of colonies of the contending organism with no inhibition zone (Table 1).

Furthermore, no growth of *M. perniciosa* was noticed on the surface of the soil or on the half of the plate containing agar medium from the set moistened with the broth culture of the isolates of bacteria or the spore suspension of the isolates of fungi. Without exception, the check, to which only sterile broth or sterile water was added, was covered with a growth of *M. perniciosa* (Fig. 1, 2).

The colonies of *M. perniciosa* were recovered on Martin's peptone dextrose agar plates to which a suspension of soil inoculated with spores of *M. perniosa* and bacteria was added.

Identification of the soil isolates concerned was not undertaken, but an attempt to identify each isolate is in progress.

b) The effect of soil pasteurization on the antifungal activity of soil microfloral orgarisms to *M. perniciosa*.— When transfers taken from the three

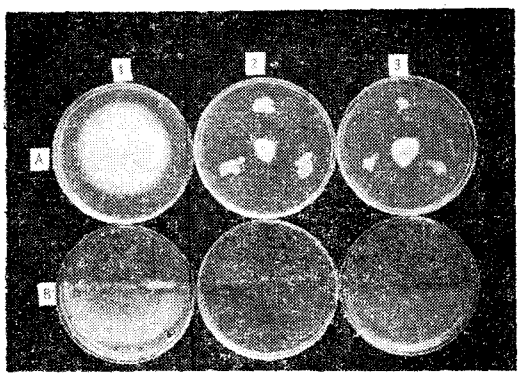

Fig. 1. Antifungal activities of isolates of bacteria obtained from casing soil against *Mycogone perniciosa*,
A) On soil extract agar plates B) on soil and PSA in Petri dish
1) Check:Without isolates of bacteria, an inoculum of *M. perniciosa* was added
2), 3) Inocula of isolates of bacteria and *M. perniciosa* were added. B1) Covering with a growth of *M. perniciosa* on soil and PSA. B2, B3) No growth of *M. perniciosa* on soil and PSA

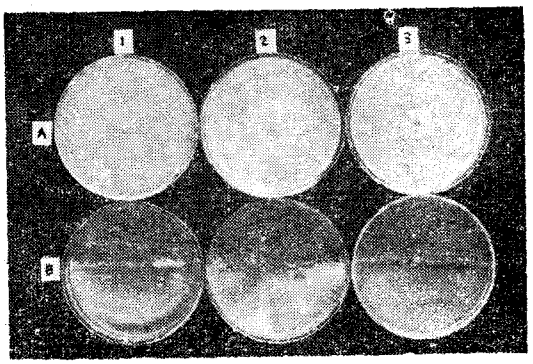

Fig. 2. Antifungal activities of isolates of fungi obtained from casing soil against *Mycogone perniciosa*.
A) On soil extract agar plates B) on soil and PSA in Petri dish.
1) Check; Without isolates of fungi, an inoculum of *M. perniciosa* was added.
2), 3) Inocula of isolates of fungi and *M. perniciosa* were added. B1) Covering with a growth of *M. perniciosa* on soil and PSA. B2, B3) Covering with growth of isolates of fungi on soil and PSA.

kinds of treated soil were placed on Martin's peptone dextrose agar plates, no *M. perniciosa* was recovered at all from the soil exposed to 80°C for 60 minutes and non-treated natural soil, but only recovered from the autoclaved soil.

Also, growth of *M. perniciosa* either was arrested several millimeters or was completely suppressed from the unknown bacterial colonies on potato sucrose agar plates at 10^{-4} and 10^{-5} dilution of soil exposed to 80°C for 60 miuntes and non-treated natural soil.

c) Test under cropping conditions— During an experiment to ascertain the results of laboratory tests, all the infected sporophores occurred within an area applied with spore suspension as spot treatment and no infected ones in the area around the spots treated.

Throughout these studies, microfloral organisms in the casing soil suppressed the development of *M. perniciosa,* only isolates of bacteria caused fungistasis in *M. perniciosa* and no antifungal activity of soil microfloral organisms was affected by soil pasteurization.

The results of our tests conflict with the results reported by Smith (1924), who stated that spread by the growth of the mycelium of this fungus in the casing was usual.

Fletcher and Ganney(1968) concluded that spread through the casing is unlikely to result in a large build-up of this disease on commercial farms. In their report, however, the reason for this phenomenon was not suggested.

Our results presented here indicate that development of *M. perniciosa* through the casing soil is impossible because of antifungal activities of microfloral organisms in the casing soil against *M. perniciosa.*

Interactions between *A. bispours* and *M. perniciosa.*

a) Antagonistic relationship between mycelium of *A. bisporus* and *M. perniciosa* on the agar media —— The results of the experiments on agar media are summarized in Table 2.

Although the antagonistic relationship between both test organisms was somewhat different in association with the varying kinds of media, *A. bisporus* ultimately overgrew the colony of *M. pernicisoa.* The results of this experiment indicate that the inhibitional effect of *A. bisporus* toward *M. perniciosa* became much more distinct in poor nutritions

Table 2. Antagonistic relationship between *A. bisporus* and *M. perniciosa* on various solid media.

Media	Types of inhibition	
	Method A	Method B
SEA(Ⅰ) SEA(Ⅱ)	*A. bisporus* overgrew the colony of the contending organism without indication of inhibition.	
MSEA	Both organisms were inhibited for about 2 weeks remaining in immediate contact with the advancing margin of colonies but the colony of *A. bisporus* later overgrew slowly the colony of *M.perniciosa.*	At first, mycelial growth of *A. bisporns* was suppressed as the resulting colony of *M. perniciosa* covered the suface of medium, but the mycelium at the edge of the colony of *A. bisporus* later branched on the colony of *M. perniciosa.*
PSA	Inhibition type usually including all of type in the case of MSEA, but growth stunt condition of both organisms remained for about 3 weeks.	Including all of inhibition type in the case of MSEA.

than in rich nutritions. Porter (1924) suggested that in the case of growth superficially over the contending organism, the underlying organism is always greatly inhibited and the richer the medium in nutrients the less marked were the inhibitions. In addition, to examine whether *A. bisporus* cause lysis of *M. perniciosa*, agar blocks taken from the portions of colony of *A. bisporus* overgrowing *M. perniciosa* were placed on the potato sucrose agar plates. This test showed that the colonies of *M. perniciosa* were not recovered at all from the transferred potato sucrose agar block, but were recovered from the other agar blocks.

In the study of the production of growth inhibiting substances on PSA and SEA (II) media by both test organisms, normal spore germination and growth of *M. perniciosa* occurred in both the control dishes and in dishes with a colony of *A. bisporus* killed by chlorlform. Similarly, a colony of *M. perniciosa* killed by chloroform did not affected the growth of *A. bisporus*.

The results of this series of experiments indicated that *A. bisporus* did not cause lysis of *M. perniciosa* in the media as close as possible to the natural substrate such as various kinds of soil extract agars. Also, production of growth inhibiting substances by both test organisms is untraceable on the agar media.

Chage and Sarazin (1936) reported that the mycelium of *A. bisporus* invaded the culture of *M. perniciosa* without becoming affected, the culture media on which it had grown prevented the germination of the spores of *M. perniciosa* and humoral products secreted by *A. bisporus* becoming diffused in the media.

According to Garibova (1968), most monospore isolates of *A. bisporus* tested suppressed the development of *M. perniciosa* in joint cultures, some isolates formed large zones of suppression and others, mainly brown ones, grew over colonies of the parasite.

From these view points, it is considered that the results presented here are partially different from the results of Chage and Sarazin (1936) and Garibova (1968).

b) Parasitic relationship between *A. bisporus* and *M. perniciosa*— When spore inoculation was applied on the surface of grain spawn and the mid layer of casing soil, no diseased sporophore was produced, whereas the diseased sporophore were only produced on casing soil inoculated on the surface with spore suspension immediately after casing.

Labrousse (1936) stated that no parasitic relationship was observed between *M. perniciosa* and actively growing mycelium of *A. bisporus* and direct infection of the sporophores of *A. bisporus* by *M. perniciosa* was invariably obtained. Also, Smith (1924) reported that infection of the spawn of *A. bisporus* was not observed to occur, and each mushroom appeared to be separately infected above ground or at soil level by spore or mycelium in the soil. No evidence of parasitism of the mushroom mycleium by *M. perniciosa* and only infection of the sporophore were also observed by Fletcher and Ganney (1968). Accordingly, in our researches, indications are that no parasitic relationship between *M. perniciosa* and the growing mycelium of *A. bisporus* exist, the parasitism of *M. perniciosa* on the mushroom is restricted to the mushroom sporophore and furthermore, results when a mushroom sporophore only develops in immediate contact with the inoculum of. *M. perniciosa*.

Also, it is considered that no infection when spores were inoculated either on the spawn surface or in the mid casing layer is utterly due to the antifungal activities of soil microfloral organisms against *M. perniciosa*.

References

Chaze, J. and A. Sarazin (1936): Nouvelles dann'ess biologiques et expérimentale sur la môle maladie to Champignon de couche (New biological and experimental data on the mole disease of the edible mushroom). Reprinted from *Ann. Sci. nat., Bot.*, Ser. **10**, 18 : 84pp. ; *Rev. appl. Mycol.* **16** : 15.

Fletcher, J.T. and G.W. Ganney (1968): Experiments on the biology and control of *Mycogone pern-*

iciosa Magn. *Mushr. Sci.* **7** : 221-237.

Garibova, L.V. (1968), :Ob antagonisticheskoi aktivnosti mitseliya kul'tiviruemogo shampin'ona *Agaricus bisporus* (J.Lange) Sing. (On antagonistic activity of the mycelium of the cultivated mushroom, *A. bisporus*) *mikol. i Fitopatol.*, **2**(2) : 81-87 ; *Rev. appl. Mycol.* **47** : 2954.

Labrousse, F. (1936): Les maladies verticilliennes du champignon de couche (Verticillium diseases of the edible mushroom). *Rev. Path. veg.*, **23** : 162-172; *Rev. appl. Mycol.* **15** : 628.

Porter, C. L. (1924): Concerning the characters certain fungi as exhibited by their growth in the presence of other fungi. *Amer. Jour. Bot.* **11** : 168-188.

Smith, F.E.V. (1924):Three diseases of cultivated mushrooms. *Trans. Brit. Mycol. Soc.* **10** : 81—97.

摘　要

本 試驗은 양송이 覆土中 微生物의 *Mycogone perniciosa* Magn.에 대한 抗菌作用및 *M. perniciosa* 와 *Agaricus bisporus* (Lange) Sing. 사이의 相互關係를 究明하기 위하여 實施되었으며 試驗한 結果,

1. In vitro test에서 *M. perniciosa* 는 覆土에서 分離된 未同定의 微生物들에 의해서 生長이 抑制되었으며 栽培試驗에서 *M. perniciosa* 의 胞子懸濁液을 覆土表面에 局所接種하였을 때 罹病버섯은 接種된 部分에서만 發生되었고 接種部分 周邊에서는 發生되지 않았다.

2 *M. perniciosa* 와 *A. bisporus* 사이의 拮抗的 關係는 in vitro test에서 培養基의 種類에 따라 多少 差異가 있었으나 *A. bisporus* 의 菌絲는 *M. perniciosa* 菌叢 위를 덮어서 자랐다. 栽培試驗에서 *M. perniciosa* 의 胞子懸濁液을 *A. bisporus* 의 穀粒種菌 表面 혹은 覆土層의 中間部位에 噴霧接種하였을 때에는 罹病버섯이 發生되지 않았으나, 覆土表面에 接種하였을 때에만 罹病버섯이 發生되었다.