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Decrease of Nematode Population by Introduction of Nematophagous Fungi into The Soil as Affected by Inoculum Concentration and Temperature *in Vitro*

線蟲 寄生 天敵 眞菌의 接種源 濃度와 溫度條件에 따른 線蟲感染 및 集團의 減少效果

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ABSTRACT Five nematophagous fungi were evaluated for their nematicidal effect *in vitro* on *Rhabditis* sp. and *Meloidogyne hapla* in soil. Inocula of *Arthrobotrys arthrobotryoides, A. conoides, A. oligospora, Dactylella lobata,* and *Fusarium oxysporum* were grown in moistened corn-sandy soil and chopped potato-sandy soil media, and incubated at 26°C for one week. The prepared inocula were incorporated in autoclaved sandy soil, mixing thoroughly at rates equivalent to 1 : 50, 1 : 100, 1 : 200, and 1 : 400, respectively, before 80g of the mixture carrying 100 *Rhabditis* sp. was put into petri plates. Nematophagous fungi effectively reduced the population of *Rhabditis* sp. in soil in a week or two, following treatment of the inocula at concentration of 1 : 50 and 1 : 100. The *Rhabditis* sp, population in soil decreased significantly at temperature ranging from 15 to 30°C. The optimum was at 25°C for nematicidial effect as high as 80—100%. The at the rate of 1 : 100 prepared inocula were incorporated in autoclaved soil, where 100 juveniles *M. hapla* were introduced per 80g soil. All fungi infected the *M. hapla* effectively in soil, causing more than 90% mortality within one week. This result indicated the potential value of these fungi as promising biocontrol agents.

KEY WORDS *Arthrobotrys* spp., *D. lobata* and *F. oxysporum*, mortality of *M. hapla* in soil

抄 錄 線蟲 寄生 天敵 眞菌의 接種源을 製造하여 接種源의 處理 濃度와 溫度에 따른 線蟲感染率을 調査하여, 適合한 條件下에서 당근 뿌리혹線蟲인 *Meloidogyne hapla*의 致死率을 살펴 보았다. 天敵寄生菌중 *A. arthrobotryoides, A. conoides, A. oligospora*와 *D. lobata* 및 *F. oxysporum*의 接種源은 옥수수—물—흙(0.5 : 1.5 : 10) 培養基나 혹은 감자—물—흙(1 : 1.5 : 10) 培養基에 26°C, 7일간 培養한 後 이를 接種源으로 使用하여, 接種源 對 土壤의 比率을 1 : 50, 1 : 100, 1 : 200 및 1 : 400으로 섞어서 處理한 뒤, 土壤 80g 當 *Rhabditis* sp. 100마리를 接種하여 1주일과 2주일 후에 調査한 結果, 1 : 50과 1 : 100 水準에서 線蟲의 致死率이 가장 높았다. 天敵寄生菌의 溫度別 效果를 調査해 본 結果, 15—30°C 사이에서 그 效果가 優秀하였으며, 特히 25°C에서의 效果가 卓越하였다. *Meloidogyne hapla*의 2齡蟲을 土壤 80g 當 100마리 處理하고, 接種源 對 土壤의 比率을 1 : 100 水準으로 처리하여 1주일 후에 觀察한 結果, *A. oligospora* 처리구는 그 효과가 약간 低調하였으나, 나머지 4菌株의 效果는 優秀하여 今後 生物的 防除에 利用可能한 菌으로 有望視되었다.

檢索語 *Arthrobotrys* spp., *D. lobata* and *F. oxysporum*, 線蟲 致死率

Investigators over the world are concentrating their efforts on integration of biological control agents in nematode management strategies (Dackman & Nordbring Hertz 1985, Dube & Smart 1987, Jaffee & Zehr 1985, Jatala 1986, Kerry 1984, Mankau 1980a, Niblak & Hussey 1986). Most of the works on root-knot nematodes in Korea are limited to the occurrence and assessement of dam-

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age. There have been very few works on screening for resistance and no trials on biological control at all. In our previous work (Jeong & Kim 1989), we have isolated seven nematophagous fungi, *A. arthrobotryoides*, *A. conoides*, *A. oligospora*, *D. lobata*, *F. oxysporum*, *Monacrosporium ellipsosporum* and *Harposporium anguillulae*, and determined the optimum condition for their grow ths and sporulations *in vitro* as a preliminary work toward biocontrol of plant parasitic nematodes.

The fungal antagonists of nematodes consist of a great variety of organisms belonging to widely divergent orders and families of fungi (Harrd 1968, Mankau 1980a, Mankau 1980b).

The parasitic abilities of nematode-attacking fungi are known to be often inversely related to saprophytic ones; i.e., efficient parasites are inefficiently saprophytic and *vice versa* (Jaffee & Zehr 1985, Jansson & Nordbring Hertz 1979). Competitive saprophytic ability is greatly influenced by substrate and environment(Jaffee & Zehr 1985). Temperature is one of the most important environmental factors influencing the initiation and development of fungal disease of invertebrates(Niblack & Hussey 1986).

Mankau(Mankau 1962) suggested use of inoculum in a form of dried spore material is the most feasible method for large-scale application and utilization of such organisms. He determined the presence of fungistatic factors to conidia and germ tubes of nematophagous fungi in a number of Southern California soils. Therefore, the inocula should be prepared and applied to nematode-infested soil as a survival structure in such a way that nematophagous fungi could successfully compete in the field soil environment.

The experiments in this report attempted to apply nematophagous fungi, obtained in our previous study, in a form of propagules as survival structure, to evaluated their biocontrol potentials and to determine optimum temperature for antagonism *in vitro*.

MATERIALS AND METHODS

Inoculum preparation of nematodes and nematophagous fungi

Nematode

The population obtained from the Baermann funnel was usually a mixture of nematode species. From the mixtured population, nematodes of *Rhabditis* sp. were removed on a fine needle and transferred to a drop of water on of Nigon's medium(Dougherty 1960) useful for culturing the nematodes. This medium contains in 1 liter distilled water 0.75g $MgSO_4 \cdot 7H_2O$, 0.75g K_2HPO, 2.75g NaCl, 3.0g KNO_3, 2.5g peptone, 1.0g lecithin, and 15.0g agar.

Inoculum of *M. hapla* was raised on the potted tomato plants cultivar [Kwang-Su(Jung-Ang Seed Co.)] in a greenhouse. About 50days later, egg masses were collected manually from the infected roots and incubated in 20ml water in 100ml flask at 26°C for 5 days for hatching of the eggs. Thus, the second-stage juveniles were supplied for the experiment.

Nematophagous fungi

Inoculum for soil infestation was cultured in moistened corn-sandy soil for *Arthrobotrys* spp. and in chopped potato-sandy soil for *D. lobata* and *F. oxysporum* media in 1 liter Erlenmeyer flasks containing 300g of 0.5 : 1.5 : 10 and 1 : 1.5 : 10 mixture of comminuted corn or chopped potato: distilled water: and sandy soil, respectively. They were autoclaved for one hour on two consecutive

days. Five mycelial agar blocks, one cm in diameter, of each fungus were aseptically introduced into the medium and allowed to colonize for one week at 26°C in incubator. The soil medium fully covered with the fungus, was spread on flat trays to allow air drying for 4—5days and was pulverized to powder in a mortar. Then, it was passed through a 2-mm-mesh sieve to remove the large pieces. This powdered inoculum was stored in air tightend bottle at room temperature.

Laboratory conditions for testing efficacy of nematophagous fungi

Inoculum concentration

From the results of preliminary experiments, potential nematophagous fungi were selected on the basis of their saprophytic growth, ability and efficacy of nematode killing action *in vitro*.

Unless otherwise indicated, nematophagous fungi used in the experiments throughout this study were *A. arthrobotryoides*, *A. conoides*, *A. oligospora*, *D. lobata*, and *F. oxysporum*. The inocula prepared in cornmeal and/or chopped potato soil medium, hereafter refered to as soil inocula, were incorporated in autoclaved sandy soil and mixed thoroughly at rates equivalent to 1 : 50, 1 : 100, 1 : 200, and 1 : 400. These mixtures were put into petri plates to carry without fungi or with one of the five nematophagous fungi, where one hundred of *Rhabditis* sp. were introduced per plate. Plates were maintained in a incubator at 26°C for 7days and 14 days. The Baermann funnel technique was used to assay the soil in each container for nematode population. Sample suspension (1ml) from Baermann funnel were placed in a Peter eelworm slide (Hawksley, England), and nematodes were counted under the compound light microscope.

Temperature

Soil inocula were incoporated in autoclaved sandy soil at rates of 1 : 100. Autoclaved soil with out fungi or with one of the five nematophagous fungi, was 80g per 9-cm-diameter glass petri plate, where 100 of *Rhabditis* sp. were introduced. Plates were maintained in incubator at 10, 15, 20, 25 and 30°C for 7 days and/or 14days. Sample suspensions extracted from the soil through Baermann funnel were placed in a Peter eelworm slide, and nematodes were counted under the compound light microscope.

Effect of nematophagous fungi against *M. hapla*

The same method used above was applied to screen for efficacy of fungi against *M. hapla* juveniles at inoculum concentration of 1 : 100. One hundred juveniles were inoculated per each plate. The seperation of juveniles from soil was done by the centrifugal sugar floatation(Tayer & Sasser 1978). Sample suspension(1ml) from centrifugation tube were placed in a Peter eelworm slide and nematodes were counted under the compound light microscope.

RESULTS

Biocontrol potentials of nematophagous fungi in soil

Effect of inoculum concentration

Five promising nematophagous fungi were isolated from the nematodes naturally infested with fungi. The nematophagous fungi effectively reduced the population of *Rhabditis* sp., which were incorporated in sterile soil after 2 weeks (Fig. 1). All of the nematophagous fungi killed more than 90% at

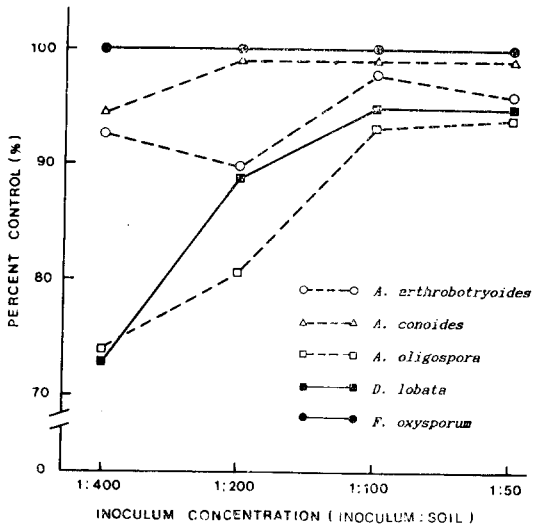

Fig. 1. Percent control of *Rabditis* sp, in autoclaved soils after two weeks following inoculation of nematophagous fungi with different inoculum concentration, at 25°C.

inoculum concentration 1 : 50 and 1 : 100. However, when the inoculum concentration were diluted to 1 : 200 or more, the percentage control of the nematode were reduced gradually except *F. oxysporum*. *F. oxysporum* was the most effective biocontrol agent in reducing population of *Rhabditis* sp. at all inoculum concentrations, and *A. conoides* was the next. When the inocula of the other three fungi were diluted more than 1 : 100 the nematophagous efficiency on *Rhabditis* sp. was reduced sharply except *A. arthrobotryoides*. This result indicated that 1 : 100 dilution was suitable to test the efficiency of nematophagous fungi in the control of the saprophytic nematodes in soil.

Effect of temperature

The effect of temperature on the nematode control by the five nematophagous fungi was indicated in Figure 2. Regardless of the species of nematophagous fungi, the optimum temperature for killing *Rhabditis* sp. was 25°C, at which the control efficiency was as high as 80—100% in autoclaved soil. Nematicidal effect was not more than 40% at 10°C. However, at 15—20°C, it was increased significantly to more than 60% upto 80%. Equivalent mortality was observed at 30°C also. Such results corresponded to the optimum temperature for growth in the dr-

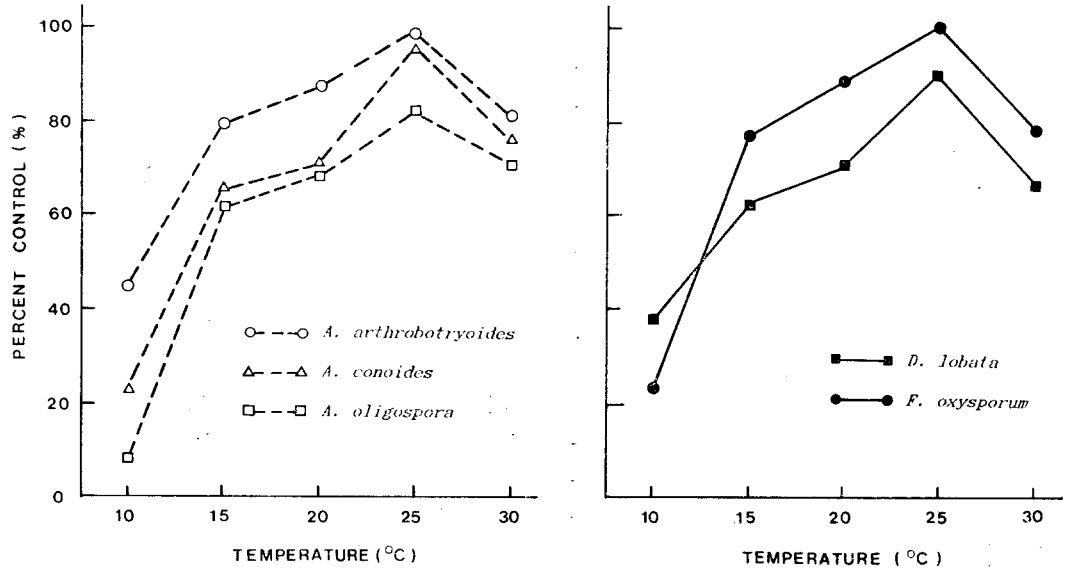

Fig. 2. Percent control of *Rhabditis* sp. in autoclaved soils after one week following inoculation of nematophagous fungi with concentration of 1 : 100, at different temperature.

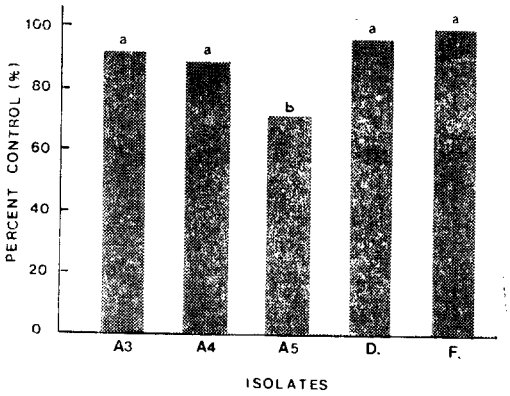

Fig. 3. Percent control of *M. hapla* in autoclaved soils after one week following treatment of nematophagous fungi with concentration of 1 : 100, at 25 °C.(p=0.05)
A_3, *A. arthrobotryoides:* A_4, *A. conoides:* A_5, *A. oligospora: D., D. lobata: F.,F. oxysporum*

evious *in vitro* test(Jeong & Kim 1989).

Effect of nematophagous fungi against *M. hapla*

In the all experiments, *A. arthrobotryoides, A. conoides, A. oligospora, D. lobata,* and *F. oxysporum* significantly reduced population of *M. hapla* juveniles in autoclaved soil. Juveniles of *M. hapla* were used to screen the effective biocontrol fungi in laboratory. The efficiency of each nematophagous fungus was shown in Figure 3. All fungi controlled the *M. hapla* effectively in autoclaved soil but *A. oligospora* was slightly less effective than other fungi, although significant statistically. The mortality of *M. hapla* due to nematophagous fungi reached to more than 90% within a week, significantly high in the nematode control.

DISCUSSION

The powdered inocula of dried preparation were evidently effective for nematophagous activity. This observation is particularly meaningful because many results available so far were based on parasitism in culture media, rather than optimum condition for fungi. However, field conditions are not always ideal for nematode and fungi. There fore, our attempt was to develop inoculum in a form of propagules as survival structure in natural ecosystem, and then to evaluate their efficacy as a preliminary test. All five nematophagous fungi was effective against both *Rhabditis* sp. and *M. hapla.*

In working with nematophagous fungi, saprophytic *Rhabditis* sp. could well be used for preliminary screening for parasitic ability of any fungus toward root-knot nematodes, which facilitates the rapid screening of the control efficacy because high population of *Rhabditis* sp. is readily available in the laboratory scale.

Rapid nematophagous activity was observed for *M. hapla* in a week after treatment (Fig. 3), of which the the level was obtainable in two weeks for saprophytic nematode, *Rhabditis* sp. (Fig. 1). This results suggested that plant parasitic nematode might be more susceptible to fungal treatment than the saprophytic ones. This phenomenon is desirable in fact because we should not destroy the saprophytic nematode population in soil. We need only to keep plant parasitic nematodes below economic thresholds.

It is generally known that saprophytic ability of nematophagous fungi are inversely proportional to parasitic ability (Jaffee & Zehr 1985, Jansson & Nordbring-Hertz 1979). However, such a generalization did not hold true for *D. lobata* and *F. oxysporum* in this experiment. Both fungi was proved as outstanding isolates for growth and sporulation based on our earlier work (Jeong & Kim 1989). They also were aggressively parasitic to nematodes. *A. oligospora* was weakly saprophytic and slightly less effective for the

control of both *Rhabditis* sp. and *M. hapla* than other fungi, but other two *Arthrobotrys* spp. were equally effective against nematodes. *F. oxysporum* isolated from egg masses of *M. hapla*, was the most aggressive among the fungi against both nematodes. *D. lobata* was rapidly growing and slightly less effective against saprophytic nematode *Rhabditis* sp., but was equally effective against root knot nematode *M. hapla* as the most promising fungus *F. oxysporum*. This might indicate host specificity of the fungus to root-knot nematode as Jansson and Nordbring(1979, 1980) noticed that nematophagous fungi revealed differences in dependence on nematodes for nutrients.

REFERENCES CITED

Dackmam, C. & B. Nordbring-Hertz. 1985. Fungal parasites of the cereal cyst nematode *Heterodera avenae* in Southern Sweden. J. Nematol. 17 : 50—55.

Dougherty, E.C. 1960. Cultivation of Aschelminths, especially Rhabditid nematodes. In: Nematology (Eds J.N. Sasser & W.R. Jenkins), pp. 297—318. University of North Carolina Press, Chapel Hill.

Dube, B. & G. C. Smart, JR. 1987. Biological control of *Meloigogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. J. Nematol. 19 : 222—227.

Harrd, K. 1968. Taxonomic studies on the genus *Arthrobotrys* corda. Mycologia 60 : 1140—1159.

Jaffee, B.A. & E.I. Zehr. 1985. Parasitic and saprophytic abilities of the nematode-attacking fungus *Hirsutella rhossiliensis*. J. Nematol. 17 : 341—345.

Jansson, H.B. & B. Nordbring- Hertz. 1979. Attraction of nematodes to living mycelium of nematophagous fungi. Journal of General Microbiology 112 : 89—93.

Jansson, H.B. & B. Nordbring-Hertz. 1980. Interactions between nematophagous fungi and plant-parasitic nematodes: attraction, induction of trap formation and capture. Nematologica 26 : 383—389.

Jatala, P. 1986. Biological control of plant-parasitic nematodes. Ann. Rev. Phytopathol. 24 : 453—489.

Jeong, M.J. 1988. Isolation of nematophagous fungi and evaluation of their biological control potential against *Meloidogyne hapla* Chitwood in pepper. M. S. Thesis, Gyeong-sang National University. 86pp.

Jeong, M.J. & H.K. Kim. 1989. Isolation of nematophagous fungi against nematode and their growth *in vitro*. Kor. J. Applied Entomology. 27 (3) : 149—158.

Kerry, B.R. 1984. Nematophagous fungi and the regulation of nematode populations in soil. Helminth. Abstr. Ser. B. 53 : 1—14.

Mankau, R. 1962. Soil fungistasis and nematophagous fungi. Phytopathology 52 : 611—613.

Mankau, R. 1980a. Biocontrol: fungi as nematode control agensts. J. Nematol. 12 : 244—252.

Makau, R. 1980b. Biological control of nematode pests by natural enemies. Ann. Rev. Phytopathol. 18 : 415—440.

Niblack, T.L. & R.S. Hussey. 1986. Evaluation of *Arthrobotrys ameroapora* as a biocontrol agent for *Heterodera glycines* on soybean. Plant Disease 70 : 448—451.

Tayer, A.L. & J.N. Sasser. 1978. Biology, Identification and control of root-knot nematodes(*Meloidogyne* species). pp. 1—106.

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