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Evaluation for Biocontrol Potentials of Nematophagous Fungi against Root-knot Nematode

뿌리혹 선충에 대한 선충 천적 기생균의 생물적 방제 효과

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ABSTRACT Five nematophagous fungi, *Arthrobotrys arthrobotryoides, A. conoides, A. oligospora, Dactylella lobata* and *Fusarium oxysporum* were evaluated for nematicidal effect on *Meloidogyne hapla* in greenhouse. Treatment of nematophagous fungi reduced the root galling by *M. hapla* and increased red-pepper growth in naturally infested pot soil. Number of galling were significantly less in all pots in 4 different inoculum densities of 5 nematophagous fungi compared to untreated plots. Especially, treatment of *F. oxysporum* resulted significant reduction of root gall of red-pepper. The increased shoot growth was significantly higher in pretreated plots by *A. arthrobotryoides, A. conoides, A. oligospora, D. lobata* and *F. oxysporum* at inoculum concentration of 1:100 but other treatments were not significantly increased shoot growth. Two promising fungi, *D. lobata* and *F. oxysporum* were selected in greenhouse test and in vitro results of previously experiment and applied to field plot naturally infested by *M. hapla* serverely. Number of galls were remarkbly fewer in plots treated with *D. lobata* and *F. oxysporum* at either 1 : 70 or 1 : 100 concentration compared to the untreated plots. The shoot growth of red-pepper was increased strikingly in the plots following the red-pepper was increased strikingly in the plots following the treatment of both fungus than greenhouse test.

KEY WORDS *Nematophagous fungi, Arthrobotrys* spp., *D. lobata, F. oxysporum,* Inoculum concentration

초 록 5종의 선충 천적 기생균 *A. arthrobotryodes. A. conoides, A. oligospora, D. lobata* 그리고 *F. oxysporum*을 이용해 온실에서 당근뿌리 혹선충 방제효과를 시험한 결과, 선충 천적 기생균을 처리했을 때 뿌리혹형성이 감소하였고 또한 고추생육이 증진되었다. 5가지 천적 진균 모두 4가지 접종원 농도로 처리했을 때 뿌리혹 형성을 무처리와 비교한 결과 현저히 감소되었다. 특히 *F. oxysporum* 처리구가 뿌리혹 감소 효과가 매우 높았다. 고추 지상부 생육은 *A. arthrobotryoides, A. conoides, A. oligospora, D. lobata* and *F. oxysporum* 접종원을 고추 정식전 처리했을 때 크게 증가하였으나 나머지 처리구에서는 무처리와 비교해서 현저한 차이는 없었다. 온실 시험과 실내시험을 통하여 방제 효과가 우수하였던 *D. lobata*와 *F. oxysporum*을 선발하여 뿌리혹 선충의 밀도가 높은 포장에서 시험한 결과, 두가지 선충 천적 기생균 모두 접종원 농도 1 : 70, 1 : 100에서 무처리에 비해 고추의 뿌리혹수가 현저히 감소하였다. 이때 고추 지상부 생육은 두처리 모두 온실 시험에서 보다 크게 증가하였다.

검 색 어 선충 천적 기생균, *Arthrobotrys* spp., *D. lobata, F. oxysporum* 접종원 농도

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Nematodes are ubiquitous wormlike animals. They are very active creatures that move sinuously. Plant parasitic nematodes, especially root-knot nematodes were economically important in crop yield(Tayer & Sasser 1978, Choi & Choo 1978). Intensive attention have been given to the control of nematode through the current methods such as soil fumigation, nematicide application and crop rotation. Recently, many investigators suggested that nematode was able to control by biological agents. All currently known enemies would probably exceed 200 species. about three-quarters would be fungi and remainder would be soil invertebrates, including many predacious nematodes, or bacterial species (Sayre 1980). Nematophagus fungi are wide spred throught the world and similar species occur in a wide range of habitats and have been tested for biocontrol of pant parasitic nematodes for long time (Barron 1977, Jansson et al. 1985). However, there are great technical difficulties in the practice of ovserving and assessing these organisms. Recently, techniques have been advanced in this area. Investigators over world are concentrating their efforts with application of biological agents in nematode management strategies (Jatala 1986). Most of the works on root-knot nematode pests in korea are limited to the occurrence, assessment of damage (Choi & Choo 1978, Choo 1978). Very few works were conducted with biocontrol of root-knot nematodes. Such a situation was prompted us to challenging on biocontrol of root-knot nematodes. Therefore this study was undertaken to evaluate biocontrol potentials of nematophagous fungi in the greenhouse and field.

MATERIALS AND METHODS

Nematophagous fungi and Inoculum

The nematophagous fungi, *A. arthrobotryoides*, *A. conoides*, *A. oligospora*, *D. lobata* and *F. oxysporum* were used in this experiment. The morphological characteristics and inoculum peparation of nematophagous fungi were described in previous report (Jeong & Kim 1988, Kim et al. 1988).

Collection of test soil

Evaluation of biocontrol of root-knot nematode was carried out in sandy loam soil collected from field heavily infested with *M. hapla*. The soil sample from 5～30cm deep were collected randomly througout a field. The field had been cultivated red-pepper for 5～6 years continuously and *Angelica dahurica* Benth et Hook for 2 years.

Greenhouse evaluation for biocontrol potentials of nematophagous fungi

Precolonization of antagonist to rhizosphere of pepper seedings; The pots(6 × 6cm) were filled with steam sterilized soil mixed with preparation of 5 nematophagous fungi at rates of 1 : 50, 1 : 100, 1 : 200 and 1 : 400. A 4 weeks old pepper seedling was planted in each pot and placed in a greenhouse. Two weeks later, pepper seedling were transplanted to root-knot nematode infested soil. This experiment repeated two times. Plants were grown for 65 days in the greenhouse, where the temperature was fluctuating from 22 to 28℃ in daytime, from 11 to 22℃ in the night, in April to June. Plants were harvested and root and shoot weights per plant were recorded, and number of galls per plant were counted by staining with phloxine B.

Direct inoculation of antagonist to soil; The pots(20 × 30 × 10cm) were filled with nematode infested soil mixed with a preparation of nematophagous fungi at corresponding concentration as above. Four pepper seedlings with 6 weeks old were planted in each pot with two replicates. Plants were grown for 65 days in the greenhouse as the same above condition. Plants were harvested for investigation root and shoot weight and number of galls per plant.

Field evaluation of biocontrol of *F. oxysporum*

Experiments were carried out in the field heavily infested with *M. hapla* to determine the efficacy of nematophagous fungi. Fifty seeds were sown to each plot (36 × 40 × 15cm) with 3 replications in randomized block design. Total soil incorporated with fungal inoculum (1 : 100 and 1 : 70) was 21.6Kg soil per plot. Seeds of pepper were planted on 29 April 1987. Plants were thinned after emergence and ten healthy plants per plots were saved. At 124 days after sowing, plants were harvested, root and shoot weight per plant were recorded, and number of galls per plant were determined.

RESULTS AND DISCUSSION

Greenhouse evaluation

Treatment of fungi reduced the root galling by *M. hapla* and increased pepper growth in naturally infested field soil. Number of galls were signigicantly less in all pots in 4 different inoculum densities of 5 nematophagous fungi compared to untreated plots(Fig. 1). Three isolates of *A. arthrobotryoides*, *A. conoides*, *A. oligospora*. *D. lobata* and *D. lobata* apppreard control value about 85 to 90% at inoculum concentration 1 : 200 to 1 : 50 in the treated preinoculation plot and no differences among inoculum density. But,

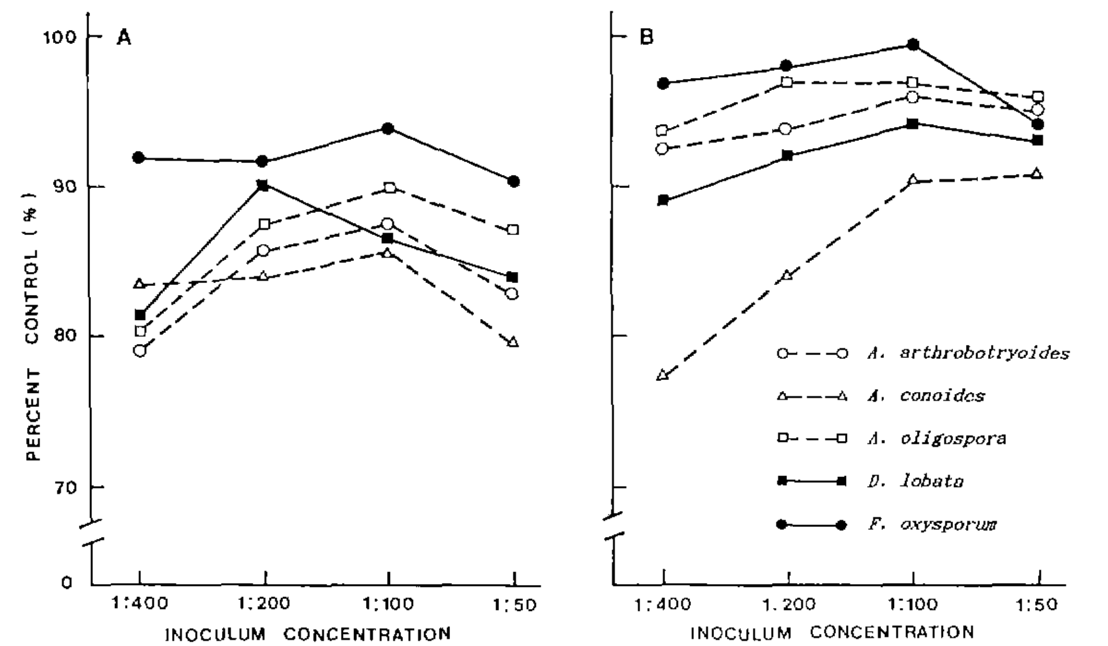

Fig. 1. Percent control of red-pepper root galling in pot soil, naturally infested by *M. hapla*, following introduction of nematophagous fungi with different inoculum concentration in greenhouse. A : pre inoculation, b : simultaneous inoculation.

when the inoculum concentration was diluted to 1 : 400, percentage control of root gall on red-pepper was reduced gradually except *F. oxysporum* and *A. conoides.* However, simultaneously treatments were significantly higher effect at all inoculum concentration and also percent control value was no difference according to Duncan's multiple range test except *A conoides.* Among the isolates, treatment of *F. oxysporum* resulted significantly reduce of root gall. Preinoculation was slightly less effective than the simultaneous inoculation. However, no significant difference was recognized between treatments of fungi simultaneously at planting and preinoculation two weeks prior to planting in greenhouse trial.

This result is different from Al-Hazmi et al. (1982). They demonstrated that. *A. conoides* was most effective when the fungus was introduced into the soil two weeks prior nematode inoculation and planting of corn. Our experiment was carried out in pots contaning field soil, where the root-knot nematodes were severely infested.

Plant biomass was based on the fresh weight of shoot and root of pepper grown for 65 days after treatments. Generally, treatment of fungi resulted in increased top weight reduced root weight (Table 1). The increased shoot growth was significantly higher in pretreated plots by

Table 1. Fresh Weight of shoot and root of red-pepper in pot soil, naturally infested by *M. hapla* following introduction of nemathophagous fungi with different inoculm concentration in greenhouse

Treatments	Shoot weight (g)				Root weight (g)			
	Inoculum concentration (Inoculum : Soil)							
	1 : 400	1 : 200	1 : 100	1 : 50	1 : 400	1 : 200	1 : 100	1 : 50
Preinoculation								
A. art.	28.0	30.8	32.5	17.2	4.45	4.08	3.80	3.63
A. con.	26.8	33.1	34.3	27.7	3.90	3.85	3.70	4.18
A. oli.	30.2	32.8	34.7	34.1	4.90	4.50	4.35	4.90
D. lob.	31.5	34.3	34.0	30.1	4.95	4.70	5.00	4.93
F. oxy.	27.1	30.2	36.3	29.6	4.40	3.93	3.98	4.58
Control	21.0	21.0	21.0	21.0	5.68	5.68	5.68	5.68
LSD.05	11.7	14.3	12.2	10.3	1.70	1.72	1.49	1.46
Simultaneous inoculation								
A. art.	31.2	32.8	34.7	28.8	4.88	4.05	4.25	4.80
A. con.	30.6	31.9	34.2	34.3	5.65	5.48	5.58	5.63
A. oli.	36.8	41.9	42.4	40.7	4.83	3.85	4.15	5.33
D. lob.	37.8	39.8	43.3	36.1	4.88	4.40	4.60	4.88
F. oxy.	31.4	31.7	46.5	31.3	4.85	4.00	4.00	4.33
Control	30.0	30.0	30.0	30.0	6.05	6.05	6.05	6.05
LSD.05	10.6	14.6	14.3	8.8	1.29	1.05	1.96	1.40

A. art.; *A. Arthrobotryoides,* A. con.; *A. conoides,* A. oli.; *A. oligospora,* D. lob.; *D. lobata,* F. oxy.; *F. oxysporum*

A. arthrobotryoides, A. conoides, A. oligospora, D. lobata and *F. oxysporum* at inoculum concentration of 1 : 100 but other treatments were not signigicantly increased shoot growth. Reduction in root weight, although variable, was also significant in plot of either treatment. However, this experiment carry out in small pot and the pot did not added any nutrient upto end of experiment. Therefora, observation of plant growth was very difficult because red-pepper growth was retared after 50 days due to deficience of nutrition.

Cho (1985) reported that tomato root weight was increased by infection *M. hapla*, although top weight was not significantly influenced. Root-knot nematode infection induced abnomal root system with profuse lateral root development. Such a phenomenon was also observed in this experiment.

In laboratory screening, all five nematophagous fungi were effective for both *Rhabditis sp.* and *M. hapla*. Rapid nematophagous fungi activity was observed for *M. hapla* in a week after treatment upto the level obtainable in two weeks *M haopla*, was the most aggressive against either nematode among the fungi. *D. lobata* was fast growing and slightly less effective against saprophytic nematode *Rhabditis* sp., but was as equally effective against root-knot nematode *M. hapla* as the most promising fungus *F. oxysporum* (Jeong & Kim 1988, Kim et al. 1988). 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. Results of this exeriment encouraged further evaluation of nematophagous fungi, *D. lobata* and *F. oxysporum* for the control of root-knot nematodes.

Field evaluation of biocontrol potentials of *D. lobata* and *F. oxysporum*.

Number of galls were remarkbly fewer and plant growth was greater in plots treated with either fungus at either concentration compared to the untreated plots. Control values at both concentration of *D. lobata* and *F. oxysporum* were 75 and 67, 66 and 77% throughout a period of 124 days in field trial (Table 2). Accordingly, plant growth was increased strikingly in the plots following the treatment of both fungi in either inoculum concentration. Corresponding response in plant height was also remarkable in the plots by the addition of both fungi (Fig. 2)

Table 2. Field evaluation of biocontrol potentials of *Dactylella lobata* and *Fusarium oxysporum*

	Inoculum Concentration	No. of Gall	Control Percent (%)
Control	–	62.8 b*	–
D. lobata	1 : 100	15.6 a	75.2
D. lobata	1 : 70	20.9 a	67.0
F. oxysporum	1 : 100	20.7 a	66.7
F. oxysporum	1 : 70	14.2 a	77.4

*Data in each column with different letters are Signigicantly differ (p=0.05) according to Duncan's multiple range test.

However, in the field soil, plant growth is affected by many environmental conditions, which in turn, influences the density fluctuation of plant parasitic nematode, nematode destroying fungi as well as other microorganisms.

Mycostatic factors may be removed by soil sterilization or the addition of a nutrient source and are considered to have absence of a suitablle substance. Mankau (1962) demonstrated the presence of a factor fungistatic to conidia of three species of nematophagous fungi, *A. arthrobotryoides, A. dactyloides and D. ellipsospora* in a number of southern California soils by the agar disc technique. Spores exposed to various field exhibited a range of 0.5～70.4% germination with most soils highly antagonistic. He illustrated that germ tubes were sometime lysed or formed trapping organs directly from the spore. A chopped alfalfa amendments altered the inhibitory properties of a soil tempo-

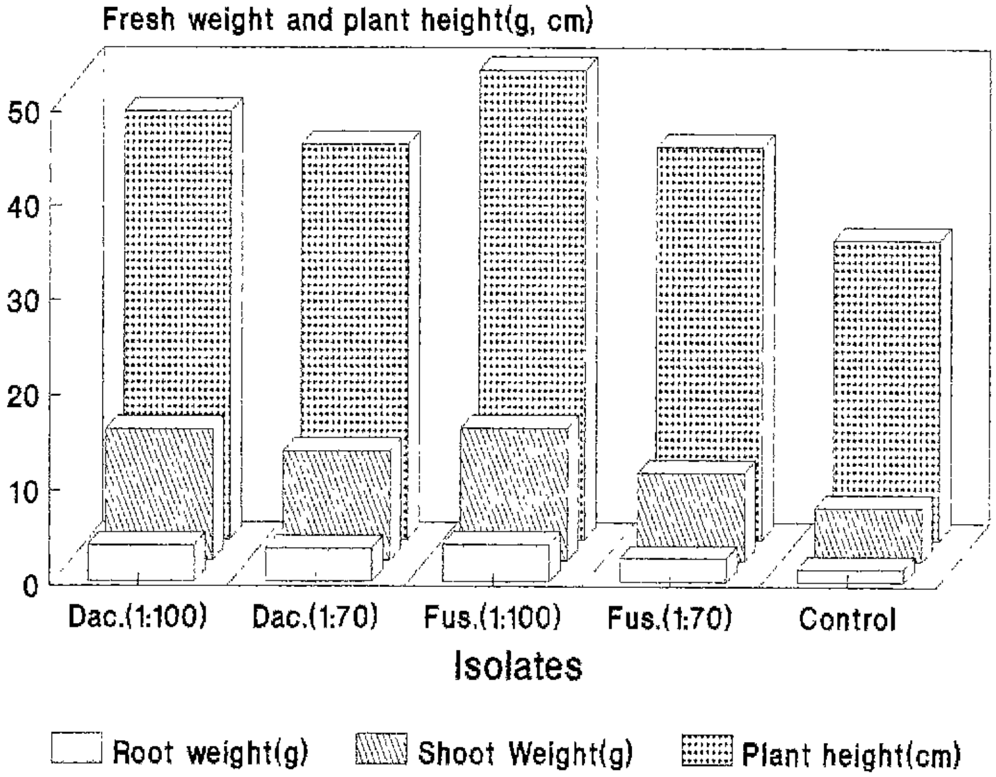

Fig. 2. Growth enhancement of red-pepper seedling in field soil treated by nematophagous fungi with different inoculum concentration. Dac : *D. lobata*, Fus : *F. oxysporum*

rarily and increased spore germination most effectively. Mitsui (1983, 1985) reported that application of rice straw to *M. hapla* infesting soil effectively increased activity of the trapping fungi, and also the addition of ammonium nitrate further increased the activity for a long period than rice straw alone in pot tests. However. in field tested, various soil amendments, dung of cattle and pig, various composts failed to raise trapping fungus population. Therefore, survivility of nematophagous fungi at root system and soil were most important factor because the nematophagous fungi protected the infection site plant parasite nematodes.

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