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BIOCONTROL OF *HETERODERA SCHACHTII* BY *PAECILOMYCES FUMOSOROSEUS* ON SUGAR BEET

by

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Summary. The survival of *Paecilomyces fumosoroseus* in soil and its pathogenic effect on *Heterodera schachtii* were studied in laboratory, pot and microplot tests. The fungus multiplied in culture tubes more at 15 and 25 °C than at 30 °C. *P. fumosoroseus* applied as a wheat culture to soil in pot tests suppressed the nematode, but had no effect when applied as a spore suspension. The fungus applied with wheat also increased tap root weight of sugar beets. In the microplot experiment, nematode reproduction was greater and nematode population reduction by *P. fumosoroseus* was enhanced in steam sterilized soil, compared to natural soil. However, the fungus had no effect on plant growth. At the end of the experiments colony forming units were much reduced in number compared to the inoculum concentration, but were not affected by oxamyl.

The sugar beet cyst nematode, *Heterodera schachtii*, is widespread in most of the sugar beet growing areas of Iran where it can cause major losses (Eshtiaghi and Barooti, 1992). Among the various control measures used against the nematode the use of fungi as biocontrol agents has increased in recent years (Hojat-Jalali and Cooseman, 1995; Ahmadi *et al.*, 1998; Saidi-Naini *et al.*, 1998). As many as 438 fungi have been isolated from eggs and juveniles of *H. schachtii* and *Paecilomyces fumosoroseus* was found associated with the nematode for the first time in the world (Fatemy, 1993; Fatemy *et al.*, 1999).

Investigation described here tested the ability of *P. fumosoroseus* to survive in soil, with and without wheat grains, at different temperatures and the control of *H. schachtii* on sugar beet in two soil types in pot and microplot experiments.

Materials and methods

Cysts of *Heterodera schachtii* Schmidt were obtained from cultures maintained on sugar beet, *Beta vulgaris* L., in pots. To extract freshly hatched juveniles, cysts were placed on 200 µm sieves in a dish containing 100 ml distilled water which was substituted after 24 hr with sugar beet root exudate. Root exudates were obtained from five months old sugar beets drained with 500 ml distilled water, the percolates then filtered through Whatman no. 42 filter paper and kept at 5 °C until required, which was always within a week.

The fungus, *Paecilomyces fumosoroseus* (Wize) Brown *et* Smith was grown on PDA for two weeks and two plugs of 10 mm diam from the colony were used to inoculate commercial wheat (*Triticum aestivum* L.) in conical flasks. The wheat grain had been soaked overnight in

tap water and autoclaved at 121 °C for 20 min on two consecutive days, with 200 g added to each flask. Inoculated flasks were kept for two weeks in the dark at 25 °C and periodically shaken. To evaluate spore density, 2 g of inoculated wheat were placed in 25 ml of sterilized distilled water contained in a 100 ml beaker and stirred for 5 min at 1500 rpm. A subsample of the spore suspension was counted in a haemocytometer.

Survival tests were carried out in 20x2 cm sterile tubes filled with 50 g of sterile sand moistened to 25% (w:v) with sterile distilled water. Tubes were inoculated with 10^6 spores either as 1 g of infected wheat or 5 ml spore suspension. The soil in each tube was mixed thoroughly and topped with cotton wool and aluminium foil before storing them for two months at 15, 20, 25 or 30 °C. There were five replicates of each treatment.

Colonies per g of soil were determined by the dilution method (Kerry *et al.*, 1993). The contents of each tube were mixed and 1 g subsample was added to 10 ml of 0.05% water agar containing 100 ppm tween 80 and mixed for 5 min at 900 rpm with a magnetic stirrer. One ml of the mixture was added to 9 ml of water agar and further diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Using a sterile pipette, 0.2 ml of the last dilution were transferred to 9 cm Petri dishes containing semi-selective medium (Fatemy, 1997) and spread evenly using a sterile glass rod. Three replicates of each dilution were kept for 3-5 days at 25 °C and then the number of colony forming units (cfu) were counted.

To study the effect of the fungus on *H. schachtii*, plastic pots, 15 cm diameter, were filled with 1500 g sterile soil and planted with a single three week old sugar beet seedling. After two weeks a 10 ml water suspension of 9000 juveniles of *H. schachtii* was poured into three holes made in the root zone of the plants. At the same time the fungus inoculum was added in the form of 30 g of colonized wheat (18×10^{10} spores), 10 g colonized wheat (6.1×10^{10} spores), or 5 ml suspension of 18.2×10^{10} spores to the pots with and without nematodes. Fungus inoculum was placed in the rhizosphere of the seedlings about 15 cm beneath the surface and incorporated thoroughly. The pots were distributed at random in a glasshouse at 13-33 °C and each treatment was replicated five times. After three months the sugar beet plants were uprooted and after washing and blotting, dry roots and tops were weighed separately. Cysts and females were collected on a 250 µm sieve (Kim and Riggs, 1995), washed into a beaker and hand picked on to a glass slide. Using a cover glass, cysts and females were crushed and juveniles were counted. The number of cfu/g soil in treatments containing both nematodes and fungi were determined as explained above.

The microplot experiment was carried out in 30x30 cm (diamxdepth) plastic containers plunged into the soil up to their edge and placed 0.50 m apart. They were filled with 10 kg of soil mixture used in the pot test, either steam sterilized or untreated. A three week old sugar beet seedling was planted in each plot and three weeks later nematodes (9500 J2/pot) were added to the allocated plots and fungus inoculum, 10 g colonized wheat containing 24×10^{10} spores were added as previously indicated. 0.5 g of oxamyl were added in the plots with sterile soil and nematodes and nematodes plus fungus. The microplots were distributed at random with each treatmet replicated five times.

The experiment was discontinued after three months: plants were removed, tap roots washed and weighed, and final nematode and fungus populations determined.

Results and discussion

In the test the fungus survived with and without wheat (Table I). However, the multiplication rate was higher at 15 and 25 °C than at 30 °C. In a similar experiment on PDA, the growth of col-

ony diametres measured after 10 days were 5.3, 24, 43, 13, and 0.0 mm at 15, 20, 25, 30, and 35 °C, respectively. However, the poor growth of fungus at 20 °C was unexpected and needs further investigation.

P. fumosoroseus may reproduce in soil better on wheat than as spore inoculum.

P. fumosoroseus applied in pots to the soil as a wheat culture suppressed both cysts and eggs of *H. schachtii* (Table II). Nematode population in the soil without *P. fumosoroseus* increased from 6 eggs/g soil as Pi to 21.3 eggs/g soil as Pf. However, the fungus inoculation as spore suspension had no effect on nematode reproduction. In general reproduction factor of *H. schachtii* was significantly lower in treatments when *P. fumosoroseus* was added as wheat culture, compared to the others.

Treatments with *P. fumosoroseus* in wheat culture also increased significantly (from 26 to 40% for 10 and 30 g inoculum, respectively) root weight of sugar beets. Densities of the fungus per g of soil decreased in all treatments three months after their inoculation.

In the microplot experiment, nematode reproduction was higher in steam sterilized soil than in natural soil, although the differences were not significant (Table III). Nematode egg numbers were much reduced in presence of wheat inoculum of *P. fumosoroseus* and repressive effect was greatest (63%) in sterilized soil; also the grain alone reduced populations of nematodes by about 38%. No suppresive effect was observed when the fungus was added to natural soil.

Oxamyl reduced (38%) the number of eggs in sterile soil, but the combination of oxamyl and *P. fumosoroseus* did not reduce nematode numbers and the fungus had no effect on sugar beet root weight. Colony forming units had decreased by 2-3 folds, compared to the inoculum

TABLE I - *Colony forming units/g soil (x 10^6) of* Paecilomyces fumosoroseus *applied to soil either as a spore suspension or on wheat culture at different temperatures.*

Treatment	Temp. °C			
	15	20	25	30
Spore suspension	106.2 c	3.1 d	175.0 a	3.0 d
Spore + wheat culture	114.0 c	27.0 d	141.0 b	10.0 d

Figures in each column followed by the same letter are not significantly different at 5% level.

TABLE II - *Effect of* P. fumosoroseus *on* Heterodera schachtii *reproduction and growth of sugar beet in pots.*

Treatment	Fresh weight top (g)	Fresh weight root (g)	Cyst/100 g soil	Eggs/g soil	Pf/Pi	Cfu/g soil x 10^6
Nematodes + 30 g fungus	19.8 ab	24.7 bcd	12.2 bc	10.3 b	1.7 b	12.6 a
30 g fungus	22.9 a	27.9 bcd				
30 g sterile wheat	19.2 ab	34.3 ab				
Nematodes + 10 g fungus	14.4 bcd	22.3 cde	7.4 c	10.5 b	1.8 b	7.53 a
10 g fungus	16.8 abc	19.1 cde				
10 g sterile wheat	15.7 bcd	25.9 bcd				
Nematodes + spore suspension	21.8 a	39.2 a	22.2 ab	23.3 a	3.9 a	0.75 a
Spore suspension	22.4 a	34.6 ab				
Nematodes	12.1 d	17.6 de	22.1 ab	21.3 ab	3.5 ab	
Control	10.1 d	12.3 e				

Figures in each column followed by the same letter are not significantly different at 5% level. 30 and 10 g fungus = infected wheat; Pf/Pi = nematode final/initial population; Cfu = Colony forming units.

TABLE III - *Effect of* P. fumosoroseus *on* H. schachtii *reproduction and growth of sugar beet in microplots.*

Treatment	Fresh weight top (g)	Fresh weight root (g)	Fresh weight tap root (g)	Egg/cyst	Egg/g soil	Pf/Pi	Cfu/g soil x 10^6
Sterilized soil							
Nematodes alone	312 ab	350 abc	12.9 b	91 bc	316 a	332 a	
Nematodes+fungus	142 b	171 b	11.5 bc	71 bc	118 a	124 a	8.0 a
Nematodes+sterile wheat	446 ab	490 ab	10.5 bc	78 bc	196 a	206 a	
Sterile wheat	470 ab	392 ab	6.1 bc				
Control	353 ab	538 ab	7.5 bc				
Nematodes+ oxamyl	156 b	215 b	9.6 bc	123 b	197 a	208 a	
Nematodes+ fungus+oxamyl	214 ab	232 b	4.8 c	194 a	337 a	355 a	13.4 a
Unsterile soil							
Nematodes	606 a	810 a	13.2 b	80 bc	228 a	240 a	
Nematodes+fungus	511 ab	487 ab	11.4 bc	113 bc	217 a	232 a	9.8 a
Nematodes+ sterile wheat	572 ab	571 ab	22.2 a	52 c	124 a	131 a	
Sterile wheat	480 ab	415 ab	5.6 bc				
Control	205 ab	245 b	7.3 b				

Figures in each column followed by the same letters are not significanly different at 5% level. Cfu = Colony forming units. P_f/P_i = nematode final/initial population densities.

concentration, at the end of the experiment, but were not affected by oxamyl application. Obviously small dose of oxamyl (0.5 g/plot) was not sufficient enough to control nematode multiplication and damage to the plants.

At the end of the experiment the population of the nematodes were much smaller in the pots than in the microplots. The confined space of pots could partly be responsible for the poorer growth of the roots and their ability to support higher nematode reproduction compared to the plots.

In vitro tests, which were carried out at temperatures between 15 to 30 °C, showed that all the females of *H. schachtii* and 75% of their eggs and more than 40 to 67% of the eggs inside cysts were infected by *P. fumosoroseus* (Fatemy, 1998). In soil there was no evidence of the fungus infecting eggs in cysts. Whether infection has occurred at an earlier stage on young females is not known. As with *Verticillium chlamydosporium*, young females of *H. schachtii* were more susceptible to infection than older females full of eggs (Crump, 1985). Furthermore, the control of beet cyst nematodes was not related to the numbers of cysts colonized by different isolates of *V. chlamydosporium* at the end of the experiment but was dependent upon the proportion of young females infected within two weeks of their emergence on the root surface (Kerry, 1989).

Although *P. fumosoroseus* appeared to be having some effects, the populations of nematodes were still large in all plots at the end of the microplot test and the large densities of the fungus in soil failed to provide adequate control even in sterilized soil. Beet cyst nematode has approximately 3-4 generations per year in Iran (Damadzadeh *et al.*, 1993). Duration of pot and

microplot tests was about 90 days, which permit at least 2-3 generations during that time. Considering these facts and the high initial population of nematodes, *P. fumosoroseus* may not have been able to infect such large numbers of females, especially before they become cyst full of mature eggs which are more resistant to infection. Therefore, an understanding of the time of infection and the spread can be important in the selection of potential biological control agents. Also, the ability to survive in soil may not be related to the levels of nematode infection unless the fungus is able to colonize and survive on the root surface (De Leij and Kerry, 1991).

The results of these experiments have raised several questions which future works should provide the satisfactory answers. More investigation on survival, proliferation, host specificity, rhizosphere competence, rate and time of application and so on is needed in order to provide sufficient information about *P. fumosoroseus* efficacy as a biocontrol agent of *H. schachtii* and/or root knot nematodes.

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