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Factors influencing the effectiveness of non-pathogenic *Fusarium solani* strain Fs5 in the suppression of root-knot nematode in tomato

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Summary. Four experiments were carried out under greenhouse conditions to evaluate the effectiveness of *Fusarium solani* strain Fs5 against the root-knot nematode *Meloidogyne javanica*. The effect of population densities of *M. javanica*, various application rates of *F. solani*, moisture regimes and levels of benzaldehyde, a volatile compound of plant origin affecting the plant-nematode-fungus interaction, were also studied. *F. solani* parasitized eggs and females of *M. javanica* and thereby reduced root-knot severity in tomato. Although the fungus was frequently isolated from root tissues, it did not produce phytotoxic symptoms; instead, there was enhanced plant growth. At higher nematode densities, inner root colonization by the fungus increased. The rates of fungal infection on *M. javanica* eggs and females also increased with increasing nematode densities and fungal inoculum levels. Nematode invasion and subsequent root-knot increased with increasing soil moisture, in both *F. solani*-treated and untreated plants. However, root-knot development was lower at all moisture regimes when *F. solani* was applied to the soil. Root colonization by *F. solani* and parasitism on female nematodes was highest at 50% moisture holding capacity (MHC) whereas egg parasitism by the fungus was greatest at 75% MHC. With increasing concentration of benzaldehyde in soil, nematode penetration and subsequent root-knot infection were progressively reduced. Root colonization by *F. solani* was greatest in soil treated with benzaldehyde at 2 μg g^{-1} of soil in the presence of *M. javanica*. Increasing benzaldehyde concentrations resulted in increased parasitism of *M. javanica* females by *F. solani* but in lower parasitism of the eggs. Treatments with *F. solani* led to better plant growth when they were combined with benzaldehyde at 2 μg g^{-1} of soil.

Key words: *Meloidogyne javanica*, endophyte, biological control, benzaldehyde.

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are plant parasites attacking a wide variety of crops worldwide (Goodey *et al*., 1965). The economic damage inflicted by this group of nematodes is enormous with crop losses estimated at nearly 13% (Sasser, 1979), the tropics and subtropics being the areas most severely affected. Yield losses are influenced by the pathogenicity of the nematode species involved, its reproductive potential, nematode population densities at planting, host susceptibility and tolerance, and a range of environmental factors. Nematicides are traditionally used for the management of nematode populations. The fact that many nematicides (e.g. fumigants) pose significant health and environmental risks has promoted a search for alternative methods of nematode management. An understanding of the biological control of nematodes in nature and an assessment of the exploitation of antagonists to nem-

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atodes is fundamental to the development of such methods. There is also a great need for information on how nematodes interact with other organisms in the soil and the rhizosphere.

Often the efficacy of a biocontrol agent is dependent on biotic or abiotic factors such as inoculum level (Leij *et al.,* 1992), nematode species (Leij, 1992) soil moisture (Nigh *et al.,* 1980; Leij and Kerry, 1992; Siddiqui and Ehteshamul-Haque, 2001) and temperature (Nigh *et al.,* 1980). Temperature has a direct effect on fungal growth and sporulation and on the development of target nematodes. Soil moisture rarely limits the growth of fungi but affects dispersal of spores or zoospores. Soil texture and structure influence nematode activity and the growth and spread of micro-organisms (Kerry, 1997). Another means to control several plant pathogens may be by using naturally occurring plant volatile compounds (Wilson *et al.,* 1999). Benzaldehyde is commonly found in fruits as a flavor compound and readily decomposes into benzoic acid and water (Do *et al.,* 1969). It is reported to act as a fungicide *in vitro* (Wilson et al., 1987) and as a nematicide in the soil (Bauske *et al.,* 1994; Shaukat and Siddiqui, unpublished data). Benzaldehyde when applied as a fumigant significantly reduced the viability of soil-borne plant pathogenic fungi, including *Rhizoctonia solani* and *Sclerotinia minor,* and reduced populations of *Pythium aphanidermatum*. However, it also markedly inhibited the growth of biocontrol fungi like *Trichoderma harzianum* and *Talaromyces flavus* (Wilson *et al.,* 1999). Therefore, a thorough understanding of the interactions between nematodes and their enemies and of the factors that influence these interactions, is essential in exploiting the potential of nematode biological control agents.

Among *Fusarium* species, *Fusarium solani* (Mart) Appel & Wollenw. Emend. Snyd. & Hans has a world-wide distribution in the soil and is one of the most destructive plant pathogens, attacking hundreds of hosts on which it chiefly causes root and fruit rots (Domsch *et al.,* 1980). Some nematological investigations have examined the toxic compounds produced by *Fusarium* spp. and their effects on many plant-parasitic and saprophytic nematodes at various stages of their life cycle (Krizkova *et al.,* 1979; Mani and Sethi, 1984). Ciancio *et al.* (1988) reported that moniliformin, fusarenone and neosolaniol reduced egg hatching in *M. incognita*. Toxins released by *Fusarium* spp. may well play a key role in inhibiting plant-parasitic nematode population in the field, when a new crop is rotated to a soil infected with *Fusarium* from a previous crop.

The present paper examines the effect of *F. solani* application levels and *Meloidogyne javanica* density on fungal root colonization by *F. solani*, root invasion by nematode juveniles, and the subsequent infection and growth of the tomato host. The effect of the moisture regime and of a naturally occurring plant volatile, benzaldehyde, on the effectiveness of *F. solani* against root-knot nematodes and on the endophytic colonization of tomato roots was also investigated.

Materials and methods

The soil used for the experiment was a sandy-loam (sand:silt:clay, 70:19:11) with pH 8.1 and a maximum water holding capacity of 38%, obtained from the experimental field located near the Department of Botany, University of Karachi. The soil was sterilized by autoclaving and placed in 21-cm-diam. plastic pots (2 kg pot^{-1}). *Fusarium solani* strain Fs5 was cultured on potato dextrose agar (PDA) for a week at 27°C. This strain, originally isolated from a *M. javanica* female, produces significant suppression of root-knot nematodes under both greenhouse and field conditions (Amer-Zareen *et al.,* 2001). Inoculum of the fungus was prepared by scraping the fungus from the surface of the medium with a sterilized bent glass rod after adding 10 ml sterilized distilled water. Four experiments were carried out under greenhouse conditions to determine the degree to which *F. solani* controlled *M. javanica*, and how a number of factors affected fungus effectiveness.

Effect of *F. solani* on population densities of *M. javanica*

The first test explored the effectiveness of *F. solani* with various population densities of *M. javanica*. The experimental design was planned as 5×2 factorial, with 5 population densities (0, 500, 1000, 2000 and 4000 J_2 $plant^{-1}$) of *M. javanica* and 2 inoculum levels (0 and 10^7 cfu ml^{-1}) of *F. solani*. The potted soil was removed to a depth of 3 cm and each pot was drenched with 35 ml conidial suspension of the *F. solani* strain containing 1.8×10^7 cfu

ml^{-1}. Soil drenched with 35 ml sterile distilled water served as a control. After drenching, the removed soil was returned to the pots and 2–3-week-old tomato plants cv. Sun 6002 (PVP) previously grown in sterile soil were planted. One week after seedling transplantation, *M. javanica* juveniles (less than 4 days old and contained in 10 ml water) were added to the soil by pipetting with 10 ml water to give concentrations of 0, 500, 1000, 2000 and 4000 juveniles per plant. Each treatment was replicated four times and the pots were kept in a randomized complete block design on the greenhouse bench.

The experiment was terminated 6 weeks after nematode inoculation, at which time plant growth parameters such as plant height, root length and f wt of shoots and roots were recorded. The galls produced by *M. javanica* on the root system were counted under low magnification (×6). The root system was divided into two equal portions and kept in a refrigerator prior to use. To determine whether nematode invasion had occurred, one of the portions was blotted dry, reweighed, wrapped in muslin cloth and dipped for 3–4 minutes in boiled 0.25% fuchsin acid with lactic acid. The roots were then washed in running tap water to remove excess stain and cooled in vials containing 1:1 glycerol:water with a few drops of lactic acid. The roots were macerated in an electric grinder for 45 seconds. The macerate was suspended in 100 ml water, and invaded females of *M. javanica* were counted in 5 samples of 5 ml each with the aid of a low-power microscope (×6). To determine fungal parasitism, two egg-masses per replicate were randomly selected. Each egg-mass was crushed in a drop of 0.01% sodium hypochlorite solution to dissolve the gelatinous matrix. Eggs were washed three times in distilled water and dispersed in 3 ml water, and 0.5 ml of each suspension was plated on 0.8% water agar. The dishes were incubated at room temperature (27±3°C). After 3 days 100–200 eggs on each dish were examined for growth of fungal hyphae from the eggs. Additionally, ten hand-picked females were surface-sterilized with 0.5% $Ca(OCl)_2$, washed thoroughly and plated on 0.8% water agar to detect parasitism.

To assess root colonization by *F. solani*, 5-mm-long root pieces (five pieces per plate) from one of the remaining sample portion, after surface disinfestation with 1% $Ca(OCl)_2$ for three minutes, were placed on PDA (Difco, Detroit, MI, USA) supplemented with penicillin (0.1 g l^{-1}) and streptomycin sulphate (0.2 g l^{-1}). After incubation at 28°C for 5 days, the colonization percentage was estimated as follows:

$$\text{Colonization (\%)} = \frac{\text{No. of root pieces colonized by } F.\ solani}{\text{Total No. of root pieces)}} \times 100$$

Interaction between various application rates of *F. solani* and population densities of *M. javanica*

The second experiment was almost identical to the first with some slight modifications in the treatments and the design. The experimental design was a 4×4 factorial with four replications. The factors included 4 population densities (0, 1000, 2000 and 4000 J_2 $plant^{-1}$) of *M. javanica* and 4 inoculum levels (0, 10^5, 10^6 and 10^7 cfu ml^{-1}) of *F. solani*. Both pathogens were introduced into the soil as described above. The experiment was terminated 6 weeks after nematode inoculation, at which time growth and disease parameters were measured as described above. Fungal colonization of the roots and parasitism rates of *M. javanica* females and eggs were also calculated. This experiment was repeated once.

Effect of moisture levels on the effectiveness of *F. solani* against *M. javanica*

A third experiment examined how soil moisture levels affected the efficacy of *F. solani* in root-knot nematode suppression. The experimental design here was a 3×2×2 factorial with two seedlings arranged in randomized blocks. The factors were three moisture levels (25, 50 and 75% of soil moisture holding capacity, MHC), absence or presence of *F. solani*, and absence or presence of nematodes. The seedlings were transplanted to 8-cm-diam. plastic pots containing 350 g sterilized soil each (two seedlings per pot). The soil of each pot was drenched with a 20-ml conidial suspension of Fs5 (1.7×10^7 cfu ml^{-1}) and kept separately at one of the three moisture levels. The weight of the moisture in the soil at maximum water holding capacity was determined and the three maximum water holding capacity percentages (25, 50 and 75%) were calculated accordingly. The pots were weighed periodically and requisite amounts of water added to keep the moisture levels constant. In another, similar set of pots, one week after transplanting, seedlings were inoculated with 2000 freshly hatched *M. javanica* juveniles. A third set of pots was treat-

ed in the same way with *M. javanica,* but these pots did not receive any fungal inoculum. Soil treated with 25 ml of distilled water without fungus or nematodes, and kept at 25%, 50% or 75% MHC, served as a control. Treatments were replicated three times in the first experiment, and four times in the repeated experiment. The plants were uprooted 6 weeks after nematode inoculation and at that time plant growth, root-knot infection, fungal colonization of the root and eggs, and extent of parasitism of the females were determined.

The influence of benzaldehyde on the effectiveness of *F. solani* against *M. javanica*

A fourth experiment examined the effect of benzaldehyde on the efficacy of *F. solani* in suppressing root-knot nematodes. The experimental design was a 3×2×2 factorial with five replications, each with two plants arranged in randomized complete blocks. The factors included three levels of benzaldehyde (0, 2, or 4 μg g^{-1}), absence or presence of *F. solani,* and presence or absence of nematodes. Tomato seedlings were transplanted to 8-cm-diam. plastic pots each containing 350 g soil. The soil of each pot was drenched with 20-ml of a conidial suspension of Fs5 (1.7×10^7 cfu ml^{-1}). The pots were randomized on the greenhouse bench. Six weeks after nematode inoculation, plants were uprooted and plant growth, disease incidence by the nematodes, fungal colonization of the roots and parasitism of eggs and females of *M. javanica* were determined as described above.

Statistical analyses

The results were analyzed with factorial analysis of variance (FANOVA) employing the STATISTICA 5.0 package (1995, StatSoft Inc. Tulsa, OK, USA). Treatment means were compared using least significant differences. In case of repeated experiments the homogeneity of the variances was tested with Bartlett's test. Since no variances between repeated experiments were detected, the analysis was performed on the pooled data.

Results

Effect of *F. solani* on population densities of *M. javanica*

Inoculation of tomato seedlings with *F. solani* resulted in a significant ($P<0.001$) reduction in the number of galls and in the extent of nematode invasion (Table 1). The fungus survived inside the host and was frequently isolated from surface-sterilized root tissue. It was not isolated from the root tissue of tomato plants grown as controls. At increasing nematode densities, fungal colonization of the root tissue increased. The fungus was also found in *M. javanica* eggs and females. Increasing nematode density resulted in increased parasitism of the eggs and females by the fungus. In the absence of root-knot nematodes and at 1000 juveniles, per plant, *F. solani* inoculum increased plant height ($P<0.05$). Fungus application with 500 or 1000 juveniles enhanced root length and fresh weight of shoots ($P<0.05$). The highest root fresh weight was recorded when 4000 juveniles per plant were added without *F. solani*.

Interaction between various application rates of *F. solani* and population densities of *M. javanica*

In general, increasing fungal inoculum led to a progressive decrease ($P<0.001$) in the number of galls and to a reduced nematode invasion at all nematode densities (Table 2). *F. solani* applied at 10^7 cfu ml^{-1} in the presence of 2000 juveniles/plant of *M. javanica* caused the greatest reduction (>38%) in galling intensity. The degree to which *F. solani* colonized the inner root tissues increased when both fungal and nematode densities increased. *F. solani* parasitized both eggs and females of *M. javanica*. Fungal parasitism increased with increasing nematode and fungal population levels. *F. solani* at 10^7 cfu ml^{-1} applied in combination with 1000 *M. javanica* juveniles/plant gave the maximum ($P<0.05$) plant height and shoot f wt. However, root length was greatest following application with *F. solani* at 10^7 cfu ml^{-1} in the presence of 1000 *M. javanica* juveniles $plant^{-1}$. Plants inoculated with 4000 juveniles of *M. javanica* and without the fungus had the greatest root f wt.

The effect of moisture level on the effectiveness of *F. solani* against *M. javanica*

Application of *F. solani* reduced galling rates at all moisture levels ($P<0.05$) (Table 3) but the reduction was greatest at 50% MHC. Nematode invasion increased with increasing moisture levels in both *F. solani*-treated and untreated plants. However, at all moisture levels, *M. javanica* den-

Table 1. Effect of *Fusarium solani* strain Fs5 on various population densities of *Meloidogyne javanica* on tomato, as shown by development of root-knot infection, *M. javanica* invasion, root colonization by *F. solani*, female and egg parasitism of *M. javanica* by the fungus, and growth of tomato plants.

Treatment	Galls/ root system	Nematode population (×g root)	*F. solani* colonization	Fungal parasitism %		Plant height (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
				Female	Egg				
M. javanica (MJ) 0	0	0	0	0	0	23.5	6.5	14.7	3.3
MJ 500	25	34	0	0	0	26.4	6.1	16.6	2.7
MJ 1000	37	50	0	0	0	25.3	5.4	16.3	3.5
MJ 2000	83	97	0	0	0	22	5.5	16	4.8
MJ 4000	123	161	0	0	0	20.7	4.3	14.6	5.5
MJ 0 + Fs5	0	0	17	0	0	29.6	6.9	21	3.5
MJ 500 + Fs5	15	29	37	5	0	26.7	7.7	18.6	3.4
MJ 1000 + Fs5	21	39	46	18	3	28.4	8.7	17	3.6
MJ 2000 + Fs5	59	94	46	23	14	23.1	5.1	17	2
MJ 4000 + Fs5	97	123	62	23	20	21.1	5.3	16.5	4.7
$LSD_{0.05}$									
Nematode	14	15	-	-	-	2.8	1.3	1.9	1.2
F. solani	9	11	30[a]	2.2[a]	1.1[a]	1.8	0.8	1.2	0.7

[a] One-way analysis was performed.

Table 2. Effect of various inoculum levels of *Fusarium solani* strain Fs5 on various population densities of *Meloidogyne javanica* on tomato, as shown by development of root-knot infection, *M. javanica* invasion, root colonization by *F. solani*, female and egg parasitism of *M. javanica* by the fungus, and growth of tomato plants.

Treatment	Galls/ root system	Nematode population (×g root)	*F. solani* colonization	Fungal parasitism %		Plant height (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
				Female	Egg				
M. javanica (MJ) 0	0	0	0	0	0	22.4	2.6	15.5	1.7
MJ 1000	33	42	0	0	0	23.6	2.1	17.9	1.9
MJ 2000	68	100	0	0	0	21.2	2.5	15.1	2.1
MJ 4000	114	172	0	0	0	17.4	1.9	13	3.5
MJ 0 + Fs5 (10^6)	0	0	10	0	0	23	2.4	15.8	2.2
MJ 1000 + Fs5 (10^6)	24	27	19	8	2	23.7	2.6	16.2	1.9
MJ 2000 + Fs5 (10^6)	63	80	27	17	3	20.8	3.2	15.6	2.9
MJ 4000 + Fs5 (10^6)	116	157	19	10	7	18	3.2	11.4	3.1
MJ 0 + Fs5 (10^7)	0	0	21	0	0	24.3	2.3	16.2	2.8
MJ 1000 + Fs5 (10^7)	27	24	35	22	16	25	3.8	18.1	2.1
MJ 2000 + Fs5 (10^7)	42	66	27	18	18	23.3	2.9	17.4	2.5
MJ 4000 + Fs5 (10^7)	98	141	38	24	16	18	2.7	13.5	2.9
MJ 0 + Fs5 (10^8)	0	0	36	0	0	24.7	2.6	15.2	2.4
MJ 1000 + Fs5 (10^8)	15	27	40	32	26	23.5	2.8	15.7	1.9
MJ 2000 + Fs5 (10^8)	46	57	52	30	21	22.9	3.6	14.3	1.9
MJ 4000 + Fs5 (10^8)	93	137	52	30	27	18.4	2.7	12.7	2.7
[a]$LSD_{0.05}$									
Nematode	3.6	5.6	6.2	3.3	1.9	0.8	0.3	1.1	0.3
F. solani	3.6	5.6	6.2	3.3	1.9	0.8	0.3	1.1	0.3

[a] ANOVA included two factors.

Table 3. Effect of *Fusarium solani* strain Fs5 at various soil-moisture levels on *Meloidogyne javanica* on tomato, as shown by the development of root-knot infection, *M. javanica* invasion, root colonization by *F. solani*, female and egg parasitism by the fungus, and growth of tomato plants.

Treatment	Galls/ root system	Nematode population (×g root)	*F. solani* colonization	Fungal parasitism %		Plant height (cm)	Shoot weight (g)	Root weight (g)
				Female	Egg			
Control + 25% MHC (A)	0	0	0	0	0	10.8	0.9	0.7
A + *M. javanica*	65	106	0	0	0	10.4	1	1
A + *F. solani*	0	0	26	0	0	11.7	1.4	0.8
A + *M. javanica* + *F. solani*	49	76	36	26	18	12.5	1.6	0.7
Control + 50% MHC (B)	0	0	0	0	0	11.2	1	0.8
B + *M. javanica*	79	132	0	0	0	11.8	1.3	1.1
B + *F. solani*	0	0	45	0	0	13.9	1.7	1
B + *M. javanica* + *F. solani*	41	95	52	29	17	13.6	1.7	1.2
Control + 75% MHC (C)	0	0	0	0	0	11.8	1.3	0.7
C + *M. javanica*	76	148	0	0	0	11.5	0.8	1.1
C + *F. solani*	0	0	37	0	0	13.4	1.7	0.8
C + *M. javanica* + *F. solani*	53	108	42	20	19	14.1	1.8	1.3
$LSD_{0.05}$								
Moisture	16	25	9[a]	6[b]	4[b]	1.4	0.7	0.5
Nematode	14	21	8	-	-	1.1	0.6	0.4
Fungus	14	21	-	-	-	1.1	0.6	0.4

[a] ANOVA included two factors.
[b] One-way analysis was carried out.

sities were lower when the soil was treated with *F. solani*. Both root colonization of *F. solani* and parasitism of females were greatest at 50% MHC. At all moisture regimes, root colonization by *F. solani* increased when *M. javanica* was added to the soil. By contrast, egg parasitism by *F. solani* was greatest at a soil moisture of 75% MHC. In general, plant height and shoot weight were reduced ($P<0.05$) by *M. javanica* infestation, but both these reductions were more than offset by *F. solani*. Root weight was greater ($P<0.05$) in soils containing both *M. javanica* and *F. solani* than in uninoculated soils or soils at 75% MHC treated with *F. solani*.

The influence of benzaldehyde on the effectiveness of *F. solani* against *M. javanica*

With increasing concentrations of benzaldehyde in the soil, nematode penetration and subsequent root-knot development were significantly ($P<0.05$) reduced (Table 4). A high level of benzaldehyde, such as 4 μg g^{-1} of soil with or without *F. solani* was more effective in suppressing root-knot nematode than benzaldehyde at only 2 μg g^{-1} of soil, or than the benzaldehyde-untreated controls. Benzaldehyde at both concentrations in combination with *F. solani* suppressed the root-knot nematode more strongly than either component alone. Fungal colonization of the root tissues of tomato increased ($P<0.05$) in the presence of the nematode. When benzaldehyde concentrations were increased, root colonization by *F. solani* was reduced in the absence of the nematode. Endophytic colonization by *F. solani* was greatest in soil treated with benzaldehyde at 2 μg g^{-1} in the presence of *M. javanica*. The highest benzaldehyde concentration increased the parasitism rate of *F. solani* on *M. javanica* females. In plants not treated with either *M. javanica* or *F. solani*, higher levels of benzaldehyde increased plant height and shoot fresh weight. *F. solani* led to better plant growth when applied in combination with benzaldehyde at 2 μg g^{-1} of soil than when applied with benzaldehyde at 4 μg g^{-1} of soil.

Table 4. Effect of *Fusarium solani* strain Fs5 at various concentrations of benzaldehyde on *Meloidogyne javanica* on tomato, as shown by development of root-knot infection, *M. javanica* invasion, root colonization by *F. solani*, female and egg parasitism by the fungus, and growth of tomato plants.

Treatment	Galls/ root system	Nematode population (×g root)	*F. solani* colonization	Fungal parasitism %		Plant height (cm)	Shoot weight (g)	Root weight (g)
				Female	Egg			
Control + benzaldehyde 0 μg g^{-1} (A)	0	0	0	0	0	11.9	1	0.7
A + *M. javanica*	85	137	0	0	0	11.2	1	1
A + *F. solani*	0	0	39	0	0	13.5	1.4	0.8
A + *M. javanica* + *F. solani*	51	96	35	16	24	13.9	1.5	0.8
Control + benzaldehyde 2 μg g^{-1} (B)	0	0	0	0	0	12.7	1.4	1.1
B + *M. javanica*	72	118	0	0	0	11.9	1.2	0.9
B + *F. solani*	0	0	36	0	0	14.2	1.5	1
B + *M. javanica* + *F. solani*	54	83	42	19	23	14.4	1.5	1.1
Control + benzaldehyde 4 μg g^{-1} (C)	0	0	0	0	0	13.2	1.4	1.2
C + *M. javanica*	61	93	0	0	0	13.2	1.2	1.1
C + *F. solani*	0	0	28	0	0	14.1	1.4	1.1
C + *M. javanica* + *F. solani*	47	74	38	17	14	13.8	1.5	1
$LSD_{0.05}$								
Benzaldehyde	14	17	9[a]	2[b]	5[b]	1.8	0.4	0.3
Nematode	12	14	7	-	-	1.5	0.3	0.2
Fungus	12	14	-	-	-	1.5	0.3	0.2

[a] ANOVA included two factors.
[b] One-way analysis was carried out.

Discussion

In the greenhouse tests, *F. solani* strain Fs5 parasitized eggs and females of *M. javanica* and thereby reduced root gall intensity. In a previous study, it was found that this strain of *F. solani* secreted thermostable toxins of a polar nature which caused heavy mortality of the juveniles of *M. javanica*, parasitized nematode eggs and females both *in vitro* and in greenhouse conditions, and protected tomato roots from nematode damage (Amer-Zareen *et al*., 2001). *Fusarium* species are considered to be facultative saprotrophs (*sensu* Garrett, 1956) that colonize both living and dead eggs. *F. solani* invades and destroys the plant cells on which nematodes feed (Fattan and Webster, 1983; Moussa and Hague, 1988). After nematode death, the fungus can be isolated from the dead females. Tebreiz and Husain (1986) reported a reduction in galls due to parasitism of *M. javanica* eggs and juveniles by *F. solani*. Similarly, *Meloidogyne incognita* reproduction in *F. oxysporum*-colonized tomato plants was 50% less than in plants that were *F. oxysporum*-free (Hallmann and Sikora, 1994). Besides the effect due to parasitism, a direct effect of *F. solani* toxins on root-knot nematodes cannot be ruled out (Amer-Zareen *et al*., 2001).

F. solani was frequently isolated from the root tissues of tomato seedlings but did not cause any harm to the plant. *Fusarium* spp. are pathogens and endophytes of crop plants (Bacon and Hinton, 1996) and produce a variety of field mycotoxins, some of which are harmful to plants while others are not (Abbas and Mirocha, 1988; Bacon and Hinton, 1996; May *et al*., 2000). The strain of *F. solani* used here (Fs5) was originally isolated from a female of *M. javanica*. The pathogenicity of the *Fusarium* species is restricted to their original host nematode (Owen, 1956). The use of endophytes for the management of plant-parasitic nematodes is a relatively new approach. Endophytes colonize the same root tissues as sedentary plant-parasitic nematodes. The fact that the endophytic *F. solani* is associated with nematodes throughout the nematode life-cycle makes this fungus a likely candidate for biocontrol strategies.

An important aspect of the ecology of soilborne fungi is their ability to survive under adverse conditions such as extreme temperatures and soil-moisture levels. *F. solani* in the present study was more effective against root-knot nematodes and it colonized root tissues better in soil at 50% MHC than at 25 or 75%. Egg parasitism by the fungus was greatest in soil at 75% MHC. Nigh *et al*. (1980) reported that temperature and moisture affected parasitism of *Heterodera schachtii* eggs by fungal parasites including *Acremonium strictum* and *F. oxysporum*. In dry soil, *F. oxysporum* was much more effective than *A. strictum*. Recently we found that biocontrol and growth of the rhizobacterium *Pseudomonas aeruginosa* were increased when a 50 or 75% MHC was maintained, whereas at 25% MHC they were reduced (Siddiqui and Ehteshamul-Haque, 2001). In the same study, *F. solani* colonized tomato roots with greater frequency in soil at 75% MHC than in soil at 25 or 50% MHC. Heckenberg and Sikora (1994) reported a significant reduction in *Globodera pallida* infection with *Agrobacterium radiobacter* when soil moisture was between 60 and 90% of field capacity. When moisture levels were held at 30% of field capacity, nematode invasion was still reduced, but not significantly.

In the present study, *F. solani* and benzaldehyde together produced a greater reduction of *M. javanica* population densities and subsequent root-knot infection than when either was applied alone. Although the extent to which *F. solani* parasitized *M. javanica* eggs was reduced by the combined application of fungus and a high level of benzaldehyde, its ability to infect *M. javanica* females and its endophytic colonization were not affected. Under conditions of low to moderate nematode infestation, *Meloidogyne* species lay their eggs on the roots, most of which are exposed to the rhizosphere. This is where the interaction between the eggs and their parasites occurs. High levels of benzaldehyde in the soil may affect the viability and growth of the antagonist in the rhizosphere, reducing the amount of interaction between the eggs and the fungus. The present study shows that for best practical results levels of benzaldehyde greater than 4 $\mu g\ g^{-1}$ should be avoided. Even so, however, benzaldehyde at dosages greater than 4 $\mu g\ g^{-1}$ may provide substantial control of other soil-borne fungal pathogens; in this study it was found to suppress significantly the soil-borne root-infecting fungi *Macrophomina phaseolina, Fusarium solani* and *Rhizoctonia solani* in mungbean (Siddiqui and Shaukat, unpublished data). By using natural fumigants that inhibit pathogens and promote antagonists, a favourable environment could be created in the soil favouring antagonists with resultant disease control. Canullo *et al.,* (1992) found that repeated treatment of soil with furfuraldehyde significantly increased populations of a *Trichoderma* species and bacteria. Benzaldehyde is a desirable alternative to chemical pesticides since it is inexpensive, biodegradable, and its eventual breakdown products (CO_2 and H_2O) are harmless to humans and the environment.

Besides abiotic factors such as temperature and moisture, the ability of *F. solani* to parasitize *M. javanica* eggs and females and its endophytic colonization potential seem to be dependent on two factors: a) the initial size of the fungal inoculum, which presumably affects the chances of its parasitism on nematodes, and b) the stimulus that the roots give to the fungus to grow. Leij and Kerry (1991) found that *Pochonia chlamydosporia* (*Verticillium chlamydosporium*), a facultative parasite of cyst and root-knot nematode, colonized extensively galled tissues more often than healthy roots. They suggested that rhizosphere colonization by the fungus could be related to the density of the nematodes in the roots. Hewlett *et al.,* (1988) also reported that *Paecilomyces lilacinus* was more frequent in the galls, and they stressed the importance of the root system in determining how far the fungus would spread. Presumably, infected root tissue leaks a greater amount of nutrients into the rhizosphere than do healthy root tissues. However, on susceptible crops like tomato, the gall size induced by *Meloidogyne* spp. at certain sites in the root is proportional to the number of nematodes developing in these sites (Dropkin, 1954). At high nematode densities large galls are induced and a significant proportion of the egg-masses may stay embedded in the gall tissues. Since *Pochonia chlamydosporia* is a rhizosphere colonist which does not invade the root tissues, it does not attack the egg masses inside the large galls (Leij *et al.,* 1992). *F. solani* on the other hand is an inner root colonizer that also attacks egg masses inside the galls. Therefore this fungus is expected to be more effective in situations of heavy nematode infestation.

The banning of chemical pesticides, including methyl bromide, as pre-plant treatments for horticultural crops in the year 2005 will cause major problems in the cultivation of tomatoes and other vegetables. Endophytes such as non-pathogenic *F. solani,* alone or in combination with compatible dosages of natural plant-derived volatile compounds, may offer a solution to this problem.

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Literature cited

Abbas H.K. and C.J. Mirocha, 1988. Isolation and purification of a hemmorrhagic factor (Wortmannian) from *Fusarium oxysporum* (N17B). *Applied and Environmental Microbiology* 54, 1268–1274.

Amer-Zareen, I.A. Siddiqui, F. Aleem, M.J. Zaki and S.S. Shaukat, 2001. Observations on the nematicidal effect of *Fusarium solani* on the root-knot nematode, *Meloidogyne javanica*. *Journal of Plant Pathology* 83, 207–214.

Bacon C.W. and D.M. Hinton, 1996. Symptomless endophytic colonization of maize by *Fusarium moniliforme*. *Canadian Journal of Botany* 74, 1195–1202.

Bauske E.M., R. Rodríguez-Kábana, V. Estuan, J.W. Kloepper, D.G. Robertson, C.F. Weaver and P.S. King, 1994. Managament of *Meloidogyne incognita* on cotton by the use of botanical aromatic compounds. *Nematropica* 24, 143–150.

Canullo G.H., R. Rodríguez-Kábana and J.W. Kloepper, 1992. Changes in soil microflora associated with control of *Sclerotium rolfsii* by furfuraldehyde. *Biocontrol Science and Technology* 2, 159–169.

Ciancio A., A. Logrieco, F. Lamberti and A. Bottalico, 1988. Nematicidal effects of some *Fusarium* toxins. *Nematologia Mediterranea* 16, 137–138.

Do J.Y., D.K. Salunkhe and L.E. Olson, 1969. Isolation, identification and comparison of the volatiles of peach fruit as related to harvest maturity and artificial ripening. *Journal of Food Science* 34, 618–621.

Domsch K.H., W. Gams and T. Anderson 1980. *Compendium of Soil Fungi*. Academic Press, London, UK, 858 pp.

Dropkin V.H., 1954. Infectivity and gall size in tomato and cucumber seedlings infected with *Meloidogyne incognita* var. *acrita* (root-knot nematode). *Phytopathology* 44, 43–49.

Fattan F.A. and J.M. Webster, 1983. Ultrastructural changes caused by *Fusarium oxysporum* f. sp. *lycopersici* in *Meloidogyne javanica* induced giant cells in *Fusarium* resistant and susceptible tomato cultivars. *Journal of Nematology* 15, 128–155.

Garrett S.D., 1956. *Biology of Root-infecting Fungi*. Cambridge University Press, London, UK, 293 pp.

Goodey J.B., M.T. Franklin and D.J. Hooper, 1965. *T. Goodey's The Nematode Parasites of Plants Catalogued under their Hosts*. 3rd edition. Commonwealth Agricultural Bureau, Farnham, UK, 214 pp.

Hallmann J. and R.A. Sikora, 1994. Influence of *Fusarium oxysporum*, a mutualistic fungal endophyte, on *Meloidogyne incognita* infection of tomato. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 101, 475–481.

Heckenberg C. and R.A. Sikora, 1994. Wirkung des pflanzengesundheitsfördenden Rhizobakteriums *Agrobacterium radiobacter* Stamm G12 gegen den Kartoffelzystennematoden *Globodera pallida* bei unterschiedlicher Temperatur und Bodenfeuchte. *Journal of Phytopathology* 142, 338–344.

Hewlett T.E., D.W. Dickson, D.J. Mitchell and M.E. Annwischer-Mitchell, 1988. Evaluation of *Paecilomyces lilacinus* as a biocontrol agent of *Meloidogyne javanica* on tobacco. *Journal of Nematology* 20, 578–584.

Kerry B.R., 1997. Biological control of nematodes: prospects and opportunities. In: *Plant Nematode Problems and their Control in the Near East Region*. (M.A. Maqbool, B.R. Kerry, ed.), *Proceedings of the expert consultation on nematode problems and their control in the near east region*, Karachi, Pakistan, 22–26 Novembre 1992. FAO Plant Production and Protection Paper, 144, FAO, Rome, Italy, 79–92.

Krizkova L., J. Dobias, P. Memic and A. Kolozsyary, 1976. Predator fungi *Dactylaria piriforme* and *Dactylaria thaumasia*: production of attractants and nematicides. *Folia Microbiologica* 21, 493–494.

Leij F.A.A.M. de, 1992. *Significance of ecology in the development of* Verticillium chlamydosporium *as a biological control agent against root-knot nematode (*Meloidogyne *spp.)*. PhD Thesis, University of Wageningen, Netherlands.

Leij F.A.A.M. de, K.G. Davies and B.R. Kerry, 1992. The use of *Verticillium chlamydosporium* and *Pasteuria penetrans* alone and in combination to control *Meloidogyne incognita* on tomato plants. *Fundamental and Applied Nematology* 15, 235–242.

Leij F.A.A.M. de and B.R. Kerry, 1991. The nematophagous fungus *Verticillium chlamydosporium* as a potential biological control agent for *Meloidogyne arenaria*. *Review of Nematology* 14, 157–164.

Mani A. and C.L. Sethi, 1984. Effect of culture filtrate of *Fusarium oxysporum* f. sp. *ciceri* and *Fusarium solani* on hatching and juvenile mobility of *Meloidogyne incognita*. *Nematropica* 14, 139–144.

May H.D., Q. Wu and C.K. Blake, 2000. Effects of the *Fusarium* spp. mycotoxins fusaric acid and deoxynivalenol on the growth of *Ruminococcus albus* and *Methanobrevibacter ruminantium*. *Canadian Journal of Microbiology* 46, 692–699.

Moussa E.M. and N.G.M. Hague, 1988. Influence of *Fusarium oxysporum* f. sp. *glycines* on the invasion and de-

velopment of *Meloidogyne incognita* on soybean. *Review of Nematology* 11, 437–439.

Nigh E.A., I.J. Thomason and S.D. Van Gundy, 1980. Effect of temperature and moisture on parasitization of *Heterodera schachtii* eggs by *Acremonium strictum* and *Fusarium oxysporum*. *Phytopathology* 70, 889–891.

Sasser J.N., 1979. Pathogenicity, host range and variability in *Meloidogyne* species. In: *Root-knot Nematodes (Meloidogyne species) Systematics, Biology and Control*. (F. Lamberti, C.E. Taylor, ed.), Academic Press, London, UK, 257–268.

Siddiqui I.A. and S. Ehteshamul-Haque, 2001. Suppression of the root rot-root knot disease complex by *Pseudomonas aeruginosa* in tomato: the influence of inoculum density, nematode populations, moisture and other plant associated bacteria. *Plant and Soil* 237, 81–89.

Tabreiz A. and S.I. Husain, 1986. Parasitism of *Meloidogyne incognita* by *Fusarium solani*. *International Nematological Network Newsletter* 3, 11–13.

Wilson C.L., J.M. Solar and A. El Ghaouth, 1999. Benzaldehyde as a soil fumigant, and an apparatus for rapid fumigant evaluation. *Horticulture Science* 34, 681–685.

Wilson C.L., J.D. Franklin and B.E. Otto, 1987. Fruit volatiles inhibitory to *Monilinia fructicola* and *Botrytis cinerea*. *Plant Disease* 71, 316–319.

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