



OBSERVATIONS ON THE NEMATICIDAL EFFECT OF *FUSARIUM SOLANI* ON THE ROOT-KNOT NEMATODE, *MELOIDOGYNE JAVANICA*

A. Zareen¹, I.A. Siddiqui², F. Aleem¹, M.J. Zaki¹ and S. Shahid Shaukat¹

¹Department of Botany, University of Karachi, Karachi, 75270, Pakistan

²PAF Intermediate College Korangi Creek Karachi, Pakistan

SUMMARY

The effects of 10 strains of *Fusarium solani* on *Meloidogyne javanica* were tested *in vitro* and in controlled conditions. Culture filtrates of the strains varied with respect to parasitism on eggs and females of *M. javanica* and nematicidal activity in terms of juvenile mortality. Mortality in boiled culture filtrates was slightly lower than that caused by un-boiled filtrates, but substantial nematicidal activity was retained, pointing to the relative thermostability of the active principle(s) involved. Aqueous and ethyl acetate extracts of *F. solani* produced higher nematicidal activity than a hexane extract indicating that the active compound(s) were polar in nature. Conidial suspensions of *F. solani* strains Fs5, Fs9 and Fs10 used as soil drench significantly reduced nematode populations in soil and root-knot disease severity, resulting in enhanced growth of tomato plants. There was no significant difference among *F. solani* strains on shoot fresh weight. Strain Fs5 was frequently reisolated from surface sterilized tomato roots. When evaluated in a field test, strain Fs5, reduced *M. javanica* reproductive potential, promoting growth of tomato plants. However, root length and fresh root weights were slightly lower in Fs5-treated plants.

Key words: *Fusarium solani*, *Meloidogyne javanica*, biological control, parasitism.

INTRODUCTION

Interactions between phytopathogenic fungi and species of *Meloidogyne* coinhabiting a particular host plant are not uncommon (Tu and Cheng, 1971; Garber *et al.*, 1979; El-Sharif and ElWakil, 1991; Hallmann and Sikora, 1994). Of the various soilborne fungi, *Fusarium solani* (Mart) Appel and Wollenw. Emend. Snyder and Hans. has a world wide distribution in soil and is consi-

dered as one of the most destructive plant pathogens, attacking hundreds of hosts, causing chiefly root and fruit rots (Domsch *et al.*, 1980).

Some nematological investigations have considered the ability of *Fusarium* spp., to produce toxic compounds, and their effects on the different life stages of many species of plant-parasitic and saprophytic nematodes (Krizkova *et al.*, 1979; Mani and Sethi, 1984), and various strains of *Fusarium* spp., have been shown to produce a variety of mycotoxins (Kurobane *et al.*, 1980; Baker *et al.*, 1981; James and Robert, 1983; Marasas *et al.*, 1984; Tatum *et al.*, 1985; Ciancio *et al.*, 1988). Ciancio *et al.* (1988) showed that moniliformin, fusarenone and neosolaniol caused reduced egg hatching in *M. incognita*. Ciancio (1995) further showed that T2-toxin, moniliformin, verrucaric acid and cyclochalasin B produced by species of *Fusarium* and other soil fungi reduced viability of *M. javanica* juveniles. Thus toxins released by *Fusarium* spp. may play a vital role in the population dynamics of plant parasitic nematodes in the field, where crops are rotated in soils previously infected with *Fusarium*. Indeed, endophytic colonization by *F. oxysporum* is reported to protect tomato roots from *M. incognita* (Hallmann and Sikora, 1994).

Strains of fungi often vary in their pathogenicity to nematode eggs and larvae (Kerry *et al.*, 1986; Amer-Zareen and Zaki, 1999). Strains of *Verticillium chlamydosporium* varied in their pathogenicity to *Heterodera avenae* eggs as well as in their growth rates, optimum growth temperature and production of chlamydospores (Kerry *et al.*, 1986). Pathogenicity to cyst nematodes by strains of *V. chlamydosporium* was influenced by nutrient level and temperature (Irving and Kerry, 1986).

The aims of the present study were (i) to investigate variation between strains of *F. solani* in toxicity to larvae, and ability to parasitize eggs and females of *M. javanica*; (ii) to test the thermostability and solubility of the nematicidal compounds produced by *F. solani* strains in various solvents and (iii) to evaluate the ability of some strains to control *M. javanica* infection in tomato under greenhouse and field conditions.

Corresponding author: S. Shahid Shaukat

Fax: +92.21.92439766

E-mail: shahidshaukat@yahoo.co.uk

MATERIALS AND METHODS

Sampling. A survey of cultivated fields in several localities of southern Sindh including Karachi University campus, Shah Faisal Colony, Korangi, Memon Goth, Linkroad, Ghara, Mirpur Sakro, Thatta and Kathore area was carried out during January 1999 to December 1999. A total of 200 plant specimens belonging to 18 species showing symptoms of root-knot nematode disease were collected together with soil samples (250 g) collected at 10 cm depth.

Isolation and identification of *F. solani*. Egg masses of root-knot nematodes were hand picked using sterilized forceps, surface sterilized in 1% Ca(OCl)₂ for 1-2 minutes, rinsed twice in sterile distilled water and plated on 0.8% water agar supplemented with penicillin (100,000 units l⁻¹) and streptomycin sulfate (0.2 g l⁻¹).

Eggs of root-knot nematodes were extracted by shaking infected roots in 2% NaOCl solution, and collected on a 400 mesh sieve. Aliquots (1 ml) of the suspension were then evenly spread on water agar plates.

Females of *M. javanica* were obtained by teasing gall root tissues with a sterilized needle under a dissecting microscope. After surface sterilization in 2% NaOCl solution, females were washed thoroughly with sterile distilled water and plated onto water agar as described above.

For *F. solani* isolation from roots, 5 mm long root pieces were surface sterilized with 1% Ca(OCl)₂ and plated onto PDA plates containing penicillin and streptomycin sulfate.

Three replicates of each sample were incubated at 30 ± 2°C for 5 days. As soon as some fungal colonies appeared, hyphal fragments were transferred onto PDA plates and fungi (*Fusarium* spp.) were identified according to Booth (1971). Ten strains of *F. solani* either isolated from *Meloidogyne* egg masses, eggs, and females or from root tissues were used (Table 1). All strains of *F. solani* were maintained on PDA at 25°C before use.

Female and egg parasitism by *F. solani* strains *in vitro*. Females of *M. javanica* were obtained from a population maintained on brinjal roots growing in earthen pots with autoclaved soil. Galls were teased with sterilized needles and after surface sterilization with 1% Ca(OCl)₂, 5 females were transferred to 2% water agar plates supplemented with penicillin and streptomycin sulfate. At the centre, each plate was inoculated with a 5 mm diam., disc of a single strain of

F. solani. Plates with a water agar disc without fungal culture were used as controls. After 7 days incubation, 10 ml sterile distilled water was added to each plate and females were collected. After surface sterilization the females were placed on 2% water agar plates and incubated at room temperature. Structures of the emerging fungi were then compared with that of the test fungus.

To test eggs parasitism, ten egg masses of *M. javanica* were randomly hand picked from greenhouse cultures. Each egg mass was crushed in a drop of 0.01% NaOCl solution to dissolve the gelatinous matrix. Eggs were then washed three times in distilled water and dispersed in 3 ml water, plating 0.5 ml of each suspension on 0.8% water agar to observe parasitism. The dishes were incubated at 30 ± 1°C. After 3 days, 100-200 eggs on each dish were examined for growth of hyphae from eggs.

Extracts from culture filtrates. Strains of the fungus were grown in Erlenmeyer flasks containing Czapek's Dox broth for 7 days in darkness at 30°C. The broth was filtered through Whatman no. 1 filter paper to obtain culture filtrate. Three strains (Fs2, Fs5 and Fs9) which showed greater nematocidal activity were selected for further investigation with ten-fold dilutions in distilled water. One set of dilutions was boiled for 5 minutes.

For extraction of the culture filtrate, one portion of the culture filtrate of each strain was lyophilized and dilutions of 0.01, 1.0 and 10 mg ml⁻¹ were prepared in sterile distilled water. Culture filtrates of *F. solani* were extracted with ethyl acetate or hexane (1:2 v/v) and the fraction was concentrated on a rotary vacuum evaporator (Eyla) under reduced pressure at 37°C. Dilutions of 0.01, 0.10, 1.0 and 10 mg ml⁻¹ of the extracts were prepared in their respective solvents. The chemical nature of the toxin was not determined.

Nematicidal activity of *F. solani* strains *in vitro*. Two ml of the culture filtrate or lyophilized powder dilutions were placed in cavity blocks and 1 ml of a suspension containing 30-45 *M. javanica* juveniles ml⁻¹ were transferred to each cavity block and kept at room temperature in three replicates. Cavity blocks with sterile distilled water were used as controls. For organic solvent fractions, 2 ml of each dilution was transferred to each cavity block and left for 48 h to allow the solvents to evaporate. One ml of the *M. javanica* juveniles suspension was then added to each cavity block. Toxicity was assessed as the mean percentage of dead nematodes. Nematodes were considered dead if they did not move when probed with a fine needle (Cayrol *et al.*, 1989).

Greenhouse evaluation of *F. solani* strains against *M. javanica*. The soil used for the experiment was a sandy-loam (sand 70%; silt 19%; clay 11%, pH 8.1) with a maximum water holding capacity of 38%, obtained from the experimental field of the Department of Botany, University of Karachi. The soil was transferred to 8 cm diam. plastic pots (350 g per pot). *F. solani* strains Fs5, Fs10 and Fs11 were maintained on PDA for a week at 30°C. Conidia and hyphae scraped from the surface of the medium using a flame-sterilized glass rod after adding 10 ml sterile distilled water.

The soil was excavated to a depth of 3 cm and 35 ml of conidial suspension of each *F. solani* strain containing 1.8×10^8 cfu ml⁻¹ was watered on each pot. Sterile distilled water served as control. After treatment, the soil surface was covered with soil and planted with three-week old tomato seedlings. One week after seedling transplant, soil in each pot was inoculated with 2000 freshly hatched second-stage *M. javanica* juveniles introduced into three holes around the seedlings. The experiment was terminated 60 days after nematode inoculation and plant growth parameters such as plant height and fresh shoot and root weight were recorded. The number of galls and *M. javanica* egg masses were counted with a stereomicroscope (×10). To determine the nematode population, soil from each replicate was pooled in a plastic container and 5 samples of 50 g were used for nematode extraction using the Baermann funnel technique. Reisolation of the *F. solani* strains from eggs and females was accomplished as described above. Each treatment was replicated five times with pots kept in a randomized design on the greenhouse bench.

Field experiment. Strain Fs5, which showed best suppression of *M. javanica* in laboratory and greenhouse conditions, was selected for test of biological control efficiency under field conditions. A field experiment was carried out in 2 × 1 meter microplots at the Department of Botany, University of Karachi. The soil was naturally infested with *Fusarium* spp. (2500 cfu g⁻¹ of soil), as assessed by the soil dilution technique (Nash and Snyder, 1962).

After removing the soil to a depth of 6 cm, a conidial suspension of Fs5 yielding 2.5×10^8 cfu ml⁻¹ was drenched at 300 ml m⁻¹ furrow. Sterile distilled water served as control. After drenching, the removed soil was returned and six three-week old tomato seedlings were planted. One week after transplantation, seedlings were inoculated with 1000 freshly hatched *M. javanica* second-stage juveniles. The juveniles were suspended in 25 ml water poured into three holes around the tomato

roots. Each treatment and control was replicated three times. The microplots were arranged in a randomized complete block design.

The plants were watered at two-days intervals and harvested 60 days after nematode inoculation. The data collected included plant height and fresh weight of shoot and root, and number of galls and egg masses produced on the entire root system. Reisolation of Fs5 from female and eggs was done as described above.

Statistical analysis. Data were analysed using analysis of variance (ANOVA) or factorial analysis of variance (FANOVA) in case of more than one factor. In the experiments that were repeated, since the error variances were similar, the analysis was performed on pooled data. Treatment means were compared following Duncan's multiple range test (Sokal and Rohlf, 1995).

RESULTS

Culture filtrates of all ten strains of *F. solani* caused substantial mortality of *M. javanica* juveniles (Table 1). The strains differed significantly ($P < 0.001$) in their ability to kill juveniles. Strain Fs5 showed highest mortality followed by strain Fs9 and Fs2. Low mortality was observed for filtrate of strain Fs1 and Fs3.

F. solani strains showed significant ($P < 0.001$) variation in parasitism of *M. javanica* females and eggs (Table 1). Strain Fs9 and Fs5 showed highest rates of parasitism on females and eggs. Strain Fs10 also showed a high rate of egg parasitism but lower juvenile mortality.

Ability of filtrates to kill *M. javanica* juveniles was tested for three strains of *F. solani* that produced highest mortality in the initial test, using various boiled and unboiled dilutions to test the thermostability of the active compounds (Table 2). In general, mortality decreased with decrease in filtrate concentration. Boiled filtrates substantially retained their effectiveness against *M. javanica* larvae, but caused significantly lower mortality when compared to unboiled filtrates. Unboiled pure culture filtrate of strain Fs5 yielded the highest mortality of *M. javanica*.

Extracts of *F. solani* culture filtrates differed significantly ($P < 0.001$) in mortality of *M. javanica* juveniles. Differences were also observed for the extraction procedure used (Table 3). Water extracts of all the three strains yielded greatest mortality followed by ethyl acetate, while hexane extract showed lowest activity. In general, increasing concentration resulted in increased juvenile deaths.

Table 1. Source of *F. solani* strains and effects on females, eggs and juveniles of *M. javanica*.

Strains	Source	Host plant	Locality*	Mortality (%)	Parasitism %	
					Female	Egg
Control	–	–	–	2 e	0 b	0 d
Fs1	egg mass	eggplant	1	28 d	5 b	1 d
Fs2	egg mass	eggplant	1	87 a	10 b	2 d
Fs3	egg mass	eggplant	2	32 cd	10 b	1 d
Fs4	egg	eggplant	3	40 c	0 b	1 d
Fs5	female	tomato	1	91 a	55 a	48 a
Fs6	egg	eggplant	3	38 c	0 b	1 d
Fs7	roots	okra	3	54 b	0 b	2 d
Fs8	egg mass	eggplant	2	63 b	5 b	1 d
Fs9	female	tomato	4	90 a	58 a	32 c
Fs10	egg	eggplant	3	56 b	48 a	42 b

Each figure is an average of 8 values obtained in two experiments each with four replicates. Figures followed by the same letter in each column are not significant ($P \leq 0.05$) according to the Duncan's multiple range test.

* 1: Gharo; 2: Linkroad; 3: Mirpur Sakro; 4: Shah Faisal colony.

Table 2. Mortality (%) of *M. javanica* juveniles in different concentrations of boiled and unboiled culture filtrates of three *F. solani* strains.

Strains	Concentration (mg ml ⁻¹)							
	Undiluted		0.1		0.01		0.001	
	Boiled	Unboiled	Boiled	Unboiled	Boiled	Unboiled	Boiled	Unboiled
Control	2	3	2	1	2	4	2	2
Fs2	76	91	66	78	33	59	24	41
Fs5	68	94	45	74	36	65	21	38
Fs9	71	89	44	68	29	47	19	41
LSD _{0.05}								
Strains	3.31	F = 491.4						
Lability	2.34	F = 240.15						
Concentration	3.31	F = 216.11						

Table 3. Mortality (%) of *M. javanica* juveniles in various culture filtrate extracts of different *F. solani* strains after 48 h exposure at $27 \pm 1^\circ\text{C}$.

Strains	Extract	Concentration (mg ml ⁻¹)				
		0	0.01	0.10	1.0	10
Fs2	water	3	21	34	49	63
	ethyl acetate	3	20	31	34	53
	hexane	1	3	6	9	24
Fs5	water	3	33	37	71	93
	ethyl acetate	3	19	25	33	87
	hexane	1	1	6	15	23
Fs9	water	3	13	22	35	60
	ethyl acetate	3	6	14	21	43
	hexane	1	2	1	9	19
LSD _{0.05}						
Strain	2.12	F = 76.44				
Extract	2.12	F = 350.83				
Concentration	2.74	F = 374.33				

Table 4. Effect of different *F. solani* strains on parasitism and development of *M. javanica* on tomato plants under greenhouse conditions.

Strains	Galls/ root system	no. of egg mass/root system	Nematodes 250 g soil	Fungal parasitism (%)		<i>F. solani</i> colonization (%)	Plant height (cm)	Shoot weight (g)	Root weight (g)
				Females	Eggs				
Control	80 a	34 a	4215 a	0 c	0 c	34 b	15.3 a	1.2 a	0.53 a
Fs 5	49 c	17 c	2590 c	46 a	38 a	48 a	20.2 b	1.9 b	0.53 a
Fs10	65 b	25 b	3870 a	14 b	6 b	39 ab	17.8 b	1.6 c	0.56 a
Fs 9	62 b	26 b	3290 b	20 b	3b c	38 ab	15.6 a	1.6 c	0.54 a
LSD _{0.05}	8.96	3.53	554.10	13.07	4.37	11	1.60	0.22	0.05

Each figure is an average of 10 values obtained in two experiments each with five replicates. Means flanked in columns by the same letter are not statistically different according to Duncan's Multiple Range test ($P < 0.05$).

Table 5. Effect of Fs5 strain of *F. solani* on development of root-knot infection, egg production and growth of tomato plants under field conditions.

Strain	Galls/ root system	no. of egg mass/root system	Fungal parasitism (%)		<i>F. solani</i> colonization (%)	Plant height (cm)	Shoot weight (g)	Root length (cm)	Root weigh (g)
			Females	Eggs					
Control	77 a	34 a	0 a	2 a	26 a	19.3 a	13.2 a	14.3 a	2.5 a
Fs5	48 b	17 b	36 b	34 b	39 b	26.1 b	22.1 b	13.5 a	2.1 a
LSD _{0.05}	25.2	15.3	16.2	18.5	9.3	6.1	6.5	1.2	0.8

Each figure is an average of 10 values obtained in two experiments each with five replicates. Means flanked in columns by the same letter are not statistically different according to Duncan's Multiple Range test ($P < 0.05$).

F. solani strains significantly ($P < 0.01$) reduced nematode populations in soil, lowering the number of galls per root system under greenhouse conditions (Table 4). Among the strains used, Fs5 was most effective resulting in 38, 50 and 38% reduction over controls in number of galls, egg mass per root system and nematode population in soil respectively. Female and egg parasitism was also highest for strain Fs5.

F. solani strains differed in their ability to colonize tomato roots. Strain Fs5 showing highest colonization. A weak negative correlation ($r = -0.25$; $P < 0.1$) between extent of root colonization and root-knot disease severity was also recorded. Plant height was significantly greater in soil treated with strains Fs5 and Fs10 compared to controls, while shoot weight significantly increased for all three strains tested. There was no significant difference among strains on root weight.

Due to a greater efficacy shown in greenhouse conditions, strain Fs5 was selected for testing its ability to control *M. javanica* under field conditions. The number of galls and egg masses observed per root system were significantly ($P < 0.001$) lower following soil treatment with Fs5 conidial suspensions (Table 5). Parasitism of eggs was significantly higher in the treated plants. A few

eggs were also found to be infected by *F. solani* in controls. Highest colonization was achieved in the roots growing in treated soil. Both, plant height and shoot weights increased in the treated plants. However, both root length and root weight slightly declined over the controls.

DISCUSSION

Considerable variation between strains was found in ability to cause nematode mortality and parasitism on eggs and females of *M. javanica*. Variation with respect to egg parasitism among fungi has been reported. Significant variation was observed among strains of *V. chlamyosporium*, a nematophagous fungus parasitizing cyst and root-knot nematodes, causing suppression of *H. avenae* (Kerry *et al.*, 1986). Similarly, Santos *et al.*, (1992), observed substantial variability in virulence among strains of *Paecilomyces lilacinus*, *Arthrobotrys conoides*, *A. musiformis*, *A. robusta*, *Monacrosporium ellisporum*, *Dactylaria thanmasia*, *Cylindrocarpon* sp., and *Trichoderma harzianum* tested against second-stage juveniles (J2), eggs and egg masses of *M. incognita* race

3. These authors assumed that variability could be due to selective adaptation to various edaphic factors at their geographic origin such as soil type or ambient temperature. A marked variation in *F. solani* strains was also reported (Burnett, 1984) and was attributed to genetic diversity (Domsch *et al.*, 1980). The variation among various strains of *F. solani* could also be attributed to their qualitative and quantitative differences in production of nematicidal compound(s).

In the present study, pure culture filtrates and various dilutions of different strains of *F. solani* caused significant mortality of *M. javanica* larvae. Many *Fusarium* spp. are reported as toxin producers (Marasas *et al.*, 1984; Ciancio 1995). In a previous report, culture filtrate of *F. solani* was far more potent than that of *F. oxysporum* f.sp. *ciceri* in inhibiting hatch, and immobilizing the second stage juveniles of *M. incognita* (Mani and Sethi, 1984). Nematicidal effects are also reported for mycelial extracts of *Alternaria* spp., *Penicillium* spp., *Aspergillus* spp., and other soil inhabiting fungi (Shukla and Swarup, 1971; Krizkova *et al.*, 1979; Singh *et al.*, 1983). Filtrates of some nematophagous fungi also showed nematicidal activity (Krizkova *et al.*, 1976). Ciancio (1995) suggested that toxins produced by *Fusarium* spp., can interact with nematodes.

The active principle(s) retained their nematicidal properties after heating. Similarly, there was no marked difference in activity between culture filtrates and their lyophilized powders. This suggests that active nematicidal compound(s) were not proteins. Giurma *et al.* (1973) showed that three species of *Nematoctonus* can produce thermostable toxins lethal to parasitized nematodes during the germination of infective conidia. Mankau (1969) also noted the thermostability of *Aspergillus niger* filtrates and their nematicidal action both *in vitro* and in soil, against the nematode *Aphelenchus avenae*. Similarly, Zuckerman *et al.* (1994) demonstrated the thermostability of a nematicidal compound (oxalic acid) isolated from a strain of *A. niger*.

Water and ethyl acetate fractions of *F. solani* caused significant mortality of *M. javanica* larvae *in vitro* whereas hexane fractions showed least activity. This suggests that the active principle(s) were soluble in water or ethyl acetate and consequently polar in nature. A number of active nematicidal compounds are reported from *Fusarium* spp. (James and Robert, 1983; Marasas *et al.*, 1984; Tatum *et al.*, 1985), and it is probable that more than one compound were involved in the nematicidal activity.

Under greenhouse conditions, three strains of *F. solani* (Fs5, Fs9 and Fs10) parasitized *M. javanica* eggs and females, and gave reduced nematode populations and increased growth of tomato plants. Similar results

were obtained with strain Fs5 when tested under field conditions. Species of *Fusarium* are consistently found associated with eggs of *M. incognita* (Santos *et al.*, 1992) and cysts and eggs of *Heterodera* spp., (Nigh *et al.*, 1980; Godoy *et al.*, 1982; Gintis *et al.*, 1983). Activity of *F. solani* as an egg and female parasite relates to its generic ability to decompose chitin and protein rather than to specific parasitism. Since opportunistic and ectoparasitic fungi do not produce structures for nematode capture, the constant movement of nematodes should interfere with sustained contact and penetration of the fungi into live juveniles (Freire and Bridge, 1985). On the other hand, females and eggs, being sedentary stages, would facilitate penetration by the fungus.

Therefore, *F. solani* strains used in this study may be regarded as opportunistic parasites or saprophytes rather than the true parasites. Chen *et al.* (1996) suggested that a fungus may not be an actual parasite if the egg parasitism index (EPI) is low, whereas fungi that show a high EPI are likely egg parasites. In addition to the egg and female parasitism, the reduction in nematode reproduction (number of egg masses) and root galling from combined inoculations may also be due to competition between *M. javanica* and *F. solani* for infection loci or feeding sites and to a reduction in root growth, as also suggested by Griffin and Thyr (1988). Study of the disease complex, *F. oxysporum* f.sp. *glycines* and *M. incognita* on soybean showed active colonization of the giant cell by the fungus, resulting in reduced juvenile development and an increased proportion of males (Moussa and Hague, 1988). Tabreiz and Husain (1986) reported a reduction in galls due to parasitism of *M. javanica* eggs and juveniles by *F. solani*. Likewise, *M. incognita* reproduction in *F. oxysporum*-colonized tomato plants was reduced 50% over *F. oxysporum*-free plants (Hallmann and Sikora, 1994).

Greenhouse and field observations suggest that the *Fusarium* species tested did not exert any adverse effect on growth of tomato plants. The strains used in greenhouse or field were obtained from eggs or females of *M. javanica* and not from infected tomato plants. Although strains did colonize tomato roots, the phytotoxic symptoms were not evident, most likely due to the fact that pathogenicity of *Fusarium* species is restricted to their original host (Owen, 1956). However, both root length and root weight were slightly reduced under field conditions, evidently due to small size and lesser number of galls. Similarly, Hallmann and Sikora (1994) also found a reduced root weight in *F. oxysporum*-colonized plants.

Although in our studies, strains of *F. solani* did reduce reproductive potential and invasion of *M. javanica* the fungus is also a well-known opportunistic pathogen

in human beings (Venditti *et al.*, 1988; Patoux-Pibouin *et al.*, 1992; Leu *et al.*, 1995; Kumar *et al.*, 1997). The fungus could provide potential toxic principle(s) worthy of further investigation. However, the application of mycotoxins for the management of plant-parasitic nematodes cannot be recommended because of various technical (storage and handling safety, soil and water contamination, hazards to animals and man) and economic considerations (Ciancio, 1995).

Strains of *Fusarium* hypovirulent or non-pathogenic on plants, antagonistic to *Meloidogyne* spp., may reduce root-knot severity providing better crop growth under field conditions. As an endophyte, *F. solani* offers an advantage over some species of nematophagous fungi that remain confined exclusively to the rhizoplane. In a susceptible host like tomato, at high nematode densities, large galls are produced in which a significant proportion of egg-masses may stay embedded in the gall tissue. These egg masses are physically protected from attack by the nematophagous fungi. On the other hand, endophytes may provide protection to plants from invasion of wilt-inducing fungi and endoparasitic nematodes (Siddiqui *et al.*, 2000).

ACKNOWLEDGEMENTS

The present work was carried out under a research grant provided by University Grants Commission Pakistan.

REFERENCES

- Amer-Zareen, Zaki M.J., 1999. Nematicidal and nematostatic properties of species of *Aspergillus*. In: *Proceedings of 2nd National Conference of Plant Pathology, Faisalabad 1999*, 195-198.
- Baker R.A., Tatum J.H., Nemeč S.Jr., 1981. Toxin production by *Fusarium solani* from fibrous roots of blight diseased citrus. *Phytopathology* **71**: 951-954.
- Booth C., 1971. The genus *Fusarium*. The Common Wealth Mycological Institute, Kew, Surrey, England.
- Burnett J.H., 1984. Aspects of *Fusarium* genetics. In: Moss M.O., Smith J.E. (eds.). *The applied mycology of Fusarium*, pp. 39-69. Cambridge University Press, Cambridge.
- Cayrol J.C., Djan C., Pijarowski L., 1989. Study on the nematocidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. *Revue Nematologie* **12**: 331-336.
- Chen S.Y., Dickson D.W., Mitchell D.J., 1996. Pathogenicity of fungi to eggs of *Heterodera glycines*. *Journal of Nematology* **28**: 148-158.
- Ciancio A., Logrieco A., Lamberti F., Bottalico A., 1988. Nematicidal effects of some *Fusarium* toxins. *Nematologia Mediterranea* **16**: 137-138.
- Ciancio A., 1995. Observations on the nematicidal properties of some mycotoxins. *Fundamental and Applied Nematology* **18**: 451-454.
- Domsch K.H., Gams W., Anderson T., 1980. *Compendium of soil fungi*. Academic Press, London.
- El-Sharif A.G., ElWakil M.A., 1991. Interaction between *Meloidogyne incognita* and *Agrobacterium tumefaciens* or *Fusarium oxysporum* f.sp. *lycopersici* on tomato. *Journal of Nematology* **23**: 239-242.
- Freire F.C.O., Bridge J., 1985. Parasitism of eggs, females and juveniles of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Verticillium chlamydosporium*. *Fitopatologia Brasileira* **10**: 577-596.
- Garber R.H., Jorgenson E.C., Smith S., Hyer A.H., 1979. Interaction of population levels of *Fusarium oxysporum* f.sp. *vasinfectum* and *Meloidogyne incognita* on cotton. *Journal of Nematology* **11**: 133-137.
- Gintis B.O., Morgan-Jones G., Rodriguez-Kabana R., 1983. Fungi associated with several developmental stages of *Heterodera glycines* from an Alabama soybean field soil. *Nematropica* **13**: 181-200.
- Giurma A.Y., Hackett A.M., Cooke R.C., 1973. Thermostable nema-toxin produced by germinating conidia of some endozoic fungi. *Transactions British Mycological Society* **60**: 49-56.
- Godoy G., Rodriguez-Kabana R., Morgan-Jones G., 1982. Parasitism of eggs of *Heterodera glycines* and *Meloidogyne arenaria* by fungi strains from cyst of *H. glycines*. *Nematropica* **12**: 111-119.
- Griffin G.D., Thyr B.D., 1988. Interaction of *Meloidogyne hapla* and *Fusarium oxysporum* f.sp. *medicaginis* on alfalfa. *Phytopathology* **78**: 421-425.
- Hallmann J., Sikora R.A., 1994. Influence of *Fusarium oxysporum*, a mutualistic fungal endophyte, on *Meloidogyne incognita* infection of tomato. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **101**: 475-481.
- Irving F., Kerry B.R., 1986. Variation between strains of the nematophagous fungus, *Verticillium chlamydosporium* Goddard II. Factors affecting parasitism of cyst nematode eggs. *Nematologica* **32**: 476-485.
- James H.T., Robert A.B., 1983. Napthaquinones produced by *Fusarium solani* strains from citrus. *Phytochemistry* **22**: 543-547.
- Kerry B.R., Irving F., Hornsey, J.C., 1986. Variation between strains of the nematophagous fungus, *Verticillium chlamydosporium* Goddard. I. Factors affecting growth *in vitro*. *Nematologica* **32**: 461-473.

- Krizkova L., Dobias J., Memic P., Kolozsyary A., 1976. Predator fungi *Dactylaria piriforme* and *Dactylaria thaumasia*: production of attractants and nematicides. *Folia Microbiologica* **21**: 493-494.
- Krizkova L., Dobias J., Podova M., Memic P., 1979. Nematicidal effect of entomophagous fungi. *Folia Microbiologica* **24**: 171-175.
- Kumar R.R., Kumar B.R., Shafiulla M., Lakshmaiah K.C., Sridhar H., 1997. *Fusarium solani* infection in a patient with acute myelogenous leukemia—a case report. *Indian Journal of Pathology and Microbiology* **40**: 555-7.
- Kurobane I., Vining L.C., McInnes A.G., Gerber N.N., 1980. Metabolites of *Fusarium solani* related to dihydrofusarubin. *Journal of Antibiotics* **33**: 1376-1379.
- Leu H.S., Lee A.Y., Kuo T.T., 1995. Recurrence of *Fusarium solani* abscess formation in an otherwise healthy patients. *Infection* **23**: 303-305.
- Mani A., Sethi C.L., 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.
- Mankau R., 1969. Nematicidal activity of *Aspergillus niger* culture filtrates. *Phytopathology* **59**: 1170.
- Marasas W.F.O., Nelson P.E., Toussoun T.A., 1984. Toxogenic *Fusarium* species. Pennsylvania State University, Park and London.
- Moussa E.M., Hague N.G.M., 1988. Influence of *Fusarium oxysporum* f.sp. *glycines* on the invasion and development of *Meloidogyne incognita* on soybean. *Revue de Nematologie* **11**: 437-439.
- Nash S.M., Snyder W.C., 1962. Quantitative estimation by plate counts of propagules of the bean root rot *Fusarium* in field soil. *Phytopathology* **52**: 567-572.
- Nigh E.A., Thomason I.J., Van Gundy S.D., 1980. Effect of temperature and moisture on parasitization of *Heterodera schachtii* eggs by *Acremonium strictum* and *Fusarium oxysporum*. *Phytopathology* **70**: 889-891.
- Owen J.H., 1956. Cucumber wilt, caused by *Fusarium oxysporum* f. *cucumerinum*. *Phytopathology* **46**: 153-157.
- Patoux-Pibouin M., Couatarmanach A., Le Gall F., Bergeron C., De Bievre C., Guiguen C., Chevrant-Breton J., 1992. *Fusarium solani* fusariosis in a leukemic adolescent. *Annals of Dermatology and Venereology* **119**: 377-380.
- Santos M.A., Ferraz S., Muchovej J.J., 1992. Evaluation of 20 species of fungi from Brazil for biocontrol of *Meloidogyne incognita* race 3. *Nematropica* **22**: 183-192.
- Shukla V.N., Swarup G., 1971. Studies on root knot of vegetables. IV. Effects of *Sclerotium rolfsii* filtrate on *Meloidogyne incognita*. *Indian Journal of Nematology* **1**: 52-58.
- Siddiqui I.A., Qureshi S.A., Sultana V., Ehteshamul-Haque S., Ghaffar A., 2000. Biological control of root rot-root knot disease complex of tomato. *Plant Soil* **227**: 163-169.
- Singh S.P., Veena P., Khan A., Sexena S.K., 1983. Inhibitory effect of some rhizosphere fungi of tomato influenced by oil cakes on the mortality and larval hatch of *Meloidogyne incognita*. *Nematologia Mediterranea* **11**: 119-123.
- Sokal R.R., Rohlf F.J., 1995. Biometry: the principles and practices of statistics in biological research. Freeman, New York.
- Tabreiz A., Husain S.I., 1986. Parasitism of *Meloidogyne incognita* by *Fusarium solani*. *International Nematological Network Newsletter* **3**: 11-13.
- Tatum J.H., Baler R.A., Bessy R.E., 1985. Three further naphthaquinones produced by *Fusarium solani*. *Phytochemistry* **24**: 3019-3021.
- Tu C.C., Cheng H.Y., 1971. Interaction of *Meloidogyne javanica* and *Macrophomina phaseolina* in kenaf root rot. *Journal of Nematology* **3**: 39-42.
- Venditti M., Micozzi A., Gentile G., Polonelli L., Morace G., Bianco P., Avvisati G., Papa G., Martino P., 1988. Invasive *Fusarium solani* infections in patient with acute leukemia. *Review of Infectious Diseases* **10**: 653-660.
- Zuckerman B.M., Mathew M., Acosta N., 1994. Control of Plant-parasitic nematodes by a nematicidal strain of *Aspergillus niger*. *Journal of Chemical Ecology* **20**: 33-43.

Received 13 February 2001

Accepted 28 June 2001