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NEMATICIDAL POTENTIAL OF CULTURE FILTRATES OF SOIL FUNGI ASSOCIATED WITH RHIZOSPHERE AND RHIZOPLANE OF CULTIVATED AND WILD PLANTS

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Abstract

Several fungi are known to regulate the nematode densities in soil by exhibiting a range of antagonistic activity including production of nematoxic compounds. Since fungi and nematodes occur together in the rhizosphere, the toxic metabolites naturally produced by fungi may be responsible for keeping a low level of nematode populations. In this study culture filtrates of several isolates of fungi, isolated from rhizosphere and rhizoplane of cultivated and wild plants exhibited significant nematicidal activity on *Meloidogyne javanica*, by killing 2nd stage juveniles at varying degrees. *Aspergillus candidus, A. fumigatus, A. niger, A. ochraceus, A. sulphureus, A. terreus, A. ustus, Cephalosporium* sp., *Chaetomium flavum, C. globosum, Cladosporium* sp., *Memnoniella echinata, Paecilomyces lilacinus, Penicillium aspermum, P. citrinum, P. purpurogenum, P. raistrickii, Scopulariopsis brumptti, Stachybotrys atra, S. parvispora, Trichoderma hamatum, T. harzianum, T. koningii, T. viride* and *Verticillium chlamydosporium (Pochonia chlamydosporia*) showed highest nematicidal activity. However, different isolates of same species of fungi showed variation in their nematicidal potential. Secondary metabolites from fungi associated with rhizopshpere and rhizoplane of crop plants offer an exciting area of research for the discovery of potential nematicidal compounds.

Introduction

Plant parasitic nematodes cause serious damages to agriculture and forestry (Siddiqui & Mehmood, 1996; Li *et al.*, 2007). Among the plant parasitic nematodes, the root knot nematodes attack wide range of host plants (Regaieg *et al.*, 2010). About 2000 plants are susceptible to their infection and they cause approximately 5% of global crop loss (Hussey & Janssen, 2002). The damages to global agricultural crops due to root knot nematodes is estimated around US$ 80 billion annually (Li, 2007; Rodrigue-Kabana & Canullo, 1992). One of the alternative of chemical pesticides for controlling the parasitic nematodes is the use of beneficial or antagonistic microorganisms which can suppress soilborne pathogens in rhizosphere (Berg *et al*., 2005). Several fungi are known to regulate the nematode densities in soil by exhibiting a range of antagonistic activity including production of nematoxic compounds (Kerryy, 2000; Lopez-Llorca & Jansson, 2006). There are several reports available about the production of nematicidal compounds by the fungi active against plant parasitic nematodes (Anke *et al.*, 2010; 1995; Hallmann & Sikora, 1996; Anke & Sterner, 1997; Chen *et al.*, 2000; Meyer *et al.*, 2000; Meyer *et al.*, 2004). Since soilborne fungi and nematodes occur together in the rhizosphere, the toxic metabolites naturally produced by fungi may be responsible for keeping a low level of nematode populations (Siddiqui & Mehmood, 1996).

The rhizosphere encompasses the millimeters of soil surrounding a plant root where complex biological and ecological processes occur (Bais *et al*., 2006). The term rhizosphere was conied by Hiltner in 1904 (Brimecombe *et al.*, 2001; Lynch, 1990). The organic susbstances released from roots to rhizosphere soil support higher microbial biomass and microbial activity than in the bulk soil (Nannipieri *et al.*, 2007). Antagonistic activities of numerous microbial populations in the rhizosphere influence plant growth and health (Weller, 1988, Weller *et al.*, 2002; Whipps, 2001, 1997; Berg *et al*., 2005). Among the microorganisms regulating nematode densities in soil, fungi hold an important position due to their parasitic, antagonistic and predatory behavior (Ehteshamul-Haque *et al.*, 1994; Whipps, 2001; 1997; Regaieg *et al*., 2010). Searching for new microbial strains as a source of biological nematicides is an important goal for those considering the significant economic damage caused by plant parasitic nematodes (Dong *et al.*, 2004). The present work describes the nematicidal activity of culture filtrates of soil fungi isolated from rhizoplane and rhizosphere of some wild and cultivated plants.

Materials and Methods

Fungal material: For the isolation of fungi from rhizosphere and rhizoplane plant samples were collected from different locations like Darsano Chano, Gharo, Karachi University Campus, Kathor, Memon Goth and Thatta from Sindh and Hub from Baluchistan. Healthy cultivated and some wild plants were dug out carefully and root samples with adhering soil were collected in polyethylene bags, brought to laboratory and stored in refrigerator. Isolation of fungi were made within 24 hours of collection.

Isolation of fungi from rhizosphere: For the isolation of fungi from rhizosphere, Volume Displacement Technique as suggested by Reyes & Mitchell (1962) was used. A dilution of soil (v/v) was prepared from 1:10 to 1:10,000. One ml aliquot of the two highest soil dilutions were poured in sterilized Petri dishes containing Potato

Dextrose Agar supplemented with penicillin (100,000 units/litres), streptomycin (0.2 g/litres) to prevent bacterial growth. Fungi grown after 5 days of incubation at room temperature (25-30°C) were identified after reference to Barnett & Hunter (1998); Booth (1971); Domsch *et al.*, (1980), Dugan (2006), Ellis (1971); Gilman (1957); Nelson *et al*., (1983); Raper & Fennel (1965); Raper & Thom (1949) and Thom & Raper (1945).

Isolation of fungi from rhizoplane: Roots were washed in running tap water and 1 cm long root pieces from tap and lateral roots were cut and then washed in sterilized distilled water. Root pieces were transferred on PDA plates containing penicillin (100,000 units/litre) and streptomycin (0.2 g/litre). Dishes were incubated for 5 days at 28°C. Fungi grown were identified as mentioned above.

Preparation of culture filtrates of fungi: Test fungi were grown in conical flasks (500 ml) containing 200 ml Czapek's Dox broth, plugged with cotton wool and autoclaved at 121°C for 20 minutes. After cooling the medium, each flask was inoculated with 5 mm disc, cut from the margin of vigorously growing culture of test fungi. Each test fungus had 5 flasks. After 15 days of incubation at room temperature (25-30°C), test fungi were filtered through Whatman No.1 filter paper. The culture filtrates were separated whereas mycelium were dried under Laminar flow hood and weighed.

***In vitro* nematicidal activity of culture filtrates:** Pure culture of root knot nematode *Meloidogyne javanica* was obtained for brinjal plants grown in earthen pots. Roots were washed under tap water. Egg masses were picked under stereomicroscope and placed in cavity blocks containing distilled water and left for hatching at room temperature (25-30°C). Juveniles hatched after 48 hours were used for the study. For the determination of the nematicidal activity, 2 ml of each undiluted (1:0) and diluted (1:10 & 1:100) culture filtrates were transferred in a small watch glass containing 20 hand picked second stage nematode (*Meloidogyne javanica*) juveniles (J_2). Whereas 2 ml of distilled water was used as control. The watch glasses were kept at room temperature (25-30°C) and nematode mortality was recorded after 24 and 48 hours under stereomicroscope (Ara *et al*., 1997). Data were analyzed and subjected analysis of variance and means were separated according to Gomez & Gomez (1984).

Results

In the present study, culture filtrates of 46 isolates of fungi belonging to 15 genera and 37 species viz., *Aspergillus* (9 species), *Cephalosporium* sp., *Chaetomium* (2 species, with 3 isolates of *C. globosum*), *Cladosporium* sp., *Curvularia clavata* (2 isolates), *Drechslera* (2 species with 2 isolates of *D. australiensis*), *Fusarium* (2 species with 2 isolates of *F. solani*), *Macrophomina phaseolina*, *Memnoniella echinata*, *Myrothecium cinctum*, *M. roridum*, *Paecilomyces lilacinus* (two isolates), *Penicillium* (7 species), *Scopulariopsis brumptii*, *Stachybotrys* (2 species, with 2 isolates of *S. atra*), *Trichoderma* (4 species, with 3 isolates of *T. viride)* and *Verticillium chlamydosporium* were tested for nematicidal activity against *Meloidogyne javanica* root knot nematode.

Of the species of *Aspergillus* tested, culture filtrates of *A. sulphureus*, *A. niger*, *A. terreus* and *A. ustus* produced more than 50% mortality of second stage larvae (J_2) of *M. javanica* after 24 hours while after 48 hours *A. candidus*, *A. sulphureus*, *A. ochraceus*, *A. fumigatus*, *A. niger*, *A. terreus* and *A. ustus* showed more than 50% mortality. *A. fumigatus*, *A. sulphureus* and *A. ustus* (88%) followed by *A. terreus* (87%) produced maximum mortality. Diluting of culture filtrates reduced their activity, *A. sulphureus* showed 73% mortality after 48 hours at 1:10 dilution (Table 1).

The *Cephalosporium* sp., produced 57 and 96% death after 24 and 48 hours exposures respectively. Of the 3 strains of *Chaetomium globosum* tested, maximum larval mortality were caused by *C. globosum* (S-1) 45% and 90% after 24 & 48 hours respectively, whereas rest of the 2 strains of the same fungus showed weak activity. The undiluted and diluted (1:10) culture filtrates of *Chaetomium flavum* produced more than 50% mortality of larvae (J_2) after 24 and 48 hours. Culture filtrate of *Cladosporium* sp. caused 92% juveniles death after 48 hours when used undiluted. Weak nematicidal activity was observed by two strains of each *Curvularia clavata* and *Drechslera australiensis* (Table 1).

Memnoniella echinata caused 63% mortality after 48 hours. Culture filtrate of *Myrothecium cinctum* produced 40 and 45% mortality after 24 and 48 hours respectively. Of the well-known nematophagous fungi tested, culture filtrate of *Paecilomyces lilacinus* (S-1) caused 65 and 95% juveniles death respectively after 24 & 48 hours. It also caused 77% mortality after 48 hours when 1:10 dilution was used whereas undiluted culture filtrate of *P. lilacinus* (S-2) caused 78% death after 48 hours. Culture filtrate of *Verticillium chlamydosporium* caused 60 and 73% larval death after 48 hours when used 1:10 diluted and undiluted respectively. Of the two isolates of *Fusarium solani* (S-1 & S-2) tested caused 95 and 85% death of nematode larvae (Table 1).

Of the *Penicillium* species tested, culture filtrates of *P. luteum* and *P. raistrickii* were found to cause more than 50% larval mortality after 24 hours. After 48 hours *P. aspermum*, *P. luteum*, *P. purpurogenum* and *P. raistrickii* produced more than 50 % mortality. Maximum mortality was observed by *P. luteum* (95%) followed by *P. raistrikii* (90%) and *P. aspermum* (80%). Culture filtrate of *P. purpurogenum* also caused 50% larval mortality after 48 hours when used in 1:10 dilution. Undiluted culture filtrates of *Stachybotrys atra* (S-2) caused 100% larval mortality after 24 hours, while *S. parvispora* caused 60% death after 48 hours. Exposure of nematode larvae to *Scopulariopsis brumptii* culture filtrate resulted in 71 and 93 % death after 24 and 48 hours respectively when used undiluted (Table 1).

Culture filtrates of *Trichoderma viride* (S-1 & S-3), *T. harzianum* and *T. koningii* caused more than 50% larval death after 24 hours. Whereas maximum mortality was produced by *T. viride* S-3 (90%) followed by *T. harzianum* (88%). After 48 hours *T. viride* (S-1 & S-3), *T. hamatum*, *T. harzianum* and *T. koningii* produced more than 50% larval mortality, with maximum mortality caused by *Trichoderma hamatum* (92%) followed by *T. viride* (S-3) (90%) and *T. harzianum* (88%) whereas at 1: 10 dilution of *T. harzianum* caused more than 50% mortality (Table 1).

Table 1. Mortality % of *Meloidogyne javanica* juveniles at different concentrations of culture filtrates of soil fungi.

Name of species		Host	Region	Locality	Concentrations			Concentrations		
					1:100	1:10	1:0	1:100	1:10	1:0
					Juveniles mortality %					
S. No.	Fungi				After 24 hours			After 48 hours		
1.	Control	-	-	-	0.0	0.0	0.0	0.0	0.0	0.0
2.	*Aspergillus candidus*	*Leucaena leucocephala*	Rhizosphere	Malir	0.0	0.0	0.0	0.0	16.6	60
3.	*A. fumigatus*	*Phaseolus vulgaris*	"	Gharo	0.0	0.0	8.3	0.0	0.0	88.3
4.	*A. nidulans* (S-1)	*Solanum melongena*	"	Hub	0.0	0.0	0.0	5.0	16.6	28.3
5.	*A. niger* (S-1)	*Cyperus totundus*	"	"	0.0	0.0	53.3	0.0	3.3	75.0
6.	*A. ochraceus*	*Solanum surranttence*	"	"	0.0	0.0	15	0.0	3.3	71.6
7.	*A. sulphureus*	*Lagenaria siceraria*	"	"	0.0	38.3	61.6	0.0	73.3	88.3
8.	*A. terreus*	*L. siceraria*	"	"	0.0	0.0	68.3	0.0	0.0	86.6
9.	*A.versicolor*	*Solanum melongena*	"	"	0.0	0.0	0.0	0.0	0.0	25
10.	*A. ustus*	*Solanum surranttence*	"	Malir	5.0	41.6	73.3	5.0	41.6	88.3
11.	*Cephalosporium* sp.	*Lagenaria siceraria*	Rhizoplane	"	0.0	11.6	56.6	0.0	23.3	96.6
12.	*Chaetomium flavum*	*Melilotus alba*	Rhizosphere	Memon Goth	0.0	63.3	70	0.0	63.3	76.6
13.	*C. globosum* (S-1)	*Vigna radiate*	"	KU	0.0	0.0	45	0.0	0.0	90
14.	*C. globosum* (S-2)	*Chenopodium album*	"	Gharo	0.0	3.3	23.3	3.3	13.3	25
15.	*C. globosum* (S-4)	*Melilotus alba*	Rhizosphere	Malir	0.0	3.3	11.6	1.6	5.0	18.3
16.	*Cladosporium* sp.	*Digera muricata*	Rhizoplane	KU	0.0	0.0	11.6	0.0	0.0	91.6
17.	*Curvularia clavata* (S-1)	*Cenchrno setigerus*	Rhizosphere	Hub	0.0	0.0	0.0	0.0	0.0	0.0
18.	*C. clavata* (S-2)	*Sorghum bicolor*	Rhizosphere	DarsanoCheno	0.0	11.6	18.3	0.0	11.6	18.3
19.	*Drechslera australiensis* (S-1)	*Citrullus lanatus*	Rhizoplane	Hub	0.0	0.0	23	2.0	4.0	34
20.	*D. australiensis* (S-2)	*Launea nudicaulis*	Rhizoplane	KU	0.0	0.0	38.3	0.0	0.0	38.3
21.	*D. hawaiiensis*	*Medicago sativa*	Rhizoplane	Malir	0.0	0.0	0.0	0.0	0.0	8.3
22.	*Fusarium oxysporum*	*Arachis hypogaea*	Rhizoplane	KU	0.0	0.0	0.0	0.0	0.0	0.0
23.	*F. solani*	*Aerva javanica*	Rhizopshere	Hub	0.0	15	85	0.0	05	95
24.	*F. solani*	*Avena sativa*	Rhizoplane	Hub	0.0	10	80	0.0	05	85
25.	*Macrophomina phaseolina*	*Abutilon indicum*	Rhizosphere	Hub	0.0	0.0	21.6	0.0	0.0	56.6

Table 1. (Cont'd.).

Name of species		Host	Region	Locality	Concentrations			Concentrations		
					1:100	1:10	1:0	1:100	1:10	1:0
					Juveniles mortality %					
S. No.	Fungi				After 24 hours			After 48 hours		
26.	*Memnoniella echinata*	*Sorghum bicolor*	Rhizosphere	Kathor	0.0	23.3	46.6	0.0	26.6	63.3
27.	*Myrothecium cinctum*	*Citrullus lanatus*	Rhizosphere	Hub	0.0	0.0	40	0.0	0.0	45
28.	*M. roridum*	*Vigna mungo*	Rhizoplane	KU	0.0	0.0	0.0	0.0	0.0	0.0
29.	*Paecilomyces lilacinus*(S-1)	*Citrullus lanatus*	Rhizosphere	Hub	0.0	6.6	65	0.0	77.3	95
30.	*P. lilacinus* (S-2)	*Cynodon dactylon*	Rhizosphere	Hub	0.0	0.0	0.0	5.0	5.0	77.7
31.	*Penicillium aspermum*	*Daucus carota*	Rhizosphere	HU	0.0	26.6	35	15	21.6	80
32.	*P. citrinum*	*Cyamopsis tetragonoloba*	*Rhizosphere*	Kathor	0.0	0.0	0.0	0.0	13.3	28.3
33.	*P. luteum*	*Gossypium arboretum*	*Rhizosphere*	KU	0.0	8.3	95	3.3.	8.3	95
34.	*P. purpurogenum*	*Vigna mungo*	*Rhizosphere*	KU	0.0	10	31.6	0.0	50	77.3
35.	*P. purpuroscence*	*Raphanus sativus*	*Rhizosphere*	Malir	0.0	0.0	0.0	0.0	0.0	0.0
36.	*P. raistrickii*	*Pennisetum americanum*	*Rhizosphere*	KU	0.0	0.0	55	0.0	6.6	90
37.	*Scopulariopsis brumptti*	*Citrullus lanatus*	*Rhizosphere*	Hub	0.0	0.0	70	0.0	55	93.3
38.	*Stachybotrys atra* (S-1)	*Daucus carota*	Rhizossphere	Memon Goth	0.0	1.6	11.6	0.0	18.3	35
39.	*S. atra* (S-2)	*Coriandrum sativum*	Rhizosphere	Memon Goth	1.6	5.0	100	1.6	5.0	100
40.	*S. parvispora*	*Zea mays*	Rhizosphere	Kathor	0.0	19	37	0.0	25	60
41.	*Trichoderma hamatum*	*Zea mays*	Rhizoplane	Kathor	0.0	0.0	11.6	0.0	0.0	91.6
42.	*T. harzianum*	*Glycine max*	Rhizosphere	KU	0.0	73	88.3	3.3	73.3	88.3
43.	*T. koningii*	*Phaseolus vulgaris*	Rhizosphere	Kathor	0.0	0.0	58.3	0.0	3.3	60
44.	*T. viride* (S-1)	*Gossypium arboretum*	Rhizosphere	KU	0.0	0.0	75	0.0	0.0	75
45.	*T. viride* (S-2)	*Cyperus rotundus*	Rhizosphere	Hub	0.0	0.0	0.0	1.6	3.3	21.6
46.	*T. viride* (S-3)	*Capsicum annuum*	Rhizoplane	Kathor	0.0	0.0	90	0.0	0.0	90
47.	*Verticillium chlamydosporium*	*Solanum melongena*	Rhizosphere	KU	0.0	18.3	33.3	0.0	60	73.3
$LSD_{0.05}$[1]					**0.6[1]**	**2.6[1]**	**11.3[1]**	**2.0[1]**	**3.4[1]**	**12.4[1]**

[1]Mean values in column showing differences greater than LSD values are significantly different at $p < 0.05$

KU= Karachi University

Discussion

Application or manipulation of nematode-antagonistic microbes is one area being investigated to find out the alternative to chemical nematicides (Meyer, 2003). In the soil, many beneficial fungi were found to inhibit the nematodes population by directly parasitizing them or by the production of toxic metabolites (Dayal, 2000). A number of fungi have been reported to secrete nematicidal metabolites and enzymes that affect nematode viability (Nitao *et al*., 1999). In this study culture filtrate of several fungi like *Aspergillus, Fusarium, Penicillium, Trichoderma, Paecilomyces, Verticillium* and *Chaetomium* showed significant nematicidal activity by killing the 2nd stage juveniles of *Meloidogyne javanica.* There are reports that many fungi produce active nematicidal compounds (Cayrol *et al.,* 1989; Anke *et al.,* 1995; Hallmann & Sikora, 1996; Anke & Sterner, 1997; Chen *et al.,* 2000; Meyer *et al.,* 2000; Meyer *et al.,* 2004). Similarly, soil-borne fungi include nematode-trapping or predacious fungi, endoparasitic fungi, parasites of nematode eggs and cysts, produce metabolites toxic to nematodes (Li *et al.,* 2007). Filtrates from cultures of *Fusarium* spp., *Paecilomyces lilacinus*, and *Pochonia chlamydosporia* were toxic to *M. incognita* second stage juveniles, inhibited hatching and/or suppressed egg or J2 populations on plants (Hallman & Sikora, 1996; Meyer *et al.,* 2004; Kerry, 2000; Nitao *et al*., 2001).

In this study, two isolates *Paecilomyces lilacinus*, an egg parasite of root knot and cyst nematode have shown potential nematoxic activity. Nematicidal efficiency of metabolites produced by *Paecilomyces lilacinus* and their specificity in controlling plant parasitic nematodes has been reported (Jatala *et al*., 1990). Nematicidal toxins produced by the species of *Fusarium, Trichoderma* and *Aspergillus niger* were found to be effective against *Meloidogyne* while *Paecilomyces* produced toxins active against root knot nematodes *Meloidogyne* and cyst nematodes *Heterodera* (Cayrol *et al*., 1989). Nematicidal activity of fungal metabolites of *Penicillium, Arthrobotrys conoides, Paecilomyces* sp., *Gliocladium deliquescens*, *Trichoderma viride* and *Trichothecium roseum* against plant parasitic nematode *Aphelenchoides composticola* Franklin have been reported (Grewal *et al*., 1989). Culture filtrates of *Aspergillus niger* and *Rhizoctonia solani* improved plant growth, reduced *Meloidogyne incognita* larval penetration, suppressed nematode reproduction and gall formation on tomato roots (Khan *et al*., 1985). In this study, *Fusarium solani*, *Paecilomyces lilacinus* and *Trichoderma* spp., showed significant nematicidal activity. There are reports that, toxins from various *Fusarium* spp., reduced nematodes viability (Nitao *et al.,* 2001; Ciancio, 1995) whereas acetic acid was an active component from culture filtrates of *Paecilomyces lilacinus* and *Trichoderma longibrachiatum* (Djian *et al.,* 1991). Meyer *et al.,* (2004) reported nematicidal potential of culture filtrates of several fungi isolated from eggs of soybean cyst nematode (*Heterodera glycines).* Culture filtrate of nematophagous fungus *Verticillium leptobactrum* inactivated the second stage juveniles and collapse of eggs of *M. incognita* (Regaieg *et al.,* 2010).

Fungi are known to posses a huge diversity of metabolic pathways and they have provided several large classes of commercial compounds, including many antibiotics used in medicine (Smedsgaard & Nielsen, 2005). Several compounds with nematicidal activity have also been reported from fungi (Li *et al.,* 2007; Anke, 2010). But no major commercial product based on these natural fungal compounds has been developed yet for wide use (Li, *et al.,* 2007). Secondary metabolites from fungi associated with rhizopshpere and rhizoplane of crop plants offer an exciting area of research for the discovery of potential nematicidal compounds.

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