

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Fungal Parasites of Root-knot Nematodes

Amer-Zareen, Imran Ali Siddiqui and M. Javed Zaki

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

Abstract: A total of 28 species belonging to 16 genera of fungi parasitic on root-knot nematodes were isolated and identified. *Aspergillus terreus*, *A. nidulans*, *A. tamarri* and *Fusarium anthophellum* are appeared to be new report on root-knot nematode and not reported hitherto. Of the fungi isolated, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Aspergillus* sp., *Acremonium butyri*, *Alternaria alternata*, *Catenaria* sp., *Cephalosporium* sp., *Cladosporium cladosporoides*, *Cladosporium* sp., *Cunninghamella elegans*, *Curvularia lunata*, *Fusarium exosporium*, *Fusarium* sp., *Ulocladium atrum* and a sterile fungus are new records on root-knot nematodes in Pakistan. Natural infestation of *Paecilomyces lilacinus* and *Arthrobotrys* sp. is also recorded first time from Pakistan.

Key words: Root-knot nematodes, nematode parasite, *Aspergillus* spp., *Fusarium anthophellum*, biological control, nematophagous fungi, antagonists

Introduction

The control of plant parasitic nematodes using trapping or parasitic fungi is of great interest because the use of nematicides or soil sterilant brings about various ecological and toxicological dangers (Jatala, 1986). At least four products have been developed commercially for the control of plant parasitic nematodes but non has used widely because control has tend to be erratic and practical application rate (Kerry, 1992). Despite such difficulties positive results obtained with biocontrol fungi of nematode in a number of other studies indicates that continued isolation and testing of fungi should lead to fungal agent that will add in nematode control (Morgan-Jones *et al.*, 1981; Susan *et al.*, 1990). Although a wide literature concerning fungi parasitic on root-knot nematodes is available worldwide (Kim *et al.*, 1992; Ma *et al.*, 1991; Jatala *et al.*, 1979; Morgan-Jones *et al.*, 1984; Viaene and Abawi, 1998), few fungal parasites of nematode have been reported from Pakistan (Munir *et al.*, 1988; Zaki and Maqbool, 1993; Zaki, 1999a; Khan *et al.*, 1994). A survey was therefore carried out to isolate fungi parasitic on root-knot nematodes (*Meloidogyne* spp.).

Materials and Methods

A survey of the cultivated fields of Karachi University campus, Shah Faisal colony, Korangi, Memon Goth, Linkroad, Ghara, Mirpur Sakro, Thatta and Kathore area was carried out during January, 1999 to September, 1999. A total number of 150 plant specimens belonging to 13 plant species showing symptoms of root knot disease were collected. Soil sample 250 g at 10 cm depth collected in a polythene bags.

Isolation and identification of fungal parasites: Egg masses of root knot nematodes were hand picked with the help of a sterilized forcep, surface sterilized in 1% Ca(OCl)₂ for 1-2 minutes, rinsed twice in sterile distilled water and plated onto 0.8 percent water agar supplemented with penicillin (100,000 units/L.) and streptomycin (0.2 g/L.).

Eggs of root knot nematodes were extracted by shaking the infected roots in 2 percent sodium hypochlorite solution, collected on a 400 mesh sieve and 1ml of the suspension was evenly spread onto water agar plates.

Meloidogyne females were obtained by teasing gall root tissues with the help of a sterilized needle under a stereoscope dissecting microscope. After surface sterilization in 2 percent sodium hypochlorite solution females were washed thoroughly with sterile distilled water and plated onto water agar plates as described earlier.

Meloidogyne eggs were extracted by sodium hypochlorite solution and washed thoroughly in sterile distilled water and kept for hatching at 25°C. For predacious or trapping fungi, one gram soil sample was sprinkled onto water agar plates and freshly

hatched juveniles suspension containing 100-200 juveniles/ml were pipetted onto water agar surface.

There were three replicates of each samples and dishes were incubated at room temperature (25-30°C) for 3-5 days. As soon as some fungal colonies appeared, hyphal fragment was transferred onto PDA plates and fungi were identified after reference to Booth (1971), Nelson *et al.* (1983), Domsch *et al.* (1980) and Thom and Raper (1945).

Results and Discussion

From 150 root and soil samples infested with root knot nematodes collected from 9 localities of vegetable crops, total of 16 genera comprising 28 species of fungi were isolated (Table 1).

Table 1: Fungal parasites associated with root-knot nematodes
Fungi

	Fungal isolated from			
	Egg masses	Eggs	Juveniles	Female
** <i>Aspergillus flavus</i>	-	-	+	+
** <i>A. Fumigatus</i>	+	-	+	-
* <i>A. nidulans</i>	-	-	+	+
* <i>A. Tamarri</i>	-	+	-	+
* <i>A. Terreus</i>	+	-	-	+
** <i>A. niger</i>	-	-	+	-
** <i>Aspergillus</i> sp.	+	-	+	-
** <i>Acremonium butyri</i>	-	+	-	-
** <i>Alternaria alternata</i>	+	-	-	-
** <i>Arthrobotry</i> sp.	-	-	+	-
<i>Catenaria</i> sp	-	-	+	-
** <i>Cephalosporium</i> sp	-	+	+	-
** <i>C. cladosporoides</i>	-	-	-	+
** <i>Cladosporium</i> sp.	+	+	+	-
** <i>Cunninghamella elegans</i>	-	+	-	-
** <i>Curvularia lunata</i>	-	+	-	-
* <i>Fusarium anthophellum</i>	-	+	-	-
** <i>F.oxysporum</i>	+	+	-	+
<i>F. Pallidoroseum</i>	+	-	-	-
<i>F.solani</i>	+	+	-	+
** <i>Fusarium</i> sp.	+	+	-	-
** <i>Paecilomyces lilacinus</i>	-	+	-	+
<i>P.verioti</i>	-	+	-	-
** <i>Penicillium</i> sp.	+	-	-	-
<i>Rhizoctonia solani</i>	+	-	-	-
** <i>Ulocladium atrum</i>	-	+	-	-
<i>Verticillium chlamydosporium</i>	+	+	-	+
** <i>Sterile fungi</i>	+	+	-	-

*New report **New record from Pakistan

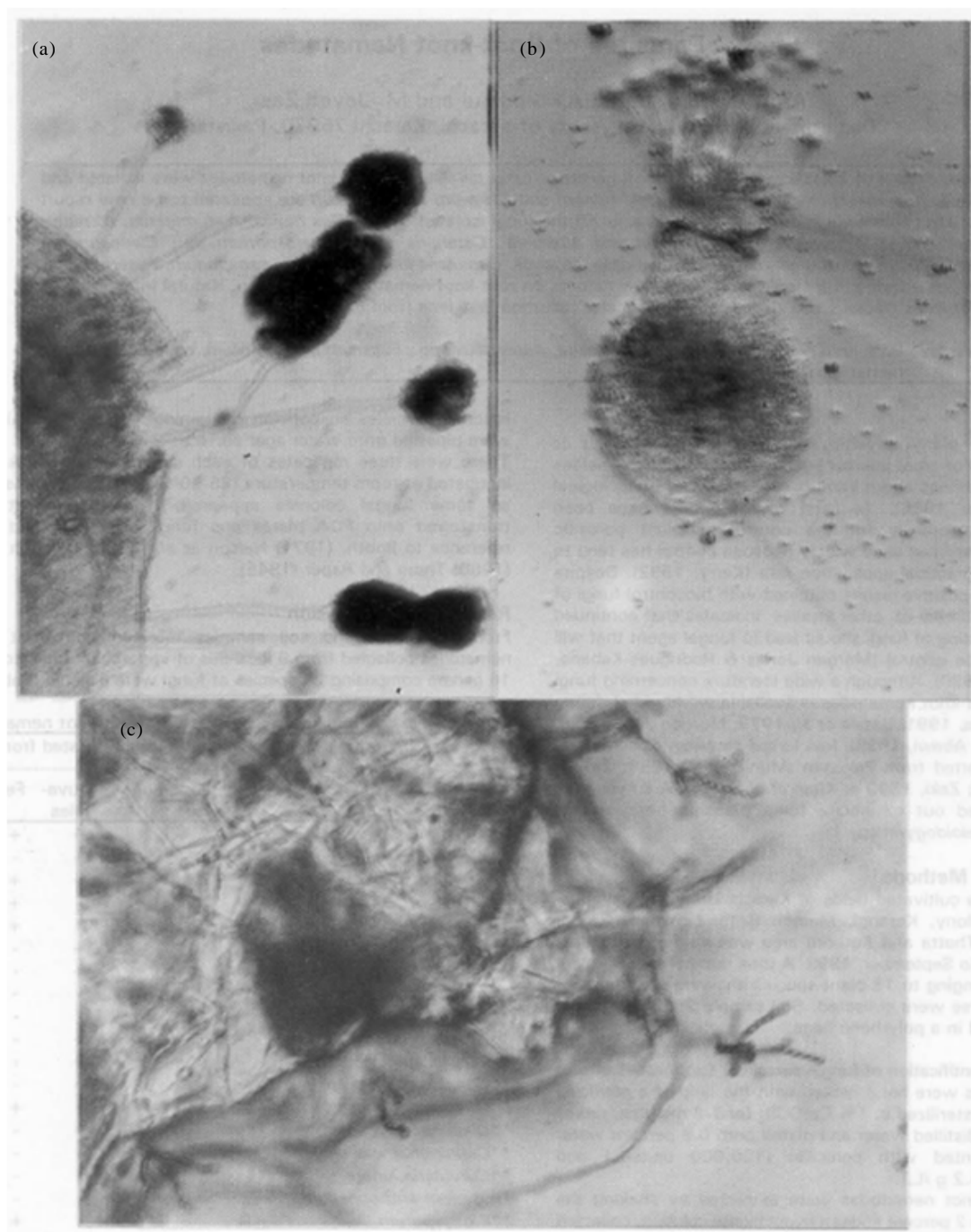


Fig. 1(a-c): Conidiophore emerging from females of *Meloidogyne* spp., a) *Aspergillus nidulans*, b) *A. terreus* and c) *Paecilomyces lilacinus*

Of these *Acremonium butyri*, *Alternaria alternata*, *Catenpria* sp., *Cephalosporium* sp., *Cladosporium cladosporoides*, *Cladosporium* sp., *Cunninghamella elegans*, *Curvularia lunata*, *Penicillium* sp., *Rhizoctonia solani* and sterile fungi were appeared to be new record on root knot nematode, *Meloidogyne* spp., in Pakistan. *Aspergillus* spp. were found most frequent, parasitizing almost all the stages of root knot nematodes. Of these *A. flavus* and *A. fumigatus* have been earlier recorded on root knot and cyst nematodes (Khan and Sexana, 1995) whereas *A. nidulans*

(Fig. 1a), *A. tamarri* and *A. terreus* (Fig. 1b) were found associated with females, eggs and egg masses and appeared to be new reports on *Meloidogyne* spp. *Fusarium* species appeared second major fungal group found associated with different forms of root knot nematodes. *F. exosporium*, *F. pallidoroseum*, *F. solani* and *Fusarium* sp. have been reported on *Meloidogyne* spp. (Ma *et al.*, 1991) but *F. anthophilum* on eggs appeared to be new report. *Paecilomyces lilacinus* (Jatala *et al.*, 1979) and *Verticillium chlamydosporium* (Morgan-Jones *et al.*, 1981) are

well known nematophagous fungi isolated from eggs, egg masses and females of root knot nematodes. Both of these fungi have been extensively studied under green house and field trials (Ehteshamul-Haque *et al.*, 1994; Zaki, 1999b; Parveen *et al.*, 1998). Culture of *P. lilacinus* used in the previous studies was supplied by Dr. P. Jatala of Peru and is deposited in Karachi University Culture Collection as KUCC-244. Natural infection of *P. lilacinus* is recorded first time recorded from Pakistan (Fig. 1c). A zoosporic fungus *Catenaria* sp. and trapping fungus *Arthrobotrys* sp. isolated from nematode suppressive soil during the present survey. Both of these fungi were associated with *Meloidogyne* larvae. *Catenaria* sp. has been reported by Zaki (1999a) but *Arthrobotrys* sp. appeared to be new record from Pakistan. During the present study, *Ulocladium atrum* was found associated with eggs of *Meloidogyne* root knot nematode. The fungus has been reported from cyst of *Globodera rostochiensis* (Munir *et al.*, 1988). There is need to test the efficacy of these fungi *in vitro* and *in vivo* for the control of plant parasitic nematodes.

Acknowledgment

This work, was carried out under University Grant Commissions grant which is sincerely acknowledged.

References

- Booth, C., 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England, ISBN-10: 851980465, Pages: 237.
- Domsch, K.H., W. Gams and T.H. Anderson, 1980. Compendium of Soil Fungi. Vol. 1, Academic Press, New York.
- Ehteshamul-Haque, S., M.J. Zaki, M. Abid and A. Ghaffar, 1994. Use of biocontrol agents with *Bradyrhizobium japonicum* in the control of root-knot nematode in chickpea. Pak. J. Nematol., 12: 149-154.
- Jatala, P., 1986. Biological control of plant-parasitic nematodes. Annu. Rev. Phytopathol., 24: 453-489.
- Jatala, P., R. Kaltenbach and M. Bocangel, 1979. Biological-control of *Meloidogyne-incognita* acrita and *Globodera pallid* on potatoes. J. Nematol., 11: 303-303.
- Kerry, B.R., 1992. The Status of Biological Control of Plant Nematodes. In: Status of Plant Pathology in Pakistan, Ghaffar, A. and S. Shahzad (Eds.). University of Karachi, Karachi, Pakistan, pp: 225-232.
- Khan, K., M.J. Zaki and M.A. Maqbool, 1994. Fungi associated with cysts of *Heterodera rea*. Med. Biol. J., 2: 31-33.
- Khan, T.A. and S.K. Saxena, 1995. Studies on the degree of parasitism and chitinolytic activity of fungi associated with root-knot and cyst nematodes in Uttar Pradesh, India. Ann. Plant Prot. Sci., 3: 137-140.
- Kim, J.I., C.H. Kim, H.U. Lee and S.C. Han, 1992. Identification and distribution of egg-parasitic and trapping fungi of root-knot nematode. Res. Rep. Rural Dev. Admin., 34: 91-95.
- Ma, C.Z., D.Y. Zhang and Z.G. Qian, 1991. Investigation on the fungi parasites of *Meloidogyne hapla* in peanut field soil. Disinsectional Microorganism, 3: 202-207.
- Morgan-Jones, G., G. Godoy and R. Rodriguez-Kabana, 1981. *Verticillium chlamydosporium*, fungal parasite of *Meloidogyne arenaria* females. Nematropica, 11: 115-120.
- Morgan-Jones, G., J.F. White and R. Rodriguez-Kabana, 1984. Fungal parasite of *Meloidogyne incognita* in an alabama soybean field soil. Nematropica, 14: 93-96.
- Munir, A., S. Iftikhar, I. Ahmed and M. Aslam, 1988. Fungi Associated with Cysts of *Globodera rostochiensis* on Potato in Kalam Valley, Swat, Pakistan. In: Advances in Plant Nematology: Proceedings of the U.S. Pakistan International Workshop on Plant Nematology, April 6-8, Karachi, Pakistan, Maqbool, M.A., A.M. Golden, A. Ghaffar and L.R. Krusberg (Eds.). National Nematological Research Centre, University of Karachi, Karachi, pp: 253-256.
- Nelson, P.E., T.A. Toussoun and W.F.O. Marasas, 1983. *Fusarium Species: An Illustrated Manual for Identification*. Pennsylvania State University Press, USA. ISBN 0271003499, Pages:193.
- Parveen, S., S. Ehteshamul-Haque and A. Ghaffar, 1998. Efficacy of *Pseudomonas aeruginosa* and *Paecilomyces lilacinus* in the control of root rot-root knot disease complex of some vegetables. Nematol. Medit., 26: 209-212.
- Susan, L.F.M., N.H., Robin and R.M. Sayre, 1990. Isolation of fungi from *Heterodera glycines* and < > bioassay for their antagonism to eggs. J. Nematol., 22: 532-537.
- Thom, C. and K.B. Raper, 1945. A Manual of *Aspergilli*. Williams and Wilkins Co., Baltimore, USA., Pages: 373.
- Viaene, N.M. and G.S. Abawi, 1998. Fungi parasitic on juveniles and egg masses of *Meloidogyne hapla* in organic soils from New York. J. Nematol., 30: 632-638.
- Zaki, M.J. and M.A. Maqbool, 1993. Occurrence of *Verticillium chlamydosporium* egg parasite of root-knot nematodes *Meloidogyne javanica* and *M. incognita* in Pakistan. Pak. J. Nematol., 11: 37-40.
- Zaki, M.J., 1999a. Biomanagement of root knot nematode of vegetables: Annual research report. Department of Botany, University of Karachi, Pakistan, pp: 54
- Zaki, M.J., 1999b. New host records of *Catenaria anguillulae* Sorokin from Pakistan. Pak. J. Biol. Sci., 2: 259-260.