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Antagonistic Effect of Some Species of *Pleurotus* on the Root-knot Nematode, *Meloidogyne javanica in vitro*

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Abstract: Antagonistic effect of five species of *Pleurotus*, including *P. ostreatus*, *P. sajor-caju*, *P. cornucopiae*, *P. florida* and *P. eryngii* on second stage juveniles (J2) of *Meloidogyne javanica* were studied, *in vitro*. On water agar, all of the species tested produced tiny droplets of toxin. Nematodes touching such droplets showed a sudden response and became colonized by the fungi after 24-48 h. Filtrates of the tested fungi grown in malt extract broth were toxic on the nematodes but this toxicity varied between species. Culture filtrates of *P. ostreatus* showed the highest nematicidal activity toward *M. javanica* J2 and the lowest toxic effect was observed in filtrates of *P. eryngii*. A linear relationship was proved between increasing toxin concentration and the percentage of dead nematodes.

Key words: *Meloidogyne javanica, Pleurotus* spp., Antagonistic, culture filtrate

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are one of the most economically important pest causing sever damages to a wide variety of crops particularly to tomato (Siddiqui and Shaukat, 2003). Their wide host ranges (Jepson, 1987) made them difficult to be controlled by rotation and resistant cultivars have variable value because of the occurrence of virulent races and species mixtures (Roberts, 1992). They also have rapid rates of multiplication on good hosts, further increasing the difficulty of preventing crop damage as small populations increase and become damaging during one growing season (Trudgill, *et al.,* 2000). Resistance genes effective against *Meloidogyne* spp. Appear not to have resulted from coevolution (Trudgill and Block, 2001). Existing management procedures could be enhance by the development of biocontrol strategies (Siddiqui and Shaukat, 2003). Nematicides are used to control various species of root-knot nematodes in developed agriculture but are mostly inappropriate for subsistence farmers in developing countries. Consequently, there is a great need to increase the control options for managing root-knot nematodes and biological control has been an active area of research.

At least 10 species of gilled fungi belonging to the genera *Hohenbuehelia, Pleurotus* and Resupinatus can attack nematodes by adhesion or toxins (Tzen and Liou, 1993). Thorn and Barron (1984) found that when nematodes came in contact with the hyphae of *Pleurotus*, they became inactive very quickly and were subsequently colonized and digested by the fungus. *Pleurotus* species display a kind of nematode capture that appears to be unique to this genus. They produce tiny appendages on the vegetative hyphae and these secrete droplets of a potent toxin (Thorn and Barron, 1984; Barron and Thorn, 1987; Peterson, 1993; Thorn and Tsuneda 1993). The toxin produced by *Pleurotus ostreatus* has been identified as trans-2-decenedioic acid (Kwock, *et al*., 1992). Barron and Thorn (1987) reported that rhabditid nematodes touching such droplets, showed a sudden response, the head region shrinked, hyphae attracted to the body orifices and homed in on the head. This mode of nematode attack has been observed in *P. cornucopiae, P. cystidiosus, P. levis, P. pulmonarius* (Thorn and Barron, 1984; Thorn and Tsuneda 1993), *P. tuberregium, P. dryinus, P. euosmus, P. eryngii* (Hibbet and Thorn, 1994) and *P. sajor-caju* (Sharma, 1994). The toxin produced by *P. cystidiosus* is present either at much lower concentration or is structurally different from that of *P. ostreatus* (Barron and Thorn, 1987). *Aphelenchoides composticola*, one of the most damaging mushroom nematodes, immobilized in *P. sajor-caju* culture filtrates within 2 to 4 h. of the exposure (Sharma, 1994).

It was suggested (Barron and Dierkes, 1977; Thorn and Barron, 1984) that nematodes may be an important nitrogen supplement for fungi, especially in wood substrates where the C:N ratio is very high and that the predatory capability of wood decay fungi has evolved to

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satisfy their nitrogen requirement. The host range for *Pleurotus* has never been fully established. Toxin droplets can be instrumental, not only in supplying a nitrogen source to *Pleurotus*, but can also function as an antifeedant that protect hyphae from fungus feeding microfauna (Barron, 2003). In this study, the nematoxic effects of some species of *Pleurotus* has been tested *in vitro*.

MATERIALS AND METHODS

The present investigation has been performed in the Department of Plant Pathology Tarbiat Moddares University in the year 2003-2004. Single-egg-mass cultures of *Meloidogyne javanica* were maintained on tomato (*Lycopersicon esculentum*) cultivar. Early Urbana in an autoclaved (140°C, 40 min) sandy soil in 18 cm-diameter pots in a glasshouse (14 h. light and 10 hrs. dark daily; temperature 24-30°C and RH 75-85%). Eggs were extracted by the NaOCl technique (Hussey and Baker, 1973). Second stage juveniles (J2s) were set to hatch from the eggs spread on a 30 μm sieve (Baker, 1985), collected daily and stored at 15°C. Juveniles aged less than 3 days were used in all experiments.

Behavior of *M. javanica* juveniles in *Pleurotus* cultures: Pure cultures of *P. cornucopiae* (ITCC No: 1724), *P. eryngii* (ITCC No: 3046), *P. ostreatus* (ITCC No: 2572), *P. sajor-caju* (ITCC No: 1725) and *P. florida* (ITCC No: 3308) were collected from Indian Type Culture Collection (ITCC) and maintained on potato dextrose agar (PDA) and transferred or subcultured weekly. Agar disks (5 mm) diameter were cut from 6-day-old cultures and each disk was placed at the center of water agar (2% w/v) plates and grown for 5-7 days at 24°C. By this time, the fungi had radiated a distance of 2-4 cm from the disks. Twenty active J2s of *M. javanica* previously surface sterilized in 5% streptomycin solution (De Ley and M-Ocampo, 2004) were placed at the edge of the colonies. Periodic observations were made under a stereoscopic microscope.

Viability of the juveniles in *Pleurotus* culture filtrates: Erlenmeyer flasks (500 mL) containing 300 mL of Malt Extract Broth (MEB), were inoculated with a 1 cmblock from a 7 days old fungal colonies growing on PDA. The fungi were cultured for three weeks on a rotary shaker at a low speed of forward and backward motion (40-50/min), at room temperature (23-24°C). The filtrates were first passed through a layer of filter paper (whatman cat. No: 1001110) and then through a 0.45 μm filter. The filtrates were used at concentrations of the original preparation (1×) and diluted 5× or 25× (Sharma, 1994).

A suspension of 100 J2 mL^{-1} of sterile water was prepared. About 50 surface sterilized J2 in 0.5 mL of water were mixed with 5 mL of the culture filtrates in a Petri-dish of 4cm dia., fresh malt extract broth or sterile water. Four replicates were tested per treatment. Viability of the J2s was determined after 4, 8 and 24 h and the percentage of paralyzed J2 was calculated (Moje, 1959). Juveniles that did not move even after touching the tail were considered to be dead. So, after the incubation period, culture filtrates were replaced with tap water and mortality of the juveniles were checked after 20-24 h.

The experiment was repeated. A completely randomized design was used. Data were subjected to analysis of variance (ANOVA) and means were compared with the Least Significant Differences (LSD) test ($p = 0.05$).

RESULTS

Behavior of *M. javanica* juveniles in *Pleurotus* cultures: Secretory processes were observed on aerial hyphae of all tested species. From the top, only the round outlines of the toxin droplets were visible (Fig. 1A). Viewed from the side, the secretions were seen as a slender stalk, leading to a rounded head (Fig. 1B). Nematodes moved without any harm over the young hyphae at the periphery of the colony, where there were no toxin droplets were absent. After touching the droplets on the older parts of the mat, they usually recoiled immediately. By one or more contact, nematodes became inactive and after 24-48 h. it was noticed that the hyphae of the fungi had grown toward the nematodes and penetrating them through body orifices especially through their mouth (Fig. 1C and D). The body contents were digested within 2-3 days (Fig. 1E). All species tested caused similar symptoms on nematodes, but the length of exposure time and the number of contacts required for appearance of these different symptoms were varied between fungi tested. the J2s showed severe reactions after contact with toxic droplets of *P. ostreatus* and inactivated quickly. Similar symptoms could be seen after prolonged exposure to a large number of droplets of *P. eryngii*.

Effect of *Pleurotus* culture filtrates on viability of *M. javanica* J2s: The effect of culture filtrates on J2s

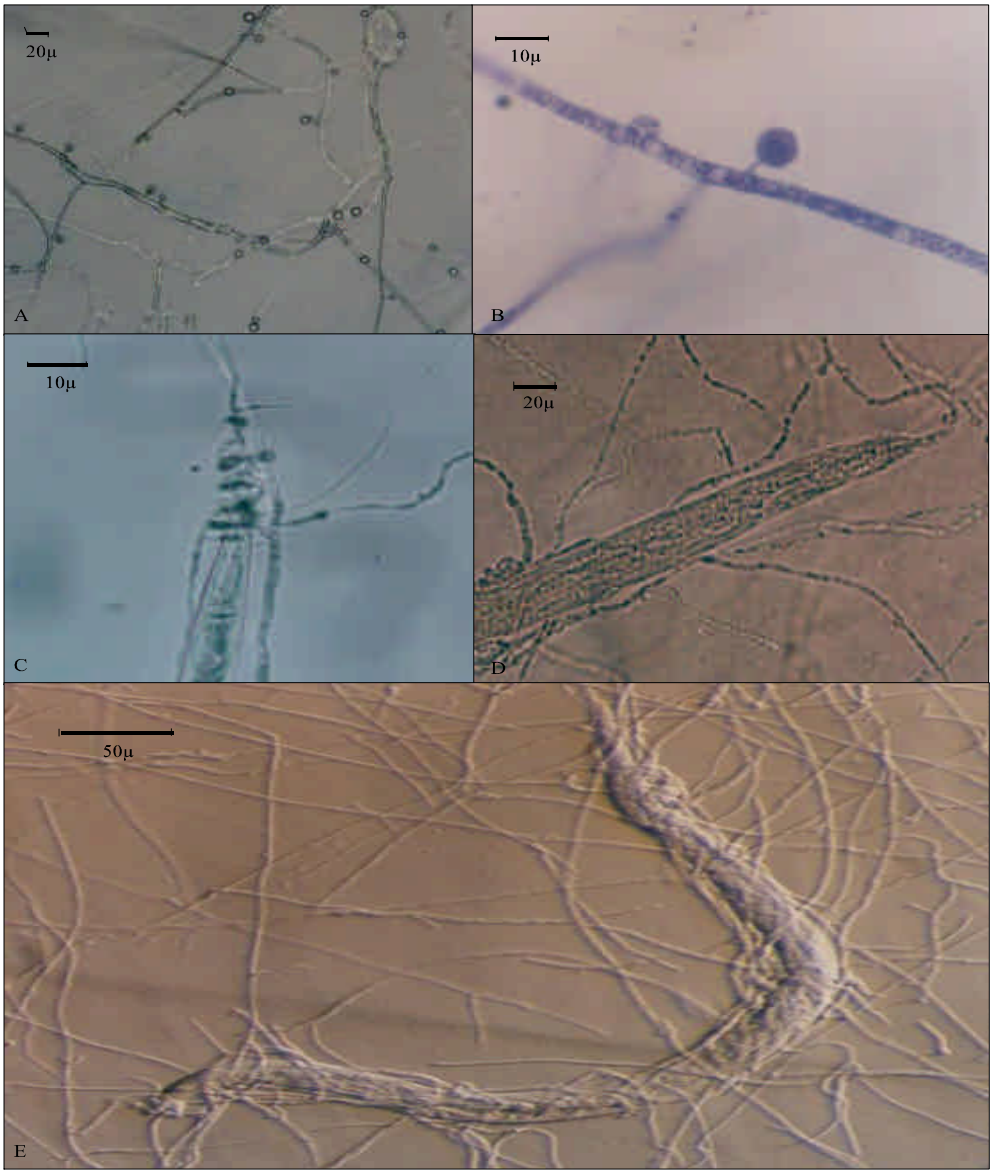

Fig. 1: Nematode trapping structure of *Pleurotus ostreatus* and nematodes on agar cultures of *Pleurotus ostreatus*. A. Toxin droplets on aerial hyphae, viewed without coverslip. B. side view of toxin droplet on stalked appendage, C. viewed with coverslip. D. Hyphae of *Pleurotus ostreatus* homing in on the mouth of nematode on water agar. E. *Meloidogyne javanica* J2 colonized by hyphae of *Pleurotus ostreatus*.

viability varied between the fungal species. Culture filtrates of *P. ostreatus* and *P. sajor-caju*, showed the highest nematicidal activity toward *M. javanica* J2s (Table 1). Culture filtrate of *P. ostreatus* in 1× malt extract broth; paralyzed 78% of J2s after an exposure period of 4 h and all of them after 24 h. A similar toxicity was observed in culture filtrates of *P. sajor-caju*. Culture filtrates of *P. cornucopiae* and *P. florida* in 1×, exhibited similar toxic activities to J2s and paralyzed 80 and 76% of J2s, after 24 h respectively. Culture filtrate of *P. eryngii* showed the lowest toxicity on J2s and there was not significant difference between 25× filtrate dilution of *P. eryngii* and controls (Table 1). A linear relationship was proved between increasing concentration and the percentage of paralyzed nematodes. Repeated experiment showed similar results (Table 2).

Table 1: Percentage of second-stage juveniles of *Meloidogyne javanica* paralyzed after a period of incubation in filtrates of fungal cultures in malt extract broth

		Incubation period (h)		
Treatments	Dilution	4	8	24
P. ostreatus	1	78.6	93.8	100
	5	34.2	44.3	54
	25	10.6	17	19.8
P. sajor-caju	1	62.9	81	97.1
	5	20.6	34.6	41.7
	25	9.4	15.8	18.7
P. cornucopiae	1	45.4	66	80.2
	5	7.9	14.5	22.6
	25	2.8	8	11.4
P. florida	1	51.2	64.7	75.7
	5	8.8	15.9	21.1
	25	1.3	2.7	9.2
P. eryngii	1	21.4	33.8	44.3
	5	6.6	10.2	11.7
	25	0.5	3.7	6.3
Malt extract broth	1	0	0.6	1.5
Sterile Water		0	0.4	0.8
LSD (p = 0.05)		6.82	7.14	6.26

Table 2: Percentage of second-stage juveniles of *Meloidogyne javanica* paralyzed after a period of incubation in filtrates of fungal cultures in malt extract broth

		Incubation period (h)		
Treatments	Dilution	4	8	24
P. ostreatus	1	83.1	97.5	100
	5	29.2	50.3	65.3
	25	9.8	16	26.7
P. sajor-caju	1	56.4	77.3	100
	5	25.7	41.7	55.1
	25	7	15.3	20
P. cornucopiae	1	43.2	58.5	86.7
	5	10.1	17.3	30.1
	25	3.1	3.7	10.7
P. florida	1	40	62	83.4
	5	12.3	20.2	33.4
	25	3	5.8	12.5
P. eryngii	1	30.2	38.1	42.2
	5	5	12.1	15.6
	25	0	2.7	5
Malt extract broth	1	0	0.6	2.3
Sterile water		0	0	2.3
LSD (p = 0.05)		7.23	6.91	6.34

DISCUSSION

In our study, all of the tested *Pleurotus* species showed toxic effects on *M. javanica* juveniles. Our observations proved the previous reports (Hibbet and Thorn, 1994) about toxic droplets. The nematodes did not show severe symptoms such as shrinkage of the head region reported previously for rhabditd nematodes (Barron and Thorn, 1987). This may be due to the structure of the buccal cavity of plant parasitic nematodes that is different from free living or bacteriophagous nematodes. As reported (Barron and Thorn, 1987), there was no relationship between the droplet density and nematicidal activity of the species. Thus the concentration of the toxin produced by the species may be different or the toxins may be structurally different in various species.

Toxin production in liquid media is influenced by a number of factors such as culture medium, aeration of media and pH (Chen, *et al.,* 2000). Variation of toxin production among strains within species has also been observed (Hallman and Sikora, 1996). Because toxin production of fungi was influenced by culture media (Chen *et al.,* 2000), the effect of toxins on nematodes may not become clear until toxin production by fungi is evaluated *in situ.* The toxin production in soil or on a root may be different from that in culture media. Further experiments should be performed to determine whether and under which conditions these fungi can produce toxins in large and effective amounts.

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