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Infectivity and virulence of indigenous entomopathogenic nematodes to mushroom phorid fly, *Megaselia sandhui* (Disney)

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ABSTRACT : Five indigenous isolates of entomopathogenic nematodes (*Steinernema abbasi*, *S. pakistanense*, *Steinernema* sp. and two isolates of *Heterorhabditis indica*) were isolated and tested *in vitro* for their infectivity and virulence against phorid fly, *Megaselia sandhui*. Use of 24-well tissue culture plates for testing infectivity was found not suitable. Sand barrier method revealed that only *H. indica* (isolate II) was promising in causing significant mortality of larvae of *M. sandhui* at nematode inoculum levels of 300 IJs and above per five larvae.

KEY WORDS: Entomopathogenic nematodes, *Heterorhabditis*, infectivity, *Megaselia sandhui*, mushroom, phorid flies, *Steinernema*, virulence

INTRODUCTION

Haryana is the leading state in seasonal mushroom production due to ever increasing demand in and around Delhi. Sciarid fly, *Bradysia tritici* (Coquillet) and phorid fly, *Megaselia sandhui* (Disney) are very serious pests of mushroom in Haryana. During October–November, phorid flies attack early crop of mushroom and become active again in February and cause up to 46 per cent infestation (Kumar and Sharma, 2002). The prevailing methods of insect pest control in mushroom production rely mainly on use of chemical insecticides (Sandhu and Arora, 1990; Aggrawal *et al.*, 2001), but this method is insane for fear of residue problem in fruiting bodies due to short life of mushroom crop. Entomopathogenic nematode (EPNs), *Steinernema feltiae* has been found to be a viable alternative to chemical pesticides in controlling mushroom flies in Europe (Scheepmaker *et al.*, 1997). Preliminary studies in Haryana revealed that *M. sandhui* was successfully infected by *Steinernema* isolates causing 30-40 per cent larval mortality (Walia *et al.*, 2004). The present study was conducted to determine the infectivity and to select the most virulent indigenous isolate of EPN against *M. sandhui*.

Five indigenous isolates of EPNs were isolated by 'insect trap method' (Bedding and Akhurst, 1975) using greater wax moth, *Galleria mellonella* larvae. Infective juveniles (IJs) were extracted from cadavers by 'White Trap' method (White, 1927), collected in sterile water and stored in 500 ml flasks in an incubator at 5-10° C. Fresh IJs were obtained by multiplying on *Galleria* larvae whenever required.

MATERIALS AND METHODS

Laboratory bioassays were conducted in a

BOD incubator at 20±1° C by two methods to test the infectivity and virulence of EPNs on *M. sandhui*. In the first test, 24-well tissue culture plates were used. The bottom of each well was lined with filter paper pieces and 20 IJs released in each well in 2 drops of water. Three-days-old larvae of *M. sandhui* were rinsed in sterile water and one larva was transferred to each well. The wells were covered with lids. There were 10 replicates for each nematode isolate. Observations were recorded on dead/live larvae 24, 48 and 72 h after release. After 72 h, the dead larvae were examined microscopically for nematode infection.

In the second test, plastic tubes (50 cc) were used. Five larvae were placed at the bottom of each tube. Different levels (50, 100, 200, 300, 400, 500) of IJs were released in tubes in 2 ml of water suspension and covered by river sand. Water alone was used as control. Utmost care was taken to minimize mortality of larvae due to other reasons by using thoroughly washed tubes, steam-sterilized sand and water. *M. sandhui* larvae were also gently washed with sterile water before use. There were nine tubes for each inoculum level of EPN isolate. Small pieces of *Agaricus bisporus* were placed in each tube as food for larvae. All the tubes were covered with lids having small holes for aeration and placed in a BOD incubator at 20±1°C. Three tubes each were removed after 24 and 48 h and per cent larval mortality was recorded. Remaining tubes were kept for observing fly emergence after nine days. At 24 and 48 h, the contents of the tube were taken out in a 30 cm petri plate, spread thinly and *M. sandhui* larvae (dead or live) or pupae were recovered in a cavity block. The dead larvae and pupae were dissected in water, and the nematodes recovered were counted.

RESULTS AND DISCUSSION

The five indigenous nematode isolates (recovered from CCS HAU farm area) were identified as: *Steinernema abbasi*, *S. pakistanense*; *Heterorhabditis indica* Poinar *et al.*, 1992 (isolate I); *H. indica*., 1992 (isolate II) (Elawad *et al.*, 1997; Shahina *et al.*, 2001; Poinar *et al.*, 1992); one isolate of *Steinernema* could not be identified conclusively and is, therefore, being referred to as *Steinernema* sp.

In the first test on the infectivity of five EPN isolates on *M. sandhui* (tissue culture plate method), most of the *M. sandhui* larvae escaped from their wells, and the rest which were present, were not sure to be in their original wells; but all were live. Obviously, the lids of the tissue culture plates were not tight enough to hold the larvae in their wells. The experiment was repeated by replacing the lids with paraflims. Two-three holes in each well were drilled with a dissecting needle in the parafilm for aeration. Other conditions remained same. This technique was successful in holding the larvae to their wells, but larval mortalities were not evident even after 48 h by any of the EPN isolates. Periodic observation under incident light of the stereomicroscope revealed that the *M. sandhui* larvae were moving along the circumference of the parafilm, ostensibly to escape. The larvae rarely moved to the base (filter paper with IJs) of the well, thus avoiding contact with the IJs. This may be the reason for non-infectivity of *M. sandhui* larvae by EPNs in this test. ELISA plates (Hay & Richardson, 1995) and tissue-culture plates (Scheepmaker *et al.*, 1998c) have been used earlier in case of sciarid and phorid flies, however, in most cases instead of wells lined with filter paper, these contained agar compost and finely chopped compost straw.

The second test simulated more or less the natural environment both for nematodes and phorid larvae. The actual mortality of larvae was higher in each case; but in this study only those larvae which revealed nematodes upon dissection have been accounted for. Data on larval mortality after 24 h did not reveal any trend. Discussed below are the observations recorded after 48 h.

S. abbasi: Nematode inoculum levels of 50, 100 and 200 did not cause any mortality of larvae. Mortality was low (7%) at 200 and 400 inoculum levels and it increased to 13 per cent at 500 inoculum level (Table 1), however, the results were non-significant. Nematode recovery

Table 1. Infectivity and virulence of five isolates of EPNs on *Megaselia sandhui* larvae after 48 h

Nematode inoculum levels (IJs/5 insect larvae)	*Steinernema abbasi*		*Steinernema pakistanense*		*Steinernema* sp.		*Heterorhabditis indica* (I)		*Heterorhabditis indica* (II)	
	Larval mortality* %	No. of IJs per larva	Larval mortality* %	No. of IJs per larva	Larval mortality* %	No. of IJs per larva	Larval mortality* %	No. of IJs per larva	Larval mortality* %	No. of IJs per larva
50	0.0 (1.8)	0.0	27.0 (30.8)	17.7	0.0 (1.8)	0.0	7.0 (10.1)	0.3	7.0 (10.1)	0.7
100	0.0 (1.8)	0.0	0.0 (1.8)	0.0	0.0 (1.8)	0.0	7.0 (10.1)	3.3	0.0 (1.8)	0.0
200	0.0 (1.8)	0.0	7.0 (10.1)	3.7	0.0 (1.8)	0.0	13.0 (18.1)	16.0	27.0 (30.8)	14.8
300	7.0 (10.1)	0.3	27.0 (30.8)	20.0	0.0 (1.8)	0.0	13.0 (14.3)	2.8	60.0 (50.8)	13.0
400	13.0 (18.3)	4.0	27.0 (26.4)	4.8	0.0 (1.8)	0.0	33.0 (30.6)	7.2	87.0 (72.3)	15.0
500	13.0 (14.3)	1.5	20.0 (21.5)	10.9	0.0 (1.8)	0.0	33.0 (35.0)	14.5	67.0 (60.0)	10.2
Uninoculated Control	0.0 (1.8)	0.0	0.0 (1.8)	0.0	0.0 (1.8)	0.0	0.0 (1.8)	0.0	0.0 (1.8)	0.0
C.D. (P=0.05)	(NS)		(NS)		(NS)		(NS)		(25.9)	

Figures in the Parentheses are angular transformed values

*Accounted only those from which nematodes recovered upon dissection, actual mortality higher

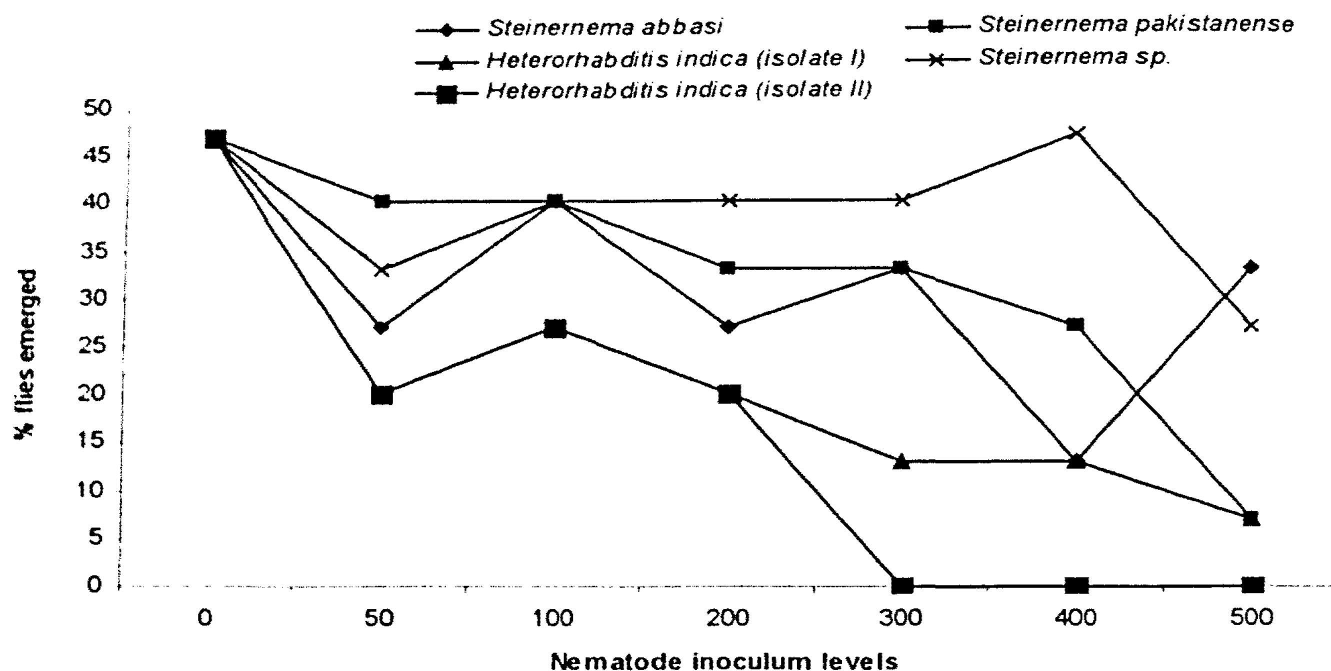

Fig.1 Effect of different EPN isolates on the fly emergence

from dead larvae did not reveal any trend. Data on fly emergence (Fig. 1) also did not comply with the increasing nematode inoculum levels, though reductions were evident in all treatments over control and ranged from 14.9-72.3 per cent.

S. pakistanense: Larval mortality was more consistent at higher (300, 400, 500) nematode inoculum levels and it ranged between 20-27 per cent (Table 1). Fly emergence was inversely proportional to nematode inoculum levels (Fig. 1). Per cent reduction in fly emergence over control ranged from 14.9 (at 50 and 100 inoculum levels) to 85.1 (at 500 inoculum level).

Steinernema sp.: This isolate of the nematode was ineffective, since no mortality of larvae was recorded even at 500 inoculum level (Table 1). Consequently, appreciable reduction in fly emergence was also not evident (Fig. 1).

H. indica (isolate I): Larval mortality revealed a clear trend- 7 per cent at low (50, 100) inoculum levels, 13 per cent at medium (200, 300), and 33 per cent at high (400, 500) inoculum levels (Table 1). A drastic reduction (85.1%) in fly emergence was recorded at 500 inoculum level. Low nematode inoculum levels also caused 57.5 and 42.5 per cent reduction in fly emergence (Fig. 1).

H. indica (isolate II): Inoculum levels of 200 and more resulted in significant mortality of larvae. Statistically, the inoculum levels of 300, 400 and 500 were on par, however, maximum larval mortality (87%) was caused by 400 nematode inoculum level. The average number of nematodes recovered from dead larva, was in general, quite high (Table 1). No fly emergence was recorded at 300, 400 and 500 inoculum levels and it was significantly less even at 200 and 50 levels (Fig. 1). The infected larvae (dark) could easily be distinguished from healthy larvae (transparent body cavity) even at low magnification.

A comparison of results on all the five EPN isolates leads to the inference that only *Heterorhabditis indica* (isolate II) resulted in significant mortality of *M. sandhui* larvae. Inoculum levels of 300, 400 and 500 were equally effective. *H. indica* (isolate I), *S. pakistanense* and *S. abbasi*, though caused appreciable larval mortalities in

descending order but the results were inconsistent. *Steinernema* sp. was totally ineffective. Richardson (1987) also reported that *Heterorhabditis* spp. may be more suitable than *Steinernema* spp. for the control of mushroom fly larvae because they can penetrate insect cuticle as well as natural openings.

The numbers of nematodes recovered from dead larvae of *M. sandhui* were highly variable. This is inconsequential for larval mortality, since even a single IJ can kill an insect. However, nematode numbers inside a dead larva may be of concern for progeny production, since more nematode numbers will lead to more adults and their further multiplication.

The data on fly emergence is based on 'actual' larval mortality, including 'considered' mortality of larvae because of confirmed nematode infection. The reduction in fly emergence in control (without nematodes) gives credence to the role of other factors (natural mortality) in causing larval mortality. Several studies pertaining to efficacy of EPNs particularly against phorid fly (Cantelo *et al.* 1977; Richardson, 1987; Scheepmaker *et al.* 1998a, 1998b; Jess and Bingham, 2004), have taken into account only reduction in fly emergence as compared to control.

All the species/isolates of EPNs may not be equally effective against many insect pests. Selection of a right combination of EPN isolate and target insect pest is desired to achieve successful results. Gouge and Hague (1995) found that the infectivity of *S. feltiae* was consistently higher than that of *S. carpocapsae* against sciarid flies. In another study, only three *Steinernema* isolates out of 10 were found to suppress *Megaselia halterata* (Long *et al.* 1998). In the present study *H. indica* (isolate II) was found to be most effective, among the five EPN isolates tested.

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