J. Biol. Control, 22(1): 7-12, 2008

Host range, pathogenicity and foraging behaviour of *Heterorhabditis indica*, *Steinernema asiaticum* and *Steinernema siamkayai* strains indigenous to Haryana

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ABSTRACT: **Indigenous strains of *Heterorhabditis indica*, *Steinernema asiaticum* and *S. siamkayai* caused 100% mortality in *Pieris brassicae* (after 48h) and *Agrotis ipsilon* (after 24h), whereas it was 80, 10 and 40%, respectively in *Helicoverpa armigera* (after 48 h) *in vitro*. On *Galleria mellonella*, mortality by *H. indica* was 100% at 30°C, 6.6% at 25°C and 33.3% at 20°C after 48h, and it increased to 100% only after 120h of exposure. IJs from insects killed at 25°C could be recovered only after transferring to 20°C. Average yield of IJs at 30, 25 and 20°C was $1.02x10^5$, $1.28x10^5$ and $1.51x10^5$ per insect larva, respectively. *S. asiaticum* resulted in 93.3% mortality at 25° and 30°, and 86.6% mortality at 20°C after 120h. Though emergence of IJs was delayed by 4 days at 30° and 20°C, the IJ yield per larva was not affected. *S. siamkayai* caused 100% mortality after 72h at all temperatures. In laboratory bioassays, both the *Steinernema* species revealed ambushing, whereas *H. indica* exhibited cruising behaviour.**

KEY WORDS: Foraging behaviour, *Heterorhabditis indica*, host range, pathogenicity, *Steinernema asiaticum*, *Steinernema siamkayai*, temperature

INTRODUCTION

Entomopathogenic nematodes (EPNs) are potential biocontrol agents of a wide range of insect pests of crops. However, different geographical isolates of the same species may differ with respect to their response to temperature, moisture and other environmental conditions. Grewal *et al.* (2002) reported differences in tolerance to heat, moisture stress and ultraviolet radiations in 15 natural populations of *Heterorhabditis bacteriophora* Poinar, 1976. *Steinernema asiaticum*, *S. siamkayai* and *H. indica* have been described from tropical and sub-tropical regions of Asian sub-continent (Poinar *et al.*, 1992; Stock *et al.*, 1998; Anis *et al.*, 2002) and these three species were isolated from Haryana (India) during surveys for EPNs over the last three years (Walia *et al.*, 2004). Hence, it was thought imperative to study their host range, pathogenicity and foraging behaviour *in vitro* for further applications.

MATERIALS AND METHODS

Isolation and Maintenance of nematodes

Heterorhabdtis indica, *S. asiaticum* and *S. siamkayai* were isolated from soil by '*Galleria* bait method' (Bedding and Akhurst, 1975). Infective juveniles (IJs) were obtained by multiplying *in vivo* on late instar larvae of greater wax moth, *Galleria mellonella* (L.) by filter paper method and used for further experimentation.

Host Range

Individual larvae (late instar) of *Agrotis ipsilon* (Hufnagel), *Helicoverpa armigera* (Hubner) and *Pieris brassicae* (L.) were exposed to 50IJs of *H. indica*, *S. asiaticum* and *S. siamkayai* separately by filter paper method and replicated five times. Per cent mortality was calculated from data recorded on dead and live insects at 24h interval for 3 days.

Pathogenicity

Heterorhabditis indica, *S. asiaticum* and *S. siamkayai* were inoculated @200IJs to 10 late instar larvae of *G. mellonella* in three separate experiments. The inoculations were made by filter paper method in 9 cm Petri-plates in triplicate. All the Petri-plates were stored in BOD incubators at 20, 25 and 30±1°C in a completely randomized design. Observations on insect mortality were recorded at 24h interval for 5 days. The dead larvae were incubated for 2 days in case of *Steinernema*, and 5 days for *Heterorhabditis* at respective temperatures and then transferred to White trap for the recovery of IJs (White, 1927). The number of IJs emerged was counted daily for 7 days.

Foraging behaviour

The study was carried out in 9cm Petri-plates containing 2% sterilized water agar (0.5cm thick layer). One gram of sterilized river sand was sprinkled on the surface of the agar. One hundred IJs each of *H. indica*, *S. asiaticum* and *S. siamkayai* were released in 0.5ml water on the surface in separate sets of Petri-plates (Campbell & Gaugler, 1993). Observations were recorded 24 h later. The species were classified as cruiser or ambusher based on more than 80% individuals showing a particular behaviour.

RESULTS AND DISCUSSION

Host Range

Heterorhabdtis indica, *S. asiaticum* and *S. siamkayai* infected the three insect hosts tested and caused 100% mortality in *P. brassicae* after 24h and in *A. ipsilon* after 48h. However, the infectivity varied on *H. armigera*, in which case 80% kill was recorded by *H. indica*, 10% by *S. asiaticum* and 40% by *S. siamkayai* after 48 h (Fig. 1). *S. pakistanense* has also been reported to be more virulent to *P. brassicae* and *A. ipsilon* than to *H. armigera* (Walia *et al.*, 2006).

Pathogenicity

Temperature influenced the pathogenicity of the three EPN species to *G. mellonella* larvae. *H. indica* caused maximum mean mortality at 30°C (81.3%), followed by 20 (63.9%) and 25°C (56.6%), irrespective of the duration. Mortality increased with time, it was negligible at 24h, but increased to more than 90% after 72 h. Interaction of temperature and time was significant. Total (100%) mortality was achieved earliest (48 h) at 30°C, while it took 120h to reach that level at 20 and 25°C (Table 1). The recovery of IJs from larvae killed at 25°C could be made only after transferring the cadavers to 20°C. Similar observations were recorded earlier on *S. pakistanense* too (Walia *et al.*, 2006). However, the average yield of IJs at 20 (1.51×10^5), 25 (1.28×10^5) and 30°C (1.02×10^5) was statistically non-significant.

Steinernema asiaticum was most effective at 25°C and least at 20°C. No mortality was observed at 30 and 20°C after 24h, while at 25°C it was 43.3%. After 72h, the insect mortality was statistically on par at the three temperatures (Table 2). Though the emergence of IJs was delayed by 4 days at 30 and 20°C, the IJs yield per larva was not affected (Fig. 2). It is inferred that the temperature affected only infection, and once the IJs invaded the host tissue, further development proceeded normally.

In case of *S. siamkayai*, 30°C was the best, followed by 25 and 20°C, in causing maximum insect mortality. The larval mortality increased significantly with time. The interaction of temperature and time revealed that maximum mortality (90%) was caused at 30°C after 24 h, and it reached 100% after 48h. However, it took 72h to reach 100% mortality level at 25 and 20°C (Table 3). The number of IJs recovered per larva at the three temperatures was statistically on par (Fig. 2).

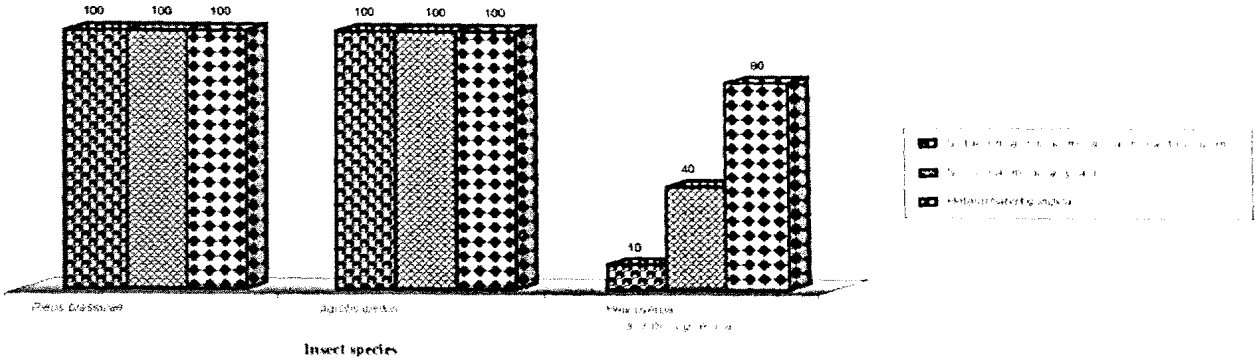

Fig. 1. Infectivity (per cent mortality) of *Steinernema asiaticum, S. siamkayai* and *Heterorhabditis indica* to three insect hosts after 48h

Table1. Pathogenicity of *Heterorhabditis indica* to *Galleria mellonella* larvae at different temperatures

Temperature (°C)	Per cent mortality after (h)					
	24	48	72	96	120	Mean (Temp.)
20	00.0 (00.0)	33.3 (46.9)	93.3 (81.1)	93.3 (81.1)	100.0 (90.0)	63.9 (59.8)
25	00.0 (00.0)	06.6 (08.8)	83.3 (75.0)	93.3 (81.1)	100.0 (90.0)	56.6 (51.0)
30	06.6 (12.3)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	81.3 (74.4)
Mean (Time)	02.2 (04.1)	46.6 (48.6)	92.2 (82.5)	95.5 (84.1)	100.0 (90.0)	
		SEM	LSD (P = 0.05)			
Temperature		2.88	8.35			
Time		3.73	10.78			
Temperature x Time		6.46	18.66			

*Mean of 3 replicates; figures in parentheses are angular transformed values

Table 2. Pathogenicity of *Steinernema asiaticum* to *Galleria mellonella* larvae at different temperatures

Temperature (°C)	Per cent mortality after (h)					
	24	48	72	96	120	Mean (Temp.)
20	00.0 (00.0)	23.3 (24.1)	76.6 (61.9)	80.0 (63.9)	86.6 (68.8)	53.3 (43.8)
25	43.3 (41.1)	76.6 (66.9)	80.0 (68.8)	90.0 (75.0)	93.3 (81.1)	76.6 (66.6)
30	00.0 (00.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	93.3 (81.1)	61.9 (54.5)
Mean (Time)	14.4 (13.7)	46.6 (43.4)	79.9 (67.2)	87.7 (73.3)	91.0 (77.0)	
		SEM±	LSD (P = 0.05)			
Temperature		3.81	11.00			
Time		4.91	14.21			
Temperature x Time		8.50	24.60			

*Mean of 3 replicates; figures in parentheses are angular transformed values

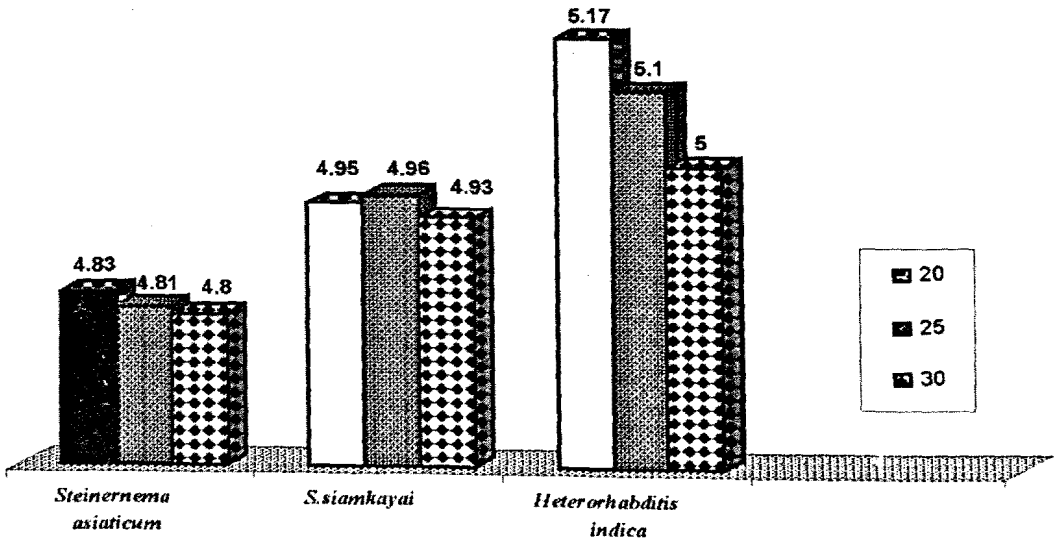

Fig. 2. Yield of IJs (log values) of three EPN species on *Galleria mellonella* at different temperatures

Table 3. Effect of temperature on pathogenicity of *Steinernema siamkayai* to *Galleria mellonella*

Temperature (° C)	Per cent mortality after (h)			
	24	48	72	Mean(Temp.)
20	63.3 (52.9)	83.3 (66.6)	100 (90.0)	82.2 (69.8)
25	86.6 (68.8)	96.6 (83.8)	100 (90.0)	94.4 (80.9)
30	90.0 (71.6)	100 (90.0)	100 (90.0)	96.6 (83.8)
Mean (Time)	79.9 (64.4)	93.3 (80.2)	100 (90.0)	
		SEM±	LSD (P = 0.05)	
Temperature		1.77	5.26	
Interval		1.77	5.26	
Interval x temperature		3.07	9.12	

*Mean of 3 replicates; figures in parentheses are angular transformed values

Temperature and moisture conditions affect the life processes of EPNs (Glazer, 2002). Species isolated from temperate regions are well adapted to lower temperatures, whereas those from tropical and sub- tropical regions are successful at a higher range of temperature. Subramanian (2004) observed highest mortality of *Corcyra cephalonica* by *H. indica* at 30 and 35°C and by *Steinernema glaseri* at 25°C. Hussaini *et al.* (2005) reported 28-32°C as the optimum temperature for 100% mortality in *G mellonella* and *A. ipsilon* after 2-3 days of initial inoculation by indigenous *Steinernema* isolates. Observations of Karunakar *et al.* (1999) and Shamseldean *et al.* (1999) too support our findings that *Heterorhabditis* penetrates, multiplies and persists better at higher (27-32° C) temperatures than those required by most *Steinernema* species (20-25° C).

Foraging behaviour

In *H. indica*, most of the IJs were seen moving on the surface of agar. Occasionally, 5-10% IJs tried to swing half of their bodies in an attempt to cross over to other soil particles. Hence, *H. indica* was classified as predominantly cruiser, whereas the two *Steinernema* species were found to be mainly ambushers since most of the IJs were nictating on the soil particles.

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(Received: 12.04.2007; Revised: 20.07.2007; Accepted: 10.08.2007)