J. Biol. Control, 19(1): 51-58, 2005

Influence of temperature on infectivity of entomopathogenic nematodes against black cutworm, *Agrotis ipsilon* (Hufnagel) and greater wax moth, *Galleria mellonella* (Linnaeus) larvae

S. S. HUSSAINI, V. SHAKEELA and M. H. DAR
Project Directorate of Biological Control (ICAR)
P. B. No. 2491, H. A. Farm Post, Bellary Road
Hebbal, Bangalore 560 024, Karnataka, India
E mail: sshblr@yahoo.com

ABSTRACT: **Effect of different temperatures on infectivity and progeny production of indigenous entomopathogenic nematode isolates, *Steinernema carpocapsae* PDBC EN 6.11, *S. abbasi* PDBC EN 3.1, *S.tami* PDBC EN 2.1, and *Heterorhabditis indica* PDBC EN 6.71, PDBC EN 13.3 were studied under laboratory condition against *Galleria mellonella* (Linnaeus) and *Agrotis ipsilon* (Hufnagel) final instar larvae. All isolates caused absolute mortality of test insect larvae, and produced progeny at 25 and 32° C. Low rate of infection or no infection was observed at lower temperatures, 15 and 8°C, respectively. Time required to kill host insects by all isolates showed difference at 15, 25 and 32° C. Shortest time for mortality of *A. ipsilon* and *G. mellonella* larvae was observed at 32° C followed by 25° C. Absolute mortality of larvae was observed with all isolates after 48 and 72 hours at 25° and 32° C, whereas the same was not achieved at 15° C even after 96 hours post exposure. Progeny production of all isolates varied with reference to temperature. Irrespective of isolates, 25°C was found suitable for infection and development of nematode populations.**

KEY WORDS: *Agrotis ipsilon*, entomopathogenic nematodes, *Galleria mellonella*, *Heterorhabditis indica*, *Steinernema abbasi*, *S. carpocapsae*, *S. tami*, temperature

INTRODUCTION

Entomopathogenic nematodes have long been used for suppressing soil insect pests like white grubs (Georgis and Gaugler, 1991) and cutworms (Simons and Guys, 1980). Black cutworm, *Agrotis ipsilon* (Hufnagel) is a soil dwelling polyphagous herbivore and is susceptible to entomopathogenic nematodes (Capinera *et al*., 1988). Soil types and temperatures are considered as important physical factors influencing nematode mobility, efficacy, reproduction, development and persistence in the field (Kung, 1990). An attempt was made in the present study to find the influence of different temperature regimes on the infectivity and reproduction of some indigenous isolates of *Steinernema* spp. and *Heterorhabditis indica* using *A. ipsilon* and *Galleria mellonella* (Linnaeus) as host insects.

MATERIALS AND METHODS

Greater wax moth culture

Greater wax moth, *G. mellonella* larvae were reared on artificial diet as per procedure described by Singh (1994). All the stages of black cutworm, *A. ipsilon* were maintained on artificial diet under laboratory condition (Blenk *et al.*, 1985).

Nematode Cultures

Steinernema abbasi PDBC EN 3.1, *S. tami* PDBC EN 2.1, *S. carpocapsae* PDBC EN 6.11, *S. glaseri*, *H. indica* PDBC EN 6.71 and PDBC EN 13.3 isolated from different agro-climatic regions were cultured and propagated on final instar larvae of *G. mellonella*. Infective juveniles were harvested through modified white traps at 24 hours interval and stored in distilled water at 25° C with intermittent aeration with an aquarium aerator. The infective juveniles stored for 120 hours under conditions mentioned above were used for the experiments.

Infectivity and developmental studies at different temperatures in lab

Bioassays for infectivity were conducted in 7cm plastic vials filled with moist sterile soil (8% w/ w) to a depth of 4 cm. Dosage of 180 and 20 IJs/ larva were used for inoculation of *A. ipsilon* and *G. mellonella*, respectively. Nematode suspension having 180 and 20 IJs/ ml was added separately to each container and were placed in incubators at 8, 15, 25 and 32°C. After acclimation for 24 hours at the temperatures mentioned, single final instar larvae of *A. ipsilon*/ *G. mellonella* was added individually to each container. Plastic vials were covered in polythene bags to prevent desiccation. Larval mortality was determined at 24, 48, 72 and 96 hours post inoculation. Each treatment consisted of 5 replications with two insects per replication. The study was conducted at Nematology Laboratory, Project Directorate of Biological Control, Bangalore during the period 2000-01.

To study the influence of temperature on development, cadavers of *A. ipsilon* and *G. mellonella* from infectivity assay were placed individually on modified white trap. The traps were incubated at original temperatures and checked daily for emergence of IJs. The emerged IJs were collected at regular intervals and the total number of infective juveniles emerged from each larva was computed by dilution count using stereo zoom microscope. Each treatment was replicated twice.

For statistical analysis, the per cent mortality values were converted to arcsine transformation and the treatment effects were subjected to analysis of variance. Significance was tested at the 5 per cent level using Duncan's multiple comparison tests to separate the means and the effects were compared.

RESULTS AND DISCUSSION

The infectivity of different entompathogenic nematode isolates against final instar larvae of *G. mellonella* and *A. ipsilon* was variable at different temperatures. Temperature had a significant effect on time of host death by all nematode species/ isolates tested. All the populations tested elicited faster and rapid rate of kill at 32° C followed by 25° C. No infection of test insects was observed at 8° C even after 7 days of exposure and this temperature was discontinued. Absolute mortality of *G. mellonella* larvae was achieved within 48 hours at 25° and 32° C except *S. tami* at 32° C whereas the same was not achieved even after 96 hours exposure at 15° C; this was further monitored up to 7 days and there was no increase in mortality while the larvae pupated (Table 1). The maximum mortality observed was only 20 per cent with *S. glaseri* and *S. tami* at 48 hours exposure period. As the exposure period increased, mortality of larvae also increased significantly and was 80 and 60 per cent at 96hours exposure with the above isolates. Other isolates, *S. carpocapsae* and *S. abbasi* resulted in 40 per cent mortality while *H. indica* only 20 per cent during the same period. The results indicated that higher temperatures, 25° and 32° C were better for infection process compared to 15° C in respect of all isolates of *Steinernema* sp. or *Heterorhabditis* sp. used in the study.

Table 1. Mean mortality of *G. mellonella* larvae by indigenous nematode isolates in soil assay at different temperatures

Nematode isolate	Mortality of *G. mellonella* larvae at different temperatures and time intervals (%)*											
	15° C				25° C				32° C			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24h	48h	72h	96h
S. abbasi	0.0 (0.4) ^{g}	0.0 (0.4) ^{g}	20.0 (23.9) ^{e}	40.0 (36.1) ^{d}	60.0 (54.0) ^{c}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	60.0 (54.0) ^{c}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}
S. tami	0.0 (0.4) ^{g}	20.0 (18.2) ^{e}	60.0 (54.0) ^{c}	60.0 (51.2) ^{c}	60.0 (51.2) ^{c}	80.0 (66.2) ^{b}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	60.0 (54.0) ^{c}	60.0 (54.0) ^{c}	60.0 (54.0) ^{c}	60.0 (54.0) ^{c}
S. carpocapsae	0.0 (0.4) ^{g}	20.0 (20.9) ^{e}	40.0 (39) ^{d}	40.0 (36.1) ^{d}	60.0 (54.0) ^{c}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	60.0 (54.0) ^{c}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}
S. glaseri	0.0 (0.4) ^{g}	20.0 (23.9) ^{e}	60.0 (51.0) ^{c}	80.0 (69.0) ^{b}	60.0 (54.0) ^{c}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	80.0 (72.0) ^{b}	100.0 (9.00)	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}
H. indica 13.3	0.0 (0.4) ^{g}	0.0 (0.4) ^{g}	20.0 (21.2) ^{e}	20.0 (18.2) ^{e}	60.0 (54.0) ^{c}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}
H. indica 6.71	0.0 (0.4) ^{g}	0.0 (0.4) ^{g}	20.0 (23.9) ^{e}	20.0 (20.9) ^{e}	60.0 (51.0) ^{c}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	80.0 (72.0) ^{b}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}	100.0 (90.0) ^{a}

	SEM ±	CD (P=0.05)
Nematode species	1.57	4.35
Temp	1.11	3.08
Time interval	1.28	3.55
Nematode species x temp	2.72	7.54
Temp x time	2.22	6.16
Nematode x time	3.14	8.71
Nematode x temp x time	5.44	15.08

*Means followed by the same letter are not significantly different.

Figures in parentheses are arcsine-transformed values.

Table 2. Mean per cent mortality of *A. ipsilon* larvae by indigenous nematode isolates in soil assay at different temperatures

Nematode isolate	Mortality of *A. ipsilon* larvae at different temperatures and time intervals (%) *											
	15 °C				25 °C				32°C			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24h	48h	72h	96h
S. abbasi	0.0 (1.3)^{g}	0.0 (1.3)^{g}	0.0 (1.3)^{g}	20.0 (21.5)^{f}	40.0 (39.0)^{d}	60.0 (51.3)^{c}	80.0 (69.0)^{b}	100.0 (90.0)^{a}	40.0 (39.0)^{d}	80.0 (66.2)^{b}	100.0 (90.0)^{a}	100.0 (90.0)^{a}
S. tami	0.0 (1.3)^{g}	0.0 (1.3)^{g}	20.0 (21.5)^{f}	40.0 (38.8)^{d}	20.0 (24.1)^{e}	40.0 (39.0)^{d}	60.0 (51.3)^{c}	100.0 (90.0)^{a}	40.0 (39.0)^{d}	60.0 (51.0)^{c}	80.0 (69.0)^{b}	100.0 (90.0)^{a}
S. carpocapsae	0.0 (1.3)^{g}	0.0 (1.3)^{g}	0.0 (1.3)^{g}	20.0 (24.1)^{e}	20.0 (24.1)^{e}	20.0 (24.1)^{e}	40.0 (39.1)^{d}	60.0 (54.0)^{c}	20.0 (21.5)^{f}	40.0 (39.0)^{d}	60.0 (54.0)^{c}	80.0 (69.0)^{b}
S. glaseri	0.0 (1.3)^{g}	0.0 (1.3)^{g}	20.0 (21.5)^{f}	60.0 (51)^{c}	20.0 (24.1)^{e}	40.0 (39.0)^{d}	60.0 (51.0)^{c}	100.0 (90.0)^{a}	40.0 (39.0)^{d}	80.0 (69.0)^{b}	100.0 (90.0)^{a}	100.0 (90.0)^{a}
H. indica 13.3	0.0 (1.3)^{g}	0.0 (1.3)^{g}	0.0 (1.3)^{g}	0.0 (1.3)^{g}	40.0 (39.0)^{d}	60.0 (51.0)^{c}	80.0 (69.2)^{b}	100.0 (90.0)^{a}	60.0 (51.0)^{c}	100.0 (90.0)^{a}	100.0 (90.0)^{a}	100.0 (90.0)^{a}
H. indica 6.71	0.0 (1.3)^{g}	0.0 (1.3)^{g}	0.0 (1.3)^{g}	0.0 (1.3)^{g}	40.0 (39.0)^{d}	40.0 (39.0)^{d}	80.0 (66.2)^{b}	100.0 (90.0)^{a}	40.0 (36.3)^{d}	80.0 (69.2)^{b}	100.0 (90.0)^{a}	100.0 (90.0)^{a}

	SEM ±	CD (P=0.05)
Nematode species	1.41	3.91
Temp	0.99	2.77
Time interval	1.15	3.2
Nematode species x temp	2.45	6.78
Temp x time	1.99	5.54
Nematode x time	2.82	7.83
Nematode x temp x time	4.89	13.56

*Means followed by the same letter are not significantly different.

Figures in parentheses are arcsine-transformed values.

Mortality of *A. ipsilon* larvae was also significantly affected by nematode species and temperature. Significant mortality was not observed at 15°C due to all isolates except *S. glaseri*, which caused 20 per cent mortality at 72 hours post exposure and maximum mortality, 60 per cent at 96 hours exposure. *Steinernema tami*, *S. carpocapsae* and *S. abbasi* caused 40, and 20 per cent, respectively and no infection was observed with *H. indica* isolates during the same period. At 25° C *H. indica* isolates caused 40 per cent mortality and *Steinernema* spp. 20 per cent 24 hours after exposure. At this temperature absolute mortality was observed only at 96 hours post exposure due to all isolates except *S. carpocapsae*, whereas at 32° C, 40-60 per cent mortality was observed irrespective of nematode isolates 24 hours post exposure and absolute mortality was obtained within 72 hours due to all isolates except *S. carpocapsae* and *S. tami* (Table 2). It has been inferred from the results that *S. glaseri* infection and progeny production were least affected at all temperature ranges studied. *Steinernema tami*, which was originally isolated from Jorhat, Assam exhibited a preference to cooler temperature and *S. abbasi* and *H. indica* isolates (originally isolated from Delhi and Chidambaram, Tamil Nadu, respectively) showed higher preference for warmer temperature. Our results support the earlier studies that *H. indica* is adapted to warmer climates (Griffin, 1993) and species and /or strains are governed by the temperature range of their original localities.

The effect of low temperature on nematode efficacy was pronounced in case of *A. ipsilon* compared to *G. mellonella*. Increasing the temperature from 8 °- 32°C resulted in significant increase in mortality and reduction in time of host death. In laboratory studies, the effect of a range of temperatures (5°-30° C) on the infectivity of *Heterorhabditis* sp. and *S. carpocapsae* against *Otiorrhynchus sulcatus* resulted in increase in infectivity with increase in temperature, resulting in 100 per cent mortality of *O. sulcatus* when *Heterorhabditis* spp. was kept at 20°C for 12 days and 65 per cent mortality with *S. carpocapsae* at 20° and 25° C (Miduturi *et al.*, 1995).

Lowered virulence of nematodes at lower temperature is attributed to the decreased mobility of nematodes, less cues being produced by the insect or by a lower sensitivity of the nematodes to host cues at lower temperatures (Chen *et al.*, 2003). Activity at low temperatures was not related to a specific taxonomic group (Westerman, 1994).

The progeny production of infective juveniles in *A. ipsilon* and *G. mellonella* at different temperatures is presented in Tables 3 and 4. Nematode yield varied with respect to temperature, species and isolates of nematodes and target

Table 3. Progeny production EPN isolates in *A. ipsilon* at different temperatures

Nematode isolate	Total no. of IJs emerged/ *A. ipsilon* larva (in lakhs) *		
	15°C	25°C	32°C
S. glaseri	0.28^{i}	0.38^{k}	0.33^{j}
S. abbasi PDBC EN 3.1	0.31^{i}	2.9^{d}	2.46^{e}
S. tami PDBC EN 2.1	0.32^{i}	2.59^{e}	1.57^{g}
S. carpocapsae PDBC EN 6.11	0.22^{i}	2.17^{f}	1.73^{h}
H. indica PDBC EN 13.3	0.12^{i}	3.78^{a}	3.14^{c}
H. indica PDBC EN 6.71	0.12^{i}	3.46^{b}	2.66^{e}

SEM ± =0.12 CD (P=0.05)= 0.34

*Means followed by the same letter are not significantly different.

Table 4. Progeny production EPN isolates in *G. mellonella* at different temperatures

Nematode isolate	Total no. of IJs emerged/ *G. mellonella* larva (in lakhs) *		
	15°C	25°C	32°C
S. glaseri	0.39[i]	0.42[i]	0.37[i]
S. abbasi PDBC EN 3.1	0.46[i]	3.8[e]	3.2[d]
S. tami PDBC EN 2.1	0.52[i]	3.65[e]	2.77[f]
S. carpocapsae PDBC EN 6.11	0.43[i]	2.62[f]	2.14[g]
H. indica PDBC EN 13.3	0.14[m]	4.71[a]	4.25[b]
H. indica PDBC EN 6.71	0.12[m]	4.54[a]	3.9[b]

SEM ± = 0.13 CD (P=0.05) = 0.37

*Means followed by the same letter are not significantly different

insects. All the isolates tested yielded maximum in *G. mellonella* larvae compared to *A. ipsilon*. Highest yields (4.71 and 4.54 lakhs IJs per larva) were observed with *H. indica* isolates 13.3 and 6.71. Among the temperatures, 25°C was found suitable for higher yields in respect of all the isolates in both the insects (Table 3 and 4). Significant differences were observed in number of progenies produced at three different temperatures studied. The time of first emergence from cadavers was longer (7 days) at 15°C than at 25° and 32°C (3-4 days) and the progeny production was least (0.12 lakh/ larva) at 15°C. It is evident from the results that temperature range for infectivity was higher than for multiplication of entomopathogenic nematodes. Though *S. glaseri* yield was the lowest among the nematodes tested, it was the least affected at varied temperatures (Table 4). This is in agreement with the earlier studies that the thermal niche breadth for establishment within hosts was the widest for *S. glaseri* (10-37°C) and the narrowest for *S. feltiae* (8-30° C). Thermal niche breadth for reproduction was widest for *S. glaseri*, (12-32° C) and the narrowest for *S. carpocapsae* (20°-30° C) (Grewal *et al.*, 1994).

ACKNOWLEDGEMENT

The authors thank Department of Biotechnology, New Delhi for providing financial assistance.

REFERENCES

Blenk, R. G., Gourger, R. J., Gallo, T. S., Jordan, L. K., and Howell, E. 1985. *Agrotis ipsilon*, pp.177-188. In: P. Singh and R. F. Moore (Eds.), *Handbook of insect rearing*, Vol. II. Elsevier, USA.

Capinera, J. L., Pelissier, D., Menout, G. S. and Epsky, N. D. 1988. Control of black cutworm, *Agrotis ipsilon* with entomogenous nematodes. *Journal of Invertebrate Pathology*, **52**: 427-435.

Chen, S., Li, J., Han, X. and Moens, M. 2003. Effect of temperature on the pathogenicity of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) to *Delia radicum*. *BioControl*, **48**: 713-724.

Georgis, R. and Gaugler, R. 1991. Predictability in biological control using Entomopathogenic nematodes. *Journal of Economic Entomology*, **84**: 714-720.

Grewal, P. S., Selvan, S. and Gaugler, R. 1994. Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction. *Journal of Thermal Biology*, **19**: 245-253.

Griffin, C. T. 1993. Temperature responses of Entomopathogenic nematodes for the success of biological control programmes, pp. 101-111. In: R.

Bedding, R. Akhurst and H. Kaya (Eds.), *Nematodes and the Biological Control of Insect Pests*. CSIRO Publications, East Melbourne, Australia.

Kung, S. P. 1990. Abiotic factors affecting the persistence of two entomopathogenic nematodes, *Steinernema carpocapsae*, and *Steinernema glaseri* (Nematoda: Steinernematidae) in the soil. *Dissertation Abstracts International B Sciences and Engineering*, **51**: 4, 1620.

Miduturi, J. S., Clercq, R., Casteels, H. and Grisse, A. 1995. Effect of temperature on the infectivity of entomopathogenic nematodes against black vine weevil (*Otiorrhynchus sulcatus* F.). *Parasitica*. **50**: 103-108.

Simons, W. R. and Guys, P. 1980. Control of insects with nematodes, pp. 275-278. In: A. K. Minks (Ed.), *Integrated control of insect pests in the Netherlands*. Center for Agricultural Publishing and Documentation, Wageningen, Netherlands.

Singh, S. P. 1994. *Technology for Production of Natural Enemies*. Project Directorate of Biological Control, Bangalore. 221pp.

Westerman, P. R. 1994. The vertical migration of *Heterorhabditis* spp. and *Steinernema* spp. at 9°C and the relationship to efficacy against *Otiorrhynchus sulcatus* at 9°C. *Bulletin of OILB SROP*, **17**: 81-85.