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RESEARCH ARTICLE

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Effect of low temperature on the activity of entomopathogenic nematodes

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ABSTRACT : The present study was investigated the effect of low temperature on the activity of the entomopathogenic nematodes, *Heterorhabditis indica* and *Steinernema glaseri*. The effects of low temperature at 5^0 to 25^0C were tested under BOD conditions. The survival and infectivity of entomopathogenic nematodes in insect host were studied. Survival of *H. indica* was significantly greater at the lowest temperature of 10^0C conversely survival of *S. glaseri* was significantly greater at a temperature of 5^0 and 10^0C. The infectivity of *H. indica* and *S. glaseri* was effective at temperature of 20^0 and 25^0 C (100 % and 100 %, respectively) for *S. glaseri* 10^0, 15^0 and 20^0C (74.00%, 100 % and 100 %, respectively).

KEY WORDS : Entomopathogenic nematodes, *Heterorhabditis indica*, *Steinernema glaseri*, *Corcyra cephalonica*, Low temperature, infectivity

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INTRODUCTION

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* with their associated symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*, respectively) are widely distributed in soils throughout the world. These nematode parasites of insects, killing them within 48h with the aid of their associated bacterial symbionts, and have a great importance as biological control agents of many insect pests.

The use of entomopathogenic nematodes in soil depends on various biotic and abiotic factors. Biotic and abiotic factors that can enhance efficacy are the choice of nematode species in relation to the target host, soil moisture and temperature are key factors affecting entomopathogenic nematode application (Kaya and Gaugler, 1993)

The entomopathogenic nematodes can have their survival, infectivity, development and reproduction affected when exposed to adverse environmental conditions, such as temperature and soil moisture. Soil temperature can greatly affect the activity of entomopathogenic nematodes. Optimum temperature for locomotion, infection and reproduction vary among nematode species. The low temperature induce inactivity in infective juveniles, they seem to be the main barrier to the use of EPNs in temperate regions. Such inactivity is characterised by decreased enzymatic activity and mobility, both reducing metabolic expenditures.

Selection of an entomopathogenic nematode species for the control of a particular insect pest is based on

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several factors that include the nematode host range, host finding or foraging strategy, tolerance of environmental factors and their effects on survival and efficacy (temperature, moisture, soil type, ultraviolet light, salinity and organic content of soil). The critical factors are moisture, temperature, pathogenicity for the targeted insects and foraging strategy (Kaya and Gaugler, 1993; Grewal *et al*., 2005).

The objective of this study is to evaluate the influence of low temperature on the activity and infectivity of *Heterorhabditis indica* and *Steinernema glaseri*.

EXPERIMENTAL METHODS

Insect host :

Corcyra cephalonica larvae were used as host for the bioassays. The larvae were held at 25±2^{0}C and feed on cumbu grains.

Nematodes :

The nematodes *viz*., *H. indica* and *S. glaseri* were obtained from Sugarcane Breeding Institute, Coimbatore and mass cultured in *C. cephlonica*. The larvae were reared on broken cumbu grains sterilized at 100^{0}C for 30 minutes, according to the procedure of Kaya and Gaugler (1997). The third stage juveniles (IJs) were harvested from water surrounding White's trap within 10 days of emergence from their hosts. A stock suspension of the IJs in distilled water was stored at 20^{0}C for 2 weeks before use in BOD incubator.

Effect of low temperature on the survival of *H. indica* and *S. glaseri* and the infectivity to *C. cephalonica* :

The effect of temperature on the survival of *H. indica* and *S. glaseri* was studied under laboratory conditions. The studies were conducted at five different controlled low temperatures *viz*., 5, 10, 15, 20 and 25^{0}C in BOD incubators. The IJs were exposed to different temperatures at the rate of 100 infective juveniles (IJs) per Petri dish. Five replications were maintained for each temperature.Observation on live IJs at different temperatures was made at 24 h intervals.

Infectivity :

Ten final instar larvae of *C. cephalonica* were released over two layers of Whatman number 1 filter paper on 9 cm dia Petri dish and the test nematodes were inoculated at a dose of 20 IJ/ larva. The Petri dishes were covered with lid and sealed with kiln flim to conserve moisture. After 48 h of inoculation, the dead larvae were counted.

Reproductivity :

Heterorhabditis indica and *S. glaseri* infected *C. cephalonica* larvae were transferred to White's trap and modified White's trap (Plaster of Paris), for the emergence of *H. indica* and *S. glaseri* IJs, respectively, which will confirm the reproductivity of the nematodes.

EXPERIMENTAL RESULTS AND ANALYSIS

The abiotic component temperature affects the activity of entomopathogenic nematode showing differential response with different nematodes. The survival, infectivity, development and reproduction of entomopathogenic nematodes adversely affected when exposed to unfavourable environmental conditions.

In the present study, the low temperatures of 5^0 to 25^{0}C caused significant mortality of juveniles of both *H.*

Table 1 : Effect of temperature on *H. indica*

Temperature (^{o}C)	Mortality of infective juveniles (%)			Infectivity percentage	Reproductivity
	1st day	3rd day	7th day		
5	2.00 (8.49)	8.40 (16.84)	10.40 (18.80)	24.00 (29.22)	-
10	1.20 (6.21)	2.60 (9.23)	5.20 (13.14)	50.00 (45.00)	+
15	0.00 (0.28)	0.6 (3.55)	0.80 (4.03)	58.00 (49.61)	+
20	0.00 (0.28)	0.00 (0.28)	0.00 (0.28)	100 (89.71)	+
25	0.00 (0.28)	0.00 (0.28)	0.00 (0.28)	100 (89.71)	+
Control	0.00 (0.28)	0.00 (0.28)	0.00 (0.28)	100 (89.71)	+
C.D. (P=0.05)	0.71	1.70	2.00	3.22	

Figures in parentheses are arcsine transformed values (-) Reproductivity not observed (+) Reproductivity observed

indica and *S. glaseri*. The different low temperatures had adverse effect on the survival of nematodes under controlled conditions in BOD. Temperatures of 5^0, 10^0 and 15^0C caused juvenile mortality of 10.40, 5.20 and 0.80 per cent for *H. indica* and 5.40, 2.00 and 0.80 per cent juvenile mortality for *S. glaseri* at the end of the 7th day, respectively. The mortality of infective juveniles were not observed at 20^0 and 25^0C for *H. indica* and *S. glaseri* caused 0.20 and 0.80 per cent juvenile mortality at the end of the 7th day, respectively.

Both *H. indica* and *S. glaseri* were tested for their infectivity at temperatures of 20^0 and 25^0C and *H. indica* caused highest infectivity of 100 per cent on *C. cephalonica* which was at par with control (100 %). *S. glaseri* was tested at temperatures of 15^0, 20^0 and 25^0C and caused 100 per cent infectivity on *C. cephalonica* and which was at par with control (100 %). Infective juveniles of *H. indica* were tested at temperatures of 5^0 and 10^0C caused 24 and 50 per cent infectivity and *S. glaseri* caused 70 and 78 per cent infectivity on *C. cephalonica,* respectively.

Both *H. indica* and *S. glaseri* multiplied well on *C. cephalonica* larvae infected by infective juveniles tested at temperatures of 10^0 to 25^0 C. However, the reproductivity of *H. indica* was not observed at 5^0C (Table 2 and 3).

The results appended shows that the emergence of infective juveniles increased as the temperature increased. In *H. indica* the highest number of infective juveniles emerged at 25^0C (13462.8 IJ/larva) and the lowest at 10^0C (215.2 IJ/larva) and no emergence was noticed at 5^0C.

In *S. glaseri* also the same trend was noticed. The highest emergence of infective juveniles (12980 IJ/larva) was noticed at 25^0C and the lowest (1804.80 IJ/larva) at 5^0C.

In the present study, low temperatures of 5^0, 10^0 and 15^0C caused highest juvenile mortality of *H. indica* and *S. glaseri* at the end of the 7th day. Kaya (1990) also reported similar results that the temperatures below 9-10^0C and above 30^0C adversely affected nematode survival and persistence. The present findings are in line with many of the earlier reports Molyneux (1984) reported that entomopathogenic nematodes had poor survival and persistence at the lowest temperature of 5^0C. Hang *et al*. (2007) observed that temperature

Table 2 : Effect of temperature on *S. glaseri*

Temperature (°C)	Mortality of infective juveniles (%)			Infectivity percentage	Reproductivity
	1st day	3rd day	7th day		
5	0.80 (4.64)	3.20 (10.23)	5.40 (13.39)	70.00 (56.91)	+
10	0.40 (1.85)	1.60 (7.17)	2.00 (7.91)	78.00 (62.10)	+
15	0.00 (0.28)	0.6 (3.55)	0.80 (4.03)	100 (89.71)	+
20	0.00 (0.28)	0.00 (0.28)	0.20 (1.37)	100 (89.71)	+
25	0.00 (0.28)	0.00 (0.28)	0.80 (4.64)	100 (89.71)	+
Control	0.00 (0.28)	0.00 (0.28)	0.00 (0.28)	100 (89.71)	+
C.D. (P=0.05)	2.27	1.89	2.94	1.86	

Figures in parentheses are arcsine transformed values (+) Reproductivity observed

Table 3: Effect of low temperature on the number of infective juveniles of *H. indica* and *S. glaseri* emerged from *C. cephalonica* (Mean of 5 replications)

Temperature (°C)	Number of infective juveniles emerged/ larva	
	H. indica	*S. glaseri*
5	-	1804.80^f
10	215.2^d	2905^e
15	1493.8^c	3525^d
20	13147.4^b	12639.2^c
25	13462.8^b	12980^b
Control (room temperature 24±3)	14047.8^a	14063^a
C.D. (P=0.05)	332.95	327.73

Column figures followed by different letters are significantly different from each other (-) Emergence of IJs not observed

ranging from 13°, 18°, 24° and 30°C or 35°C on the virulence, development, reproduction and mortality of two Korean isolates of *S. glaseri* Dongrae strain and *S. longicadum* Nonsan strain on *G. mellonella*. Both the nematode species caused mortality of *G. mellonella* at all the temperatures.

The present study showed that temperature range of 20° and 25°C is highly suitable for the survival of both *H. indica* and *S. glaseri* at the end of the 7th day and no mortality of infective juveniles were noticed. Contradictory results were obtained by Molyneux (1985) who reported that *S. glaseri* survived extremely well at elevated temperature and cold temperature resulting in longer survival of *S. glaseri* and it survived many months at temperature of 10-28°C.

In the present study, *H. indica* and *S. glaseri* recorded 100 per cent infectivity at temperatures of 20° and 25°C on *C. cephalonica*. *S. glaseri* caused lowest infectivity at temperatures of 5° and 10°C on *C. cephalonica* showing cold tolerance. Similar results to *S. glaseri* pathogenicity were greater at higher temperature ranges (15-35°C) than at the lowest tested temperature of 5°C as reported by Kung *et al*. (1991). Yul *et al*. (2002) observed that *S. carpocapsae* was pathogenic to *G. mellonella* larvae at temperatures of 13°, 18°, 24°, 30° and 35°C with highest mortality at 37°C, and with lethal time being directly proportional to the temperature increase. Radova and Trnkova (2010) reported that *S. feltiae* showed very good tolerance to cold and ability to infect *Tenebrio molitor* at the lowest temperatures (15 and 10°C). Chen *et al.* (2003) confirmed in their study that S. *feltiae* was the only species that destroyed *Delia radicum* larvae at 10°C.

Lowest temperatures of 8° and 18°C caused low mortality of *S. glaseri* on *C. cephalonica*. It was shown that infectivity of these nematodes at cold temperatures (5-15°C) was improved by propagation at cold temperature, while infectivity at warm temperature (0-25°C) was enhanced by propagation at warm temperatures (Jagdale and Gordon, 1997). These result shows indication that the entomopathogenic nematodes can be used to control insect pests that occur in temperate regions.

H. indica and *S. glaseri* multiplied and reproduce well on *C. cephalonica* larvae infected at temperatures of 10° to 25°C. However, *S. glaseri* reproduced even at 5°C. Milstead (1981) reported that *H. bacteriophora* caused mortality and reproductivity of *G. mellonella* at 12°C. Yamanaka *et al*. (2000) reported that *S. glaseri* developed but not survived on *G. mellonella* at above 35°C. The nematode development and reproduction at 15-30°C and progeny production was greatest at 28°C.

The highest number of infective juveniles of *H. indica* emerged at 25°C (13462.8 IJ/larva) and lowest number of infective juveniles emergence was observed at 10°C (215.2 IJ/larva) for *S. glaseri* indicating the delay in the emergence of juveniles at low temperatures. Grewal *et al*. (1994) reported that exposure to low and high temperatures generally prolonged the time for emergence of both Heterorhbditids and Steinernematids. Brown and Gaugler (1997) also reported that low temperatures significantly delayed the emergence of *S. feltiae, S. carpocapsae, S. glaseri* and *H. bacteriophora.*

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