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Effect of temperature on the life cycle of entomopathogenic nematodes, *Steinernema abbasi* and *Heterorhabditis indica*

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ABSTRACT: ***In vitro*** **studies on the effect of temperature on the life cycle of** ***Steinernema abbasi*** **and** ***Heterorhabditis indica*** **revealed that IJs were able to penetrate** ***Galleria mellonella*** **at temperature range between 20° and 30°C for both the species. The maximum number of IJs of** ***S. abbasi*** **and** ***H. indica*** **emerge from** ***G. mellonella*** **larvae at 30°C and 25°C, respectively, and time (days) taken for emergence of first and second generation individuals of** ***S. abbsi*** **and** ***H. indica*** **on** ***G. mellonella*** **were observed to be 3-4 and 7-8 days and 3-4 and 6-10 days, respectively. The number of male and female amphimictic generation was 25IJs for both EPNs. However the sex ratio was 1: 14.9 at 25°C, 1: 11.6 at 20°C and 1: 11.3 was minimum at 25°C (12.5: 32.5) followed by 20°C (1: 14.7) and 30°C (1: 16.6)** ***S. abbasi*** **and** ***H. indica*****, respectively. As the temperature decreased, the emergence of IJs also decreased significantly.**

KEY WORDS: Entomopathogenic nematodes (EPNs), *Heteorhabditis indica*, life cycle, *Steinernema abbasi,* temperature.

INTRODUCTION

General concern on environment pollution and related hazards by extensive use of chemical pesticides and their gradual withdrawal from the market have led to vigorous research pursuits seeking alternative means of pest and disease control. Among the various eco-friendly option available, entomopathogenic nematodes (EPNs) belonging to genera, *Steinernema* and *Heterorhabditis* have become the subject of intensive research and have been used for inundative, augmentative or inoculative biological control of crop pests during the past two decades (Gaugler and Kaya, 1990; Bedding *et al*., 1993; Parkman and Smart 1996). Keeping in view the diverse agro-climatic conditions in the country, studies on ecological parameters governing the efficiency of these bio-control agents must be undertaken prior to their mass multiplication, formulations and field applications. Since these play a crucial role in the success of many bio-control programmes, the effect of temperature on the life cycle of *S. abbasi* and *H. indica* was studied *in vitro* on *Galleria mellonella* at three different temperatures.

MATERIALS AND METHODS

Nematode culture

The culture of two species of *Steinernema* and two species of *Hetororhabditis* were isolated by insect trap method as suggested by Bedding and Akhurst (1975) from Haryana are available in the Department of Nematology, CCS HAU, Hisar, The cultures are maintained on greater wax moth, *Galleria mellonella.* These cultures were isolated by Dr. Kumkum Walia, Sr. Scientist, CCS, HAU, Hisar, Hariyana.

Effect of temperature on the life cycle of *S. abbasi* and *H. indica*

The life cycle of *S. abbasi* and *H. indica* were studied *in vitro* on *G. mellonella* of three different temperatures. According to the climatic conditions of CCS, HAU, the ideal temperature for rearing *G. mellonella* were observed as 20°, 25° and 30°C. The optimum temperature for penetration and development varies depending upon the species or isolate. The experiments were conducted in 10cm Petridishes lined with filter paper. IJs of EPNs @ 20 per insect larva were released on the filter paper in 1ml of water. Twenty last instar (4^{th}) larvae of *G. mellonella* were placed in each Petriplate. The Petriplates were enclosed in polythene bags and placed in BOD incubators at three different controlled temperatures, viz., 20°, 25° and 30°C. Seven replications were maintained for each temperature. Observation on mortality of *Galleria mellonella* larva was made. The dead larvae were dissected in water at 24h after inoculation and the number of IJs per larva was counted and also the time required (in days) for development of first and second generation adults and sex ratio were recorded.

Effect of temperature on penetration of *S. abbasi* and *H. indica* on *G. mellonella* and mortality of *G. mellonella*

To study the effect of temperature on penetration of *S. abbasi* and *H. indica* using last instar larvae of *G. mellonella* as host, studies were conducted at three different controlled temperatures, *viz.*, 20°, 25°, 30°C in B.O.D. incubators and at room temperature (22° - 30.5°C). The larvae were exposed to IJs (20IJs larva^{-1}) at the rate of 20 larvae per Petri dish for 24h by filter paper exposure method. Seven replications were maintained for each temperature. Observations on mortality of *G. mellonella* larvae at different temperatures were made. The dead larvae were dissected in Ringer's solution at 24h after inoculation and the number of IJs penetrated per larva was counted by using 1 ml of inoculated solution, which was diluted in 10ml of water and from this 0.1 ml was taken out and observed under a microscope by using tally counter.

$$\text{No. of IJs penetrated} = \frac{\text{Solution taken for observation}}{\text{Total solution}} \times 100$$

Effect of temperature on the time taken for IJ penetration and number of IJs emerged from *G. mellonella*

To observe the time taken for IJ penetration and number of IJs emerged from *G. mellonalla,* studies were conducted at three different controlled temperatures, *viz*., 20°, 25° and 30°C, in BOD incubators and at room temperature (22°-30.5°C). Last instar larvae of *G. mellonella* were exposed to IJs (20IJs larva^{-1}) of *S. abbasi* and *H. indica* at the rate of 20 larvae per petri dish (5cm dia.) by filter paper exposure method. Seven replications were maintained for each temperature. Two days after the exposure of IJs, dead *G. mellonella* larvae were transferred to White's trap for *S. abbasi* and *H. indica* counting. Observations on the time taken for IJ penetration and number of IJs emerging from *G. mellonella* were made at different temperatures and at different intervals. Since the occurrence of penetration was observed after 24h, the inoculation was observed at a time interval of 24h.

Statistical analysis

The data on dead and live IJs were made and then per cent survival of IJs of nematodes was calculated by using the following formula:

$$\text{Per cent survival (\%)} = \frac{\text{Total number of live IJs} - \text{Total number of dead IJs}}{\text{Total number of live IJs}} \times 100$$

Arc sine transformation was used on data presented in percentages. The survival data were subjected to analysis of variance (ANOVA). All comparison was at 0.05% significance level.

RESULTS AND DISCUSSION

Effect of temperature on the life cycle of EPNs

Steinernema abbasi

In vitro studies were conducted on the effect of temperature on the life cycle of *S. abbasi*. The result revealed that the penetration of *S. abbasi* on *G. mellonella* occurred in a temperature range between 20°C to 30°C (Table 1). The maximum penetration of IJs was observed at 25°C followed by 20°C and 30°C. Maximum number of IJs penetrated on *G. mellonella* after 96h exposure at 25°C. The present finding is in close agreement with that of Hussaini *et al*. (2005). They observed that temperature ranging 25° - 32°C was suitable for infectivity and virulence of *S. carpocapsae* and *S. abbasi.* Yang and Li (1988) reported that *S. feltiae* to develop to adult at 12° - 30°C and the optimum temperature was 25°C. Below 12°C and over 35°C, the nematodes lost their infectivity.

Maximum number of IJs of *S. abbasi* emerged from *G. mellonella* larvae at 30°C. As the temperature decreased, IJ emergence from *G. mellonella* decreased significantly. Brown and Gaugler (1997) reported that low temperature significantly delayed emergence of *S. carpocapsae* and *S. glaseri,* but had no effect on rate of emergence of *S. feltiae* at 25°C and 75% relative humidity. Further, present studies

Table 1. Effect of temperature on the penetration of *S. abbasi* on *G. mellonella*

Temperature (°C)	Number of IJs penetrated larva^{-1}						
Time (h)	24	48	72	96	120	144	168
20	0.00	3.42	13.00	14.57	0.00	0.00	0.00
25	0.00	6.57	16.00	18.57	0.00	0.00	0.00
30	0.00	4.14	12.85	15.42	0.00	0.00	0.00
SEM±	–	0.56	0.75	0.95	–	–	–
CD (P = 0.05)	–	1.65	2.23	2.29	–	–	–

Table 2. Effect of temperature on life cycle of *S.* on *G. mellonella*

Observation (n = 7)	Temperature (°C)		
	20	25	30
No. of IJs penetrated/larvae	19.35	19.41	19.33
Duration of first generation	4 days	3 days	3 days
Duration of Second generation	8 days	7 days	5 days
No. of IJs emergence/larvae	38.00	133.9	142.6
No. of males and female amphimictic generation	3.0: 35	10.9: 123.9	7.2: 107.4
Sex ratio	1: 11.6	1: 11.3	1: 14.9

* Average of 7 larvae observed

Table 3. Effect of temperature on the penetration of *H. indica* on *G. mellonella*

Temperature (°C)	Number of IJs penetrated larva^{-1}					
Time (h)	24	48	72	96	144	168
20	0.00	3.42	10.08	18.42	0.00	0.00
25	0.00	6.57	11.12	18.98	0.00	0.00
30	0.00	4.16	11.85	19.32	0.00	0.00
SEM±	–	0.47	0.95	0.75	–	–
CD (P = 0.05)	–	1.45	2.80	5.17	–	–

Studies on effect of temperature on life cycle of *H. indica* further revealed that the highest number of IJs larvae^{-1} emerged at 25°C followed by 20 and 30°C (Table 4).

revealed that time (days) taken for emergence of first generation of individual of *S. abbasi* was 3-4 days, whereas second generation took 7-8 days to emerge from the larvae (Table 2).

Observations on male and female ratio were recorded by counting the total no. of male and female IJs in 1ml of diluted solution. Based on morphological changes, the first and the second generation were differentiated. The amphimictic generation was maximum at 25°C (10.9: 123.9) followed by 30°C (7.2: 107.4) and minimum at 20°C (3.0: 3.5). Highest sex ratio was observed at 30°C (1: 14.9) followed by 20°C (1: 11.6) and was minimum at 25°C (1: 11.3). Ganguly (2003) reported that time (days) taken for first generation males and females of steinernematids was 2-4 days and 5-7 days for emergence of second generation males and females.

Heterorhabditis indica

Studies on the effect of temperature on the life cycle of *H. indica* revealed that the penetration of *H. indica* on *G. mellonella* took place between 20° and 30°C (Table 3). The maximum penetration of IJs was observed at 25°C, followed by 20°C and 30°C. Highest number of IJs penetrated *G. mellonella* after 96h of exposure at 25°C. The present finding was in close agreement with that of Karunakar *et al*. (1999). They reported that more number of IJs of *H. indica* penetrated the host at temperatures above 27.5°C and Hussaini *et al.* (2005) also reported 25° - 32°C was suitable for infectivity and virulence of *H. indica*.

The present investigations also revealed that time (days) taken for emergence of first generation of individual was 3 - 4 days, whereas the individual of second generation took 7- 8 days for emergence. The number of male and female of amphimictic generation was maximum at 25°C (12.5: 132.5) followed by 30°C (6.5: 108.4) and minimum at 20°C (2.8: 41.2) and the sex ratio was (1: 10.6) at 25°C followed by (1: 14.7) at 20°C and (1: 16.6) at 30°C.

The present study is closely in agreement with Subramanian *et al.* (2000), who reported that number of male and female amphimectic generation *H. indica* was maximum at 25°C (11.8: 139.8) followed by at 30°C (66: 110.2) and minimum at 20°C (2.6: 38). The highest sex ratio was observed at 30°C (1: 16.7) followed by at 20°C (1: 14.6) and at 25°C was minimum (1: 11.8). Earlier Shamseldean

Table 4. Effect of temperature on the life cycle of *Heterorhabditis* on *G. mellonella*

Observation (n = 7)	Temperature (°C)		
	20	25	30
No. of IJs penetrated larvae^{-1}	7.5	10.28	8.10
Duration of first generation	4 days	3 days	3 days
Duration of Second generation	10 days	7 days	6 days
No. of IJs emergence larvae^{-1}	44	145.0	114.9
No. of males and female amphimictic generation	2.8:41.2	12.5:132.5	6.5:108.4
Sex ratio	1:14.7	1:10.6	1:16.6

CD (P = 0.05)	Emergence	Sex ratio
Nematode (A)	2.84 (NS)	1.32 (NS)
Time (B)	5.32*	2.47*
Temperature (C)	3.48*	1.62*
A × B	7.52*	3.56*
A × C	4.92*	NS
B × C	9.21 (NS)	4.75*
A × B × C	13.30 (NS)	NS

Average of 7 larvae observed; NS= significant; Significant at 5%

Table 5. Effect of temperature on the penetration of *Steinernema abbasi* and *Heterorhabditis indica*

Temperature (°C)	Number of IJs penetrated larva^{-1}	
20	7.75	19.35
25	8.46	19.82
30	8.107	19.33
SEM	0.55	0.93
CD (P = 0.05)	1.58	2.76

Significant at 5%; NS = non significant; CD (P = 0.05); Nematode (A) = 2.57 (NS); Time (B) = 3.63; temperature (C) = 3.15*; A × B = 5.14*; A × C = 4.45*; B × C = 6.30*; A × B × C = 8.90*

et al. (1996) observed that *H. indica* exhibited higher reproduction efficiency at high temperature than other entomopathogenic nematodes. The comparative virulence of *S. abbasi* and *H. indica* on *G. mellonella* revealed that *H. indica* was more virulent then *S. abbasi* at all the three temperatures 20, 25 and 30°C. The results indicate that *H. indica* is more tolerant to temperature gradient than *S. abbasi* (Table 5).

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