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RESEARCH ARTICLE :

Effect of temperature on life cycle of entomopathogenic nematode, *Heterorhabditis indica* poinar

AMIT U. PASCHAPUR, K. VIJAYALAKSHMI, B.S. SUNANDA AND VINOD PAWAR

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SUMMARY : Experiment was conducted to study the effect of temperature on infection of host insect and time taken for emergence of IJs of *H. indica* from the cadavers of host insect (*Galleria mellonella*) under controlled laboratory conditions. The results indicated that the mean time taken by the nematode to cause infection in the host insect was significantly less at three temperatures, *viz.*, 30^{0}C, room temperature (25-28^{0}C) and ambient atmospheric temperature (23-34^{0}C) which ranged from 24-30 hours. At 20^{0}C temperature, the infection occurred after 44 hrs of inoculation, indicating the maximum time required for *H. indica* to cause infection. At test temperatures of 10^{0}C and 40^{0}C nematode did not cause the infection to the host insect due to lethal high and lethal low temperature effects. From the results it was evident that at ambient atmospheric temperature (23-34^{0}C), the mean time taken for emergence of IJs was 226 hrs (9.42 days). While, the mean time taken for emergence at 30^{0}C and room temperature (25-28^{0}C) were 236 hrs (9.83 days) and 246 hrs (10.25 days), respectively. The mean time taken for emergence at 20^{0}C, was 286 hrs (11.92 days) and all the above treatments were significantly on par with each other.

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Author for correspondence :
AMIT U. PASCHAPUR
Division of Entomology, ICAR-IARI, NEW DELHI, INDIA
Email : amitp3929@gmail.com

See end of the article for authors' affiliations

BACKGROUND AND OBJECTIVES

Entomopathogenic nematodes are soft bodied, non-segmented roundworms that are obligate or sometimes facultative parasites of insects. EPNs have been described from 23 nematode families (Koppenhofer, 2007). Of all of the nematodes studied for biological control of insects, the Steinernematidae and Heterorhabditidae have received the most attention because they possess many of the attributes of effective biological control agents (Kaya and Gaugler, 1993; Grewal *et al.*, 2005 and Koppenhofer, 2007).The only free-living stage in a life cycle of entomopathogenic nematode is an infective juvenile (IJ_3) produced when host nutrients are depleted. The only function of the infective juveniles is to locate and parasitize new hosts (Poinar, 1990 and Grewal *et al.*, 1993). Infective juveniles may have mechanisms to survive under

adverse environmental conditions. Persistence in soil, infectivity (Griffin and Downes, 1991 and Kung and Gaugler, 1991), development, maturation and reproduction of entomopathogenic nematodes are influenced by temperature (Zervos and Webster, 1991 and Grewal *et al.*, 1993). Variation among species of entomopathogenic nematodes for temperature tolerance has been reported (Wright, 1992 and Grewal *et al.*, 1993). Temperature is an environmental factor of great biological significance. As an environmental factor, temperature is variable both in space and time. EPNs isolated from diverse geographical regions and climate provides an opportunity to compare adaptations to a wide variety of thermal regimes. Keeping this in view, the Experiments were carried out under controlled laboratory conditions to study the effect of temperature on life cycle of IJs of *Heterorhabditis indica*.

RESOURCES AND METHODS

The life cycle of *H. indica* was studied *in vivo* on forth instar *Galleria mellonella* larvae at six different test temperatures. The experiment was conducted in 90 mm Petri-plates (9 cm) lined with filter paper. IJs of *H. indica* with different inoculum levels *viz.*, 50, 150, 250, 350, 450 IJs/ml and control (without EPNs) were prepared. One ml of EPN suspension was inoculated on the filter paper. Ten fourth instar larvae of *G. mellonella* were placed in each Petri-plate. The Petri-plates were placed in BOD incubators at four different test temperatures *viz.*, 10^0C, 20^0C, 30^0C, 40ºC and other plates were incubated at controlled room temperature (25-28^{0}C) and at uncontrolled ambient atmospheric temperature (23-34^{0}C). Six replications were maintained for each temperature and inoculum level. Observations on time taken for mortality of *Galleria mellonella* after every 12 hours of inoculation of EPNs and time taken for emergence of IJs from dead cadavers every 12 hours after placement on white trap were taken.

The data were subjected to analysis of variance (ANOVA). All the comparisons were tested at 5% level of significance.

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads:

Effect of temperature and inoculum levels on infection of *H. indica* in the larvae of *G. mellonella*:

The results obtained from the studies on effect of temperature on infection of *H. indica* in larvae of *G. mellonella* are presented in Table 1 and Fig. 1. The results indicated that the mean time taken by the nematode to cause infection in the host insect was significantly less at three temperatures, *viz.*, 30^{0}C, room temperature (25-28^{0}C) and ambient atmospheric temperature (23-34^{0}C) which ranged from 24-30 hours and they were on par with each other and varied significantly with other three test temperatures. At 20^{0}C temperature, the infection occurred after 44 hrs of inoculation, indicating the maximum time required for *H. indica* to cause infection. At test temperatures of 10^{0}C

Table 1: Effect of temperature and inoculum levels on infection of *H. indica* in *Galleria mellonella* larvae

Temperature (^{0}C)	Time taken in hours for infection of *Galleria mellonella* larvae						
	Inoculum level (IJ's/ml)						
	50	150	250	350	450	Control	Mean
10	0	0	0	0	0	0	0
20	72	60	48	48	36	0	44
30	36	36	24	24	24	0	24
40	0	0	0	0	0	0	0
25-28	36	36	24	24	24	0	24
23-34	48	36	36	36	24	0	30
Mean	32	28	22	22	18	0	
		P – value		F		S.E.±	C.D. (P=0.05)
Temperature		1.13E-07		**		4.04	11.26
Inoculum level		0.000189		**		4.04	11.26
Temperature x inoculum		-		**		9.90	27.60

and 40^{0}C nematode did not cause the infection to the host due to lethal high and lethal low temperature effects. The results indicated that, the most optimum temperature required for quicker infection was 30^{0}C, ambient atmospheric temperature (23-34^{0}C) and room temperature (25-28^{0}C), which took significantly less time to cause infection.

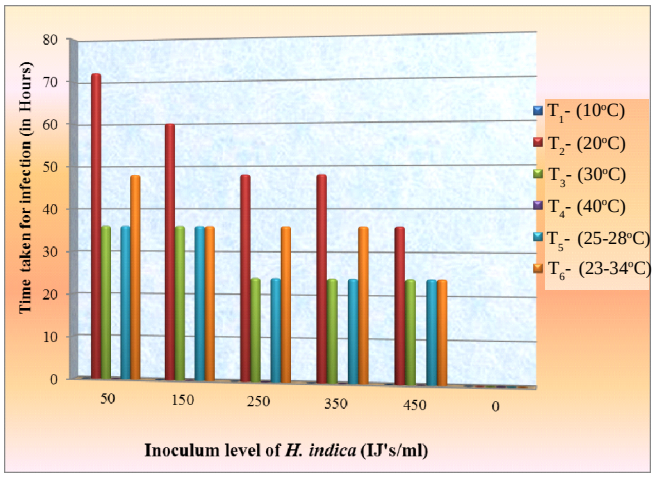

Fig. 1 : Time taken for infection of *Galleria mellonella* larvae by *Heterorhabditis indica*

The findings of different inoculum levels of IJs of *H. indica* on infection of *G. mellonella* showed that, The mean time taken for infection of *G. mellonella* with 150, 250, 350 and 450 IJs/ml were significantly on par with each other and varied from 18-22 hours, but with inoculum levels of 50IJs/ml the nematodes took maximum time of 32 hrs to cause infection.

The interaction effect of temperature and inoculum levels of *H. indica* on infection of *G. mellonella* showed that, EPNs took longer time of 72 hrs to cause infection at 20^{0}C at inoculum level of 50 IJs/ml. While, they took minimum time of 24 hrs for causing infection at 30^{0}C and room temperature (25-28^{0}C) with an inoculum levels of 250 IJs/ml.

Effect of temperature and inoculum levels on emergence of *H. indica* from larvae of *G. mellonella* :

The results pertaining to the studies on effect of temperature on emergence of IJs of *H. indica* from larvae of *G. mellonella* are presented in Table 2 and Fig. 2. From the results it was evident that at ambient

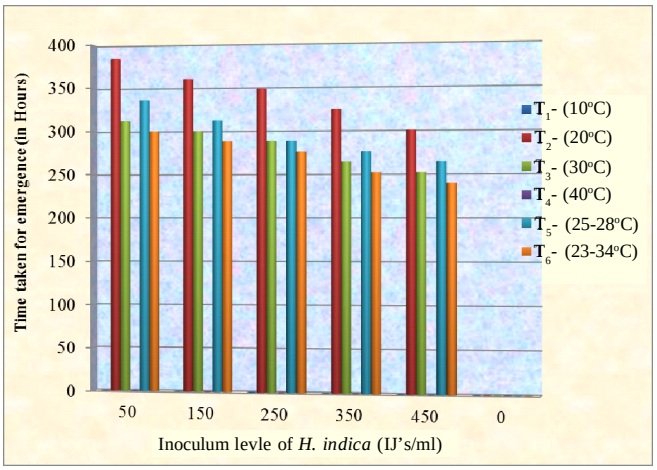

Fig. 2 : Time taken for emergence of *H. indica* from *G. mellonella* larvae

Table 2: Effect of temperature on emergence of *Heterorhabditis indica* from *Galleria mellonella* larvae

Temperature (^{0}C)	Time taken in hours for emergence from *Galleria mellonella* larvae						
	Inoculum level (IJ's/ml)						
	50	150	250	350	450	Control	Mean
10	0	0	0	0	0	0	0
20	384	360	348	324	300	0	286
30	312	300	288	264	252	0	236
40	0	0	0	0	0	0	0
25-28	336	312	288	276	264	0	246
23-34	300	288	276	252	240	0	226
Mean	222	210	200	186	176	0	
			P – value	F		S.E.±	C.D. (P=0.05)
Temperature			8.61E-09	**		26.54	73.99
Inoculum level			2.97E-05	**		26.54	73.99
Temperature x inoculum			-	**		65.02	181.24

atmospheric temperature (23-34^{0}C), the mean time taken for emergence of IJs was 226 hrs (9.42 days). While, the mean time taken for emergence at 30^{0}C and room temperature (25-28^{0}C) were 236 hrs (9.83 days) and 246 hrs (10.25 days), respectively. The mean time taken for emergence at 20^{0}C, was 286 hrs (11.92 days) and all the above treatments were significantly on par with each other and varied significantly from the other two test temperatures, *viz*., 10^{0}C and 40 ^{0}C. At 10^{0}C and 40^{0}C the emergence of IJs did not occur. Instead the IJs desiccated in dead cadavers due to lethal high and lethal low temperature effects, respectively.

The effects of different inoculum levels, *viz.*, 50, 150, 250, 350, 450IJs/ml and control (without IJs) on emergence of IJs of *H. indica* from larvae of *G. mellonella* showed that, the mean time taken for emergence of IJs at five different inoculum levels did not show any significant variation on emergence from the host larvae and it varied from 176 to 222 days (7.33 to 9.25 days) and were on par with each other, indicating that temperature is a major factor which plays an important role in life cycle of EPNs and the inoculum levels have very meagre role to play.

The interaction effect of temperature and inoculum levels on the emergence of *H. indica* showed that at extreme low and high temperature (10^{0}C and 40^{0}C), there was no emergence of infective juveniles and at the remaining temperature and inoculum levels, also no significant difference was observed and the time taken for emergence of IJs from these treatments ranged from 240 to 384 hours and they were on par with each other. The above results indicated that the optimum temperature required to complete life cycle of *H. Indica* ranges from 20-34^{0}C.

The above results are in accordance with the results obtained by, Yamanaka *et al.* (2000), who reported that *S. glaseri* developed and reproduced at temperature range of 15-30ºC and progeny production was greatest at 28ºC and emergence time was fastest at 20 and 25ºC. Ganguly and Singh (2001) reported that *S. thermophilum* can infect and develop at a wide range of temperatures (20-35ºC), optimum being 25-35ºC, with better performance at 30-35ºC. Gokte-narkhedkar *et al*. (2005) reported that at temperature range of 23-40ºC expression of symptoms of EPN was observed in *Corcyra cephalonica* and at temperature range of 23-40ºC the emergence of the IJs of *H. indica* from cadavers took only 8-9 days at RH 85-100%. Lalramliana *et al*. (2005) noticed no emergence of IJs of *H. indica* from dead cadavers of *G. mellonella* below 10ºC and above 30ºC. Studies conducted by Sunanda (2009) showed that maximum number of IJs of *S. abbasi* (142.6 IJs) and *H. indica* (145 IJs) emerged from *G. mellonella* larvae at 30°C and 25°C, respectively. Experiments conducted by Maketon *et al.* (2011) showed that *H. indica* completed two generations of its life cycle in *G. mellonella* larvae within 10 days at 25°C. Studies by Muthulakshmi *et al*. (2012) observed emergence of IJs of *H. indica* from cadavers of *Corcyra cephalonica* after 192 hours (8 days).

The temperature range for infection depends on the "native home" of the nematode. The results indicated that EPNs can be used as an effective bioagent when the temperature fluctuates around 20-34^{0}C and its efficacy cannot be obtained at too low (10^{0}C) and too high (40^{0}C) temperature. Since the present studies clearly revealed the greater efficacy of *H. indica* against the greater wax moth at temperature ranges of 20-30^{0}C, it gives an insight that, if this nematode is used during the periods where the temperature fluctuates around 25-30^{0}C, it would result in effective management of crop pests.

Future studies on the identification of local EPN isolates which could tolerate high temperatures would help in effective management of pests infecting at high temperature regimes.

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Authors' affiliations :

K. VIJAYALAKSHMI, B.S. SUNANDA AND VINOD PAWAR, National Institute of Plant Health Management, Rajendranagar, HYDERABAD (TELANGANA) INDIA

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