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Short Communication

Effect of Temperature on the Reproductive Potential of Indigenous and Exotic Species of Entomopathogenic Nematodes inside *Galleria mellonella* L. LarvaeMuhammad Raheel^{1,*}, Nazir Javed², Sajid Aleem Khan², Hafiz Muhammad Aatif³ and Sohail Ahmed⁴¹University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan²Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan³College of Agriculture, BZU Bahadur Subcampus, Layyah, Pakistan⁴Department of Entomology, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

The influence of different temperature ranges on the reproductive potential of native and exotic species of entomopathogenic nematodes (EPNs) was checked on *Galleria mellonella* larvae. The native species included *Steinernema asiaticum* and *Heterorhabditis indica* whereas exotic species were *S. feltiae* and *H. bacteriophora*. *G. mellonella* larvae were exposed to 300 IJs of each species. After inoculation at different temperatures, the reproductive potential of EPNs increased with increasing temperature and was found to be the best at 25°C. No EPNs species could reproduce at 5°C. *S. feltiae* started reproduction at 10°C, while all remaining species reproduced at 15°C or higher temperatures. Maximum numbers of IJs were recovered from *H. bacteriophora*. Time taken for first emergence of IJs from the host was shortest at 25°C i.e. 7-8 days in case of *S. asiaticum* and *S. feltiae*, while 11-13 days for *H. bacteriophora* and *H. indica*. Maximum emergence time was taken by *S. feltiae* at 10°C.

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Authors' Contributions

MR and NJ designed the project. MR performed experimental work. SAK, HMA and SA collected, statistically analyzed and interpreted the data. HMA helped in rearing and maintaining EPN culture, and wrote the article. NJ supervised the work.

Key words

Entomopathogenic nematodes, EPNs, *Galleria mellonella*, *Heterorhabditis bacteriophora*, *H. indica*, Reproduction, *Steinernema feltiae*, *S. asiaticum*, Temperature.

Entomopathogenic nematodes (EPN) of both families Steinernematidae and Heterorhabditidae have already been effectively utilized to manage the numerous insect pests of agricultural importance (Aatif *et al.*, 2015, 2016; Kaya and Gaugler, 1993). These nematodes have shown best performances against soil inhabiting insect pests having cryptic behavior (Wright, 1992; Gaugler, 1988). Closely associated with reproduction of heterorhabditid and steinernematid nematodes are symbiotic bacteria of the genera *Xenorhabdus* and *Photorhabdus* in the absence of which, the nematodes fail to kill insect or to reproduce (Ehlers, 1996). The bacteria live within the intestine of infective juvenile-stage (IJ) nematodes. When IJs of EPNs find host insect, they enter in the body of host through mouth, anus or spiracles (Poinar, 1979; Mason and Hominick, 1995). After getting inside the insect, EPNs penetrate into the haemolymph, and release bacteria. The bacteria proliferate and make favorable environment for nematode reproduction by providing nutrients and producing antimicrobial substances (Akhurst, 1982). Many abiotic factors affect EPNs but temperature is the most critical one. The infectivity, development and reproduction of EPNs are influenced by temperature (Grewal *et al.*, 1994). Temperature was variable in both time and space. It was noted that the lower temperature caused the inactivity of the IJs (Rutherford *et al.*, 1987). This is due to various enzymes and activity of various metabolic pathways (Fan and Hominick, 1991).

The range of temperature for the development and reproduction of heterorhabditis and steinernematids spp. and for their strains were different (Molyneux, 1983). The objective of the present study was to examine the reproductive potential of four species of EPNs at various temperatures. The study of *S. asiaticum* in this experiment would be novel.

Materials and methods

Heterorhabditis bacteriophora, *H. indica*, *Steinernema feltiae* and *S. asiaticum* were used for experimentation. Two species of EPNs (*H. bacteriophora* and *S. feltiae*) were taken from University of Readings, Readings, UK. *S. asiaticum* was obtained from NNRC, Karachi and *H. indica* was obtained from Nematology Laboratory, Plant Pathology Department, University of Agriculture, Faisalabad.

The larvae of *G. mellonella* were reared on cereal diet in Nematology Laboratory, Plant Pathology Department, University of Agriculture, Faisalabad (Wiesner, 1993).

EPNs were cultured on *G. mellonella* larvae. The freshly born EPNs were kept in shallow clear plastic containers with lids with suspension being no more than 1 cm in depth to ensure sufficient oxygen availability at 10°C. All hatched juveniles were again cultured every 4 months. For EPNs, only those freshly produced *in vitro* (less than 2 weeks old) were used.

For the evaluation of reproductive potential of these four species of EPNs, larvae of *G. mellonella* were inoculated with EPNs species. Larvae were exposed to 300 IJs of each species in a 5cm petri dish. After inoculation petri plates containing inoculated larvae were incubated at various temperatures i.e., 5, 10, 15, 20 and 25°C using different incubators. Dead larvae

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were transferred to white traps (Woodering and Kaya, 1988; White, 1927) for the recovery of new progenies of IJs. After 7-10 days when new progenies of EPNs came out from dead insect cadaver, their counting was carried out under stereomicroscope (Olympus 5240). Data were recorded on the basis of EPNs reproductive potential inside host. The experiment was conducted under CRD design and there were three replications for each treatment.

All data collected was subjected to statistical analysis by analysis of variance and regression analysis using statistical package Genstat 6.0 for Windows (VSN International Ltd, UK). Graphs were prepared using the Excel for Windows 2007.

Results

ANOVA for establishment of EPNs at various temperature ranges showed that effect of treatments and species was significant ($p > 0.05$). Interaction of treatments and species was also significant (Table I). The ratio of IJs which were successfully established inside *G. mellonella* was temperature dependent and differed among species. Our results showed that IJs of any species of EPN could not establish itself inside *G. mellonella* larvae at 5°C, while at 10°C only *S. feltiae* showed its establishment. The highest percentage of establishment was achieved with *S. feltiae* at 15°C, *H. indica* at 25°C and with *S. asiaticum* at 25°C (Table I).

Table I.- Effect of different temperatures on the nematode establishment of four species of entomopathogenic nematodes.

Treatment	<i>S. feltiae</i>	<i>H. bacteriophora</i>	<i>H. indica</i>	<i>S. asiaticum</i>
5°C	0.00 H	0.00 H	0.00 H	0.00 H
10°C	5.00 D	0.00 H	0.00 H	0.00 H
15°C	11.8 A	2.00 EF	1.20 FG	0.60 GH
20°C	4.20 D	2.60 E	6.00 C	4.80 D
25°C	2.80 E	4.80 D	7.20 B	6.00 C

Numbers followed by different letters are significantly different from each other at $p < 0.05$. Data is mean of three replications.

Table II.- First emergence of infective juveniles from larvae of *Galleria mellonella* at various temperatures.

EPN Species	Days to first emergence at different temperatures (°C)				
	5	10	15	20	25
<i>S. feltiae</i>	-	70	20	12	8
<i>H. bacteriophora</i>	-	-	38	11	13
<i>H. indica</i>	-	-	41	13	11
<i>S. asiaticum</i>	-	-	47	10	7

Time taken for the first emergence of IJs from host was shortest at 25°C *i.e.* 7-8 days in the case of *S. asiaticum* and *S. feltiae*, while 11-13 days were spent for *H. bacteriophora*

and *H. indica*. Maximum time taken for emergence of IJs at 10°C in *S. feltiae* was 70 days (Table II). There was an inverse relationship between time and temperature for first emergence of IJs.

The reproduction rate of *H. bacteriophora* was significantly higher than all three EPNs species. It was followed by *H. indica* and *S. feltiae* while the reproduction rate of *S. asiaticum* was the lowest among all the species evaluated. There was no reproduction of any species at 5°C, while at 10°C only *S. feltiae* could reproduce. All other species like *H. bacteriophora*, *H. indica* and *S. asiaticum* started reproduction at 15°C. The rate of reproduction of *S. feltiae* was lower at higher temperature (25°C), while the reproductive potential of all other species showed increasing trends with increase of temperature (Fig. 1).

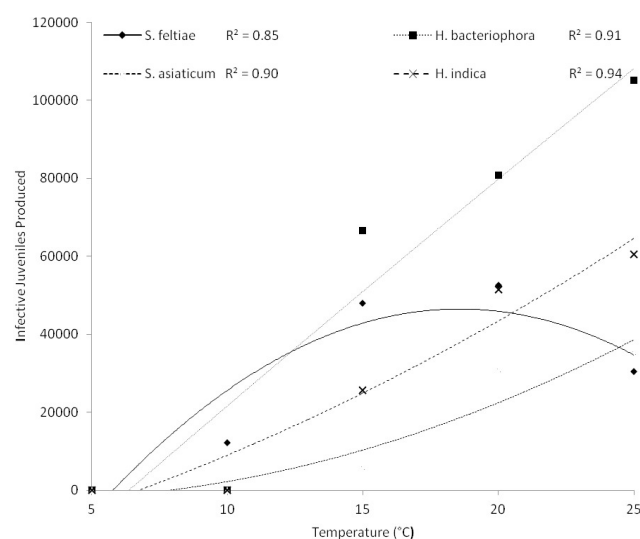


Fig. 1. Relationship of infective juveniles production of four species of entomopathogenic nematodes at various temperatures inside *Galleria mellonella* larvae.

Discussion

On the basis of the recent work, it is suggested that the differences among four species regarding their establishment and reproductive rates are either due to their mutualistic bacteria or temperature or the combined effect of both of them, because in the absence of symbiotic bacteria, the nematodes fail to kill insect or to reproduce (Ehlers, 1996). The conserved nature of nematode thermal niche breadth would support the hypothesis that temperature activity ranges of different species represent the climatic conditions of their original locality. Tolerance to warm temperatures by *H. indica* and *S. asiaticum* suggest tropical origins. Our results collaborate the findings of Grewal *et al.* (1994) who reported *S. scapterisci*, *S. riobravus* and *Steinernema* sp. are warm adopted species whereas *S. scapterisci* was stored better at 10 and 25°C than at 5°C. In contrast, *S. feltiae* is a species of temperate origin and can infect insects between 8-30°C and reproduce between 10-25°C. Thermal adjustment of EPNs can be supported through effective survival strategies. *S. carpocapsae* is not active

at lower temperature (Molyneux, 1986), cannot reproduce below 15°C, but still is found in cooler areas. Grewal *et al.* (1994) reported that *S. carpocapsae* can penetrate the insect at 10°C, rest within living host for prolonged times and when temperature increases it restarts its normal growth. Therefore, it may be capable of overwintering in the hosts. Variations among temperature adaptation may result in host specialization among EPNs. The species adjusted to lower temperature reproduction like *S. feltiae* would be effective insect parasites which are active in winter. Species adjusted to warmer temperature reproduction like *H. indica* and *S. asiaticum* would kill insects which are active during summer. The recent study revealing the optimum temperature for reproduction will be helpful in mass production of EPNs and their use in IPM programs in Pakistan.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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