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Effect of temperature on the infectivity of entomopathogenic nematodes (Steinernematidae and Heterorhaditidae) isolated from Mizoram, northeastern India

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Abstract

The study investigates the effect of temperature on the infectivity of two entomopathogenic nematodes, viz. *Steinernema* sp. and *Heterorhabditis indica*, locally isolated from Mizoram, northeastern India, using last instar larvae of greater wax moth, *Galleria mellonella*, as an insect host. Two hundred infective juveniles/larva of wax moth were exposed to different temperatures, viz. 10, 15, 20, 25, 30 and 35°C. It is observed that temperature play a significant role in infectivity of the two nematodes. No establishment of IJs were observed at 10°C, in addition 15°C in *H. indica*, and 35°C. *Steinernema* sp. appeared to be best adapted to temperatures between 15 and 30°C with an optimum temperature range of 25-30°C, whereas *H. indica* appeared to be adapted to temperatures between 20 and 30°C with an optimum temperature of 30°C.

Key words: Biological control; ecological characters; infection; nematodes.

Introduction

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* Travassos and *Heterohabditis* Poinar are obligate parasites of a wide range of insect species that have a wide geographical distribution throughout the world.[1] They are characterized by their unique association with symbiotic bacteria carried in their digestive tract; *Xenorhabdus* spp. in steinernematids, and *Photorhabdus* spp. in heterorhabditids.[2] The bacteria released by steinernematid and heterorhabditid nematodes rapidly multiply and the kills the host by septicaemia within 24-48 hours[3] which render them a good biological control agent worldwide. The success of nematode applications for insect control in soil depends on the infective juveniles (IJs) ability to disperse and persist until it can locate a host.[4-6] Numbers of studies have revealed that IJs of different EPNs differ in their ecological and behavioural traits with regard to their persistence and survival in the soil.[4]

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It has been reported that the temperature plays an important role in affecting the infectivity, time of death, development, reproduction and storage of EPNs.[7-9] Furthermore, isolates of EPNs from specific regions exhibit considerable variations in terms of their host range, reproduction, infectivity and conditions for survival.[10] The biocontrol potentials of EPNs are, therefore, influenced by different abiotic and biotic factors, beside others.[4,11] It is for these reasons that, for their use in biological control, locally adapted species or isolates from native habitats need a characterization in terms of their optimum biological requirements.[12] The study investigate the ranges of temperature favourable for the successful infection of locally isolated EPNs for future use as biological control agents.

Material and Methods

Entomopathogenic nematodes tested in the present study are *Steinernema* sp. and *Heterorhabditis indica* locally isolated from Mizoram, India. The EPNs were reared in the laboratory on late instar larvae of *Galleria mellonella* (0.19–0.25 gm) following the method of Woodring & Kaya.[13] The IJs that emerged from wax moth larvae cadavers were collected using modified white traps. Freshly emerged IJs were used in all the experiment.

The experiments were conducted using petridish assay in a single-well tissue culture plates lined with Whatman No. 1 filter paper at different selected temperatures, viz. 10, 15, 20, 25, 30 and 35°C. For each temperature, plates containing 200 IJs were kept for 30 minutes prior to exposure of insect to stabilise the nematodes. Afer 30 minutes, the third instar larvae of *G. mellonella* was placed inside each culture plate and sealed with parafilm. There were five replicates for each temperature. Insect mortality was recorded 24 hours time interval till 120 hours. The numbers of IJs established/larva were counted by dissecting the dead larvae in Ringers solution after 72 hours of insect dead.

Statistical analysis

The data were analyzed statistically and are represented as mean ± standard error of the mean (SEM). The significance of the difference was determined by the one-way analysis of variance (ANOVA). P values < 0.05 were accepted as statistically significant.

Results

The results of percent larval mortality caused by the two nematode species are presented in Fig. 1 A & B. No larval mortality was observed at 10, in addition 15 for *H. indica*, and 35°C throughout the experiment period. The temperatures were found to be positively correlated with the time of mortality. Except at 15°C, *Steinernema* sp. caused insect larval mortality as early as 24 hours after inoculation (HAI) achieving 100% mortality, however, at 15°C the first mortality was observed only after 48 HAI with 100% mortality. In case of *H. indica*, insect mortality started as early as 24 HAI achieving 40% and 60% mortality at 20 and 25°C respectively. At 30°C the insect mortality was observed at 24 HAI with 100% mortality.

Temperature had significant effects on establishment of IJs of *Steinernema* sp. on wax moth larvae ($F = 3.239$; $df = 3, 16$; $p < 0.05$). *Steinernema* sp. appeared to be best adapted to temperatures between 15 and 30°C with a more optimum temperature range of 25–30°C. Its establishment was not observed at 10 and 35°C (Fig. 2A). Establishment of *H. indica* IJs was significantly affected by temperature ($F = 5.318$; $df = 2, 12$; $p < 0.05$). IJs did not establish at 10, 15 and 35°C. It appeared to be adapted to temperatures between 20 and 30°C with an optimum temperature of 30°C (Fig. 2B).

Discussion

Different entomopathogenic nematode species (EPNs) have different environmental requirements depending upon place of their ori-

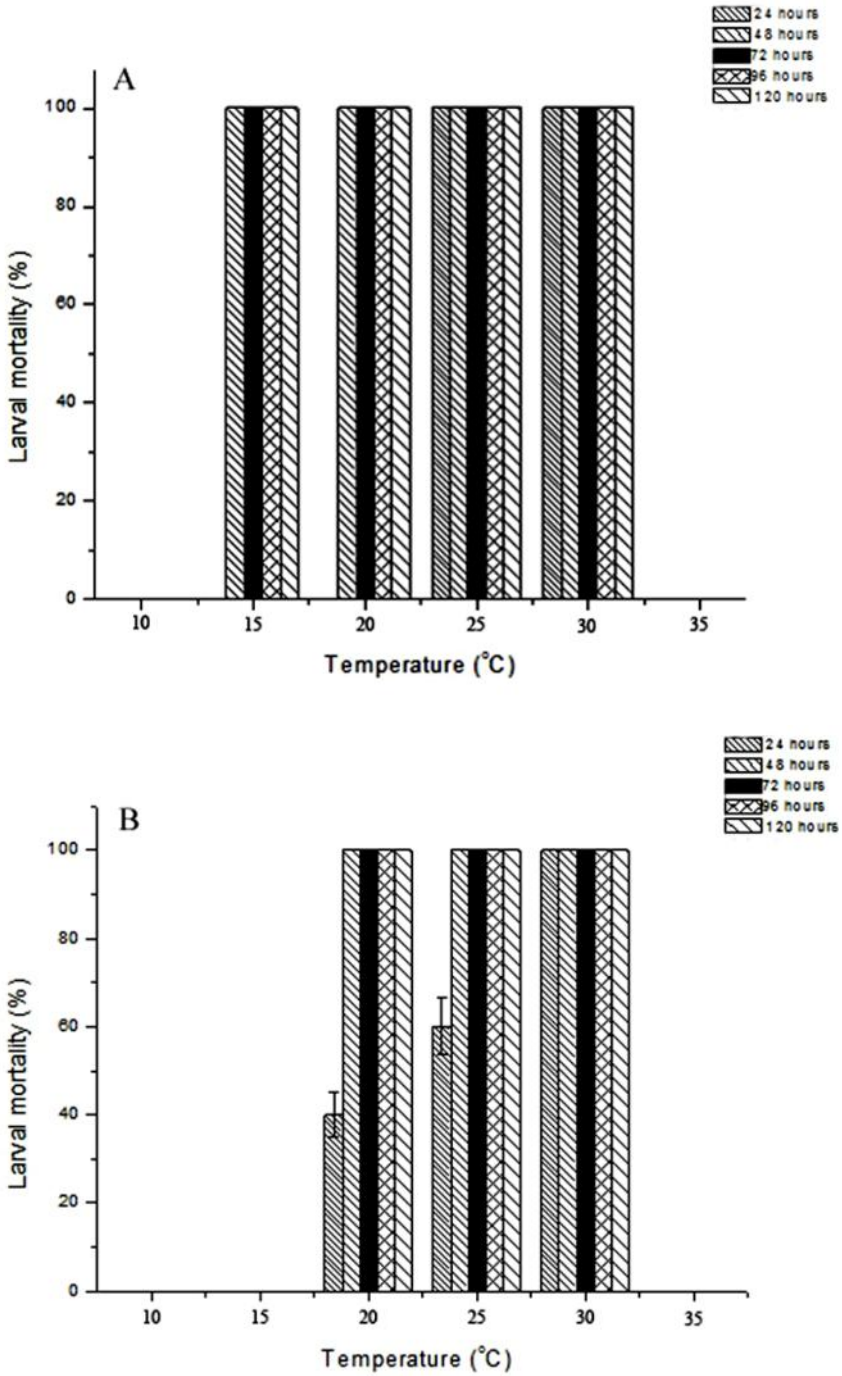

Figure 1. Larval mortality of *G. mellonella* induced by EPNs at different temperatures. A. *Steinernema* sp. and B. *Heterorhabditis indica*.

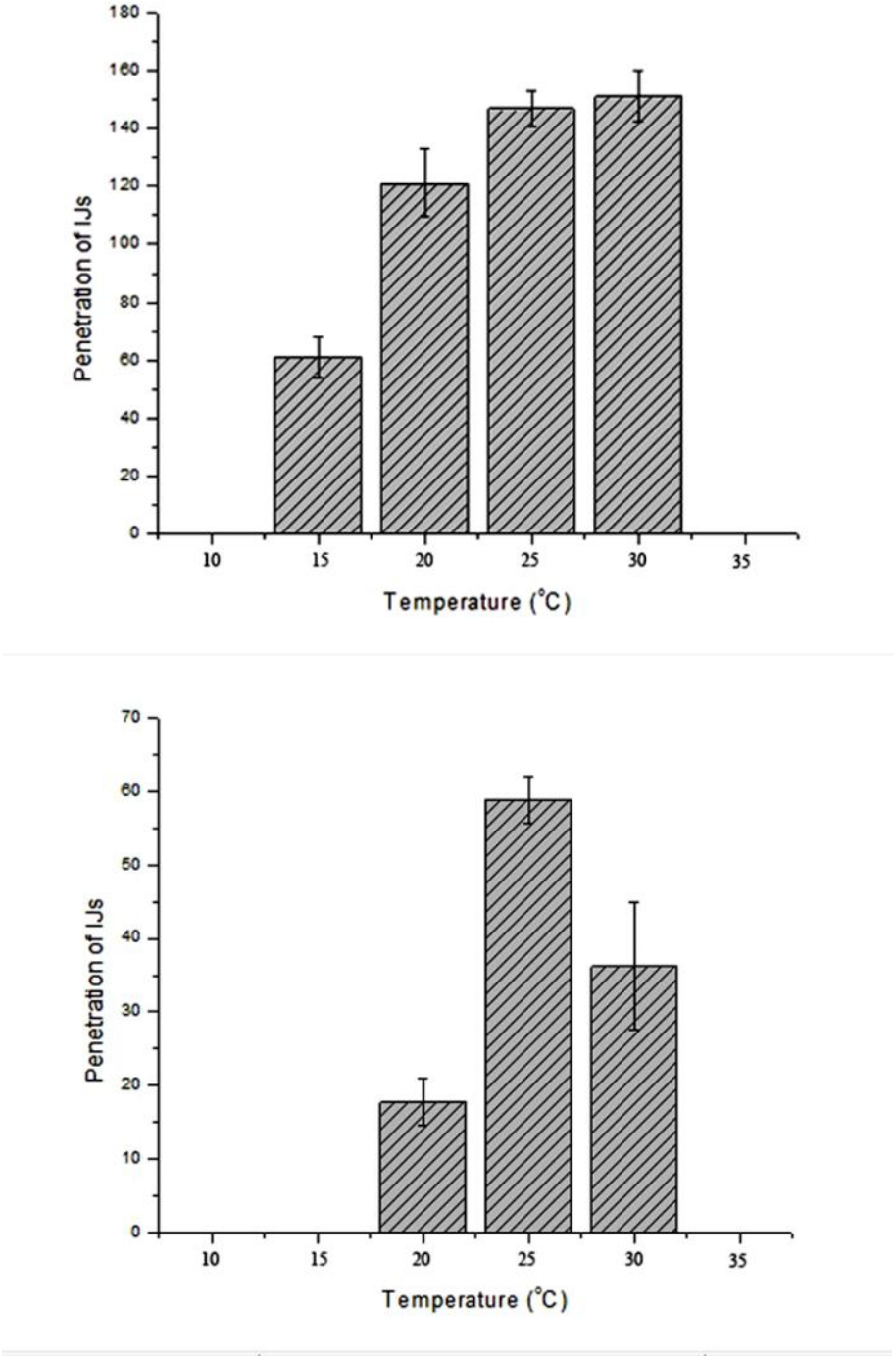

Figure 2. IJs establishment at different temperatures A. *Steinernema* sp. and B. *Heterorhabditis indica*. Data are presented as mean±SE .

gin.[8,14,15] It is an utmost important that EPN species descriptions need to supplement with data on ecological characterization.[8] The present study characterized the temperature requirements of locally isolated two EPNs, viz. *Steinernema* sp. and *Heterorhabditis indica*. The result indicates that the time taken for infective juveniles to cause insect mortality was significantly influenced by temperature. The study also revealed that IJs of the two species did not establish at 10°C and in addition 15°C in *H. indica*. Grewal *et al*.[16] reported that exposure to low temperatures generally prolongs the time of emergence for both steinernematids and heterorhabditids. This finding also gains support from Karunakar *et al*.[17] who studied the temperature effects on *Steinernema feltiae*, *S. glaseri* and *H. indica* and reported that no mortality of insect host was observed at 10°C. The unsuccessful infection of *H. indica* at 15°C in the present study may be attributed to the warmer climatic conditions where the species were isolated. In the present investigation it was observed that the *Steinernema* sp. could establish at a temperature of 15 to 30°C and the optimum temperature ranges appeared to be 20 to 30°C, whereas *H. indica* IJs appeared to be adapted to temperatures between 20 and 30°C with an optimum temperature of 30°C. Among the two studied nematodes it is obvious that *Steinernema* sp. can tolerate lower temperature as compare to *H. indica.* In a related study on the effect of different temperatures on infectivity and growth of *H. indica*, Sosamma[18] reported that the fastest mortality of host occurs at 22–24°C. Similarly, Hussaini *et al*.[19] observed that at temperature between 25 and 32°C, *S. carpocapsae* caused 100% mortality of *Galleria mellonella* and *Agrotis ipsilon* larvae, but no infection or low mortality at 8–18°C. Further, Figueroa and Roman[20] reported that *S. glaseri* performs well at temperatures between 20–30°C to control *Diaprepes abbreviatus*. A slight difference in the temperature requirements of *Steinernema* sp. as observed in the present study may be attributed to different hosts or different origins of the isolates.

The two nematode tested in the present study did not cause insect mortality, as well as no establishment of IJs, at 35°C. With the exception of high temperature adapted species like *S. abbasi, S. riobrave, S. puertoricencse* and *S. thermophilum*,[9,21] most species of *Steinernema* has not been found to develop and reproduce at temperature higher than 27°C.[4] Comparisons of thermal thresholds to the geographical origins of rhabditid nematodes suggest a relationship between temperature niche breadth and locality and support the notion of temperature adaptation of rhabditid nematodes to their environment; e.g. tolerance to warmer temperatures by *S. riobravis, S. scapterisci, Steinernema* sp. (strain M87/45), *Heterorhabditis* sp. (strain Trinidad) and *H. indica* (strain 52) reflects their tropical origins.[16,21,23]

It can be concluded that *Steinernema* sp. and *Heterorhabditis indica* collected from Mizoram can be applied successfully for controlling insect pests in the prevailing local climatic conditions and, further, can be used to control insect pests that occur in other tropical climate regions.

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