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ABSTRACT: Investigations were initiated to evaluate optimum temperature requirements for infectivity and development of an indigenous entomopathogenic nematode, *Steinernema thermophilum* Ganguly & Singh, 2000 (IARI-EPNI), under laboratory conditions, using greater wax moth larvae (*Galleria mellonella*) as insect host. The nematode-inoculated insect larvae were exposed to 5 different temperatures, viz. 10, 20, 25, 30 and 35°C. At 35°C, the larval mortality up to 100% was achieved, within 36 hrs. after inoculation (HAI) while at 25 and 30°C, it occurred within 48 HAI, and at 20°C within 216 HAI. At 10°C, the larval mortality was considerably affected, with only 16% mortality even at 216 HAI i.e. 9 days after inoculation (DAI). The IJs started emerging from the cadaver at 7, 6 and 5 DAI at 25, 30 and 35°C, respectively. The emergence continued for 3-5 weeks at 25-35°C, with maximum harvest within 16 DAI at these 3 temperature conditions. The number of infective juveniles (IJs) produced per cadaver was maximum at 30 and 35°C. It has been demonstrated that *S. thermophilum* can infect and develop at a wide range of temperature (20-35°C), the optimum being 25-35°C, with better performance at 30-35°C. Hence, it may prove to be useful bioagent against insect pests of crops especially in tropical and subtropical parts of the world.

Key words: *Steinernema thermophilum*, temperature, infectivity, mortality, development, reproduction, wax moth larvae, *Galleria mellonella*.

Various abiotic and biotic factors are known to influence the biocontrol efficacy of entomopathogenic nematodes (Bedding et al., 1983; Kaya, 1990; Koppenhofer et al., 1995; Womersley; 1990). Therefore, a detailed knowledge of ecology of the biocontrol agents, is essential for its successful use. Koppenhofer & Kaya (1999) also emphasized the need to supplement species descriptions with data on ecological characterization and have developed protocols for such studies. Among the abiotic factors, temperature plays a vital role affecting infectivity and development (Grewal et al., 1994; Mracek & Webster, 1993; Mracek et al., 1997). Further, different nematode species have different temperature requirements depending upon place of origin (Koppenhofer & Kaya, 1999; Koppenhofer et al., 2000). Therefore, it is essential to understand the optimum environmental conditions required for nematode species before testing their field efficacy.

In August, 1999 an indigenous strain of *Steinernema* sp. (IAI-EPNI) was isolated by the senior author from the rhizosphere of mungbeen (*Phaseolus aureus*) at IARI Farm, New Delhi (later described as *S. thermophilum* by Ganguly & Singh 2000). It was named "thermophilum" due to its capability to complete its life cycle at 30°C, a temperature at which most of the other species do not perform efficiently. Being indigenous, it can adapt well to local agroclimatic conditions and may prove to be useful bioagent against insect pests of crops. Therefore, in the first instance optimum temperature requirements for infectivity, development and reproduction were investigated and the results are reported here.

MATERIALS AND METHODS

Freshly emerged pure population of infective juveniles (IJs) of *S. thermophilum* (strain IARI-
EPNI), were maintained on rice moth larvae (Corcyra cephalonica) by repeated sub culturing.

The lid of a 35 x 10 mm petri dish was lined with double layered Whatman No. 1 filter paper. The nematodes @ 50 IJs/200 µl sterile water were evenly distributed on the filter paper and then incubated in BOD incubators at 5 different temperatures (10, 20, 25, 30 & 35°C). After 30 minutes, one larva of wax moth (Galleria mellonella) was placed on the filter paper. The petriplates were then covered with the base and placed in polythene bags containing wet paper towel to keep sufficient moisture conditions. These were then placed back at their respective temperatures. There were 15 replicates each, along with uninoculated controls.

The petriplates were examined every 12 hour for insect mortality and first emergence of nematode progeny from the cadavers. The petriplates having cadavers with emerging progeny were placed on to the White Traps i.e. in bigger petriplate (100mm x 15mm) containing sterile water, for extracting the emerged IJs, at respective temperatures. The nematodes that migrated to the outer petriplates containing water, were harvested and stored in tissue culture flasks at 20°C. The period of emergence and the total number of IJs emerged at each temperature was recorded.

**RESULTS**

**Insect mortality:** The nematodes infected the insect larvae and induced mortality and the percent mortality at different intervals, after inoculation, varied with temperature. Mortality was faster at 25-35°C than at 10-20°C. At 12 HAI, no insect mortality was recorded at any temperature. The insect mortality started within 24 HAI at 30 and 35°C and within 36 HAI at 25°C. At 36 HAI, there was 75, 83 and 100% mortality at 25, 30 and 35°C, respectively. At 25-35°C, cent per cent mortality of G. mellonella was achieved within 48 HAI. At 10-20°C, the mortality was not only less but also delayed. There was only 25% mortality within 120

Fig.1 (A-E). Effect of temperature on infectivity and development of Steinernema thermophilum. A: time until death of wax moth larvae; B: time until first emergence of infective juveniles (IJ) from the cadaver; C: percentage of wax moth larval cadavers producing nematode progeny; D: duration of IJ emergence; E: number of IJ emerging per cadaver. No emergence of nematode progeny at 10°C. Means sharing a letter are not significantly different (P<0.05; n=15).

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Table I. Effect of temperature on mortality of wax moth larvae, *Galleria melonella* at different hours after inoculation (HAI) with *Steinernema thermophilum* (IARI-EPNI)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>*Percent mortality of <em>G. mellonella</em> larvae at different HAI</th>
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<tbody>
<tr>
<td>10</td>
<td>0 0 0 0 0 25 38 75 88 100</td>
</tr>
<tr>
<td>20</td>
<td>0 0 0 0 0 25 38 75 88 100</td>
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<td>35</td>
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*Each observation is mean of 15 replicates; - Observations on mortality not required; E- Emergence of nematode progeny from the cadaver.

HAI, which increased to 100% at 216 HAI at 20°C (Table 1; Fig 1 A).

**First emergence from the cadaver:** The time taken for first emergence of nematode progeny from the cadavers, was 7, 6 and 5 days after inoculation (DAI) at 25, 30 and 35°C, respectively (Fig. 1B). The emergence was significantly delayed at 20°C since the IJs started emerging from the cadavers only at 25th DAI. While at 10°C nematode progeny did not emerge from the cadavers even at 30 day after inoculation.

**Percent cadavers producing progeny:** Cent per cent cadavers produced nematode progeny at 25, 30 and 35°C (Fig. 1 C). While at 20°C, despite delayed insect mortality, the progeny was formed but the percent cadavers producing progeny was remarkably less (38%). The IJs could not develop and reproduce at 10°C, since none of the cadaver produced nematode progeny at this temperature.

**Period of emergence:** The emergence of nematode progeny from the cadavers continued for a maximum period of 30 days at 35°C followed by 22, 14 and 10 days at 30, 25 and 20°C respectively (Fig. 1 D). The duration of emergence at 35°C was almost at par with that at 30°C but was significantly higher than that of 25 and 20°C.

**Number of IJs produced:** The number of IJs produced per cadaver exhibited an increasing trend with increasing temperature from 20 to 35°C with maximum at 35°C (Fig. 1 E). The IJs produced at 30 and 35°C were 73 x 10³ and 77 x 10³, respectively which were significantly (at 5% level) higher than those at 25 (55 x 10³) and 20°C (22 x 10³).

**DISCUSSION**

*S. thermophilum* could infect the insect host and induce mortality at a wide range of temperature varying from 10 to 35°C, the optimum being 20-35°C. But, the time taken for mortality and percent mortality was greatly influenced by temperature as was evident from the delayed mortality at 10 and 20°C. The infective juveniles could develop, reproduce and produce progeny at a temperature range of 20-35°C, the optimum being 25-35°C. Higher temperature requirements for development and reproduction than that of infectivity has also been reported earlier for *S. monticolum* (Koppenhofer et al., 2000), *S. rarum* (Koppenhofer & Kaya, 1999) and other *Steinernema* species (Grewal et al., 1994)

Even though, *S. thermophilum* could efficiently develop, reproduce and produce progeny at a range of 20-35°C, its performance improved with the
increasing temperature up to 35°C. Most of the species of Steinernema do not develop and reproduce at temperatures higher than 27°C (Kaya, 1990). However, there are very few species like S. abasi, S. riobrave and S. puertoricense which are reported to prefer high temperature. The thermal activity range of S. thermophilum showed closer resemblance with S. abasi than the other two species (S. riobrave and S. puertoricense), since both of these produced more number of IJs at 35°C than at 30°C, while S. riobrave and S. puertoricense produced maximum number of IJs at 30°C.

It was also evident that the high temperature optima (25-35°C) of S. thermophilum corresponded with the semi-arid and sub-tropical climatic conditions of Delhi, the place of its origin, where the temperature varies between 28-40°C (summer) and 5-25°C (winter). The correlation between temperature requirements of different species and their respective home temperatures has been documented (Cabanillas et al., 1994; Kaya, 1990; Roman & Figueroa, 1994; Elawad et al., 1997; Koppenhofer & Kaya, 1999; Koppenhofer et al., 2000), and it was indicated that the temperature optima for cold adapted species (S. monticolum) was lower than that of the species reported from moderately warm (S. rarum) or hot tropical (S. abasi, S. riobrave and S. puertoricense) regions of the world.

S. thermophilum being indigenous, can adapt well in the Indian agro-climatic conditions and may prove to be useful bioagent against local insect pests. In field application, however, soil temperature below 25°C may reduce its biocontrol efficacy since nematode infectivity and development on a highly susceptible host, even under optimum laboratory conditions, was remarkably reduced at 20°C. Whether or not, it can infect and reproduce at temperature higher than 35°C, needs to be further investigated. However, with an infectivity range between 10 and 35°C (optimum 20-35°C) and reproduction range between 20 and 35°C (optimum 25-35°C), it may form an important component of Integrated Pest Management schedules, in future, not only in India but also other tropical and subtropical parts of the world.

REFERENCES


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