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Effect of temperature on survival of *Steinernema seemae*, *S. masoodi* and *S. carpocapsae* (Rhabditida: Steinernematidae) and their subsequent infectivity to prepupa of *Helicoverpa armigera* (Hübner)

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Abstract
The survival and infectivity of three indigenous entomopathogenic nematodes, *Steinernema seemae*, *S. masoodi* and *S. carpocapsae* at different temperatures (15, 20, 25, 30, 35, 40 and 45°C) were studied against prepupa of *Helicoverpa armigera* (Hübner). Percent survival of nematodes decreased with increases in temperature. However, 46.6% of the populations were able to survive and tolerate the sub-lethal temperature (45°C) treatment for 6 h. Out of the populations that survived, 43.3% infectivity was observed against *H. armigera* prepupa. The survivors did not infect at 25 and 30°C, rather their activity was found optimum. These heat tolerant isolates could play a vital role in the management of susceptible stages of *H. armigera* at high temperature regimes and for the management of other insect pests of agricultural importance, which pupate in the soil.

Keywords: *Entomopathogenic nematode (EPN)*, Helicoverpa armigera, *infectivity, survival, temperature*

Introduction

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* are obligate pathogens of insects in nature (Poinar 1979). They are potential alternatives to chemical control for many insect pests (Gaugler & Kaya 1990). They have been recovered from soils of a wide variety of climatic regions in India (Karunakar et al. 1999; Ganguly & Singh 2001; Hussaini et al. 2004). Studies on *S. abbasi*, *S. tami*, *S. carpocapsae*, *S. feltiae*, *S. glaseri* and *S. thermophilum* have revealed that optimum temperature and moisture requirement for their infectivity and survival vary from species to species (Karunakar et al. 1999; Ganguly & Singh 2001; Ganguly & Gavas 2004; Hussaini et al. 2004) and on the nematodes' climatic origin from where it is isolated.

Cooler temperatures have not been detrimental to nematode survival (Kaya 1990), however, temperatures above 30°C tend to inhibit their development in a host (Milstead 1981) and temperature above 35°C over an extended period of time is detrimental to infective

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juveniles (Schmiege 1963). Mortality and/or reduced infectivity of juveniles under field conditions is one of the most important factors restricting their application in sub-tropical ecosystems where temperatures can be very high. The present investigation was undertaken to know the upper limit of heat tolerance and infectivity of two new species, *S. seemae* (Ali et al. 2005) and *S. masoodi* (Ali et al. 2005) and *S. carpocapsae* (Weiser 1955) Wouts et al., 1982 against prepupal stage of pod borer, *Helicoverpa armigera* (Hübner), which after completing its larval stage goes into the soil for pupation.

Materials and methods

Nematode and insect cultures. Steinernema seemae and *S. masoodi* were obtained by baiting of soil samples brought from Hamirpur and Kanpur (Bithoor locality) districts of Uttar Pradesh (Ali et al. 2005). *S. carpocapsae* was procured from a Nematology laboratory, Project Directorate of Biological Control, Bangalore, which was originally isolated from Kanpur itself. All these local isolates were cultured on fully grown *Galleria mellonella* larvae as per the procedure described by Woodring & Kaya (1988). Emerged infective juveniles (IJs) were surface sterilized in 0.1% Hyamine solution and stored in distilled water in tissue culture flasks. Up to one-week-old cultures were used in the experiments. The test insect, *H. armigera* larvae were collected from pigeonpea/chickpea fields and reared on semi-synthetic diet as described by Armes et al. (1992) up to pre-pupal stage.

Heat tolerance assays. The survival of *S. seemae*, *S. masoodi* and *S. carpocapsae* were assessed under seven controlled temperatures regimes of 15, 20, 25, 30, 35, 40 and 45°C. A known quantity of 1000 IJs of each isolate was placed in 100 g sterilized sandy loam soil at 9% moisture level (w/w) in a small earthen pot (10 × 7 cm) and incubated in BOD incubator at particular temperature for 6 h. After the required period of temperature treatment, pots were taken out and distilled water was added to maintain the moisture level in the soil.

Nematode survival and pathogenicity. The soil from each of the earthen pots was sieved and IJs were extracted with the help of modified Cobb sieving and decantation (Cobb 1918) and Baermann's funnel method (Baermann 1917). The surviving IJs in soil were counted three times under Leica MS 5 stereoscopic binocular microscope in a Syracuse counting dish and mean values were worked out.

Pathogenicity was assessed by the ability of EPN to kill prepupa of *H. armigera*. Bioassays were conducted by releasing heat-treated nematodes on *H. armigera* prepupa, placed on pieces of semi-synthetic diet in earthen pot covered with muslin cloth and left for 3 days at 30 ± 1°C. Observation was taken every 24 h to check the mortality of prepupa. Each nematode isolate was tested separately at different temperatures. There were 10 replicates in each treatment. The percent data was transformed to arc sine prior to analyses.

Results and discussion

Infective juveniles of indigenous populations of *S. seemae, S. masoodi* and *S. carpocapsae* were able to survive at all the tested temperatures. A temperature regime of 20–35°C was found better suited for survival of EPN studied, as it ranged from 60–80% (Figure 1B). The best temperature was 25°C wherein 81% of survival was noted both in *S. seemae* and *S. carpocapsae* and 78% in *S. masoodi*. The next best storage temperature was found to be 30°C. In this case 78, 71 and 76% survival was recorded in *S. seemae, S. masoodi* and *S. carpocapsae*, respectively.

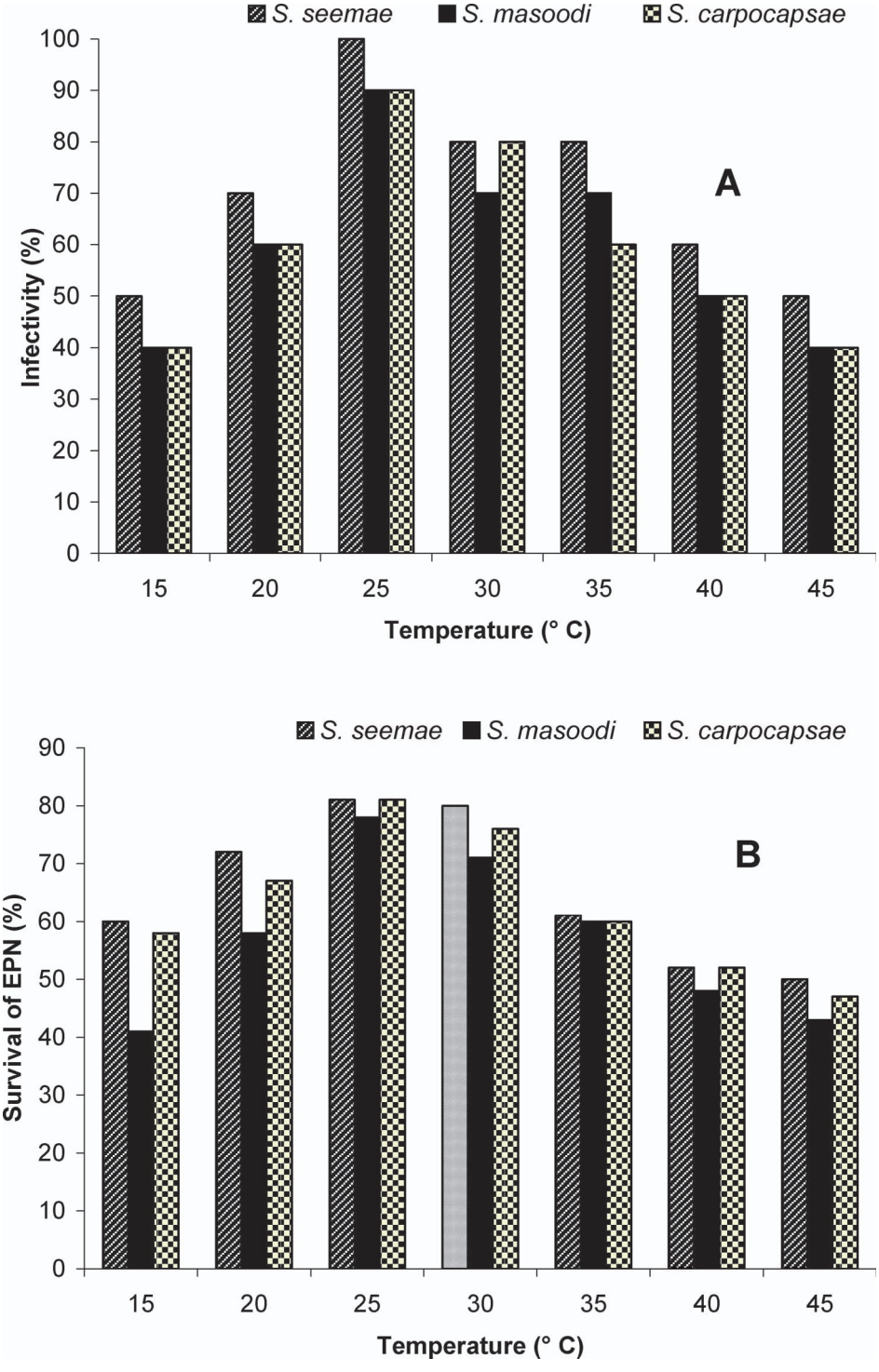

Figure 1. (A) Effect of different temperature on the infectivity of *Steinernema seemae*, *S. masoodi* and *S. carpocapsae* against *H. armigera*. (B) Effect of different temperature on the survival of *Steinernema seemae*, *S. masoodi and S. carpocapsae* in soil.

It was interesting to note that *S. seemae* had better tolerance (50–81%) to this temperature and next in order was *S. carpocapsae* (47–81%) and *S. masoodi* (41–78%).

The effect of temperatures on nematode performance varies with nematode species and strains (Kaya 1990; Grewal et al. 1994). Generally, IJs become sluggish at lower temperature (<10–15°C) and will be inactivated at higher temperatures (>30–40°C). Extended exposure to temperature below 0°C and above 40°C is lethal to most entomopathogenic nematode species, but the effect depends on the duration of exposure. In the soil environment, IJs are normally buffered from temperature extremes or usually have enough time to move down into deeper soil layers where the buffering effect is stronger.

Highest infectivity of prepupa of *H. armigera* was observed by *S. seemae* (100%) followed by *S. carpocapsae* (82%) and *S. masoodi* (70%) at 25°C (Figure 1A). *S. seemae* was found highly virulent than *S. carpocapsae* and *S. masoodi* at all tested temperature regimes. Infectivity of nematodes was affected at lower and upper limits of temperature. Infectivity of *S. masoodi* was found on par with *S. carpocapsae* (40%) at 15 and 45°C, respectively. Infectivity rate of *S. masoodi* and *S. carpocapsae* was on par at 15, 20, 25, 40 and 45°C. Though both species, *S. seemae* and *S. masoodi* have been isolated from locality experiencing high temperature, but the former (*S. seemae*) seems to tolerate it better than the later (*S. masoodi*), so much so that at 45°C, its infectivity was 50% against 40% in the later and *S. carpocapsae.* Ishibashi et al. (1981) reported that DD-136 caused 100% mortality of 5th instar larvae of *Spodoptera litura* (Fab.) at 25–30°C in two days and it was 90% and 75% at 20 and 15°C after five days, respectively. The optimal infection, reproduction and multiplication took place between 25 and 28°C (Kaya 1977; Molyneux 1986). *S. feltiae* when tested against pre-pupa, pupa and adult of *S. litura* at 10 000, 1000 and 100 nematodes, resulted in complete mortality of the insect. The pupa was less susceptible than the pre-pupa or adult (Narayanan & Gopalakrishnan 1987).

Exposure to extremes of temperature is damaging to nematodes, but the extent and nature of damage depends on the duration of exposure. All infective juveniles of *S. carpocapsae* exposed to 41°C for 1 h were killed (Schmiege 1963), while *S. carpocapsae* Arkansas isolate survived for two weeks in soil at 40°C (Gray & Johnson 1983). From the biological control perspective, the point at which irreversible heat or chill coma is induced in the nematodes is more important than the thermal death point. In general, nematodes in deeper layers of soil will not be exposed to high lethal temperatures (Kaya 1990); such temperatures are most likely to be encountered following foliar application, where they interact with the lethal effects of ultra-violet radiation and high temperature.

Nematodes may act in a synergistic manner along with bacteria to provide control of lepidopteran pests that pupate in the soil. Innundative applications of *S. riobrave* to control 6th instar larvae and pupae of *H. zea* in maize were found to be more effective under flood irrigation, causing over 90% mortality (Feaster & Steinkraus 1996). Given the ability of *S. riobrave* to persist under severe environmental conditions, it may become a sustainable IPM component in flood-irrigated cropping system. Soil surface and subsurface applications of nematodes can also affect corn earworm populations because larvae drop to the soil for pupation (Cabanillas & Raulston 1996).

H. armigera larvae after completing its feeding on aerial parts like leaves, pods, squares, bolls, etc., also drop down to soil and pupae in earthen cocoons made for this purpose. There is every likelihood that these will come into contact with EPN and die, if EPN are surviving in the soil. This will check further multiplication of the pest. This approach may have application for commercial crop protection, but the larvae must complete their development before being controlled and drop down to soil, thereby reducing insect pest pressure for the next cropping cycle – an approach that is feasible only in a long-term integrated pest management strategy for a wider area and diverse cropping system where *H. armigera* is the pest for most of the crops grown.

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