Effect of temperatures on infectivity of Entomopathogenic Nematodes

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Date of Reciept: 07.07-2014; Accepted: 25.07.2014

ABSTRACT

The infectivity of eight native isolates of EPNs, *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *S. ramanai* (IISR-EPN 03), *S. carpocapsae* (IISR-EPN 06), *Oscheius* spp. (IISR-EPN 04, 05 & 08) and *O. gingeri* (IISR-EPN 07) were tested against lepidopteran insect pest, *Galleria mellonella* larva and multiplication into host at different temperatures *viz*., 20, 25, 30 and 35°C. The temperature significantly affected the pathogenicity and multiplication ability of all tested EPNs. Among the test temperature, maximum mortality of larvae was found at 30°C followed by 25°C, whereas the least mortality of insects was recorded at 20 and 35°C. Maximum number of infective juveniles of EPNs were multiplied at 30°C, followed by 25°C, whereas, minimum multiplication was recorded at 35°C. However, no multiplication of EPNs was recorded at 20°C. Therefore, the optimal temperature for infection and multiplication of EPNs was 30°C.

Key words: EPN, Temperature.

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Chemo intensive pest management modules were being widely advocated for management of the insect pests. The dependence on pesticides is still in practice in spite of associated problems like, development of insect resistance to insecticides, pest resurgence, outbreak of secondary pests and other socio-economic problems. Therefore, there is a need to identify suitable alternative methods for the management of insect pests which is ecofriendly and economically viable. Among these methods, entomopathogenic nematodes (EPNs) are potent biological control agents against insect pests of crops due to their wide host range, easy to handle, short life cycle, economically produced at large scale and environmentally safe (Gupta *et al.,* 2011).

Third stage infective juveniles penetrate into insect body through natural openings and they release the symbiotic bacteria and caused septicaemia and death of the insect within 24 to 72 hrs. Temperature is one of the most important factors limiting the success of EPNs. Parvez *et al.* (2014) correlated % mortality of shoot borer with number of EPN under laboratory condition. Hence, the present study was carried out to test the infectivity of eight native EPNs namely, *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *S. ramanai, S. carpocapsae*, *Oscheius* spp. (IISR-EPN 04, 05 & 08) and *O. gingeri* (Pervez *et al*., 2013) against larva of greater wax moth *G. mellonella* (GWML) and their multiplication at different temperatures.

Materials and Methods

Infective juveniles (IJs) of tested EPNs were obtained from nucleus culture of nematodes maintained in the Nematology Laboratory, IISR, Kozhikode. All tested EPNs were cultured as per the procedure described by Kaya and Stock (1997). Fresh harvested IJs were surface sterilised with 0.1% Hyamine solution and used for bioassay.

GWML reared on artificial diet and were sorted out and those of same size were taken for the present study.

Infectivity of test EPNs against GWML was tested in six well plates. One larva of test insect was kept in each well into which 100 IJs (in 100 µl water) of each test EPNs were inoculated and kept at 20, 25, 30 and 35^{0}C in BOD and the mortality was recorded at 72 hrs. For each EPNs and temperature combination evaluated separately and replicated 12 times along with control (only water).

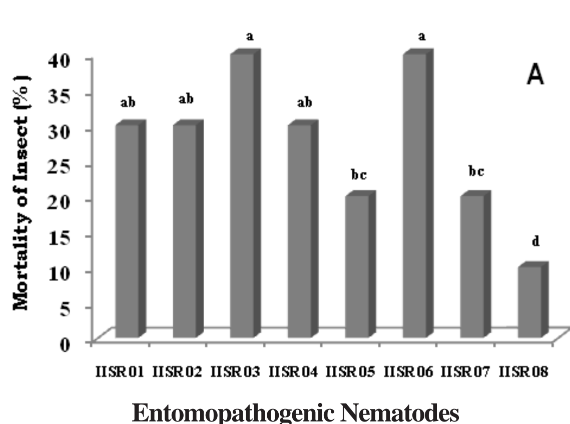
EPN infected dead GWML were removed from the well and kept on White trap at test temperature regimes *viz.,* 20, 25, 30 and 35°C for emergence of infective juveniles. IJs were collected daily, till the emergence stopped in about 15 days. From this collection, the total emerged populations of EPNs were counted thrice under a stereoscopic binocular microscope with the help of Syracuse counting dish and mean values were worked out.

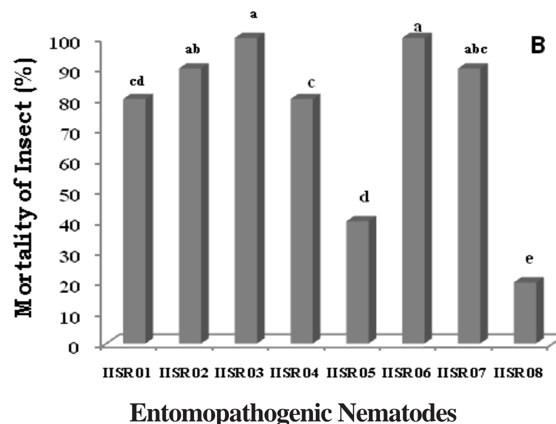
Results and Discussion

The result showed that, % mortality of GWML and multiplication of infective juveniles of all test EPNs were influenced by temperatures. All the test EPNs were pathogenic against GWML at test temperatures but the rate of mortality was significantly different (P <0.05) between EPNs and temperature regimes. At all temperatures studied, *S. ramani* (IISR-EPN 03) showed highest virulence, whereas *Oscheius* sp. (IISR-EPN 08) the lowest one. No mortality of GWML was recorded in the control. Among the test temperatures, maximum mortality of insect was found at 30^{0}C followed by 25°C, whereas the least mortality of GWML was recorded at 20 and 35^{0}C (Fig. 1A&D) (Seal *et al.,* 2010).

Exposure to extremes of temperature was found damaging for nematodes but the extent and nature of damage depends on the duration of exposure. Obviously, the success of a biocontrol programme using EPNs will largely depend on the performance of nematodes at these temperatures. Pathogenicity studies shown considerable inter and intraspecific variations in infectivity of different isolates of EPNs. Saravanapriya and Subramanian (2007) also reported similar results, which were attributed to the variation in the ability of the IJs to find and enter a host as well as the different host susceptibility among various insects (Banu *et al.,* 2003) or insect stages. However, there was considerable variation in the infectivity of EPNs and no single species or strain was suitable for controlling all or even most insect species. Hussaini *et al.* (2005) reported role of anti-desicants and abiotic factors on survival and pathogenicity of EPN on *Plutella xylostella*.

Temperature affected the production of IJs and the level of multiplication significantly varied (df = 7, 18; F= 42.41; p < 0.0001) within tested EPN isolates and temperature regimes. Among the test temperatures, maximum number of IJs was obtained at 30°C followed by 25°C. However, no multiplication of EPNs was recorded at 20°C, similarly at 35°C except multiplication of *O. gingeri*





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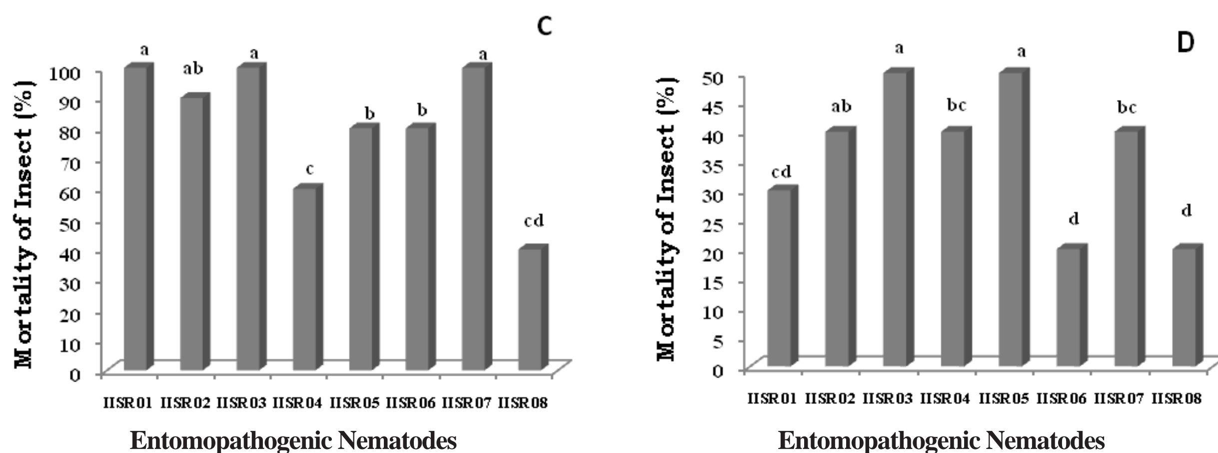

Fig. 1. Effect of temperature on the infectivity of EPNs against GWML. (**A**) - at 20°C; **(B)** - at 25°C; **(C)** - at 30°C and **(D)** - at 35°C. **IISR 01-** *Heterorhabditis* sp. (IISR- EPN 01); **IISR 02 -** *Steinernema* sp. (IISR-EPN 02); **IISR 03** - *S. ramanai* (IISR- EPN 03); **IISR 04 -** *Oscheius* sp. (IISR-EPN 04); **IISR 05 -** *Oscheius* sp. (IISR-EPN 05); **IISR 06 -** *S. carpocapsae* (IISR- EPN 06); **IISR 07** – *O. gingeri* (IISR-EPN 08) and **IISR 08 -** *Oscheius* sp. (IISR-EPN 08).

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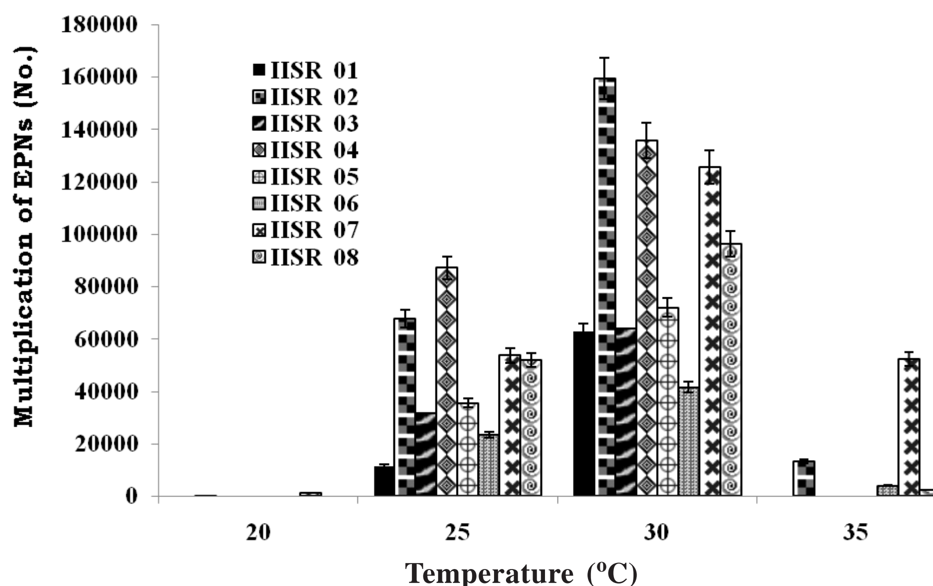

Fig. 2. Effect of temperature on multiplication of infective juveniles of EPNs. **IISR 01-** *Heterorhabditis* sp. (IISR- EPN 01); **IISR 02 -** *Steinernema* sp. (IISR-EPN 02); **IISR 03** - *S. ramanai* (IISR- EPN 03); **IISR 04 -** *Oscheius* sp. (IISR-EPN 04); **IISR 05 -** *Oscheius* sp. (IISR-EPN 05); **IISR 06 -** *S. carpocapsae* (IISR- EPN 06); **IISR 07** – *O. gingeri* (IISR-EPN 07) and **IISR 08 -** *Oscheius* sp. (IISR-EPN 08).

(IISR-EPN 07) (52,410 IJs/larva). Among the tested EPNs, highest number of multiplication of infective juveniles of *Steinernema* sp. (IISR-EPN 02) (1,59,361 IJs/larva), followed by *Oscheius* sp. (IISR-EPN 04) (1,35,894 IJs/larva), whereas, minimum number of IJs of *S. ramani* (IISR-EPN 03) (62,597 IJs/larva) multiplied at 30°C (Fig. 2).

Low temperatures seem to be the main barrier for EPN use in temperate regions (Pervez *et al.*, 2008). Low temperature induced inactivity of infective juveniles and was characterised by decreased enzymatic activity and mobility, both reducing metabolic expenditures. However, Pranav *et al.* (2012) also emphasized the role of temperature on activity of *Xenorhabdus* sp. associated with *S. thermophilum. S. carpocapsae* development did not occur at 10°C and above 33°C, whereas between 15°C and 27°C the EPNs developed and reproduced, with the optimum at 25°C. The optimum temperature for activity of *S. carpocapsae* was determined in the temperature range from 22 to 24°C, *H. bacteriophora* from 22 to 26°C and *S. feltiae* at 25°C. However, Blackshaw and Newell (1987) reported that the optimum temperature required for *H. heliothidis* for infection and penetration was 28°C, while the range was 12 to 32°C. The present finding on the temperature preference of the nematodes for infection agree with the published literature.

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Hence, it is concluded that temperature can affect infectivity and multiplication of EPNs. At lower and higher temperatures affected EPNs activities. Among test temperature, maximum infectivity and multiplication of EPNs were recorded from 25 – 30°C. Therefore, this study gave a clue that culturing of EPNs with optimum temperature at 30°C, when maximum numbers of IJs required for research purposes or field application.

Acknowledgements: The authors express their gratitude to the Director, Indian Institute of Spices Research, Kozhikode (Kerala) for providing the facilities and to Mr. K. Jayarajan for statistical analysis.

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