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Susceptibility of three lepidopteran pests to five entomopathogenic nematodes and *in vivo* mass production of these nematodes

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Abstract
An investigation was conducted in pots to access the susceptibility of three lepidopteran pests, namely, gram pod borer, *Helicoverpa armigera*, greater wax moth, *Galleria mellonella*, and rice moth, *Corcyra cephalonica*, to two recently described species, *Steinernema masoodi*, *S. seemae*, and three indigenous *S. carpocapsae*, *S. glaseri* and *S. thermophilum* entomopathogenic nematodes (EPN). The suitability of these lepidopterans for the *in vivo* mass production of the nematodes was also estimated. Among the five species of EPN, *S. masoodi*, *S. seemae* and *S. carpocapsae* were found most pathogenic to *C. cephalonica*, bringing about mortality within 24 h, followed by *H. armigera* (36, 38 and 48 h, respectively) and *G. mellonella* (30, 36 and 48 h, respectively). The other species of EPN, viz., *S. glaseri* and *S. thermophilum* was the least pathogenic, which killed the larvae of *C. cephalonica* in 29 and 36 h, respectively, *G. mellonella* in 48 h, and *H. armigera* in 38 and 56 h, respectively. *Galleria mellonella* was found the most suitable host for the mass production of infective juveniles (IJs) of *S. seemae*, which yielded higher IJs than *S. carpocapsae*. *Helicoverpa armigera* was the next best suitable alternate host, which produced maximum IJs in case of *S. seemae* followed by *S. masoodi*, *S. carpocapsae*, *S. glaseri* and *S. thermophilum*. Rice moth, *Corcyra cephalonica* was the least suitable host. The susceptibility of *H. armigera* to five tested EPN species and susceptibility of *G. mellonella* and *C. cephalonica* to *S. masoodi* and *S. seemae* are new records.

Keywords: *Entomopathogenic nematodes, lepidopteran,* H. armigera*, mass production*

Introduction

During the last decade, chemo-intensive integrated pest management modules are being widely advocated for the management of insect pests. However, dependence on pesticides is still widespread in spite of associated problems such as the development of insect resistance to insecticides, pest resurgence, and 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. Among these methods, entomopathogenic nematodes (EPN) are a potent candidate that can be used as biopesticides against lepidopteran insect pests.

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EPN are symbiotically associated with the bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. When these bacteria are released into the insect haemocoel through EPN, they cause septicaemia and death of the insect within 24–29 h (Kaya & Gaugler 1993). Entomopathogenic nematodes, especially Steinernematidae, have a great potential as biological control agents against insect pests because of their wide host range (Poinar 1990; Cabanillas et al. 1994).

EPN can be mass produced *in vivo* where the insect serves as a small biological reactor. *Galleria mellonella* L. has been widely used for *in vivo* mass production of EPN, while other insect like *Chilo sacchariphagus indicus* (Kapur) have also been used to study the infectivity and multiplication of *Steinernema feltiae*, *S. glaseri* and *Heterorhabditis bacteriophora* (Karunakar et al. 1992).

This study was undertaken to broaden the list of tested insect pests to which these EPN (both newly described and earlier species) are pathogenic and *in vivo* mass production techniques. The comparative pathogenicity of five species of EPN, namely, *Steinernema masoodi* (Ali, Shaheen, Pervez and Hussain), *S. seemae* (Ali, Shaheen, Pervez and Hussain), *S. carpocapsae* [(Weiser) Wouts, Mráček, Gerdin and Bedding], *S. glaseri* [(Steiner) Wouts, Mráček, Gerdin and Bedding] and *S. thermophilum* Ganguly and Singh against final instar larvae of lepidopteran pests namely, pod borer, *Helicoverpa armigera* (Hübner), greater wax moth, *Galleria mellonella* L. and rice moth, *Corcyra cephalonica* L. were studied. The *in vivo* mass production potential of the entomopathogenic nematodes on the above named lepidopteran larvae was also undertaken.

Materials and methods

The experiments were conducted in earthen pots. One-hundred grams of sterilized soil was put in each pot and moisture maintained according to the field capacity of the soil. One-thousand freshly harvested infective juveniles of each species of *Steinernema* from the gram pod borer, *H. armigera*, were sterilized with 0.1% hyamine solution and were inoculated in a single larva of the test insect. Each species of EPN was tested against final instar larva of wax moth, *G. mellonella*, which were reared on an artificial diet (David & Kurup 1988), while field collected larvae of pod borer, *H. armigera*, were used as the laboratory host for multiplication of all five test species of EPN, which were reproduced in the laboratory on larvae of the rice moth, *C. cephalonica*. Test species of entomopathogenic nematodes were tested against the three lepidopteran insect larvae separately and singly. All experiments were conducted at room temperature during April 2004 and replicated 15 times along with control. Observations were made at 6-h intervals. Nematode-infected dead larvae of test insects were removed from the earthen pots, kept on to white trap for their emergence from the body and were collected daily up to a fortnight, till the emergence of IJs was stopped, from insect cadavers by the modified white trap method (White 1927). From this collection, the populations of entomopathogenic nematodes were counted three times under a Leica MS 5 stereoscopic binocular microscope with the help of a Syracuse counting dish and mean values were calculated.

Results and discussion

Among the five species of entomopathogenic nematodes tested, *S. masoodi*, *S. seemae* and *S. carpocapsae* were found to be most pathogenic to *C. cephalonica*, causing mortality within 24 h. Other species of EPN, viz., *S. glaseri* and *S. thermophilum* were less pathogenic as these killed the larvae of *C. cephalonica* in 29 and 36 h, respectively. The mortality of the pod borer,

H. armigera, by *S. seemae*, *S. carpocapsae* and *S. glaseri* was observed within 36, 38 and 48 h, respectively. *Steinernema thermophilum* and *S. masoodi* were the least pathogenic to *H. armigera* larva as these caused about mortality in 48 and 56 h, respectively. On the other hand, *S. seemae* and *S. masoodi* killed the larva of *G. mellonella* within 30 and 36 h, respectively, followed by *S. carpocapsae*, *S. glaseri* and *S. thermophilum* within 48 h (Figure 1A). It was observed that EPN-infected larvae of tested insects turned brownish, greyish and light yellow.

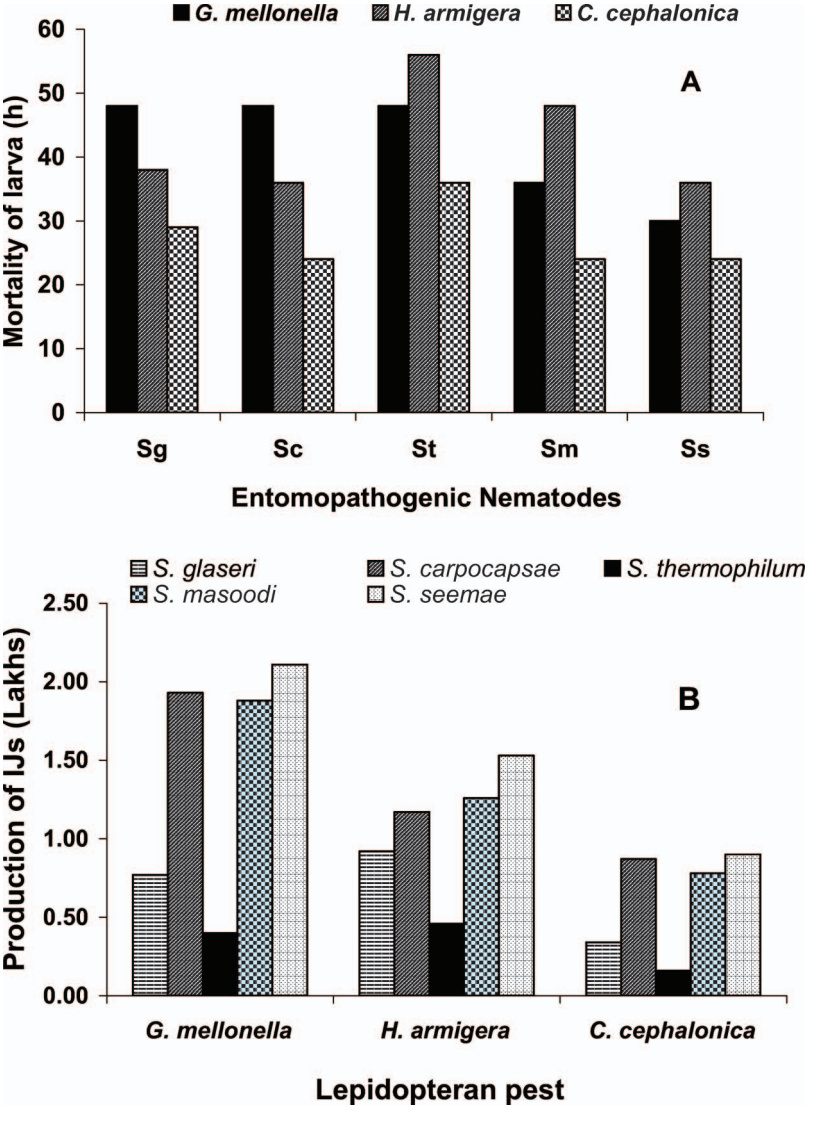

Figure 1. (A) Mortality of the three lepidopteran pests by the five entomopathogenic nematodes. Sg, *S. glaseri*; Sc, *S. carpocapsae*; St, *S. thermophilum*; Sm, *S. masoodi*; Ss, *S. seemae*. (B) *In vivo* mass production of infective juveniles of the five entomopathogenic nematodes on the three lepidopteran species.

With respect to yield of IJs, it was observed that *G. mellonella* was the most suitable host for the highest mass production of infective juveniles (IJs) of *S. seemae*, which yielded 2.1×10^5 IJs/larva, followed by *S. carpocapsae*, 1.9×10^5 IJs/larva (Figure 1B). Lowest number of *S. thermophilum* (0.4×10^5 IJs/larva) was recovered from the *G. mellonella*. *Helicoverpa armigera* was the next best suitable alternate host, which yielded *S. seemae* (1.5×10^5 IJs/larva), *S. masoodi* (1.2×10^5 IJs/larva), *S. carpocapsae* (1.1×10^5 IJs/larva), *S. glaseri* (0.9×10^5 IJs/larva) and *S. thermophilum* (0.4×10^5 IJs/larva). Rice moth, *C. cephalonica*, was the least suitable host, which yielded, 0.9×10^5 IJs of *S. seemae* and 0.8×10^5 IJs of *S. carpocapsae* per larva. The lowest number of *S. glaseri* (0.3×10^5 IJs/larva) followed by *S. thermophilum* (0.1×10^5 IJs/larva) emerged from the body of *C. cephalonica* (Fig. 1B).

S. glaseri took a minimum time of 37 h to kill *C. cephalonica*, followed by 42 h for *G. mellonella* and 52 h for *H. armigera* (Figure 1B). The least multiplication of *S. glaseri* was observed in *C. cephalonica*, which agrees with the studies of Karunakar et al. (1999).

S. masoodi is capable of killing the larva of *G. mellonella* within 36 h and yield of IJs on *H. armigera* was the second highest. EPN, *S. masoodi* and *S. carpocapsae* were found to be on a par with respect to mass production of IJs on the test insects.

These results indicate that all three species of lepidopteran insect larvae are susceptible to five EPN species tested, viz., *S. carpocapsae*, *S. glaseri*, *S. thermophilum*, *S. seemae* and *S. masoodi*. In the present study, *S. glaseri* took 29 h to kill *C. cephalonica*, 48 h to both *H. armigera* and *G. mellonella*. Variation in yield of IJs, mortality time and their ability to support large populations cannot be correlated with the body size of tested insects. Susceptibility of *S. seemae* and *S. masoodi* to *H. armigera*, *G. mellonella* and *C. cephalonica* larvae is reported for the first time.

It can be concluded that *S. seemae* was more pathogenic than other species of entomopathogenic nematodes to the larvae of three lepidopteran pests. The most suitable host for multiplication are *G. mellonella* and *H. armigera*, and these insects can be selected as the alternate host for *in vivo* production of IJs under laboratory conditions. The recently described EPN *S. seemae* is also promising, being the most pathogenic, giving highest IJs yield when infected to *G. mellonella*. These EPN have great potential as candidate in biological control programme of lepidopteran pest management.

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