EFFICACY OF SOME ENTOMOPATHOGENIC NEMATODES AGAINST INSECT PESTS OF GINGER AND THEIR MULTIPLICATION

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Summary. The efficacies of eight native entomopathogenic nematodes (EPNs) were tested against larvae of hairy caterpillar, *Euproctis* sp., and larvae and pupae of the shoot borer, *Conogethes punctiferalis*, and their multiplication was assessed. The penetration of these nematodes in the shoot borer larvae was also assessed. Of the tested EPNs, all isolates, except IISR 08 of *Oscheius* sp., caused 100% mortality to larvae of hairy caterpillar. *Heterorhabditis* sp. (IISR 01), *Steinernema* sp. (IISR 02) and *Oscheius* sp. (IISR 07 and 08) caused 100% mortality also to shoot borer larvae. *Oscheius* sp. (IISR 07) was the most virulent against the shoot borer pupae, causing 100% mortality, followed by *Steinernema* sp. (IISR 02) and *Oscheius* sp. (IISR 05) which killed 67% of the pupae. The multiplication of infective juveniles (IJs) of EPNs was greater in shoot borer larvae than in hairy caterpillar. The greatest number of infective juveniles was observed for *Steinernema* sp. (IISR 02) followed by *Oscheius* sp. (IISR 05), with the fewest found in *Steinernema* sp. (IISR 03) and *Oscheius* sp. (IISR 07) (10,432 and 14,373 IJs/ larva, respectively). The largest number of IJs that penetrated into shoot borer larvae (15.5 IJs/larva) were of *Steinernema* sp. (IISR 03) followed by *Heterorhabditis* sp. (IISR 01), and the fewest (2.8 IJs/larva) were of *Oscheius* sp. (IISR 08).

Key words: Bio-control, *Conogethes punctiferalis*, *Euproctis* sp., reproduction, *Zingiber officinale*.

Ginger (*Zingiber officinale* Roscoe) is a perennial herb belonging to the family Zingiberaceae of which the rhizome or rootstalk is used as spice and medicine. India is a leading producer of ginger in the world, with an average yield of 3,583 kg/ha during 2009-2010. Ginger is cultivated in most of the states in India, with the states of Kerala, Meghalaya, Arunachal Pradesh, Mizoram, Sikkim, Nagaland and Orissa together contributing 70% to the country's total production. From 1975 to the 1980s, India was the major producer of ginger with a share of 30-35% of the world production. However, in the later part of the 90s, ginger production declined.

Among the several insect pests reported on ginger, the shoot borer, *Conogethes punctiferalis* Guenee, is the most severe. The larvae bore into pseudostems and feed on the internal shoot resulting in yellowing and drying of infected pseudostems. The presence of bore holes on the pseudostem, through which frass is extruded, and the withered central shoot are characteristic symptoms of pest infestation (Devasahayam and Koya, 2005). Studies on yield loss caused by the pest in Kerala state indicated that when 50% of the pseudostems in a plant are affected, there is a significant yield reduction per plant (Koya *et al*., 1986). Yield losses of 25% have also been reported when 23 to 24% of a plant's pseudostems are infested. The pest was reported to cause 40% yield loss in Kottayam and Idukki districts in Kerala state (Nybe, 2001).

Chemically intensive management is being widely advocated for insect pests. In view of the ban on pesticides in Kerala, there is a need to identify suitable alternative methods for managing these insect pests.

Entomopathogenic nematodes (EPNs) have great potential as biological control agents of insect pests of crops due to their wide host range, ease of handling, short life cycle and environmental safety (Gaugler and Kaya, 1990; Shapiro *et al*., 2002; Ali *et al*., 2005, 2008; Pervez *et al*., 2007). EPNs are symbiotically associated with bacteria of the genera *Xenorhabdus* and *Photorhabdus* (Akhurst, 1982; Boemare, 2002). Third stage infective juveniles of EPNs penetrate into the host's body through natural openings, release the symbiotic bacteria and thereby cause septicaemia and death of the insect (Kaya, 1990; Shapiro and McCoy, 2000).

Investigations were conducted in 2010-2011, to test the efficacy of eight native EPN isolates against fully grown larvae of the hairy caterpillar, *Euproctis* sp., and larvae and pupae of the shoot borer, *C. punctiferalis*, and their multiplication in the hosts was assessed. The rate of penetration of these nematodes into the shoot borer larvae was also assessed.

MATERIALS AND METHODS

Nematode and insect cultures. Infective juveniles of eight isolates of EPNs, *Heterorhabditis* sp. (IISR 01), *Steinernema* sp. (IISR 02), *Steinernema* sp. (IISR 03), *S. carpocapsae* (Weiser) Wouts, Mráček, Gerdin *et* Bedding and *Oscheius* spp. (IISR 04, 05, 07 and 08) were obtained from stock cultures of nematodes maintained in

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the Nematology Laboratory, Indian Institute of Spices Research, Calicut. All of these EPN species were cultured on fully grown *Galleria mellonella* L. larvae as per the procedure described by Woodring and Kaya (1988). The infective juveniles (IJs) were surface sterilised in 0.1% hyamine solution for 2 minutes and stored in distilled water in tissue culture flasks for study. The shoot borer larvae were collected from ginger fields of the Indian Institute of Spice Research (IISR) Experimental Farm, P'muzhi, and hairy caterpillar was collected from a farmer's field in a village near Thamarassary, Calicut District. The larvae were sorted and those of similar size were chosen for the study.

Efficacy of EPNs against hairy caterpillar and shoot borer larvae. The efficacy of EPNs against *Euproticis* sp. and *C. punctiferalis* was tested in six well plates (3.5 cm diameter) lined with filter paper at the bottom of the plate. One larva of the tested insect was kept in each well and 100 infective juveniles (IJs) of each tested species of EPN in 0.5 ml water were added and the mortality of the larvae recorded after 72 h. Each species of EPN was tested separately. The experiment was conducted at 28 °C in a BOD incubator and replicated using twenty and twelve larvae per EPN of *Euproctis* and *C. punctiferalis*, respectively, along with uninoculated controls. The percentage mortality and mean values were determined.

Dead larvae of the test insects infected by EPNs were removed from the wells and kept on White traps (White, 1927) for emergence of EPNs from the body of the insect. IJs were collected daily until the emergence stopped in about 15 days. The total emerged EPNs were counted three times under a stereoscopic binocular microscope, with the help of a Syracuse counting dish, and mean values were determined.

Efficacy of EPNs against pupae of the shoot borer. The efficacy of EPNs was tested against shoot borer pupae in plastic containers (5.5 cm diameter) lined with filter paper at the bottom of the containers. One pupa was placed in each container and 500 IJs of the tested species of EPN in 0.5 ml water were released in each container. Observations on their mortality were made after 7 days. The experiment was conducted at room temperature and each treatment was replicated six times along with a control (water only).

Number of IJs penetrating into larvae of the shoot borer. The penetration of IJs into shoot borer larvae was tested in Petri plates. One larva of the shoot borer was added to each Petri plate and the EPN species, at a concentration of 500 IJs in 0.5 ml water, were inoculated individually over the filter paper at the bottom of the Petri plates. The Petri plates were kept at 28 °C for 72 h and treatments were replicated six times. After that, enzymatic digestion of dead larvae was done using 3 ml pepsin (1%) in a tube, which was placed in a shaker incubator (30 °C) at 120 rpm for 1 h. The tubes were then shaken well by hand and returned to the shaker for an-

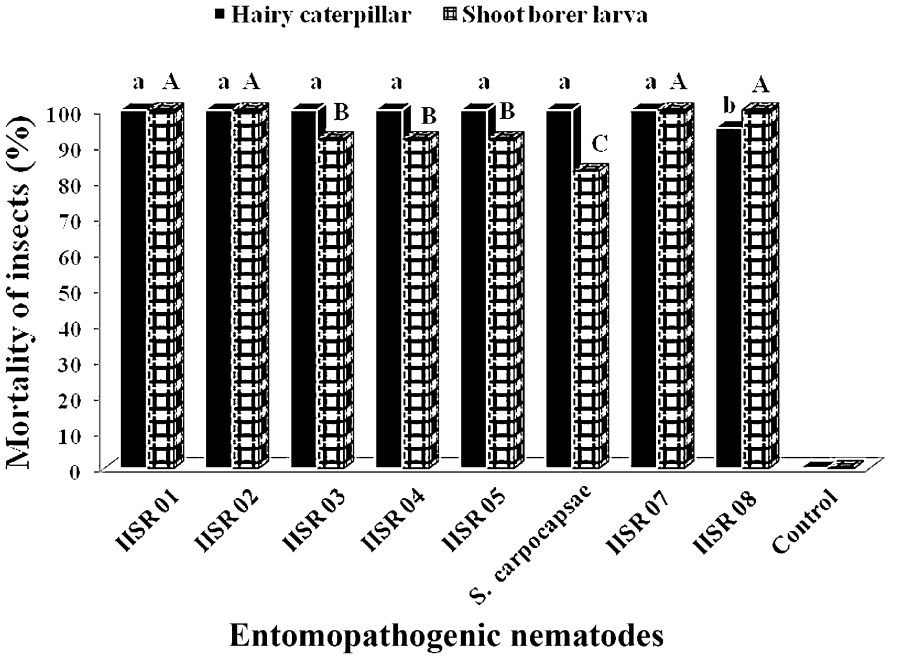

Fig. 1. Mortality of hairy caterpillar (*Euproctis* sp.) and shoot borer (*Conogethes punctiferalis*) larvae caused by IJs of different isolates of entomopathogenic nematodes. Bars with different letters indicate significant differences according to Duncan's multiple range test at P = 0.05. Lower case letters for hairy caterpillar larvae and capital letters for shoot borer larvae.

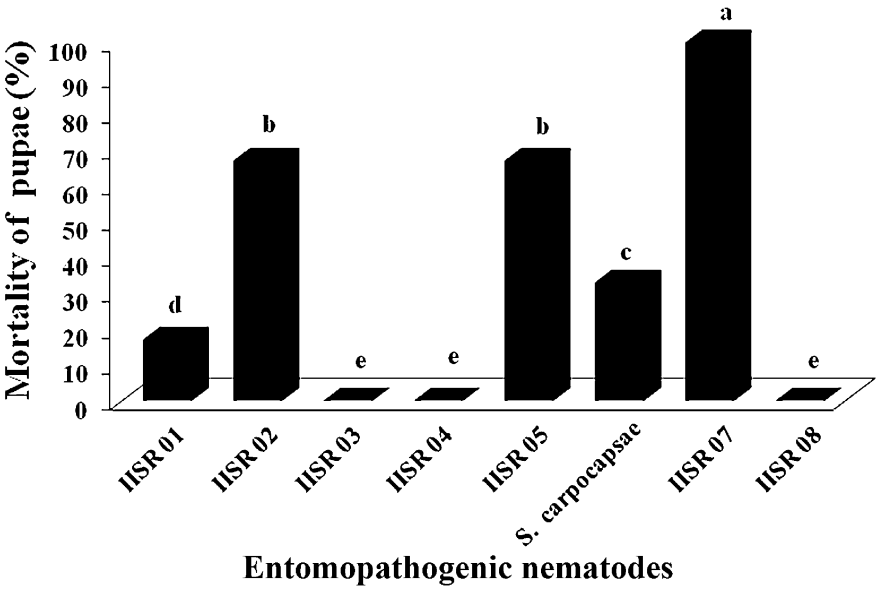

Fig. 2. Mortality of shoot borer (*C. punctiferalis*) pupae caused by IJs of different isolates of entomopathogenic nematodes. Bars with different letters indicate significant differences according to Duncan's multiple range test at P = 0.05.

other 20 min after which 7 ml of Tween 80 were added to each tube, which were then shaken very well and kept at 5 °C for up to 48 h until the nematodes were counted. The penetration rate was then determined.

Statistical analysis. All data were subjected to analysis of variance (ANOVA) and means compared according to Duncan´s multiple range test. Before analysis, data of penetration and multiplication of the nematodes were square root-transformed and those of percentages of insect mortalities were arcsine transformed. All means were transformed back to the original units for presentation.

RESULTS AND DISCUSSION

Efficacy of EPNs against hairy caterpillar and shoot borer larvae. There was no significant difference in hairy caterpillar larva mortality among the EPN isolates. Out of the eight tested EPNs isolates, seven isolates caused 100% mortality of hairy caterpillar and only *Oscheius* sp. (IISR 08) caused less (almost 95%) mortality (Fig. 1).

All the tested EPN isolates caused mortality of shoot borer larvae, but the level of mortality varied significantly (P <0.05) between the EPN isolates. Among the tested EPN isolates, *Heterorhabditis* sp. (IISR 01), *Steinernema* sp. (IISR 02) and *Oscheius* sp. (IISR 07 and 08)

Table I. Multiplication of different isolates of entomopathogenic nematodes (EPNs) on hairy caterpillar (*Euproctis* sp.) and shoot borer (*C. punctiferalis*) larvae.

EPN	No. of IJs/larva	
	Hairy caterpillar larva	Shoot borer larva
Heterorhabditis sp. (IISR 01)	1,720 de	59,020 cd
Steinernema sp. (IISR 02)	3,067 cde	82,987 a
Steinernema sp. (IISR 03)	15,600 b	10,432 f
Oscheius sp. (IISR 04)	-	73,817 ab
Oscheius sp. (IISR 05)	15,600 b	68,200 bc
Steinernema carpocapsae	5,460 c	51,086 d
Oscheius sp. (IISR 07)	4,740 cd	14,373 f
Oscheius sp. (IISR 08)	33,750 a	35,298 e

Mean values, in the same column, followed by different letter are significantly different according to Duncan's multiple range test at P = 0.05.

caused 100% mortality to shoot borer larvae, while *Steinernema* sp. (IISR 03) and *Oscheius* spp. (IISR 04 and 05) caused 92% and *S. carpocapsae* 83% mortality (Fig. 1). There was no mortality of shoot borer or hairy caterpillar larvae in the controls after 72 h.

Laboratory screening of EPNs for infectivity can be an important step in developing a biological control programme for a particular pest (Ricci *et al*., 1996). One of the main reasons for failure of EPNs as biological control of insect pests is the wrong choice of nematode species or strain (Georgis and Gaugler, 1991) as results can vary greatly even among strains of the same species (Taylor *et al*., 1998; Shapiro *et al*., 2002). The variation in mortality percentage within steinernematids, heterorhabditids and the *Oscheius* group indicated that no one group was superior to the others (Gaugler and Kaya, 1990; Ali *et al*., 2008).

Multiplication of EPNs. Almost all the EPN isolates multiplied on the tested insects, but the level of multiplication varied significantly between EPN isolates (Table I). Larvae of the shoot borer were better hosts ($P < 0.05$) than those of hairy caterpillar for the multiplication of infective juveniles (IJs) of the EPNs.

In the shoot borer larvae (Table I), the greatest number (82,986 IJs/larva) of infective juveniles was observed for *Steinernema* sp. (IISR 02), followed by *Oscheius* sp. (IISR 05) (73,187 IJs/larva), which were at par, and the fewest IJs were observed for *Steinernema* sp. (IISR 03) and *Oscheius* sp. (IISR 07) (10,432 and 14,373 IJs/larva, respectively). *Oscheius* sp. (IISR 05), *Heterorhabditis* sp. (IISR 01) and *S. carpocapsae* showed intermediate levels of multiplication.

In the larvae of hairy caterpillar, the significantly largest number of IJs (33,750/larva) was recorded for *Oscheius* sp. (IISR 08), followed by *Steinernema* sp. (IISR 03) and *Oscheius* sp. (IISR 05), which had the same number (15,600/larva). The reproduction of the remaining EPNs was significantly much less. No multiplication of *Oscheius* sp. (IISR 04) was recorded in hairy caterpillar (Table I).

EPNs can be mass produced *in vivo* where the insect serves as a small biological reactor. Greater wax moth, *Galleria mellonella* L., has been widely used for *in vivo* mass production of EPNs. Among other insects, *Chilo sacchariphagus indicus* Kapur produced 907.5 IJs of *S. glaseri*/mg larva (Karunakar *et al*., 1992, 1999), *Helicoverpa armigera* Hubner and *Corcyra cephalonica* Stainton produced 1.5 and 0.9×10^5 IJs/larvae of *Steinernema* sp., respectively, (Ali *et al*., 2008), and *Athalia proxima* Klug and *Spodoptera litura* Fabricius produced 0.62 and 0.79×10^5 IJs/larvae of *Steinernema* sp., respectively (Pervez *et al.,* 2007; Pervez and Ali, 2009). These insects have been used for multiplication of various species of *Steinernema, Heterorhabditis* and *Oscheius* with varying yields of infective juveniles depending upon the size of larvae of the test insect.

Efficacy of EPNs against pupae of shoot borer. There were significant differences ($P < 0.05$) in the mortality of shoot borer pupa with the various EPNs. Among the EPNs tested, *Oscheius* sp. (IISR 07) was the most virulent isolate against the shoot borer pupae, causing 100% mortality, followed by 67% mortality by *Steinernema* sp. (IISR 02) and *Oscheius* sp. (IISR 05). No mortality was recorded with *Steinernema* sp. (IISR 03) and *Oscheius* sp. (IISR 04 and 08) (Fig. 2).

In this study, pupae of shoot borer were found not to be as susceptible as larvae. Despite the number of IJs inoculated per pupa being five times that per larva, only EPN isolate *Oscheius* sp. (IISR 07) killed all pupae, while four isolates gave 17-67% mortality, and three were not effective at all.

Number of IJs penetrating into larvae of the shoot borer. The numbers of IJs of the different isolates of the

Table II. Number of IJs of different isolates of entomopathogenic nematodes (EPNs) penetrating into shoot borer (*C. punctiferalis*) larvae.

EPN	No. of IJs/larva
Heterorhabditis sp. (IISR 01)	10.7 ab
Steinernema sp. (IISR 02)	8.2 bcd
Steinernema sp. (IISR 03)	15.5 a
Oscheius sp. (IISR 04)	4.3 cd
Oscheius sp. (IISR 05)	4.8 cd
Steinernema carpocapsae	9.3 bc
Oscheius sp. (IISR 07)	6.0 bcd
Oscheius sp. (IISR 08)	2.8 d

Mean values followed by different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

tested EPNs that penetrated into the body of the shoot borer larvae showed significant differences ($P < 0.05$). Among the tested species, the largest number of IJs that penetrated into shoot borer larvae (15.5 IJs/larva) were of *Steinernema* sp. (IISR 03), followed by *Heterorhabditis* sp. (IISR 01) (10.7 IJs/larva), whereas a significantly smaller number (2.8 IJs/larva) was observed for *Oscheius* sp. (IISR 08) (Table II).

The rate of penetration could be used as a real measure of host susceptibility. Dunphy and Webster (1988, 1991) reported that the difference in the toxicity of bacterial symbionts is related to the difference in their cell wall substances, which may have led to the relative destruction of host hemocytes and finally to the death of the host. The variation in efficiency of the various entomopathogenic nematodes may be due to variation in the bacterial symbionts (Forst *et al*., 1997; Boemare and Givaudan, 1998; Boemare, 2002).

Entomopathogenic nematodes had not previously been used for the biological control of insect pests of ginger, and this is the first report of their possible use as biological control agents against key pests of ginger in India. Among the tested EPNs, *Steinernema* sp. (IISR 02) and *Oscheius* sp. (IISR 07) appear very promising as they were the only isolates found effective against both hairy caterpillar larvae and shoot borer larvae and pupae. However, more studies are necessary to obtain insights on feeding and preferential behaviour, multiplication of these EPNs on the insects and the mode of action of their associated symbiotic bacteria. This would open a new hope of utilizing EPN in the management of insect pests of ginger.

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