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RESEARCH ARTICLE

Eco-friendly management of cardamom root grub (*Basilepta fulvicorne* Jacoby) through entomopathogenic nematodes

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ABSTRACT: Studies on the infective juvenile (IJs) of entomopathogenic nematodes attachment, penetration into root grub and infectivity of eight native EPNs namely, *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *S. ramanai* (IISR-EPN 03), *S. carpocapsae* (IISR-EPN 06), *Oscheius gingeri* (IISR-EPN 07) and *Oscheius* spp. (IISR-EPN 04, 05, and 08) were tested against cardamom root grub. Among the tested EPNs, maximum number of *O. gingeri* (IISR-EPN 07) IJs attached, followed by *Heterorhabditis* sp. (IISR-EPN 01) after 3 h of inoculation. Maximum number of *Heterorhabditis* sp. (IISR-EPN 01) IJs penetrate into grub, followed by *O. gingeri* (IISR-EPN 07). The lowest rate of penetration was found in the *Oscheius* sp. (IISR-EPN 05). In case of infectivity of EPNs, *Heterorhabditis* sp. (IISR-EPN 01) and *O. gingeri* (IISR-EPN 07) caused 100 % mortality to root grub, followed by *S. ramanai* (IISR-EPN 03) (83%). Whereas, *Steinernema* sp. (IISR-EPN 02), *S. carpocapsae* (IISR-EPN 06) and *Oscheius* sp. (IISR-EPN 08) brought about 67% mortality. However, *Oscheius* spp. (IISR-EPN 04 and 05) kill only 50% root grub after 72 h. Infectivity of these EPN against cardamom root grub is being reported for the first time. This opens a new non chemical option for management of insect pests of cardamom.

Key words: Entomopathogenic nematodes, cardamom, root grub, Biocontrol

One of the major constraints in agriculture production in India is sustained losses due to insect pests, diseases and other reasons, but in many instances it has been used to denote insects alone. One or more insect pests always associated with every crop, which cause economic loss to crop, their control is one of the major requirements for increase in crop productivity.

Root grub (*Basilepta fulvicorne* Jacoby) (Coleoptera: Chrysomelidae) is a major pest of cardamom nurseries and plantations of India (Varadarasan *et al*., 1988). The grubs feed on the roots leading to yellowing of leaves and gradual death of shoots. The yield loss is estimated to be 10-70% depending on the level of infestation (Varadarasan, 2013). Excessive and indiscriminate use of pesticides for the management of this pest could result in pesticide residues in the produce affecting human health and also causing other ecological hazards. There has been a renewed interest in developing environment-friendly pest management schedules in agriculture. Entomopathogenic nematodes (EPNs) have got little attention by researchers though they have a great potential in reducing pest population and with little manipulation their role can be enhanced (Ali *et al*., 2005).

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* (IISR-EPN 03), *S. carpocapsae* (IISR-EPN 06), *O. gingeri* (IISR-EPN 07) and *Oscheius* spp. (IISR-EPN 04, 05, and 08) against cardamom root grub. The attachment to and rate of penetration of these EPNs into tested insect was also undertaken.

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MATERIALS AND METHODS

Entomopathogenic nematodes sources

Infective juveniles (IJs) of tested EPNs were obtained from nucleus culture of nematodes maintained in the Nematology Laboratory, ICAR-Indian Institute of Spices Research (IISR), Kozhikode. All tested EPN were cultured as per the procedure described by Kaya and Stock (1997). Fresh harvested IJs were surface sterilized with 0.1% Hyamine solution and stored in distilled water in tissue culture flasks for study.

Insect sources

Greater wax moth, *Galleria mellonella* reared on artificial diet as per the procedure described by David and Kurup (1988). The test insect, cardamom root grub was collected from cardamom fields at ICRI Experimental Farm and farmers fields of Idukki District.

IJs attachment to root grub

Attachment of test EPNs to root grub tested in petri plates. One root grub was kept in each plate and 100 IJs of tested EPNs were released and kept at room temperature. The treatments were replicated 10 times. The number of IJs attached to the insect was counted by the washing the insect in a counting dish with distilled water after 3, 6, 12 and 24 h.

IJs penetration into root grub

The penetration rate assay was conducted as described by Caroli *et al*. (1996). About 100 IJs of respective EPNs

were inoculated in the petri plate containing one grub/ plate and kept at room temperature. Each treatment consisted of ten replicates. Number of penetrated IJs was determined by dissecting the dead grub in Ringer's solution after 72 h.

Infectivity of EPNs against root grub

Infectivity of EPNs against root grub was tested in petri plates. For this, single grub was kept in plastic pot containing sterilized soil with small piece of cardamom root and 500 IJs in 1 ml water of each tested species of EPNs were inoculated. The mortality of root grub was recorded after 72 h. The experiment was conducted at room temperature and replicated twelve times along with control. The mortality of root grub was calculated into per cent.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and means compared according to Duncan´s multiple range test (DMRT). Before analysis, data of penetration and infectivity of EPNs 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

The study indicated that, all the tested EPNs were pathogenic to cardamom root grub but attachment, penetration of IJs and per cent mortality of tested insect was varied from species to species.

IJs attachment to root grub

All tested EPNs IJs attached into root grub, whereas significant differences (df = 7, 39; F= 7.39; *P* = 0.003) was found in the attachment of IJs. Among the tested EPNs, maximum number of *O. gingeri* (IISR-EPN 07) (6 IJs/grub) IJs attached into grub (11.5 IJs/grub), followed by *Heterorhabditis* sp. (IISR-EPN 01) (3 IJs/grub) after 3 h of inoculation, whereas no attachment of *Steinernema* sp. (IISR-EPN 02), *S. carpocapsae* (IISR-EPN 06) and *Oscheius* sp. (IISR-EPN 04 and 08) was found after 3 h. However, maximum attachments of all tested EPNs were found after 12 and 24 h post exposure (Fig. 1).

Table 1. Number of IJs penetrated in the root grub.

EPNs	No. of IJs/grub
Heterorhabditis sp. (IISR-EPN 01)	11.50[a]
Steinernema sp. (IISR-EPN 02)	8.30[bc]
S. ramanai (IISR-EPN 03)	8.60[bc]
Oscheius sp. (IISR-EPN 04)	3.10[e]
Oscheius sp. (IISR-EPN 05)	2.30[ef]
S. carpocapsae (IISR-EPN 06)	7.20[cd]
Oscheius sp. (IISR-EPN 07)	9.20[b]
Oscheius sp. (IISR-EPN 08)	6.00[d]

IJs penetration into root grub

All tested EPNs IJs penetrated into root grub, whereas significant differences (df = 6, 32; F= 6.34; *P* = 0.003) was found in the penetration of IJs. Among the tested EPNs, maximum number of *Heterorhabditis* sp. (IISR-EPN 01) IJs penetrate into grub (11.5 IJs/grub), followed by *O. gingeri* (IISR-EPN 07) (9.2 IJs/grub). The lowest rate of penetration was found in the *Oscheius* sp. (IISR-EPN 05) (2. 3 IJs/grub) (Table 1).

Infectivity of EPNs against root grub

The entire test EPNs were pathogenic against root grub but the rate of mortality was significantly varied (P <0.05). Among the tested EPNs, *Heterorhabditis* sp. (IISR-EPN 01) and *O. gingeri* (IISR-EPN 07) caused 100 % mortality to root grub, followed by *S. ramanai* (IISR-EPN 03) (83%). Whereas, *Steinernema* sp. (IISR-EPN 02), *S. carpocapsae* (IISR-EPN 06) and *Oscheius* sp. (IISR-EPN 08) brought about 67 % mortality. However, *Oscheius* spp. (IISR-EPN 04 and 05) kill only 50% root grub after 72 h. No mortality of root grub was recorded in the controls (Fig. 2).

Pathogenicity studies have shown considerable inter and intraspecific variations in infectivity of different isolates of entomopathogenic nematodes (Menti *et al.,* 2000) which have been attributed to the variation in the ability of the IJ to find and enter a host (Griffin *et al.*, 1989) as well as the different host susceptibility among various insects (Pervez *et al*., 2012). However, there is considerable variation in the infectivity of EPNs and no single species or strain is suitable for controlling all or even most insect species (Simoes and Rosa, 1996).

Penetration of the IJs of *Heterorhabditis* sp. showed highest penetration and was superior to *Steinernema* spp. and *Oscheius* spp., never the less the rate of penetration is also depends on the IJs infection strategies (cruiser and abuser). These results might suggest that the new isolates were of different species or natural variability within root grub. Similarly, Tomalak (2004) observed variation in IJs penetration between native isolates of EPNs originating from collection sites located within a short distance, suggesting that variation in infection can be observed between EPNs of different species but also between strains of the same EPN species (Mwaitulo, 2011).

Therefore, it is concluded that cardamom root grub was susceptible to tested EPNs, there were differences among these EPNs in their ability to kill the insect. Among test EPNs, *Heterorhabditis* sp. (IISR-EPN 01) and *O. gingeri* (IISR-EPN 07) appears to be the most promising EPN against root grub. The infectivity of these EPNs against cardamom root grub is reported for the first time which opens up new vistas in eco-friendly insect pest management in cardamom. Therefore, further work is required to confirm these results under field conditions.

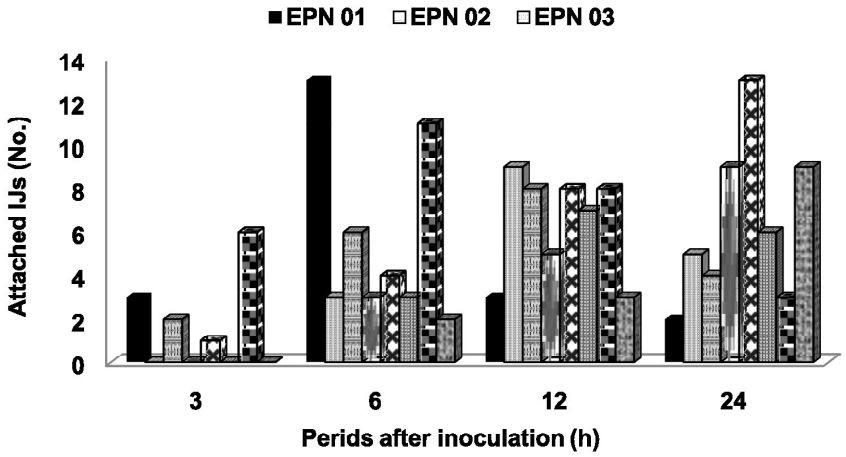

Fig. 1. Attachment of IJs to cardamom root grub

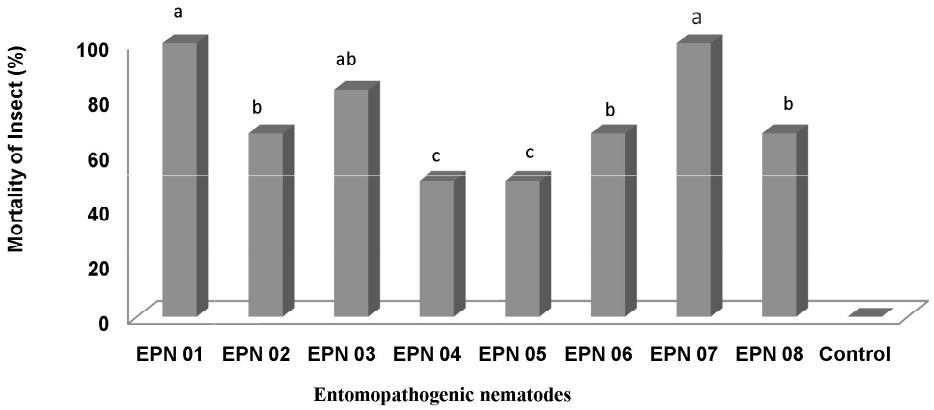

Fig. 2. Mortality of cardamom root grub

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