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Penetration and infectivity of entomopathogenic nematodes against *Lema* sp. (Chrysomelidae: Coleoptera) infesting turmeric (*Curcuma longa* L.) and their multiplication

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Abstract

Penetration and infectivity of eight native isolates of entomopathogenic nematodes (EPNs), *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 evaluated against larvae of leaf feeder (LF) *Lema* sp. infesting turmeric. Among the tested EPNs, *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* (IISR-EPN 07) were more pathogenic to LF larva as they brought about 100% mortality to the insect within 48 h post exposure, followed by *Heterorhabditis* sp. (IISR-EPN 01) and *Oscheius* sp. (IISR-EPN 08) after 72 h of exposure. *S. ramanai* (IISR-EPN 03) and *Oscheius* spp. (IISR-EPN 04 and 05) took 96 and 120 h, respectively, to kill the test insect. *Lema* sp. larva was the most suitable host for multiplication of infective juveniles (IJs) of *O. gingeri* (IISR-EPN 07), which yielded 11, 480 IJs larva^{-1}, followed by *Steinernema* sp. (IISR-EPN 02) (8, 658 IJs larva^{-1}) and *S. carpocapsae* (IISR-EPN 06) (6, 810 IJs larva^{-1}), however, *Heterorhabditis* sp. (IISR-EPN 01) less multiplied. The maximum number of *Steinernema* sp. (IISR-EPN 02) IJs penetrated into test larva (17.5 IJs larva^{-1}), followed by *S. carpocapsae* (IISR-EPN 06) (10.2 IJs larva^{-1}) and the fewest (2.8 IJs larva^{-1}) were of *Oscheius* sp. (IISR-EPN 08). The infectivity of the above EPNs against LF is being reported for the first time which opens up a new hope of utilizing them in insect pest management in turmeric.

Keywords: bio-control, *Curcuma longa,* infectivity, *Lema* sp., multiplication

Introduction

Turmeric "the golden spice" is one of the essential spices used as an important ingredient in culinary, medicinal, cosmetic, dyeing and colouring all over the world. More than 70 insect pests have been found to infected turmeric crop (Devasahayam *et al*. 2012). Among them, leaf feeder (LF), *Lema* sp. (Chrysomelidae: Coleoptera) is an important insect pest infesting turmeric. *Lema* sp. feed on the leaf, scraping the chlorophyll, resulting in the formation of white patches on the leaf which develop into holes on maturation of leaves. The damage reaches its peak during monsoon period. The only effective method to manage *Lema* spp. is the use of insecticides causing ecological hazards. There is a need to identify suitable

alternative methods for managing this insect pest.

Entomopathogenic nematodes (EPNs) are emerging as a potent biocontrol agent against a variety of insect pests infesting different crops (Shapiro *et al*. 2002; Pervez *et al.* 2012). There is scope of using EPNs in turmeric, which will take care of this insect pest and reduce the damage caused by them.

Some EPNs are more efficacious against a particular insect group than against another insect group. In addition, proper match of the nematode to the host entails virulence, host finding and ecological factors. If a nematode does not possess a high level of virulence towards the target pest, there is little hope of success (Georgis 2004). Bedding *et al*. (1983) indicated the importance of screening several nematode species against the target insect in the laboratory before commencing field evaluations. Therefore, investigations were conducted in 2012-13, to test the penetration and infectivity of eight native EPNs *viz*., *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) against LF larva and their multiplication was also assessed.

Materials and methods

Nematode and insect cultures

Infective juveniles (IJs) of tested EPNs were obtained from nucleus culture of nematodes maintained in the Nematology Laboratory, Indian Institute of Spices Research (IISR), Kozhikode. All tested EPNs were cultured as per the procedure described by Kaya & Stock (1997). The IJs were surface sterilised in 0.1% Hyamine solution and stored in distilled water in tissue culture flasks for study. However, only fresh nematode culture was used in the bioassays.

Greater wax moth, *Gallaria mellonella* reared on artificial diet as per the procedure described by David & Kurup (1988) and leaf feeder *Lema* sp. larvae were collected from turmeric fields, IISR-Experimental Farm, Peruvannamuzhi, Kozhikode District. The larvae were sorted out and those of same size were used for the study.

Infectivity assay

Infectivity of EPNs against LF larvae was tested in six well plates. One larva of tested insect was kept in each well and treated 100 IJs in 50 µL water with the respective EPN. The mortality of *Lema* sp. was recorded at 24 h interval up to 120 h. Each isolate of EPN was tested separately. The experiment was conducted at room temperature and replicated 12 times along with control. The mortality was calculated expressed in percentage.

Multiplication of EPNs

The EPN infected insects were kept on the White trap (White 1927) for emergence of IJs. 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.

Penetration rate assay

The penetration rate assay was conducted as described by Caroli *et al*. (1996). About 100 IJs of respective EPNs were inoculated in the well plate containing one LF larva/well. Each treatment consisted of ten replicates. Number of penetrated IJs was determined by dissecting the dead cadaver in Ringer's solution after 72 h.

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 insect mortalities (%) were arcsine transformed. All means were transformed back to the original units for presentation.

Results and discussion

The results indicated that all tested EPNs were pathogenic to LF larvae. However, percentage mortality, rate of penetration and multiplication of IJs varied from species to species.

Infectivity assay

All tested EPNs caused 100% mortality to LF larva but mortality time was varied. Among the EPNs, *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* (IISR-EPN 07) were more pathogenic to LF larva, as they brought about 100% mortality to the insect within 48 h post exposure, followed by *Heterorhabditis* sp. (IISR-EPN 01) and *Oscheius* sp. (IISR-EPN 08) after 72 h of exposure. However, *Oscheius* sp. (IISR-EPN 04) and *Oscheius* sp. (IISR-EPN 05) took maximum time (120 h) to kill the test insect. No mortality was observed in control (Fig. 1).

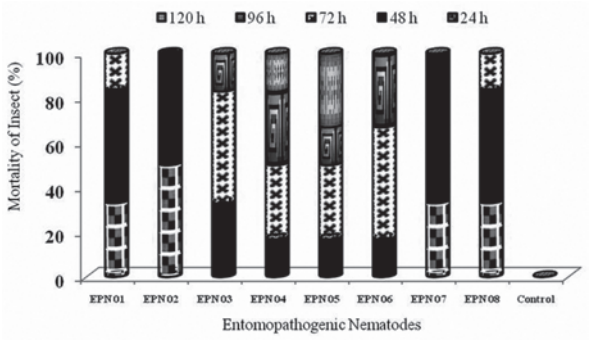

Fig. 1. Mortality of *Lema* sp. caused by different entomopathogenic nematodes. EPN 01=*Heterorhabditis* sp. (IISR- EPN 01); EPN 02=*Steinernema* sp. (IISR-EPN 02); EPN 03=*S. ramanai* (IISR-EPN 03); EPN 04=*Oscheius* sp. (IISR-EPN 04); EPN 05=*Oscheius* sp. (IISR-EPN 05); EPN 06=*S. carpocapsae* (IISR-EPN 06); EPN 07=*O. gingeri* (IISR-EPN 07) and EPN 08=*Oscheius* sp. (IISR-EPN 08).

EPNs demonstrated great variation in their infectivity in different hosts and none of the species/strains of EPMS infected all the insect species and some species or strains were reported as highly host specific (Georgis & Manweiler 1994). Gaugler *et al.* (1989) found a similar variation among geographically distant strains of *S. carpocapsae*. Hence, laboratory screening of EPNs for infectivity is an important component for developing a biological control programme for a particular pest (Ricci *et al*. 1996). Our study is supported by earlier workers and indicated the variation in mortality percentage within Steinernematids, Heterorhabditids and Rhabditid groups. This suggested that, neither group was superior to the other.

Multiplication of EPNs

All the EPNs isolates multiplied on the tested insects, but the level of multiplication varied within EPN isolates. Among the EPNs, *O. gingeri* (IISR-EPN 07) and *Steinernema* sp. (IISR-EPN 02) multiplied maximum number (11,480 IJs larva^{-1} and 8,658 IJs larva^{-1}, respectively), followed by *S. carpocapsae* (IISR-EPN 06) (6,810 IJs larva^{-1}). Whereas, infective juveniles of *Heterorhabditis* sp. (IISR-EPN 01) were produced in lesser number (675 IJs larva^{-1}) (Fig. 2).

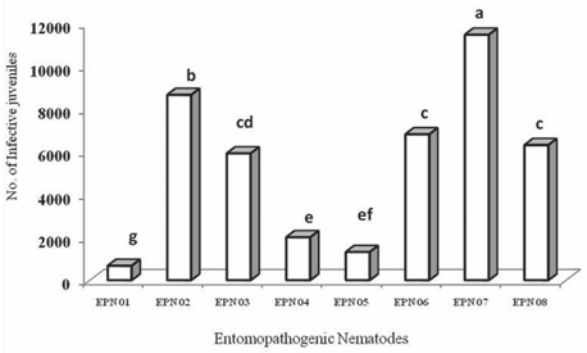

Fig. 2. Multiplication of different entomopathogenic nematodes on leaf feeder. EPN 01=*Heterorhabditis* sp. (IISR- EPN 01); EPN 02=*Steinernema* sp. (IISR-EPN 02); EPN 03=*S. ramanai* (IISR-EPN 03); EPN 04=*Oscheius* sp. (IISR-EPN 04); EPN 05=*Oscheius* sp. (IISR-EPN 05); EPN 06=*S. carpocapsae* (IISR-EPN 06); EPN 07=*O. gingeri* (IISR-EPN 07) and EPN 08=*Oscheius* sp. (IISR-EPN 08).

EPNs can be multiplied on *Chilo sacchariphagus indicus* (Karunakar *et al.* 1999), *G. mellonella, Helicoverpa armigera* and *Corcyra cephalonica* (Ali *et al*. 2008), *Athalia proxima, Spodoptera litura* and *Conogethes punctiferalis* (Pervez *et al*. 2007; 2009; 2012) with varying yields of IJs depending upon the size of larvae of the test insects and reproductive behaviour of the EPN species (Ali *et al*. 2005; Pervez *et al*. 2010).

Penetration rate assay

All tested EPNs penetrated into LF larvae, whereas significant differences (df=7, 40; F=5.39; p=0.003) were found in the penetration of IJs. Among the tested species, the maximum

number of *Steinernema* sp. (IISR-EPN 02) IJs penetrated into larva (17.5 IJs larva^{-1}), followed by *S. carpocapsae* (IISR-EPN 06) (10.2 IJs larva^{-1}). However, the fewest number (2.8 IJs larva^{-1}) of *Oscheius* sp. (IISR-EPN 08) IJs penetrated into the test insect body (Table 1).

Table 1. Number of IJs penetrated in *Lema* sp. larvae

Entomopathogenic Nematodes	No. of IJs larva^{-1}
Heterorhabditis sp. (IISR-EPN 01)	6.89^{bcd}
Steinernema sp. (IISR-EPN 02)	17.50^{a}
S. ramanai (IISR-EPN 03)	7.21^{bcd}
Oscheius sp. (IISR-EPN 04)	3.89^{cd}
Oscheius sp. (IISR-EPN 05)	3.63^{cd}
S. carpocapsae (IISR-EPN 06)	10.14^{b}
O. gingeri (IISR-EPN 07)	8.46^{bc}
Oscheius sp. (IISR-EPN 08)	2.79^{d}

Penetration of the IJs of Steinernematids showed highest penetration and was superior to heterorhabditids. The penetration was least in the case of *Oscheius* spp. except *O. gingeri*. Our results supported by previous study (Sankarnarayanan *et al*. 2011; Pervez *et al*. 2012). The differences in penetration of IJs in the present study might be due to the infection strategies of nematodes. The rate of penetration could be used as a real measure of host infection. These results suggested that the native isolates were of different species or natural variability within LF larva.

In the present study, *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* (IISR-EPN 07) were found to be superior to other tested IJs in terms of penetration, mortality of LF and multiplication. EPNs have not been previously used for the biological control of LF larva infesting turmeric, and this is the first report of their possible use as a biological control agent against insect pest infesting turmeric. Therefore, more investigations on their mass production, formulation and performance under field conditions should be encouraged.

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