Research Article

doi:10.19071/jpc.2017.v45.i3.3342
Journal of Plantation Crops, 2017, 45(3):180-189

Screening of native entomopathogenic nematodes against semi-looper (*Synegia* sp.) infesting black pepper (*Piper nigrum* L.)

Rashid Pervez*, S.J. Eapen[1] and Rajkumar[2]

Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi-110 012, India
[1]ICAR-Indian Institute of Spices Research, Kozhikode - 673 012, Kerala, India
[2]ICAR-Central Plantation Crops Research Institute, Kasaragod - 671 124, Kerala, India

(Manuscript Received: 12-09-17, Revised: 28-10-17, Accepted: 09-11-17)

Abstract

Several insect pests damage and caused economic losses to black pepper (*Piper nigrum* L.). Among them semi-looper (*Synegia* sp.) is an important pest especially in younger vines of black pepper. Pesticides are currently being used to manage this pest leading to environmental and health concerns. Screening for virulence of eight native entomopathogenic nematodes were tested against the semi-looper (SL). The suitability of this insect for multiplying EPNs, attachment to and penetration into host was also studied. Among test EPNs, *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* (IISR-EPN 07) were more virulent to semi-looper as they caused 100 per cent mortality to SL within 72 h. Maximum number of infective juveniles (IJs) attached into larva at 6 and 12 h post exposure. Maximum number of *Steinernema* sp. (IISR-EPN 02) IJs penetrated into larva (12.5 IJs per larva), followed by *Heterorhabditis* sp. (IISR-EPN 01) (8.7 IJs per larva) and only 5.9 IJs per larva of *O. gingeri* (IISR-EPN 07) penetrated into the test insect body. Maximum multiplication of IJs was observed with *O. gingeri* (IISR-EPN 07), which yielded 9,324 IJs per larva within 15 days, followed by *Oscheius* sp. (IISR-EPN 04) (8,638 IJs per larva) and *Oscheius* sp. (IISR-EPN 08) (8,236 IJs per larva). The infectivity of EPNs against semi-looper (*Synegia* sp.) is being reported for the first time which opens up a new vista in eco-friendly insect pest management in black pepper.

Keywords: Black pepper, entomopathogenic nematodes, semi-looper, *Synegia* sp.

Introduction

Black pepper production in Kerala is in steady decline due to several reasons, among which insect pests is a major constraints. Among them, semi-looper *Synegia* sp. (Lepidoptera: Geometridae) infestation causes reduction in pepper yield. The larvae of *Synegia* sp. feed on tender leaves and spikes of younger vines of black pepper during monsoon season (Devasahayam, 2008). The larvae are reported to damage up to 52 per cent of leaves and 17 per cent of spikes in Kozhikode district of Kerala (Premkumar and Devasahayam, 1989).

Pesticides are currently being used to manage this pest leading to environmental and health concerns and result in the suppression of other naturally occurring biocontrol agents as well as developing resistance against pesticides. There is a need to identify suitable alternative methods for managing this insect pest. Recently, entomopathogenic nematodes (EPNs) are emerging as potent biocontrol agents against a variety of insect pests of crops due to their wide host range, easy to handle, short life cycle, environmentally safe and economically multiplied at large scale (Ali *et al*., 2005; 2008; Pervez *et al*., 2007; Pervez *et al*., 2012). EPNs are symbiotically associated with bacteria which are released into the insect hemocoel, causing causing septicemia and death of the insect (Pervez *et al*., 2015a).

**Corresponding Author: rashidpervez2003@gmail.com*

Some EPNs are more virulent against a particular insect group than against another insect group. In addition, proper combination of the EPN to the host entails virulence, host finding and ecological factors. If EPN 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 of several EPNs against the target insect in the laboratory before commencing field evaluations.

EPNs have been found effective against a variety of insect pest infesting spice crops (Pervez *et al*., 2012; 2014 a,b; 2016). There is scope of using EPN in black pepper, which will take care of insect pest and reduce the damage caused by them and thus these will be an ideal alternative to chemicals, economical and will fit in long term pest management system without risk to non-target organisms.

Objective of the study was to screen the virulence of native EPNs against semi-looper (*Synegia* sp.) infesting black pepper. Attachment, penetration and suitability of this insect for multiplication of infective juveniles (IJs) of EPNs, LC_{50} and LT_{50} of the promising EPNs were also calculated.

Materials and methods

Sources of entomopathogenic nematode

The IJs of eight native EPNs were obtained from nucleus culture of nematodes maintained in the Nematology Laboratory, ICAR-Indian Institute of Spices Research (IISR), Kozhikode (Table 1). All tested EPNs were cultured on greater wax moth, *Gallaria mellonella* larvae. The IJs were surface sterilized in 0.1 per cent hyamine solution and stored in distilled water in tissue culture flasks for study.

Insect sources

Greater wax moth, *G. mellonella* larvae cultured as per the procedure described by Kaya and Stock (1997). The test insect, semi-looper (*Synegia* sp.) was collected from ICAR-IISR Experimental Farm, Peruvannamuzhi, Kozhikode and ICAR-CPCRI Experimental Farm, Kasaragod. Larvae of same size were taken for present study.

Infectivity of EPNs against semi-looper

Infectivity of EPNs against semi-looper (*Synegia* sp.) was tested in Petri plates. For this, single insect was kept in each plate with small piece of black pepper leave and 100 IJs in 0.5 mL water of each tested species of EPNs (Table 1) were inoculated and their mortality was recorded at 24 h interval. Each species of EPN was tested separately and singly. The experiment was conducted at room temperature and replicated six times along with control. The mortality was calculated according to following formula.

Mortality (%) = D/N x 100
where, D = number of dead larvae and
N = total number of larvae

Determination of lethal concentrations (LC_{50})

Lethal concentrations were calculated using four densities *viz*., 25, 50, 75 and 100 IJs of three promising EPNs, *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* (IISR-EPN 07) against semi-looper (*Synegia* sp.). Five insect were kept per plate with small piece of black pepper leaves and suspension of each density of tested EPN was inoculated into Petri plates lined with filter paper at the bottom of the plate. Mortality of semi-looper was recorded at 72 h. The experiment was conducted at room temperature and replicated six times along with controls. The percentage mortality calculated using Abbott's formula (Abbott, 1925) and mean values were worked out. The LC_{50} values were calculated using regression analysis. Correlation between concentrations and mortality of insect was also determined.

Determination of lethal time (LT_{50})

Lethal time was calculated using one EPN density @ 100 IJs per Petri plate. All experimental condition same as to determine LC_{50} except the mortality of the larvae recorded at 24, 48, 72 and 96 h of post inoculation. The percentage mortality calculated using Abbott's formula (Abbott, 1925) and mean values were worked out. The LT_{50} values were calculated using regression analysis. Correlation between exposure time and mortality of semi-looper (*Synegia* sp.) was also calculated.

Attachment of IJs to semi-looper

Attachment of three promising EPNs *viz*., *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp.

Table 1. Entomopathogenic nematodes used in bioassay

Family	Genus	Isolates
Heterorhabditidae (Poinar, 1976)	*Heterorhabditis* sp. (Poinar, 1976)	*Heterorhabditis* sp. (IISR-EPN 01) (Pervez *et al*., 2014a)
Steinernematidae (Chitwood and Chitwood, 1937)	*Steinernema* spp. (Steiner, 1923)	*Steinernema* sp. (IISR-EPN 02) (Pervez *et al*., 2014a) *S. ramanai* (IISR-EPN 03) (Pervez *et al*., 2011) *S. carpocapsae* (IISR-EPN 06) (Pervez *et al*., 2015b)
Rhabditidae (Poinar, 1976)	*Oscheius* spp. (Andrassy, 1976)	*Oscheius* sp. (IISR-EPN 04) (Pervez *et al*., 2014a) *Oscheius* sp. (IISR-EPN 05) (Pervez *et al*., 2014b) *O. gingeri* (IISR-EPN 07) (Pervez *et al*., 2013) *Oscheius* sp. (IISR-EPN 08) (Pervez *et al*., 2014b)

(IISR-EPN 02) and *O. gingeri* (IISR-EPN 07) to semi-looper (*Synegia* sp.) tested in six well plates. One larva of semi-looper was kept in each well and 100 IJs of tested EPNs were released and kept at room temperature. The treatments were replicated six times. The number of IJs attached to the semi-looper was counted by the washing the insect in a counting dish with distilled water after 3, 6, 12 and 24 h.

Penetration of IJs in to semi-looper

The penetration rate assay was conducted as procedure described by Pervez *et al.* (2014b). About 100 IJs of promising EPNs *viz*., *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* (IISR-EPN 07) were inoculated in the well plate containing one larva per well and kept at room temperature. Each treatment replicated six times. Number of penetrated IJs was determined by dissecting the dead cadaver after 72 h.

Multiplication of EPNs

EPN infected dead larva were removed from the Petri plate and kept on the White trap (White, 1927) for emergence of IJs from the body of larva. IJs were collected daily, till the emergence stopped in about 15 days. Collected IJs were counted three times under a stereoscopic binocular microscope with the help of Syracuse counting dish and mean values were worked out.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and means compared according to Duncan´s multiple range tests. Before analysis, data of mortality and multiplication of the 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 results indicated that, all tested EPNs were virulent against semi-looper (*Synegia* sp.). Although, attachment, penetration, mortality (%) and multiplication number of IJs varied from EPN to EPN. However, concentrations and exposures time had a significant effect on the mortality of semi-looper.

Infectivity of EPNs against semi-looper

All test EPNs were virulent against semi-looper, but insect mortality time was varied. Among test EPNs, *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* (IISR-EPN 07) were more virulent to semi-looper as they caused 100 per cent mortality to the SL within 72 h, followed by *S. ramanai* (IISR-EPN 03), *S. carpocapsae* (IISR-EPN 06) and *Oscheius* sp. (IISR-EPN 08). The other species took 120 h, to kill the test insect. No mortality was observed in control (Fig. 1).

Some EPN species or strains were reported as highly host specific and demonstrate great variation in their infectivity considerably in different hosts and none of the species or strains of EPN infected all the insect pests (Shapiro and Mc Coy, 2000; Ali *et al.,* 2008). Gaugler *et al.* (1989) found a similar variation among geographically distant strains of

S. carpocapsae. Our studies supported by earlier workers, the variation in mortality percentage within Steinernematids, Heterorhabditids and Rhabditid groups indicated that, neither group was superior to the other. The variation in efficiency of the various EPNs may be due to the difference in the bacterial symbionts (Boemare, 2002) and preferential feeding behaviour (Bilgrami and Gaugler, 2009; Pervez, 2011). Hence, laboratory screening of EPNs for infectivity an important component of developing a biological control programme for a particular pest (Ricci *et al*., 1996).

Determination of LC_{50}

Result shows that, mortality per cent of semi-looper increases with the increase the number of IJs. Among the test densities, maximum mortality was found in 100 IJs per plate, whereas minimum mortality was in 25 IJs per plate. Mortality was also observed when treated @ 25 IJs per plate for all test EPNs except *O. gingeri* (IISR-EPN 07), whereas it was observed at 50 IJs per plate (Fig. 3). A significant and positive correlation was found between IJs density and insect mortality. Probit analysis indicated that, *Steinernema* sp. (IISR-EPN 02), and *Heterorhabditis* sp. (IISR-EPN 01) required less number (51 and 54 IJs per larva, respectively), whereas *O. gingeri* (IISR-EPN 07) need maximum number (78 IJs per larva) to provoke 50 per cent mortality of semi-looper at 72 h post inoculation (Fig. 2).

Determination of LT_{50}

Semi-looper mortality per cent increases with the increase the exposure time (Fig. 4). All tested EPNs induced mortality of semi-looper within 24 h. A significant and positive correlation was found between exposure time and mortality. Time assay response revealed that *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* (IISR-EPN 07) took less time (31 and 38 h, respectively) to kill 50 per cent semi-looper, whereas *Heterorhabditis* sp. (IISR-EPN 01) took more time to kill insect (42 h) (Fig. 3).

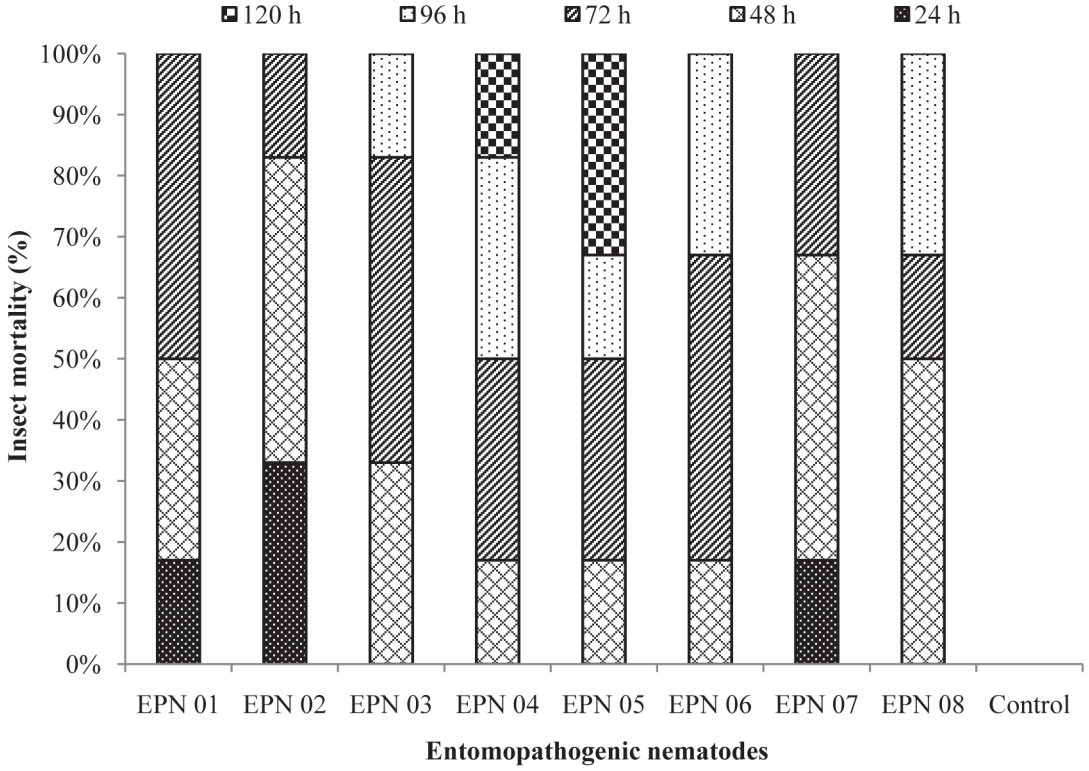

Fig. 1. Mortality of semi-looper *Synegia* sp. EPN 01 - *Heterorhabditis* sp.; EPN 02 - *Steinernema* sp.; EPN 03 - *S. ramanai*; EPN 04 - *Oscheius* sp.; EPN 05 - *Oscheius* sp.; EPN 06 - *S. carpocapsae*; EPN 07 - *O. gingeri* and EPN 08 - *Oscheius* sp.

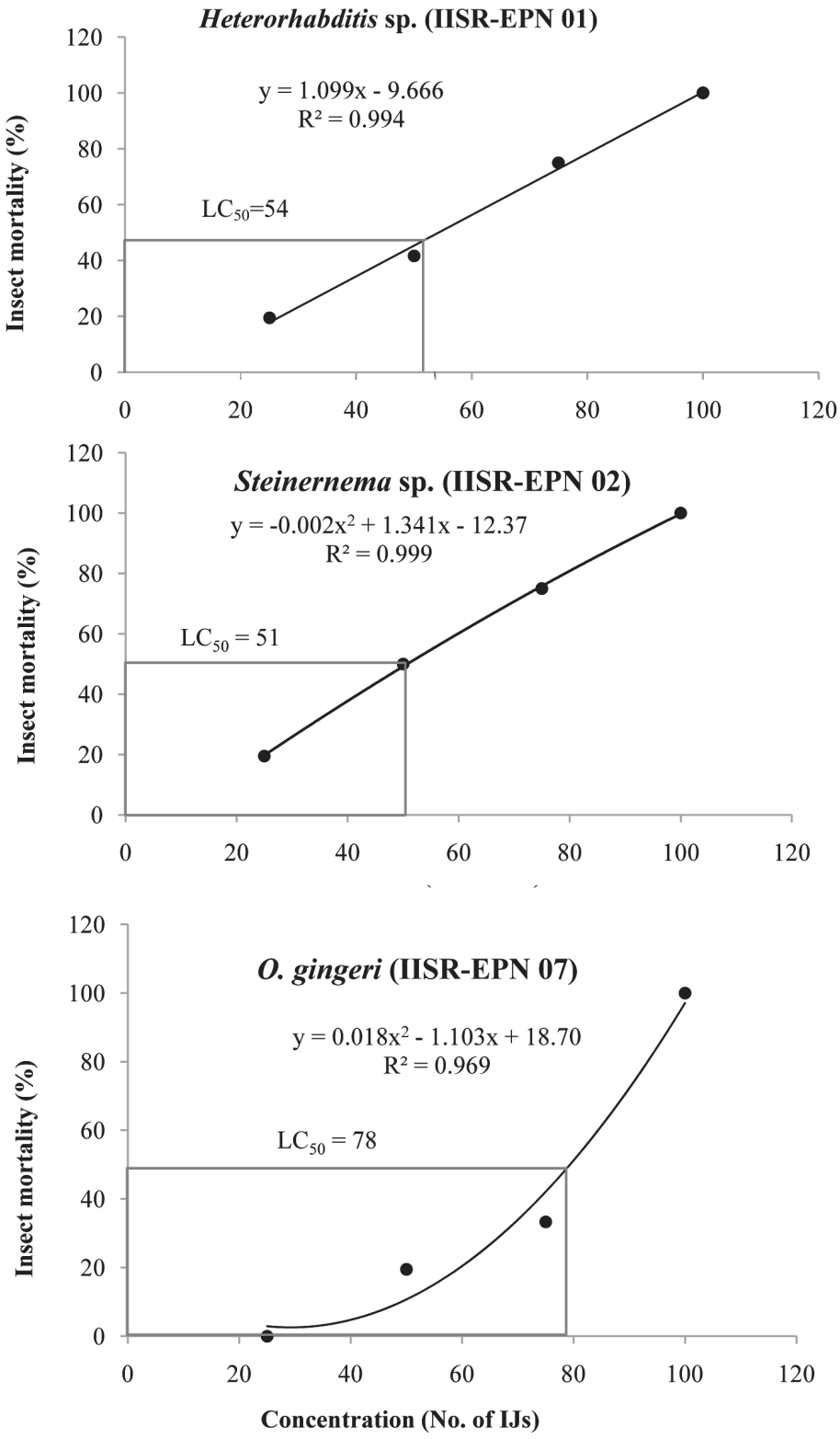

Fig. 2. LC_{50} value of the entomopathogenic nematodes against semi-looper *Synegia* sp.

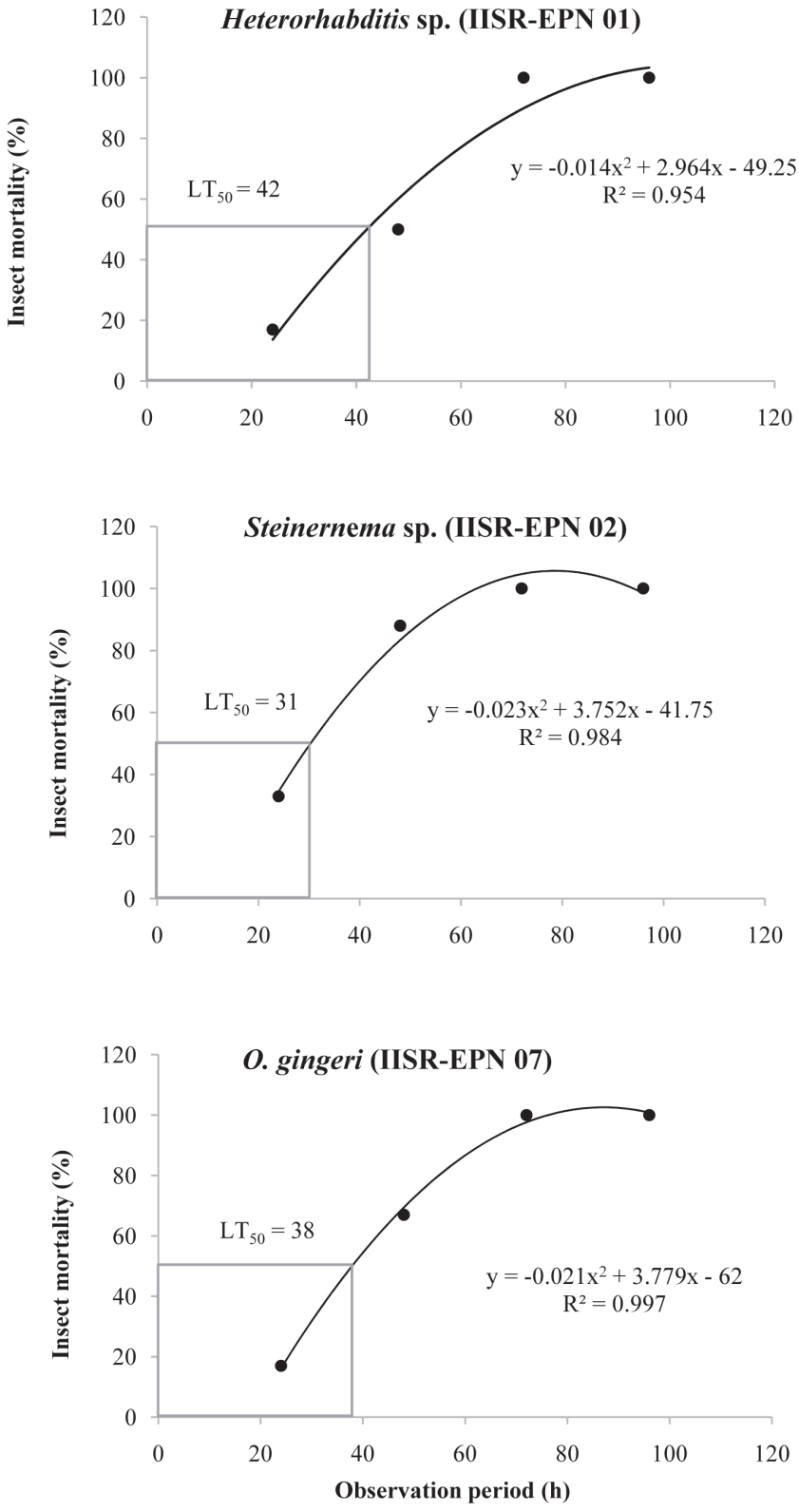

Fig. 3. LT_{50} value of the entomopathogenic nematodes against semi-looper *Synegia* sp.

Although, the semi-looper was susceptible to test EPNs, there were differences among these EPNs in their ability to mortality of the insect. Among test EPNs, *Steinernema* sp. (IISR-EPN 02) was most virulent against semi-looper. The mortalities were higher, LC_{50} values were lower and LT_{50} values were shorter for this EPN. These differences may be due to difference of the origins of the strains (Mbata and Sapiro-Ilan, 2005; Canhilal, 2012) and preferential feeding behaviour (Bilgrami and Gaugler, 2009; Pervez, 2011).

Attachment of IJs to semi-looper

All tested EPNs IJs attached to SL, whereas significant differences (df = 7, 41; F= 5.69; p = 0.003) was recorded in the attachment of IJs. Among the test EPNs, the maximum number of IJs attached to larva at 6 and 12 h post exposure (Fig. 4).

Penetration of IJs into semi-looper

All tested EPNs IJs penetrated into semi-looper, whereas significant differences (df = 6, 38; F= 6.12; p = 0.003) was found in the penetration of IJs. Among the tested species, the maximum number of *Steinernema* sp. (IISR-EPN 02) IJs penetrated into larva (12.5 IJs per larva), followed by *Heterorhabditis* sp. (IISR-EPN 01) (8.7 IJs per larva). However, the minimum number (5.9 IJs per larva) of *O. gingeri* (IISR-EPN 07) IJs penetrated into the test insect body (Table 2).

Table 2. Number of IJs penetrated in to semi-looper *Synegia* sp.

Entomopathogenic Nematodes	Number of IJs per larva
Heterorhabditis sp. (IISR-EPN 01)	8.7[ab]
Steinernema sp. (IISR-EPN 02)	12.5[a]
Oscheius gingeri (IISR-EPN 07)	5.9[c]

Values followed by different letters are significantly different

Penetration of the IJs of *Steinernema* spp. and *Heterorhabditids* sp. showed highest penetration and was superior to *Oscheius* sp. The present findings supported by previous study (Sankaranarayanan *et al*., 2011; Pervez *et al*., 2011; 2014a,b). The differences in penetration of IJs in the present study might be due to the infection strategies of EPNs. The rate of penetration could be used as a real measure of host infection. These results might suggest that different species of EPN or natural variability within semi-looper. However, Tomalak (2004) observed variation in IJs penetration between native EPNs originating from collection sites located within a short distance, suggesting that variation in infection can be

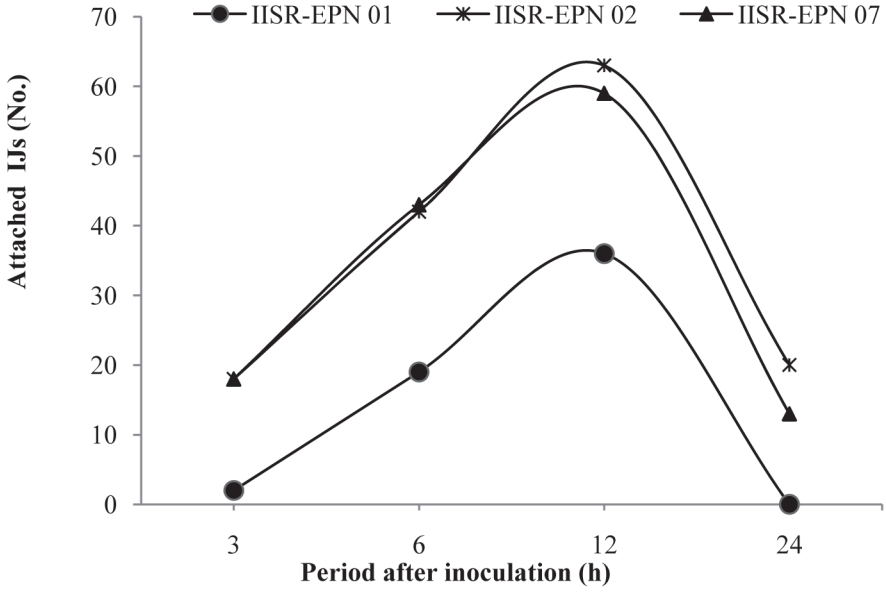

Fig. 4. Entomopathogenic nematodes attached to semi-looper *Synegia* sp. IISR-EPN 01- *Heterorhabditis* sp. (IISR- EPN 01); IISR-EPN 02 - *Steinernema* sp. (IISR-EPN 02) and IISR-EPN 07 – *O. gingeri*

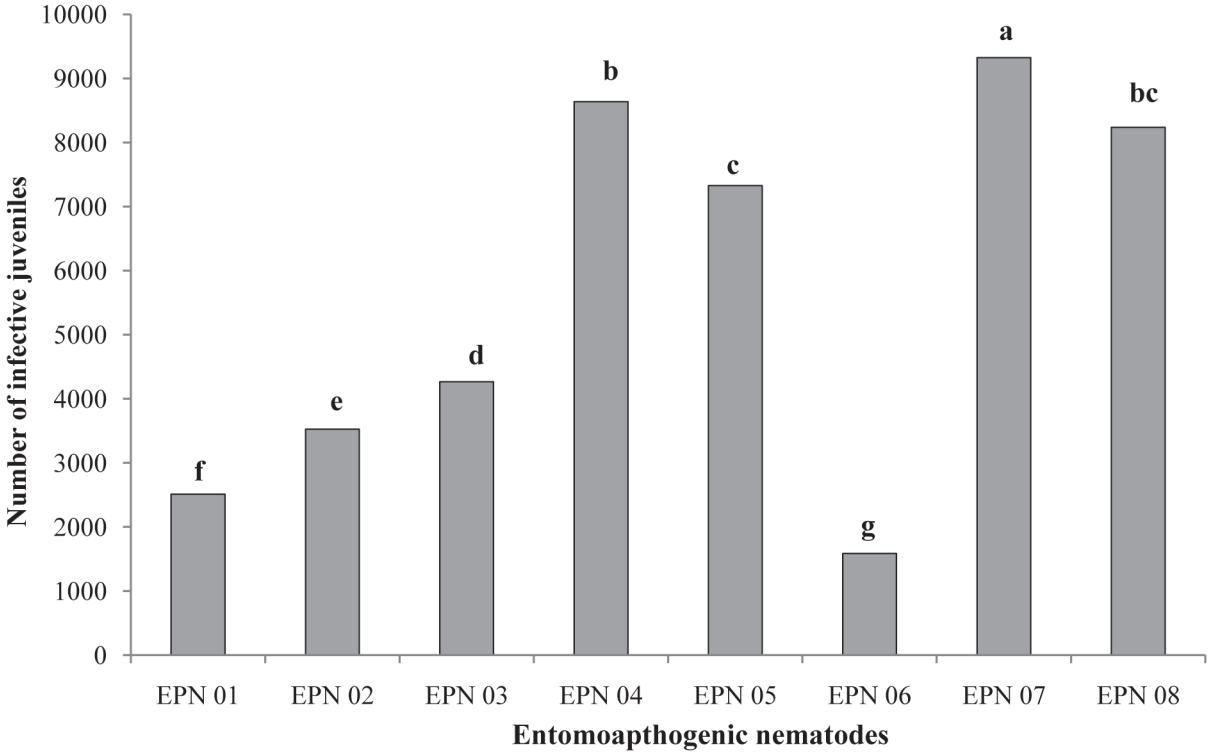

Fig. 5. Multiplication of infective juveniles (IJs) of entomopathogenic nematodes. EPN 01- *Heterorhabditis* sp. (IISR- EPN 01); EPN 02 - *Steinernema* sp. (IISR-EPN 02); EPN 03 - *S. ramanai*; EPN 04 - *Oscheius* sp. (IISR-EPN 04); EPN 05 - *Oscheius* sp. (IISR-EPN 05); EPN 06 - *S. carpocapsae*; EPN 07 – *O. gingeri* and EPN 08 - *Oscheius* sp. (IISR-EPN 08). Bars with different letters are significantly different

observed between EPNs of different species but also between isolate of same EPN species (Mwaitulo *et al.*, 2011; Pervez *et al*., 2014).

Multiplication of EPNs

All tested EPNs were multiplied on the semi-looper but rate of multiplication of IJs varied. Among the tested EPNs, maximum multiplication of IJs was observed with *O. gingeri*, which yielded 9,324 IJs per larva, followed by *Oscheius* sp. (IISR-EPN 04) (8,638 IJs per larva) and *Oscheius* sp. (IISR-EPN 08) (8,236 IJs per larva). However, *S. carpocapsae* (IISR-EPN 06) yielded the least number of IJs (1,586 IJs per larva) (Fig. 5).

Mass produced of EPNs *in vivo* where the insect serves as a small biological reactor. Greater wax moth, *G. mellonella* has been widely used for *in vivo* mass production of EPNs (Ali *et al*., 2005; 2008). Other insects, such as spotted borer, *Chilo sacchariphagus indicus* (Karunakar *et al.,* 1992; 1999), gram pod borer, *Helicoverpa armigera* and rice moth, *Corcyra cephalonica* (Ali *et al*., 2008; Pervez, 2010), mustard saw fly, *Athalia proxima* (Pervez *et al*., 2007), green bug, *Nezara viridula* (Pervez *et al.,* 2008), tobacco caterpillar, *Spodoptera litura* (Pervez and Ali, 2009), legume pod borer, *Maruca vitrata* (Pervez and Ali, 2011), shoot borer, *Conogethes punctiferalis* and hairy caterpillar, *Euproctis* sp. (Pervez *et al*., 2012) and turmeric leaf feeder, *Lema* sp. (Pervez *et al*., 2014) also suitable for multiplication of various species of *Steinernema, Heterorhabditis* and *Oscheius* with varying yields of IJs depending upon the size of larvae of the test insects and reproductive behaviour of the EPN species (Pervez and Ali, 2013; Pervez, 2010; 2011).

Conclusion

The infectivity of EPNs against semi-looper (*Synegia* sp.) is reported for the first time which will be valuable in designing eco-friendly insect pest management in black pepper. Among the EPNs studied, *Steinernema* sp. (IISR-EPN 02) was more virulent against semi-looper (*Synegia* sp.) infesting black pepper. Further work is required to confirm

these results in the green house and field conditions with this promising EPN against semi-looper (*Synegia* sp.).

Acknowledgements

The authors express their gratitude to Director, ICAR-Indian Institute of Spices Research, Kozhikode and Director, ICAR-Central Plantation Crops Research Institute, Kasaragod for providing all the facilities. Thanks also extended to Mr. K. Jayarajan, Assistant Chief Technical Officer, ICAR-IISR, Kozhikode (Kerala) for statistical analysis of data.

References

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**: 265-267.

Ali, S.S., Ahmad, R., Hussain, M.A. and Pervez, R. 2005. *Pest Management of Pulses Through Entomopathogenic Nematodes*. Indian Institute of Pulses Research, Kanpur, Army Press, Lucknow, India, 159 p.

Ali, S.S., Pervez, R., Hussain, M.A. and Ahmad, R. 2008. Susceptibility of three lepidopteran pests to five entomopathogenic nematodes and *in vivo* mass production of these nematodes. *Archives of Phytopathology and Plant Protection* **41**(4): 300-304.

Bedding, R.A., Molyneux, A.S. and Akhurst, R.J. 1983. *Heterorhabditis* spp., *Neoaplectana* spp., and *Steinernema kraussei*: Interspecific and intraspecific differences in infectivity for insects. *Experimental Parasitology* **55**: 249-257.

Bilgrami, A.L. and Gaugler, R. 2009. Feeding behaviour, In: *Nematodes Behaviour* (Eds.) Bilgrami, A.L. and Gaugler, R. CABI Publishing, UK, pp. 91-126.

Boemare, N. 2002. Biology, taxonomy and systematic of *Photorhabdus* and *Xenorhabdus*. In: *Entomopathogenic Nematology,* (Ed.) Gaugler, R. UK: CABI, pp. 35-56.

Canhilal, R. 2012. Comparison of efficacy of nine new heterorhabditid isolates (Rhabditida: Heterorhabditidae) in *Tenebrio molitor* (Coleoptera: Tenebrionidae). *African Journal of Microbiology Research* **6**(7): 1597-1602.

Chitwood, B.G. and Chitwood, B.G. 1937. *An Introduction to Nematology*. Monumental Printing Company, Batimore, Maryland, 213p.

Devasahayam, S. 2008. Biological control of insect pests. In: *Organic Spices*. (Eds.) Parthasarathy, V.A., Kandiannan, K. and Srinivasan, V. New India Publishing Agency, New Delhi. pp.133-152.

Gaugler, R, McGuire, T. and Campbell, J. 1989. Genetic variability among strains of the entomopathogenic nematode *Steinernema feltiae*. *Journal of Nematology* **21**: 247-253.

Georgis, R. 2004. Current and prospective markets for entomopathogenic nematodes. *International Journal of Nematology* **14**: 1-8.

Karunakar, G., David, H. and Easwaramoorthy, S. 1992. Influence of temperature on infectivity, penetration and multiplication of *Steinernema feltiae, S. glaseri* and *Heterorhabditis indicus* on mortality of the host and multiplication of infective juveniles in sugarcane inter node borer*, Chilo sacchariphagus indicus*. *Journal of Biological Control* **6**: 26-28.

Karunakar, G., Easwaramoorthy, S. and David, H. 1999. Susceptibility of nine lepidopteran insects to *Steinernema glaseri, S. feltiae* and *Heterorhabditis indicus* infection. *International Journal of Nematology* **9**: 68-71.

Kaya, H.K. and Stock, S.P. 1997. Techniques in insect nematology. In: *Manual of Techniques in Insect Pathology,* (Ed.) Lacey, L. A. Academic Press, San Diego, CA. pp. 281-324.

Mbata, G.N. and Sapiro-Ilan, D.I. 2005. Laboratory evaluation of virulence of heterorhabditid nematodes to *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). *Journal of Economic Entomology* **34**(3): 676-682.

Mwaitulo, S., Haukeland, S., Saethre, M.G., Laudisoit, A. and Maerere, A.P. 2011. First report of entomopathogenic nematodes from Tanzania and their virulence against larvae and adults of the banana weevil *Cosmopolites sordidus* (Coleoptera: Curculionidae). *International Journal of Tropical Insect Science* **31**(3): 154-161.

Premkumar, T. and Devasahayam S. 1989. Record of *Synegia* sp. (Lepidoptera: Geometridae) infesting black pepper (*Piper nigrum* L.). *Journal of the Bombay Natural History Society* **86**: 112-113.

Pervez, R., Ali, S.S. and Ahmad, R. 2007. Efficacy of some entomopathogenic nematodes against mustard saw fly and *in vivo* production of these nematodes. *International Journal of Nematology* **17**(1): 55-58.

Pervez, R., Ali, S.S. and Ahmad, R. 2008. Efficacy of entomopathogenic nematodes against green bug, *Nezara viridula* (L.) and their *in vivo* mass production. *Trends in Biosciences* **1**(1&2): 49-51.

Pervez, R. and Ali, S.S. 2009. Infectivity of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) by certain native entomopathogenic nematodes and their penetration in test insect and *in vivo* production. *Trends in Biosciences* **2**(2): 70-73.

Pervez, R. 2010. Biocontrol potential of entomopathogenic nematodes against different instar larvae of gram pod borer, *Helicoverpa armigera* infesting chickpea. *Current Nematology* **21**(2): 17 -21.

Pervez, R. 2011. Attraction behaviour of entomopathogenic nematodes towards different lepidopteran insect pests. *Current Nematology* **22**(1&2): 81-84.

Pervez, R. and Ali, S.S. 2011. Efficacy, penetration and *in vivo* production of entomopathogenic nematodes against legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Pyrilidae). *Trends in Biosciences* **4**(1): 103-105.

Pervez, R., Eapen, S.J., Devasahayam, S. and Jacob, T.K. 2011. A new species of entomopathogenic nematode *Steinernema ramanai* (Rhabditida: Steinernematidae) from ginger (*Zingiber officinale* Rosc.). In: *Proceedings of 13th Indian Agricultural Scientists and Farmers Congress,* Bioved Research Society, Allahabad, pp. 10.

Pervez, R., Eapen, S.J., Devasahayam, S. and Jacob, T.K. 2012. Efficacy of some entomopathogenic nematodes against insect pests of ginger and their multiplication. *Nematologia Mediterranea* **40**(1): 39-44.

Pervez, R. and Ali, S.S. 2013. Entomopathogenic nematodes as a potent biopesticide against insect pests, In: *Newer Approaches to Biotechnology*, (Ed.) Behra, K. K., Narendra Publishing House Publishers and Distributors, New Delhi, pp. 213-235.

Pervez, R., Eapen, S.J., Devasahayam, S. and Jacob, T.K. 2013. A new species of entomopathogenic nematode *Oscheius gingeri* sp. from ginger rhizosphere. *Archives of Phytopathology and Plant Protection* **46**(5): 526-535.

Pervez, R., Eapen, S.J., Devasahayam, S. and Jacob, T.K. 2014a. Natural occurrence of entomopathogenic nematodes associated with ginger (*Zingiber officinale* Rosc.) ecosystem in India. *Indian Journal of Nematology* **42**(2): 238-245.

Pervez, R., Jacob, T.K., Devasahayam, S. and Eapen, S.J. 2014b. Penetration and infectivity of entomopathogenic nematodes against *Lema* sp. infesting turmeric. *Journal of Spices and Aromatic Crops* **23**(1): 71-75.

Pervez, R., Eapen, S.J., Devasahayam, S. and Dinsha, M. 2015a. Characterization of entomopathogenic nematode *Steinernema carpocapsae* from ginger (*Zingiber officinale* Rosc.) rhizosphere in India. *The Journal of Plant Protection Sciences* **6**(1): 13-20.

Pervez, R., Revathi, J., Eapen, S.J., Devasahayam, S and Jacob, T.K. 2015b. Isolation and identification of symbiotic bacterium associated with the entomopathogenic nematode, *Heterorhabditis* sp. (IISR-EPN 01) from ginger rhizosphere. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **6**(2): 339-343.

Pervez, R., Eapen, S.J., Devasahayam, S. and Jacob, T.K. 2016. Eco-friendly management of cardamom root grub (*Basilepta fulvicorne* Jacoby). *Indian Phytopathology* **69**(4): 260-265.

Poinar, G.O. Jr. 1976. Description and biology of a new insect parasitic rhabditoid, *Heterorhabditis bacteriophora* N. Gen, N. sp. (Rhabbditida: Heterorhabditidae N. Fam.) *Nematologica* **21**: 463-470.

Ricci, M., Glazer, I. and Gaugler, R. 1996. Entomopathogenic nematodes infectivity assay: comparison of laboratory bioassay. *Biocontrol Sciences and Technology* **6**: 235-245.

Sankaranarayanan, C., Singaravelu, B., Somasekhar, N. and Santhalakshmi, G. 2011. Penetration and pathogenicity of entomopathogenic nematodes to sugarcane early shoot borer, *Chilo infuscatellus* Snellen (Lepidoptera: Crambidae). *Journal of Biological Control* **25**(1): 1-4.

Shapiro, D.I. and McCoy, C.W. 2000. Infectivity of entomopathogenic nematodes to *Diaprepes abbreriath* (Coleoptera: Curculionidae) in the laboratory. *Journal of Economic Entomology* **93**: 1090-1095.

Steiner, G. 1923. *Aplectana kraussei* n. sp., eine in der Blattwespe Lyda sp. Parasitiernde Nematodenform, nebst Bemerkungen uber das Steitenorgan der parasitischen Nematoden. Zbl. Bakt. *Parasitenk. Infektionskrank, Abstarct* **2**(59): 14-18.

Tomalak, M. 2004. Infectivity of EPNs to soil-dwelling developmental stages of the tree leaf beetles *Altica quercetorum* and *Agelastica alni*. *Entomologia Experimentalis et Applicata* **110**: 125-133.

White, G.F. 1927. A method for obtaining infective nematode larvae from cultures. *Science* **66**: 302-303.