International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 1129-1132

Received: 11-09-2017
Accepted: 14-10-2017

R Sharmila
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Subramanian
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

R Rajesh
Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
R Sharmila
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil nadu, India

Comparative virulence of using filter paper and leaf disc bioassay for entomopathogenic nematodes

R Sharmila, S Subramanian and R Rajesh

Abstract
Entomopathogenic nematodes mainly symbiotic association with gram positive bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. Entomopathogenic nematodes (EPNs) are beneficial nematodes parasiting insect pests and are being effectively used as a bio pesticide against a wide variety of insect pests. The present study was investigated the effect of using filter paper and leaf disc bioassay on the activity of the entomopathogenic nematodes, *Heterorhabditis indica* and *Steinernema glaseri* under laboratory conditions. The result showed that virulence of *H. indica* followed by *S. glaseri* was found to be more virulent to *Spodoptera litura* and *Helicoverpa armigera*. *S. glaseri* followed by *H. indica* were found to be more virulent to *Agrotis ipsilon* and *Anomala communis* based on LC_{50} and LD_{50} values in the filter paper method. In the leaf disc bioassay method found that *S. litura* larvae were found to be susceptible to *H. indica* and *S. glaseri*. The LC_{50} and LT_{50} of *H. indica* were significantly lower than that of *S. glaseri*. Compared to filter paper method, leaf disc bioassay requires lower LC_{50} of 7.01 IJ/larva and least LT_{50} of 21.22 h/larva to cause mortality to *S. litura*. The results concluded that mortality of insects recorded on leaf disc assay method was also lower when compared to filter paper exposure.

Keywords: Entomopathogenic nematodes, *Heterorhabditis indica*, *Steinernema glaseri*, *Corcyra cephalonica*, virulence, leaf disc bioassay

Introduction
Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabdidae with their associated symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*, respectively) are widely distributed in soils throughout the world. These nematode parasites of insects, killing them within 48h with the aid of their associated bacterial symbionts, and have great importance as biological control agents of many insect pests.

The virulence of entomopathogenic nematodes depend on the species and strain of the nematodes, variation in the species of symbiotic bacteria, bacterial inoculum carried by the nematodes and their activity. The behavioural, morphological and physiological defence strategies of insects affect the ability of the nematodes to infest the host which in turn influence the virulence of the nematodes (Kaya, 1990) [7].

The application of entomopathogenic nematodes in biological control was traditionally used to control soil pests until a few years ago. But the research from the last two decades indicates their marked potential against foliar pests under unique conditions. Earlier attempts have demonstrated that entomopathogenic nematodes at high concentrations, together with favourable abiotic components (high humidity and optimal temperature) can be highly effective biological control agents of insects in commercial agriculture (Laznik *et al*., 2012) [10].

Selection of an entomopathogenic nematode species for the control of a particular insect pest is based on several factors that include the nematode host range, host finding or foraging strategy, tolerance of environmental factors and their effects on survival and efficacy (temperature, moisture, soil type, ultraviolet light, salinity and organic content of soil). The critical factors are moisture, temperature, pathogenicity for the targeted insects and foraging strategy (Grewal *et al*., 2004) [5]. The objective of this study is to compare the virulence of using filter paper and leaf disc bioassay for entomopathogenic nematodes *viz*., *Heterorhabditis indica* and *Steinernema glaseri*.

Materials and Methods

Virulence of entomopathogenic nematode

Heterorhabditis indica and *S. glaseri* were selected for testing virulence against *Helicoverpa armigera*. Dose – mortality relationship and time mortality tests were conducted in 9 cm diameter Petri dishes lined at the bottom with a Whatman No. 1 filter paper and moistened with 1ml sterile distilled water. Infective juveniles were evenly applied over the filter paper. The dosages used were 0, 5, 10, 20, 40, 80 and 100 infective juveniles per larva, with 10 larvae per insect per replicate and four replicates for each level. The treatments were given as follows. The insect mortality data were recorded from 12 hours after inoculation, at an interval of 12 h up to 72 h and LC_{50} and LT_{50} values were arrived by Probit analysis (Finney, 1962) [3].

Leaf disc bioassay for entomopathogenic nematodes against *Helicoverpa armigera*

Insect mortality bioassays were conducted with two species of entomopathogenic nematodes *viz*., *H. indica* and *S. glaseri*. Castor leaves were washed using sterile distilled water and cut into 9 cm diameter discs. The discs were placed inside the petri dishes which act as a source of nourishment for the developing *H. armigera* larvae and placed in 9 cm diameter petri dishes. Addition of 2 ml of nematode suspension containing 200 IJ/ml was inoculated on the leaf disc. The petri dishes were incubated at room temperature for 72 hours. Treatments are arranged in a Completely Randomized Design with 5 replicates. Control experiments were maintained by providing as 0.5 ml distilled water to wet the filter papers before placing the *H. armigera* larvae of castor leaf discs in the petri dishes. Data on mortality were collected after 24, 48, and 72 hours from the initial of the experiment. LC_{50} and LT_{50} values were arrived by Probit analysis.

Results

Virulence of *H. indica* and *S. glaseri*

Heterorhabditis indica was found to be highly virulent to *H. armigera* with the LC_{50} value of 5.92 IJ/larva. The LC_{50} value of the insect pests was not significantly different from each other as the fiducial limits were overlapping. The LT_{50} of *H. indica* caused 50 per cent larval mortality of *H. armigera* in a minimum period of 13.42 h/larva at 72h interval.

Steinernema glaseri was virulent to larvae of *H. armigera* with the LC_{50} value of 9.01 IJ/larva. However, significant difference was not observed among the LC_{50} values of the insect because of overlapping fiducial limits. The median lethal time of LT_{50} value of 17.06 h/larva for *S. glaseri* tested on *H. armigera* (Table 1 and 2).

Leaf disc bioassay for *H. indica* and *S. glaseri* against *H. armigera*

The leaf disc bioassay found that *H. armigera* larvae were found to be susceptible to *H. indica* and *S. glaseri*. The degree of susceptibility to nematode infection varied from species to species and also on the exposure time. Also, a positive correlation was found between the concentration of infective juveniles and time taken for larval mortality for *H. indica* and *S. glaseri*. The effect of time on the mortality of *H. armigera* larvae as caused by *H. indica* and *S. glaseri* was significant. The LC_{50} of *H. indica* was significantly lower than that of *S. glaseri*. The LC_{50} values of *H. indica* and *S. glaseri* were 7.01 IJ/larva and 8.39 IJ/larva respectively. The LT_{50} of *H. indica* was significantly lower when compared to that of *S. glaseri*. The LT_{50} for *H. indica* and *S. glaseri* was at 21.22 and 22.51 h/larva respectively.

The results indicated that *H. indica* requires the least exposure time (21.22 h/larva) to causing 50 per cent larval mortality of *H. armigera*, whereas *S. glaseri* require the highest exposure time (22.51 h/larva) to cause 50 per cent mortality of *H. armigera* larvae. Both the LC_{50} and LT_{50} values of *H. indica* and *S. glaseri* for larvae of *H. armigera* insect are significantly different from each other (Table 3 and 4).

Discussion

Virulence of *H. indica* and *S. glaseri*

The virulence was determined in the present study by median lethal concentration (LC_{50}) and Median lethal time (LT_{50}) of *H. indica* and *S. glaseri* against for insect pest of *H. armigera*. The present investigation indicated that *H. indica* and *S. glaseri* were more virulent to *H. armigera*. The findings showed that LC_{50} and LD_{50} of *H. indica* was low for *H. armigera*. *Heterorhabditis indica* was found to be highly virulent to *H. armigera* with the lowest LC_{50} value at 48 h interval. Umamaheswari *et al*. (2004) [22] and Saravanapriya and Subramanian (2007) [20] reported that *H. indica* as highly virulent against *H. armigera* (LC_{50}-3.5 and 7 IJ/larva) and caused 50 per cent mortality in a minimum time of 13.42 h/larva which confirm the present findings. Divya *et al.* (2010) [2] reported early instar larvae of *H. armigera, S. litura* and *G. mellonella* were more susceptible to *H. indica*, which confirm the present findings. In the present study *S. glaseri* caused the lowest LC_{50} of 9.01 IJ/larva for *H. armigera* followed by 9.01 IJ/larva for *H. armigera* and 18.03/IJ larva for *A. ipsilon*. Prabowo (2012) [17]; Mahmoud *et al.* (2007) [12]; Prabhu and Sudheer (2008) [18] found an increase in the concentration of *Steinernema* sp. increased the mortality of *S. litura.* Gupta *et al*. (1987) [6] reported 66 per cent mortality of cutworm, *S. litura* in tobacco. Vyas and Yadav (1992) [23] recorded cent per cent larval mortality in a laboratory bioassay of *S. glaseri* against *A. ipsilon* and *S. litura*. *S. glaseri* was effective against sedentary pests. The dosage mortality response against different stages of four lepidopterans insects were reported by Saravanapriya and Subramanian (2007) [20].

The LT_{50} of *H. indica* to cause 50 per cent mortality of *H. armigera* larvae in a minimum period 23.93 h/ larva. A higher virulence of *H. indica* (LT_{50} 33.76 h) @ 300 IJ/ *T. molitor* was reported by Noosidum *et al.* (2012) [15]. Kulkarni *et al.* (2011) recorded susceptibility (LC_{50}) of 34, 200 and 4.57 IJ/ larva of cutworm (*A. ipsilon*). In the present study, LT_{50} of *S. glaseri* was minimum against *S. litura* (16.27 h/larva) followed by *H. armigera* (17.06 h/larva), *A. ipsilon* (23.98 h/larva) and *A. communis* (36.93 h/larva). The maximum LT_{50} was recorded in *A. communis* (36.93 h/larva). Prabhu and Sudheer (2008) [18] found that LC_{50} values were increasing in proportion to the age of the insect. Shahina and Tabassum (2010) [21] reported 90 per cent mortality in *S. exigua* by *S. pakistanese* after 24h exposure period. Nyasani *et al.* (2007) [16] observed 50 per cent mortality in the least (20.27 h) exposure time to *H. indica* than *S. karii* (38.12 h) in diamond back moth (DBM) larvae. Virulence of entomopathogenic nematodes was also strongly affected by different larval stages of white grubs as reported by Ma *et al*. (2013) [11]. Similarly, Kalia *et al*. (2014) [8] reported that the LT_{50} values of *S. thermophilum* were 41.40 h with *S. litura* and 33.6 h with *G. mellonella*. Lalramliana (2012) [9] reported that virulence is considered as an important factor in determining the time and dose of subsequent

entomopathogenic nematodes application, which may be useful in reducing the cost of entomopathogenic nematode application in the field.

Leaf disc bioassay for *H. indica* and *S. glaseri* against *H. armigera*

In this study, *H. armigera* larvae were found to be susceptible to *H. indica* and *S. glaseri*. The effect of time taken for mortality of *H. armigera* larvae as caused by *H. indica* and *S. glaseri* were significant. The LC_{50} of *H. indica* was significantly lower than *S. glaseri*. The LC_{50} values of *H. indica* and *S. glaseri* were 7.01 and 8.39 IJ/larva. The LT_{50} of *H. indica* was significantly lower when compared to *S. glaseri*. The LT_{50} for *H. indica* and *S. glaseri* were recorded at 21.11 h and 22.5 h/larva respectively. The present finding was in agreement with Mahar *et al*. (2004) [13] and Mason and Wright (1997) [14], who reported that the percentage mortality of the Diamond Back Moth (DBM) larvae was lowest for nematode isolates and highest at 72 h for all entomopathogenic nematodes, indicating that *H. indica* requires the least exposure time to cause 50 per cent mortality of *H. armigera*, whereas *S. glaseri* require the highest exposure time to cause 50 per cent mortality in the *H. armigera* larvae.

The exposure time has an implication on the efficacy of the entomopathogenic nematode isolate to control *H. armigera* larvae in the field as field effectiveness of entomopathogenic nematodes is limited by desiccation, extreme temperature, UV radiation and relative humidity in the microclimate (Mason and Wright, 1997) [14]. Virulence of entomopathogenic nematodes depend on the species and strain of the nematode, variation in the species of symbiotic bacteria inoculum carried by the nematodes and their activity (Wout's, 1991; Glazer *et al*., 1991) [24, 4].

The results concluded that mortality of *H. armigera* recorded on leaf disc assay method was also lower when compared to filter paper exposure. The possible reason could be that the insects have no chance to escape from nematode attack.

Table 1: LC_{50} Values calculated from dosage response assays conducted with different nematodes species and last instar larvae of *H. armigera*

Nematode species	Incubation period (h)	LC_{50}	Fiducial limit (95 %)	
			UL	LL
H. indica	24	5.81	4.98	9.32
	48	5.92	5.05	9.47
	72	7.39	12.80	20.98
	96	7.77	22.39	39.58
S. glaseri	24	8.45	6.05	11.81
	48	9.01	6.18	11.91
	72	9.03	12.70	25.91
	96	10.48	15.62	29.54

Table 2: LT_{50} Values calculated from dosage response assays conducted with different nematodes species and last instar larvae of *H. armigera*.

Nematode species	Incubation period (h)	LT_{50}	Fiducial limit (95 %)	
			LL	UL
H. indica	24	12.42	19.98	27.44
	48	13.42	20.57	27.83
	72	18.69	25.46	32.33
	96	88.66	33.66	43.31
S. glaseri	24	16.27	13.51	19.60
	48	17.06	14.17	20.55
	72	23.98	20.61	27.90
	96	26.93	32.61	41.83

Table 3: Median lethal concentration (LC_{50}) of *H. indica* and *S. glaseri* against larvae of *H. armigera* in leaf disc bioassay.

Nematode	Chi^2	b	±SE	LC_{50} (IJ/larva)	Fiducial limits	
					Lower	Upper
H. indica	1.59	2.05	0.31	7.01	5.26	9.35
S. glaseri	0.37	2.01	0.27	8.39	6.36	11.08

Table 4: Median lethal time (LT_{50}) of *H. indica* and *S. glaseri* against larvae of *H. armigera* in leaf disc bioassay.

Nematodes	Chi^2	b	±SE	LT_{50} (h)	Fiducial limits	
					Lower	Upper
H. indica	5.14	3.19	0.38	21.22	17.58	25.62
S. glaseri	6.49	3.25	0.39	22.51	18.78	26.97

Acknowledgement

We thank the Dr. K. Sankaranarayan, Senior Scientist from Sugarcane Breeding Institute, Coimbatore for providing the EPN cultures to carry out the research work. The corresponding author expresses her gratitude to University Grants Commission, New Delhi for financial support by providing fellowship for the Doctoral Programme.

References

1. Alm SR, Yeh T, Hanula LL, Georgis R. Biological control of Japanese Oriental and black turfgrass ataenius beetle (Coleoptera: Scarabaeidae) larvae with Entomopathogenic nematodes (Nematoda: Steinernematidae, Heterorhabditidae). Journal of Economic Entomology, 1992; 85:1660-1665.
2. Divya K, Sankar M, Marulasiddesha KN. Efficacy of Entomopathogenic nematode, *Heterorhabditis indica*

against three lepidopteran insect pests. Asian Journal of Experimental Biological Science, 2010; 1:183-188.
3. Finney DJ. Statistical Methods in Biological Assay**.** Charles Griffen and Co., Ltd., London, U.K. 1962, 661.
4. Glazer I, Galper S, Sharou E. Virulence of the nematode (Steinernematids and Heterorhabditis) –bacteria (*Xenorhabdus* sp.) complex to the Egyptian cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae). Journal of Invertebrate Pathology, 1991; 57:97-100.
5. Grewal PS, Power KT, Grewal SK, Suggars A, Haupricht S. Enhanced consistency in biological control of white grubs (Coleoptera: Scarabaeidae) with new strains of Entomopathogenic nematodes. Biological control, 2004; 30:73-82.
6. Gupta M, Desai P, Pawar AO. The feasibilities of using DD-136 strain of *Neoplectana carpocapsae* Weiser for the control of insect pests. Plant Protection Bulletin, 1987; 39:16-19.
7. Kaya HK. Soil Ecology. In: Entomopathogenic Nematodes in Biological Control (Gauglerr, R. and Kaya, H.K. Eds.) CRC Press, Boca Raton, Florida, USA. 1990, 93-115.
8. Kalia, V, Sharma G, Shapiro-Ilan DI, Ganguly S. Biocontrol potential of *Steinernema thermophilum* and its symbiont *Xenorhabdus indica* against Lepidopteran pests: virulence to egg and larval stages. Journal of Nematology. 2014; 46(1):18-26.
9. Lalramliana AK. Efficacy of indigenous entomopathogenic nematodes from Meghalaya, India against the larvae of taro leaf beetle, *Aplosonyx chalybaeus* (Hope). Journal of Parasitic Diseases. 2012a; 36:149-154.
10. Laznik Z, Vidrih M, Trdan S. Effect of different fungicides on viability of entomopathogenic nematodes *Steinernema feltiae* (Filipjev), *S. carpocapsae* (Weiser) and *Heterorhabditis downesi* (Stock, Griffin & Burnell) (Nematoda: Rhabditida) under laboratory conditions. Chilean of Journal of Agricultural Research. 2012; 72:62-67.
11. Ma J, Chen SL, Moens M, Han RC, De Clereq P. Efficacy of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) against the chive gnat, *Bradysia odoriphaga*. Journal of Pest Science. 2013; 86:551-561.
12. Mahmoud MF, Mandour NS, Pomazkov YI. Efficacy of the entomopathogenic nematode *Steinernema feltiae* cross N 33 against larvae and pupae of four fly species in the laboratory. Nematologia Mediterranea, 2007; 35:221-226.
13. Mahar AN, Muntr M, Laghari KB. Production and pathogenicity of four Steinernematide in diamond back moth, *Plutella xylostella* (Lepidoptera: Yaponomeutidae), Asian Journal of Plant Sciences. 2004; 3:484-488.
14. Mason JM, Wright DJ. Potential for the control of *Plutella xylostella* larvae with entomopathogenic nematodes. Journal of Invertebrate Pathology. 1997; 70:234-243.
15. Noosidum A, Hodson AK, Lewis EE, Chandrapatya A. A new entomopathogenic nematode from Thailand and its virulence against *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and *Oryctes rhinoceros* MacLeay (Coleoptera: Scarabaeidae). 2nd International Symposium of Bio-Pesticides and Ecotoxicological Network (2nd IS-BIOPEN). Isbiopen, 24-26 September. 2012, 73-78.
16. Nyasani JO, Kimenju JW, Olubayo FM, Wilson MJ. Potential of using entomopathogenic nematodes in the management of diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in Kenya. African Crop Science Conference Proceedings. 2007; 8:1063-1066.
17. Prabowo H. Pemanfaatan nematoda patogen *Steinernema* spp. isolate malang dan nusa tenggara barat dalam pengendalian *Spodoptera litura* L. yang ramah lingkungan. Bumi Lestari Journal of Environment. 2012; 12:350-356.
18. Prabhu S, Sudheer MJ. Evaluation of two native isolates of entomopathogenic nematodes *Steinernema* sp. and *Heterorhabditis indica* from Andhra Pradesh against *Amsacta albistriga* walk in groundnut. Journal of Biopesticides. 2008; 1:140-142.
19. Qiu L, Lacey MJ, Bedding R. Energy metabolism and its relation to survival and infectivity of infective juveniles of *Steinernema carpocapsae* under aerobic conditions. Nematology, 2000; 2:551-559.
20. Saravanapriya B, Subramanian S. Pathogenicity of Entomopathogenic nematode to certain foliar insect pests. Annals of Plant Protection Science. 2007; 15:219-222.
21. Shahina F, Tabassum AK. Virulence of *Steinernema pakistanense* against different insect species in laboratory condition. Pakistan Journal of Nematology. 2010; 28:279-284.
22. Umamaheswari R, Sivakumar M, Subramanian S. Virulence of native entomopathogenic nematodes to *Spodoptera litura* (Lepidoptera: Noctuidae). Current Nematology, 2004; 15:97-100.
23. Vyas RV, Yadav DN. Infectivity of entomopathogenic nematodes to the two soil dwelling lepidopterans. Annals of Biology. 1992; 8:59-63.
24. Wout's WM. *Steinernema* (Neoplectana) and *Heterorhabditis* species. In: Manual of Agricultural Nematology (Nickle, W.R.Ed) Marcel Dekker. Inc New York, U.S.A. 1991, 555-597.