Laboratory evaluation of entomopathogenic nematodes against American serpentine leaf miner, *Liriomyza trifolii* (Burgess)

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ABSTRACT

A laboratory study was conducted during 2011 to 2013 for evaluating the pathogenicity of entomopathogenic nematodes (EPNs) against *Liriomyza trifolii.* Seventy two soil samples were collected from three districts of Kerala, namely, Thrissur, Ernakulam and Kottayam for the isolation of EPNs. Four numbers of EPNs, *viz*., EPN Isolate - 1, 2, 3 and 4 were obtained from collected soil samples. All the isolated EPNs were identified as *Steinernema carpocapsae* Weiser. The efficacy of soil isolated EPNs was compared with *Steinernema bicornutum* Tallosi, Peters & Ehlers and *Heterorhabditis indica* Poinar, Karunakar and David by leaf disc bioassay method. The treated EPNs were effective in causing mortality to *L. trifolii* maggots inside the mines. But *S. carpocapsae* Isolate – 1 (Kannara) was found to be more effective against *L. trifolii* larvae with lowest LC_{50} (1.79/ maggot) value (24 h). The pathogenicity of EPNs against *L. trifolii* revealed the scope of their utilization in IPM programmes.

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INTRODUCTION

Liriomyza trifolii commonly known as the American serpentine leaf miner, is one of the predominant and economically important phytophagous pest of several vegetable crops. The damage is caused by the maggots, feeding on the mesophyll tissues leaving the epidermis intact. Heavy infestation causes desiccation and drying of leaves (Chandler and Thomas, 1983). Outbreak of *L. trifolii* adversely affected the yield in cowpea (Singh and Meroett, 1980) and the infestation of the pest caused 70 per cent loss of tomato yield (Zoebisch *et al*., 1984). Krishnakumar (1998) reported the yield loss by the infestation of *L. trifolii* as 15 to 70 per cent in French bean, 41 per cent in cucumber and 35 per cent in tomato from Karnataka. Jacob and Mathew (2014a) reported an infestation index of 55 per cent in ashgourd and 45 per cent in cowpea by *L. trifolii*.

The entomopathogenic nematodes (EPNs) belonging to the families, Steinernematidae and Heterorhabditidae were reported to suppress *L. trifolii* (Harris *et al*., 1990; Olthof and Broadbent, 1991). The potency of *S. carpocapsae* and *S. feltiae* in the suppression of *L. trifolii* was studied earlier (Hara *et al*., 1993; LeBeck *et al*., 1993; Sher *et al*., 2000; Tomalak *et al*., 2005). The utilization of EPNs against *L. trifolii* is being practiced outside India. But knowledge on the effectiveness of the native isolates of EPNs against *L. trifolii* was less. Hence a laboratory evaluation was carried out in the Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University, Vellanikkara, during 2011-2013, for the pathogenicity of the local isolates of EPNs, *Steinernema carpocapsae* along with *Steinernema bicornutum* and *Heterorhabditis indica* against *L. trifolii.*

MATERIALS AND METHODS

Laboratory rearing of Insects

The test insect, *L. trifolii* was reared on nine days old cowpea (*Vigna unguiculata* L.)

seedlings both in polythene bags and in rearing cages (Jacob and Mathew, 2014b). The laboratory reared second instar larvae of *L. trifolii* were used for the study. The last instar larvae of *G. mellonella* reared in the laboratory in artificial diet (Singh, 1994) were used as the trap insect to isolate EPNs from soil.

Collection of soil samples

Seventy two soil samples were collected from two locations each from three district panchayats, namely, Thrissur, Ernakulam and Kottayam. Soil samples were collected from three spots each in each site (after removing the top soil with vegetation) with a shovel from a depth of 10 to 20 cm. Samples were collected from undisturbed area where the agricultural operations were not carried out to ensure the absence of pesticides.

Isolation, extraction and identification

Soil samples were transferred to plastic jars of one litre capacity for the isolation of EPNs. Each soil sample was baited with five numbers of last instar larvae of *G. mellonella* and was covered with muslin cloth to facilitate aeration and to prevent the escape of larvae. Pieces of muslin cloth were placed on the top of soil also to prevent larvae from coming out of the soil samples and to ensure contact with third stage juveniles of EPNs, if any, in the soil, in the containers. The mortality of *G. mellonella* was observed for one week. The dead larvae were collected daily from the soil. After surface sterilization with Sodium hypochlorite solution (1%), cadavers were transferred to dry filter paper kept in Petri dishes for incubation. After two to three days, the dead larvae were transferred to White's trap (White, 1927) for emergence of nematodes. The harvested nematodes were kept in sterile distilled water in a beaker. The isolated EPNs were identified from Division of Nematology, Indian Agricultural Research Institute, New Delhi.

Maintenance of the soil isolated EPNs

The soil isolated EPNs were maintained in sterilized soil and in aerated sterile distilled water. The extraction of nematodes was done using distilled water and the extracted nematodes were mixed with sterilized soil in plastic jar, when the storage was done in soil. EPNs were also stored in distilled water with intermittent aeration using aquarium aerator and change of water, in glass beakers. The EPNs could live in distilled water when aeration was provided. The nematodes were maintained healthy and virulent by inoculating healthy larvae of *G. mellonella* once in three weeks.

Bioefficacy of entomopathogenic nematodes

The soil isolated EPNs were tested for their efficacy against *L. trifolii* in the laboratory along with *Steinernema bicornutum* obtained from Banana Research Station, Kannara, Thrissur and *Heterorhabditis indica* obtained from Indian Cardamom Research Institute (ICRI), Myladumpara, Idukki. The experiment was carried out at room temperature (27±1ºC). Five different doses were tested, *viz*., 10, 15, 20, 25 and 30 IJs per larva. The IJs were counted individually and placed on to the small circular filter paper disc placed at the bottom of the penicillin vials. Cowpea leaf bit containing healthy maggots were placed on the filter paper at a rate of one maggot per vial. Three replications were maintained for each treatment and ten maggots were used per replication. The cumulative mortality obtained after 12, 18 and 24 HAT were analyzed using one way ANOVA after arc-sine transformation. The data was subjected to Probit Analysis (SPSS 17.00) to determine the LC_{50} values at 24 HAT.

RESULTS AND DISCUSSIONS

Identification of entomopathogenic nematodes

The four EPNs isolated from the soil samples of Kannara (Isolate – 1 and Isolate - 2 and Vellanikkara (Isolate - 3 and Isolate – 4) of Thrissur district were identified as *Steinernema carpocapsae*.

Bioefficacy of entomopathogenic nematodes

The maggots inside the leaf mines and the pre pupae emerged from mines were killed by the infective juveniles (IJs). The colour of the infected maggots changed to dark brown to black. Pupae from infected pre pupae had a shrivelled appearance. The pupae of *L. trifolii* were not infected by EPNs. The mortality caused by EPN isolates increased with increase in the concentration of IJs.

At 12 HAT, the mortality caused by application of 10 IJs (T_1), 15 IJs (T_2) and 20 IJs/ maggot (T_3) of *S. carpocapsae* Isolate-1 was statistically on par (Table 1) (F= 45.108; df= 5,15; P <0.05) In the case of *S. carpocapsae* Isolate – 2, a minimum of 15 IJs were required to cause mortality in 12 h. The higher concentration, T_5 was significantly superior to all other treatments tested (F= 6.113; df= 5,15; P <0.05). No significant difference was observed between the treatments applied in the case of *S. carpocapsae* Isolate - 3 and all treatments were on par (F= 7.044; df= 5,15; P <0.05). T_1-T_3 were statistically on par and were inferior to T_4 and T_5 (F= 50.624; df= 5,15; P <0.05) for *S. carpocapsae* Isolate - 4. The lower concentrations (10 IJs/ maggot and 15 IJs/ maggot) of *S. bicornutum* were observed to be on par. T_2 was on par with T_4 and the higher concentration, T_5. Application of T_3 was significantly superior from the two lower concentrations tested, namely, T_1 (10 IJs/ maggot) and T_2 (15 IJs/ maggot) (F= 14.289; df= 5,15; P <0.05) . The lower concentrations of *H. indica*, namely, T_1-T_3 were on par. T_4 and T_5 were significantly superior from all other treatments tested (F= 34.138; df= 5,15; P <0.05) . Hence among the EPNs evaluated at 12 HAT, *S. carpocapsae* Isolate-1 caused highest mortality of 83.33 per cent at 12 h after treatment.

There was increase in the mortality caused by the EPNs tested at 18 HAT (Table 2). In *S. carpocapsae* Isolate-1, application of 15 IJs/ maggot (T_2) and 20 IJs/ maggot (T_3) were on par. No significant difference in mortality was observed between the higher concentrations T_3-T_5 (F= 54.867; df= 5,15; P <0.05). In the case of *S. carpocapsae* Isolate–3, no significant difference was observed between the lower concentrations. The higher concentrations were on par and were significantly superior to T_1 and T_2 (F= 55.581; df= 5,15; P <0.05). In the case of *S. carpocapsae* Isolate-4, T_1 and T_2 were on par. No significant difference was observed between the treatments, T_3 and T_4. The highest concentration (T_5) was significantly superior to all other treatments (F= 112.333; df= 5,15; P <0.05). The lowest concentration of *S. bicornutum*, T_1 was significantly inferior to all other treatments (F= 32.866; df= 5,15; P <0.05) which were on par in effectiveness. The first three concentrations of *H. indica*, namely, T_1, T_2 and T_3 caused lower mortalities compared to other EPNs tested. T_5 was significantly superior to other treatments (F= 38.300; df= 5,15; P <0.05). Considering the mortalities caused by the different EPNs, *S. carpocapsae* Isolate-1 ranked first in mortality ranging from 50 in the lowest concentration to 96.67 in the highest concentration.

At 24 HAT (Table 3), the lowest concentration (T_1) of *S. carpocapsae* Isolate - 1, was significantly inferior (F= 133.991; df= 5,15; P <0.05) to all other concentrations applied. A dose of 20 IJs/ maggot and above caused cent per cent mortality. As in the case of *S. carpocapsae* Isolate - 1, the lowest concentration of Isolate - 2 was significantly inferior to all other concentrations tested (F= 21.084; df= 5,15; P <0.05). The four lower concentrations were observed to be statistically on par for *S. carpocapsae* Isolate–3 (F= 88.482; df= 5,15; P <0.05). *S. carpocapsae* Isolate-4 the three higher concentrations were on par and were significantly superior to the two lower concentrations (F= 54.897; df= 5,15; P <0.05). For *S. bicornutum* the treatments, T_2-T_4 were on par and no significant difference was observed between T_4 and T_5 (F= 36.239; df= 5,15; P <0.05). The lower concentrations of *H. indica* (T_1-T_3) were on par. The treatments, T_4 and T_5 were significantly superior to T_1, T_2 and T_3 (F= 223.909; df= 5,15; P <0.05). Among the EPNs tested against *L. trifolii* larvae, the lowest concentration which caused 100 per cent mortality was with that of *S. carpocapsae* Isolate - 1.

Table 1. Mortality of ***Liriomyza trifolii*** **(Mean ±SD)** caused by entomopathogenic nematodes at 12 HAT, 18 HAT and 24 HAT

Treatment	**Per cent of insect mortality (Mean ±SD)**					
	Steinernema carpocapsae				***S. bicornutum***	***H. indica***
	Isolate - 1	**Isolate – 2**	**Isolate – 3**	**Isolate – 4**		
12 Hours after treatment (HAT)						
T_1 - 10 IJs/ maggot	30.00±10.00^{a}	0.00±0.00^{a}	13.33±5.77^{a}	13.33±5.77^{a}	3.33±5.77^{a}	6.67±5.77^{ab}
T_2 - 15 IJs/ maggot	40.00±26.46^{ab}	13.33±7.73^{ab}	10.00±10.00^{a}	20.00±10.00^{a}	13.33±15.28^{ab}	3.33±5.77^{a}
T_3 - 20 IJs/ maggot	60.00±10.00^{b}	26.67±25.11^{ab}	23.33±15.28^{a}	23.33±5.77^{a}	36.67±11.55^{c}	10.00±0.00^{ab}
T_4 - 25 IJs/ maggot	66.67±11.55^{bc}	40.00±24.57^{ab}	13.33±5.77^{a}	46.67±15.28^{b}	26.67±5.77^{bc}	26.67±5.77^{b}
T_5 - 30 IJs/ maggot	83.33±5.77^{c}	56.67±25.17^{b}	6.66±5.77^{a}	66.67±5.77^{c}	33.33±15.28^{bc}	73.33±15.28^{c}
Computed F (5,15) value	45.108	6.113	7.044^{a}	50.624	14.289	34.138
18 Hours after treatment (HAT)						
T_1 - 10 IJs/ maggot	50.00±10.00^{a}	6.67±5.77^{a}	20.00±0.00^{a}	23.33±5.77^{a}	10.00±0.00^{a}	6.67±5.77^{a}
T_2 - 15 IJs/ maggot	70.00±17.32^{ab}	46.67±35.12^{ab}	23.33±5.77^{ab}	26.67±5.77^{a}	36.67±15.28^{b}	13.33±5.77^{ab}
T_3 - 20 IJs/ maggot	86.67±11.55^{bc}	40.00±36.06^{ab}	40.00±17.32^{bc}	46.67±5.77^{b}	40.00±10.00^{b}	16.67±5.77^{ab}
T_4 - 25 IJs/ maggot	96.67±5.77^{c}	50.00±26.46^{ab}	56.67±5.77^{c}	56.67±11.55^{b}	36.67±5.77^{b}	40.00±10.00^{b}
T_5 - 30 IJs/ maggot	96.67±5.77^{c}	73.33±15.28^{b}	53.33±5.77^{c}	76.67±5.77^{c}	56.67±15.28^{b}	90.00±10.00^{c}
Computed F (5,15) value	54.867	9.136	55.581	112.333	32.886	38.300
24 Hours after treatment (HAT)						
T_1 - 10 IJs/ maggot	73.33±11.55^{a}	26.67±15.28^{a}	63.33±5.77^{a}	53.33±5.77^{a}	43.33±5.77^{a}	16.67±5.77^{a}
T_2 - 15 IJs/ maggot	90.00±11.55^{b}	73.33±28.87^{b}	83.33±11.55^{a}	53. 33±5.77^{a}	53.33±11.55^{ab}	16.67±11.55^{a}
T_3 - 20 IJs/ maggot	100.00±0.00^{b}	66.67±32.15^{ab}	80.00±17.32^{a}	80.00±10.00^{ab}	65.66±5.77^{ab}	23.33±5.77^{a}
T_4 - 25 IJs/ maggot	100.00±0.00^{b}	70.00±20.00^{ab}	80.00±17.32^{a}	96.67±5.77^{b}	80.00±20.00^{bc}	53.33±11.55^{b}
T_5 - 30 IJs/ maggot	96.67±5.77^{b}	93.33±5.77^{b}	100.00±0.00^{b}	86.67±15.28 ^{b}	93.33±5.77^{b}	100.00±0.00^{c}
Computed F (5,15) value	113.991	21.084	88.482	54.897	36.239	223.909

S. bicornutum – Steinernema bicornutum ; *H. indica – Heterorhabditis indica;* Means followed by same letters within a column are not statistically different at $P < 0.05$ level (ANOVA followed by Duncan post- hoc test)

Median lethal concentration

At 24 HAT, the lowest LC_{50} was observed in *S. carpocapsae* Isolate – 1 (Table 4). Hence *S. carpocapsae* Isolate - 1 was selected as the most effective isolate among those isolated from soil for conducting further experiments.

Table 4. *LC_{50}* value (IJs) of entomopathogenic nematodes at 24 HAT

Treatments	LC_{50} (IJs/ maggot)	Heterogenity ($\chi 2$)	df
Steinernema carpocapsae Isolate - 1	1.79	15.00	12.00
S. carpocapsae Isolate - 2	13.95	20.00	16.00
S. carpocapsae Isolate - 3	2.71	20.00	16.00
S. carpocapsae Isolate - 4	10.06	15.00	12.00
Steinernema bicornutum	11.73	15.00	12.00
Heterorhabditis indica	21.99	15.00	12.00

Four numbers of EPN isolates were obtained from 72 soil samples collected from three districts, namely, Thrissur, Ernakulam and Kottayam. Soil is reported as a natural reservoir of EPNs (Akhurst, 1986; Gaugler, 1988) offering excellent conditions for nematode survival and activity. Native soil isolated entomopathogens were used in the present study as the indigenous isolates of EPNs only could provide more efficient biological control because of the adaptation to local climate and population regulators of insect pest as opined by Bedding (1990). The EPNs were reported to have been isolated from all continents (except Antarctica) and all regions of the world (Hominick, 2002; Adams *et al*., 2006). A check list of insect parasitic nematodes of India (Gantait and Sanyal, 2007) showed that a total of 72 species under three orders were reported so far from India. This list does not contain *S. carpocapsae* from Kerala and thus it forms a new report for Kerala.

Bioefficacy of entomopathogenic nematodes

The use of EPNs belonging to families Steinernematidae and Heterorhabditidae against leaf miners were reported earlier (Harris *et al*., 1990; Olthof and Broadbent, 1991). In the present study, mortality of *L. trifolii* maggots was observed to occur before 12 HAT and was directly proportional to time and concentration. It ranged from 3.33 to cent per cent depending upon the concentration of EPNs. Steinernematid and Heterorhabditid nematodes were reported to cause mortality ranging from 48 to 98 per cent to larvae of *L. trifolii* (Hara *et al*., 1993; Sher *et al*., 2000; Tomalak *et al*., 2005)**.**

In most of the cases, maggots were observed to be dead inside the mines itself. This showed the ability of EPNs to enter into the mines in search of the prey. The oviposition sites made by adult females of leaf miner and the tear in the mines were reported as the major entry points of EPNs (Harris *et al*., 1990; LeBeck *et al*., 1993). The *Steinernema* sp. studied (*S. carpocapsae* Isolates 1-4 and *S. bicornutum*) were observed to be efficient in causing mortality to *L. trifolii* larvae. The result is in agreement with Harris *et al*. (1990) who reported 64 per cent mortality to leaf miner larvae in the laboratory with *S. carpocapsae*. Variation in effectiveness was observed between *S. carpocapsae* Isolates and *S. bicornutum* in the laboratory. This might be due to the variation in the pathogenicity of the symbiotic bacteria associated with different species of genus *Steinernema* as reported by Akhurst and Boemare (1990). Lewis *et al.* (2006) reported this difference as the distinction in their behavior in relation to emergence, foraging strategy and search for hosts, along with the physiological differences and changing tolerance to abiotic factors that occurs among the several EPN strains.

Median lethal concentrations

LC_{50} at 24 HAT values varied among the soil isolates of *S. carpocapsae*. This variation could again be supported by the findings of Akhurst and Boemare (1990). The time taken in hours to cause 50 per cent mortality for all the EPNs was worked out at different concentrations. Compared to other isolates, *S. carpocapsae* Isolate - 1 had low LC_{50} value at all time intervals. In the laboratory evaluation, EPNs were observed to be potent in controlling the maggots of *L. trifolii*. The efficacy of the EPNs could be utilized for the management of insect pests in polyhouses and field conditions. The pathogenicity of the EPNs against the foliar pests could be effectively utilized in the IPM programmes.

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