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M Nithiskarani
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu India

B Anita
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu India

P Vetrivelkalai
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu India

S Jeyarajan Nelson
Department of Agriculture Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu India

Correspondence
M Nithiskarani
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu India

Biocontrol potential of entomopathogenic Nematodes, *Steinernema glaseri* (Steiner, 1929) and *Heterorhabditis indica* (Poinar, Karunakar & David, 1992) against brinjal ash weevil (*Myllocerus subfasciatus*)

M Nithiskarani, B Anita, P Vetrivelkalai and S Jeyarajan Nelson

Abstract
Brinjal ash weevil (*Myllocerus subfasciatus*) is an important pest of brinjal (*Solanum melongena* L.) in India and Southeast Asia. The probable efficacy of two species of entomopathogenic nematodes (EPNs), *Steinernema glaseri* and *Heterorhabditis indica*, against the third instars of the brinjal ash weevil was tested under laboratory conditions at different concentrations. It was observed that the third instars were susceptible to the entomopathogenic nematodes. Among the two nematode species, *Steinernema glaseri* was more effective against third instar stage than *H. indica*. In soil column test, 95.00% and 87.5% mortality of *Myllocerus subfasciatus* was observed due to infection by *S. glaseri* and *H. indica* respectively. Both the EPNs species were able to replicate in third instars of ash weevil.

Keywords: *Solanum melongena*, *Steinernema glaseri*, *Heterorhabditis* indica, efficacy, concentration

1. Introduction
Brinjal (*Solanum melongena* L.) is one of the stable foods in India and other countries of South East Asia. It is also considered as a poor man's vegetable because it is grown throughout the year. In India, brinjal is cultivated in 733 lakh hectares with a productivity of 19.2 tons per ha (Damodhar, 2018) [2]. Brinjal is more susceptible to attack by insect pests and diseases. Ash weevil, *M. subfasciatus* adult feeds on the leaf in 'C' shape and all grub stages causes more damage to the plants. The infected plant shows stunting and finally get wilted (Shanmugam *et al*., 2018) [11]. For the control of insect pest, spraying of chemical insecticides is widely followed. But, nowadays many of the insecticides are banned for their harmful effect on the environment. So, entomopathogenic nematodes were evaluated as an alternative source for the management of ash weevil grubs.
EPNs belonging to the genera *Steinernema* and *Heterorhabditis* with their bacterial symbionts of *Xenorhabdus* and *Photorhabdus* are effective against several insect hosts. Since, all the ash weevil grub stages are completed in the soil, it is a necessary to manage the pests in the soil. For the control of grubs in soil, EPNs can be released in the soil before the grub stage enter into the roots which will help in preventing damage to the crop. The success of EPNs on the targeted pests in the field condition was observed on various soil borne insect pests like white grubs, *Holotrichia* spp. (Sankaranarayanan *et al*., 2006) [10], citrus root weevil, *Diaprepes abbreviates* (Jenkins *et al*., 2007) [5], blackvine weevil, *Otiorhynchus sulcatus* (Ansari and Butt, 2011) [1] and diamond back moth, *Plutella xylostella* (Zolfagharian *et al*., 2014) [14]. Primarily, this study was conducted with the objective of evaluating the virulence of EPNs against brinjal ash weevil.

2. Materials and methods
2.1. Collection of ash weevil grubs (*Myllocerus subfasciatus*)
Grubs of *Myllocerus subfasciatus* were collected from infected brinjal fields. Ash weevil grub infected brinjal field exhibited symptoms of 'C' shape cutting in the leaf margin, unhealthy appearance, stunting and finally wilting symptoms. Grubs were collected by uprooting the infected plants. Some grubs were also found inside the root. The collected ash weevil grubs were maintained in a pot grown with brinjal and potato as a food source. Only healthy and active grubs were used for the experiment.

2.2. Culturing of entomopathogenic nematodes

Initial inoculum of EPNs was obtained from Department of Nematology, TNAU, Coimbatore and further multiplied using the Petri plate method. The multiplied EPNs were collected by two methods *viz*., modified white trap and white trap method (Gaugler, 2002) [5]. A total of ten *Corcyra cephalonica* larvae were inoculated with *S. glaseri* and *H. indica* infective juveniles (IJs) in Petri dishes with a filter paper disc (90mm diameter, Whatmann No.1). The multiplied EPNs were harvested by modified white trap method for *S. glaseri* and a white trap method for *H. indica*. The harvested EPNs were stored in a canted tissue culture flask and maintained at a temperature of 19ºC in a BOD. Fresh IJs were used for the efficacy assays.

2.3. Culturing of *Corcyra cephalonica*

Artificial diet was prepared for *Corcyra cephalonica* using Cumbu (2kg) and groundnut (200g) mixed in a tray. Eggs of the *Corcyra cephalonica* were spread on the artificial diet. After 30-35 days the third instar larvae of *Corcyra cephalonica* emerged and used for multiplication of EPNs.

2.4. Laboratory experiments for evaluation of EPNs efficacy

2.4.1. Petri plate assay

The pathogenicity of EPN against the third instar ash weevil grubs was studied *in vitro*. Ten grubs were placed in Petri plates with Whatmann No. 1 filter paper. EPN were inoculated at different concentrations *viz*., 0, 500, 1000, 1500, and 2000 IJs/plate. Later Petri plates were sealed with kiln film and observation was recorded after 12, 24, 36, and 72 hours.

The infected cadavers were collected and kept in white traps for the extraction of EPNs. After three days, *S. glaseri and H. indica* were collected and the population of IJs emerging from the insect cadavers was assessed. The experiments were conducted in a completely randomized design with five treatments and four replications.

2.4.2. Soil column assay

To confirm the laboratory studies, a soil column assay was conducted in plastic containers with soil. Ten third instar grubs of ash weevil grubs were released in plastic containers and *S. glaseri* and *H. indica* were inoculated at a rate of 500, 1000, 1500 and 2000 IJs. Uninoculated container was used as control.

The experiment was conducted in a completely randomized design with five treatments and four replications. Observation of ash weevil grub mortality was recorded for four days. The numbers of surviving grubs were counted 24, 48, 72 and 96 hours of exposure to infective juveniles.

Statistical analysis

The data obtained from the present experiment data were analyzed statistically using ANOVA and Duncan's Multiple Range Test (DMRT) (Panse, V. G. Sukhatme, 1967) [7].

3. Results and Discussion

Laboratory experiments

Experiments were conducted under laboratory conditions to assess the efficacy of EPN, S. glaseri and H. indica against brinjal ash weevil grubs at different concentrations. It was observed that both S. glaseri and H. indica IJs at all concentrations caused grub mortality.

3.1. Efficacy of S. glaseri against ash weevil grubs

Petri plate assay

The highest grub mortality of 100% was observed at a concentration of 2000 IJs 72 and 96 h after inoculation. The mortality of grubs was found to be positively correlated with the concentration of IJs and time of exposure. After 24 hours, 2.5 to 30% mortality was observed at all concentrations. The percentage of mortality ranged between 27.5 to 80.0 after 48 h of exposure and 42.5 to 100 percent after 72 h of exposure of entomopathogenic nematodes.

Sand column assay

Under sand column assay, no mortality was observed 24h after inoculation of EPN. Grub mortality was observed only 48 h after EPN inoculation. The highest grub mortality of 95% was observed at a concentration of 2000IJs, 96 h after inoculation. The mortality of grubs was found to be positively correlated with the concentration of IJs and time of exposure. The percentage of mortality ranged between 12.50 to 50.0 after 48 h of exposure, 32.50 to 70.0 after 72 h and 57.50 to 95.0 after 96 h of exposure.

3.2. Efficacy of H. indica against ash weevil grubs

Petri plate assay

The highest grub mortality of 95% was observed at a concentration of 2000IJs 96 h after inoculation. The mortality of grubs was found to be positively correlated with the concentration of IJs and time of exposure. After 24 hours, 2.5 to 25% mortality was observed at all concentrations. The percentage of mortality ranged between 25.0 to 62.50 after 48 h of exposure, 40.0 to 87.50 after 72 h of exposure and 50.0 to 95.0 after 96 h of exposure of entomopathogenic nematodes.

Sand column assay

Under sand column assay, no mortality was obtained 24 h after inoculation of EPN. Grub mortality was observed only 48 h after EPN inoculation. The highest grub mortality of 87.5% was observed at a concentration of 2000IJs 96 h after inoculation. The mortality of grubs was found to be positively correlated with the concentration of IJs and time of exposure. The percentage of mortality ranged between 12.5 to 55 after 48 h of exposure, 27.50 to 65.0 after 72 h and 42.50 to 87.50 after 96 h of exposure to entomopathogenic nematodes.

It is observed that 95% mortality was observed in S. glaseri compared to 87.5% mortality due to H. indica at 2000 IJs after 96 h. Among the two EPN, S. glaseri exhibited more efficacy compared to H. indica for the management of ash weevil grubs M. subfasciatus.

Multiplication of EPNs in host cadavers

In this study, it was observed that EPNs were found to multiply within the host insect upto 5386±425.0 after three days per insect cadaver of S. glaseri and 5995.0±355.0 by H. indica.

EPN has been successfully used to manage soil borne insect pests. Many scientists have reported that EPNs gave good control of insect pests Subramanian *et al*., (2000) [13], Sankaranarayanan *et al*. (2006) [11], Nagesh *et al*. (2016) [6], Patil *et al*. (2016) [9], Sharifi *et al*. (2014) [12].

In the present study, EPN *S. glaseri* and *H. indica* were found to be successfully infect brinjal ash weevil *M. subfasciatus* and cause death of grubs within 96 hours. Among the two *S. glaseri* was found to be more effective than *H. indica*.

Earlier, Nagesh *et al*. (2016) [6] studied the efficacy of *S. abbasi*, *S. carpocapsae*, *S. glaseri*, *H. bacteriophora* and *H. indica* against ash weevil in different soil types and have

reported significant control of insects. However, in contrast to the present study they have recorded more infectivity by *H. indica* compared to *S. glaseri*. Similarly, Patil *et al*. (2016) [9] also reported that the mortality rate of ash weevil *M. subfasciatus* was high due to infectivity of *H. indica* was high compared to *S. glaseri*. In the present study infectivity of *S. glaseri* shows higher than *H. indica* against ash weevil *M. subfasciatus*. Gowda *et al*. (2016) [4] have also tested *S. carpocapsae* and *H. indica* against brinjal ash weevil and reported that among the two EPNs, *S. carpocapsae* was more effective than *H. indica*. Similar results were obtained in the present study.

According to Sharifi *et al*. (2014) [12] when the EPNs are able to reproduce inside their host, then it is considered as a successful insect pathogen and a good biocontrol agent. The present study showed successful reproduction of infective juveniles inside their host ash weevil grubs, *M. subfasciatus*, suggesting that EPNs, *S. glaseri* and *H. indica* can be used as biocontrol agents.

The results indicated that two entomopathogenic nematodes *viz*., *S. glaseri* and *H. indica* have biocontrol potential against *M. subfasciatus* grub. There is a vast scope for development of suitable cost effective and different formulations for management of *M. subfasciatus* in the field level.

Table1: Efficacy of *Steinernema glaseri* against ash weevil grubs (*Myllocerous subfasciatus*)

Dosage	Petri plate assay				Sand column assay			
	Percentage grub mortality				Percentage grub mortality			
	24h	48h	72h	96h	24h	48h	72h	96h
T1 500IJs	0.25 (2.50)	2.75 (27.50)	4.25 (42.50)	5.75 (57.50)	0.00 (0.00)	1.25 (12.50)	3.25 (32.50)	5.75 (57.50)
T2 1000IJs	1.00 (10.00)	3.25 (32.50)	5.25 (52.50)	7.50 (75.00)	0.00 (0.00)	3.25 (32.50)	5.50 (55.00)	7.50 (75.00)
T3 1500IJs	2.00 (20.00)	5.50 (55.00)	6.25 (62.50)	8.50 (85.00)	0.00 (0.00)	4.00 (40.00)	6.50 (65.00)	8.50 (85.00)
T4 2000IJs	3.00 (30.00)	8.00 (80.00)	10.00 (100.00)	10.00 (100.00)	0.00 (0.00)	5.00 (50.00)	7.00 (70.00)	9.50 (95.00)
T5 control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.00 (10.00)
SEd	0.24	0.69	0.11	0.09	0.00	0.19	0.15	0.11
CD	0.72	2.04	0.33	0.29	0.00	0.58	0.44	0.33

Figures in the parentheses show percentage grub mortality

Table 2: Efficacy of *Heterorhabditis indica* against ash weevil grubs (*Myllocerous subfasciatus*)

Dosage	Petri plate assay				Sand column assay			
	Percentage grub mortality				Percentage grub mortality			
	24h	48h	72h	96h	24h	48h	72h	96h
T1 500IJs	0.25 (2.50)	2.50 (25.00)	4.00 (40.00)	5.00 (50.00)	0.00 (0.00)	1.25 (12.5)	2.75 (27.50)	4.25 (42.50)
T2 1000IJs	0.75 (7.50)	4.50 (45.00)	5.25 (52.50)	6.25 (62.50)	0.00 (0.00)	3.25 (32.5)	5.00 (50.00)	6.75 (67.50)
T3 1500IJs	1.75 (17.50)	5.25 (52.50)	6.25 (62.50)	7.75 (77.50)	0.00 (0.00)	5.00 (50.00)	6.25 (62.50)	7.5 (75.00)
T4 2000IJs	2.50 (25.00)	6.25 (62.50)	8.75 (87.50)	9.50 (95.00)	0.00 (0.00)	5.50 (55.00)	6.50 (65.00)	8.75 (87.50)
T5 control	0.00 (0.00)	0.00 (0.00)	0.00(0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.00 (20.00)
SEd	0.26	0.17	0.15	0.12	0.00	0.17	0.14	0.19
CD	0.76	0.49	0.45	0.36	0.00	0.50	0.40	0.57

Figures in the parentheses show percentage grub mortality

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