FIELD EFFICACY OF THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA CARPOCAPSAE* (WEISER, 1955) AGAINST BRINJAL SHOOT AND FRUIT BORER, *LEUCINODES ORBONALIS* GUENEE

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Summary. The brinjal shoot and fruit borer, *Leucinodes orbonalis*, is the major pest of brinjal. Field trials were conducted for three consecutive years (2005-07) to assess the efficacy of an isolate of the entomopathogenic nematode *Steinernema carpocapsae* against this pest under field conditions. Three rates of the entomopathogenic nematode (1, 1.5 and 2 billion/ha) were evaluated by spraying infective juvenile stages 10-12 times, at ten-day intervals, during the brinjal growth cycle, starting at the 5-10% flowering stage. *Steinernema carpocapsae* caused significant reduction in fruit borer damage and increased yield in the first two years.

Keywords: Biological control, egg plant, field trial, *Solanum melongena*.

Eggplant, *Solanum melongena* L., is an important vegetable in India, with 26% of world production. Irrespective of the wide range of races, accessions and cultivars grown, the shoot and fruit borer, *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae), is the predominant destructive pest in south and southeast Asia (Ahmad, 1977), including India. The severity of infestation varies according to season and sometimes the entire crop can be destroyed (Alam *et al.*, 2003). The damage is observed initially on the plant shoots prior to flowering and later on the fruits. Indiscriminate spraying of chemical pesticides often fails to control the pest, besides being expensive and toxic to beneficial organisms and consumers. These problems have necessitated a search for alternative non-chemical methods of pest management.

Management of insect pests by biological control is an alternative strategy that results in pesticide-free produce with no hazard to the environment. Among the different agents for biological control, entomopathogenic nematodes (EPN) are gaining importance, because they possess many positive attributes of an effective biological control agent. EPN often have broad-spectrum effectiveness, short life cycles, amenability to mass production, recycling ability, persistence etc. (Gaugler, 1981; Kaya and Gaugler, 1993). In vitro studies carried out by Subramanian (2000) and Hussaini *et al.* (2002) showed that *Steinenerma* spp. are potential biological control agents of brinjal shoot and fruit borer. The insect's eggs are laid singly on the lower surface of leaves, mostly near the lateral veins. On hatching, the larva enters the plant through the nodal region of the terminal or lateral bud, and feeds in a circular manner on the outer portion of the stem. This results in the death of the terminal portion of the shoot. With initiation of flowering and hardening of shoots, larvae move to buds, flowers and young fruits. Fully grown larvae fall to the ground and pupate in the soil or among dried leaves by forming a silken cocoon. Laboratory observations indicate that the larva takes about 24 hour to enter the pupal stage. Observations undertaken under field conditions showed that about 14-28% of the half-opened flowers are infested with early larval stages (first, second and third instar). In the present study, larvae infesting flowers and those that have fallen onto the soil prior to pupation are targeted for control by *Steinernema carpocapsae* (Weiser). We present the results of a three-year field study undertaken to assess the efficacy of *S. carpocapsae* on brinjal shoot and fruit borer. A local isolate of *S. carpocapsae* (PDBC -11) was selected for the study. This strain was reported to have potential for the control of the brinjal borer (Hussaini *et al.*, 2002).

MATERIALS AND METHODS

The field experiments were conducted during the December-August growing season in 2004-05, 2005-06 and 2006-07, at the Indian Institute of Horticultural Research, Bangalore, Karnataka. The brinjal variety Arka Neelakanth was transplanted in an area of 500 m^2 at a spacing of 60 cm × 45 cm. The soil type where the experiment was conducted was a loamy red soil. NPK fertilizers at 120 kg: 80 kg: 50 kg/ha were applied to the crop. Half of the nitrogen was applied at transplanting and half 30 days after transplanting, while P and K were wholly applied at transplanting. The experiments were arranged according to a randomized block design comprising five treatments, each replicated five times. Each replicate was a plot of 20 m^2 with 40 plants. The

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dosages of the nematode *S. carpocapsae* at 1, 1.5 and 2 billion per hectare were compared with the standard check, Cypermethrin 25% EC at 0.5 ml/l (sprayed at fortnightly intervals), and an untreated control. The nematode isolate, denoted as PDBC isolate 11, was collected from the Bangalore area and is available at Project Directorate of Biological Control, Bangalore, Karnataka, India. The first sprayings of *S. carpocapsae* and cypermethrin were started when 5-10% of the plants were starting to flower. A total of 10-12 sprays of *S. carpocapsae* were given at ten-day intervals during the evening hours. The spray schedule and number of sprays were standardized based on earlier field studies carried out at the IIHR farm (Anonymous, 2004). The volume of water sprayed for the five plots receiving each treatment (100 m^2) was calculated based on a standard recommendation of 500 l/ha. Spraying was carried out with a gator GR-5 rocking sprayer (ASPEE) having a duro-mist spray nozzle. Separate sprayers were used for the EPN spraying and chemical spraying. In addition to foliar application, the soil below the plant was also moistened with EPN suspension so that final instar larvae and pre-pupal stages of the insect were also exposed to nematode suspension (pupation is reported to take place in the soil). Uniform foliar applications were made to ensure that the whole of each plant was drenched with the EPN suspension. The top soil (to a depth of 1 cm) of the root zone area of the plant (up to 10 cm diameter) was also uniformly wetted with the suspension from the sprayer. However, the proportions of the EPN applied to the foliage and soil were not determined. The field was irrigated before and 24 hrs after spraying, to increase the activity of the EPN. The crop was irrigated at the rate of 4-5 litres of water/m^2/day by drip irrigation with 1 bar pressure. Since there was no severe incidence of any disease or pests, no fungicides or insecticides were used as plant protection measures.

Steinernema carpocapsae was multiplied in the laboratory on wax moth, *Galleria mellonella* L. Larvae and infective juvenile stages (IJs) were collected, filtered and embedded in 10 cm × 10 cm high density sponge (Anonymous, 2008). These sponge sheets were enclosed in plastic covers, kept at room temperature of 24-27 °C and used for spraying in the field. EPN embedded sponges less than a week old were used. The IJs of *S. carpocapsae* needed for each treatment (1, 1.5 and 2 billion/ha) were embedded in separate sponge sheets. Prior to spraying, sponge sheets were immersed in a flat bottom plastic basin (1 m diameter) containing one litre of water for one hour, allowing the IJs to move from the sponge sheets to the water in the basin. This procedure was repeated two or three times to recover most of the IJs. The water in the basin was well stirred with an aerator and 1 ml of the suspension was taken with a pipette and checked under a stereo microscope to count the number of active IJs. This procedure was followed for each treatment separately. The required quantity of water as spray fluid was added to the suspensions before spraying the recommended dosage of nematodes in 500 l/ha of water for each treatment. No adjuvant was used in the spray suspensions.

The effect of *S. carpocapsae* on the insect was assessed by recording the fruit damaged at harvest. A total of eight to twelve harvests were made per growth season. Weight of healthy and damaged fruits from each plot was recorded separately at each harvest. The data on percentage of fruit damaged at each harvest was pooled to determine the mean percentage borer damage in the different treatments. The marketable yield obtained in each treatment was expressed as weight per hectare to compare the effects of different treatments.

Based on the numbers of damaged and undamaged fruits, the percentages of fruits damaged at each harvest were calculated. The harvest data was pooled and mean percentages of fruits damaged were also calculated. The resultant values were converted into arcsine-transformed values and subjected to analysis of variance (ANOVA). Treatment means were compared with the F test at a level of significance of $P = 0.05$.

RESULTS AND DISCUSSION

Steinernema carpocapsae significantly reduced borer incidence in each year of experiment. In the first year, a total of eight harvests was made. Fruits damaged varied from 3.2 to 17.8%, with mean fruit damage percentages of 9.7, 8.7 and 7.5% in *S. carpocapsae* sprayed at 1, 1.5 and 2 billion IJ/ha, respectively (Table I). These levels of damage did not differ significantly from each other or the chemical treatment (7.1%), but were significantly less than the untreated control, in which infestation varied from 14% to 38.6% with a mean of 20.9% fruit damage.

In the second year trial, a total of nine harvests was made. There were 8.6, 7.4 and 7.9% of fruits damaged in plots sprayed with *S. carpocapsae* at 1, 1.5 and 2 billion IJs/ha, respectively, compared to 8.9% in the plots treated with cypermethrin and 22.4% in the untreated control (Table II). *Steinernema carpocapsae* treatments were at par with each other and cypermethrin and significantly superior to the control.

Similar results were recorded in the third year experiment (Table III), when a total of twelve harvests was made. The mean percentages of fruits damaged at harvests were statistically at par with each other and significantly less than in the control.

The efficacy of the treatments resulted in significant yield increases in the first two years (Table IV). In the first year, marketable yields of 28.7, 29.3 and 32.5 t/ha of brinjal were harvested from plots sprayed with 1, 1.5 and 2 billion *S. carpocapsae* per ha, respectively, which were at par with each other and significantly more than in the control (23.8 t/ha). Use of cypermethrin produced 31.1 t/ha of brinjal. The results were similar in the second year but the total yield was less than in year

Table I. Effect of *Steinernema carpocapsae* against brinjal shoot and fruit borer, *Leucinodes orbonalis.* Per cent fruit damaged (2004-2005).

Treatment	Per cent fruit damage at each harvest								
	I harvest	II harvest	III harvest	IV harvest	V harvest	VI harvest	VII harvest	VIII harvest	Mean
S. carpocapsae at 1 billion/ha	17.8b	8.5a	4.5a	7.4a	10.2a	9.2a	8.0a	7.2a	9.7b
S. carpocapsae at 1.5 billion/ha	15.3a	5.8a	5.4a	9.0a	6.8a	10.0a	9.9a	7.8a	8.7 b
S. carpocapsae at 2 billion/ha	10.7a	6.9a	3.2a	7.0a	10.2a	7.9a	9.0a	5.2a	7.5a
Cypermethrin at 0.05%	11.1a	8.0a	4.6a	7.3a	6.6a	7.1a	7.3a	4.5a	7.1a
Control	38.6c	19.3b	14.0b	16.1b	18.5b	19.5b	21.2b	20.0b	20.9c
CD at P= 0.05	5.4	4.1	3.9	2.5	3.6	6.3	3.4	5.0	1.30
CV	16.05	17.36	17.09	10.5	14.4	25.82	22.14	13.25	5.14

Figures in columns followed by the same letter are not significantly different.

Table II. Effect of *S. carpocapsae* against brinjal shoot and fruit borer, *L. orbonalis*. Per cent fruit damaged (2005-2006).

Treatment	Per cent fruit damage at each harvest									
	I harvest	II harvest	III harvest	IV harvest	V harvest	VI harvest	VII harvest	VIII harvest	IX harvest	Mean
S. carpocapsae at 1 billion/ha	11.9a	9.5a	17.7b	6.6a	10.3a	5.2a	7.7b	6.4a	3.2a	8.6a
S. carpocapsae at 1.5 billion/ha	11.4a	9.2a	9.8a	8.1a	11.8a	2.6a	4.6a	7.4a	1.5a	7.4a
S. carpocapsae at 2 billion/ha	10.7a	7.7a	13.7a	6.5a	11.2a	9.9b	7.4a	4.3a	3.2a	7.9a
Cypermethrin at 0.05%	13.9a	11.4b	10.6a	7.8a	9.7a	9.2b	4.3a	8.3a	1.5a	8.2a
Control	18.3b	22.6c	21.4c	23.4b	19.4b	20.4c	29.7c	23.5b	21.0b	22.4b
CD at P = 0.05	3.50	3.39	5.02	2.64	2.97	3.18	3.08	4.09	2.77	1.77
CV	12.30	13.72	16.83	10.67	10.59	16.5	23.3	16.3	15.73	12.32

Figures in columns followed by the same letter are not significantly different.

Table III. Effect of *S. carpocapsae* against brinjal shoot and fruit borer, *L. orbonalis*. Per cent fruit damaged (2006-2007).

Treatment	Per cent fruit damage at each harvest												
	I harvest	II harvest	III harvest	IV harvest	V harvest	VI harvest	VII harvest	VIII harvest	IX harvest	X harvest	XI harvest	XII harvest	Mean
S. carpocapsae at 1 billion/ha	34. 3a	11.6a	17.7a	14.8a	10.0a	8.7a	5.5a	13.4a	5.9	5.4a	5.7	7.5a	12.17 a
S. carpocapsae at 1.5 billion/ha	34.9b	11.6a	23.7b	12.8a	7.2a	12.3a	7.7a	15.0a	6.0	6.8a	7.7	3.3a	12.93bc
S. carpocapsae at 2 billion/ha	26.4a	11.1a	15.9a	14.9a	9.1a	10.5a	5.3a	8.0a	3.9	4.4 a	9.0	5.3a	10.35 a
Cypermethrin at 0.05%	42.6c	16.2b	26.4b	19.9b	12.6b	11.9a	5.6a	17.1a	6.7	8.8a	8.3	4.8a	14.90 b
Control	45.8d	27.9c	38.6c	32.3c	31.8c	33.6b	55.2b	55.2b	12.0	13.3b	11.8	13.2b	25.28 c
CD at P=0.05	7.90	4.06	5.71	4.81	4.03	4.07	8.01	17.53	NS	4.33	NS	4.53	2.5
CV	15.40	12.72	14.05	13.66	13.23	13.13	27.47	51.15	33.54	19.45	18.31	21.90	11.57

Figures in columns followed by the same letter are not significantly different.

1. Means of 13.9, 12.9 and 13.7 t/ha of fruits, respectively, were recorded in the plots treated with 1, 1.5 and 2 billion *S. carpocapsae* per ha, which were significantly more than the 10.7 t/ha recorded in control plots. Fruit yield in all treatments increased markedly in the third year of the trial and no treatment differed significantly from any other. Such annual yield variation (nearly fourfold in control plots) in field experiments with *S. carpocapsae* against shoot and fruit borer of brinjal were also observed by Punjab Agricultural University and Kerala Agricultural University, located 2000 and 400 km from the study location, respectively (Anonymous, 2007, 2008). Although specific reasons for this variation could not be ascertained, these places experience higher temperatures and more rainfall than Bangalore.

Timing of application of entomopathogenic nematodes with reference to the life cycle of the target insect is a key factor to increase efficacy (Hussaini and Singh, 1998). Larvae of brinjal shoot and fruit borer, after completing their development, fall to the soil, where they remain as pre-pupae for nearly 24 hours before forming a cocoon for pupation. Moreover, we have observed that about 18-28% of half-opened flowers of eggplant at any time of flowering under field conditions are harbouring early instar stages of *L. orbonalis* larvae. Factors such as temperature and sunlight are reported to affect the activity of IJs (Gaugler and Bousch, 1978; Gaugler *et al.*, 1992; Grewal *et al.*, 1994). Spraying of IJs at dusk is reported to reduce the negative effects of sunlight by maintaining high RH (Lello *et al.*, 1996). Also, the use of local isolates, which are adapted to local temperatures, was reported to give a high level of efficacy against the target pest (Wright and Mason, 1997). In the present study, spraying was made in the evening hours and a local isolate that was reported promising was used. These factors and the irrigation of the field before and after spraying might have combined to contribute to the effectiveness of *S. carpocapsae* against *L. orbonalis*.

Earlier studies by Hussaini *et al.* (2002) reported that application of *S. carpocapsae* reduced fruit damage in terms of number of fruits bored by *L. orbonalis* and increased the yield of brinjal. The results reported in this article indicate that *S. carpocapsae* is a potential biological control agent of brinjal shoot and fruit borer and could be utilized either alone or in combination with other biological control agents, such as the egg parasitoid *Trichogramma chilonis* Ishii, to develop a bio-control based management strategy that could further reduce pest damage.

Repeated chemical spraying to combat *L. orbonalis* often results in unsatisfactory control. Hence, farmers are willing to adopt alternative control measures such as biological control. This measure is safe and effective, although the cost of the treatments may be greater than that of chemical pesticides. Nevertheless, there is a good scope for using biological control agents such as *S. carpocapsae* for the management of brinjal borer, especially

Table IV. Effect of treatments with *S. carpocapsae* on yield of brinjal infested by *L. orbonalis*.

Treatment	Yield (t/ha)		
	Year I 2004-05	Year II 2005-06	Year III 2006-07
S. carpocapsae at 1 billion/ha	28.7a	13.9a	42.2
S. carpocapsae at 1.5 billion/ha	29.3a	12.9a	43.2
S. carpocapsae at 2 billion/ha	32.5a	13.7a	42.8
Cypermethrin at 0.05%	31.1a	13.4a	43.3
Control	23.8b	10.7b	40.9
CD at P = 0.05	4.3	1.72	NS
CV	8.53	10.12	3.34

Figures in columns followed by the same letter are not significantly different.
NS = Non-significant.

on organic farms. However, timely availability of good quality bio-control agents is one of the factors that are presently hindering biological control in India.

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