Indian Journal of Entomology, 70(4): 346-349 (2008)

PATHOGENICITY AND *IN VIVO* CULTURING OF A LOCAL ISOLATE OF *STEINERNEMA CARPOCAPSAE* AGAINST *SPODOPTERA LITURA* (FAB.)

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ABSTRACT

Experiments were conducted in the laboratory to find out the pathogenicity of local isolate of *Steinernema carpocapsae* against 3rd, 4th and 5th instar larvae of tobacco caterpillar, *Spodoptera litura* (Fab.). Study revealed cent per cent mortality after 96 hours in all the tested larval instars and at all the doses. The LC_{50} values varied between 11.41 and 27.17 infective juveniles per larva in all the instars. The LT_{50} values of 3rd, 4th and 5th instar larva of *S. litura* were observed to be 24.8, 31.5 and 38.8 hours, respectively. Fifth instar larvae of *S. litura* at a dose of 160 infective juveniles per larva produced maximum infective juveniles (3.29×10^5) whereas minimum number of infective juveniles (0.97×10^5) was obtained from 3rd instar larvae at a dose of 10 infective juveniles per larva.

Key words: *Steinernema carpocapsae*, entomopathogenic nematodes, *Spodoptera litura*, pathogenicity

Tobacco caterpillar, *Spodoptera litura* is one of the serious insect pests of cole crops causing yield loss upto 45 per cent (Sachan and Gangwar, 1990). Sole reliance on pesticides for its management is not sustainable and alternate and effective eco-friendly methods have to be adopted as this trend has gained momentum in recent years. Biological control of crop pests is an ideal alternative and safe to non-target organisms. Use of Entomopathogenic Nematodes (EPNs) against crop pests is one such alternative which has shown promise in recent years. The impressive attributes of EPN have stimulated strong commercial interest in nematodes as biological insecticides. These include their wide spectrum of insecticidal activity, ability to kill most hosts within short periods and efficient mass culturing techniques (Kaya and Gaugler, 1993). Entomopathogenic nematodes (EPN) of the genera Steinernematidae and Heterorhabtidae are soil born organisms equipped with a bacterium, which kills the insect host (Han and Ehlers, 2000). The EPN are safe to users and the environment (Ehlers and Hokkanen, 1996). The pathogenicity of *S. carpocapsae* (DD-136) on *S. litura* larvae was studied under various conditions in filter paper bioassay.

MATERIALS AND METHODS

A survey was conducted and a total of 230 samples were collected from fruit crops, ornamental plants, vegetables, oil seeds, cereals, barren land in different locations of Jammu division (Udheywalla, Marh, Chatta, R.S. Pura, Dhainsar, Samba, Hiranagar, Kookachalk, Nagri, Kathua, Chadwal, Udhampur, Changran, Pancherry and Aknoor) during 2005-06 and 2006-07. Each soil sample (approximately 1kg) was a composite of 5 random sub samples taken distantly located from each other in an area of 10 m^2 and at a depth of 0-20 cm. Samples were taken with a hand shovel, placed in polyethylene bags to prevent water loss and brought to laboratory for further studies.

Entomopathogenic nematodes were recovered from soil samples using the insect baiting method as described by Bedding and Akhurst (1975). Insect baits (five last instar *Galleria mellonella* larvae) were placed in 250g plastic containers with moistened soil obtained from each sample. Containers were covered with a lid, turned up side down and kept at room temperature (25 ± 2^0C). Water was added to samples if they appear dry at any point during their baiting. Larvae of *G. mellonella* were checked daily and its mortality was recorded up to 10 days. Dead larvae from each sample were rinsed thrice with sterile distilled water and incubated on modified white trap method for the extraction of entomopathogenic nematodes. The entomopathogenic nematodes that emerged from the larvae were collected in test tubes using sterile distilled water and were allowed to settle at the bottom of test tube. The supernatant suspension was separated out

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and the process was repeated 4-5 times for clear nematode suspension. Re-inoculation of extracted entomopathogenic nematodes were done in petriplates on Whatman No. 1 filter paper and re-isolated by using Koch's postulates technique to confirm the pathogenic status of entomopathogenic nematodes on fresh *G. mellonella.*

Progeny production of various isolates of entomopathogenic nematodes in *S. litura* were determined at different inoculum of entomopathogenic nematodes *viz.*, 10, 20, 40, 80, 160 and 320 infective juveniles/larva on moist filter paper (Whatman No.1) in 10 cm diameter petridishes. Samples were checked daily one week after inoculation for the emergence of infective juveniles. Each day emergence was observed; infective juveniles were rinsed into a vial and stored at 15°C. Nematodes were counted in the entire sample or by serial dilution when number grew higher than 100 infective juveniles per day.

Another experiment was conducted in the laboratory to find out the pathogenicity of local isolate of *S. carpocapsae* against 3rd, 4th and 5th instar larvae of *S. litura.* Six well tissue culture plates were used and each well was padded with single layer of Whatman No. 1 filter paper. Single 3rd, 4th and 5th instar larva of test insect was placed in each well and infective juveniles were inoculated @ of 10, 20, 40, 80, 160 and 320/ larva in 0.5 ml water. The mortality data were recorded 24, 48, 72 and 96 h post exposure and the LC_{50} and LT_{50} were calculated by probit analysis using SPSS software (version13).

RESULTS AND DISCUSSION

In present findings, EPN were recovered from only 3 out of 230 samples (1.3%), the recovery highlights the importance of conducting a more intensive survey. Kumar *et al.* (2003) also reported the presence of entomopathogenic nematodes in 5 samples out of 105 samples. Lorio *et al.* (2005) also noticed similar observations wherein they also found *Steinernema* spp. only in 12.3% samples. The results of the pathogenicity of *S. carpocapsae* against 3rd, 4th and 5th instar larvae of *S. litura* are presented in Table 1, which shows the highest mortality after 24 hours (87.50%) in 3rd instar larvae at a dose of 320 infective juveniles larva. However, no mortality was recorded in 5th instar larva at a dose of 10 infective juveniles / larva. Cent per cent mortality after 48 hours was observed in 3rd and 4th instar larva at a dose of 320 infective juveniles while the lowest mortality (32.50%) was found in 5th instar larvae at a dose of 10 infective juveniles/larva. After, 72 hours, cent percent mortality was recorded in 3rd, 4th and 5th instar larvae inoculated with 160 and 320 infective juveniles/larva, whereas after 96 hours cent percent mortality was recorded in all the instars and against all the doses tested. Our findings are akin to the work of Sivakumar *et al.* (1998). However, the

Table 1. Mortality of 3rd, 4th and 5th instar larvae of *S. litura* to *S. carpocapsae* (JMU) at different time intervals

Concentration (IJ's/larva)	Per cent mortality of insect larvae after exposure											
	24h			48h			72h			96h		
	3rd Instar	4th Instar	5th Instar	3rd Instar	4th Instar	5th Instar	3rd Instar	4th Instar	5th Instar	3rd Instar	4th Instar	5th Instar
10	22.50	10.00	0.00	52.50	47.50	32.50	75.00	67.50	57.50	100.00	100.00	100.00
20	37.50	17.50	7.50	60.00	55.00	42.50	87.50	80.00	70.00	100.00	100.00	100.00
40	52.50	32.50	22.50	72.50	62.50	55.00	95.00	92.50	82.50	100.00	100.00	100.00
80	65.00	45.00	30.00	85.00	75.00	7.00	100.00	100.00	90.00	100.00	100.00	100.00
160	77.50	62.50	45.00	92.50	87.50	82.50	100.00	100.00	100.00	100.00	100.00	100.00
320	87.50	77.50	67.50	100.00	100.00	97.50	100.00	100.00	100.00	100.00	100.00	100.00
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

present findings do not corroborate with those of Vyas and Yadav (1992) where they recorded cent per cent mortality after 72 hours.

The LC_{50} values for 3rd, 4th and 5th instar larvae (Fig. 1) depicted that 11.41 infective juveniles/larva could cause 50% mortality of 3rd instar larva. The LC_{50} values of 4th and 5th instar were found to be 15.62 and 27.17 infective juveniles/larva respectively. The LC_{50} values of 3rd and 4th and 4th and 5th instar larvae do not differ significantly as their fiducial limits are overlapping with each other. However, the LC_{50} values of 3rd and 5th instar larvae differ significantly from each other, as their fiducial limits are not overlapping. The LT_{50} values of 3rd, 4th and 5th instar larvae of *S. litura* and their corresponding fiducial limits were observed to be 24.8, 31.5 and 38.8 and (3.6 - 44.9), (17.4 - 44.5) and (33.4 - 44.2) hours respectively. The LT_{50} values of 3rd, 4th and 5th instars of *S. litura* were non significant from each other as the fiducial limits are overlapping. The present study corroborate with that of Sivakumar *et al.* (1998) while testing the pathogenicity of *S. carpocapsae* against *S. litura.*

The progeny production of *S. carpocapsae* (JMU) was studied in the laboratory. The number of infective juveniles produced from each larva was recorded. Maximum infective juveniles (3.29×10^5) were produced from 5th instar larva at a dose of 160 infective juveniles/larva (Fig. 2). However, minimum number of infective juveniles (0.97×10^5) was obtained from 3rd instar larva at a dose of 10 infective juvenile/larva.

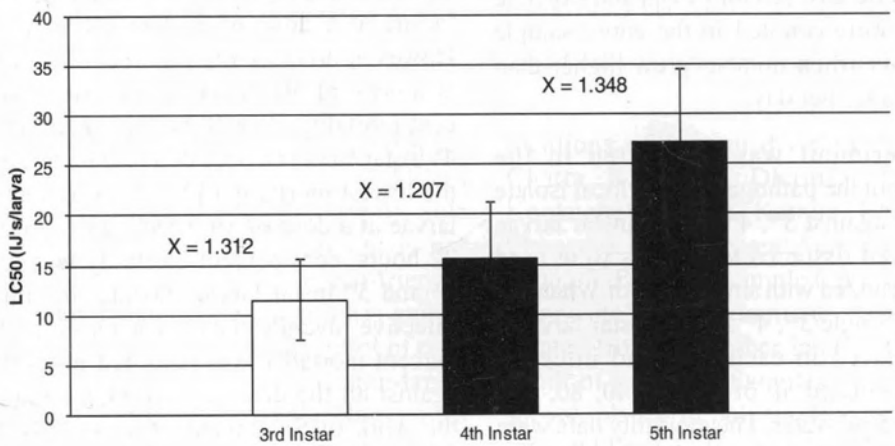

Fig. 1 L.C.50 values of *Steinemema carpocapsae* (JMU) against 3rd to 5th instar larvae of *Spodoptera litura* Error bars indicate 95% fiducial limits and X=slope calculated according to probit analysis

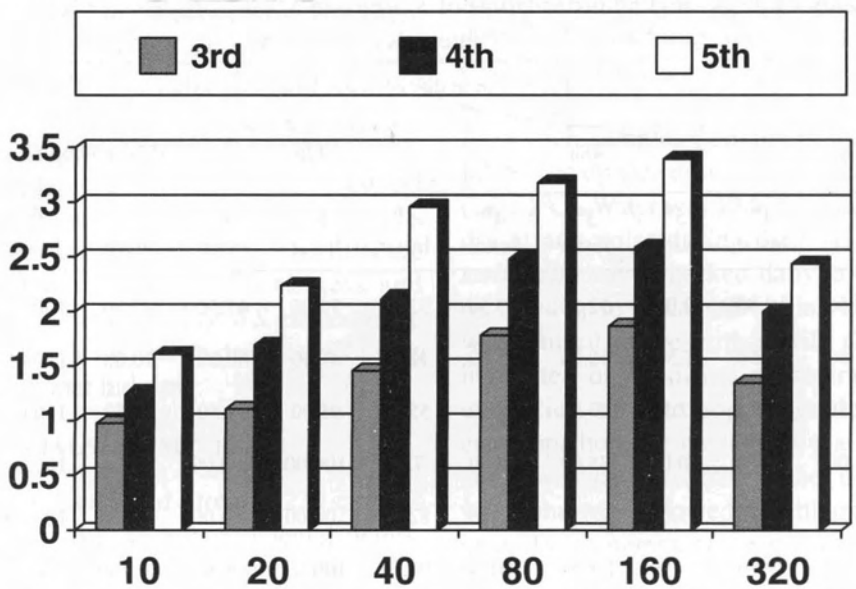

Fig.2. Progeny production of *Steinernema carpocapsae* (JMU) in different instars of *Spodoptera litura* (in lakhs)

The data further showed that at each dose, the 5th instar larva produced more infective juveniles as compared to 3rd and 4th instars. The production increased with increase in dose upto 160 infective juveniles/larva but at the dose of 320 infective juveniles the production decreased in all the instars.

In general nematode yield is proportional to host size (Flander *et al.*, 1996). *In vivo* production, yields are dependent on nematode doses (Boff *et al.*, 2000). In present experiment, the production of infective juveniles also showed similar trend. A dose that is too low, results in low host morality and the dose that is too high results in high level of failed infections due to competition with secondary invaders.

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(Manuscript Received: August 2008)