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Virulence of *Steinernima* and *Heterorhabditis* against black Cutworm, *Agrotis segetum* (Lepidoptera: Noctuidae) in Potato Crop

Neelam Kumari[1*], Deepak C. Kalia[1] and V.K. Chandla[2]

[1]*Department of Bio-sciences, Himachal Pradesh University Shimla, India*
[2]*Department of Plant Protection, ICAR- CRRI Shimla, India*

**Corresponding author*

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A B S T R A C T

The common cutworm (turnip moth) *Agrotis segetum* (Denis & Schiffermuller) is nearly cosmopolitan insect pest of potato. The efficacy of the entomopathogenic nematodes (EPN) *Steinernema carpocapsae* and *Heterorhabditis indica* was evaluated against different developmental stages of *A. segetum* under laboratory conditions. In laboratory studies, significant mortality of second instar larvae was recorded by *H. indica* (73.3-100 per cent) and 63.3-100 per cent by *S. carpocapsae* after 48 hrs of treatment at EPNs suspension of 50-300 IJs/ml (1 ml per larva). In the preliminary bioassay study, all the tested EPNs were capable to cause maximum mortality within three to five days of treatment. The highest mortality (100 per cent) of fourth instar larvae and pupae was observed at more than 200 IJs/ml nematodes suspension of *S. carpocapsae* and *H. indica* after five days of treatment. At nematodes suspension 50 IJs/ml, 56.7 per cent mortality of fourth instar was recorded by *S. carpocapsae* and 76.7 per cent by *H. indica* after five days of treatment. The pupae of *A. segetum* were less susceptible to EPNs infections as compared to larvae due to reduced number of portal entries.

Introduction

Among soil pests, *Agrotis segetum* (Denis & Schiffermueller) belonging to family Noctuidae and order Lepidoptera, is the major pest of potato in Himachal Pradesh. The cutworms (larvae of *Agrotis* spp.) cut off the tender stalks of young plants of potato at their bases or a few centimeters above the ground during night and feed on them. Sometimes, heavily infested fields look as if they have been grazed and losses caused by them during epidemic year cross over 30 per cent (Chaudhary, 1953). In young crops, cutworms usually damage tender shoots and branches. However, after tuberisation their damage is confined to tubers in standing crop. The five species of cutworm *viz.*, *A. ipsilon* (Hufnagal), *A. interacta* (Walk.), *A. flammatra* (Denis & Schiffermueller), *A. spinnifera* (Hufnagal) and *A. segetum* (Denis & Schiffermueller) have been reported on potato crop (Chaudhary, 1953 and Nirula, 1961). Saxena and Misra (1983) reported 12 per cent tuber damage in potato crop by cutworm in Shimla (India).

At present, synthetic insecticides are the primary means for the control of cutworms. But due to the implementation of the Food Quality Protection Act, it has become necessary to look forward towards the biological control measures for the management of cutworm. There are many factors that in theory make entomopathogen nematodes (EPN) the ideal biological control agents. Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are widely distributed in soils throughout the world. Additionally, some species of nematodes can actively seek out their hosts and kill them rapidly. The studies on evaluation of effectiveness of these nematodes in potato crop in the mid and higher hills of Himachal Pradesh were limited. In the present study entomopathogenic nematodes, *S. carpocapsae* and *H. indica* were evaluated against cutworms (second, fourth instar larvae and pupae) in laboratory conditions.

Materials and Methods

Procurement and Mass Multiplication of EPN Entomopathogen nematodes

The entomopathofenic nematodes**,** *S. carpocapsae* and *H. indica* were procured from Protect Directorate of Biological Control (PDBC), Bangalore, India. The waxworm, *Galleria mellonella* is the insect of choice for in vivo production because it is produced commercially in large numbers (Ehlers R., 2001). The basic method for small-scale production is described in Kaya and Stock (1997). White trap having inverted watch glass (60mm) placed in Petri dish (150 × 20 mm) and filled with 15 ml sterile water. A moist filter paper placed on watch glass. *G. mallonella* cadavers are placed onto this filter paper. After two week days infective juveniles (IJ) started emerging from the cadavers and migrated into the water of Petri dish. IJ are the third stage juveniles of entomopathogenic nematodes and only this infective stage of EPN is capable of surviving outside the host and moving from one insect to another.

Laboratory bioassay

Larval mortality bio-assays were carried out in petri dishes (35 x 10 mm) lined with double layer of Whatman no. 1 filter paper (Kaya and Stock (1997). The nematode suspensions of 50 to 300 infective juveniles (IJs) in 1 ml of deionized water were added to the filter paper in petri plates. Control plates were treated with distilled water only. Five replicates were considered for each treatment and Petri dishes were kept at 27 ± 1 °C. Dead larvae were recognized according to change in their body color. Cadavers were transferred to White trap to confirm nematode infection. The experiment was repeated thrice. The untreated controls were identical to the treatment except that no IJs were added. The larval mortality was recorded at every 24 hrs for a week. The cause of larval death was confirmed by change in body colour of the cadaver due to symbiotic bacteria. The raw values were square root transformed and percent data were arcsine transformed. All data were subjected to the one-way ANOVA. Mean difference was tested using Duncan's multiple range test (DMRT). MSTAT-C software was used for analysis.

Results and Discussion

Bioassay of EPNs against 2nd instar cutworm, *A. segetum*

The study on per cent mortality of second instar cutworm at EPNs suspension of 50-300 IJs/ml (1 ml per larva) showed 73.3-100 per cent mortality by *H. indica* followed by 63.3-100 per cent by *S. carpocapsae* after 48 hrs of treatment. The mortality increased with

increased in the numbers of EPNs from 50-300 IJs/ml. At nematodes suspension of 50 IJs/ml, 63.3 per cent by *S. carpocapsae* after 48 hrs of treatment (Fig. 1), whereas 73.3 per cent was recorded by *H. indica* (Fig. 2). At nematodes suspension of 100 IJs/ml, maximum mortality (96.6 per cent) was observed by *H. indica* which was followed 80.0 per cent by *S. carpocapsae*, whereas maximum mortality (100 per cent) was found at nematodes suspension more than 200 IJs/ml in all the treatments. Among all the treatments, *H. indica* proved as most effective one due to the presence of dorsal tooth that can locate the host plant easily and caused early mortalilty of target pests. The results are in agreement with Hussaini (1980), who reported that the infective juveniles of *Heterorhabditis* spp. gained entry by abrading the inter-segmental membranes of the insect by using dorsal tooth (Table 1).

Preliminary bioassay of EPNs on 4th instars cutworm and pupae, *A. segetum*

The highest mortality (100 per cent) of fourth instar larvae and pupae was observed at more than 200 IJs/ml nematodes suspension of *S. carpocapsae* and *H. indica* after five days of treatment. At nematodes suspension 50 IJs/ml,, 56.7 and 76.7 per cent mortality was recorded respectively, *S. carpocapsae* and *H. indica* after five days of treatment. At nematodes suspension 100 IJs/ml, 70.0 and 83.3 per cent mortality was recorded respectively, *S. carpocapsae* and *H. indica* at five days of treatment (Table 2).

The pupae of *A. segetum* were less susceptible to EPNs infections as compared to larvae due to reduced number of portal entries. At nematodes suspension 200 IJs/ml, 86.7 and 93.6 per cent mortality of pupae was recorded in *S. carpocapsae* and *H. indica* respectively as compared to control (3.33-6.67 per cent) (Table 2). Kaya and Gangler (1993) also reported that the pupae were less susceptible to nematodes infections than the larvae because of the reducing numbers of the portal entries. Ebsaa and Koppenhofer (2011) reported the efficacy of commercial products containing the entomopathogenic nematodes *H. bacteriophora*, *S. carpocapsae*, *S. feltiae* and *S. riobrave* against fourth instar black cutworm. The results are in agreement with the findings of Badr *et al.*, (2009), investigated the effects of entomopathogenic nematodes, *S. carpocapsae* and *H. indica* against *A. ipsilon*, this study proved that *S. carpocapsae* and *H. indica* were highly virulent at nematodes suspension of 100 IJs/ml that caused 100 per cent mortality of third instar larvae and pupae.

Table.1 Susceptibility of 2nd instar larvae of *A. segetum* to different concentrations of EPNs

(IJs/ ml)	Mortality % (48 hrs)	
	Sc	Hb
50	63.3 (52.73)	**73.3 (58.91)**
100	80.0 (63.46)	**96.6 (79.41)**
200	93.3 (75.03)	**100 (90.04)**
250	100(90.04)	**100 (90.04)**
300	100 (90.04)	**100 (90.04)**
Control	0.0 (0.0)	**6.67 (14.91)**
S.Em.±	2.01	**2.35**
C.D.(0.05)	**8.78**	**6.35**

Figures in parentheses are arc sine transformed values; Sc = *S. carpocapsae;* Hb = *H. indica*

Table.2 Susceptibility of 4th instar larvae and pupae of *A. segetum* to different concentrations of EPNs

Nematode Concentrations (IJs/ larvae)	Mortality % (5 DAT)			
	Larvae		Pupae	
	Sc	Hb	Sc	Hb
50	56.7 (48.47)	76.7 (61.14)	50.0 (45.02)	**63.3 (52.73)**
100	70.0 (56.81)	83.3 (65.91)	60.0 (50.79)	**76.7 (61.16)**
200	90.0 (71.60)	96.7 (79.57)	86.7 (68.64)	**93.3 (75.03)**
250	100.0 (90.04)	100.0 (90.04)	100.0 (90.04)	**100.0 (90.04)**
300	100.0 (90.04)	100.0 (90.04)	100.0 (90.04)	**100.0 (90.04)**
Control	0.00 (0.0)	3.33 (10.51)	0.00 (0.0)	**6.67 (14.97)**
S.Em.±	1.70	2.30	1.25	**3.64**
C.D.(0.05)	**5.25**	**7.08**	**3.88**	**9.37**

Fig.1 The percentage mortality of 2nd instar larvae of *A. segetum* by different concentrations of infective juveniles of *S. carpocapsae* (Sc)

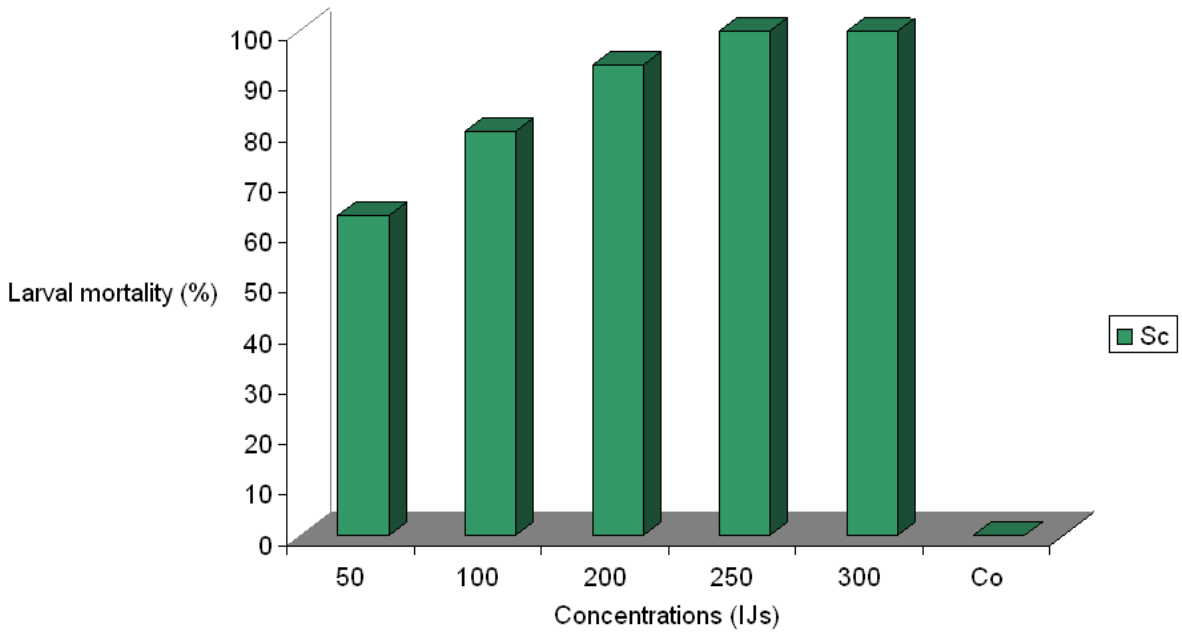
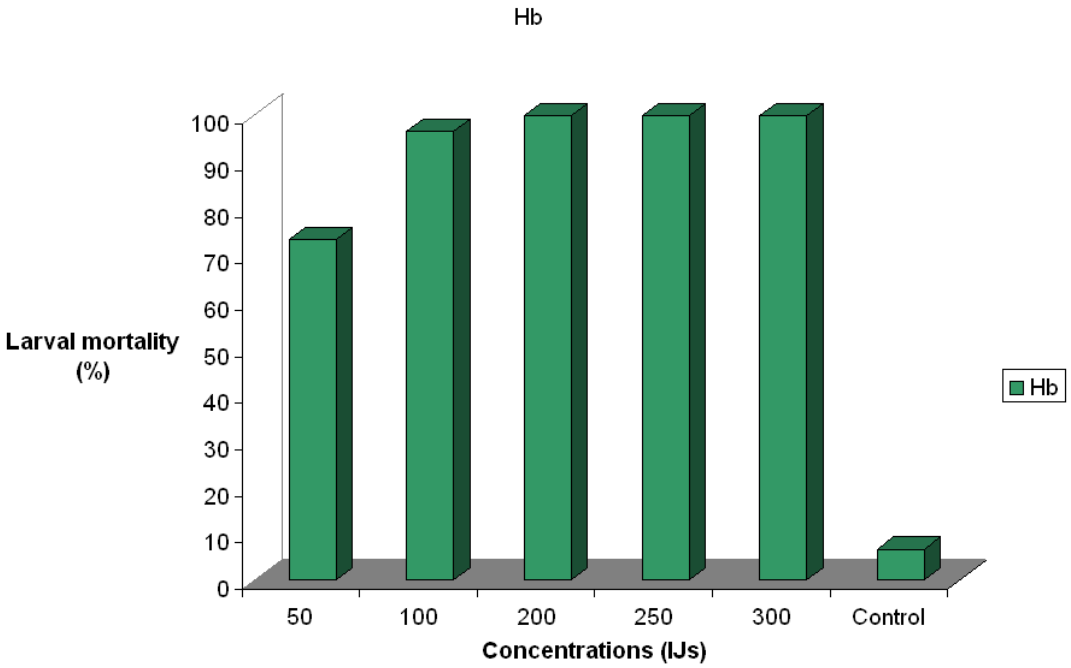


Fig.2 The percentage mortality of 2nd instar larvae of *A. segetum* through different concentrations of infective juveniles of *H. indica* (Hb)



Hussaini (1980) reported the efficacy of different formulations (talc based, alginate capsules and water) of *S. carpocapsae*, *S. abbasi* and *H. indica* against *A. ipsilon* in a filter paper and soil assay. The results showed that alginate formulations caused the maximum mortality of 33-47 per cent and 60-80 per cent after 96 hrs for the filter paper and soil assay, respectively. The survival of infective juveniles was highest in water followed by talc and alginate for *Steinernema* spp. The larval mortality of target pests was due to the toxin released by the symbiotic bacteria associated with the EPNs inside the haemocoel of insect's larvae. Hussaini (1980) also reported that once in the haemocoel of the insect the infective juveniles released cells of the symbiotic bacterium (*Xenorhabdus* spp. for *Steinernema* spp. and *Photorhabdus* spp. for *Heterorhabditis* spp.) which it carried in its intestine. The insect haemolymph provided rich nutrients medium for the bacterial growth and these bacteria released toxins/ exoenzymes, which killed the insect through septicemia. Divya, K and Sankar M (2009) proved that EPN *H. indica* is a potential option for possible management, capable of nematode production with efficacy notable against cotton bollworm, *Helicoverpa armigera* and tobacco caterpillar, *Spodoptera litura*.

EPNs have been successfully employed as efficient biocontrol agents against the larvae of some noctuids, including *A. ipsilon* (Hufnagel) and *A. segetum* in some Asian or European countries (Georgis *et al.*, 2006; Kaya *et al.*, 2006; Lacey & Georgis, 2012). The study provides evidence that *S. carpocapsae* and *H. indica* under controlled laboratory conditions are able to exert an excellent degree of control over different stages of *A. segetum*. It is therefore recommended that entomopathogenic nematodes, *S. carpocapsae* and *H. indica* could be used as one of the best bio-control agent for the bio-intensive management of cutworms in potato crop.

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