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# Virulence of *Heterorhabditis bacteriophora* (Poinar) against cutworm, *Agrotis segetum* (Denis and Schiff.)

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**ABSTRACT: Surveys for entomopathogenic nematodes (EPNs) were carried out in eight localities covering three districts (Kangra, Sirmaur and Solan) of Himachal Pradesh, India. EPNs were recorded in two localities – Baijnath (Kangra) and Rajgarh (Sirmour), and were identified as *Heterorhabditis bacteriophora*. The potential of this nematode to kill the larvae of the cutworm, *Agrotis segetum,* was investigated at 10-40IJs / cm$^2$ in Petri plates and in soil (at 1000-5000 IJs Kg$^{-1}$) experiments. Studies on the comparative susceptibility of 3$^{rd}$, 4$^{th}$ and 5$^{th}$ instar larvae of *A. segetum* indicated that the susceptibility of insect larvae to *H. bacteriophora* decreased with the increase in age. Insect mortality increased with the increase in the exposure time and inoculum level of the EPN. In the soil, 1000 IJs of *H. bacteriophora* Kg$^{-1}$ were found to be sufficient to initiate the infection and kill up to 61.3 of 5$^{th}$ instar larvae of *A. segetum* after 7 days of exposure.**

**KEY WORDS**: Entomopathogenic nematode*, Heterorhabditis bacteriophora, Agrotis segetum,* cutworm, survey

## INTRODUCTION

Cutworms belonging to the genus *Agrotis* (Lepidoptera: Noctuidae) are polyphagous and ubiquitous insects and have been recorded as serious pests from many parts of the world, including India (Gulab *et al*., 2001; Mrowczynski *et al*., 2003; Napiorkowska and Gawowska, 2004). These insects cause considerable damage to different crops by cutting seedlings at the ground level and spoil more than they actually consume, thereby reducing the yield drastically. Mainly two species of cutworm, *viz*., *Agrotis ipsilon* (Hufn.) and *A. segetum* (Denis and Schiff.) are prevalent in Himachal Pradesh, India (Verma and Verma, 2002). Of these, *A. segetum* is predominant in mid and high hills and causes 3-18% infestation in vegetables and field crops (Anonymous, 2003).

Various methods are adopted for the control of cutworms. Biological control by entomopathogenic nematodes (EPNs) appears to be one of the sustainable alternatives to manage this pest. EPNs of the genera *Steinernema* and *Heterorhabditis* are capable of controlling a variety of economically important insect pests (Klein, 1990; Shapiro-Ilan *et al*., 2002). The infective juveniles (IJs) of EPNs enter the host insect and release their symbiotic bacteria (*Photorhabdus* spp. or *Xenorhabdus* spp.), which cause septicemia leading to the death of the insects in a couple of days. The distribution of EPNs has been reported from various regions of the world (Poinar, 1990). High virulence, ease of culturing and high reproductive potential have led to successful integration of these nematodes into pest management programmes for the control of soil borne pests (Kaya and Gaugler, 1993).

In Himachal Pradesh (India), though there are favorable conditions for the occurrence and use of EPNs, no systematic attempt has so far been made to exploit them as biocontrol agents against cutworms. The present study was, therefore, undertaken to isolate the naturally occurring EPNs and to test their virulence against *A. segetum*.

## MATERIALS AND METHODS

### Isolation of EPNs from the soil

One hundred twenty-seven soil samples were collected from eight localities covering three districts, Kangra, Sirmaur and Solan, of Himachal Pradesh. About 1 kg of soil sample composed of three subsamples was collected from the rhizosphere of plants/fruit trees, farm yard manure and spent mushroom compost. The samples were placed in polyethylene bags, labeled, tied with rubber bands and

brought to the laboratory for the isolation of EPNs. The larvae of lesser wax moth, *Achroia grisella* (F.) were used as trap hosts for EPNs. Ten 5th instar larvae were placed at the bottom of a jar (10cm diameter). After thoroughly mixing the soil sample, about 250g of soil was put over the larvae in each jar. The jars containing soil samples and trap host were kept at 25±1°C. After 5–7 days, dead larvae were collected and examined for infection and the presence of EPNs, if any.

**Identification of the isolated EPNs**

Temporary and permanent mounts of the nematodes were prepared for taking measurements. The morphological characters of different stages were observed and compared with original descriptions, drawings and taxonomic keys (Nguyen and Smart, 1996). Cultures of *A. segetum* and *H. bacteriophora* were maintained at the Department of Entomology of the university.

**Virulence of *H. bacteriophora* to *A. segetum***
**Petri plate bioassay**

The virulence of *H. bacteriophora* was evaluated against 3rd, 4th and 5th instar larvae of *A. segetum*. A Whatman No.1 filter paper (9.5cm diameter) was placed in a Petri plate (9 cm diameter). The EPN concentrations were 10, 20, 30 and 40IJs / $cm^2$ for 3rd and 4th instar larvae and 10, 20, 30, 40 and 50IJs / $cm^2$ for 5th instar larvae of the cutworm. The inoculum was evenly applied over filter paper in one ml of distilled water. In control, only one ml of distilled water was added. Two larvae of *A. segetum* of the test instar were put in each Petri plate. The cutworms were provided with cabbage leaves as food. There were five replicated Petri plates per treatment kept at room temperature (21.8-30.3°C). The data on the mortality of the cutworm were recorded at 24 hour intervals for 96 hours. Corrected per cent mortalities were calculated by Abbott's formula (Abbott, 1925).

**Soil bioassay**

For determining the infective dose of *H. bacteriophora* required to obtain larval mortality of *A. segetum*, about 1 kg of sterilised soil was put in a container. To this soil, different EPN concentrations – 1000, 5000 and 10000 IJs $kg^{-1}$ in 10 ml distilled water – were added, followed by thorough mixing of the soil. In control containers, only 10 ml of distilled water were added. This soil containing nematodes was transferred to a plastic jar, having twenty 5th instar larvae of *A. segetum* at its bottom. There were four replicated jars per treatment. Cabbage leaves were also added in the jars as food to the larvae. These jars were covered with a muslin cloth and kept at room temperature (21.8-30.3°C). The data on mortality of the insect were recorded after 3, 5 and 7 days. Corrected per cent mortalities were calculated as above.

## RESULTS AND DISCUSSION

Out of the eight localities surveyed, nematodes were encountered only at Baijnath (Distt. Kangra, litchi crop) and Rajgarh (Distt. Sirmaur, stone fruits). At Baijnath, two out of eight and at Rajgarh, four out of 19 samples were found to be EPN positive (Table 1).

The taxonomic studies were carried out on EPNs isolated during the survey. The nematode was identified as *H. bacteriophora* at both the localities. This nematode is reported to be present in a number of countries including India (Singh, 1990; Poinar *et al*., 1992; Glazer *et al*., 1993; Hazir *et al*., 2003; Atwa, 2004; Mekete *et al*., 2005; Mráček *et al*., 2005,). In India, *H. bacteriophora* was reported earlier from Himachal Pradesh (Singh, 1990) and Tamil Nadu (Sivakumar *et al*., 1989). However, now it is being reported for the first time from Kangra district of Himachal Pradesh. In both the orchards (litchi and stone fruits), where EPNs were encountered, organic matter content was quite high in the basins of the plants/fruit trees and white grub larvae and other arthropods were also encountered in some of the basins while sampling, thus confirming earlier findings by Miduturi *et al*. (1996), who reported the occurrence of *H. bacteriophora* in soils having high organic matter. No EPNs could be recorded in FYM and spent mushroom compost. Further, at both the places where EPNs were found, soil texture was of sandy loam type. According to Kung *et al*. (1990), soil texture affects the survival of EPNs, which was higher in sandy soils. Interestingly, the nematodes were trapped from the basins of the fruit trees only. Probably, in perennial crops such as fruit trees, agronomic practices are less compared to field crops and the fauna on which the EPNs survive suffer fewer disturbances. The presence of white grubs and other arthropods, in the localities where EPNs were recorded, is likely to be a supporting factor for the build up of EPNs. Earlier, Singh and Gupta (2006) reported the efficacy of *H. bacteriophora* against white grubs (*Brahmina coriacea* (Hope) and *Holotrichia* sp.)

**Pathogenic effect of *H. bacteriophora* on *A. segetum***
**Petri plate bioassay**

The data recorded are presented in Tables 2-5. In 3rd instar larvae of the cutworm, no mortality could be recorded at any of the EPN concentration for up to 24 h and at 10 or 20IJs/$cm^2$ until 48 h of exposure (Table 2). The mean mortality after 96h of larval exposure at different concentrations ranged between 30.0 and 75.0%. There were no significant differences between mortalities at the highest two EPN concentrations except after 48h of exposure. The mean larval mortality increased significantly with the increase of exposure period and ranged from 42.5 to 100% when the larvae were exposed from 48 to 96 h to *H. bacteriophora*.

**Table 1. Distribution of EPNs in different localities of Kangra, Solan and Sirmaur districts of Himachal Pradesh**

| District | Locality | Sampling site description | | | | | |
|---|---|---|---|---|---|---|---|
| | | Crop / Tree / substrate sampled | No. of samples analyzed | Frequency of occurrence (%) | Soil texture | Organic matter | Irrigated/ Unirrigated |
| Kangra | Palampur | *Zea mays* | 4 | - | Silty clay loam | Low | Irrigated |
| | | *Triticum aestivum* | 6 | - | Silty clay loam | Low | Irrigated |
| | | *Camellia* sp. | 15 | - | Silty clay loam | High | Unirrigated |
| | | *Citrus* spp. | 4 | - | Silty clay loam | High | Irrigated |
| | | *Actinidia deliciosa* | 4 | - | Silty clay loam | High | Irrigated |
| | | *Psidium guajava* | 4 | - | Silty clay loam | Low | Irrigated |
| | | *Prunus persicae* | 2 | - | Silty clay loam | High | Irrigated |
| | | *Pisum sativum* | 5 | - | Silty clay loam | High | Irrigated |
| | | *Lycopersicon esculentum* | 2 | - | Silty clay loam | High | Irrigated |
| | | *Brassica oleracea* var. *capitata* | 3 | - | Silty clay loam | High | Irrigated |
| | | *B. oleracea* var. *botrytis* | 3 | - | Silty clay loam | High | Irrigated |
| | | *Allium cepa* | 2 | - | Silty clay loam | High | Irrigated |
| | | *B. juncea* | 2 | - | Silty clay loam | High | Irrigated |
| | | *Solanum tuberosum* | 6 | - | Silty clay loam | High | Irrigated |
| | | *Sesamum indicum* | 1 | - | Silty clay loam | Low | Irrigated |
| | | *Pinus roxburgii* | 4 | - | Silty clay loam | High | Unirrigated |
| | | *Populus* spp. | 4 | - | Silty clay loam | High | Unirrigated |
| | | *Ficus carica* | 2 | - | Silty clay loam | Low | Unirrigated |
| | | Spent mushroom compost | 4 | - | - | High | - |
| | | Farmyard manure (FYM) | 2 | - | - | High | - |
| | | *Rosa* spp. | 2 | - | Silty clay loam | Low | Irrigated |
| | | *Dianthus caryophyllus* | 2 | - | Silty clay loam | Low | Irrigated |
| | | *Tagetes erecta* | 4 | - | Silty clay loam | Low | Unirrigated |
| | Nagrota | *B. oleracea* var. *capitata* | 2 | - | Sandy loam | Low | Irrigated |
| | Yol | *Cajanus cajan* | 2 | - | Sandy loam | Low | Irrigated |
| | Baijnath | *Litchi chinensis* | 4 | 25.0 | Sandy loam | High | Irrigated |
| | Barot | *S. tuberosum* | 3 | - | Sandy loam | High | Irrigated |
| Sirmaur | Kheradhar | *S. tuberosum* | 6 | - | Sandy loam | High | Irrigated |
| | Rajgarh | Stone fruits | 19 | 21.1 | Sandy loam | High | Irrigated |
| Solan | Nauni | Stone fruits | 4 | - | Sandy loam | High | Irrigated |

**Table 2. Efficacy of *H. bacteriophora* against third instar larvae of *A. segetum* in Petri plates**

| EPN concentration (No. of IJs / cm$^2$) | Corrected % larval mortality (after hours of exposure) | | | | Mean |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | |
| 10 | 0.0 (2.0) | 0.0 (2.0) | 20.0 (19.2) | 100.0 (87.9) | 30.0 (27.8) |
| 20 | 0.0 (2.0) | 0.0 (2.0) | 60.0 (53.6) | 100.0 (87.9) | 40.0 (36.4) |
| 30 | 0.0 (2.0) | 70.0 (62.2) | 100.0 (87.9) | 100.0 (87.9) | 67.5 (60.0) |
| 40 | 0.0 (2.0) | 100.0 (87.9) | 100.0 (87.9) | 100.0 (87.9) | 75.0 (66.5) |
| Mean | 0.0 (2.0) | 42.5 (38.5) | 70.0 (62.2) | 100.0 (87.9) | |
| $CD_{0.05}$ | EPN concentration (C) | | 7.74 | | |
| | Exposure period (E) | | 7.74 | | |
| | C × E | | 15.48 | | |

Figures in parentheses are arc-sine transformed values

In 4th and 5th instar larvae (Tables 3 and 4), similar trends were observed and there was no mortality at 24 h of exposure period at any of the EPN concentration and mortality increased with the increase in concentration and exposure period. In case of 3rd and 4th instar larvae, complete kill of the larvae was achieved when exposed to 40IJs / cm$^2$ whereas in 5th instar larvae complete kill of the larvae could be achieved only at the highest concentration of 50IJs / cm$^2$.

**Table 3. Efficacy of *H. bacteriophora* against fourth instar larvae of *A. segetum* in Petri plates**

| EPN concentration (No. of IJs / cm$^2$) | Corrected percent larval mortality (after hours of exposure) | | | | Mean |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | |
| 10 | 0.0 (2.0) | 0.0 (2.0) | 20.0 (19.2) | 60.0 (53.6) | 20.0 (19.2) |
| 20 | 0.0 (2.0) | 10.0 (10.6) | 50.0 (45.0) | 80.0 (70.8) | 35.0 (32.1) |
| 30 | 0.0 (2.0) | 20.0 (19.2) | 70.0 (62.2) | 90.0 (79.4) | 45.0 (40.7) |
| 40 | 0.0 (2.0) | 100.0 (87.9) | 100.0 (87.9) | 100.0 (87.9) | 75.0 (66.5) |
| Mean | 0.0 (2.0) | 32.5 (29.9) | 60.0 (53.6) | 82.5 (72.9) | |
| $CD_{0.05}$ | EPN concentration (C) | | 13.24 | | |
| | Exposure period (E) | | 13.24 | | |
| | C × E | | 26.48 | | |

Figures in parentheses are arc-sine transformed values

**Table 4. Efficacy of *H. bacteriophora* against fifth instar larvae of *A. segetum* in Petri plates**

| EPN concentration (No. of IJs / cm$^2$) | Corrected % larval mortality (after hours of exposure) | | | | Mean |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | |
| 10 | 0.0 (2.0) | 0.0 (2.0) | 0.0 (2.0) | 20.0 (19.2) | 5.0 (6.3) |
| 20 | 0.0 (2.0) | 0.0 (2.0) | 20.0 (19.2) | 30.0 (27.8) | 12.5 (12.8) |
| 30 | 0.0 (2.0) | 0.0 (2.0) | 30.0 (27.8) | 60.0 (53.4) | 22.5 (21.3) |
| 40 | 0.0 (2.0) | 10.0 (10.6) | 50.0 (45.0) | 90.0 (79.4) | 37.5 (34.2) |
| 50 | 0.0 (2.0) | 30.0 (27.8) | 70.0 (62.2) | 100.0 (87.9) | 50.0 (45.0) |
| Mean | 0.0 (2.0) | 8.0 (8.9) | 34.0 (31.2) | 60.0 (53.5) | |
| $CD_{0.05}$ | EPN concentration (C) | | | 11.93 | |
| | Exposure period (E) | | | 10.67 | |
| | C × E | | | 23.87 | |

Figures in parentheses are arc-sine transformed values

Interaction effect of different stages (S) of *A. segetum* to EPN concentration (C) and to exposure periods (E) are given in Tables 5a and 5b. If the age of the cutworm is considered, maximum mean per cent mortality was recorded in 3rd instar larvae (53.1), followed by 4th (43.8) and 5th instar larvae (19.4) which were significantly different from each other. At lower concentrations of 10 and 20IJs / $cm^2$, there was no significant difference in per cent mortality in all the instars. However, at EPN concentrations of 30 and 40IJs / $cm^2$ significant differences were observed between 4th and 5th instar larvae.

When the EPN concentration is considered, statistically different mean per cent mortalities of 19.2, 29.1, 50.8 and 62.5 were recorded at 10, 20, 30 and 40IJs / $cm^2$, respectively. Similarly, as the exposure period increased, mean per cent mortality also increased significantly. There was no difference in per cent mortality at the exposure period of 24h in all the EPN concentration. At 48 hours exposure, a significantly higher per cent mortality was recorded in the Petri plates inoculated with 40IJs / $cm^2$ (70.0). At 96h of exposure, cutworm mortality (83.3%) increased significantly at concentration of 30IJs / $cm^2$ as compared to the lower EPN concentration and was on par with the mortality (96.7%) obtained with 40IJs / $cm^2$. At all concentrations of EPNs, the mean mortality of the cutworm increased with the increase in exposure period.

**Table 5a. Interaction effects of different stages of *A. segetum*, EPN concentration and exposure periods in Petri plates**

| *A. segetum* stage | Corrected % larval mortality at EPN concentration (No. of IJs / $cm^2$) of | | | | Mean | Corrected % larval mortality after (hours) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | | 24 | 48 | 72 | 96 |
| 3rd | 30.0 (27.8) | 40.0 (36.4) | 67.5 (60.0) | 75.0 (66.5) | 53.1 (47.7) | 0.0 (2.0) | 42.5 (38.5) | 70.0 (62.2) | 100.0 (88.0) |
| 4th | 20.0 (23.5) | 35.0 (29.9) | 45.0 (38.5) | 75.0 (66.5) | 43.8 (39.6) | 0.0 (2.0) | 32.5 (30.0) | 60.0 (53.6) | 82.5 (72.9) |
| 5th | 5.0 (6.3) | 12.5 (12.8) | 22.5 (21.3) | 37.5 (34.2) | 19.4 (18.7) | 0.0 (2.0) | 2.5 (4.2) | 25.0 (23.5) | 50.0 (45.0) |
| $CD_{0.05}$ | Stage (S) | | | | 5.47 | | | | |
| | S × C | | | | 10.94 | | | | |
| | S × E | | | | 10.94 | | | | |

Figures in parentheses are arc-sine transformed values

**Table 5b. Effect of EPN concentration and exposure period on mortality of *A. segetum* in Petri plates**

| EPN concentration (No. of IJs / $cm^2$) | Corrected % larval mortality (after hours) | | | | Mean |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | |
| 10 | 0.0 (2.0) | 0.0 (2.0) | 13.3 (13.5) | 63.3 (56.4) | 19.2 (18.5) |
| 20 | 0.0 (2.0) | 6.7 (7.8) | 43.3 (39.3) | 66.7 (59.3) | 29.2 (27.1) |
| 30 | 0.0 (2.0) | 53.3 (24.9) | 66.7 (59.3) | 83.3 (73.6) | 50.8 (40.0) |
| 40 | 0.0 (2.0) | 70.0 (62.2) | 83.3 (73.6) | 96.7 (85.1) | 62.5 (55.7) |
| Mean | 0.0 (2.0) | 32.5 (24.2) | 51.7 (46.4) | 77.5 (68.6) | |
| $CD_{0.05}$ | EPN concentration (C) | | | 6.32 | |
| | Exposure periods (E) | | | 6.32 | |
| | C × E | | | 12.63 | |

Figures in parentheses are arc-sine transformed values

**Table 6. Efficacy of *H. bacteriophora* against *A. segetum* in the soil**

| EPN concentration (IJs $Kg^{-1}$ soil) | Corrected % larval mortality (after days) | | | Mean |
|---|---|---|---|---|
| | 3 | 5 | 7 | |
| 1000 | 11.3 (18.7) | 33.2 (35.1) | 61.3 (51.5) | 35.3 (35.1) |
| 5000 | 33.4 (35.2) | 71.9 (57.0) | 85.4 (68.6) | 63.6 (53.6) |
| 10000 | 42.0 (46.1) | 76.8 (61.5) | 91.6 (74.5) | 70.1 (60.8) |
| Mean | 28.9 (33.4) | 60.6 (51.2) | 79.43 (64.9) | |
| $CD_{0.05}$ | EPN concentration (C) | | 5.85 | |
| | Exposure periods (E) | | 5.85 | |
| | C × E | | 11.70 | |

Figures in parentheses are arc-sine transformed values

**Soil bioassay**

Table 6 shows that larval mortalities of 11.3, 33.4 and 42.0% of *A. segetum* were recorded at EPN concentrations of 1000, 5000 and 10000 IJs $kg^{-1}$ soil, respectively, after 3 days of exposure. The mortality increased significantly with the increase in exposure time and after 7 days, the nematode population was 61.3, 85.4 and 91.6% at 1000, 5000 and 10000IJs $kg^{-1}$ soil, respectively. The least EPN concentration of 1000IJs of *H. bacteriophora* per $kg^{-1}$ soil was sufficient to initiate infection and gave 30% mortality of *A. segetum* larvae. Our results show that *H. bacteriophora* has potential to be used as a biological control agent for *A. segetum*.

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