Virulence of native entomopathogenic nematodes against major lepidopteran insect species of tomato (Solanum lycopersicum L.)

Neelam Thakur*, Preety Tomar, Simranjeet Kaur, Poonam Kumari
Department of Zoology, Akal College of Basic Sciences, Eternal University, Baru Sahib, Sirmour, India.

ARTICLE INFO

Article history:
Received on: July 19, 2021
Accepted on: September 13, 2021
Available online: March 18, 2022

Key words:
Agrotis segetum, bio-efficacy, Helicoverpa armigera, Heterorhabditis bacteriophora, Spodoptera litura

ABSTRACT

In the present investigation, the bio-efficacy of indigenous entomopathogenic nematode (EPNs) Heterorhabditis bacteriophora was evaluated against the third and fourth instar larvae of major lepidopteran insect pests, viz. pod borer (Helicoverpa armigera), tabacco cutworm (Spodoptera litura), and cutworm (Agrotis segetum). Lepidopteran insect pests are responsible for causing high damage to agricultural and horticultural crops every year and it is becoming difficult to control these pests in the fields. The persistent use of chemical pesticides against these insect pests has resulted in development of resistance along with degradation of soil and human health. The two larval stages were exposed to 50, 100, 150, and 200 infective juveniles (IJs) for different time intervals and they all achieved high mortality after 120 hours. The results from the present laboratory experiment revealed that against H. armigera, the lethal concentration 50 (LC$_{50}$) of third instar larvae was 60.14 IJs/larvae and fourth instar larvae was 57.90 IJs/larvae, respectively. The LC$_{50}$ values of S. litura observed were 59.95 and 50.91 IJs/larvae in third and fourth instar larvae, respectively, after 120 hours of exposure. The pathogenic effect of H. bacteriophora against the third and fourth instar larvae of A. segetum showed LC$_{50}$ = 54.86 and 57.90 IJs/larvae, after 120 hours. It was further evaluated that there was an increase in mortality with the advancement of larval instars. The present findings indicate that native species of EPNs show high virulence against the local insect pest of tomatoes under laboratory conditions.

1. INTRODUCTION

Tomato (Solanum lycopersicum L.) is one of the most important commercial cash crops belonging to family Solanaceae. It is estimated from the earlier available data that the annual world production of tomatoes is around 177 million tons and our country contribute about 18.3 million tons from an area of 0.76 million hectares [1]. Tomatoes originated in Peru, South America, and is a short duration cash crop from which farmers can generate high income in a short period [2]. In India, there are many factors which are responsible for the low yields of tomato from which insect pests play a major role. The pest range of tomato is very wide which comprises almost 100–200 species [3], which attack tomatoes at all growth stages. It was found that the tomato crop is more susceptible to pest attacks as compared to other vegetable crops, mainly due to its softness and tenderness. It is attacked by sucking pests, whiteflies, leaf hoppers, and aphids which are responsible for the poor plant growth and also act as a vector of many viral diseases. This crop is infested with various insect pests that cause economical yield losses year after year [4]. The major damage is caused by the tomato fruit borer, Helicoverpa armigera Hubner (Lepidoptera: Noctuidae) [5].

The tomato fruit borer (H. armigera) is the most important insect pest affecting the productivity of tomatoes in different states of India and is responsible for causing resistance against a maximum number of insecticides [6]. It was reported by the World Resource Institute in 1994 that more than 500 insects and mites species are resistant to one or more insecticides [6]. According to the investigation carried by many scientists worldwide, this is the most important polyphagous agricultural insect pest [7]. Helicoverpa armigera is a serious insect pest which reduces the crop production year after year in tomato, cotton, corn, soybean, and groundnuts [8]. The immature larval stages generally make a hole in tomatoes and ultimately the fruit falls down to the earth which shows

*Corresponding Author
Neelam Thakur, Department of Zoology, Akal College of Basic Sciences, Eternal University, Sirmour, India. E-mail: neelam.panwar2 @gmail.com

© 2022 Thakur et al. This is an open access article distributed under the terms of the Creative Commons Attribution License -NonCommercial-ShareAlike Unported License (http://creativecommons.org/licenses/by-nc-sa/3.0/).
the infectivity of tomatoes that affects the yield [9]. Nowadays, a large number of chemicals are used to control *H. armigera* on tomatoes and other crops but this insect develops resistance against a wide range of insecticides [10]. *Spodoptera litura* is a very destructive and polyphagous insect pest not only in India but also in China, Japan, Korea, Australia, Sri Lanka, and South East Asia, and is commonly known as cutworm or tobacco caterpillar [11]. *Spodoptera litura* attack a large number of plants belonging to different botanical origin in India [12,13]. In the field condition, this pest is responsible for causing 26%–100% yield losses in about 150 plant species. This insect pest skelatalizes the leaves of tomatoes in the early stages and cause insensitive defoliation in their stages, thus resulting in the reduction of photosynthetic capacity of infected plants [14]. *Agrotis segetum* is a polytopagous and serious insect pest in India commonly known as common cutworm and black cutworm [15–17]. They usually live in the soil, on vegetables seedlings, and grains, especially in corn, potatoes, beans, peppers, eggplant, okra, lettuce, tobacco, sugar beet, and cabbage fields [18]. This insect pest causes significant damage to many crops by cutting plants at their seedling stage on the ground level and destroes the farmer field badly. Generally, two species of cutworms, *A. segetum* and *Agrotis ipsilon*, are commonly present in Himachal Pradesh, India [19].

Nowadays, among researchers, interest has been developed on the use of entomopathogenic nematodes (EPNs) as bio-control agents as they have a wide host range, actively find target host, eco-friendly in nature, and are able to recycle itself in the soil environment [20]. It is clear from earlier experiments that these nematodes are also safe to other non-target organisms [21,22] and are extraordinarily effective to target host insect, such as dipteran, lepidopteran, isopteran, hemipteran, hymenopteran, and coleopteran [23], which kills the target host insect within 24–48 hours after infection. These are the group of lethal obligate parasites that are mainly beneficial to horticultural and agricultural crops and are used as bio-control agents against insect pests [24,20]. More than 90% of insects spend part of their life in the soil and EPNs are more effective against soil dwelling insect pests and against insects present in all types of habitats [25].

The third-stage infective juveniles (IJ3) of EPNs are the only pathogenic stage and are also called dauer juvenile. The dauer larvae of different EPN species showed different behavioral strategies for host finding outside the body of cadaver [26,27]. Among EPNs, *Steinernema carpocapsae* is an important species used as a bio-control agent and has a widely distributed and broad host range. It is lethal in their nature against more than 250 species of insects belong to 10 different orders [28–30]. Researchers in India also tried to use entomopathogenic bacteria in field trial by using the imported exotic species of *S. carpocapsae, S. feltiae* and *H. bacteriophora* [31–33]. After a series of surveys and studies, it was concluded that the indigenous EPN species are more adapted to local niche and are better bio-control agents against local insect pests [34]. The increasing pest resistance problems require the development of those strategies which are economic, eco-friendly, and highly effective. Keeping this in view, the present investigation is planned to explore the insecticidal potential of EPNs.

### 2. MATERIAL AND METHODS

The rearing of rice moth (*Corcyra cephalonica* Stainton) larvae was carried out on the artificial diet consisting of crushed maize (sterilized) and yeast. About 1–2 kg crushed maize along with 5% yeast powder (*Saccharomyces cerevisiae*) was mixed properly and kept inside the rearing box with eggs. Larval population was checked and the last instar larvae were collected manually for their future usage as bait. These baits were further used for rearing and isolation of EPNs from the collected soil samples.

The rearing of greater wax moth (*Galleria mellonella* Linnaeus) larvae were carried out by transferring into the rearing box containing artificially prepared diet as given by Kulkarni et al. [35]. The prepared diet was placed inside the rearing box so that the inoculated larvae could feed on it. The last instar larvae of wax moth were collected manually and kept for their future use in the laboratory. The larvae were used as bait along with rice moth for the isolation and *in vivo* culturing of EPNs.

### 2.2. Maintenance of the Laboratory Culture of Target Insect Pests

The culture of the test insect collected from University Agricultural fields and farmer’s field was maintained under controlled conditions at 28°C ± 1°C. The newly emerged adults were kept in a glass vials (30 ml) which was covered with a muslin cloth. The adults were fed with 10% sugar solution in a cotton swab to stimulate egg laying. The newly hatched larvae were reared in mass on artificial diet. The pupae were transferred to a new jar and after emergence the adults were transferred to separate chimneys again for egg laying.

*Helicoverpa armigera* larvae were fed on artificial diet which was based mainly on the chickpea flour. The diet was prepared by mixing chickpea flour with other ingredients, viz. water, agarose, ascorbic acid, yeast, and vitamins. For the preparation of diet, the method was followed with slight modifications, viz. addition of carbendazim and different compositions of vitamin mixture. The different ingredients of diet along with their weight/volume are represented in Table 1. The final mixture was stirred well until it started to solidify into a gel. The artificial diet thus prepared can be preserved up to 2–3 months in the refrigerator at 5°C–7°C.

Larvae of tobacco cutworm were collected fed on castor leaves in beaker and plastic jars till adult emergence. Emerging adults were then transferred to chimney for oviposition where moths were fed on 15% sucrose solution. Paper was also placed inside the chimney for providing sufficient resting space to the adult moths. Each chimney was covered with muslin cloth. After 3–4 days, females laid eggs in clusters over the muslin cloth and on the paper. Eggs were collected daily. The eggs were collected and stored in new plastic jars with their oviposition substrates for hatching. Newly hatched larvae were transferred into plastic jars with castor leaves up to second instar. Third instar larvae were
Table 1: Different ingredients of chickpea flour-based artificial diet of *H. armigera*.

| S. No | Ingredients                  | Weight/volume |
|-------|------------------------------|---------------|
| 1.    | Agar                         | 7.3 g         |
| 2.    | Ascorbic acid                | 1.9 g         |
| 3.    | Carbendazim                  | 200 mg        |
| 4.    | Chickpea flour               | 60 g          |
| 5.    | Distilled water              | 445 ml        |
| 6.    | Formaldehyde (10%)           | 1.9 ml        |
| 7.    | Methyl-p-hydroxybenzoate     | 1.2 g         |
| 8.    | Sorbic acid                  | 0.57 g        |
| 9.    | Streptomycin                 | 140 mg        |
| 10.   | Sucrose                      | 5.0 g         |
| 11.   | Vitamin mixture              | 700 mg        |
| 12.   | Yeast                        | 5.7 g         |

collected and reared again in a single jar. Castor leaves were given as a diet to the voraciously feeding larvae of *S. litura*.

Larvae of *A. segetum* were collected from university agricultural fields and from farmers’ fields maintained in the laboratory according to the method standardized by Verma [36]. First, instar larvae of *A. segetum* were cultured on soft cabbage leaves in plastic containers. The larvae were reared up to third instar, and after third instar, the larvae showed cannibalism with each other. Keeping in view its cannibalism, the third instar larvae were shifted into another jar filled with soil and sand up to 10 cm. Fresh leaves of cabbage were given in jars as natural diet for the developing larvae. The fully developed larvae pupated in the soil and sand mixture and emerged later on about 15 days. The adults were identified on the basis of their morphological character and were transferred to their glass chimneys for mating. Paper was placed in chimney for easy repose of moths. In the glass chimney, 10% sucrose solution was kept in a petri plate. The eggs were collected with the help of hair brush in the laboratory on moist filter paper placed in the petri plate for hatching.

2.3. In Vivo Culturing of EPNs

A large petri plate (100 mm) was lined with Whatman filter paper and about 1 ml of nematode suspension containing 500 IJs was poured on that petri plate. About 20 anesthetized last instar larvae of rice moth and 10 last instar larvae of wax moth (Approx. 20 nematodes/larva) were then added to the petri plate in order to infect the larvae with IJs. These petri plates were then labeled with the name of the isolate and date. Petri plates were kept inside transparent plastic bags to conserve moisture and incubated at 25°C ± 1°C temperature. After 5–7 days, the plates were checked for infected larvae and transferred to new White traps [37,38] for extraction of IJs. The harvesting was carried out by pouring the nematode suspension into the beaker for 4–5 day until the nematode population ceases.

2.4. Bio-efficacy of an Indigenous EPN (*H. bacteriophora*)

For petri plate bioassay, Whatman filter paper no. 1 (9.5 cm dia.) was placed in the sterile petri dish (9 cm dia.). The nematodes concentrations were adjusted to 50, 100, 150, and 200 IJs. Healthy and same instars of laboratory reared larvae (target insects) were used to study the bio-efficacy of EPNs under laboratory conditions. These larval stages were exposed to 1–2 ml nematode suspension at 50, 100, 150 and 200 IJs/10 insect pest larvae on to a filter paper placed in the petri dish. There were 10 larvae of target insect per petri plate and each treatment was replicated five times. In control, only 1–2 ml of distilled water was applied. The target insect larvae were provided with their artificial/natural diet, after that petri dish was incubated at 25°C ± 1°C. The insect mortality was checked after 24, 48, 72, 96, and 120 hours of inoculation.

2.5. Statistical Analysis

The data obtained over the insect mortality was subjected to probit analysis. At the same time, median lethal concentration (LC50) was determined using maximum likelihood method [39].

3. RESULTS

In this investigation, different concentrations of *H. bacteriophora* were applied for biological control of third and fourth instar of insect pests of tomato, viz. *H. armigera*, *S. litura*, and *A. segetum*. The results obtained from the 2 years pooled data of this investigation were given under the following headings.

3.1. Bio-Efficacy of an Indigenous EPN (*H. bacteriophora*) Against *H. armigera*

The effect of *H. bacteriophora* on two larval instar of *H. armigera* at different exposure times is presented in Table 2 and Figure 1. It was observed from the table that the LC50 value (60.14 IJs/larvae) was lowest after 120 hours of exposure and indicated the highest mortality against third instar of *H. armigera* with 95% fiducial limit (FL) which ranged from 41.80 to 86.52 IJs/larvae. Even after 96 hours of exposure, LC50 = 87.86 IJs/larvae, 95% FL: 58.94–130.94 IJs/larvae, followed by 72 hours (LC50 = 154.92 IJs/larvae, 95% FL: 93.79–255.88 IJ/larva), and 48 hours (LC50 = 285.55 IJs/larvae, 95% FL: 153.43–531.45 IJs/larvae), respectively. The highest value of LC50 = 1,125.60 IJs/larvae, 95% FL: 427.03–2,967.12 IJs/larvae was observed after 24 hours of exposure.
Table 2: Pathogenicity of *H. bacteriophora* against third and fourth instar larvae of *H. armigera* in petri plate assays.

| Treatments | Incubation period (hours) | LC$_{50}$ (IJs/larva) | FL (95%) | Upper limit | Lower limit | $\chi^2$ | Std. error | p-value | LC$_{50}$ (IJs/larva) | FL (95%) | Upper limit | Lower limit | $\chi^2$ | Std. error | p-value |
|------------|--------------------------|------------------------|----------|-------------|-------------|---------|-----------|---------|------------------------|----------|-------------|-------------|---------|-----------|---------|
| *H. bacteriophora* (50, 100, 150 and 200 IJ) | 24 | 1,125.60 | 2,967.12 | 427.03 | 0.93 | 1.18 | 0.05 | 884.59 | 2,269.34 | 344.81 | 0.97 | 1.08 | 0.05 |
| | 48 | 285.55 | 531.45 | 153.43 | 0.99 | 0.96 | 0.02 | 232.93 | 443.60 | 122.31 | 0.92 | 0.90 | 0.03 |
| | 72 | 154.92 | 255.88 | 93.79 | 0.96 | 0.89 | 0.02 | 125.44 | 203.45 | 77.34 | 0.96 | 0.86 | 0.02 |
| | 96 | 87.86 | 130.94 | 58.94 | 0.66 | 0.87 | 0.01 | 78.01 | 119.38 | 50.96 | 0.67 | 0.86 | 0.01 |
| | 120 | 60.14 | 86.52 | 41.80 | 0.17 | 0.92 | 0.01 | 57.90 | 81.31 | 41.23 | 0.64 | 0.97 | 0.01 |

| Treatments | Incubation period (hours) | LC$_{50}$ (IJs/larva) | FL (95%) | Upper limit | Lower limit | $\chi^2$ | Std. error | p-value | LC$_{50}$ (IJs/larva) | FL (95%) | Upper limit | Lower limit | $\chi^2$ | Std. error | p-value |
|------------|--------------------------|------------------------|----------|-------------|-------------|---------|-----------|---------|------------------------|----------|-------------|-------------|---------|-----------|---------|
| *H. bacteriophora* | 72 | 154.92 | 255.88 | 93.79 | 0.96 | 0.89 | 0.02 | 125.44 | 203.45 | 77.34 | 0.96 | 0.86 | 0.02 |
| | 96 | 87.86 | 130.94 | 58.94 | 0.66 | 0.87 | 0.01 | 78.01 | 119.38 | 50.96 | 0.67 | 0.86 | 0.01 |
| | 120 | 60.14 | 86.52 | 41.80 | 0.17 | 0.92 | 0.01 | 57.90 | 81.31 | 41.23 | 0.64 | 0.97 | 0.01 |

L.C$_{50}$ = Lethal concentration 50; $\chi^2$ = Pearson’s $\chi^2$ of the slope; Std. error = Standard error.

which indicated the lowest mortality caused by *H. bacteriophora* in third instar larvae of *H. armigera*. It was also observed that all the concentrations of EPNs are effective against third instars and the mortality rates after 96 and 120 hours are statistically at par.

In another experiment, where *H. bacteriophora* was applied against the fourth instar showed the lowest LC$_{50}$ = 57.90 IJs/larvae (95% FT: 41.23–81.31 IJs/larvae) after 120 hours of application, followed by LC$_{50}$ = 78.00 IJs/larvae (95% FT: 50.96–119.38 IJs/larvae) after 96 hours of exposure. Highest LC$_{50}$ was observed after 24 hours of exposure was 884.59 IJs/larvae (95% FT: 344.81–2,269.34 IJs/larvae). Moreover, the p-value < 0.05 indicated that the results were significant. Furthermore, the comparison of LC$_{50}$ of both the instars proved that fourth instar larvae were much more susceptible to IJs infection as compared to third instars.

3.2. Bio-Efficacy of *H. bacteriophora* Against *S. litura*

The impact of different doses of *H. bacteriophora* on two larval instar of *S. litura* at different exposure times was observed (Table 3 and Figure 1). It was concluded from the table that the lowest LC$_{50}$ value was observed after 120 hours of exposure, i.e., 59.95 (95% FL: 41.96–85.63 IJs/larvae). The LC$_{50}$ of IJs after 96, 72, and 42 hours ranged from 81.46 IJs/larvae (95% FL: 54.24–122.33 IJs/larvae), 154.92 IJs/larvae (95% FL: 93.79–255.88), and 314.45 IJs/larvae (95% FL: 167.31–591.01 IJs/larvae), respectively. The highest LC$_{50}$ = 1,125.64 IJs/larvae, 95% FL: 427.03–2,967.12 IJs/larvae) was observed after 24 hours of exposure. Application of *H. bacteriophora* against the fourth instar showed the lowest LC$_{50}$ = 50.91 IJs/larvae (95% FT: 34.26–75.66 IJs/larvae) after 120 hours of exposure, followed by LC$_{50}$ = 76.59 IJs/larvae (95% FT: 50.74–115.61 IJs/larvae) after 96 hours of exposure. The highest LC$_{50}$ was again observed after 24 hours of exposure and was 958.58 IJs/larvae (95% FT: 369.02–2,490.04 IJs/larvae).

3.3. Bio-Efficacy of *H. bacteriophora* Against *A. segetum*

The effect of *H. bacteriophora* on different larval instar of *A. segetum* at different time intervals is presented in Table 3 and Figure 1. The results denoted that the LC$_{50}$ value (57.90 IJs/larvae) was lowest after 120 hours of exposure and indicated the highest mortality against third instar of *H. armigera* with 95% FL ranged from 41.23 to 81.31 IJs/larvae. Even after 96 hours of exposure, LC$_{50}$ = 78.00, 95% FL: 50.96–119.38 IJs/larvae, followed by 72 hours (LC$_{50}$ = 125.44, 95% FL: 77.34–203.45 IJs/larvae), and 48 hours (LC$_{50}$ = 232.93, 95% FL: 122.31–443.60 IJs/larvae), respectively. The highest value of LC$_{50}$ = 884.59 IJs/larvae (95% FT: 344.81–2,269.34 IJs/larvae) was observed after 24 hours of exposure signifying the lowest mortality caused by *H. bacteriophora* in third instar larvae. In fourth instar larvae, the lowest LC$_{50}$ = 54.86 IJs/larvae (95% FT: 39.90–75.42) was after 120 hours of application, followed by LC$_{50}$ = 74.09 IJs/larvae (95% FT: 46.37–118.39 IJs/larvae) after 96 hours of exposure. The highest LC$_{50}$ was again observed after 24 hours of exposure and was 766.42 IJs/larvae (95% FL: 286.26–2,051.94 IJs/larvae).

From the table, it is apparent that all these doses show their significant effect at different time intervals and remarkably vary from the control. It is also observed that among all the insect species, *S. litura* fourth instars were more prone to EPN infection, followed by *A. segetum* and *H. armigera*. The p-value < 0.05 indicated that the results were significant.

4. DISCUSSION

The bio-efficacy of different doses of EPNs (*H. bacteriophora*) on different larval instar of *H. armigera*, *S. litura*, and *A. segetum* at different exposure times was observed in this investigation. It is clear from the present study that with the increase in time and larval instar high mortality has been caused by *H. bacteriophora*. In case of *H. armigera*, the lowest LC$_{50}$ values was reported against third instar (60.14 IJs/larvae), followed by fourth instar (57.90 IJs/larvae) after 120 hours of exposure. Vashishth [40] evaluated the effect of different doses of *Heterorhabditis indica* and *H. bacteriophora* (10, 20, 30, and 40 IJs) against third, fourth, and fifth instar larvae of *H. armigera*, *S. litura*, *A. segetum*, and *Photorhabdus luminescens* in petri plates at the Department of Entomology, College of Agriculture, CSK HPKV, Palampur. Grewal et al. [41] concluded that different strains of same EPNs might act differentially on different insect pests. Gokte-Narkhedkar et al. [42] evaluated the efficacy of 10 isolates of *H. indica* against *H. armigera* and *S. litura*. Third instar larvae of *H. armigera* were found to be more prone to EPN infection as compared to that of *S. litura* and two isolates of genus *Heterorhabditis* (CICR-Su and CICR-SUB) were most virulent on *H. armigera*. Only
Figure 1: Laboratory culturing of bait insects, EPNs isolation, and bio-efficacy of EPNs against lepidopteran insect pests of tomato.
Table 3: Pathogenicity of *H. bacteriophora* against third and fourth instar larvae of *S. litura* in petri plate assays.

| Treatments           | Incubation period (hours) | LC₅₀ (IJ/larva) | FL (95%) | Std. error | p-value | LC₅₀ (IJ/larva) | FL (95%) | Std. error | p-value |
|----------------------|---------------------------|-----------------|----------|------------|---------|-----------------|----------|------------|---------|
| *H. bacteriophora*   | 24                        | 1,125.64        | 2,967.12 | 427.03     | 0.93    | 1.18            | 0.05    | 958.58     | 2,490.04| 369.02 | 0.83 | 1.10 | 0.05 |
| (50, 100, 150 and 200 IJ) | 48                        | 314.45          | 591.01   | 167.31     | 0.86    | 0.98            | 0.03    | 251.93     | 453.71 | 139.88 | 0.84 | 0.95 | 0.02 |
|                      | 72                        | 154.92          | 255.88   | 93.79      | 0.96    | 0.89            | 0.02    | 125.44     | 203.45 | 77.34 | 0.96 | 0.86 | 0.02 |
|                      | 96                        | 81.46           | 122.33   | 54.24      | 0.75    | 0.86            | 0.01    | 76.59      | 115.61 | 50.74 | 0.83 | 0.86 | 0.01 |
|                      | 120                       | 59.95           | 85.63    | 41.96      | 0.23    | 0.93            | 0.01    | 50.91      | 75.66  | 34.26 | 0.41 | 0.94 | 0.01 |

LC₅₀ = Lethal concentration 50; χ² = Pearson’s χ² of the slope; Std. error = Standard error.

Table 4: Pathogenicity of *H. bacteriophora* against third and fourth instar larvae *A. segetum* in petri plate assays.

| Treatments           | Incubation period (hours) | LC₅₀ (IJ/larva) | FL (95%) | Std. error | p-value | LC₅₀ (IJ/larva) | FL (95%) | Std. error | p-value |
|----------------------|---------------------------|-----------------|----------|------------|---------|-----------------|----------|------------|---------|
| *H. bacteriophora*   | 24                        | 884.59          | 2,269.34 | 344.81     | 0.97    | 1.08            | 0.05    | 766.42     | 2,051.94| 286.26 | 0.89 | 0.99 | 0.05 |
| (50, 100, 150 and 200 IJ) | 48                        | 232.93          | 443.60   | 122.31     | 0.92    | 0.90            | 0.03    | 306.16     | 732.12 | 128.03 | 0.69 | 0.88 | 0.05 |
|                      | 72                        | 125.44          | 203.45   | 77.34      | 0.96    | 0.86            | 0.02    | 139.36     | 256.13 | 75.82 | 0.54 | 0.85 | 0.03 |
|                      | 96                        | 78.00           | 119.38   | 50.96      | 0.67    | 0.86            | 0.01    | 74.09      | 118.39 | 46.37 | 0.88 | 0.85 | 0.02 |
|                      | 120                       | 57.90           | 81.31    | 41.23      | 0.64    | 0.97            | 0.01    | 54.86      | 75.42  | 39.90 | 0.33 | 1.06 | 0.009 |

LC₅₀ = Lethal concentration 50; χ² = Pearson’s χ² of the slope; Std. error = Standard error.

one isolate (CICR-BBFNS2) multiplied in *S. litura*. They also recorded the presence of high number of bacteria in *H. armigera* which helped in the successful multiplication of EPNs. Glazer and Navon [43] also tested the pathogenicity of *Heterorhabditis* sp. against third instar larvae of *H. armigera* and observed LD₅₀ of 49 IJs/larvae. Vashisth et al. [44] observed the efficacy of three indigenous *Heterorhabditis* spp. from Northwest Himalayas against *H. armigera* under optimum laboratory conditions and they also compared its efficacy with commercial formulation of *H. indica*. The native strain showed 73.3% mortality after 96 hours of exposure time, whereas the commercial strain of *H. indica* showed about 80.0% mortality.

There was increase in mortality with the advancement of instar. The present experiment results are similar to earlier investigation carried by Kary et al. [45], showing that *H. bacteriophora* have a greater effect on the last instar larvae of *H. armigera* under laboratory conditions. However, Jothi and Mehta [46] noticed the pathogenicity of *H. bacteriophora* against third and fourth instar larvae of *H. armigera* at inoculation dose from 1 to 100 IJs. They further recorded maximum mean mortality (59.0%) against third instar, followed by fourth instar (56.4%).

Similar observations were recorded by Rosa and Simoes [47], who tested 28 isolates of *H. bacteriophora* and were responsible to cause mortality up to 100% after exposure period of 96 hours.

In South India, Prabhuraj and Patil [48] observed that *H. indica* produced up to 45% mortality against fourth instar larvae of *H. armigera* at inoculation 20–40 IJs/larva after an exposure time of 60 hours. Andaló et al. [49] applied *Heterorhabditis amazonensis* MC01 against *H. armigera* pupae under laboratory and field conditions, which resulted in 80% mortality in laboratory as well as in field. Divya et al. [50] conducted a laboratory bioassay study on *H. indica* against third instar larvae of *S. litura*. They also noticed that after time exposure of 28.76 hours at inoculation, 100 IJs/larva caused 100% mortality. Raveendranath et al. [51] investigated the bio-efficacy of *H. indica* against third instar larvae of *S. litura* by using soil column assay.

The LC₅₀ value of *S. litura* observed was 59.95 and 50.91 IJs/larvae in third and fourth instar larvae after 120 hours of exposure, respectively. Our findings are in conformity with the previous findings carried out by various researchers. Divya et al. [50] noticed that at inoculation 100 IJs of *H. indica*/larva caused 100% mortality after time exposure of 50 hours against fourth instar larvae of *S. litura*. Abdel-Razek and Abd-Elgawad [52] observed 100% mortality within 24 hours with genus *Heterorhabditis* strain ELG and *Heterorhabditis* strain ELB against *Spodoptera littoralis* is under laboratory conditions. Kumar et al. [53] reported highest LC₅₀₈ of *Heterorhabditis* sp. against last instar larvae of *S. litura* at inoculation doses as 75.7, 56.3, and 39.2 IJs/larvae, after time exposure 72, 96, and 120 hours, respectively. Park et al.
conducted a laboratory experiment to examine the efficacy of some EPNs against S. litura. In that study, H. bacteriophora caused 100% mortality at 47 hours against third and fourth instar larvae. Hussaini et al. [55] also noticed the better efficacy of H. bacteriophora as compared to H. indica in case of S. litura. They observed 40.0% and 20% death with H. bacteriophora and H. indica after 72 hours of treatment. According to earlier studies conducted by various scientists, these values were close to the LC₅₀ doses 8.3, 9.2, and 19.1 IJs/larvae of H. bacteriophora against fourth instar larvae of S. litura [56–58]. In the previous studies, larvae of Spodoptera frugiperda were found to be highly susceptible to EPN infection [59].

The pathogenic effect of H. bacteriophora against the third instar larvae of A. segetum summarized and LC₅₀ = 54.86 IJs/larvae was observed after 120 hours. These findings are in agreement with the findings of Chandel and Kapoor [60], who observed 100% mortality at 10–40 IJs/cm² showed by H. bacteriophora against third instar larvae of A. segetum in petri plate bioassay. Kumari et al. [61] used S. carpocapsae and H. indica against different developing stages of A. segetum under laboratory conditions. Results revealed that H. indica caused 73.3%–100% mortality and S. carpocapsae was responsible for 63.3%–100% mortality after 48 hours at dose level 50–300 IJs/ml.

The calculated LC₅₀ of H. bacteriophora against 4th instar of A. Segetum was 57.90IJs/larvae. Chandel and Kapoor [60] also conducted bioassay experiment against fourth instar larvae of A. segetum and recorded that the mortality varied from 60.0%–100% at 10–40 IJs/cm² at 96 hours of time exposure. Fetoh et al. [62] investigated under laboratory experiment the efficacy of Egyptian strain of H. bacteriophora in case of fourth instar larvae of A. ipsilon. According to them, 80% ± 4.0% to 100% ± 0.0% mortality after inoculated with 25–100 IJs/ml of H. bacteriophora is highly virulent in case of A. ipsilon.

Bareth et al. [63] observed the bio-efficacy of H. bacteriophora in case of last larval instar of Agrotis flammetera and A. ipsilon. They also reported LT₅₀ value after time exposure 5.78 and 6.13 hours at inoculation 100 IJs/cm². Kappor et al. [64] reported the nematodes bio-efficacy against fifth instar larvae of A. segetum that resulted in 30.55%–68.33% mortality at inoculation 1,000–10,000 IJs/kg soil after time exposure of 7 days. Chandel and Kapoor [60] also observed that H. bacteriophora dose of 1,000 IJs/kg soil was sufficient to start infection against the larvae of A. segetum. They further reported 61.3%–91.6% mortality in case of fourth instar larvae at inoculation 1,000–10,000 IJs/kg soil. These findings gives considerable support to the various investigations carried out with EPNs against A. segetum [65], where H. bacteriophora caused encouraging results.

6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

7. AUTHORS’ CONTRIBUTIONS

SK and PK conducted the experiment and wrote the manuscript. PT helped in conducting the survey to collect the insects from fields and with statistical analysis. NT developed the concept and drafted the manuscript. All authors have read and reviewed the manuscript.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

REFERENCES

1. FAO. 2018. Available via http://www.fao.org/statisticaldatabases/agriculture.

2. Wener J. Guide to tomato production in home gardens [Online]. 2000. Available via www.agrisupportonline.com

3. Lange WH, Bronson L. Insect pests of tomatoes. Annu Rev Entomol 1981;26:345–71.

4. Kumar CA, Shivaraju C. Bioefficacy of newer insecticide molecules against tomato fruit borer, Helicoverpa armigera (Hubner). Karnataka J Agric Sci 2009;22:588–90.

5. Sajjad M, Ashfaq M, Suhail A, Akhtar S. Screening of tomato genotypes for resistance to tomato fruit borer (Helicoverpa armigera (Lepidoptera: Noctuidae)) in Brazil. PLoS One 2013;8:e80134.

6. Mittro K. Status of insecticide resistance in insect pests. Commonwealth Publishers, New Delhi, India, 2000.

7. Tay WT, Soria MF, Walsh T, Thomazoni D, Silvie P, Behere GT, et al. A brave new world for an old world pest: Helicoverpa armigera (Lepidoptera: Noctuidae) in Brazil. PLoS One 2013;8:e80134.

8. Fitt GP. The ecology of Heliothis species in relation to agroecosystems. Annu Rev Entomol 1989;34:17–53.

9. Garcia FM. Analysis of the spatio–temporal distribution of Helicoverpa armigera Hb. in a tomato field using a stochastic approach. Biosys Eng 2006;93:253–9.

10. Kranthi KR, Jadhav D, Wanjari R, Kranthi S, Russell D. Pyrethroid resistance and mechanisms of resistance in field strains of Helicoverpa armigera (Lepidoptera: Noctuidae). J Econ Entomol 2001;94:253–63.

11. Hill DS. Agricultural insect pests of the tropics and their control. Cambridge University Press Archive, Cambridge, UK, 1998.

12. Dhir B, Mohapatra H, Senapati B. Assessment of crop loss in groundnut due to tobacco caterpillar, Spodoptera litura (F.). Indian J Plant Prot 1992;20:215–7.

13. Rao GR, Wightman J, Rao DR. World review of the natural enemies and diseases of Spodoptera litura (F.)(Lepidoptera: Noctuidae). Int J Trop Insect Sci 1993;14:273–84.

14. Yadav S, Patil J, Sharma H. Bio-efficacy of Steinernema carpocapsae against Spodoptera litura under laboratory condition. Int J Pure Appl Biosci 2017;5:165–72.

15. Ram G, Misra S, Dhamayanti K. Relative susceptibility of advanced hybrids and promising cultivars of potato, Solanum tuberosum L. to greasy cutworm, Agrotis ipsilon (Hufn.) in North-eastern plains. J Entomol Res 2001;25:183–7.

16. Mrowczynski M, Wachowiak H, Boron M. Cutworms—a dangerous pest in the autumn of 2003. Ochrana Roslin 2003;47:24–6.

17. Napiekowska K, Gawowska J. Increase of harmfulness of caterpillars (Hadeniinae and Noctuinae, Lepidoptera: Noctuidae) on cabbage and other cole crops. Prog Plant Prot 2004;44:978–80.

18. Ministry of Agriculture of Turkey. The agricultural control technical recommendations. p 3, 2008.
19. Verma K, Verma A. Cutworm species associated with different crops in Himachal Pradesh. Insect Environ 2002;8:1–23.
20. Kaya HK, Gaugler R. Entomopathogenic nematodes. Ann Rev Ent 1993;38:181–206.
21. Akhurst R, Boemare N, Gaugler R, Kaya H. Entomopathogenic nematodes in biological control. pp 75–90, 1990;
22. Bathon H. Impact of entomopathogenic nematodes on non-target hosts. Biocontrol Sci Technol 1996;6:421–34.
23. Labaude S, Griffin CT. Transmission success of entomopathogenic nematodes used in pest control. Insects 2018;9:72.
24. Chitra P, Sujatha K, Jeyasankar A. Entomopathogenic nematode as a biocontrol agent–recent trends–a review. Int J Adv Res Biol Sci 2017;4:9–20.
25. Miles C, Blethen C, Gaugler R, Shapiro-Ilan D, Murray T. Using entomopathogenic nematodes for crop insect pest control. Pacific NW Ext 2012;54:1–9.
26. Ramos-rodriguez O, Campbell J, Christen J, Shapiro-Ilan D, Lewis E, Ramaswamy S. Attraction behaviour of three entomopathogenic nematode species towards infected and uninfectr hosts. Parasitol 2006;134:729–38.
27. San-Blas E, Gowen SR. Facultative scavenging as a survival strategy of entomopathogenic nematodes. Int J Parasitol 2008;38:85–91.
28. Poinar Jr G. Nematodes for biological control of insects. CRC Press, Boca Raton, FL, p 340, 1979.
29. Shahina F, Mehreen G. Phylogenetic relationship of Pakistani entomopathogenic nematode strains on the basis of ITS-1, 5.8 S and ITS-2 D2-D3/28S (LSU) rDNA region and 12S rDNA mitochondrial gene. Pak J Nematol 2014;32:173–94.
30. Kaushik C. Molecular characterization of Steinernema pakistanense (Nematoda Steinernematidae) strain isolated from India. J Zool Stud 2016;3:22–6.
31. Rao Y, Rao PP, Varma A, Israel P. Tests with an insect parasitic nematode DD-136 (Nematoda: Steinernematidae) against the rice stem borer, Tryporyza incertulas Walker. Indian J Entomol 1971;45:275–7.
32. Hussaini S. Progress of research work on entomopathogenic nematodes in India. In: Current status of research on entomopathogenic nematodes in India: Workshop on the Entomopathogenic Nematodes in India held on 22 and 23rd January, 2003. Project Directorate of Biological Control, Indian Council of Agricultural Research Bangalore, Karnataka, India, pp 27–68, 2003.
33. Yadav AK. Effects of storage temperature on survival and infectivity of three indigenous entomopathogenic nematode strains (Steinernematidae and Heterorhabditidae) from Meghalaya, India. J Parasit Dis 2016;40:1150–4.
34. Koppenhöfer AM, Kaya HK. Ecological characterization of Steinernema rara. J Invert Pathol 1999;73:120–8.
35. Kulkarni N, Kushwaha DK, Mishra VK, Paunikar S. Effect of economical modification in artificial diet of greater wax moth Galleria mellonella (Lepidoptera: Pyralidae). Indian J Entomol 2012;74:36974.
36. Verma K. Bioecology and management of turnip moth (Lepidoptera, Noctuidae) attacking vegetable crops. Ph.D. Thesis, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan, pp 1–121, 1996.
37. White G. A method for obtaining infective nematode larvae from cultures. Science (Washington) 1927;66:302–3.
38. Orozco RA, Lee MM, Stock SP. Soil sampling and isolation of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). J Visualized Exp 2014;89:e52083.
39. Ross G. Maximum likelihood programme. The Numerical Algorithms Gr Rothmested Experiment Station, Harpendon, UK, pp 1–184, 1977.
40. Vashisth S. Distribution and biocontrol potential of entomopathogenic nematodes against some lepidopterous pests. CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India, 2014.
41. Grewal PS, Ehlers RU, Shapiro-Ilan DJ. Nematodes as biocontrol agents. CABI, Wallingford, UK, pp 1–523, 2005.
42. Gokette-Narkhedkar N, Bhakre K, Nawarkar P, Chilliwer P, Fand BB, Kranthi S. Parasitic potential of entomopathogenic nematode Heterorhabditis indica against two Lepidopteran insect pests of cotton, Helicoverpa armigera (Hubner) and Spodoptera litura (Fabricius). Phytoparasitica 2019;47:31–41.
43. Glazer I, Navon A. Activity and persistence of entomoparasitic nematodes tested against Heliothis armigera (Lepidoptera: Noctuidae). J Econ Entomol 1990;83:795–800.
44. Vashisth S, Chandel Y, Chandel R. Comparative efficacy of indigenous heterorhabditid nematodes from north western Himalaya and Heterorhabditis indica (Poinar, Karunakar & David) against the larvae of Helicoverpa armigera (Hubner). Int J Pest Manage 2019;65:16–22.
45. Kary NE, Gollizadeh A, Dastjerdi HR, Mohammad M, Aghahi S, Omrani M, et al. A laboratory study of susceptibility of Helicoverpa armigera (Hubner) to three species of entomopathogenic nematodes. Mun Entomol Zool 2012;7;372–9.
46. Jothi B, Mehta U. Pathogenicity of three species of entomopathogenic nematodes against cotton bollworm, Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae). In: Biological control of lepidopteran pests. Proceedings of the Symposium of Biological Control of Lepidopteran Pests, Society for Biocontrol Advancement, 2002 July 17–18, Bangalore, India, 2003, pp 199–203.
47. Rosa J, Simões N. Evaluation of twenty-eight strains of Heterorhabditis bacteriophora isolated in Azores for biocontrol of the armyworm, Pseudalatta unipuncta (Lepidoptera: Noctuidae) Biol Control 2004;29:409–17.
48. Prabhuraj A, Patil B. Pathogenicity of Heterorhabditis indicus against Helicoverpa armigera (Hubner). Indian J Entomol 2006;68:36.
49. Andaló V, Faria Lsd, Carvalho FJ, Assis GAd, Santos V, Mendes SM, et al. Entomopathogenic nematodes for the control of Helicoverpa armigera (Hübner)(Lepidoptera: Noctuidae) pupae. Arq Inst Biol 2021;81:1–8.
50. Divya K, Sankar M, Marulasiddehsa K. Efficacy of entomopathogenic nematode, Heterorhabditis indicus against three lepidopteron insect pests. Asian J Exp Biol Sci 2010;1:183–8.
51. Ravendranath S, Krishnayya P, Rao P, Murthy K, Hussaini S. Bioefficacy of entomopathogenic nematodes, Steinernema carpocapsae and Heterorhabditis indica against third instar larvae of Spodoptera litura. Indian J Nematol 2007;37:145–8.
52. Abdel-Raeez A, Adb-Elgawad M. Investigations on the efficacy of entomopathogenic nematodes against Spodoptera littoralis (Biod.) and Galleria mellonella (L.). Arch Phytopathol Plant Prot 2007;40:414–22.
53. Kumar MR, Parihar A, Siddiqui A. Effect of entomopathogenic nematode, Heterorhabditis sp. against Spodoptera litura. Annil Plant Prot Sci 2003;11:406–7.
54. Park SH, Yu YS, Park JS, Choo HY, Do Bae S, Nam MH. Biological control of tobacco cutworm, Spodoptera litura Fabricius with entomopathogenic nematodes. Biotechnol Bioprocess Eng 2001;6:139–43.
55. Hussaini S, Nagesh M, Shaakeela V. Survival of infective juveniles of entomopathogenic nematodes under storage and their infectivity against Galleria mellonella and Spodoptera litura. Indian J Plant Prot 2005;33:68.
56. Baskaran RM, Sivakumar C, Venugopal M. Biocontrol potential of native entomopathogenic nematodes in controlling red hairy caterpillar (Amastica albistirga)(Lepidoptera: Arctiidae) of groundnut (Arachis hypogaeae). Indian J Agric Sci 1994;64:655–7.
57. Shinde S, Singh N. Susceptibility of diamond back moth, Plutella xylostella (L) to entomopathogenic nematodes, pp 956–9, 2000.
58. Sivakumar C, Jayaraj S, Subramanian S. Observations on an Indian population of the entomopathogenic nematode, Heterorhabditis bacteriophora Poinar, 1976. J Biol control 1988;2:112–3.
59. Acharya R, Hwang HS, Mostafiz MM, Yu YS, Lee KY. Susceptibility of various developmental stages of the fall armyworm, Spodoptera frugiperda, to entomopathogenic nematodes. Insects 2020;11:868.
60. Chandel Y, Kapoor S, SK. Virulence of *Heterorhabditis bacteriophora* (Poinar) against cutworm, *Agrotis segetum* (Denis and Schiff.). J Biol Cont 2009;409–15.

61. Kumari V, Singh N, Shinde S, Meena S, Lata RK. Factors affecting propagation of *Heterorhabditis bacteriophora* Poinar, an entomopathogenic nematode in *Plutella xylostella* (L.). J Biopestic 2019;12:1–6.

62. Fetoh BESA, Khaled AS, El-Nagar TF. Combined effect of entomopathogenic nematodes and biopesticides to control the greasy cutworm, *Agrotis ipsilon* (Hufn.) in the strawberry fields. Egypt Acad J Biol Sci A Entomol 2009;2:227–36.

63. Bareth SS, Shinde VKR, Mathur YS, CPS Y. Bioefficacy of entomopathogenic nematodes against cutworms and wireworms. Indian J Appl Entomol 1997;11:73–80.

64. Kappor S, Chandel YS, Kumar S. Pathogenic effects of entomopathogenic nematode, *Heterorhabditis bacteriophora* against some insect-pests of agricultural importance. In: Proceedings of 2nd Congress on Insect Science held at Punjab Agricultural University, Ludhiana, India from 21–22 Feb, pp 344–5, 2008.

65. Singh M, Gupta P. Occurrence of entomopathogenic nematodes in Himachal Pradesh, India and their pathogenicity against various insect species. Pest Manage Econ Zool 2006;14:179–89.

How to cite this article:
Thakur N, Tomar P, Kaur S, Kumari P. Virulence of native entomopathogenic nematodes against major lepidopteron insect species of tomato (*Solanum lycopersicum* L.) J Appl Biol Biotech 2022; 10(Suppl 1):6–14.