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

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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 broad 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. 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

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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 polypagous 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 destroys 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 (IJ_3) 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

This work was conducted in the Department of Zoology and Entomology, Eternal University, Sirmour (30.7537° N, 77.2965° E, 1,900 m altitude), Himachal Pradesh, India.

2.1. Laboratory Culturing of Bait Insects

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 instars 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, agrose, 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-ρ-hydroxybenzoate	1.2 g
8.	Sorbic acid	0.57 g
9.	Streptomycine	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 (LC_{50}) 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 LC_{50} 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, LC_{50} = 87.86 IJs/larvae, 95% FL: 58.94–130.94 IJs/larvae, followed by 72 hours (LC_{50} = 154.92 IJs/larvae, 95% FL: 93.79–255.88 IJ/larva), and 48 hours (LC_{50} = 285.55 IJs/larvae, 95% FL: 153.43–531.45 IJs/larvae), respectively. The highest value of LC_{50} = 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)	Third instar larvae of *H. armigera*						Fourth instar larvae of *H. armigera*					
		LC_{50} (IJ/larva)	FL (95%) Upper limit	FL (95%) Lower limit	χ^2	Std. error	*p*-value	LC_{50} (IJ/larva)	FL (95%) Upper limit	FL (95%) Lower limit	χ^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

LC_{50} = Lethal concentration 50; χ^2 = Pearson's χ^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) after120 hours of application, followed by LC_{50} = 78.01 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 Figure1). 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% FL: 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. Vashisth [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 *Plutella xylostella* 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

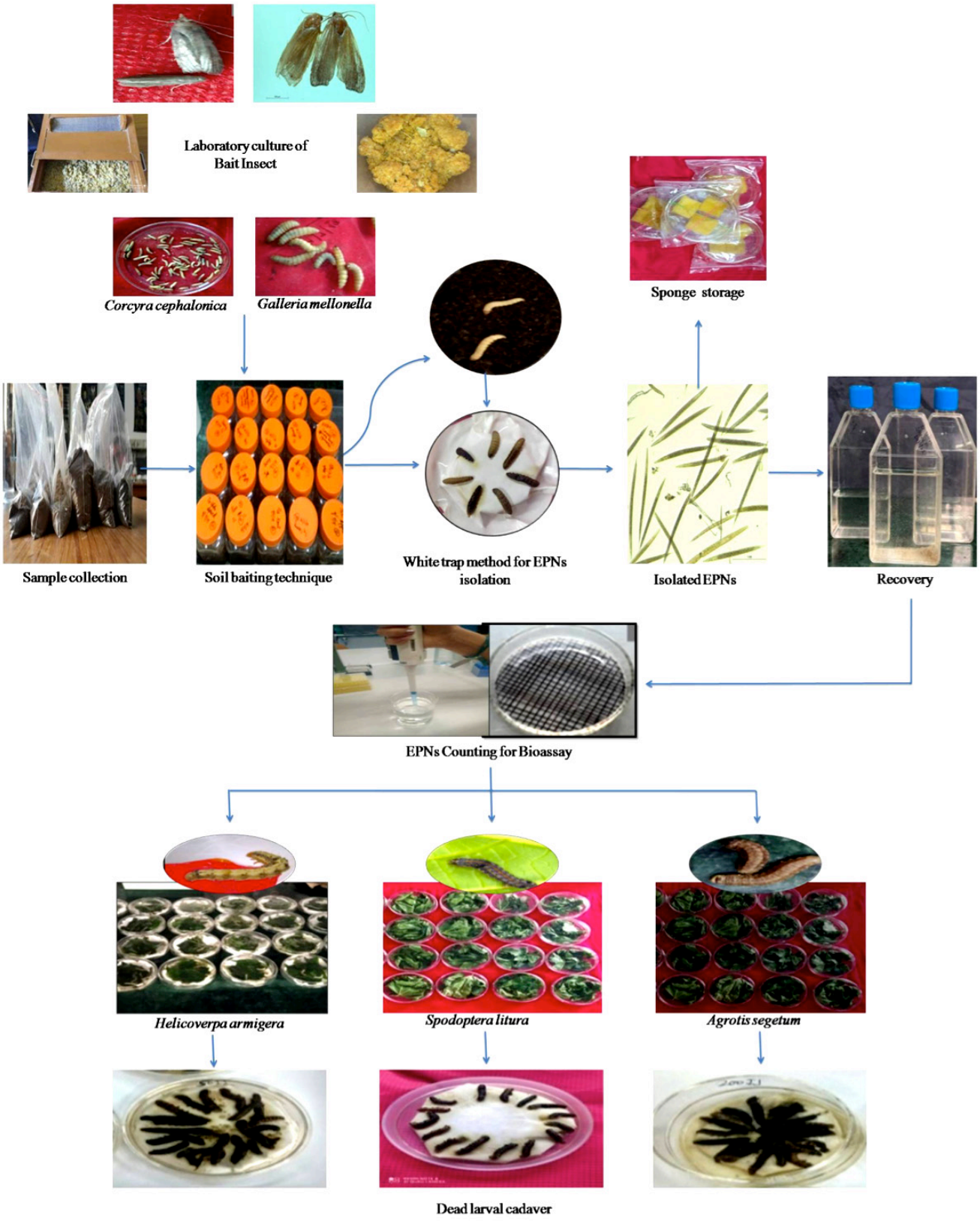

Figure 1: Laboratory culturing of bait insects, EPNs isolation, and bio-efficacy of EPNs against lepidopteran insect pests of tomato.

Table 3: Pathogenicity of *H. bacteriophora* against third and fourth instar larvae of *S. litura* in petri plate assays.

		Third instar larvae of *S. litura*						Fourth instar larvae of *S. litura*					
			FL (95%)						FL (95%)				
Treatments	Incubation period (hours)	LC_{50} (IJ/larva)	Upper limit	Lower limit	χ^2	Std. error	*p*-value	LC_{50} (IJ/larva)	Upper limit	Lower limit	χ^2	Std. error	*p*-value
H. bacteriophora (50, 100, 150 and 200 IJ)	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
	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

LC_{50} = Lethal concentration 50; χ^2 = Pearson's χ^2 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.

		Third instar larvae of *A. segetum*						Fourth instar larvae of *A. segetum*					
			FL (95%)						FL (95%)				
Treatments	Incubation period (hours)	LC_{50} (IJ/ larva)	Upper limit	Lower limit	χ^2	Std. error	*p*-value	LC_{50} (IJ/ larva)	Upper limit	Lower limit	χ^2	Std. error	*p*-value
H. bacteriophora (50, 100, 150 and 200 IJ)	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
	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

LC_{50} = Lethal concentration 50; χ^2 = Pearson's χ^2 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 LD_{50} 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 LC_{50} 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*/larvae 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 littoral* is under laboratory conditions. Kumar *et al.* [53] reported highest LC_{50}s 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.*

[54] 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 LC_{50} 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 LC_{50} = 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/cm^2 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/cm^2 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 flammetra* and *A. ipsilon*. They also reported LT_{50} value after time exposure 5.78 and 6.13 hours at inoculation 100 IJs/cm^2. 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.

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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.

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