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Biocidal potential of indigenous isolates of Entomopathogenic Nematodes (EPNs) against tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

Preety Tomar and Neelam Thakur*

Abstract

Background: Entomopathogenic nematodes (EPNs) have been regarded as the most convenient strategy for insect pest management. The native strains of EPNs: *Heterorhabditis bacteriophora* EUPT-SD, *H. bacteriophora* EUPT-R, *H. bacteriophora* EUPT-KN, *H. bacteriophora* EUPT-K and *H. bacteriophora* EUPT-H isolated from mid-Himalayan region of Himachal Pradesh were tested in laboratory for their multiplication and virulence against 3rd and 4th larval instars of the tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), the serious polyphagous pest affecting a wide range of agricultural crops worldwide.

Results: All the EPN strains were effective against 3rd and 4th larval instars of *S. litura*. Insect mortality reached 90–96% after 96 h at nematode concentrations of 150 infective juveniles (IJs)/ml. The insect mortality was also recorded at low concentrations of IJs, but the most exposure period was required. High virulence was shown by *H. bacteriophora* EUPT-SD 96 and 94%, followed by *H. bacteriophora* EUPT-R 92 and 90%, *H. bacteriophora* EUPT-KN 92 and 90%, *H. bacteriophora* EUPT-K 92 and 90% and *H. bacteriophora* EUPT-H 92 and 90%, respectively, against 3rd and 4th larval instars in terms of reproductive potential and killing. All the insects were alive in the absolute control.

Conclusion: Utilization of EPNs for the management of *S. litura* may be the best method to overcome the insect resistance problems and to manage the population of this insect pest. It may be an effective method and may be a partial substitute of synthetic insecticides, thus minimizing the excessive use of synthetic chemicals. The results demonstrated the potential of indigenous EPNs isolates against *S. litura*, but before further recommendation, multiplication field trials need to be conducted to confirm their efficacy at farm level.

Keywords: Entomopathogenic Nematodes, *Heterorhabditis bacteriophora*, *Spodoptera litura*, Virulence, Reproductive potential

Background

The tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), is a serious insect pest that feeds upon a wide variety of crops having very high fecundity with elevated reproduction rates and migratory behaviour. Young larvae feed on leaves, and 4th and 5th larval instars cause maximum damage by completely skeletonized the leaves, which declined the photosynthesis ability of infected plants (Yadav et al. 2017). In Himachal Pradesh, the control strategies applied to manage the population of these insect pests are generally chemicals based which are hazardous to living beings and costly. Applications of chemical-based synthetic insecticides are the solution only for shorter duration that ultimately resulted in negative impacts over

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the biodiversity (Thakur et al. 2022a). However, insect pest population was triggered by biological opponents such as parasitoids, pathogens and predators. The microorganism-based biocides are the best method to overcome all negative issues (Thakur et al. 2020) that ultimately promote the sustainability through natural farming practices (Thakur et al. 2022b).

EPNs were reported as an eminent biocontrol agent against a broad spectrum of insect pests accompanied with *S. litura* (Tomar et al. 2022a). EPNs in families: Steinernematidae and Heterorhabditidae, premise in several soil types (Hominick 2002). Host range of steinernematids and heterorhabditids is very broad, and it includes over 200 different insect species (Hasan et al. 2009). The infective juveniles (J3) have the potential to invade the insect body via natural routes and the natural body openings, *i.e.* mouth, spiracles, cuticle and anus (Askary and Ahmad 2020). The juvenile stage along with its bacterial endosymbiont kills the insects efficiently through invading haemocoel by regurgitating endosymbiont (Tomar et al. 2022b). These endosymbiont releases toxic compounds inside insect body, which finally kills the insects via toxaemia (Gaugler 2002). As the insect killed, the body becomes soft but not decayed due to the production of antibiotics and secondary metabolites by the bacteria. Infection with steinernematids resulted in ochre, black or yellow brown colour cadaver, while heterorhabditids infected cadaver showed brick-red, purple, red, sometimes green or orange colourations (Sundarababu and Sankaranarayanan 1998). The insect management by the use of EPNs depends upon the developmental stages of insects (Jackson and Brooks 1995). Environmental pollution caused by synthetic chemicals is a major public concern nowadays for which a greener and cleaner tactic is required by the use of entomopathogenic microbial biopesticides. The applications of EPNs are an influential, efficient and eco-friendly approach for insect pest management (Thakur et al. 2021). Earlier EPNs along with entomopathogenic fungus and bacteria have been reported to cause the highest mortality against *Spodoptera* larvae (Tomar et al. 2022c) in bioassay as well as in polyhouse and field conditions (Thakur et al. 2022c). EPNs are mass produce, formulate and applied easily, therefore being used throughout the world against the soil dwelling and foliar crop insect pests (Tomar and Thakur 2022). In the present investigation, EPNs (*Heterorhabditis bacteriophora*) collected from various regions of mid-Himalaya were comprehensively compared and evaluated for its biocidal and reproductive potential against *S. litura* larvae. Furthermore, this study established the most virulent strain of *H. bacteriophora* for the eradication of *S. litura* problem under integrated pest management (IPM) programs.

Methods

Culturing and multiplication of entomopathogenic nematodes

The EPNs were isolated from the soil samples collected from five districts namely Solan, Shimla, Kangra, Kullu and Hamirpur districts of Himachal Pradesh. The collected soil samples were kept into the zip lock polythene bags labelled with information such as soil type, locality, type of fruit orchard, etc. The samples were brought to the Zoology laboratory and kept at low temperature and were processed within 3–5 days. The soil samples were taken out from the bags, and the debris was removed. The soil was filled into the plastic containers and the last instar of the greater wax moth, and *Galleria mellonella* larvae were added into these containers. The dead cadavers were removed from the containers every day and subjected to white trap. Nematodes were collected via white trap methodology (White 1927). Further, the EPNs were nurtured using *G. mellonella* larvae at 27 ± 1 °C with RH 55 ± 10% in the laboratory (Orozco et al. 2014). EPNs stock was perpetuated and stored in distilled water (Woodring and Kaya 1988). The collected isolates were named as EUPT-SD from district Solan, EUPT-R from district Shimla, EUPT-KN from district Kangra, EUPT-K from district Kullu and EUPT-H from district Hamirpur, respectively.

Identification of entomopathogenic nematodes

Nematode identification was done on the basis of their morphological characteristics. For this, the nematodes were killed; fixed and morphological observations were recorded based upon their taxonomical keys (Poinar Jr 1975).

Collection of the host insect (*Spodoptera litura*)

Larvae of *S. litura* were collected directly from the agricultural fields, while adults were collected using light traps. The larvae were transferred to the plastic vials (30 ml), and the adults were kept into a chimney. The castor leaves were provided to the larvae, and sucrose solution was given to the adults for feeding. A piece of paper was also kept inside the chimneys where females lay eggs. The eggs were further transferred to the moist tissue paper where they hatched into 1st instar larvae. The emerged adults were further transferred on the tender castor leaves upon, which they feed and grow into further instars. This way the culture was propagated in the laboratory at 55 ± 10% RH and 27 ± 1 °C.

Biocidal predisposition of nematodes towards *Spodoptera litura*

The insect killing competence of different strains of *H. bacteriophora* collected from various localities was

evaluated under a laboratory bioassay experiment against 3rd and 4th larval instars of *S. litura.* The laboratory-reared larvae *S. litura* were kept over the Whatman filter paper No. 1 inside a Petri plate along with diet (castor leaves). The nematode suspensions collected from five different districts named: Solan (EUPT-SD), Shimla (EUPT-R), Kangra (EUPT-KN), Kullu (EUPT-K) and Hamirpur (EUPT-H), were applied into the Petri plate at different concentrations such as 30, 60, 90, 120 and 150 IJs/ml along with absolute control. The insect death rate was noticed after every 24 up to 96 h. The treatments were replicated 5 times, and the experiment was accomplished for twice. The data recorded over the two experiments were pooled and subjected to the statistical analysis.

Nematodes multiplication on *Spodoptera litura*

The dead insect cadavers were collected from the bioassay experiment and were kept in the white trap. The trap consists of two different sized Petri plates in which the smaller one of 60 mm diameter was lined with one Whatman filter paper No. 1 that was placed inside a larger Petri plate of 100 mm diameter. The larger Petri plate was filled with distilled water in order to maintain the moisture content on the filter paper. The dead cadavers recovered from the bioassay study were washed with distilled water twice and then kept over the moist filter paper. The Petri plate was covered and kept inside the incubator. The emerging nematodes were moved into the water in the surrounding region. These were harvested from the water and kept into the storage bottles. The harvesting was done up to 10 days until all the EPNs were collected. The data on the nematode emergence and reciprocation on 3rd and 4th larval instars were recorded and subjected to statistical analysis.

Statistical analysis

Statistical analyses were performed on the data collected over the insect mortality and nematode reciprocation. The analysis of variance (ANOVA) was used to conclude the EPNs' biocontrol efficacy and reproduction rates against *S. litura*. Corrected mortality was calculated using Abbott's formula (Abbott 1925). Probit analysis was performed, and the median lethal concentration (LC_{50}) was calculated. The calculated data were signified as means ± standard error.

Results

Entomopathogenic nematodes (EPNs) were isolated via soil baiting technique using *G. mellonella* larvae, followed by the white trap methodology; nematode isolation was done from various localities among five districts of Himachal Pradesh. The morphological measurements of the mounted specimens were taken, and the isolated EPNs were identified as *H. bacteriophora* based upon their morphological observations. The isolated nematodes were further multiplied on *G. mellonella* larvae and were kept into the roux bottles, and their insecticidal activities were evaluated (Fig. 1).

Biocontrol potential of different strains of *H. bacteriophora* collected from the different localities was assessed in the laboratory. The comparative analysis on the bioefficacy study showed that locally available *H. bacteriophora* strain EUPT-SD caused maximum larval mortality 96% ($F=20.52$, df = 5, $P<0.05$) and 94% ($F=23.76$, df = 5, $P<0.05$) amongst 3rd and 4th larval instars of *S. litura* in the inoculum of 150 IJs/ ml after 96 h. of nematode inoculation (Fig. 2). A significant difference amongst the larval mortality was caused by five different nematode inoculums. Considering the data based upon probit analysis, the calculated median lethal concentrations $LC_{50}=36.76$ IJs (95% FL: 29.14–46.37) slope 1.98 ± 0.25 amongst 3rd instar larvae and $LC_{50}=37.83$IJs (95% FL: 29.70–48.19) slope 1.87 ± 0.25 for the 4th instar larvae after 96 h (Table 1). The Shimla isolate *H. bacteriophora* strain EUPT-R caused 92% and 90% high larval mortality in the nematode inoculum concentration of 150 IJs after 96 h. The recorded mortality data showed significant variations upon exposure of different nematode inoculum concentration ($F=16.74$, df = 5, $P<0.05$) amongst the 3rd and ($F=16.74$, df = 5, $P<0.05$) 4th larval instars (Fig. 2). Based upon the probit of killing, LC_{50} value for 3rd instar *S. litura* larvae were 41.33 IJs with 95% FL: 32.58–52.41, slope 1.88 ± 0.25 and $LC_{50}=43.36$IJs with 95% FL: 33.83–55.57, slope 1.77 ± 0.24 for 4th instar larvae after 96 h (Table 1).

The EPN isolates from district Kangra, *H. bacteriophora* strain EUPT-KN, also exhibited 92 and 90% larval mortality amongst both the instars, respectively (Fig. 2). The insect mortality increased as the inoculum concentration raised and also the exposure period enhanced. Statistically significant results were obtained from the bioassay experiment ($F=19.93$, df = 5, $P<0.05$) in 3rd and ($F=26.15$, df = 5, $P<0.05$) in 4th larval instars. In the log probit analysis, the LC_{50} value for 3rd instar larvae was 46.81 IJs with 95% FL: 37.63–58.22, slope 2.04 ± 0.25 and for 4th instar larvae was 50.06 IJs with 95%FL: 33.83–55.57, slope 1.99 ± 0.24 after 96 h (Table 1). Similarly, the Kullu isolate, *H. bacteriophora* strain EUPT-K, also caused the highest 92 and 90% mortality amongst both larval instars, respectively. Significant variations were observed after treatment with different concentrations of nematode juveniles ($F=16.63$, df = 5, $P<0.05$) among 3rd instar larvae and ($F=24.92$, df = 5, $P<0.05$) for 4th instar larvae (Fig. 2). The mortality rate further increased with

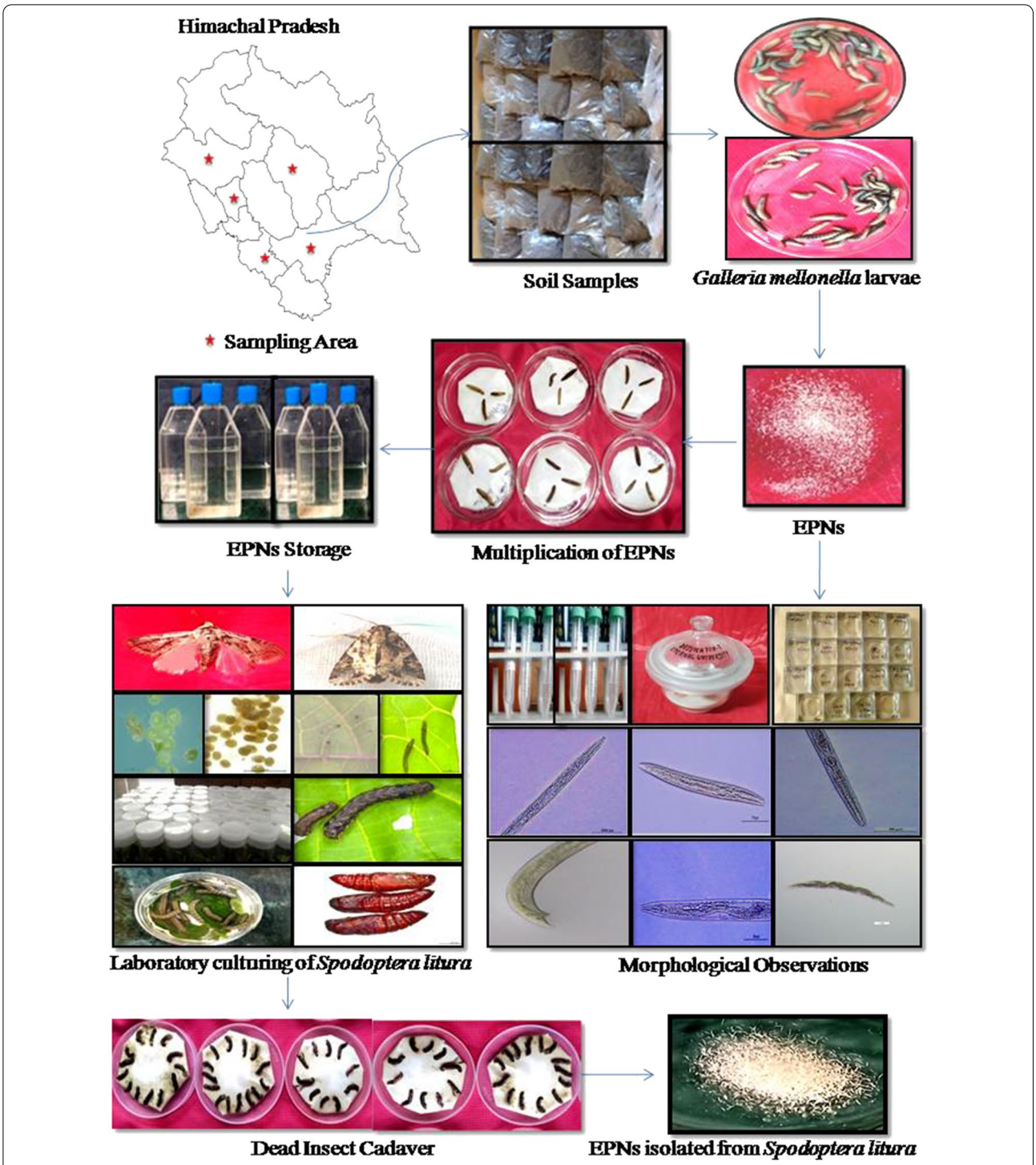

Fig. 1 Isolation, identification and bioassay of entomopathogenic nematode strains in the laboratory

the increase in the inoculum concentration. The calculated LC_{50} = 44.86IJs with 95% FL: 36.306–55.43, slope 1.99 ± 0.27 among 3rd instar larvae and LC_{50} = 52.98IJs with 95% FL: 41.65–67.383, slope 1.79 ± 0.24 among 4th instar larvae after 96 h (Table 1).

The Hamirpur isolate *H. bacteriophora* strain EUPT-H again showed maximum larval mortality 92% (F = 21.30, df = 5, P < 0.05) and 90% (F = 29.15, df = 5, P < 0.05), respectively, for both larval instars of *Spodoptera* (Fig. 2). All the insects were alive in the absolute

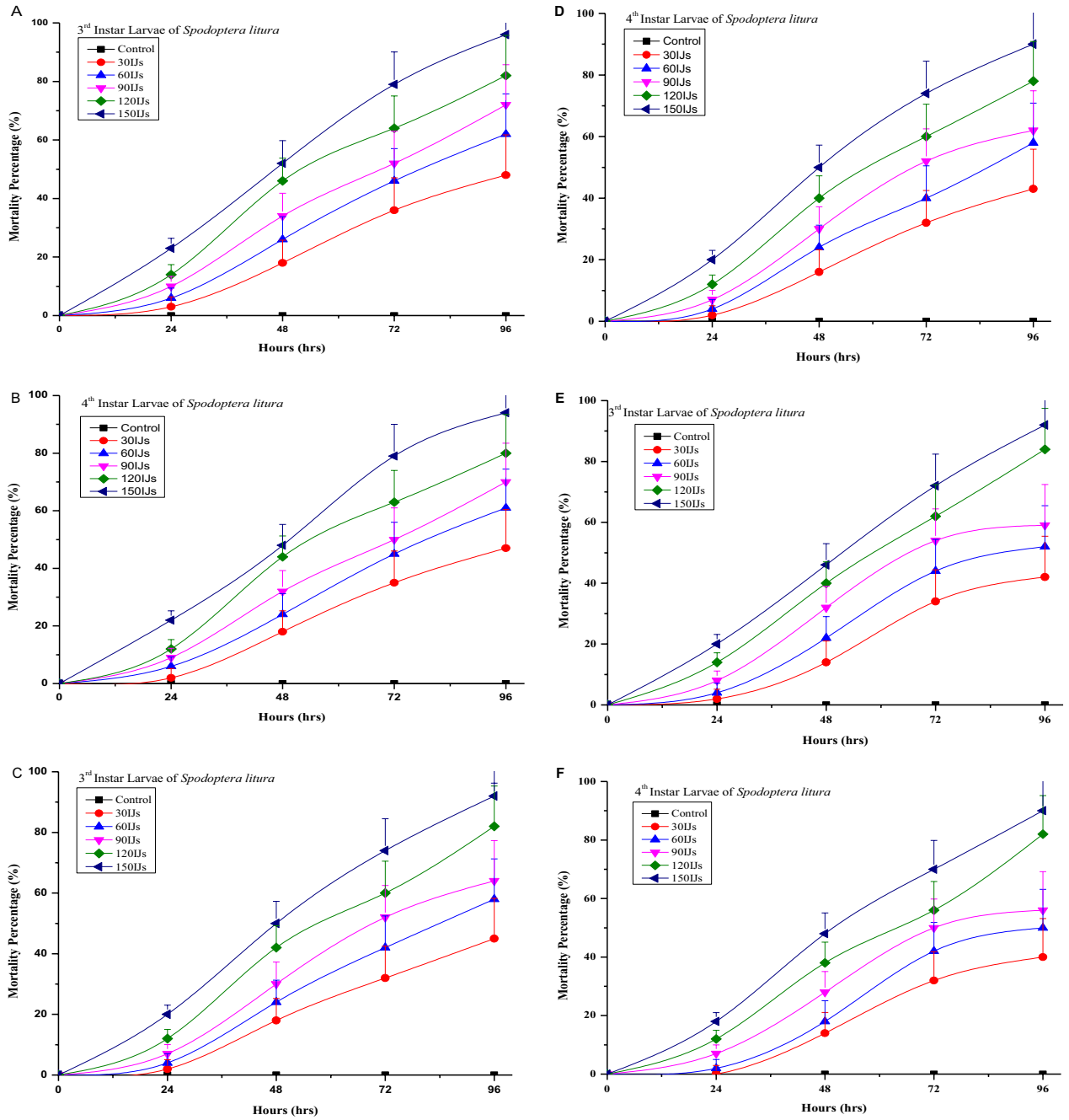

Fig. 2 Percent mortality caused by *Heterorhabditis bacteriophora* strains in 3rd and 4th larval instars of *Spodoptera litura* (**A** and **B**) Mortality caused by *Heterorhabditis bacteriophora* EUPT-SD; (**C** and **D**) Mortality caused by *Heterorhabditis bacteriophora* EUPT-R; (**E** and **F**) Mortality caused by *Heterorhabditis bacteriophora* EUPT-KN; (**G** and **H**) Mortality caused by *Heterorhabditis bacteriophora* EUPT-K; and (**I** and **J**) Mortality caused by *Heterorhabditis bacteriophora* EUPT-H

control. The calculated LC_{50} value was 42.78IJs with 95% FL: 33.92–53.94, slope 1.93 ± 0.25 for 3rd instar larvae and LC_{50} = 46.80IJs with 95% FL: 37.38–58.59, slope 1.97 ± 0.27 against 4th instar larvae after 96 h (Table 1). Significant variations were observed between the insect mortality data upon treatment with different concentrations of infective juveniles.

EPNs multiplication in insect larvae was also evaluated through comparing the number of emerging nematodes from each larval instar. The data were collected regularly

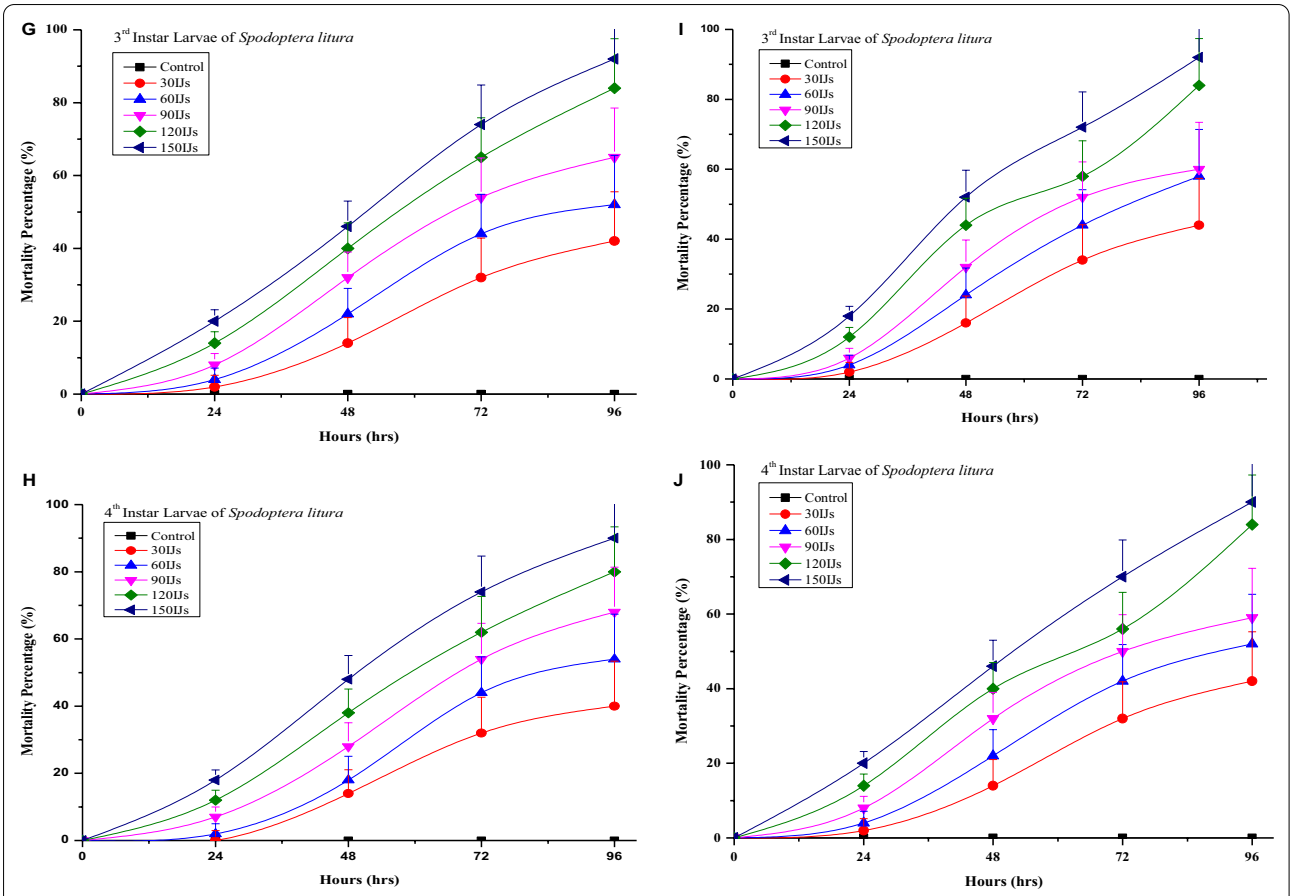

Fig. 2 continued

until all the EPNs were harvested from the white trap. The data recorded from the present study denoted that the later instar (4th instar larvae) produced more number of nematodes as compared to the younger instar (3rd instar larvae) (Figs. 3 and 4). Among the different strains of EPNs, the highest number of nematodes was procured from the *H. bacteriophora* EUPT-H and *H. bacteriophora* EUPT-SD upon treatment with 150 IJs/ml concentration against 4th instar larvae of *S. litura*.

Discussion

Different strains of EPNs, *H. bacteriophora* strains, were isolated from different mid-Himalayan regions of Himachal Pradesh, and the virulence of these strains was evaluated against the 3rd and 4th larval instars of *S. litura* by conducting a bioassay experiment. The results revealed that the native population of *H. bacteriophora* strain EUPT-SD caused the maximum larval mortality 96 and 94% amongst both larval instars. Other isolates including *H. bacteriophora* strain EUPT-R, *H. bacteriophora* strain EUPT-KN, *H. bacteriophora* strain EUPT-K and *H. bacteriophora* strain EUPT-H showed almost similar effect causing 92 and 90% mortality, respectively. All the treatments showed significant mortality rates in the insects. Earlier, many researchers have reported the biocontrol potential of EPNs against the *S. litura* larvae (Yan et al. 2020). The present investigation is also supported by Park et al. (2001) who reported that small instars were more prone EPNs to infection due to their sensitive skin. Acharya et al. (2020) who evaluated the virulence potential of four EPN species such as *H. indica, H. bacteriophora*, *Steinernema longicaudum* and *S. carpocapsae* against all larval instars of tobacco cutworm and reported more mortality amongst younger larval instars than the older ones. These findings are similar to the present findings. Similar findings were observed by Burana et al. (2022). Obtained results are also in line with the earlier findings of Sun et al. (2021) who evaluated the virulence potential of *Heterorhabditis* and *Steinernema* against 5th instar the *S. litura* larvae and recorded over 90% mortality in *S. litura* after the exposure of 72 h. Javed et al. (2022) also recorded similar observations.

Table 1 Log probit analysis for the assessment of mortality in 3rd and 4th larval instars of the tobacco cutworm, *Spodoptera litura* by different entomopathogenic nematode strains (EPNs)

EPN strains	Susceptibility (hrs)	LC_{50} (IJs/ml)	95% fiducial limit		Slope ± SE	Pearson's $\chi 2$	*p*-value
			LL	UL			
Against 3rd instar larvae							
Heterorhabditis bacteriophora EUPT-SD	24	472.83	338.17	661.12	1.68 ± 0.39	0.730	0.010
	48	150.79	111.36	204.17	1.42 ± 0.25	0.700	0.005
	72	62.61	47.04	83.35	1.48 ± 0.24	0.092	0.003
	96	36.76	29.14	46.37	1.98 ± 0.25	0.020	0.001
H. bacteriophora EUPT-R	24	472.80	342.89	651.94	1.88 ± 0.45	0.670	0.010
	48	177.16	127.65	245.89	1.32 ± 0.25	0.470	0.007
	72	72.23	54.25	96.18	1.47 ± 0.24	0.330	0.003
	96	41.33	32.58	52.41	1.88 ± 0.25	0.020	0.002
H. bacteriophora EUPT-KN	24	439.57	322.37	599.39	1.92 ± 0.44	0.820	0.010
	48	183.47	135.32	248.75	1.45 ± 0.26	0.930	0.005
	72	68.04	50.03	92.54	1.36 ± 0.24	0.630	0.004
	96	46.81	37.63	58.22	2.04 ± 0.25	0.001	0.001
H. bacteriophora EUPT-K	24	439.57	322.37	599.39	1.92 ± 0.44	0.820	0.010
	48	183.47	135.32	248.75	1.45 ± 0.26	0.930	0.005
	72	68.86	51.94	91.31	1.49 ± 0.24	0.550	0.003
	96	45.48	36.73	56.33	2.10 ± 0.26	0.750	0.009
H. bacteriophora EUPT-H	24	542.35	384.06	765.87	1.78 ± 0.45	0.690	0.010
	48	155.84	117.07	207.43	1.52 ± 0.26	0.630	0.004
	72	71.40	51.59	98.83	1.29 ± 0.23	0.400	0.005
	96	42.78	33.92	53.94	1.93 ± 0.25	0.002	0.001
Against 4th instar larvae							
H. bacteriophora EUPT-SD	24	463.84	335.28	641.69	1.79 ± 0.41	0.709	0.010
	48	174.93	125.72	243.41	1.31 ± 0.25	0.640	0.007
	72	65.43	49.34	86.77	1.49 ± 0.24	0.050	0.003
	96	37.83	29.70	48.19	1.87 ± 0.25	0.040	0.002
H. bacteriophora EUPT-R	24	472.80	342.89	651.94	1.88 ± 0.45	0.667	0.013
	48	177.22	129.70	242.16	1.40 ± 0.26	0.606	0.006
	72	73.51	55.42	97.49	1.49 ± 0.24	0.250	0.003
	96	43.36	33.83	55.57	1.77 ± 0.24	0.040	0.002
H. bacteriophora EUPT-KN	24	527.74	375.91	740.88	1.82 ± 0.45	0.350	0.016
	48	189.32	141.55	253.21	1.53 ± 0.27	0.380	0.005
	72	78.47	56.75	108.49	1.29 ± 0.24	0.420	0.005
	96	50.06	40.13	62.43	1.99 ± 0.24	0.001	0.001
H. bacteriophora EUPT-K	24	527.74	375.91	740.88	1.82 ± 0.45	0.350	0.020
	48	189.32	141.55	253.21	1.53 ± 0.27	0.380	0.005
	72	79.95	58.86	108.59	1.37 ± 0.24	0.490	0.004
	96	48.80	39.14	60.86	2.00 ± 0.25	0.016	0.001
H. bacteriophora EUPT-H	24	439.57	322.37	599.39	1.92 ± 0.44	0.230	0.012
	48	183.47	135.32	248.75	1.43 ± 0.26	0.930	0.005
	72	78.47	56.75	108.49	1.29 ± 0.24	0.420	0.005
	96	46.80	37.38	58.59	1.97 ± 0.27	0.002	0.001

* LC_{50}: Median Lethal Concentration; S. E.: Standard Error; Pearson's χ2: Pearson's Chi Square Value

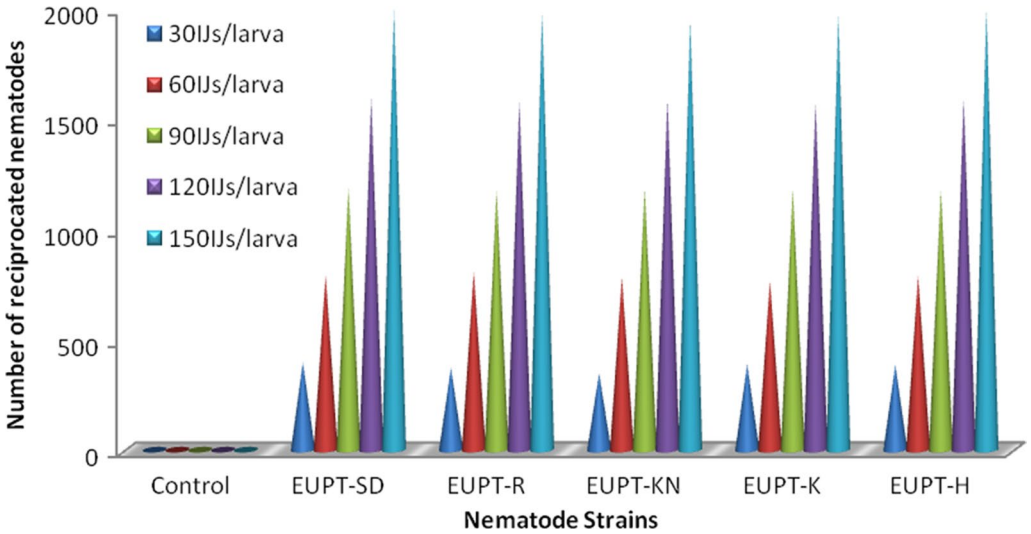

Fig. 3 Multiplication of nematode juveniles on 3rd instar larvae of *Spodoptera litura* after treatment with different concentrations of nematodes strains collected from various localities of Himachal Pradesh

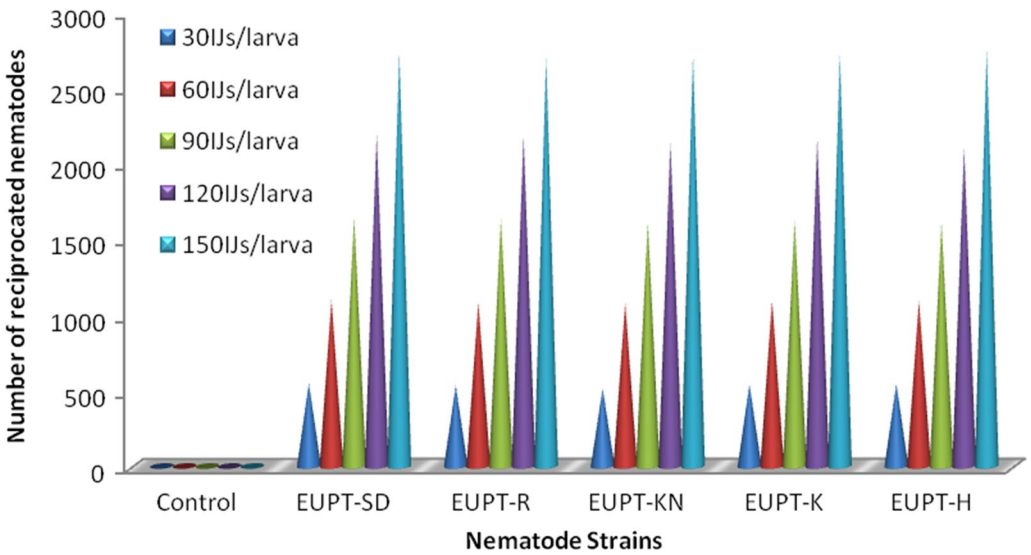

Fig. 4 Multiplication of nematode juveniles on 4th instar larvae of *Spodoptera litura* after treatment with different concentrations of nematodes strains collected from various localities of Himachal Pradesh

During the present investigation, it was also observed that increased nematode inoculum concentration and the enhanced exposure period resulted in maximum larval mortality. The results are in conformity with the earlier research work carried by Holajjer et al. (2014). The results are also supported by the findings of Acharya et al. (2020). They observed that the insect mortality increased with the raise in the nematode inoculum and time interval. The lowest median lethal concentration LC_{50} = 36.76IJs of 3rd instar larvae and LC_{50} = 37.83IJs of 4th instar larvae was recorded, when different concentrations of *H. bacteriophora* strain EUPT-SD were inoculated to the larval instars. Similar observations were recorded by Umamaheswari et al. (2006) who reported LC_{50} = 3.53 IJs/larva upon treatment with six different isolates of EPNs. Burana et al. (2022) reported LD_{50} = 15.84 dauer juvenile/larva in 1st instar and LD_{50} = 40.34 dauer juvenile/larva among 3rd instar larvae upon treatment with *S. siamkayai*. The results are also supported by Dichusa et al. (2021) who had reported the

insect mortality range between 0 and 100% upon treated with seven species of EPNs with LC_{50} value 7.13 ± 1 IJs/larva. Thakur et al. (2022a) recorded $LC_{50} = 59.95$ IJs/larvae in 3rd instar larvae and $LC_{50} = 50.91$ IJs/larvae among 4th instar larvae of *S. litura* under the laboratory conditions.

The study on the nematode reciprocation denoted that larger instars of *S. litura* produced more population of EPNs and the young instars produced much lower nematode concentration. The present finding supported by the earlier findings of Park et al. (2001) who reported the highest number of nematodes from 5 and 6th larval instars of *S. litura*. Holajjer et al. (2014) reported that the number of nematode inocula added to infect the *S. litura* larvae does not affect the population of nematode juveniles and does not interfere in reproduction and multiplication of the EPNs. Safdar et al. (2018) observed maximum 25,786 nematodes produced from 5th instar of *S. litura,* followed by younger instar (4th instar larvae) containing 17,500 nematodes, followed by 3rd instar larvae 12,642 nematodes and 2nd ones 9652 nematodes. The reproduction potential of the EPNs fluctuates with respect to species, isolates, invasion rates, size of the host, host susceptibility and diverse environmental conditions as humidity and temperature (Askary and Ahmad 2021).

Conclusion

It has been concluded from the present study that the native strains of EPNs were highly virulent and can cause maximum larval mortality amongst the 3rd and 4th larval instars of *S. litura*. Moreover, the insect mortality rate increased as the exposure time exceeded. It was further observed that the youngest instar larvae (3rd instar) were more susceptible towards the EPNs infection than the oldest instar larvae (4th instar). Further, the nematode reciprocation was much high in the largest instar larvae than the smallest ones. So it can be demonstrated that utilization of EPNs for the management of *S. litura* is the best method to overcome the insect resistance problems and to eradicate this insect pest population. It is an effective method and may be a partial substitute in place of synthetic insecticides, thus minimize the excessive use of synthetic chemicals.

Abbreviations

H. bacteriophora: *Heterorhabditis bacteriophora*; Df: Degree of freedom; IJs: Infective juveniles; EPNs: Entomopathogenic nematodes; BCAs: Biocontrol agents.

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Author contributions

NT gave the concept. PT performed the experiment, wrote the manuscript and did the statistical analysis. Both the authors have read the manuscript.

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Declarations

Ethical approval

Not applicable.

Consent for publication

The authors declare that the submitted manuscript is the authors' original research work and it has not been submitted for publication elsewhere.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Competing interests

The authors declare that there is no conflict of interest.

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