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Indigenous entomopathogenic nematode as biocontrol agents for insect pest management in hilly regions

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Abstract

The present investigation emphasized on the development and use of entomopathogenic nematodes (EPNs) as a bio-insecticide. The success in controlling insect pests in the soil environments increased the production and use of the adapted indigenous EPNs species for insect management in the fields. EPNs as biocontrol agents were capable for high virulence, easy for application, safe for non-target animals and eco-friendly in nature. These nematodes have ubiquitous nature. These occur in low population in their natural habitat which was mass multiplied in the laboratory. In the present investigation, 5 concentrations (30, 60, 90, 120 and 150IJs) of *Heterorhabditis bacteriophora* strain S_{15} were applied against the 3rd and 4th instar larvae of four major agricultural insect pests, namely *Helicoverva armigera* (Hubner), *Spodoptera litura* (Fabricus), *Agrotis segetum* (Denis and Schiffermüller) and *Mythimna separata* (Walker) under laboratory conditions at different time exposure (24, 48, 72 and 96 h). It was observed that the 3rd and 4th larval instars of all 4 insects (*H. armigera*, *S. litura, A. segetum* and *M. separata*) were highly susceptible for the pathogenesis caused by *H. bacteriophora* strain S15. Amongst all insects, both the larval instars of *M. separata* are highly susceptible for EPNs infection with highest 96% and 98% mortality in highest dose @150IJs. In 3rd instar larvae of other insects such as *H. armigera*, *S. litura* and *A. segetum* larval mortality ranges from 84%, 92% and 94% respectively. Among 4th instar larvae of *H. armigera*, *S. litura* and *A. segetum* the pathogenicity varies from 88%, 94% and 96%, respectively. The recorded median lethal concentration (LC_{50}) in 3rd instar larvae of *H. armigera*, *S. litura, A. segetum* and *M. separata* varies from 36.15, 30.05, 30.97 and 23.8IJs/larva. Similarly in 4th instar larvae of *H. armigera*, *S. litura, A. segetum* and *M. separata*, LC_{50} ranged from 31.41, 28.64, 26.92 and 20.64IJs/larva respectively. Statistically significant variations were observed in the data recorded on the mortality, in all the treatments. EPNs are the best weapon to overcome insect resistance problems and must be employed to manage insect population.

Keywords

Agrotis segetum, Biocontrol, *Helicoverpa armigera*, *Mythimna separata*, *Spodoptera litura*

Introduction

Over the next few decades, the world's population predicted to be reached about 10 billion (1). Most of the people in India depend upon agriculture to fulfil their every day needs. The main concern of the agricultural industry is

to attain highest production in such a way that it is ecologically sustainable and worthwhile, but the major problem with modern agriculture is the losses caused by insect pest. About one million or more insect species are recognized and they all are universal and adaptable in nature (2). According to FAO removal of natural forest with advanced agricultural strategies like greenhouses for the production of monoculture provides suitable habits and habitat for the pest population. Entomopathogenic nematodes (EPNs) are microscopic roundworms belonging to families Heterorhabditidae and Steinernematidae under phylum Nematoda (3). The term entomopathogen has originated from greek words 'entomon' refers to insect and 'pathogen' that causing diseases. EPNs are actually beneficial nematodes that exhibit holoparasitic mode of survival (3, 4). They can survive in almost all kinds of habitats except Antarctica (5). The use of EPNs is considered a best integrated pest management (IPM) approach to control such insect population. They have been reported as the efficient bio-control agents against agricultural and forest insect pests (6). The EPNs from genus *Heterorhabditis* and *Steinernema* were considered as deadly fatal for a number of agricultural insect pests (7, 8).

Seventeen species of *Heterorhabditis* and hundred species of *Steinernema* have been sorted out that are found to be lethal for insect pests (3, 9). Parasitism by nematode worm in insects may result in sterility, reduced fecundity, delayed development, aberrant behaviour or death of the host, which prevents the degree of insect damage to crops (10). They are considered as the best substitute of chemical insecticides, due to their high potential of infecting the insects hidden even in mysterious places, high multiplication ability as well as their eco-friendly nature (4, 11). Nematodes got signal from the insects through odour and ordure inside soil (12-14). In EPNs the third stage juvenile resides freely in soil with non-foraging behaviour, is responsible for causing the pathogenicity in their host and is commonly regarded as dauer juvenile. The dauer juvenile penetrate into the host body through spiracles or through natural body openings and releases its symbiotic bacteria inside the haemocoel of insect (4). The bacterial cells duplicates and generate severe toxins that have the high insecticidal potential and can kill the host insect within 1-2 days (15). The infected host dies soon after infection due to sepsis (16). Within the insect cadaver these bacteria nourish the EPNs and promote the growth and reproduction of EPNs (5). Major insect pests of agricultural crops are belonging to order lepidoptera including *Helicoverva armigera* (Hubner), *Spodoptera litura* (Fabricus), *Agrotis segetum* (Denis and Schiffermüller) and *Mythimna separata* (Walker). Most of these insect pests are polyphagous and cause considerable damage to the agricultural crops.

Cotton bollworm (*H. armigera*) is reported to infest wide range of crops such as tomato, cotton, corn, soybean and groundnuts. It is considered as very serious insect pest of agricultural crops that cause considerable damage in productivity (17). Tobacco cutworm (*S. litura*) causes about 26-100% losses in yield every year as it attack more than 150 plants of various botanical origin in India including acacia, beetroot, banana, cotton, cabbage, lettuce, peanuts, strawberry and tomatoes (18, 19). Common cutworm (*A. segetum*) usually affects the growing seedlings of various crops including beans, cabbage, eggplant, lettuce, okra, peppers, potatoes, sugar beet and tomatoes (20-23). Armyworm or rice ear-cutting caterpillar (*M. separata*) known to cause extensive foliar damage (44%) to the crops including wheat, maize, rice, oats, sorghum and sugarcane (24).

In the present study indigenous EPNs species *H. bacteriophora* strain S_{15} from hilly regions of Himachal Pradesh were tested for its pathogenic effect against the major lepidopteran insect pests (*H. armigera*, *S. litura, A. segetum* and *M. separata* under the laboratory conditions.

Materials and Methods

Collection of soil samples

Soil samples were collected from the fruit orchards of district Solan, Shimla, Kangra, Kullu and Sirmaur, Himachal Pradesh. Soil samples were collected randomly from the rhizospheric soil of the fruit trees at a depth of about 15-30 cm. Collected samples were brought in the polythene bags marked with proper information such as locality, type of fruit orchard and date of sampling. In order to maintain their moisture content, the samples were kept at 5 °C in the laboratory and were processed within 3-4 days.

Laboratory culturing of bait insect

Galleria mellonella (Linnaeus) larvae and adults were collected from the Eternal University apiary. The culture was maintained into the rearing boxes containing natural diet (wax) and artificial diet. The artificial diet was also prepared by mixing the ingredients such as wheat flour (100 mg), wheat bran (100 g), milk powder (100 g), maize flour (200 g), dried yeast (50 g), honey (175 ml) and glycerine (175 ml). The eggs were inoculated into the diet and culture was maintained.

Isolation of entomopathogenic nematodes from the soil

Isolation of EPNs was done through soil baiting technique (25). The collected soil samples were processed and debris was removed. One hundred and fifty gram of soil was taken in well labelled plastic container. About 4-5 last instars larvae were placed into the plastic container and containers were placed in the dark. The samples were checked regularly after each 24h to find out the mortality of insect. Dead insect cadavers were collected and extraction of EPNs was done through white trap method (25, 26).

Collection of host insects and their rearing in the laboratory

Agricultural insect pests *H. armigera*, *S. litura, A. segetum* and *M. separata* were collected from agricultural fields of Eternal University and from farmer's field at different localities. The culture of all these host insects were maintained using the earlier methodology described for the laboratory rearing of *H. armigera* (27), *S. litura* (28), *A. segetum* (29, 30) and *M. separata* (31) respectively.

Bioefficacy of entomopathogenic nematodes against different insect pests

Biocontrol potential of EPNs was tested against different insects under the laboratory conditions. Different concentrations of infective juveniles (IJs) of EPNs such as 30, 60, 90, 120, 150IJs along with control were tested against 3rd and 4th larval instars of all polyphagous insects and the mortality was checked 24, 48, 72 and 96 h after exposure. Each treatment was replicated five times and in control two millilitre water was applied. The experiments were repeated twice and the pooled data was subjected to statistical analysis.

Statistical analysis

The data obtained on the mortality caused by EPNs in insect larvae was subjected to the statistical analysis. Analysis of variance (ANOVA) was measured using arcsine transformation by applying three factor analyses over percent mortality. Probit analysis was performed to assess the median lethal concentration (LC_{50}) value of EPNs required to cause 50% mortality using OP stat software.

Results

Screening the effectiveness of entomopathogenic nematodes Heterorhabditis bacteriophora strain S15 against insect pests in the laboratory

EPNs were isolated from the soil samples using insect baiting technique and white trap method. The emerging EPNs from the dead cadaver were recovered. The population of these isolated EPNs is quite lower in the recovery, so these recovered EPNs were mass multiplied in the laboratory using last instars of *G. mellonella*. The multiplied EPNs were recovered and stored in storage bottles for further bioassay study in the laboratory.

The insecticidal potential of isolated EPNs *H. bacteriophora* strain S_{15} was evaluated against 3rd and 4th instar larvae of insect pests. Different concentrations such as 30, 60, 90, 120 and 150IJs and at different time interval were applied to ensure their insecticidal efficiency. The 3rd and 4th instar insect larvae of all the four insects (*H. armigera*, *S. litura, A. segetum* and *M. separata*) were found highly susceptible for EPNs (*H. bacteriophora*) infection (Fig. 1). The data obtained on this investigation is summarised under following subheads:

Susceptibility and mortality in Helicoverpa armigera

The results obtained over the effectiveness of *H. bacteriophora* strain S_{15} against 3rd and 4th instar larvae of *H. armigera* revealed that EPNs are responsible for causing significant mortality in both the instars. It is clear from the (Fig. 2) that in 3rd instar larvae, at highest dose @150IJs, highest 84% mortality was observed after 96 h of infection followed by 76%@120IJs. Similarly, against 4th instars highest 88% insect mortality was observed in the highest dose (150IJs) followed by 82% in dose of 120IJs after 96 h of infection. In control, zero mortality was recorded against 3rd and 4th instar of *H. armigera* even after 96 h of time exposure. The table (Table 1) represented the LC_{50} values of

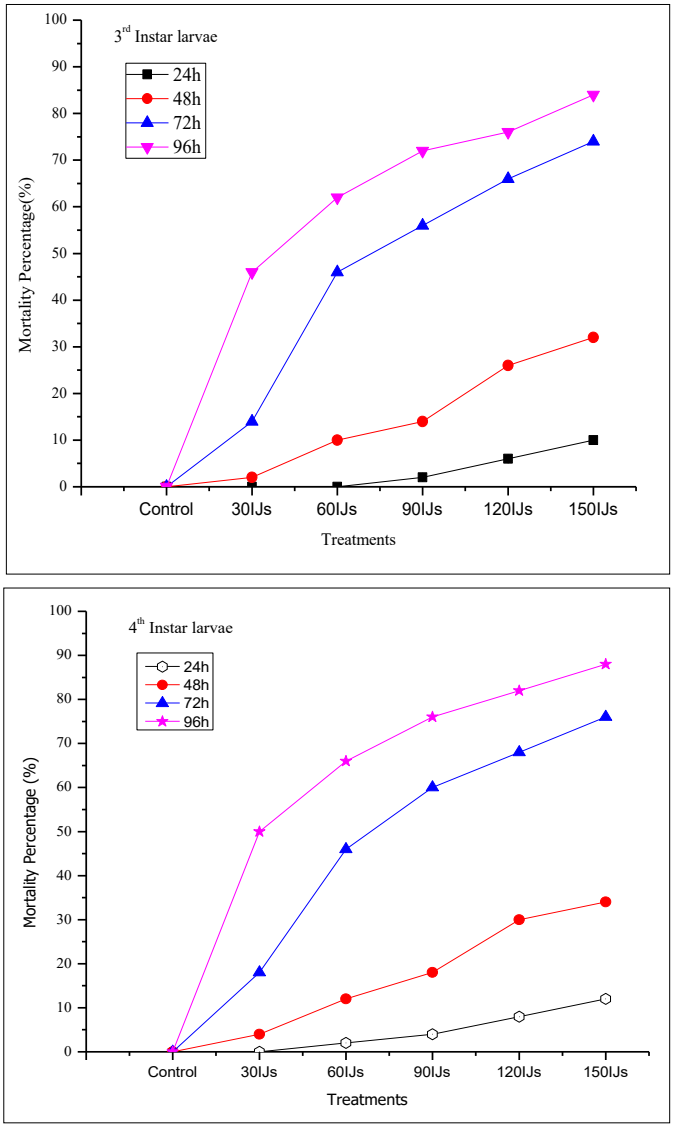

Fig. 2. Percent mortality caused by *Heterorhabditis bacteriophora* strain S_{15} in 3rd and 4th instar larvae of *Helicoverpa armigera.*

both the instars at different time intervals and doses. The calculated LC_{50} of 3rd instar was 36.15IJs/larva (95% fiducial limit (FL): 26.87-48.64) and 4th instar was 31.41IJs/larva (95%FL: 23.72-41.61). It was cleared from the table that after 48 h of infection significant mortality was observed as $p < 0.01$.

Susceptibility and mortality in Spodoptera litura

The efficiency of *H. bacteriophora* strain S_{15} was also observed against 3rd and 4th instar larvae of *S. litura* showed that in 3rd instar larvae, 92% larval mortality was caused at highest dose @150IJs followed by 80% @120IJs, after 96 h of infection. 4th instars larvae also showed 94% mortality at dose @150IJs followed by 86% in dose of @120IJs after 96 h. Lowest dose @ 30IJs showed lowest mortality 52% in 3rd instars and 54% in 4th instar larvae after 96 h exposure. The data obtained over the mortality was presented in (Fig. 3). In control, no mortality was recorded against 3rd and 4th instar of *S. litura* even after 96 h of time exposure. The lowest LC_{50} values of 3rd instar larvae were 30.05IJs/larva (95% FL: 22.61-39.95) and 4th instar larvae is 28.64IJs/larva (95%FL: 22.05-37.20) after 96 h of exposure. It is evident from the table (Table 1) that all the treatments

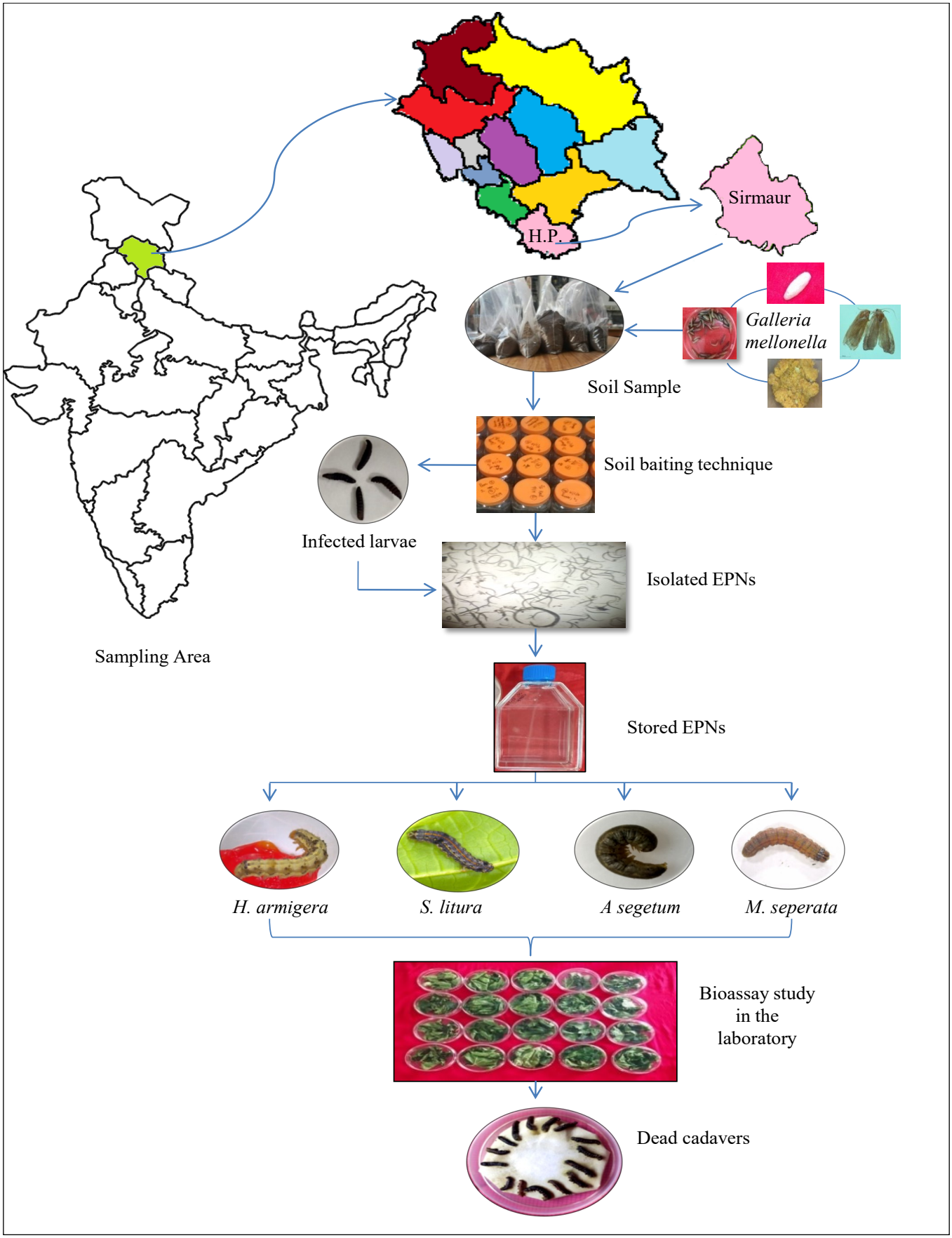

Fig. 1. Isolation of entomopathogenic nematodes and their applications against lepidopteran insect pests under the laboratory.

were effective and showed significant mortality as we have a p value less than 0.01. During the present bioassay study it was very clear that with the increase of time interval and doses in both the instar larvae, insect mortality percentage also increased significantly.

Susceptibility and mortality in Agrotis segetum

The results achieved over the efficacy of *H. bacteriophora* strain S_{15} against 3rd and 4th instar larvae of *A. segetum* indicated that significant mortality was caused by EPNs in both the larval instars. It is evident from the (Fig. 4) that

Table 1. Log probit analysis to evaluate the larval mortality by *H. bacteriophora* strain S_{15} against lepidopteran insect pests

Larval instar	Host insects	Susceptibility (h)	LC_{50}	95% fiducial limit		Slope ± *SE*	Pearson's χ2	*p*-value
				Lower limit	Upper limit			
3rd instar	*H. armigera*	24	3236.70	1648.82	6353.77	1.07±0.48	0.25	0.080
		48	243.58	193.30	306.93	2.23±0.37	0.84	0.004
		72	77.06	64.00	92.77	2.34±0.26	0.52	0.001
		96	36.15	26.87	48.64	1.48±0.24	0.91	0.004
	S. litura	24	982.41	625.85	1,542.11	1.46±0.46	0.46	0.030
		48	208.81	172.20	253.19	2.73±0.42	0.17	0.003
		72	70.66	58.20	85.78	2.24±0.25	0.74	0.001
		96	30.05	22.61	39.95	1.60±0.25	0.25	0.003
	A. segetum	24	546.30	387.44	770.30	1.83±0.47	0.16	0.02
		48	166.65	135.80	204.50	2.29±0.32	0.64	0.002
		72	73.49	59.61	90.60	2.05±0.25	0.89	0.001
		96	30.97	24.47	39.19	2.04±0.26	0.75	0.001
	M. separata	24	388.57	292.00	517.06	2.09±0.46	0.23	0.010
		48	170.76	139.13	209.59	2.30±0.32	0.97	0.002
		72	63.19	50.73	78.73	1.96±0.24	0.94	0.001
		96	23.84	18.44	30.82	1.98±0.27	0.73	0.002
4th instar	*H. armigera*	24	1340.89	806.91	2228.24	1.34±0.47	0.44	0.004
		48	239.11	186.26	306.97	1.97±0,33	0.90	0.004
		72	71.57	59.07	86.71	2.26±0.25	0.88	0.001
		96	31.41	23.72	41.61	1.61±0.25	0.92	0.003
	S. litura	24	593.02	412.69	852.15	1.69±0.04	0.76	0.020
		48	178.58	147.71	215.89	2.59±0.36	0.08	0.002
		72	59.47	47.91	73.83	2.00±0.24	0.76	0.001
		96	28.64	22.05	37.20	1.81±0.26	0.40	0.002
	A. segetum	24	431.35	318.78	583.66	2.01±0.46	0.18	0.010
		48	154.64	127.23	187.96	2.39±0.31	0.87	0.002
		72	68.90	55.82	85.04	2.05±0.25	0.91	0.001
		96	26.92	21.02	34.47	1.99±0.27	0.48	0.002
	M. separata	24	390.55	289.51	526.84	1.87±0.39	0.84	0.010
		48	159.17	128.94	196.50	2.18±0.30	0.89	0.002
		72	56.34	45.34	70.01	2.00±0.24	0.73	0.001
		96	20.64	15.79	26.97	1.99±0.28	0.34	0.002

the larval mortality ranged from 56%, 72%, 80%, 88% and 94% against 3rd instar larvae while it is ranged from 56%, 74%, 82%, 90% and 96% in 4th instar larvae after 96 h of time exposure at 30, 60, 90, 120 and 150IJs of EPNs concentration. In control, zero mortality against 3rd and 4th instar larvae in *A. segetum* was recorded even after 96 h of time exposure. The LC_{50} value was also determined in table (Table 1) to check the significance. In 3rd instar of *A. segetum* the calculated LC_{50}=30.97IJs/larva with 95%FL: 24.47-39.19 whereas, in 4th instar larvae LC_{50}=26.92IJs/larva with 95%FL: 21.02-34.47. The treatments were found significant as p=<0.01 in all the treatments except 24 h exposure at dose 30IJs against 3rd instars.

Susceptibility and mortality in Mythimna separata

The efficacy of *H. bacteriophora* strain S_{15} was also evaluated against 3rd and 4th instar larvae of *M. separata* under laboratory conditions. The results revealed that EPNs causing high mortality in oriental armyworm larvae. The data obtained over the mortality against 3rd and 4th instar larvae was discussed (Fig. 5). The highest mortality 96% was observed in highest dose @150IJs after 96 h of infection in 3rd instar larvae. While 98% mortality was observed against 4th instars in the highest dose @150IJs after 96 h exposure. The calculated LC_{50} of 3rd instar larvae was 23.84IJs/larva (95%FL: 18.44-30.82) and 4th instar larvae was 20.64IJs/larva (95%FL: 15.79-26.97) (Table 1). The p value < 0.01 represents the significant mortality in larvae at all treatment doses.

Discussion

Bio-control is the method of repressing or managing the population of various insect pests using their natural adversary. Prolonged applications of chemical pesticides not only affect the environment but also show adverse impact on human health too. EPNs have been reported as conventional and intensified method in insect pest management.

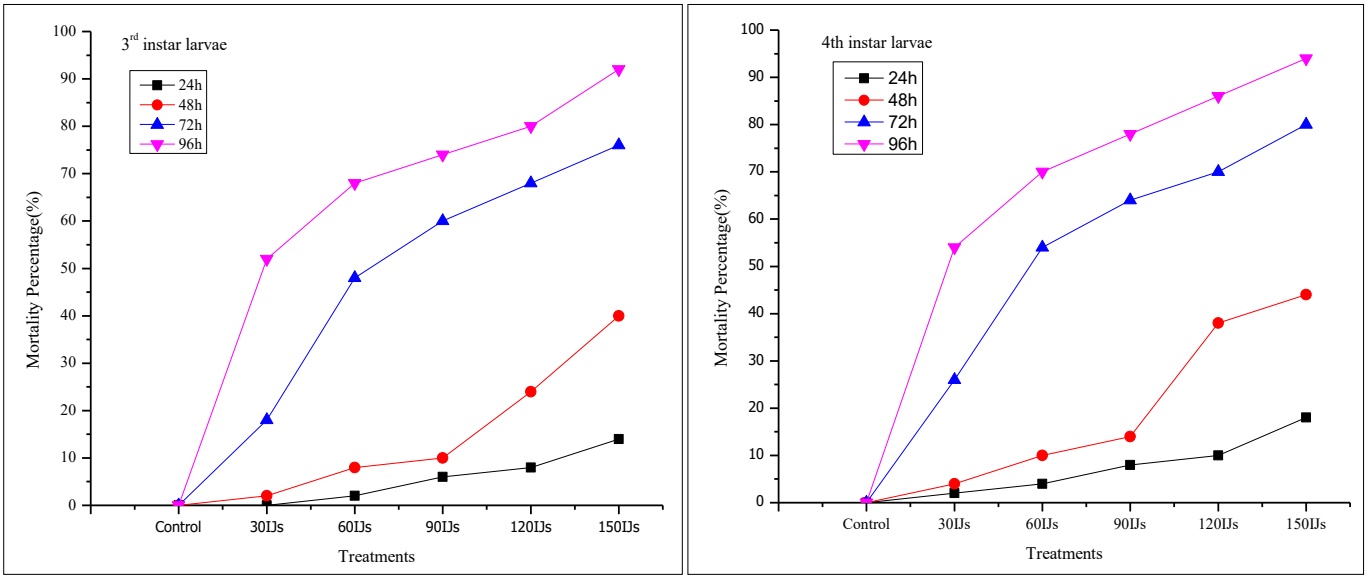

Fig. 3. Percent mortality caused by *Heterorhabditis bacteriophora* strain S_{15} in 3rd and 4th instar larvae of *Spodoptera litura.*

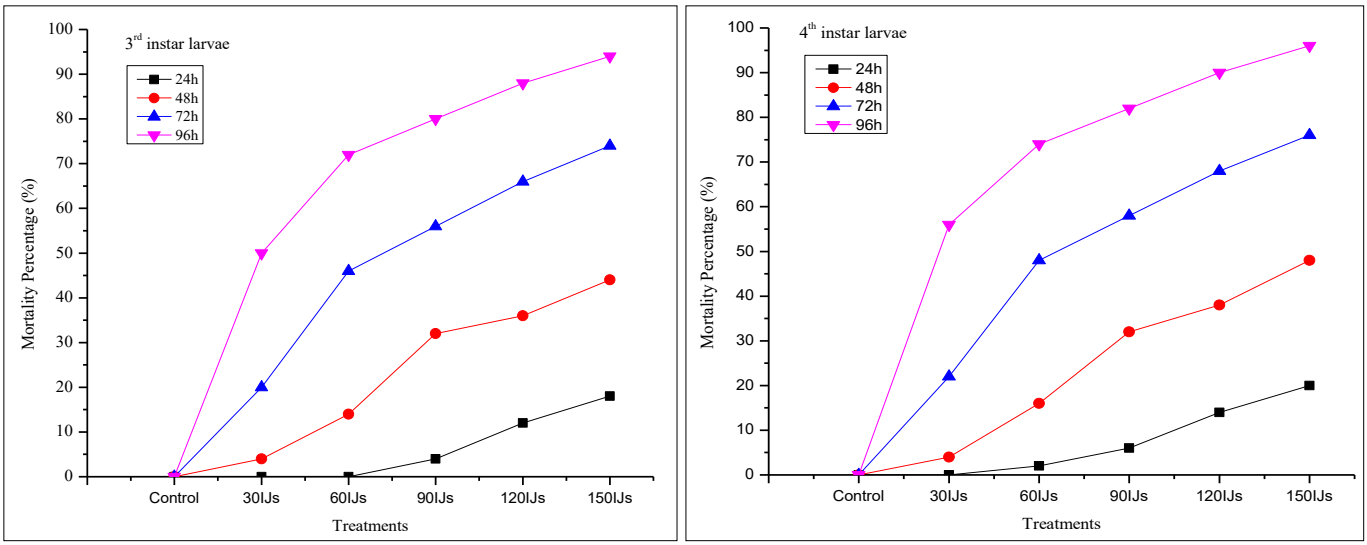

Fig. 4. Percent mortality caused by *Heterorhabditis bacteriophora* strain S_{15} in 3rd and 4th instar larvae of *Agrotis segetum.*

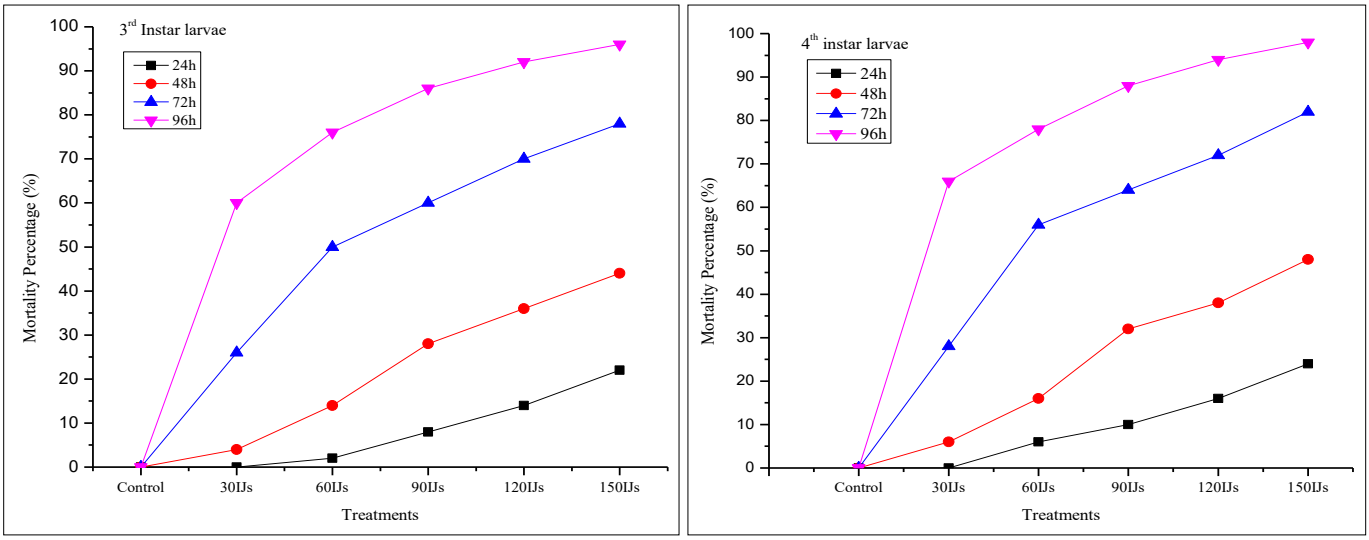

Fig. 5. Percent mortality caused by *Heterorhabditis bacteriophora* strain S_{15} in 3rd and 4th instar larvae of *Mythimna separata.*

The results obtained from this study provide insights on the insect pest management strategy using indigenous EPNs. The *H. bacteriophora* strain S_{15} were found highly virulent against all four insect pests viz. *H. armigera, S. litura, A. segetum* and *M. separata* under the laboratory conditions after 96 h of time exposure. Earlier it was reported that both genera of EPNs (*Steinernema* and *Heterorhabditis*) were highly lethal against the lepidopteran insect pests (32, 33).

In this study, 84% mortality was observed in 3rd instar larvae and 88% mortality was observed against 4th

instar larvae of after 96 h of infection with *H. bacteriophora* strain S_{15}. Earlier, similar study on EPNs susceptibility by *H. armigera* was carried out against three EPNs species viz. *Steinernema carpocapsae, Steinernema feltiae* and *H. bacteriophora.* They observed that among all 3 EPNs species *H. bacteriophora* is highly pathogenic and caused 83% mortality in *H. armigera*. They also reported 71.63% mortality by *S. feltiae* and 30% mortality by *S. carpocapsae* (34). Similar observations were also recorded by the native strain of EPNs (*H. bacteriophora*) that showed 73.3% mortality after 96 h of exposure time against *H. armigera* (35). Our results are in conformity with the previous work (36) in which they applied *Heterorhabditis amazonensis* MC01 against *H. armigera* pupae under laboratory and field conditions that resulted in 80% mortality in laboratory as well as in the field. In the present investigation, the calculated LC_{50} of 3rd instar larvae was 36.15IJs/larva (95%FL: 26.87-48.64) and 4th instar larvae was 31.41IJs/larva (95%FL: 23.72-41.61). Similar experiment was conducted by Glazer and Navon and reported LD_{50} 49IJs/larvae (37).

The *H. bacteriophora* strain S_{15} susceptibility by *S. litura* was evaluated and it is evident from the results that in 3rd instar larvae 92% larval mortality was caused, whereas 94% mortality was observed in 4th instars. Similar observations were recorded in the earlier investigation in which EPNs were responsible for causing 90-100% mortality in 2nd, 3rd and 4th instar larvae of *S. litura* (38). Previously, EPNs susceptibility was also tested against different larval instars of *S. frugiperda* and it was reported that all the instars were highly susceptible for EPNs infection (39, 40). Earlier,0-100% mortality was recorded in *S. litura* even after 24 h of EPNs exposure (41).

In this study, the LC_{50} values of 3rd instar larvae of *S. litura* were 30.05IJs/larva (95%FL: 22.61-39.95) and 4thinstarwas 28.64IJs/larva (95%FL: 22.05-37.20) after 96 h of EPNs exposure. The present investigation is also in agreement with the earlier studies carried out to find out the median lethal concentrations (LC_{50}). LC_{50}=6.81/larva and 8.45IJ/larva was recorded when EPNs (*Heterorhabditis indica* and *Steinernema glaseri*) were applied against *S. litura* under laboratory conditions (42). Similar observations were recorded in the previous work in which LC_{50}=7.13 were recorded to kill 50% population of *S. litura* (41).

In this investigation, efficacy of *H. bacteriophora* strain S_{15} against 3rd and 4th instar larvae of *A. segetum* was evaluated that resulted in causing 94% and 96% mortality in both the instars. Earlier, similar observations were made in which about 100% pathogenicity was observed in 3rd instar larvae of *A. segetum* when treated with *H. bacteriophora* (43). Pathogenicity of *S. carpocapsae* and *H. indica* was also evaluated against the different larval instars of *A. segetum.* The results are in close proximity with our findings, it was reported that about 73-100% pathogenicity was caused by *H. indica* while 60-100% mortality was caused by *S. carpocapsae* (44). EPNs were considered to have very high insecticidal potential and were considerably manage the population of cutworm (45).

In the current investigation, the calculated LC_{50} of 3rd instar larvae of *A. segetum* was 30.97IJs/larva with 95% FL: 24.47-39.19 whereas, in 4th instar larvae LC_{50}=26.92IJs/larva with 95%FL: 21.02-34.47. In *Agrotis ipsilon*, LC_{50} of 6.81 IJs/larva was reported, which was quite lower than our observations (46). The nematodes *S. carpocapsae* were applied against last instar larvae of *A. segetum* and the calculated LC_{10}= 9.9IJs, LC_{50}= 54.13IJs and LC_{90}= 246.2IJs per larva were recorded (47) which is in close proximity with our findings.

The insecticidal potential of *H. bacteriophora* strain S_{15} was also evaluated against *M. separata* under laboratory conditions. It is evident from the results that highest mortality 96% and 98% was observed in highest dose @150IJs after 96 h of infection in 3rdand 4thinstar larvae. In our findings, the calculated LC_{50} of 3rd instar larvae was 23.84IJs/larva (95%FL: 18.44-30.82) and 4th instar larvae was 20.64IJs/larva (95%FL: 15.79-26.97). The insecticidal potential of *H. indica, S. carpocapsae, Steinernema abbasi* and *Steinernema siamkayai* was also evaluated against *M. separata* under laboratory conditions and 100% larval mortality was recorded (31). They further reported significant differences in LC_{50} and LC_{90} value after exposure of *M. separata* towards different species of EPNs.

Conclusion

The main emphasis of this research was focused on developing and using entomopathogenic nematodes as a bio-insecticide. The success in controlling insect pests in the soil environments increased the production and use of the adapted species (indigenous) for insect management in the fields. EPNs (Rhabditida: Heterorhabditidae) in this study have arisen as admirable biological control agents against soil-inhabiting lepidopteran insect pests. This bio-control agent is capable for high virulence, easiness for application, safe for non-target animals and also exempted from registration in various countries. Actually, there is an urgent need in this research to attain more basic information on examination of these new reported nematode isolates. These nematodes showed ubiquitous nature and occur in low population in their natural habitat. More research is required for understanding the various factors that can regulate and manipulate their population to pledge epizootic against insect pests. Overall statistically significant differences in the mortality were observed in all treatments during this study. EPNs are the best weapon to overcome insect resistance problems and must be employed to manage insect population. Further future studies required more nematode based formulations for the management of wide range of insect pest to check their potential in field conditions.

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Authors contributions

PT conducted the experiment and wrote the manuscript. NT and ANY gave concept and drafted the manuscript. All the authors have read and reviewed the manuscript.

Compliance with ethical standards

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References

1. Kaneda T, Greenbaum C, Kline K. World population data sheet shows older populations growing, total fertility rates declining. Population Reference Bureau. 2020. Available from: https://www.prnewswire.com/news-releases/prbs-2021-world-population-data-sheet-released-301357333.html
2. Thakur N, Tomar P, Kaur S, Jhamta S, Thakur R, Yadav AN. Entomopathogenic soil microbes for sustainable crop protection, In: Yadav AN.; editor. Soil Microbiomes for Sustainable Agriculture Springer. 2021;529-71. https://doi.org/10.1007/978-981-15-6949-4_1
3. Bhat AH, Chaubey AK, Askary TH. Global distribution of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*. Egypt J Biol Pest Co. 2020;30:1-15. https://doi.org/10.1186/s41938-020-0212-y
4. Kaya HK, Gaugler R. Entomopathogenic nematodes. Ann Rev Ent. 1993; 38(1):181-206. https://doi.org/10.1146/annurev.en.38.010193.001145
5. Hominick WM. Biogeography. In: Gaugler R., editor Entomopathogenic Nematology. CABI Publications, UK. 2002;1:115-43.
6. Campos-Herrera R. Nematode pathogenesis of insects and other pests: ecology and applied technologies for sustainable plant and crop protection In: Campos-Herrera R., editor Sustainability in plant and crop protection. Springer. 2015; p. 1-531. https://doi.org/10.1007/978-3-319-18266-7
7. Georgis R, Koppenhöfer AM, Lacey LA, Bélair G, Duncan LW, Grewal PS et al. Successes and failures in the use of parasitic nematodes for pest control. Biol Control. 2006;38(1):103-23. https://doi.org/10.1016/j.biocontrol.2005.11.005
8. Grewal PS, Selvan S, Gaugler R. Nematodes: niche breadth for infection, establishment and reproduction. J Therm Biol. 1994;19:245-53.
9. Gulcu BA, Cimen HA, Raja RK, Hazir SE. Entomopathogenic nematodes and their mutualistic bacteria: their ecology and application as microbial control agents. Biopestic. 2017;13(2):79-112.
10. Kaya HK, Nelsen C. Encapsulation of steinernematid and heterorhabditid nematodes with calcium alginate: a new approach for insect control and other applications. Environ Entomol. 1985;14(5):572-74. https://doi.org/10.1093/ee/14.5.572
11. Thakur N, Kaur S, Tomar P, Thakur S, Yadav AN. Microbial biopesticides: current status and advancement for sustainable agriculture and environment, In: Rastegari AA, Yadav AN, Yadav N, editors. Trends of Microbial Biotechnology for Sustainable Agriculture and Biomedicine Systems: Diversity and Functional Perspectives. Elsevier, Amsterdam. p 243-82. https://doi.org/10.1016/B978-0-12-820526-6.00016-6
12. Dillman AR, Guillermin ML, Lee JH, Kim B, Sternberg PW, Hallem EA. Olfaction shapes host–parasite interactions in parasitic nematodes. In: Proceedings of the National Academy of Sciences. 2012;109(35):E2324-E2333. https://doi.org/10.1073/pnas.1211436109
13. Hallem EA, Dillman AR, Hong AV, Zhang Y, Yano JM, DeMarco SF, Sternberg PW. A sensory code for host seeking in parasitic nematodes. Current Biol. 2011;21(5):377-83. j.cub.2011.01.048
14. Grewal P, Gaugler R, Selvan S. Host recognition by entomopathogenic nematodes: behavioral response to contact with host feces. J Chem Ecol. 1993;19(6):1219-31. https://doi.org/10.1007/BF00987382
15. Burnell A, Stock SP. *Heterorhabditis, Steinernema* and their bacterial symbionts—lethal pathogens of insects. Nematol. 2000;2(1):31-42. https://doi.org/10.1163/156854100508872
16. Forst S, Dowds B, Boemare N, Stackebrandt E. *Xenorhabdus* and *Photorhabdus* spp.: bugs that kill bugs. Annu Rev Microbiol. 1997;51(1):47-72. https://www.annualreviews.org/doi/10.1146/annurev.micro.51.1.47
17. Fitt GP. The ecology of *Heliothis* species in relation to agroecosystems. Annu Rev Entomol. 1989;34(1):17-53.
18. 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(2):215-17.
19. Rao GR, Wightman J, Rao DR. World review of the natural enemies and diseases of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Int J Tropic Insect Sci. 1993;14(3):273-84. https://doi.org/10.1017/S1742758400014764
20. Ram G, Misra S, Dhamayanthi K. Relative susceptibility of advanced hybrids and promising cultivars of potato, *Solanum tuberosum* L. to greasy cutworm, *Agrotis ipsilon* (Hufn.) in Northeastern plains. J Entomol Res. 2001;25(3):183-87.
21. Mrowczynski M, Wachowiak H, M B. Cutworms -a dangerous pest in the autumn of 2003. Ochrana Roslin. 2003;47:24-26.
22. Napiorkowska K, Gawowska J. Increase of harmfulness of caterpillars (Hadeninae and Noctuinae, Lepidoptera: Noctuidae) on cabbage and other cole crops. Progress in Plant Prot. 2004;44:978-80.
23. Arab Republic of Egypt. Ministry of Agriculture and Land Reclamation. Technical Recommendations for Agricultural Pest Control. p. 1-285. http://www.apc.gov.eg/Files/Releases/Recom.20-English.pdf
24. Hill M, Atkins A. Incidence of the armyworm, *Mythimna separata* Walker (Noctuidae: Lepidoptera), and its introduced parasite, *Apanteles ruficrus* Halliday (Braconidae: Hymenoptera), in maize. New Zeal J Agr Res. 1983;26(1):135-38. https://doi.org/10.1080/00288233.1983.10420963
25. White G. A method for obtaining infective nematode larvae from cultures Science (Washington). 1927;66(1709):302-03. https://doi.org/10.1126/science.66.1709.302-b
26. Orozco RA, Lee M-M, Stock SP. Soil sampling and isolation of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). J Visual Exp. 2014;(89):e52083. https://dx.doi.org/10.3791/52083
27. Kumar L, Bisht RS, Singh H, Kumar M. Studies on growth and development of *Helicoverpa armigera* (Hub.) on various hosts and artificial diet under laboratory conditions. Int J Curr Microbiol Appl Sci. 2017;6(12):1627-37. https://doi.org/10.20546/ijcmas.2017.612.183
28. Seth R, Sharma V. Growth, development, reproductive competence and adult behaviour of *Spodoptera litura* (Lepidoptera: Noctuidae) reared on different diets. 2002;15-22. https://www.osti.gov/etdeweb/biblio/20246472

29. Verma K. Bioecology and management of turnip moth (Lepidoptera, Noctidae) attacking vegetable crops. Doctoral dissertation, Ph. D. Thesis, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan, 1996 p. 121.

30. Manjula K, Kotikal Y. Biology of turnip moth, *Agrotis segetum* (Denis and Schiffermüller) on palak, *Beta vulgaris* var. *bengalensis.* Hort J Entomol Zool Stud. 2018;6(6):1183-86.

31. Patil J, Vijayakumar R, Linga V, Sivakumar G. Susceptibility of Oriental armyworm, *Mythimna separata* (Lepidoptera: Noctuidae) larvae and pupae to native entomopathogenic nematodes. J Appl Entomol. 2020;144(7):647-54. https://doi.org/10.1111/jen.12786

32. Hassani-Kakhki M, Karimi J and Hosseini M. Efficacy of entomopathogenic nematodes against potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) under laboratory conditions. Biocontrol Sci Technol. 2013;23(2):146-59. https://doi.org/10.1080/09583157.2012.745481

33. Vashisth S. Distribution and biocontrol potential of entomopathogenic nematodes against some lepidopterous pests. Doctoral dissertation, Ph. D. Thesis, CSKPV Krishi Vishavavidyalaya, Palampur. Himachal Pradesh. 2014;1-182. http://hdl.handle.net/10603/203884

34. Kary NE, Golizadeh A, Dastjerdi HR, Mohammadi D, Afghahi S, M, et al. A laboratory study of susceptibility of *Helicoverpa armigera* (Hübner) to three species of entomopathogenic nematodes. Munis Entomol Zool. 2012;7(1):72-379.

35. Vashisth S, Chandel Y, R Chandel. 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(1):16-22. https://doi.org/10.1080/09670874.2018.1453099

36. Andaló V, Faria LS, Carvalho FJ, Assis GA, Santos V, Mendes SM, Gonring AH Entomopathogenic nematodes for the control of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) pupae. Arq Inst Biol. 2021;88:1-8. https://doi.org/10.1590/1808-1657000742019

37. Glazer I, Navon A. Activity and persistence of entomoparasitic nematodes tested against *Heliothis armigera* (Lepidoptera: Noctuidae). J Econ Entomol. 1990;83(5):1795-1800. https://doi.org/10.1093/jee/83.5.1795

38. Yan X, Shahid Arain M, Lin Y, Gu X, Zhang L, Li J, Han R. Efficacy of entomopathogenic nematodes against the tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). J Econ Entomol. 2020;113(1):64-72. https://doi.org/10.1093/jee/toz262

39. 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(12):868. https://doi.org/10.3390/insects11120868

40. Acharya R, Yu YS, Shim JK, Lee KY. Virulence of four entomopathogenic nematodes against the tobacco cutworm, *Spodoptera litura* Fabricius. Biol Control. 2020;150:104348. https://doi.org/10.1016/j.biocontrol.2020.104348

41. Dichusa CA, Ramos R, Aryal S, Sumaya NP, Sumaya NH. Survey and identification of entomopathogenic nematodes in the province of Cotabato, Philippines, for biocontrol potential against the tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Egypt J Biol Pest Co. 2021;31(1):1-10. https://doi.org/10.1186/s41938-021-00390-w

42. Radhakrishnan S, Shanmugam S. Bioefficacy of entomopathogenic nematodes against *Spodoptera litura* (Lepidoptera: Noctuidae) in Bhendi. Int J Curr Microbiol Appl Sci. 2017;6:2314-19. https://doi.org/10.20546/ijcmas.2017.607.273

43. Chandel Y, Kapoor S, Kumar S. Virulence of *Heterorhabditis bacteriophora* (Poinar) against cutworm, *Agrotis segetum* (Denis & Schiff.). J Biol Cont. 2010;23(4):409-15. https://doi.org/10.18311/jbc/2009/3696

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

45. Chandel RS, Verma KS, Rana A, Sanjta S, Badiyala A, Vashisth The ecology and management of cutworms in India. Oriental Insects. 2021;1-26. https://doi.org/10.1080/00305316.2021.1936256

46. Radhakrishnan S, Shanmugam S, Ramasamy R. Bio control efficacy of Entomopathogenic nematodes against Black Cutworms, *Agrotis ipsilon* (Hufnagel) (Noctuidae: Lepidoptera) in potato. Chem Sci Rev Lett. 2017;6(21):219-24. https://www.researchgate.net/publication/318458813

47. Ebrahimi L, TanhaMaafi Z, Sharifi P. First report of the entomopathogenic nematode, *Steinernema carpocapsae* from Moghan region of Iran and its efficacy against the turnip moth, *Agrotis segetum* Denis & Schiffermuller (Lepidoptera: Noctuidae), larvae. Egypt J Biol Pest Co. 2019;29(1):1-6. https://doi.org/10.1186/s41938-019-0168-y

48. FAO. 2018 http//www.fao.org/statisticaldatabases/agriculture.

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