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RESEARCH

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Eco-friendly management of *Spodoptera litura* (Lepidoptera: Noctuidae) in tomato under polyhouse and field conditions using *Heterorhabditis bacteriophora* Poinar, their associated bacteria (*Photorhabdus luminescens*), and *Bacillus thuringiensis* var. *kurstaki*

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Abstract

Background Insect pests cause substantial agriculture losses annually, and their regulation through chemical pesticides instigates a prolonged detrimental impact on the environment and human health. The upsurge in difficulties such as pesticide residue, soil degradation and pest resistance acted as the pacesetter for research on biological control with a prime focus on entomopathogens. To aid in knowing the biocontrol potential of these creatures, the present work deals with the applications of entomopathogenic nematode *Heterorhabditis bacteriophora* EUPT-SD, and entomopathogenic bacteria (EPBs) *Photorhabdus luminescens* and *Bacillus thuringiensis* var. *kurstaki* against the 4th larval instar of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in the laboratory experiment, polyhouse as well as field condition.

Results The results demonstrated that EPB, *P. luminescens*, caused the highest 100% larval mortality at the highest concentration of 5×10^2 CFU/ml after 96 h of infection, followed by *B. thuringiensis* var. *kurstaki* which resulted in 98% at a concentration of 5×10^2 CFU/ml and 92% mortality at the treatment with *H. bacteriophora* EUPT-SD (140IJs/ml) under laboratory bioassay study. In the polyhouse and field evaluation tests, again the *P. luminescens* was recorded as the most effective, followed by *B. thuringiensis* and *H. bacteriophora*, respectively. It was found that the treated plots experienced lesser damage when compared to non-treated plots.

Conclusion Applications of these pathogens are nature friendly and are a proficient alternative to synthetic chemical insecticides. It is suggested from the present investigation that the use of *P. luminescens* was the best biocontrol agent to overcome the local pest problems of this region because it is safe for animals, humans, non-target insect pests, plants, as well as for the environment.

Keywords *Spodoptera litura*, Bioassay, Entomopathogens, Management, Tomato

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Background

Occurrences of abiotic and biotic stresses are the key factors in the crops' losses (Pandey et al. 2017). The main obstacles to crop productivity are the losses due to insect pests. The prominent management strategies against these pests are the dependence on chemicals spray (Brodeur et al. 2017). Enormous applications of pesticides for minimizing losses caused by these organisms showed the prejudicial effects on the beneficial living organisms. It also added pesticide residues in the food and promotes environmental pollution.

Environment sustainability and better human health are the crucial components that can be achieved through biological control methods to replace harmful chemical pesticides. Biopesticides have a wide range of benefits with less side effect (Thakur et al. 2020). In recent agriculture practices, sustainability is the prime aim which is achieved by utilizing organisms present in the environment in such a way that they can help in improving crop health, increasing yields and reducing pollution (Thakur et al. 2022). Researchers nowadays are interested in the usage of entomopathogenic nematodes (EPNs) as a biocontrol agent due to their wide host range, eco-friendly nature, active host finding strategy, easy mass culturing and compatibility with standard irrigation equipment along with the ability to recycle them in the soil environment (Thakur et al. 2021; Tomar et al. 2022a). The nematodes live in a symbiotic association with the bacteria *Xenorhabdus* and *Photorhabdus* belonging to the family Enterobacteriaceae, which are gram-negative with non-fermentative rodlike appearance (Koppenhöfer 2007). These bacteria are virulent against Lepidoptera, Diptera, Coleoptera and Hemiptera orders of class Insecta (Vitta et al. 2018) as they produce one to many groups of bioactive secondary metabolites. *Bacillus thuringiensis* (*Bt*) along with several microbial species proves to be successful industrial and academic efforts. It is the spore-forming bacteria that is omnipresent and forms crystal proteins. These crystal proteins are also known as δ-endotoxin and are toxic to insects.

Spodoptera litura (Fabricius, 1775) (Lepidoptera: Noctuidae), a lesser armyworm, beet armyworm, tobacco caterpillar and cutworm, is a serious pest in India, Pakistan, China, Japan (Ghaffar et al. 2002) and South Asia (Qin et al. 2004). These polyphagous insect pests are very destructive and cause damage to various host crops such as cotton, potato, capsicum, clover, tomato, okra, soybean and onion (Saleem et al. 2016). In the field conditions, the pest caused 26–100% losses in yield among approximately 150 species of plants (Rao et al. 1993). The young larvae of the pest enter deep into the fruits and contaminate the fruits, which affects the yield and causes losses economically (Czepak et al. 2013). This tobacco caterpillar has developed resistance against different synthetic pesticides. The present study was planned to evaluate the potential of EPNs and their associated bacteria, against *S. litura* larvae under the laboratory, polyhouse and field conditions.

Methods

Bioassay experiment

EPNs were isolated from rhizospheric soils of field crops in Solan district of Himachal Pradesh (H.P.), India, through the soil baiting technique (Orozco et al. 2014). The extracted nematodes were identified as *Heterorhabditis bacteriophora* Poinar, 1976 morphologically and molecularly and denoted as *H. bacteriophora* EUPT-SD. Nematodes collected by the white traps were lifted in the disinfected double distilled water at a temperature of 15 ± 1 °C for 6–14 days before usage (Shapiro-Ilan et al. 2002). To attain the viable pathogenic infective juveniles, these identified nematodes were then replicated by in vivo culturing using the last instar of *Corcyra cephalonica* Stainton, 1865 (Malan et al. 2014). The collected nematode suspension was poured over the container that had small pieces of the sterilized synthetic sponges at a temperature of 10–15 °C (Ramakuwela et al. 2015).

Nutrient agar–bromothymol blue–tetrazolium chloride (NBT) microbiological media was used for the isolation of EPNs-associated bacteria (*P. luminescens* EUPT-SD). Bromothymol blue (25 mg/ml) and triphenyl tetrazolium chloride (TTC) 40 mg/ml were added at the rate of 100 µl to 100 ml media. Nutrient broth, bromothymol blue (BTB) and agar were mixed in distilled water to make a volume of 1000 ml. Initially, the pH of the medium was adjusted to 7.0–7.2 and the medium in 50–100 ml aliquots was then steam-autoclaved at the 15 psi of steam for about 20 min. The stock of TTC was filtered, sterilized and added @100 µl in 100 ml to the media. Ampicillin 100 µg/ml was added to the media at the level of 100 µl of ampicillin stock solution, i.e., 100 mg ampicillin sodium salt/ml with sterilized Milli-Q water. Cholesterol 10 µg/ml was added to the medium for the proper growth and reciprocation of the EPNs. Hundred infective juveniles of nematodes were picked with the help of a sharp needle. The collected juveniles were centrifuged (5000 rpm for 5 min) for the pellet formation. This pellet was then dissolved in 1% sodium hypochlorite solution for surface sterilization for about 5 min. After that 1 ml distilled water was applied for washing and centrifuged for 5 min at 5000 rpm. Autoclaved sterile mortar and pestle were used, and crushed IJs were used to make a loop. Streaking was done on the NBT agar media in the petri plates with sterile needles. Petri plates were incubated (28 ± 1 °C) for 48 h. Single bacterial colonies obtained were purified by re-streaking on the same medium and then incubation

again (28 ± 1 °C) for 48 h (Akhurst 1980). The cultures of bacteria isolated from the individual colonies were streak purified on NBT agar medium and incubated (28 ± 1 °C). The plates with the bacterial growth were maintained at 4 °C after 48 h and these plates were mentioned as the master plates. The fully grown bacterial colonies were preserved in nutrient broth (NB) as well as in 25% glycerol stock for future use. Commercially available formulations of *B. thuringiensis* var. *kurstaki* Mahastra (Strain: DOR BT-1, 0.5% W.P.) were bought from the marketplace and used for the experiment.

Solanum lycopersicum Linnaeus, 1753 (Heemsohna) seeds were procured from the market and grown as a nursery in the Departmental biological control polyhouse with temperature 19–24 °C (avg.), 70–75% humidity and 11 h light/13 h dark light regime. Post-germinated tomato seedlings (28–30 days) were shifted into the plastic pot (20 × 20 × 14 cm) filled with rice husk and soil. Irrigation was applied twice daily and the seedlings were nourished with the Agrobium's® (NPK-8:25:25, 3–4 g/plant) at 15 days intervals. This was done to establish the insect breeding stalk as more numbers of insects were required for the bioassay experiments.

Adult and the larvae of *S. litura* were gathered from the University Agricultural Farms of Eternal University. As a feed, castor leaves were used and kept at 28 ± 1 °C temperature for the development (Patil et al. 2014). The adults were shifted to the chimney for egg laying that already contained sucrose syrup for the feeding (Santharam 1985). The egg masses were found in clusters usually after 3–5 days of mating. The breeding stock of *S. litura* was retained by relocating the larvae of *S. litura* on the tomato seedlings under the plastic pots with 05larvae/plant. The pot was placed in entomological cages (40 cm^3) for confinement. The *S. litura* life cycle lasted approximately 33–39 days under polyhouse conditions.

Bioassay experiment

Heterorhabditis bacteriophora EUPT-SD, P. luminescence and B. thuringiensis var. kurstaki applications

Bioefficacy of different treatments of EPNs (*H. bacteriophora* EUPT-SD), *P. luminescens* and *B. thuringiensis* var. *kurstaki* were evaluated against *S. litura* (4th instar), alone and also in combination (Table 1) in the laboratory. Bioefficacy tests were performed using 3 treatments at the 5 different concentrations along with the absolute control in the Petri plate. The nematode concentrations were 20, 40, 60, 80 and 100 IJs/ml. Laboratory-reared active 4th instar larvae were used to check the bioefficacy in the laboratory. These larvae were kept under Petri dishes (10 larvae/plate) lined with Whatman filter paper and nematode suspension 1–2 ml was sprinkled into the plates that contained 05 concentrations, namely 20, 40, 60, 80 and 100 IJs/ml. Diet (natural/artificial) was provided to the larvae for feeding, and after that the Petri dish was incubated at 25 ± 1 °C. Mortality among the target insect pest was examined after 24, 48, 72 and 96 h of inoculation. At the same time, colonies of *P. luminescens* and *B. thuringiensis* were grown on nutrient agar (NA) media and nutrient broth (NB) was prepared. From the prepared broth, *P. luminescens* concentration was adjusted to 1×10^2, 2×10^2, 3×10^2, 4×10^2 and 5×10^2 CFU/ml and used against 4th instar larvae. The *B. thuringiensis* var. *kurstaki* concentration was also adjusted to 1×10^2, 2×10^2, 3×10^2, 4×10^2 and

Table 1 Biocontrol agent used against *Spodoptera litura* larvae (4th instar) in bioassay experiment under laboratory, under polyhouse and field conditions

Heterorhabditis bacteriophora **(IJs/ml)**	***Photorhabdus luminescence*** **(CFU/ml)**	***Bacillus thuringiensis*** **var. *kurstaki* (CFU/ml)**
Different concentrations used against 4th instar larvae of Spodoptera litura in bioassay experiment under laboratory conditions		
20	1×10^2	1×10^2
40	2×10^2	2×10^2
60	3×10^2	3×10^2
80	4×10^2	4×10^2
100	5×10^2	5×10^2
Absolute control	Absolute control	Absolute control
Different concentrations used against S. litura larvae (4th instar) in polyhouse and field conditions		
80	2×10^2	2×10^2
100	3×10^2	3×10^2
120	4×10^2	4×10^2
140	5×10^2	5×10^2
Absolute control	Absolute control	Absolute control

5×10^2 CFU/ml and was used against the 10 larvae of target insect. In control, only distilled water (1–2 ml) was used. The diet was provided to feed them and the Petri dish was again incubated at 25 ± 1°C. Insect mortality was tested after 24, 48, 72 and 96 h of inoculation. Every treatment was replicated 05 times and insect larval mortality was measured after every 24 h of application. The experiment was performed twice, and data were pooled for statistical analysis.

Polyhouse phase

Heterorhabditis bacteriophora EUPT-SD, P. luminescens and B. thuringiensis var. kurstaki applications

Spodoptera litura vulnerability against nematodes *H. bacteriophora* EUPT-SD was examined by accomplishing an experiment on the tomato at its vegetative stages. This experiment was performed in a departmental polyhouse (44×36 m) with an area of 22×11 m that was divided into two different blocks that contained 26 plots of 2 m^2. The pre-grown tomato seedlings (20 per plot) were transplanted into these plots with an average distance of 20 cm. The cultured larvae of *S. litura* (4th instar) were transferred to each plant at the rate of 5 larvae per plant and 280 adults were also exposed to each block 24 h prior to the applications of a biocontrol agent. After 24 h of insect inoculation, the different suspensions of infective juveniles (combined with 0.3% polysorbate 20) were applied using a sprayer on abaxial and adaxial surfaces. Each treatment was replicated five times and the percentage data on the leaf damage and larval mortality was examined every 24 h for 15 days. The level of damage was calculated by adopting a graphical method, earlier described by Tomar et al. (2022d). The trial was executed under a randomized block design and the statistical analysis was performed by calculating the analysis of variance (ANOVA).

Field phase

Heterorhabditis bacteriophora EUPT-SD, P. luminescens EUPT-SD and B. thuringiensis var. kurstaki applications

A field experiment was conducted in the agricultural field, located at (30.76° N and 77.29° E at 1027 m altitude), with 14 to 25 °C mean temperature and 50 to 64% RH. In transplanting, 100 m^2 areas were prefabricated first and then the soil was allowed to recuperate before plowing. Agrobium's NPK compost was applied to this selected area. The selected region was divided into two blocks, each with 50 m^2 area. These two blocks were further distributed into 50 plots of 4 m^2 area. Each of these plots was transplanted with tomato seedlings 20 seedlings per plot with a plant-to-plant distance of 20 cm. After 28 days of uprooting, the pupae (800) and adult (280) were inoculated in the field, each block containing 400 pupae and 140 adults. At the phonological growth stage of the plant, 3 applications of EPNs and EPB after mixing with 0.3% polysorbate 20, were used in accordance with the variation in population density of *S. litura.* The 1st foliar application of these pathogens was performed after 1–2 weeks of pest inoculation. The second application was performed at the vegetative stage, 1–2 weeks after the 1st application. The 3rd spray was applied at the reproductive stage (1–2 weeks after the 2nd application). These applications of these pathogens were performed in the evening time during the complete cropping period until harvesting as per the fluctuations in the *S. litura* population. The experiment was executed under a randomized block design, and the statistical variables such as damage and mortality were assessed by the method prescribed by Tomar et al. (2022a).

Statistical analysis

The analysis was done by calculating the analysis of variance (ANOVA). The experiment was performed twice and the statistical analysis was performed on OPSTAT software. The treatment of bioagents included 5 applications of *H. bacteriophora* EUPT-SD, *P. luminescence* and *B. thuringiensis* var. *kurstaki* 5 along with control. The statistical relationship among all the treatments was also calculated using Pearson's correlation analysis.

Results

Bioassay experiment

A laboratory bioassay experiment was performed in order to evaluate the insecticidal potential of *H. bacteriophora* EUPT-SD, *P. luminescens* and *B. thuringiensis* var. *kurstaki* against *S. litura* larvae (4th instar). Among the three treatments, the most effective treatments were the applications of *P. luminescens* that caused the highest mortality (100%) in the highest concentration of 5×10^2 CFU/ml among *S. litura* larvae after 96 h of infection, although the highest concentrations of *B. thuringiensis* var. *kurstaki* (5×10^2 CFU/ml) and *H. bacteriophora* EUPT-SD (140IJs/ml) resulted in 98 and 92% larval mortality after 96 h of exposure (Fig. 1). Insect mortality represented considerable variations among the different concentrations of *P. luminescens* ($F = 34.16$, DF = 5, $P < 0.05$) with the mortality percentages of 42, 64, 88, 100 and 100% 96 h post-application. Among different concentrations of *B. thuringiensis* var. *kurstaki*, the mortality percentages were 34, 56, 76, 84 and 98% 96 h post-application with $F = 20.22$, DF = 5, $P < 0.05$. The different concentrations of *H. bacteriophora* EUPT-SD showed the mortality percentages 34, 44, 60, 76 and 92% after 96 h of nematode application with $F = 43.97$, DF = 5, $P < 0.05$. The data demonstrated that all the treatments were highly significant and caused considerable mortality among insects.

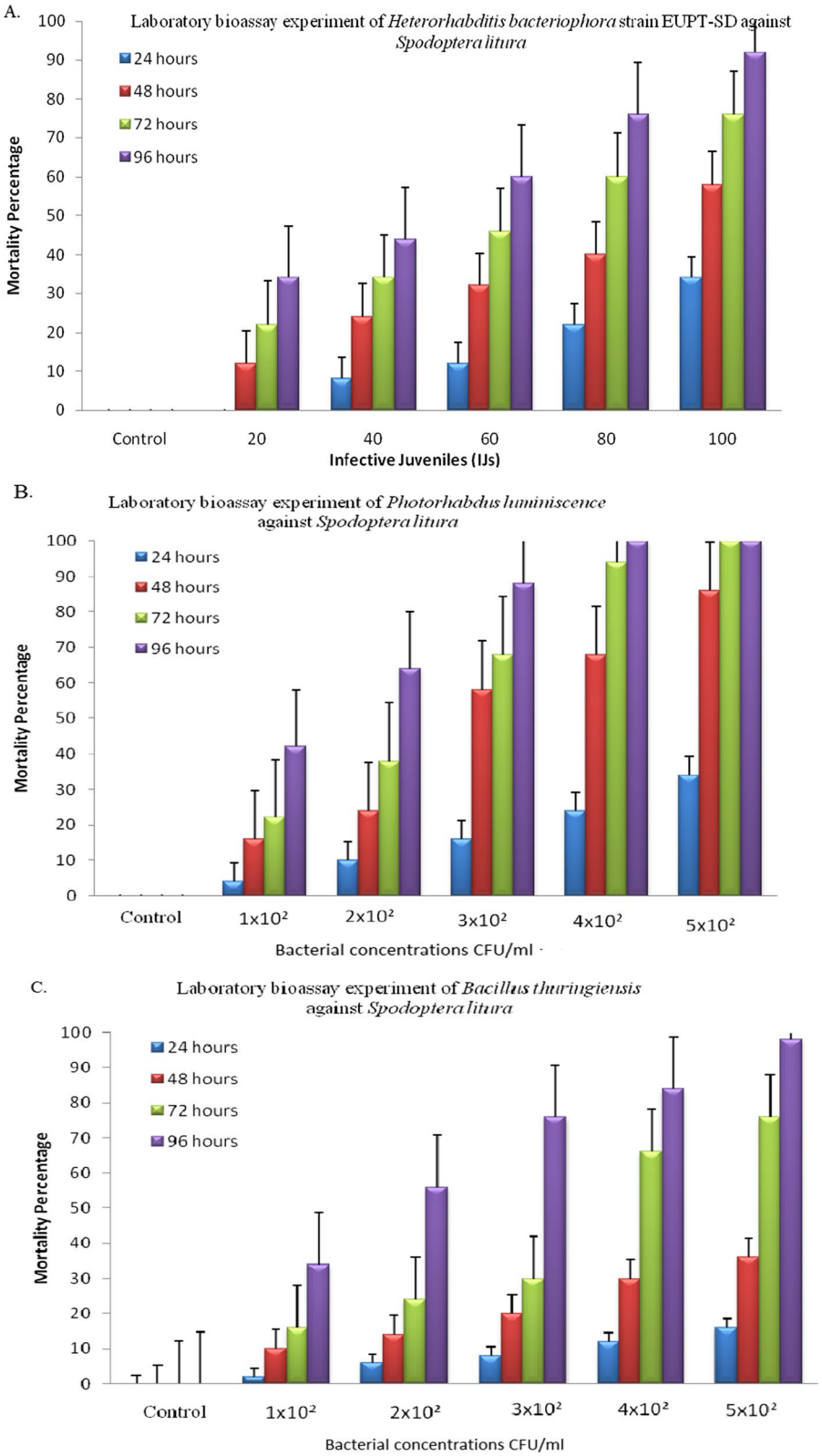


Fig. 1 Larval mortality by (**A**) *Heterorhabditis bacteriophora* strain EUPT-SD; (**B**) *Photorhabdus luminescens* and (**C**) *Bacillus thuringiensis* against *Spodoptera litura* larvae under the laboratory conditions

Polyhouse phase

Foliar applications of *H. bacteriophora* EUPT-SD in tomato plants resulted in lower damage percentage when compared to absolute control ($F=60.48$, DF = 4, $P<0.05$). The plot treated with the highest nematode concentration showed minimum damage (Fig. 2). The damage percentage was also increased to 32.4 and 37.2%, after spray 1st, which further declined after spray 2nd, and reached minimum after spray 3rd. The data collected over the larvae mortality demonstrated that considerable variation was observed among the IJs concentration with percent mortality 48, 78, 85 and 90% ($F=70.52$, DF = 4, $P<0.05$) after 5 days of nematodes application (after the third spray) (Fig. 2). Initially, it was observed that under the polyhouse conditions *H. bacteriophora* EUPT-SD concentration of 140IJs/ml provides high protection to the tomato plants.

Applications of *P. luminescens* resulted in reduced percent damage when compared to absolute control ($F=67.10$, DF = 4, $P<0.05$). The highest bacterial inoculum showed minimum damage (Fig. 2). Although in the lowest inoculum concentrations, the damage percentage was also increased to 30 and 31%, after 1st spray that further declined after 2nd spray and minimum after 3rd spray. The data collected over the larvae mortality demonstrated that application of different concentrations of *P. luminescence* caused a high mortality that ranged from 58, 80, 98 and 100% ($F=127.15$, DF = 4, $P<0.05$) after the third spray (Fig. 2). It was observed that the highest concentration of *P. luminescens* provides high protection to the plants.

Various concentrations of *B. thuringiensis* var. *kurstaki* were also applied against *S. litura*, resulting in a lower damage percentage in comparison with absolute control ($F=62.39$, DF = 4, $P<0.05$). The damage percentage in *Bt*-treated plots was also increased to 36 and 38%, after spray 1st, which further declined after spray 2nd, and reached minimum after spray 3rd. The data collected over the larvae mortality demonstrated that significant variation was observed among the percent mortality 49, 78, 86 and 98% ($F=106.23$, DF = 4, $P<0.05$) after five days of nematodes application (after the third spray) (Fig. 2). It was found that under the polyhouse conditions *B. thuringiensis* var. *kurstaki* concentration is highly effective. Maximum damage with no mortality was observed in the plots treated with absolute control.

Field phase

The insecticidal potential of all the entomopathogens was also evaluated under the field conditions. The foliar applications of *H. bacteriophora* EUPT-SD were applied at different concentrations against the larvae and pupae of *S. litura* under field conditions that showed significant variations in the damage ($F=42.84$, DF = 4, $P<0.05$) (Fig. 3). The highest damage (100%) was recorded in the control. In the EPNs concentrations of 120IJs/ml and 140IJs/ml, the damage percentage ranged from 40 and 35% after 3rd nematode application. Initially, much low larval mortality was recorded that increased with the increase in the nematode application. Maximum larvae were killed after 3rd application of EPNs. A damage percentage of 35% remained constant up to harvesting, signifying the biocontrol potential of EPNs.

The foliar application of *P. luminescens* showed the least damage percentage of 30% after 1st spray, increased to 31% after 2nd spray and remained constant after 3rd spray ($F=79.44$, DF = 4, $P<0.05$) (Fig. 3). The highest damage was recorded in the control (100%). The maximum larval mortality was recorded among the highest concentration that resulted in 70% larval mortality after the 1st spray and 100% mortality after the 2nd and 3rd spray, respectively. The different concentrations of *Bt* were also applied against *S. litura* under field conditions. The data recorded demonstrated the significant variations in the damage $F=61.34$, DF = 4, $P<0.05$ (Fig. 3). The damage percentage ranged from 38 and 39% after the 3rd spray of the highest bacterial concentration. The highest damage (100%) was recorded in the control. Initially, 66% larval mortality was recorded after 1st spray that increased to 91% after 2nd spray and 96% after 3rd spray. Damage percentage of 39% remained constant up to harvesting, establishing the biological pest control.

Discussion

Solanaceous crops such as tomato are widely cultivated in India and are the cash crops of many farmers which demonstrate their economic importance in their life. Huge losses to the economy were caused by armyworm larvae (*S. litura*) in the tomato field. Although applications of chemical pesticides might be reduced the larval population but also resulted in resistance among larvae. This resulted in the rapid outburst of the *Spodoptera* population in the field. Furthermore, relocation among the *Spodoptera* moths also increased their survivability and population in the field. Owing to the great losses by *Spodoptera* larvae, interest in adopting an eco-friendly method has developed. The application of a biocontrol agent reflected a better opportunity in the management of armyworm population, although there are some biotic factors (bacteria, fungi, virus, weeds, animal interference) and abiotic stresses (temperature, humidity, application time) that limit the effectiveness of these pathogens against susceptible pests. In the present investigation, applications of three biocontrol agents (*H. bacteriophora* EUPT-SD, *P. luminescence* and *B. thuringiensis*) were

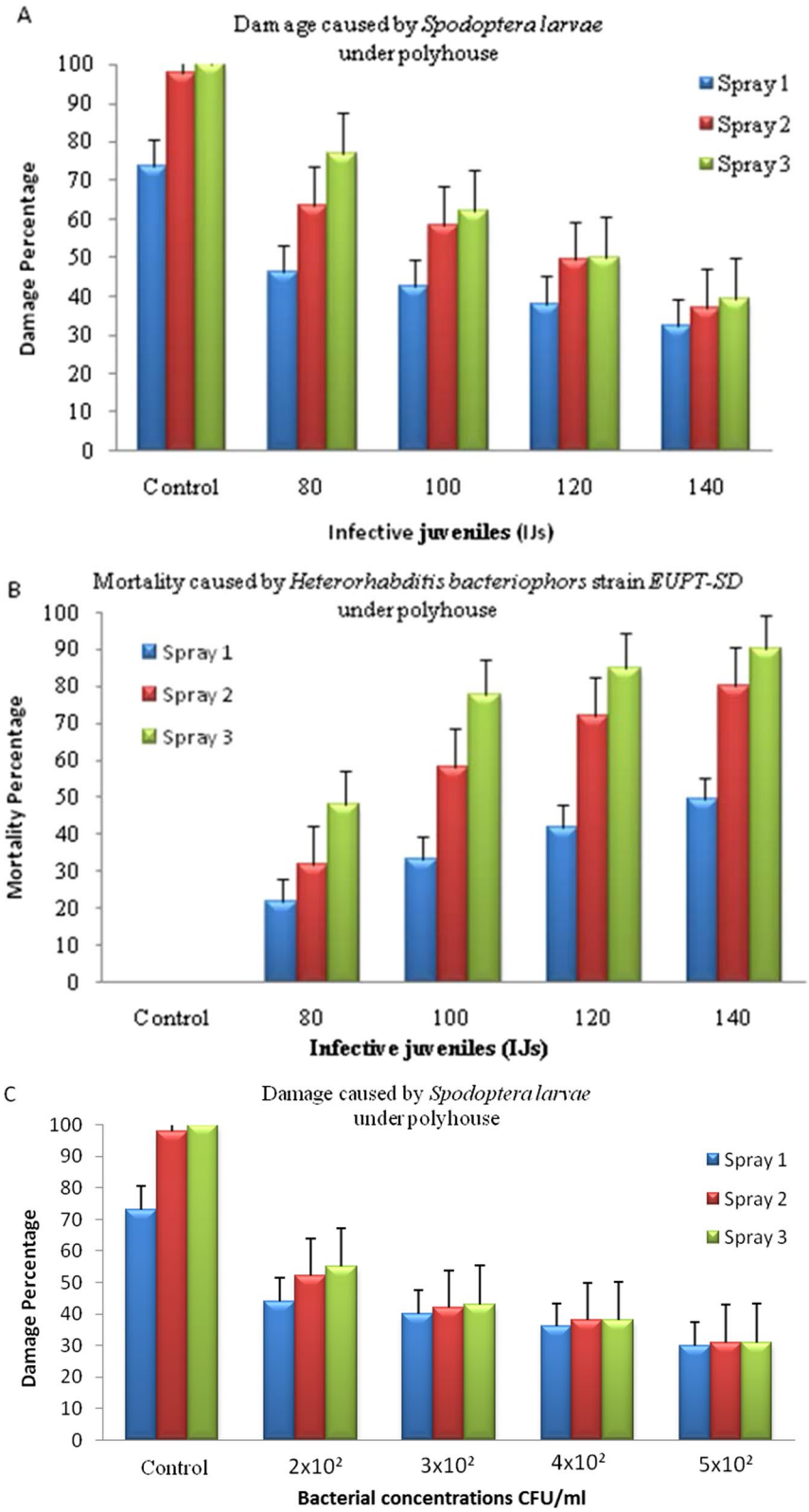
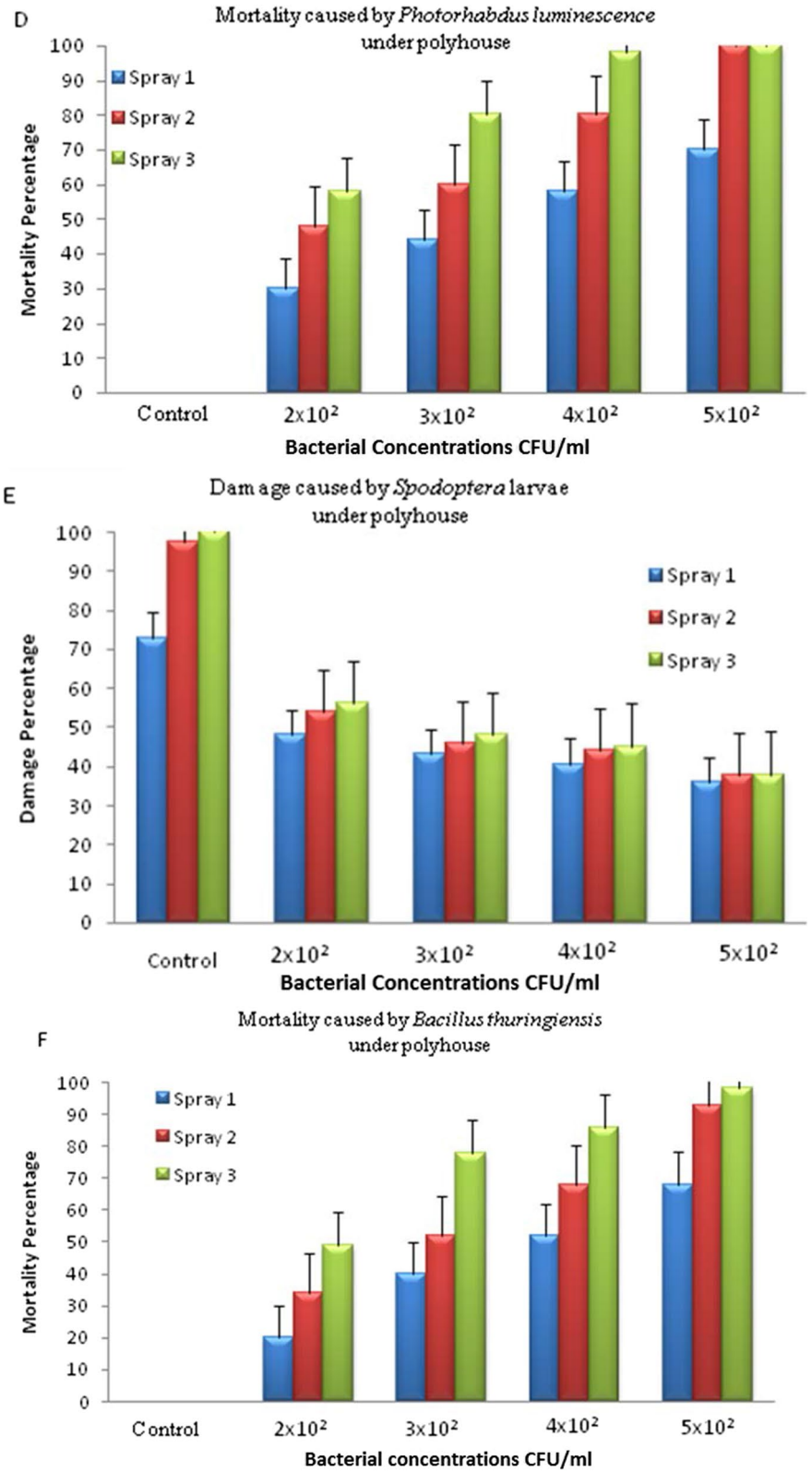


Fig. 2 Polyhouse evaluations; (**A**, **C** & **E**) damage; (**B**, **D** & **F**) larval mortality by *Heterorhabditis bacteriophora* strain EUPT-SD, *Photorhabdus luminescence* and *Bacillus thuringiensis* against *Spodoptera litura*



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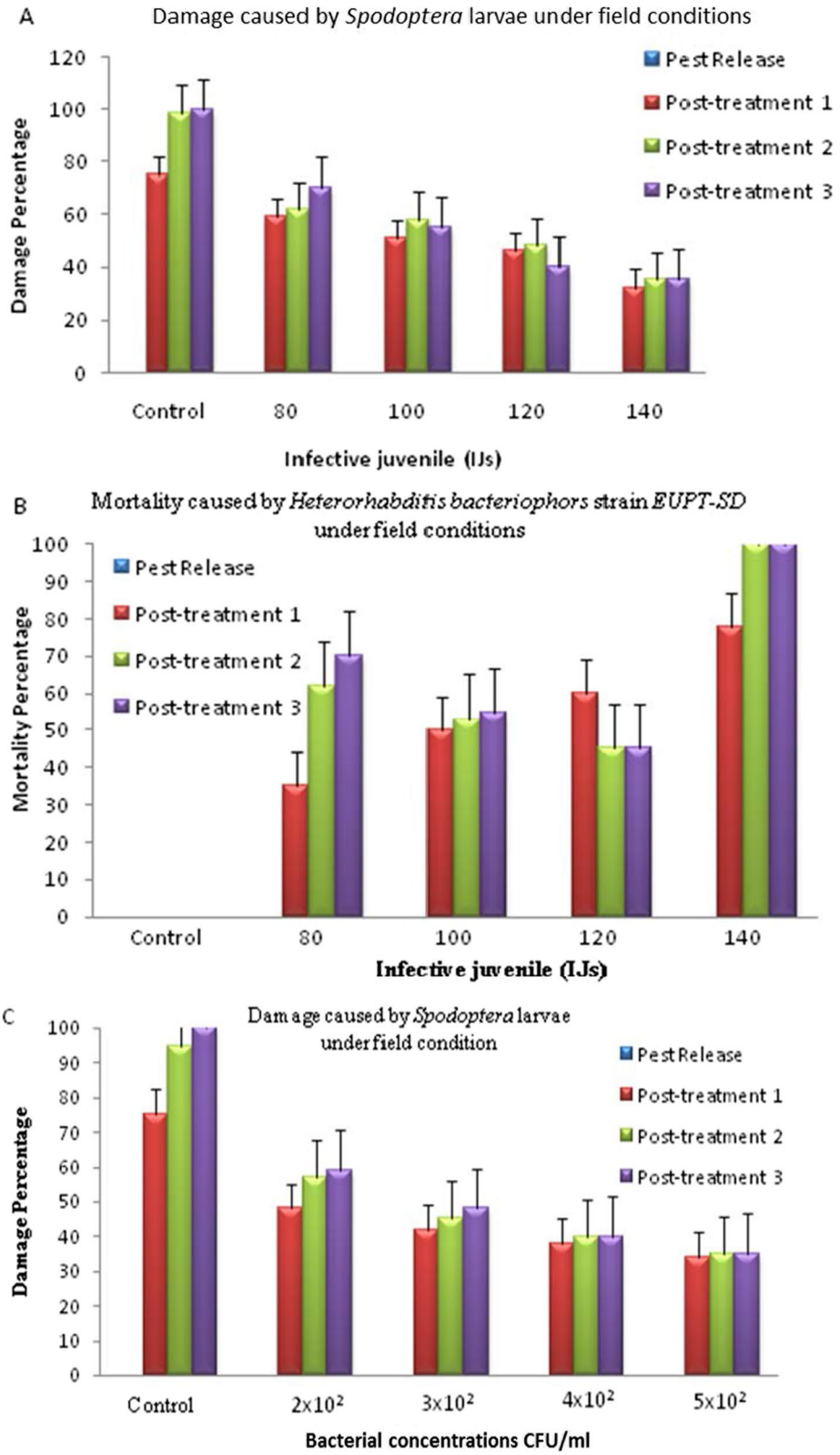

Fig. 3 Field evaluations; (**A**, **C** & **E**) damage; (**B**, **D** & **F**) larval mortality, by *Heterorhabditis bacteriophora* strain EUPT-SD, *Photorhabdus luminescence* and *Bacillus thuringiensis* against *Spodoptera litura*

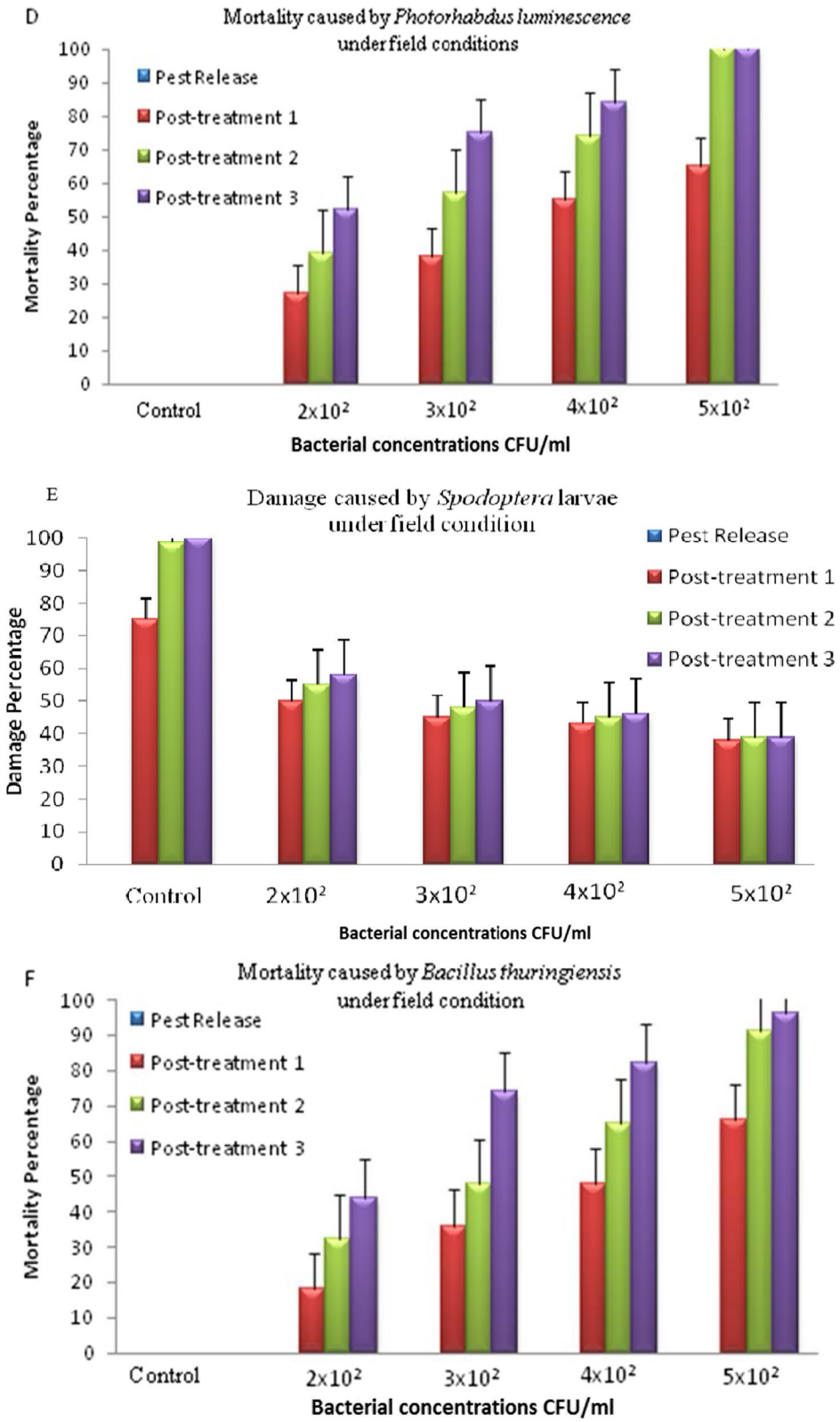

Fig. 3 continued

evaluated against *S. litura* larvae (4th instar) in the laboratory, polyhouse and field conditions. Among the three treatments, the most effective treatments were the applications of *P. luminescens* that caused the highest mortality (100%) in the highest concentration of 5×10^2 CFU/ml among *S. litura* larvae after 96 h of infection.

Earlier, Salazar-Gutiérrez et al. (2017) applied *Photorhabdus luminescens* strain SL0708 and its cell-free filtrate against *Spodoptera frugiperda* Smith, 1797 and *Galleria mellonella* Linnaeus, 1758 larvae in a bioassay experiment. They reported 100% larval mortality after 48 h of bacterial application at the rate of $1 \times 10^3 - 1 \times 10^4$ CFU/larva. It was recorded that *H. bacteriophora* EUPT-SD (140IJs/ml) resulted in 92% larval mortality after 96 h of exposure. The insecticidal potential of *Photorhabdus* and *Xenorhabdus* also was demonstrated by Adithya et al. (2020). Earlier, Yan et al. (2020) also reported the insecticidal effect of EPNs against tobacco cutworm. Sun et al. (2021) evaluated the biocontrol potential of *Heterorhabditis* and *Steinernema* nematodes against *S. litura* larvae (5th instar) and recorded 90% mortality in *S. litura* after 72 h. Similar observations were recorded by (Burana et al. 2022). Tomar et al. (2022b) reported that application of EPNs is an effective strategy in managing the 3rd and 4th larval instars of *S. litura* in laboratory conditions. Tomar et al (2022c) reported that the native strain of EPNs was highly effective in managing the lepidopteran insect population including *S. litura*.

The highest concentration of *B. thuringiensis var. kurstaki* (5×10^2 CFU/ml) resulted in 98% larval mortality after 96 h of exposure. The study was also supported by the earlier investigation of Sajid et al. (2020) who recorded 100% larval mortality among *S. litura* (2nd instar larvae) after being treated with *B. thuringiensis*. Similarly, the effectiveness of *B. thuringiensis* granules (water dispersible) along with starch and adjuvant Tween20 against *Spodoptera* larvae was also described by Vimala Devi et al. (2021). Virulence of *Bt* cry proteins was also explained against lepidopteran insect pests by Singh et al. (2021).

Under polyhouse evaluation test, foliar applications of *H. bacteriophora* EUPT-SD at the rate of 140 IJs/ml in tomato plants resulted in lower damage percentage and high larval mortality (90%) than with absolute control. Earlier, Andaló et al. (2010) evaluated the efficacy of *Steinernema arenarium* Artyukhovsky, 1967 and *Heterorhabditis* sp. RSC02 against *S. frugiperda* under the laboratory and polyhouse conditions towards maize crop. They recorded 100 and 97.6% insect mortality under laboratory conditions and 77.5 and 87.5% mortality under greenhouse conditions, respectively. Tomar et al. (2022b) reported that applications of EPNs are highly effective in controlling the insect population under polyhouse and also in field conditions. Applications of *P. luminescens* and *B. thuringiensis* var. *kurstaki* resulted in 100 and 98% larval mortality after the third spray under polyhouse conditions. The present study also supported by Thakur et al. (2022c) evaluated the individual and combined impact of entomopathogens *H. bacteriophora*, *B. bassiana* and *B. thuringiensis* against *Spodoptera* larvae and reported that applications of these entomopathogens were highly effective in managing the insect population under the laboratory as well as in greenhouse conditions.

Under field conditions also significant control in the damage and high larval mortality was recorded after treatment with *H. bacteriophora* EUPT-SD, *P. luminescence* and *B. thuringiensis* var. *kurstaki* against *Spodoptera* larvae. Earlier, Mohan et al. (2003) reported that 100 percent cabbage butterfly larvae were killed upon treatment with foliar applications of *Photorhabdus* bacteria. Similarly, Uma et al. (2010) applied cellular and cell-free filtrate against aphids and reported a significant reduction in aphid nymph population. Navon (2000) also reported the application of *B. thuringiensis* in controlling the agricultural insect pests.

Conclusions

Entomopathogens have been known to manage insect populations for years. During the present study, entomopathogenic nematode *H. bacteriophora* EUPT-SD, and entomopathogenic bacteria *P. luminescence* EUPT-SD and *B. thuringiensis* var. *kurstaki* were applied against the 4th instar larvae of *S. litura* under the laboratory, polyhouse and field conditions. The obtained results demonstrated that entomopathogenic bacteria *P. luminescence* caused a significant mortality rate, followed by *B. thuringiensis* var. *kurstaki* and then *H. bacteriophora* EUPT-SD under laboratory bioassay study. In addition, they were in the polyhouse experiments and in field conditions. So the treated plots experienced lesser damage when compared to non-treated plots. The applications of these pathogens are nature-friendly and are a proficient alternative to synthetic chemical insecticides.

Abbreviations

H. bacteriophora	*Heterorhabditis bacteriophora*
IJs	Infective juveniles
EPNs	Entomopathogenic nematodes
CFU	Colony-forming unit
%	Percent
Hrs	Hours
EPA	Environment protection agency

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Author contributions
NT gave the concept. NT and PT conducted the experiment and wrote the manuscript with statistical analysis. JK, SK, AS and SJ helped in conducting the survey to collect the insects from various fields. ANY, HSD, RT and ST gave their valuable suggestions during the field experiments. All authors have read and reviewed the manuscript.

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Declarations

Ethics approval and consent to participate
All procedures performed in studies are in accordance with the ethical standards of the institutional and/or national research committee. We further declare that no animal was harmed during this study.

Consent for publication
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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