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Synergistic effect of entomopathogens against *Spodoptera litura* (Fabricius) under laboratory and greenhouse conditions

Neelam Thakur[1], Preety Tomar[1], Sakshi Sharma[1], Simranjeet Kaur[1], Sushma Sharma[2], Ajar Nath Yadav[3] and Abd El-Latif Hesham[4*]

Abstract

Background: Entomopathogens such as nematodes, bacteria and fungi are well recognized for their biocontrol potential. This study was carried out to examine the insecticidal properties of the *Heterorhabditis bacteriophora* Poinar, *Beauveria bassiana* Balsamo-Crivelli, *Bacillus thuringiensis* Berliner, individually and in combination against 3rd instar larvae of *Spodoptera litura* Fabricius (Noctuidae: Lepidoptera) under controlled laboratory and greenhouse conditions at Eternal University, Baru Sahib, Sirmaur, Himachal Pradesh.

Results: The results demonstrated that the combined applications of the tested entomopathogens resulted in 100% insect mortality under the laboratory conditions. Among the individual concentrations, applications of 200 IJs/ml were noticed highly virulent with (98%) mortality, followed by *B. thuringiensis* (96%) and then by *B. bassiana* (92%). However, single treatments were also evaluated that further showed a highest mortality in the target pest by *H. bacteriophora*, followed by *B. thuringiensis*. Among the combined treatments by *H. bacteriophora* plus *B. thuringiensis* (200 IJs + 1×10^{12} CFU/cm^2) more effective caused (100%) mortality were noticed in the laboratory and (28%) under the greenhouse conditions than *H. bacteriophora* plus *B. bassiana* (200 IJs + 1×10^{10} conidia/cm^2) that caused (100%) mortality and (34%) damage under both, laboratory and greenhouse conditions.

Conclusion: Laboratory bioassay and greenhouse evaluation tests demonstrated that the combined sprayed treatments showed reliable and fast synergism. This study could be recommended to the farmers to control the pest.

Keywords: *Bacillus thuringiensis*, *Beauveria bassiana*, Bio-efficacy, *Heterorhabditis bacteriophora*, *Spodoptera litura*

Background

Spodoptera litura (Fabricius,1755) (Lepidoptera: Noctuidae), known as tobacco caterpillar, beet armyworm, lesser armyworm, small mottled willow, cutworm and pigweed caterpillar, is the most serious insect pest in the countries like Japan, China, India, Pakistan (Ghaffar et al. 2002) and South Asia (Qin et al. 2004). It is a very destructive and polyphagous insect pest that causes damage to various crops such as potato, cotton, capsicum, tomato, soybean, okra, clover and onion (Saleem et al. 2016). The larvae feed on leaves of the cultivated plants that lead to complete defoliation in the early stage causing severe crop damage in India (Firake and Behere 2020). Commercially important vegetable Capsicum (*Capsicum annuum* Linnaeus) (Solanales: Solanaceae) grown worldwide is highly infested by *S. litura* (Baikar and Naik 2016). Constant use of pesticides leads to environmental contamination and pesticide residues in all foodstuffs all over the world (WHO 2017). This leads to develop safer, novel, biodegradable biopesticides as insecticidal alternatives (Chaudhary et al. 2017).

*Correspondence: hesham_egypt5@agr.bsu.edu.eg
[4] Department of Genetics, Faculty of Agriculture, Beni-Suef University, Beni-Suef 62521, Egypt
Full list of author information is available at the end of the article

Entomopathogenic nematodes (EPNs) are considered as non-chemical alternatives of pesticides. They belong to family Heterorhabditidae and Steinernematidae and mostly genera *Heterorhabditis* and *Steinernema* (Razia and Sivaramakrishnan 2014). Infective juvenile of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae), kills their host insect within 24–72 h (Vashisth et al. 2013). Juvenile reaches the hemocoel of the host insect, releases its bacteria that multiply in hemolymph and liberates many factors virulent against host insect including antimicrobial compounds, hydrolytic enzymes, complexes of toxins and hemolysins (Ribeiro and Vaz 2019).

Entomopathogenic fungus (EPF) such as *Beauveria bassiana* Balsamo-Crivelli, 1912 (Hypocreales: Cordycipitaceae), is a pathogenic against many insects pest widely (Butt et al. 2001) and is alternated to synthetic insecticides (Maina et al. 2018) that infect the insect by entering through their cuticle. *B. bassiana* is non-obligatory saprophyte in nature that can also survive as endophytes of plants (Bing and Lewis 1992). It produces a number of mono-nucleated single celled submerged conidia, aerial conidia and blastospores (Holder et al. 2007). The conidia of the fungus come in close contact with the host insect cuticle for further germination and proliferation (Ortiz-Urquiza and Keyhani 2013). It is used for the management of bacterial or viral diseases caused by grasshoppers and locusts (Quesada-Moraga 2002).

Entomopathogenic bacteria (EPB) *Bacillus thuringiensis* (*Bt*) Berliner, 1915 (Bacillales: Bacillaceae) along with several microbial species proven as successful biocontrol agent. It is the spore forming bacteria which is omnipresent and forms crystal proteins. These crystal proteins are δ-endotoxin and are toxic for insects. It is found in grain storage facilities, insect-rearing facilities, sericulture farms and grain dust from flour mills (insect-rich environments). During spore formation, it also produces proteins which are released into the environment after crystallizing it degrades the cell wall. It expedites insect death, when these crystals are ingested by insect. The Cry proteins have diversifying nature and mainly target insects belonging to order Diptera (flies and mosquitoes), Lepidoptera (butterflies and moths), Coleoptera (beetles and weevils) and Hymenoptera (wasps and bees) (Bravo et al. 2007). EPNs, EPF and EPB are used as biopesticides against a variety of insects (Thakur et al. 2021). Although there are several synthetic chemical insecticides available in the market, *S. litura* develop resistance against various pesticides so there is an urgent need to develop various management strategies that are eco-friendly and user safe. In this investigation, a mixture of the EPNs (*H. bacteriophora*), EPB (*B. thuringiensis*), EPF (*B. bassiana*), each singly and in combination, was applied as control measures against *S. litura* (3rd instar) under controlled laboratory and greenhouse conditions.

Methods

Rearing of *Spodoptera litura*

This work was performed in the Department of Zoology and Entomology, Eternal University, Sirmour (30.7537° N, 77.2965° E, 1900 m altitude), state of Himachal Pradesh, India. Adults and larvae of *S. litura* (target insect) were collected from University Agricultural fields and farmer's fields near Eternal University. Castor leaves were provided to feed the larvae. The culture was maintained at 28 ± 1 °C temperature and 70% relative humidity (Patil et al. 2014). Emerged adults were transferred to chimneys for oviposition that contained 15% sucrose solution for feeding of moths (Santharam 1985). Female laid eggs in cluster after 3–4 days.

Entomopathogenic nematode EPNs

Heterorhabditis bacteriophora were cultured on *Corcyra cephalonica* Stainton, 1866 (Lepidoptera: Pyralidae) larvae (Chaudhuri and Senapati 2017). These baits were further used for mass multiplication of EPNs (Orozco et al. 2014). Emerged nematodes were accumulated from the white traps and stored in disinfected distilled water at 14 ± 1 °C for 5–15 days before use (Shapiro-Ilan et al. 2002). The suspension containing nematodes was also poured over the sterilized synthetic sponge pieces and stored at 10 °C for future use (Ramakuwela et al. 2015).

Entomopathogenic Fungi and Bacteria

Commercially available formulations of *B. bassiana* Daman (1.0% W.P. Strain: IPL/BB/MI/01, International Panaacea Limited, New Delhi, India) and *B. thuringiensis* var. *kurstaki* Mahastra (0.5% W.P., Strain: DOR BT-1, International Panaacea Limited, New Delhi, India) were purchased from the local market for the bioassay experiment.

Bio-efficacy studies under the laboratory

Bio-efficacy of different treatments of EPNs (*H. bacteriophora*), EPF (*B. bassiana*) and EPB (*B. thuringiensis*) were applied against *S. litura* larvae (3rd instar), alone and in combination (Table 1) under laboratory conditions. Bioassay test was executed using 5 different treatments at different concentrations along with absolute control under a polystyrene tray having 48 wells per tray. The experiment was replicated 5 times, and the insect larval mortality was assessed every 24 h. of applications.

Table 1 List of tested entomopathogens applied against 3rd instar larvae of *Spodoptera litura* under laboratory conditions

Heterorhabditis bacteriophora	*Beauveria bassiana*	*Bacillus thuringiensis*	*H. bacteriophora* + *B. bassiana*	*H. bacteriophora* + *B. thuringiensis*
50 IJs/ml	1×10^4 conidia/ml	1×10^3 CFU/ml	50 IJs + 1×10^{10} conidia/ml	50 IJs + 1×10^{12} CFU/ml
100 IJs/ml	1×10^6 conidia/ml	1×10^6 CFU/ml	100 IJs + 1×10^{10} conidia/ml	100 IJs + 1×10^{12} CFU/ml
150 IJs/ml	1×10^8 conidia/ml	1×10^9 CFU/ml	150 IJs + 1×10^{10} conidia/ml	150 IJs + 1×10^{12} CFU/ml
200 IJs/ml	1×10^{10} conidia/ml	1×10^{12} CFU/ml	200 IJs + 1×10^{10} conidia/ml	200 IJs + 1×10^{12} CFU/ml

Table 2 Tested biocontrol pathogens and the concentrations applied against 3rd instar larvae of *Spodoptera litura* under greenhouse conditions

Heterorhabditis bacteriophora	*Beauveria bassiana*	*Bacillus thuringiensis*
50IJs/cm^2	1×10^4 conidia/cm^2	1×10^3 CFU/cm^2
100IJs/cm^2	1×10^6 conidia/cm^2	1×10^6 CFU/cm^2
150IJs/cm^2	1×10^8 conidia/cm^2	1×10^9 CFU/cm^2
200IJs/cm^2	1×10^{10} conidia/cm^2	1×10^{12} CFU/cm^2
Control	Control	Control

Table 3 Combined concentrations of entomopathogens' applied against 3rd instar larvae of *Spodoptera litura* under greenhouse conditions

Treatments	Biocontrol agent	Concentrations
T1	*H. bacteriophora* + *B. bassiana*	150 IJs + 1×10^{10} conidia/cm^2
T2	*H. bacteriophora* + *B. bassiana*	200 IJs + 1×10^{10} conidia/cm^2
T3	*H. bacteriophora* + *B. thuringiensis*	150 IJs + 1×10^{12} CFU/ cm^2
T4	*H. bacteriophora* + *B. thuringiensis*	200 IJs + 1×10^{12} CFU/ cm^2
T5	Absolute control	Water only

Bioassay studies under greenhouse conditions

Single applications of H. bacteriophora, B. bassiana and B. thuringiensis

The vulnerability of *H. bacteriophora*, *B. bassiana* and *B. thuringiensis* against 3rd instar larvae of the target pest was assessed in capsicum plants under greenhouse conditions at 30 ± 2 °C temperature and 60% relative humidity. The capsicum plants were grown under polyhouse conditions with a randomized block design. The area was divided into small plots (3 × 3 m) containing 16 capsicum plants per plot. As the plants attained vegetative stage, the larvae (3rd instar) were transferred 12 h. prior to the treatment applications. The IJs, conidia and spores were mixed in water individually. Surfactant Tween 20 (0.3%) was added and applied with hand sprayer. Data over leaf damage and mortality were noted after 24 h up to 5 consecutive days. Four different concentrations of each biocontrol agent: *H. bacteriophora* (50 IJs, 100 IJs, 150 IJs and 200 IJs/cm^2), *B. bassiana* (1×10^4, 1×10^6, 1×10^8 and 1×10^{10} conidia/cm^2) and *B. thuringiensis* (1×10^3, 1×10^6, 1×10^9 and 1×10^{12} CFU/cm^2), were applied along with control (absolute) (Table 2) in each plot. The experiment was replicated thrice per treatment. The study was conducted for 2 consecutive years, and the data over the mortality were pooled for the statistical analysis.

Combined applications of H. bacteriophora, B. bassiana and B. thuringiensis

Experiment was accomplished to evaluate the efficiency of combined concentrations of entomopathogens against *S. litura* larvae (3rd instar) under the greenhouse conditions (Table 3). During this experiment also, insect larvae were relocated 12 h. before treatment application. The combined concentrations of IJs + fungal conidia (150 IJs + 1×10^{10} conidia/cm^2 and 200 IJs + 1×10^{10} conidia/cm^2) and IJs + bacterial spores (150 IJs + 1×10^{12} CFU/cm^2 and 200 IJs + 1×10^{12} CFU/cm^2) were suspended in water containing Tween 20 (0.3%) and applied over the capsicum leaves. Result variables such as leaf damage and larval mortality were noted 24 h post treatment applications for 5 days after 3 consecutive sprays. The data calculated over the leaf damage percentage and larval mortality were recorded.

Statistical analysis

Statistical analysis of variance ANOVA was enumerated. ANOVA was evaluated using OPSTAT software developed by HAU, Haryana. Statistical significance was calculated at level $P < 0.05$. Statistical differences among the treatments were considered.

Results

Bio-efficacy of entomopathogens under laboratory

Pathogenic potential of different tested treatments of entomopathogens was assessed against 3rd instar larvae of *S. litura*, alone and in combination under controlled conditions. The data obtained over the mortality are represented in Fig. 1, Table 4. Mortality percentage increased in all the treatments as exposure time increased. In *H. bacteriophora*, the highest larval mortality (98%) was recorded at the highest treatment of 200 IJs/ml after 96 h. of nematode exposure. However, (48%) mortality was observed after 96 h., in the lowest concentration (50 IJs/ml) of nematode (Fig. 1A). Significant differences were recorded in the mortality percentage by EPNs ($F=16.91$, df = 4, P value < 0.001). In treatments with *B. bassiana*, maximum mortality (92%) in treatment 1×10^{10} conidia/ml and minimum mortality (45%) (Table 5) were detected in treatment 1×10^{4} conidia/ml after 96 h. of inoculation (Fig. 1B). Statistically significant differences were observed in insect mortality rates ($F=20.43$, df = 4, $P<0.001$). The larvae treated with different concentrations of *B. thuringiensis* recorded (96%) insect mortality at the highest concentration 1×10^{12} CFU/ml, followed by the low concentrations (Table 6). Minimum mortality rate (54%) was noticed in the lowest concentration 1×10^{3} CFU/ml after 96 h. of incubation (Fig. 1C). Considerable differences in the percent mortality were recorded in all treatments of *B. thuringiensis*, ($F=32.35$, df = 4, P value < 0.001).

Combined concentrations of treatments showed differential impact over the mortality. Treatments of EPNs and EPF, 200 IJs + 1×10^{10} conidia/ml gave a maximum of (100%) mortality rate, followed by (96%) in the treatment of 150 IJs + 1×10^{10} conidia/ml (Table 7). Minimum mortality rate (74%) was perceived at the lowest concentration 50 IJs + 1×10^{10} conidia/ml (Fig. 1D). Significantly increased mortality percent were recorded after treated with combined concentrations of *H. bacteriophora* + *B. bassiana* ($F=160.10$, df = 4, P value < 0.001). In another experiment of combined concentrations of EPNs and EPB, 150IJs + 1×10^{12} CFU/ml and 200 IJs + 1×10^{12} CFU/ml, both high concentrations caused (100%) larval mortality after 96 h. of exposure (Table 8). Even minimum mortality rate (78%) was recorded at the concentration 50 IJs + 1×10^{12} CFU/ml (Fig. 1E). The combined mixture of *H. bacteriophora* + *B. thuringiensis* showed synergistic impact on *S. litura* larvae (3rd instar) and significant differentiation in the larval mortality was recorded ($F=238.12$, df = 4, P value < 0.001) under the laboratory bioassay study.

Bio-efficacy of entomopathogens single and combined mixture under greenhouse conditions

Pathogenic potential of entomopathogens were also evaluated under greenhouse conditions. Capsicum plants sprayed with *H. bacteriophora* IJs showed significantly reduced damage in contrast to control. Minimum damage percentage was detected at plot treated with 200 IJs/cm^2 concentration. Even though the damage percentages were quite higher (35%) but significantly lower than the control ($F=10.39$, df = 4, P value < 0.001) (Fig. 2A). In treatment with *B. bassiana* also, (43%) damage was recorded at the highest concentration (1×10^{10} conidia/cm^2) after 3rd application of the biocontrol agent. However, it showed protective effect on capsicum plant against non-treated plants ($F=11.79$, df = 4, P value < 0.001) (Fig. 2B). Another experiment with *B. thuringiensis*, (41%) damage was recorded after 3rd spray of 1×10^{12} CFU/cm^2. Significantly reduced damage percentage was recorded at the highest treatment in contradiction of control ($F=10.39$, df = 4, P value < 0.001) (Fig. 2C).

Combined mixture of concentrations containing *H. bacteriophora* plus *B. bassiana* and *H. bacteriophora* plus *B. thuringiensis* reduced the damage percentage and synergistic impact of these entomopathogens, in contradiction of non-treated plants. Significant variations were recorded. Least damage (28%) was detected in concentration 200 IJs + 1×10^{12} CFU/cm^2, while (34%) damage was encountered in 200 IJs + 1×10^{10} conidia/cm^2. The highest (96%) damage was detected in the control (Fig. 2D).

Additionally, the data recorded over the mortality in *S. litura* larvae (3rd instar) via *H. bacteriophora* also demonstrated that application of 200 IJ/cm^2 caused significant mortality in insect larvae after 3rd spray. The percent mortality ranged between (35 and 93%) at 5 days after 3rd application of infective juveniles ($F=28.71$, df = 4, $P<0.001$) (Fig. 3A). Applications with *B. bassiana* also showed variation in the percent mortality in contradiction to control. In treatment *B. bassiana*, insect mortality percentage ranged between (30 and 88%) ($F=26.01$, df = 4, P value < 0.001) (Fig. 3B). Regarding the applications of different concentration of *B. thuringiensis*, treatment of 1×10^{12} CFU/ml resulted in a significant mortality rate in insect larvae. Mortality rates increased from (38 to 90%) after 3rd spray; in contrast, no larval deaths were observed in the control ($F=25.33$, df = 4, P value < 0.001) (Fig. 3C).

Capsicum plants treated with combined concentrations of *H. bacteriophora* plus *B. bassiana* showed improved insect mortality. Furthermore, *H. bacteriophora* plus *B. thuringiensis* also demonstrated increased insect mortality rates. The least mortality (41%) was detected at 150 IJs + 1×10^{10} conidia/cm^2, and the maximum one (98%) was evidenced at 200 IJs + 1×10^{12} CFU/cm^2.

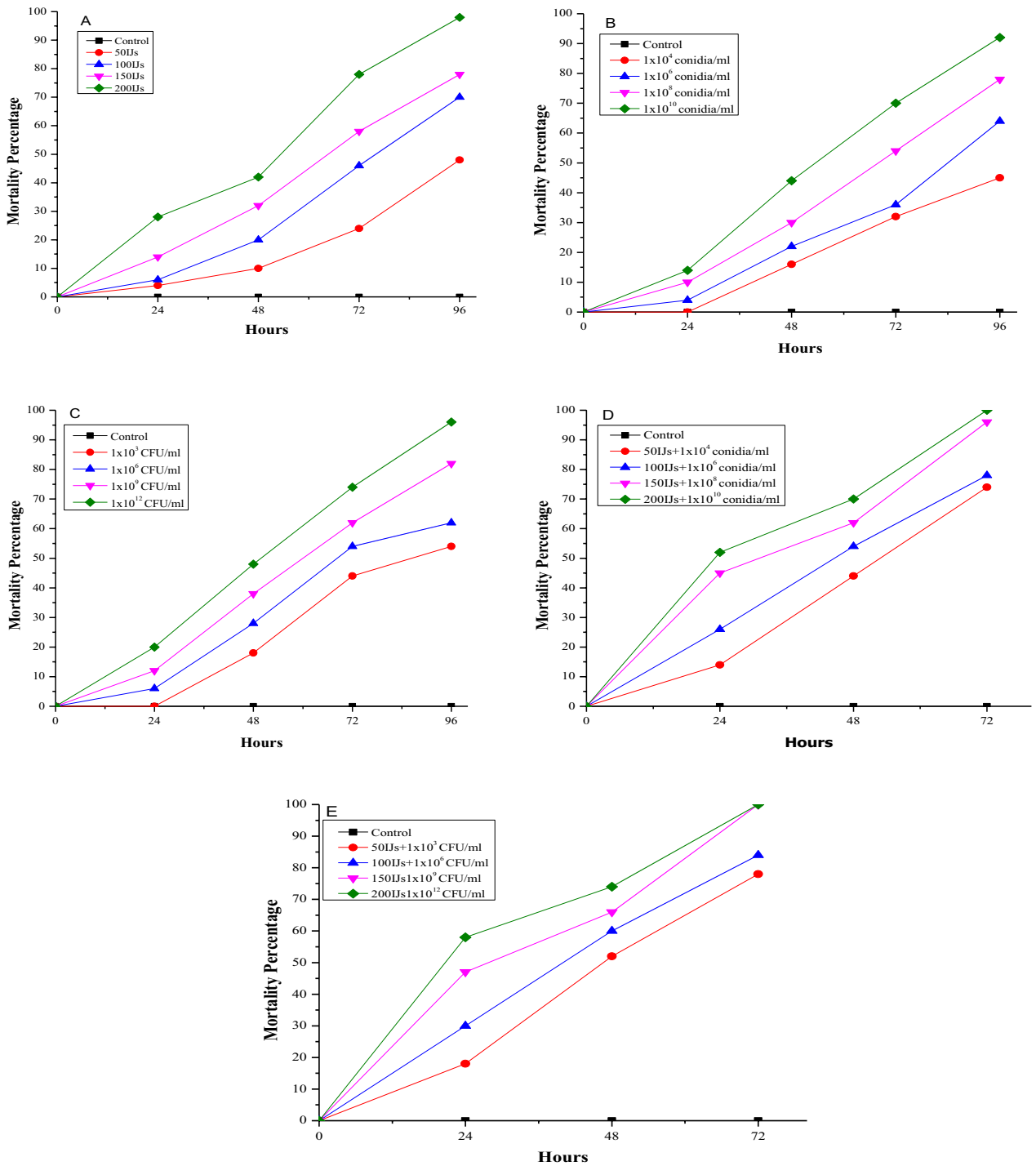

Fig. 1 Application of entomopathogens as a biocontrol agent against 3rd instar larvae of *Spodoptera litura* under laboratory conditions. Insect mortality percentage by using **A** *Heterorhabditis bacteriophora*, **B** *Beauveria bassiana*, **C** *Bacillus thuringiensis*, **D** *H. bacteriophora* + *B. bassiana*, **E** *H. bacteriophora* + *B. thuringiensis*

Statistically significant results were achieved in the combined concentrations of all entomopathogens ($F = 108.39$, df = 4, $P < 0.001$) (Fig. 3D). All larvae were alive in control. The combined mixture of entomopathogens was found synergistic and had potential of reducing the insect population.

Table 4 Pathogenic potential of *Heterorhabditis bacteriophora* against 3rd instar larvae of *Spodoptera litura* under laboratory conditions

Concentrations (IJs)	Corrected % mortality against 3rd instar larvae after treatments (h)			
	24	48	72	96
50	04.00 (01.92)	10.00 (03.32)	24.00 (04.97)	48.00 (06.98)
100	06.00 (02.39)	20.00 (04.53)	46.00 (06.81)	70.00 (08.41)
150	14.00 (03.82)	32.00 (05.70)	58.00 (07.66)	86.00 (09.32)
200	28.00 (05.34)	42.00 (06.52)	78.00 (08.88)	98.00 (09.95)
Control	00.00 (01.00)	00.00 (01.00)	00.00 (01.00)	00.00 (01.00)
Mean	10.40 (02.89)	20.80 (04.21)	41.20 (05.86)	60.40 (07.13)

Figures in parentheses are transformed values (arc sine transformation) CD ($P=0.05$)

Table 5 Pathogenic potential of *Beauveria bassiana* against 3rd instar larvae of *Spodoptera litura* under laboratory conditions

Concentrations (conidia/ml)	Corrected % mortality against 3rd instar larvae after treatments (h)			
	24	48	72	96
1×10^4	00.00 (01.00)	16.00 (04.08)	32.00 (05.32)	45.00 (06.98)
1×10^6	04.00 (01.93)	22.00 (04.72)	36.00 (06.02)	64.00 (08.41)
1×10^8	10.00 (03.32)	30.00 (05.54)	54.00 (07.39)	78.00 (09.32)
1×10^{10}	24.00 (03.82)	44.00 (06.70)	70.00 (08.42)	92.00 (09.95)
Control	00.00 (01.00)	00.00 (01.00)	00.00 (01.00)	00.00 (01.00)

Figures in parentheses are transformed values (arc sine transformation) CD ($P=0.05$)

Table 6 Pathogenic potential of *Bacillus thuringiensis* against 3rd instar larvae of *Spodoptera litura* under laboratory conditions

Concentrations (CFU/ml)	Corrected % mortality against 3rd instar larvae after treatments (h)			
	24	48	72	96
1×10^3	00.00 (01.00)	18.00 (04.33)	44.00 (06.70)	54.00 (06.76)
1×10^6	06.00 (02.39)	28.00 (05.34)	54.00 (07.41)	62.00 (07.92)
1×10^9	12.00 (03.57)	38.00 (06.22)	62.00 (07.92)	82.00 (09.10)
1×10^{12}	20.00 (04.58)	48.00 (06.98)	74.00 (08.66)	96.00 (09.85)
Control	00.00 (01.00)	00.00 (01.00)	00.00 (01.00)	00.00 (01.00)

Figures in parentheses are transformed values (arc sine transformation) CD ($P=0.05$)

Table 7 Potential of *Heterorhabditis bacteriophora* + *Beauveria bassiana* against 3rd instar larvae of *Spodoptera litura* under laboratory conditions

Concentrations (IJs + conidia/ml)	Corrected % mortality against 3rd instar larvae after treatments (h)		
	24	48	72
$50 + 1 \times 10^{10}$	14.00 (03.82)	44.00 (06.70)	74.00 (08.66)
$100 + 1 \times 10^{10}$	26.00 (05.17)	54.00 (07.41)	78.00 (08.88)
$150 + 1 \times 10^{10}$	52.00 (06.77)	62.00 (07.92)	96.00 (09.85)
$200 + 1 \times 10^{10}$	66.00 (07.27)	70.00 (08.42)	100.00 (10.05)
Control	00.00 (01.00)	00.00 (01.00)	00.00 (01.00)

Figures in parentheses are transformed values (arc sine transformation) CD ($P=0.05$)

Table 8 Potential of *Heterorhabditis bacteriophora* + *Bacillus thuringiensis* against 3rd instar larvae of *Spodoptera litura* under laboratory conditions

Concentration (IJs + CFU/ml)	Corrected % mortality against 3rd instar larvae after treatments (h)		
	24	48	72
$50 + 1 \times 10^{12}$	18.00 (04.33)	52.00 (07.27)	78.00 (08.88)
$100 + 1 \times 10^{12}$	30.00 (05.57)	60.00 (07.80)	84.00 (09.22)
$150 + 1 \times 10^{12}$	56.00 (06.92)	66.00 (08.18)	100.00 (10.05)
$200 + 1 \times 10^{12}$	70.00 (07.70)	74.00 (08.66)	100.00 (10.05)
Control	00.00 (01.00)	00.00 (01.00)	00.00 (01.00)

Figures in parentheses are transformed values (arc sine transformation) CD ($P=0.05$)

Discussion

In the present study, the pathogenicity potential of *H. bacteriophora*, *B. bassiana* and *B. thuringiensis* was evaluated individually and in combined treatment against *S. litura* (3rd instar) under controlled laboratory and greenhouse conditions. Foliar spray of *H. bacteriophora* was quite effective in managing 3rd instar larvae under both controlled conditions. Hussaini (2005) noticed better efficacy in *H. bacteriophora* than *H. indica* against *S. litura*. He reported 40 and 20% mortality with *H. bacteriophora* and *H. indica* after 72 h. of exposure. Divya et al. (2010) conducted in laboratory bioassay study on *H. indica* against *S. litura* (3rd instar). Yadav et al (2017) also reported that EPNs (*S. carpocapsae*) were responsible for causing 100% larval mortality in *S. litura* at a concentration of 400 IJs after 96 h. of exposure. Khan et al. (2020) evaluated the biocontrol potential of EPNs against four lepidopteran insect pests including *S. litura*. They reported EPNs as significant biocontrol agents which is highly virulent against insect population. Sun et al. (2021) recorded 100% mortality

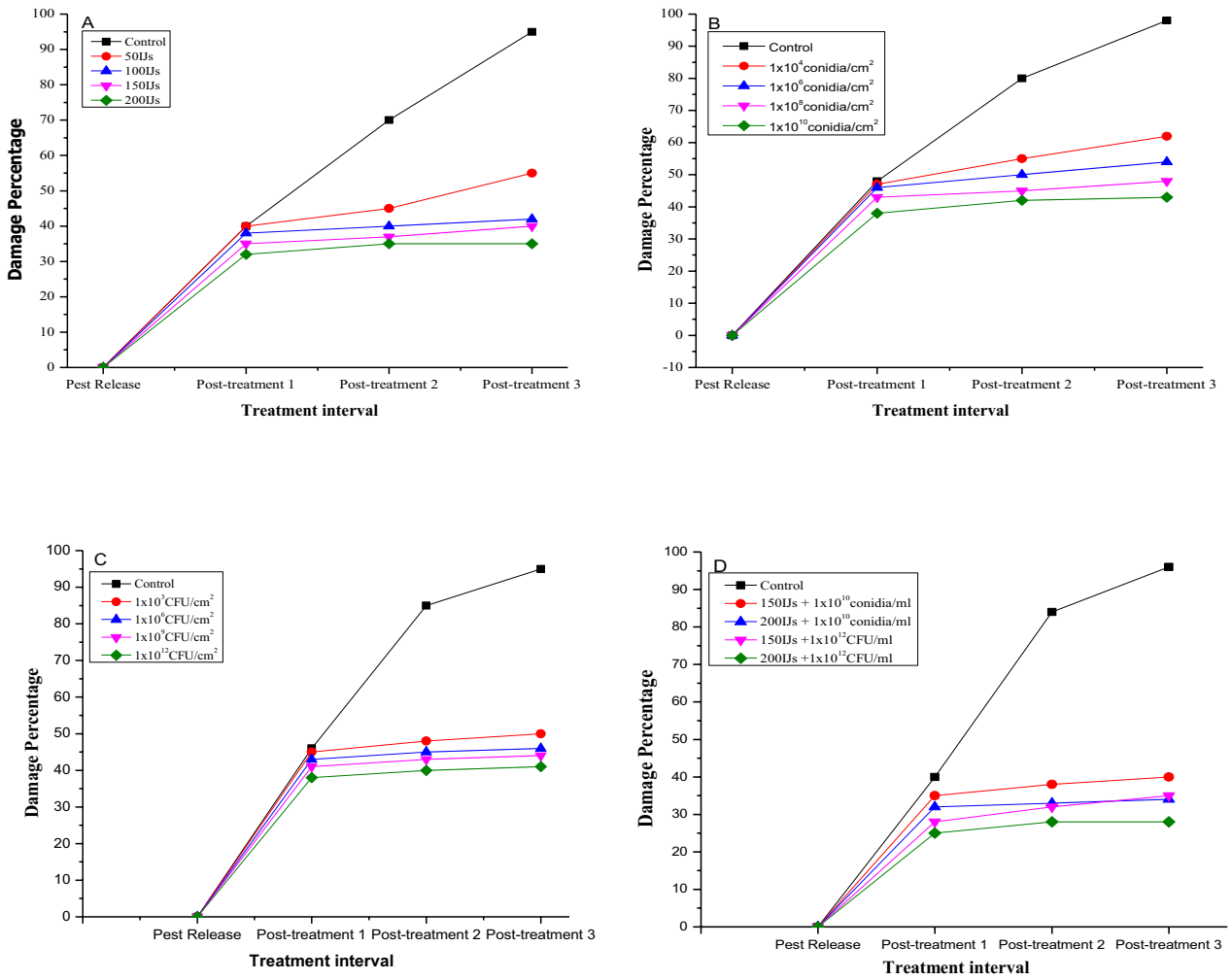

Fig. 2 Application of entomopathogens on capsicum plants against *Spodoptera litura* (3rd instar) As: **A** *Heterorhabditis bacteriophora*, **B** *Beauveria bassiana*, **C** *Bacillus thuringiensis*, **D** *H. bacteriophora* + *B. bassiana* and *H. bacteriophora* + *B. thuringiensis*

within 48 h in *S. litura* larvae when exposed to ten different isolates of *Steinernema* and *Heterorhabditis*.

B. bassiana showed significant mortality against *S. litura* under the laboratory conditions. The treatments were also effective in managing the insect in the greenhouse. Similar observations were recorded by Indriyanti et al. (2017) at Salatiga. They reported that *B. bassiana* is a powerful and eco-friendly biopesticides in managing the *S. litura* population. Moorthi et al. (2011) found that foliar sprays of *B. bassiana* were highly virulent against *S. litura*. Erawati et al. (2018) reported that application of *B. bassiana* against *S. litura* resulted in 50–60% less leaf damage than control and recommended it as effective biocontrol agent for the management of leaf-eating larvae. Ullah et al. (2019) also described the biocontrol prospective of EPF against *S. litura*. El-Husseini (2019) also reported *B. bassiana* as an effective biocontrol agent against *Spodoptera exigua* under the laboratory as well as open field conditions. Fergani and Refaei (2021) conducted a bioassay experiment by using *B. bassiana* against different developmental stages of *Spodoptera littoralis* in the laboratory conditions. They recommended *B. bassiana* as an effective biological control agent for *Spodoptera* management.

Another treatment of *B. thuringiensis* also evidenced high insect mortality in controlled at greenhouse conditions. Similar study was carried by Sajid et al. (2020) who reported 100% mortality in *S. litura* (2nd instars) by the applications of *B. thuringiensis*. Vimala Devi et al. (2021) reported that water dispersible granules of *B. thuringiensis* mixed with Tween20 and starch were highly effective against *S. litura* larvae. Singh et al. (2021) reported that cry proteins of *B. thuringiensis* were highly virulent against lepidopteran insect pest

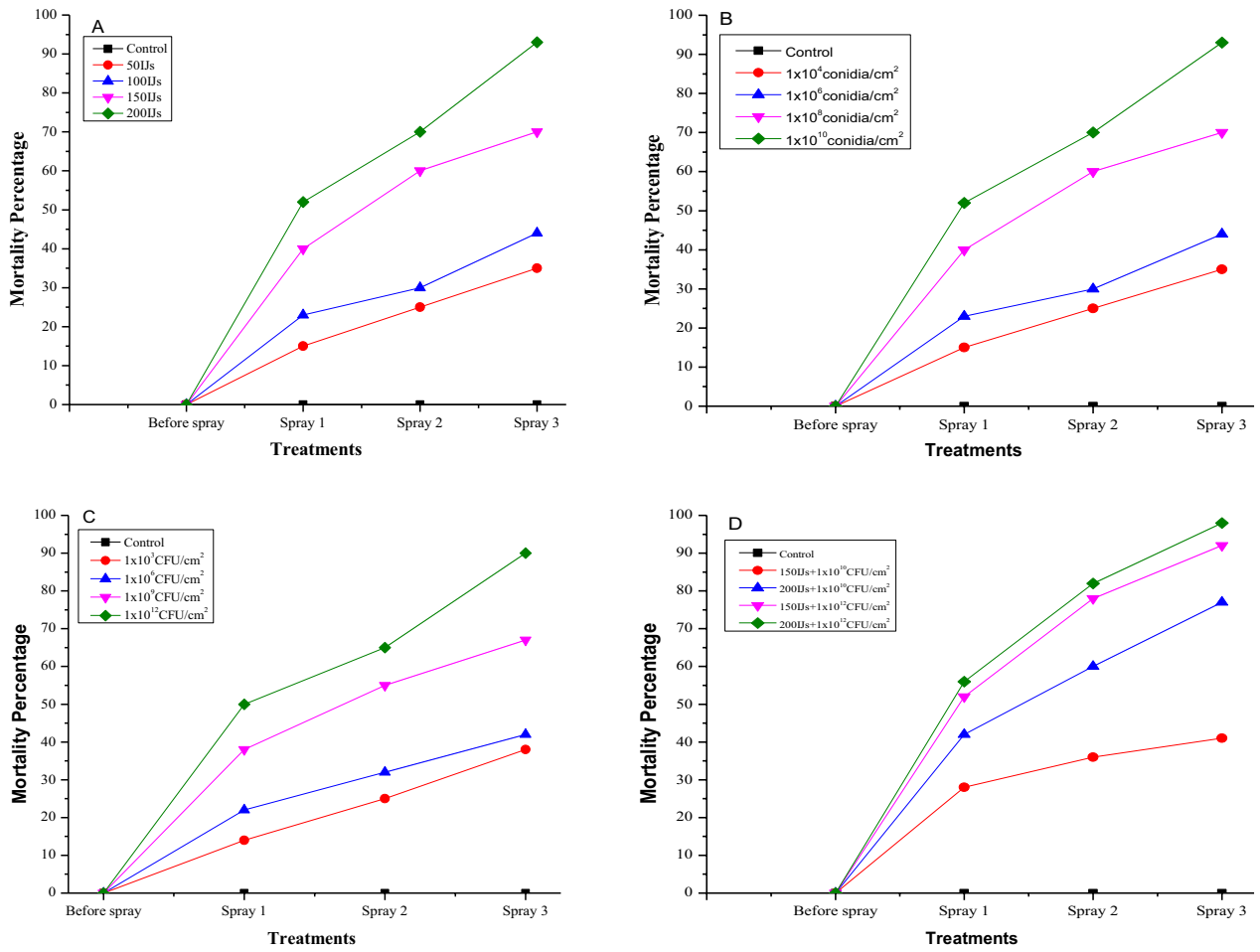

Fig. 3 Application of entomopathogens on larval mortality in capsicum plants against *Spodoptera litura* (3rd instar) As: **A** *Heterorhabditis bacteriophora*, **B** *Beauveria bassiana*, **C** *Bacillus thuringiensis*, **D** *H. bacteriophora* + *B. bassiana* and *H. bacteriophora* + *B. thuringiensis*

including *S. litura.* The combined treatment of the mixture containing *H. bacteriophora* plus *B. bassiana* and *H. bacteriophora* + *B. thuringiensis* demonstrated the effectiveness of these treatments in insect management with reduced damage percentage. The combined mixtures of treatments were recorded highly synergistic. Karthikeyan et al. (2016) demonstrated that the combination of *B. thuringiensis* with *X. bovienii* resulted in 100% larval mortality after 60 h. Synergistic and additive connections were detected in the combined mixture of treatments. *H. downesi* and *S. carpocapsae* were the EPNs used to manage destructive insect pests. Beside this, commercial strains of *M. brunneum* and *B. bassiana* were also employed. Sáenz-Aponte et al. (2020) also demonstrated that the combined applications of *H. bacteriophora* along with fungi including *B. bassiana* and *M. anisopliae* were highly effective in controlling the diamond back moth in the greenhouse and in field conditions.

Conclusion

This study was carried to investigate the pathogenicity prospective of entomopathogens against polyphagous insect species, *S. litura* under laboratory and greenhouse conditions. The outcome of this study concluded that the combined mixture of native EPNs, EPF and EPB showed high virulence toward *S. litura* in bioassay experiment and under greenhouse. The highly reliable and fast synergism observed in combined concentration containing *H. bacteriophora* plus *B. bassiana* and *H. bacteriophora* plus *B. thuringiensis* under controlled conditions and in greenhouse conditions. The study suggested that the synergistic blend of these 3 entomopathogens could be best biological control means to overcome local pest problems.

Abbreviations

EPB: Entomopathogenic bacteria; EPF: Entomopathogenic fungus; EPNs: Entomopathogenic nematodes; *B. thuringiensis*: *Bacillus thuringiensis*; *B.*

bassiana: *Beauveria bassiana*; *H. bacteriophora*: *Heterorhabditis bacteriophora*; *S. litura*: *Spodoptera litura*; *S. carpocapsae*: *Steinernema carpocapsae*; *S. feltiae*: *Steinernema feltiae*.

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Author contributions
PT, SS and SK conducted the experiment and wrote the manuscript. SS, ANY and AE-LH collaborated with useful suggestions in biocontrol. NT gave concept. All authors have read and approved the final manuscript.

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Availability of data and materials
All data generated and analyzed for the current study are presented in this manuscript, and the corresponding authors have no objection to the availability of data and materials.

Declarations

Ethical approval and consent to participate
All procedures performed in studies 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. Informed consent was obtained from all individual participants included in the study. The manuscript has been read and approved by all authors.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Zoology, Akal College of Basic Sciences, Eternal University, Sirmour 173101, India. [2]Department of Plant Pathology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Sirmour 173101, India. [3]Department of Genetics Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Sirmour 173101, India. [4]Department of Genetics, Faculty of Agriculture, Beni-Suef University, Beni-Suef 62521, Egypt.

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