Bio-efficacy of entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora* against the Cabbage butterfly *Pieris brassicae* (L.), under laboratory conditions

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Research Article

Keywords: Cabbage, Biocontrol, P. brassicae, S. feltiae, H. bacteriophora

Posted Date: March 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-304688/v1

Abstract

BACKGROUND: Keeping in view the serious health and environmental apprehensions associated with the use of pesticides, entomopathogenic nematodes have the potential to supersede larvicidal activity for the management of various insect pests.

RESULT: The lab experiments were conducted to test the pathogenicity of two EPNs species *S. feltiae*and *H. bacteriophora* at different (IJs/cm^2) concentrations against cabbage pests. Based on the pathogenicity of strains, only two isolates effectively show larvicidal activity. The native isolate was obtained from soil samples, collected from Rajgarh, Hamachi Pradesh, India. Petri dish bioassay use nematodes *S. feltiae* HR1 and *H. bacteriophora* HR2 species dose (0, 10, 20, 40, 80, 160/ IJs/cm^2). The highest (%) 2^{nd} instar larval mortality was recorded in treatments with *H. bacteriophora* and *S. feltiae* @ 160 /IJs/cm^{-2} were (72.08, 67.42 percent). And 4^{th} instar larval mortality was recorded in treatment with *H. bacteriophora*, and *S. feltiae* @ 160 /IJs/cm^{-2} were (85.38,69.50 percent). The next best treatments in order of their efficacy's pupae mortality were *H. bacteriophora* and *S. feltiae* @ 160 /IJs/cm^{-2} (74.12, percent) both are seam result, *H. bacteriophora* and *S. feltiae* @ 80 /IJs/cm^{-2} (62.12, 58.58 percent). Larvicidal activity after 48- and 72-hours exposure, the *S. feltiae* and *H. bacteriophora* (1.0, 1.30, 1.60, 1.90, 2.20 /IJs/cm^2) showed potent larvicidal activity with $LC_{50,}$ LC_{75} and LC_{90} of all instars and pupae show high mortality. The strain inhibits the larval and pupal development 48 to 72 hr exposer time with LC_{50} range from 11.30 to 39.94, LC_{75} 18.15 to 73.54, LC_{90} 61.80 to 99.21.

CONCLUSION: These studies demonstrate the challenge for cabbage butterfly *P. brassicae*. The local indigenous strains of EPNs (*S. feltiae* HR1, *H. bacteriophora* HR2) as a good biocontrol agent against, cruciferous vegetables crop pest *P. brassicae.*

Background

Cruciferous vegetables are the family Cruciferae (also known as family Brassicaceae) with many genera, species, and cultivars being raised for vegetable production such as Cabbage (*Brassica oleraceae* L. var. *capitata*) and (*Brassica oleracea* var. *botrytis*) is an important vegetable crop grown in almost all area of India. Cabbage butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae), is one of the major limiting factors of cabbage production in the Himalayas, causing severe losses to the crop by larval feeding on leaves. Young larvae scrap the leaf surface whereas older larvae consume leaves from the margins inward leaving only the main veins. In case of heavy infestation, the entire plant is eaten up. For the past few decades, chemical insecticides have been a general practice used by cabbage growers for the management of *P. brassicae* in the Himalayas but the adverse effect of chemicals on the environment such as groundwater contamination, pesticide resistance, toxicity hazards and destruction of biodiversity of useful natural enemies demand an effective alternative method of crop insect pest management that should be eco-friendly and safe for non-target organisms. Entomopathogenic nematodes (EPNs) that belong to families, *Steinernematidae* and *Heterorhabditidae* are lethal parasites of insect/pests, safe to humans, other vertebrates, non-target organisms, easy to apply, and cause no hazardous effect on the environment (Askary and Abdel Gawad 2017).

Entomopathogenic nematodes (EPNs) that belong to the families Heterorhabditidae and Steinernematidae are soil-inhabiting organisms that are obligate insect parasites in nature (Kaya and Gaugler 1993). These nematodes have evolved a mutualistic association with bacteria in the genera *Photorhabdus* is associated with *Heterorhabditis* spp., is carried in the intestine of infective juveniles (IJs) (Bird and Peters 2002; Arthurs et al. 2004; Silva et al. 2002). *Xenorhabdus* is connected with *Steinernema* spp. and confined to a specific vesicle within the intestine of the IJs. Nematodes locate their potential host by following insect cues (Lewis et al. 2006). After IJs locate a host, they infect it through an orifice such as the mouth, anus, or spiracles or by penetrating the cuticle (particularly in *Heterorhabditis* spp.). Once IJs enter the host, they shed their outer cuticle (Sicard et al. 2004) and begin ingesting hemolymph, which triggers

the release of symbionts by defecation (in *Steinernema* spp.) or regurgitation (in *Heterorhabditis* spp.) (Martens et al. 2004; Grewal et al. 2005). The nematode–bacteria complex kills the host within 24–48h through septicemia or toxaemia (Dowds and Peters 1971; Forst and Clarke 2002). Bacteria recolonize the nematodes, which emerge as IJs from the depleted insect cadaver in search of fresh hosts (Poinar 1990).

Over 100 species of EPNs have been identified globally (approximately 80% are steinernematid) and at least 13 of these species have been commercialized (Shapiro-Ilan et al. 2014). Commonly, the innate virulence against different pest species varies among EPN species. Moreover, differences among EPN species in host-seeking strategy and tolerance to environmental conditions like temperature and desiccation can determine the field efficacy of EPNs (Martens et al. 2004). EPNs have been broadly used in the biological control of a variety of economically important pests occupying different habitats (Grewal et al. 2005). However, EPN formulation to retard desiccation or the addition of adjuvants to increase leaf coverage and persistence of the IJs has enhanced the use of EPNs against foliar pests (Williams and Watters 2000; Arthurs *et al.* 2002; Head et al. 2004).

The objective of the present study was to provide fundamental information necessary for the utilization of indigenously isolated EPNs as biological control agents. The study dealt with two nematode species such as *S. feltiae* and *H. bacteriophora* (Poinar) and their pathogenicity against *P. brassicae* under laboratory conditions

Methods

A *Pieris brassicae* (L.) eggs natural infestation was collected from the department of Entomology Research farm. The eggs were maintained from under Nematology laboratory in Department of Entomology, UHF Nauni, Solan, HP, India, that used *S. feltiae* and *H. bacteriophora* (Poinar) for the pest's management.

Rearing of cabbage butterfly, *Paris brassicae* (L.)

The eggs were found in clusters on the lower sides of the leaves of few plants. They were collected from respective crop fields and brought in the laboratory and placed in a BOD incubator calibrated at 25 ± 1 °C coupled with 65 + 5 % relative humidity and the photoperiod of 12 h L: 12 h D. After calculating the percent of hatching from eggs, one hundred newly hatched larvae were collected and reared in plastic vials (10 x 12 cm) along with selective food in the form of leaves.

Entomopathogenic nematodes

In this study, two isolates of *S. feltiae* and *H. bacteriophora* were used directly in the experiments without culturing. The native isolate was obtained from soil samples, collected from Rajgarh, Himachal Pradesh, India using *G. mellonella* larvae as nematode baiting traps. This isolate was cultured based on the method (Kaya and Gaugler 1993) at 21 ± 1 °C on the last instar larvae of *G. mellonella*. Infective juveniles (IJs) that emerged during the first ten days were collected from white traps stored at 4 °C in distilled water for up to 14 days. The nematodes were acclimatized at room temperature for about 30 minutes before being used in the experiments.

Effect of Nematode Concentration

Bioassays were conducted in a petri dish (9 cm). Each unit was filled with 20 g of sand soil (Table 1). Soil moisture was adjusted to 7% ($^{w}/_{w}$). IJs were uniformly applied to the soil surface at 0, 5, 10, 20, 40, 80, and 160 IJs/cm^2 in 1 ml of distilled water. The final soil moisture was 10% ($^{w}/_{w}$). The containers were then kept at room temperature for 1 h before every instar's ten *T. absoluta* larvae per container were placed on the soil surface. There were four replicates for each concentration. The containers were kept for 72 h under controlled conditions in a growth chamber. Then, the larvae were separated from the substrate by gentle sieving and were individually maintained in controlled conditions until adult

emergence. Three days later, 25 % of the dead larvae were selected randomly and dissected under a stereomicroscope to confirm nematode infection. The experiment was conducted twice.

Larvicidal activity

Each nematode species was added at different concentrations (1.00, 1.30, 1.60, 1.90, 2.20 / IJs/cm^2) into the 9 cm petri dish in triplicate with 2 ml of dechlorinated sterile water and 10 larvae of tested *P. brassicae* strains. The larvae were provided with young cabbage leaves. One Petri plate without EPNs suspension was used as a control. After 24, 48, and 72 h the number of dead larvae was calculated. The strains that killed more than 50 % of the larvae were considered pathogenic. 24 Two nematode isolates were examined quantitatively for larvicidal activity against *P. brassicae*, using various concentrations of EPNs suspensions. The infected larvae were observed under a stereo zoom microscope for each concentration at 72 h exposure time.

Statistical Analysis

Insect mortality was control-corrected (Abbott 1925) and Arcsine transformed when required to meet assumptions of normality and homogeneity of variances. In all experiments, control-corrected mortality was subjected to one-factor analysis of variance (ANOVA)

$$\text{Corrected mortality (\%)} = \frac{\text{(\% mortality in treatment-\% mortality in control)}}{\text{100 - \% mortality in the control}} \times 100$$

The corrected percent mortality data thus obtained for different concentrations of *P. brassicae* (L.) at different concentrations were subjected to probit analysis as per the method given by Finney 1971. Concentration-mortality response data was conducted. Also, LSD *(P<* 0.05) values were calculated to differentiate means among treatments.

Results

Petri dish bioassay of *P. brassicae* life stages

The data on the efficacy of entomopathogenic nematodes for the control of larvae *P. brassicae* are presented in (Table 1) and graphically depicted in Fig. 4, Result from Table 19.1 revealed that the percent larval mortality rate significantly increased with the increase in the time periods, being maximum at 72 hrs with highest mean value, followed by 48 hrs and then 24 hrs, respectively. Percent larval mortality of *P. brassicae* treated with all treatments was significantly more as compared to untreated control.

All the entomopathogenic agents were found to be significantly superior over untreated control when observations were recorded 72 hrs after the treatment. The highest per cent 2nd instar larval mortality was recorded in treatments with *H. bacteriophora* @ 160 /IJs/cm^{-2} were (72.08 per cent), *S. feltiae* @ 160 /IJs/cm^{-2} (67.42 per cent) followed by *H. bacteriophora* and *S. feltiae* @ 80 /IJs/cm^{-2} (56.92 per cent) seam results, *H. bacteriophora* @ 40 /IJs/cm^{-2} were (49.37 per cent), *S. feltiae* @ 160 /IJs/cm^{-2} (52.31 per cent) (Fig 1). The next best treatment was 4th instar larval mortality was recorded in treatment with *H. bacteriophora* @ 160 /IJs/cm^{-2} were 85.38 percent larval mortality was recorded which was followed by *S. feltiae* @ 160/IJs/cm^{-2} (69.50 percent) (Fig 2). The next best treatments in order of their efficacy's pupae mortality were *H. bacteriophora* @ 160 /IJs/cm^{-2} (74.12 per cent), *S. feltiae* @ 160 /IJs/cm^{-2} (74.12 per cent), *H.*

bacteriophora @ 80 /IJs/cm^{-2} (62.12 per cent), *S. feltiae* @ 80 /IJs/cm^{-2} (58.58 per cent) (Fig 3). There was no larval mortality observed in the untreated control.

The treatment after 48 hrs, the result showed that the highest percent 2nd instar larval mortality was recorded in treatments with *H. bacteriophora* @ 160 /IJs/cm^{-2} were (58.58 percent), *S. feltiae* @ 160 /IJs/cm^{-2} (55.26 percent) followed by *H. bacteriophora*and *S. feltiae* @ 80 /IJs/cm^{-2} (50.81, 47.86 percent) seam results, *H. bacteriophora* and *S. feltiae*@ 40 /IJs/cm^{-2} were (43.54 percent) results in the seam (Fig 1). The next best treatments were 4th instar larval mortality was recorded in treatment with *H. bacteriophora* @ 160 /IJs/cm^{-2} was 74.12 percent larval mortality was recorded which was followed by *S. feltiae* @ 160/IJs/cm^{-2} (63.78 percent) (Fig 2). The next best treatments in order of their efficacy's pupae mortality were *H. bacteriophora* @ 80 /IJs/cm^{-2} (63.78 per cent), *S. feltiae* @ 80 /IJs/cm^{-2} (55.26 per cent). The next best treatments in order of their efficacy's pupae mortality were *H. bacteriophora* @ 160 /IJs/cm^{-2} (61.74 per cent), *S. feltiae* @ 160 /IJs/cm^{-2} (60.08 per cent), *H. bacteriophora* @ 80 /IJs/cm^{-2} (53.75 per cent), *S. feltiae* @ 80 /IJs/cm^{-2} (52.31 per cent). There was no larval mortality observed in the untreated control (Fig 3).

The treatment after 24 hrs, the result showed that the highest percent 2nd instar larval mortality was recorded in treatments with *S. feltiae* @ 160 /IJs/cm^{-2} (46.42 percent), *H. bacteriophora* @ 160 /IJs/cm^{-2} were (44.98 percent), followed by *H. bacteriophora*and *S. feltiae* @ 80 /IJs/cm^{-2} (40.65, 37.71 percent), *H. bacteriophora* and *S. feltiae* @ 40/IJs/cm^{-2} were (36.20, 34.70 percent. The next best treatments was 4th instar larval mortality was recorded in treatment with *H. bacteriophora* @ 160 /IJs/cm^{-2} was 61.74 percent larval mortality was recorded which was followed by *S. feltiae* @ 160 /IJs/cm^{-2} (53.75 percent). The next best treatments in order of their efficacy's pupae mortality were *H. bacteriophora* @ 160 /IJs/cm^{-2} (50.81 per cent), *S. feltiae* @ 160 /IJs/cm^{-2} (47.86 per cent), *H. bacteriophora* @ 80 /IJs/cm^{-2} (46.12 per cent), *S. feltiae* @ 80 /IJs/cm^{-2} (43.54 per cent). There was no larval mortality observed in the untreated control. Were moderately effective in order of their efficacy there was no high significant differences exist among the remaining treatments.

Table 1. Cabbage butterfly *Pieris brassicae* (L.) mortality (%) of different stages of larvae and pupae against entomopathogenic nematodes

Treatments	Stage	*Pieris brassicae* mortality (%) 24 hrs after treatment						
		S. feltiae			*H. bacteriophora*			
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	Mean
Control	2nd instar	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4th instar	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
	Pupae	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
10 / IJ's	2nd instar	17.50	32.50	42.50	20.00	30.00	42.50	30.83
		(24.52)	(34.70)	(40.65)	(26.18)	(33.04)	(40.59)	(33.28)
	4th instar	22.50	32.50	40.00	32.50	52.50	65.00	40.83
		(28.21)	(34.70)	(39.15)	(34.70)	(46.42)	(53.75)	(39.48)
	Pupae	20.00	32.50	45.00	22.50	35.00	45.00	33.33
		(26.18)	(34.70)	(42.09)	(28.21)	(36.20)	(42.09)	(34.91)
20 / IJ's	2nd instar	25.00	37.50	50.00	27.50	40.00	50.00	38.33
		(29.87)	(37.71)	(44.98)	(31.53)	(39.15)	(44.98)	(38.03)
	4th instar	35.00	45.00	55.00	45.00	67.50	77.50	54.16
		(36.20)	(42.09)	(47.86)	(42.09)	(55.26)	(61.74)	(47.54)
	Pupae	27.50	37.50	52.50	35.00	47.50	57.50	42.91
		(31.53)	(37.71)	(46.42)	(36.20)	(43.54)	(49.30)	(40.78)
40 / IJ's	2nd instar	32.50	47.50	62.50	35.00	47.50	57.50	47.08
		(34.70)	(43.54)	(52.31)	(36.20)	(43.54)	(49.37)	(43.27)
	4th instar	47.50	57.50	67.50	55.00	72.50	85.00	64.16
		(43.86)	(49.37)	(55.41)	(47.86)	(58.42)	(67.47)	(53.73)
	Pupae	40.00	50.00	60.00	45.00	57.50	70.00	53.75
		(39.15)	(44.98)	(50.81)	(42.09)	(49.30)	(56.76)	(47.18)
80 / IJ's	2nd instar	37.50	55.00	70.00	42.50	60.00	70.00	55.83
		(37.71)	(47.86)	(56.92)	(40.65)	(50.81)	(56.92)	(48.47)
	4th instar	55.00	67.50	77.50	67.50	80.00	90.00	72.91
		(47.86)	(55.26)	(61.74)	(55.26)	(63.78)	(74.12)	(59.67)
	Pupae	47.50	62.50	72.50	52.50	65.00	77.50	62.91
		(43.54)	(52.31)	(58.58)	(46.42)	(53.75)	(62.12)	(52.78)
160 / IJ's	2nd instar	52.50	67.50	85.00	50.00	72.50	87.50	69.16
		(46.42)	(55.26)	(67.47)	(44.98)	(58.58)	(72.08)	(57.46)
	4th instar	65.00	80.00	87.50	77.50	90.00	97.50	82.91
		(53.75)	(63.78)	(69.50)	(61.74)	(74.12)	(85.38)	(68.04)
	Pupae	55.00	75.00	90.00	60.00	77.50	90.00	74.58
		(47.86)	(60.08)	(74.12)	(50.81)	(61.74)	(74.12)	(61.45)
LSD (p< 0.05)	2nd instar	4.64	4.17	6.44	5.80	7.24	10.03	
	4th instar	5.35	6.03	6.05	4.37	8.46	9.80	
	Pupae	5.80	5.69	8.78	4.95	4.39	8.54	

Figures in parentheses are arc sine transformed values

Bioassay of Log probit analysis larvicidal activity

A toxicity assay was conducted to estimate the lethal concentration of entomopathogenic nematodes to deferent instar larvae of *Pieris brassicae*. The $LC_{50,}$ LC_{75} and LC_{90} for the *S. feltiae*and *H. bacteriophora* (1.0, 1.30, 1.60, 1.90, 2.20 /IJs/cm^2), 2nd instar for 72 h were evaluated as LC_{50} of 18.12 IJs/cm^2, 19.26 IJs/cm^2 and LC_{75} of 42.53 IJs/cm^2, 43.20 IJs/cm^2, and LC_{90} of 63.12 IJs/cm^2, 82.61IJs/cm^2 respectively (Fig 5,7) (Table 2). 2nd instar for 48 h were evaluated as LC_{50} of 37.27 IJs/cm^2, 39.94 IJs/cm^2 and LC_{75} of 46.96 IJs/cm^2, 62.35 IJs/cm^2, and LC_{90} of 87.26 IJs/cm^2, 98.73 IJs/cm^2 respectively (Fig 4,6) (Table 2).

4th instar larvae of *P. brassicae*, for 72 h were evaluated as *S. feltiae* and *H. bacteriophora* LC_{50} of 16.23 IJs/cm^2, 11.30 IJs/cm^2 and LC_{75} of 63.45IJs/cm^2, 18.15IJs/cm^2, and LC_{90} of 95.73IJs/cm^2, 61.80 IJs/cm^2, respectively (Fig 9,10). And followed by for 48 h were evaluated as LC_{50} of 26.93 IJs/cm^2, 24.02 IJs/cm^2 and LC_{75} of 49.69 IJs/cm^2, 43.14 IJs/cm^2, and LC_{90} of 96.71 IJs/cm^2, 86.70 IJs/cm^2respectively (Fig 8,10) (Table 2).

Pupae of *P. brassicae*, for 72 h were evaluated as *S. feltiae* and *H. bacteriophora* LC_{50} of 16.73 IJs/cm^2, 13.47 IJs/cm^2 (Fig 13, 15) and LC_{75} of 73.54 IJs/cm^2, 55.26 IJs/cm^2, and LC_{90} of 98.67 IJs/cm^2, 99.21 IJs/cm^2 (Fig 12,14) respectively, and followed by pupae for 48 h were evaluated as *S. feltiae* and *H. bacteriophora* LC_{50} of 36.18 IJs/cm^2, 25.54IJs/cm^2 and LC_{75} of 55.26 IJs/cm^2, 42.74 IJs/cm^2, and LC_{90} of 97.34 IJs/cm^2, 73.12 IJs/cm^2 (Fig 12, 14) respectively, were noted by using (OPSTAT) software, the best larvicidal activity was obtained during the 72 h of exposure. The *S. feltiae* and *H. bacteriophora,* strains showed potent larvicidal activity with low concentration even at 48 and 72 h of exposure to compare other strains (Table 2).

Table 2. Log probit analysis of larvicidal activity of tested of Cabbage butterfly *Pieris brassicae*(L.) mortality LC_{50}, LC_{75} and LC_{90} of different stages of larvae and pupae against entomopathogenic nematodes

Nematode sp	Stage of Insect	Exposure Time (hr)	LC_{50}(IJs/cm^2) 95%LCl-UCL	LC_{75}(IJs/cm^2) 95%LCl-UCL	LC_{90} (IJs/cm^2) 95%LCl-UCL	Intercept	Slope±SE	χ^2 value	*P*-value
S. feltiae	2nd instar	48	37.27 (21.34±65.11)	46.96 (26.88±82.03)	87.26 (49.95±52.42)	-1.25	1.3±0.22	0.57	0.005
		72	18.12 (11.61 ± 28.30)	42.53 (27.24 ± 66.40)	63.12 (40.43 ± 98.54)	-1.21	1.5±0.22	1.54	0.006
	4th instar	48	26.93 (17.91±40.48)	49.69 (33.06±74.70)	96.71 (64.34±45.37)	-1.50	01±0.23	0.16	0.003
		72	16.23 (11.01 ± 23.92)	63.45 (43.05 ± 93.53)	95.73 (64.95 ± 96.10)	-1.37	1.3±0.23	0.11	0.004
	Pupae	48	36.18 (23.28±56.24)	55.26 (35.54±85.87)	97.34 (62.63±51.36)	-1.49	1.5±0.22	0.99	0.003
		72	16.73 (11.04 ± 25.37)	73.54 (48.51 ± 85.48)	98.67 (52.83 ± 73.44)	-1.28	01±0.23	4.92	0.005
H. bacteriophora	2nd instar	48	39.94 (25.15±63.44)	62.35 (39.25±99.02)	98.73 (62.16±52.82)	-1.46	1.5±0.22	0.47	0.003
		72	19.26 (12.63 ± 29.38)	43.20 (28.32 ± 65.89)	82.61 (54.16 ± 78.99)	-1.29	02±0.23	2.87	0.005
	4th instar	48	24.02 (14.90±39.08)	43.14 (26.42±70.45)	86.70 (53.09±41.58)	-0.83	1.5±0.23	1.15	0.024
		72	11.30 (07.21 ± 17.73)	18.15 (11.57 ± 28.47)	61.80 (39.41 ± 96.92)	-0.76	01±0.26	1.25	0.046
	Pupae	48	25.54 (15.97±40.84)	42.74 (26.72±68.34)	73.12 (45.72±65.93)	-1.27	1.5±0.22	0.40	0.005
		72	13.47 (08.98 ± 20.20)	55.26 (36.85 ± 82.86)	99.21 (66.16 ± 88.77)	-1.24	1.5±0.23	0.83	0.006

Discussion

Therefore, overall results on efficacy indicated that treatment 4th instar larvae with *H. bacteriophora*@ 160 /IJs/cm^{-2} (85.38 percent) was found to be superior as compared to other treatments. However, treatment with *S. feltiae* @ 160 /IJs/cm^{-2} was found to be the next effective treatment in order of efficacy. Hence, the results of the present study corroborate the finding of Yoon *et al. (*1999) who reported that *P. brassicae* causing larval net mortality of 86.2 and 66.5 percent under laboratory and net house conditions respectively. Similar results were also reported by Fuentes and

Carballo (1995). Obtained results confirm the findings of an increase in inoculum level of IJs, time consumed for larval mortality decreased but when the larval size increased the time consumed in larval mortality also increased.

The mortality was determined through different concentrations for both 48 and 72 h exposure. The mortality rate depends on the concentration and exposure time. However, the highest mortality range was observing *H. bacteriophora* treatment at very low concentrations for 48 and 72 h. Even though *S. feltiae* showed slow mortality in 48 and 72 h exposure time, they restrained the larval development at the early pupal stage. The results are similar to the findings of EPNs pathogenicity of *H. bacteriophora* against vine mealybug, in South African vineyards. Sabry *et al*. (2016). The data revealed that the mortality rate increases with the increase in time interval *viz*., 24, 48, and 72h. These results are in agreement with Shivankar and Rao (2003) who reported that the mortality rate increased with an increase in time period.

Conclusions

The results of this study revealed that local EPN isolates were able to find and kill Cabbage butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae), larvae stages under laboratory conditions and their efficacy increased with exposure time. The efficacy of local indigenous EPN isolates was significantly superior to that of the exotic species. This is the first study carried out in Rajghar on the good biocontrol potential of indigenous strains (HR1 and HR2) EPNs against *P. brassicae*. The results of this study form the basis for further research. High EPN efficacy obtained under laboratory conditions cannot easily be extrapolated to field efficacy. Therefore, future field experiments on tomato crops are justified to fully determine the potential of local EPN isolates against *P. brassicae* in Himachal Pradesh and Indian, conditions.

Abbreviations

EPN: Entomopathogenic nematodes; IJs: Infective juveniles; OPSTAT: Operational Status

Declarations

Ethics approval and consent to participate.

Not applicable

Consent for publication.

Not applicable

Availability of data and material.

All data generated or analysed during this study are included in this article.

Competing interests.

We, the authors do not have competing interests.

Funding.

No funding from any source.

Authors Contribution.

All authors jointly designed the experiment. KI conducted the laboratory bioassays, performed data analysis and drafted the manuscript with inputs from all authors. MS, MW, MK, NT and AR collaborated closely with KI in the whole process

especially during data analysis. All authors read and approved the final manuscript.

Acknowledgements.

We would like to acknowledge the Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, HP, India-173230 for support of this research. We are also grateful to Department of entomology, Nematology laboratory used in this study.

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Figures

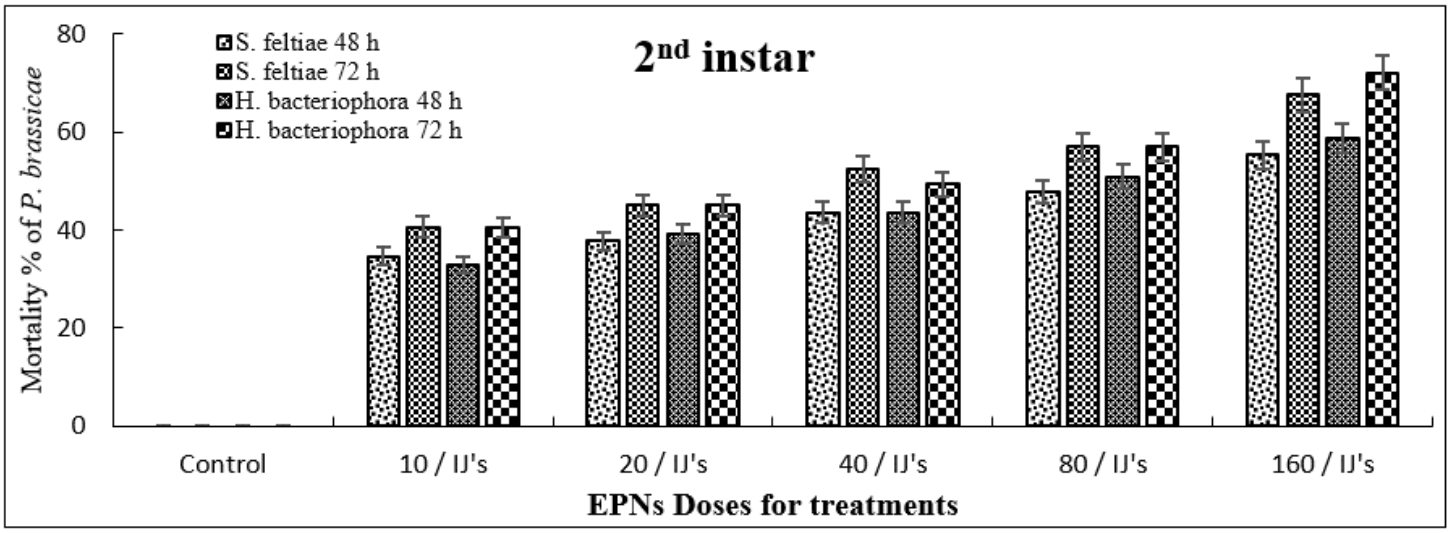

Figure 1

Efficacy of S. feltiae and H. bacteriophora against 2nd instar of P. brassicae

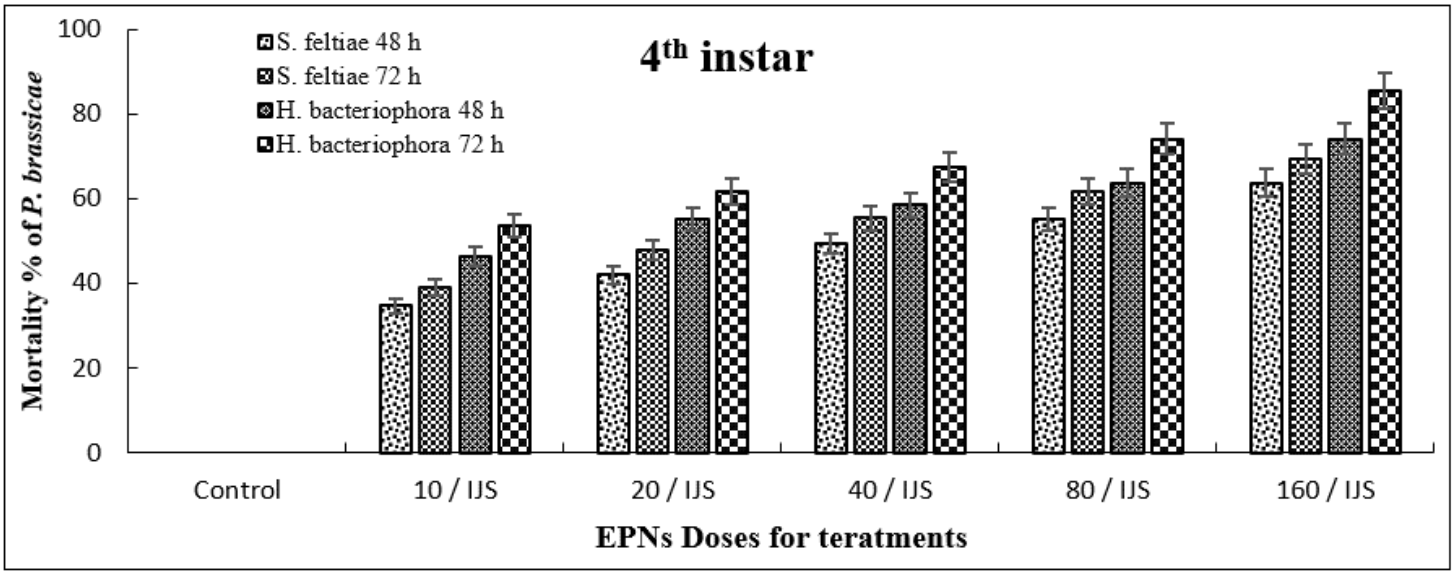

Figure 2

Efficacy of S. feltiae and H. bacteriophora against 4thinstar of P. brassicae

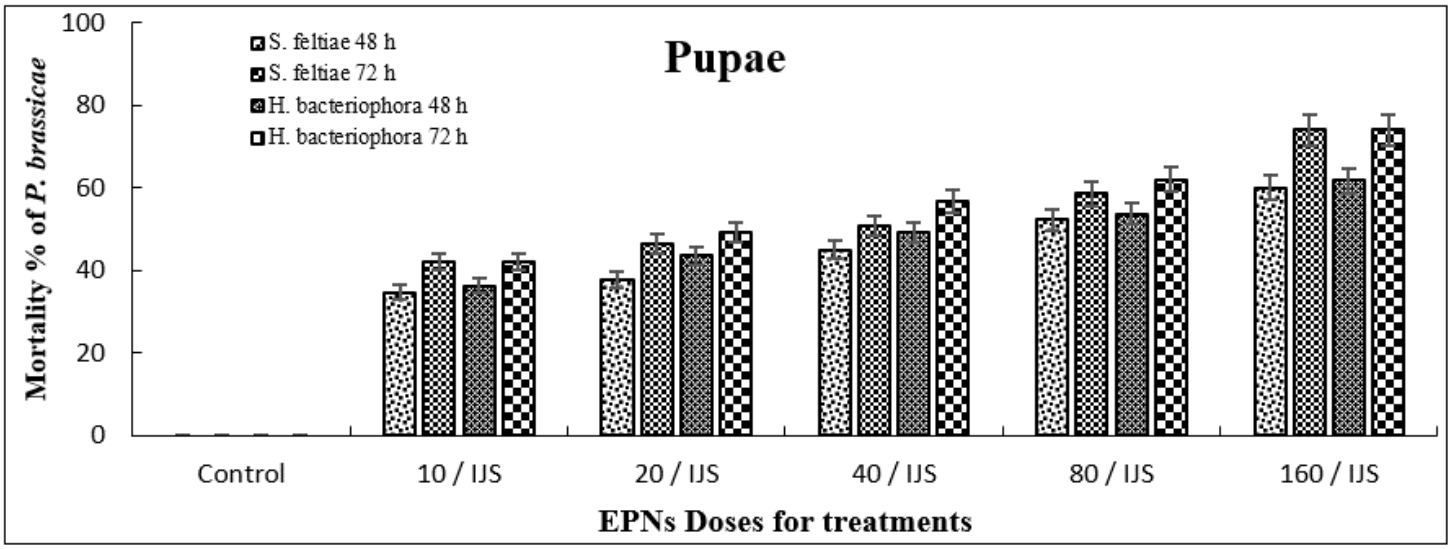

Figure 3

Efficacy of S. feltiae and H. bacteriophora against pupae stage of P. brassicae

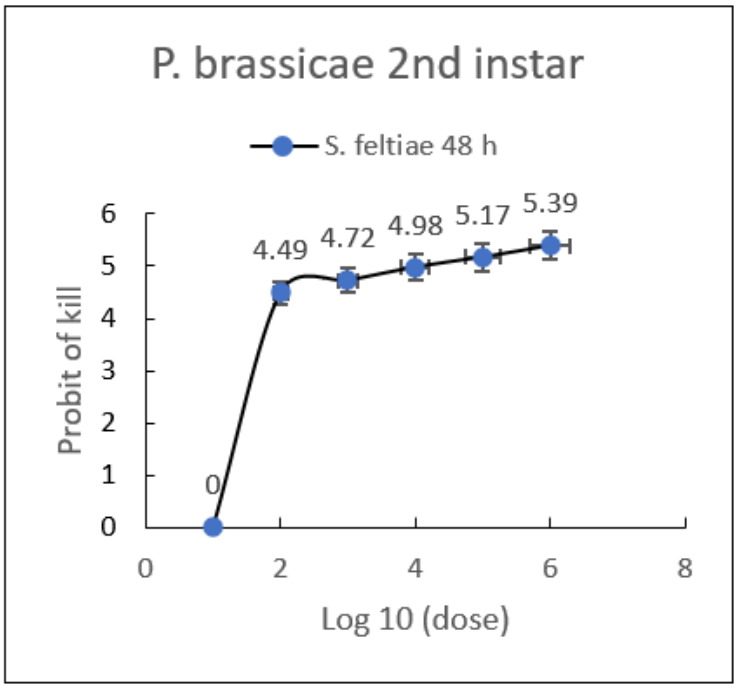

Figure 4

2nd instar Log Dose-Response Curve

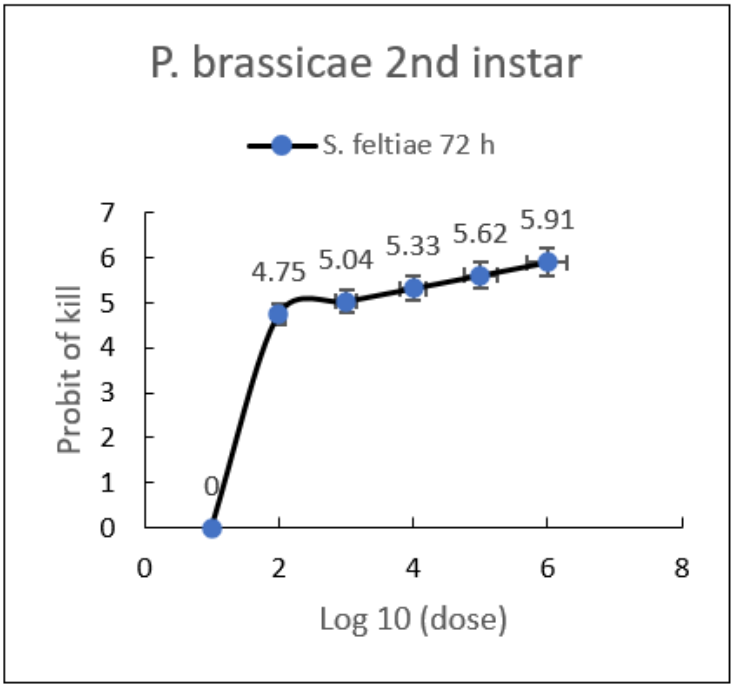

Figure 5

2nd instar Log Dose-Response Curve

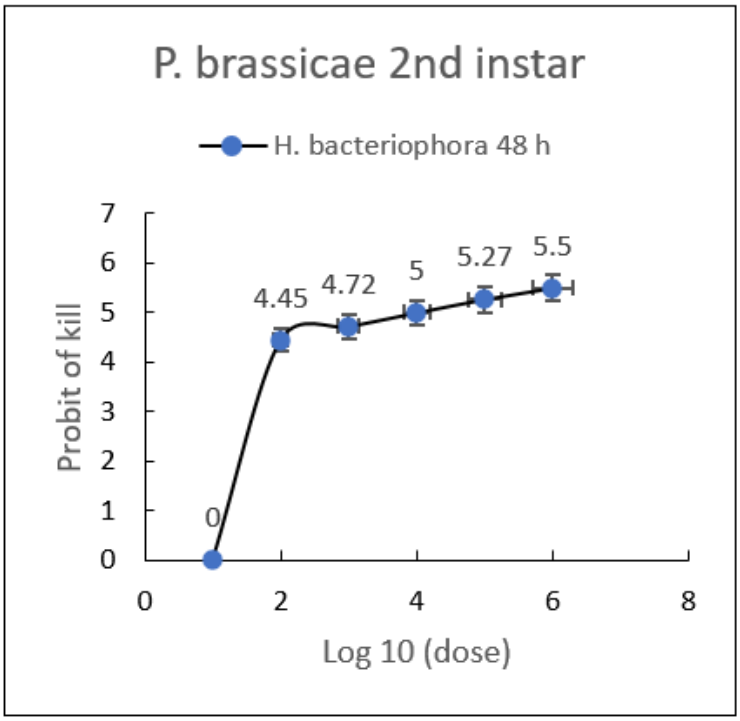

Figure 6

2nd instar Log Dose-Response Curve

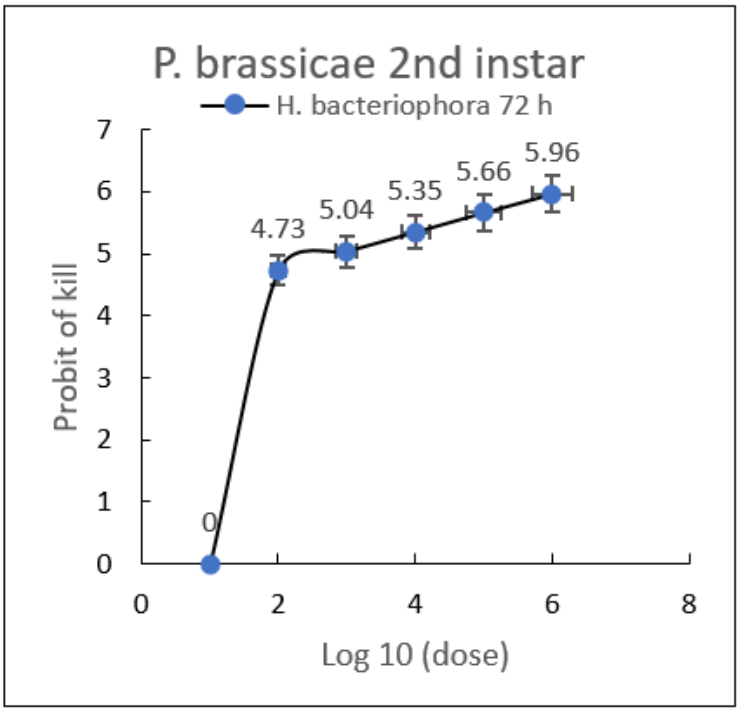

Figure 7

2nd instar Log Dose-Response Curve

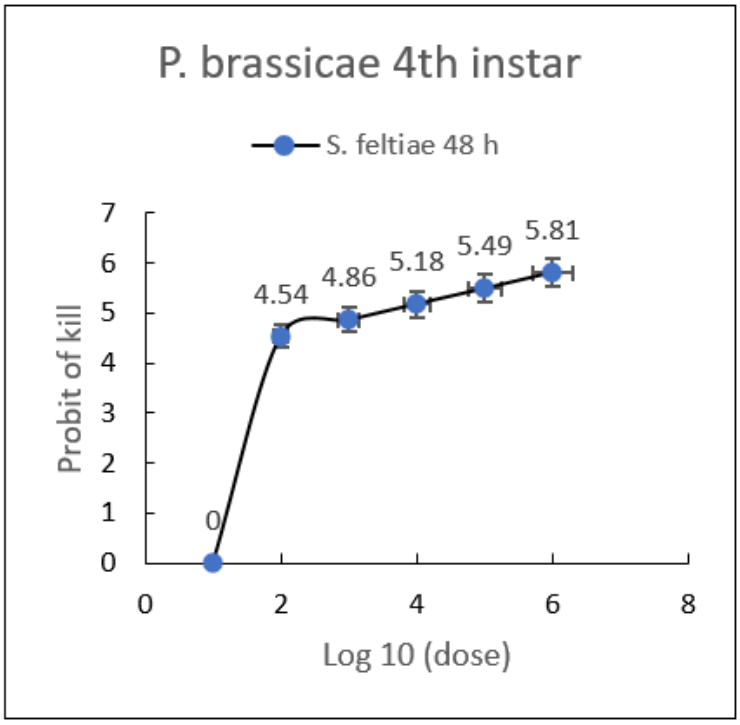

Figure 8

4th instar Log Dose-Response Curve

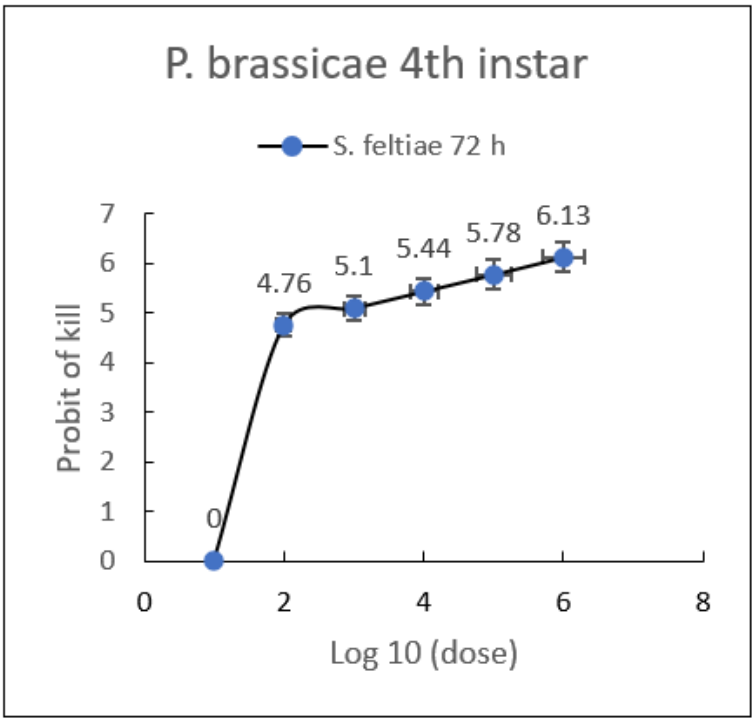

Figure 9

4th instar Log Dose-Response Curve

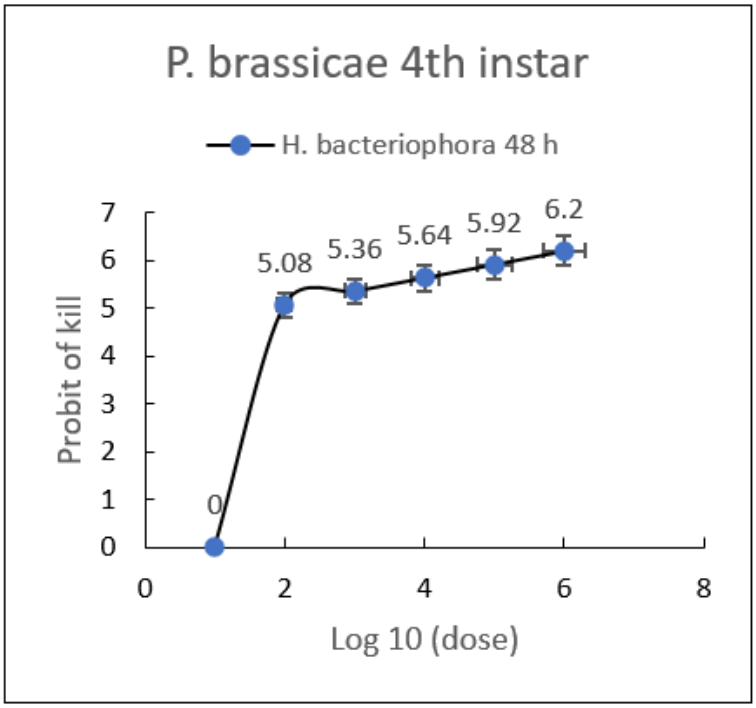

Figure 10

4th instar Log Dose-Response Curve

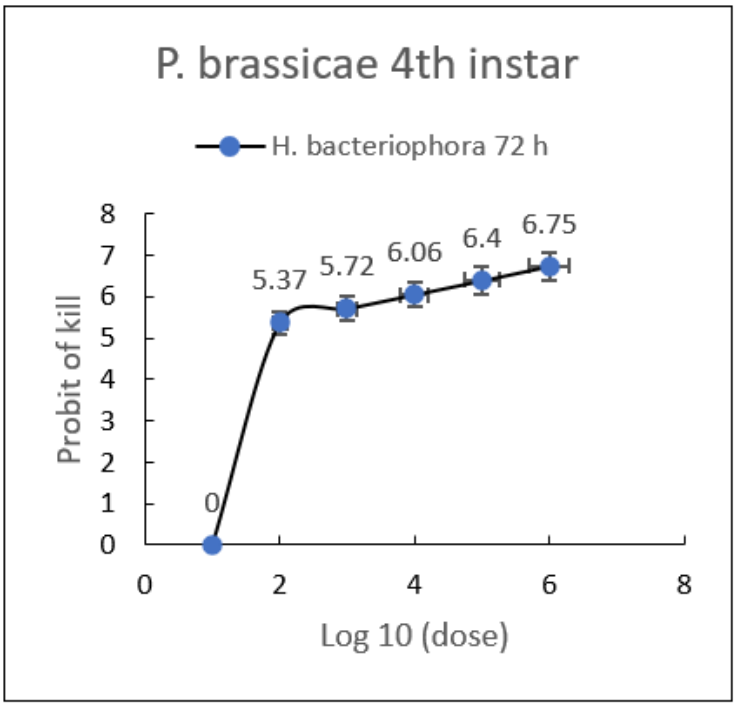

Figure 11

4th instar Log Dose-Response Curve

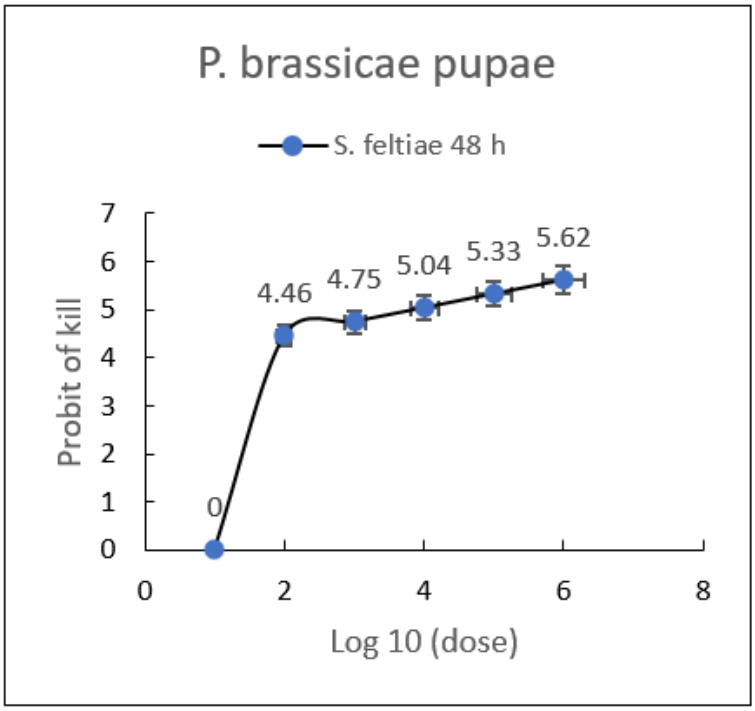

Figure 12

Pupae Log Dose-Response Curve

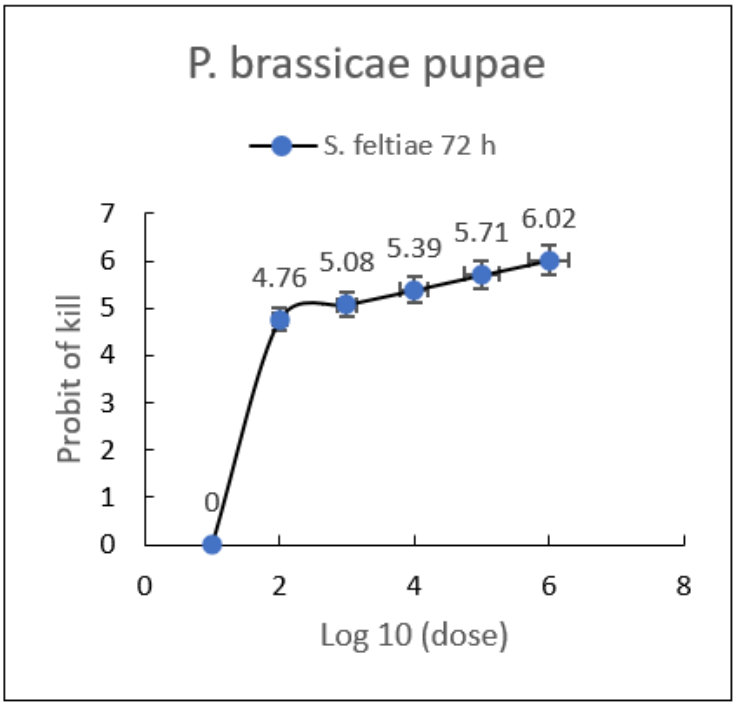

Figure 13

Pupae Log Dose-Response Curve

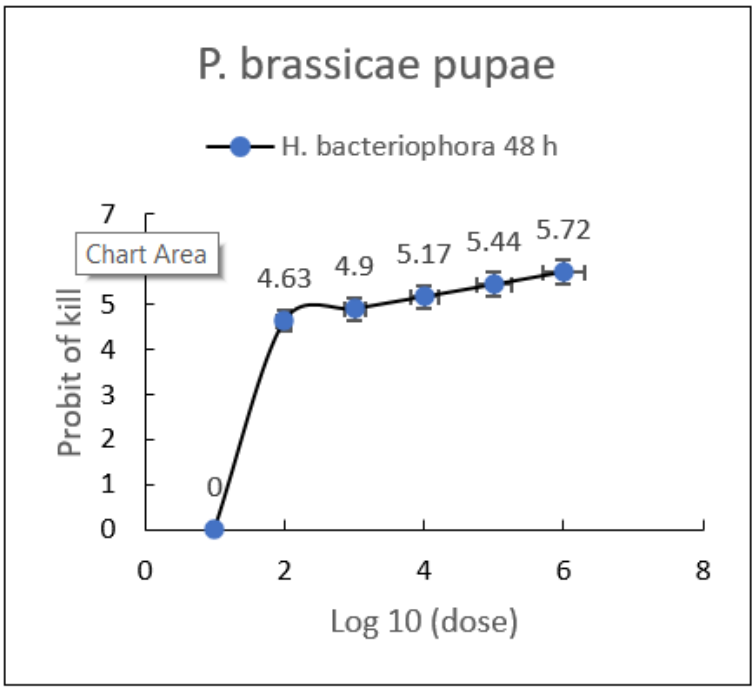

Figure 14

Pupae Log Dose-Response Curve

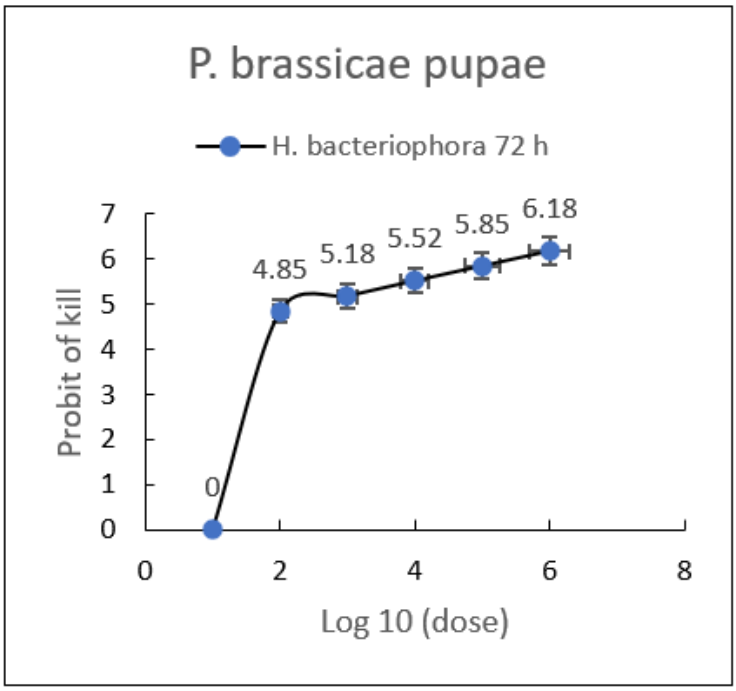

Figure 15

Pupae Log Dose-Response Curve