Effect of insecticides on generative potential of Entomopathogenic Nematode, *Steinernema abbasi* PN-1 in *Spodoptera litura* Fabricius

Abstract

This study aimed to evaluate the effects of different insecticides at varying concentrations on the progeny production of the entomopathogenic nematode *Steinernema abbasi* PN-1 in *Spodoptera litura* larvae, with a focus on determining the insecticides' compatibility with biological control agents. *S. litura* larvae were treated with a mixture of *S. abbasi* PN-1 and insecticides (fipronil, emamectin benzoate, cyantraniliprole, indoxacarb, and chlorantraniliprole) at three different concentrations (lower, medium, and higher). Progeny production of *S. abbasi* PN-1 was assessed and compared across treatments, with control groups consisting of larvae treated with *S. abbasi* PN-1 alone. The study found that progeny production was significantly influenced by the type and concentration of insecticides. Fipronil exhibited the least negative impact on progeny production across all concentrations, followed by emamectin benzoate, cyantraniliprole, indoxacarb, and chlorantraniliprole, with the latter showing the most significant inhibitory effects. Higher concentrations of insecticides resulted in a greater reduction in progeny production, indicating a dose-dependent relationship. The results suggest that certain insecticides, particularly at higher concentrations, can negatively affect the progeny production of *S. abbasi* PN-1, potentially compromising the effectiveness of EPNs in integrated pest management (IPM) programs. The study highlights the importance of selecting compatible insecticides and their appropriate concentrations to enhance the success of biological control strategies involving EPNs.

Key words: Biological control, *Steinernema abbasi* PN-1, Insecticides and *Spodoptera litura*

1. Introduction

The Entomopathogenic nematodes (EPNs) are soil inhabiting, delicate or soft bodied, non-segmented roundworms that are obligate or facultative parasites of insect pests. Entomopathogenic nematodes (EPNs) are members of the Steinernematidae and Heterorhabditidae families. The genera Steinernema and Neosteinernema belong to the Steinernematidae family, while Heterorhabditis is represented by the genus Heterorhabditis

[1]. Nematodes of the genera Heterorhabditis and Steinernema are found worldwide, infecting about 250 distinct insect species belonging to ten orders [2]. The EPNs have simple life cycle, *viz.*, egg, four juvenile stages (separated by moults) and adult stages. The infective stage is J3 (third juvenile stage), and it is known as infective juveniles (IJs). The IJs are non-feeding, free-living active stage capable of withstanding adverse environmental circumstances and the non-availability of host for an extended time period. Nowadays EPNs are mostly utilized in environments where chemical substances fail, such as galleries of boring insects, soil, or situations where pesticide resistance has developed [3]. Farmers in India use a variety of insecticides, including those from both novel classes (such as insect growth regulators, diamides, spinosyns, and avermectins) and conventional classes (such as organophosphates, carbamates, and pyrethroids), to manage insect pests [4]. However, many pesticides have become ineffective due to the development of resistance in pests and their negative impacts on the environment and human health [5]. Therefore, it is essential to reduce the frequency of spraying or the amount of active ingredient in pesticide applications, integrating them with other management strategies like biological control. Combining different control agents can enhance the effectiveness of integrated pest management (IPM) strategies, providing a quicker, more cost-effective, and efficient method for controlling insect pests. When two control agents act independently on a target host, their combined effects can be antagonistic, potentiating, or additive, depending on whether one agent's toxicity is influenced by the other agent [6-7]. Numerous studies have examined the EPNs compatibility with a variety of insecticides to combat insect pests [8-9]. This study has been conducted to determine the effect of selected insecticides (targeting common lepidopteran pests) on generative potential of *Steinernema abbasi* PN-1 under laboratory conditions.

2. Material and methods

2.1 Insect and Nematode culture:

The EPN *Steinernema abbasi* PN-1 was obtained from the Department of Entomology, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The EPN was cultured on late-instar larvae of the greater wax moth, *Galleria mellonella* Linnaeus. A fresh 2-day-old infective juvenile (IJ) solution was used for experiments. Egg masses of *Spodoptera litura* were collected from castor and guava trees at the CRC, Pantnagar, Uttarakhand. Upon hatching, the young larvae were placed in rearing boxes and provided with fresh, sterilized soft castor leaves regularly. The rearing boxes were cleaned and sterilized daily

with ethanol (70%). A running culture of *S. litura* was maintained and used for laboratory experiments. Larvae of similar size and weight were selected for the study.

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2.2 Progeny production of *S. abbasi* PN-1 in *S. litura* treated with a mixture of *S. abbasi* PN-1and insecticide.

The commonly used insecticides for lepidopteran pests were selected. The 7 days old larvae of *S. litura* were placed in a 9cm petri dish lined with filter paper. The larvae were provided with one castor leaf (5cm× 5cm) treated with selected doses of insecticides (based on a preliminary trial) for one minute and left for 3 minutes to air-dry. The Leaves were applied with 1 ml suspension containing 500IJs/ml of EPN. The Petri dishes were sealed tight immediately and incubated at 27±2 °C and 75±10 per cent RH under laboratory conditions. Control Petri dishes provided with EPN alone. Ten larvae were maintained in each petri dish, with three replicates maintained. The larvae which were dead were washed three times and placed on a white trap according to treatments [10]. The new IJs started emerging from the cadaver after 4-5 days were collected in a beaker. The IJs were collected daily until the production ceased. The beaker with harvested IJs was kept undisturbed for some time to settle down IJs and after that supernatant suspension was discarded. This process was repeated 3-4 times by adding distilled water until the clear nematode solution was formed. The IJs are counted under a stereomicroscope.

2.3 Statistical analysis

Using SPSS 10.0 for Windows software, the CD and SE(m) values are calculated.

3. Results and discussion

3.1 Progeny production of *S. abbasi* PN-1 with lower concentrations of insecticide.

Progeny production of *S. abbasi* PN-1 in *S. litura* treated with a mixture of *S. abbasi* PN-1and insecticide as shown in Table 1. The progeny production of *S. abbasi* PN-1 in *S. litura* treated with different insecticides was significantly different. The maximum progeny production (9517.33 IJs/larva) was recorded in fipronil which was followed by emamectin benzoate, cyantraniliprole, indoxacarb and chlorantraniliprole where the progeny production was 9412.3, 9386.3, 9375 and 9216.3 IJs/larva respectively. The treatment of *S. abbasi* PN-1 alone yielded 9564.33 IJs/larva. Fipronil showed a significantly higher progeny production compared to emamectin benzoate, cyantraniliprole, and chlorantraniliprole. There was no significant difference between fipronil and indoxacarb or between fipronil and the control

group. Among the tested insecticides fipronil has the least negative effect on the progeny production of *S. abbasi* PN-1 compared to other insecticides tested.

Table 1: Progeny production of *S. abbasi* PN-1 in *S. litura* treated with a mixture of *S. abbasi* PN-1and insecticides.

Treatments	Progeny production/larvae
T1: *S. abbasi* +Fipronil (0.0013%)	9517.33 (97.56) *
T2: *S. abbasi* +Emamectin benzoate (0.00012%)	9412.33 (96.89) *
T3: *S. abbasi* +Indoxacarb (0.008%)	9375 (97.02) *
T4: *S. abbasi* +Chlorantraniliprole (0.0015%)	9216.33 (96) *
T5: *S. abbasi* +Cyantraniliprole (0.003%)	9386.33 (96.88) *
T6: Control (*S. abbasi*)	9564.33 (97.8) *
CD	0.627
SE(m)	0.201

S. abbasi PN-1 @ 500IJs/ml, *values in the parenthesis are square root transformed values.

3.2 Progeny production of *S. abbasi* PN-1 with medium concentrations of insecticide.

Progeny production of *S. abbasi* PN-1 in *S. litura* treated with a mixture of *S. abbasi* PN-1and insecticide as shown in Table 2. The progeny production of *S. abbasi* PN-1 in *S. litura* treated with different insecticides was significantly different. The maximum progeny production (9414 IJs/larva) was recorded in fipronil which was followed by emamectin benzoate, cyantraniliprole, indoxacarb and chlorantraniliprole where the progeny production was 9287, 9267, 9033.6 and 8923 IJs/larva respectively. The treatment of *S. abbasi* PN-1 alone yielded 9564.33 IJs/larva. Fipronil showed a significantly higher progeny production compared to all the treatments. Among the tested insecticides fipronil showed less negative effect followed by emamectin benzoate, cyantraniliprole, indoxacarb and chlorantraniliprole showed a more negative effect on progeny production of *S. abbasi* PN-1.

Table 2: Progeny production of *S. abbasi* PN-1 in *S. litura* treated with a mixture of *S. abbasi* PN-1and insecticides.

Treatments:	Progeny production/larvae
T1: *S. abbasi* +Fipronil (0.0025%)	9414 (96.38) *
T2: *S. abbasi* +Emamectin benzoate (0.00024%)	9287 (94.48) *
T3: *S. abbasi* +Indoxacarb (0.0016%)	9033.67 (95.46) *
T4: *S. abbasi* +Chlorantraniliprole (0.003%)	8923 (93.38) *

T5: *S. abbasi* +Cyantraniliprole (0.005%)	9267.33 (94.48) *
T6: Control (*S. abbasi*)	9564.33 (97.80) *
CD	0.461
SE(m)	0.148

S. abbasi PN-1 @ 500IJs/ml, *values in the parenthesis are square root transformed values.

3.3 Progeny production of *S. abbasi* PN-1 with higher concentrations of insecticide.

Progeny production of *S. abbasi* PN-1 in *S. litura* treated with a mixture of *S. abbasi* PN-1and insecticide as shown in Table 3. The progeny production of *S. abbasi* PN-1 in *S. litura* treated with different insecticides was significantly different. The maximum progeny production (9289.6 IJs/larva) was recorded in fipronil which was followed by emamectin benzoate, indoxacarb, cyantraniliprole and chlorantraniliprole where the progeny production was 9112.3, 8927, 8926.3 and 8719.6 IJs/larva respectively. The treatment of *S. abbasi* PN-1 alone yielded 9564.33 IJs/larva. Fipronil showed a significantly higher progeny production compared to all the treatments. Among the tested insecticides fipronil showed less negative effect compared to all the treatments.

All the treatments with lower concentration insecticides have less effect on progeny production in comparison to the higher concentrations of insecticides with *S. abbasi* PN-1. Among the tested insecticides fipronil showed less negative effect followed by indoxacarb, cyantraniliprole, emamectin benzoate and chlorantraniliprole showed a more negative effect on progeny production of *S. abbasi* PN-1. Some insecticides are less toxic to EPNs may be because of the presence of the butyrylcolinesterase in the synapse of EPNs. Selkirk *et al*. [11] reported the action of butyrylcholinesterase as a first line of defense in nematodes and it may protect against early attack by inhibitors to acetylcholinesterase therefore, in contrast to such inhibitor compounds, it is directly involved in the defense. Bhat *et al.* [12] reported that sometime IJs are sheathed with previous molt cuticle and it may not allow the penetration of some insecticides and other substances into the IJs body. The findings were similar to studies conducted [13], where they tested the effect of insecticide treated larvae of *Hyphantria cunea* (Fall webworm) on the progeny production of *S. carpocapsae* and they observed no negative effect of insecticides on progeny production of *S. carpocapsae* under laboratory conditions. Ozdemir *et al*. [14] studied the progeny production of *S. feltiae* and *H. bacteriophora* exposed to insecticide and observed that imidacloprid recorded no negative effect on progeny production but spinosad recorded a negative effect on progeny production both the EPN species in *G. mellonella.*

Table 3: Progeny production of *S. abbasi* PN-1 in *S. litura* treated with a mixture of *S. abbasi* PN-1and insecticides.

Treatments	Progeny production /larvae
T1: *S. abbasi* +Fipronil (0.005%)	9289.67 (97.03) *
T2: *S. abbasi* +Emamectin benzoate (0.00036%)	9112.33 (95.05) *
T3: *S. abbasi* +Indoxacarb (0.0024%)	8927 (96.34) *
T4: *S. abbasi* +Chlorantraniliprole (0.0045%)	8719.67 (94.46) *
T5: *S. abbasi* +Cyantraniliprole (0.007%)	8926.33 (96.27) *
T6: Control (*S. abbasi*)	9564.33 (97.80) *
CD	0.089
SE(m)	0.6

S. abbasi PN-1 @ 500IJs/ml, *values in the parenthesis are square root transformed values.

4. Conclusion:

This study evaluated the effects of different concentrations of insecticides on the progeny production of *Steinernema abbasi* PN-1 in *Spodoptera litura*. Our findings indicate that the progeny production of *S. abbasi* PN-1 was significantly affected by the type and concentration of the insecticides used. Among the insecticides tested, fipronil consistently showed the least negative impact on progeny production across all concentrations, followed by emamectin benzoate, cyantraniliprole, indoxacarb, and chlorantraniliprole, with the latter exhibiting the most substantial inhibitory effects. The negative impact on progeny production increased with the concentration of the insecticides, underscoring the sensitivity of *S. abbasi* PN-1 to higher doses of chemical treatments. The presence of butyrylcholinesterase and the sheathing of infective juveniles (IJs) with the previous molt cuticle may provide some level of protection against certain insecticides. These findings contribute to the understanding of the interaction between entomopathogenic nematodes (EPNs) and insecticides, offering insights for integrated pest management (IPM) strategies where the compatibility of biocontrol agents and chemical treatments is crucial. The study suggests that careful selection and concentration of insecticides are essential to minimize the negative effects on beneficial EPNs, thereby enhancing their efficacy in biological control programs.

Data availability statement

All the data have been provided in the main text of the manuscript.

Ethics approval statement

Not Applicable

Consent to participate

Not Applicable

Consent to publish

Not Applicable

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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