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COMPATIBILITY OF ENTOMOPATHOGENIC NEMATODE, *STEINERNEMA ABBASI* PN-1 WITH INSECTICIDES AND THEIR COMBINED EFFECT ON TOBACCO CATERPILLAR, *SPODOPTERA LITURA* (FABRICIUS)

P. Vireesha*, R. Pandey, M. Shashikala, N. Mehra and S. Kumar

Department of Entomology, College of Agriculture, G. B. P. U. A. & T., U. S. Nagar - 263 145, Uttarakhand, India.

*Corresponding author E-mail : veerubabbur16@gmail.com

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ABSTRACT

Use of biological control agents and pesticides would continue to play important role in integrated pest management programme in view of complexity of pest problem, therefore selection of pesticides must be based on its compatibility against other method of pest management. This study aimed to determine the interaction between insecticides, Chlorantraniliprole, fipronil cyantaniliprole, indoxacarb and emamectin benzoate with *Steinernema abbasi* PN-1 and their combined effect against *Spodopetra litura* under laboratory condition. The maximum mortality of infective juveniles of *S. abbasi* PN-1 was recorded in Chlorantraniliprole followed by emamectin benzoate, indoxacarb, cyantaniliprole and fipronil at 72 hours after treatment. In compatibility studies, it is concluded that tested insecticides fipronil is more compatible followed by cyantaniliprole, indoxacarb, emamectin benzoate and Chlorantraniliprole. The combined efficacy of *S. abbasi* PN-1 with insecticides given additive interactions after 42 hours of treatments and the more antagonistic interaction is found at 36 hours after treatments this may be due to delayed action of nematodes in the presence of insecticides. This study can be a practical strategy for managing *S. litura* and could pave the way to using new control technologies in protecting crops from pest.

Key words : *Steinernema abbasi* PN-1, *Spodoptera litura*, Insecticides, Additive interactions, Antagonistic interaction.

Introduction

Spodoptera litura Fabricius, a polyphagous pest belonging to the Noctuidae family, is a widespread pest that affects over 120 host plants, such as cotton, maize, tobacco, groundnut, vegetables, legumes and fodder crops (Ahmad and Mehmood, 2015). The younger instar larvae consume the mesophyll and leave behind the vein pattern of the leaf on the plant. As they grow, caterpillars feed on entire leaves, as well as fruits and flowers and at high larval density complete defoliation is possible (Xue *et al*., 2010). Farmers spray a wide range of insecticides, including those from novel (insect growth regulators, diamides, spinosyns, and avermectins) and conventional (organophosphates, carbamates and pyrethroids) classes, to manage this pest in India (Natikar and Balikai, 2017). Many pesticides are currently ineffective in controlling *S. litura* because of the development of pesticide resistance and their detrimental effects on the environment and human health (Pavela, 2007; Gandhi *et al*., 2016). Thus, it is crucial to reduce the frequency of spraying or the amount of active ingredient in pesticide applications and to mix them with other management strategies like biological control (Laznik and Trdan, 2014). Combining different control agents can increase the effectiveness of IPM strategies and offer a quicker, less expensive, and more efficient way to manage *S. litura*. When two control agents act independently on a target host, their combined actions can be antagonistic, potentiating, or additive depending on whether one component's toxicity is altered by the other (Robertson *et al*., 2017).

The Bio-pesticides, including nuclear polyhedrosis

viruses (NPVs), entomopathogenic nematodes (EPNs) and *Bacillus thuringiensis* have been reported as effective tools in integrated pest management due to their pest selectivity and lower toxicity to beneficial arthropods (Sharma *et al*., 2022). EPNs, particularly those belonging to the families Steinernematidae and Heterorhabdiridae, have been successfully used as safe biocontrol agents in pest management (Acharya *et al*., 2020). Numerous studies have examined the EPNs' compatibility with a variety of insecticides to combat *S. litura*. The EPNs have a significant potential for controlling numerous insect pests of economic significance in agriculture, according to reports on the toxicity of some conventional and non-conventional insecticides with novel chemistries (Khan *et al*., 2018; Khan *et al*., 2021). This study has been conducted to determine the effect of selected insecticides (targeting common lepidopteran pests) on the survival rates and virulence of *Steinernema abbasi* under laboratory conditions.

Materials and Methods

Insect and Nematode culture

The egg masses of *S. litura* were collected from castor trees in CRC, Pantnagar, Uttarkhand, India. After hatching of the eggs, young larvae were provided with fresh and well-sterilized soft castor leaves in the rearing boxes. The rearing boxes are cleaned and sterilized daily and provided with fresh leaves.A running culture of *S. litura* was maintained and used for experiments in the laboratory.

The EPN *S. abbasi* PN-1 was collected from the Department of Entomology, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttrakhand, India. The EPN was cultured on late instar larvae of the greater wax moth, *Galleria mellonella* Linnaeus (Woodring and Kaya, 1988). The two days old Fresh IJs solution was used for all the experiments.

Toxicity of insecticides to EPN *S. abbasi* PN-1

The commonly used insecticides for lepidopteran pests were selected for the compatibility test. The compatibility test of *S. abbasi* PN-1 with insecticides was done at the field recommended dose of the selected insecticides (Table 1). The stock solution of insecticides was prepared in distilled water. The sterilized glass test tube was filled with 1 ml of prepared insecticide solution and 10 µl of infective juveniles (IJs) was added to the insecticide solution at 10IJs/µl. The test tubes were gently tapped for thoroughly mixing of treatment solutions. The distilled water without chemicals is considered as control and test tubes are incubated at 25 ± 2°C. All the treatments were repeated four times under the same conditions. Mortality of the IJs was determined by observing under a stereomicroscope at 48, 72 and 96 hours of post treatment and non-responding IJs upon touching with a needle were considered dead. The tested insecticide which gives less than 25 per cent mortality of IJs was considered as least toxic and used for the combined efficacy test.

Efficacy of insecticides and EPN mixtures against *S. litura*

Five insecticides were selected from the compatibility study and the combined efficacy of insecticides with *S.abbasii* PN-1 was evaluated against *S. litura*. 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 one castor leaf washed in distilled water. A treatment containing the EPN alone was served as a negative control for Co-toxicity factor calculations. Ten larvae were maintained in each petri dish, with three replicates maintained and larval mortality was recorded in a 12 hours interval of post-treatment.

Statistical analysis

The mortality data of *S. litura* and *S. abbasi* PN-1 IJs were transformed into mean per cent mortality and a one-way analysis of variance was used for statistical analysis of these data. Tukey's test at $P<0.05$ was used to analyze the difference between the treatments (Littell *et al*., 2002).

The combined action of the *S. abbasi* PN-1 and insecticides was estimated by using Richer's (1987) formula: $E = (X + Y) – XY /100$

Where, E = expected mortality, X and Y = mortality percentages resulting from EPN and insecticides, respectively.

The calculated expected effect was compared to the observed effect obtained from combined efficacy study according to Mansour *et al*. (1966):

$$\text{Co-toxicity Factor} = \frac{\text{Observed effect (\%)} – \text{Expected effect (\%)}}{\text{Expected effect (\%)}} \times 100$$

If the co-toxicity factor ≥ +20 was considered

Table 1 : List of insecticides (CIBRC, 2022) selected for compatibility test with *S. abbasi* PN-1.

Insecticides	Trade name	IRAC mode of action classification	Recommended dose a.i. (gm/ha)
Chlorantraniliprole 18.50%SC	Coragen	Ryanodine receptor modulators (Diamides)	30
Cyantraniliprole 10.26% OD	Benevia	Ryanodine receptor modulators (Diamides)	60
Emamectin benzoate 5% SG	Proclaim	Glutamate-gated chloride channel (GluCl) allosteric modulators (Avermectins)	11
Fipronil 5% SC	Mahaveer SC	GABA-gated chloride channel blockers (Phenylpyrazoles)	45-50
Indoxacarb 15.80% EC	Indogoldplus	Voltage-dependent sodium channel blockers (Oxadiazines)	30

synergetic/potentiation, while (–20 to+20) indicated an additive effect and ≤ –20 was considered antagonism.

Results

Evaluation of selected insecticides toxicity at field recommended dose against *S. abbasi* PN-1

The five insecticides were tested to check compatibility with *S abbasi* PN-1 at field recommended dose. Mortality of the IJs was determined by observing under a stereomicroscope at 48, 72 and 96 hours of post treatment. At 48 hours after treatment, the mean per cent mortality of *S. abbasi* PN-1 at different concentrations was significantly different (F=84.45; P=0.00). The maximum mortality (9.6%) was recorded in Chlorantraniliprole, while no mortality was observed in control. At 72 hours after treatment, the IJs mortality ranged from 5.6 to 15 per cent, which was significantly different at different concentrations (F =314.4; P = 0.00). The maximum mortality (15%) was recorded in chlorantraniliprole which was significantly higher than all other treatments. At 96 hours after treatment, the IJs mortality ranged from 20.6 to 8 per cent, which was significantly different at different concentrations (F = 409.86; P = 0.00). The maximum mortality (20.6%) was recorded in chlorantraniliprole which was significantly higher than all other treatments. This was followed by emamectin benzoate, indoxacarb, cyantaniliprole and fipronil in which 12.6, 12.3, 11 and 8 per cent mortality was recorded, respectively; while no mortality was observed in control.

Table 2 : Toxicity of selected insecticides at field recommended dose against *S. abbasi* PN-1.

Treatment	Mean per cent Mortality		
	48HAT	72HAT	96HAT
T_1: Fipronil 5% SC	2.33^{b}	5.66^{b}	8^{b}
T_2: Emamectin benzoate 5% SG	3^{bc}	7.66^{c}	12.66^{d}
T_3: Indoxacarb 15.80% EC	3.33^{bc}	8.33^{c}	12.33^{cd}
T_4: Cyantraniliprole 10.26% OD	4.33^{c}	7.33^{c}	11^{c}
T_5: Chlorantraniliprole 18.50%SC	9.66^{d}	15^{d}	20.66^{e}
T_6: Control	0^{a}	0^{a}	0^{a}
F value	80.45	314.4	409.86
P value	0.00	0.00	0.00

Mean followed by the same letters in the column do not differ by Tukey's test ($p<0.05$).

Combined efficacy of *S. abbasi* PN-1 with insecticides against *S. litura*

The efficacy of a mixture of insecticide and *S. abbasi* PN- 1 was tested against *S. litura* and mortality was recorded at 36h and 48h after treatment. The combined efficacy of *S. abbasi* PN-1 and insecticides against *S. litura* is presented in Tables 3 and 4.

At 36 hours after treatment the mean per cent mortality rate of *S. litura* larvae exposed to different concentrations was significantly different. The maximum mortality of 93.33per cent was recorded with an additive interaction (-6.66), when *S. abbasi* PN-1 was applied with emamectin benzoate 5% SG at 0.00036 per cent dose. The least mortality of 46.33 per cent with an antagonistic interaction (-53.3) was recorded in *S. abbasi* PN-1+ indoxacarb 15.80% EC at 0.008 per cent dose. The 33.3 per cent larval mortality was observed when *S. abbasi* PN-1 was applied alone.

At 46 hours after treatment the mean per cent mortality rate of *S. litura* larvae exposed to different concentrations was significantly different. The maximum mortality of 100per cent was recorded with an additive interaction (3.8) when

Table 3 : Determination of Co toxicity factor for *S. abbasi* PN-1 with insecticides at 36h after treatment.

Co toxicity factor for *S. abbasi* PN-1 with Cyantraniliprole 10.26% OD (Cyantraniliprole in per cent)										
Treatments:	**T_1: SA**	**T_2: 0.003%**	**T_3:0.005%**	**T_4:0.007%**	**T_5:0.003% +SA**	**T_6:0.005% +SA**	**T_7:0.007% +SA**	**T_8: Control**	**F value**	**P value**
***Mean per cent Mortality**	33.33^{b}	43.33^{bc}	53.33^{cd}	66.66^{de}	53.33^{cd}	60^{cde}	76.66^{e}	0^{a}	44.69	0
Co toxicity factor	-	-	-	-	-14.28	-12.9	-1.42	-	-	-
Interactions	-	-	-	-	Additive	Additive	Additive	-	-	-
Co toxicity factor for *S. abbasi*PN-1 with Fipronil 5% SC (Fipronil in per cent)										
Treatments:	**T_1: SA**	**T_2: 0.0013 %**	**T_3:0.0025%**	**T_4:0.005%**	**T_5:0.0013% +SA**	**T_6:0.0025% +SA**	**T_7:0.005% +SA**	**T_8: Control**	**F value**	**P value**
Mean per cent Mortality*	33.33^{b}	43.33^{b}	76.66^{c}	86.66^{c}	46.66^{b}	83.33^{c}	86.66^{c}	0^{a}	102.02	0
Co toxicity factor	-	-	-	-	-53.33	-16.66	-13.33	-	-	-
Interactions	-	-	-	-	Antagonism	Additive	Additive	-	-	-
Co toxicity factor for *S. abbasi*PN-1 with Emamectin benzoate 5% SG (Emamectin benzoate in per cent)										
Treatments:	**T_1: SA**	**T_2: 0.00012 %**	**T_3:0.00024 %**	**T_4:0.00036 %**	**T_5:0.00012 %+SA**	**T_6:0.00024 %+SA**	**T_7:0.00036 %+SA**	**T_8: Control**	**F value**	**P value**
Mean per cent Mortality*	33.33^{b}	56.66^{c}	76.66^{de}	86.66^{ef}	63.33^{cd}	80^{ef}	93.33^{f}	0^{a}	116.92	0
Co toxicity factor	-	-	-	-	-36.66	-20	-6.66	-	-	-
Interactions	-	-	-	-	Antagonism	Antagonism	Additive	-	-	-
Co toxicity factor for *S. abbasi*PN-1 with Indoxacarb 15.80% EC (Indoxacarb in per cent)										
Treatments:	**T_1: SA**	**T_2: 0.008 %**	**T_3:0.0016 %**	**T_4:0.0024 %**	**T_5:0.008 %+SA**	**T_6:0.0016 %+SA**	**T_7:0.0024 %+SA**	**T_8: Control**	**F value**	**P value**
Mean per cent Mortality*	33.33^{b}	40^{b}	56.66^{c}	80^{d}	43.33^{b}	56.66^{c}	83.33^{d}	0^{a}	103.2	0
Co toxicity factor	-	-	-	-	-56.66	-43.33	-16.66	-	-	-
Interactions	-	-	-	-	Antagonism	Antagonism	Additive	-	-	-
Co toxicity factor for *S. abbasi*PN-1 with Chlorantraniliprole 18.50%SC (Chlorantraniliprolein per cent)										
Treatments:	**T_1: SA**	**T_2: 0.0015 %**	**T_3:0.003 %**	**T_4:0.0045 %**	**T_5:0.0015 %+SA**	**T_6:0.003 %+SA**	**T_7:0.0045 %+SA**	**T_8: Control**	**F value**	**P value**
***Mean per cent Mortality**	33.33^{bc}	30^{b}	43.33^{cd}	66.66^{e}	46.66^{bd}	53.33^{cd}	70^{e}	0^{a}	72.42	0
Co toxicity factor	-	-	-	-	-53.33	-46.66	-30	-	-	-
Interactions	-	-	-	-	Antagonism	Antagonism	Antagonism	-	-	-

Mean followed by same letters in the column do not differ by Tukey's test ($p<0.05$); *Mean per cent mortality after 36 HAT, SA: *S. abbasi* PN-1 @ 500IJs/ml.

Table 4 : Determination of Co toxicity factor for *S. abbasi* PN-1 with insecticides at 46h after treatment.

Co toxicity factor for *S. abbasi* PN-1 with Cyantraniliprole 10.26% OD (Cyantraniliprole in per cent)										
Treatments:	**T_1: SA**	**T_2: 0.003%**	**T_3:0.005%**	**T_4:0.007 %**	**T_5:0.003 %+SA**	**T_6:0.005 %+SA**	**T_7:0.007 %+SA**	**T_8: Control**	**F value**	**P value**
***Mean per cent Mortality**	63.33^{c}	46.66^{b}	63.33^{c}	80^{ef}	66.66^{cd}	76.66^{d}	90^{f}	0^{a}	111.42	0
Co toxicity factor	-	-	-	-	-17.12	-11.42	-2.87	-	-	-
Interactions	-	-	-	-	Additive	Additive	Additive	-	-	-
Co toxicity factor for *S. abbasi*PN-1 with Fipronil 5% SC (Fipronil in per cent)										
Treatments :	**T_1: SA**	**T_2: 0.0013 %**	**T_3:0.0025 %**	**T_4:0.005 %**	**T_5:0.0013 %+SA**	**T_6:0.0025 %+SA**	**T_7:0.005 %+SA**	**T_8: Control**	**F value**	**P value**
Mean per cent Mortality*	63.33^{c}	50^{b}	83.33^{d}	90^{de}	66.66^{c}	86.66^{de}	96.66^{e}	0^{a}	148	0
Co toxicity factor	-	-	-	-	-18.36	-7.69	0.34	-	-	-
Interactions	-	-	-	-	Additive	Additive	Additive	-	-	-
Co toxicity factor for *S. abbasi*PN-1 with Emamectin benzoate 5% SG (Emamectin benzoate in per cent)										
Treatments:	**T_1: SA**	**T_2: 0.00012 %**	**T_3:0.00024 %**	**T_4:0.00036 %**	**T_5:0.00012 %+SA**	**T_6:0.00024 %+SA**	**T_7:0.00036 %+SA**	**T_8: Control**	**F value**	**P value**
Mean per cent Mortality*	63.33^{b}	63.33^{b}	80^{c}	93.33^{d}	70^{b}	86.66^{cd}	100^{e}	0^{a}	228.38	0
Co toxicity factor	-	-	-	-	-19.12	-6.47	3.8	-	-	-
Interactions	-	-	-	-	Additive	Additive	Additive	-	-	-
Co toxicity factor for *S. abbasi*PN-1 with Indoxacarb 15.80% EC (Indoxacarb in per cent)										
Treatments:	**T_1: SA**	**T_2: 0.008 %**	**T_3:0.0016 %**	**T_4:0.0024 %**	**T_5:0.008 %+SA**	**T_6:0.0016 %+SA**	**T_7:0.0024 %+SA**	**T_8: Control**	**F value**	**P value**
Mean per cent Mortality*	63.33^{c}	43.33^{b}	66.66^{cd}	83.33^{e}	66.66^{cd}	80^{de}	93.33^{e}	0^{a}	102.83	0
Co toxicity factor	-	-	-	-	-15.84	-8.86	-0.59	-	-	-
Interactions	-	-	-	-	Additive	Additive	Additive	-	-	-
Co toxicity factor for *S. abbasi*PN-1 with Chlorantraniliprole 18.50%SC (Chlorantraniliprolein per cent)										
Treatments:	**T_1: SA**	**T_2: 0.0015 %**	**T_3:0.003%**	**T_4:0.0045 %**	**T_5:0.0015 %+SA**	**T_6:0.003 %+SA**	**T_7:0.0045 %+SA**	**T_8: Control**	**F value**	**P value**
***Mean per cent Mortality**	63.33^{cdf}	36.66^{b}	53.33^{c}	76.66^{ef}	60^{cdf}	73.33^{de}	83.33^{f}	0^{a}	86.76	0
Co toxicity factor	-	-	-	-	-21.85	-11.52	-8.86	-	-	-
Interactions	-	-	-	-	Antagonism	Additive	Additive	-	-	-

Mean followed by same letters in the column do not differ by Tukey's test ($p<0.05$); *Mean per cent mortality after 46 HAT, SA: *S. abbasi* PN-1@ 500IJs/ml.

S. abbasi PN-1 was applied with emamectin benzoate 5% SG at 0.00036 per cent dose. The least mortality of 60 per cent with an antagonistic interaction (-21.85) was recorded in *S. abbasi* PN-1+ chlorantaniliprole at 0.0015% dose. The 63.33 per cent larval mortality was observed when *S. abbasi* PN-1 was applied alone.

Compatibility studies of *S. abbasi* PN-1 with insecticides proved that among the tested insecticides fipronil is more compatible followed by cyantaniliprole, indoxacarb, emamectin benzoate and chlorantraniliprole less compatible. The combined efficacy of *S. abbasi* PN-1 with insecticides given additive interactions after 42 hours of treatments and the more antagonism interaction is found at 36 hours after treatments this may be due to delayed action of nematodes in the presence of insecticides.

Discussion

From the present finding, it is proved that 8 to 20.66 per cent mortality of infective juvenile was recorded in various insecticides at 96 HAT at recommended doses. The findings were similar toKhan *et al.* (2021), where they tested the toxicity of selected insecticides at field recommended dose against *S. carpocapase* and *H. indicus* and reported that Chlorfenapyr recorded highest mortality (14.3 and 16.4%, respectively) and Methoxyfenozide least mortality of IJs (9.3 and 11.3%, respectively) at 96 hours after treatment. Negrisoli *et al.* (2010) reported that among the tested insecticides, chlorpyrifos, deltamethrin, lufenuron, deltramethrin + triazophos, diflubenzuron, gama cyhalothrin, lambda cyhalothrin, spinosad, cypermethrin, triflumuron and permethrin were compatible with *H. indica*, *S. carpocapsae* and *S. glaseri*.

Kumar *et al.* (2015) reported that *S. abbasi* (CISH EPN-1) was very sensitive to dichlorvas (100% mortality of IJs), profenofos (90.6%), chlorfenpyr (84.6%), chlorantaniliprole (49.6%) and quinolphos (63.3%) but no mortality observed in spinosad and bifenthrin treated IJs at 72 hours after treatment.Sabino *et al.* (2019) reported that the *H. amazonensis* was compatible with all three tested insecticides thiamethoxam, imidacloprid and chlorantraniliprole. Khan *et al.* (2018) tested the compatibility of the two EPNs *S. carpocapsae* and *H. indica* with spinosad (Tracer® 240SC) and reported that the highest concentration (800 ppm) of the spinosad showed 14 per cent and 13 per cent mortality in *H. indica* and *S. carpocapsae* respectively at 48 hours after treatment.

Some insecticides are less toxic to EPNs may be because of the presence of the butyrylcolinesterase in the synapse of EPNs 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 (Selkirk *et al.*, 2001). 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(Campbell and Gaugler, 1991; Bhat *et al.*, 2016).

The findings of the combined efficacy of *S. abbasi* PN-1and insecticides against 7 days old larvae of *S. litura* were similar to Khan *et al.* (2021), where they tested the combined efficacy of *S. carpocapase* and *H. indicus* with insecticides against *S. litura* and reported additive interaction of EPNs with all the tested insecticides at 96 hours after treatment. Kasi *et al.* (2022) reported the synergistic interaction of *S. felatia* and *H. bacteriophora* with Spinosad, indoxacarb and chlorantraniliprole against *S. frugiperda* and *T. absoluta* at 96 hours after treatment.

Aioub *et al.* (2021)studied the combined efficacy of EPNs, *Steinernema* sp and *Heterorhabditis* sp and insecticide against *Pieris rapae* and reported different interactions between EPNs and insecticide at different concentrations and different times. Özdemir *et al.* (2020) tested the effect of insecticide and fungicide on survival, progeny production and infectivity of *S. feltiae* and *H. bacteriophora* against *G. melonell* larvae. Reported that tested chemical altered progeny production and infectivity of EPNs on *G. melonell*.

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