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Research Article

Compatibility of *Steinernema carpocapsae* and *Heterorhabditis indica* with insecticides registered against *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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ABSTRACT: The gram pod borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae) is considered as a key pest of pigeonpea and is also a major polyphagous pest of several agricultural and horticultural crops in India. Combining chemical insecticides with Entomopathogenic Nematodes (EPNs) could be an effective alternative to reduce the use of harmful chemicals. Experiments were conducted to determine the compatibility of *Steinernema carpocapsae* and *Heterorhabditis indica* with registered insecticides used for *H. armigera* control in pigeon pea, under laboratory conditions. Compatibility of the insecticides with EPNs was evaluated by observing infective juveniles (IJs) survival and virulence of *Galleria mellonella* at 24 and 48 h after dipping in insecticide solutions. It was observed that, insecticides showed moderate effect on IJs survival. IJs were able to infect *G. mellonella* larvae after exposure to these chemicals, but their progeny production was significantly ($P < 0.05$) reduced. Both nematode species showed differential sensitivity to the tested insecticides, with *H. indica* exhibiting better tolerance than *S. carpocapsae*. The studies revealed that the chemicals showed a strong sub lethal effect on the nematode reproductive potential, limiting seriously their possible recycling in the field.

KEY WORDS: Compatibility, *Galleria mellonella, Helicoverpa armigera, Heterorhabditis indica, Steinernema carpocapsae*

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INTRODUCTION

Helicoverpa armigera (Hubner) is an important polyphagous pest causing severe damage to several agricultural and horticultural crops (Reed and Pawar, 1982; Zalucki *et al.,* 1986; Fitt, 1989; Gowda, 2005). This pest feeds nearly 182 species of plants spanning 47 families and among them 56 plant species are severely damaged (Pawar *et al.*, 1986). An estimated US$ 2 billion is annually lost due to this pest (Sharma, 2005), of which US$ 550 million accounts for loss in chickpea and Pigeonpea (ICRISAT, 1992).

Over use of chemical insecticides are common when *H. armigera* incidence is severe. Hence, with increased awareness among farmers to adopt safer control measures there is increasing emphasis on integrated pest management (IPM) where biological control agents can be deployed for management of *H. armigera*. Entomopathogenic nematodes (EPNs) belonging to the families of Steinernematidae and Heterorhabditidae are considered as suitable biocontrol agents, because of their ability to infect, kill and reproduce inside the some species. This has been well established with *H. armigera* and *H. zea* both in laboratory and field conditions (Glazer and Navon*.,* 1990; Naser *et al.,* 2012; Hussain *et al.,* 2014; Kallia *et al.,* 2014; Cabanillas *et al.,* 1994).

The integration of EPNs in IPM technique for combating *H. armigera,* will reduce the dependence on many conventional insecticides, thereby preventing harmful effects on human and soil (Dent, 2000). However, before an IPM technique for the control of this pest can be brought out, it is important that the compatibility of the nematodes with insecticides registered against this pest needs to be established. Moreover, chemical insecticide developing industries often do not test product toxicity to entomopathogens, only safety for predators and parasitoids is established (Alves *et al.,* 1998). Hence, research is needed to know whether the insecticides are having any deleterious effect on EPNs before combining them with insecticides. The present investigation was carried out to evaluate compatibility of *S. carpocapsae* and *H. indica* with chemical insecticides registered against *H. armigera* under laboratory conditions, so as to enable the integration of these control methods into effective management strategies. This paper reports the effect of direct exposure to insecticide solutions on the sur-

vivability, infectivity and reproduction of *S. carpocapsae* and *H. indica*.

MATERIALS AND METHODS

Insect culture

Wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), was reared under laboratory conditions at 25 ± 2°C on standard artificial diet. The moths collected in plastic jars were fed with honey solutions. They were allowed to lay eggs on a tissue paper lining. The eggs were allowed to hatch in a separate plastic jar covered with muslin cloth. Newly hatched larvae (0-24 h old) were released on artificial diet containing plastic jar covered with muslin cloth. After reaching the last larval instar, healthy larvae were used for the experiment.

Insecticides

To determine the compatibility of *Steinernema carpocapsae* Weiser, 1955 (Wouts, Mracek, Gerdin and Bedding, 1982) and *Heterorhabditis indica* (Poinar, Karunakar and David, 1992) infective juveniles (IJ) with seven commercial insecticides registered for *Helicoverpa armigera* in pigeonpea were evaluated. Details about the insecticides are listed in Table 1 and 2.

Nematode culture

Steinernema carpocapsae and *Heterorhabditis indica* obtained from the Department of Insect Systematics, ICAR- National Bureau of Agricultural Insect Resources (NBAIR) Bengaluru, India, were used in this study. Nematodes were propagated in parallel at room temperature on final instar Galleria larvae (Kaya and Stock 1997). IJs emerging from the larvae within 3 days from the first day of emergence were collected. Nematode viability was 100%, unless otherwise stated. New batch of IJs were used in all the experiments.

Effect of insecticides on EPNs survival

To determine compatibility of EPNs with registered insecticides for *H. armigera* in pigeonpea, a laboratory assay was conducted. Before the assay, seven registered insecticides aliquot suspension was prepared by using, active

Table 1. Characteristics of insecticides registered in India for the management of *Helicoverpa armigera* in Pigeonpea

Name		Formulation	Mode of action[a]	Chemical group	Concentration Kg or Liter/ha[b]	Spray volume Liter/ha[c]
Technical	Commercial					
Emamectin benzoate	Proclaim®	5% SG	Ggcca	Avermectins	0.220	500-750
Flubendiamide	Fame®	39.35% SC	IRRA	Diamide	0.1	500
Indoxcarb	King Dox®	14.5% SC	SDM	Oxadiazines	0.35-0.40	500-1000
Lambdacyhalo-thrin	Ballista Super®	5% EC	SDM	Pyrethroids	0.40-0.50	400-600
Profenophos	Attach®	50% EC	CI	Organophosphates	1.5-2.0	500-1000
Profenophos + Cypermethrin	Prolife Super®	40% EC+4% EC	CI and SDM	Organophosphates+ Pyrethroid	1.0-1.5	500-1000
Monocrotophos	Monoplus®	36% SL	CI	Organophosphates	1.25	500-1000

[a]Mode of action, Ggcca = GABA gated chloride channels activators, IRRA = Insect Ryanodine Receptors agonist, SDM = Sodium channel modulator, CI = Cholinesterase inhibitor
[b]Corresponding to terrestrial application.
[c]Corresponding to aerial application.

Table 2. Insecticides registered in India for *Helicoverpa armigera* in pigeonpea, toxicity classification of insecticides; the effect of the treatments on entomopathogenic nematodes infectivity of *Galleria mellonella* larvae was classified according Peters and Poullot (2004), based on IOBC guideline

Treatment [a]	*Steinernema carpocapsae*				*Heterorhabditis indica*			
	24 h		48h		24h		48h	
	E%[b]	C[c]	E%	C	E%	C	E%	C
Proclaim®	100.0	4	100	4	100.0	100.0	100.0	4
Fame®	0.0	1	0.0	1	0.0	1	0.0	1
King Dox®	0.0	1	0.0	1	0.0	1	0.0	1
Ballista Super®	6.7	1	33.4	2	20.0	1	26.7	1
Attach®	46.7	2	60.0	2	13.4	1	60.0	2
Prolife Super®	46.7	2	66.7	2	53.4	2	66.7	2
Monoplus®	33.4	2	40.0	2	40.0	2	46.7	2

[a] a.i./ha recommended for aerial application.
[b] Treatment effects: E% = 100 - (100 - corrected mortality) × (100×Red). % corrected mortality was null in all treatments and therefore it was not considered in E% calculation.
[c] Toxicity classification of insecticides by IOBC: 1– non-toxic (<30%), 2– slightly toxic (30 to 79%), 3- moderately toxic (80% to 99%) and 4 – harmful (>99%).

ingredient (a.i.) /ha recommended for field application and their spray volume (Table 1), in which each insecticide a.i. was calculated for 10 ml spray volume. Each calculated insecticide a.i. was dissolved in 9 ml distilled water and mixed with approximately 10000 fresh IJs containing 1 ml distilled water. After preparing each insecticide final suspension, 2 ml of aliquots of each suspension was placed in 24 well plates and 2 ml distilled water were used for the control treatment. Plates were sealed with Para film to avoid evaporation and these plates were incubated at 25 ± 1° C for 24 and 48 h. After exposure, the number of dead and live IJs was counted under a stereomicroscope by taking three 10 µl samples for each plate and percentage survival was calculated. Nematode viability was determined by observing motility and they were considered dead if not responding to probing with a fine needle. There were five wells per insecticide, nematode species, exposure time and assay was repeated once.

Effect of insecticide on EPNs virulence

To study the nematode infectivity, which is the capability to cause nematode death, first IJs were exposed to different insecticides as mentioned above. IJs infectivity and mortality were tested using *Galleria* as a host. After exposure, multi well plate were filled with 2 ml of distilled water and placed to rest for 30 min at 25 ± 1° C. Supernatant liquid (approximately 2 ml) was then withdrawn and the rinsing in distilled water, process repeated for four times. After the last rinsing, a volume of 150 µl (approximately 150 IJs) were retrieved from the bottom of each tube and distributed in petri dish (9 cm diameter) containing filter paper previously wetted with 850 µl distilled water for each treatment. Each plate received three last instar *G. mellonella* larvae, incubated at 25 ± 1°C till death of the larvae. After death, insect cadaver were transferred to Petri dish (9 cm diameter) containing dry filter paper and maintained in darkness for 24 h, finally they were dissected in order to verify nematode's presence. There were five replicates per insecticide, nematodes species, Exposure time and the assay was repeated once.

Effect of insecticide on EPNs reproduction

To assess the nematode reproduction for each nematode species, above mentioned nematodes infectivity method was followed. After death of wax moth larvae, three cadavers were rinsed in sterile distilled water to remove nematodes from their surface body. Then cadavers were incubated at 25 ± 1°C in dark at room temperature for 7 and 10 days for *S. carpocapsae* and *H. indica* respectively. The total number of IJs that emerged from each larva was determined. There were five replicates per insecticide, nematodes species, exposure time and assay was repeated once**.**

Statistical analysis

Percentage data were normalized using arcsine transformation and numerical data (progeny production) were square root transformed prior to analysis. Analysis was undertaken on the transformed data and back transformed data only is presented. Insecticides, time and nematode species and their interactive effects on nematode survival, infectivity, and progeny production data were subjected to analysis of variance (ANOVA) using PROC GLM (SAS version 9.3; SAS institute). When ANOVA was significant, comparisons of relevant means were made using the Tukey's significance test values at the 5% level of significance. The effect of the treatments on EPNs infectivity of Galleria larvae was classified according Peters and Poullot (2004), based on the International Organization for Biological Control (IOBC) guideline and the formula.

RESULTS AND DISCUSSION

Effect of insecticide on EPNs survival

Irrespective of the nematode species, Prolife Super® and Attach® were the insecticides that caused the lowest survival (< 50%) of the two EPNs tested. Survivability of *H. indica* and *S. carpocapsae* IJs was significantly different when exposed to the different insecticides. The survivability of *S. carpocapsae* was lowest in mixture with Monoplus® (15.8%), Prolife Super® (24.3%) and Attach® (36.7%) while for *H. indica* was lowest in mixture with Prolife Super® (31.8%) and Attach® (45.5%) 48 h after their exposure to these insecticides. However, *H. indica* survivability was not significantly different between 24 and 48 h after their exposure to Monoplus® 52.2% and 51.2%, respectively. Among the nematode species, irrespective of insecticides, the per cent survival of *H. indica* was significantly ($P < 0.05$) higher compared to the *S. carpocapsae* (Fig. 1). When exposure time of IJs was extended up to 48 h, the survivability of the nematode species were significantly ($P < 0.05$) reduced. Insecticides and exposure period significantly influenced nematode survival.

Effect of insecticide on EPN infectivity

Infectivity, that is, capacity of *S. carpocapsae* and *H. indica* to cause *G. mellonella* larval death was statistically ($P < 0.05$) different after being exposed to the insecticides. Independently from nematodes, infectivity was higher after exposure to Fame® (100 %), King-Dox® (100%) and Ballista Super® (85.0%). Infectivity of *S. carpocapsae* was equal to the control in treatments with Fame® (100%) and King-Dox® (100%), the lowest infectivity registered when exposed to Prolife Super® (33.3%) for 48 h. Similarly, *H. indica* was highly infective after being exposed to Fame® (100%), King-Dox® (100%) and Ballista Super® (73.3%),

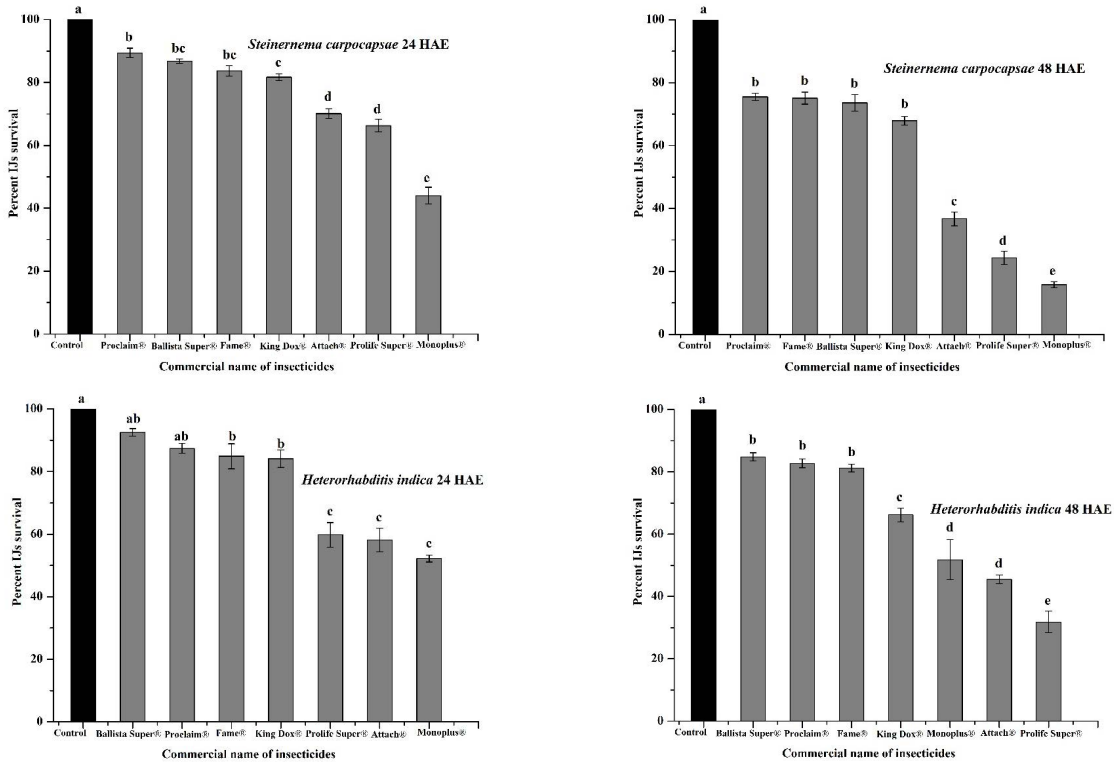

Fig. 1. Percentage survival (Mean ± SE) of *Steinernema carpocapsae* and *Heterorhabditis indica* after 24 and 48 hours after exposure (HAE) to registered insecticides for the management of *Helicoverpa armigera*. Different letters on the top of error bars indicates statistically different values for different insecticide a.i./ha recommended for aerial application at ($P < 0.05$) using Tukey's test. Error bars indicate standard error (n = 5).

Prolife Super® (33.3%) caused the lowest infectivity of this nematode. However, both nematodes species failed to infect *G. mellonella* when exposed to Proclaim® (Table 3).

Table 3. The infectivity (Mean ± SE) capacity of *Steinernema carpocapsae* and *Heterorhabditis indica*, to *Galleria mellonella* larvae after 24 and 48 hours exposure in *Helicoverpa armigera* registered insecticides

Treatment [a]	Infectivity (%)			
	S. carpocapsae		*H. indica*	
	24 h	48 h	24 h	48 h
Proclaim®	0.0 ± 0.0d[b]	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0d
Attach®	53.3± 8.1c	40.0 ± 12.4b	86.6 ± 8.1ab	40.0 ± 12.4bc
Prolife Super®	53.3± 8.1c	33.3±10.5bc	46.6 ± 13.3c	33.3 ± 10.5cd
Monoplus®	66.6±10.5bc	60.0 ± 12.4b	60.0 ± 6.6bc	53.3 ± 8.1bc
Ballista Super®	93.3±6.6ab	66.6 ± 10.5ab	80.0 ± 13.3abc	73.3 ± 12.4ab
Fame®	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
King Dox®	100.0± 0.0a	100.0± 0.0a	100.0± 0.0a	100.0± 0.0a
Control	100.0±0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
P value	<0.0001			
Insecticides (I)	0.9101			
Nematode (N)	0.0001			
Time (T)	0.3921			
N×I	0.9101			
N×T	0.0197			
T×I	0.3259			
N×I×T				

[a] a.i./ha recommended for aerial application.

[b] Means of five replications. Means followed by the same letter in a column are not significantly different at $P < 0.05$, as determined by Tukey's test.

Effect of insecticide on EPN reproduction

Reproduction of both nematode species was significantly ($P < 0.05$) affected by insecticides and time of exposure. For both nematodes, the number of progeny produced per milligram body weight of Galleria larvae was significantly ($P < 0.05$) reduced when IJs were exposed to insecticides before the inoculation. Reproduction was significantly ($P < 0.05$) reduced in both nematode species, after exposure to all the insecticides when compared to control. Reproduction of *S. carpocapsae* being the lowest, registered when exposed to Prolife Super® (699 IJs/ mg body weight) for 24 h. Similarly, *H. indica* reproduction was not significantly different between Fame®, Ballista Super® and King Dox®. Monoplus® (516 IJs/ mg body weight) caused the lowest reproduction of this nematode when IJs exposed for 24 h (Fig. 2).

It has been well established that the combined use of biological control agents like EPN and insecticides are important to IPM programs against many agricultural pests (Koppenhofer and Grewal, 2005). To study the adverse effects of insecticides on natural enemies (Nabil El-Wakeil *et al.,* 2013) and especially on EPNs, it's pre-requisite to validate the compatibility of EPNs with insecticides, because it has one of the important agriculture inputs commonly available to farmers when pests approach the economic threshold level. Commercial formulations of Fame®, King-Dox® and Ballista Super® showed no adverse effects on the survival and infectivity of both nematodes species, whereas the IJs exposed to Proclaim® recorded 75.70% and 82.71% survival in *S. carpocapsae* and *H. indica,* respectively at 48 h of exposure, and it had adversely affected the infectivity of Galleria larvae. The present findings are in line with those of Yan *et al.* (2012) who opined that the viability of *S. carpocapsae* IJs were not affected by Proclaim®, but their infectivity was impaired.

According to Fetoh *et al*. (2009) emamectin benzoate had no adverse effects on *S. carpocapsae* and a mixture of *S. carpocapsae* with formulated emamectin benzoate significantly increased mortality to greasy cutworm (*Agrotis ipsilon*), compared to *S. carpocapsae* alone. In our study, however, viability of *S. carpocapsae* and *H. indica* was not affected by Proclaim® (Emamectin benzoate), but their infectivity was zero in both the species of EPNs. Therefore, generalisations on EPNs tolerance to insecticides were inaccurate, because different findings among studies may be related to differences in chemical composition and formulation of the product (Koppenhofer and Grewal, 2005; Negrisoli Jr *et al*., 2010). Therefore, studies of the interac-

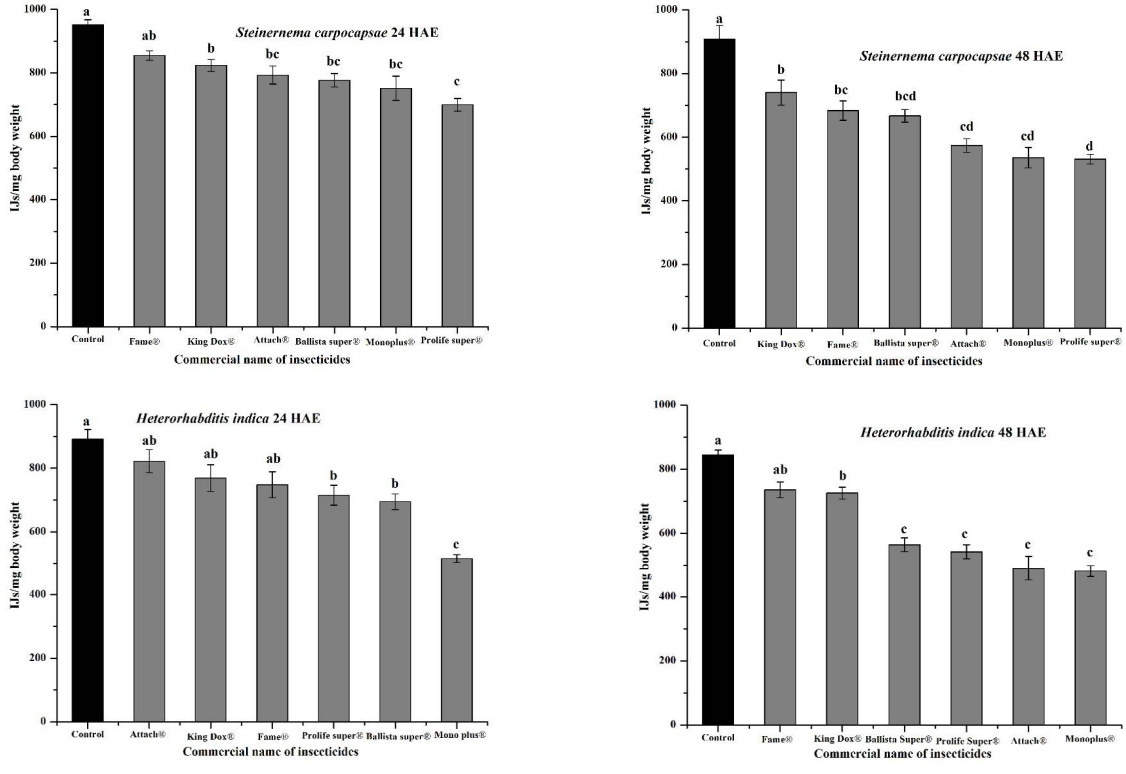

Fig. 2. The progeny production (Mean ± SE) capacity of *Steinernema carpocapsae* and *Heterorhabditis indica,* in *Galleria mellonella* larvae at 24 and 48 hours after exposure in *Helicoverpa armigera* registered insecticides. Different letters on the top of error bars indicates statistically different values for different insecticide a.i./ha recommended for aerial application at ($P < 0.05$) using Tukey's test. Error bars indicate standard error (n = 5).

tion and compatibility between insecticides and EPNs are recommended before field applications in IPM programs.

Among the organophosphate group (OP) of insecticides, when IJs were exposed to Attach® and Monoplus® we could notice, lower survivability. Similarly, Zimmerman and Cranshaw (1990) also showed significant reduction of *S. feltiae* survival when exposed to OP compounds. Hence inhibition of acetylcholine esterase activity may be slightly toxic to both EPN strains. After exposure of IJs to these OP compounds, we also recorded moderate IJs infection of Galleria larvae and reduced reproduction rate. However, Rovesti and Deseo, 1990 reported that nematode reproduction in Galleria larvae under *in vitro* conditions was not affected by exposure to OP compounds and carbamates.

In the present study, IJs exposure to pyrethroid group of insecticide Ballista super® resulted in 73.6% and 84.0% survival of *S. carpocapsae* and *H. indica,* respectively, and also showed moderate effect on infectivity and reproduction rate. However, Negrisoli Jr *et al*. (2010) observed 88% survival in *S. carpocapsae* when exposed to Cypermethrin. Negrisoli Jr. *et al.* (2008) reported that, pyrethroids registered higher mortality of *S. carpocapsae* (28.4%) compared to *H. bacteriophora* (5.6%) when exposed to Decis, similarly in our studies, *H. indica* showed higher survival as compared to *S. carpocapsae.* These results indicated that, insecticide tolerance also depends on the EPN species.

The present studies show that *S. carpocapsae* and *H. indica* can be successfully included in IPM of *H. armigera*. It may reduce the dependence on chemical insecticides and thus contribute to slowing down the development of insecticide resistance and preventing adverse effects on public health and the environment. The results of this work expand our knowledge on compatibility of EPN with registered insecticides for the control of *H. armigera*. Knowledge of the potential reproduction losses attributable to the used insecticides will be help to predict the required application rate of nematodes in IPM programs against *H. armigera*.

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