Pakistan Journal of Nematology (2019) 37 (1):45-52

ISSN 0255-7576 (Print)
ISSN 2313-1942 (Online)
www.pjn.com.pk

http://dx.doi.org/10.18681/pjn.v37.i01.p45-52

Combined efficacy of insecticides and entomopathogenic nematodes in the management of cotton leaf worm *Spodoptera littoralis*

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Abstract

Effects of certain insecticides *viz*., Coragen, Nomolt, Ekio and Magic smart with two species of entomopathogenic nematodes, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* were tested against cotton leaf worm *Spodoptera littoralis*. All tested insecticides caused less mortality to the tested entomopathogenic nematodes. Magic smart significantly surpassed other insecticides causing the mortality of 11.6 and 13.8 % to the entomopathogenic nematodes *S. carpocapsae* and *H. bacteriophora*, respectively as compared to Coragen that caused slight mortality, 2.5 and 4.4%, respectively and did not differ significantly than the check. Nomolt and Ekio caused 5.4 and 8.8 % mortality of *S. carpocapsae* as well as 8.8 and 12.0 % to *H. bacteriophora*, respectively. These results show that the tested insecticides have less effect on entomopathogenic nematodes especially *S. carpocapsae*. *S. carpocapsae* exposed to Coragen, Nomolt, Ekio and Magic smart resulted in 5th instar larval mortality of cotton leaf worm by 92.5, 85.0, 80.0 and 75.0 %, respectively whereas *H. bacteriophora* caused mortality by 83.4, 77.2, 71.0 and 60.6 %, respectively. The experimental findings showed that combined efficacy of *S. carpocapsae* or *H. bacteriophora* with either of the insecticides *viz*., Coragen, Nomolt, Ekio and Magic smart at the quarter lethal concentration (LC_{25}) is effective in causing mortality of *S. littoralis*.

Keywords: *Spodoptera littoralis*, *S. carpocapsae*, *H. bacteriophora*, insecticides, mortality

Spodoptera littoralis Boisduval, the cotton leaf worm, is one of the most destructive lepidopterous pests in cotton production and has caused significant yield losses. The entomopathogenic nematodes *Steinernema* and *Heterorhabditis* are mostly used as biopesticides in pest management worldwide (Chitra *et al*., 2017; Gilblin-Davis *et al*., 2015). They have been proven to be virulent and lethal to the *S. littoralis* and their combined effect produces synergism and an additive effect and increases the percentage mortality of the *S. littoralis*.

Many investigations on the use of entomopathogenic nematodes and its compatibility with insecticides to control this pest has been carried out that yielded very exciting results in improving pest management in agriculture. Garcia *et al*., (2008) observed a 75 % mortality of the fall armyworm with a concentration of 400 infective juveniles of the *Heterorhabditis indica in vitro*. Bernardi *et al*., (2010) reported that a mixture of entomopathogenic nematodes (EPNs) and insecticides mixtures for the control of the *Spodoptera frugiperda*, showed a significant increment in the mortality of the *S. frugiperda*. This implies that there can be a synergistic and additive effect between both the EPNs and the insecticide. Negrisoli *et al*., (2010) evaluated the efficacy of mixtures of EPNs and insecticides to control *S. frugiperda* in corn crops and found that the interaction between EPN species and insecticides were synergistic

Published by Pakistan Society of Nematologists
Received:28 Sep, 2018
Accepted:20 Nov, 2018

and additive. Atwa (2013) observed that EPNs *viz*., *H. bacteriophora* and *Steinernema* were compatible with most of the pesticides tested against *Spodoptera littoralis* (Boisd.) and therefore EPNs insecticide mixtures can be used in an integrated pest management system. Hassan (2016) in a conference paper reported effect of some factors on viability and bio-insecticidal potency of the entomopathogenic nematodes. In an investigation of the agrochemicals on nematode viability and virulence on rice Chavan *et al*., (2018) reported that *H. indica* showed compatible with all the tested agrochemicals.

The objective of the present study was to evaluate the combined effects of two entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* and four insecticides *viz*., Coragen, Nomolt, Ekio and Magic smart at different combinations. These were also assayed for their synergistic and addictive effects as efficacy control for the *S. littoralis* in cotton under laboratory conditions.

Materials & Methods

Rearing of Cotton leaf worms: *Spodoptera littoralis*, cotton leaf worms were reared in Plant Protection Department Laboratory, Faculty of Agriculture, Minia University on castor leaves for several generations according to the method outlined by Kaya & Stock (1997).

Rearing of Greater wax moth larvae: *Galleria mellonella* L., Greater wax moth larvae were reared in incubator at 28± 2 ºC, 60% relative humidity and 12 h photoperiod, according to the methodology modified by Parra (1998).

Rearing of Entomopathogenic nematodes: Entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* were obtained from Laboratory of Nematology, Faculty of Agriculture Cairo University and reared on *Galleria mellonella* for several generations in Zoology Research Laboratory, Plant Protection Department, Faculty of Agriculture, Minia University according to Woodering & Kaya (1988). Infective juveniles of nematodes stored in incubator at 25◦ C in plastic bottles arranged horizontally according to Hassan (2016).

Insect rearing and entomopathogenic nematodes multiplication, as well as bioassays, were performed at Pesticide Research Laboratory, Plant Protection Department, Faculty of Agriculture, Minia University, Egypt.

The tested insecticides: The tested insecticides were Coragen (chlorantaniliprole); Nomolt (teflubenzuron); Ekio (novaluron) and Magic Smart (lufenuron) were obtained from Pesticide Research Laboratory, Plant Protection Department, Faculty of Agriculture, Minia University.

These particular insecticides have been selected for evaluation because these insecticides represent different mode of actions against insects especially from Lepidoptera and these insecticides are commonly used in Egypt against cotton leaf worms *Spodoptera littoralis.*

The effect of mixing entomopathogenic nematodes with insecticides: Entomopathogenic nematodes @ of 2500 IJs/ml (the field rate) with the tested insecticides with their field usage rate (Coragen 20% SC at 60 ml/400 L., Nomolt 15% SC 50ml/100 L., Ekio10% EC 60 ml/100 L. and Magic Smart 5% EC at 160 ml/400 L.) were applied for evaluating IJs viability and infectivity after being exposed to insecticides (Negrisoli *et al*., 2010).

One liter of each insecticide formulation was prepared proportionally to the double concentrations that would be applied in the field. From these solutions, 1 mL aliquots of each insecticide prepared solution were placed in glass test tube distilled and latter 2500 IJs per each nematode species contained in 1 mL of distilled water were added to the above mentioned tubes. Each tube represented one of the five replicates. These tubes were held in incubator at 22 ±1 C; RH of 70±10% with 12 h photoperiod.

Nematode mortality was evaluated 48 h after their exposure to the insecticide. Suspension (0.1 ml) of each tube was pipetted out and placed in a watch glass; 100 IJs were observed under the stereo microscope, nematodes were considered dead which did not react when touched with a probe. Mortality percentages were corrected according to Abbot' formula (Abbot, 1925). Nematode infectivity tesst were carried by filling the tubes of the previous test with 3 ml of distilled water and placed to rest for 30 min in incubator at 15º C. Supernatant liquid (approximately 3 ml) was then withdrawn and rinsing process repeated for three times. After the last rinsing, a volume of 0.2 mL (approximately 400 IJs) were drawn from the bottom of each tube and distributed in five Petri dishes (9 cm diameter) containing filter paper previously wetted with 1.8 ml distilled water for each treatment with each Petri dish as one replicate. Each dish received ten fourth instar larvae of *S. littoralis*, kept in incubator at 22 ± 1º C and R.H. of 70±10 % with 12 h photoperiod for five days. Dead larvae were counted, transferred to Petri dishes (9 cm diameter) containing dry filter paper and maintained into darkness for more three days.

Finally they were dissected to verify nematode's presence (Negrisoli *et al*., 2010). The experiment's statistical design was completely random.

Data analysis: Nematodes and cotton leaf worm mortality data were subjected to variance analysis and differences between treatment means were estimated by Chi square test at 0.05 probability. The effect of the treatments on nematodes infectivity over *S. littoralis* larvae was classified according to Peters & Poullot (2004), based on IOBC guidelines:

E%=1-(It/ Ic) x 100:
Whereas It= mortality in treatment; Ic= mortality in control treatment.
Classification of insecticides effect on nematodes infectivity:

1= non effect (E<30 %); 2= slightly effect (E=30-79%); 3= moderately effect E >79.

Combined effect of nematodes and insecticides: To determine the combined effect of nematodes and insecticides, mixtures of insecticides and nematodes at the concentration of LC_{25} for each were applied against *S. littoralis* 5th larval instar and mortality percentages were estimated. Estimation of LC_{25} of nematodes was made by testing seven concentrations @ 2000, 5000, 10000, 20000, 30000, 40000 and 50000 infective juveniles (IJs) of each *H. bacteriophora* and *S. carpocapsae* nematodes/liter in distilled water. One ml of nematode suspension of each concentration was dispersed on five larvae of *S. littoralis* (5th instar) supplied with piece of castor leaf in Petri dishes 15 cm in diameter. As for insecticides different concentrations of each insecticide were tested. Mortality of larvae by nematodes or insecticides was corrected by Abbot's formula (Abbot, 1925). LC_{25} were estimated according to Finney (1971).

Mixtures of insecticides and nematodes at the concentration of LC_{25} for each were prepared. One ml of each mixture was applied on larvae supplied with castor leaf in Petri dishes. Each treatment was replicated four times. Average of mortality was estimated and corrected with control treatment. Co-toxicity factor (C.F.) was determined according to Mansour *et al*., (1966) as follows:

Co-toxicity factor = Observed % mortality - Expected % mortality / expected % mortality × 100

A positive C.F. value of 20 or more was considered synergism. Negative value of 20 or more was considered antagonism. Any value lies between -20 and + 20 was taken as an additive effect.

Results and Discussion

Magic insecticide significantly surpassed other insecticides causing the highest mortality 11.6 % to *Steinernema carpocapsae* whereas Coragen caused least mortality to this nematode and did not differ significantly than the check. Ekio and Nomolt gave 8.8 and 5.4% mortality of *S. carpocapsae*, respectively. These results showed that insecticide Coragen has least effect on entomopathogenic nematodes *S. carpocapsae* (Table 1).

The insecticide Magic smart significantly surpassed others in efficacy causing the highest mortality of 13.8 % of *Heterorhabditis bacteriophora* whereas Coragen caused least mortality of this nematode (4.4%) and did not differ significantly than the check. Ekio and Nomolt gave 12.0 and 8.8 % mortality of *H. bacteriophora*, respectively (Table 1).

The results showed that *H. bacteriophora* was more sensitive to the tested insecticides than *S. carpocapsae*. Our results are in accordance to the Gaugler (2002) who reported that heterorhabditid are more sensitive to chemical challenges, including pesticides than steinernematids.

It is usually desirable to tank mix one or more inputs to save time and money. Infective juveniles are tolerant of short exposures (2-6 h) to most agrochemicals including herbicides, fungicides, acaricides and insecticides and therefore, can often be tank-mixed (Krishnayya & Grewal, 2002; De Nardo & Grewal, 2003). Steinernematid and heterorhabditid nematodes can survive exposure to many chemical pesticides. However, infective juveniles are highly susceptible to several nematicides likely to be found in the agro ecosystem (Laznik *et al*., 2012).

Glazer *et al*., (1997) examined genetic selection as a means of enhancing resistance of *H. bacteriophora* strain HP88 to the nematicides: fenamiphos (organophosphate), oxamyl (carbamate) and avermectin (biologically-derived product). After 11 rounds of selection, resistance to the nematicides as well as several traits relevant to bio-control efficacy including virulence, heat tolerance and reproduction potential were examined.

The entomopathogenic nematode *S. carpocapsae* exposed to Coragen gave the highest mortality of *S. littoralis* (92.5%) and did not significantly differ than check treatment (95% mortality) and the treatment effect (E, value) was 2.6 which showed that insecticide had no effect on *S. carpocapsae* infectivity. *S. littoralis* exposed to Nomolt gave 85% mortality with E, value 10.5. Ekio showed 15.8 E value with 80% mortality of cotton leaf worms as compared to control (95% mortality of cotton leaf worms). On the other hand, nematodes exposed to Magic smart significantly differed with other insecticides and check treatment that caused least mortality 75% of *S. littoralis* and treatment effect value was 23.7 that was found within the range of non-effect on nematode infectivity (Table 2) as described by Peters & Poullot (2004).

Similar observations were made by Rovesti & Deseo (1990) who reported that Chitin synthesis inhibitors did not affect *S. carpocapsae* viability. These insecticides did not cause noticeable inhibition in reproduction and development of *S. carpocapsae* as reported by Hara & Kaya (1982).

Entomopathogenic nematode *H. bacteriophora* exposed to Coragen gave high mortality to *S. littoralis* (83.4%) but it was significantly different from the check (96.0%) and the estimated treatment effect of insecticide on nematode infectivity (E value) was 13.1 (non-effect). This nematode exposed to Nomolt gave 77.2 % mortality of *S. littoralis* and E value 19.6 (Non-effect). Ekio caused moderated loss of infectivity of *H. bacteriophora* (71.0% mortality of *S. littoralis*) with E, value of 26.0 (non-effect on nematode infectivity) (Table 2). *H. bacteriophora* exposed to Magic smart significantly caused least mortality 60.6 % to *S. littoralis*. The estimated treatment effect of Magic smart on *H. bacteriophora* infectivity on *S. littoralis* was 37.5 that mean slight effect of Magic smart on *H. bacteriophora* infectivity. In the probit data analysis the estimated LC_{50} of insecticides viz., Coragen, Nomolt, Ekio and Magic smart were 24.72, 3.88, 19.84 and 13.98

mg/l while the LC_{25} values were 11.84, 0.61, 5.00 and 3.91 mg/l for these insecticides, respectively. On the other hand the estimated LC_{50} of *S. carpocapsae* and *H. bacteriophora* were 27531 and 19780 infective juveniles per liter (IJs), while the LC_{25} for these nematodes were 6003 and 4343 IJs, respectively (Table 3).

Data of the joint action of *S. carpocapsae* with each tested insecticides viz., Coragen, Nomolt, Ekio and Magic smart to *S. littoralis* at LC_{25} is shown in Table 4. Data indicates that 4 tested mixtures decreased the toxicity of *S. littoralis* according to co-toxicity factor. Co-toxicity coefficient (CC) of two tested mixtures of EPN and insecticide (*S. carpocapsea* + Coragen and *S. carpocapsea* + Nomolt) were of synergistic effect while other two joint action of EPN and insecticide (*S. carpocapsea* + Ekio and *S. carpocapsea* + Magic smart) produced additive effect. In case of *H. bacteriophora* the effect of joint action data at LC_{25} with Coragen was synergism in nature while the other three combinations produced additive effect (Table 4).

Table 1. Effect of certain insecticides on the mortality of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* at 22± 1ºC and RH 70 ± 10%.

Treatment	Nematode Mortality %	
	S. carpocapsae	***H. bacteriophora***
Control (only distilled water)	0.0 c	0.0* c
Nematodes exposed to Coragen	2.5 c	4.4 bc
Nematodes exposed to Nomolt	5.4 abc	8.8 abc
Nematodes exposed to Ekio	8.8 ab	12.0 ab
Nematodes exposed to Magic smart	11.6 a	13.8 a

1-Mortality percentages were corrected according to Abbot's formula
2-Mortalities followed by the same letters didn't significantly differ according to Chi square test.

Table 2. Effect of certain insecticides on the infectivity (average ±SE) of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* measured by *Spodoptera littoralis* 5th larval instar mortality at 22± 1ºC and RH 70 ± 10%.

Treatment	% mortality of cotton leaf worms		Treatment effect (E%)		Toxicity classification	
	S.car	***H.bac***	***S.car***	***H.bac***	***S.car***	***H.bac***
Control	95.0 a	96.0a	-	-	-	-
Coragen	92.5 a	83.4b	2.6	13.1	1	1
Nomolt	85.0 b	77.2bc	10.5	19.6	1	1
Ekio	80.0 bc	71.0bc	15.8	26.0	1	1
Magic	75.0 c	60.6c	23.7	37.5	1	2

1- Mortalities followed by the same letters didn't significantly differ according to Chi square test.
2-E% = 1- (It / Ic) x 100; It = mortality in treatment; Ic = mortality in control treatment.
Classification of insecticides effect on nematodes infectivity: 1= non effect (E = < 30%); 2= slightly effect (E= 30-79%); 3= moderately effect (E = >79).

Table 3. Probit data established from toxicity line equations of tested insecticides and entompathogenic nematodes on *S. littoralis* 5th larval instar.

Treatments	Toxicity line equation	Slope	D.F.	LC $_{50}$	LC $_{25}$
Coragen (chlorantraniliprole)	Y=2.0946+2.0821	2.0946	3	24.72 mg / l	11.84
Nomolt (teflubenzuron)	Y= 0.8311X+4.5101	0.8311	6	3.88 mg / l	0.61
Ekio (novaluron)	Y=1.1205X+3.546	1.1205	4	19.84 mg / l	5.00
Magic smart (Lufenuron)	Y=1.2103X+3.6137	1.2103	7	13.98 mg /l	3.91
S. carpocapsae	Y=1.0129X+0.5029	1.0129	5	27531.0 IJs / l	6003.0 IJs / l
H. bacteriophora	Y=1.0175X+0.6286	1.0175	5	19780.0 IJs /l	4343.0 IJs /l

Table 4. Combined effect of *Steinernema carpocapsae* mixtures with certain insecticides on *S. litoralis* 5th instar larvae under laboratory conditions.

Mixture of Nematode + insecticide	LC_{25} + LC_{25} IJs /liter + mg /l	Mortality %			Remarks
		Expected	Observed	Co-toxicity coefficient	
S. carpocapsae + Coragen	6003.0 + 11.84	50	65	+30	Synergism
S. carpocapsae + Nomolt	6003.0 + 0.61	50	60	+20	Synergism
S. carpocapsae + Ekio	6003.0 + 5.00	50	55	+10	Additive
S. carpocapsae + Magic smart	6003.0 + 3.91	50	45	-10	Additive
H. bacteriophora + Coragen	4343.0 + 11.84	50	60	+20	Synergism
H. bacteriophora + Nomolt	4343.0 + 0.61	50	55	+10	Additive
H. bacteriophora + Ekio	4343.0 + 5.00	50	45	-10	Additive
H. bacteriophora+ Magic smart	4343.0 + 3.91	50	42	-16	Additive

Conclusion

Effects of four insecticides viz., Coragen, Nomolt, Ekio and Magic smart with two species of entomopathogenic nematodes, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* were tested against cotton leaf worm *Spodoptera littoralis*. Magic smart significantly surpassed other insecticides causing the highest mortality of *Heterorhabditis bacteriophora*; so it can be concluded that Heterorhabditids are more sensitive to chemical challenges, including pesticides than Steinernematids. The results also showed that combined efficacy of *S. carpocapsae* or *H. bacteriophora* with either of the insecticides viz., Coragen, Nomolt, Ekio and Magic smart at the quarter lethal concentration (LC_{25}) is effective in causing mortality of *S. littoralis*. The results presented herein indicated that EPN may be suitable for use as a bioagent in combination with chemicals in the management of cotton leaf worm, *Spodoptera littoralis*.

Acknowledgement

This study was performed at Department of Plant Protection, Faculty of Agriculture, Minia University.

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