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Tank-mix Compatibility of the Entomopathogenic Nematode, *Heterorhabditis indica*, with Selected Chemical Pesticides

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Abstract: *Heterorhabditis indica* have the potential for biological control of crop pests. Compatibility studies need to be conducted to analyze which chemical pesticides are compatible with this bioagent. The aim of this work was to evaluate the compatibility between *Heterorhabditis indica* and the chemical pesticides (9 insecticides, 4 fungicides and 3 herbicides) that are most commonly used on the various crops. The infective juveniles (IJs) of *H. indica* were exposed to the recommended rates of pesticides applied in 24-well plates at room temperature up to 72 h and then viability and infectivity of IJs determined. Survival and virulence evaluation of IJs exposed to the highest recommended doses of the pesticides against 4th instar larvae of *Galleria mellonella* confirmed the retention of virulence, except in the IJs treated with chlorpyriphos. The results obtained from this study revealed that it may be possible to use *H. indica* with most pesticidal formulations tested here (except chlorpyriphos) as tank-mix for application in the field.

Keywords: Compatibility, *Heterorhabditis indica*, pesticides, tank-mixing.

INTRODUCTION

Biological control is an important tool in the reduction of serious problems caused by chemical control such as elimination of the population of natural enemies, human and environmental contamination and induced resistance in pest populations [4]. Use of entomopathogenic nematode, *Heterorhabditis indica,* is an important biological control strategy due to its symbiotic association with bacteria from the genera *Photorhabdus* which cause rapid death of the insect [3].

The nematodes may be applied in crops with various agricultural inputs such as fertilizers and chemical products applied on the leaves in a single operation. Some of these products may reduce the survival and infectivity of these nematodes [7]. In an integrated pest management system, biological control agents and selective pesticides are used together. Pesticides may influence the activity of these biological control agents [1]. Hence, this study was undertaken to evaluate the compatibility of commonly used pesticides and *H. indica* with an aim to develop an integrated pest management programme involving this nematode.

MATERIALS AND METHODS

H. indica was cultured on last instar larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), according to the method of Dutky *et al.* [5]. The IJs were harvested using White traps as described by White [17] at 25±2^{o}C and maintained in an aqueous suspension (500 IJs / mL) at 16 ± 1 °C and stored for up to one week before being used in the experiment.

The chemical pesticides used in this experiment are mentioned in Table 1, 2 and 3.

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Table 1: List of insecticidal formulations used in the study

S. No.	Technical name	Trade name	Group	% a.i.	Dosage/ ha [a.i (gm)][1]		Source
					HRD[2]	LRD[3]	
1.	Bifenthrin	Imperial	Synthetic pyrethroid	10 EC	100	50	Adama India Pvt. Ltd.
2.	Acetamiprid	Dhanpreet	Neonicotinoid	20 SP	20	10	Dhanuka Agritech Limited
3.	Chlorpyrifos	Cross	Organo-phosphate	20 EC	1000	100	Afield Crop Care
4.	Carbosulfan	Aatank	Oogano-carbamate	25 EC	200	312.5	Dhanuka Agritech Limited
5.	Cypermethrin	Cypercine -10	Synthetic pyrethroid	10 EC	70	50	Cropcine Agro Chemicals
6.	Deltamethrin	Decis	Synthetic pyrethroid	2.8 EC	12.5	3	Bayer Crop Science Ld.
7.	Lambda-cyhalothrin	Karate	Synthetic pyrethroid	5 EC	10	25	Syngenta India
8.	Imidachloprid	Media	Neonicotinoid	17.8 SL	10	70	Dhanuka Agritech Limited
9.	Spinosad	Tracer	Biopesticide	45 SC	100	56	Bayer Crop Science Ltd.

[1]http://ppqs.gov.in/
[2]Highest recommended dose
[3] Lowest recommended dose

Table 2: List of herbicidal formulations used in the study

S. No.	Technical name	Trade name	Group	% a.i.	Dosage/ ha [a.i (kg)][1]		Source
					HRD[2]	LRD[3]	
1.	2,4-D Sodium salt	Suspend	Unclassified	80 WP	2.6	0.5	Canary Agro Chemicals Private Limited
2.	Atrazine	Foost	Triazine	50 WP	2.0	0.5	Bayer Crop Science Ltd.
3.	Isoproturon	Isoguard	Urea	50 WP	1.0	1.0	Gharda Chemicals
4.	Pendimethalin	Stomp	Dinitroaniline	30 EC	1.5	0.7	Cynamid

[1]http://ppqs.gov.in/
[2]Highest recommended dose
[3] Lowest recommended dose

Table 3: List of fungicidal formulations used in the study

S. No.	Technical name	Trade name	Group	% a.i.	Dosage/ ha [a.i (gm)][1]		Source
					HRD[2]	LRD[3]	
1.	Carbendazim	Bavistin	Benzimidazole	50 WP	1250	750	Bayer Crop Science Ld.
2.	Hexaconazole	Contaf	Triazole	5 EC	75	10	Rallis india Ltd.
3.	Mancozeb	Win M-45	Zinc	75 WP	1500	1125	Volkschem Crop Science Private Limited

[1]http://ppqs.gov.in/
[2]Highest recommended dose
[3] Lowest recommended dose

Stock solution at double the recommended concentration of the pesticide was prepared in distilled water. The suspension of infective juveniles was prepared in distilled water with a concentration of 2000 IJ/ml and one ml of nematode suspension was transferred to each well of 24-well culture plate (Orange Scientific Cat no. 132024). One ml solution of pesticide was added to the nematode suspension in each well so that final pesticide concentration in the well equals to the recommended concentration. The recommended doses of pesticides were as per the Central Insecticide Board and Registration Committee, Faridabad, Haryana, India . Distilled water without chemical was used as control. The treatments were replicated four times. The plates were kept at 25 °C in BOD incubator. The mortality of IJs was recorded up to 72 h, by taking three 50 µl aliquots of nematode suspension from each well and observed under the stereo zoom microscope. Virulence test was performed only for insecticide treated IJs. For virulence assay, the insecticide treated IJs after 24 h of incubation in insecticide solution were used. Fifty live IJs of *H. indica* were applied in 5 cm petridish lined with double layer of moist filter paper containing single fifth instar *Galleria* larvae. Five similar dish containing single insect larvae were considered as one unit and replicated four times. Petri dishes were kept at room temperature (24–26°C) in darkness. The insect mortality was recorded after 24 h and 48 h. Statistical analyses were performed using SPSS 20.0. The percent IJ mortality was corrected by Abbott's formula and arcsine transformation was performed before statistical analysis. Insect mortality data from nematode virulence experiment was not corrected since no any mortality was observed in control. One-way ANOVA was used for analysis and assess significant differences among treatments.

RESULTS AND DISCUSSION

Nematode survival

The results showed that *Heterorhabditis indica* was quite tolerant to most of the chemical pesticides tested (Table 1, 2 and 3). No mortality of infective juveniles of *H. indica* was observed in all pesticides after 24 h while less than 5% mortality was observed in all pesticides except chlorpyriphos after 48 h of exposure. After 72 h, less than 10% mortality was observed in most pesticides except chlorpyriphos (Fig. 1 and 2).

Nematode virulence

Insecticides showed only a marginal effect on the virulence of *H. indica* (Fig 2, 4). Mortality of *Galleria* larvae ranged from 40% to 95% after 24 h of inoculation while mortality ranged from 60 to 100% after 48 h of inoculation . The mortality of *Galleria* larvae was more than 75% in all treatments except in treatment with IJs treated with chlorpyriphos after 24 h while more than 90% mortality was recorded in all the treatments except in treatments with chloropyriphos treated IJs after 48 h of inoculation.

Nematode survival rates observed in this study were similar to that reported by Alumai and Grewal [2] who reported that pesticides directly applied with *S. carpocapsae* at recommended dose had no effect on its viability at room temperature after 3 h of exposure. Similar way the botanical insecticides or chemical insecticides at recommended doses had no effect on nematode survival after 72 h of exposure as reported by Mohamoud *et al.* [13]. In parallel to the results recorded in this study, imidachloprid showed no effect on mortality of *H. bacteriophora*, *H. indica* [16] and *S. carpocapsae* [11]. Studies carried out by other authors also show low IJ mortality of *S. carpocapsae* when exposed to chlorpyrifos [2, 8, 18]. Thiomethoxam was also reported compatible with *H. megidis*, *S. feltae* and *S. glasseri* [10]. Insecticides like fipronil had also reported compatible with *H. bacteriophora* and *S. carpocapsae* and dinotefuran with *H. sonorensis* and *S. riobrave* [14]. Mahmoud *et al.* [12] reported that the imidacloprid (25% WP) at 0.18 g/l, thiamethoxam (25% WG) at 0.5 g/l, Neem Azal (1% azadirachtin) and Neemix (4.5% azadirachtin) at the rate of 1% have no adverse effect on survival of *S. carpocapsae* and *H. bacteriophora* after 72 hours of exposure. There are some reports of incompatibility between EPN and insecticides. Organophosphates like monocrotophos had adverse effect on *S. carpocapsae* [15] and *H. indica* [16] .

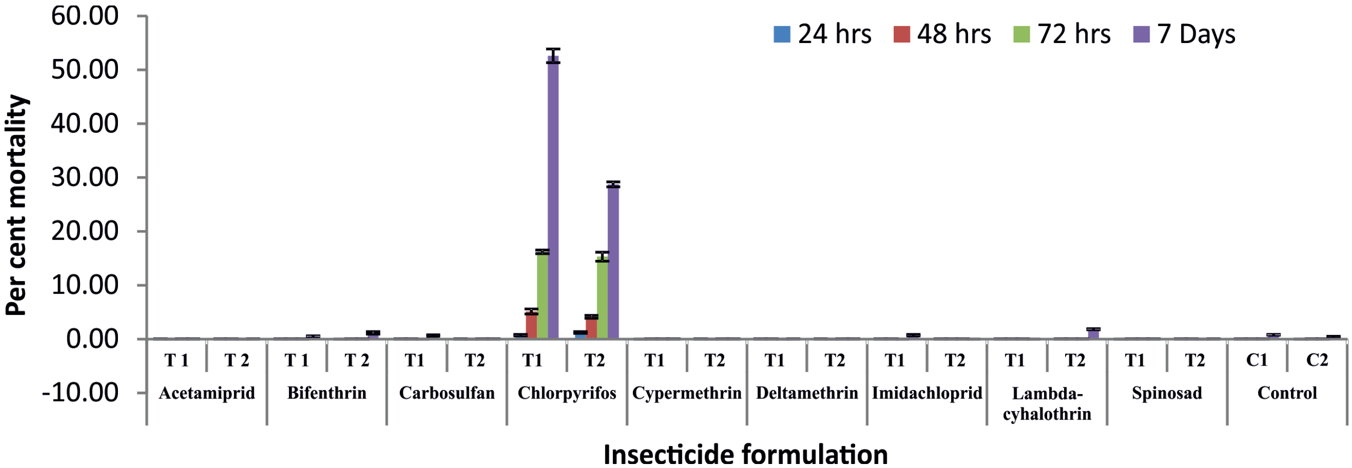

Fig. 1: Effect of different doses of insecticidal formulations on infective juveniles (IJs) of *Heterorhabditis indica in vitro* (at 27°C ±1°C)

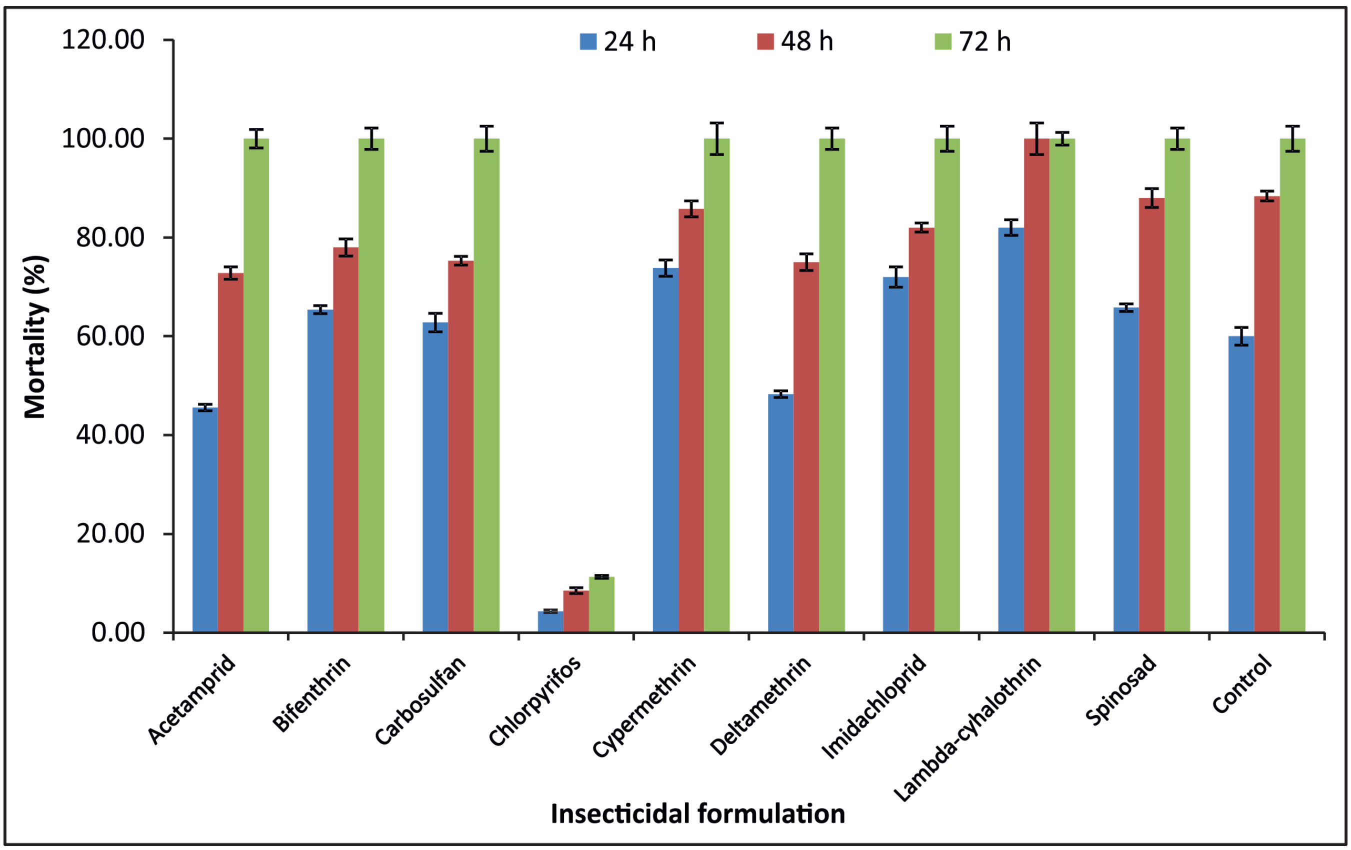

Fig. 2: Mortality of 4th instar larvae of *Galleria mellonella* infected with IJs of *H. indica* exposed to the highest recommended doses of insecticidal formulations for 48 h *in vitro* (at 27°C ±1°C)

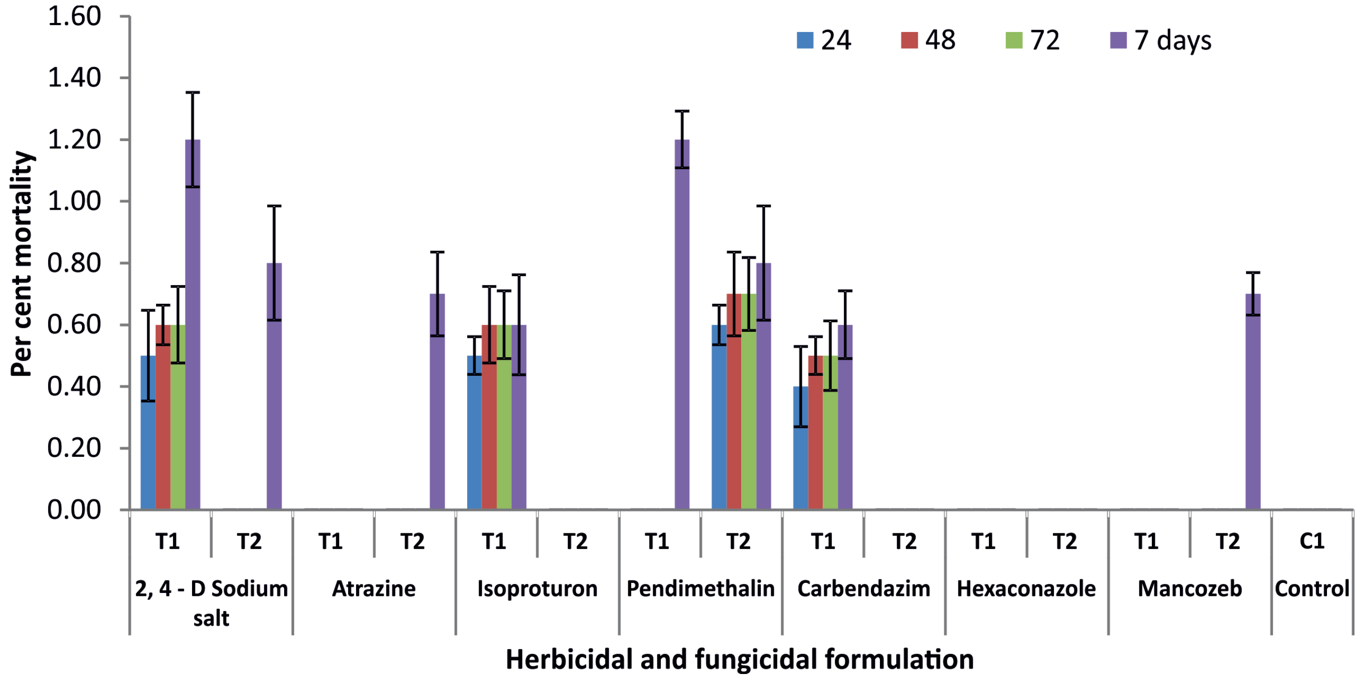

Fig. 3: Effect of different doses of herbicidal and fungicidal formulations on infective juveniles (IJs) of *Heterorhabditis indica in vitro* (at 27°C ±1°C)

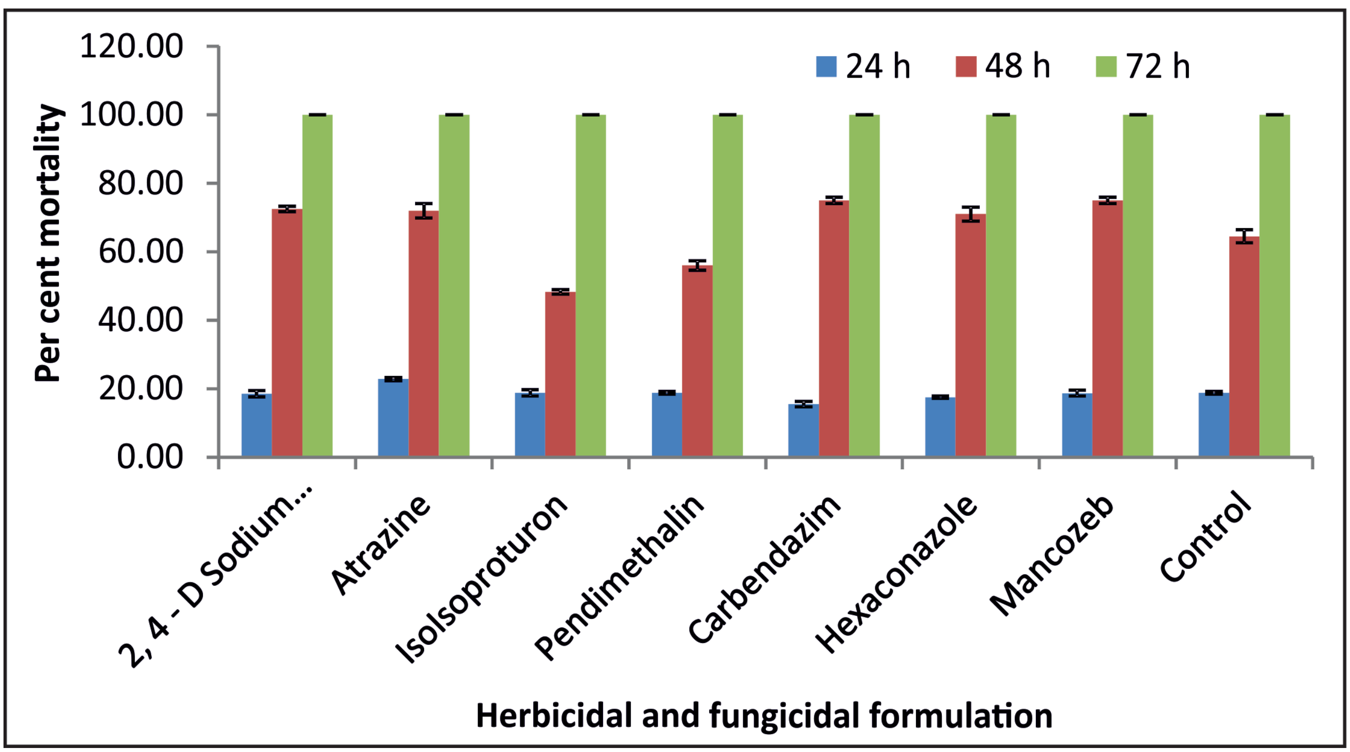

Fig. 4: Mortality of 4th instar larvae of *Galleria mellonella* infected with IJs of *H. indica* exposed to the highest recommended doses of herbicidal and fungicidal formulations for 48 h *in vitro* (at 27°C ±1°C)

CONCLUSIONS

The results of this study increase our knowledge of EPN-pesticides interactions by demonstrating that all the chemical pesticides except chlorpyriphos used in this study are not toxic to *H. indica*. This study suggests that *H. indica* can be successfully included in integrated pest management involving pesticides in agro-ecosystems.

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