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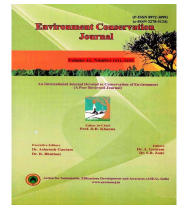

Compatibility studies of *Heterorhabditis indica* with newer insecticides under laboratory condition

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ABSTRACT

Entomopathogenic nematodes (EPNs) have been identified as promising biocontrol agents for controlling economically important insect pests of agricultural and horticultural crops. The compatibility of entomopathogenic nematode *Heterorhabditis indica* with 7 CIB registered insecticides was investigated under laboratory conditions. The effect of these insecticides on nematode survival at recommended concentrations was observed after 12, 24, 48, 72 hours upon direct exposure. EPN *H. indica* was compatible with Imidacloprid 17.8% SL as maximum per cent of live *H. indica* were observed after 72 h of exposure to this insecticide. Similarly, *H. indica* was compatible with Fipronil 5% SC up to 48 h of exposure whereas, less than 70% live EPN were there in Thiamethoxam 25% WG, Diafenthiuron 50% WP and Cypermethrin 25% EC resulting these insecticides to be least compatible. Emamectin benzoate 5% SG and chlorpyriphos 20% EC were incompatible with *H. indica* after 48 h of exposure. The result of this experiment will help in reducing the dependence on chemical insecticides and thus slowing down the development of insecticide resistance and preventing adverse effects on public health and the environment.

Introduction

The aftermath of pesticides on agricultural production has been inescapable. Pesticides have been proved to be harmful to living beings, human health as well as the environment. This is because chemical pesticides are directly linked with the pathogenesis of Parkinson's and Alzheimer's diseases and various disorders of the respiratory and reproductive tracts (Baltazar *et al.,* 2014). In addition to this, oxidative stress is another important mechanism through which many pesticides affect living beings. This oxidative stress leads to DNA damage and later cause malignancies and other disorders (Sabarwal *et al.,* 2018). Excessive pesticide residues on crop produce could have harmful, acute or long-term effects on end-users' health and lead to biomagnification and bioaccumulation. The pesticides enter aquatic organisms or plants through water and later enter food chain to affect other organisms. This has also been observed in earlier case studies on food web components from Zhoushan Fishing Ground, China (Zhou *et al.,* 2018); plankton and fish of Ignacio Ramirez reservoir, Mexico (Favari *et al.,* 2002); organisms of Greenland biota (Vorkamp *et al.,*

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2004); organisms of Antarctic biota (Goerke *et al.,* 2004), etc. Pesticides may be hazardous to non-target species such as bees, wild animals, birds and fish. Most of the pesticides in an application fail to reach the site of action in the target organism. To preclude these detrimental sequels of pesticides, there has been an increased call for a substitute management method, i.e., biological control agents which works quietly in nature.

Entomopathogenic nematodes (EPNs) from the genera *Heterorhabditis* have been identified as promising biocontrol agents for controlling economically important insect pests of a wide range of agricultural and horticultural crops (Ehlers and Hokkanen, 1996; Hazir *et al.,* 2003; Lacey and Georgis, 2012). EPNs are often applied to sites that frequently receives other chemical inputs such as pesticides, fertilizers, herbicides that may mesh with nematodes. It is usually advisable to know if EPNs can be tank-mixed or applied at once with another pesticide to save time and money and become compatible with integrated pest management systems which will reduce the use of chemical pesticides. Therefore, the main aim of the present study was to test the compatibility of CIB registered insecticides with entomopathogenic nematode *Heterorhabditis indica*.

Material and Methods

Treatment details

Multiplication and culturing of EPN

Pure culture of *H. indica* was maintained separately in late instar larvae of *Galleria mellonella* in Entomology Section, College of Agriculture, Nagpur, PDKV, Akola. Initially, measured amounts of suspension with standard count of IJs/100µl from the EPN isolate were taken and larvae of *G. mellonella* were inoculated by direct contact method. Larvae killed by nematodes were placed on white traps for harvesting of nematode population. Emerging infective juvenile stages of nematodes were collected and re-infected to fresh *G. mellonella* larvae and this process of inoculation and re-infection of larvae was repeated until the pure culture of nematode populations with infective juveniles were obtained. This pure culture was used for treatments for further studies.

Table 1. CIB&RC list of label claim insecticides updated on 01.01.2021 (http://ppqs.gov.in/divisions/cib-rc/major-uses-of-pesticides) # Per ha dose is for dilution in 500 litres of water.

Treatment	Trade name	Technical name	Group	a.i.%	Dose (per lit)	Source
1.	Actara®	Thiamethoxam	NN	25 WG	0.2gm	Syngenta India Ltd.
2.	Pegasus®	Diafenthiuron	Thiourea	50 WP	2.5gm	Syngenta India Ltd.
3.	Confidor®	Imidacloprid	NN	17.80 SL	0.1ml	Bayer Crop Science Ltd.
4.	Regent®	Fipronil	Py	5 SC	1.5ml	Bayer Crop Science Ltd.
5.	Proclaim®	Emamectin benzoate	Avermectin	5 SG	0.4gm	Syngenta India Ltd.
6.	Cymbush®	Cypermethrin	SP	25 EC	0.6ml	Syngenta India Ltd.
7.	Excel®	Chlorpyriphos	OP	20 EC	5ml	Moti Insecticides Pvt Ltd.
8.	Control	Distilled water	-	-	-	-

Storage of EPN

The infective juveniles of the EPN isolate *H. indica* were stored in conical/tissue culture flasks in distilled water at room temperature (Songbi and Itamar, 2005). The nematode concentrations were kept in the range of 10,000 IJs/ml of distilled water.

Experimental setup

The present work was carried on the compatibility of *Heterorhabditis indica* with insecticides in the post-graduate laboratory, Entomology Section, College of Agriculture, Nagpur, PDKV, Akola. 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 container. One ml pesticide solution was added to the nematode suspension in each container so that the final pesticide concentration was equal 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 chemicals was used as a control. There were four replicates in each treatment. The plates were kept at 25 ± 1°C (Alonso, 2018).

Data collection

The mortality of IJs was recorded after 12, 24, 48 and 72 hours. The observations were taken with 100 µl aliquots from each container and observed under the stereo zoom microscope. The observations were recorded for nematode mortality. Straight IJs having no motion and not responding to prodding were counted as dead. The interpretations of observations on the compatibility of nematodes with chemicals were made based on the record of the proportion of nematodes dead stage. The compatibility was classified in to four categories as, highly compatible: 86-100% survivals, compatible: 71-85% survivals, least compatible: 51-70% survivals and incompatible: < 50% survivals.

Statistical analysis

The data obtained were statistically analysed by using one factor analysis (CRD) with the help of OPSTAT software.

Results and Discussion

The result showed that the survival percentage of *H. indica* with different pesticides after 12, 24, 48 and 72 h of exposure were in decreasing trend. As the exposure of time increased, the rate of survival decreased (Table 2).

Observations after 12 h

After 12 h of exposure, among all combinations tested, Thiamethoxam 25% WG showed maximum per cent of survivals (92.85), i.e., highly compatible with *H. indica* followed by Imidacloprid 17.8% SL (90.85%), Fipronil 5% SC (89.35%) and Diafenthiuron 50% WP (88.05%). Cypermethrin 25% EC (84.8%), Chlorpyriphos 20% EC (78.8%) were found relatively less compatible as compared to above-mentioned insecticides. Similarly, Emamectin Benzoate 5% SG showed the least compatibility, having a minimum per cent of survivals (67.60) and were at par with each other in the order mentioned above.

Observations after 24 h

As far as insecticides' effect was concerned, the insecticide Imidacloprid 17.8% SL having per cent survivals (88.05) was highly compatible with *H. indica* and found significantly superior along with the 86.35% survivals at Fipronil 5% SC after 24 h of exposure. The effect of Emamectin Benzoate 5% SG on *H. indica* having 67.3% survivals didn't vary significantly from Chlorpyriphos 20% EC having 65.6% survivals and found least compatible with *H. indica*.

Table 2: Survival percentage of *H. indica* at 12, 24, 48 and 72 hrs of exposure to Thiamethoxam 25% WG (T_1), Diafenthiuron 50% WP (T_2), Imidacloprid 17.8% SL (T_3), Fipronil 5 SC% (T_4), Emamectin Benzoate 5% SG (T_5), Cypermethrin 25% EC (T_6), Chlorpyriphos 20% EC (T_7) and water as control (T_8). Survival (%) = [(Total no. of nematode IJs - No. of died IJs of nematodes) / Total no. of nematode IJs] x 100.

Treatment	12 h	24 h	48 h	72 h
IJs + Thiamethoxam 25%WG	92.85 (75.63)	82.45 (65.23)	59.75 (50.62)	58.75 (50.03)
IJs + Diafenthiuron 50% WP	88.05 (70.00)	82.85 (65.53)	64.30 (53.29)	60.38 (50.99)
IJs + Imidacloprid 17.8% SL	90.85 (72.47)	88.05 (69.77)	86.15 (68.13)	71.80 (57.96)
IJs + Fipronil 5% SC	89.35 (71.04)	86.35 (68.36)	75.60 (60.38)	66.73 (54.76)
IJs + Emamectin benzoate 5% SG	67.60 (55.30)	67.30 (55.21)	27.10 (31.23)	11.85 (20.00)
IJs + Cypermethrin 25% EC	84.80 (67.14)	81.45 (64.62)	58.55 (49.91)	54.78 (47.73)
IJs + Chlorpyriphos 20% EC	78.80 (62.82)	65.60 (54.10)	39.95 (38.99)	8.55 (16.25)
IJs + Distilled water (Control)	100.00 (90.00)	99.40 (85.73)	98.50 (82.93)	97.88 (81.61)
S.E(d)±	2.80	1.92	2.55	2.31
S.E(m)±	1.98	1.36	1.80	1.63
C.D. @5%	5.81	4.00	5.31	4.80
C.V. (%)	5.61	4.12	6.64	6.90

(Figures in the bracket are arcsine transformation; **F test highly significant at 1% level of significance)

Table 3: Summary of compatibility status observed in the present study

Treatment	12hr	24hr	48hr	72hr
IJs + Thiamethoxam 25% WG	Highly compatible	compatible	Least compatible	Least compatible
IJs + Diafenthiuron 50% WP	Highly compatible	compatible	Least compatible	Least compatible
IJs + Imidacloprid 17.8% SL	Highly compatible	Highly compatible	Highly compatible	compatible
IJs + Fipronil 5% SC	Highly compatible	Highly compatible	compatible	Least compatible
IJs + Emamectin benzoate 5% SG	Least compatible	Least compatible	Incompatible	Incompatible
IJs + Cypermethrin 25% EC	compatible	compatible	Least compatible	Least compatible
IJs + Chlorpyriphos 20% EC	compatible	Least compatible	Incompatible	Incompatible

Observations after 48 h

Data about mean per cent survivals presented in Table 2 showed that after 48 h of exposure, maximum per cent survivals were observed in Imidacloprid 17.8% SL followed by Fipronil 5% SC, i.e., 86.15%, 75.6% survivals, respectively. While minimum per cent survivals were recorded in Emamectin Benzoate 5% SG (27.10%) and it was found to be incompatible with *H. indica*.

Observations after 72 h

After 72 h of exposure, the treatment T_3, i.e., Imidacloprid 17.8% SL, having per cent survival 71.80 was compatible with *H. indica* and found significantly superior along with the insecticide Fipronil 5% SC having per cent survival 66.72. Diafenthiuron 50% WP (60.37%), Thiamethoxam 25% WG (58.75%) and Cypermethrin 25% EC (54.75%) were found least compatible with *H. indica* as compare to the insecticides mentioned above and were at par with each other in the given order. Emamectin Benzoate 5% SG and Chlorpyriphos 20% EC were incompatible with *H. indica* showing 11.85% and 8.55% survivals, respectively and were statistically the same. The compatibility status has been summarized in Table 3. Earlier works related to the compatibility of EPN *H. indica* with insecticides are very scant. The present study was supported by an earlier work of Priya and Subramanian (2008), where it has been reported that *H. indica* was compatible with carbofuran, carbosulfan and imidacloprid. Earlier work suggests that neonicotinoid insecticides have fewer adverse effects on nematode survival, pathogenicity, and infectivity (Koppenhöfer *et al.,*2003). Thiamethoxam and imidacloprid belong to the same insecticide group, i.e., neonicotinoid. Thiamethoxam which is moderately toxic is from second generation, whereas imidacloprid which is highly toxic is from first generation neonicotinoid. The mode of action of both these chemicals is the same. But interestingly, the compatibility level of Thiamethoxam decreased in the present study that requires further investigation. Thiamethoxam was also reported compatible with *H. megidis*, *S. feltae* and *S. glasseri*. Organophosphates like monocrotophos, chlorpyrifos have an adverse effect on *S. carpocapsae* and *H. indica* (Chavan *et al.,* 2018). Some reports demonstrated that certain insecticides, particularly organophosphates and carbamates, possess nematocidal properties (Atwa, 1999). Prolonged exposure to some plant protection products can affect the efficiency and reproduction of the nematodes (Negrisoli Jr *et al.,* 2010). Patil et al. (2017) showed that the IJs exposed to Proclaim® recorded 82.71% survival in *H. indica* at 48 h of exposure. However, the mortality of EPN species was less than our present study, which may be due to their lower doses of insecticides and may be related to differences in chemical composition and formulation of the product.

Conclusion

The results of this study increased our knowledge of EPN and insecticide interactions. *H. indica* was found to be compatible with most of the insecticides tested except Emamectin benzoate 5% SG and chlorpyriphos 20% EC. *H. indica* can be successfully included in IPM of economically important crop pests. It may reduce the dependence on chemical insecticides, development of insecticide resistance and adverse effects on public health and the environment. The results of this work expand our knowledge on the compatibility of EPN with registered insecticides for the control of crop pests. Knowledge of the survival per cent with respect to the used insecticides will be helpful to predict the required application rate of nematodes in IPM programs.

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Conflict of interest

The authors declare that they have no conflict of interest.

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