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Compatibility of entomopathogenic nematode *Heterorhabditis indica* (Nematoda: Heterorhabditidae) with agrochemicals used in the rice ecosystem

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Abstract
The compatibility of entomopathogenic nematode *Heterorhabditis indica* with 14 insecticides, 6 fungicides and 7 herbicides commonly used in rice ecosystems was investigated under laboratory conditions. The effect of these chemicals on nematode viability and virulence upon direct exposure to chemicals at recommended concentrations was studied. *H. indica* was tolerant to most of the insecticides tested with less than 10% nematode mortality observed in all insecticides except monocrotophos (19.5%) and cartap hydrochloride (100%) after 72 h of exposure. Less than 10% mortality was observed in all fungicides tested except tricyclazole (14.5%) and carbendazium + mancozeb (21.5%) after 72 h of exposure to fungicides. Nematode mortality in case of herbicides ranged from 3.5% to 18% after 72 h of exposure. Insecticides showed only a marginal effect on the virulence of *H. indica*. Mortality of *Galleria mellonella* larvae in all the treatments was more than 90% except in treatments with chlorpyrifos (80%) and monocrotophos (60%) treated infective juveniles after 48 h. These results show that *H. indica* is compatible with all the tested agrochemicals except monocrotophos and cartap hydrochloride among insecticides, tricyclazole and carbendazium+mancozeb among fungicides, and pendimethalin among herbicides.

Keywords: Compatibility, *Heterorhabditis indica*, agrochemicals, rice

Introduction
Entomopathogenic nematodes (EPN) *Steinernema* and *Heterorhabditis* are lethal parasites of insects [1]. EPN have great potential as biological control agents against a wide range of economically important insect pests and have been found effective against several insect pests of rice [2-17]. Compatibility of EPN with agrochemicals have been studied and reviewed by several authors [18, 19]. Infective juveniles (IJs) of EPN have been found to be tolerant to short exposures (2-6 h) of most insecticides, acaricides, fungicides and herbicides [18, 19, 20] and therefore EPN can also be tank-mixed and applied together with pesticides. EPN are easy to apply in field, as they are easily sprayed using standard equipments including pressurized, mist, fan, electrostatic and aerial sprayers and can be combined with almost all chemical control compounds [21]. EPN-pesticide compatibility can be strain-specific [22]. Some chemicals used as inert ingredients or adjuvants in formulations can be toxic to nematodes hence compatibility of each formulation with the specific nematode species should be evaluated [23].
Hence, the objective of this study was to investigate the compatibility of EPN with commonly used insecticides, botanicals, fungicides and herbicides in rice ecosystem. The effect of agrochemicals on nematode viability and virulence upon direct exposure to pesticides at recommended concentrations was studied.

Materials and Methods
The EPN species *Heterorhabditis indica* [24] used in the experiment was obtained from EPN culture collections maintained at Nematology lab, ICAR-IIRR, Hyderabad. Fifth instar larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) which were reared on artificial diet [25] were used as laboratory host for the multiplication of nematodes. The basic *in vivo* production method outlined by Woodring and Kaya [26] was followed for the multiplication of the nematodes.

The agrochemicals commonly used in rice ecosystems were selected for this study (Table 1). The pesticides: 11 insecticides *viz.* imidachloprid (17.8% SL), monocrotophos (36% SL), chlorpyrifos (20% EC), acephate (75% SP), fipronil (5% SC), dinotefuran (20% SG), buprofezin (25% SC), thiamethoxam (25% WG), pymetrozine (50% WG), cartap hydrochloride (50% SP) and ethiprole+imidacloprid (40%+40% WG)), botanicals- azadirachtin (1% and 0.3% EC); 6 fungicides *viz.* carbendazium (50% SP), hexaconazole (5% EC), tricyclazole (75% SP), propiconazole (25% EC), mancozeb (75% WP) and carbendazium+mancozeb (12%+3% WP) and 6 herbicides *viz.* cyhalofop butyl (10% EC), 2,4-D sodium (80% WP), penoxsulam (21.7% EC), pendimethalin (30% EC), bispyribac sodium (10% EC) and butachlor (50% EC) etc were tested.

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 [27]. 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 after 6 h, 12 h, 24 h, 48 h and 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 except cartap hydrochloride treated IJs since the nematode mortality was around 100% at 24 h in cartap hydrochloride. Out of three azadirachtin formulations only Nema azal was tested in virulance assay. 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 Tukey's HSD test at P=0.05 was used to assess significant differences among treatments.

Table 1: List of the agrochemicals tested with active ingredient, formulation and recommended dose

Trade name	Active ingradient (%)	F	RFD (ml-g/ha)	C	Manufacturer
Chemical Insecticides					
Confidor	Imidachloprid (17.8)	SL	100-125	0.25	Bayer Cropscience Ltd
Monostar	Monocrotophos (36)	SL	1250	2.5	Swal Corporation Ltd
Dursban	Chlorpyriphos (20)	EC	1250	2.5	Dow Agroscience
Starthene	Acephate (75)	SP	666-1000	2	Swal Corporation Ltd
Regent	Fipronil (5)	SC	1000-1500	3	Bayer Cropscience Ltd
Token	Dinotefuran (20)	SG	150-200	0.4	Indofil Chemicals Company Ltd
Kaal	Buprofezin (25)	SC	800	1.6	GSP Cropscience Pvt. Ltd.
Actara	Thiamethoxam (25)	WG	100	0.2	Syngenta
Chess	Pymetrozine (50)	WG	300	0.6	Syngenta
Caldan SP	Cartap hydrochloride (50)	SP	1000	2	Dhanuka Agritech Ltd.
Glamore	Ethiprole + Imidacloprid (40+40)	WG	125	0.25	Bayer Cropscience Ltd
Botanical insecticides					
Neem azal	Azadirachtin (1)	EC	100-1500	3	E.I.D.-Parry (India) Ltd.
Nimbicidine	Azadirachtin (0.03)	EC	2000	4	T. Stanes & Company Ltd.
Multineem	Azadirachtin (0.03)	EC	2000	4	Karnataka Agro Chemicals Bangalore (India) Pvt. Ltd
Fungicides					
Benfil	Carbendazium (50)	SP	250-500 g	1.0	Indofil Industries Ltd
Contaf	Hexaconazole (5)	EC	1000 ml	2.0	Rallis India Ltd
Baan	Tricyclazole (75)	WP	300-400 g	0.6	Indofil Industries Ltd
Tilt	Propiconazole (25)	EC	500 ml	1.0	Syngenta
Indofil M-45	Mancozeb (75)	WP	1.5-2 kg	2	Indofil Industries Ltd
Companion	Carbendazium 12%+Mancozeb 3% (63)	WP	750	1.5	Indofil Industries Ltd
Herbicides					
Clincher	Cyhalofop Butyl (10)	EC	750-800 ml	1.6	Dow Agrosciences India Pvt. Ltd
Superhit	2,4-D Sodium (80)	WP	1.25 kg	3	Insecticides (India) Ltd
Granite	Penoxsulam (21.7)	EC	93.7-104.2	0.21	Dow Agrosciences India Pvt. Ltd
Swalpendi	Pendimethalin (30)	EC	3.5-5 lit	10	Swal Corporation Limited
Nominee	Bispyribac Sodium (10)	EC	200 ml	0.4	Pi Industries Ltd
Butachlor	Butachlor (50)	EC	2.5-4 lit	8	Jai Shree Rasayan Udyog Ltd

F: Formulation type, RFD: Recommended Field dose, C: Concentration of chemicals g-ml/lit, SC – suspension concentrate; SL – soluble liquid; WP – wettable powder; WG – water dispersible granules; EC – emulsifable concentrate; SP – Soluble powder

Results and Discussion

Nematode survival

Nematode survival was significantly affected by agrochemicals. The results show that *Heterorhabditis indica* was quite tolerant to most of the agrochemicals tested (Table 1). No mortality of infective juveniles was observed in all agrochemicals after 6 h while less than 5% mortality was observed in all agrochemicals except cartap hydrochloride after 12 h of exposure. Significant differences were observed among insecticides after 24 h (F =2300, df = 13, 55, P=0.05), 48 h (F =4271, df = 13, 55, P=0.05) and 72h (F =1985, df = 13, 55, P=0.05). After 72 h, less than 10% mortality was observed in most insecticides except ethiprole+imidacloprid, Neem azal, monocrotophos and cartap hydrochloride (Table 2).

Nematode survival rates observed in this study were similar to that reported by Alumai and Grewal [28] 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 and co-author [29]. In parallel to the results recorded in this study, imidachlopridate showed no effect on mortality of *H. bacteriophora* [30], *H. indica* [31] and *S. carpocapsae* [32]. Studies carried out by other authors also show low IJ mortality of *S. carpocapsae* when exposed to chlorpyrifos [28, 33, 34]. Thiomethoxam was also reported compatible with *H. megidis*, *S. feltae* and *S. glasseri* [35] and with *S. carpocapsae* [28]. Insecticides like fipronil had also reported compatible with *H. bacteriophora* and *S. carpocapsae* [36] and dinotefuran with *H. sonorensis* and *S. riobrave* [37]. Mahmoud and co-author [38] 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* [39] and *H. indica* [31]. Acephate had been reported to be toxic to *H. bacteriophora* [18]. Generally, insecticides belonging to organophosphates and carbamates have been found to be toxic to *Steinernema* spp. and *Heterorhabditis* spp. though with notable differences between the various active ingredients [18, 20, 40-42]. Cartap hydrochloride had been found to be highly toxic to *S. carpocapsae* [43], *H. indica* and *Metarhabditis amsactae* [17].

There were significant differences among the fungicides at all time intervals: 24 h (F =20, df = 5, 23, P=0.05), 48 h (F =25, df = 5, 23, P=0.05) and 72h (F =33, df = 5, 23, P=0.05). Less than 10% mortality was observed with all fungicides tested except tricyclazole (14.5%) and carbendazium +mancozeb (21.5%) at 72h (Table 2). Similar results were observed with carbendazium on the *S. feltae* [20] and with propiconazole on *H. bacteriophora* [18] and *S. carpocapsae* and *S. feltae* [33]. Laznic and Tradan [44] reported that EPN *S. feltae* was compatible with most commonly used fungicides.

All tested herbicides showed marginal effect on mortality of *H. indica*. Significant differences were observed among treatments after 24 h (F =12, df = 6, 27, P > 0.05), 48 h (F =23, df = 6, 27, P =0.05) and 72h (F =14, df = 6, 27, P=0.05). Mortality ranged from 3.5% to 18% among herbicides after 72 h (Table 2). Glyphosate had little effect on mortality and was compatible with *H. bacteriophora* [18], *S. carpocapsae* and *S. feltae* [20]. Gupta and Siddiqi [45] reported that 2,4-D Sodium was not compatible with *S. carpocapsae* while Laznic and Tradan [46] reported no any detrimental effect on viability of *S. feltae* when exposed to 2,4-D Sodium. Similar to the results obtained in this experiment, pendimithalin was not compatible with *H. bacteriophora* [18] but in contrast to this, Rovesti and Deseö [20] reported that pendimithalin was compatible with *S. carpocapsae* and *S. feltae*.

Table 2: Mean mortality (% ± SE) of infective juveniles of *Heterorhabditis indica* after exposure to agrochemicals after 24, 48 and 72 hours.

Group	Agrochemicals	24 h	48 h	72 h
Insecticide	Imidachloprid	2.75 ± 0.275^{bc}	6.25 ± 0.363^{cd}	9.5 ± 0.558^{c}
	Monocrotophos	5.75 ± 0.491^{b}	9.75 ± 0.275^{b}	19.5 ± 0.885^{b}
	Chlorpyriphos	0.25 ± 0.250^{bc}	0.25 ± 0.285^{e}	1.75 ± 0.409^{d}
	Acephate	0.00 ± 0.000^{bc}	2.75 ± 0.278^{e}	4 ± 0.553^{d}
	Fipronil	0.25 ± 0.250^{bc}	2.75 ± 0.491^{e}	2.75 ± 0.432^{d}
	Dinotefuran	0.5 ± 0.289^{c}	1.75 ± 0.166^{e}	3 ± 0.167^{d}
	Buprofezin	0 ± 0.000^{bc}	1.25 ± 0.233^{e}	1.25 ± 0.264^{d}
	Thiamethoxam	0 ± 0.000^{bc}	0.25 ± 0.285^{e}	2.25 ± 0.408^{d}
	Pymetrozine	0 ± 0.000^{bc}	1 ± 0.273^{e}	2.25 ± 0.432^{d}
	Cartap hydrochloride	99.5 ± 1.366^{a}	100 ± 0.000^{a}	100 ± 0.000^{a}
	Ethiprole + Imidacloprid	2.75 ± 0.275^{bc}	8.25 ± 0.365^{bc}	10.5 ± 0.673^{c}
	Azadirachtin (Neem azal)	1.25± 0.205^{c}	4 ± 0.408^{de}	10.5 ± 0.278^{c}
	Azadirachtin (Nimbicidine)	0.5 ± 0.289^{c}	0.75 ± 0.285^{e}	1 ± 0.306^{d}
	Azadirachtin (Multineem)	0.25 ± 0.250^{bc}	0.5 ± 0.329^{e}	1 ± 0.145^{d}
Fungicides	Carbendazium	7 ± 0.333^{cd}	7.5 ± 0.289^{cd}	7.5 ± 0.558^{cd}
	Hexaconazole	1 ± 0.332^{e}	2.5± 0.289^{cd}	2.5 ± 0.291^{d}
	Tricyclazole	14 ± 0.823^{ab}	14 ± 1.508^{b}	14.5 ± 0.211^{b}
	Propiconazole	1.5 ± 0.288^{de}	1.5 ± 0.289^{d}	2.5 ± 0.291^{d}
	Mancozeb	8.5 ± 0.289^{bc}	9 ± 0.749^{bc}	9.5 ± 0.559^{bc}
	Carbendazium 12% + Mancozeb 3%	14.5 ± 1.34^{a}	21.5 ± 1.310^{a}	21.5 ± 1.016^{a}
Herbicides	Cyhalofop Butyl	3.5 ± 0.288^{bcd}	6 ± 0.820^{bc}	6 ± 1.258^{b}
	2,4-D Sodium	0.5 ± 0.288^{b}	11.5 ± 0.994^{ab}	12 ±0.265^{b}
	Penoxsulam	2.5 ±0.288^{cd}	3 ± 0.334^{c}	4 ± 0.475^{b}
	Pendimethalin	8 ± 0.817^{a}	18 ± 0.479^{a}	18 ± 0.481^{a}
	Bispyribac Sodium	3 ± 0.332^{cd}	4.5 ± 554^{bc}	5.5 ± 0.873^{b}
	Butachlor	0.5 ± 0.288^{d}	1 ± 0.3341^{c}	3.5 ± 0.557^{b}

Means followed by the same letter within the group are not statistically different according to the Tukey HSD test at P=0.05

Nematode virulence

Insecticides showed only a marginal effect on the virulence of *H. indica* (Fig 1). Mortality of *Galleria* larvae ranged from 40% to 95% after 24 h of inoculation (F =3, df = 11, 47, P=0.05) while mortality ranged from 60 to 100% after 48 h of inoculation (F =5, df = 11, 47, P=0.05). The mortality of *Galleria* larvae was more than 75% in all treatments except in treatment with IJs treated with monocrotophos after 24 h while more than 90% mortality was recorded in all the treatments except in treatments with chloropyriphos and monocrotophos treated IJs after 48 h of inoculation. Kulkarni and co-authors [47] reported that the IJs of *S. carpocapsae* were tolerent to the chemical insecticides like imidacloprid and thiomethoxam and biopesticides like Neemgold (azadirachtin), spinosad and Agropest (Bt) even at concentration higher than recommended doses in terms of survival and further infectivity. According to Navarro and co-authors [37] combinations of imidacloprid were antagonistic to the virulence of *S. riobrave* but additive with respect to the virulence of *H. sonorensis* while dinotefuran had an additive effect on virulence of both EPN species against *Helicoverpa zeae*.

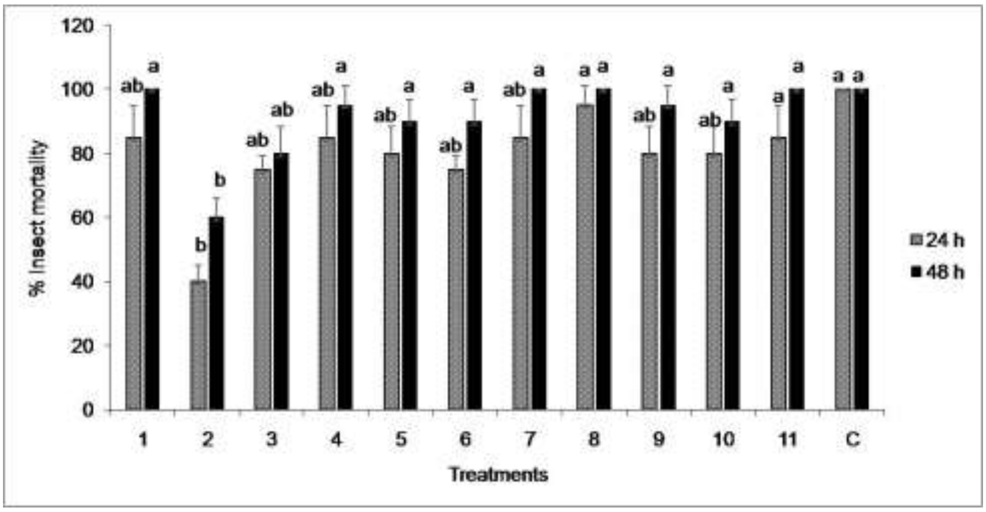

Fig 1: Mean mortality (% ± SE) of insect *Galleria melonella* after exposure to insecticide treated infective juveniles of *Heterorhabditis indica* for 24 and 48 hours. Bars with the same letters within exposure periods are not statistically different according to the Tukey HSD test at P=0.05. (1- imidachloprid, 2-monocrotophos, 3-chlorpyrifos, 4-acephate, 5-fipronil, 6-dinotefuran, 7-buprofezin, 8-thiamethoxam, 9-pymetrozine, 10-ethiprole+imidacloprid, 11-Neemazal and C- control)

Conclusion

Majority of agrochemicals at recommended concentrations caused less than 10% nematode mortality of entomopathogenic nematode *Heterorhabditis indica* after 72 h of exposure. *H. indica* was found to be compatible with most of agrochemicals tested except monocrotophos and cartap hydrochloride among insecticides, tricyclazole and carbendazium+mancozeb among fungicides and pendimethalin among herbicides. Hence, our results suggest that entomopathogenic nematode *H. indica* is compatible with the majority of the agrochemicals registered to use in rice ecosystem.

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