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COMPATIBILITY OF NIMBECIDINE AND TRICEL WITH ENTOMOPATHOGENIC NEMATODE, *HETERORHABDITIS BACTERIOPHORA*

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Abstract–A laboratory study was conducted to study of compatibility of entomopathogenic nematode, *Heterorhabditis bacteriophora* with insecticides currently used against cutworm (*Agrotis ipsilon*). To evaluate the nematode survival rate, infective juveniles (IJs) *H. bacteriophora* were exposed to Nimbecidine and Tricel at three different concentrations for 3 h,6 h, 12 h and 24 h. The surviving nematodes were used to determine the infectivity, penetration rate and reproduction potential in last instars cutworm larvae. Data showed that the survival rate of nematodes exposed to Nimbecidine and Tricel was 53% and 50% respectively. *H. bacteriophora* was found to be virulent with the *A. ipsilon* mortality (60 % to 70%).Penetration rateof IJs of *H. bacteriophora* in *A. ipsilon* larvae reached 55.5% when exposed to Nimbecidine at 0.125%. *H.bacteriophora* was more susceptible to chlorpyriphos than Nimbecidine. *H. bacteriophora* infectious ability and reproduction rate in *A. ipsilon* was found to be reduced in exposure of chlorpyriphos.

INTRODUCTION

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* are being used as a biocontrol agent against a wide range of insect pests of agricultural crops.Infective juveniles (IJs) of entomopathogenic nematodes have been found to be tolerant to short exposures (2-6 h) of most acaricides, fungicides, herbicides, and insecticides (Rovesti *et al*., 1988) and can therefore be applied simultaneously with many agrochemicals. Earlier studies have addressed the consequences of direct exposure to solutions of insecticides on the behavior and infectivity of selected entomopathogenic nematode species (Hara and Kaya, 1982, 1983; Zhang *et al*., 1994; Gordon *et al*., 1996). Some pesticides can reduce entomopathogenic nematode viability and infectivity (Zimmerman and Cranshaw, 1990; Patel and Wright, 1996; Head *et al*., 2000; Krishnayya and Grewal, 2002; Rovesti and Deseo, 1990; Gaugler and Campbell, 1991). Moreover, entomopathogenic nematode species differ in their susceptibility and sensitivity to different formulations of the same chemical pesticide (Grewal, 2002). However, it has also been suggested that exposure to certain chemicals may stimulate nematode movement and enhance host finding behaviour and penetration of the host (Ishibashi and Takii, 1993). Therefore, the compatibility of different agrochemicals and EPN isolates should be assessed to achieve good pest management.

In India, *Agrotisipsilon* Hufnagel, *A.interacta* Walk, *A. flammatra* Schiff., *A.spinnifera* Hb. and *A.segetum* Schiff. have been reported causing 12-40% loss in potato tuber yield in different parts. *A. ipsilon* has been reported from almost all potato growing regions of Assam and other states of North East India and the extent of damage due to it varied from 53.5 to 57.5% of plant and 34.6 to 40.1% of tuber on weight basis (Borah *et al*., 2009). Biopesticides such as neem products are one of the best biocontrol options for cutworm management. Azadirachtin causes several effects on insects, viz., growth disruption, feeding, oviposition disruption, reduction in fitness, and fecundity, thus making it more susceptible to microbial infection (Defago *et al*., 2011; Mondal and Mondal, 2012). Entomopathogenic nematodes (EPNs) have also good application prospects for the control of the black cutworm, *Agrotisipsilon* (Hussaini *et al*., 2000; Yan *et al*., 2014; Ibrahim, 2019).

Considering the pest status of cutworm, *A. ipsilon* in Assam, a laboratory experiment was conducted to evaluate thesurvival and virulence of *H. bacteriophora* to appraise their compatibility with the tested insecticides, viz., Nimbecidine and chlorpyriphos.

MATERIALS AND METHODS

Adult females and males of greater wax moths, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) were put in a plastic box (17×13×7 cm) and fed them with 10% honey syrup. Eggs were laid on the paper in 3 days, cut and placed onto a new rearing box containing artificial diet. The larvae were maintained inside the rearing box and fresh diets were added every three days. The fifth instar larvae were stored at 15 °C to use for subsequent studies within 2 weeks.

The EPN isolate, *Heterorhabditis bacteriophora* recovered from lower Brahmaputra valley zone, Assam, India (Devi *et al*., 2020) were used in the experiments. A drop containing 500 IJs of *H. bacteriophora* was applied into 5 cm diameter Petri dish lined with Whatman® #1 filter paper. Then, ten numbers of fifth instar larvae of *G. mellonella* were placed into a Petri dish and keptat 25±2 °C and 60±5% RH. Three days later, the cadavers were transferred to a white trap (Kaya and Stock, 1997). Newly emerged infective juveniles (IJs) of *H. bacteriophora* were kept refrigerated in tap water at 5 ^{0}C for one week before the experiments were carried out (Ehlers, 2001).

The last instar larvae of cutworm, *Agrotisipsilon* were collected from infested potato field.

The selected pesticides that were used in this study were procured from the market.

Compatibility Study

Nimbecidine (Azadirachtin 0.03% EC) and Tricel (Chlorpyriphos 20% EC), the commercial formulations were tested for compatibility with entomopathogenic nematode, *H. bacteriophora*. Nimbecidine as suspension in distilled water at concentrations of 0.5%, 0.25%, 0.125% and Tricel at 0.2%, 0.1%, 0.05% was prepared from the recommended field concentrationdose. Stock solutions of these chemicals were made in water at double strength of the necessitated dose. Two hundred IJs of *H. bacteriophora* in 0.5 ml of water were mixed with 0.5 ml of each stock solution in each cavity block. These cavity blockswere kept at room temperature (20-25 °C). In control treatment water was used. There were three replications of each treatment. Infective Juveniles which did not show any movement after prodding were considered dead. Number of living IJs was counted at 3,6,12 and 24 hours under a stereomicroscope.

Survival data was transformed (angular) and statistical analyses were performed by two-way factorial completely randomized design by OPSTAT. Significance at 5% level was considered significant.

Infectivity assay

To evaluated for infectivity against last in star larvae of *A. ipsilon* the experiment was carried out in Petri dishes (5.5 cm diameter) containing a double layer of filter paper. After 6 h exposure to pesticides the viable 200 IJs of *H. bacteriophora* were rinsed with sterile water 3 times to remove the rest of the pesticide and added to the filter paper surface. Thereafter, onelast instarcutworm larvae were placed in each Petri dish. The IJs unexposed to the pesticides were used in control treatments. Atotal of ten replicates were performed for each treatment. Experimental units were incubated underroom temperature (21-30 °C). The Petri dishes were placed in plastic containers lined with moistened paper towels, and closed to ensure high humidity levels (RH ± 95%). The data on the mortality of the cutworm were recorded for 96 hours according to body colour change of the cadaver. Dead insects were dissected under a stereo microscope to confirm nematode infection.

For statistical analysis, the per cent mortality values were converted to arcsine transformation and the treatment effects were subjected to analysis of variance. Significance was tested at the 5 percent level using Duncan's multiple comparison tests to separate the means and the effects were compared. All means were transformed back to the original units for presentation.

Invasion/penetration study

Invasion/penetration was evaluated by using the filter paper method. About 200 IJs suspended in different concentration of pesticides for 6 hwere inoculated into a Petri dish containing a piece of filter paper placed at the bottom of the dish. Then, the final instar of cutworm larva was released into the dish, and the dish was incubated in the dark at room temperature (21-30 ^{0}C). The IJs unexposed to the pesticides were used in control treatments. The Petri dishes were placed in plastic containers lined

with moistened paper towels, and closed to ensure high humidity levels. The experiments were replicated five times for each treatment. Observations on number of penetrated nematodes were taken 48h after nematode inoculation by dissecting the cutworm larvain Ringer's solution. The penetration rate was calculated as an average.

All data were subjected to analysis of variance (ANOVA) and means compared according to Duncan´s multiple range tests. Before analysis, penetration rate of the IJs of *H.bacteriophora* was square root transformed. All means were transformed back to the original units for presentation.

Reproductive rate of *H. bacteriophora* in the *A.ipsilon* larvae

H.bacteriophora 200 IJs suspended in different concentration of pesticides for 6 h were inoculated per Petri-dish by the filter paper method. One last instar larvae were placed in each Petri-plate. After application of the IJs, the Petri-plates were kept for 96 hours at room temperature (21-30 ^{0}C). Control treatment received only distilled water. There were five replicates for each treatment. Cadavers were collected and rinsed in distilled water to remove surface nematodes and placed individually on White traps. Production of the IJs from *A. ipsilon* larvae began at 7-9 days after IJs host exposure. After 15 days of infection all IJ that emerged from the cadaver over this period in the water were harvested and the total nematode suspension was put in a 50 ml tissue culture flask. To assess the total production during the harvest period, the contents of the flask were mixed thoroughly with air bubbles from an aquarium pump and from this suspension 5 samples of 10 µl were counted under a stereomicroscope using a counting slide and average was recorded.

All data were subjected to analysis of variance (ANOVA) and means compared according to Duncan´s multiple range tests. Before analysis, multiplication of the EPNs was square root transformed. All means were transformed back to the original units for presentation.

RESULTS AND DISCUSSION

The survival rates of 200 IJs of *H.bacteriophora* exposed in different concentrations of Nimbecidine and Tricel for 3,6,12 and 24 hours were evaluated. Irrespective of concentration of Nimbecidine and Tricel, the survival percentage of IJs was found to be decreased with increased in exposure period of insecticides. The results showed that survival rates of *H. bacteriophora* in Nimbecidine and Tricel was found to be 63% for 3 hours, 55% for 6 hours, 43% for 12 hours and 20% for 24 hours (Table 1). Irrespective of time of exposure, the survival rate of the IJs of *H. bacteriophora* was higher when exposed to Nimbecidine at 0.5%,0.25% and 0.125% as compared to Tricel at 0.2%, 0.1% and 0.05%. Survival rates of IJs of *H. bacteriophora* exposed to Nimbecidine was found to be 40%, 47% and 53% at 0.5%, 0.25% and 0.125% concentration respectively. Survival rates of IJs of *H.bacteriophora* exposed to Tricel range from 38%, 43% to 50% at 0.2%, 0.1% and 0.05% concentration respectively. Interaction effect of concentration and exposure time was also significant. More than 50% survival rate of *H.bacteriophora* was observed when Nimbecidine and Tricel at all concentrations were exposed to 3-6 hours.

Table 1. Survival percentage of Infective juveniles of *Heterorhabditis bacteriophora* at different concentration of pesticides and exposure period (Mean of three replications)

Concentration (C)		Exposure period (H)				Mean
		3h	6h	12h	24h	
Nimbecidine	0.5%	60(7.7)	50(7.0)	40(6.2)	12(3.3)	40(6.2)
	0.25%	65(8.0)	56(7.4)	45(6.6)	22(4.6)	47(6.8)
	0.125%	70(8.3)	62(7.8)	50(7.0)	30(5.4)	53(7.2)
Tricel	0.2%	58(7.8)	51(7.1)	34(5.7)	10(3.1)	38(6.0)
	0.1%	60(7.7)	56(7.4)	42(6.4)	20(4.4)	43(6.6)
	0.05%	65(8.0)	60(7.7)	48(6.8)	28(5.2)	50(7.0)
Mean	63(7.9)	55(7.5)	43(6.6)	20(4.5)		

CD (0.05): Concentration: 0.23
Exposure time: 0.19
Concentration X Exposure time: 0.46
(Figures in parenthesis are angular transformed values)

Stark (1996); Yan *et al*. (2012) and Laznik and Trdan (2014) showed that azadirachtin did not cause any harm to survival or infectivity of entomopathogenic nematodes. Low EPN mortality was reported by Chen *et al.,* (2003) and Gutierrez *et al*. (2008) and Kruk and Dziêgielewska (2020) with chlorpyrifos where as some conventional organophosphates including chlorphenvinphos and dichlorvos and their mixtures were reported to cause high mortality of *H. amazonensis.* Zimmerman and Cranshaw (1990) showed significant reduction of *S. feltiae* survival when exposed to organophosphate compounds. Studies have shown that delayed exposures of certain insecticides at higher concentrations can cause variable mortality in *S. carpocapsae* and *H. indica* (Khan *et al*., 2018). No negative results were obtained for survival and infectivity of the IJs of *H.bacteriophora* and *S.feltiae* after exposure in 6,12 and 24 h to the recommended dosage of imidacloprid (Le Vieux and Malan, 2015). Head *et al*. (2000) reported that direct exposure of *S. feltiae* to neem oil (azadirachtin) have negligible effects on EPN survival comparatively to the control. The soap surfactant in commercial neem products caused 23-25% mortality of *S.feltiae* (Krishnayya and Grewal, 2002). The compatibility of botanicals to EPNs was recorded by Hussaini *et al*. (2001), viz., Neem Suraksha to two species of *Steinernema* and three of *Heterorhabditis*; Koppenhofer and Grewal (2005) viz., neem to *S.carpocapsae*; Raheel *et al*. (2017),viz., neem to *S.feltiae*, *S.asiaticum*, *H.bacteriophora*, and *H.indica*. Koppenhofer and Grewal (2005); Radova (2011); Laznik and Trdan (2014) observed the compatibility of the infective juveniles of entomopathogenic nematodes in most of the pest control compounds for 2 to 6 h, and they can be tank-mixed before use and applied, without any loss of survival and virulence. The compatibility may differ with species, strains, doses, and adjuvants used in formulations (Koppenhofer and Grewal, 2005; Shamseldean *et al*., 2013). Some of the recent work in which EPNs compatibility with plant protection products was accessed, showed that compatibility is species specific (Stark, 1996; Krishnayya and Grewal, 2002; De Nardo and Grewal, 2003; Radova, 2011; Laznik *et al*., 2012 ; Atwa *et al*., 2013). Laznik and Trdan (2014) concluded that compatibility of EPN with pesticides is not only a species-specific, but also a strain-specific characteristic.

Though infectivity of IJs of *H. bacteriophora* to *A.ipsilon* was observed in all concentrations of Nimbecidine (40-70%), followed by Tricel (30-60%) after 6h of exposure, lowest mortality (40 and 30%) was recorded at 0.5 and 0.2% concentration of Nimbecidine and Tricel respectively which is significantly different from the control (Fig. 1). More than 60% larval mortality of cutworm was caused by *H.bacteriophora* after their exposure to the insecticides at lower concentration. Nimbecidine exposed at lower concentration (0.25% and 0.125%) did not affect the virulence of the strains as the infectivity of *H. bacteriophora* was 50% and 70%. There were no significant differences on the virulence of *H.bacteriophora* exposed to Nimbecidine at 0.5% and Tricel at 0.1% at the same exposure level. Sankar *et al*., (2009) observed a variation in response of combination of EPNs and neem formulation (Nimor) that took 48h for 100% mortality of *G.mellonella*. Meyer *et al*. (2012) observed that Neem Azal-U though caused significant mortality of *H.bacteriophora*, but virulence was not affected. In another study Nitjarunkul *et al*. (2015) found that the survival rates of *S.carpocapsae* combined with neem was more than 94.5%, but the virulence of the nematode was decreased by longer soaking periods against *G.mellonella* larvae. Alumai and Grewal (2004) and Reddy and Chowdary (2021) and emphasized that the combined use of EPNs with a sublethal dose of chemical should be considered. At such a dose, the stress factor imposed on the target pest by the chemical should inhibit feeding, while rendering the host more susceptible to the IJs.A hypothesized reason for synergism caused by neem products on entomopathogens that the growth retardation due to azadirachtin causes elongation of the inter-molt period, thus enabling more time for the pathogen to attack the cuticle.

It is evident from the Table 2 that the highest penetration rate of IJs of *H. bacteriophora* (55.5%)

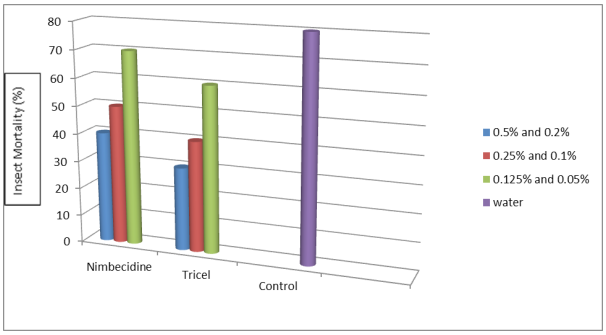

Fig. 1. Mortality of *Agrotisipsilon* larvae infected by infective juveniles of *Heterorhabditis bacteriophora* treated with different dosage of Nimbecidine and Tricel for 6 hours exposure period

Table 2. Number of Infective juveniles of *H.bacteriophora* penetrated into *A. ipsilon* after exposure in pesticides

Entomopathogenic nematodes IJs	(180/Larva) after exposure to Insecticide	Penetration rate (%)
Nimbecidine	0.5%	44.9(9.0)
	0.25%	51.6(9.6)
	0.125%	55.5(10.0)
Tricel	0.2%	39.9(8.4)
	0.1%	47.7(9.3)
	0.05%	51.6(9.6)
Control		73.0(11.4)
CD(P=0.05)		(0.96)

(Figures in parenthesis are angular transformed values)

exposed to Nimbecidine (0.125%) for 6 hours was found in dead *A.ipsilon* larvae. After exposure to Nimbecidine 0.25% and 0.5% for 6 hours, the penetration rate of *H. bacteriophora* was 51.6% and 44.9 % respectively. The least penetration rate (39.9%, 47.7%, 51.6 %) of *H.bacteriophora* was found when Tricel at 0.2%, 0.1%, and 0.05% was exposed for 6 hours. There was no significant difference between the penetration rate of IJs when exposed to Nimbecidine 0.25% and Tricel 0.05% at 6 hours of exposure.These insecticides at lower concentration did not affect the ability of *H.bacteriophora* to penetrate cutworm larvae. However, for both insecticides the average number of penetrating IJs was significantly different to the average number observed in the controls.

Reproduction rate of IJs observed inside cutworm larvae after 6 h exposure time in each concentration of insecticides varied significantly (Fig. 2). Reproduction was significantly reduced in *H.bacteriophora*, after 6 hours exposure to Nimbecidine and Tricel at all concentrations when compared to control. Reproduction of

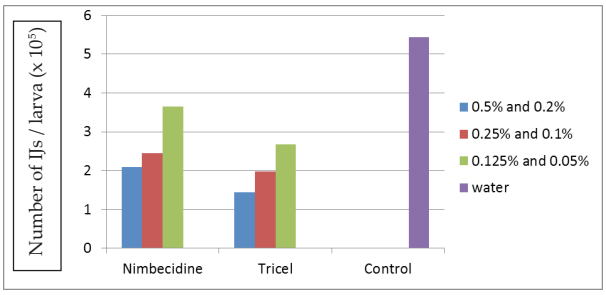

Fig. 2. Reproductive rate of *H.bacteriophora* following exposure to Nimbecidine and Tricel at different concentration.

H.bacteriophora was lower (1.45 x 10^5, 1.98 x 10^5, 2.68 x 10^5), when exposed to Tricel at 0.2%, 0.1%, 0.05% respectively. However, *H. bacteriophora* reproduction was higher (3.65 x 10^5) at 0.125% concentration of Nimbecidine.Rovesti and Deseo (1990) reported that nematode reproduction in *Galleria* larvae was not affected by exposure to organophosphate compounds and carbamates.

CONCLUSION

The experiments conducted on compatibility of *H.bacteriophora* with reduced concentration/dose of Nimbecidine and Tricel showed significant potential for *A. ipsilon* management. Results indicated that both the insecticides were relatively harmless or leasttoxic to *H.bacteriophora,* with better compatibility with Nimbecidine at lower dose of recommended dose at field level may be included in Integrated Pest Management (IPM) Programs to reduce costs and application time against cutworm. Besides, EPN mixtures with reduced volumes of insecticides will help in lowering the risks of environmental pollution and to public health. The combinations of biopesticides such as neem products with entomopathogenic nematodes can create a new excellent commercial bioinsecticide formulation giving boost to organic agriculture. More EPNs should be investigated for their compatibility with insecticides against certain insect pests of agricultural economic significance and before recommendations can be made for inclusion of chemical pesticides in IPM systems, such pesticides should be individually evaluated.

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