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Safety of botanical and microbial pesticide mixtures to *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae)

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ABSTRACT: **Laboratory experiments were conducted to study the effects of botanical (neem, sweet-flag and pongamia) and microbial pesticide mixtures (*Ha*NPV, *Bacillus thuringiensis* (=Spicturin) and spinosad) on adult mortality and adult emergence of the egg parasitoid, *Trichogramma chilonis*. In contact toxicity test, neem + sweet-flag and neem + sweet-flag + pongamia at 0.12 and 0.18 per cent concentrations registered up to 33.89 per cent mortality of adult parasitoids while the same concentrations had 79.58 to 81.00 per cent adult emergence. Experiments with mixtures of microbial pesticides revealed that spinosad at 75 g a. i./ ha and its combination with *Ha*NPV or Spicturin or with both registered cent per cent adult mortality in 24 hours. Also, spinosad or its combinations registered poor adult emergence of this parasitoid, which ranged from 13.08 to 13.83 while *Ha*NPV and Spicturin registered adult emergence of 93.33 and 93.58 per cent. The possibilities of using the botanical mixtures or microbial pesticide mixtures along with this parasitoid are discussed.**

KEYWORDS: Botanical insecticides, *Ha* NPV, Spicturin, Spinosad, *Trichogramma chilonis*

INTRODUCTION

Trichogramma chilonis Ishii is a potential egg parasitoid used in integrated pest management programme. Continuous and indiscriminate use of synthetic chemicals in plant protection has resulted in toxicity to non-target beneficial organisms. Realising the adverse effects of chemical insecticides, attention is now diverted in favour of non-chemical methods of pest management. The safety of these biopesticides to non-target organisms over conventional pesticides has already been reported by several scientists (Raguraman, 1994; Schmutterer, 1990; Ignacimuthu and Jayaraj, 2005). Raguraman and Singh (1997) stated that botanical mixtures are the best alternative to conventional pesticides to deal with problems of resistance, resurgence and residues. But reports on safety of botanical mixtures and microbial pesticide mixtures to non-target insects are lacking. The objective of the present study was to obtain a precise laboratory assessment on the safety of

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botanical mixtures (neem, sweet-flag and pongamia) microbial pesticide mixtures (*Ha*NPV, *B. thuringiensis* and spinosad) to *Trichogramma.*

MATERIALS AND METHODS

Plant materials

Seeds of neem (*Azadirachta indica* A. Juss.) and pongamia (*Pongamia glabra* Pierre) collected from farm premises of Agricultural College and Research Institute (AC & RI), Madurai and rhizomes of sweet flag (*Acorus calamus* Linn.) obtained from local market were chosen for this study.

Extraction and formulation of plant materials

The extracts of seed kernels of neem and pongamia and rhizomes of sweet flag were prepared using ethanol as a solvent and EC formulations were made by the following procedure. Hundred grams of seed kernel or rhizome powder was stirred with 500ml of ethanol for 3 hours in a magnetic stirrer and filtered through Whatman No.1 filter paper. The residue material was restirred with 500 ml of ethanol for 1h and filtered. The filtrate was pooled and freed of ethanol in a distillation unit at 50°C under reduced pressure. The extract was formulated to 60EC using an organic solvent (cyclohexanone) and an emulsifier (Tween-80®) at 30 and 10 per cent, respectively. The following formulations were prepared from the extracts as detailed above.

1. Neem + Sweet flag NS (I) 60 EC in 2:1 (v/v)
2. Neem + Sweet flag NS (II) 60 EC in 1:2 (v/v)
3. Neem + Sweet flag + Pongamia NSP 60 EC in 1:1:1 (v/v)

Microbial pesticides

Helicoverpa armigera nucleo polyhedro virus (*Ha*NPV) obtained from Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore; commercial formulations of *Bacillus thuringiensis* var. *galleriae* (Spicturin®) and spinosad (Success®) supplied by E.I.D. Parry Agrochemicals Ltd., Chennai were used in this study in different combinations.

Toxicity Tests

Laboratory experiments were conducted at the Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai during 2003. *Trichogramma chilonis* cards obtained from the Office of the Agricultural Officer, Vinayagapuram, Madurai, Tamil Nadu were used as stock culture and further multiplied on *Corcyra cephalonica* eggs in the laboratory at 25±1°C and 70±5 per cent relative humidity. Two experiments were conducted one being the test on contact toxicity and the other on adult emergence when exposed to botanical mixtures and microbial pesticide mixtures. The concentrations or doses used are given in tables. For contact toxicity study, 0.5ml of solution or suspension was pipetted out into the glass test tube (size: 15cm long and 2.5cm diam), which was later constantly rotated and rolled on a flat surface so as to form a thin and uniform coating inside the walk of test tube. Each tube served as a replicate with three such replications per treatment. Thirty freshly emerged (3-5 h old) adult parasitoids were transferred into each test tube using a small aspirator. The tubes were then plugged with cotton wads. A streak of honey (50%) provided on the walls of tubes served as food for the parasitoids during 24 hours of exposure. Mortality of the parasitoids was recorded 24 hours after release. In the second experiment, on adult emergence, egg strips containing 100 parasitized eggs were treated with test concentrations and the emergence of *Trichogramma* was recorded. Each egg card served as a replicate with three such replications per treatment. Both the experiments were conducted in a complete randomized block design and the data collected on adult mortality and adult emergence were subjected to statistical analyses after angular transformations and the means were separated by DMRT (Gomez and Gomez, 1984). The mortality data were then categorized to know the level of safety of the products tested following the method of Hassan (1989).

Mortality (%)	Category
Less than 50	Harmless
50 to 79	Slightly harmful
80 to 99	Moderately harmful
More than 99	Harmful

RESULTS AND DISCUSSION

Safety of botanical mixtures

Results on mortality of *Trichogramma* adults due to botanical mixtures are presented in Table 1. NS (I) and NS (II) at concentrations 0.12 and 0.18 per cent registered mortalities from 20.64 to 25.44 per cent at 24 hours after treatment (HAT). But, NSP II 0.18 per cent concentration recorded 33.89 per cent mortality while it was 37.70 per cent for NSKE (5%). However, the mortality due to botanical mixtures is significantly different from untreated check. A slight dose-dependent mortality is observed. Experiment on adult emergence revealed that the botanical mixtures including the NSKE spray recorded a slightly considerable reduction in adult emergence, which ranged from 77.92 to 81.00 per cent when compared to untreated check (Fig. 1).

The choice of the concentrations of the botanical mixtures used in this study is based on the earlier reports from laboratory and field studies against *Helicoverpa armigera* and *Earias vitella* (Rao, 2001). The present findings are concomitant with the findings of Rao (2001) who reported that NSP (0.12%) reduced the emergence of *Trichogramma* adults to an extent of 33.33 per cent. Raguraman and Singh (1999) also reported 50 per cent mortality of *T. chilonis* due to exposure to neem oil (2%) in 24 hours. But, Klemm and Schmutterer (1993) reported that *T. principium* accepted neem treated eggs in the laboratory. Raguraman (1994) reported that an aqueous extract of NSKE (5%) effected more than 50 per cent reduction in parasitization and was mildly toxic to *T. chilonis* adults. However, Srineevasa and Patil (1998) reported that neem products were fairly safe to *Trichogramma* spp.

Table 1. Adult mortality of *T. chilonis* exposed to botanical mixtures

Treatment	Concentration (%)	Adult mortality 24 h after treatment (%)*
Neem + sweet flag (2:1) (NS I)	0.12	20.64 (26.99)^{b}
Neem + sweet flag (2:1) (NS II)	0.18	21.79 (27.77)^{bc}
Neem + sweet flag (1:2) (NS III)	0.12	20.95 (27.22)^{b}
Neem + sweet flag (1:2) (NS IV)	0.18	25.44 (30.29)^{c}
Neem + sweet flag + pongamia (1:1:1) (NSP I)	0.12	25.44 (30.29)^{c}
Neem + sweet flag + pongamia (1:1:1) (NSP II)	0.18	33.89 (35.60)^{d}
Neem seed kernel extract (NSKE)	5	37.70 (37.87)^{d}
Untreated check	-	0.00 (0.58)^{a}

*Mean of three replications

Figures in parentheses are arcsine-transformed values.

In a column, means followed by the same alphabet are not significantly different (P=0.05) by DMRT.

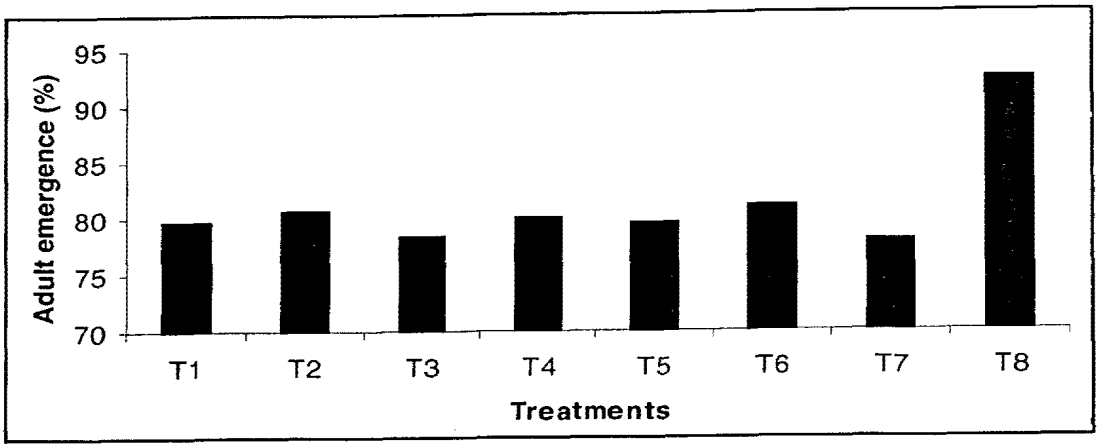

Fig. 1. Effect of botanical mixtures on adult emergence of *T. chilonis*

Safety of microbial pesticide mixtures

In the microbial combinations, cent per cent mortality of *T. chilonis* adults was observed in Spinosad, Spinosad + *Ha*NPV, Spinosad + Spicturin and Spinosad + *Ha*NPV + Spicturin (Table 2). But *Ha*NPV and Spicturin recorded 8.05 and 23.61 per cent adult mortality, respectively while it was 67.59 in endosulfan and all the treatments were significantly different from untreated check. Experiment on adult emergence revealed that Spinosad and its combination with other microbials recorded significantly very poor adult emergence up to 13.75 per cent. Higher levels of adult emergence were recorded in Spicturin and *Ha*NPV treated eggs with 93.58 and 93.33 percentages, respectively and were on par with untreated check (Fig. 2).

Among the microbials tested, even though Spicturin and *Ha*NPV were not toxic to *T. chilonis* when treated individually, in the combination of

Table 2. Adult mortality of *T. chilonis* when exposed to pesticides

Treatment	Dose / concentration	*Adult mortality 24 h after treatment (%)
Spinosad 25 SC	75g a.i/ ha	100.00 (89.42)^{e}
Spinosad + *Ha*NPV	75g a.i./ ha + 1.5x 10^{12} POBs/ ha	100.00 (89.42)^{e}
Spinosad + Spicturin	75g a.i./ ha + 1 lit/ ha	100.00 (89.42)^{e}
Spinosad + *Ha*NPV + Spicturin	75g a.i./ ha + 1.5x 10^{12} POBs/ ha + 1 lit/ ha	100.00 (89.42)^{e}
*Ha*NPV	3x10^{12} POBs/ ha	8.05 (16.41)^{b}
Spicturin	2 lit/ ha	23.61 (29.04)^{c}
Endosulfan 35 EC	0.07%	67.59 (53.39)^{d}
Untreated check	-	0.00 (0.58)^{a}

* Mean of three replications

Figures in parentheses are arcsine-transformed values.

In a column, means followed by the same alphabet are not significantly different (P = 0.05) by DMRT.

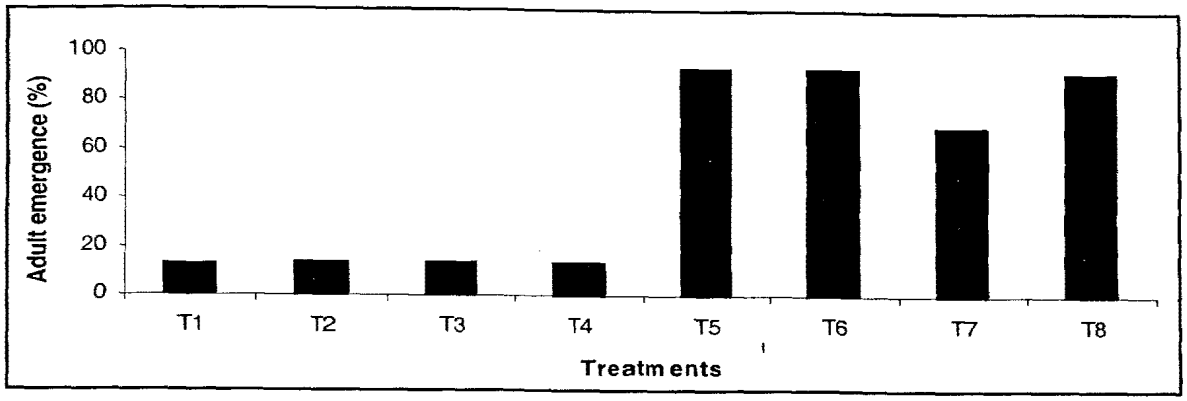

Fig. 2. Effect of microbial pesticide mixtures on adult emergence of *T. chilonis*

Spinosad they showed harmful effects to *T. chilonis*. This might be due to the increased contact toxicity of Spinosad over Spicturin and *Ha*NPV. The present findings are in accordance with findings of Ruberson and Tillman (1999) who reported Spinosad to have toxic effects on *T. pretiosum*. According to Tillman and Mullrooney (2000) and Suh *et al.* (2000) fresh spray of Spinosad could kill *Trichogramma* and other parasitoids and honeybees. Scholz and Zalucki (2000) reported that foraging *Trichogramma pretiosum* in maize plots was reduced approximately by two-thirds on Spinosad- treated egg masses. Recently, Penagos *et al.* (2005) predicted based on lethal and sub-lethal effects of Spinosad up to 200 ppm on egg and larval parasitoids that there are several field factors to work in favour of the parasitoids to avoid the ill effects of Spinosad. *Bt* was also compatible with *Trichogramma* sp. as feeding honey with high concentrations of *Bt* had not affected their survival (Pawar, 1996).

From this study, it can be summarized that NS (I-IV), NSP (I-II), NSKE, *Bt*, and *Ha*NPV were not harmful (<50% mortality) to adults of *T. chilonis* when tested under laboratory condition. But Spinosad and their combination with *Ha*NPV and Spicturin were found to be moderately harmful or even harmful to *T. chilonis* (Table 3). A detailed investigation is required to effectively integrate the

Table 3. Safety categorization of botanicals and microbial pesticides to *T. chilonis*

Effect	Category (%)			
	< 50	50-79	80-99	> 99
Reduction in adult emergence	NS (I), NS (II), NSP, NSKE *Ha*NPV, Spicturin	Endosulfan	Spinosad Spinosad + Spicturin Spinosad + *Ha*NPV Spinosad + *Ha*NPV+ Spicturin	None
Adult mortality	NS (I), NS (II), NSP, NSKE *Ha*NPV, Spicturin	Endosulfan		Spinosad Spinosad+Spicturin Spinosad + *Ha*NPV Spinosad + *Ha*NPV + Spicturin

botanical and microbial pesticides when both are used in respective mixtures along with *Trichogramma* release in the field. However, it is suggested that timing the application of neem or its mixtures with other botanicals and Spinosad and its microbial mixtures would conserve this parasitoid in field.

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