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Ecotoxicological performances and biochemical effect of selected pesticides on *Trichogramma chilonis* ishii. (Hymenoptera: Trichogrammatidae)

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Abstract
In this context the toxic effect of the selected insecticide, BIOVECTRA, which contains *Bacillus thuringiensis*, SICORIN 10® (Cypermethrin), SUFOS® (Monocrotophos), Thiodan® (Endosulfan) and Vijay Neem® (Neem oil + Azadirachtin) as active ingredients on the egg parasitoid, *Trichogramma chilonis* ishii were studied. Toxicological bioassays were performed in different pesticides on *T. chilonis*. Lethal doses LC_{50} of *B. thuringiensis* (52.70mg), cypermethrin (0.960ppm), monocrotophos (7.76ppm), endosulfan (20.87ppm) and Vijay Neem (621.71ppm) were estimated in bioassays, with residual contact bioassay. Maximum significant inhibition of egg hatchability was observed after Cypermethrin treatments followed by Monocrotophos and Endosulfan. Maximum adult emergence was recorded with Vijay Neem treatment followed by *B. thuringiensis*. Proteins, carbohydrates and lipids constituents were maximum inhibited by the cypermethrin, monocrotophos and endosulfan treatments. Our outcome strongly suggested that botanical pesticides and microbial pesticides which are safe and sound to the non-target organisms.

Keywords: *Bacillus thuringiensis,* biochemicals, Egg hatchability, Neem oil, *Trichogramma chilonis,*

1. Introduction
Modern industrial and agricultural techniques involve the use of million tones of fertilizers and pesticides. The fast revolution in the agricultural segment, the release and discharge of a large number of pollutants, especially chemical fertilizer and pesticides pose alarming health hazards [1]. Insecticides are considered as the most effective means of protecting crop against insects' damage as they provide rapid control of whole pest-complexes [2]. Despite its benefits, the agrochemicals do create environmental problems. The pesticides reach the aquatic environments through runoff from the land or through direct application [3]. When pesticides are ingested or otherwise carried by target species, they will stay in the environment and their bio-concentration increases in food chain [4].
Environmental damage due to pesticides is a serious problem. The complex chemistry of pesticides, persistence in the environment, toxicity to animals and human beings and bioaccumulation-risks make pesticidal pollution, a critical problem [5]. Therefore, ecological recovery and rehabilitation of aquatic ecosystem have become major objectives of many research programmes [2]. Among the various predators, *Trichogramma chilonis,* is a Hymenopteran parasitoid and has a high bio-control potential against lepidopteran larvae [6]. Implementing biological control practices have been reported to reduce the chemical use, environmental pollution and the pesticide exposure to animals and human beings [7].
Pesticides are constantly being applied to agriculture, but little is known about the impact on terrestrial biota during natural exposure [8]. Terrestrial exposure assessments for pesticides are generally based on laboratory studies performed in water alone [9]. The agricultural industry and urban pesticide users are increasingly relying upon insecticides and shifting to more potent members of the class. Yet little information is available on residue of these substances in aquatic system [10].
Trichogramma chilonis is an egg parasitoid that attacks more than 400 pest species, mostly lepidopteran insects. It has been successfully used in inundative and inoculative biological control programmes worldwide [11]. Detrimental effects of pesticides on *Trichogramma* have been limited to very few studies. Consoli *et al*. [12] determined the effects of pesticides on *T. pretiosum* by dipping the parasitized host eggs in the pesticide solutions.

Lingathurai [13] reported that crude extracts, fractions and compounds of plant origin did not inhibit *T. chilonis* development. The utilization of this parasitoid is extensively developed on corn, rice, sugarcane, cotton, vegetables and pines.

T. chilonis is widely distributed in the Indian subcontinent and is responsible for large-scale mortality of the American boll worm *Helicoverpa armigera* in several crops and efficiently controls other lepidopteran pests [14]. The aim of this study was to investigate the different types of market available (microbial, synthetic and botanical pesticides) on egg parasitoids, particularly *T. chilonis* toxicity and its biochemical effects.

2. Material and Methods

2.1. Insects

In the laboratory the parasitoids were multiplied on *Corcyra* eggs. The eggs laid by *Corcyra* moths were collected and sieved to remove the moth scales etc. The pure eggs, thus obtained and were exposed to ultra-violet light in UV chamber to kill the host embryo but at the same time permit parasitisation. The quantity of the sterilized eggs was assessed in a measuring cylinder volumetrically. The eggs in volume of six cc were then sprinkled uniformly over a 144 gsm card of 30 x 18 cm size. The card was divided in to two halves of 30 x 9 cm (LxB). The trichocard were purchased from Sun Agro Biotech Ltd. Porur, Chennai for conducting the experiments.

2.2. Pesticides

Bacillus thuringiensis var, Cypermethrin 10% EC, Monocrotophos 36% EC, Endosulfan 35% EC and a botanical pesticide Vijay Neem® (Table 1) were obtained from Pesticide Market, Gummidipoondi, Thiruvallur District, Tamil Nadu, India.

2.3. Egg hatchability

To assess the effect of different pesticides on the eggs of *T. chilonis,* the eggs along with stalk collected on brown paper strips were sprayed with the formulated pesticides. The test substances were diluted (w/v) in distilled water, by using magnetic shaker, to allow complete homogenization. The dilution level was as per standard method Gupta *et al.,* 2011 [15] by using atomizer. In each treatment 150 stalked eggs were used, with three replications of 50 eggs each. The untreated check was maintained by spraying water with soap solution (1 ml per 100 ml water). The number of grubs hatched from each treatment was recorded and percent hatchability (Number of hatched larvae / Total number of eggs X 100) was determined for four days.

2.4. Adult emergence and parasitisation

The parasitised egg cards were cut into manageable size (1cm) and the three day old, parasitized eggs (appearing black and plumpy) were sprayed with recommended dose of test substances (Table 1). For the untreated check, only distilled water was sprayed using atomizer. The treated egg cards were shade dried for 10 min. and then kept inside a test tube (20 X 1.5 cm). The treatments were replicated thrice. The number of parasitoids emerged from each treatment was recorded and the percent emergence was estimated. The percent adult emergence was assessed by the following formula,

Percent adult emergence = [(Ac-At)/Ac] X 100, where Ac is adult emerged in control group and at is adult emerged in treated group.

Table 1: Pesticides used to evaluate the percent parasitism, parasitoid emergence and percent of egg hatchability of *T. chilonis.*

Pesticides	Trade name	Chemical group	Rate/L
Bacillus thuringiensis	Biovectra	Biopesticide	50g
Cypermethrin	Sicorin 10 ®	Synthetic pesticide	100g
Monocrotophos	Sufos ®	Synthetic pesticide	360g
Endosulfan	Thiodan ®	Synthetic pesticide	350g
Azadirachtin	Vijay Neem®	Botanical pesticide	3g

Fresh egg cards sprayed with tested pesticides at concentrations of 20, 40 and 80 mg/ml for *B.t* and 20,40 and 80ppm for each of Cypermethrin, Monocrotophos, Endosulfan and Neem then they were put in to small vials (l0 X I cm) and one-day old *Trichogramma* was released in each vial containing card, then covered with muslin cloth.

The parasitoids were allowed to parasitize the host eggs for 24 h, after which they were removed. Droplets of honey were added directly to the wall of the vial as food for the adults. On 4th day after adult release, the percent of parasitisation was recorded and parasitized eggs appeared black and plumpy.

2.5. Insect tissue preparations

For enzyme preparations, 50 insects (5-10mg) were used for experiment. Insects were homogenized with 200 µl distilled water at 4 °C. The homogenate was spin at 14,000 rpm for 2 min at 4 °C in a microfuge. Then it was filtered through the filter paper.

2.6. Determination of biochemical constituents

50 insects per treatment were used for biochemical estimations to three sub-lethal concentrations of five pesticides for 7 days. Control insects were (Water sprayed and unsprayed) also maintained throughout the study. The Samples were used to analyze the total carbohydrate as per methodology adopted by Trevelyan and Harrison [16], protein by Bradford [17] and lipid by Bligh and Dyer [18] method. Thirty replicates were maintained in each treatment and the obtained values were expressed in mg/ml

2.7. Statistical analysis

Means and variances of the treatments were analyzed by one-way ANOVA using SPSS 11.5 and EPA 1.5 software. The means were separated by protected least significant difference (LSD) at significance level 0.05.

3. Results

T. chilonis toxicity, survival and biochemical constituents results were recorded after treated the pesticides. Table 2 shows that the different pesticides, *Bacillus thuringiensis* at 20, 40 and 80mg/ml and Cypermethrin, Monocrotophos, Endosulfan and Vijay Neem were treated at concentrations of 20, 40 and 80ppm concentrations. The highest inhibitions in egg hatchability were observed in Cypermethrin at 80ppm concentration (82.4%), 40ppm (78.9%) and 20ppm (74.3%) respectively. Monocrotophos showed percentage of 78.2%, 77.5% and 62.3% at 20, 40 and 80ppm concentrations respectively. Endosulfan inhibited the treatment to 76.6%, 64.8% and 51.2% for 20, 40 and 80 ppm respectively. Control showed inhibition in the egg hatchability to 4.2 % level. Vijay Neem recorded the lowest percent egg hatchability. After the

treatment with the concentrations of 20, 40 and 80 ppm the results were observed 21.6%, 28.5% and 32.2% for the egg development inhibition. These results were obtained significant differences in egg hatchability inhibitions between the pesticides and their concentrations.

Table 2: Toxicity of the tested insecticides on egg development /hatchability of *T. chilonis*

Treatment	Concentrations	Hatchability (%)
Bacillus thuringiensis	20 mg	22.4 ± 1.08^{ab}
	40 mg	48.8 ± 2.24^{c}
	80 mg	62.1 ± 4.91^{cd}
Cypermethrin	20 ppm	74.3 ± 3.26^{d}
	40 ppm	78.9 ± 4.81^{d}
	80 ppm	82.4 ± 5.23^{de}
Monocrotophos	20 ppm	62.3 ± 3.55^{cd}
	40 ppm	77.5 ± 6.67^{d}
	80 ppm	78.2 ± 4.82^{d}
Endosulfan	20 ppm	51.2 ± 7.14^{cd}
	40 ppm	64.8 ± 4.64^{cd}
	80 ppm	76.6 ± 3.69^{d}
Vijay Neem	20 ppm	21.6 ± 1.02^{ab}
	40 ppm	28.5 ± 1.10^{b}
	80 ppm	32.2 ± 1.46^{bc}
Control		4.2 ± 0.02^{a}

Values are the mean ± S.D of 30 replicates for each treatment. Each replicate consisted of random eggs. In a column, significant differences according to Tukey's test at $P \leq 0.05$ levels are indicated by different letters and data followed by the same letters are not significantly different from each other.

The *B. thuringiensis* at concentrations of 20, 40 and 80mg/ml gives 73.4%, 51.2% and 37.9% respectively. Vijay Neem exhibit highest percent parasitoid emergence was observed. Vijay Neem gives 67.8%, 71.5% and 78.4% at 20, 40 and 80ppm concentration respectively. Cypermethrin 80 ppm showed 17.6 % of adult emergences (Table 3). These results are indicated that neem based on botanical pesticide for an effective control method of insect pests.

Table 3: Toxicity of the tested insecticides on the percent emergence of the parasitoid, *T. chilonis*

Treatment	Concentrations (ppm)	Adult emergence (%)
Bacillus thuringiensis	20 mg	73.4 ±7.17^{c}
	40 mg	51.2 ± 2.34^{b}
	80 mg	37.9 ± 3.53^{ab}
Cypermethrin	20 ppm	25.7 ± 6.59^{a}
	40 ppm	21.1 ± 0.66^{a}
	80 ppm	17.6 ± 3.17^{a}
Monocrotophos	20 ppm	37.7 ± 1.32^{ab}
	40 ppm	22.5 ± 1.72^{a}
	80 ppm	21.8 ± 1.26^{a}
Endosulfan	20 ppm	48.8 ± 2.65^{b}
	40 ppm	35.2 ± 1.11^{ab}
	80 ppm	23.4 ± 1.02^{a}
Vijay Neem	20 ppm	78.4 ± 5.42^{c}
	40 ppm	71.5 ± 4.88^{c}
	80 ppm	67.8 ± 6.35^{bc}
Control		95.8 ± 6.63^{cd}

Values are the mean ± S.D of 30 replicates for each treatment. Each replicate consisted of random eggs. In a column, significant differences according to Tukey's test at $P \leq 0.05$ levels are indicated by different letters and data followed by the same letters are not significantly different from each other.

The LC_{50} and LC_{90} values, confidence limit (95%) and Chi-square value at 96 h exposure to pesticides are depicted in Table 4. Cypermethrin recorded a very low concentration between treatments (0.960 ppm) that gave 50 % mortality of parasitoids (Table 4). The LC_{50} and LC_{90} values were 0.960 and 502.17 ppm, respectively. Ascending increase in toxicity level of treated pesticides against *T. chilonis* were Cypermethrin > Monocrotophos > Endosulfan > *B.t* > Vijay Neem. So, the treatment of Vijay Neem exhibited the safest with very low side effects to non-target/ beneficial insect *T. chilonis.*

Table 4: Probit analysis of different pesticides on *T. chilonis*

Pesticides	LC_{50}	95%Confidence Limit		LC_{90}	95%Confidence limit		Chi- square χ^2
		Lower	Upper		Lower	Upper	
B. thuringiensis	52.70	42.70	68.70	260.60	157.55	730.88	1.731*
Cypermethrin	0.960	-	-	502.17	-	-	0.004*
Monocrotophos	7.76	0.01	17.02	293.80	112.37	61513.81	1.538*
Endosulfan	20.87	9.20	29.33	249.302	125.48	2050.37	0.001*
Vijay Neem	621.71	-	-	90837.03	-	-	0.127*

LC_{50} and LC_{90} values are expressed as percentage (n=30). *B.t* unit as mg and other pesticides unit as ppm. 95% lower and upper Confidence limits are shown in parenthesis * χ^2 values are significant at $P \leq 0.05$ levels.

Results indicated the concentration dependent response on all biochemical inhibition, for the different pesticides. Table 5 showed that the biopesticide, *B. thuringiensis* pesticide inhibited the protein, carbohydrate and lipid level. At concentration of 80 mg the total protein (8.10 mg/ml), total carbohydrate (2.11 mg/ml) and lipid (0.35 mg/ml) were reduced as compared to control. Also Cypermethrin showed inhibition in protein, carbohydrate and lipid levels at the same concentration (Table 6). At the concentration of 80 ppm, total protein (9.21mg/ml), total carbohydrate (2.15 mg/ml) and lipid (0.55 mg/ml) reduced as compared to untreated (Control).

Table 5: Effect of *Bacillus thuringiensis* on biochemical constituents of *T. chilonis*

Treatment	Concentration (mg/l)	Biochemical constituents		
		Total Protein (mg/ml)	Total Carbohydrate (mg/ml)	Total Lipid (mg/ml)
B. thuringiensis	20	10.56 ±0.95^{ab}	3.06 ± 0.03^{ab}	0.61 ± 0.04^{ab}
	40	9.21 ± 0.86^{b}	2.79 ± 0.03^{b}	0.47 ± 0.03^{b}
	80	8.10 ± 0.71^{b}	2.11 ± 0.02^{c}	0.35 ± 0.02^{c}
Control		12. 05 ± 0.91^{a}	3.64 ± 0.03^{a}	0.84 ± 0.06^{a}

Means within columns and row followed by the same letter are not significantly different (Tukey's test $P \leq 0.05$)

Table 6: Effect of Cypermethrin on biochemical constituents of *T. chilonis*

Treatment	Concentrations (ppm)	Biochemical constituents		
		Total Protein (mg/ml)	Total Carbohydrate (mg/ml)	Total Lipid (mg/ml)
Cypermethrin	20	8.79 ± 0.62^{ab}	1.96 ± 0.10^{b}	0.43 ± 0.03^{b}
	40	11.95 ± 0.79^{a}	3.98 ± 0.25^{a}	0.96 ± 0.07^{a}
	80	9.21 ± 0.62^{ab}	2.15 ± 0.18^{ab}	0.55 ± 0.03^{ab}
Control		12. 05 ± 0.91^{a}	3.64 ± 0.03^{a}	0.84 ± 0.09^{a}

Means within columns followed by the same letter are not significantly different (Tukey's test $P \leq 0.05$)

Table 7 showed the Monocrotophos inhibition of the protein, carbohydrate and lipid levels. At concentration of 80 ppm, total protein (4.21 mg/ml), total carbohydrate (0.64 mg/ml) and lipid (0.15 mg/ml) reduced. Table 8 observed that Endosulfan inhibited the protein, carbohydrate and lipid levels. At concentrations of 80 ppm, the total protein (2.11 mg/ml), total carbohydrate (0.37 mg/ml) and lipid (0.07 mg/ml) reduced as compared to control. Vijay Neem inhibited the protein, carbohydrate and lipid levels. Reduction of total protein, total carbohydrate and lipid were 8.10 mg/ml, 2.11 mg/ml and 0.35 mg/ml respectively at a concentration of 80 ppm. Control exhibited the protein, carbohydrate and lipid to 12.05 mg/ ml, 3.64 mg/ ml and 0.84 mg/ml respectively. This was revealed untreated control (Table 9).

Table 7: Effect of Monocrotophos on biochemical constituents of *T. chilonis*

Treatment	Concentrations (ppm)	Biochemical constituents		
		Total Protein (mg/ml)	Total Carbohydrate (mg/ml)	Total Lipid (mg/ml)
Monocrotophos	20	10.27 ± 0.92^{ab}	3.12 ± 0.21^{ab}	0.81 ± 0.05^{a}
	40	7.11 ± 0.53^{b}	1.45 ± 0.12^{b}	0.36 ± 0.001^{ab}
	80	4.21 ± 0.25^{bc}	0.64 ± 0.04^{bc}	0.15 ± 0.003^{b}
Control		12. 05 ± 0.91^{a}	3.64 ± 0.03^{a}	0.84 ± 0.06^{a}

Means within columns followed by the same letter are not significantly different (Tukey's test $P \leq 0.05$)

Table 8: Effect of Endosulfan on biochemical constituents of *Trichogramma chilonis*

Treatment	Concentrations (ppm)	Biochemical constituents		
		Total Protein (mg/ml)	Total Carbohydrate (mg/ml)	Total Lipid (mg/ml)
Endosulfan	20	9.05 ± 0.71^{ab}	2.39 ± 0.02^{ab}	0.42 ± 0.003^{ab}
	40	5.98 ± 0.52^{b}	1.05 ± 0.10^{b}	0.16 ± 0.007^{b}
	80	2.11 ± 0.12^{bc}	0.37 ± 0.01^{bc}	0.07 ± 0.003^{bc}
Control		12. 05 ± 0.91^{a}	3.64 ± 0.03^{a}	0.84 ± 0.06^{a}

Means within columns followed by the same letter are not significantly different (Tukey's test $P \leq 0.05$)

Table 9: Effect of Vijay Neem on biochemical constituents of *T. chilonis*

Treatment	Concentrations (ppm)	Biochemical constituents		
		Total Protein (mg/ml)	Total Carbohydrate (mg/ml)	Total Lipid (mg/ml)
Vijay Neem	20	10.41 ± 0.95^{ab}	3.02 ± 0.04^{ab}	0.58 ± 0.03^{ab}
	40	9.05 ± 0.81^{b}	2.44 ± 0.02^{b}	0.45 ± 0.02^{b}
	80	7.93 ± 0.68^{bc}	1.98 ± 0.01^{bc}	0.32 ± 0.02^{bc}
Control		12. 05 ± 0.91^{a}	3.64 ± 0.03^{a}	0.84 ± 0.06^{a}

Means within columns followed by the same letter are not significantly different (Tukey's test $P \leq 0.05$)

4. Discussion

The present experiment comprises different pesticides on the effect of egg hatchability and adult emergence of *T. chilonis*. *T. chilonis* mostly inhibited by the tested chemical pesticides like cypermethrin, monocrotophos and endosulfan. Botanical pesticide also inhibits the *T. chilonis* development. Highly inhibitory activity was showed all the treatment. A biorational insecticide should not only first be effective in controlling the target pest but should be relatively innocuous to the target

pest's natural enemies [19]. Neem preparations have been reported to conserve insect parasitoids and predators and thus are of immense value in integrated pest management [20]. The results showed that the mortality rate was positively increased with the concentration of pesticides. Synthetic pesticide treatment was extremely inhibited the mortality rate.

The insect biochemical (protein, carbohydrates and lipids) are primary metabolites for access their energy, supporting and many physiological processes. In our experimental insect parasitoid previously reported these sources. They reported that carbohydrates increase the longevity of *Trichogramma* [21]. The significance of this finding was supported in these experiments where clearly obtained a significant inhibition in the survival and biochemical changes of this insect. The success of biological control in many systems, including *T. carverae* system, is determined by the reproductive success of parasitoids. This result supports the previous findings when insects are exposed to the synthetic pesticides and caused a reduction of growth hormone level [22]. Effects of the insecticide on the behavior of *Trichogramma* were entirely lying on nervous transmissions efficiency [23, 24].

Pyrethroid insecticides interrupt the normal nervous function. Deltamethrin and cypermethrin containing α-cyano group, is classified as a type II pyrethroid [25]. Its principal molecular mode of action is the alteration of the sodium channel transmission and sodium current during membrane excitation [26]. The inhibitory effects have also been described for calcium channels [27]. ATPases [28] and the receptors for acetylcholine like lipid complex of protein [29] and Gaba [30]. These multiple actions on nervous transmissions were difficult to point and they were disrupted by pesticides. All chemical insecticides act on the nervous system of the insect, but their target is different. Organophosphorus' target is acetylcholinesterase, while pyrethroids' main target is sodium channel [31]. Feldhege and Schmutterer [32] found that lower concentration of 10 mg/l azadirachtin (Margosan O) on a whitefly parasitoid, *Encarsia formosa Gahan*, was relatively nontoxic to the parasitoid and emergence from puparia, but a higher concentration of 20 mg/l azadirachtin resulted in a slight but significant reduction of *E. formosa*. Our results have been corroborated by Hoelmer [33], who observed that neem seed extract did not affect the emergence of *E. formosa* and *E. transvena* after dipping of parasitized *B. tabaci* puparia as compared to untreated control.

Cypermetrin, endosulfan, Monocrotophos and *B. thuringiensis* were found to be highly detrimental to both adults as well as immature stages of adult. Stansly and Liu [34] found that the neem extract, the insecticidal soap and two sugar esters derived from *Nicotiana gossei* Domain had little or no effect on *Encarsia pergandiella* Howard. However, Cypermethrin as a pyrethroid was found to be toxic topically and residually to these stages [35]. Medina [36] reported that under laboratory conditions, three novel insecticides, spinosad (Tracer R), tebufenozide (Mimic R) and azadirachtin (Align R) were not toxic to the eggs and pupae of *C. carnea* [37].

Furthermore, it was demonstrated that the use of synthetic pyrethroid to protect cotton from phytophagous insects leads to the elimination of natural enemies counteracting the benefits of the insecticide use [38]. On the other hand, *Trichogramma* species are largely used in biological control by inundative and inoculative releases [39] and their natural populations also contribute to the control of Lepidopteron pests [40].

Considering the above results on *T. chilonis* and proved to be safer than chemical insecticides and thus can be successfully used in IPM programmers requiring a self-sustaining parasitoid population. Although these biorational insecticides have still seen limited usage, they could represent important tools for pest management. The selectivity of these botanical insecticides should also be studied under field conditions as their realized effects can only be accurately assessed there.

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