

## EVALUATION OF TOXICITY OF SOME NOVEL PESTICIDES TO PARASITISM BY *TRICHOGRAMMA CHILONIS* (HYMENOPTERA: TRICHOGRAMMATIDAE)

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### ABSTRACT

A study was conducted in Entomology laboratory at Nuclear Institute of Food and Agriculture, Peshawar, Pakistan during 2011 to assess the toxicity of fifteen (15) pesticides, on parasitism by *Trichogramma chilonis* (Ishii) for which eggs were treated with pesticides. The mean parasitism based on square root transformed values for field dose was for two most highly toxic Spiromesifen (2.406) and Abamectin (2.606), while moderately toxicity was shown by Spinetoram (2.932), Fipronil (13.177), Acetamiprid (3.549), Heloxyfop-P-Methyl (4.206), and Myclubutanil (4.493). The 2X dose results showed that harmful pesticides were Spiromesifen (1.173), Fipronil (1.260), Abamectin (2.211) and Spinetoram (2.278), while Acetamiprid (2.932), Heloxyfop-P-Methyl (3.974), Myclubutanil (4.061), mixture of Trifloxystrobin and Tebuconazole, Spirotetramat (4.072) were moderately toxic. The 0.5X concentration results indicated that Abamectin (2.682) was harmful to parasitism, while Spinetoram (2.739), Spiromesifen (2.880), Fipronil (4.527) and Myclubutanil (4.537) were found to be slightly harmful to parasitism by *T. chilonis*.

**KEYWORDS:** *Trichogramma chilonis*; parasitism; pesticides; adverse impact; KPK; Pakistan.

### INTRODUCTION

Several species of the parasitic wasp belonging to the genus *Trichogramma* are reared and released for controlling insect pests of corn, rice, cotton, sugar-beet, tomatoes, vegetables, and orchards (7, 19) in about 50 countries. In spite of intensive research work on *Trichogramma*, as an egg parasitoid of lepidopteran insects, application of chemicals adversely affects the efficacy of *Trichogramma* (8). Over all impacts of pesticides not only include lethal

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effect but the sublethal effects should be given due importance to assess complete effects of pesticides on natural enemies (4). Sublethal effects can drastically decrease the efficacy of biological control agents (5). The adverse impacts of non-selective, broad spectrum pesticides are commonly recognized as either responsible for high mortality or adversely affect the efficacy of natural enemies as biological control agents (16).

Therefore, assessment of lethal as well as sublethal effects is primarily aimed, to incorporate the pesticides in integrated pest management (20). So that only these pesticides (novel pesticides) should be selected which have minimum sublethal effects on biological control agents (4).

The use of novel or selective insecticides with biological control is highly recognized as these are in favor of beneficial conservation strategy (2). Effect of pesticides on parasitism by *Trichogramma* is one of the important sub-lethal effects, which severely decreases the performance of this tiny wasp to control pests.

*Trichogramma chilonis* an important egg parasitoid plays an important role in suppressing lepidopterous pests in Pakistan. It is therefore, compulsory to evaluate its integration with the use of pesticides in IPM mode. The objective of this study was to assess the effect of some pesticides, on the parasitic efficiency of *T. chilonis* to observe the compatibility of these chemicals for successful integration with this tiny parasitoid in pest management.

## MATERIALS AND METHODS

This study was conducted in Entomology Laboratory, Nuclear Institute of Food and Agriculture, Peshawar, Pakistan during the year 2011. Fifteen novel/selected pesticides including six insecticides i.e. HaNPV *Helicoverpa armigera* Nuclear Polyhedroses Virus, Chlorantraniliprole, Spirotetramat, Acetamiprid, Spinetoram, and Fipronil, two miticides (Spiromesifen and Abamectin); three herbicides (Heloxyfop-P-Methyl, Bispyribac Sodium and Nicosulfuron) and four fungicides (Myclubutanil, mixture of Pyraclostrobin + Metiram, mixture of Chlorothalonil + Procymidone, and mixture of Trifloxystrobin + Tebuconazole) were evaluated.

### **Rearing of *Sitotroga cerealella* on wheat grain**

*Sitotroga cerealella* adults were collected from the infested grains daily by an electric suction apparatus into oviposition jars (10cm x 15cm in dimension) having mesh no 35-40 fixed to their bottom. Then the jars were placed on a tray containing starch for oviposition. The host eggs laid on the starch were collected daily using sieves (mesh no 50, 70). The collected host eggs were spread on sterilized wheat grain in plastic jar (14-cm x 22-cm) for larval and subsequent adult emergence. The host eggs were used both to increase progeny of *T. chilonis* as well as to maintain *S. cerealella* culture.

### **Rearing of *T. chilonis***

*S. cerealella* eggs (approximately 800-1000) were sprinkled on glued card (4-cm x 7-cm), followed by subsequent drying and putting to parasitism in glass jars (5cm diameter x 12cm height). This Jar contained approximately 20-30 pairs of adult of *T. chilonis*, in the laboratory at average conditions of 24°C, 65±10% RH, and 16:8h (L: D). After 24 hours of exposure, the parasitized cards were shifted to another glass jar of the same size and were incubated at the 23 °C, 70±10% RH, until adult emergence.

### **Preparation of different concentrations of pesticides solution**

Commercially available formulated pesticides (Table 1) were diluted by tap water to prepare three types of concentrations, i.e., field recommended concentration, FRC(X), 2X, and 0.5X for using in laboratory with the formulae:  $C_1 V_1 = C_2 V_2$ , where  $C_1$  and  $V_1$  are the concentrations and volume of commercial pesticides formulation and  $C_2$  and  $V_2$  are the concentrations and volume of required pesticides solution, respectively.

### **Evaluation of pesticides effects on parasitism**

The dried card of fresh *Sitotroga* eggs were cut to small cards (1x4-cm), containing each approximately 20 to 35 moth eggs and were dipped in field recommended concentration, FRC X, 2X, and 0.5X of

various pesticides in tap water (untreated control) for 1-2 seconds. These cards were again dried at room temperature for at least 1-hour, and were transferred to a glass vial (1-cm diameter x 7-cm length). Freshly emerged pair of *T. chilonis* of less than 24 hours was

**Table 1.** Label descriptions of pesticides used in the experiments.

Trade name and formulation	Active ingredient	Chemical class	Field rate /acre
Coragen 200 SC	Chlorantraniliprole	Anthranilicdiamide	80-ml
HaNPV	HaNPV ( $7.5 \times 10^{12}$ OB/L)	Biopesticides	80-ml
Movento 240 SC	Spirotetramat	Tetramic acids	125-ml+adjuvant 250-ml
Radiant 120 SC	Spinetoram	Spinosyns	100-ml
Mospilon 20 SP	Acetamiprid	Neonicotinoid	125-gm
Regent 50 SC	Fipronil	Phenylpyrazol	480- ml
Oberon 240 SC	Spiromesifen	Tetronic acids	250-ml
Abamectin 1.8	Abamectin	Avermectin or Glycoside	480-ml
Clover 200 WP	Bispyribac Sodium (20% w/w)+ Adjuvant (Polyethoxylated Fatty alcohol, 99.50 %w/w)	Pyrimidinylthiobenzoates	80-gm
Sun 750 WDG	Nicosulfuron	Sulfonylurea	30-gm
Percept 10.8 EC	Heloxyfop-P-Methyl	Aryloxyphenoxypropionate	350- ml
Systhane 20 EW	Myclobutanil	Triazole	35-ml
Nativo 750 WG	Trifloxystrobin (25% w/w) + Tebuconazole (50% w/w)	Mandelamide + Triazole	65-gm
CabrioTop 600 WDG	Pyraclostrobin (5% w/w)+ Metiram (55% w/w)	methoxy-carbamates + Ethylene bisdithiocarbamates	250-gm
Protocol 500 WP	Chlorothalonil (33.3% w/w) + Procymidone (16.7% w/w)	Organochlorine fungicide+ Dicarboximide fungicide	500-gm

introduced into each vial. The vial was kept in the laboratory at stated conditions for completion of parasitization, followed by removal of parasitizing female. The vials containing parasitized cards were incubated at 23°C, 70±10% RH, until development of pupae. Ten replications were carried out for each concentration under each pesticide. Oviposition data for 24-hour period were recorded by counting the total parasitized eggs (black eggs) on a card in each vial after seven days of exposure.

### Statistical analysis

The data were undergone assumption of normality (19) and found that present data did not follow the normality or homogeneity of variance,

Therefore square root transformation was performed on the parasitism data prior to analysis that needed to be normalized, followed by subjected to two way ANOVA (Analysis of variance) Tukey's post hoc HSD test ( $P=0.5$ ) was used for separation and comparison of means for statistical significance (1) with computer based statistical software package M.Stat-C.

## RESULTS AND DISCUSSION

The result of ANOVA indicated significant differences for interaction of pesticide and dose (Table 2) determined by Tukey test. It indicated that different pesticides as well as doses had significant effect on parasitism by *Trichogramma chilonis*.

Table 2. Analysis of variance for parasitism (Tukey test,  $p \leq 0.05$ ).

K value	Source	D. F.	S. S.	M. S.	F value	P
2	Pesticide (P)	14	578.515	41.322	1467.919	0.00
-3	Error	135	3.800	0.028		
4	Dose (D)	03	86.803	28.934	1258.014	0.00
6	P x D	42	200.483	4.773	207.391	0.00
-7	Error	405	9.473	0.023		
	Total	599	1052.681			

### Effect of field (X) dose

The results based on mean parasitism of square root transformed data revealed for field dose (Table. 3), that the most toxic among pesticides was Spiromesifen, with mean parasitism 2.406 (class 3), followed by Abamectin, 2.606 (class 3), Spinetoram, 2.932 (class 2), Fipronil 3.177 (class 2), Acetamiprid 3.549 (class 2), Heloxyfop-P-Methyl 4.206 (class 2), and Myclubutanil 4.493 (class 2). The rest of pesticides including HaNPV, Spirotetramat, Chlorantraniliprole, Bispyribac Sodium, Nicosulfuron, mixture of Pyraclostrobin + Metiram, mixture of Chlorothalonil + Procymidone, and mixture of Trifloxystrobin + Tebuconazole did not reveal significant effect on parasitism compared with control and are strongly recommended for integration with biological control by *Trichogramma*.

### Effect of 2X dose

The 2X concentration (Table 3) showed that the most harmful one was Spiromesifen, with mean parasitism 1.173 based on transformed data, It was followed by Fipronil (1.260), Abamectin (2.211), Spinetoram (2.278), Acetamiprid (2.932), Heloxyfop-P-Methyl (3.974), Myclubutanil (4.061), mixture of Trifloxystrobin and Tebuconazole (4.072), Spirotetramat (4.300). Other pesticides were found having no adverse impact on parasitism by *Trichogramma chilonis* as compared to control.

**Table 3. Pesticides with doses, and their respective mean parasitism based on square root transformed value.**

	Doses				
	2X	X	0.5X	Control	
Spiromesifen	1.173 Z	2.406 XY	2.880 VW	5.297 HIJKL	2.939 I
Acetamiprid	2.932UV	3.549 T	4.897 NO	4.998 MNO	4.094 F
Abamectin	2.211 Y	2.606 WX	2.682 VWX	5.548 CDEFGH	3.262 H
Chlorantraniliprole	5.336 FGHIJK	5.027 LMNO	5.327 GHIJK	5.547 CDEFGH	5.309 C
Spinetoram	2.278 Y	2.932 UV	2.739 VW	4.998 MNO	3.237 H
Myclubutanil	4.061 RS	4.493 PQ	4.537 P	5.239 IJKLM	4.583 E
Chlorothalonil + Procymidone	5.353 EFGHIJK	5.583 CDEFGH	6.030 B	5.373 EFGHIJK	5.585 B
Fipronil	1.260 Z	3.177 U	4.527 P	5.708 C	3.668 G
Bispyribac Sodium	5.628 CDEF	5.618 CDEFG	5.382 DEFGHIJK	6.251 AB	5.720 A
Nicosulfuron	5.388 DEFGHIJK	5.325 GHIJK	5.098 KLMNO	5.418 CDEFGHIJ	5.307 C
HaNPV	5.690 C	5.600 CDEFG	5.521 CDEFGHI	6.331 A	5.785 A
Trifloxystrobin + Tebuconazole	4.072 RS	4.866 O	6.080 AB	6.266 AB	5.321 C
Spirotetramat	4.300 PQR	5.383 DEFGHIJK	5.583 CDEFGH	6.283 AB	5.387 C
Heloxyfop-P-Methyl	3.974 S	4.206 QRS	5.636 CDE	6.281 AB	5.024 D
Pyraclostrobin + Metiram	5.185 JKLMN	5.672 CD	5.699 C	6.274 AB	5.707 AB
Mean	3.923 D	4.430 C	4.841 B	5.721 A	-

Note. Means based on square root transformed data, followed by same alphabetic do not differ significantly from one another ( $P=0.05$ , Tukey HSD test).

### Effect of 0.5X dose

In 0.5X concentration trial result the most toxic chemical (Table. 3) was Abamectin (moderately harmful), which led to 2.682 mean parasitism based on transformed data. It was followed by slightly harmful

pesticides, i.e., Spinetoram (2.739), Spiromesifen (2.880), Fipronil (4.527), Myclobutanil (4.537). Other chemicals were considered safe to parasitism by *Trichogramma chilonis*.

### Parasitism reduction (%) relative to control

The evaluation of percent parasitism reduction relative to control are based on the toxicity categories (TC) of IOBC/ WPRS (7): 1= harmless (<30%); 2 = slight harmful (30-79%); 3 = moderately harmful (80-99%); 4 = harmful (>99%) (Fig. 1). Unfortunately, there are very few published literatures available on the effect of chemicals on the biological control agents. Additionally most of the pesticides literatures deals with *Trichogramma* are based on the mortality of immature stages, determination of sublethal effects were ignored considerably in the past.

The present studies demonstrated that two miticides namely Spiromesifen and Abamectin were found as the most harmful to parasitism as compared to the rest of pesticides, as Spiromesifen has no literature related to parasitism by beneficial.

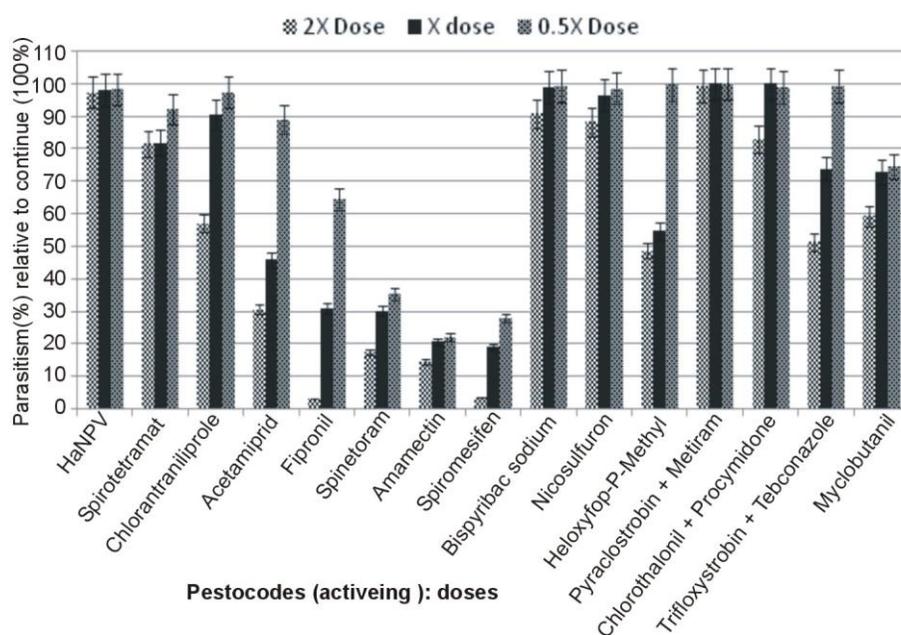


Fig. 1. Parasitism (%) relative to control (100%) by *Trichogramma chilonis* in response to different pesticides dose treatment: Reduction in parasitism relative to control.

Acetamiprid and contradict Abamectin in the present study were found slightly and moderately harmful, respectively to parasitism at field rate, as somewhat similar effects were observed by Moura *et al.* (12) where Acetamiprid and Abamectin treated surface reduced the parasitism efficiency of the exposed *Trichogramma pretiosum* females and proved as moderately harmful and harmful, respectively. Moreover according to Vianna *et al.* (21) Abamectin had significantly reduced the parasitism in *T. apretiosum*.

The HaNPV (microbial insecticide) was found very safe to the parasitism by *T. chilonis* in present research. Vianna *et al.* (21) also reported that *Bacillus thuringiensis* (microbial insecticide) and growth regulators caused no significant effect on either parasitism or emergence of the *T. pretiosum*. According to Gandhi *et al.* (6), biopesticides cause less effect on parasitism than synthetic chemicals

The data demonstrated that Spinetoram (Spynosyn) proved as a slightly harmful to parasitism by *T. chilonis*. As confirmed by Carmo *et al.* (3) who found that Spinosad, belonging to same group Spynosyn, as the Spinetoram, caused moderate toxicity to parasitism by *Telonomus remus* on first day of parasitism. Moreover Sattar *et al.* (17) proved that Spinosad (0.2% concentration) resulted in 15.40% parasitism of *T. chilonis*.

Trifloxystrobin, Pyraclostrobin, and Tebucanazole, were found safe to parasitism to *T. chilonis*. Similar results were obtained by Carmo *et al.* (3) who observed that Trifloxystrobin, Pyraclostrobin, and Tebucanazole fungicides had no significant difference in either parasitism or emergence of *T. remus* compared to control and were categorized as harmless to adults on both first and second days of parasitism.

It was further observed that Chlorantraniliprole was harmless to parasitism which has been confirmed by Moscardini *et al.* (11) who recommended that concentration of Chlorantraniliprole had less adverse effect on the foraging ability (frequency of ovipositor probing) of *Anargus nilaparvatae*. Moreover, Chlorantraniliprole was ranked as harmless to the egg viability and production in flowering bug,

*Oriusinsidiosus*. However, Liu *et al.* (9) have observed sublethal impact of the same chemical on beneficial insects remains unknown.

The result revealed that Metiram, Chlorothalonil, and Procymidone had no adverse impact on parasitism by *T. chilonis*. Petersen (13) also concluded that Metiram (80%), 0.42% in 6 $\mu$ l/cm<sup>2</sup> had no-significant effect on reduction percentage in egg production and hatchability, while Chlorothalonil 500 (0.30% in 6 $\mu$ l/cm<sup>2</sup>) and Procymidone 50 % (0.15% in 6 $\mu$ l/cm<sup>2</sup>) had no adverse impact on parasitism in *Alleocharabelineata*.

Heloxyfop-p-methyl was proved as harmless to parasitism. Peterson (13, 14) also found that Heloxyfop-R, 108g/L(0.255% in 6 $\mu$ l/cm<sup>2</sup>) was safe to egg production of rove beetle *Alleocharabelineata*.

Spirotetramat (Movento) chemical was found harmless to *T. chilonis* in present study too. Similarly, Spirotetramat was harmless to *Trichogramma acrytophlebiae* (in Nagarajaon citrus,), Moens *et al.*, (10) has stated that little research has been conducted to assess the side effects of spirotetramat on natural enemies. According to Planes *et al.*, (15), direct application of spirotetramaton on larvae and adults of *Cryptolaemus Montrouzieri* did not adversely affected the survival, life span, fecundity, egg hatching, and offspring survival.

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