Research Article

Comparative Toxicity of Insecticides against *Trichogramma chilonis* (Hymenoptera; Trichogrammatidae) under Laboratory Conditions

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Abstract | *Trichogramma* (Hymenoptera; Trichogrammatidae) is a genus of parasitic wasps that are powerful biocontrol agents against a variety of insect pests. These biological control agents retain their importance due to their easy mass rearing, high searching ability and effectiveness against many crop insect pests. In this study the toxicological effect of seven insecticides viz Flubendiamide (Belt 480 SC)@ 500 ul/l, Pyriproxyfen (Priority 10.8 EC) @ 2000 µl/l, Chlorantraniliprole + Thiamethoxam (Voliam Flexi 300 SC) @ 800µl/l, Nitenpyram (Pyramid 10% AS) @ 2000 µl/l, Lufenuron (Match)@ 2000 µl/l, Chlorantraniliprole (Coragen 20% SC) @ 200 µl/l and Flonicamid (Ulala50% WG) @ 60 mg/l were evaluated on the viability of parasitized eggs, survival and parasitism efficacy of *Trichogramma chilonis* under controlled conditions at toxicology laboratory Entomological Research Institute Ayub Agriculture Research Institute, Faisalabad. The viability of eggs was evaluated by exposing egg cards to insecticides by dipping them in insecticide solution for 10 seconds while adult survival was investigated through dipped surface residue bioassay method. The results revealed that new chemistry insecticides viz., Flubendiamide, Flonicamid and Chlorantraniliprole were comparatively safer to egg parasitiod followed by Chlorantraniliprole while Nitenpyram and Pyriproxyfen was found toxic to *Trichogramma*. Flubendiamide, Chlorantraniliprole and Flonicamidwas proved comparatively less toxic against *T. chilonis* with emergence percentage while in the case of survival of *T. chilonis*wasps, the results revealed that Pyriproxyfen and Nitenpyram had less knockdown effect as compared to other insecticides while Lufenuron was most toxic at 4 h post treatment and at 24 h all the insecticides were equally toxic to the parasitoid at adult stage.

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1. Introduction

Trichogrammatids are tiny endoparasite wasps, 0.2-1.5 mm in size. They belong to common group of egg parasitoids and used for biological control (Chailleux *et al.*, 2012, 2013; Tabone *et al.*, 2010) as an important tool in the integrated pest management. The species of Trichogrammatids are extensively used natural enemies insect in the world as they are easy to mass rare and attack many insect pests of important crops (Sarwar and Salman, 2015; Parra *et al*., 2010). They have the capacity to increase in number rapidly and are very destructive parasitoids of insect eggs especially eggs of butterflies and moths. They sometimes show 100% parasitism during optimum environmental conditions periods and the increasing parasitizing females showed the optimum temperature range 25-30°C (Nadeem *et al*., 2009; Reznik *et al*., 2009). It has been shown that two species i.e. *T. nubilale* Ertle and Davis are mostly active for controlling corn pests of Lepidoptera order (Wang *et al*., 1999). *T. chilonis* attacks on the lepidopterans of more than 400 pest species. It has been effectively used in the inoculative and inundative biocontrol programme around the world (Wang *et al*., 1999).

The genus *Trichogramma* is very effective taxa in studies of biological control programs. Members of *Trichogramma* genus are among one of the 80 genera in the *Trichogrammatidae* family and act as eggparasitoids of insects. The species of *Trichogramma* which are mostly collected from orchard and crops are *T. brevicapillum, T. thalense, T. atopovirilia, T. pretiosum* and *T. chilonis, T. platneri, T. deion, T. nubilale, T. exiguum, T. minutum and T. fuentesi*. The adult female wasps laid eggs into the eggs of moths. But few species of the *Trichogramma* parasitize also the eggs of beetles (Coleoptera), flies (Diptera), other wasps (Hymenoptera), true bugs (Heteropteran), lacewings and their relatives (Neuropteran) (Sarwar and Salman, 2015). Species of *Trichogramma* are mostly used against insect pests of different crops as biological control agent through release and augmentation. *Trichogramma* species saves more than 16 million ha crops by destroying the pests through parasitization (Singhamuni *et al*., 2015).

In integrated pest management program, use of insecticide remains a major control technique because it is cost-effective, efficient and easy for applying. However, use of chemicals along with reducing the number of target pests, affects the action of beneficial insects too (Wang *et al*., 2018; Umar *et al*., 2021; Saleem *et al*., 2021). Chemical control with *Trichogramma* have been measured incompatible because of the negative impact of insecticides. Toxic insecticides and *Trichogramma* are carefully planned in the same crop and these are mostly incompatible (Saber, 2011). Insecticides are used in many countries for the control of insect pests of soybean, vegetables, pine, rice, corn, sugarcane, cotton and sugar beet (Saleem el al., 2021). The natural population of *T. chilonis* contributed for the control of pests' population. Therefore, the effects of environmental pollutions on this specie by the insecticides also showed significant risk for the protection of biodiversity (Delpuech *et al*., 1998; Shehzad *et al*., 2021). *T. chilonis* are mostly exposed in the foraging process to insecticides in the lepidopterous pests of IPM program. Studies on the harmful effects of pesticides on *Trichogramma* have been inadequate (Lingathurai *et al*., 2015).

The females of *T. chilonis* discriminate the parasitized host eggs from unparasitized ones, they are mostly parasitized by heterospecific females (*Trichogrammabactrae* Nagaraja). They focus on lying of eggs and stay in the host eggs that are unparasitize. On the of insecticides no information is available in *T. chilonis* on their host discernment behavior (Wang *et al*., 2016). Pesticide resistance, severe pesticide regulation, secondary pest outbreak and concern about environment quality and human health have extended the awareness in IPM that emphasizes biocontrol and minimizing the use of insecticides. Current research was conducted to evaluate the toxic effect of seven insecticides viz., Flubendiamide, Pyriproxyfen, Chlorantraniliprole + Thiamethoxam, Nitenpyram, Lufenuron, Chlorantraniliprole and Flonicamid against immature and adult stages of *T. chilonis* after four and twenty-four hour of exposure to the insecticides and compatibility of the insecticides with this biological control agent.

2. Materials and Methods

The experiment was carried out at the toxicology laboratory of Entomological Research Institute, AARI, Faisalabad under controlled conditions to evaluate the toxic effect of different insecticides on the viability of parasitized eggs, survival and parasitism efficacy of *T. chilonis* by using different bioassay methods.

2.1 Insecticides

Seven insecticides viz., Flubendiamide (Belt 480 SC) @ 50 ml/acre, Pyriproxyfen (Priority 10.8 EC) @ 500ml/acre, Chlorantraniliprole + Thiamethoxam (Voliam Flexi 300 SC) @ 80 ml/acre, Nitenpyram (Pyramid 10% AS) @ 200ml/acre, Lufenuron (Match)@ 200ml/acre Chlorantraniliprole (Coragen

20% SC) @ 80ml/acre and Flonicamid (Ulala50% WG) @ 60g/acre were evaluated against *T. chilonis*at adult and egg stages. Lab doses were calculated through field recommended doses using Abbot's formula (given below). Each treatment was replicated three times under CRD design. The concentrations of insecticides were measured with the help of micro pipette and properly mixed with 1 liter distilled water in a beaker (1000 ml) to make solutions. For control treatment, only distilled water was used. Following equation was used to prepare the required solution (Abbott, 1925).

$$\text{Insecticide required}\left(\mu\frac{l}{mg}\right) = \frac{\text{Conc. of stock solution} \times \text{solvent required (ml)}}{\text{Conc. of insecticide (\%)} \times 1000} \times 100$$

2.2 Egg card bioassay

The card was directly exposed to the five treatments in beakers to evaluate the viability of eggs and their efficacy of parasitism. The bioassay was conducted on the 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th day old parasitized *Sitotroga cerealella* eggs. Five randomly selected cards containing 40 parasitized eggs were dipped into each treatment for 10 seconds. After that, the dipped egg cards were dried in an ambient room. Each treated egg card was placed in a small glass petri dish (5 cm diameter and 0.5 cm deep) until healthy parasitic wasp was emerged. Number of emerged wasps were then counted for percent emergence.

2.3 Dipped surface residue bioassay

The effect of insecticide residues on the survival of adult *T. chilonis* was investigated by using a dipped surface residue bioassay. The experiment was carried out in ventilated glass bio-assay chambers (15 cm x 4 cm). The whatman filter paper (25 mm) was dipped into the each treatment, then dried and placed in a glass bioassay tube. Twenty adults of *T. chilonis* were released in each tube. After 4 hours and 24 hours of exposure to treatments, the number of alive and dead wasps was counted. Each treatment was replicated 3 times.

2.4 Data analysis

The following formula was used to determine the percentage of wasps emerged.

$$\text{Percent emergence} = \frac{\text{Total number of emerged adult}}{\text{Total number of parasitized eggs}} \times 100$$

While in case of percentage mortality was calculated by using following formula.

$$\text{Percent Mortality} = \frac{\text{Number of died adults}}{\text{Total number of adult}} \times 100$$

Then the mean of variance was statistically analysis by using Statistix 8.1 software.

Table 1: Percentage egg viability and Percent emergence of *Trichogramma chilonis*.

Treatments	1-day old egg parasitoid Mean±SE	2 days old egg parasitoid Mean±SE	3 days old egg parasitoid Mean±SE	4 days old egg parasitoid Mean±SE	5 days old egg parasitoid Mean±SE	6 days sold egg parasitoid Mean±SE	7 days old egg parasitoid Mean±SE
Control	37.80±0.52a	23.40+0.40a	32.00±0.30a	35.60±0.79a	29.20±0.94a	24.60±0.72a	20.80±0.75a
Flubendiamide (Belt 480 SC) @ 500 ul/l	17.80±0.91c	17.80+0.91a	21.00±1.18b	20.80±0.63b	26.20±0.37a	12.00±0.67c	0.40±0.25c
Pyriproxyfen (Priority 10.8 EC) @ 2000 μl/l	10.60±1.21ef	6.40+0.41cd	16.80±0.98bc	4.80±0.31d	1.20±0.31d	13.40±0.86bc	0.60±0.05c
Chlorantraniliprole +Thiamethoxam (Voliam Flexi 300 SC) @ 800 μl/l	10.20±1.10f	5.00+0.38d	4.00±0.86e	3.40±0.42e	2.20±0.41d	15.00±0.31ab	3.80±0.17b
Nitenpyram (Pyramid 10% AS) @ 2000 μl/l	15.80±0.95c	11.20±0.73bc	11.80±0.44cd	9.00±0.86c	23.80±1.17c	13.80±0.53bc	0.20±0.09d
Lufenuron (Match) @ 2000 μl/l	12.80±0.97de	8.00±0.62cd	14.20±0.79d	29.00±1.50a	13.80±0.35bc	12.20±0.71c	0.00±0.04e
Chlorantraniliprole (Coragen 20% SC) @ 200 μl/l	13.40±0.50b	16.40±1.15b	23.00±0.60b	25.40±0.87ab	17.40±0.67bc	13.20±0.89bc	0.00±0.04e
Flonicamid (Ulala 50% WG) @ 60 mg/l	20.80±0.61b	7.40±0.52cd	8.40±0.56e	2.20±0.40e	24.80±0.42ab	16.00±0.61a	0.40±0.15d

3. Results and Discussion

3.1 Percent emergence of Trichogramma chilonis

Exposure of Lufenuron and Chlorantraniliprole (Coragen 20% SC) to parasitoid, *T. chilonis*, Showed lowest emergence (0.00% to 8.00% and 0.00% to 13.20%) respectively whereas, the highest mean emergence, when no treatment was used (control) was observed as 20.80±0.75a (56%) to 37.80±0.52a (96%) was observed. The emergence in 5 days old parasitoid eggs after the treatment of Chlorantraniliprole+Thiamethoxamwas@ 800 µl/l was found as 2.20±0.41d (6%)(P=0.00; F=12.9) and for Flubendiamide@ 500 ul/l, Pyriproxyfen@ 2000 µl/l, Nitenpyram@ 2000 µl/l, Lufenuron@ 2000 µl/l, Chlorantraniliprole@ 200 µl/land Flonicamid 60 mg/l it was found as 0.40±0.25c (1%) 0.60±0.05c (2%), 0.20±0.09d (1%), 0.00±0.04e (0%), 0.00±0.04e (0%)and 0.40±0.15d (1%)(P=0.0068, F=3.49) in 7 days old eggs that was proved to have lowest effect on the *T. chilonis* emergence and showed harmless to the egg parasitoid. New Chemistry insecticides viz., Flubendiamide, Flonicamid and Chlorantraniliprole observed to be most safe to egg parasitiod while Chlorantraniliprole+Thiamethoxam and Lufenuron found safer as compared to Nitenpyram and Pyriproxyfen.

3.2 Survival rate

In this study, all the insecticides differed significantly regarding survival of adults of *T. chilonis* 4 hours post application (Table 2). Pyriproxyfen resulted in maximum survival72.34 % of adults of egg parasitoid after 4 hour of treatments application followed by Nitenpyram (68.22% survival) which was found least toxic insecticides. On other hand Lufenuron was found most toxic insecticide resulted in lowest survival (27.44%) of the adult parasitoids which was significantly different survival percentage rate from Chlorantraniliprole (44.89%) and Flonicamid (41.55%).

Results after 24 hours of treatments application revealed that Flubendiamide (8.37%) was least toxic to the survival of *T. chilonis* adults followed by Chlorantraniliprole + Thiamethoxam (4.03%) and Nitenpyram (4.03%) while Nitenpyram, Lufenuron and Flonicamid showed 100% moratlity percentage so all insecticdes were found toxic to the adults, survival percentage ranged as compared to 82.43% in control.

From these results, it is clear that Pyriproxyfen and Nitenpyram had less knockdown effect as compared to other insecticides while Lufenuron was most toxic at 4 h post treatment and at 24 h all the insecticides were equally toxic to the parasitoid at adult stage.

Unsystematic use of pesticides is important aspect which causing the resurgence of insect pest and mortality of natural enemies. The present study indicates that Lufenuron was moderately toxic to *T. chilonis*and this has already been observed in previous studies of (Sattar *et al*., 2011). It may be compatible due to a difference in exposure period or dose rate, but the adult Trichogramma's effect on Lufenuron is likewise very less toxic than the other pesticides after 4 hours. Which are favored by other scientists (Hussain*et al*., 2012) resulted that Lufenuron applications were safer. In the same way (Anand and Mallapur, 2016) stated it is less toxic. But after 24 hours, 100% mortality was observed and it was found that Lufenuron was not safer for *Trichogrammachilonis* adult.

Flubendiamide (Belt 480 SC) treatment was moderately toxic. Our findings are quietly in line to that of Sattar *et al*. (2011) and Hussain *et al*. (2012). Sattar *et al*. (2011) resulted in study that Flubendiamide was moderately toxic. Similarly, Hussain *et al*. (2012) revealed that Flubendiamide was less toxic.

Table 2: Survival (%) of *T. chilonis* adults at different post treatment intervals of insecticides.

Insecticides/Treatments	Percentage survival	
	After 4 h	After 24 h
Flubendiamide (Belt 480 SC) @ 500 ul/l	46.44 D	8.37 B
Pyriproxyfen (Priority 10.8 EC) @ 2000 µl/l	72.34 B	0.00 D
Chlorantraniliprole +Thiamethoxam (Voliam Flexi 300 SC) @ 800 µl/l	46.33 D	4.03 C
Nitenpyram (Pyramid 10% AS) @ 2000 µl/l	68.22 C	4.03 C
Lufenuron (Match) @ 2000 µl/l	27.44 F	0.00 D
Chlorantraniliprole (Coragen 20% SC) @ 200 µl/l	44.89 D	0.00 D
Flonicamid (Ulala50% WG) @ 60 mg/l	41.55 E	0.00 D
Control	92.77 A	82.43 A

Chlorantraniliprole (Coragen 20% SC) was comparatively less toxic after exposure to egg parasitoid *T. chilonis*. Some other scientists also same resulted that it was less toxic for egg parasitoid (Hussain *et al*., 2012; Uma *et al*., 2014).

When *T. chilonis* adults treated with nitenpyram, it causes knockdown effect and 100% mortality was observed after 24 h of treatments. Our results were in accordance with (Ko *et al*., 2015) also found 95% mortality and also with (Preetha *et al*., 2009; Zhao *et al*., 2012).

Chlorantraniliprole + Thiamethoxam (Voliam Flexi 300 SC) was found moderately toxic in this study. Similarly, (Baehaki *et al*.,2017) reported that Chlorantraniliprole + thiamethoxam were moderately toxic. All adult behavior after 4 h showed less toxicity whereas, after 24 hours was proved to be highly toxic. However new chemistry insecticides with various modes of action were discovered to be most commonly utilized for their target host and their effect on the host was greater than that of their natural enemies. Therefore, they are rare bio control agents and best fit IPM programme.

As percent survival is concerned, our findings relates to those of Hussain *et al*. (2010) who stated that at Adult *T. chilonis* exposed to Imidacloprid, Emamectin benzoate, and Lufenuron had 70.0, 27.6, and 18.4% survival after 3 hours, respectively, however all pesticides were harmful to adult *T. chilonis* after 24 hours.

Conclusions and Recommendations

Flubendiamide (Belt 480 SC), chlorantraniliprole (Coragen 20% SC) and Flonicamid (Ulala 50% DF) proved comparatively less toxic against *T. chilonis* with emergence percentage. Chlorantraniliprole + Thiamethoxam (Voliam Flexi 300 SC) and Lufenuron showed moderate toxicity, Pyriproxyfen and Nitenpyram proved highly toxic. All insecticides proved highly toxic against adult stage after twenty-four hours exposure to insecticides and after four hours exposure to insecticides showed less toxicity as per mortality percentage data.

Novelty Statement

The biological control measures are major pillar of integrated pest management (IPM) and broad spectrum insecticides are major thread for the survival and efficacy of parasite so the results of current study will help the judicial l use of insecticides.

Author's Contribution

QA and DH design supervises the trial. KH, MS and AA execute the trial. TN, MFA, MZ and NAA wrote the research article. MUQ, KH and AA statistical analyzed the data. MZ provided helpful material for experiment.

Statement of conflict of interest

The authors have declared no conflict of interest.

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