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Efficacy of new generation insecticides against *Trichogramma chilonis* Ishii and *Trichogramma pretiosum* Riley

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Abstract

The experiment was conducted at Bio-control Laboratory, Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal during 2014 to investigate the toxic effect of modern insecticides (Spiromesifen, Rynaxypyr, Cyazypyr, Thiocloprid and Tolfenpyrad) on pupal stage of hymenopteran parasitoids. The trial was arranged in Factorial Complete Randomised Design consist of ten treatments excluding control with three replication. The present experiment resulted that the 100 per cent mortality was obtained in application of Tolfenpyrad 15% EC @ on *Trichogramma chilonis* Ishii and *T. pretiosum* Riley. All new molecules, spiromesifen, rynaxypyr, cyaxypyr and thiacloprid were observed harmless towards trichogramma species and use as a IPM component. The interaction between insecticidal treatment and parasitoids species was non-significant. The first report of new formulation of rynaxypyr 35 WG was observed harmless effect on *T. chilonis* and *T. pretiosum*

Keywords: *Trichogramma* spp., rynaxypyr 35 WG, Modern Insecticides, Toxicity, Natural enemy

1. Introduction

Several species of *Trichogramma* are reared and released around the world annually on an estimated 80 million acres of agricultural crops and forests in 30 countries^[1, 2]. Hymenoptera is one of the most dominant entomophagous order which widely used in biological control agent in world. Among the trichogrammatids (Trichogrammatidae; Chalcidoidea), *Trichogramma* are minute parasite wasps that attack on eggs of many lepidopteran insects. The genus contains 130 species of which 20 species have been reported from India^[3]. In spite the important role of the biological control agents in agriculture, chemical control is still indispensable but, the use of non-selective insecticides greatly reduces the beneficial potential of the biocontrol agents, particularly parasitic Hymenoptera that are often far more susceptible to insecticides than their hosts. A range of harmful effects of insecticides on *Trichogramma* spp. have been described by different workers^[4, 5, 6]. Pesticides may affect natural enemies' effectiveness either by causing direct mortality or by influencing their reproduction, behaviour, foraging or movement^[7, 8, 5]. Biological control agents are an important tool in integrated pest management programme. However adverse effect on natural enemies varies with the intrinsic relative toxicity of compound, formulation of concentration applied, timing and special pattern of application and environmental factors. Better results can be obtained in the IPM programme by judiciously combining natural enemies and selective pesticides that will control the pest without causing much harm to natural enemies^[9, 10].

In the recent past a number of new generation insecticide molecules have been developed which are generally claimed to be rather selective and relatively safer to various groups of non-target organisms. However, a particular insecticide selective to a particular species may be toxic to others. In recent years, some highly toxic broad spectrum insecticides have been banned or their used restricted. Whereas, a number of new molecule have been introduced which are generally considered to be rather selective and relatively safer group to non-target organism including natural enemies. However, a particular insecticide selective to a particular species may be toxic to others. The present programme has been undertaken in laboratory to find out the toxicity of several new generation insecticides to the pupal stage of two species of Trichogrammatids egg parasitoid *Trichogramma chilonis* Ishii and *Trichogramma pretiosum* Riley.

2. Materials and methods

2.1. Mass culture of *Corcyra cephalonica* and egg parasitoids, *Trichogramma* spp.

The rice meal moth *Corcyra cephalonica* Stainton (Galleriidae; Lepidoptera) was reared in the Agricultural Entomology Laboratory, BCKV, Mohanpur, West Bengal, India. Sterilized crushed maize grains were used as feed for the host *C. cephalonica*. The rice meal moth, *C. cephalonica* was used as host for *Trichogramma pretiosum* Riley and *T. chilonis* Ishii.

2.2. Feed Preparation for *Corcyra cephalonica*

For the mass multiplication of *C. cephalonica*, crushed maize grains were sterilized in oven at 100° C for 30 minutes before use to eliminate others insect pests. The sterilized grains, after cooling, were sprayed with 0.2% formalin to prevent the growth of mould as well as to conserve grain humidity and air dried. The grains were poured into rearing boxes @ 2.5 kg / box and each box was inoculated with 0.5cc *Corcyra* eggs and was closed by placing the lid as shown in fig.1. After about 40 days, moths started emerging, that continued for 60 days. Moths were collected daily and were transferred to specially designed oviposition cages placed over a petriplate for egg laying. The eggs were collected every day, debris like dust and scales were removed by blowing with fan.

2.3. Mass culture of *Trichogramma* species

Collected *C. cephalonica* eggs were used for the mass multiplication of *Trichogramma* spp., at laboratory. Eggs of *Corcyra* (not more than 24h old) were sterilized under UV light (25 watt) for 30 minutes to kill the developing embryo. These were sprinkled uniformly over paper card (15.5cm x 8cm), smeared with a uniform thin layer of 50% aqueous solution of pure gum Arabica with the help of a camel hair brush. Egg cards were offered to the newly emerged parasitoids for parasitisation inside a glass tube. Superparasitism was avoided by regulating host parasitoid density. The parasitoids were provided with 50% honey solution as food. The temperature of the rearing room was maintained at 27±1 °C. The egg card was removed from the tube after 24hrs and a fresh egg card was offered again for parasitisation and the process was continued till 20-25% of the parasitoids were alive. Blackening of *Corcyra* eggs in the card indicated parasitisation as shown in fig.2. and the cards

were either kept as such for the emergence of adult parasitoids or utilized for the experiment.

2.4. Evaluation of insecticides against hymenopteran parasitoids

The five different new concentration of insecticides [Cyazypyr 10% OD and Rynaxypyr 35% WP (M/S E.I. DuPont India Pvt. Ltd.), Spiromesifen 24% SC and Thiacloprid 21.7% SC (Bayer crop science Limited), Tolfenpyrad 15% EC (PI Industries Ltd.)] were tested against the pupal stage of *Trichogramma* spp. under laboratory condition. Stock solutions of formulated insecticides were prepared in fresh tap water. The five insecticides with different concentration like Cyazypyr 10% OD @ 0.015 and 0.010 a.i./ha., Rynaxypyr 35% WP @ 0.007 and 0.0052 a.i./ha, Spiromesifen 24% SC @ 0.030 and 0.024 a.i./ha, Thiacloprid 21.7% SC @ 0.021 and 0.016 a.i./ha and Tolfenpyrad 15% EC @ 0.0049 and 0.0037 a.i./ha were evaluated on pupal stage of both egg parasitoids.

2.5. Collection of data

On the fifth day after parasitisation when all the parasitized eggs have turned black indicating that, the parasitoids inside have entered pupation, the egg cards were made into pieces, each piece containing at least 30 parasitized eggs. These small cards were dipped in different insecticide concentrations, removed immediately and dried under fan. In control treatment, egg cards were dipped in water. The egg cards were kept separately in glass vials to allow the emergence of adult parasitoids from the insecticide treated host eggs. The mouth of the vials was covered with muslin cloth tied with rubber bands. The pupal mortality was recorded after adult emergence and no adult has emerged from pupae were considered as dead as shown in fig.4. Each treatment was replicated three times and each replication containing 30 parasitized eggs (fig.3) which observed under a binocular Stereo Microscope.

2.6. Statistical analysis

The per cent mortality was calculated from the recorded data of emergence of adult and the value was subjected for the calculation of corrected % mortality by using Abbott's ^[11] formula.

$$\text{Mortality \%} = \frac{\text{Total emergence of adult from pupa after treatment}}{\text{Total emergence of adult from pupa in control}} \times 100$$

$$\text{Corrected \%} = \left(1 - \frac{\text{N in T after treatment}}{\text{N in Co after treatment}}\right) \times 100$$

Where: n = Insect population, T = treated, Co = control
The percent mortality data were transformed into angular transformation to normalize the data. The data on corrected mortality was analysed for test of significance following Factorial Complete Randomised Design were performed in SPSS 16.0 version.

3. Results

The toxicity of five promising new generation insecticides were tested against *Trichogramma chilonis* Ishii and *Trichogramma pretiosum* Riley pupae at laboratory condition

and result was presented in Table 1 based on mortality and toxic category of insecticides.

The maximum emergence of adults were observed in spiromesifen followed by rynaxypyr, cyaxypyr and thiacloprid whereas tolfenpyrad was found most toxic due to least adults of *Trichogramma* spp. emerged from pupae. Application of spiromesifen 24 SC @ 1ml/L and 1.33 ml/L were recorded minimum mortality of *T. chilonis* (1.11% and 2.22% mortality) and *T. pretiosum* pupae (2.22% and 3.33% mortality) respectively. Application of Tolfenpyrad 15% EC @ 0.33ml/L and 0.25 ml/L were found statistically more toxic

to pupal stage of *T. chilonis* (100% mortality) and *T. pretiosum* (100% mortality) as compared to control in laboratory condition. Adult emergence increased in spiromesifen insecticides followed rynaxypyr, cyazypyr and thiacloprid which considered as 'harmless' category while tolfenpyrad observed negative effect on adult emergence and labelled as 'hamful' category.

The mortality of both the species of *Trichogramma* in all treatments were found significant lowest except tolfenpyrad. However, both concentrations of Cyazypyr and Thiacloprid observed statistically at par effect. Rynaxypyr, cynaxypyr, cyazypyr, spiromesifen, thiacloprid were found minimum mortality ranges from 1.11 to 12.22% which considered as harmless (Toxicity class 1) while 100% mortality found in tolfenpyrad considered as hamful (Toxicity class 4).

Considering both *Trichogramma* spp. all insecticidal treatments together was non-significant indicating that the two species of *Trichogramma* i.e. *Trichogramma chilonis* and *Trichogramma pretiosum*, did not showed any significant difference in respect of their pupal mortality. The effect of interaction between insecticidal treatments and *Trichogramma* spp. were non-significant which indicates that the two species was affected on the similar way when exposed to insecticides.

4. Discussion

Trichogramma spp. are promising bioagent which parasitized eggs of more than 400 species of insect belonging to different orders [12]. However indiscriminate use of insecticides is considered as important factors causing mortality of natural enemies causing resurgence of pest. The present investigation revealed that all new generation insecticides were found safer except tolfenpyrad on pupal stage of both the species of *Trichogramma*. Cyazypyr 10 OD (1 and 1.5 ml/L) and Thiacloprid 21.7% SC (0.75 ml/L and 1ml/L) were observed that no adverse effect on mortality of pupal stage of both species and considered as harmless insecticides. Similar result were reported by Moura *et al* [13] evaluated residual action of insecticides on *Trichogramma pretiosum*, and concluded that thiacloprid was harmless. Ram [14] evaluated several new generation of insecticides on *T. pretiosum* and reported that spiromesifen 24% SC @ 0.019 and 0.010 a.i., rynaxypyr 20% SC @ 0.008 and 0.004 a.i., cyazypyr 20% SC @ 0.024 a.i., flubendiamide 20% WG @ 0.004 a.i., had showed no mortality on the pupae as well as totally safe to same species of natural enemies. Mode of action of spiromesifen is inhibitors of lipid synthesis which presently used and recorded negligible mortality as compared to other insecticides against both species of egg parasitoids and labelled as harmless. Similar result was reported by Bielza *et al* [15] that spiromesifen were harmless to adult stage of *Eretmocerus mundus* (Hymenoptera). Thiacloprid was observed as harmless response on *Trichogramma* spp. in present study. Veire and Tirry [16] revealed that thiacloprid showed harmless against nymph of *Macrolophus caliginosus* and harmful response on nymph of *Orius laevigatus*. In the present study tolfenpyrad 15% EC @ 0.33 ml/L and 0.25 ml/L were found 100% mortality and registered as harmful to

both *Trichogramma* spp. under laboratory condition. Mode of action of tolfenpyrad is mitochondrial complex 1 electron transport inhibitor. Kamde [17] assessed new molecules against cabbage pest and its natural enemies and found Tolfenpyrad 15 EC @ 150 g a.i./ha and other molecules were found non-significant effect on population of spider and coccinella in different doses of insecticides.

Cyazypyr 10 OD @ 1 and 1.5 ml/L were recorded no adverse effect on mortality of both *trichogramma* species which indicated safe to natural enemies. Similar result was concluded by Mondal [18] that cyazypyr @ 90 and 60 g a.i./ha were safe to *T. pretiosum* Riley pupae (23.17% and 20.73% mortality) respectively. Misra [19] reported that application of Cyazypyr 10% OD (cyantraniliprole compound) @ 90 and 105 g a.i./ha in tomato field were recorded as safe to coccinellid predators.

In present investigation a new formulation of rynaxypyr 35% WG was used for which no literature is available till date, showed harmless against *T. chilonis* and *T. pretiosum*. As such, a present finding with rynaxypyr is first report of its category which was safer to natural enemies. Rynaxypyr 20% SC and other anthranilic diamide insecticides were reported safe to pupal stage of *T. chilonis* Ishi [20] and rynaxypyr @ 125 ml/ha also found non-toxic to pollinator and natural enemies in corn field [21]. Dinter *et al* [22] evaluated the toxicity of new anthranilic diamide insecticides (chlorantraniliprole (DPX-E2Y45 or Rynaxypyr), Coragen (200 g Rynaxypyr/L; DPX-E2Y45 20SC) and Altacor (350 g Rynaxypyr/kg; DPXE2Y45 35WG) against pollinators and concluded that the application of chlorantraniliprole and its formulated products, Coragen and Altacor were safe for both pollinators, furthermore suggested that the compounds were incredibly less susceptible towards non target organisms like pollinator and other beneficial organism. So, these chemical based formulation and products are advisable for farmers field.

The two parasitoids species i.e. *T. chilonis* and *T. pretiosum* didn't showed any significance difference in their susceptibility to the insecticides as a whole. Furthermore the interaction of insecticidal treatment and parasitoids species were non-significant which indicating similar level of susceptibility of the parasitoids to the insecticidal treatments.

5. Conclusion

The present study showed that new generation insecticides like rynaxypyr, cynaxypyr, cyazypyr, spiromesifen, thiacloprid are extremely harmless towards *Trichogramma* spp. These insecticides are may be included in future as sole components of IPM programmed to diminished the 3 R's factor i.e. resistance, resurgence and replacement that made the pest control more complicated

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Fig 1: Mass rearing box of *Corcyra cephalonica*



Fig 2: Blackening of Trichocard after 5th day of Parasitization



Fig 3: Treatment with three replications on pupal stages of *Trichogramma spp*



Fig 4: Emerged and non-emerged adult of *Trichogramma spp* from Parasitized eggs of *C. cephalonica*.

Table 1: Toxic effect of new generation insecticides on pupal mortality of *T. chilonis* and *T. pretiosum*.

Treatments	<i>T. chilonis</i>		<i>T. pretiosum</i>		Average % pupal mortality	Toxicity Class
	Mortality%	Corrected Mortality	Mortality%	Corrected Mortality		
Control (Water)	0.00 (0.00 ^e)*	-	1.11 (3.51 ^e)*	-	-	-
Rynaxypyr 35% WG @ 0.20g/L	6.67 (14.96 ^{bc})	6.67	7.78 (16.12 ^{bcd})	5.62	6.15 (15.54 ^{bc})	1
Rynaxypyr 35% WG @ 0.15g/L	4.44 (12.00 ^{bcd})	6.67	5.56 (13.48 ^{abcd})	5.62	6.15 (12.74 ^{cd})	1
Cyazypyr 10% OD @ 1.5ml/L	7.78 (16.12 ^b)	10.00	7.78 (16.12 ^{bcd})	8.99	9.50 (16.12 ^{bc})	1
Cyazypyr 10% OD @ 1ml/L	8.89 (17.28 ^b)	6.67	11.11 (19.43 ^{bc})	8.99	7.83 (18.35 ^{bc})	1
Spiromesifen 24% SC @ 1.25ml/L	2.22 (7.01 ^{cde})	0.00	3.33 (8.49 ^{cde})	5.62	2.81 (7.75 ^{de})	1
Spiromesifen 24% SC @ 1ml/L	1.11 (3.51 ^{de})	0.00	2.22 (7.01 ^{de})	2.25	1.13 (5.26 ^e)	1
Thiacloprid 21.7% SC @ 1ml/L	11.11 (19.43 ^b)	13.33	12.22 (20.42 ^b)	12.36	12.85 (19.93 ^b)	1
Thiacloprid 21.7% SC @ 0.75ml/L	8.89 (17.28 ^b)	6.67	11.11 (19.43 ^{bc})	12.36	9.52 (18.35 ^{bc})	1
Tolfenpyrad 15% EC @ 0.33ml/L	100.00 (90.00 ^a)	100	100.00 (90.00 ^a)	100	100.00 (90.00 ^a)	4
Tolfenpyrad 15% EC @ 0.25ml/L	100.00 (90.00 ^a)	100	100.00 (90.00 ^a)	100	100.00 (90.00 ^a)	4

* Figure in parentheses indicates the angular transformation.

^a Means followed by the same letter in column do not differ statistically by Tukey HSD test ($P \leq 0.05$).

According to IOBC, Class 1 = harmless (mortality < 30%); Class 2 = slightly harmful (30% - 79%); Class 3 = moderately harmful (80% - 99%); Class 4 = harmful (> 99%).

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