IMPACT OF CHLORONICOTINYL INSECTICIDE, IMIDACLOPRID ON EGG, EGG-LARVAL AND LARVAL PARASITOIDS UNDER LABORATORY CONDITONS

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Abstract: Laboratory studies were carried out to compare the toxicity of the chloronicotinyl insecticide, imidacloprid on parasitoids. The studies took place at the Tamil Nadu Agricultural University, Coimbatore from 2006–2007. Imidacloprid was tested against three beneficial insects *viz*., an egg parasitoid, egg larval parasitoid and a larval parasitoid representing two families of Hymenoptera: Trichogrammatidae (*Trichogramma chilonis* Ishii) and Braconidae (*Chelonus blackburni* Cameron; *Bracon hebetor* Say) that attack insect pests of cotton. The toxicity of imidacloprid was evaluated by treating the parasitized eggs using an atomizer in the case of *T. chilonis* and glass scintillation vial residue bioassay method for the adults of *C. blackburni* and *B. hebetor.* The toxicity of imidacloprid to parasitoids was compared with another neonicotinoid, named thiamethoxam, and a standard check, methyl demeton. Imidacloprid 17.8 SL did not cause any adverse effects on the adult emergence and parasitization of *T. chilonis*. At the recommended dose of imidacloprid [25 g active substance (a.s.)/ha], 90.67 and 85.32 per cent adult emergence and parasitization was recorded, respectively. The recommended dose of imidacloprid caused 56 per cent mortality and was found to have moderate impact on the adults of *C. blackburni.* On the other hand, it was found to be toxic to the parasitoid *B. hebetor*, causing 70 per cent mortality at 48 hours after treatment (HAT). The data presented here will provide pest managers with specific information on the compatibility of selected insecticides with natural enemies attacking pests of cotton, *Gossypium hirsutum* L.

Key words: imidacloprid, *Chelonus blackburni, Trichogramma chilonis, Bracon hebetor,* toxicity

INTRODUCTION

The selection of an effective natural enemy for introduction into the field is a major issue in the development of biological control programs. Trichogrammatid egg parasitoids are considered to be the most useful biological control agents for inundative releases against lepidopterous pests (Stinner 1977; King *et al*. 1986; Ravensberg and Berger 1988; Alba 1990; Singh and Jalali 1994). In India, mass releases of *Trichogramma* spp. have been used successfully in the control of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) on cotton. *Bracon hebetor* Say is a minute Braconidae wasp that is an internal parasite to the caterpillar host. The gut enzymes from the *B. hebetor* wasp quickly destroy the blood proteins in the moth larvae. This ability makes the *B. hebetor* wasp an effective biocontrol agent (Stanley and Tunaz 2007).

These key natural enemies are important in suppressing insect pest populations. The conservation of these natural enemies is a valuable Integrated Pest Management (IPM) approach in cotton. Natural enemies do not eliminate pest populations. Instead, these enemies establish an equilibrium with pest insect populations that are generally below damage thresholds. When pest densities exceed these thresholds, an occasional insecticide treatment is needed. Thus, pesticide intervention is considered essential in some situations to control high infestations of specific pests so as to reduce economic damage and pest spread, to other crops. Selective insecticides that target pest species could play a role in conserving this wide diversity of natural enemies associated with cotton. Several insecticides that are widely used to suppress various pests can disrupt the effectiveness of these beneficial agents. It is less clear to what degree insecticides are disruptive with other non-target organisms. Improved understanding of pest-natural enemy-insecticide interactions will assist in formulating more effective Integrated Pest Management strategies. Therefore, more recent evaluations of foliar insecticide toxicity against selected natural enemies seems well-timed.

The toxic effects of pesticides on beneficial insects, particularly parasites, have been studied in India (Paul *et al*. 1976; Kareem *et al*. 1977; Sithanantham and Paul 1977). Schuld and Schmuck (1997) observed no adverse effects of imidacloprid on *Trichogramma* spp., the egg parasitoid of *Cydia pomonella* Meyrick. Guifen *et al.* (1997) stated that imidacloprid and buprofezin caused less mor-

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tality of the parasitoid, *Trichogramma japonicum* Gahan. They recommended these chemicals when the parasitoid is at the egg stage of the life cycle. Kumar (1998) observed no significant adverse effect of imidacloprid on adult emergence of *Trichogramma chilonis* Ishii. A similar finding was reported by Suganthy (2003), whereas Carvalho *et al*. (2003) reported that imidacloprid showed an intermediate effect on the parasitism of *Trichogramma pretiosum* Riley. Shanmugam *et al*. (2006) indicated that imidacloprid 17.8 SC was moderately toxic with a 53 per cent reduction in parasitism of *T. chilonis*. Bethke and Redak (1997) studied the effect of imidacloprid on the parasitism of *Encarsia formosa* Gahan on whitefly, *Bemisia argentifolii* Bellows and Perring and observed a low level of parasitization (< 10%). Gonzalez *et al.* (1997) concluded that imidacloprid was moderately toxic to the pupae of *Eretmocerus mundus* (Mercet) parasitizing *B. tabaci*. The information available on pesticide toxicity to parasitoids other than *Trichogramma* is scarce. Our study is a report of toxicity of neonicotinoid, imidacloprid to egg parasitoid, *T. chilonis*, egg larval parasitoid, *C. blackburni* Cameron and larval parasitoid, *B. hebetor*.

MATERIALS AND METHODS

Egg parasitoid, *T. chilonis* Ishii

Corcyra cephalonica was reared in the laboratory according to the method described by Upadhyay *et al*. (2001). The emerged *Corcyra* adults were collected in the morning and allowed inside an oviposition cage of 21x25 cm size, with a wire mesh on the bottom and lateral sides for ventilation. Adults were provided with a 50 per cent honey solution as food. Eggs were collected at the bottom on a blotting paper kept in tray and cleaned with sieves or an egg separator. The cleaned eggs were sprinkled over half ground cumbu grains, at the rate of one cc per 2.5 kg of grains, fortified with ten grams of yeast in a plastic basin (45x30x10 cm) and covered with kada cloth. Care was taken to keep the culture free of storage mites and diseases by mixing five grams of wettable sulphur 80 WP and streptomycin sulphate at 0.5 per cent, respectively. The emerged adults were collected and used again for culturing both host (*C. cephalonica*) and parasitoid (*T. chilonis*). The culture was maintained at room temperature (28±2°C and 80±5% RH).

T. chilonis was mass cultured on the eggs of rice moth, *C. cephalonica*. The fresh *Corcyra* eggs were collected in the early morning and sterilized under UV radiation of 15 Watts for 20 minutes at a distance of 15 cm to avoid the emergence of *C. cephalonica* larvae. The sterilized eggs were then pasted on paper cards of 16x32 cm size containing thirty, 7x2 cm rectangles. These egg cards were placed in polythene bags along with a nucleus card at 6 : 1 ratio for parasitization. After parasitisation, the egg cards were cut into one cm^2 bits and the per cent of three day old, parasitized eggs (eggs appearing black and plumpy) were used for conducting the experiment.

Egg and larval parasitoid, *C. blackburni*

The egg larval parasitoid, *C. blackburni* was purchased from Project Directorate of Biological Control (PDBC), Bangalore and used for the study.

Larval parasitoid, *B. hebetor*

The larval parasitoid, *B. hebetor* were obtained from Biocontrol Laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore for conducting the bioassay.

Insecticide bioassays

The bioassay method described by Jalali and Singh (1997) was adopted for *T. chilonis* with modifications. The parasitized egg cards (16x32 cm) were sprayed with insecticides of respective treatments at the rate of 2.5 ml per card using an atomizer. Distilled water was used in the untreated check. The treated egg cards were shade dried for 10 min. After complete drying, three 7x2 cm cards, representing three replications were cut carefully from the treated cards and kept in test tubes. The test tubes were 15 cm length and 2.5 cm dia. Using a hand lens, observations were recorded on the number of parasitoids which emerged after 24 and 48 h of treatment in one cm^2 area. Per cent emergence was worked out using the formula:

$$\text{Per cent of emergence} = \frac{\text{No. of wasps emerged}}{\text{Total No. of eggs in one cm}^2} \times 100$$

Fresh eggs were provided to these parasitoids at 6 : 1 ratio and the number of parasitized eggs (eggs appearing black and plumpy) were recorded after 24 and 48 h of treatment and the parasitization per cent was worked out using the formula:

$$\text{Parasitization per cent} = \frac{\text{No. of parasitized eggs}}{\text{Total No. of } \textit{Corcyra} \text{ eggs}} \times 100$$

The bioassay method described by Mccutchen and Plapp (1988) for *Chrysoperla carnea* Stephens was adopted with modifications. The insecticidal concentrations were prepared using acetone and water in a ratio of 80 : 20.

Glass scintillation vials of 20 ml capacity were evenly coated with 0.5 ml of the insecticide formulations dissolved in acetone : water and dried by rolling the vials in between the palms. After ensuring that the vials are dried, three day old adults of *C. blackburni* were released at the rate of 10 vial and covered with muslin cloth secured with a rubber band. After a 1 h exposure, the wasps were transferred to test tubes and fed a honey solution. Observations were recorded on the mortality of wasps at 12, 24 and 48 h after treatment. For the untreated check, acetone:water (80 : 20) alone was used. Mortality per cent of adults was worked out using the formula:

$$\text{Mortality per cent} = \frac{\text{No. of adults dead}}{\text{Total number of wasps}} \times 100$$

Glass scintillation vial bioassay was used to find the toxicity of insecticides to *B. hebetor* as that of *C. blackburni.* The adults of *B. hebetor* were released into the vials at the rate of 10 vial, covered with muslin cloth and secured with a rubber band. After an h of exposure, the *B. hebetor* adults were transferred to clean test tubes and they were provided with a honey solution. Mortality observations were made at 24 and 48 h after treatment and mortality per cent of the adults was worked out as in the case of *C. blackburni*.

Insecticides

For the bioassays, we used imidacloprid 17.8 SL (Mahamaya AgriScience Services, Haryana). This imidacloprid was compared with another imidacloprid formulation (Tatamida 17.8 SL, Saraswati Agrochemicals India Pvt. Ltd., Jammu), thiamethoxam (Actara 25 WG, Syngenta India Ltd., Mumbai) and the conventional insecticide methyl demeton (Metasystox 25 EC) which is used for the control of sucking pests. Commercial formulations of insecticides were used for conducting bioassays.

Statistical analysis

The percentage mortality in the test insects was corrected relative to the control mortality using Abbott's formula (Abbott 1925). The experiments were conducted in a Completely Randomized Design (CRD) and the percentage values were converted to arcsine percentage. The mean values of treatments were then separated by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez 1976, 1984).

RESULTS

Toxicity of imidacloprid to *T. chilonis*

The effect of imidacloprid on the adult emergence and parasitization of *T. chilonis* summarized in table 1 indicated that all the doses of imidacloprid exerted a lesser impact on the emergence of adults. The adult emergence percentage ranged from 80.86 to 93.81 among the insecticidal treatments. Thiamethoxam recorded 93.81 per cent adult emergence which was on par with untreated control (98.73%). The imidacloprid at the recommended dose (25 g a.s. ha^{-1}) recorded 90.67 per cent adult emergence. The insecticides exerted little impact on the parasitization of *T. chilonis* also. The higher dose of imidacloprid (50 g a.s./ha) reduced the parasitization to some extent with a recording of 72.36 per cent parasitization when compared to the untreated check (93.36%). The other doses of imidacloprid (15 and 25 g a.s./ha) and thiamethoxam recorded a parasitization per cent of 90.66, 85.32 and 88.59, respectively. These recorded per cents were on par with the untreated check and superior to the standard check, methyl demeton which recorded 64.14 per cent.

Table 1. Selective toxicity of imidacloprid 17.8 SL on the parasitoid, *T. chilonis* Ishii. (Mean of three observations)

Treatments	Per cent of adult emergence	Per cent of parasitization
Imidacloprid 17.8 SL 15 g a.s./ha	92.85 ab (74.92)	90.66 a (74.28)
Imidacloprid 17.8 SL 25 g a.s./ha	90.67 b (73.10)	85.32 ab (67.84)
Imidacloprid 17.8 SL 50 g a.s./ha	88.89 bc (70.76)	72.36 bc (58.33)
Imidacloprid 17.8 SL 25 g a.s./ha (Tatamida®)	91.80 ab (73.70)	87.60 ab (69.56)
Thiamethoxam 25 WG 25 g a.s./ha	93.81 a (76.14)	88.59 a (70.47)
Methyl demeton 25 EC 125 g a.s./ha	80.86 c (64.14)	64.14 c (53.23)
Untreated check	98.73 a (83.96)	93.36 a (77.35)

In a column, means followed by a common letter are not significantly different at p = 0.05 by Duncan's multiple range test (DMRT); Figures in parentheses are arcsine P transformed values
a.s. – active substance

Toxicity of imidacloprid to *C. blackburni*

The toxicity studies of imidacloprid to the adults of *C. blackburni* revealed that the adult mortality per cent increased gradually at different intervals in all the insecticidal treatments. All the insecticides were significantly toxic to *C. blackburni*. The least mortality of *C. blackburni* adults was observed when using the lower dose of imidacloprid (15 g a.s./ha) which showed 13.33, 30.00 and 36.67 per cent mortality at 6, 12 and 24 HAT, while the recommended dose of imidacloprid (25 g a.s./ha) recorded an adult mortality per cent of 23.33, 36.67 and 50.00 at 6, 12 and 24 HAT, respectively. Among the imidacloprid treatments, the highest mortality per cent was accounted for by imidacloprid at 50 g a.s./ha of 30.00, 46.67 and 63.33 per cent, respectively at 6, 12 and 24 HAT. The check, methyl demeton at 125 g a.s./ha registered the mortality percentage of 36.67, 53.33 and 76.67 at 6, 12 and 24 HAT, respectively (Table 2).

Toxicity of imidacloprid to *B. hebetor*

All the insecticides were significantly toxic to *B. hebetor* (Table 3). The higher dose (50 g a.s./ha) of imidacloprid caused 39.29 and 96.43 per cent adult mortality at 24 and 48 HAT, respectively. Imidacloprid, at the normal dose of 25 g a.s./ha caused 30.00 and 70.00% mortality at 24 and 48 HAT. The lower dose of imidacloprid (15 g a.s./ha) and thiamethoxam (25 g a.s./ha) recorded a mortality of 13.33 and 43.33 at 24 HAT and 23.33 and 93.33 per cent at 48 HAT, respectively. Fifty per cent mortality of *B. hebetor* adults were observed at 24 HAT and hundred per cent mortality at 48 HAT in the check methyl demeton (125 g a.s./ha). There was no mortality in the untreated check.

Table 2. Selective toxicity of imidacloprid 17.8 SL on the parasitoid, *C. blackburni*. (Mean of three observations)

Treatments	6 HAT		12 HAT		24 HAT	
	per cent of mortality	corrected mortality	per cent of mortality	corrected mortality	per cent of mortality	corrected mortality
Imidacloprid 17.8 SL 15 g a.s./ha	13.33 b (21.40)	13.33	30.00 b (33.20)	27.58	36.67 b (37.27)	34.48
Imidacloprid 17.8 SL 25 g a.s./ha	23.33 d (28.88)	23.33	36.67 c (37.27)	34.48	50.00 d (45.00)	48.27
Imidacloprid 17.8 SL 50 g a.s./ha	30.00 f (33.20)	30.00	46.67 e (43.09)	44.83	63.33 f (52.73)	62.06
Imidacloprid 17.8 SL 25 g a.s./ha (Tatamida®)	20.00 c (26.56)	20.00	36.67 c (37.27)	34.48	46.67 c (43.09)	44.83
Thiamethoxam 25 WG 25 g a.s./ha	26.67 e (31.09)	26.67	40.00 d (39.23)	37.93	53.33 e (46.91)	51.72
Methyl demeton 25 EC 125 g a.s./ha	36.67 g (37.27)	36.67	53.33 f (46.91)	51.72	76.67 g (61.12)	75.86
Untreated check	0.00 a (0.19)	0.00	3.33 a (10.49)	0.00	3.33 a (10.49)	0.00

In a column, means followed by a common letter are not significantly different at p = 0.05 by DMRT; Figures in parentheses are arcsine P transformed values; a.s. – active substance

Table 3. Selective toxicity of imidacloprid 17.8 SL on the parasitoid, *B. hebetor*

Treatments	Per cent of mortality at 24 HAT	Per cent of mortality at 48 HAT
Imidacloprid 17.8 SL 15 g a.s./ha	13.33 b (21.39)	23.33 b (28.87)
Imidacloprid 17.8 SL 25 g a.s./ha	30.00 c (33.19)	70.00 c (56.81)
Imidacloprid 17.8 SL 50 g a.s./ha	39.29 d (38.81)	96.43 e (79.35)
Imidacloprid 17.8 SL 25 g a.s./ha (Tatamida®)	33.33 c (35.26)	70.00 c (56.79)
Thiamethoxam 25 WG 25 g a.s./ha	43.33 e (41.17)	93.33 d (75.10)
Methyl demeton 25 EC 125 g a.s./ha	50.00 f (45.00)	100.00 f (90.00)
Untreated check	0.00 a (0.19)	0.00 a (0.19)

In a column, means followed by a common letter are not significantly different at p = 0.05 by DMRT; Figures in parentheses are arcsine P transformed values; a.s. – active substance

DISCUSSION

Increased pesticide use in the agro-ecosystem endangers human health and was identified as a single factor causing massive ecological disruption. Thus, assessment on the safety of insecticides to natural enemies was essential. Insecticide which is used for the control of pests, should not kill natural enemies and cause complex problems like resistance and resurgence in pest populations. The insecticidal effect on non-target organisms are categorized according to the recommendations of the International Organisation for Biological Control, West Palaearctic Regional Section (IOBC/WPRS) working group (Hasan 1989; Nasreen *et al.* 2000) as harmless (< 50% mortality), slightly harmful (50 to 79% mortality), moderately harmful (80 to 89% mortality) and harmful (> 90% mortality) when tested at the field recommended dose. Imidacloprid had little impact on the egg parasitoid, *T. chilonis.* Imidacloprid, at the recommended dose (25 g a.s./ha) recorded a 90.67 per cent adult emergence and 85.32 per cent parasitization. The present finding was in tune with Suganthy (2003) who concluded that there was no significant adverse effect on *T. chilonis* by imidacloprid (confidence®). Our findings deviated from the findings of Shanmugam *et al*. (2006) who stated that imidacloprid and thiamethoxam were moderately toxic, recording intermediate parasitization.

Imidacloprid was found to be slightly toxic to *C. blackburni*. The recommended dose of imidacloprid (25 g a.s./ha) registered 50.00 per cent adult mortality 24 h after treatment, whereas the higher dose (50 g a.s./ha) recorded a 63.33 per cent mortality. Suganyakanna (2006) reported that another chloronicotinyl compound, acetamiprid caused significant adverse effects and caused high mortality in *C. blackburni* adults. Imidacloprid was found to be slightly harmful to the larval parasitoid, *B. hebetor* which recorded a 70 per cent mortality after 48 h of treatment at the recommended dose. The toxicity of thiamethoxam at the recommended dose was equal to the higher dose of imidacloprid (50 g a.s./ha), revealing the most toxic nature of thiamethoxam to *B. hebetor*. Thus, imidacloprid 17.8 SL was found to be harmless to *T. chilonis,* but slightly harmful to *C. blackburni* and *B. hebetor,* according to IOBC's classification. Care should be taken while spraying imidacloprid in the field when *C. blackburni* and *B. hebetor* are there and these parasitoid releases should not coincide with the insecticide spray. Imidacloprid, however, was found harmless to *T. chilonis*.

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POLISH SUMMARY

WPŁYW INSEKTYCYDU CHLORONIKOTYNOWEGO IMIDACLOPORIDU NA PARAZYTOIDY JAJ, JAJ I LARW ORAZ LARW W WARUNKACH LABORATORYJNYCH

W latach 2006–2007 na Uniwersytecie Rolniczym w Tamil Nadu, Coimbatore, wykonano badania laboratoryjne, w celu porównania toksyczności insektycydu chloronikotynowego imidacloprid dla parazytoidów. Testowano działanie imidaclopridu przeciwko trzem pożytecznym owadom, mianowicie parazytoidowi jaj, jaj i larw oraz larw reprezentujących dwie rodziny Hymenoptera: Trichogrammatidae (*Trichogramma chilonis* Ishii) i Braconidae (*Chelonus blackburni* Cameron; *Bacon hebctor* Say), atakujących szkodniki bawełny. Toksyczność imidaclopridu oceniono traktując jaja parazytoidów przy użyciu atomizera w przypadku *T. chilonis* i biotestem scyntylacyjnym w szkalnej probówce do określania pozostałości w przypadku dorosłych osobników *C. blackburni* i *B. hebctor*. Toksyczność ta dla parazytoidów została porównana z innym związkiem – neonicotinoid thiamethoxam oraz standardową kontrolą o nazwie: methyl demeton. Imidacloprid 18,8 SL nie spowodował niekorzystnego działania na pojaw dorosłych osobników i pasożytowanie *T. chilonis*. Przy zalecanej dawce imidaclopridu (25 g s.a./ha) stwierdzono, odpowiednio 90,67 i 85,32% pojawu osobników dorosłych oraz parazytoidów. Zalecana dawka imidaclopridu spowodowała 56% śmiertelności i miała umiarkowany wpływ na osobniki dorosłe *C. blackburni*, natomiast dla parazytoidu *B. hebctor*, była toksyczna, powodując 70% śmiertelności po 48 godzinach. Zaprezentowane w pracy dane dostarczają służbie ochrony roślin specyficznej informacji dotyczącej kompatybilności wybranych insektycydów z wrogami naturalnymi atakującymi szkodniki bawełny, *Gossypium hirsutum* L.