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Effect of novel insecticides to *Trichogramma chilonis* and *Snellenius maculipennis*, the potential parasitoids of castor semilooper

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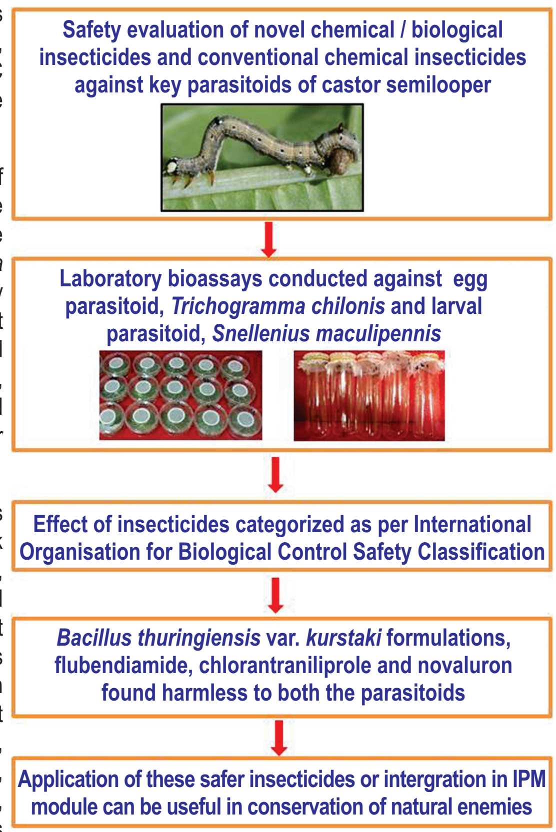
Abstract

Aim : The present investigation was undertaken to evaluate the toxicity of seven novel chemical and biological insecticides [flubendiamide 39.35 SC, chlorantraniliprole 18.5 SC, emamectin benzoate 5 SG, novaluron 10 EC, indoxacarb 15.8 EC and two *Bacillus thuringiensis* var. *kurstaki* (Btk) formulations] and five conventional chemical insecticides (dimethoate 30 EC, malathion 50 EC, monocrotophos 36 SL, profenofos 50 EC and lambda-cyhalothrin 5 EC) against the parasitoids of castor semilooper.

Methodology : The toxic effect of insecticides was evaluated by treating the parasitized eggs using an atomizer in the case of egg parasitoid, *Trichogramma chilonis,* while topical bioassay and dry film residue method was used against pupal and adult stages of larval parasitoid, *Snellenius maculipennis*, respectively. The effect was categorized as per International Organisation for Biological Control Safety Classification.

Results : The toxicity of insecticides against *T. chilonis* revealed that Btk formulations, flubendiamide, chlorantraniliprole, indoxacarb and novaluron had lesser effect on adult emergence and categorized as harmless (< 30% mortality). Topical bioassay with pupae of *S. maculipennis* revealed that Btk formulations, novaluron, flubendiamide, chlorantraniliprole, emamectin benzoate, indoxacarb, dimethoate and profenofos were rated as harmless (< 30% mortality). Dry film residue method using adults of *S. maculipennis* revealed that Btk formulations, novaluron, flubendiamide and chlorantraniliprole were rated as harmless (< 30% mortality).



Interpretation : Btk formulations, flubendiamide, chlorantraniliprole and novaluron were found harmless to both egg parasitoid, *T. chilonis* and larval parasitoid, *S. maculipennis.* These safer insecticides can be incorporated in IPM module to unify the safe and sustainable use of chemical and bio-control methods.

Introduction

Among biological constraints in the castor production, undoubtedly insect pests dominate the scenario. In India more than 100 species of insects are recorded on castor at different phenological stages of the crop. However, about half a dozen of them are of economic importance in India. Among them semilooper, *Achaea janata* L. (Lepidoptera : Noctuidae) is the most important pest in the rainfed castor belts of Southern India and is also known to occur regularly throughout the country wherever the crop is grown (Duraimurugan *et al.,* 2017).

The older larvae are voracious feeders which often totally defoliate the crop at the peak level of infestation. It is estimated that yields are reduced by 30-50% due to castor semilooper alone (Rao *et al.,* 2012). Farmers frequently utilize insecticides to overcome the damage inflicted by insect pests. However, unwise use of broad spectrum insecticides is unsafe to natural enemies. Castor semilooper is attacked by an array of parasitoids, among them *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) and *Snellenius (Microplitis) maculipennis* (Szépligeti) (Hymenoptera : Braconidae) are the most potential parasitoids in the castor ecosystem. *T. chilonis* is the most powerful egg parasitoid attacking semilooper eggs and the rate of parasitism is reported to be up to 92%, while *S. maculipennis* is a solitary larval endoparasitoid of semilooper which parasitizes up to 96% of larvae in the field under favourable conditions (Basappa, 2003; Prabhakar and Prasad, 2005). One of the key approaches in Integrated Pest Management (IPM) programme is appropriate selection of an insecticide which will reduce the pest with minimum disruptive effect on its natural enemies (Patra *et al.,* 2017).

Therefore, more understanding of pest-natural enemy-insecticide interaction is needed to formulate more effective IPM strategies (Preetha *et al.,* 2010; Pearsons and Tooker, 2017). In the present study, laboratory bioassays were carried out to determine the effect of a set of novel and conventional insecticides against *T. chilonis* and *S. maculipennis* with an objective to search for comparatively less toxic insecticide against the parasitoids to be incorporated into IPM programme.

Materials and Methods

Parasitoids : The egg parasitoid, *T. chilonis* was obtained from National Institute of Plant Health Management (NIPHM), Hyderabad and used for the study. Laboratory culture of *S. maculipennis* was established from the field collected parasitized larvae of castor semilooper from Research Farm, ICAR-Indian Institute of Oilseeds Research, Hyderabad during Kharif 2014. The parasitized larvae were collected and individually placed in a plastic container (9 cm x 4 cm) having a small circular hole on the lid covered with fine brass mess. The parasitized larvae were maintained in a container at room temperature and observed for emergence of pupa (cocoon) at the posterior end of the parasitized larvae (Fig. 1 A and B). The pupa was held until adult parasitoids emerged. A cotton swab soaked in 50% honey solution was kept in the container for *S. maculipennis* adults. The pupae (Fig. 1C) and adult (Fig. 1D) parasitoids (1-day-old) were used for the experiments.

Insecticides : Commercial formulations of five newer chemical insecticides (flubendiamide 39.35 SC, chlorantraniliprole 18.5 SC, emamectin benzoate 5 SG, indoxacarb 15.8 EC and novaluron 10 EC), two formulations of bioinsecticide, *Bacillus thuringiensis* var. *kurstaki* (Delfin and DOR Bt-1) and five conventional insecticides (profenofos 50 EC, monocrotophos 36 SL, malathion 50 EC, dimethoate 30 EC and lambda-cyhalothrin 5 EC) were used in the experiment.

Bioassay techniques : Bioassay method described by Jalali and Singh (1993) was used to evaluate the toxic effect of insecticides to *T. chilonis* eggs under laboratory conditions. The bioassay was conducted in completely randomized design with twelve insecticide treatments along with an untreated control and replicated twice. The parasitized egg cards of 3-day old were cut into one cm^2 bits and sprayed with insecticides at field recommended concentrations using an atomizer. In each treatment, about 0.5 ml of spray fluid was used for each card. For untreated check, only distilled water was sprayed. The treated egg cards were shade dried at room temperature. After drying, each card was put in a test tube (10 x 2.5 cm) and kept at ambient conditions (27±2°C, 60-70% RH). The adult parasitoids emerged from each treatment was recorded up to 9 days using a hand lens and per cent adult emergence was worked out by the following formula :

No. of wasps emerged/Total no. of eggs in 1 cm^2 x 100.

The mortality of the eggs was assessed on the basis of adult emergence.

Topical bioassay method described by Mgocheki and Addison (2009) was used to assess the effect of insecticides to pupae (cocoons) of *S. maculipennis* under laboratory conditions. Ten one-day old pupae were selected and placed on castor leaf which was placed on plastic container (9 cm x 4 cm) as described earlier. The recommended field concentration of respective insecticide was sprayed on the pupae using an atomizer and allowed to dry for an hour. The container kept at ambient conditions (27±2°C, 60-70% RH) and observed for emergence of the adults. Thirty pupae for each treatment (three replicates each of 10 pupae) were used in completely randomized design. The mortality of the pupae was assessed on the basis of the failure of adult emergence.

Dry film residue method described by Desneux *et al.* (2006) was used to evaluate the toxic effect of insecticides to adults of *S. maculipennis* under laboratory conditions. Recommended concentration of insecticides was prepared using water. Glass vials of 40 ml capacity were uniformly coated with 0.5 ml of insecticide solution and dried by manual rotating of the vial horizontally on a table. Adults of *S. maculipennis* were released into the vials @ 10 per vial and covered with muslin cloth. After one-hour of exposure, they were released in test tubes (10 x 2.5

cm) and 50% honey solution was given as feed and observations on the adult mortality were recorded at 24 and 48 hrs after treatment (HAT). For untreated check, water alone was used. Mortality per cent of adults was worked out by the following formula :

Mortality (%) = No. of adults dead/Total number of adults x 100.

The insecticidal effect on life stages of *T. chilonis* and *S. maculipennis* was classified as per International Organisation for Biological Control, West Palaearctic Regional Section (IOBC/WPRS) Working Group (Nasreen *et al.,* 2000) as harmless (<30% mortality), slightly harmful (30–79% mortality), moderately harmful (80–99% mortality) and harmful (>99% mortality).

Statistical analysis : The per cent mortality values were converted to arcsine percentage and subjected to analysis of variance (ANOVA) followed by means separation using least significant difference (LSD) test at 5% level of significance.

Results and Discussion

The adult emergence of *T. chilonis* ranged from 31.9 to 90.1% among the insecticidal treatments. *Bacillus thuringiensis* var. *kurstaki* (Btk) formulations *viz.,* Delfin and DOR Bt-1 recorded 90.1 and 88.5% adult emergence and found at par with each other, while 98.2% adult emergence was recorded in untreated control. The novel insecticides *viz.,* flubendiamide, chlorantraniliprole, indoxacarb, novaluron and emamectin benzoate recorded 67.6 to 78.8% adult emergence. The conventional insecticides *viz.,* malathion, dimethoate, lambda-cyhalothrin, profenofos and monocrotophos resulted 31.9 to 60.7 % adult emergence (Table 1). Based on the criteria suggested by IOBC to assess the toxicity of insecticides to natural enemies, Btk formulations, flubendiamide, chlorantraniliprole, indoxacarb and novaluron had lesser effect on *T. chilonis* and were categorized as harmless (<30% mortality), while emamectin benzoate, dimethoate, profenofos, malathion, monocrotophos and lambda-cyhalothrin were rated as slightly harmful (30-79% mortality).

The results revealed that there were significant differences between the insecticidal treatments with respect to percentage pupal mortality of *S. maculipennis.* The per cent mortality of pupa ranged between 0.0 to 53.3% among the insecticidal treatments. Among them, both the formulations of Btk (Delfin and DOR Bt-1) and untreated control caused no mortality. Novaluron, flubendiamide and chlorantraniliprole recorded pupal mortality of 6.7 to 13.3% and were found at par with each other. It was followed by emamectin benzoate, indoxacarb, and dimethoate recorded 16.7 to 20.0% pupal mortality. Profenofos, monocrotophos and malathion recorded pupal mortality of 26.7, 33.3 and 36.7%, respectively. Lambda-cyhalothrin recorded highest mortality of 53.3% (Table 1). Based on the criteria suggested by IOBC to evaluate the toxicity of insecticides to natural enemies, Btk formulations, novaluron, flubendiamide, chlorantraniliprole, emamectin benzoate, indoxacarb, dimethoate and profenofos were rated as harmless (<30%

Table 1 : Effect of novel and conventional insecticides to egg parasitoid, *Trichogramma chilonis* and larval parasitoid, *Snellenius maculipennis*

Treatments	Concentration	Chemical class	*T. chilonis*	*S. maculipennis*		
			Adult emergence (%)	Pupal mortality (%)	Adult mortality (%)	
					24 HAT	48 HAT
T_1 - Flubendiamide 39.35SC	0.2 ml l^{-1}	Phthalic acid diamide	78.8 (62.6)^c	6.7 (12.4)^b	13.3 (21.2)^b	23.3 (28.8)^b
T_2 - Chlorantraniliprole 18.5SC	0.3 ml l^{-1}	Anthranilic diamide	73.5 (59.0)^{cd}	13.3 (21.2)^{bc}	20.0 (26.1)^{bc}	26.7 (30.8)^b
T_3 - Emamectin benzoate 5SG	0.5 g l^{-1}	Avermectin	67.6 (55.3)^{de}	16.7 (23.9)^{cd}	26.7 (31.0)^{cd}	43.3 (41.2)^c
T_4 - Novaluron 10EC	1.0 ml l^{-1}	Insect Growth Regulator (Benzoylphenyl urea)	70.9 (57.4)^d	6.7 (12.4)^b	0.0 (0.5)^a	13.3 (21.2)^b
T_5 - Indoxacarb 15.8EC	1.0 ml l^{-1}	Oxadiazine	73.5 (59.0)^{cd}	16.7 (23.9)^{cd}	40.0 (39.2)^d	66.7 (54.8)^d
T_6 - *Bacillus thuringiensis* var. *kurstaki* (Delfin)	1.0 g l^{-1}	Biological insecticide	90.1 (71.7)^b	0.0 (0.5)^a	0.0 (0.5)^a	0.0 (0.5)^a
T_7 - *Bacillus thuringiensis* var. *kurstaki* (DOR Bt-1)	1.0 g l^{-1}	Biological insecticide	88.5 (70.3)^b	0.0 (0.5)^a	0.0 (0.5)^a	0.0 (0.5)^a
T_8 - Dimethoate 30EC	1.7 ml l^{-1}	Organophosphate	60.7 (51.2)^{ef}	20.0 (26.1)^{cde}	56.7 (48.8)^e	90.0 (78.6)^e
T_9 - Malathion 50EC	1.0 ml l^{-1}	Organophosphate	33.6 (35.4)^h	36.7 (37.2)^f	96.7 (83.6)^f	100.0 (89.5)^f
T_{10} - Monocrotophos 36SL	1.4 ml l^{-1}	Organophosphate	56.4 (48.7)^{fg}	33.3 (35.2)^{ef}	100.0 (89.5)^f	100.0 (89.5)^f
T_{11} - Profenofos 50EC	1.0 ml l^{-1}	Organophosphate	52.2 (46.2)^g	26.7 (31.0)^{def}	96.7 (83.6)^f	100.0 (89.5)^f
T_{12} - Lambda-cyhalothrin 5EC	1.0 ml l^{-1}	Synthetic pyrethroid	31.9 (34.4)^h	53.3 (46.9)^g	100.0 (89.5)^f	100.0 (89.5)^f
T_{13} - Untreated check	-	-	98.2 (82.4)^a	0.0 (0.5)^a	0.0 (0.5)^a	0.0 (0.5)^a
CD (p=0.05)	-	-	4.30	9.53	8.74	10.10

Figures in parentheses are arcsine transformed values; In a column means followed by a common letter are not significantly different at p = 0.05 by LSD; HAT - hours after treatment mortality), while monocrotophos, malathion and lambda-cyhalothrin were rated as slightly harmful (30-79% mortality) to pupa of *S. maculipennis.*

The adult mortality of *S. maculipennis* varied from 0.0 to 100% among the treatments at 48 HAT (Table 2). No adult mortality occurred in Btk formulations (Delfin and DOR Bt-1) and untreated control. It was followed by lower per cent adult mortality of *S. maculipennis* was observed with novaluron (0.0 and 13.3% mortality at 24 and 48 HAT, respectively), flubendiamide (13.3 and 23.3% at 24 and 48 HAT, respectively) and chlorantraniliprole (20.0 and 26.7% at 24 and 48 HAT, respectively). Emamectin benzoate, indoxacarb and dimethoate recorded a mortality of 43.3, 66.7 and 90.0% at 48 HAT, respectively. Malathion, profenofos, monocrotophos and lambda-cyhalothrin caused 100% adult mortality at 48 HAT (Table 2.). Grading of toxicity of insecticides to natural enemies recommended by IOBC revealed that Btk formulations, novaluron, flubendiamide and chlorantraniliprole were rated as harmless (<30% mortality). Emamectin benzoate and indoxacarb were rated as slightly harmful (30-79% mortality) while dimethoate was classified as moderately harmful with 80-99% mortality. Profenofos, monocrotophos, malathion and lambda-cyhalothrin, which caused 100% mortality to adults at 48 HAT, were rated as harmful.

Natural enemies are important in suppressing insect pest populations. Conversely, the use of insecticides because of its magnificent and quick results in suppressing the pests in almost all situations has temporarily ignored the long lasting benefit of bio-control agents and enhanced the sole reliance on chemical control. This has resulted in deleterious effects such as insecticide resistance, pest resurgence and secondary pest outbreaks due to elimination of natural enemies (Roy *et al.,* 2017). IPM programmes would be more successful if the insecticides were effective only against the insect pests and relatively safer to natural enemies. A step-wise assessment, moving from laboratory to field is recommended in the screening of insecticides against bio-control agents (Croft, 1990). In the present investigation, toxicity of newer, biological and conventional insecticides was studied against egg parasitoid, *Trichogramma chilonis* and larval parasitoid, *Snellenius maculipennis,* and it was found that Btk formulations, novaluron, flubendiamide and chlorantraniliprole were harmless to the principal parasitoids of castor semilooper. Srinivasan and Babu (2000) evaluated the toxicity of various *B. thuringiensis* products (Delfin, Biobit, Spicthurin and Halt) against three species of egg parasitoids *viz., T. chilonis, T. japonicum* and *T. brasiliensis* and found that all the Bt preparations were safe for the growth and development of parasitoids. Firake *et al.* (2017) reported that *B. thuringiensis* var. *kurstaki* were found harmless to three important

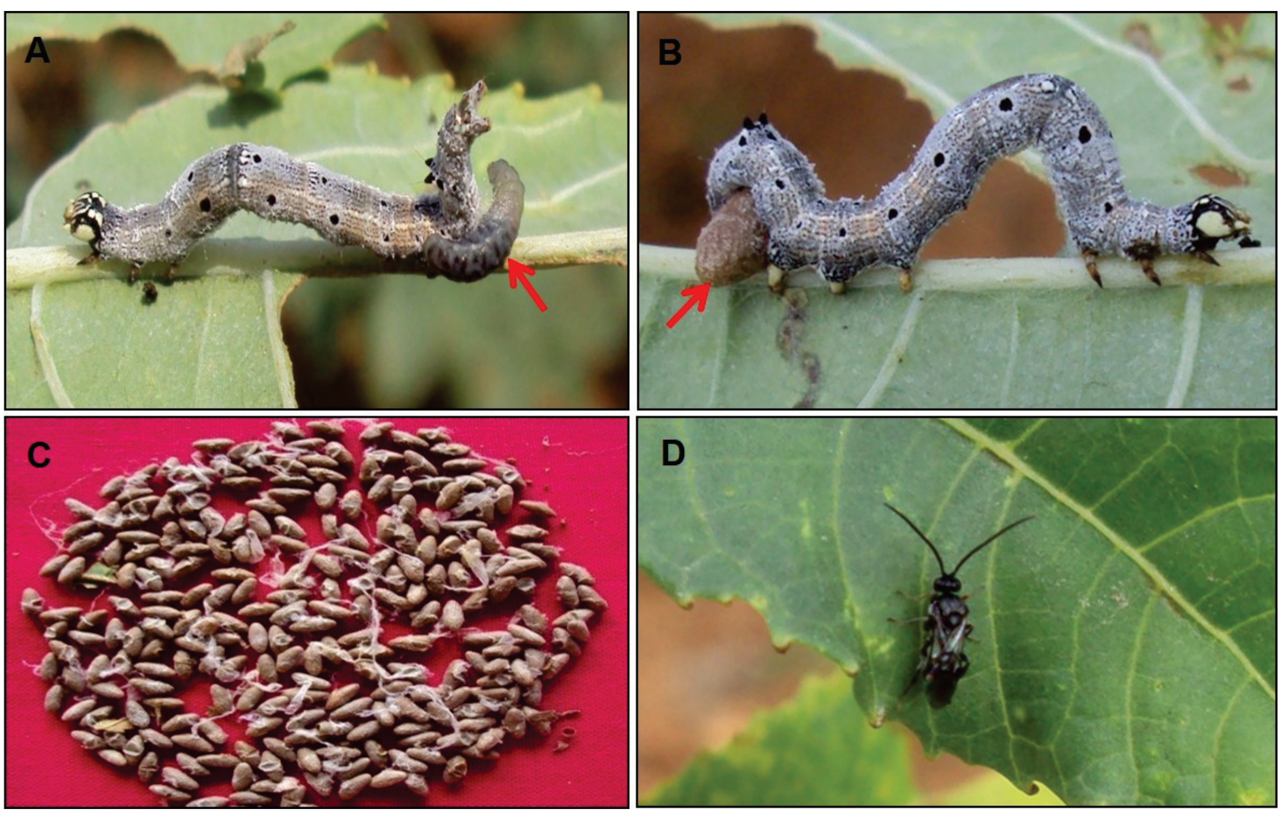


Fig. 1 : Life stages of solitary larval endoparasitoid, *Snellenius maculipennis* of castor semilooper : (A) Parasitoid larva emerge from the host; (B) Formation of pupa (cocoon) just after the parasitoid larva emerged from the host; (C) Parasitoid pupae (cocoons) and (D) Adult parasitoid

parasitoids of cabbage butterflies *viz., Hyposoter ebeninus, Cotesia glomerata* and *Pteromalus puparum*. The results are in accordance with the present findings. Gashawbeza (2011) found that novaluron was safer to parasitoid of diamondback moth, *Diadegma* sp. as compared to lambda-cyhalothrin and profenofos which is in line with the present findings. Our findings are also consistent to those of Rodrigo *et al.* (2017) who reported that flubendiamide and chlorantraniliprole were harmless to the parasitoids, *T. chilonis* and *Copidosoma truncatellum.*

Among insecticide classes, increasing toxicity of organophosphates and synthetic pyrethroids to natural enemies have been reported in the literature (Theiling and Croft, 1988; Easwaramoorthy *et al.,* 1994; Rao *et al.,* 2002; Bueno *et al.,* 2008; Uma *et al.,* 2014). In general, the organophosphate and synthetic pyrethroid insecticides are the cheapest and widely available conventional insecticides to the castor growers, thus they are frequently over used. Nevertheless, previous studies as well as our results showed that these conventional insecticides were not safe for natural enemies (Wang *et al.,* 2008; Carmo *et al.,* 2010; Uma *et al.,* 2014). The use of chemical or biological insecticides with high selectivity characters and reducing the frequency of application of hazardous insecticides are simple methods for conserving natural enemies. Hence, the data presented here will be useful in developing IPM module to unify the sustainable and safe use of chemical and bio-control methods.

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