

Indirect Toxicity of Treated Prey with Two Chitin Synthesis Inhibitors, on Pre-imaginal Stages of *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) under Laboratory and Field Conditions

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ABSTRACT

A study on the indirect toxicity of two chitin synthesis inhibitors namely, Cascade and Match, against larval and pupal stages of the predatory species *Coccinella undecimpunctata* L., reared on treated 2nd instar larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) under laboratory and field conditions was carried out. The experiments were conducted using a contact method, under the laboratory conditions of 25°C±1, 65% R.H. and 16L: 8D photoperiods. Both compounds were tested using four serial concentrations *i. e.*, 1.5, 3.1, 6.3 and 12.5 ppm. Results indicated that Cascade and Match treated preys increased larval duration of the predator compared to the control, Cascade did not affect the larval duration as much as Match did at any of the four tested concentrations. Both compounds decreased larval feeding capacity of the predator in comparison to the control at all larval instars. In that respect, Cascade reduced significantly the feeding capacity more than Match did at all larval instars. Effect of Cascade and Match on pupal duration was significant at the lower concentrations and had no effect at higher ones compared to the control. *C. undecimpunctata* pupal weight increased gradually with increasing the concentration of Match and was significantly differing from the control. It could be concluded that both tested chitin synthesis inhibitors can be used in Integrated Pest Management in cotton growing areas with acceptable safety to *C.undecimpunctata*.

Key words: *Coccinella undecimpunctata*, *Spodoptera littoralis*, Chitin Synthesis Inhibitors, Cascade, Match, Toxicity.

INTRODUCTION

The ladybird, *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) is a polyphagous predator that feeds, especially on aphid insects with interesting potential as a control agent in Integrated Pest Management (IPM) (Moura *et al.*, 2006 and Cabral *et al.*, 2008). Cascade and Match are benzoylphenylureas insecticides that belong to the group of insect cuticular chitin synthesis inhibitors and act on the incorporation of N-acetyl glucosamine monomer into chitin in the integument, resulting in the formation of abnormal new cuticle and insect mortality (Oberlander and Silhacek, 1998). The two insecticides were selected on the basis of their potential usage in the cotton leaf worms; *Spodoptera littoralis* (Boisd.) and *Helicoverpa armigera* (Hbn.) (Lepidoptera: Noctuidae), control program on cotton plants in Egypt.

Side effect assessment of pesticides on natural enemies is an important contribution of a successful IPM program. The hallmarks of various chitin synthesis inhibitors are abortive molting and defects of egg hatching, which seems to be a direct consequence of a disruption of the chitin deposition that derives in the abnormal cuticle formation (Merzendorfer, 2013). These compounds have been tested successfully against several species of insect pests (Pineda *et al.*, 2007; Gelbic *et al.*, 2011 and Khajepour *et al.*, 2012).

In the field, beneficial arthropods can be exposed to insecticides in several ways by direct contact with spray droplets through uptake of residues when contacting with contaminated plant surfaces and by ingestion of insecticide contaminated preys, nectar or honeydew (Longley and Stark, 1996 and Youn *et al.*, 2003). They are frequently exposed to various pesticides because of their close association with the host. Their side effects were studied in the laboratory to maximize compatibility of chemical and biological control methods (Preetha *et al.*, 2009). Integration of biological control agents with pesticides in IPM programs would be most effective if the pesticides used are efficacious against the pest species and relatively safe for beneficial arthropods such as parasitoids and predators (Sayed *et al.*, 2014). Moreover, release of the natural enemies along with selected insecticide, which has less effect on them, is effective in suppressing the population density of the pest (Carvalho *et al.*, 2002; Chen and Liu 2002 and El Arnaouty *et al.*, 2010). Selection of pesticides suitable for use in integrated control programs is urgently needed (Sterk *et al.*, 1999). Several studies have shown the possibility of integrating insecticides into IPM due to their selective properties (Feldhege and Schmutterer 1993). Side effects on beneficial organisms are increasingly important in the development of new pesticides and the re-registration of old active ingredients (Sterk *et al.*, 2001).

The present study aimed to assess the indirect

toxicity of two chitin synthesis inhibitors *i.e.*; cascade and match against *C. undecimpunctata* through feeding on treated 2nd instar larvae of *S. littoralis*.

MATERIALS AND METHODS

Insecticides used

Common name: **Flufenoxuron**

Trade name: Cascade 10% EC

Chemical name: 1-[4-(2-chloro- α,α,α -trifluoro-*p*-tolxyloxy)-2-fluorophenyl]-3-(2,6-difluorobenzoyl) urea

Common name: **Lufenuron**

Trade name: Match 5% EC

Chemical name: N-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylaminocarbonyl]2,6-difluoro-benzamide.

The two tested compounds were obtained from Sumitomo Chemical Co. Ltd.

Insects

Egg masses of *S. littoralis* were obtained from the Dept. of the Cotton Leaf Worm, Plant Protection Research Institute, Giza, Egypt. *S. littoralis* was reared in the laboratory on fresh leaves of castor bean, *Ricinus communis* (Family: *Euphorbiaceae*). Culture of *C. undecimpunctata* was obtained from Kahaa Research Station, Egypt and reared on the cowpea aphid species, *Aphis craccivora* Koch., according to the method described by (Tabozada and El Arnaouty, 2015) as natural food source, under the laboratory conditions of 25°C $\pm$ 1, 65% R.H.

Experimental design

Four concentrations (12.5, 6.3, 3.1 and 1.5 ppm) of the two compounds; Cascade and Match with a control (distilled water) were tested as indirect toxicity against *C. undecimpunctata* fed on treated 2nd instar larvae of *S. littoralis*. Feeding capacity of the predator's larvae was measured by the sublethal concentration (1.5 ppm) of the two compounds. Indirect toxicity was estimated using contact method of the 2nd instar larvae of *S. littoralis* according to Tabozada *et al.* (2014) for 5 minutes and then the larvae were transferred to the Petri dishes containing the predator larva. Each predator larva was placed separately in a Petri dish with sufficient amount of the treated 2nd instar larvae of *S. littoralis* and replicated thirty times. Larval duration, predation capacity, pupal duration and adult emergence rate were recorded daily.

Another experiment was carried out under field conditions at Qaha station, Qalubia governorate, Egypt to estimate toxicity of the two tested compounds. The cotton area was divided into three plots each (2 x 175 m). Cotton seeds, Giza 86 variety, were planted in two seasons (2013 & 2014), plot 1

was treated by Cascade, plot 2 was treated by Match and plot 3 was left untreated as control. At seedlings stage, leaves were counted at two levels, while at full growing stage, they were at three levels. Sampling was done in early morning every other day. Upper and lower surfaces of the leaves were covered completely by spraying the Cascade and Match. Each plot consisted of 25 plants. Each pair and impair numbers of plant was detached by plastic label around the stem and all present stages of the predator were counted.

Statistical analysis

Means and standard deviations were calculated and the data were compared using one way ANOVA. Significance among means was compared by LSD values at 0.05 level. All analyses were performed using statistical Software (SPSS, 2002).

RESULTS AND DISCUSSION

Data presented in table (1) showed that both insecticides increased relatively larval duration of the predator compared to the control. Cascade reduced significantly this period than match at all tested concentrations. Moreover, larval duration was increased gradually with increasing the concentration of the two tested compounds, reached 21.11 $\pm$ 0.12 and 20.12 $\pm$ 0.11 days at 12.5 ppm of match and cascade, respectively. Tabozada *et al.* (2015) reported that no significant difference was found between Cascade and Match on both larval and pupal durations of the larval parasitoid, *Bracon brevicornis* but Cascade had higher toxic effect than Match against the adult.

Both Match and Cascade decreased larval feeding or predation capacity of *C. undecimpunctata* than that of control at all larval instars, reaching 53.0 $\pm$ 0.35, 58.6 $\pm$ 0.44, 77.5 $\pm$ 0.60 and 87.6 $\pm$ 0.81 individuals of *S. littoralis* 2nd instar larvae for 1st, 2nd, 3rd and 4th instars, respectively, while Cascade significantly reduced these parameters than Match at all the four instars (Table 2). The same finding was recorded by Lanzoni *et al.* (2012) who indicated that exposure of the larval stage of the coccinellid, *Adalia bipunctata* L. to Match significantly reduced all the demographic parameters in comparison with control.

Data presented in table (3) indicated that the effect of Match and Cascade on pupal period did not significantly differ at the lower concentrations of 1.5 and 3.1 ppm, and achieved lower pupal period than the control (5.30 $\pm$ 0.06 days). At higher concentrations of 6.3 and 12.5 ppm, of both compounds, pupal period did not differ than the control, except Cascade at 6.3 ppm (4.99 $\pm$ 0.09 days). Also, at these concentrations, Match achieved longer pupal durations than Cascade. Same results were obtained by Rahmani *et al.* (2013) who indicated that

Table (1): Effect of Match and Cascade treated prey (*S. littoralis*) on larval period of the predator *C. undecimpunctata*

Concentration (ppm)	Larval duration (Mean±SE)			F value	Sig.
	Match	Cascade	Control		
1.5	19.30±0.10 ^{C_a}	19.21±0.08 ^{C_b}	18.55±0.10 ^c	27.07	0.00
3.1	20.16±0.18 ^{BC_a}	19.37±0.06 ^{BC_b}	18.55±0.10 ^c	30.31	0.00
6.3	20.26±0.22 ^{AB_a}	19.48±0.11 ^{B_b}	18.55±0.10 ^c	31.22	0.00
12.5	21.11±0.12 ^{A_a}	20.12±0.11 ^{A_b}	18.55±0.10 ^c	1810.33	0.00
Control	18.55±0.10 ^D	18.55±0.10 ^D	18.55±0.10		
F value	18.532	25.402			
Sig.	0.00	0.00			

-Values followed by the different uppercase and capital letter are significantly different from each other at the same column- the different lowercase and small letter are significantly different from each other at the same row according to Tukey's test.

Table (2): Effect of Match and Cascade on larval predation capacity of the predator *C. undecimpunctata* on treated 2nd instar larvae of *S. littoralis*

Larval instar	Predation capacity (Mean±SD)			F value	Sig.
	Match	Cascade	Control		
1 st	40.2±0.94 ^C	32.6±0.83 ^B	53.0±0.35 ^A	162.63	0.00
2 nd	48.5±0.91 ^C	38.5±0.81 ^B	58.6±0.44 ^A	165.54	0.00
3 rd	65.8±1.75 ^C	50.6±1.30 ^B	77.5±0.60 ^A	102.50	0.00
4 th	75.5±0.96 ^C	65.8±0.74 ^B	87.6±0.81 ^A	101.72	0.00

Table (3): Effect of Match and Cascade treated prey (*S. littoralis*) on pupal period of the predator *C. undecimpunctata*

Concentration (ppm)	Pupal period (Mean±SD)			F value	Sig.
	Match	Cascade	Control		
1.5	4.80±0.06 ^{C_a}	4.56±0.10 ^{D_a}	5.30±0.06 ^b	24.086	0.00
3.1	4.94±0.06 ^{C_a}	4.85±0.08 ^{CD_a}	5.30±0.06 ^b	16.540	0.00
6.3	5.54±0.08 ^{B_a}	4.99±0.09 ^{BC_b}	5.30±0.06 ^a	4.402	0.021
12.5	5.72±0.07 ^{A_a}	5.14±0.11 ^{AB_b}	5.30±0.06 ^a	5.633	0.008
Control	5.30±0.06 ^A	5.30±0.06 ^A	5.30±0.06		
F value	19.885	10.702			
Sig.	0.00	0.001			

Table (4): Effect of Match and Cascade treated prey (*S. littoralis*) on pupal weight of the predator *C. undecimpunctata*

Concentration (ppm)	Pupal weight (mg) (Mean±SD)			F value	Sig.
	Match	Cascade	Control		
1.5	12.12±0.081 ^{D_a}	12.18±0.078 ^{B_a}	12.72±0.050 ^b	12.326	0.00
3.1	12.23±0.046 ^{CD_a}	12.31±0.061 ^{B_a}	12.72±0.050 ^b	12.212	0.00
6.3	12.31±0.043 ^{BC_a}	12.30±0.062 ^{B_a}	12.72±0.050 ^b	7.658	0.002
12.5	12.45±0.051 ^{AB_{ab}}	12.38±0.052 ^{B_a}	12.72±0.050 ^b	5.375	0.011
Control	12.72±0.050 ^A	12.72±0.050 ^A	12.72±0.050		
F value	10.533	5.623			
Sig.	0.00	0.001			

Table (5): Indirect toxicity on *Coccinella undecimpunctata* different stages after spraying the cotton plants with cascade and match, under field conditions in the two seasons 2013 & 2014

Mean of four replicates after treatment	1 st season, 2013								2 nd season, 2014							
	egg		larva		pupa		adult		egg		larva		pupa		adult	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
2 days	0	1	3	3	4	4	4	5	0	2	0	2	1	1	3	5
4 days	2	2	3	4	2	4	4	5	0	3	2	2	1	3	2	4
6 days	0	2	4	5	2	5	3	6	2	3	2	3	3	3	2	5
8 days	2	3	2	4	4	4	5	5	2	2	4	3	3	3	5	6
Mean±SE	1±0.2	2±0.1	3±0.1	4±0.1	3±0.1	4.2±0.1	4±0.1	5.2±0.1	1±0.2	2.5±0.1	2±0.1	2.5±0.1	2±0.1	2.5±0.1	3±0.1	5±0.1
Control	4±0.11		5±0.09		5±0.09		7±0.08		3±0.12		6±0.09		5±0.09		8±0.08	

(a: cascade b: match)

Match decreased pre-adult development time significantly in *Hippodamia variegata* L. but it had no effect on adult's longevity. In Match treatment, pupal weight was increased gradually by increasing the concentration but it was significantly different than the control (12.72 ± 0.050 mg/pupa). All concentrations of Cascade did not significantly differ in their effects on the pupal weight but it was significantly different than the control (Table 4). Some studies have been carried out to assess the susceptibility of *C. undecimpunctata* to different insecticides but all in some way showed adversely affected this species (Omar *et al.*, 2002). Chitin synthesis inhibitors interfere with formation of chitin and control immature stages of many insect pests with relatively low harm to beneficial arthropods (Liu and Stansly 2004).

After spraying cotton plants with both chitin synthesis inhibitors under field conditions, different stages of the predator were recorded during the two seasons, with the mean numbers of 1 ± 0.2 , 3 ± 0.1 , 3 ± 0.1 and 4 ± 0.1 in the 1st season and 1 ± 0.2 , 2 ± 0.1 , 2 ± 0.1 and 3 ± 0.1 in the 2nd season for egg, larva, pupa and adult stages, respectively with Cascade. Respective mean numbers were 2 ± 0.1 , 4 ± 0.1 , 4.2 ± 0.1 and 5.2 ± 0.1 in the 1st season and 2.5 ± 0.1 , 2.5 ± 0.1 and 5 ± 0.1 in the 2nd season with Match compared to the control at plot 3 (Table 5). Individuals of 2nd, 3rd and 4th instars larvae and pupae of *C. undecimpunctata* were found attached to the upper surface of leaves. Numbers of predator different stages were observed on the upper and lower surfaces of the cotton leaves after treatment, while no egg-masses or newly hatched larvae of *S. littoralis* were recorded (Fig.1.). Ahmed *et al.* (2011) found that in the field, sprayed leaves with imidacloprid proved to be least toxic and in residual film method, Acetamiprid was also the least toxic but it was most toxic in glass vial method against *C. undecimpunctata*.

The present study showed that both chitin synthesis inhibitors are suitable candidates in IPM programs in cotton fields due to their comparatively low toxicity and high safety to the coccinellid predator, *C. undecimpunctata*.

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