

Side effects of sublethal concentration of two Neonicotinoids; Thiamethoxam and Thiacloprid on the larval parasitoid, *Bracon brevicornis* (Hymenoptera: Braconidae).

Abstract

Laboratory bioassays were carried out to assess the responses of the larval parasitoid *Bracon brevicornis*, exposed as larvae, to two neonicotinoids, including thiacloprid and thiamethoxam at sublethal dose. The immature and adults stages of the larval parasitoid *B. brevicornis* were investigated on the treated 2nd larvae instars of *Spodoptera littoralis* at periods zero time, 1st, 3rd and 5th days of parasitism by these two neonicotinoids with contact method. no significant difference between the two tested compounds on larval and pupal durations. Thiacloprid have high toxic effect than Thiamethoxam on the larval parasitoid, *B. brevicornis*. Thiacloprid achieved low number of emerged adults /parasitoid's female, low emerged females, shorter male and female longevity. We can concluded that using of Thiamethoxam for controlling sucking insect pests is more safer for the larval parasitoid, *B. brevicornis* than Thiacloprid. We also suggest that Thiamethoxam share programs of integrated pest management.

Key words: *Bracon brevicornis*, Neonicotinoids, Thiamethoxam, Thiacloprid.

1.Introduction

The larval parasitoid, *Bracon brevicornis* (Hymenoptera: Braconidae) appear to be good candidates for using in IPM programs. The integrated use of natural enemies particularly *B. brevicornis* for management the cotton pests with different selective pesticides appear possible to conserve the parasitoids (1, 2, 3, 4, 5).

Efforts have been made to develop nicotinyl insecticides with high affinity to the insect nicotinic acetylcholine receptor (nAChR), resulting in the development of a new group of insecticides (6, 7, 8). Neonicotinoids interact with nicotinic acetylcholine receptors at the central and peripheral nervous system, resulting in excitation and paralysis, followed by death. The neonicotinoids act specifically on sucking pests and have a mild or no effect on parasitoids and predators, and as such, they fit well in various IPM programs. Hence nAChR proved to be an important site for the development of a new group of insecticides able to control homopteran pests and others in various agricultural systems (9).

The neonicotinoids are effective against whiteflies and aphids in cotton fields (10). The use of neonicotinoid insecticides (also termed chloronicotinyl insecticides) against sucking pests is now increasing rapidly. The first commercial compound was imidacloprid. Others being introduced are acetamiprid, nitenpyram, thiamethoxam, and thiacloprid. (11). Neonicotinoids acts on the nerve cells to suppress muscle contraction thus inhibiting the larvae from feeding that can be used for the control program especially in vegetables by an acute contact and stomach effect. It binds to the postsynaptic nicotinerbic receptor, which such compound is applied during the sensitive period of insect development to conserve and increase the role of biological control. Side effects of insecticides were studied in the laboratory to maximize compatibility of chemical and biological control methods (12).

Beneficial insect species play an indispensable role in controlling various crop(s) pests worldwide (13). The integration of biological control agents with pesticides for integrated pest management (IPM) would be most effective if the pesticides used were efficacious against the pest species and relatively safe for beneficial arthropods such as parasitoids and predators (14). In crop protection,

beneficial insect species are frequently exposed to various pesticides because of their close association with the host (pests) (13).

Identification of selective pesticides suitable for use in integrated control programs is urgently needed (15). Several studies have shown the possibility of integrating insecticides into IPM due to their selective properties (16, 17). Testing side effects on beneficial organisms is increasingly important in the development of new pesticides and the re-registration of old active ingredients and to find compounds suitable for use in IPM programs (18). Studying only the lethal effects of pesticides may not completely achieve the targets, because sublethal effects of pesticides on these organisms may not be sustainable for crop protection if not investigated and evaluated properly (19).

The present study was conducted to assess the effect of two Neonicotinoids *i.e.*; Thiamethoxam and Thiacloprid on the parasitoid; *B. brevicornis* at sublethal dose by treating of 2nd larval instar of *Spodoptera littoralis* from different periods of parasitism. The two tested insecticides were selected on the basis of their potential usage for sucking pests control program on the cotton plants.

2. Material and methods

2.1. Tested pesticides:

The two tested compounds are Neonicotinoids; Thiamethoxam (Actara 25%) obtained from Syngenta Chemical Co. Ltd. and Thiacloprid (Calypso 24%) obtained from Maytrade Chemical Co. Ltd.

2.2. Tested insects:

2.2.1. *Spodoptera littoralis*

Egg masses of the cotton leafworm, *S. littoralis* were obtained from the Division of the Cotton Leafworm, Plant Protection Research Institute, Giza, Egypt. Culture was reared in the laboratory on fresh leaves of castor bean plant of 25°C and 65% R.H.

2.2.2. *Bracon brevicornis*

Larval parasitoid *B. brevicornis*, that culture was reared under laboratory conditions at 20°C±1, 65% R.H. on the 2nd larvae instars of *S. littoralis*. Culture of the larval parasitoid was obtained from infested bollworm of the cotton plants at Kaha Research Station.

2.3. Experimental design:

Direct and indirect toxicity of these two compounds with concentration of 1.5 ppm was measured on the parasitoid; *B. brevicornis* under laboratory conditions of 20°C±1 and 65% R.H. Larval and pupal durations, parasitism rate, adult emergence and sex ratio were observed daily. The larval parasitoid, *B. brevicornis* culture was reared under the same laboratory conditions on the 2nd larval instars of *S. littoralis*. Direct toxicity to the parasitoid adults was measured by spray method on the inner surface of the glass tubes while indirect toxicity was estimated by contact method to the 2nd larval instars of *S. littoralis* at zero time, 1st, 3rd and 5th days of parasitism. Contact method on the 2nd larval instars of *S. littoralis* was applied by spraying of two tested compounds solution using micropipette in petri dish in order to simulate of insecticide spray on plant leaves. Then, the larvae were deposited in these petri dishes for 5 minutes and then transferred to the test tubes. In each treatment, ten replicates of the parasitoid pairs were exposed to ten hosts, then number of emerged adults/parasitoid's female and sex ratio were calculated.

2.4. Statistical analysis :

Means and standard deviations were calculated for each experiment and the data were compared using the one way ANOVA test and the significance among means were compared by LSD values at 0.05 level. The two means at the same character was compared by T- values at 0.01 and 0.05 levels. All analyses were conducted using statistical Software (20).

3. Results

3.1. Direct toxicity on *B. brevicornis*:

Under laboratory conditions of $20^{\circ}\text{C}\pm 1$, 65% R.H., the mortality (%) of *B. brevicornis* adults treated with Thiamethoxam and Thiacloprid by spray method with concentration of 1.5 ppm for both compounds were 1.3 ± 0.3 and 1.6 ± 0.2 , respectively.

3.2. Indirect toxicity on *B. brevicornis*:

Side effects of Thiamethoxam and Thiacloprid on the larval parasitoid, *B. brevicornis* immature stages at all periods of parasitism were not differed significantly with the control. Also, no significant difference between the two tested compounds on larval and pupal durations. The larval duration of *B. brevicornis* with Thiamethoxam at zero time, 1st, 3rd, 5th of parasitism were 5.05, 5.2, 5.15 and 5.2 days, respectively. But with Thiacloprid, these periods were 5.05, 5.15, 5.1 and 5.1 days, respectively. The larval period with control was 5.2 days. The pupal duration of *B. brevicornis* with Thiamethoxam at zero time, 1st, 3rd, 5th of parasitism were 5.3, 5.25, 5.3 and 5.35 days, respectively, while with Thiacloprid, were 5.3, 5.2, 5.25 and 5.3 days, respectively. The pupal period with control was 5.35 days (Table 1).

Sex ratio (proportion of females/ proportion of males + females) of adult parasitoid significantly affected by the two compounds at earlier periods of parasitism (zero time and one day of parasitism for both compounds and 3rd day of parasitism with Thiacloprid only) . This ratio in control was 0.57 while at zero time, 1st, 3rd and 5th days after parasitism by treated host larvae with Thiamethoxam were 0.54, 0.55, 0.56 and 0.57 While these ratios with Thiacloprid were recorded as 0.52, 0.53, 0.54 and 0.55, respectively (Table 2). Also, there are significant differences between the two tested compounds on sex ratio whereas there is reduction in female adult emergence resulted from treated larvae by Thiamethoxam with the exception of treated larvae 5th day after parasitism.

The total number of the parasitoid individuals that treated with the two compounds and completed their development were significantly differed with the untreated individuals (22.5 individuals) specially with treatment of earlier periods of parasitism (Table 2). Treatment with Thiacloprid at zero time, one day and five day of parasitism were highly reduced this number than the treatment with Thiamethoxam.

As presented in Table (3), treated host's larvae by Thiamethoxam and Thiacloprid at zero time, 1st, 3rd and 5th day of parasitism indicated that male longevities of the parasitoid were 14.0 and 12.0, 12.8 and 11.5, 13.6 and 10.5, and 13.8 and 10.8 days, respectively. While female longevities were 14.5 and 14.0, 13.6 and 13.2, 14.4 and 12.6, and 15.0 and 12.9 days, respectively. The female and male longevities of the untreated parasitoid recorded as 16.1 and 14.5 days. It is clear that treatment with the two compounds affected the female and male longevities. Moreover, all treatments with Thiacloprid had high effect on the male longevity of the parasitoid than the effect of Thiamethoxam. While the same effect

149 with female longevity was obtained only with treatment of 3rd and 5th days of
150 parasitism.

151 **4. Discussion**

152 Indirect toxicity of Thiamethoxam and Thiacloprid to the larval parasitoid,
153 *B. brevicornis* indicated that the larval and pupal periods were not differed
154 significantly with the control at all periods of parasitism. Female and male
155 longevities were decreased than that of untreated individuals specially with
156 Thiacloprid. An agreement with the our results reported by (4) who mentioned
157 that longevity of parasitoids surviving a sub-lethal dose was reduced and potential
158 fecundity of females was decreased.

159 Sex ratio of adult parasitoid significantly affected by the two compounds
160 whereas the ratio of females was highly decreased with the earlier periods of
161 parasitism. The same findings was obtained by (21) with studying the side effects
162 of sublethal concentrations of two chitin synthesis inhibitors on two hymenopteran
163 parasitoids. This result is due to the fact that the pre-imaginal stages of parasitoid
164 females are less tolerant for these compounds than those of males. An agreement
165 with this result reported by (22) with the Neonicotinoid, Emamectin against the
166 parasitoid, *B. brevicornis*. On the contrary, reports by (23, 24, 25) mentioned that
167 the sex ratio of *H. hebetor* offspring was not significantly affected by either
168 insecticide.

169 The neonicotinoids have a mild or no effect on parasitoids and predators (9).
170 On the contrary, exposure of larval and adult stages of the coccinellid *Adalia*
171 *bipunctata* to thiamethoxam significantly reduced all the demographic parameters
172 in comparison with control (26). Also, many neonicotinoids such as thiacloprid and
173 thiamethoxam were highly toxic and caused considerable mortality for adults of
174 *Encarsia formosa* (27).

Generally, Thiacloprid have high toxic effect than Thiamethoxam on the larval parasitoid, *B. brevicornis*. Thiacloprid achieved low number of emerged adults /parasitoid's female, low emerged females, shorter male and female longevity. We can concluded that using of Thiamethoxam for controlling sucking insect pests is more safer for the larval parasitoid, *B. brevicornis* than Thiacloprid.

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280 Table (1): Side effects of sublethal concentration of Thiamethoxam and Thiacloprid on
 281 pre-imaginal stages of *B. brevicornis* after treated 2nd larval instars of *S. littoralis*.

Treatment	Larval periods (days±SD)			Pupal periods(days±SD)		
	Thiamethoxam	Thiacloprid	T-test	Thiamethoxam	Thiacloprid	T-test
Zero time	5.05±0.28	5.05±0.28	0	5.3±0.25	5.3±0.34	0
One day	5.2±0.42	5.15±0.33	0.287	5.25±0.26	5.2±0.35	0.557
Three days	5.15±0.41	5.1±0.39	1	5.3±0.34	5.25±0.35	0.317
Five days	5.2±0.42	5.1±0.45	1	5.35±0.24	5.3±0.26	1
Untreated	5.2±0.25	5.2±0.25	—	5.35±0.24	5.35±0.24	—
F-value	0.316	0.259		0.233	0.329	

282 No any significant differences among means at the same column and between the
 283 two means at the same row.

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296 Table (2): Side effects of sublethal concentration of Thiamethoxam and
297 Thiocloprid on adult emergence and sex ratio of *B. brevicornis* after treated 2nd
298 larval instars of *S. littoralis*.

Treatment	No. of emerged adults $\pm$ SD/parasitoid's female			Sex ratio $\pm$ SD		
	Thiamethoxam	Thiocloprid	T-test	Thiamethoxam	Thiocloprid	T-test
Zero time	16.2 $\pm$ 1.32b	13.7 $\pm$ 1.95b	4.29**	0.54 $\pm$ 0.020a	0.52 $\pm$ 1.559a	4.15**
One day	13.2 $\pm$ 1.23a	11.9 $\pm$ 0.99a	2.25*	0.55 $\pm$ 0.018ab	0.53 $\pm$ 0.035ab	2.73*
Three days	13.6 $\pm$ 1.17a	13.7 $\pm$ 1.16b	-0.26	0.56 $\pm$ 0.007bc	0.54 $\pm$ 0.027ab	3.38**
Five days	17.7 $\pm$ 2.79c	15.5 $\pm$ 2.12c	2.66*	0.57 $\pm$ 0.005c	0.55 $\pm$ 0.031bc	1.77
Untreated	22.5 $\pm$ 1.18d	22.5 $\pm$ 1.18d	—	0.57 $\pm$ 0.009c	0.57 $\pm$ 0.009c	—
F-value	51.388	71.217		8.115	7.456	

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300 -Values followed by the different letter at the same column are significantly different from each
301 other according to Tukey's test.

302 -Values of T-test followed by (*) and (**) at the same column are significantly different at $\geq$
303 0.01 and 0.05, respectively.

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Table (3): Side effects of sublethal concentration of Thiamethoxam and Thiachloprid on adult longevity of *B. brevicornis* after treated 2nd larval instars of *S. littoralis*.

Treatment	Male longevity (days±SD)			Female longevity (days±SD)		
	Thiamethoxam	Thiachloprid	T-test	Thiamethoxam	Thiachloprid	T-test
Zero time	14.0±0.67 ^{bc}	12.0±0.67 ^b	7.75 ^{**}	14.5±0.85 ^b	14.0±0.47 ^b	1.46
One day	12.8±0.79 ^a	11.5±0.71 ^b	3.88 ^{**}	13.6±0.84 ^a	13.2±0.92 ^a	0.89
Three days	13.6±0.70 ^b	10.5±0.53 ^a	9.86 ^{**}	14.4±0.97 ^b	12.6±0.70 ^a	5.51 ^{**}
Five days	13.8±0.79 ^{bc}	10.8±0.63 ^a	20.12 ^{**}	15.0±0.67 ^b	12.9±0.74 ^a	11.70 ^{**}
Untreated	14.5±0.85 ^c	14.5±0.85 ^c	—	16.1±0.88 ^c	16.1±0.88 ^c	—
F-value	6.690	53.808		11.837	34.587	

-Values followed by the different letter at the same column are significantly different from each other according to Tukey's test.

-Values of T-test followed by (*) and (**) at the same column are significantly different at ≥ 0.01 and 0.05, respectively.