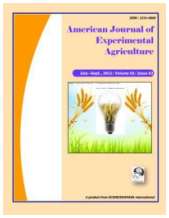

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Side Effects of Sublethal Concentration of Two Neonicotinoids; Thiamethoxam and Thiacloprid on the Larval Parasitoid, *Bracon brevicornis* (*Hymenoptera: Braconidae*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author EOT designed the study and wrote the first draft of the manuscript. Author SMS performed the statistical analysis, managed the literature searches, wrote the protocol and wrote the final draft of the manuscript. Author SAE wrote the protocol, managed the analyses of the study and reviewed the final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To assess the responses of the larval parasitoid *Bracon brevicornis*, exposed as larvae parasitized on *Spodoptera littoralis* larvae, to two neonicotinoids.
Study Design: 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.
Place and Duration of Study: A laboratory experiments were carried out during the summer 2013 at Faculty of Agriculture, Cairo University, Egypt.

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Methodology: Two neonicotinoids including thiacloprid and thiamethoxam at sublethal dose under constant laboratory conditions by spray method (direct toxicity) to the parasitoid adults and by contact method (indirect toxicity) on the parasitoid, *B. brevicornis* for five minutes to the 2nd larval instars of *S. littoralisas* as a host at zero time, 1st, 3rd and 5th days of parasitism.
Results: No significant difference was found between the two tested compounds on larval and pupal durations. thiacloprid have higher 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.
Conclusion: 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.

Keywords: Bracon brevicornis; spodoptera littoralis, neonicotinoids; thiamethoxam; thiacloprid; insecticide; sublethal dose; toxicity; parasitoid.

1. INTRODUCTION

The larval parasitoid, *Bracon brevicornis* (Wesmael) (*Hymenoptera: Braconidae*) appear to be good candidates for using in integrated pest management (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 [1-5].

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 [6-8]. Neonicotinoids interact with nAChR 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 act 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. They bind to nAChR, 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 IPM programs 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 sub lethal 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* Boisd. (*Lepidoptera: Noctuidae*) from different periods of parasitism in

order to select the safest on the abovementioned parasitoid in IPM programmes. The two tested insecticides were selected on the basis of their potential usage for sucking pests control program on the cotton plants in Egypt.

2. MATERIALS AND METHODS

2.1 Tested Pesticides

The two tested compounds are neonicotinoids; thiamethoxam (Actara 25%), 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-N-nitro-4*H*-1,3,5-oxadiazin-4-imine as water dispersible granules and obtained from Syngenta Chemical Co. Ltd, Switzerland and thiacloprid (Calypso 24%), [3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene] cyanamide as water dispersible granules and obtained from Bayer Crop Science, Germany.

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 on fresh leaves of castor bean plant, *Ricinus communis* (Family: *Euphorbiaceae*) at laboratory conditions of 25ºC and 65% relative humidity (R.H.)

2.2.2 *Braconb revicornis*

The culture larval parasitoid, *B. brevicornis* was reared under laboratory conditions of 20ºC±1, 65% R.H. on the 2nd larvae instars of *S. littoralis.* The 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 toxicities of these two compounds at a concentration of 1.5ppm were measured on the parasitoid; *B. brevicornis* under laboratory conditions of 20ºC±1 and 65% R.H. Larval and pupal durations, parasitism rate, adult emergence and sex ratio were daily observed. The larval parasitoid, *B. brevicornis* culture was reared under the same laboratory conditions on the 2nd larval instars of *S. littoralis.*

The direct toxicity on the parasitoid adults was measured by spray method along the interior side of each glass tube (7.5x2cm), that were left to dry for one hour. Droplets of 10% honey solution with a pair of male and female in each glass tube and be plugged tightly with a piece of cotton and be kept at the same conditions. Insects treated by each insecticide were 50 pairs of the parasitoid with 10 individuals per tube and repeated ten times (totally 100 individuals). 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 [20]. 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 [21]. 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 and the data were compared using one way ANOVA 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 [22].

3. RESULTS

3.1 Direct Toxicity on *B. brevicornis*

Under laboratory conditions of 20ºC±1, 65% R.H., the mortality (%) of *B. brevicornis* adults treated with thiamethoxam and thiacloprid by spray method with a concentration of 1.5ppm 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 did not differ significantly with the control. Also, there was no significant difference between the two tested compounds on larval and pupal durations. The larval duration of *B. brevicornis* with thiamethoxam at periods of parasitism ranged between 5.05 and 5.2 days. But with thiacloprid, these periods ranged between 5.05 and 5.15

days. The pupal duration with thiamethoxam at periods of parasitism were range ranged between 5.25 and 5.35 days while with thiacloprid, they ranged between 5.2 and 5.3 days (Table 1).

Sex ratio (proportion of females/proportion of total individuals) 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 the control was 0.57 while at zero time, 1st, 3rd and 5th days after parasitism by treated host larvae with thiamethoxam ranged between 0.54 and 0.57 While these ratios with thiacloprid ranged between 0.52 and 0.55 (Table 2). Also, there are significant differences were found between the two tested compounds on sex ratio whereas there was a reduction in female adult emergence resulted from treated larvae by thiamethoxam with the exception of the treated larvae 5th day after parasitism.

The total number of the treated parasitoid with the two compounds which completed their development differed significantly from untreated ones (22.5 individuals), particularly, at earlier periods of parasitism (Table 2). This number with thiacloprid was highly reduced at zero time, first day and fifth day of parasitism than 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 ranged from 14.0 to 10.5 days. While female longevities varied from 12.6 to 15.0 days. The female and male longevities of the untreated parasitoid were 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 with female longevity was obtained only with treatment of 3rd and 5th days of parasitism.

Table 1. Side effects of sublethal concentration of thiamethoxam and thiacloprid on 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	

No any significant difference among means at the same column and between the two means at the same row at P ≤ 0.01 and 0.05

Table 2. Side effects of sublethal concentration of thiamethoxam and thiacloprid on adult emergence and sex ratio of *B. brevicornis* after treated 2nd larval instars of *S. littoralis*

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

-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 P ≤ 0.01 and 0.05, respectively

Table 3. Side effects of sublethal concentration of thiamethoxam and thiacloprid on adult longivity of *B. brevicornis* after treated 2nd larval instars of *S. littoralis*

Treatment	Male longevity (days±SD)			Female longevity (days±SD)		
	Thiamethoxam	Thiacloprid	T-test	Thiamethoxam	Thiacloprid	T-test
Zero time	14.0±0.67^{bc}	12.0±0.67b	7.75**	14.5±0.85b	14.0±0.47b	1.46
One day	12.8±0.79^{a}	11.5±0.71b	3.88**	13.6±0.84a	13.2±0.92a	0.89
Three days	13.6±0.70^{b}	10.5±0.53a	9.86**	14.4±0.97b	12.6±0.70a	5.51**
Five days	13.8±0.79^{bc}	10.8±0.63a	20.12**	15.0±0.67b	12.9±0.74a	11.70**
Untreated	14.5±0.85^{c}	14.5±0.85c	_	16.1±0.88c	16.1±0.88c	_
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 P≤0.01 and 0.05, respectively

4. DISCUSSION

Indirect toxicity of thiamethoxam and thiacloprid to the larval parasitoid, *B. brevicornis* indicated that the larval and pupal periods were not differed significantly with the control at all periods of parasitism. Female and male longevities were decreased than that of untreated individuals specially with thiacloprid. An agreement with the our results reported by [4] who mentioned that longevity of parasitoids surviving a sub-lethal dose was reduced and potential fecundity of females was decreased.

Sex ratio of adult parasitoid significantly affected by the two compounds whereas the ratio of females was highly decreased with the earlier periods of parasitism. The same findings was obtained by [20] with studying the side effects of sublethal concentrations of two chitin synthesis inhibitors on two hymenopteran parasitoids. This result is due to the fact that the pre-imaginal stages of parasitoid females are less tolerant for these compounds than those of males. An agreement with this result reported with the neonicotinoid, emamectin against the parasitoid, *B. brevicornis* [21]. On the contrary, reports by [23-25] mentioned that the sex ratio of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) offspring was not significantly affected by either insecticide.

On the contrary, exposure of larval and adult stages of the coccinellid *Adalia bipunctata* L. (*Coleoptera: Coccinellidae*) to thiamethoxam significantly reduced all the demographic parameters in comparison with control [26]. Also, many neonicotinoids such as thiacloprid and thiamethoxam were highly toxic and caused considerable mortality for adults of *Encarsia formosa* Gahan (*Aphelinidae: Hymenoptera*) [27]. 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].

5. CONCLUSION

Thiacloprid have higher toxic effects 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. Also, other studies should be done for these compounds on other insect parasitoids and predators in order to knowing the effectiveness of these compounds on more natural enemies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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