



26 Key words: *Bracon brevicornis*, *Spodoptera littoralis*, neonicotinoids,  
27 thiamethoxam, thiacloprid, insecticide, sublethal dose, toxicity, parasitoid.

## 28 1. INTRODUCTION

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

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

43 The neonicotinoids are effective against whiteflies and aphids in cotton  
44 fields (10). The use of neonicotinoid insecticides (also termed chloronicotinyl  
45 insecticides) against sucking pests is now increasing rapidly. The first commercial  
46 compound was imidacloprid. Others being introduced are acetamiprid, nitenpyram,  
47 thiamethoxam, and thiacloprid. (11). Neonicotinoids act on the nerve cells to  
48 suppress muscle contraction thus inhibiting the larvae from feeding that can be  
49 used for the control program especially in vegetables by an acute contact and  
50 stomach effect. They bind to nAChR, which such compound is applied during the  
51 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 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 2<sup>nd</sup> larval instar of *Spodoptera littoralis* Bois. (Lepidoptera: Noctuidae) from different periods of parasitism in order to select the safest from the both 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.

## 2.MATERIAL AND METHODS

## 2.1. Tested pesticides:

The two tested compounds are **neonicotinoids**; thiamethoxam (Actara 25%) obtained from Syngenta Chemical Co. Ltd, **Switzerland** and thiacloprid (Calypso 24%) 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 in the laboratory on fresh leaves of castor bean plant of 25°C and 65% relative humidity (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 2<sup>nd</sup> 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 2<sup>nd</sup> larval instars of *S. littoralis*. Direct toxicity to the parasitoid adults was measured by spray method on the inner surface of the glass tubes **with 10 individuals per tube and repeated ten times (totally 100 individuals)**. **Indirect** toxicity was estimated by contact method to the 2<sup>nd</sup> larval instars of *S. littoralis* at zero time, 1<sup>st</sup>,

3<sup>rd</sup> and 5<sup>th</sup> days of parasitism (20). Contact method on the 2<sup>nd</sup> 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 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 (22).

### 3. RESULTS

#### 3.1. Direct toxicity on *B. brevicornis*:

Under laboratory conditions of 20°C±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 periods of parasitism were ranged between 5.05 and 5.2 days. But with thiacloprid, these periods were ranged between 5.05 and 5.15 days. The pupal duration with thiamethoxam at periods of parasitism were ranged between 5.25 and 5.35 days while with thiacloprid, were ranged between 5.2 and 5.3 days (Table 1).

**Table 1. Side effects of sublethal concentration of thiamethoxam and thiacloprid on pre-imaginal stages of *B. brevicornis* after treated 2<sup>nd</sup> 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  $P \leq 0.01$  and 0.05.

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 3<sup>rd</sup> day of parasitism with thiacloprid only) . This ratio in control was 0.57 while at zero time, 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> days after parasitism by treated host larvae with thiamethoxam were ranged between 0.54 and 0.57 While these ratios with thiacloprid were were

ranged between 0.52 and 0.55 (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 5<sup>th</sup> day after parasitism.

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

| Treatment  | No. of emerged adults $\pm$ SD/parasitoid's female |                  |        | Sex ratio $\pm$ SD |                    |        |
|------------|----------------------------------------------------|------------------|--------|--------------------|--------------------|--------|
|            | thiamethoxam                                       | Thiacloprid      | T-test | thiamethoxam       | thiacloprid        | 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              |        |

-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  $P \leq 0.01$  and  $0.05$ , respectively.

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, 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> day of parasitism indicated that male longevities of the parasitoid were ranged from 14.0 to 10.5 days. While female longevities were 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 3<sup>rd</sup> and 5<sup>th</sup> days of parasitism.

**Table 3. Side effects of sublethal concentration of thiamethoxam and thiacloprid on adult longevity of *B. brevicornis* after treated 2<sup>nd</sup> 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 <sup>bc</sup>  | 12.0±0.67b  | 7.75**  | 14.5±0.85b                 | 14.0±0.47b  | 1.46    |
| One day    | 12.8±0.79 <sup>a</sup>   | 11.5±0.71b  | 3.88**  | 13.6±0.84a                 | 13.2±0.92a  | 0.89    |
| Three days | 13.6±0.70 <sup>b</sup>   | 10.5±0.53a  | 9.86**  | 14.4±0.97b                 | 12.6±0.70a  | 5.51**  |
| Five days  | 13.8±0.79 <sup>bc</sup>  | 10.8±0.63a  | 20.12** | 15.0±0.67b                 | 12.9±0.74a  | 11.70** |
| Untreated  | 14.5±0.85 <sup>c</sup>   | 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  $P \leq 0.01$  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, 24, 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.

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