Effectiveness of two chitin synthesis inhibitors; Flufenoxuron and Lufenuron on *Spodoptera littoralis* (Lepidoptera: Noctuidae) and side effects of sublethal concentrations of them on two hymenopteran parasitoids.

Essam. K. Tabozada[1], S. A. El-Arnaouty[2], Samy M. Sayed[2,3]

[1]Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, 12619. Egypt.
[2]Department of Economic Entomology and pesticides, Faculty of Agriculture, Cairo University, Giza, 12613. Egypt.
[3] Department of Biotechnology, Faculty of Science, Taif University, Taif, 21974, KSA.
samy_mahmoud@hotmail.com

Abstract: Laboratory bioassays were carried out to evaluate the effectiveness of two Insect Growth Regulators (IGRs); Flufenoxuron (Cascade 10%) and Lufenuron (Match 5%) on *Spodoptera littoralis* and their safety on natural enemies through studying the direct and indirect toxicity on the egg parasitoid, *Trichogramma evanescens* and the larval parasitoid *Bracon brevicornis*. The results indicated a high toxic effect on the treated 2nd larval instars of *S. littoralis* with Flufenoxuron that was observed by using contact method rather than dipping method. LC_{50} values indicated that Flufenoxuron was more toxic than Lufenuron after treated the 2nd larval instars of *S. littoralis* whereas the mortality percentages when calculated with probit paper indicated that LC_{50} were 0.151 and 0.647 ppm for Flufenoxuron while they were 1.121 and 1.512 ppm for Lufenuron with dipping and contact methods, respectively. No effects for the two tested IGRs were observed on the adults of the two tested parasitoids while Flufenoxuron has more indirect toxicity than Lufenuron. Parasitism rate, adult emergence and adult longevity of *T. evanescens* and *B. brevicornis* were decreased with Flufenoxuron than treated by Lufenuron. The results suggest that Lufenuron is a potentially compound for controlling *S. littoralis* and share programs of integrated pest management (IPM) because it is safer for parasitoids under investigation.
[Essam. K. Tabozada, S. A. El-Arnaouty, Samy M. Sayed. **Effectiveness of two chitin synthesis inhibitors; Flufenoxuron and Lufenuron on *Spodoptera littoralis* (Lepidoptera: Noctuidae) and side effects of sublethal concentrations of them on two hymenopteran parasitoids.** *Life Sci J* 2014;11(10):239-245]. (ISSN:1097-8135). http://www.lifesciencesite.com. 33

Key Words: *Trichogramma evanescens, Bracon brevicornis, Spodoptera littoralis,* Insect Growth Regulators, Flufenoxuron, Lufenuron.

1. Introduction

Spodoptera littoralis (Lepidoptera: Noctuidae) is a worldwide pest of several crops including cotton plants (Pineda *et al*., 2007). The Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera, Pyralidae), is a well known pest for stored cereals (Rees, 2003). It has been largely used as a substitute host for the rearing of predators and parasites aimed for biological control and research (Rahman *et al*., 2004). Parasitoids can have a major impact in natural and agricultural ecosystems where they influence or regulate the population density of many of their hosts (Godfray, 1994). The larval parasitoid, *Bracon brevicornis* (Hymenoptera: Braconidae) is an extremely polyphagous ectoparasitoid, attacking Crambidae and Pyralidae in stored products and in the field; in the field other lepidopterous families may also be attacked (van Achterberg and Walker, 1998). The egg parasitoid, *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) appear to be good candidates for using in IPM programs. *Trichogramma* have been studied in more than 50 countries and commercial releases are successfully performed in approximately 32 million hectares every year (Smith, 1996)

Intensive and successive foliar applications of broad spectrum chemical insecticides for controlling larval stages led to environmental pollution, in addition to the relation between the insect resistance development and the natural balance disturbance (Ekrem *et al*., 1996). The integrated use of natural enemies particularly *T. evanescens* and *B. brevicornis* for management the cotton pests with different selective pesticides appear possible to conserve the two parasitoids (Tillman & Mulrooney, 2000; Anne *et al.,* 2001; Dora *et al*., 2004). Insect growth regulators insecticides are compounds that are active directly on the immature stages (larvae or nymphs) and adults of certain insect pests, and there are three distinct categories of insect growth regulators: juvenile hormone mimics, chitin synthesis inhibitors, and ecdysone agonists (Yu, 2008). Chitin synthesis inhibitors interfere with formation of chitin and control immature stages of many pests with relatively low harm to beneficial arthropods (Consoli *et al.,* 2001; Liu & Stansly, 2004). The hallmarks of various chitin synthesis inhibitors are abortive

molting and defects in egg hatching, which seem 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; Khajepour *et al*., 2012)

Flufenoxuron and Lufenuron are benzoylphenylureas insecticides which is a 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 death of the insect (Oberlander & Silhacek 1998). Side effects of insecticides were studied in the laboratory to maximize compatibility of chemical and biological control methods. This will help minimize any negative impact on the natural enemies (Van de Vierre *et al*., 1996). Assessment of the effects that pesticides have on the natural enemies is therefore an important part of a successful IPM program (Preetha *et al*., 2009).

The objectives and hypothesis of this work are; 1- The effectiveness of two chitin inhibitors insecticides *i. e.*; Lufenuron and Flufenoxuron on *S. littoralis*. 2- Direct and indirect sublethal toxicity assessment of two insecticides on two parasitoids of *S. littoralis*; *T. evanescens* and *B. brevicornis.* The two tested insecticides were selected on the basis of their potential usage for lepidopteran control program on the cotton plants.

2. Material and Methods

1- Insecticides:

The two tested compounds were obtained from Sumitomo Chemical Co. Ltd. Flufenoxuron (Cascade10%); 1-[4-(2-chloro-α,α,α-trifluoro-*p*-tolyloxy)-2-fluorophenyl]-3-(2,6-difluorobenzoyl) urea and Lufenuron (Match5%); 1-[2,5-Dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-3-(2,6-difluorobenzoyl)urea.

2- Insects:

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; *Ricinus communis* under laboratory conditions of 25°C and 65% R.H. Egg cards of *E. kuehniella* were obtained from Kahaa Research Station, Qaliobia, Egypt. Colonies were stored and reared under laboratory conditions of 20°C ±1and 65% R.H. The egg parasitoids, *T. evanescens* were obtained from Kahaa Research Station, Egypt. Culture was reared on *E. kuehniella* egg cards under laboratory conditions of 20°C±1 and 65% R.H. The larval parasitoid *B. brevicornis,* culture was reared under laboratory conditions of 20°C±1and 65% R.H. on the 2nd larval instars of the cotton leafworm, *S. littoralis*. Larval parasitoid was obtained from infested bollworm of the cotton plants attacked by *Helicoverpa armigera* larvae at Kahaa Research Station.

3- Bioassays:

3.1- Effectiveness of insecticides on *S. littoralis*.

Four concentrations (12.5, 6.3, 3.1 and 1.5 ppm) of the two compounds; Flufenoxuron and Lufenuron were tested against newly molted 2nd larval instars of the cotton leafworm, *S. littoralis* with replication of 40 larvae/ treatment and a control (distilled water). Mortalities of the larvae and pupae were corrected by that of the control according to Abbott's formulas (Abbott, 1925). Toxicity data were illustrated in the form of LCP line, resulting from a plot of concentrations (ppm) versus the corresponding cumulative mortality percentages on probit scale as described by Finney (1952). Data were analyzed according to the method described by Finney (1971) to determine the LC_{50} values of each compound for each stage.

3.2- Side effects of two insecticides on both parasitoids.

Direct and indirect toxicity and effects on the progeny of these two compounds with a concentration of 1.5 ppm was measured for two parasitoids; *T. evanescens* and *B. brevicornis* under laboratory conditions of 20°C±1 and 65% R.H. Parasitism rate, adult emergence and sex ratio were observed daily. The direct toxicity on the egg parasitoid, *T. evanescens* adults was measured according to Sayed *et al*. (2014) by spray method along the interior side of each glass tube (7.5 x 2 cm), 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 80 pairs of *T. evanescens*. The indirect toxicity was tested on this parasitoid by treatment of *E. kuehniella* egg cards at zero time, 1st, 4th and 7th days of parasitism. The indirect toxicity by spray method on the *E. kuehniella* eggs card was applied by hand spryer and left for one hour to dry. Egg cards were divided into small grids contained new freshly deposited eggs (400-500 eggs/grid) and were replicated ten times for each treatment.

The larval parasitoid, *B. brevicornis* culture was reared under the same laboratory conditions on the 2nd larval instars of *S. littoralis*. The direct toxicity for larval parasitoid was measured also with the same spray method. Insects treated by each insecticide were 80 pairs of *B. brevicornis*. The

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 the insecticide solution using a microapplicator in Petri dish in order to simulate of insecticide spray on plant leaves in the field. 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 five hosts of *S. littoralis* (2nd larval instars).

Statistical analysis:

Means and standard errors were calculated for each experiment and the data were compared using the ANOVA test and the significance between means was compared by LSD values at 0.05 level using SAS program (SAS, 1988).

3. Results

Lethal and sublethal effects on 2nd larval instars of S. *littoralis*:

As shown in Tables 1 and 2, the mortality rate was increased after feeding of the treated 2nd larval instars of *S. littoralis* with Flufenoxuron and Lufenuron by dipping castor bean leaves and contact in thin layer film methods. High concentration of the tested compounds caused high mortalities and significantly differed with the untreated larvae. LC_{50} values indicated that Flufenoxuron was more toxic than Lufenuron after treated the 2nd larval instars whereas the mortality percentages when calculated with probit paper indicated that LC_{50} were 0.151 and 0.647 ppm for Flufenoxuron while they were 1.121 and 1.512 ppm for Lufenuron with dipping and contact methods, respectively. Lowest larval mortality percentages with Flufenoxuron (52 and 54% by dipping and contact methods, respectively) were recorded at 1.5 ppm (Table 1). While these percentages with Lufenuron at 1.5 ppm were 46 and 52%, respectively (Table 2). High adults emergence percentages were 30 % with dipping method and 26% with contact method by Lufenuron at 6.3 ppm (Table 2), while that were 28 % by Flufenoxuron at 3.1 ppm with both methods (Table 1).

Direct toxicity to the parasitoids:

The mortality(%) of *B. brevicornis* adults by Flufenoxuron and Lufenuron with spray method with concentration of 1.5 ppm for both compounds were 1.4 ± 0.2 and 1.2±0.2, respectively. Also, these rates for *T. evanescens* adults by Flufenoxuron and Lufenuron were 1.8 ± 0.2 and 1.3 ± 0.2, respectively.

Indirect toxicity on *T. evanescens*:

The lowest female longevity (days ± SE) of *T. evanescens* was obtained with Flufenoxuron treatment at zero time and with treated by Lufenuron after 1st day of parasitism which were 11.4 ± 0.16 and 12.4 ± 0.14, respectively (Table 3). While male longevity at the same period (zero time, 1st day) were 12.5 ± 0.14 and 14.2 ± 0.10 days, respectively. In addition, highest female longevity was obtained with 7th days of parasitism by Lufenuron (15.9 ± 0.09) but it was obtained with 4th days of parasitism by Flufenoxuron (13.0 ± 0.13). Adult longevity for untreated *T. evanescens* were 15.5 ± 0.09 for females and 17.3 ± 0.07 for males (Table 3). Sex ratio (proportion of males/ proportion of males + females) affected with these compounds at all periods of parasitism. These ratios at zero time, 1st, 4th and 7th days after parasitism with Lufenuron were 0.45, 0.44, 0.435 and 0.43, respectively. While with Flufenoxuron they were 0.46, 0.45, 0.45 and 0.44, respectively (Table 3). Data presented in Table (4) indicated that the high parasitism rate (%) was obtained with treatment of 7th days after parasitism by both Lufenuron and Flufenoxuron (68.17 and 60.37, respectively), while the lowest rate was obtained at zero time by Flufenoxuron and Lufenuron (44.85 and 53.25, respectively). All treatments were significantly differed with the ratio that obtained on the untreated *E. kuehniella* eggs card (87.5). Adult emergence (%) was increased gradually from zero time to after 7 days of parasitism for the two tested compounds (Table 4). This ratio with treatment of Lufenuron was higher than that treated by Flufenoxuron (83, 67.3 %, respectively). While lowest percentage was at zero time by Flufenoxuron than that treated by Lufenuron (38, 42 %, respectively).

Indirect toxicity on *B. brevicornis*:

In zero time old parasitized larvae, higher *B. brevicornis* longevity (Table 5) was recorded according to other treatments except the untreated host (16.5 and 19.5 days for male and female, respectively). Male longevity at zero time of parasitism were 12.3 and 13.9 days while female longevity were 16.6 and 17.2 days with Flufenoxuron and Lufenuron, respectively. Minimum adult longevity was observed in three day old of parasitism with Lufenuron and in five day of parasitism with Flufenoxuron. Sex ratio affected with these compounds at all periods of parasitism, especially in earlier old of parasitism. These ratios at zero time, 1st, 3rd and 5th days after parasitism with Lufenuron were 0.47, 0.465, 0.45 and 0.44, respectively. While with Flufenoxuron were observed as 0.48, 0.465, 0.46 and 0.44 , respectively (Table 5). Treatment with Lufenuron and Flufenoxuron after 5th days of parasitism achieved high parasitism rates which were 58.78 and 52.87%, respectively (Table 6). The lowest parasitism rates were obtained at zero time by Lufenuron and Flufenoxuron (49.50 and 43.75%,

respectively). These ratios were increased gradually with treatments from zero time to 5th day of parasitism and were significantly differed with the ratio that obtained with the untreated *S. littoralis* larvae (82.2%). The higher adult emergences (%) as indicated in Table (6) were achieved with treatment of 5th days after parasitism by Lufenuron and Flufenoxuron (87.5 and 58.2, respectively).The lowest of this percentage was observed at zero time by Flufenoxuron and Lufenuron (21.5 and 25.6 %, respectively).

Table 1: Pupation and adult emergence of the cotton leafworm 2nd larval instars, *S. littoralis* treated with Flufenoxuron by dipping and contact methods.

Treatment (ppm)	Pupation%		Pupal deformation%		Emergence%		Adult deformation	
	dipping	contact	dipping	contact	dipping	contact	dipping	contact
12.5	20	20	6	8	20	20	80	80
6.3	26	26	14	10	26	26	74	74
3.1	28	30	28	8	28	28	72	72
1.5	26	22	14	14	26	26	74	74
Mean±SE	25±1.05	24.5±1.09	15.5±0.24	10±0.2	25±1.05	25±1.05	75±0.18	75.5±0.18
Untreated	100	100	0	0	100	100	0	0

Table 2. Pupation and adults emergence of the cotton leafworm 2nd larval instars, *S. littoralis* treated with Lufenuron by dipping and contact methods.

Treatment (ppm)	Pupation%		Pupal deformation%		Emergence%		Adult deformation	
	dipping	contact	dipping	contact	dipping	contact	dipping	contact
12.5	28	26	4	2	28	26	72	74
6.3	30	26	2	8	30	26	70	74
3.1	24	22	12	14	24	22	76	78
1.5	22	22	14	12	22	22	78	78
Mean±SE	26±0.99	24±1.13	8±0.27	9±0.24	26±1.05	24±1.05	74±0.18	76±0.18
Untreated	100	100	0	0	100	100	0	0

Table 3. Adults longevity and sex ratio of *T. evanescens* after treated *E. kuehniella* eggs cards by Flufenoxuron and Lufenuron.

Development stages	Adult longevity (days±SE)				Sex ratio	
	Lufenuron		Flufenoxuron			
	Male	Female	Male	Female	Lufenuron	Flufenoxuron
Zero time	14.9±0.11	14.7±0.11	12.5±0.14	11.4±0.16	0.45	0.46
One day	14±0.10	12.5±0.14	14.2±0.10	12.4±0.14	0.44	0.45
Four days	15.1±0.11	13.3±0.12	14.8±0.10	13±0.13	0.435	0.45
Seven days	15.9±0.09	13.9±0.12	15.2±0.09	12.9±0.14	0.43	0.44
Untreated	17.3±0.07	15.5±0.09	17.3±0.07	15.5±0.09	0.42	0.42

Table 4. Parasitism rate and adult emergence of *T. evanescens* after treated *E. kuehniella* eggs cards by Flufenoxuron and Lufenuron.

Development stages	Parasitism rate (%±SE)		Adult emergence (%±SE)	
	Lufenuron	Flufenoxuron	Lufenuron	Flufenoxuron
Zero time	53.25±0.46^{c}	44.85±1.30^{c}	42±1.30^{c}	38±1.54^{c}
One day	65.5±0.32^{b}	56.97±0.43^{c}	58.2±0.42^{c}	50.2±0.57^{c}
Four days	66.92±0.30^{b}	58.95±0.41^{c}	67±0.33^{b}	58±0.42^{c}
Seven days	68.17±0.28^{b}	60.37±0.38^{c}	83±0.26^{a}	67.3±0.33^{b}
Untreated	87.5±0.19^{a}	87.5±0.19^{a}	86.2±0.22^{a}	86.2±0.22^{a}

At the same column, means followed by similar letters are not significantly different ($P \leq 0.05$).

Table 5. Adults longevity and sex ratio of *B. brevicornis* after treated 2nd larval instars of *S. littoralis* by Flufenoxuron and Lufenuron.

Development stages	Adult longevity (days±SE)				Sex ratio	
	Lufenuron		Flufenoxuron			
	Male	Female	Male	Female	Lufenuron	Flufenoxuron
Zero time	13.9±0.24	17.2±0.13	12.3±0.35	16.6±0.22	0.47	0.48
One day	12.8±0.37	15.8±0.30	11.3±0.51	16.3±0.26	0.465	0.465
Four days	11.1±0.51	14.6±0.26	10.8±0.57	12.5±0.30	0.45	0.46
Seven days	12.4±0.36	14.7±0.26	10.0±0.58	12.2±0.30	0.44	0.44
Untreated	16.5±0.08	19.5±0.19	16.5±0.08	19.5±0.19	0.434	0.434

Table 6. Parasitism rate and adult emergence of *B. brevicornis* after treated 2nd larval instars of *S. littoralis* by Flufenoxuron and Lufenuron.

Development stages	Parasitism rate (%±SE)		Adult emergence (%±SE)	
	Lufenuron	Flufenoxuron	Lufenuron	Flufenoxuron
Zero time	49.50±0.99^{c}	43.75±1.28^{c}	25.6±1.89^{c}	21.5±1.93^{c}
One day	55.25±0.85^{b}	49.77±0.98^{c}	37.51±1.56^{c}	27.95±172^{c}
Four days	57.35±0.82^{b}	51.83±0.96^{c}	48.6±1.27^{c}	37.25±1.56^{c}
Seven days	58.78±0.80^{b}	52.87±0.94^{c}	87.5±0.51^{a}	58.2±0.82^{b}
Untreated	82.2±0.35^{a}	82.2±0.35^{a}	88.3±0.52^{a}	88.3±0.52^{a}

At the same column, means followed by similar letters are not significantly different ($P \leq 0.05$).

4. Discussion

High concentrations of Flufenoxuron and Lufenuron caused high mortalities for the 2nd larval instars of *S. littoralis* and significantly differed with the untreated larvae. LC_{50} values indicated that Flufenoxuron was more toxic than Lufenuron after treating the 2nd larval instars with dipping and contact methods. It was also shown that the effect of chitin synthesis inhibitors increased with increasing of its concentration when earlier instars were treated with these compounds (Tabouzada *et al.*, 2006). Moreover, the treated larvae of *S. littoralis* with Lufenuron were unable to complete the moulting process and died in the old larval cuticle (Gelbic *et al*., 2011). The larval mortality response caused by these CSIs in the present investigation is in agreement with previous investigations (Whiting *et al*., 2000; Saenz-de-Cabenzon *et al*., 2004). This effect is mainly induced by inhibiting chitin formation (Abdel Rahman *et al*., 2007).

Our results indicated that Flufenoxuron has more indirect toxicity than Lufenuron on the two tested parasitoids. Adult longevity of *T. evanescens* and *B. brevicornis* was more decreased with Flufenoxuron than with treated by Lufenuron. Previous observation reported that longevity of parasitoids surviving a sub lethal dose was reduced (Anne *et al.*, 2001).

In all treatments, parasitism rates of *T. evanescens* were significantly different than from those obtained on untreated *E. kuehniella* eggs. The same result was achieved for *B. brevicornis* when compared with the untreated *S. littoralis* larvae. On the contrary, Consoli *et al.* (2001) stated that lufenuron and triflumuron did not affect the parasitization capacity of the parasitoid, *Trichogramma galloi*.

Sex ratio of *T. evanescens* and *B. brevicornis* were affected by these compounds throughout the parasitism periods, especially in earlier stages of parasitism. 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. Adult emergences of both *T. evanescens* and *B. brevicornis* gradually increased from zero time to 7 days after parasitization for the two tested compounds in our investigation. Previous investigations stated that insect growth regulators such as lufenuron were found comparatively safer with a least effect on *Trichogramma* adult emergence (Consoli *et al*., 1998; Hassan, 1998). Although insecticides are considered as toxic to adult parasitoids, different investigations indicated that the pre-imaginal development stages within host eggs appear to be well protected from many insecticides (Singh & Varma, 1986; Brar *et al*., 1991; Consoli *et al*., 1998). Generally, strategies in integrated pest management require the development of selective and safe pesticides. The more specifically these pesticides act, and the less are their adverse side effects on beneficial insects, the more they are suited to control arthropod pests (Merzendorfer, 2013). Moreover, release of the natural enemies along with selected insecticides, which have no effect on them, is effective in depressing the population density of the pest (Preetha *et al*., 2009). Therefore, Although

Lufenuron achieved a toxicity on *S. littoralis* larvae less than Flufenoxuron, we can suggest that Lufenuron is a potentially compound that can be used for control of *S. littoralis* within integrated pest management programs (IPM) because it is safer for parasitoids under investigation; *T. evanescens* and *B. brevicornis.*

Corresponding author:
Samy M. Sayed; Department of Economic Entomology and pesticides, Faculty of Agriculture, Cairo University, Giza, 12613. Egypt.
E-mail: samy_mahmoud@hotmail.com.

References

1. Abbott, W. S., 1925. A method of computing effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265 - 270.
2. Abdel Rahman, S. M., E. M. Hegazy and A. E. Elwey, 2007. Direct and latent effect of two chitin synthesis inhibitors to *Spodoptera littoralis* (Boisd) larvae. *American-Eurasian J. Agric. & Environ. Sci.,* 2 (40): 457- 464.
3. Anne, A., M. C. Anne, P. N Jean and P. A. Jean, 2001. Selectivity assessment of chlorfenvinphos revaluated by including physiological and behavioral effects on an important beneficial insect. *Environ. Toxicol. and Chemi*., 20 (11): 2530 - 2536.
4. Brar, K. S., G. C. Varma and M. Shenhmar, 1991. Effect of Insecticides on *Trichogamma chilonis* Ishii. (Hymenoptera: Trichogrammatidae) an egg perasitoid of sugarcane borers ad cotton bollworms. *South west. Entomol*., 16: 43 - 48.
5. Consoli, F. L., J. R. P. Parra and S. A. Hassan, 1998. Side effects of insecticides used in tomato fields on the egg parasitoids *Trichogramma pretiosum* Riley (Hym., Trichogrammatidae), a natural enemy of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae). *Journal of Applied Entomology,* 122: 43 - 47.
6. Consoli, F. L., P. S. M. Botelho and J. R. P. Parra, 2001. Selectivity of insecticides to the egg parasitoid *Trichogramma galloi* Zucchi 1988, (Hymenoptera: Trichogrammatidae). *Journal of Applied Entomology,* 125: 37- 43.
7. Dora, I. P., C. Juan, H. Olivia and W. Trevor, 2004. Lethal and sub lethal effects of the naturally derived insecticide Spinosad on parasitoids of *S. frugiperda* (Lepidoptera: Noctuidae). *Biocontrol Sci. and Technol*., 15(1): 81 - 95.
8. Ekrem, A., C. Moshe and R. David, 1996. Within plant distribution of Thrips and their Predators: Effects of cotton variety and developmental stage. *Department of Entomology. Fac. Agric., Univ. of Çukurova, Turkey*, 86(6): 45 - 49.
9. Finney, D. J., 1952. Probit analysis. 2nd edi., *a statistical treatment of the sigmoid response curve*. Cambridge Univ. Press, England, 41: 719 - 721.
10. Finney, D. J., 1971. Probit analysis. 3rd ed., Cambridge Univ. Press, London.
11. Gelbic, I., M. M. Adel and H. M. Hussein, 2011. Effects of nonsteroidal ecdysone agonist RH-5992 and chitin biosynthesis inhibitor lufenuron on *Spodoptera littoralis* (Boisduval, 1833). *Cent. Eur. J. Biol.*, 6(5): 861 - 869.
12. Godfray, H. C. J., 1994. Parasitoids. *Behavioral and Evolutionary Ecology.* Princeton University Press, Princeton, New Jersey, U.S.A.
13. Hassan, S. A., 1998. The suitability of *Trichogramma cacoeciae* as an indicator species for testing the side effect of pesticides on beneficial arthropods compared to other hymenopterus parasitoids. *IOBC/WPRS Bull*., 21: 89 - 92.
14. Khajepour, S., H. Izadi and M. J. Asari, 2012. Evaluation of two formulated chitin synthesis inhibitors, hexaflumuron and lufenuron against the raisin moth, *Ephestia figulilella. Journal of Insect Science,* 12:102.
15. Liu, T. X. and P. A. Stansly, 2004. Lethal and sub lethal effects of two Insect Growth Regulators on Adult *Delphastus catalinae* (Coleoptera: Coccinellidae), a Predator of Whiteflies (Homoptera: Aleyrodidae). *Biological Control*, 30: 298 - 305.
16. Merzendorfer, H., 2013. Chitin synthesis inhibitors: old molecules and new developments. *Insect Science,* 20: 121 - 138.
17. Oberlander, H. and D. L. Silhacek, 1998. New perspectives on the mode of action of benzoylphenyl urea insecticides. In: Ishaaya I, Degheele D, Editors. *Insecticides with Novel Modes of Action*. pp: 92 - 105. Springer.
18. Pineda, S., M. I. Schneider, G. Smagghe, A. M. Martínez, P. Del Estal, E. Viñuela, J. Valle and F. Budia, 2007. Lethal and sublethal effects of methoxyfenozide and spinosad on *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, 100(3):773 - 780.
19. Preetha, G., J. Stanley, S. Suresh , S. Kuttalam and R. Samiyappan, 2009. Toxicity of selected insecticides to Trichogramma chilonis: Assessing their safety in the rice ecosystem. *Phytoparasitica*, 37: 209 - 215.
20. Rahman, M. M, H. L. S. Roberts and O. Schmidt, 2004. The development of the endoparasitoid *Venturia canescens* in Bt-

tolerant, immune induced larvae of the flour moth *Ephestia kuehniella*. *Journal of Invertebrate Pathology*, 87: 129-131.
21. Rees, D., 2003. *Insects of stored products*. CSIRO Publishing, London. 181 pp.
22. Saenz-de-Cabenzon, F. J., V. Marco, F. G. Zalom and I. Perez Moreno, 2004. Effect of lufenuron on *Lobesia botrana*. *Pest. Manag. Sci*., 61(11): 1133 - 1137.
23. SAS, 1988. *SAS user guide: statistics*. SAS Institute, Cary, N. C.
24. Sayed, S. M., S. A. El Arnaouty and E. O. Tabozada, 2014. Effects of the Neonicotinoid compound, Emamectin on *Bracon brevicornis* (Hymenoptera: Braconidae) with parasitization on two lepidopteran hosts. *Life Science Journal*, 11(1): 232-235
25. Singh, P. P. and G. C. Varma, 1986. Comparative toxicities of some insecticides to *Chrysoperla carnea* (Chrysopidae:Neuroptera) and *Trichogramma brasiliensis* (Trichogrammatidae: Hymenoptera), two arthropodal natural enemies of cotton pests. *Agric. Ecosys. Environ*., 15: 23 - 30.
26. Smith, S. M., 1996. Biological control with *Trichogramma*: Advances, successes and potential of their use. *Annual Review of Entomology*, 41: 375−406.
27. Tabouzada, E. O. K., S. A. Emara and F. F. Shalaby, 2006. Effect of flufenoxuron and lufenuron on larval stages of the cotton leafworm, *Spodoptera littoralis* and the Greasy cutworm, *Agrotis ipsilon*. *J. of Appl. Sci. Egypt*, 21(12): 253 - 265.
28. Tillman, P. C. and J. E. Mulrooney, 2000. Effect of Selected Insecticides on the Natural Enemies *Coleomegilla maculata* and *Hippodamia convergens* (Coleopter: Coccinellidae), *Geocoris punctipes* (Hemiptera: Lygaeidae), and *Bracon mellitor, Cardiochiles nigriceps,* and *Cotesia marginiventris* (Hymenoptera: Braconidae) in Cotton. *Journal of Economic Entomology*, 93 (6): 1638 - 1643.
29. van Achterberg, C. and A. K. Walker, 1998. Braconidae, pp. 137-185. In: *African cereal stem borers: Economic importance, taxonomy, natural enemies and control* (Ed. A. Polaszek). CAB International / CTA, 530 p.
30. Van de Vierre, M. Smagghe and G. Degheele, 1996. Laboratory test method to evaluate the effect of 31 pesticides on the predatory bug, *Orius laevigatus* (Het. Anthocordae). *Entomophaga,* 41: 235 - 243.
31. Virgala, M. B. R. and E. N. Botto, 2010. Biological studies on *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae), egg parasitoid of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). *Neotrop. Ent*., 39(4): 612 - 617.
32. Whiting, D. C., L. E. Jamieson and P. G. Connolly, 2000. Pre-and postharvest effect of Lufenuron on *Epiphyas postvittana* (Lepid.: Tortricidae). *Journal of Economic Entomology*, 93(3): 673 - 679.
33. Yu, S. J., 2008. *The Toxicology and Biochemistry of Insecticides*. CRC Press, Taylor & Francis Group, Boca Raton, FL.

6/6/2014