

Physiological Insecticidal Activity of Triflumuron as Insect Growth Regulator Against *Spodoptera littoralis* (Boisd.)

El-Sabrou, A. M.* and H. M. Zahran

Applied Entomology Department, Faculty of Agriculture, University of Alexandria, Egypt.

* Corresponding author: elsabroutahmed@alexu.edu.eg



ABSTRACT

Effects of triflumuron (Baycidal 25%) on newly fifth larval instars of *Spodoptera littoralis* were studied by a topical bioassay for the doses of 0.01, 0.1, 1, 5 and 10 $\mu\text{l/larva}$. The growth inhibition percentages were calculated during the experiment until 72 hours from the treatment, and were ranged between 8.7 to 58.53 %. Larval mortality percentages ranged between 40 and 82.5 %, while it was 2.5% in the control. The results showed reduction in the pupae weight values of *S.littoralis*, these values were between 239.25 to 178.28 mg while it weighed 250.8 mg in the control. Inhibition of adult formation percentages were calculated from larval stage until the adult formation, were ranged between 57.5, 92.5%, while it was 5% in the control. The histological studies illustrated heavily destruction of the reproductive tract of male when triflumuron were applied. Sex ratio of male (SR) was affected and ranged between 47.3 to 100%. Also, on the other hand, the lethal dose of triflumuron (LD_{50} = 0.006 $\mu\text{l/Larva}$) on chitin formation caused inhibition in the larval growth of the cotton leafworm. The chitin formation ratio value displayed 44.96 mg/gm for the control, while it was 15.38 mg/gm with LD_{50} = 0.006 $\mu\text{l/Larva}$.

Keywords: Triflumuron, *Spodoptera littoralis*, Growth inhibition, Chitin formation, Pupal weight and Sex ratio.

INTRODUCTION

Cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is a polyphagous caterpillar damaging plants of economic importance in Southern Europe, Africa and the Middle East (Abo-El-Ghar *et al.*, 1986 and El-Sabrou, 2013). In insect pest management, the purpose of research is to maintain the pest population below a level of economic loss.

In insects, ecdysteroids regulate many developmental and physiological processes (Gäde *et al.*, 1997) and are considered as potential specific target sites for pest control (Dinan, 1989 and Khebbab, *et al.*, 2008). The major groups of insect growth regulators (IGR) compounds include chitin inhibitors (e.g., diflubenzuron), molting hormone analogues (e.g., ecdysteroids), juvenile hormone analogues (e.g., methoprene) and anti-juvenile hormones (e.g. Precocenes) (El-Sabrou, 2009). Non-steroidal ecdysteroid agonists and induces an incomplete molt in several insect orders. Most IGR compounds mimic the physiological process of the natural insect molting hormone 20-hydroxyecdysone (20E) by binding to the ecdysteroid receptor complex in a competitive manner with ecdysteroids (Wing, 1988). Although this non-steroidal ecdysteroid agonist was developed with an aim of disturbing the larval development, substantial effects were detected on Lepidoptera reproduction Khebbab, *et al.*, (2008).

IGR compounds have many mode of action are affected through the stimulation of specific deterrent receptors, through the chitin formation inhibition receptors, or by an interaction of the activities of both deterrent and phagostimulant receptors, depending on the individual species. Growth regulatory and sterilitant effects are poorly understood at the cellular level, although interference with the synthesis and release of regulatory morphogenetic neuropeptides is involved (Huang *et al.* 2004). IGR are known to cause reproductive sterility in insects. Some of these compounds inhibit ovarian growth, testes growth and development, while others appear to induce fundamental changes in the chemical structure of

nucleic acids. Another groups of compounds known as insect growth regulators (IGRs) can be used to stimulate development at inappropriate times or inhibit it at other times (El-Zoghaby, 1992 and El-Sabrou, 2009).

The present study was designed to evaluate the insecticidal activity of triflumuron applied topically on newly fifth larval instars of *S. littoralis*, on adult reproduction, because sexual maturation in this Lepidoptera species was implemented during pupae development, therefore triflumuron (Baicydial 25%) was applied as IGR on the larval stage, longevity, chitin formation, pupal weight, inhibition of adult formation, sex ratio,.. overall on physiological activity and biology of *S. littoralis*, which is the major pest of cotton in many parts of the world, particularly Egypt.

MATERIALS AND METHODS

1. Insect rearing:

A susceptible strain of *Spodoptera littoralis* (Lepidoptera: Noctuidae), was reared under controlling conditions in laboratory of 25 ± 2 °C and 70 ± 5 % R.H. on castor oil leaves, *Ricinus communis* L., (Family: Euphorbiaceae), according to El-Zoghby (1980). Egg-masses were confined in sterilized jars and tapped with muslin covers. Upon hatching, fresh and clean castor oil leaves were provided as food. Jars were daily cleaned out where fresh leaves were substituted for the used ones. Upon pupation, pupae were sexed prior to moth emergence. Adult moths were provided by 10 % sugar solution in which a cotton cord was immersed for feeding. In addition, two leaves of *Nerium oleander* were provided as oviposition sites. Deposited egg-masses were daily collected and the hatched larvae were reared again for another generation.

2. Baycidal® 25% as Insect Growth Regulator (IGR)

This chemical compound is triflumuron (Trifluron), dispersable concentrate wp 25% (w/v) and acts as IGR (chitin synthesis inhibitors). The chemical name is 2-chloro-N-[[4- (trifluoromethoxy) phenyl] carbamoyl] benzamide (IUPAC Name), with Molecular Weight 358.69971 g/mol, and Molecular Formula: $\text{C}_{15}\text{H}_{10}\text{ClF}_3\text{N}_2\text{O}_3$, but the commercial name is Baycidal® 25%, this product were magnification by

Bayer company, It was provided by King Abdulaziz University (KAU) in Jeddah, Saudi Arabia.

3. Bioassay

Baycidal 25% compound was used in topical application tests. Preliminary tests were carried out to determine the suitable concentrations for each compound. Tested dosages were applied on the dorsal segment of mesothorax of newly molted fifth instar larvae by means of Eppendorf (Varipette 4710) at the rate of 1 microliter per larva. In triflumuron tests, the concentrations were prepared in water as a solvent and 40 larvae were used for each dose. The tested doses of triflumuron as IGR were 0.01, 0.1, 1, 5 and 10 µl/larva. Control was set up using the solvent only. For each treatment were tested, four replicates were carried, in each replicate 10 larvae were released in a plastic dish (10 cm in diameter). The treated larvae were allowed to feed for untreated castor bean leaves, which change every 24 h.

4. Criteria parameters for the test:

1. Larval mortality (%).
2. Longevity of larval stage (day).
3. Growth inhibition (%) was calculated according to Badawy *et al.*, (2005) during the experiment until 72 hours from the treatment:

$$\text{Growth inhibition (\%)} = [(C_L - T_L) / C_L] \times 100,$$

where C_L is the larval weight gained in the control and T_L is the larval weight gained in the treatment.

4. The pupal weights 24 h after pupation of *S. littoralis* larvae treated by Baycidal 25% were recorded (Ramos-López *et al.*, 2010 and Schmidt *et al.*, 1997).
5. Inhibition of the adult formation (%) was calculated from larval stage until the adult formation.
6. Sex-ratio of adults male (SR) produced after treatment.
5. Dissection of males reproductive tracts and its histological sections:

Both treated and control male adults were dissected after 2 days from emergence. The male (2days-old) reproductive tracts were dissected from these insects under a binocular microscope (10× magnification) in Ringer's solution. The Ringer's solution consists of (0.42

g KCl, 0.002 g NaHCO₃, 0.9 g NaCl, 0.48 g CaCl₂). This solution was removed and the freshly dissected testes were placed in a small covered. The testes of adults males resulted from *S. littoralis* larvae treated by triflumuron were dissected and kept in 10 % formalin after dissection. Histological procedures were achieved at the Pathology Department, Faculty of Medicine, University of Alexandria, according to the method of Junqueira & Carneiro (1980).

6. Measurement of chitin body wall:

This experiment was conducted on the newly molted fifth instar larvae of cotton leafworm. Thirty larvae were treated by lethal dose (LD₅₀= 0.006µl/larva) of triflumuron in three replications. The procedures followed were after Hughes *et al.*, (1989). The ruptured larvae were weighed in the same age with the control larvae, anaesthetized by chilling, decapitated and dissected along the ventral surface. The gut, fat body and other internal tissues were removed. After rinsing under water, the body wall of each larva was placed in 3 ml of 10 % (w/v) potassium hydroxide (KOH) at 100°C for 4 hours, then allowed to stand overnight at room temperature. The remaining chitin from each larva was waited thoroughly with cold water. The trachea and spiracles were removed and the chitin extracts were oven-dried overnight at 80°C. After equilibration to room temperature, the extracts were weighed individually. In this way, the ratio of chitin dry weight to the larval fresh weight could be determined for the individual larva, as follows:

$$\text{Ratio of chitin formation} = \frac{\text{Chitin dry weight}}{\text{Larval fresh weight}} \text{ Hughes } et al., (1989).$$

Statistical analysis:

Statistical analysis was fulfilled using (ANOVA) one-way F-test and calculated the LSD test statistically significant at $p \leq 0.05$ according to Snedecor & Cochran (1974) by MINITAB® release 14.1 statistical software.

RESULTS AND DISCUSSION

Many biological effects of triflumuron as IGR on *Spodoptera littoralis* were topically applied in Table (1):

Table (1): Comparison between the different studied groups according to larval mortality (%), larval longevity (day), growth inhibition% after 72h from the treatment, pupal weight (24h) (mg) and total mortality(%)

Doses (µl/Larva)	No. of treated larvae	Larval stage Mean ± SEM			Pupal stage Mean ± SEM	Inhibition of adult formation (%) Mean ± SEM
		Larval Mortality (%)	Larval longevity (day)	Growth inhibition (%)	Weight of pupae (mg)	
0.01	40	40.00±4.082 ^c	11.92±0.15 ^b	8.70±1.53 ^c	239.25±8.89 ^{ab}	57.50±2.50 ^c
0.1	40	45.00±5.00 ^c	12.37±0.47 ^{ab}	10.11±0.84 ^c	228.38±11.28 ^{ab}	62.50±2.50 ^c
1	40	65.00±6.45 ^b	12.50±0.51 ^{ab}	17.69±3.75 ^{bc}	213.95±7.99 ^{bc}	80.00±4.082 ^b
5	40	77.50±4.78 ^{ab}	12.90±0.32 ^a	34.43±10.41 ^b	194.25±9.60 ^{cd}	90.00±0.00 ^a
10	40	82.50±4.78 ^a	13.27±0.19 ^a	58.53±11.70 ^a	178.28±10.53 ^d	92.50±2.50 ^a
Control	40	2.50±2.50 ^d	8.00±0.00 ^c	---	250.80±3.65 ^d	5.00±2.88 ^d

F: F test f (ANOVA)

1-Effects on larval stage:

Statistical analysis of triflumuron found in tables (1&2) emphasized that there were significant differences between the obtained means of larval mortality percentages (L.S.D_{0.05}= 14.117, F. calculated = 38.93*(<0.001), while F. tabulated_{0.05} = 2.77), but the

means larval longevity (days) showed the same trend (L.S.D_{0.05}= 34.97, F. cal. = 0.971* (<0.001) while F. tab. 0.05 = 2.77).

The tested doses of triflumuron as IGR in Table (1) were 0.01, 0.1, 1, 5 and 10 µl/larva. The larval mortality percentages ranged between 40 and 82.5 %,

while it was 2.5% in the control. All the previous larval mortality in different times during larval stage were calculated by larval longevity elongated to 13.27 days, the longevity (days) of larval stage were calculated when the fifth instar larvae of *S. littoralis* were fed up to the end of its stage before the pupation, these values were 11.92, 12.37, 12.5, 12.9 and 13.27 days, in respect, for the tested doses, while it was 8 days when compared by control. The present results about the larval longevity carried by triflumuron illustrated significant differences between the obtained means of larval mortality, (Table 1).

The means of growth inhibition percentages in Table (1) showed that there were significant differences (L.S.D_{0.05}= 21.836, F. calculated = 8.33*(<0.001), while F. tabulated_{0.05} = 3.06). The growth inhibition percentages were calculated according to the formula of Badawy *et al.*, (2005) during the experiment until 72 hours from the treatment, and were recorded 8.7, 10.11, 17.69, 34.43 and 58.53 % in the tested doses, in respect. The present results were generally in accordance with those obtained by El-Zoghby & El-Ansary (1993) who studied the effects of Cascade and Fastac (IGR) against the cotton leafworm and evaluated their injury to the honey bee. Also, the data were in accordance with Fisk & Wright (1992) who reported similar conclusions on the acylurea insect growth regulators, flufenoxuron (formulated as Cascade) and teflubenzuron (formulated as Nomolt).

Inhibition of adult formation percentages were calculated in Table (1) from larval stage until the adult formation, were 57.5, 62.5, 80, 90 and 92.5% in respect, for the tested doses, while it was 5% in the control. The means of total mortality percentages were showed significant differences (L.S.D_{0.05}= 26.783, F. cal. = 144.49*(<0.001) while F. tab._{0.05} = 2.77).

Daily observations of the development of the larvae treated with triflumuron proved that there are many larval-pupal intermediates and some pupae were reduced in size at higher dose (10 µl/Larva) when compared with control and other lower doses. These observations have been observed by Hopkins & Kramer (1992) and Root & Dauterman (1996). The larvae failed to pupate may be explained as anti-ecdysone activity of some IGRs may be due to interaction with the ecdysone receptor (EcR) using a reporter-gene assay and a cell differentiation assay of an ecdysone-responsive cell line according to Oberdörster *et al.*, (2001).

Higher dosages of chlorfluazuron when applied to newly molted fifth instars had a devastating effect on the *Spodoptera litura* (F.) population by killing them during larval, pupal, and adult stages (Perveen & Miyata, 2000).

2-Effect on the weight of pupae

It was clearly noticed that the weights of pupae were gradually decreased by increasing to the doses. Higher dose gave low weight value, the weights of pupae were determined in mgs were 239.25, 228.38, 213.95, 194.25 and 178.28 mg in the tested doses, in respect, while it was 250.8 mg in the control. It was noticed that, the weights of pupae were decreased when applied triflumuron at the doses of 0.01, 0.1, 1, 5 and 10

µl/larva, respectively (Tab. 1). The means of pupae weights were showed significant differences (L.S.D_{0.05}= 26.783, F. cal. = 9.35*(<0.001) while F. tab._{0.05} = 2.77). The present results were in accordance with Ramos-López *et al.*, (2010) who applied the extract of *Ricinus communis* L. against *Spodoptera frugiperda* and studied the following parameters: the larval and pupal longevity; the larval and pupal viability, as well as the pupal weights at 24 h. Schmidt *et al.*, (1997) and Baskar *et al.*, (2011) who calculated the larval duration after treatment the larvae to become pupae. Pupal duration was calculated from pupation to the day of emergence of adults.

3-Effect of triflumuron on sex-ratio:

The sex ratio (SR) of male was affected and ranged between 47.3 to 100%, when applied triflumuron as IGR. It may be concluded that triflumuron has hormone-like effects which had been expressed on the fecundity, fertility and the inhibition of development in the cotton leafworm. The previous results in Fig.(1) in sex-ratio according with El-Zoghaby, (1992) when tested the flavoanone Glycoside (trifolin), isolated from the flowers of *Ononis vaginalis* against *S. littoralis* on the development, reproduction.

4-Effect of triflumuron on chitin formation:

The chitin formation ratio was calculated according to the formula of Hughes *et al.*, (1989). The measurements of chitin from the body wall of *S. littoralis* larvae are detailed; the larval fresh weight before removing its viscera was 0.467 gram in the control. While it recorded 0.195 gram with triflumuron as IGR at the lethal dose of 50% (LD₅₀ =0.006 µl/larva).

Ratio of chitin formation = Chitin dry weight/Larval fresh weight= mg/ g according to Hughes *et al.*, (1989)

The value recorded 44.96 mg/g for the control, while it was 15.38 mg/g for LD₅₀ of triflumuron. The obtained results indicated that triflumuron slightly retarded the chitin formation in the fifth instar larvae of the cotton leafworm, also, emphasized that triflumuron inhibited the larval growth of the cotton leafworm, which indicates that the tested triflumuron could be considered a larval growth inhibitor and also an inhibitor of chitin synthesis.

These interpretations are in accordance with the findings of many authors such as Hughes *et al.*, (1989) who worked on the inhibition of growth and development of the tobacco hornworm, Also, the results of Root & Dauterman, (1996) confirmed the present results. They found that the high doses of cyromazine caused ruptures in the cuticle earlier than the lower doses. They also reported that the slower larval growth could mean less food consumed and accordingly, a smaller ingested dose of the chemical. Another explanation was offered by Hopkins & Kramer, (1992). They attributed the higher incidence of ruptures to the weaker cuticle which is the result of the diversion of limited sclerotization precursor pool from the pathway of sclerotization to that of melanization. Many studies were carried out to illustrate the mode of action of the insect growth regulators. For example, the

morphological and ultrastructural changes that occur in the epidermis during insect growth and development are dependent upon the regulation of gene expression with different concentrations of 20E in the absence or presence of JH. Any interference by IGRs antagonists) would result in the disruption or abnormal (larval-pupal intermediates) development of the target insect Khebbeb, *et al.*, (2008) with a hormone action in the homeostasis of one or more of these hormones with

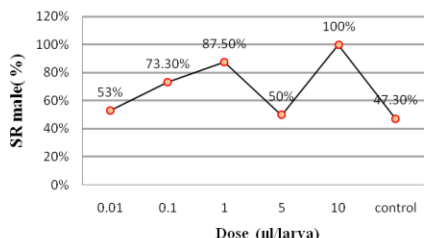


Figure (1): Effect of triflumuron on sex ratio (SR) of adult male of *S. littoralis*.

5-Effect of triflumuron on male reproductive tract of *S. littoralis*

By dissecting the reproductive tract of the males (2 days-old) produced from the treated larvae with triflumuron, it was noticed that the testes were reduced in size when compared with the normal testes. The histological sections of testes were extracted from the treated with triflumuron and control reproductive system of males adults at a thick of 3-5µm were cut. The slides were checked by microscope showing the longitudinal sections of the testes of a male with many vacuoles when compared with the normal testes (Fig. 3; A). The occurrence of some of permanent copulations in moths may be due to malformation of the general



exogenous sources of the hormone or with synthetic analogs (agonists or

Nasr *et al.*, (2010) evaluated the lethal and sublethal effects of two IGRs buprofezin and pyriproxyfen on larvae of cotton leafworm *Spodoptera littoralis*. The compounds were low toxic against the larvae at 0.05, 0.1, 0.25, 0.5, and 1.0-fold of the field application rate. However, the overall mortalities within 6 days of feeding at 2.0-fold were 46.67% and 100% for buprofezin and pyriproxyfen, respectively.

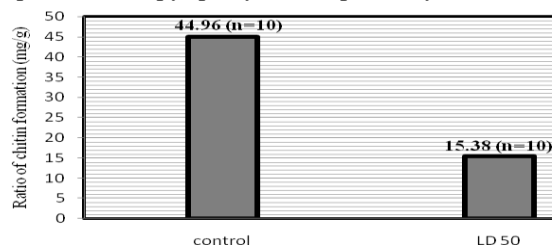


Figure (2): Assaying of the chitin of body walls of the cotton leafworm larvae, which treated by triflumuron as IGR.

reproductive system with many vacuoles (Va) as (Fig. 3; B) and premature sclerotisation (Navon & Levinson, 1976). The malformation of male reproductive tracts in our results, thus, it appears that tebufenozide, topically applied on newly exuvied pupae of *E. kuehniella*, reduces reproductive potential of adults, in particular by falling fertility, fecundity and biochemical composition of gonads Khebbeb, *et al.*, (2008). Perveen & Miyata, (2000) confirmed the present results, when topically applied sublethal doses of chlorfluazuron (LD₁₀: 1.00 µg/larva or LD₃₀: 3.75 µg/larva) on *S. litura*, but reduced it by affecting its reproduction, reducing significantly the fecundity, fertility, and hatchability of adults under laboratory conditions

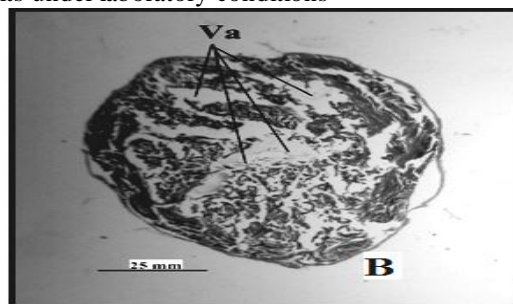


Figure (3): (A): Longitudinal section of testes of a male moth obtained from control larvae; (B): Longitudinal section of testes of a male moth obtained from larvae treated with triflumuron.

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النشاط الإبادي الحشري الفيسيولوجي للترافلوميورون كمنظم نمو حشري ضد دودة ورق القطن. أحمد محمد الصبروت و حسام الدين مجدي زهران قسم علم الحشرات التطبيقي – كلية الزراعة جامعة الأسكندرية

يهدف البحث إلى دراسة تأثيرات التراي فلوميورون (بايسيدال ٢٥%) على بداية العمر البرقي الخامس لدودة ورق القطن عن طريق التقييم الحيوي السطحي بالجرعات التالية ٠,٠١، ٠,٠١، ٠,٠١، ١٠، ٥٠، ١٠٠ ميكروغرام/يرقة. حيث تم حساب النسبة المئوية لتنشيط النمو لليرقات أثناء التجربة حتى ٧٢ ساعة من المعاملة والتي تراوحت من ٨,٧ إلى ٥٨,٥٣%. أما النسبة المئوية لموت اليرقات تراوحت من ٤٠ إلى ٨٢,٥% بينما كانت ٢,٥% في الكنترول. أظهرت النتائج انخفاض في قيم أوزان عذارى دودة ورق القطن و هذه القيم تراوحت من ٢٣٩,٢٥ إلى ١٧٨,٢٨ جرام بينما كانت وزن ٢٥٠,٨ جرام في الكنترول. تم حساب النسبة المئوية لتنشيط خروج الحشرات الكاملة من طور البرقي حتى خروج الحشرة الكاملة و التي تراوحت بين ٥٧,٥ و ٩٢,٥% بينما كانت تساوي ٥% في الكنترول. أوضحت الدراسة الهيستولوجية حدوث تدمير شديد في الجهاز التناسلي للذكور المعاملة. كما تأثرت النسبة الجنسية في الذكور و التي تراوحت من ٤٧,٣% إلى ١٠٠% كما حدث تنشيط في تكوين الكيتين أثناء فترة نمو يرقات دودة ورق القطن عند تعرضها إلى الجرعة المسببة لموت ٥٠% من اليرقات LD₅₀ و هي ٠,٠٠٦ ميكروغرام/يرقة. و كانت قيمة معدل تكوين الكيتين ٤٤.٩٦ ملجرام/ جرام في اليرقات المعاملة بال LD₅₀ بينما كانت ١٥,٣٨ ملجرام/جرام.