

# Minimized Doses of Three IGI's – Compounds on the Following Generations of *Spodoptera Littoralis*, Boisd (Lep.; Noctuidae)

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## ABSTRACT

The efficiency of three insect growth inhibitors (IGIs); namely: Lufenuron, Flufenoxuron and Chlorfluazuron against the cotton leafworm *Spodoptera littoralis* was studied. The least efficient minimized doses were determined for measuring their delayed effect on the fitness components and reproductivity of the insect-pest; throughout sex following generations. Chlorfluazuron achieved superior toxic efficacy overall the other tested IGI's. In some cases of used lower concentrations, bleeding took place in the newly moulted larvae. The used lower concentrations of Flufenoxuron caused a case of antifeeding effect due to paralysis of the musculature of larval mouth parts. The tested IGI's, slightly and insignificantly affected the durational periods of treated insect pest. The lowest measured weights of larvae, pupae; the least number of oviposited eggs and hatch percent was detected for the treatment of Flufenoxuron least minimized dose. The consequent treatment of the least efficient dose of each tested IGI to a more or less extent, reflected its drastic effect on the of developing stages. Besides the revealance of (F<sub>1</sub>) female-moths possessing shortened atrophied ovarioles with undifferentiated oöcytes. The calculated values of sterility and induced dominated lethals (genes) reaced its extremity at the end of 2<sup>nd</sup> generation in case of Flufenoxuron, the 6<sup>th</sup> Lufenuron and the 5<sup>th</sup> Chlorfluazuron causing the complete termination of the life cycle of the insect.

## INTRODUCTION

Cotton is the main cash crop in Egypt. The cotton leafworm, *Spodoptera littoralis* (Boisd.) is considered as one of the most serious and destructive phytophagous lepidopterous insect-pests in Egypt, not only for cotton plants but also for other field crops and vegetables.

Owing to the endless and various problems that have been arisen by using insecticides (e.g., the development of pest resistance, rapid resurgence of target species and outbreaks of secondary pests), the need to develop novel alternatives or functional combinations of pest control techniques is emphatically a product of this decade. Attention was therefore paid to control insects using different non traditional insecticides, e.g., IGRs compounds which are considered nowadays one of the mainly component of IPM program. Term insect growth

regulators (IGR5) describe a new class of bio-rational compounds, which act by inhibiting synthesis of chitin in larvae ingested them. The mode of action of IGI was studied by Grosscurt and Andrson, 1980). The compounds of this group are active against larvae of many lepidopterous species (Ascher and Nemny, 1976 and 1985; Abde1Naby, 1990, Mesbah *et al.*, 1990 a, b, Masoud, 1990, Meshab *et al.*, 1991, Tayeb *et al.*, 1991, Haroun, 1993 and Mesbah *et al.*, 1999).

Therefor the present work was conducted to study the efficiency of three insect growth inhibitors (IGIs) upon the 4<sup>th</sup> larval instar of the cotton leafworm. The latent effect of their least efficient doses on the fitness components of following generations and resulted deformities in female reproductive system.

## MATERIALS AND METHODS

### I- Rearing technique:

A susceptible strain of *S. littoralis* was maintained under laboratory hygrothermic conditions of 25° ± 1°C and 75 ± 5% RH., following the described rearing technique by El-Defrawi *et al.* (1964). After pupation, the resulting pupae were collected and sexed. The emerged moths were fed on 10% sucrose solution in cages supplied with oleander twigs (*Nerium oleander*). The deposited egg-masses were daily collected and left up to hatching. The newly hatched larvae were transferred to clean 1L glass jars supplied with fresh castor bean leaves.

### II- The Tested Compound:

#### 1. Lufenuron (Match<sup>®</sup> 5% Ec)

**Emperical formula:** C<sub>17</sub>H<sub>8</sub>Cl<sub>2</sub>F<sub>7</sub>N<sub>2</sub>O<sub>3</sub>

**Chemical name:** N-[[[2,5-dichloro-4- (1,1,2,3,3,3-hexafluoropropoxy)- (phenyl) amino] carbonyl]- 2,6-difluorobenzamide.

#### 2. Flufenoxuron: (cascade<sup>®</sup> 10% EC)

**Emperical formula:** C<sub>12</sub>H<sub>11</sub>Cl F<sub>6</sub>N<sub>2</sub>O<sub>3</sub>

**Chemicalname:** 1-[4-[2-chloro-4-(trifluorometly) phenoxy]2- fluoro Phenyl]-3 (2,6-difluorobenzoyl) urea.

**Chlorfluazuron** (Atabron<sup>®</sup>, IKI-7899-5% EC)

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**Empirical formula:**  $C_{20}H_9Cl_3F_5N_3O_3$

**Chemical name:** 1-(2,6-difluorobenzoyl)-3-[4(chloro-5-trifluoromethyl-2-pyridyloxy)phenyl] urea.

### III- The treatment of the cotton leafworm with tested IGIs:

The 4<sup>th</sup> instar larvae were used in all performed treatments. The-tested progressive concentrations ranged from 0.1 to 2 ppm for Lufenuron, 0.1 to 3 ppm for Flufenoxuron and 0.05 to 1.0 ppm for chlorfluazuron. Castor leaves were treated by dipping in the prepared concentrations for 30 seconds, while those used for control were dipped in tap water. The leaves were left over to dry before being offered to the larvae. Treatments were replicated three times; each replicate contained ten larvae; larvae were fed on the treated castor leaves in 250 ml glass jars. Mortality counts were recorded after 2, 3, 4 and 5 days post treatment. Mortality percentages were corrected by Abbott's formula (1925).

### IV- The delayed effect of the tested IGIs:

For studying the delayed effects of evaluated IGIs on the fitness components of the treated 4<sup>th</sup> instar larvae, castor leaves were dipped for 30 seconds in the diluted serial concentrations which represented the  $LC_{16}$ ,  $LC_{25}$  and  $LC_{50}$  of each tested compound. Treatments were done in three replicates; each contained 20 larvae. The parameters of larval duration, larval and pupal weight, moth emergence, number of deposited eggs and hatchability were recorded. The percentages of malformed larvae, pupae, adults; and adults longevities were also detected.

### V- The latent effect of the least minimized doses of the tested IGIs:

The experimental procedure following parent generation (P1) treatments was run after moths emergence from the raised pupae after the performed treatments of each of evaluated IGIs. Two crosses were carried out for each IGI treatment as follow:

- 1- Treated females x Treated males ( $\text{♀t} \times \text{♂t}$ ).
- 2- Wild females x wild males ( $\text{♀w} \times \text{♂w}$ ).

The wild males and females were picked up from the reared culture of untreated control. The resulted cultures from the above mentioned crosses were kept under the same rearing hygrothermic conditions of  $27 \pm 2^\circ\text{C}$  and  $76.7 \pm 5\%$  R.H. The laid eggs by each female were daily collected, counted and transferred to  $\frac{1}{2}$  L glass jars till hatching. Also, for examining the effect of tested chemicals on the progeny of the 1<sup>st</sup> generation ( $F_1$ ) as well as the following generations, the newly hatched larvae from deposited eggs of the aforementioned

crosses of (P1) were reared on the same used natural diets till pupation.

For determining the latent effect of the least efficient doses of Lufenuron, flufenoxuron and chlorfluazuron, another experimental procedure was initiated for achieving the possible prevention of mass larval mortality post treatment and enhancing the continuity of life cycle till moths emergence, egg deposition and hatchability. For that goal, the 4<sup>th</sup>-instar larvae of the susceptible laboratory strain were, firstly treated as previously shown, with each of these IGIs at the rate of deduced  $LC_{16}$  value after 72 hrs and 96 hrs from treatment of (P1) larvae. Later, the total number of incipient normal-fertile moths of both sexes was considered the main parameter for the determination of the desired least efficient dose of each tested IGI compound. The same experimental procedure was sequentially repeated using, merely, the half and quarter of  $LC_{16}$  concentration to gain a big number of normal-fertile adult- moths for the completion of the study. To attain high rates of normal- fertile moths, exceeding 70% of treated population, the selected concentrations of Lufenuron, Flufenoxuron and Chlorfluazuron were greatly minimized up to 0.1, 0.003 and 0.02 ppm, respectively. And these concentrations were regarded as least efficient doses for measuring the latent effect of the tested IGIs on the fitness components of the insect. Thereafter, the larvae of susceptible laboratory strain were subjected to a pressure of sequential treatments with the determined minimized doses of each IGI compounds throughout a period of 6 generations. The induced dominant lethals of ( $F_1$ ) generation or/and sterility of the following generations had been estimated according to the equation given by Topozada *et al.* (1966).

$$\% \text{ sterility} = 100 - \left( \frac{ab}{AB} \times 100 \right)$$

#### Where:

- (a) is the number of eggs laid/female in the treatment,
- (b) is the hatch % in the treatment, (A) is the number of eggs laid/female in control and (B) is the hatch % in control; and induced dominant lethal was calculated according to Abrahamson and Hexskovitz (1957) as follow:

$$\left[ \frac{\% \text{ unhatched treated} - \% \text{ unhatched control}}{100 - \% \text{ unhatched control}} \times 100 \right]$$

## RESULTS AND DISCUSSION

### Efficiency of tested (IGIs) upon the 4<sup>th</sup> larval-instar of cotton leafworm.

From the demonstrated results in Table 1, it could be shown that Chlorfluazuron achieved superior toxic efficacy compared to the tested other tested IGI's. The calculated LC<sub>50</sub> values of lufenuron, flufenoxuron and chlorfluazuron were 1.56, 0.88 and 0.27 ppm, respectively, after 72 hours from treatment. Similar results were mentioned in the works of Ascher and Nemny (1976) who concluded that diflubenzuron has contact toxicity, as well as the known stomach poison action when tested on the larvae of *S. littoralis*. and determined that the residual effect of SIR. 8514 on alfalfa, fed to *S. littoralis* larvae was more toxic than that of diflubenzuron.

All the evaluated insect growth inhibitors blocked metamorphosis revealing an anti-moulting effect, particularly, after 72 hours post treatment. The treated larvae with tested IGIs were unable to complete the moulting process, which partially took place in all treated individuals. In some cases of used lower concentrations bleeding took place as droplets from different sites of weak synthesized integument, especially in the dorsal side of the body. The lower concentrations of flufenoxuron caused a temporary antifeeding effect and paralysis of the musculature of larval mouth parts. The survived larvae at the end of treatments, exhibited weakened synthesized integument. At the higher concentrations development was blocked and the larvae completely failed to moult. Such observations were also shown by Hassan (1987), Massoud (1990), Mesbah *et al.* (1991) and Haroun (1993).

### The latent. effect of least efficient doses of tested IGI's on the fitness components of *S. littoralis*

The included results in Table 2 declare the detected effects post treatment of 4<sup>th</sup> instar larvae of each of parent generation and consequent ones with the tested IGIs at the least minimize dose (LC<sub>4</sub>). All the tested IGI's slightly and insignificantly affected the period of larval duration compared with untreated larvae.

Lufenuron showed its effect on the calculated percentage of revealed malformed pupae or/and adults (Table, 2). The highest significant larval weight of 739 mg was recorded for the treated larvae with 0.02 ppm chlorfluazuron, versus the lowest weight of 479 mg, recorded for those fed on leaves treated with 0.003 pm flufenoxuron.

The weights of raised pupa were insignificantly affected and the highest weight of 267 mg was recorded for chlorfluazuron, versus the lower weight noticed for flufenoxuron (21.5 mg). Consequently, that was reflected on the reproductivity of emerged moths and embryonic viability of deposited eggs. The least number of oviposited eggs (290.75) and percentage of hatched eggs (77.29) was detected for flufenoxuron compared to 1094.33 eggs and 95.29% in the untreated control, and flufenoxuron was the utmostly, efficient, in comparison to lufenuron and chlorfluazuron (Table 2). Moreover, the calculated period of total life cycle was greatly shortened by Flufenoxuron treatment (22 days) versus that, prolonged period up to 27 days in the untreated check, and 31 & 33 days in case of lufenuron & chlorfluazuron, respectively.

Furthermore, the continuous use of the least minimize dose (LC<sub>4</sub>) of each tested IGI compound along the following generations post parent one was adopted because these least concentrations have no killing effect on the treated larvae. Herein Table 2 show and summarize the delayed latent effect of each of these tested doses (LC<sub>4</sub>) on the following inspected six generations. Similarly the insignificant effects of these minimized concentrations on the durational periods of larvae, raised pupae and total life cycle of the insect was ascertained.

In comparison to the untreated control, the estimated values of the weight of larvae and raised pupae, the number of deposited eggs and hatchability, to a more or less extent, were affected in the different following generations; whereas, the treatment of flufenoxuron throughout the 1<sup>st</sup> and 2<sup>nd</sup> generations indicated lower weight, lowest number of deposited eggs/female and lowest percentage of hatchability;

**Table 1. Effect of tested IGIs compounds on the 4<sup>th</sup> instar of larvae' of the cotton leafworm.**

tested compound	N.E.D (response)/ Log (dose). Regression	LC <sub>50</sub> (ppm)	95% Fiducial limits for LC <sub>50</sub>		LC <sub>25</sub>	LC <sub>16</sub>	LC <sub>8</sub>	LC <sub>4</sub> *
			Lower	Upper				
Lufenuron	Y = -0.307 + 1.584 X	1.65	1.17	2.56	0.6	0.40	0.200	0.120
Flufenoxuron	Y = 0.037 + 0.695 X	0.88	0.30	1.90	0.1	0.01	0.008	0.003
Chlorfluazuron	Y = 0.944 + 1.659 X	0.27	0.21	0.35	0.1	0.07	0.040	0.020

\* LC. represent the used least minimized dose, and calculated using regression equation.

and the survived larvae and pupae in both generations were weak small in size and became unable to complete their life cycle and died at the beginning of 3<sup>rd</sup> generation. That might be due to the rapid occurrence of the unprofitable cumulative effects of sequential treatments of flufenoxuron with its least minimized dose. Which revealed in the extremely increased percentage of sterility and values of induced dominant lethals genes-70.6 & 100%, respectively (Table 3). Comparatively, the needed time for revealing the going on incidence of delayed latent effects of the tested least minimized doses of lufenuron and chlorfluazuron, to a great extent, was prolonged than that recorded for flufenoxuron.

Lufenuron and Chlorfluazuron showed somewhat similar gradual effects on the inspected parameters of fitness components of the treated insect. Comparatively the continuous treatment of 4<sup>th</sup>-instar larvae with the least minimized doses of both IGIs from the beginning of 1<sup>st</sup> generation up to the end of the experiment indicated calibrating significant values of the studied parameters, particularly the weights of larvae and pupae along the period, extending from 1<sup>st</sup> generation up to the 6<sup>th</sup> one (Table 2). Sequentially, that fluctuating weight of derived pupae in different generation to a more or less extent, significantly affected the rates of deposited eggs and hatchability. That fact was ascertained by the calculated significant values of the induced dominant lethal and percentage of sterility throughout the time of following developing generation (Tables 3). Rein, chlorfluazuron least minimized dose completely inhibited the development of the insect at the end of 5<sup>th</sup> generation (Table 2), due to the attainment of percentage sterility amounted to 79.8% and relatively faster induction of dominant lethals comprising 100% (Table 3).

Similar findings about the efficiency and mode of action of some evaluated IGIs compounds were mentioned in the works of Wolfenburger et al. (1977) For the tobacco bud worm, *Heliothis virescens*; Dimetry, Nadia and Ramadan (1979); Radwan et al. (1980) and Mostafa, Omayma and El-Attal (1984) for the cotton leaf worm *S. littoralis*.

Also, Hassan (1987) showed that all the tested insect growth inhibitors gave gradual increase of *S. littoralis* larval mortality along 9 days after treatment. The medium or/and lowered concentrations of these insect growth inhibitors, more or less, affected the forms of pupal deformation, moth incipency, number of laid eggs, egg-hatch and embryonic viability. Masoud (1990) stated that treatment with the tested IGIs at the rates of

their calculated LC<sub>25</sub> and LC<sub>50</sub> values almost gave malformed moths. The deduced percentages of malformed adults ranged between 25 and 100. Adults emergency was completely inhibited after treatment with chlorfluazuron; XRD-473 and aimx at the level of their calculated LC<sub>50</sub> values.

Mesbah *et al.* (1991) stated that all the evaluated IGIs: diflubenzuron, triflumuron and chlorfluazuron increased larval mortality along 9 days after treatment; and affected the measured weight of resulted pupae; and revealed pupal malformation, particularly their medium or/and lower concentrations; were sufficient to interrupt the continuity of insect life cycle. Haroun (1993), stated also that treatments of IGI least efficient doses along nine following generations significantly affected the reproductivity of cotton leafworm. The calculated percentages of eggs hatchability or/and moth's sterility were greatly affected. Identical findings were reported by Gouda (2002). When lufenuron, at 25 and 100 ppm, was tested against *E. cautella* larvae by Al-Jboory *et al.* (1998) the mortality percentages were about 52 and 83%, respectively, life-spans of the developed larvae and pupae were obviously prolonged and adult longevity was shortened. Similar results were determined by Tariq *et al.* (1999) against newly hatched larvae of *S. cretica*.

#### **Effect of tested minimized doses on the revealed deformities in female reproductive system:**

Generally the treatment with the least minimized doses of the tested IGIs, abnormally prolonged the total length of the female reproductive system (FRS) and increased the quantity of the accumulated fats surrounding it.

Results in (Table 4) show the revealed significant effects on the measured total lengths of the reproductive systems of the treated females in comparison to those of untreated ones. The length of the ovarioles of parent generation females was more increased than that of the control; and in case of chlorfluazuron comprised 10.5 cm and was merely as twice as that of the control (5.0 cm). That length included both the ovariole regions of vitellogenesis (7.3 cm) and germarium (3.2 cm). Moreover, a big quantity of fats was obviously seen surrounding the dissected reproductive system causing a reduction in number of deposited eggs by these females. The length of the ovarioles in lufenuron treatment was shorter 7 cm., indicating a germarium region of 3cm & vitellogenesis region of 4 cm. While, that estimated length post flufenoxuron treatment was rather reduced to 5.5 cm with a germarium region of 1.2 cm and vitellogenesis region of 4.3 cm only. In each of these affected ovarioles post IGIs treatments the vitellogenesis





**Table 3. The calculated values of induced dominant lethals and percentage of sterility of the sequentially treated generation of *S. littoralis* with least minimized doses of IGIs.**

Treatment	The used least minimized dose ppm	Induced dominant lethals of parent	Sterility percentage					
			F1	F2	F3	F4	F5	F6
Lufenuron	0.100	8.40	46.311	48.5	58.5	62.2	85.4	100.0
Flufenoxuron	0.003	17.70	69.700	70.6	—	—	--	—
Chlorfluazuron	0.020	0.70	57.500	60.9	63.20	69.2	79.8	
Control (Check)	--	-	8.900	7.1	8.50	6.5	4.9	5.7
Significance	--	*	**	**	N.S	**	**	--

**Table 4. Effect of least minimized doses of tested IGIs on the detected changes of the length in female reproductive system of the consequently treated during the following generations of cotton leafworm *S. littoralis*.**

Treatment	The used least minimized dose (ppm)		Length (cm)						
			Parent	F1	F2	F3	F4	F5	F6
Lufenuron	0.100	a	7.0	6.7	7.0	7.0	7.5	7.0	6.5
		b	4.0	4.0	4.7	5.3	3.4	2.8	0.5
		c	3.0	2.5	2.3	1.8	3.6	4.2	6.0
Flufenoxuron	0.003	a	5.5	5.3	5.0	--	--	--	--
		b	4.3	2.9	1.5	--	--	--	--
		c	1.2	2.4	3.5	--	--	--	--
Chlorfluazuron	0.02	a	10.5	6.7	6.0	7.0	6.5	7.0	--
		b	7.3	4.5	4.2	4.2	3.0	2.5	--
		c	3.2	2.2	1.8	2.8	3.5	4.5	--
Control (check)		a	5.0	5.6	5.0	5.2	5.6	5.3	5.6
		b	3.3	4.3	3.5	4.2	4.4	4.3	4.3
		c	1.7	1.3	1.5	1.0	1.2	1.0	1.3

a) Total length ovarioles

b) vitellogensis region

c) germarium region

region was found including small undifferentiated oöcytes.

Similarly, for the emerged F1 adult females, treatments with chlorfluazuron, lufenuron and/or flufenoxuron showed an appearance of atrophied ovarioles with shortened lengths; including the undifferentiated oöcytes. The sequential use of such compounds for many following generations resulted in a distinct reduction of the total length of the female reproductive system, with an increased length of the ovariole's germarium region including the immature eggs. Therefore that would be practically valuable when such compounds are involved in IPM Program of this insect pest. Also it was detected that the consequent use of these least minimized doses drastically reduced the size of the developing eggs within the affected ovarioles.

The mean total length of the reproductive system of emerged female-moths of last generation (LG) was ratherly decreased up to distinctly shortened length amounted to 5 cm, for flufenoxuron least minimized dose, with a germarium region of 3.5 cm; vitellogensis region of 1.5 cm., and greatly decreased number of small and blue-greenish deposited eggs.

The following use of chlorfluazuron till the 5<sup>th</sup> generation (L.G.) indicated a mean length of the reproductive system reached 7 cm (germarium region-4.5 cm and vitellogensis 2.5 cm) with reddish eggs in germarium region. Also the sequential use of lufenuron least minimized dose till the 6<sup>th</sup> generation (L.G.) gave shortened length of female reproductive system comprised 6.5 cm and characterized by obvious influenced regions of the ovarioles, containing small

greenish immature eggs besides a big lot of fats surrounding these atrophied ovarioles.

Similar trend of results was detailed in the works of numerous authors who tested many chemical compounds of insect growth inhibitors or mutagenic agents during the sensitive period of larval metamorphic development showing irreversible morphological abnormalities which often led to death before maturation (Radwan *et al.*, 1980, El-Sayed, 1981, Mesbah *et al.*, 1982-1983, Carpenter *et al.*, 1983, Souka, 1984, El-Kady, 1988, El-Kady *et al.*, 1989 and 1991).

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## الملخص العربي

التأثير الممتد المفعول للجرعات فائقة التخفيض لثلاث مثبطات نمو حشرية على الأجيال المتعاقبة لدودة القطن

**Spodoptra. Littoralis (Lep. Noc.)**

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أظهرت التركيزات فائقة التخفيض من مثبطات النمو الحشرية المختبرة تأثير محدود وغير معنوي على فترات تطور المراحل المختلفة للحشرة. كما اتضح أن أقل وزن لليرقات والعداري وأقل عد من البيض الموضوع ونسبة الفقس يحدث عند استخدام التركيز فائق التخفيض من مركب الفلوفينوكسيرون كذلك أرى تتابع المعاملة بالتركيزات الفائقة التخفيض لهذه المركبات إلى حدوث تأثيرات متباينة للدرجات المختلفة بالإضافة إلى حدوث نقص في طول فروع المبيض لإناث الفراشات الناتجة من اليرقات المعاملة بالإضافة إلى الزيادة التدريجية والمتراكمة في القيم المحسوبة لنسبة القيم مما أدى إلى حدوث الموت الكامل وتوقف التطور في نهاية الجيل الثاني في حالة مركب الفلوفينوكسيرون، والجيل الخامس والسادس بالنسبة لمركب الكلورفلوزيرون والفلوفينورون على الترتيب.

أجريت الدراسة لتقييم ثلاثة تجهيزات من مثبطات النمو الحشرية وجرعاتها فائقة التخفيض على سلالة حساسة لدودة ورق القطن. وهي اللوفينورون، الفلوفينوكسيرون والكلورفلوزيرون. وذلك بقياس تأثيراتها الكامنة والممتدة المفعول على مكونات المواءمة للحشرة باستخدام تركيزات فائقة التخفيض لكل منها (0.1 ، 0.003 ، 0.02 جزئ في المليون، على الترتيب) خلال المعاملة بهذه التركيزات على مدى ستة أجيال متتالية. أظهر مركب الكلورفلوزيرون كفاءة عالية فاقت المركبات الأخرى. كما سببت التركيزات المنخفضة لمركب الفلوفينوكسيرون التأثير المانع للتغذية نتيجة حدوث شلل في عضلات أجزاء فم اليرقات.