Zagazig J. Agric. Res., Vol. 41 No. (5) 2014 1077-1087

Zagazig Journal of Agricultural Research

http:/www.journals.zu.edu.eg/journalDisplay.aspx?JournalId=1&queryType=Master

TOXICITY AND BIOLOGICAL EFFECTS OF CHLORFLUAZURON ON THE COTTON LEAFWORM, *Spodoptera littoralis* (BOISD.)

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ABSTRACT

Chlorfluazuron is an insect growth regulator used for controlling the major insect pests of several crops. The present work was conducted to study the susceptibility of laboratory strain of 5th instar larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) to chlorfluazuron using three methods of application (topical application, injection and oral administration). Data showed that the topical method exhibited high level of toxicity with LD_{50} value (0.87 µg a.i. /larva) followed by injection (3.59 µg a.i. /larva) then oral method which recorded (13.20 µg a.i. /larva). Topical application caused a prolongation in larval duration, while injection and oral methods showed no significant differences in larval duration compared with the control. All methods of administration caused reduction in pupal weight and significant deformation percentages in larval and pupal stages, whereas topical application caused the highest percentage in larval and pupal deformation compared with the other methods and control. Topical application, also, caused a significant reduction in longevity of female moth, hatchability and sterility compared with the control. Meanwhile, oral and injection methods caused reduction in fecundity compared with the control, when 5th instar larvae of *S. littoralis* treated with sub-lethal dose (LD_{25}) of chlorfluazuron. The activity could be arranged as follows: topical application, injection and oral administration.

Key words: Topical application, injection, oral, *Spodoptera littoralis*, chlorfluazuron, malformation.

INTRODUCTION

Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) is one of the most destructive pests of several crops such as cotton *Gossypium hirsutum* L.; peanut, *Arachis hypogaea* L.; soybean, *Glycine max* L., and vegetables in Africa, Asia and Europe (Bayoumi *et al.*, 1998 and El-Aswad *et al.*, 2003). In addition, its direct damage reducing photo- synthetic area, its larval presence, feeding marks and excrement residues reduce marketability of vegetables and ornamentals (Plusch-Kell *et al.*, 1998).

The potential of acylureas as insect pest control agents has received considerable attention in the last two decades. Group disrupts the moulting process of insect larvae by inhibiting chitin deposition in their cuticles during growth and development (Retnakaran and Hackman, 1985). This inhibition is considered to induce morphological disruptions during moulting, which is a typical characteristic of this class of insecticides. These compounds in some species of Lepidoptera, induced morphological disruptions that result in ecdysis failure, black-ended, ruptured integuments and fluid loss, depending on the species (Omatsu *et al.*, 1991). Topical treatment of diflubenzuron on male and female adult of boll weevils, *Anthonomus grandis*, stable flies, *Stomoxys calcitrans* and house flies, *Musca domestica*, causes a significant inhibition in the fecundity, fertility and egg hatch (Taft and Hopkins, 1975; Wright and Spates, 1976).

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Retnakaran *et al.* (1989) stated that chlorfluazuron (Atabron) is a highly effective treatment against insect pests because of its ability to disrupt chitin deposition during the moulting process. Much research were carried out to determine ways to reduce and maintain the pest population below the economic injury level.

This study is conducted to evaluate the relationship between toxicological and biological activities of chlorfluazuron as affected by different routes of administration to the 5th instar larvae of *S. littoralis.*

MATERIALS AND METHODS

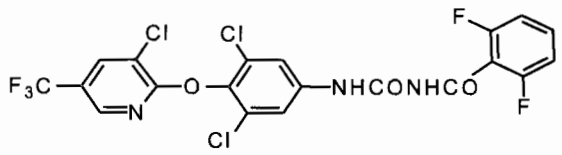
Test Compound

Atabron 5% E.C.

Common name: chlorfluazuron

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

Structural formula



Insect Rearing

The culture of a susceptible Egyptian cotton leaf worm *S. littoralis* (Boisd.) was initiated from freshly collected egg masses supplied from the Division of Cotton Pests, Branch of Plant Protection Research Institute at Zagazig, Sharkia Governorate.

The egg masses were kept in glass jars covered with muslin and fastened with rubber bands under laboratory conditions of 25±2 °C and 70±5 R.H. till hatching. The newly hatched larvae were transferred into 2 kg capacity rearing jars where enough fresh castor-oil leaves bottomed with sheets of towel paper to absorb excess humidity. Fresh castor-oil leaves, *Ricinus communis* were provided to the larvae daily according to El-Defrawi *et al.* (1964).

The accumulated feces and debris were cleaned out daily. After pupation, pupae were collected and placed in clean jars until adult emergence. Newly emerged moths were sexed and kept in mating jars (5 males and 5 females for each jar), saturated 15% sugar solution cotton wool were placed and changed daily. Jars were supplied with fresh leaves of tafla, *Nerium oleander* as an oviposition site. Egg masses were collected daily and transferred into the rearing jars.

Toxicity of Oral, Topical and Injection Administration of Chlorfluazuron to *S. littoralis* 5th Larval Instar

The newly moulted fifth instar larvae were used for these experiments. The larvae were treated by three methods (topical application, injection and oral administration). For topical application technique, several doses were applied to thoracic dorsum segment, using 1 µl droplets of acetone –dilutions (Perveen, 2000). For oral administration, 1µl of the insecticide acetonic solution was delivered directly in the foregut of the treated larvae through the buccal cavity, using a microsyring fitted with a polished straight needle to avoid bleeding. While for injection technique 1µl of the acetone solution was injected in the first proleg (Shepared, 1958). Before treatments, the larvae were anesthetized using diethyl ether.

In all cases control tests were carried out using acetone only, and were found to have no effect. Five replicates were carried out for each treatment, and 10 larvae were used in each replicate. The used doses were ranged between 15-0.0015 µg a.i. /larva.

The mortality percentages in untreated and treated larvae were recorded after 24 hr., and calculated per each dose, corrected using Abbott's formula (Abbott, 1925) if necessary. The dosages mortality regression lines were statistically analyzed by Biostat 2007 (Professional Bulid 3200) and the LD_{25}, LD_{50} and LD_{95} values were recorded.

Biological Response of *S. littorlais* Larvae Exposed to Chlorfluazuron

Topical application, injection and oral administration techniques were used, as mentioned before, to evaluate the biological changes resulting from treating the newly moultd 5th instar larvae with sublethal doses of chlorfluazuron ($\simeq$ LC_{25} value).The IGR doses

were 0.5, 1.5 and 7 µg a.i. /larva for topical application, injection and oral administration techniques respectively. Larvae were fed on untreated leaves until pupation.

Daily inspections were carried out till adult emergence. Larval duration, pupal weight, % larval mortality, % pupal mortality and adult emergence were estimated.

Percentage of pupation and moth emergence were based on the number of normal pupae or moths obtained. For mating experiments, the emerged moths either from treated or untreated larvae (control) were sexed and put in glass jars and provided with leaves of tafla *Nerium oleander* (L.) that served as an oviposition site and provided with a piece of cotton dipped in 15% sugar solution for feeding and changed daily till egg-mass depositions throughout their longevity .The eggs were counted and put in clean jars with untreated castor bean leaves till hatching.

Adult fecundity was determined by recording the number of laid eggs and newly hatched larvae also were recorded to calculate the hatchability percentage. The percentage of sterility was calculated according to the equation of Tappozada *et al.* (1966) as follows:

Sterility (%) = 100-[a × b/A ×B] × 100

Where:

a is the number of eggs laid /female in treatment. b is the (%) of hatchability in treatment. A is the number of eggs laid /female in control. B is the (%) of hatchability in control.

The following formulae have been used to calculated other biological parameters:

Pupation (%)=[Number of pupae /Total number of larvae] x 100

Deformed pupation (%) = [Number of deformed pupae / Total number of pupae] x 100

Emergence (%) =[Number of moths / Total number of pupae] x 100

Fecundity=Number of deposited eggs per female

Fertility (%) (Hatchability)=[Number of hatched eggs/Total number of eggs laid per female] x100

Deformed moths (%) = [Number of deformed moths /Total number of moths] x 100

Deformation effect of chlorfluazuron was scored according to the intensities of morphological defects in next larval instars or stages (El-Tantawy and Salem, 1976).

The mean abnormality rating was calculated for the graded scoring based up on the following formula (Redfern *et al.*, 1970; Staal, 1972):

$$AR=^{1}n_1+^{2}n_2+^{3}n_3+^{4}n_4+^{5}n_5+^{6}n_6\ldots/n_0+n_1+n_2+n_3+n_4+n_5+n_6\ldots$$

Where:

AR is the mean abnormality rating. n is the number of individuals responding at each abnormality rating of types (0-6).

Statistical Analysis

Means and standard errors were analyzed using commercial statistical software. One way analysis of variance (ANOVA) was used for significant differences ($P<0.05$) between mean values. (Costat statistical software program methods, 2005).

RESULTS AND DISCUSSION

Toxicity of Oral, Topical Application and Injection Administration of Chlorfluazuron to *S. littoralis* 5th Larval Instar

Chlorfluazuron was tested on the fifth instar larvae of *S. littoralis* using three methods of application *i.e.*, topical, injection and oral. The LD_{50} values of chlrofluazuron were 0.87, 3.59 and 13.20 µg a.i. /larva, whereas the LD_{95} values were 145.33, 105. 54 and 93.06 µg a.i. /larva for topical, injection and oral, respectively (Table 1). The activity could be therefore being arranged as follows: topical < injection < oral (Fig. 1).

The slopes of the regression lines for these methods of application were calculated as 0.7403, 1.1206 and 1.9403 for topical application, injection and oral, respectively (Table 1).

These results showed that chlorfluazuron was highly toxic for *S. littoralis* when the larvae were treated with topical application.

This result is in agreement with that obtained by El-Tantawy and Salem (1976), who pointed that topical application proved to be highly effective route than injection and oral methods against *S. littoralis* when treated with PH 60-40.

Table 1. Toxicity of topical application, injection and oral administration of chlorfluazuron to the 5th instar larvae of *S.littoralis*

Chlorfluazuron (µg a.i. /larva) Route of administration	5th instar larvae			
	LD_{25}	LD_{50}	LD_{95}	Slope
Topical application	0.38	0.87	145.33	0.7403
Oral	5.61	13.20	93.06	1.9403
Injection	0.76	3.59	105.54	1.1206

A laboratory colony of *S. littoralis* was used.

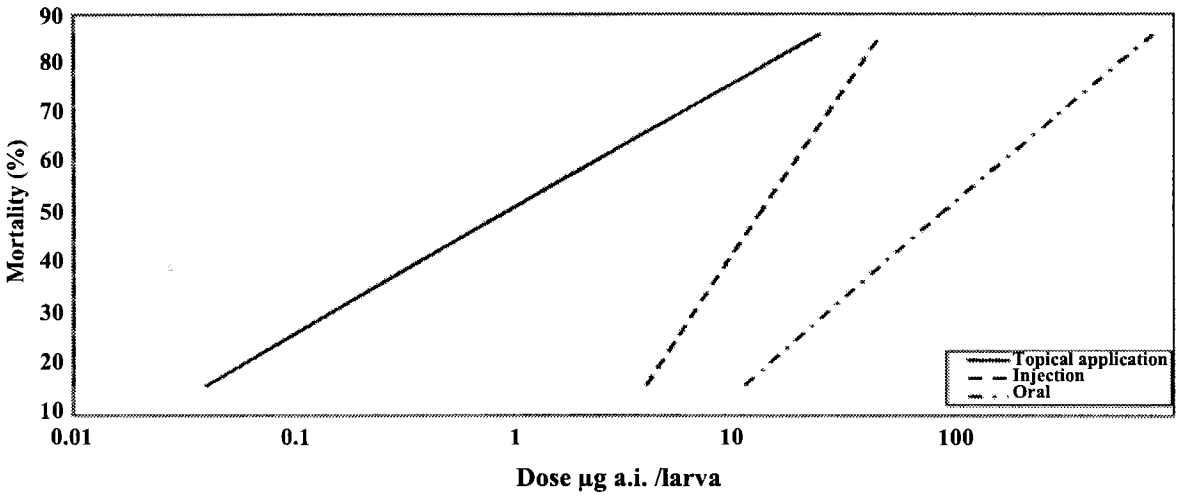


Fig. 1. Toxicity of topical application, injection and oral administration of chlorfluazuron to the 5th instar larvae of *S. littoralis*

Gujour and Mehrotra (1993) found that treatment of pupae of *S. littoralis* by topical application at 0.625 and 6.25 µg of chlorfluazuron, diflubenzuron, penfluron and triflumuron caused 70, 20, 100, 50 and 90, 50, 100, 100% mortality for the first and second dose, respectively.

Perveen (2000) showed that the toxicity tests of chlorfluazuron by topical application had a cumulative effect that extended throughout the larval, pupal and adult stages, when applied to newly moulted fifth instar larvae of *S. litura* at LD_{10} and LD_{30}.

Biological Response of *S. littoralis* Larvae Exposed to Chlorfluazuron

Larval duration

Data in Table 2 showed prolongation in total larval duration with all methods compared to control duration (6.65±0.126) days. Topical application recorded the longest larval duration (9.17±0.426) days. Meanwhile, no significant differences were noticed in larval duration when larvae were treated with injection and oral methods.

In the laboratory, Kocąk and Kilinçer (2001) mentioned that the larval duration was increased, when the 6th instar larvae of *S. littoralis* were treated with methoprene by topical application.

Larval deformation

All methods used showed significant deformation percentage compared to the control. Topical application caused the highest larvae deformation percentage (34.56±4.67%), while; oral method gave the lowest one (5.11±1.53%).

Different types of deformation were also classified into six scores based on the external characters or degree of development as illustrated in Table 3 and Fig. 2.

Table 2. Larval and pupal biological parameters as affected by exposing the 5th instar larvae of *S. littorlais* to sublethal dose of chlorfluazuron using different routes of administration

Parameters (Mean ±S.E.)	Larval duration (day)	Larval deformation (%)	Pupation (%)	Pupal weight (g)	Pupal deformation (%)	Adult emergence (%)
Route of administration						
Topical application (0.5 µg a.i./larva)	9.17 ^{a}±0.426	34.56^{a} ±4.67	62.71^{c} ±5.53	0.2236^{c} ±0.014	26.07^{a} ±5.89	87.06^{a}±3.30
Injection (1.5 µg a.i./larva)	7.55 ^{b}±0.43	17.25^{b} ±3.16	41.85^{d} ±3.55	0.2961^{b}±0.028	13.40^{ab} ±4.73	71.85^{b}±6.82
Oral (7 µg a.i. /larva)	7.76 ^{b}±0.31	5.11^{c} ±1.53	81.00^{b} ±2.81	0.2659^{bc}±0.013	3.73^{b} ±1.07	48.33^{a}±4.31
Control (0.0 µg a.i. /larva)	6.65 ^{c}±0.126	0.00^{d}	100.00^{a}	0.3562^{a}±0.017	0.00^{c}	100.00^{a}
P	***	***	***	**	***	***

-Newly moulted 5th instar larvae of a laboratory colony of *S. littoralis* were used.

- *: significant at p<0.05, ** highly significant at p<0.01 and ***: very highly significant at p<0.001.

-Means followed by similar letters indicate insignificant differences at 0.05 level of probability.

Table 3. Scoring system for larvae and pupae transformation of *S. littoralis* after treating the 5th instar larvae with sublethal dose of chlorfluazuron by different methods of application

Scores	Abnormality characteristics	Routes of administration		
		Topical application (0.5 µg a.i. /larva)	injection (1.5 µg a.i./larva)	Oral (7 µg a.i./larva)
0	Normal larvae and pupae	+	+	+
1	Pupal – larval intermediate; pupae with larval head and thoracic legs.	+	+	+
2	Larval –pupal intermediate; larva with pupal head and larva with pupal abdomen.	+	+	-
3	Larval –failed to pupate.	-	-	-
4	Partially ecdysed larvae with big batches of new cuticle without normal coloration.	+	-	-
5	Larvae with partially extrusion of the alimentary canal from the anus.	+	-	-
6	Larvae failed in molting and completely paralyzed and became inactive, shrank and gradually stopped feeding.	+	-	-

+ refers to appearance of the score in the treatment.

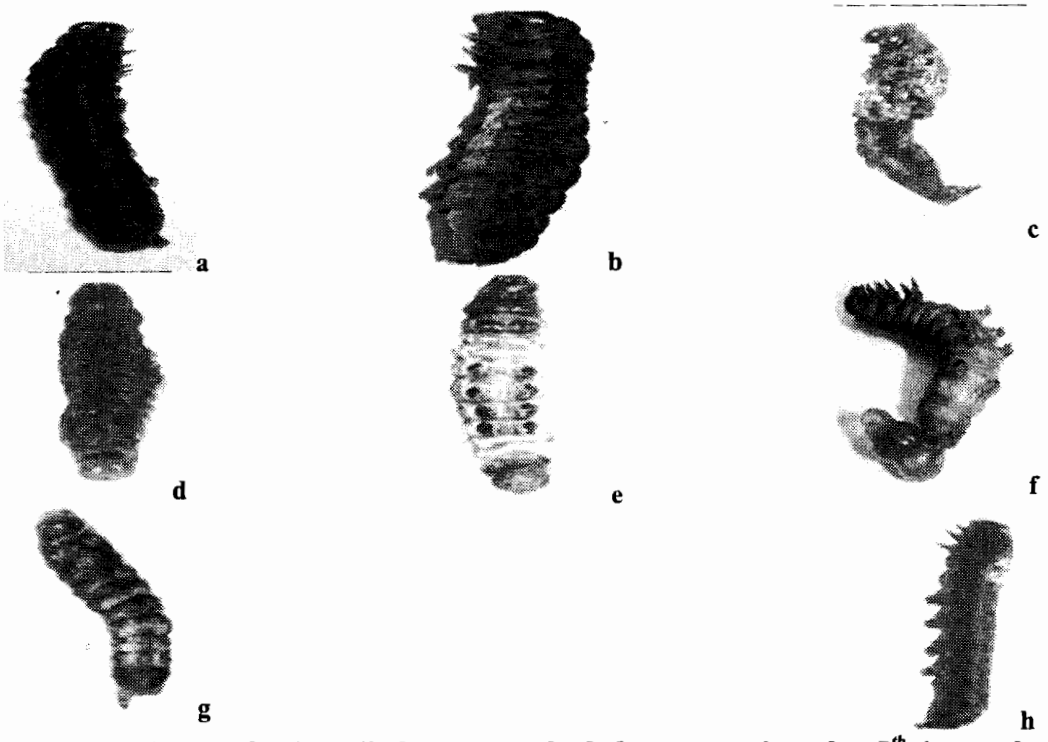


Fig. 2. Abnormalities in *S. littoralis* larvae resulted from exposing the 5th instar larvae to chlorfluazuron, using topical, injection and oral administrations, (a): score 0 normal, (b, c): score 6, (d, e, f, g) : score 5 and (h): score 4

Score (0)

Normal larvae the larval deformations include three different types.

Score (6)

Larvae became inactive, shrank and gradually stopped feeding showed in Fig. 2b.

Larvae failed in molting and completely paralyzed (Fig. 2c).

Score (5)

Larvae with partially extrusion of the alimentary canal from the anus (Fig. 2f).2d-Ventral view.2e- Dorsal view. Larvae with deformed pro-legs score (2g).

Score (4)

Partially moulted larvae with a big batches of new cuticle without normal coloration (Fig. 2h).

Guyer and Neumann (1988) found that the moulting was apparently normal but the larvae after moulting did not develop properly and eventually starved to death when larvae of *Spodoptera littoralis* and *Heliothis zea* were treated by injection method with diflubenzuron.

The present results are in agreement with those obtained by Omatsu *et al.* (1991) who showed different types of morphological abnormalities during the larval, pupal and adult stages when moulted fifth instar of *S. litura* was treated with sublethal dosages of chlorfluazuron.

Pupal Stage

Pupation percentage

Results in Table 2 cleared that the injection technique caused the highest reduction in pupation percentage (41.85±3.55%) followed by topical application, which recorded (62.71 ± 5.53%) while oral method gave 81.00±2.81% for pupation percentage compared with the control (100%).

Pupal weight

Data in Table 2 indicated that all methods of administration caused reduction in pupal weight compared with the control (0.3562±0.017 g) whereas, topical application recorded (0.2236 ± 0.014 g) followed by oral and injection methods (0.2659 ± 0.013 and 0.2961 ± 0.028 g), respectively.

The same results were obtained by Perveen (2000) who showed reduction in the body

weight in the larvae and pupae after treatment of 5^{th} instar larvae of *S. littoralis* with sublethal dose of chlorfluazuron.

Pupal deformation

Data in Table 2 showed that the topical application caused the highest pupal deformation percentage (26.07 ± 5.89%), while oral method recorded the lowest pupal deformation percentage (3.73±1.07%).

The same trend of results was recorded by Moftah and El-Awami (2004) who found that the highest numbers of abnormal pupae and adults were recorded when *Spodoptera littoralis* were treated with teflubeozuron by topical application and feeding method in the laboratory.

Pupal deformation types which detected in this study were photographed in Fig. 3 and described as follows:

Score (0)

Normal pupae.

Score (3)

Larvae failed to pupate (3 b and c).

Score (2)

Larval-pupal intermediate; larva with pupal head and larva with pupal abdomen (3d and e).

Score (1)

Pupal-larval intermediate; pupae with larval head and thoracic legs (3f and g).

Result in Table 4 showed that the abnormality rating was varied according to the methods of applying the growth regulator. The means in abnormality rating (AR) recorded were 3.71, 1.33 and 1.00 for topical application, injection and oral methods, respectively for the 5^{th} instar larvae of *S. littoralis* treated with chlorfluazuron. The calculations were carried out using the formula presented by Redfern *et al.* (1970) and Staal (1972). Topical application gave the highest abnormality rating, while the injection method gave a moderate effect, but oral administration gave the lowest rate.

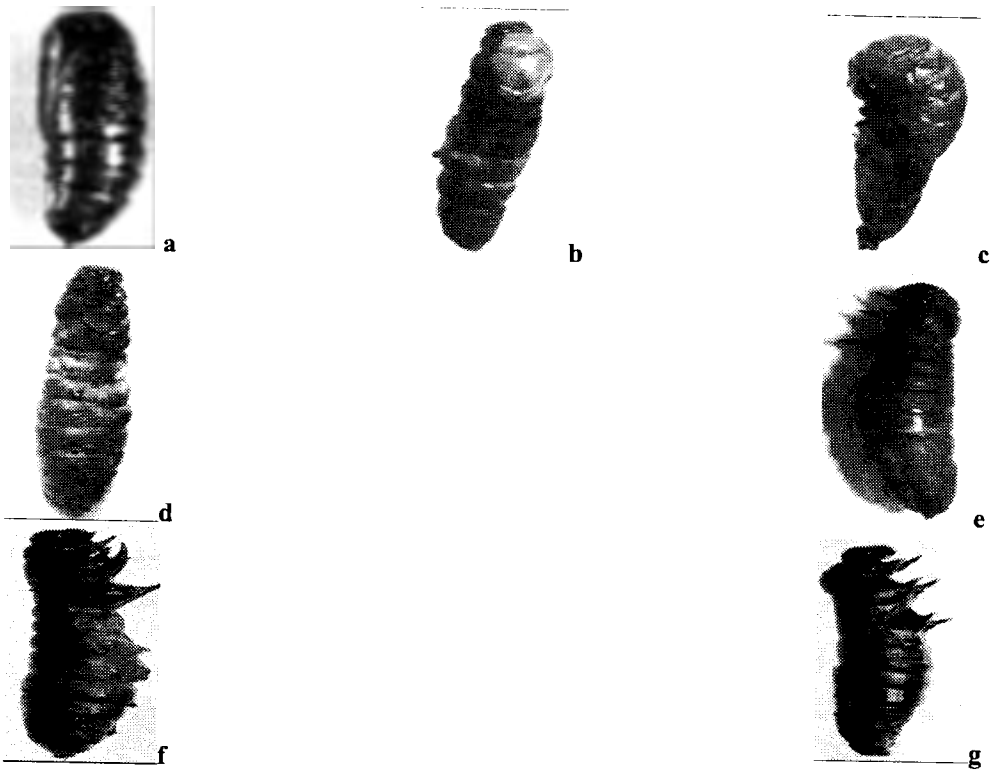


Fig. 3. Abnormalities in *S. littoralis* pupae resulted from exposing the larvae to chlorfluazuron, using topical, injection and oral administration, (a): score 0 normal, (b, c): score 3, (d, e): score 2 and (f, g): score 1

Table 4. Abnormality rating of different scores for larvae and pupae transformation of *S. littoralis* when the 5th instar larvae were treated with sublethal dose by different methods of application

Route of administration	Scores	No. of abnormal (malformed) individuals at indicated scores						Abnormality rating (AR)
		1	2	3	4	5	6	
Topical application (0.5 µg a.i. /larva)		1	1	-	3	1	1	3.71
Injection (1.5 µg a.i. /larva)		2	1	-	-	-	-	1.33
Oral (7 µg a.i. /larva)		1	-	-	-	-	-	1.00

Adult emergence percentage

Results obtained in Table 2 showed that the treated 5th larvae by oral method at LD_{25} (7 µg a.i./larva) achieved 48.33 ± 4.31emergence percentage. While, the emergence percentages recorded for injection and topical methods were 71.85 ± 6.82 and 87.06 ± 3.30(%), respectively compared to the control.

Adult Stage

Male longevity

Data in Table 5 showed reduction in the longevity of male moth comparing to the control that recorded 12.65±0.27days. The injection method recorded the higher reduction 9.77±1.11 days, while the oral method gave the lowest decrease (11.61± 1.98 days) when the 5th instar larvae was treated with the LD_{25} of chlorfluazuron.

Female longevity

Longevity of female moth that includes pre-oviposition, oviposition and post- oviposition periods are shown in Table 5. Results cleared that, all methods of application caused decreased in oviposition, post oviposition and longevity of female while, injection and oral methods did not affect the pre- oviposition. Female longevity in the three tested treatments was shorter as compared to the control which gave 10.03 ± 0.47days.

Topical application recorded the highest reduction in the longevity of female (4.83±0.36 days) followed by oral and injection methods that manifested 6.29±0.61 and 8.4± 0.24 days, respectively.

These results agree with those of Pineda *et al.* (2009) who found reduction in adult longevity through oral exposure of *Spodoptera littoralis* (Boisd.) for the treatment with methoxyfenozide and azadirachitin.

Fecundity of female moth

The obtained data showed a highly significant reduction of fecundity by injection administration LD_{25} (1.5µg a.i. /larva) which recorded 372.97 ± 78.8 eggs /female moth followed by oral and topical application which recorded 521.2 ± 67.07 and 791.4±59.32 eggs/ female moth, respectively compared with control 1749.6±278.8 eggs/ female moth (Table 5).

These results are in agreement with the findings of Aldebis *et al.* (1998) who showed reduction in fecundity of *Spodoptera littoralis* when they treated the 5th instar larvae with flufenoxuron by oral method. The same effect was not observed for adults that had been treated as larvae by topical application.

Hatchability (Fertility %)

Data in Table 5 indicated that reduction in hatchability percentages were noticed by the three used methods and the topical application gave the highest reduction 46.04±6.94% compared with control 94.00±1.213%.

Our results are confirmed with the results of Santiago-Alvarez *et al.* (1997) who observed reduction in egg hatchability of *Spodoptera littoralis* after treating the larvae with (LD_{60}) of flufenxuron by topical application.

Sterility (%)

Results in Table 5 showed that the topical application caused the highest sterility 71.91± 5.19% followed by oral and injection which recorded 44.92±4.68 and 27.5±6.77%, respectively compared with control.

Table 5. Adult biological parameters as affected by exposing the 5th instar larvae of *S. littorlais* to sublethal dose of chlorfluazuron using different routes of administration

Parameters (Mean± S.E.)	Adult longevity (day)		Oviposition periods (day)			Fecundity (egg no./female)	Hatchability (%)	Sterility (%)
Route of administration	Male	Female	Pre-oviposition	Oviposition	Post-oviposition			
Topical application (0.5 µg a.i. /larva)	$10.57^{bc} \pm 0.35$	$4.83^{d} \pm 0.36$	$1.69^{c} \pm 0.27$	$2.00^{b} \pm 0.44$	$1.14^{b} \pm 0.25$	$791.40^{b} \pm 59.32$	$46.04^{b} \pm 6.94$	$71.91^{a} \pm 5.19$
Injection (1.5 µg a.i. /larva)	$9.77^{c} \pm 1.11$	$8.4^{b} \pm 0.24$	$2.61^{ab} \pm 0.11$	$3.06^{b} \pm 0.28$	$2.73^{a} \pm 0.17$	$372.97^{c} \pm 78.8$	$66.03^{b} \pm 8.67$	$27.5^{c} \pm 6.77$
Oral (7 µg a.i. /larva)	$11.61^{ab} \pm 1.98$	$6.29^{c} \pm 0.61$	$2.45^{b} \pm 0.26$	$2.56^{b} \pm 0.46$	$1.28^{b} \pm 0.46$	$521.22^{bc} \pm 67.07$	$72.29^{b} \pm 6.16$	$44.92^{b} \pm 4.68$
Control (0.0 µg a.i. /larva)	$12.65^{a} \pm 0.27$	$10.03^{a} \pm 0.47$	$2.66^{a} \pm 0.32$	$4.17^{a} \pm 0.49$	$3.20^{a} \pm 0.54$	$1749.64^{a} \pm 278.8$	$94.00^{a} \pm 1.213$	.....
P	*	**	**	***	***	***	*	***

-Newly moulted 5th instar larvae of laboratory colony of *S.littoralis* were used.
- *: significant at p<0.05, ** highly significant at p<0.01 and ***: very highly significant at p<0.001.
-Means followed by similar letters indicate insignificant differences at 0.05 level of probability.

The data clarified that when chlorfluazuron was applied to the 5th instar larvae of *S. littoralis* using different methods of applications; topical application was the most efficient on basis of the LD_{50} values compared to oral administration or injection. Also, in respect to biological responses, topical application was the most effective on most parameters. The three used techniques caused different deformation levels in larval and pupal stages and topical application caused the highest percentage of deformations.

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السمية والتأثيرات البيولوجية لمركب الكلورفلوزيرون على دودة ورق القطن

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تناولت الدراسة معاملة العمر اليرقى الخامس لدودة ورق القطن بثلاثه طرق معاملة مختلفة وهى: المعاملة الموضعية، عن طريق حقن الأرجل البطنية وعن طريق الفم باستخدام مبيد الكلورفلوزيرون (منظم نمو حشرى)، كذلك تناولت الدراسة تأثير طرق المعاملة الثلاثة بالمركب على بعض الصفات البيولوجية من المعاملات حيث فترة العمر اليرقى، العذارى، الحشرات الكاملة، كذلك تشوهات اليرقات والعذارى وكذا ما قبل وبعد فترة وضع البيض للإناث بالإضافة إلى نسبة الخصوبة والفقس والعقم، أوضحت النتائج أن المعاملة الموضعية كانت أكثر فاعلية حيث وصلت الجرعة نصف المميتة للأفراد إلى ٠,٨٧ ميكروجرام مادة فعالة لكل يرقة، بينما كانت الجرعة نصف المميتة للمعاملة عن طريق الحقن والفم ٣,٥٩-١٣,٢٠ ميكروجرام مادة فعالة لكل يرقة على التوالي، ومن الناحية البيولوجية أوضحت النتائج أن فترة الطور اليرقى تأثرت بدرجة معنوية بكل من طرق المعاملة الثلاثة، حيث طالت فترة الطور اليرقى إلى٩,١٧ يوما عند المعاملة الموضعية فى حين أدت المعاملة بالحقن إلى إطالة فترة العمر اليرقى إلى ٧,٥٥ يوما بينما كانت فى المعاملة عن طريق الفم ٧,٧٦ يوما مقارنة بتجربة المقارنة ٦,٦٥ يوما، وأيضاً أثرت طرق المعاملة بدرجة معنوية على نسبة التعذر ووزن العذراء حيث أدت المعاملة الموضعية إلى خفض وزن العذارى إلى ٠,٢٩٦١ جرام فى حين أدت المعاملة بالحقن إلى خفض وزن العذارى إلى ٠,٢٢٣٦ جرام، بينما المعاملة عن طريق الفم أدت إلى الخفض بنسبة ٠,٢٦٥٩ جرام مقارنة بتجربة المقارنة حيث كانت ٠,٣٥٦٢ جرام، كما أوضحت المعاملات الثلاث تأثيرا معنويا فى ظهور تشوهات فى اليرقات والعذارى المعاملة حيث أدت المعاملة الموضعية إلى ظهور أكبر نسبة تشوهات فى اليرقات والعذارى والتى وصلت إلى ٣٤,٥٦ و٢٦,٠٧% على التوالى بالمقارنة بطرق المعاملة الأخرى وتجربة المقارنة. تبين ان المعاملة الموضعية كان لها تأثير كبير فى قصر عمر إناث الحشرة الكاملة، كما أثرت كل طرق المعاملة على متوسط عدد البيض الموضوع لكل أنثى حيث أوضحت النتائج أن طريقة الحقن بالأرجل البطنية كان لها تأثير كبير فى خفض عدد البيض إلى ٣٧٢,٩٧ بيضة لكل أنثى مقارنة بتجربة المقارنة ١٧٤٩,٦٤بيضة لكل أنثى بينما خفضت المعاملة عن طريق الفم والمعاملة الموضعية عدد البيض إلى ٥٢١,٢٢ و٧٩١,٤٠ بيضة لكل أنثى على التوالى، كذلك أدت المعاملة الموضعية إلى خفض نسبة الفقس إلى ٤٦,٠٤٪ وزيادة نسبة العقم للحشرة الكاملة بنسبة ٧١,٩١٪ بالمقارنة بتجربة المقارنة وطرق المعاملة الأخرى. لذلك فقد أمكن ترتيب طرق المعاملة من حيث الفاعلية على العمر اليرقى الخامس كالآتى: المعاملة الموضعية ، الحقن والمعاملة عن طريق الفم.

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