The Latent Effect of Least Minimized Doses of Three IGIs Compounds on Certain Biophysiological Parameters and Formation of Larval Body Wall of *Spodoptera littoralis*, (Boisd.) (Noctuidae, Lepidoptera)

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ABSTRACT

The present article demonstrates the effects of least minimized doses of tested IGIs compounds on the estimated values of haemolymph total proteins and fats; activity of GOT and GPT enzymes and formation of the body wall of the treated larvae of the cotton leaf worm (*S. littoralis*). The results decleared that Flufenoxuron applied at its least minimized dose (LC_4) ratherly decreased the calculated values of mean cuticle dry weight, cuticular chitin and cuticular protein; coincided with increased percentage of cuticular inhibition, besides the revealed unprofitable drastic effects on the studied biological and physiological parameters of the insect i.e., haemolyph total protein; fats and activity of GOT and GPT enzymes. Whereas, these drastic effects interupted larva moulting and normal development of the following stages of this insect and furtherly, inhibited the continuity of the life cycle at the end of 2nd generation. The inspected less effectuality of the least minimzed doses of the tested Chlorfluazuron and Lufenuron were merely the same, and were greatly pronounced at the end of the 5th and 6th generations in respect, indicating also the complete inhibition of the life cycle of the treated insect.

INTRODUCTION

Cotton, the main cash crop in Egypt, s liable to attack with many pests starting from seedlings to harvest, which cause a great damage to the plants growing during the season. Amongst, the cotton leafworm, *Spodoptera littoralis* (Boisd.) is the most serious and destructive phytophagous lepidopterous insect pest in Egypt, for cotton plants as well as other hosts of field and horticultural crops. The need to develop novel alternatives 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. Insect Growth Inhibitors (IGIs) which are considered nowadays one of the mainly components of IPM programs.

The mode of action of these compounds was studied by many investigators (Grosscurt and Anderson, 1980 and Mesbah *et al.*, 1990a,b, 1991, and 1999a). Such compounds that disrupt the metabolism of chitin appeared to offer the prospect of abroad spectrum of insect control. Particularly, the cuticle which provides a skeletal support and serves as a barrier protecting them from the potentially hostile environment.

In this respect, the efficiency of some new insect growth inhibitors (IGIs) against *S. littoralis*; besides their effects on the cuticular dry weight, chitin content and cuticular protein of the larvae was recorded in the works of Mulder and Gizswijt (1973) Soli *et al* (1976), Auda (1986), Mesbah *et al* (1991 and 1999a), Tayeb *et al* (1992) and Haroun (1993).

Therefore, the present work was initiated to study the efficiency of tested least minimized doses of three IGIs compounds on certain objectives: the formation of larval body wall, haemolymph proteins and fats and the activity of GOT and GPT enzymes.

MATERIALS AND METHODS

Rearing technique:

A susceptible strain of *S. littoralis* was maintained under laboratory hygrothermic conditions of 25° ± 1°C and 75 ± 5% R.H. 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.

The Tested Compound:

1. Lufenuron (Match® 5% EC)

Chemical name:

(RS)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)=(phenyl)3]-2,6-difluorobenzamide.

2. Flufenoxuron: (Cascade® 10% EC)

Chemical name:

1-[4-(2-chloro-α,α,α (trifluoro-p-tolyloxyl)2-fluoroPhenyl]-3(2,6-difluorobenzoyl) urea.

3- Chlorfluazuron (Atabron,® IKI-7899-5% EC)

Chemical name:

1-[3,5-dichloro-4-(3-chloro-5-trifluoromethyl-2-pyridyloxy phenyl]- =3-(2,6-difluorobenzoyl) urea.

Effect of IGIs on chitin synthesis of S. littoralis larvae

For studying the effects of the tested compounds on chitin biosynthesis of 4th-instar larvae, dilution of each separately tested IGI at the rate of its deduced least efficient dose (LC_4) were used. These minimized doses of Lufenuron, Flufenoxuron and Chlorfluazuron were 0.1, 0.003 and 0.02 ppm, respectively. These concentrations were calculated based on preliminary bioassay experiments that have been done to determine the values of LC_{50} for each tested compounds. Castor-bean leaves were dipped for 30 seconds in each of the prepared dilutions. After leaf dryness the larvae were fed on the treated leaves for 24 hours. Along 4 days post treatment the treated 4th-instar larvae were daily supplied with fresh untreated leaves. Sampling was started after the 7th day post - larval treatment. Therefore, a group of 15 treated larvae were taken from each treatment and subdivided into 5 replicates of 3 larvae, which had been placed and kept in a small vial in a deepfrezer (-20°C). The same procedure was followed with control group which has been radomisely selected from the batch of the untreated larvae.

Preparation of the cuticle

Larvae were taken out of the deepfrezer and allowed to come to room temperature before dissection, placed in distilled water; the head of each larvae was cut off; the dorsal side of the body was longitudinally opened and the digestive system, as well as the rest of the body contents were accurately and completely removed. The cuticle was rinsed twicely in cold distilled water before drying. The dry weight was determined by drying cuticles at 110°C to a constant weight. Total chitin was determined by

heating the cuticle in sealed tubes containing 2.5% Sodium hydroxide (NaOH) at 110°C for two hours. The residue of boiled cuticle in NaOH was washed successively in water, hydrochloric acid, ethanol and diethyl ether, respectively (Hackman and Goldberg, 1971). The remaining insoluble material, assumed to be chitin, was then dried to a constant weight (chitin value). Total protein was determined by subtracting this value from the cuticular dry weight. The following equation was used to calculate the rate of chitin synthesis inhibition: $\frac{a-b}{a} \times 100$

Where: a = chitin weight of untreated larvae; b = chitin weight of the treated larvae.

Statistical analysis was run to check the significance of differences between treatments (Goulden, 1952) the least significant differences (L. S.D.) were determined according to Duncan (1955).

Enzyme activities

Preparing samples for enzyme activity

Castor-bean leaves were dipped for 30 seconds in the prepared aqueous solutions of each of the least minimized doses of the tested IGIs, left for one hour in the room temperature to dry before being offered to the 4th instar larvae for 48 hrs. Thereafter, larvae were transferred to fresh untreated leaves for another 24 hrs. for the biochemical assays, the sampled larvae were placed in clean jars and let to starve for four hours. The starved larvae were homogenized in distilled water (5 larvae/5 ml distilled water) using ataflon homogenizer surrounded with a jacket of crushed ice for 3 minutes. Homogenates were centrifuged at 3500 r.p.m for 10 minutes at 5°C and the supernatants were used directly for enzyme assay.

Transaminases (GOT & GPT) assay

The activity of glutamic oxaloacetic transaminase (GOT) and glutamic pyravic tranaminase (GPT) enzymes were determined spectorophotometically in the supernatant of the cotton leafworm using commercial product Kits supplied from Diamond Co. Egypt.

GOT activity was measured according to the following principle:

$$\text{L-Aspartate} + \alpha\text{-keto glutarate} \xleftrightarrow{\text{GOT}} \text{L-glutamate} + \text{Oxaloacetate}$$

While, GPT activity was determined according to the following principal:

$$\text{L-alanine} + \alpha\text{-keto glutarate} \xleftrightarrow{\text{GPT}} \text{L-glutamic} + \text{pyruvic}$$

The product of each reaction was used as an indicator for the enzyme activity.

Procedure

The tube sample contains 0.1 ml of supernatant, 0.5 ml of substrate (L-Asparate) while the blank tube sample contains 0.1 ml distilled water and 0.5 ml substrate solution. Samples and blank tubes were incubated for 30 min. at 37°C using water bath incubator. After incubation, 0.5 ml of the colour reagent (2,4 dinitrophenylhydrazine) was added and mixed. The tubes were allowed to stand for exactly 20 min. at 20- 25°C, then amounts each of five ml of 0.4 mol/NaOH were added to stop the reaction. The colour absorbance values were spectrophotometrically measured against blank after five min. at 550 nm. The same procedure that done determining GOT activity was followed for determination of GPT activity by using the other substrate (L-alanine).

The same activity of GOT or/and GPT transaminse enzyme is expressed as activity unit/liter of the cotton leafworm homogenate U/1 (as the kit included conversion table).

Determination of total haemolymph fats

The estimation of haemolymph total fats was carried out according to Folch *et al.* (1957) with some modifications as follows: 5 ml methanol (Reagent grade) were added to one ml of larval haemolymph and the mixture was shook for five minutes. Later, 10 ml of chloroform were added to the mixture and was shook again for three minutes. The suspension was filtered and the final filtrate was washed with 0.88% KCl solution equal to $\frac{1}{5}$ of the filtrate volume (instead of using water as mentioned in Folch method to remove the nonlipid materials). The phases of fractionation process were allowed to separate in a 20 ml separating glass funnel left overhight at 4°C, then the chloroform layer containing the lipid fraction was removed, transferred to a known weighed glass watch after filtration through filter paper containing unhydrous potassium sulphate to absorb any moisture. The glass watch with the filtered chloroform-lipid fraction was left till dryness and then weighed. The deduced difference by substracting the weights of the glass watch with or/and without lipid fraction indicated the weight of fat contents in one ml of the haemolymph.

Determination of total haemolymph proteins

According to Lowery *et al.* (1951) the following procedure was used: A volume of 0.1 ml of the larval haemolymph was taken, completed to 10 ml by adding distilled water. That final volume was divided to three replicates; each was shook for one minute. Then, 0.1 ml was taken from each replicate; mixed with three ml of reagent (C). Herein, reagent (C) is a

mixture of 50 ml reagent (A) (2% Na_2CO_3 in 0.1 ml 1N- NaOH) and 1 ml of reagent (B) (0.5% $CuSO_4$ in 1% sodium potassium tartrate). Ten minutes later, an amount of 0.3 ml folin reagent (solution of sodium tungstate and sodium molybdate in phosphoric and hydrochloric acid) was added to the mixture. After 30 minutes the absorption was measured by spectophotmeter at 750 nm. The mixture of distilled water plus reagent (C) and folin reagent were used as blank. For the standard curve, the plots of standard albumine concentration (X) against optical density (Y) proved to be linear with a K value of 4.2×10^{-3} slope. The concentration of protein in the sample was calculated from the standard curve by the following equation: mg protein = $\frac{Absorbance}{K}$

RESULTS AND DISCUSSION

Effect of least minimized doses of the tested IGIs on the formation of larval body wall of S. littoralis

Cuticular dry weight

The normal or/and interrupted formation of the body wall of the treated larvae was determined in the full grown larval stage. Whereas, the 4th-instar larvae were fed on leaves treated with the least minimized doses of the tested IGIs. Later the dry weight of their cuticules was estimated when they reached the stage of full grown larvae (mature larvae). From Table (1) it is clear that the cuticular dry weight of the untreated larvae, to a more or less extent, was comparatively higher than that of the treated ones showing gradual increase from the beginning of parent generation (8.6 mg), reached the maximum (16.3 mg) in the 3rd one, then lowered and become merely equal to that of parent generation (8.8-8.9 mg) in the following 4th, 5th and 6th ones. Similar trend of results was detected for the other estimated parameters of cuticular chitin and cuticular protein of larval body wall. It was also noticed that the cuticular dry weight of 3rd generation of those untreated larvae (16.3 mg) was as much as twice of those treated 3rd generation ones either with Lufenuron or with Chlorfluazuron least test concentrations (7.6 and 7.8, respectively).

The lowest estimated values of reduced cuticular dry weight was observed for Flufenoxuron least test concentration since that weight comprised 4.2 mg for the larvae of parent generation, 6.3 mg and 5.9 mg for the treated larvae of first and second generations in respect. Whereas, the larvae didn't complete their life cycle in the 3rd generation. In this

concern, the least tested concentrations of Lufenuron indicated somewhat lowered values of measured larval cuticular dry weight than that of Chlorfluazuron in the treated 5th generation of the insect. Deul *et al.* (1978) studied the effect of diflubenzuron on the dry weight of KOH-treated cuticles of *P. brassica* 5th-instar larvae and showed that the increase in weight during development was followed by a decrease just before and at pupation. Mesbah *et al* (1999a), found that all performed insecticide/IGI treatments significantly reduced the measured cuticular dry weight. The highest rate of reduction was observed for Profenofos/Chlorfluazuron, followed by Methomyl/Diflubenzuron and Hexaflumuron.

Cuticular chitin

The demonstrated results in Table (1) show the revealed effects of the tested least minimized doses of IGIs on cuticular chitin. All the tested least minimized doses of IGIs compounds significantly decreased the calculated means of cuticular chitin, compared to untreated control. The significant values of reduced weight of cuticular chitin was observed for Flufenoxuron (0.003 ppm), along the extending period from parent generation till the end of the 2nd one-2.5, 4.9 and 4.2 mg, respectively, compared to the fluctuating values of 5.8 mg in parent generation, 9.5 mg in the 3rd and 6.5 in the 6th generation of untreated larvae. The least tested dose of Chlorfluazuron (0.02 ppm) indicated somewhat higher significant values of reduced weight of cuticular chitin calibrated from 5.2 mg in parent generation up to 6.2 mg in the 5th one. Lufenuron dose (0.1 ppm) was more efficient in reducing significantly the weight of cuticular chitin than Chlorfluazuron, which calibrated from 5.5 mg in parent generation up to 3.6 and 4.7 mg in the 5th and 6th ones. Similarly, numerous workers confirmed the adverse effect of tested IGIs compounds on chitin synthesis. (Mulder and Gizswijt, 1973; Ishaaya and Casida, 1974; Solli *et al*, 1976; Grossurt and Anderson, 1980; Neumann and Guger, 1983; Auda, 1986; Massoud, 1990; Tayeb *et al*. 1992 and Haron, 1993). Auda (1986), reported on the significant difference between untreated and treated 6th-instar larvae of *S. littoralis* with Chlorfluazuron, which inhibits almost completely the normal increase of chitin content and in a lesser amount the protein content. Mesbah *et al*. (1999a) determined the higher reduction in chitin biosynthesis for Profenofos/Chlorfluazuron and Hexaflumuron at both tested levels of calculated LC_{16} and LC_{25}. The tested concentrations of Chlorpyrifos/Hexaflumuron, Hexaflumuron and Methyomyl/Diflubenzuron gave less significant reduction.

Cuticular protein

The exhibited results in Table (1) also declear the latent effect of least minimized concentration of inspected IGIs on the calculated values of cuticular protein of treated larvae. In general, the calculated values of cuticular protein for the untreated larvae were, more or less, higher than these calculated values in the adopted IGIs treatments, throughout, going on generations post parents one. Comparatively, Flufenoxuron least minimized dose decreased the estimated values of cuticular protein 1.8 mg in parent generation, continued that decrease up to 1.4 and 1.2 mg in 1st and 2nd ones, before the incidence of complete inhibition of life cycle development at the end of 2nd generation (Table 1). Chlorfluazuron and Lufenuron least minimized doses gave merely equal effects during the period of parent generation and the following three ones, but from the beginning of 4th generation and during the 5th one, Chlorfluzuron showed a higher cumulative latent effect and reduced the values of cuticular protein up to 0.9-1.5 mg, before the complete inhibition of development at the end of 5th generation. Consequently, that phenomenon of life cycle inhibited development was recorded for Lufenuron at the end of 6th generation of treated larvae. Such results were in agreement with those of Ker (1977) on locust, Grosscurt and Anderson (1980) on *Leptinotorsa decmlineata* and Cox and Willis (1985) on *Hyalophora cecropia*. In addition, Massoud (1990) and Tayeb *et al.* (1992) investigated the effect of the tested LC_{25} values of the IGIs: Polo, Chlorfluazuron and XRD-473 on cuticular dry weight, chitin and protein and percentage of chitin inhibition in 4th-instar larvae of *S. littoralis* and showed that all the conducted treatments strongly affected the normal synthesis of cuticular chitin and protein of body wall after three days post-treatment.

Mesbah *et al.* (1999a) found that the evaluated IGIs and/or IGI/insecticides combinations decreased the rates of cuticular protein build up. Chlorfluazuron, Diafenthiuron, Profenofos/Chlorfluazuron and Hexaflumuron gave the higher significant reduction of percentages of protein inhibition, which ranged between 40.6% and 53.4%. The least significant reduction of the percentages of protein inhibition (14.8, 23.7 and 25.8) were observed for each of tested Profenofos/Chlorfluazuron, Methomyl/Diflubenzuron and Hexaflumuron at the level of LC_{25}, respectively.

Cuticle inhibition

Results in Table (1) clarify the highly significant differences between all the run IGIs treatments and control. The tested least minimize dose of Flufenoxuron ascertained the previous detected strong latent effects, since

the calculated percentage of cuticle inhibition reached a highest value (from 60.1% in the parent generation, to 83.5% in the 2nd generation) which in sequence elucidated the faster incidence of life cycle inhibition after comparative short interval of both treated generations post parent one. The tested least doses of Chlorfluazuron and Lufenuron proved to be the secondly effective ones showing less strong latent effects on the rate of chitin inhibition as previously revealed for the other studied biological parameters. Herein, the calculated values of chitin inhibition amounted to 50.4 and 54.4% in parent generation; increased to 79.9 and 75.1% in the 5th and/or 6th generations due to treatment by Chlorfluazuron and Lufenuron, respectively.

Finally, from the above cited results it could be concluded that Flufenoxuron at its least minimized dose gave rather decrease of the calculated mean cuticle dry weight, cuticular chitin and cuticular protein, coincided with increased percentage of cuticular inhibition. These drastic effects interrupted the larval moulting and normal development of the insect and furtherly prevent the treated larvae of 2nd generation from completing their development. The lesser effect of the least minimized doses of both tested Chlorfluazuron and Lufenuron was merely the same, but that effect was greatly pronounced in the 5th and/or 6th generations in respect which also indicated discountinued and incomplete life cycles.

Effect of least minimized doses of the tested IGIs on the estimated values of haemolymph total proteins and fats of the treated larvae

Results in Table (2) illustrate the detected effects of the used least minimized doses of tested IGIs on the fluctuating levels of total haemolymph proteins and fats of the treated 4th-instar larvae. Generally, the estimated levels of total haemolymph proteins and fats were higher in the larvae fed on the untreated leaves.

These values of total haemolymph proteins and/or fats, to a more or less extent, were lower according to the tested IGI compound. Herein, the level of total haemolymph proteins was the lowest in the treated parents larvae with Chlorfluazuron (21.5 mg/ml). Nevertheless, that lowered level started to rise up to 27.9 mg/ml in 2nd generation (Table, 2) and furtherly to 62.5 mg/ml in 4th generation. Vice versa, the level of total haemolymph fats was the highest (113.3 mg/g) in the larvae of treated parent with the same compound, then followed by a decreased values of 84.67 mg/g in the 2nd and 53.67 mg/g in the 4th ones. That prominent irregular fluctuations of both estimated values of total haemolymph proteins and/or fats along the following generations of developing treated larvae declare the unprofitable changes

in going on biophysiological processes which ended with the inhibition of development at the beginning of 5th generation of the insect (Table 2).

The same trend of results was revealed for the other evaluated IGIs compounds: Flufenoxuron and Lufenuron, i.e., the comparatively lowered values of estimated total haemolymph proteins informed of higher values of total haemolymph fats in the treated larvae of raised generations (Table 2). In this respect, Flufenuxuron gave a faster drastic effect on both estimated values of total haemolymph protein and/or fats, which amounted to 31.98 and 64.67 mg/ml; and 21.5 and 70.67 mg/ml in parent and 2nd generation, respectively, indicating a complete tetrmination of insect development at the end of 2nd generation. That phenomenon of development inhibition due to the irregular changes of proteins and fats synthesis was observed for the treated larvae with lufenuron at the end of 6th generation (Table 2).

Therefore, it could be concluded that the comparatively reduced amounts of total haemolymph proteins was in parallel to relatively higher but still lower amounts of total haemolymph fats than the untreated larvae, due to treatments of IGIs least minimized doses which gave faster or/and, relatively slower drastic effects reflected on the synthesis of total haemolymph proteins and fats, which are usually utilized in lipoproteins assimilation for yolk deposition in the developing oöcytes in ovarioles of females and completion the development of formed spermatocytes in the testes of males. Sequentially that reflects on the reproductive potential of the treated insect and causes a final sterility of emerged adult-moths or distinct depression of the viability of the deposited eggs; ending in a complete termination of life cycle continuity. Moreover, the statistical analysis of data showed highly significant differences between the measured values of total haemolymph proteins and fats due to the performed treatments of tested IGIs.

Identical results were detailed in the work of Mesbah *et al.* (1999b) Adb El-Lattif (2000). Ahmed *et al.* (1991) found that the decreasing of worm growth and reduction of soluble protein were correlated to the increase of Diflurobenzuron concentrations. Also the *in vitro* effect of such compound exhibited an increase in activities of transaminases (GOT and GPT) of the immatures and adult of *L. terrestris*.

Effect of least minimized doses of the tested IGIs on the activity of GOT and GPT enzymes in the treated larvae of S. littoralis

The transaminase enzymes, glutamic oxaloacetic transminase (GOT) and glutamic pyruvic transminase (GPT) have been correlated with protein

synthesis in many tissues in the developing insects. Changes in their level are used as indictors of the going on metabolism of proteins and amino acids. Therefore, the following results of this part explain the effect of each of the tested IGIs least minimized dose on the activity of the inspected transaminase enzymes.

From Table (3), it is clear that the determined amount of GOT in the untreated larvae indicated significant increased values in the parents and along the periods of the 2nd, 4th and 6th generations, respectively. While, in the other performed treatments these values were significantly more or less decreased or/and increased than that detected for untreated larvae along the same period of inspections. The amount of the inspected GOT enzyme showed increased values in the treated 2nd and 4th generations with Lufenuron and Chlorfluazuron. Also, in case of Chlorfluazuron least minimized dose the detected amount of GOT in the treated larvae reached 325.67 µl/ℓ in 4th generation, that amount was as much as three time of that of the 4th generation larvae treated with lufenuron least dose (103.49 µl/ℓ) compared to that detected for untreated larvae (325.65 µl/ℓ). The determined amounts of GOT for the treated larvae with flufenxuron were somewhat lower (122.11 and 183.47 µl/ℓ) in the parents and second generation, in respect. Again, the larvae in this treatment did not complete their life cycle and died before giving a 3rd sequential one.

The lowest amount of GOT enzyme was determined in Lufenuron treatment of the parental generation (77.93 µl/ℓ). Nevertheless, that amount started to rise up in the treated larvae of the 2nd generation and comprised 324.6 µl/ℓ, then decreased sharply up to 103.49 and reached the least value of 75.46 µl/ℓ in those treated larvae of the 6th generation.

Table (3) illustrate that all the tested IGIs least minimized doses, significantly, rather decreased the inspected amounts of that enzyme in the consequently treated larvae by Flufenoxuron, Lufenuron and Chlorfluazuron along the period of generations development than that of the untreated larvae. Whereas, the least detected amounts of that enzymes were recorded for Flufenoxuron treatment in comparison to both treatments of Lufenuron and Chlorfluazuron, respectively. It is also shown in Table 3, the significant least reduction of detected GPT amount (24.45 µl/ℓ) was achieved at the LC_4 level (0.003 ppm) of tested Flufenoxuron. This least dose of Flufenoxuron gave the highest accumulated effect since the larvae did not complete the 3rd sequential generation. Identical results of conducted research works for evaluating the efficacy of various IGIs compounds on the

enzymes activity of numerous species of insect-pests, in particular the lepidopteran ones, are mentioned in the works of many authors.

A close relation was found in insects between protein synthesis and transaminase's levels synthesis and probably involved in the synthesis of amino acids during metamorphosis, thereby, GOT and GPT are often used as indicators of the metabolism of protein and amino acids (Wigglesworth, 1973).

Abdel-Hafez *et al.* (1988) reported that Diflubenzuron and Triflumuron drastically inhibited protein synthesis of treated larvae of *S. littoralis*. El-Kordy *et al.* (1995) mentioned that Pyriproxyfen, Flufenoxuron, and Tebufenozid could be considered as inhibitor agents for protein synthesis in *S. littoralis*. They stated that Pyriproxyfen, Flufenoxuron and Tebufenozid, caused a significant reduction in the level of the total protein. GOT enzyme was significantly increased, while there was a significant reduction in the level of GPT after treatment.

Finally, all the tested minimized doses of the evaluated compounds decreased the activity and the detected amounts of GOT and GPT enzymes in the consequent treatment of larvae. That result explains the occurred and observed reduction of the haemolymph protein due to the going on effect of such compounds.

Table (1): **The delayed latent effect of least minimized doses of the tested IGIs on cuticular dry weigl t, cuticular chitin, cuticular protein and chitin inhibition of t e treated 4th instar larvae of *S. littoralis*.**

Treatment	The used least minimized dose ppm	Mean cuticular dry weight (mg)	Mean cuticular chitin (mg)	Mean cuticular protein (mg)	Mean chitin inhibition (%)
		Parent generation			
Lufenuron	0.100	8.1a	5.5a	2.7ab	54.4ab
Flufenoxuron	0.003	4.2c	2.5b	1.8b	60.1a
Chlorfluazuron	0.020	8.1ab	5.2a	2.9b	50.4b
Control (check)	-	8.6bc	5.8a	2.8a	-
Significance		*	**	**	**
		1st generation			
Lufenuron	0.100	7.1ab	5.3ab	1.8	59.9b
Flufenoxuron	0.003	6.3	4.9	1.4	73.1a
Chlorfluazuron	0.02(	8.1ab	5.9a	2.2b	54.1b
Control (check)	-	8.9	5.3	3.6	-
Significance		N.S	*	N.S	**
		2nd generation			
Lufenuron	0.100	5.9b	4.7ab	1.7	61.9c
Flufenoxuron	0.003	5.9b	4.2b	1.2	83.5a
Chlorfluazuron	0.020	7.9ab	6.0a	1.9	71.9b
Control (check)	-	9.5a	6.0a	3.5	-
Significance		*	*	N.S	**
		3rd generation			
Lufenuron	0.100	7.6b	5.9b	1.7b	70.7b
Flufenoxuron	0.003	-	-	-	-
Chlorfluazuron	0.020	7.89b	5.5b	2.3b	76.7a
Control (check)	-	16.3	9.5a	6.8a	-
Significance		**	**	*	**
		4th generation			
Lufenuron	0.100	7.7	4.9	2.8	73.9b
Flufenoxuron	0.003	-	-	-	-
Chlorfluazuron	0.020	6.6	5.7	0.9	79.3a
Control (check)	-	8.8	5.2	3.6	-
Significance		N.S	N.S	N.S	**
		5th generation			
Lufenuron	0.100	5.9a	3.6ab	2.3	73.9b
Flufenoxuron	0.003	-	-	-	-
Chlorfluazuron	0.020	7.8b	6.2b	1.5	79.9a
Control (check)	-	8.9ab	6.3ab	2.6	-
Significance		*	*	N.S	**
		6th generation			
Lufenuron	0.100	6.4	4.7b	1.7b	75.1
Flufenoxuron	0.003	-	-	-	-
Chlorfluazuron	0.020	-	-	-	-
Control (check)	-	8.9	6.5a	2.4a	-
Significance		N.S	**	**	

N.S = Non significant * = Significant at 5% ** = highly significant at 1%

Table (2): The measured haemolymph total protein (a) and total fats (b) in the tested larvae of *S.littoralis* during the following generations of treatment with the least minimized doses of the tested IGIs.

Treatment	Concent. ppm	Parent generation		2nd generation		4th generation		6th generation	
		a	b	a	b	a	b	a	b
Lufenuron	0.100	30.56 b	245.00 ab	25.00 bc	72.67 bc	30.44 c	115.00 b	20.15 b	91 b
Flufenoxuron	0.003	31.98 b	64.67 c	21.50 c	70.67 c	--	--	--	--
Chlorfluazuron	0.020	21.15 c	113.30 b	27.90 ab	84.67 bc	62.50 a	53.67	--	--
Control (check)	--	36.50 a	382.70 a	34.00 a	179.67 a	53.57 b	284.33 a	32.50 a	295 a
Significancy		**	**	**	*	**	**	**	**

Table (3): The determined concentrations of GOT (a) and GPT (b) enzymes (unit/liter) in the treated larvae of *S.littoralis*, during the consequent generation of insects treated with the last minimized dose of the tested IGIs.

Treatment	The used least minimized dose (ppm)	Parent generation		2nd generation		4th generation		6th generation	
		a	b	a	b	a	b	a	b
Lufenuron	0.100	77.93 c	33.32 ab	324.60 a	107.00 b	103.49 a	34.92 b	75.46	39.63
Flufenoxuron	0.003	122.11 c	22.45 bc	183.47 b	24.45 c	--	--	--	--
Chlorfluazuron	0.020	217.36 b	39.20 c	310.63 a	128.00 c	357.67 b	264.55 a	--	--
Control (check)	--	361.24 a	42.60 a	358.42 c	124.30 bc	325.65 a	187.00	216.50	146.00
Significancy		**	**	**	**	**	**	**	**

* = Significant at 5%
** = High significant at 1%

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الملخص العربى

التأثير الكامن للجرعات فائقة التخفيض لثلاث مركبات من مثبطات النمو الحشرية على بعض القياسات البيوفسيولوجية وتكوين جدار جسم يرقه دودة ورق القطن *S.littoralis (Boisd.)*

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أثبتت نتائج الدراسة الحالية تأثير الجرعات فائقة التخفيض لمثبطات النمو الحشرية المختبرة على القيم المقدرة بنسب البروتين والدهون الكلية فى يرقة دودة ورق القطن كذلك على نشاط أنزيمى GPT, GOT ومكونات جدار جسم اليرقات المعاملة. وقد أوضحت النتائج أن الجرعات فائقة التخفيض من مركب الفلوفينوكسيرون خفضت إلى حد كبير القيم المحسوبة لوزن الكوتكل الجاف ووزن الكيتين وكذلك وزن البروتين بالإضافة إلى فاعليته الكبيرة فى خفض معدل تخليق الكيتين بالمقارنة مع المركبين الآخرين الكلورفلوزيرون واللوفينورون. كما أثر أيضاً على نشاط كل من أنزيمى GOT، GPT. بالإضافة لما سبق كان لمركب الفلوفينوكسيرون تأثير سريع وقوى على المقاييس البيوفسيولوجية التى أثرت على معدلات التطور الطبيعى وأدت فى تأثيراتها إلى فشل الاستمرارية فى دودة الحياة فى نهاية الجيل الثانى. بينما أدى استعمال كل من مركبى الكلوروفلوزيرون واللوفينورون إلى أحداث نفس النتيجة ولكن بمعدلات أبطأ فى نهاية الجيل الخامس أو الجيل السادس على الترتيب. كما سبق يتضح جدوى

استخدام هذه المركبات (الفلوفينوكسيرون والكلوروفلوزيرون واللوفينورون) ضمن برامج المكافحة المتكاملة للآفة لمالها من تأثير فى إحداث الموت لليرقات عن طريق الفشل فى عمليات تكوين جدار الجسم والانسلاخ بالإضافة إلى التأثير الكامن لتركيزاتها فائقة التخفيض (التى تتمثل فى متبقياتها بعد استعمالها) فى التطبيقات الحقلية على العمليات الفسيولوجية والذى يؤدى إلى موت نسبة كبيرة وخفض تعداد الحشرة تدريجياً على مدى الأجيال المتعاقبة نتيجة للتعرض لهذه المركبات المختبرة نظرا لكفاءتها وفعاليتها.