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Disruptive Effects of Selected Chitin Synthesis Inhibitors on Cotton Leaf Worm *Spodoptera littoralis* (Boisd.)

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Abstract

Spodoptera littoralis is one of the most devastating pests of cotton and other vegetables in Egypt as well as Mediterranean and middle east countries. Chitin synthesis inhibitors (CSIs) are quite distinctive in their mode of action and potentially act on the target species. The susceptibility of laboratory 2nd&4th instar larvae of cotton leafworm *Spodoptera littoralis* (Boisd.) to CSIs (Flufenoxuron, Chlorfluazuron and Triflumuron) was evaluated. The biochemical responses of *S. littoralis* larvae was detected using the lc_{50} of each compound. Flufenoxuron exhibited high level of toxicity with low LC_{50} value (0.069 ppm) followed by Chlorfluazuron (1.95 ppm) and then triflumuron (75.28) for the 2nd instar larvae while, the lc_{50} was (0.14 ppm) for flufenoxuron followed by 0.42 ppm for chlorfluazuron and then 1661.58 ppm for triflumuron with 4thinstar larvae. Second instar was more susceptible than the older ones except with chlorfluazuron the 4th instar more susceptible than the 2nd instar. Those CSIs exhibited variations of activities for each enzyme. Both of them increased the activity of AST (Aspartate Transaminase) on the contrary they decreased the activity of ALT (Alanine Transaminase). Flufenoxuron increased the activity of both alkaline phosphatase, alpha & beta esterases, chitinase and phenol oxidase, while it decreased the activity of acid phosphatase. Chlorfluazuron increased the activity of both alpha & beta esterases, chitinase and phenol oxidase, while it decreased the activity of both acid & alkaline phosphatases. Triflumuron increased the activity of both alpha esterase, acid phosphatase, chitinase and phenol oxidase, while it decreased the activity of both beta esterase and alkaline phosphatase. So, from this study, it can be concluded that the overall effects of CSIs use, including mortality and sublethal effects in insects, can facilitate the development of truly selective insecticides that can be employed in integrated pest management strategies.

Key words: *Spodoptera littoralis*, (CSI)s, Sublethal, Biochemical Parameters.

INTRODUCTION

Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval), is the most devastating agricultural lepidopterous pests of cotton and vegetable plants (Hatem *et al*., 2009). The intensive application of organophosphates, carbamates and pyrethroids insecticides has given rise to *S. littoralis* populations resistant to all of these groups (Abou-Taleb, 2010). Selective insecticides with modes of action differed from those insecticide groups are highly desirable in integrated pest management (IPM) programs. Among these insecticides are insect growth regulators (IGRs) and ivermectin insecticide group. IGRs were developed to mimic, block or otherwise interact with the hormonal system of insects (Oetken *et al*., 2004). These include the juvenile hormone analogues, the ecdysone agonists and the chitin synthesis inhibitors (CSIs) (Graf, 1993; Tunaz and Uygun, 2004).

The use of insecticides may result in multiple sublethal effects on insect pests (Singh and Marwaha, 2000 and Sabri *et al* 2017). These sub-lethal effects as well as mortality must be considered when investigating the total effects of insecticides (Yin *et al*., 2008). Moreover, these sublethal doses may interfere with some biochemical process such as the related enzymes activity. One of the related enzymes to IGR's mode of action are phenoloxidases that related to critical steps of melanization reactions, which are crucial for the sclerotization of a new cuticle after ecdysis (Andersen, 2005).

Therefore, the aim of the present work is to clarify toxicity and the role played by the sublethal dose LC_{50} of three IGRs belonging to CSIs on the 4thinstar larvae of *S. littoralis* in order to better understand of the action of these compounds on lepidopterous larvae through studying their effect on biochemical parameters. These parameters are attained by estimating activity of some enzyme affecting in cuticle formation.

MATERIALS AND METHODS

- Rearing technique of S. littoralis:

S. littoralis strain used in this study is a laboratory susceptible strain reared in the Plant Protection Research Institute at Dokki, Giza Governorate according to El Defrawi *et al.*, 1964.The culture was maintained under optimum conditions ($27^0C\pm2$ and 70 ± 5 RH) and reared on fresh castor bean leaves, the 2nd& 4th larval instar which used in this study.

- Tested IGRs:

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1- Flufenoxuron 10% DC:
Chemical name:
N-[[4-[2-chloro-4-(trifluoromethyl) phenoxy]-2-fluorophenyl] carbamoyl]-2,6-difluorobenzamide.

2- Chlorfluazuron 5% EC:
Chemical name:
N-[[3,5-dichloro-4-[3-chloro-5-(trifluoromethyl) pyridin-2 yl] oxyphenyl] carbamoyl]-2,6-difluorobenzamide.

3- Triflumuron 48% SC:
Chemical name:
2-chloro-N-[[4-(trifluoromethoxy)phenyl] carbamoyl] benzamide

- *Bioassay tests:*

Leaf dipping bioassay method was used to determine the median lethal concentration (LC_{50}) value of the tested IGRs. Series concentrations (in water) of the tested formulated compounds were prepared. Castor bean leaves were dipped for ten seconds in each concentration then left to dry. The treated leaves were offered to the 2nd and newly molted 4th instar larvae for 24 hr, then replaced by untreated ones daily. Mortality percentage were recorded after 96 hr, the obtained data subjected to ldp line analysis and the toxicity index then estimated. According to the mode of action of IGRs, the mortality of larvae began after three days of treatment, thus in this paper we try to study the biochemical changes. So, samples for analysis been taken 96 hr post treatment with LC_{50} of tested IGRs and before the onset of the mortality and appearance of symptoms of molting process failure.

- Preparation of samples for biochemical studies:

The preparation of samples involved the use of the 2nd& 4th instar larvae of *S. littoralis* after 96 hr. of all treatments at LC_{50} level and control. The larvae placed in clean jars away of food and starved. The starved larvae were homogenized in distilled water using a Teflon homogenizer surrounded with jacket of crushed ice for three minutes. Homogenates were centrifuged at 500 r.p.m. for 10 minutes at 5 to remove haemocyte and supernatants were used directly for the biochemical analysis.

- Enzymes measurements:

1. Transaminases were determined according to (Reitman and Frankle, 1957)
2. Phosphatases were demonstrated according to (Powell and Smith, 1954)
3. α- and β-esterases were detected according to (Van Asperen, 1962)
4. Chitinase activity was determined according to (Bade and Stinson, 1981)
5. Phenoloxidase was detected according to (Ishaaya, 1971)
6. Total carbohydrates were determined according to (Dubois *et al*., 1956)
7. Total proteins were determined according to (Bradford,1976)
8. Total lipids were determined according to (Knight *et al*.,1972)

Statistical analysis:

Statistical significance was assessed by Duncan's and Tukey's test at $p< 0.05$(Snedecor& Cochran 1980).

RESULTS AND DISCUSSION

-Toxicological studies:

The median lethal concentrations with their confidence limits on 2nd and 4th instar larvae of *S. littoralis* of tested CSIs (flufenoxuron, Chlorfluazuron and Triflumuron) after 96 hrs of exposure is represented in Table (1).

Table 1: Susceptibility of 2nd and 4th instar larvae of cotton leafworm, *Spodoptera littoralis* (Boisd.) to tested CSIs after 96 hrs. from treatment.

Tested compound	2nd instar larvae			4th instar larvae		
	Lc_{50}	Slope	Toxicity index %	Lc_{50}	Slope	Toxicity index %
Flufenoxuron	0.069 0.009 - 0.26	0.368 ±0.074	100	0.142 0.003 - 1.165	0.219±0.065	100
Chlorfluazuron	1.952 0.682 - 4.67	0.487±0.103	3.535	0.421 0.014 - 2.097	0.279±0.070	33.49
Triflumuron	75.277 17.72 -180.58	0.484±0.109	0.092	1661.580 1006.0 - 3368.7	0.831±0.155	0.0085

Among the tested CSIs, flufenoxuron was the highest toxic action followed by Chlorfluazuron while, Triflumuron was the least toxic action, the LC_{50} values were 0.069, 1.952, 75.277 ppm for 2nd instar larvae, respectively and 0.142, 0.421,1661.580 ppm for 4th instar larvae, respectively. There is positive correlation between concentration and toxic acceptability in all tested compounds. Second instar was more susceptible than older ones except with chlorfluazuron the 4th instar more susceptible than 2nd instar. These results which proved the toxic acceptability of these larvae to the tested IGRs agreed with Ghoneim *et al.*, (2012) were reported that flufenoxuron was the most toxic action followed by Chlorfluazuron while Triflumuron were the least toxic action against 4th instar larvae for lab and field strain of *Spodoptera littoralis*. Khedr *et al.* (2005) studied the toxicological effects of five insect growth regulators (IGRs), namely Cascade [flufenoxuron], Atabron [chlorfluazuron], Consult [hexaflumuron], Match [difenzoquat] and Mimic [tebufenozide], against 2nd and 4th instar larvae of *Spodoptera littoralis* under laboratory conditions. They concluded that chlorfluazuron was the most potent IGRs against 2nd and 4th instar larvae of *S. littoralis* and the 4th instar larvae proved to be more sensitive to all the tested IGRs than the 2nd one. Anwar and abd-El-Mageed (2005) concluded that flufenoxuron most effective IGR against 2nd and 4th instar larvae of laboratory strain of *S. littoralis* followed by lufenuron, chlorfluazuron, hexaflumuron, diflubenzuron and tebufenozide. On the other hand, El-Sheikh *et al.*, (2011) assessed the toxicity effects and field persistence of the insect growth regulators lufenuron, flufenoxuron and triflumuron in the laboratory using 2nd and 4th larval instars of *Spodoptera littoralis*. Laboratory bioassays indicated that lufenuron was more effective on both 2nd and 4th larval instars, as well as killing both larval instars faster than flufenoxuron or triflumuron.

- Biochemical studies:

1- Determination of AST(GOT)& ALT(GPT) activities:

Data in table 2 indicate that triflumuron produced a significantly higher increase in AST(GOT) activity than the control it was 45.32%, followed by chlorfluazuron 21.94%, while the lowest increase in AST(GOT) activity was induced by flufenoxuron 17.98%. But a decrease in ALT(GPT) activity was observed, according to data in table 2, flufenoxuron exhibited the most observed decrease -67.70 % than in the control, followed by triflumuron -60.71 % and chlorfluazuron was -11.67 %.

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Table 2: AST (GOT) and ALT (GPT) activity in hemolymph of the 4th instar larvae *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each insecticide.

Tested compound	AST(GOT)		ALT(GPT)	
	Mean enzyme activity ($U\times10^3$/g.b.wt)	Change %	Mean enzyme activity ($U\times10^3$/g.b.wt)	Change %
Flufenoxuron	1093.33 ab	17.98	5.73 c	-67.70
Chlorfluazuron	1130 ab	21.94	15.67 b	- 11.67
Triflumuron	1346.67 a	45.32	6.97 c	-60.71
Control	926.67 b		17.74 a	
LSD 0.05	313.65		2.04	

To some extent, improvement of AST(GOT) activity in haemolymph is in agreement with the reported data for the same insect species after treatment with several IGRs or insecticides, e.g. hexaflumuron (Sokar 1995), flufenoxuron, pyriproxyfen or teflubenzuron (El-Kordy et al., 1995), hexaflumuron alone or its binary mixture with chlorpyriphos (Mohamed and Azab 2002), pyriproxyfen, flufenoxuron or chlorfluazuron (Abdel-Aal 2003), and flufenoxuron (Zohry 2006). Similar to *S. littoralis*, some IGRs rising the AST(GOT) activity in *Pectinophora gossypiella* (Anan 1993), and in *M. domestica* (Assar *et al.*, 2010) by pyriproxyfen, besides in *Culex pipiens* by Cyromazine (Assar *et al.*, 2012). On the other hand, declined level of ALT(GPT) in haemolymph of *S. littoralis* larvae by CSIs, in the present study, in agreement with decreased activity *S. Littoralis* by several IGRs and CSIs, for example, chlorfluazuron (Ahmed *et al.*, 1990), triflumuron (Abdel-Hafez *et al.*, 1988), hexaflumuron (Sokar 1995), teflubenzuron, flufenoxuron and pyriproxyfen (El-Kordy *et al.*, 1995), flufenoxuron, chlorfluazuron and pyriproxyfen, (Abdel-Aal 2003), and flufenoxuron (Zohry 2006). Decreasing ALT(GPT) activity has been reported for *P. gossypiella*, *E. insulana* and *A. ipsilon* by pyriproxyfen (Anan 1993; Etebari *et al.*, 2007), *M. domestica* by hexaflumuron or lufenuron (Assar *et al.*, 2010) and *C. pipiens* by Cyromazine (Assar *et al*., 2012). The inhibited activity of ALT(GPT) in haemolymph of *S. littoralis* larvae by CSIs, in this study can be understood since pyruvate is the precursors of Krebs cycle compounds, related to the mitochondrial oxidation phenomenon and ATP products (Azmi *et al.*, 1998). Anyhow, diverse effects of the tested CSIs on GPT activity in larvae could be due to their effects on the synthesis or functional levels of this enzyme directly or indirectly by varying the cell cytomorphology (Nath 2000), or the neurosecretory hormonal pattern.

In the present investigation, the transaminases increasing activity could be attributed to the presence of reversible binding between the tested CSIs and enzymatic site of action, on the enzyme surface. This is based on the fact that the protein synthesis and transaminase levels were affected by the hormonal control of neurosecretory hormones and protein synthesis, which involved in the regulation of transaminase levels (Etebari *et al.*, 2005; Abulyazid *et al.*, 2005). However, the exact mode of the tested CSIs action on transaminase regulation is still controversial up till now.

The opposite was true with Khedr *et al*., (2005) when they studied the biochemical effects of five insect growth regulators (IGRs), namely Cascade[flufenoxuron], Atabron [chlorfluazuron], Consult [hexaflumuron], Match [difenzoquat] and Mimic [tebufenozide], against 2nd and 4th instar larvae of *Spodoptera littoralis* under laboratory conditions. In case of the 4th instar, the tested IGRs increased the activity of the two enzymes after 2 and 5 days of treatment.

2-Determination of acid &alkaline phosphatase activity:

Table 3: Alkaline phosphatase and acid phosphatase activity in haemolymph of the 4th instar larvae *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each insecticide.

Tested compound	Alkaline phosphatase		Acid phosphatase	
	Mean enzyme activity ($U\times10^3$/g.b.wt)	Change %	Mean enzyme activity ($U\times10^3$/g.b.wt)	Change %
Flufenoxuron	5593.33 a	18.50	104 b	-6.87
Chlorfluazuron	3403.33 c	-27.90	100 b	-10.45
Triflumuron	4603.33 b	-2.47	146.33 a	31.04
Control	4720 b		111.67 b	
LSD 0.05	645.27		14.27	

The obtained data in table (3) show that the significantly high activity of alkaline phosphatase (ALK-P) was noticed in flufenoxuron 18.50 % higher than in control. On the contrast, chlorfluazuron caused reduction in (ALK-P) activity -27.90 % followed by triflumuron -2.47% compared to control. At the same respect, flufenoxuron and chlorfluazuron caused significant decrease in acid phosphatase (AC-P) activity ranging between -6.87% and -10.45% compared to the control except for triflumuron, which increased the enzyme activity 31.04% higher than in the control.

These results are in agreement with those obtained on *S. littoralis* by (El-Barky *et al.*, 2008; El-Sheikh, 2012) using spinetoram with significant decrease in both acid and alkaline phosphatases. On the other hand, some increase in the activity of acid phosphatase in the same insect by (Sokar, 1995) using hexaflumuron. Sridhara and Bhat (1963) stated that the increase or decrease of both phosphatase enzymes development is reflected in increase or decrease in acid-soluble phosphorus content. This result disagrees with Hafez and El-Naby (2014) when they studied the resistance levels in laboratory and field strain of *Spodoptera littoralis* (Boisd.) against two IGR's (lufenuron and tebufenozide). They concluded that there was a positive correlation between resistance and Alkaline phosphatase and alpha esterase enzyme, while there was negative correlation between resistance level and Acid phosphatase & trehalase activity.

3- Determination of alpha & beta esterase activity:

Values of alpha and beta esterases are tabulated in Table (4).All tested compounds caused an increase in alpha esterase activity, triflumuron recorded the highest value of activation 10.99% followed by flufenoxuron then chlorfluazuron which have 10.11% and 5.35% above the control level, respectively. On the other hand, both flufenoxuron and chlorfluazuron increased the activity of beta esterase enzymes 29.33% and 18.33% higher than in the control, except for triflumuron, which decreased the enzyme activity (-14.14%).The obtained results matched with Abdel-Megeed *et al.*(2000) findings in Gharbia, alpha -esterase activity increased but decreased in Menofia when they treated field strains of *S. littoralis* in (Gharbia and Menofia) with tebufenozide, benzoylphenyl urea, chlorfluazuron, flufenoxuron, profenofos + chlorfluazuron, chlorpyrifos + diflubenzuron and profenofos + chlorfluazuron. The increase in alpha and beta-esterase activity in the Menofia field strain was higher than that of the laboratory strain. Chlorfluazuron and flufenoxuron increased the activity of beta-esterase. The beta-esterase activity of Gharbia increased by the end of the cotton season. Assar *et al.*, (2016) concluded that both alpha and beta esterases in *S. littoralis* was highly inhibited with hexaflumuron. It is clearly noticed that IGR's may be cause different levels of significant changes in alpha and beta esterases on *S. littoralis* (Bakr *et al.*, 2013).

4- Determination of chitinase and phenoloxidase enzymes activity:

Results given in Table (5) indicated that all tested insecticides led to increase in chitinase activity ranging between 32.70%, 51.28% and 72.06% for flufenoxuron, chlorfluazuron, and triflumuron, respectively more than control. Phenoloxidase activity was highly significant activated with three tested insecticides 32.89%, 29.65%, 24.04% with chlorfluazuron, flufenoxuron and triflumuron, respectively as compared with control. It is clearly noticed that pronounced changes in the same insect by Abd El- Mageed and Shalaby (2011) using mixtures of IGRs with insecticides. Amin and Mohamady (2009) were noticed activation of chitinase and phenoloxidase after 6 and 12 hr from methoxyfenozide administration. Increasing in activation of chitinase and phenoloxidase enzymes stated also with hexaflumuron on *S. littoralis* (Assar *et al*., 2016) and (Sabry and Khedr 2014) with flufenoxuron and teflubenzuron. On other hand, phenoloxidase activity was reduced by using teflubenzuron on *S. littoralis* (Mostafa, 1993).

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Table 4: Alpha and Beta esterases activity in haemolymph of the 4th instar larvae *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each tested insecticide.

Tested compound	Alpha esterase		Beta esterase	
	Mean enzyme activity (ug α-naphthol/min/g.b.wt)	Change %	Mean enzyme activity (ugβ-naphthol/min/g.b.wt)	Change %
Flufenoxuron	377.33 a	10.11	164.67 a	29.33
Chlorfluazuron	361 ab	5.35	150.67 a	18.33
Triflumuron	380.33 a	10.99	109.33 b	-14.14
Control	342.67 b		127.33 b	
LSD 0.05	30.15		19.12	

Table 5: Chitinase and phenoloxidase activity in haemolymph of the 4th instar larvae of *Spodoptera littoralis*(Boisd.) after treatment with LC_{50} of each tested insecticide.

Tested compound	Chitinase		Phenoloxidase	
	Mean enzyme activity (µg NAGA/min/g.b. wt)	Change %	Mean enzyme activity (O.D. unit/min/g.b. wt)	Change %
Flufenoxuron	259.67 C	32.70	8.79 a	29.65
Chlorfluazuron	296 b	51.28	9.01 a	32.89
Triflumuron	336.67 a	72.06	8.41 a	24.04
Control	195.67 d		6.78 b	
LSD 0.05	34.90		1.24	

Table 6: Determination of total proteins, carbohydrates and lipids in haemolymph of the 4th instar larvae of *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each tested insecticide.

Tested compound	Total proteins		Total carbohydrates		Total lipids	
	(mg/g.b. wt)	Change %	(mg/g.b. wt)	Change %	(mg/g.b. wt)	Change %
Flufenoxuron	70.60 b	-16.48	10.67 a	69.10	6.63 a	53.12
Chlorfluazuron	68.70 b	-18.73	9.20 a	45.80	5.74 b	32.56
Triflumuron	69.53 b	-17.75	10.14 a	60.70	6.09 ab	40.65
Control	84.53 a		6.31 b		4.33 c	
LSD 0.05	0.60		2.10		10.60	

5- Determination of total proteins, carbohydrates and lipids:

Data in table (6) show that all tested CSIs have an observed significantly decrease in total proteins 70.60, 68.70, 69.53 for flufenoxuron, chlorfluazuron, triflumuron respectively, compared with 84.53 for control. This result agreed with Dahi *et al.*, (2011) observed a conspicuous depletion in total protein content in both 4th and 6th treated larval instar withLC_{50} of the novel insecticide pyridalyl. The total haemolymph protein content of 4th instars of *S. littoralis* was decreased with hexaflumuron and teflubenzuron (Assar *et al.*, 2016). Similar results were obtained by (Mostafa, 1993) and (Sokar, 1995) for the total haemolymph protein of the same species treated with teflubenzuron and hexaflumuron, respectively. More or less, similar inhibition of protein content was obtained by (El-Barky *et al.*, 2008) when using spinetoram on *S. littoralis*. The protein pool of the haemolymph functions as a reserve source of protein synthesis need for growth and development of the adult stage during pupal life (Florkin and Jeanuiaux, 1964). Wilkinson (1976) stated that protein help to synthesize microsomal detoxifying enzymes which assist in the detoxificaition of toxicants that enter into the insect body. Proteins are the most important components of biochemical of insect that bind the foreign compounds. In general, the problem of protein synthesis is intimately related to metabolism of nucleic acids.

Total carbohydrates in table (6) show a significantly increase 10.67, 9.20, 10.14 for flufenoxuron, chlorfluazuron, triflumuron respectively, while it was 6.31 for control. These observations agreed with Assar *et al.*, (2016) with hexaflumuron and teflubenzuron on the same insect. The same results stated by Abd El-Mageed and Shalaby (2011) using mixtures of IGRs with insecticides. The increase in total carbohydrates also noticed in pupal stage after treatment with Teflubenzuron (El-Sheikh 2006). Our results disagree with Osman and Abou-Zeid (2015), they noticed decrease of total carbohydrate when they used the plant extract of *Capsicum annuum L*, and Organophosphorous insecticide Profenofos (selecron) and the mixture of them for controlling 4th instar larvae of cotton leaf worm under the semi field circumstances. These contradictory findings may be attributed to the differences in species sensitivity, the potency of the IGRs, or the developmental stage. Also, the disturbance in carbohydrate content can be understood in the light of the ability of the organism to modify the synthesis of certain metabolite and disrupt the functionality of the organism (Rodriguez-Ortega*et al.*, 2003).

The obtained results in this work recorded significantly increase in total lipids values 6.63, 5.74, 6.09 for flufenoxuron, chlorfluazuron, triflumuron respectively, while it was 4.33 for control. Similar increase in total lipids recorded by Abdel-Aal (2003) in case of flufenoxuron on late 6th instars of *S. littoralis*; whereas in case of chlorfluazuron significantly decreased the lipid content. Different results were obtained by Assar *et al.*, (2016) were stated the reduction in total lipids with hexaflumuron and teflubenzuron as IGR, s against 4th instar larvae of *S. littoralis*. Similar reduction in total lipids was recorded by (El-Sheikh *et al.*,2013). Therefore, the exceptional cases of increasing lipid content in *S. littoralis* treated with CSIs may indicate its pronounced interference with not only the synthesis of lipids but also their mobilization as promoted to convert into other metabolites or fatty acids.

Conclusion:

Insect growth regulators are used widely in the world as a potential tool of bio-intensive IPM (BIPM) considering their efficacy against target pests as well as safety to the natural enemies and environment. In insects, the use of haemolymph as a medium for controlling insect pests has been made because the changes occurring in the haemolymph are quickly transferred to other portions of insect's body. On the basis of overall findings, it can be concluded that flufenoxuron and chlorfluazuron are toxic to some developmental stages of *S. littoralis*, as well as caused many biochemical effects at its sublethal level. Such as, the disturbance of transaminase activities by the present CSIs, in the current study, may be lead to disturbance of protein metabolism and synthesis of some specific compounds. Thus, these CSIs will disrupt many physiological functions and ultimately lead to death. The results of evaluating the sublethal effect of CSIs suggested that substantial physiological events in the life of *S. littoralis* larvae are involved in responding to the action of the insecticide. The overall effects of CSIs use, including mortality and sublethal effects in insects, can facilitate the development of truly selective insecticides that can be employed in integrated pest management strategies.

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Highlights and contributions

- ***The results of this paper will provide new finding and thus can improve in field of Agriculture, Pest Science and Integrated pest management of cotton pests.***
- ***The use of insect growth regulators particularly chitin synthesis inhibitors such as flufenoxuron or chlorfluazuron in program of integrated pest management of cotton pest Spodoptera littoralis in Egypt.***
- ***How to safely overcome the problems of infection of cotton plants with lepidopterous pests***