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BIOCHEMICAL IMPACTS OF THREE CHITIN SYNTHESIS INHIBITORS AND THEIR BINARY MIXTURES WITH CHLORPYRIFOS OR BETA-CYFLUTHRIN ON *Spodoptera littoralis* (BOISD.)

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ABSTRACT

The joint action of the binary mixtures of chlorpyrifos (organophosphorus) or beta-cyfluthrin (pyrethroid) plus three chitin synthesis inhibitors (flufenoxuron, chlorfluazuron and lufenuron) on the 4th instar larvae of the laboratory strain of the cotton leafworm *Spodoptera littoralis* (Boisd.) at LC_{25} level were evaluated. In general, according to co-toxicity factor data clearly indicated that all tested mixtures decreased the toxicity except for the combination between chlorpyrifos and lufenuron, it produced additive effect (-19.15). The tested mixtures of Beta-cyfluthrin with all tested insect growth regulators (IGRs) were high antagonism (ranging between - 55.56 and - 84.13) than the tested mixtures of IGRs with chlorpyrifos. The effect of both tested individual insecticides and it's mixtures on the activity of esterases (α- & β-esterase), transaminase (AST and ALT) chitinase, alkaline phosphatase, total proteins, total lipids and glucose was determined colorimetrically.

INTRODUCTION

Mixtures are available as pre-mixes from the pesticide companies or they are tank-mixed by the farmers. Ideally, the insecticides having different modes of action are mixed on the assumption that they would complement the action of each other for killing the target pest. When two compounds are mixed, they can either be potentiating or additive or antagonistic in an insect species. These effects can be varied on different insect species or strains depending upon their physiology and the mechanism(s) of resistance developed. If a mixture is potentiating, it is a useful tool in enhancing control efficacy and combating insecticide resistance. In this case, there may be potential for reducing the application rate of one or both components of the mixture. If a mixture is antagonistic, it should not be used, because it will reduce the efficiency of pest control and aggravate the resistance problem (Swelam and Sayed 2006). Synergism between pyrethroids and organophosphates (OPs) or carbamates has already been demonstrated in the control of agricultural pests (Ozaki *et al.,* 1984; Bynum *et al.,* 1997; Martin *et al.,* 2003).The occurrence of insect resistance to an insecticide is mainly due to the action of enzymes, which either insensitive to the insecticide or able to degrade it to non toxic metabolites. Because of their dissimilar modes of action, pyrethroids and organophosphates (OPs) have commonly been mixed since mid 1980s to manage pest complex of cotton and other crops (Mushtaq 2004). Insect growth regulators (IGRs), have a much slower mode of action than synthetic chemical insecticides. IGRs include juvenile hormone

(JH) mimics and chitin synthesis inhibitors (CSIs). CSIs, such as lufenuron, inhibit the production of chitin, a major component of the insect exoskeleton. Insects treated with CSIs become unable to synthesize new cuticle, and therefore unable to successfully molt into the next stage. The efficiency of insecticides and their mixtures with the IGRs against the cotton leafworm attracted several investigators (Ravi and Verma 1997; El-Aswad 2007). The growers resort to the use of insecticide mixtures in an effort to obtain acceptable control of pests. Availability of cheaper, generic insecticides has further popularized the application of pre-and tank mixed mixtures (Ahmed 2009). Insecticide mixtures are usually used in the field to enhance the spectrum of control when multiple pests are attacking simultaneously.

The objectives of this study were to evaluate the potentiation in the used binary mixtures of chlorpyrifos (organophosphorus) and beta-cyfluthrin (pyrethroid) plus insect growth regulators (IGRs) as against a laboratory strain of the cotton leafworm *Spodoptera littoralis* (Boisd.) which has its importance as one of the most destructive phytophagous lepidopterous pests under laboratory conditions, and also assessment the effect of both tested individual insecticides and it's mixtures on the activity of esterases (α- & β-esterase), transaminase (AST and ALT) chitinase, alkaline phosphatase, total proteins, total lipids and glucose was determined colorimetrically.

MATERIALS AND METHODS

I- Tested Insecticides:

A- Insect growth regulators

1- Flufenoxuron (Ageron 10 % DC) ® as chitin synthesis inhibitor

Chemical name: *N*–[[[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl] amino] carbonyl]-2,6-difluorobenzamide.

2- Chlorfluazuron (Capris 5 % DC) ® as chitin synthesis inhibitor

Chemical name: N–[[[3,5-dichloro-4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy] phenyl] amino] carbonyl]-2,6-difluorobenzamide.

3- Lufenuron (Match 5 % DC) ® as chitin synthesis inhibitor

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

B- Organophosphorus insecticide

Chlorpyrifos (Dora 48% EC) ® as cholinesterase inhibitor

Chemical name: O,O-diethyl O-(3,5,6-tirchloro-2-pyridinyl) phosphorothioate.

C- Synthetic pyrethroid insecticide

Beta-cyfluthrin (Bulldock 12.5% S.C.) ® as sodium channel modulator

Chemical name : Cyano (4-fluoro-3-phenoxyphenyl) methyl3-(2,2dichloroethenyl) 2,2- dimethylcyclopropanecarboxylate

II- Cotton leafworm strain:

A laboratory strain of the cotton leafworm *S. littoralis* (Boisd.) was maintained under constant conditions of 25°C±1 and 70 ± 5% RH and kept of any contamination with chemicals till the time of study in order to obtain a susceptible and homogenous strain as described by El-Defrawi *et al.,* (1964).

III- Determination of the joint action:

The joint action of tested pesticide mixtures was studied by mixing concentrations equivalent to LC_{25} values at the ratio of 1:1. Five replicates with twenty larvae for each were used in each treatment. Mortality counts were recorded after four days of treatment. The combined action of the different mixtures was expressed at the co-toxicity factor (CF), estimated according to the equation given by Mansour *et al.,* 1966. (CF) were determined by dividing the observed mortality percentage mince expected mortality by expected mortality percentage.

$$\text{Co-Toxicity factor} = \frac{\text{\% Observed mortality} - \text{\% Expected mortality}}{\text{\% Expected mortality}} \times 100$$

The co-toxicity factor was employed to differentiate the results into three categories: A positive factor of 20 or more is considered as potentiation, a negative of 20 or more is considered as antagonism, while intermediate values (-20 & +20) indicated additive effect.

IV- Biochemical studies:

This part of study was conducted in order to determine of some enzymes activities in 4th instar larvae of laboratory strain of *S. littoralis* after treatment with tested insecticides and its mixtures

A- Preparing samples for enzyme assays:

Castor-bean leaves were dipped for 30 seconds in an aqueous solution of each of the tested compounds at the LC_{50} level (treated with LC_{25} + LC_{25} of each mixture), then left to dry for 1 hour in room temperature before being offered to the 4th instar larvae of laboratory strain. Larvae were fed for 24 hours on the treated leaves, and then transferred to fresh untreated leaves for three days. Haemolymph was obtained from approximately fifty larvae by removing one of the prolegs by forceps and applying gentle. Pressure was on the larvae with the fingers and takes the haemolymph by syringe. The haemolymph was collected in cold tubes and stored in a refrigerator until the enzyme activities were determined (Sooker *et al.,* 1999; Abd El-Mageed *et al.,* 2008).

B- Biochemical measurements:

Alpha esterases (α-E) and beta esterases (β-E) were determined according to the method of Van Asperen (1962). Chitinase was assayed according to the method described by Ishaaya and Casida (1974). Alkaline phosphatase (ALK-P) activities were determined according to the method described by Powell and Smith (1954). The activity of AST and ALT were determined according to the method of Reitman and Frankel (1957). Total proteins were calorimetrically determined according to Bradford (1976), while total lipids were assayed by the method of Knight *et al.,* (1972). Glucose was determined colorimetrically according to Trinder (1969).

All biochemical measurements were conducted in the micro-chemical analysis unit at Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.

V- Statistical analysis:

Data were calculated as mean ± SD and analyzed using analysis of variance technique (ANOVA) followed by least significant difference (LSD). Probability of 0.05 or less was considered significant. All statistical analysis was done with CoHort Software 2004.

RESULTS AND DISCUSSION

I-The joint effect of the tested mixtures of insecticides:

The joint action data of the binary mixtures of chlorpyrifos (organophosphorus) or beta-cyfluthrin (pyrethroid) plus three insect growth regulators (IGRs) on the 4th instar larvae of the laboratory strain of cotton leafworm *S. littoralis* (Boisd.) at LC_{25} level are shown in Table (1) and Figure (1). Data clearly indicated that all tested mixtures after four days decreased the toxicity according to co-toxicity factor except for the combination between chlorpyrifos and lufenuron, it produced additive effect (-19.15). The tested mixtures of beta-cyfluthrin with all tested IGRs were higher antagonism than the tested mixtures of IGRs with chlorpyrifos. While, flufenoxuron and chlorfluazuron when used in admixture with chlorpyrifos were -24.32 and -21.43, respectively. On the other hand, tested IGRs when mixed with beta-cyfluthrin, the co-toxicity factor ranging between - 55.56 and - 84.13.

II- Biochemical impacts:-

A- Determination of non specific esterases activity:

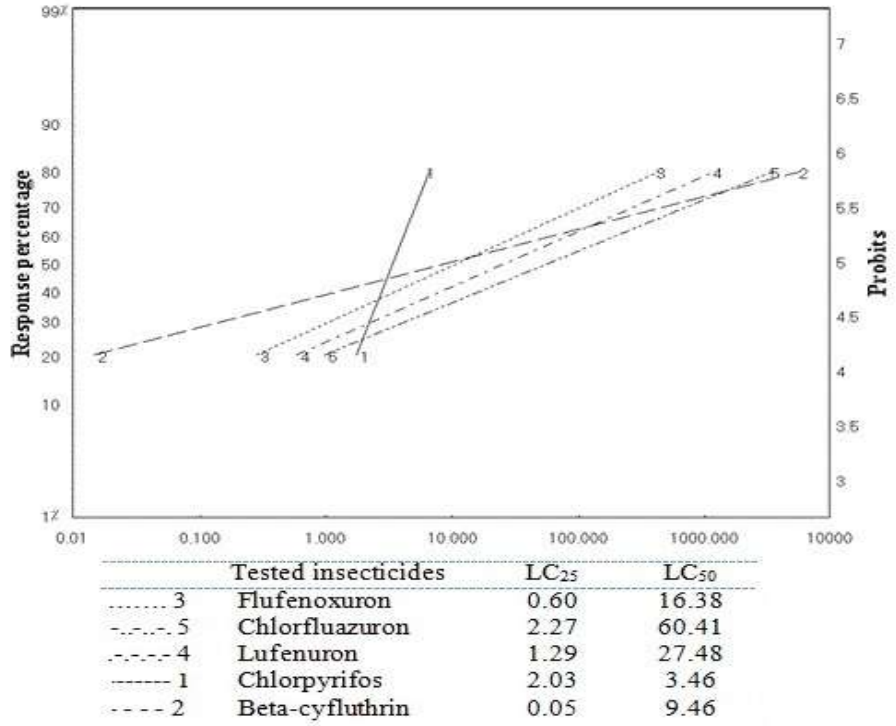
1- Alpha esterase (α-E):

Data in Table (2) revealed that the highest significant decrease of alpha esterase (α-E) activity was noticed in flufenoxuron treatment (-47.27%) followed by chlorpyrifos, chlorfluazuron and lufenuron, -29.95, -12.50 and -8.98% lower the check level, respectively. While beta-cyfluthrin was recorded a non significant decrease in enzyme activity with value -1.43% lower than check.

The data declared that chlorpyrifos + chlorfluazuron, chlorpyrifos + flufenoxuron, beta-cyfluthrin + chlorfluazuron and beta-cyfluthrin + flufenoxuron gave the same trend of response but with low level of reduction in the enzyme activity with values of -22.27, -20.57, -20.31 and -11.07%, respectively. No significant differences in lufenuron when mixed with chlorpyrifos or beta-cyfluthrin as compared with check.

2- Beta esterase (β-E):

Data in Table (2) indicated that beta-cyfluthrin gave the highest increase in beta esterase (β-E) activity higher than check, it was 36.67 %, while flufenoxuron was recorded the highest decrease in β-E activity, gave -29.04 %. All tested mixtures gave variable levels of increase or decrease in β-E activity ranged between -12.96 and 16.67 % as compared with check.



	Tested insecticides	LC_{25}	LC_{50}
..... 3	Flufenoxuron	0.60	16.38
-..-..-. 5	Chlorfluazuron	2.27	60.41
.-.-.-.- 4	Lufenuron	1.29	27.48
------- 1	Chlorpyrifos	2.03	3.46
- - - - 2	Beta-cyfluthrin	0.05	9.46

Fig.1: Log concentration probit lines of 4th instar larvae of cotton leafworm *S. littoralis* to tested insecticides.

Table 1: The joint action between LC_{25} of tested insecticides with LC_{25} of three tested IGR's on the 4th instar larvae of cotton leafworm, *S. littoralis*.

Treatments		% Expected mortality	% Observed mortality	Co-toxicity factor	Category
Chlorpyrifos	+ Flufenoxuron	37.00	28.00	- 24.32	Antagonism
	+ Chlorfluazuron	56.00	44.00	- 21.43	Antagonism
	+ Lufenuron	47.00	38.00	- 19.15	Additive
Beta-cyfluthrin	+ Flufenoxuron	43.00	12.00	- 72.09	Antagonism
	+ Chlorfluazuron	63.00	10.00	- 84.13	Antagonism
	+ Lufenuron	54.00	24.00	- 55.56	Antagonism

Table 2: Non specific esterases activity in haemolymph of the 4th instar larvae of cotton leafworm, *S. littoralis* after treatment with single and mixture compounds.

Tested compounds	Alpha esterase (α-E)		Beta esterase (β-E)	
	µg α-naphthol/min/ml (Mean ± SD)	% of Check	µg β-naphthol/min/ml (Mean ± SD)	% of Check
Single (treated with LC_{50} of each compound)				
Flufenoxuron	135.00 ±7.00 f	-47.27	49.67 ±4.71 h	-29.04
Chlorfluazuron	224.00 ±2.65 c	-12.50	64.70 ±1.61 ef	-7.57
Lufenuron	233.00 ±3.00 b	-8.98	66.67 ±1.33 e	-4.76
Chlorpyrifos	179.33 ±4.04 e	-29.95	55.40 ±2.33 g	-20.86
Beta-cyfluthrin	252.33 ±3.06 a	-1.43	95.67 ±4.12 a	36.67
Mixed (treated with LC_{25} + LC_{25} of each mixture)				
Chlorpyrifos + Flufenoxuron	203.33 ±4.93 d	-20.57	73.00 ±2.33 cd	4.29
Chlorpyrifos + Chlorfluazuron	199.00 ±2.65 d	-22.27	60.93 ±2.44 f	-12.96
Chlorpyrifos + Lufenuron	255.67 ±3.79 a	-0.13	81.67 ±3.21 b	16.67
Beta-cyfluthrin + Flufenoxuron	227.67 ±2.52 bc	-11.07	74.40 ±3.20 cd	6.29
Beta-cyfluthrin + Chlorfluazuron	204.00 ±3.61 d	-20.31	69.73 ±2.63 de	-0.39
Beta-cyfluthrin + Lufenuron	250.33 ±2.08 a	-2.21	75.50 ±2.75 c	7.86
Check	256.00 ±5.57 a		70.00 ±2.59 de	
LSD 0.05	6.72		4.91	

% of Check = (Test - Check) / Check x 100
Values followed by the same letter (s) are not significantly different according to Duncan's test.

B- Determination of transaminase enzymes activity:

1- Aspartate transaminase (AST):

Data in Table (3) showed that chlorfluazuron gave the highest reduction in aspartate transaminase (AST) activity with value of -42.34 % lower than check , while the previous compound was caused -19.38 and -17.18 lower than check when mixed with chlorpyrifos or beta-cyfluthrin, respectively. Also the other tested IGRs gave the same trend of response in the enzyme activity when mixed with chlorpyrifos or beta-cyfluthrin.

2- Alanine transaminase (ALT):

Our results (Table 3) revealed that all tested compounds caused significant reduction in alanine transaminase (ALT) activity with ranged between -13.36 to -48.29% lower than check. The same trend of response in decrease of the enzyme activity was recorded with all tested mixtures except for the case of chlorpyrifos + lufenuron which led to an increase in enzyme activity (96.58% higher than check).

C- Determination of chitinase activity:

Results indicated that all tested IGRs caused significant increase in chitinase activity (Table 4), the enzyme activity reached its maximum in chlorfluazuron (49.36% higher than check), while when mixed with

chlorpyrifos or beta-cyfluthrin gave the same trend of response but with low level of the enzyme activity (19.61 and 1.83%), respectively.

Table 3: Transaminases activity in haemolymph of the 4th instar larvae of cotton leafworm, *S. littoralis* after treatment with single and mixture compounds.

Tested compounds	Aspartate amino transaminase (AST)		Alanine amino transaminase (ALT)	
	U x 10^3/ml (Mean ± SD)	% of Check	U x 10^3/ml (Mean ± SD)	% of Check
Single (treated with LC_{50} of each compound)				
Flufenoxuron	326.00 ±5.29 f	-32.78	80.33 ±1.53 cdef	-17.47
Chlorfluazuron	279.67 ±9.61 h	-42.34	50.33 ±2.52 h	-48.29
Lufenuron	306.00 ±5.29 g	-36.91	73.67 ±3.21 f	-24.31
Chlorpyrifos	334.00 ±6.56 ef	-31.13	75.00 ±3.00 ef	-22.94
Beta-cyfluthrin	399.67 ±5.51 cd	-17.59	84.33 ±3.51 cd	-13.36
Mixed (treated with LC_{25} + LC_{25} of each mixture)				
Chlorpyrifos + Flufenoxuron	344.67 ±5.69 e	-28.93	62.33 ±3.21 g	-35.96
Chlorpyrifos + Chlorfluazuron	391.00 ±6.08 d	-19.38	78.00 ±4.00 def	-19.86
Chlorpyrifos + Lufenuron	474.67 ±17.47 a	-2.13	191.33 ±10.26 a	96.58
Beta-cyfluthrin + Flufenoxuron	452.33 ±7.77 b	-6.74	88.00 ±3.00 c	-9.59
Beta-cyfluthrin + Chlorfluazuron	401.67 ±8.50 cd	-17.18	79.67 ±3.21 def	-18.14
Beta-cyfluthrin + Lufenuron	410.67 ±5.69 c	-15.33	82.33 ±2.08 cde	-15.41
Check	485.00 ±21.79 a		97.33 ±6.43 b	
LSD 0.05	17.10		7.49	

% of Check = (Test - Check) / Check x 100
Values followed by the same letter (s) are not significantly different according to Duncan's test.

D- Determination of Alkaline phosphatase activity (Alk-P):

Flufenoxuron and lufenuron gave approximately same reaction on alkaline phosphatase activity in individual form with values of 49.98 and 35.87 %, respectively (Table 4), while mixed process between chlorpyrifos + lufenuron lead to increase the Alk-P activity (84.77%). On the other hand, the mixture between chlorpyrifos + flufenoxuron caused decreases the Alk-P (-30.45). But a negligible decrease in Alk-P activity was observed in case of chlorfluazuron when treated either individual or mixture form with chlorpyrifos (-6.52 and -1.11%), respectively.

E- Determination of total lipids:

Data in Table (5) revealed that all tested compounds give significant reduction in total lipids either in individual or mixture forms with ranged between -27.91and -55.39%.

Table 4: Chitinase and Alkaline phosphatase activity in haemolymph of the 4th instar larvae of cotton leafworm, *S. littoralis* after treatment with single and mixture compounds.

Tested compounds	Chitinase		Alkaline phosphatase	
	µg N.Acetyl glucose amine x 10^3/min/ml (Mean ± SD)	% of Check	U x 10^3/ml (Mean ± SD)	% of Check
Single (treated with LC_{50} of each compound)				
Flufenoxuron	399.33 ±10.60 b	29.10	46.00 ±2.00 c	49.98
Chlorfluazuron	462.00 ±14.11 a	49.36	28.67 ±1.15 f	-6.52
Lufenuron	347.33 ±3.06 d	12.28	41.67 ±2.08 d	35.87
Chlorpyrifos	293.33 ±11.59 g	-5.17	36.33 ±2.52 e	18.45
Beta-cyfluthrin	319.67 ±14.57 ef	3.34	69.00 ±4.36 a	124.98
Mixed (treated with LC_{25} + LC_{25} of each mixture)				
Chlorpyrifos + Flufenoxuron	362.67 ±6.43 cd	17.24	21.33 ±2.31 g	-30.45
Chlorpyrifos + Chlorfluazuron	370.00 ±6.93 c	19.61	30.33 ±2.31 f	-1.11
Chlorpyrifos + Lufenuron	357.00 ±2.65 cd	15.41	56.67 ±3.51 b	84.77
Beta-cyfluthrin + Flufenoxuron	328.00 ±6.24 e	6.04	20.33 ±1.53 g	-33.71
Beta-cyfluthrin + Chlorfluazuron	315.00 ±4.58 ef	1.83	15.33 ±1.53 h	-50.02
Beta-cyfluthrin + Lufenuron	330.33 ±10.12 e	6.79	24.00 ±2.00 g	-21.75
Check	309.33 ±11.37 f		30.67 ±2.08 f	
LSD 0.05	15.80		4.10	

% of Check = (Test - Check) / Check x 100
Values followed by the same letter (s) are not significantly different according to Duncan's test.

F- Determination of total protein:

In this study, we could classify all tested compounds into two categories, the first one includes: Lufenuron, chlorpyrifos, chlorpyrifos + flufenoxuron, beta-cyfluthrin + flufenoxuron and beta-cyfluthrin + chlorfluazuron and caused a reduction in total protein. The second category includes the other compounds which caused increase in total protein, beta-cyfluthrin and chlorpyrifos + lufenuron are the most effect of them (Table 5).

G- Determination of glucose:

From the results obtained in Table (5) it could be noticed that the highest significant activity of glucose was noticed in flufenoxuron treatment (204.05%) this effect was reduce when mixed with chlorpyrifos or beta-cyfluthrin, also there are no significant difference in glucose activity between chlorfluazuron, chlorpyrifos + chlorfluazuron, beta-cyfluthrin + flufenoxuron and beta-cyfluthrin + chlorfluazuron as compared with check.

Review the results; it is worth to note that all tested mixtures decreased the toxicity compared its individual component according to co-toxicity factor. The efficiency of different tested compounds against the 4th instar larvae of the cotton leafworm *S. littoralis* varied tremendously according to the chemical structure of the tested mixtures. The data also revealed that tested enzymes may be playing an important role in insecticidal poisoning according to the type of component of tested insecticides mixtures. But the previous studies don't provide complete a biochemical basis for the effect of

mixed process on toxicity. Detoxification enzymes in insects are generally demonstrated as the enzymatic defense against foreign compounds and play significant roles in maintaining their normal physiological functions (Li and Liu, 2007). The primary routes of insecticide resistance in all insects are alterations in the insecticide target site or changes in the rate at which the insecticide is detoxified. So far esterases, are known to be involved in the detoxification of the major groups of insecticides (Zhou *et al.,* 2002; Herron, *et al.,* 2004 and Pethuan, *et al.,* 2007). Esterase played important roles in the insecticide resistance of the pest (Wu ShiChang *et al.,* 1995). Esterase activity in treated samples at lower pesticide concentration and control samples showed an increased activity, while the activity was decreased with high concentration. This indicates that xenobiotic elicit increased activity of esterases at lower concentrations in *Spodoptera litura*.

Table 5: Total lipids, Total proteins and Glucose level in haemolymph of the 4th instar larvae of cotton leafworm, *S. littoralis* after treatment with single and mixture compounds.

Tested compounds	Total lipids		Total protein		Glucose	
	Mg/ml (Mean ± SD)	% of Check	Mg/ml (Mean ± SD)	% of Check	µg/ml (Mean ± SD)	% of Check
Single (treated with LC_{50} of each compound)						
Flufenoxuron	11.49 ±0.70 f	-55.27	27.00 ±2.65 bc	3.85	1124.00 ±75.15 a	204.05
Chlorfluazuron	14.71 ±1.05 d	-42.74	28.00 ±1.00 bc	7.69	380.33 ±26.08 e	2.88
Lufenuron	17.19 ±0.98 bc	-33.09	20.00 ±2.00 d	-23.08	391.33 ±9.50 de	5.86
Chlorpyrifos	15.33 ±0.85 cd	-40.33	22.33 ±2.08 d	-14.12	454.00 ±29.46 d	22.81
Beta-cyfluthrin	13.83 ±1.03 de	-46.17	30.67 ±1.53 b	17.96	390.67 ±27.23 de	5.68
Mixed (treated with LC_{25} + LC_{25} of each mixture)						
Chlorpyrifos + Flufenoxuron	14.08 ±1.01 de	-45.19	20.67 ±1.53 d	-20.50	842.00 ±53.86 b	127.77
Chlorpyrifos + Chlorfluazuron	11.46 ±0.81 f	-55.39	28.33 ± 1.53bc	8.96	385.33 ±31.90 e	4.24
Chlorpyrifos + Lufenuron	17.09 ±0.82 bc	-33.48	36.33 ±3.51 a	39.73	631.33 ±29.54 c	70.78
Beta-cyfluthrin + Flufenoxuron	18.52 ±0.82 b	-27.91	19.33 ±1.53 d	-25.65	358.67 ±23.25 e	-2.98
Beta-cyfluthrin + Chlorfluazuron	17.26 ±0.78 bc	-32.81	22.00 ±2.00 d	-15.38	379.33 ±33.50 e	2.61
Beta-cyfluthrin + Lufenuron	12.30 ±1.01 ef	-52.12	27.00 ±2.65 bc	3.85	414.67 ±26.10 de	12.17
Check	25.69 ±2.80 a		26.00 ±2.65 c		369.67 ±29.50 e	
LSD 0.05	1.99		3.64		61.60	

% of Check = (Test - Check) / Check x 100
Values followed by the same letter (s) are not significantly different according to Duncan's test.

Our finding agree with the results were found by Bakr *et al.,* (2013) found that lufenuron exhibited a severe reduction in the activities of the detoxification enzymes, phosphatase and esterases (α and β), as compared to the control in 4th larval instar of *S. littoralis*. Therefore, the tested IGR lufenuron , may be not detoxify by these enzymes. Kandil *et al.,* (2012) reported that the tested IGRS (lufenuron, chlorfluazuron and chromafenozide) against *Pectinophora gossypiella*, reduced the glucose, protein and

carbohydrate contents. Also, the tested IGRS elicited inhibitory effect on alanine amino transferase (ALT) and aspartate amino transferase (AST) were treated with estimated LC_{50} values. Likewise, Hamdy and Azab (2002) found that the enzyme activity of α-esterase in *S. littoralis* after treatment with IGRs was increased with chlorfluazuron. Bakr *et al.,* (2010), studied the effect of the sublethal concentrations LC_{25}, LC_{50} and LC_{90} of flufenoxuron (Cascade) on the activity of detoxification enzymes, acid phosphatase and the non-specific esterases (α, β esterases), 4th larval instar of *S. littoralis.* Their results showed that the activity of all enzymes decreased significantly in treated larvae at different times intervals post treatments. Assar *et al.,* (2012) found that match induced inhibitory effect on the housefly, *Musca domestica* at 1000 ppm. Consult had no effect on the total activity of AST. With respect to the total ALT activity, match and consult elicited inhibitory effect on the total ALT activity. El-Kordy *et al.,* (1995) found that GOT (Official name: AST) enzyme was significantly increased, while there was a significant reduction in the level of GPT (Official name: ALT) on the 4th and 6th instars larvae of *S. littoralis* after the treatment flufenoxuron. Abdel-Hafez *et al.,* (1993) cited that the IGR/insecticide mixtures or their residual gave variable decrease in the activity of alkaline phosphatase and GOT much lower than control, while GPT enzyme exhibited much increase in its activity in relation to control. Farag (1978) found that, alkaline phosphatase showed a slight decrease in OP-resistant strains of *S. littoralis* rather than the laboratory strains. Assar *et al.,* (2010) found that the total protein content and total concentration of amino acids decreased in the housefly treated with match and consult. Ghoneim *et al.,* (2012) found that proteins in treated *Schistocerca gregaria* by insect growth regulators (IGRS) were generally exhibited. Hamadah *et al.,* (2012) found a predominant inhibitory in lipid content of *S. gregaria* nymphs that treated with pyriproxyfen, tebufenozide or lufenuron. Assar *et al.,* (2012) who found that treating the 4th instar larvae of *Culex pipiens* with (cyromazine) chitin synthesis inhibitor (CSI) caused high decrease in glucose quantity. Al-Shannaf *et al.,* (2012) found that insect growth regulators (chlorfluazuron and pyriproxyfen) caused highly significant increases in the activity of chitinase enzyme (130 % times in larvae of American bollworm, *H. armigera*). Although a high level of resistance has been observed against the lufenuron (Sudhakaran 2002) yet it has proved as an effective insecticide against *S. littoralis* . Feroban proved to be the most effective of tested mixtures due to it contain chloropyrifos with twice concentrate (47.5%) compare with chlorosan (contain with chloropyrifos 24%). Also, feroban contain lufenuron (2.5%) which have a much slower mode of action (residual toxicity) and inhibit the production of chitin, therefore unable to successfully molt into the next stage (Abd El-Mageed and Shalaby, 2011)

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التأثير البيوكيميائى لثلاث مثبطات لتخليق الكيتين منفردة أو خلطاً مع مركبى كلوربيريفوس أو بيتا ثيفلوثرين على دودة ورق القطن

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أجرى هذا البحث بهدف دراسة تاثير عملية الخلط على فعالية ثلاث منظمات نمو حشرية تتبع مجموعة مثبطات تخليق الكيتين وهى فلوفينوكسيرون، كلورفلوزيرون و ليفينيورون مع مركبين احدهما يتبع مجموعة المبيدات الفوسفورية وهو كلوربيريفوس والثانى يتبع مجموعة البيرثرينات المصنعة وهو بيتا ثيفلوثرين على العمر اليرقى الرابع لدودة ورق القطن. أشارت النتائج إلى أن كل المخاليط المختبرة قد خفضت من فعالية منظمات النمو الحشرية المختبرة وفقا لدليل السمية وقد أعطى الخليط (بيتا ثيفلوثرين + كلورفلوزيرون) أعلى تأثير تضادي بينما أعطى الخليط (كلوربيريفوس + ليفينيورون) تأثير إضافة وقد لوحظ أن المخاليط التى اشتملت على مركب بيتا ثيفلوثرين أظهرت أعلى تأثير تضادى مقارنة بالمخاليط التى اشتملت على مركب كلوربيريفوس. وكان الهدف الثانى من الدراسة محاولة تفسير الدور الذى تلعبه بعض الانشطة الانزيمية فى زيادة او تثبيط فعل المبيدات سواء على الصورة المنفردة او المخلوطة من خلال تقدير مدى تأثير تلك المركبات على نشاط إنزيمات الإستيريزات (الفا و بيتا إستيريز) و الانزيمات الناقلة لمجاميع الامين (الاسبرتات والالانين) و إنزيمات الكيتينيز والفوسفاتيز القاعدى علاوة على تقدير البروتين الكلى والدهون الكلية والجلوكوز.

قام بتحكيم البحث

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