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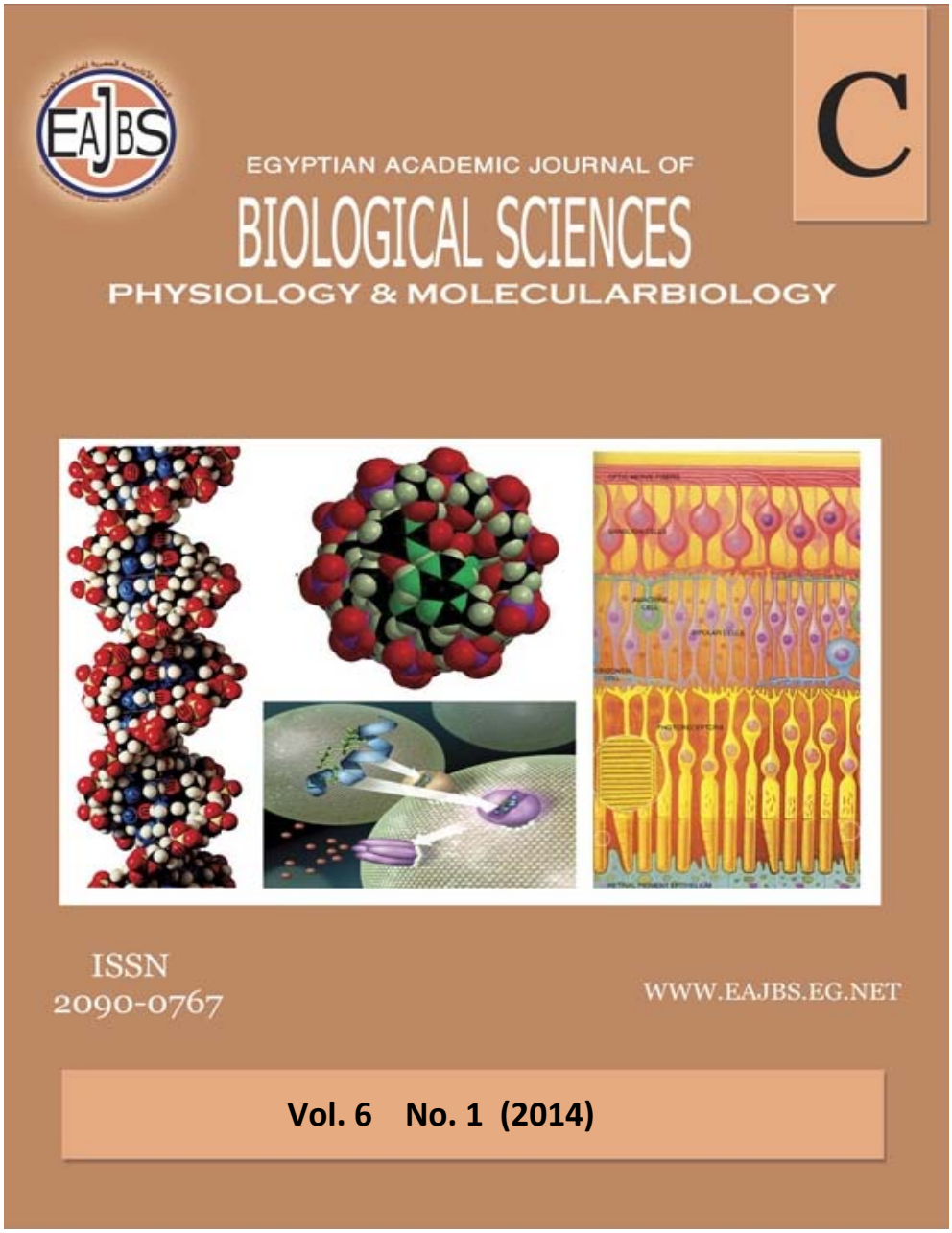

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Relationship between resistance level and some biochemical parameters to *Spodoptera littoralis* against some insect growth regulators (IGRs)

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ABSTRACT

Resistance levels in laboratory and field strain of *Spodoptera littoralis* (Boisd.) against two IGR's (lufenuron and tebufenozide) were studied. Resistance levels were higher in fayoum than Sharkia and laboratorystrain, in the two IGR's. There was a positive correlation between resistance and Alkaline phosphatase and α-esterase enzyme, while there was negative correlation between resistance level and Acid phosphatase & trehalase activity, while in amylase enzyme Fayoum strain was lower than Sharkia and laboratory strain. Fayoum strain showed higher activity in case Invertase enzyme than other two strain.

INTRODUCTION

Spodoptera littoralis is a pest of econamic importance in Egypt, where cotton is infested during May-July following migration from previous host plant clover Trifolium alexandrinum. Ishaaya and Klein (1990) found that, Spodoptera littoralis larvae collected from a cotton field that was heavily sprayed with conventional insecticides showed strong resistance to organophosphates and pyrethroids and a mild tolerance to benzoylphenylureas. IGR's differ widely from the commonly used insecticides, as they exert their insecticidal effects through their influence on development, metamorphosis and reproduction of the target insects by disrupting the normalactivity of the endocrine system (Oberlander, etal., 1997).Insect growth regulators (IGR's) received great attention as a hope for the future of insect control because of their mode of action which is different from conventional insecticides. Among these IGR's, Tebufenozide (mimic) belongs to a class of insect growth regulators (IGRs), bisacylhydrazine ecdysteroid agonists, mimicking the natural insect moulting hormone 20-hydroxyecdysone (20-E) (Dhadialla, *et al*., 1998).Also, Lufenuron (Match) is an acylurea insect growth regulator which inhibits chitin synthesis by prevents Lepidoptera larvae from molting from one stage to another. It acts by preventing the formation of the new cuticle. These compounds interfere with cutical deposition, by the inhibition of chitin synthesis (Riddiford and Truman,1978).The intensive use of broad-spectrum insecticides against S. littoralis has led the development of resistance to many registered pesticides for its control (Aydin and Gu¨ rkan, 2006) including IGRs (Temerak, 2002).

The ineffectiveness of insect growth regulators in controlling insect pests, is attributed to the increased levels of enzymatic detoxification, (Biddinger, *et al*., 1996). There is a relationship between the increase of insecticide resistance and the activity of detoxification enzymes (Xin-Ju and Hui-Min, 2011). General esterases are a large and diverse group of hydrolases that hydrolyze numerous substrates, including esters, and have no specific substrate (Walker & Mackness, 1983). A member of the esterase cluster probably plays a role in the detoxifcation of xenobiotic esters (Gacar and Tasksn, 2009). Increased esterase activity is a major mechanism of insecticide insensitivity or even resistance in many insect species (Zhou, *et al*., 2002). Detoxification enzyme, acid phosphatase (ACP) in insects is generally demonstrated as the enzymatic defense against foreign compounds and play significant roles in maintaining their normal physiological functions (Li, and Liu, 2007). Phosphatases are capable of transphosphorylation in addition to hydrolysis. Phosphatases play an important role in the metabolism of carbohydrates, phospholipids and nucleotides. Acid phosphatase is important in biological processes that need high level of energy, such as development, growth, gamete's maturation and histolysis (Ray, *et al*., 1984). The aim of this study was to assess the resistance levels of S. littoralis against the tested IGRs: moulting hormone agonist, mimic (tebufenozide) and chitin synthesis inhibitors, benzoylphenylurea derivative, match (lufenuron). In addition, to clarify the relationship between the increase of insecticide resistance and the activity of detoxification enzymes.

MATERIALS AND METHODS

Insects:

A laboratory strain of the cotton leaf worm *Spodoptera littoralis* was obtained from the Central Agricultural of Pesticide Laboratory that established under constant conditions of 25°C ± 1 and 70 ± 5 % R.H. and out of any contamination with chemicals till the time of study. The strain was reared in the laboratory as describedby El-Defrawi (1964) under the previous optimum condition during the experiment. Field starin was collect form cotton field from fayoum and Sarkia governorates.

Compounds:

Match® (Lufenuron) is an acylurea insect growth regulator which inhibits chitin synthesis and thereby prevents Lepidoptera larvae from molting from one stage to another. It acts by preventing the formation of the new cuticle.

Mimic™ 240LV (Tebufenozide) is an insecticide that has a novel mode of action as it "mimics" the action of the insect molting hormone in caterpillars.

Bioassay:

For the detection of the median lethal concentration (LC_{50}) values of IGR's (lufenuron and tebufenozide), a castor-bean leaves were dipped for 15 seconds in each aqueous concentration of the tested compound then left to dry. The treated leaves were offered to newly molted 4^{th}instar larvae for 48hr. then rplaced by untreated leaves for 24 hr. The average of mortality percentage was corrected using Abbott's formula (1925). The corrected mortality percentage of each compound was statistically computed according to Finney (1971). Resistance indexes (RI) are calculated by dividing each LC_{50} values of each strain on that of the strain with the lower LC_{50} value for each insecticide.

preparation of samples for biochemical studies:

The biochemical assay was done on the larvae homogenates of cotton leaf

worm *Spodoptera littoralis* that collected from different Governorates. After centrifugation the supernatant was used directly for enzyme assay.

Enzymes measurements:

Carbohydratase assayedwas based on the digestion of trehalose, starch, and sucrose by trehalase, amylase, and invertase, respectively, according to the method described by Ishaaya and Swirski (1976).

Chitinasewas assayed using 3,5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexoaminase liberated on chitin digestion according to the method described by Ishaaya and Casida (1974).

Alpha- and Beta- esterases activities (α-E & β-E) were determined according to the method of Van Asperen (1962) using alpha- and beta-naphthyl acetate (α - and β -NA) as substrates.

Acid- and alkaline-phosphatase activities were estimated according to the method described by Powell and Smith (1954) using disodium phenyl phosphate as substrate. The activity is expressed as ug phenol released / mg body weight.

RESULTS AND DISCUSSION

Toxicological study:

Resistance Ratio (RR) compared with Lab strain.

The resistance spectrum of the tested strains to the IGR insecticide Lufenuron as shown in Table (1) and Fig. (1), indicated that the Sharkia strain exhibited remarkable modurate level of tolerance to the tested insecticide (RR=12.63). On the other hand, Fayoumfield strain exhibited relatively remarkable, high levels of resistance to the tested insecticide (RR=22.82).

Table 1: Toxicity data and resistance ratios of IGR's Lufenuron against 4th instar larvae of *S. littoralis* lab and field-strains.

Strains	LC_{50}	Lower limit	Upper limit	RR	Slope	LC_{90}
Laboratory	0.067	0.04	0.102	1	0.737	3.679
Sharkia	0.846	0.34	1.928	12.63	0.582	134.745
Fayoum	1.529	0.935	2.502	22.82	0.486	837.475

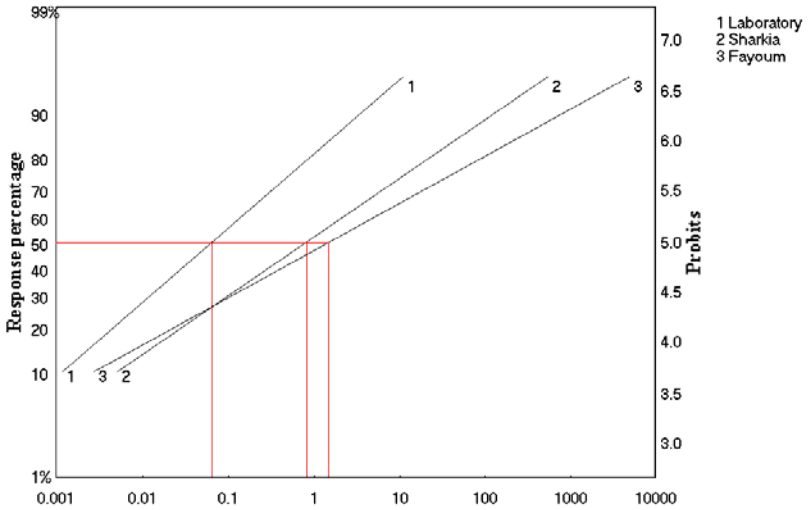

Fig.1: Log-probit concentration lines of insecticide Lufenuron on the 4thlarval instar of *S. littoralis* of laboratory and field strains.

The tested strains to the IGR insecticide Tebufenozide as shown in Table (2) and Fig. (2), indicated that the tested laboratory strain was the most susceptible strain to the insecticide (LC50 = 1.21 ppm) compared to the other field strains so it has resistance ratio (RR) value of (1).

Table 2: Toxicity data and resistance ratios of IGR's Tebufenozideagainst 4th instar larvae of *S. littoralis* lab and field-strains.

Strains	LC_{50}	Lower limit	Upper limit	RR	Slope	LC_{90}
Laboratory	1.196	0.658	1.977	1	0.488	506.347
Sharkia	7.17	4.036	12.703	5.995	0.397	12029.07
Fayoum	25.291	12.762	57.832	21.146	0.327	$2.11x\ 10^5$

Resistance Ratio (RR) compared with Laboratory

Sharkia strain exhibited remarkable moderate level of tolerance to the tested insecticide (RR=5.93). On the other hand, Fayoum strain exhibited relatively remarkable, high levels of resistance to the tested insecticide (RR=20.94).These results revealed that Lufenuron was more toxic against the 4thlarval instars of *S. littoralis*at LC50 and LC_{90} than Tebufenozide and the resistance level to both IGR's could be arranged desending as the follow Fayoum, Sharkia and laboratory strain.

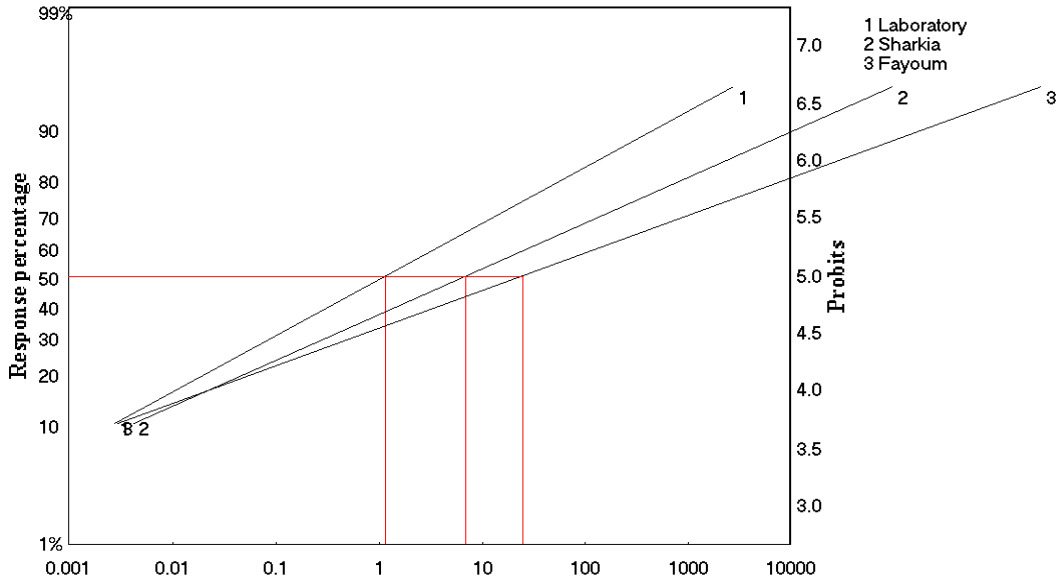

Fig. 2: Log-probit concentration lines of Tebufenozide insecticide on the 4th larval instar of *S. littoralis* of laboratory and field strains.

Biochemical study:

As showin Table (3) trehalase activity was negativly correlated with resistance level in both IGR's while in the case amylase Fayoum was lower than Sharkia and laboratory strain, as for Invertase Fayoum strain showed higher activity than other two strains. In all gavernate comparison with Laboratory strain. Carbohydrates, very efficiently utilized by insects and most species derive the main part of their nourishment from these nutrients depends. Amylase, invertase and trehalase were found to be the most important enzymes that play the major

role in the digestion and metabolism of carbohydrates in insects (Wigglesworth, 1972; and Wyatt, 1967). These enzymes have received a great deal of attention in concern with digestion and utilization of carbohydrates in insect. However, little is known about their physiological and biochemical contribution to insecticidal toxicity (Ishaaya & Ascher, 1977).

Table 3: Carbohydrates enzyme activities in the homogenates of 4th instar larvae of field and lab. strains of cotton leafworm.

Strains	Trehalase				Amylase				Invertase			
	(µg glucose / min. / g.b.wt.)											
	Activity			%	activity			%	Activity			%
Laboratory	79.85	±	0.365	100.00	33.23	±	0.998	100.00	159.63	±	0.384	100.00
Sharkia	66.35	±	0.716	83.12	40.10	±	0.768	120.70	152.85	±	0.656	95.71
Fayoum	60.10	±	0.651	75.33	28.70	±	0.657	86.40	170.40	±	0.787	106.70

% = percentage relative to control

Table (4) show the response in larval chitinase activity in all governat showed increase in the enzyme activity in the *S.littorals* in Sharkia governorate 121.4 % relative to control (Laboratorystrain), and in the same Table (3) found that the high level of alkaline phosphatase (AlP) and low level of acid phosphatase accompanied resistance to tested insecticides as compared with Laboratorystrain. Acid phosphatase (AcP) plays an important role in the detoxification process of toxic compounds entering the body (Zheng, *et al*., 2007). 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).

Table 4: Chitinase enzyme, acid and alkaline phosphatase enzyme activities in the homogenates of 4th instar larvae of field and lab. strains of cotton leafworm.

Strains	**Chitinase**				**Alkaline Phosp**				**Acid Phosp.**			
	µg NAGA / min./ g.b.wt.				(µg phenol / min. / g.b.wt.)							
	activity			%	Activity			%	Activity			%
Laboratory	4.20	±	0.657	100.0	15.30	±	0.384	100.0	3.20	±	0.651	100.0
Sharkia	5.10	±	0.768	121.4	27.50	±	0.656	179.7	3.04	±	0.768	95.0
Fayoum	4.20	±	0.657	100.0	34.40	±	0.653	224.8	2.60	±	0.716	81.2

% = percentage relative to control

Table (5) show that increase inα-esterase enzyme activity in all governorats was positively correlated with resistance level while no correlation was observed in the case of β- esterase and different degree in β- esteraseenzyme activityin all governoratrelative to Laboratorystrain sharkia and fayoum (126.6,98.1), respctivity.

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).The insect esterases can either cause broad-spectrum resistance to various nsecticides through rapid binding and slow turnover of insecticide molecules (i.e.,sequestration) or cause narrow-spectrum resistance to a very restricted range of insecticides containing a common ester linkage, such as malathion, through rapid metabolism of the insecticides (Karunaratne, *et al*.,1995).

Our results from biochemical survey suggested that the Fayoum strain exhibited relatively remarkable high levels of resistance to the tested insecticide; Fayoum strain haslow level of acid phosphatase accompanied the level of resistance to test insecticides comparison with Laboratorystrain. Acid phosphatase (AcP) plays an important role in the detoxification process of toxic compounds entering the body.

Table 5: Esterase enzyme activities in the homogenates of 4th instar larvae of field and lab. strains of cotton leafworm

Strains	α- Esterase				β - Esterase			
	(μg α- or β-naphtho/ min. / g.b.wt.)							
	Activity			%	Activity			%
Laboratory	444.3	±	0.384	100.0	805.5	±	0.651	100.0
Sharkia	707.5	±	0.656	159.2	1019.5	±	0.768	126.6
Fayoum	934.5	±	0.653	210.3	790.5	±	0.716	98.10

% = percentage relative to control

Lufenuron was more toxic against the 4th larval instars of *S. littoralis*at LC_{50} and LC90 than Tebufenozide. Our finding agree with the results were found by Bakr, *et al*., (2010). They studied the effect of the sub lethal doses LC_{25}, LC_{50} and LC_{90} of flufenoxuron (Cascade) on the activity of detoxification enzyme, acid phosphatase, of 2nd and 4thlarval instars of *S. littoralis*. Their results showed that the activity of enzyme decreased significantly in treated larvae at different times intervals post treatments.

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ARABIC SUMMARY

العلاقة بين مستوى المقاومة وبعض القياسات البيوكيميائية لدوة ورق القطن ضد بعض منظمات النمو الحشرية

محمد محى الدين عبد الحافظ - سامح مصطفى عبد النبى
معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقى – الجيزة

مستويات المقاومة المختبرة للسلالة المعملية وبعض السلالات الحقلية لدودة ورق القطن ضد مركبين من منظمات النمو الحشرية (التيبيوفينوزايد و لوفينورون). كانت مستويات المقاومة فى محافظة الفيوم أعلى من محافظة الشرقية والسلالة المعملية المختبرة. وكانت هناك علاقة إيجابية بين المقاومة وإنزيم الفوسفاتيزالقلوى وإنزيم الفا إستيريزفى حين كانت هناك علاقة سلبية بين مستويات المقاومة وإنزيم الفوسفاتيز الحامضى وإنزيم التريهالوز، بينما إنزيم الأميليز فى محافظة الفيوم كان أقل فى مستوى النشاط عن محافظة الشرقية والسلالة المعملية، بينما وجد أن سلالة محافظة الفيوم أظهرت مستوى أعلى فى النشاط لإنزيم الإنفرتيز عن غيرها من السلالات.