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POSSIBLE MECHANISMS OF ORGANSPHOSPHORUS RESISTANCE IN FIELD STRAINS OF COTTON LEAFWORM, Spodoptera littoralis (BOISD.)

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ABSTRACT

Comparative biochemical and toxicological studies of Spodoptera littoralis (Boisd.) to the organophosphorus (OP) insecticides were made to detect any changes that might be linked to the development of resistance. The resistant cotton leafworm which originated from the field colonies of Kafr El-Sheikh(KF) & Sharkia (SH) governorate had greatly elevated levels of lipids, non-specific esterases, acid-and alkaline phosphatases than the susceptible strain. On the other hand, acetylcholinesterase activity was significantly lower in resistant larvae and less sensitive to in vitro inhibition with OP insecticides than that of the susceptible strain.

INTRODUCTION

The cotton leafworm, Spodoptera littoralis Boisd,, is one of the most important lepidopterous insects that attack cotton in Egypt. Many reports indicate clearly that in Egypt, the cotton leafworm is now developing resistance to organophosphorus (OP) insecticides e.g. El-Sayed et al. 1975a & b; Dittrich et al., 1979; El-Sayed et al 1980-1981; Abbassy et al., 1982; Ayad and El-Dakroury, 1984 and Issa et al., and Abassy et al (1988). A knowledge of the factors contributing to the of resistance mechanism in cotton leafworm could conceivably be useful in devising alternative methods of chemical control. The present paper reports the results. of comparative biochemical and toxicological studies of two field strains of cotton leafworm that were resistant to OP insecticides.

MATERIALS AND METHODS

Insects.-The laboratory susceptible strain of S. littoralis (Boisd.) was used as a standard against which resistance could be measured. The susceptible (s) strain, which was obtained from the laboratory of plant protection Institute. Ministry of Agriculture , Egypt , was reared in the laboratory without any exposure to insecticides. The resistant field strains KF and SH were obtained from Kafr El-Sheikh and Sharkia Governorates, respectively. In all experiments larvae were reared according to the method of El-Defrawi et al. (1964).

Insecticides.-The following organophosphorus compounds were used for measurement of resistance Sulprofos, Bolstar (E.C. 72%), O-ethyl-O-(4-methyl thiophenyl)-S-n-propylphosphorodithioate; Chlorpyrifos, Dursban (E.C. 40.8%), O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)phosphorothioate;Profenofos,Curacr-on(E.C.72%),O-ethyl-O-(2-chloro-4-bramophenyl)-S-propylphosphorothioate.

Resistance measurement.-A microliter of acetone solution of each insecticide was applied topically on the dorsum of the 4th instar larva ten larvae were treated with each concentrations and three replicate were used. Treated insects were kept on fresh leaves of castor bean and mortality was recorded 24 hr after application. The data were corrcted according to Abbott's formula (1925) and analyzed according to the method of Litchfield and Wilcoxon (1949).

Measurements of AChE activity and Inhibition.-All AChE determinations were carried out using acetylthiocholine as a substrata and DTNB as a sulfhydryl reagent (Ellman et al. 1961).

Determinations of esterase Activity.- All determinations were done according to the method of Van' Asperen (1962) using - and B-naphthyl acetate as substrates.

Determination of phosphatases. - Acid-and alkaline phosphatases were determined according to the method of Kind and King (1954) as modified by Belfield and Goldberg (1971) using phenyl phosphate as substrata.

Determination of total and unsaturated lipids. - Total lipids and unsaturated lipids were determined according to the methods of Bennet and Thomas (1963) and Knight et al (1972) , respectively.

Protein determination. -Colorimetric method of Weichselbavm (1976) was used for the determination of total protein in the 4th in star larvae homogenate.

All of the determination were done by using the 4th instar larval homogenates.

A homogenate was made by grinding 4th instar larvae with 0.25 M sucrose (0.4 mg/ml). The cold homogenate was centrifuged and the supernatant was used as the source of enzymes.

RESULTS AND DISCUSSION

Toxicity studies:-

Resistance to OP insecticides was found to be widespread and severe in field collected populations of cotton leafworm , S. littoralis as shown in Table (1). Thus Kafr El-Sheikh (KF) and Sharkia (SH) field strains had remarkably higher LD_{50} values than that of susceptible strain. They were resistant to all OP insecticides tested with maximum resistance factor of X36.2 in KF strain and a minimum factor of X14.7 in SH strain.

Esterase activity.- Comparison of the esterase activity of larval homogenates revealed that some physiological differences between the tested strains (Table 2). The homogenates of field larvae demonstrated higher specific activity for both - and B-naphthyl acetate. The higher activity was more pronounced in KF strain (395 µM/mg protein/min. $X10^{-3}$). Such mechanism has been suggested for certain OP resistant strains of Musca domestica L. (Mengle and Casida, 1960), Heliothis virescens (Whitten and Bull, 1970), green preach aphid, Myzus persicae / Sudderuddin, 1973 and S. littoralis (Abdel-Aal & Riskallah , 1978 and Abbassy et al, 1988).

Table 1: Resistance level of KF and SH strains of S. littoralis. compareing with susceptible stran.

Insecticide	LD50 (Mg/larva S. strain	Field strains KF	SH	Resistance factor KF/S	SH/S
Chlorpyrifos	1.7	35	25	20.6	14.7
Profenofos	1.45	52.5	32	36.2	22.4
Sulprofos	4.0	142.5	-	35.6	-

Table 2: Esterase activity of larval homogenates from resistant and susceptible cotton leafworm.

Substrate	Specific activity M/mg protein/min$X10^{-3}$ -naphthyl acetate	B-naphthyl acetate
Susceptible strain	200b	292.5b
KF strain	395a	564.5a
SH strain	367a	519a

Means followed by the same letter are not significantly different.

Phosphatases:

Results in Table (3) showed that the specific activity of both acid and alkaline phosphatases was significantly higher in the field strains than that in susceptible strain. KF strain had the highest level of the alkaline phosphatase (3978 µM/mg protein/min. $x10^{-6}$). Thus , the differences in phosphatases activity between susceptible and field strains suggest the important rate of phosphatases in the OP. resistance. This relationship has been reported by many authors such as Welling et al (1971) , Lewis and Sawicki (1971) Belal et al (1985) and Abbassy et al. (1989).

Table (3): Phosphatases activity of larval homogenates from susceptible and field resitant strains.

Strains	Acid phosphatase		Alkaline phosphatase	
	Specificativity µM /phenylphosphate /mg protein/min	% of susceptible strain	S.A. µM phenylphosphate /mg protein/min	%of susceptible strain
S	603	100 c	1845	100 b
KF	1783	289.69 bc	3978	215.6a
SH	1982	328.69 ab	2859	154.96 a

Means followed by the same letters are not significantly different.

Acetylcholinesterase.- Results in Table (4) show that the specific activity of larval AChE was significantly higher in the susceptible strain (72. µM acetylthiocholine/mg protein/min) than that in the field strains.

Table 4: Specific activity and in vitro inhibition of larval AChE in susceptible and field strains.

Strains	S.A.	I_{50} (µg a.i. of profenofs)	Resistance factor I_{50} fields strain/I_{50} S
Susceptible	72 a	4 a	-
KF	84 ab	13 bc	3.25
SH	25 b	8 ab	2

S.A. : µMole/Actylthiocholine/mg protein/min.

According to the I_{50} values for profenofos (Table 4), It was found that the AChE of the field strains is less sensitive to the in vitro inhibition by profenofos than that of the susceptible strain. The I_{50} value for propenofos to AChE in KF and SH strains are only 3.25 and 2.0 fold.

respectively, than those in susceptible strain. The insensitivity of AChE inhibiter is not the main factor of OP-resistance in these strains. A mutant AChE, insensitive to inhibition by OP insecticides, was reported for S. littoralis by Zaazou et al. (1973), Abbassy et al (1978), Dittrich et al (1979), Voss (1980), and El-Nawawy et al. (1981).

Based on these results it appears that the elevated activity of esterases, phosphatases and the lower activity and sensitivity of AChE contribute to the resistance of S. littoralis to the OP insecticides.

Total and unsaturated lipids. - Results in Table (5) revealed that the quantities of total and unsaturated lipids were significantly higher in the larvae of field strains than that in susceptible larave. The highest level of total lipids was found in the larvae of KF strain. The same positive correlation was observed by Bennet and Thomas (1963) on bollweevil, Khalid et al (1975) and Abbassy et al (1982) on S. littoralis larvae. These findings suggest that lipids may play a role in the mechanism of the OP resistance.

Table 5: Total and unsaturated lipids in S. littoralis 4th instar larvae of susceptible and field strains.

Strain	Total lipids mg/g. body weight	Unsaturated lipids mg/g. body weight
KF	31.08 ab	4.40 a
SH	10.29 bc	5.68 a
S	9.63 c	1.136

Means followed by the same letters are not significantly different.

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الملخص العربى

يهدف هذا البحث الى دراسة صفة المقاومة فى السلالات الحقلية لدودة ورق القطن المجموعة من محافظتى كفر الشيخ والشرقية وذلك لفعل المبيدات الفوسفورية العضوية ومحاولة دراسة أسباب حدوث هذه المقاومة عن طريق دراسة الأختلافات البيوكيماوية المختلفة فى كل من هذه السلالات الحقلية والسلاله المعملية الحساسة. وأوضحت النتائج مايلى:-

(١) أن يرقات السلالات الحقلية اكثر مقاومة لدرجة معنوية للفعل السام للمبيدات المختبرة وهى مالبروفوس ، وكلوروبيرفوس، بروفينوفوس بالمقارنة بالسلالة المعملية الحساسة.

(٢) أن نشاط الأستيريزات الكلية والفوسفاتيز الحامضى وكذلك القلوى كان مرتفعاً معنوياً فى السلالات الحقلية بالمقارنة بالسلالة الحساسة.

(٣) كان نشاط انزيم الأستيل كولين استيريز فى يرقات السلالة الحقلية منخفضاً معنوياً فى متجانس يرقات السلالة الحقلية وذلك بالمقارنة بالسلالة المعملية الحساسة.

كما كان حساسية الأنزيم لتثبيط بهذه المبيدات خارجياً منخفض فى السلالات الحقلية عن انزيم السلالة الحساسة.

(٤) وجد أن يرقات السلالات الحقلية تحتوى على نسبة أكبر من المحتوى المحتوى الدهنى الكلى وأيضا الدهون غير المشبعة. عن التى توجد فى السلالة المعملية وعلى ذلك فإن هذه العوامل مجتمعة تلعب دوراً هاماً فى تفسير صفة المقاومة.