Biochemical Studies of Na^+,K^+-ATPase and Acetylcholinesterase Sensitivity to Phenothrin and Thiodicarb Among Different Egyptian Field Populations of *Spodoptera littoralis*

Ismail, Seham M[1].

ABSTRACT

Enzymatic activity of Na^+,K^+-ATPase and AChE of cotton leafworm *Spodoptera littoralis* collected from different four Egyptian field populations ranged from heavily-sprayed fields and cultivated fields were investigated and compared with a laboratory susceptible population. The highest levels of Na^+,K^+-ATPase and AChE activities were found in Alexandria Governorate Egypt. The moderate leveals was found in El-Boheira Governorate, Egypt. Na^+,K^+-ATPase and AChE and were isolated from brain of *S. littoralis* larvae (4^{th} instar). The sensitivity of Na^+,K^+-ATPase and AChE activity to Phenothrin and Thiodicarb respectively were measured by the I_{50} values. The I_{50} values of Phenothrin on the Na^+,K^+-ATPase activity were 0.01, 0.20, 0.36, 0.61 and 0.82µM for lab strain; Borg El-Arab; Abeis; Damanhour and Abou El-Matamir respectively. The I_{50} of Thiodicarb on AChE activity were 0.22, 0.43, 0.54, 0.71 and 0.96µM for lab strain and four field strains respectively. The inhibition constant (K_i) values were determined for Na^+,K^+-ATPase and AChE inhibitors. Values of K_i in the case of Phenothrin were 5, 18, 20, 30 and 45µM for lab strain; Borg El-Arab; Abeis; Damanhour and Abou El-Matamir respectively on Na^+,K^+-ATPase activity. Similarly, Thiodicarb were 20, 28, 30, 40 and 50µM for lab strain and four field strains respectively on AChE activity. The results of the present study may add some forward steps to uses this enzymes indicate of effect this insecticides under study, in the IPM programs of the cotton leafworm.

INTRODUCTION

The Egyptian cotton leafworm *Spodoptera littoralis* is the major pest attacking several crops and vegetables in Egypt, this pest cause the greatest part of cotton yield losses (Smagghe and Degheele, 1997; Amin *et al*., 2001; & Quero *et al*., 2002). Number of insecticides currently in widespread use: Organophosphates, Carbamates and Pyrethroids are usually used in Egypt (Devonshire and Moores 1982; & Argentine *et al*., 2002), to suppress the *S. littoralis* populations, however, most of them dose not give satisfactory results, probably because of development of resistance, (Ishaaya and Klein, 1990; & El-Aw *et al*., 2002). From this point the need for insect control is essential through chemical control (Pesticides) (Casida and Quistad 2005) so in the present study we began to study a two target in the insect to the knowleage about insecticide susceptibility.

In this work, we describe the development of a biochemical assay system for measuring the sensitivity of Na^+,K^+-ATPase and Acetylcholinesterase (AChE) to Phenothrin and Thiodicarb respectively. We also provide enzyme kinetic data for the Na^+,K^+-ATPase and AChE in this four field strains Abeis and Borg El-Arab (Alexandria Governorate) Damanhour and Abou El-Matamir (El-Boheira Governorate), and compared them with data obtained of lab strain.

MATERIALS AND METHODS

Insect:

a- The susceptible laboratory strain of *Spodoptera littoralis* was provided from centeral lab of pesticides, Agricultural Research Center (ARC) Cairo, Egypt which was reared for several years.

b- The field strain was obtained by the collection of the egg masses from cotton fields at Abeis and Borg El-Arab (Alexandria Governorate) Damanhour and Abou El-Matamir (El-Boheira Governorate); the 4^{th} larval instar used for assessments.

Chemical:

Phenothrin (Pyrethroids) provided as technical grade insecticides from U.S.A. Environmental Protection Agency (EPA), USA. Ouabain is a cardiac glycoside which specifically inhibits the Na^+,K^+-ATPase (McIIwain,1963). A pure sample was obtained from Sigma Chem., Co. ST. Loius. Thiodicarb (Carbamate) provided as technical grade insecticides from JinHung Fine Chem., Co. LTd. Koria. Stock solutions of these compounds were prepared in pure acetone.

Bioassay tests:

Fresh leaves of castor were dipped for 1min in different concentrations of the tested insecticides, all insecticides concentrations were prepared in acetone solution. Control plants were dipped in acetone solution. Treated and control plants were air-dried for 3hrs. The treated leaves were placed in clean glass container at the laboratory conditions of 27±2°C and 65-70%RH. Ten larvae (Lab and Field strains) were used for each test with three replicate at least. Number of alive and dead larvae per replicate was counted 24 and 48hr, after treatment. Concentration-mortality percentages were calculated and corrected for natural mortality according

[1]Center Laboratory of Pesticides, Sabahia, Alexandria, A.R.C.
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to Abbott equation (Abbott, 1925). LC_{50} values were calculated by using the probit-analysis method of Finney (1971).

Na^+,K^+-ATPase Preparation and Activity Assay:

Head capsoul from *S. littoralis* fourth-instar larvae dissected and homogenized in a solution of 0.32M sucrose, 1mM EDTA and 40mM Tris-HCl buffer (pH 7.4). The homogenate was filtered through two layers of cheese cloth. Mitochondrial ATPase was prepared according to the method reported by Koch (1969), by differential centrifugation of the homogenate at 8000Xg for 10min. The supernatant was then centrifuged at 20000Xg for 30min. The formed pellets were then suspended in the buffer and stored at (- 20^{o}C) for use.

The ATPase activity was measurements according to the method reported by Koch (1969), with slight modification by Morshedy (1980) using Tris-HCl buffer instead of imidazole buffer. Absorbancy of inorganic Phosphate (Pi) was measured at λ750nm (Taussky and Shorr, 1953). The method was based on the spectrophotometric determination of the inorganic Phosphate (Pi) liberated from the hydrolysis reaction of the ATP, mediated by the enzyme.

The ATPase activity wae measured in a total volume of 1ml. The mitochondrial preparation was mixed with a reaction mixture (700µl) containing 100mM Na^+, 20mM K^+, 5mM Mg^{2+} chlorides, 40mM Tris-HCl buffer (pH 7.4), and 5mM ATP. The volume was completed to 850µl with buffer. The mixture was incubated for 15min, in a shaking water bath at 37^{o}C. The reaction was stopped by adding 150µl trichloroacetic acid (TCA, 30%). Hydrolyzed Pi was determined according to the method, described by Taussky and Shorr, (1953). The activity of Mg^{2+}-ATPase was measured after the addition of 1mM ouabain, whereas the activity of Na^+,K^+-ATPase was calculated as the difference between the total ATPase and Mg^{2+}-ATPase activities.

AChE Preparation and Activity Assay:

Head capsoul from *Spodoptera littoralis* (fourth instar larvae) was dissected and homogenized in Tris-HCl buffer (pH 7.4) at 30 larvae/30ml buffer, with polytron mixer (at 50% power for 50sec.), then subjected to low speed centrifuged at 5,000 rpm for 15min at 4^{0}C. The resulting supernatant was centrifuged at 15,000rpm for 20min at 4^{0}C. The supernatant centrifuged at 25,000rpm for 1hr at 4^{0}C. Pellets were resuspended in 1ml of Tris-HCl buffer (pH 7.4) and stored at (-20^{0}C) for used as enzyme source.

The AChE activity measurements were done according to method reported by Ellman *et al.,* (1961). This method is based on the hydrolysis of acetylthiocholine iodide (ATChI) as substrate by enzyme to produce thiocholine and acetic acid. Thiocholine reacts with 5,5-dithio bis-(2-nitrobenzoic acid), "DTNB" to produce the yellow anion of 5-thio-2-nitrobenzoic acid. The rate of color production as a function of enzyme activity is measured spectrophotometrically at λ412nm. Enzyme specific activity was computed as mg protein/hr.

The protein content in prepared homogenates of *S. littoralis* was assayed spectrophotometrically by the method of Lowery *et al*., (1951) at λ750nm using Bovine Serum Albumin (BSA) as a standard protein.

***In Vivo* and *In Vitro* Inhibition and Kinetics of Na^+,K^+-ATPase and AChE:**

The inhibition of Na^+,K^+-ATPase and AChE activity were determined in all tested sources using the LC_{50} values of each of the two tested insecticides (Phenothrin and Thiodicarb) as inhibitors. The inhibitor for each of Na^+,K^+-ATPase and AChE were evaluated to determine enzyme kinetic parameters. The method of Dixon and Webb (1964) was adopted to draw the Dixon-plots by plotting 1/V versus concentrations of the inhibitor at two concentrations of the substrate. ATP (the substrate of ATPase) concentrations were 3.0 and 5.0mM, while acetylcholine iodide (the substrate of AChE) was used at concentrations of 5 and 10mM.

Estimation of I_{50} value was carried out by preincubating the enzyme with the inhibitor for 30min. Using the following concentrations 0.1; 1; 5; 10; 50 and 100µM. K_i (the inhibition constant) values for each inhibitor were estimated from Dixon-plot.

Michaelis-Menten Kinetics (K_m & V_{max}) values were calculated by a linear regression of 6 points on each Lineweaver and Burk Plot (1934).

RESULTS AND DISCUSSION

Toxicity of Insecticides Against *Spodoptera* Larvae:

Toxicity results of the insecticides expressed in terms of LC_{50} are given in Table (1). Phenothrin LC_{50} values after 24hr are 0.004, 0.011, 0.031, 0.052 and 0.071ppm for lab strain; Borg El-Arab; Abeis, Damanhour and Abou El-Matamir strains respectively. While LC_{50} values after 48hr for Phenothrin are 0.001, 0.003, 0.011, 0.031 and 0.051ppm for lab strain and the four field strains respectively. Also Thiodicarb LC_{50} values after 24hr are 0.009, 0.08, 0.05, 0.07 and 0.09ppm for lab strain and the four field strains respectively, while LC_{50} values after 48hr are 0.006, 0.002, 0.02, 0.04 and 0.06ppm for Thiodicarb against lab strain and the four field strains of *Spodoptera* larvae respectively.

It is clear that the toxicity was higher with the Phenothrin and Thiodicarb for lab strain, Borg El-Arab

Table 1. Toxicity of Phenothrin and Thiodicarb on *S. littoralis* larvae

Spodoptera strain locations	LC_{50} (ppm) Phenothrin		LC_{50} (ppm) Thiodicarb	
	24hr	48hr	24hr	48hr
Laboratory	0.004	0.001	0.009	0.006
Borg El-Arab	0.011	0.003	0.08	0.002
Abeis	0.031	0.011	0.05	0.02
Damanhour	0.052	0.031	0.07	0.04
Abou El-Matamir	0.071	0.051	0.09	0.06

and Abeis, while toxicity was low for Damanhour and Abou El-Matamir. Also Phenothrin was more toxic than Thiodicarb in controlling of *S. littoralis.*The present results emphasize that during many years of selection pressure in the field, the resistance and/or tolerance levels to the insecticides had increased due to the intensive application of such insecticides for controlling *S. littoralis* in cotton fields. These results fully agreeded with Davis *et al*., (1975), who reported that synthetic Pyrethroids was more toxic other tested insecticides in controlling many species of insects. Hosny *et al*., (1977) mentioned that synthetic Pyrethroids were most superior toxicants against the cotton leafworm better than the tested Organophosphorus insecticides. Moustafa *et al.*, (1979) proved that synthetic Pyrethroids were not only superior to Organophosphorus but also to Chlorinated hydrocarbons and Carbamate insecticides in controlling of cotton leafworm. Kaygisiz (1980) and McDonald (1981) reported that synthetic Pyrethroids were the most effective against 4th instar larvae of *S. littoralis*. Ishaaya and Klein (1990) found that *S. littoralis* larvae collected from a cotton field that was heavily sprayed with conventional insecticides showed strong resistance to Organophosphates. Korkor *et al*., (1995) reported that synthetic Pyrethroids were the most effective insecticides against Bollworms. Mascarenhas *et al*., (1998) found that several field strains of beet armyworm, *Spodoptera exigue* (Hubner), exhibited reduce susceptibility to Chlorpyrifos and Thiodicarb.

Specific Activities of Na^+,K^+-ATPase and AChE:

Table(2) summarized the specific activity of Na^+,K^+-ATPase; Mg^{+2}-ATPase and AChE Fig (1&2) show the specific activity of the ATPases, isolated from Lab strain and different field strains of *S. littoralis*. The maximum value of specific activity of Na^+,K^+-ATPase was found in Lab strain and Borg El-Arab, whereas that the values of Na^+ ,K^+- and Mg^{2+}-ATPases activities in brain preparations of the *Spodoptera*, were recorded. Total activities of ATPase were greatest (45.86±0.13 & 41.85±0.11 respectively) in Lab strain and Borg El-Arab, and least in the Abou El-Matamir (28.51±0.43). Total ATPase activities were modest in Abeis and Damanhour (the values are 38.85±0.06 & 32.94±0.17 respectively). Also observed the Na^+,K^+-ATPase activity was more than the Mg^{2+}-ATPase activity, in all different sources.

Data presented in Table (2) and Fig(3) show the specific activity of the AChE in the brain of the 4th larval instar of lab strain and all tested field strains of *S. littoralis*. The results show that there were significant differences in AChE specific activity between the strains. AChE activity were higher in the lab strain, Borg El-Arab, and Abeis (the values are 31.86±0.05, 26.56±0.37 &20.17±0.15 λ_{max} 412 mg^{-1} Protein hr^{-1} respectively) than Damanhour and Abou El-Matamir (the values are 14.28±0.12 & 10.61±0.09 λ_{max} 412 mg^{-1} Protein hr^{-1} respectively).

Table 2. Na^+,K^+-ATPase and AChE specific activities *Spodoptera* brain larve (4th instar) in different local strains

Spodoptera strain locations	Specific activities ± S.D			
	Total ATPase	Na^+,K^+-ATPase	Mg^{+2}-ATPase	AChE
Laboratory	45.86 ± 0.13	36.82 ± 0.04	9.04 ± 0.01	31.86 ± 0.05
Borg El-Arab	41.85 ± 0.11	30.92 ± 0.13	7.33 ± 0.10	26.56 ± 0.37
Abeis	38.85 ± 0.06	28.30 ± 0.14	6.60 ± 0.06	20.17 ± 0.15
Damanhour	32.94 ± 0.17	25.76 ± 0.15	0.50 ± 0.03	14.28 ± 0.12
Abou El-Matamir	28.51 ± 0.43	24.21 ± 0.52	4.17 ± 0.08	10.61 ± 0.09

Na^+,K^+-ATPase specific activity (Pi µmole mg^{-1} Protein hr^{-1})

AChE specific activity (λ_{max} 412 mg^{-1} Protein hr^{-1})

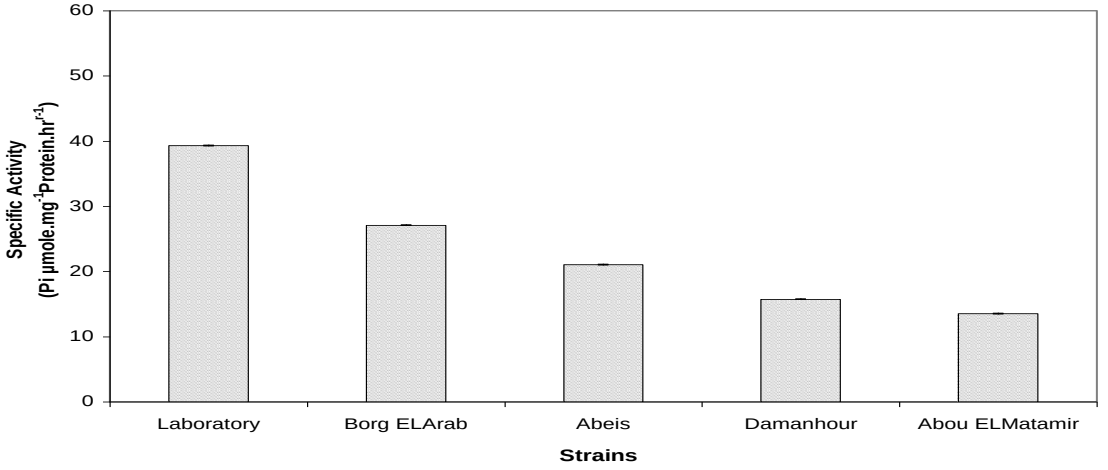

Fig1. ATPase specific activity in *Spodopetra* brain larvae (4th instar) in different local strains

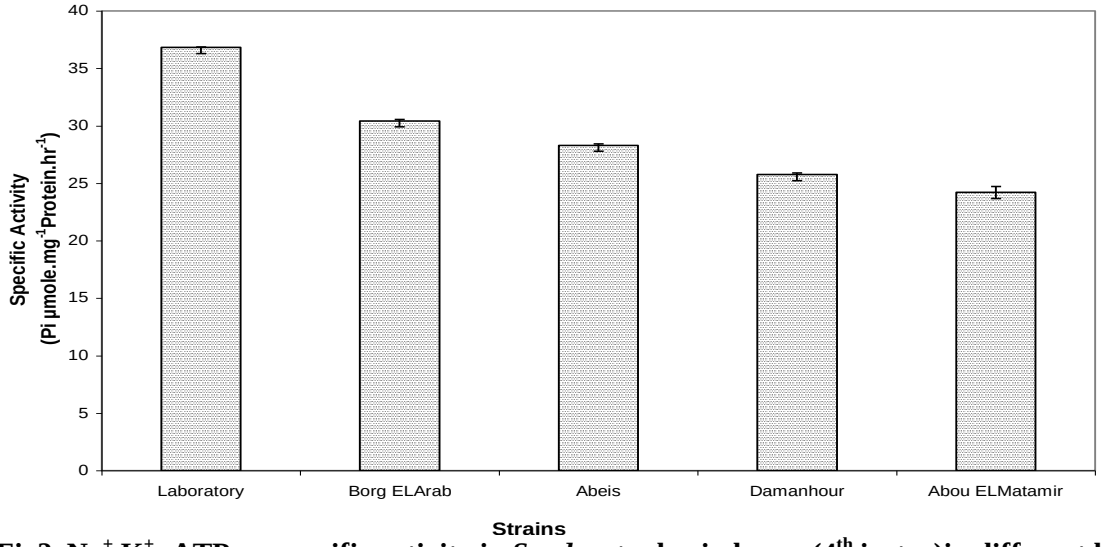

Fig2. Na^+,K^+- ATPase specific activity in *Spodopetra* brain larvae(4th instar)in different local strains

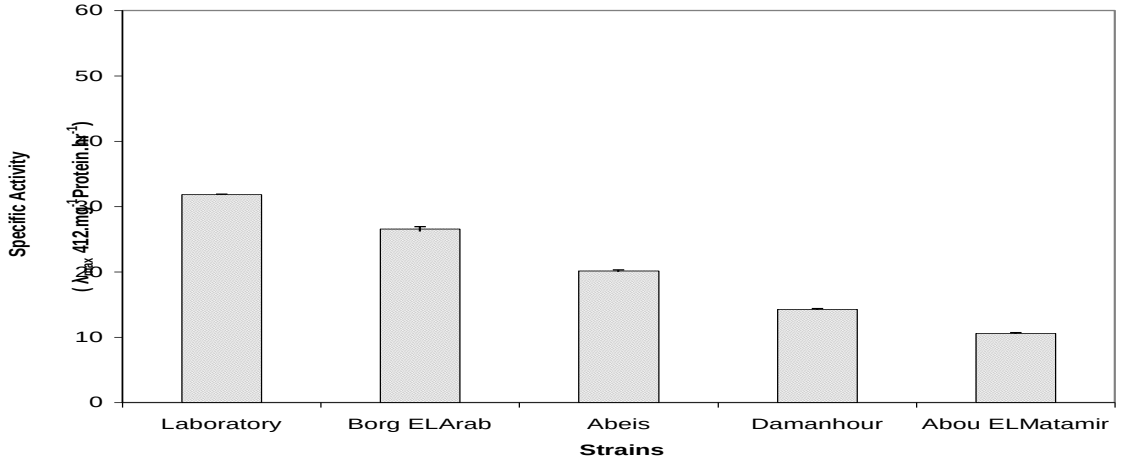

Fig3. AChE specific activity in *Spodeptera* brain larvae(4thinstar) in different local strains

In Vivo Inhibition of Brain *S. littoralis* Na^+,K^+-ATPase and AChE Activity:

The *in vivo* inhibitory effect of the LC_{50} values of two insecticides against to the *Spodoptera littoralis* 4th instar lab and field strains larval Na^+,K^+-ATPase and AChE is shown in the data given in Table (3). The data revealed that Phenothrin exhibition significant reduction in Na^+,K^+-ATPase activity. Percentages of Na^+,K^+-ATPase inhibition were 87.3, 84.2, 74.1, 71.4 and 65.5% for lab strain; Borg El- Arab; Abeis; Damanhour and Abou El-Matamir, respectively. On the other hand, in the case of AChE, the significant reduction in its activity was recorded for Thiodicarb, the percentages of AChE inhibition were 82.5, 77.4, 73.6, 68.1 and 56.7% for lab strain and four field strains respectively.

Table 3. *In vivo* inhibition of brain *S. littoralis* Na^+,K^+-ATPase and AChE activities by two compounds

Spodoptera strain locations	%Inhibition of enzymes (LC_{50})ppm	
	Na^+,K^+-ATPase	AChE
	Phenothrin	Thiodicarb
Laboratory	87.3	82.5
Borg El-Arab	84.2	77.4
Abeis	74.1	73.6
Damanhour	71.4	68.1
Abou El-Matamir	65.5	56.7

Kinetic Parameters of Na^+,K^+-ATPase and AChE Inhibition:

The kinetic studies were conducted to evaluate the effects of Phenothrin on Na^+,K^+-ATPase activity and Thiodicarb on AChE activity in both tested strains brain of *S. littoralis* 4th larvae. Table (4) shows the obtained Lineweaver-Burk (L-B) plots for Na^+,K^+-ATPase and AChE in lab strain and all four tested field strains and the statistical analysis of the obtained values of K_m (Michaelis-Menten,constant) and V_{max} (maximum velocity) of the Na^+,K^+-ATPase and AChE. The K_m values for Na^+,K^+-ATPase and AChE were generally higher in all four tested field strains than lab strain. The changes in K_m values of Na^+,K^+-ATPase and AChE between the tested field strains indicate changes in the affinities, our result are strongly emphasized by the recent kinetic studies of Gonzalez *et al.*, (1990) found that the calculated K_m of 0.22mM for AChE of gastropod *Concholepas concholepas.*

The present results show that the V_{max} values of Na^+,K^+-ATPase and AChE are obviously higher. This points to the highe substrat turnover which may reflect the physiological importance of the Na^+,K^+-ATPase in the function of the nervous tissue of the *S. littoralis* larval brain (El-Aw and Hashem, 2001). The V_{max} values were generally higher in all tested field strains than lab strain. This fact indicateds that the number of active sites on the Na^+,K^+-ATPase and AChE of the 4th larvae brain was increased in the field strains. Such change may be followed by decrease in the insect susceptibility which could be altered by field application of the Pyrethroides and Carbamate insecticides.

The *in vitro* inhibition of brain *S. littoralis* Na^+,K^+-ATPase and AChE activities:

To characterize more details about the *in vitro* inhibition of Na^+,K^+ATPase and AChE by the inhibitors, the K_i value of each inhibitor was estimated from the graphical method of Dixon and Weep, (1964) Fig. (4&5) and Table (5). The K_i values were 5, 18, 20, 30 and 45uM for lab strain; Borg El-Arab; Abeis; Damnhour and Abou El-Matamir respectively in the case of Phenothrin while the K_i values were 20, 28, 30, 40 and 50uM for lab strain and four field strains respectively in case of Thiodicarb. The obtained data proved that each of Phenothrin and Thiodicarb showed competitive inhibition on Na^+,K^+-ATPase and AChE activity. The present results are accordance with those reported by Zhu and Brindley (1992) who reported competitive inhibition of AChE purified from *Lygus Hesperus* by six OPs compounds.

Table 4. Michaelis-Menten kinetics of the Na^+,K^+- ATPase and AChE of larval brain of *S. littoralis* collected from different locations

Spodoptera strain locations	Na^+,K^+- ATPase		AChE	
	K_m(mM)	V_{max}(mM)	K_m(mM)	V_{max}(mM)
Laboratory	0.17	5.9	1.7	0.59
Borg El-Arab	0.30	3.3	1.8	0.56
Abeis	0.36	2.8	3.3	0.30
Damanhour	0.40	2.5	3.6	0.28
Abou El-Matamir	0.50	2.0	3.9	0.26

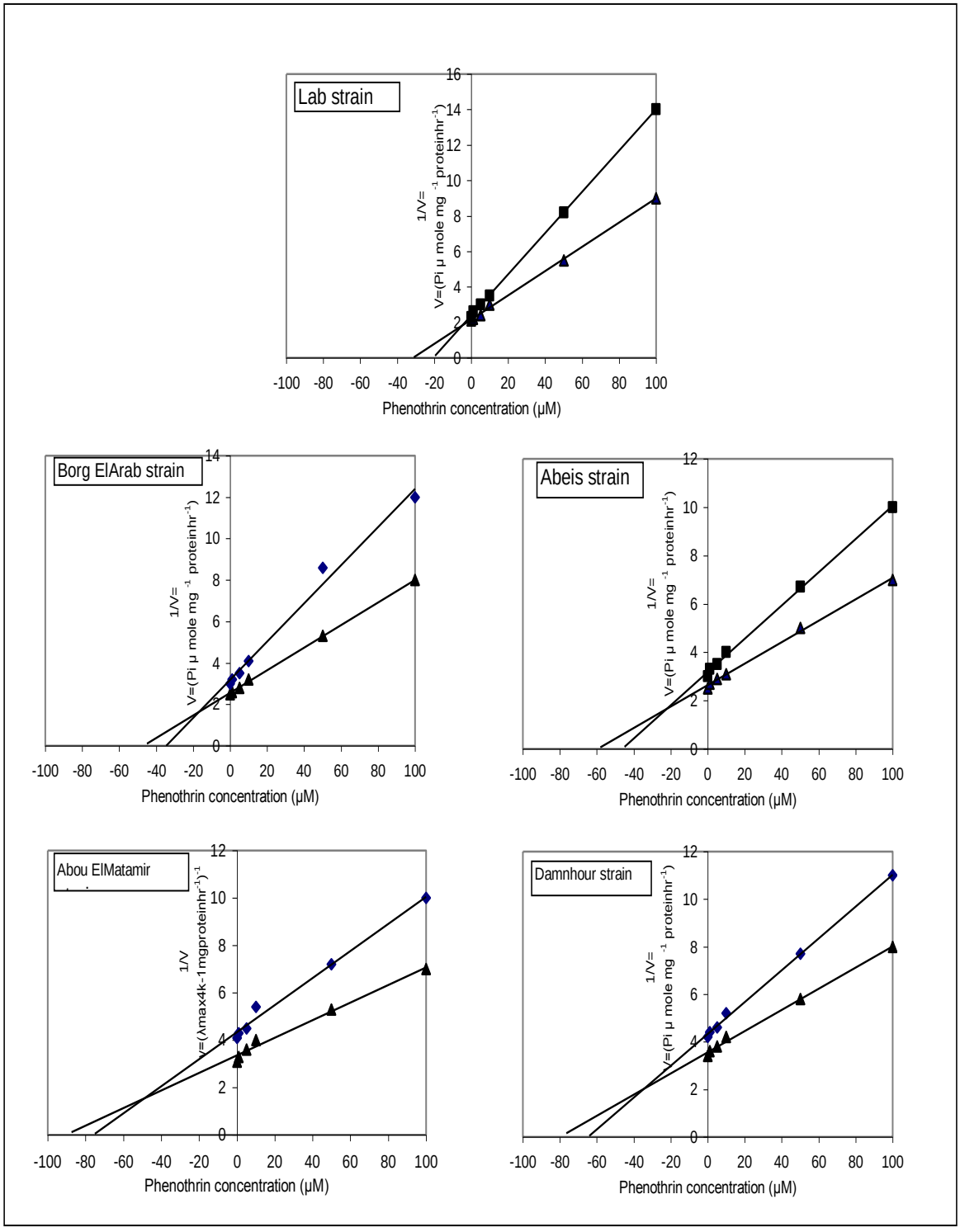

Fig 4.Dixon plot of the effect of Phenothrin on Spodopterabrain larvae(4^{th} instar) Na^+,K^+-ATPase activitiy at 3mM(□)and 5mM(Δ) ATP

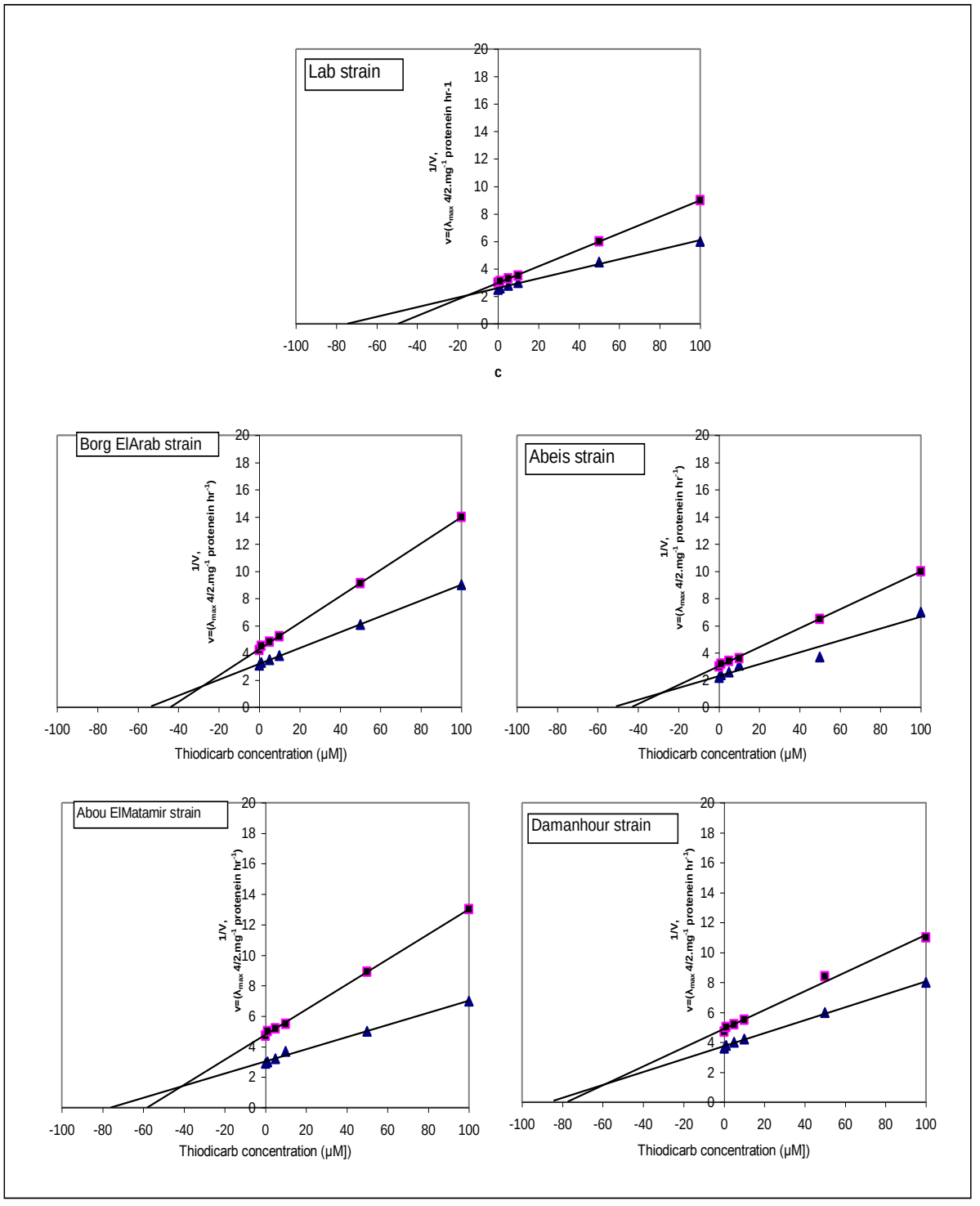

Fig 5. Dixon plot of the effect of Thiodicarb on Spodoptera brain larvae (4th instar) AChE activity at 5mM(□) and 10mM(Δ) of [ASChI]

Table 5. *In vitro* inhibition of brain *Spodoptera* larvae Na^+,K^+-ATPase and AChE activities by certain insecticides

Spodoptera strain locations.	Phenothrin		Thiodicarb	
	I_{50} (µM)	K_i (µM)	I_{50} (µM)	K_i (µM)
Laboratory	0.01	5	0.22	20
Borg El-Arab	0.20	18	0.43	28
Abeis	0.36	20	0.54	30
Damanhour	.061	30	0.71	40
Abou El-Matamir	0.82	45	0.96	50

Table (5) show the *in vitro* interaction of Phenothrin and Thiodicarb on Na^+,K^+-ATPase and AChE activity of *S. littoralis* 4th instar brain respectively. The I_{50} values for Phenothrin against of Na^+,K^+-ATPase were 0.01, 0.20, 0.36, 0.61 and 0.82uM for lab strain; Borg El-Arab; Abeis; Damanhour and Abou El-Matamir respectively. While the I_{50} values for Thiodicarb against of AChE were 0.22, 0.43, 0.54, 0.71 and 0.96uM for lab strain and four field strains respectively.

In comparing the inhibition potency of Phenothrin and Thiodicarb against Na^+,K^+-ATPase and AChE activity respectively within the different strains, it is clear that Phenothrin showed to be the strong inhibitor for *S. littoralis* On the other hand, the I_{50} values of each Phenothrin and Thiodicarb in lab strain and Borg El-Arab is more succeptible than that of Abeis, Damnhour and Abou El-Matamir. These results are in agreement with many investigators. Desaiah *et al*., (1975) who reported that inhibition of Na^+,K^+-ATPase, by three synthetic Pyrethroids were the most effective insecticides against Cockroaches and Fish. Also Saleh *et al*., (1984) and Korkor *et al*., (1995) reported synthetic Pyrethroids were the most effective insecticides against Bollworms.

In this work, we describe the development of a biochemical assay system for measuring the sensitivity of Na^+,K^+-ATPase and AChE to Pyrethroids and Carbamate insecticides respectively, our primary goal was to develop an assay that could characterize Na^+,K^+-ATPase and AChE variants in individual sharpshooters that were under insecticides selection pressure. We also provide enzyme kinetic data for the Na^+,K^+-ATPase and AChE in this insect for field strains and compare them with data for the lab strain.

Finally, it may be concluded that Phenothrin (Pyrethroides) is more convenient than Thiodicarb (Carbamate) for the control program of *S. littoralis* according to its slow effect in inducing resistance. But the induced resistance may be of great concern in the use of synthetic Pyrethroids and Carbamate, for the control programe of cotton leafworm.

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الملخص العربي

دراسة مدى حساسية أنزيم الصوديوم–بوتاسيوم أدينوسين تراى الفوسفاتيز و أنزيم الأسيتايل كولين أستريز لمبيدى الفينوثرين و الثيودىكارب فى العشائر الحقلية المصرية لدودة ورق القطن

سهام منصور اسماعيل

تم دراسة الأختلافات فى نشاط أنزيمين من أهم الأهداف البيولوجية فى الحشرة وهما أنزيم الصوديوم–بوتاسيوم أدينوسين تراى الفوسفاتيز (Na^+,K^+-ATPase) وأنزيم الأسيتايل كولين أستريز(AChE) وأيضا مستوى حساسية يرقات العمر الرابع لدودة ورق القطن للمبيدين فينوثرين وثيودى كارب حيث تم أستخلاص كلا الأنزيمين من رأس يرقات العمر الرابع لدودة ورق القطن وذلك مابين أربع عشائر مختلفة جمعت من الحقول المصرية وتمت مقارنتها بعشيرة معملية حساسة، تركزت الدراسة على العشائر المنتشرة فى المناطق التى ترش بمعدل كثيف من المبيدات (دمنهور– أبوالمطامير– أبيس) وأيضا فى المناطق الصحراوية المنزرعة حديثا (برج العرب) والتى تنتشر فيها زراعة القطن. ولقد أوضحت النتائج أن قيم التركيزات النصف مميتة (LC_{50}) أظهرت أختلافا محسوسا حيث كانت سلالة برج العرب أكثر السلالات حساسية يليها سلالة أبيس بينما دمنهور وأبوالمطامير كانت أكثر تحملا وذلك فى حالة الأنزيمين، وقد أوضحت النتائج المتحصل عليها أن أعلى معدل نشاط نوعى للأنزيمين كان فى محافظة الأسكندرية (برج العرب– أبيس) بينما كان هذا المعدل أقل فى حالة محافظة البحيرة (دمنهور– أبوالمطامير) وذلك بالمقارنة بالسلالة المعملية الحساسة، كذلك فقد تم تقدير قيم الـ I_{50} (تركيز المبيد اللازم لتثبيط 50% من النشاط الأنزيمى) فوجد بالنسبة لتأثير الفينوثرين على نشاط أ نزيم-Na^+,K^+ ATPase أوضحت النتائج أن قيمة I_{50} كانت 0.01, 0.20 , 0.36, 0.61 و 0.82 ميكرومولر وذلك بالنسبة للسلالة الحساسة ,برج العرب, أبيس, دمنهور وأبوالمطامير على التوالى بينما كانت قيم الـ I_{50} لمبيد ثيودى كارب على نشاط أنزيم AChE هى 0.22 0.43 0.54 0.71 و0.96 ميكرومولر وذلك بالنسبة للسلالة الحساسة و الأربع سلالات الحقلية المختبرة على التوالى كذلك تم تقدير ثابت التثبيط K_i فكانت فى حالة الفينوثرين 5 , 18, 20 , 30 و 45 ميكرومولر وذلك بالنسبة للسلالة الحساسة,برج العرب, أبيس, دمنهور وأبوالمطامير على التوالى بينما فى حالة ثيودى كارب 20 28 30 40 50 ميكرومولر وذلك بالنسبة للسلالة الحساسة و الأربع سلالات الحقلية المختبرة على التوالى. من هذه النتائج يمكن أن تكون هذه الأنزيمات دلالة على امكانية أستخدام هذه المبيدات فى مكافحة دودة ورق القطن وذلك من قبم النشاط الأنزيمى لهما.