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Susceptibility of *Spodoptera littoralis*, Field Populations in Egypt to Chlorantraniliprole and the Role of Detoxification Enzymes

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***Abstract*—**The cotton leafworm, *Spodoptera littoralis* (Boisduval) is a major insect pest of vegetables and cotton crops in Egypt, and exhibits different levels of tolerance to certain insecticides. Chlorantraniliprole has been registered recently in Egypt for control this insect. The susceptibilities of three *S. littoralis* populations collected from El Behaira governorate, north Egypt to chlorantraniliprole were determined by leaf-dipping technique on 4th instar larvae. Obvious variation of toxicity was observed among the laboratory susceptible, and three field populations with LC_{50} values ranged between 1.53 µg/ml and 6.22 µg/ml. However, all the three field populations were less susceptible to chlorantraniliprole than a laboratory susceptible population. The most tolerant populations were sampled from El Delengat (ED) Province where *S. littoralis* had been frequently challenged by insecticides. Certain enzyme activity assays were carried out to be correlated with the mechanism of the observed field population tolerance. All field populations showed significantly enhanced activities of detoxification enzymes compared with the susceptible strain. The regression analysis between chlorantraniliprole toxicities and enzyme activities revealed that the highest correlation is between α-esterase or β-esterase (α-β-EST) activity and collected field strains susceptibility, otherwise this correlation is not significant ($P > 0.05$). Synergism assays showed the ED and susceptible strains could be synergized by known detoxification inhibitors such as piperonyl butoxide (PBO), triphenyl phosphate (TPP) and diethyl-maleate (DEM) at different levels (1.01-8.76-fold and 1.09-2.94 fold, respectively), TPP showed the maximum synergism in both strains. The results show that there is a correlation between the enzyme activity and tolerance, and carboxylic-esterase (Car-EST) is likely the main detoxification mechanism responsible for tolerance of *S. littoralis* to chlorantraniliprole.

***Keywords*—**Chlorantraniliprole, detoxification enzymes, Egypt, *Spodoptera littoralis.*

I. Introduction

THE cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), is a serious Lepidopterous pest attacking cotton plantations, beside other economic field and vegetable crops in Egypt. It causes serious damage to the farmers, in all seasons [1]. In field crops such as cotton, *S. littoralis* may cause considerable damage by feeding on leaves, fruiting points, flower buds and, occasionally, also on the bolls [1]. In vegetables such as tomatoes, larvae bore into the fruit which is thus rendered unsuitable for consumption. Numerous other crops are attacked, mainly on their leaves [1]. Over the past 25 years, the intensive use of broad-spectrum insecticides against *S. littoralis* has led to development of resistance [2]. In this scenario, using new types of insecticides could be useful as an alternative for the integrated management approach [3].

The intensive use of several chemical pesticides caused a serious resistant problem, with special respect to organophosphates, carbamates, pyrethroids and neonicotinoids. Alternative programs should be adopted for insecticides to manage this problem; especially for those insecticides with unique mode of action. Sattelle et al. [4] mentioned that chlorantraniliprole (Rynaxypyr) as a ryanodine receptor insecticide has outstanding activity on a range of Lepidopteran pests and other orders like, Coleoptera, Diptera, Isoptera and Hemiptera. Lahm et al. [5] stated that chlorantraniliprole activates the unregulated release of internal calcium stores, leading to Ca^{2+} depletion, feeding cessation, lethargy, and muscle paralysis, finally insect death.

The objectives of this study were to investigate the susceptibility of *S. littoralis* field populations to chlorantraniliprole in Egypt, and to evaluate the detoxification activities of mixed function oxidases (MFO), glutathione S-transferases (GST) and esterases (EST), as well as to determine the synergism effects of synergists to help explain possible mechanisms involved in the tolerance to chlorantraniliprole.

II. Materials and Methods

A. Insects

1) Laboratory Strain

A laboratory strain (Lab) of *S. littoralis* larvae was obtained from the Agriculture Genetic Engineer Research Institute (AGERI), Agriculture Research Center, Ministry of Agriculture, and reared under laboratory conditions for several years without exposure to insecticides. The colony was kept at a temperature of 27±2 ºC and 65±5 RH [6]. Larvae were reared on castor oil leaves (*Ricinus communis* L.). When the larvae pupated they were put into cage, and supplied with a piece of cotton moistened with 10% sugar solution. Leaves of *Nerium oleander,* where placed for adults to deposit their eggs. The egg masses were collected daily, and then neonates were transferred to fresh castor oil leaves.

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2) Field Populations

Egg masses of *S. littoralis* were collected from cotton fields at three locations within El Beheira Governorate (from El Rahmaniah (ER) (31° 6' N, 30° 38' E), Shabrakhit (SB) (31° 2' N, 30° 42' E) and ED (30° 49' N, 30° 31' E) districts) at June 2014. These locations were previously known to be exposed to insecticides from different groups during the cotton growing seasons. After hatching the egg-masses were reared until the 4th instar larvae.

B. Chemicals

Chlorantraniliprole (CAP) [95.3% technical product] was provided by DuPont Crop Protection. Ammonium sulfamate, DEM, dimethyl formamide (DMF), dithiothreitol (DTT), Fast blue β-salt, Folin reagent, O-dinitrobenzene (DNB), phenylthiourea (PTU), PBO, reduced glutathione (GSH), reduced nicotinamide adenine dinucleotide phosphate (NADPH), sodium dodecyl sulfate (SDS), sulfanilamide, trichloroacetic acid (TCA), TPP, Triton X-100, α-Naphthol (α-Na), α-Naphthyl Acetate (α-NA), β-Naphthol (β-Na), β-Naphthyl acetate (β-NA), ρ-Nitroaniline (ρ-NA), ρ-Nitroanisole (ρ-Na), ρ-Nitrophenol (ρ-NP), and other chemicals were purchased from Sigma Chemical Co., St. Louis, Mo, USA.

C. Bioassays

The leaf-dipping bioassay method was used to determine the median lethal concentration (LC_{50}) values for laboratory and field populations of *S. littoralis,* 4th instar larvae. A series of concentrations of CAP (95.3%) ranged from 0.835 mg/L to 270 mg/L were prepared in water containing 0.01% Triton X-100 as a wetting agent [7] and 1% DMF.

Castor oil leaves were cut into discs (2 cm^2). Each disc was dipped into the test dilution for 10s, held vertically to allow excess dilution to drip off, and placed on a rack to dry. After 2 hr, discs were offered to the larvae and left under controlled conditions (27±2 ºC) for 24 hr. Three replicates with 10 larvae in each one were carried out for each treatment with cheese cloth and tied with rubber bands. Thereafter, survivors were transferred with fresh untreated castor oil leaves to clean cups after each 24 hr and kept under the same conditions. Mortality counts were recorded daily for four days. Percent mortality was calculated for each concentration and corrected for natural mortality according to Abbott equation [8].

D. Synergism Assays

For analysis of the effects of synergists on toxicity of CAP, toxicity with a concentration range of synergists was first determined to choose a suitable concentration that would have no effect on *S. littoralis* larval mortality. Concentrations up to 100 mg/liter of DEM, PBO and TPP had no effect on larval mortality. Castor oil leaves treated with that concentration of each synergist with a final acetone concentration of 1% and triton X-100 of 0.01%, were exposed to 4th instar for 12 h, and then larvae were assayed for toxicity against CAP as described above. Mortality counts were recorded, corrected according to Abbott equation [8] and subjected to probit analysis [9] within the 95% confidence limits, LC_{50}, slopes and $(Chi)^2$ were established and computerized by Ldp-line program [10]. The synergism ratio (SR) was determined by dividing the LC_{50} of insecticide alone by the LC_{50} of insecticide with synergist.

E. Enzyme Preparation

The isolated midguts were homogenized on ice in ice-cold 100 homogenization buffer (0.1 M phosphate buffer, pH 7.6, containing 1 mM EDTA, 1 mM DTT, 1 mM PTU and 20% glycerol). Forty isolated midguts were homogenized in 1 mL buffer; the homogenate was centrifuged at 4°C, 10,000 g for 30 min using sigma 3K30 rotor NO. 12158, sigma laboratories centrifuge 3K30, the solid debris and cellular material were discarded. The supernatant was transferred into a clean Eppendorf tube, placed on ice and used immediately for MFO, GST, EST assays and total protein concentration assay.

F. Enzyme Assays

1) GST

GST activity was measured according to the method of Asaoka and Takahashi [11] using ethanolic solution of DNB as a substrate with slight modification as done with El Shahawi and Al Rajhi [12]. The standard assay mixture (1ml) contained 1.5 mM GSH, 100 mM phosphate buffer pH_7, 50 µl of enzyme source, and the reaction was started by the addition of 0.5 mM DNB. After incubation at 37 ^oC for 20 min, the reaction was terminated by the addition of 0.1 ml acetic anhydride. The reaction was left for 5 min at room temperature, and then mixed with 1 ml of 1% (W/V) sulfanilamide in 20% (W/V) HCl followed by 1 ml of 0.02% (W/V) N-(1-naphthyl) ethylenediamine dihydrochloride. After diazo-coupling for 20 min at room temperature, 0.1 ml of 1% (W/V) ammonium sulfamate (freshly prepared) was added to the mixture. The mixture was left for 5 min, and then the absorbance at 540 nm was recorded using Sequoia-Turner Model 340 Spectrophotometer. An assay mixture without enzyme was used as the blank.

2) MFO

The assay of MFO was conducted using the procedures developed by Rose, et al. [13] with slight modification [14]. One hundred µL of 2 mM ρ-Na solution and 90 µL enzymes were added to a clean Eppendorf tubes. The mixture was incubated for 3 min at 27 °C and the reaction initiated by the addition of 10 µL of 9.6 mM NADPH. All mixtures were incubated for 20 min at 37 °C. Reactions were stopped by addition of 100 µl TCA 15% and samples were then centrifuged at 5000 rpm for 20 min using IEC-CRU 5000 cooling centrifuge and supernatants were collected. To develop the colorimetric reaction, 100 µl of supernatant is mixed with 100 µl of 1 M NaOH in each well of transparent 96-well Costar microplate (Corning Life Sciences, Lowell, MA). The absorbance of the reaction product, ρ-NP, was measured at 405 nm by using an ELISA plate reader (STAT FAX-2100). An assay mixture with denaturating enzyme instead of live enzyme was used as the blank wells and was subtracted as background. Specific activities for the ρ-NA

substrate was calculated based on the ρ-NP standard curve which was carried out by determining the color absorbance for a series of ρ-NP concentrations ranged from 0.4 nanomoles to 50 nanomoles in 100 ml 30% ethanol. It was fitted using the least squares method (K =0.0485 nm ρ-NP/ well), and expressed in nm ρ-NP/min.mg protein. All reaction readings were conducted in triplicate.

3) EST

The rate of degradation of α and β-NA were measured by the method of Van Asperen [15] with slight modifications. Enzyme extracts were eight times diluted with homogenization buffer. 10 µl of the diluted enzyme extract was added to 90 µl homogenization buffer and incubated for 20 min at 37 °C after addition of 50 µl of 0.5 mM α and β-NA in ethanol.

The reaction was stopped and color developed by adding 50 µl dye solution (l% fast blue B salt: 5% SDS = 2: 5, v/v) for 20 min. The absorbance was read at 545 nm for α- and β-Na by using an ELISA plate reader (STAT FAX-2100). An assay mixture with denaturating enzyme instead of live enzyme was used as the blank wells and was subtracted as background. Specific activities for the α and β-NA substrates was calculated based on the α-Na and β-Na standard curves which were carried out by determining the color absorbance for a series of α-Na and β-Na concentrations ranged from 0.4 nanomoles to 25 nanomoles for α-Na and 0.4–100 nanomoles for β-Na in 150 ml 50% ethanol. Those were fitted using the least squares method (K=0.0998 nmol α-Na/ well and 0.0281 β-Na, respectively), and expressed in nmol α- Na/min.mg and β-Na/min.mg protein, respectively. All reaction readings were conducted in triplicate.

G. Protein Assay

Protein estimation has been carried out according to Lowry et al. [16]. The standard curve was established by using different concentrations of bovine serium albumin (BSA).

H. Statistical Analysis

The mortality data were subjected to Probit analysis [9], values of LC_{50}, 95% confidence limits, slopes and $(Chi)^2$ were established and computerized by Ldp-line program [10]. Significant differences of LC_{50} were determined by non-overlapping 95% confidence limits. Resistance factors (RF) were estimated at the LC_{50} level as RF=LC_{50} of collected population/LC_{50} of Laboratory strain. Insecticide resistance level was described by using RFs as reported by Keiding [17], [18]: Susceptibility (RF=1), Decreased susceptibility (RF=3–5), Low resistance (RF=5–10), Moderate resistance (RF=10–40), High resistance (RF=40–160), and Very high resistance (RF>160).

The detoxification enzymes activity data were subjected to an ANOVA analysis followed by t-test, treatment means were compared by least significant difference (LSD) at 5% level of probability using SAS, 9.4 software [19]. Also, correlation coefficients and probability values for the relationship between the activity of each metabolic enzymes and the CAP toxicity were determined. All biochemical data were statistically analyzed using SAS, 9.4 software [19].

III. Results

A. Toxicity of CAP against 4th Instar Larvae of S. Littoralis

The probit analysis of the susceptibility of 4th instar larvae of *S. littoralis* after feeding on CAP treated castor-oil leaves under laboratory conditions is presented in Table I. Laboratory strain was very susceptible to CAP compared to the other three field populations. All *S. littoralis* collected field population from different locations in El Beheira Governorate during June 2014 had reached Decreased susceptibility to CAP, which has been registered at 2014 in Egypt. Values of LC_{50} (mg/L) are different at 4th day after treatment especially between laboratory and ED populations. LC_{50} values of CAP on *S. littoralis* populations were 1.53 mg/L, 3.63 mg/L, 3.85 mg/L and 6.22 mg/L at the 4th day after treatment for the Laboratory, SB, ER and ED populations, respectively. Data show drastic difference of the LC_{50} values between laboratory and ED strains, it is 4.07 fold tolerance of the ED strain than laboratory at the same period after treatment. There are no obvious differences in susceptibility between the laboratory, SB and ER, according to their confidence limits. However, SB and ER strains, which are close on geographical distance; exhibited 2.37 and 2.52 fold difference, respectively, compared with laboratory strain.

TABLE I
TOXICITY OF CAP TO 4TH INSTAR LARVAE OF *S. LITTORALIS*, LABORATORY AND COLLECTED FIELD POPULATIONS FROM EGYPT

Strain	n[a]	LC_{50}[b]:µg/ml (95% CL)	Slope ± SE	χ^2 (df)	RF[c]
Laboratory	240	1.53 (0.69-2.58)	0.98 ± 0.17	0.36 (5)	1.00
SB	240	3.63 (2.18-4.87)	1.00 ± 0.15	15.04 (6)	2.37
ER	240	3.85 (2.48-5.64)	1.20 ± 0.14	8.58 (6)	2.52
ED	240	6.22 (3.95-9.50)	1.08 ± 0.14	7.17 (6)	4.07

[a]Number of 4th instar larvae used. [b]LC_{50} was calculated for each treatment after 96 hr. [c]RF = LC_{50} of a population/LC_{50} of the Lab strain.

B. Synergistic Effect of Selected Inhibitors on the CAP Toxicity

TPP, DEM and PBO were normally considered as inhibitors of Carboxyl-Esterase (Car-EST), GST and MFO. The effects of these three inhibitors on CAP toxicity on the 4th instar of lab and ED field strains are shown in Table II. TPP shows remarkable synergism to *Spodoptera* 4th instar larvae, especially ED field strain pretreated with the three synergists and showed the potency of TPP as synergist. There is correlation in the synergistic effects of TPP on the laboratory and field strains, with synergism ratios (SR) values of 2.94 and 8.76, respectively, indicating the role of Car-EST in the mechanism of resistance to CAP.

C. Correlation Coefficient of Detoxification Enzyme Activities of 4th S. littoralis instar larvae and CAP Toxicity

The detoxification enzyme activities were measured in three fields collected populations and laboratory susceptible strain. There were narrow variations of detoxification enzyme activities among field populations. Maximum 2.65-fold

difference in GST activity was observed from field populations (Table III) in comparison with the laboratory strain, the highest GST activity was found in SB strain (0.0233 O.D540nm/min/mg protein), the lowest activity in lab strain (0.0088 O.D540nm/min/mg protein) (Fig. 1 (A)). The correlation between GST activity and toxicity of CAP on cotton leafworm did not fit to the linear regression models (P = 0.6835, r = 0.31646) (Fig. 1 (E)).

TABLE II
EFFECTS OF SYNERGISTS ON TOXICITY OF CAP TO 4TH INSTAR LARVAE OF *S. LITTORALIS* LABORATORY AND ED FIELD STRAINS

Strain	Treatment	n[a]	LC_{50}[b]:µg/ml (95% CL)	Slope ± SE	χ^2 (df)	SR[c]
Laboratory	CAP	240	1.53 (0.69-2.58)	0.98 ±0.17	0.36 (5)	--
	CAP+TPP	240	0.52 (0.08-1.07)	1.00 ± 0.26	0.10 (4)	2.94
	CAP+DEM	240	1.35 (0.38-2.68)	0.73 ± 0.17	3.27 (5)	1.13
	CAP+PBO	240	1.41 (0.62-2.38)	0.10 ± 0.18	0.35 (5)	1.09
ED	CAP	240	6.22 (3.95-9.50)	1.08 ± 0.14	7.17 (6)	--
	CAP+TPP	240	0.71 (0.20-1.34)	1.08 ± 0.25	2.94 (4)	8.76
	CAP+DEM	240	6.15 (3.65-9.82)	0.93 ± 0.12	4.27 (6)	1.01
	CAP+PBO	240	4.34 (2.81-6.36)	1.19 ± 0.14	9.40 (6)	1.43

[a]Number of 4th instar larvae used. [b]LC_{50} was calculated for each treatment after 96 hr. [c]SR (synergism ratio) = LC_{50} of a strain treated with insecticide alone divided by the LC_{50} of the same strain that was treated with insecticide plus a synergist.

TABLE III
DETOXIFICATION ENZYME ACTIVITIES OF *S. LITTORALIS*, 4TH INSTAR LARVAE FROM DIFFERENT SITES

Strain	GST		MFO		α-EST		β-EST	
	Activity[a]	Rate[b]	Activity	Rate	Activity	Rate	Activity	Rate
Laboratory	0.0088±0.0000[d]	--	0.227±0.016[d]	--	1.730±0.061[d]	--	5.411±0.342[d]	--
ER	0.0116±0.0001[c]	1.31	0.629±0.003[b]	2.77	2.985±0.030[c]	1.73	11.793±0.087[b]	2.18
ED	0.0159±0.0001[b]	1.80	0.452±0.007[c]	1.99	3.426±0.015[b]	1.98	12.117±0.176[b]	2.24
SB	0.0233±0.0001[a]	2.65	0.785±0.018[a]	3.45	3.708±0.098[a]	2.14	13.870±0.028[a]	2.56

Means followed by the same letters are not significantly different according to the $LSD_{0.05}$. [a]Enzyme activity represented as means ± SE (nm/min/mg protein) for MFO, α-EST, and β-EST, and ($O.D_{540}$nm/min/mg protein) for GST. [b]Rate = the enzyme activity in field strain /the activity in lab strain.

Maximum 3.45-fold difference in MFO activity was observed from field populations (Table III) in comparison with lab strain, the highest MFO activity was found in SB strain (0.785 nm ρ-NP/min/mg protein), the lowest activity in lab strain (0.227 nm ρ-NP/min/mg protein) (Fig. 1 (B)). The correlation between MFO activity and toxicity of CAP on cotton leafworm did not fit to the linear regression models (P=0.6654, r=0.33462) (Fig. 1 (F)).

Maximum 2.14 and 2.56-fold difference in α and β-EST activities respectively, were observed among the field populations (Table III) in comparison with lab strain, the highest α-EST and β-EST activity were from SB strain (3.708 and 13.870 nm α- and β-Na/min/mg protein, respectively), the lowest one from lab strain (1.730 nm and 5.411 nm α- and β-Na/min/mg protein) (Figs. 1 (C) and (D)). The correlation between α-EST and β-EST activity and toxicity of CAP on cotton leafworm did not fit to the linear regression models. Where, P=0.2450, r=0.75498 for α-EST (Fig. 1 (G)) and P=0.2984, r=0.70159 for β-EST (Fig. 1 (H)).

The highest detoxification enzyme activities were not observed in the most tolerant populations. The highest correlation was between the α-EST or β-EST activity and collected field strain susceptibility, otherwise this correlation is not significant (P>0.05).

IV. DISCUSSION

This study showed that CAP has a high potency against *S. littoralis* larvae under laboratory condition. The ability and speed to exert the larval toxicity to this insecticide may be due to the ability for feeding cessation on its initial state of exposure. It is remarkable to note that the ability and speed to cease feeding have become one of the critical indicators of insecticidal potency for discovering and developing novel chemical classes of insecticides. CAP was among the fastest – acting insecticides for feeding cessation [20], but not the fastest to kill worms like bagworm, *Metisa plana* [21]. The scanning Electron Microscopy (SEM) micrograph showed that CAP had destroyed the internal organs of the larvae. The stomach poisoning effect of CAP was manifested in the deformation of the surface, and the disintegration of the intestine. When the cells and the internal organs are damaged, the larvae become lethargic and ultimately die [22]. CAP was also reported to have caused the fastest feeding cessation on *Plutella xylostella*, *Trichoplusia ni*, *Spodoptera exigua* and *Helicoverpa zea* as compared to emamectin benzoate, indoxacarb, methoxyfenozide and metaflumizone [23].

In order to reduce pesticide hazards and the development of resistant population, insect control should be accomplished with fewer applications at far lower doses. This approach was the principle of insecticides against S. littoralis larvae, in order to stimulate our activities in assessing to toxicity of CAP at lower doses, in addition to record the field strains susceptibility from different locations to CAP which has been registered recently in 2014 in Egypt. The initial step in this matter is to record different locations larval susceptibility compared to the lab strain.

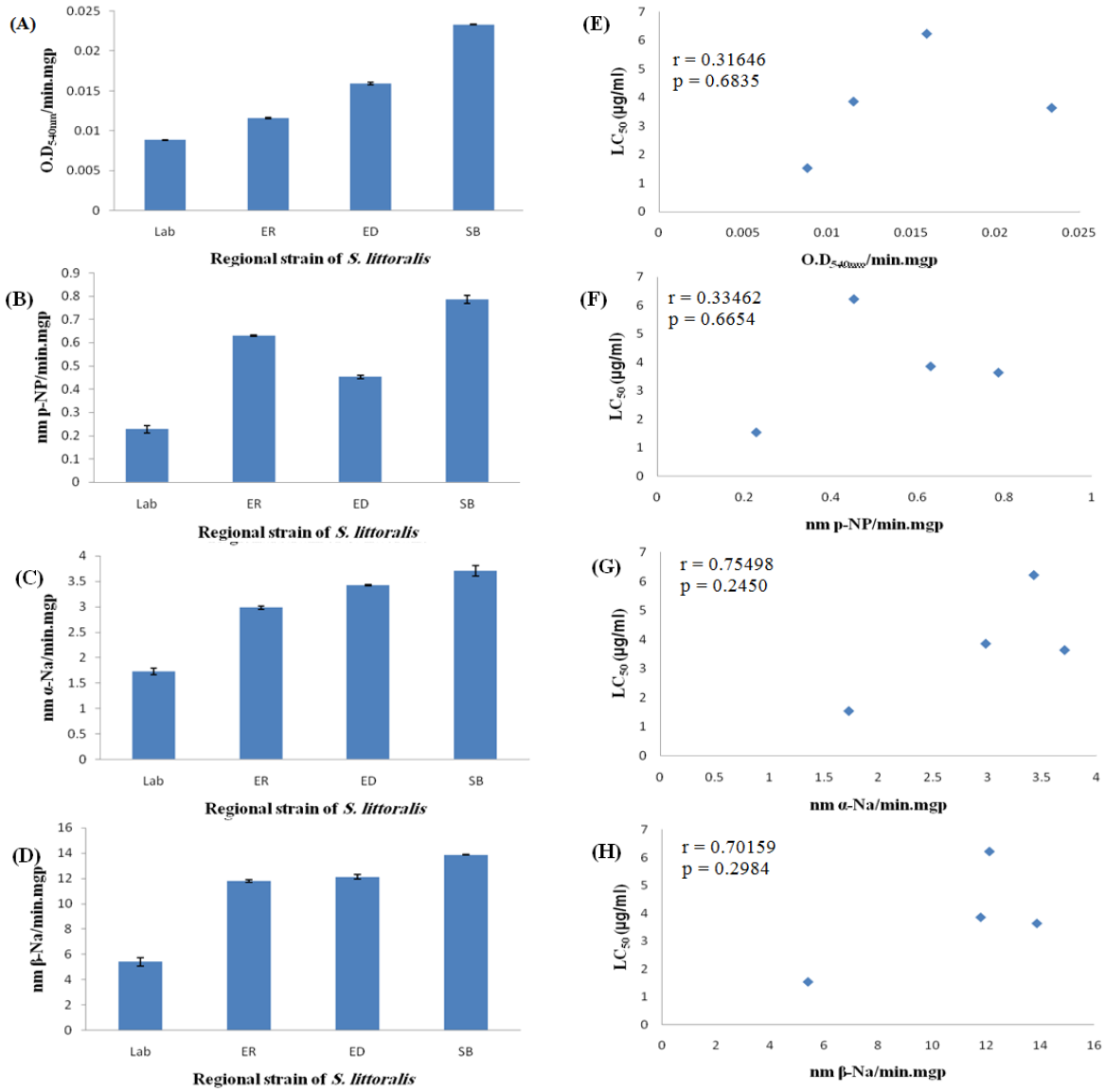

Fig. 1 Detoxification enzyme activities of field populations and the relationship between enzyme activities and toxicity of CAP on *S. littoralis* (A) and (E): GST. (B) and (F): MFO. (C) and (G): α-EST. (D) and (H): β-EST, (A), (B), (C) and (D) are enzyme activity of field population. (E), (F), (G) and (H) are the relationship between enzyme activities and LC_{50} values

The selected locations were previously known to be exposed to insecticides from different groups during the cotton growing seasons. When comparing the LC_{50} values of the lab strain with that of the three field locations (ER, SB and ED), it was found that significant deference between LC_{50} values of lab and ED strains according to their confidence limits. Values between lab strain and the three field locations are within 2-4 fold of magnitude of the field versus the lab strains. Even there are not big differences in the slope values between the field and lab strains.

To assess the possibility of CAP interaction with *S. littoralis* larvae possible developing of resistant strains, studies were extrapolated to investigate this interaction, lab vs. field strains (ER, SB and ED). The most tolerant strain was collected from ED district. Remarkable toxicity potency was observed in one of pretreated *Spodoptera* with TPP, DEM and PBO were normally considered as inhibitors of carboxylesterase (Car-ESTs), GSTs, and MFOs, respectively. The results showed that the selected lab and field strains could be synergized by known metabolic inhibitors such as TPP, and relatively less extent to DEM and PBO. The synergistic ratio of CAP against *Spodoptera* larvae with TPP were 2.94 and 8.76 for laboratory and ED field strains, respectively. This was in contrast with CAP resistance in the diamondback moth, *Plutella xylostella* (linnaeus) [24]. They found that difference in synergism for TPP, DEM and PBO against this insect. Probably this was due the practical use of CAP under field conditions to control this insect.

The high efficiency, fast action, long lasting activity and environmental benignancy of CAP make well – received in the near future by crop protection authorities in Egypt. The use of this insecticide will be potentially widespread in the future, after careful examinations practically and toxicologically. Laboratory assay showed low variation of *S. littoralis* larval susceptibility to CAP existed in the field populations of this insect, however all collected field populations showed higher tolerance to this new chemical than the laboratory susceptible population. Many results suggested that effective resistance management strategies should be developed and implement for continued success of CAP to control *S. littoralis* in Egypt. Continuous monitoring of resistance development, through careful reactions with the responsible enzyme systems, in addition to elucidation of cross – resistance and resistance mechanism provide practical and useful information on rational choice of insecticides.

Monitoring resistance is very important to manage the susceptibility of the target insect with integrated pest management strategies. This required the real understanding of the pest mechanism of resistance, in order to implement a successful pest control programs. The biochemical bases of resistance understanding, controls the proper way of the problem management [25]-[31]. Introducing the molecular bases of resistance mechanisms usually sheds lights on this problem. May molecular approaches been adopted, such as polymerase chain reaction (PCR)-based mutation analysis and microarray methods for the proper understanding of such mechanism [32]-[34].

Understanding the biochemical mechanisms conferring insecticide resistance has been shown to be necessary for resistance management tactics. One of the most important factors of insect resistance is the increase in metabolic processes leading to higher detoxification of insecticides by enzymes such as MFOs, ESTs and GSTs [35].

HU, et al. [24] suggested that there is a strong correlation between the enzyme activity and CAP resistance to *P. xylostella* L., and GST is likely the main detoxification mechanism responsible for resistance, and or specific toxicity, although P450 and Car-EST cannot be ruled out. However, the individual GST enzyme involved in CAP resistance has not, to a relative extent, been identified. Therefore, further studies in more details, such as the real mode of action and resistance might be attributed to increases in the amount of one or more GST enzymes; either as a result of gene amplification or more commonly, through increases in the transcriptional rate are needed.

In this study, the field populations of *S. littoralis* were mainly sampled from El Behira governorate and the nearby areas. In this area, year-round spraying of insecticides was necessary to suppress serious damage by *S. littoralis*, and cotton pests. The resistance situation of this insect was the most serious in Egypt. CAP was one of the few insecticides which had good control effects. This insecticide was not used or less frequently used in El Behira governorate before the sampling was made, and the populations of *S. littoralis* from this area were relatively more tolerant than susceptible laboratory strain. The metabolic enzyme activities were measured. However, the highest metabolic enzyme activities were not observed in the most tolerant populations. The variation of tolerance to CAP in *S. littoralis* may be due to heterogeneity, environmental dissimilarity, different insecticide application history or other factors.

The correlation between LC_{50} values and metabolic enzymes activity was analyzed. In our findings, the highest correlation is between the α-EST or β-EST activity and collected field strain susceptibility (r=0.75498 and 0.70159, respectively) otherwise this correlation is not significant (P=0.2450 and 0.2984>0.05, respectively). This finding is in agreement with synergism assay data, where the most synergism produced was by TPP which inhibits Car-EST, confirming the rule of Car-EST in mechanism of CAP resistance.

V. Conclusion

In summary, all findings of the present study demonstrated that Car-EST enzymes might involved in the tolerance observed in field collected populations and is likely the main detoxification mechanism responsible for resistance of CAP, although P450 and GST cannot be ruled out. However, the individual Car-EST enzyme involved in CAP resistance has not been identified. Therefore, further study in more detail, such as resistance might be attributed to increases in the amount of one or more Car-EST enzymes, either as a result of gene amplification or more commonly through increases in transcriptional rate, and so on, are needed.

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