Toxicity Of Some Insecticides Against Laboratory And Field Strains Of Cotton Leaf worm With Determination Of Some Detoxification Enzymes

El-Deeb; Dalia A.¹, Ahmed F. Bedair¹, Ahmed A. Barrania²

¹Central Agricultural Pesticides Laboratory, Sabahia Station, Alexandria Egypt.²Plant Protection Research Institute, Etay El-baroud Agric. Res. Station. Agric. Res. Center, Egypt.

Abstract: Bioassay experiments were carried out to monitor the resistance levels in the cotton leafworm (CLW), *Spodoptera littoralis* (Boisduval) field strain (Shobrakhete city, El-Behera governorate) to some insecticides compared to a laboratory strain. The activities of total esterases, glutathione *S*-transferases (GST) and alkaline phosphatases (ALP) in the two strains were also compared. Results revealed that, the 2^{nd} instar larvae of the field strain demonstrated varied levels of resistance to cypermethrin, chlorpyrifos and emamectin benzoate with resistance ratios (RR) 61.3, 68.1 and 14.7, respectively. On the other hand, the 2^{nd} instar larvae field strain showed tolerance ratios 8.1, 7.0, 5.6, 4.9 and 3.5 to spinetoram, lufenuron, chlorantraniliprole, methoxyfenozide and fipronil, respectively. The 4^{th} instar larvae showed high resistance levels to chlorpyrifos and cypermethrin where the resistance ratios were 157.9 and 120.4, respectively. The 4^{th} instar larvae field strain showed tolerance level of 11.8 to spinetoram. On the other hand, the 4^{th} instar larvae of field strain showed tolerance to chlorantraniliprole, emamectin benzoate, methoxyfenozide, lufenuron and fipronil with resistance ratios 8.1, 8.4, 9.0, 7.6 and 3.5, respectively. The field strain showed higher esterases, glutathione *S*-transferases and alkaline phosphatases activity compared to the laboratory strain. Therefore, the use of some insecticides for cotton leafworm control should be reconsidered.

Keywords: Insecticide resistance, Laboratory and field strains, Spodoptera littoralis, Detoxification Enzymes

1.Introduction

Egyptian cotton leafworm (CLW), Spodoptera littoralis (Boisduval) is a major pest of many economic crops in Egypt, Middle East and Southern Europe (Mazier et al., 1997). The control of this polyphagous insect pest relies mainly on the use of chemical insecticides. However, the extensive and the misuse of insecticides, have contributed to the development of resistance in this pest to many insecticide groups (Abo-Elghar et al., 2005; Abou-Taleb, 2010). In addition, the destructive feeding habits, multiple generations of CLW annually and the availability of host crops around the year have made the control of CLW an increasing agricultural problem (Hofte and Whiteley, 1989).

Diversification and rotating between insecticides with different modes of action can prevent or delay the development of resistance in this insect to insecticides.In addition, the continuous monitoring of resistance is important for every resistance management program (**Prabhaker** *et al.*, **1996; Temerak, 2002)**. At the same time, searching for an effective alternatives and pest control strategies to preclude increasing selection pressure of the insect population to insecticides is so important.

Insects can resist insecticides by different mechanisms, including enhanced metabolism, nerve insensitivity, reduced penetration and target site insensitivity (Attia, 1999; Abo Elghar et al., 2005; Ahmad et al., 2007). Several enzyme families are involved in insecticide detoxification, sequestration and excretion and have differing relative importance within the various taxonomic groups (Panini et al., 2015). Among the metabolic enzymes are glutathione S-transferase (GST) and total esterases (EST). Glutathione Stransferases catalyze the glutathione conjugation reaction with reduced glutathione (GSH) (Armstrong, 1997; Listowsky et al., 1998). Esterases are a large group of phase 1 metabolic enzymes that are able to metabolize a variety of exogenous and endogenous

Substrates (Hollingworth and Dong, 2008). Phosphatases have been included in the list of detoxifying enzymes of insecticides; mostly of organophosphorus (Oppenoorth, 1985).

Therefore, the present study investigated the susceptibility of CLW field strain (collected from Shobrahkete city) compared with the laboratory strain to selected insecticides of diverse chemistries with different modes of actions. Also, the activities of GST, EST, ALP, in both strains were measured.

2. Materials and Methods

2.1.Experimental insect:

2.1.1.Laboratory strain: Cotton leafworm, *Spodoptera littoralis*, larvae used for testing program was obtained from Plant Protection Research Institute and reared in the laboratory on castor bean leaves at 25 ± 2 °C and 65 ± 5 % RH according to **Eldefrawi** *et al.*, (1964).

2.1.2.Field strain: Cotton leafworm egg masses were collected from cotton fields of Shobrakhete city, El-Behera governorate during 2018 cotton season and transferred to the laboratory. The resulting larvae for test purposes were reared in the laboratory on castor bean leaves at a temperature of 25 ± 2 °C and 65 ± 5 % RH.

2.2.Tested insecticides: Emamectin benzoate (Proclaim[®] 5%SG) and lufenuron (Match[®] 5%EC) were produced by Syngenta. Chlorpyrifos (Dursban 48%EC), spinetoram (Radiant[®] 12%SC) and methoxyfenozide (Runner[®] 24%SC) were produced by Dow Agrosciences Co. Alpha-cypermethrin (Alpha-cypermethrin[®] 10% EC) was produced by Tagros Chemicals India Limited. Chlorantraniliprole (Coragen[®] 20% SC) was produced by DuPont Agri-

cultural Chemicals Ltd.). Fipronil (Rado- $X^{\mbox{\tiny (I\!\!\!\!\)}}$ 80% WG), was produced by Jiangsu Tuoqiu Agrochemical Co.

2.3.Bioassay studies: Toxicity of the formulated insecticides against 2nd and 4th instar larvae of S. littoralis (Laboratory and field strains) was evaluated. Homogenous pieces of the castor oil leaves were dipped in a series of each insecticide concentrations for 10 sec., held vertically to allow excess solution to drip off and dried at room temperature. Treated castor oil leaf pieces were transferred to a plastic cups, and a ten larvae per replicate of starved (2 hrs.) larvae 2nd and 4th instar larvae were added. Each concentration was replicated four times. Mortality percentages were recorded after 4 days of treatment, corrected according to Abbott equation (Abbott, 1925) and subjected to probit analysis (Finney, 1971). The LC₅₀ values, there 95% confidence limit and slope \pm SE were calculated. Toxicity of tested insecticides against field strain was compared to the laboratory strain by calculating resistance ratio (RR) (RR = LC_{50} against field strain / LC₅₀ against laboratory strain).

2.4.Assay of EST, GST and ALP activities in the CLW field and laboratory strains:

Total larvae of the 2^{nd} instar and the collected midguts of the 4^{th} instar larvae (Laboratory and field strains) were used for enzyme measurements. The 2^{nd} instar larvae or midguts of 4^{th} instar larvae were collected, rinsed in glass distilled water and homogenized in glass homogenizer (1: 10 w/v) in GDW. The homogenate was centrifuged at 15,000 rpm for 30 min at 4°C using Cryofuge 20-3, Heraeus Christ centrifuge. The supernatant was served as the enzymes source.

Esterase activity was determined using α naphthyl acetate as a substrate according to the assay method described by **Van Asperen (1962)**. Reaction mixture with a total volume of 900 µl contained: 865 µl solution of 1.55 mM fast blue RR salt and 100 mM sodium phosphate buffer (pH 7.6), 30 µl of enzyme source and 5 µl of 90 mM α -naphthyl acetate in ethanol. The reaction mixture was vortexed and changes in absorption at 450 nm were monitored on Sequoia-Turner Model 340 spectrophotometer for up to 5 minutes. An assay mixture without enzyme was used as a blank. Enzyme activity was calculated as Δ OD min⁻¹ mg protein⁻¹.

Glutathione S-transferase activity was determined by using1-chloro, 2,4-dinitrobenzen (CDNB) as a substrate (Kao *et al.*, 1989). The assay mixture consisted of 50 mM CDNB in 95% ethanol, 50 mM GSH and 20 μ l of enzyme source in 3 ml of 50 mM phosphate buffer (pH 7.5). Changes in absorbance were measured at 340 nm for up to 3 min and the enzyme activity in terms of μ mol of CDNB conjugated min⁻¹ mg of enzyme protein⁻¹ was calculated using the extinction coefficient of 9.6 mM⁻¹ cm⁻¹. Protein concentration was measured according to (Lowry *et al.*, 1951) using bovine serum albumin (BSA) as a standard.

Alkaline phosphatase activity was determined according to the method of **Dgkc (1972)**, using Diamond Diagnostic kit (Diamond Co. Egypt). In this method 20 μ l of the enzyme source was added to 1ml of 0.9 M diethanolamine buffer pH 9.8 containing 0.6 mM magnesium ions and 1 mM p-nitrophenyl phos-

phate, then mix in the cuvette, incubate for 30 seconds in the spectrophotometer (Milton Roy Spectronic 601), start stopwatch simultaneously and read again after exactly 1, 2 and 3 minutes at 405 nm. ALP specific activity was calculated as IU/mg protein/min.

3.Results and Discussion

3.1.Toxicity of tested insecticides against the 2nd and 4th instars larvae of CLW field and laboratory strains: Toxicity of selected insecticides against the 2nd and 4th-larval instars of CLW laboratory and field strains was carried out and results are presented in Tables (1and 2). Data in Table (1) showed that, the 2^{nd} instar larvae of the field strain demonstrated varied levels of resistance to cypermethrin, chlorpyrifos and emamectin benzoate. The LC₅₀ values of cypermethrin, chlorpyrifos and emamectin benzoate against the field strain were 1.164, 100.05 and 0.044 mg / L compared to 0.019, 1.470 and 0.003 mg/L against the laboratory strain, respectively. Therefore, resistance levels in the field strain towards these insecticides were 61.3, 68.1 and 14.7, respectively. The 2nd instar larvae field strain showed tolerance ratios 8.1, 7.0, 5.6, 4.9 and 3.5 to spinetoram, lufenuron, chlorantraniliprole, methoxyfenozide and fipronil, respectively (Table 1).

The 4th instar larvae showed higher resistance against chlorpyrifos and cypermethrin where the resistance ratios were 157.9 and 120.4, respectively. The 4th instar larvae field strain showed resistance level of 11.8 to spinetoram. On the other hand, the 4thinstar larvae of field strain showed tolerance to chlorantraniliprole, emamectin benzoate, methoxyfenozide, lufenuron and fipronil with resistance ratios 8.1, 8.4, 9.0, 7.6 and 3.5, respectively (Table 2).

Results of the present study are compatible with many previous studies. High levels of resistance to pyrethroids, OPs and carbamates had been observed in the 2^{nd} and 4^{th} larval instars of CLW field strains (El-Guindy *et al.*, 2002a and 2002b). In addition, Abou-Taleb (2010) mentioned that, the 2nd and 4th instar larvae showed high resistance levels to the OP insecticide chlorpyrifos and the pyrethroid insecticide esfenvalerate. Shoaib et al., (2014) recorded high resistance levels to the pyrethroid deltamethrin and moderate levels of tolerance to the organophosphate chlorpyrifos in Gharbia and Kafr-Elsheik field strains of CLW compared to the laboratory strain. Furthermore, Elrakaiby (2018) found that, the 2nd and 4th instars larvae of the field (Collected from Almahmoudia city, El-Behera governorate) strain demonstrated high levels of resistance to chlorpyrifos and cypermethrin compared to the laboratory strain.

Concerning emamectin benzoate, results of the present study are in accordance with Abdel-Hay et al., (2014). They recorded different resistance levels in four CLW field strains to emamectin benzoate where resistance ratios ranged from 8.6-fold in Da-kahlia strain to 13.1-fold in Sharkia strain. According to Mokbel et al., (2017) the selection of CLW with emamectin benzoate for six consecutive generations resulted in the development of 7-fold resistance. Abou -Taleb (2010) recorded tolerance ratios about 6.8 and 8.7 to lufenuron and 7.1 and 6.2 to methoxyfeno-zide in CLW field strain at 2008 and 2009 cotton sea-

Insecticide	Strain	LC ₅₀ (mg L ⁻¹)	Confidence limits	Slope ± SE	RR [*]
Cypermethrin	Lab.	0.019	0.014 - 0.025	0.90 ± 0.07	-
	Field	1.164	0.846 - 1.578	0.83 ± 0.07	61.3
Chlorpyrifos	Lab.	1.470	1.145 - 1.873	1.06 ± 0.09	-
	Field	100.05	82.83 - 122.29	1.36 ± 0.11	68.1
Spinetoram	Lab.	2.760	2.241 - 3.382	1.25 ± 0.10	-
	Field	22.41	18.16 - 27.61	1.32 ± 0.13	8.1
Chlorantraniliprole	Lab.	0.091	0.069 - 0.119	1.04 ± 0.09	-
	Field	0.514	0.366 - 0.721	0.74 ± 0.07	5.6
Emomostin honzosto	Lab.	0.003	0.002 - 0.004	1.16 ± 0.10	-
Emamectin Denzoate	Field	0.044	0.034 - 0.057	1.09 ± 0.10	14.7
Fipronil	Lab.	0.004	0.003 - 0.006	1.18 ± 0.13	-
	Field	0.014	0.011 - 0.018	1.07 ± 0.10	3.5
Lufenuron	Lab.	0.399	0.331 - 0.478	1.54 ± 0.14	-
	Field	2.79	2.19 - 3.55	1.20 ± 0.10	7.0
Methoxyfenozide	Lab.	0.408	0.337 - 0.490	1.51 ± 0.14	-
	Field	1.982	1.404 - 2.614	1.08 ± 0.13	4.9

 Table (1): Median lethal concentrations of some insecticides against laboratory and field strains of Spodoptera littoralis 2nd instar larvae

sons, respectively. These results are compatible with results of the present study.

3.1.Activity of esterases and GST in the laboratory and field strains of CLW:

Esterases activity was significantly higher in the field strain than the laboratory strain in both 2^{nc} and 4th instar larvae. Esterases activity in the 2nd instar field strain (0.689 Δ OD / mg protein / min) was 3.04 fold the esterases activity in the 2nd instar of laboratory strain (0.227 \triangle OD / mg protein / min). In addition, the field strain 4th instar larvae showed esterases activity (1.437 \triangle OD / mg protein / min) 3.18 times the 4^{th} instar laboratory strain esterases activity (0.452 Δ OD / mg protein / min) (Table 3). Results of the present study are in accordance with many previous studies. Esterases have been associated with insecticide resistance in many insect species as a consequence of quantitative and/or qualitative changes, resulting in the overproduction of the enzymes or in modifications of their structures (Li et al., 2007). The α - and β esterase activity in the Menofia field strain of CLW

was higher than in the laboratory strain (Abd El-Mgeed *et al.*, 2000). In addition, Farag (2005) revealed that most of the tested CLW field strains showed a high activity of esterase than laboratory strain. Furthermore, El-Hassawy *et al.*, (2014) recorded high levels in esterases enzymes activity in the selected strains of CLW to chlorpyrifos, profenofos, cypermethrin, spinosad and flufenoxuron compared to the laboratory susceptible strain.

Glutathione *S*-transferases activity in the filed strain is 5.36 and 3.80 times the GST activity in the laboratory strain in both 2nd and 4th instar larvae, respectively (Table 4). While GST activities in the 2nd and 4th instar field strain were 168.83 and 217.32 μ mole/min/mg protein, GST activities in the 2nd and 4th instar laboratory strain were 31.50 and 57.25 μ mole / min/mg protein, respectively. Several studies have shown that there is a relationship between the GST activity and insecticide resistance in various insect species. GST-based resistance is generally due to an increased amount of enzyme, resulting either from gene amplification or overexpression (Vontas *et al.*,

Table (2): Median lethal concentrations of some insecticides against laboratory and field strains of *Spodoptera littoralis* 4th instar larvae

Insecticide	Strain	LC ₅₀ (mg L ⁻¹)	Confidence limits	Slope ± SE	RR [*]
Cypermethrin	Lab.	0.146	0.106 - 0.199	0.82 ± 0.07	-
	Field	17.58	13.32 - 22.92	0.95 ± 0.08	120.4
Chlorpyrifos	Lab.	14.76	12.00 - 18.11	1.25 ± 0.10	-
	Field	2330.78	1910.2 - 2900.9	1.27 ± 0.11	157.9
Spinetoram	Lab.	16.36	13.06 - 20.14	1.31 ± 0.14	-
	Field	193.70	159.3 - 234.4	1.45 ± 0.14	11.8
Chlorantraniliprole	Lab.	2.052	1.498 - 2.75	0.94 ± 0.09	-
	Field	16.66	12.79 - 21.67	0.99 ± 0.08	8.1
Emomostin honzooto	Lab.	0.052	0.038 - 0.070	0.84 ± 0.07	-
Emamectin benzoate	Field	0.439	0.309 - 0.616	0.73 ± 0.07	8.4
Finnonil	Lab.	0.030	0.023 - 0.039	1.02 ± 0.09	-
Fipronii	Field	0.106	0.080 - 0.139	0.98 ± 0.08	3.5
Lufenuron	Lab.	1.113	0.940 - 1.322	1.67 ± 0.15	-
	Field	8.508	6.906 - 10.65	1.56 ± 0.14	7.6
Methoxyfenozide	Lab.	4.958	4.146 - 5.943	1.57 ± 0.14	-
	Field	44.39	35.96 - 55.77	1.54 ± 0.14	9.0

Table (3): Total esterases activity	in the field and
laboratory strains of S.	littoralis 2 nd and
4 th instar larvae	

Larval	Specific activity (∆OD/mg protein / min) ± SE	Field/ Laboratory
mstar	Laboratory strain Field strain	ratio
2 nd	$0.227 \ b \pm 0.01 \ 0.689 \ a \pm 0.02$	3.04
4 th	$0.452 \ b \pm 0.03 \ 1.437 \ a \pm 0.70$	3.18

Numbers within a row with a letter in common are not significantly different according to analysis of variance (ANOVA) test (LSD at P < 0.05).

2002; Ranson and Hemingway, 2005). GSTs also can detoxify pyrethroids in insects by sequestering the insecticide (Kostaropoulos et al., 2001). Results of the present study are in agreement with Attia (1999), who reported higher GST activity in the CLW field strain compared with that of laboratory strain. Yu et al., (2003) also reported that, detoxification enzyme activities of microsomal oxidases, GST and hydrolases were higher in field strains of S. frugiperda has high resistance levels to carbamate, organophosphate and pyrethroid insecticides) than in the susceptible strain. El-Hassawy et al., (2014) recorded a significant increase in the GST enzymes activity in the selected strains of CLW to chlorpyrifos, profenofos, and cypermethrin compared to the laboratory susceptible strain. GST activity in the deltamethrin selected strain of Helicoverpa armigera was 2.7fold higher than in the susceptible strain (Martin et al., 2002). Elrakaiby (2018) recorded higher levels of GST and esterases activities in the 2nd and 4th instars larvae of the field strain (Collected from Almahmoudia city, El-Behera governorate) compared to the laboratory strain.

Table (5) shows that, the activity of ALP in the 2^{nd} instar of field strain were 2.82-fold of the laboratory strain. Regarding 4^{th} instar, ALP activity in the field strain were 2.04-fold its activity in the laboratory strain. Phosphatases (APs) are classically described as homodimeric nonspecific metalloenzymes which catalyze phosphomonesterase reactions (**Trowsdale** *et al.*, **1990**). Phosphatases have been included in the list

Table (4): Glutathione S-transferase activity in thefield and laboratory strains of S. litto-ralis 2nd and 4th instar larvae

Larval instar	Specific activ protein /	Field/ Laboratory	
	Laboratory strain	Field strain	ratio
2 nd	$31.50\ b\pm1.30$	168.83 a ± 8.11	5.36
4 th	$57.25\ b\pm2.23$	$217.32 a \pm 7.36$	3.80

Numbers within a row with a letter in common are not significantly different according to analysis of variance (ANOVA) test (LSD at P < 0.05).

Table (5): Alkaline phosphatase activity in the
field and laboratory strains of S. litto-
ralis 2nd and 4th instar larvae

Larval instar	Specific a (IU / hr/mg pr	Field/ Laboratory	
	Laboratory strain	Field strain	ratio
2 nd	$254.2 \text{ b} \pm 11.2$	$717.4 \text{ a} \pm 14.8$	2.82
4 th	$645.4\ b\pm22.5$	$1315.7 a \pm 17.5$	2.04

Numbers within a row with a letter in common are not significantly different according to analysis of variance (ANOVA) test (LSD at P < 0.05).

of detoxifying enzymes of insecticides; mostly of organophosphorus (**Oppenoorth**, **1985**), however, fenvalerate and cypermethrin resistant larvae of *Helicoverpa armigera* showed higher activities of esterases, phosphatases and methyl paraoxon hydrolase compared with susceptible larvae (**Srinivaset** *al.*, **2003**). **Abou-Taleb** (**2010**) showed that, the 2nd and 4th instar larvae exerts higher ALP activity compared to the laboratory strain.

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سمية بعض المبيدات على سلاله معمليه و سلاله حقليه من ديدان ورق القطن مع تقدير بعض انزيمات ازاله السميه داليا أحمد الديب¹ و أحمد فتحى بدير¹ و أحمد عبد الحكيم برانية² المعمل المركزى للمبيدات - محطة الصباحية - الأسكندرية - مصر ²معهد بحوث وقاية النبات - إيتاى البارود - مركز البحوث الزراعية - البحيرة - مصر الملخص العربي

تم اجراء تجارب التقييم الحيوي لتحديد مستويات المقاومه لديدان ورق القطن (سلاله معمليه و سلاله حقليه) . كما تم تقدير نشاط انزيمات الاستير ازيس ، الجلوتاثيون اس ترانسفير از ، و الالكلين فوفسفاتيز في كل من السلاله المعمليه و السلاله الحقليه.

اظهرت النتائج ان العمر اليرقي الثاني في السلاله الحقليه اظهر مستويات مختلفه من المقاومه لكل من سيبرميثرن ، الكلوروبيريفوس ، ايمامكتين بنزوات و ميثوكسيفينوزيد بمعامل سميه 61.3 ، 68.1 ، 14.7 و 29.4 على التوالي. بينما اظهر العمر اليرقي الثاني للسلاله الحقليه نسبه تحمل 8.1 ، 7، 5.6 و 3.6 لكل من سيبنتورام ، لوفينرون ، كلوانترابول و الفيبرونيل على التوالي. ان العمر اليرقي الرابع في السلاله الحقليه اظهر مستويات مقاومه عاليه لكل من الكلوروبيريفوس (157.9 و 150.) و يلدونيل على التوالي. ان العمر اليرقي الرابع في السلاله الحقليه اظهر من كلوانترابول (8.1) ، ايمامكتين بنزوات (8.4) ، ميثوكسيفينوزيد (9) ، لوفينرون (7.6) و الفيبرونيل (6.5) .

اظهرت السلاله الحقليه مستويات نشاط اعلى من السلاله المعملية في انزيمات الأستيرازيس ، الجلوت اثيون اس ترانسفيراز ، و الالكلين فو فسفاتيز