

Differential Toxicity of Some Insecticides Against Egg and Larval Stages of Cotton Leafworm and Role of Two Detoxification Enzymes

Hamdy K. Abou-Taleb¹

ABSTRACT

Toxicity of some insecticides against egg masses and two larval instars of cotton leafworm (CLW), *Spodoptera littoralis*, field and laboratory strains was determined. The activities of glutathione *S*-transferases (GST) and alkaline phosphatases (ALP) in the two strains also were compared. Results revealed that, spinosad, spinetoram, emamectin benzoate and lufenuron have low ovicidal activity. Chlorpyrifos and methomyl at 10 ppm achieved 80.4 and 83.6% mortality of treated egg masses, respectively. On the other hand, spinosad, spinetoram and emamectin benzoate at the same concentration caused 18.9, 19.4 and 28.1% mortality of treated egg masses, respectively. While lufenuron at 25 ppm caused 54.9% mortality of treated egg masses, lufenuron at 100 ppm achieved 100% mortality of treated egg masses at 100 ppm. Although spinosad, spinetoram and emamectin benzoate have low ovicidal activity compared with methomyl and chlorpyrifos, they have a comparable residual toxicity with methomyl and chlorpyrifos against the neonates. The residual toxicity of lufenuron against neonates is low at all tested concentrations. The 2nd and 4th instar larvae of the field strain exerts high resistance levels towards methomyl, chlorpyrifos and esfenvalerate. Regarding the 2nd instar, resistance ratios in 2008 and 2009 cotton seasons were 43.9 and 50.8 towards methomyl, 27.6 and 24.7 towards chlorpyrifos and 76.4 and 79.2 towards esfenvalerate. For the 4th instar resistance ratios in 2008 and 2009 cotton seasons were 62.8 and 63.6 towards methomyl, 43.9 and 49.2 towards chlorpyrifos and 112.4 and 114.8 towards esfenvalerate. On the other hand, field strain shows low or no levels of tolerance to spinosad, spinetoram, emamectin benzoate, lufenuron and methoxyfenozide. Activities of GST and ALP in the field strain were higher compared with that in the laboratory strain. In conclusion, the alternation between these insecticides can avoid increasing selection pressure of CLW populations to these insecticides.

INTRODUCTION

Cotton leafworm (CLW), *Spodoptera littoralis*, is one of the most destructive agricultural lepidopterous pests. It can attack numerous economically important crops all the year round. The chemical control of *S. littoralis* has been extensively reported in relation especially to cotton in Egypt (Issa *et al.*, 1984a & b and Abo Elghar *et al.*, 1986). Extensive use of insecticides, multiple generations of CLW per annum, and the

availability of host crops, around the year, have contributed to the development of resistance in this pest to many insecticide groups (Abo Elghar *et al.*, 2005).

In Egypt, management of CLW has depended on preserving and extending the insecticide efficacy based on rotating various insecticides including organophosphates, carbamates, insect growth regulators, and pyrethroids every year (Temerak, 2002). In addition, the continuous monitoring of resistance is fundamentally important to every resistance management program (Prabhaker *et al.*, 1996). At the same time, searching for an effective alternatives and/or pest control strategies to avoid increasing selection pressure of the insect population to insecticides is so important. More consideration needs also to be given to the possibility of controlling the pest at other stages of its development, when it may be more susceptible to the chemicals used for control. Several studies had been conducted to evaluate the ovicidal activity against many insect species (Wells and Guyer, 1962; Dittrich, 1967; Mitri and Kamel, 1970; El-Guindy *et al.*, 1983; Renkleff *et al.*, 1995; Canela *et al.*, 2000).

Different mechanisms of resistance to organophosphate, carbamate and pyrethroid insecticides have been identified in CLW, including enhanced metabolism, nerve insensitivity, reduced penetration and target site insensitivity (Attia, 1999; Abo Elghar *et al.*, 2005). Among the metabolic enzymes are glutathione *S*-transferase (GST) and alkaline phosphatase (ALP). Glutathione *S*-transferases are enzymes widespread in both prokaryotic and eukaryotic cells and catalyze the glutathione conjugation reaction with reduced glutathione (GSH) (Listowsky *et al.*, 1998; Armstrong, 1997). 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 Abou-El-Matameer city) compared with the laboratory strain to selected insecticides of diverse chemistries. Also, the activities of GST and ALP in both strains were investigated. In addition, the ovicidal activity of some insecticides was carried out.

¹Plant Protection Research Institute, ARC, Bacous, Sabahia, Alexandria Egypt.

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MATERIALS AND METHODS

Experimental insect:

a. 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 in 1-liter muslin covered mason jars. The number of larvae per jar differed according to the larval instar. All larvae were transferred every day into clean disinfected jars containing fresh food and the numbers adjusted according to the larval instar. When the larvae pupated they were sexed and 12 pupae put into each jar. When the moths emerged they were supplied with a piece of cotton moistened with 10% sugar solution and 2 *Nerium oleander* leaves, on which they deposited their eggs. The egg masses were collected daily and as they hatched on the oleander leaves the larvae were transferred to fresh castor oil leaves. The colony was kept at a temperature of 25 ± 2 °C and 65 ± 5 % RH (Eldefrawi *et al.*, 1964).

b. Field strain: Cotton leafworm egg masses were collected from cotton fields of Abou-El-Matameer city, El-Behera governorate during 2008 & 2009 cotton seasons 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.

Tested insecticides: Emamectin benzoate (Proclaim® 5%SG) and lufenuron (Match® 5%EC) were supplied by Syngenta. Chlorpyrifos (Dursban 48%EC), spinosad (Tracer® 24%SC), spinetoram (Radiant® 12%SC) and methoxyfenozide (Runner® 24%SC) were supplied by Dow Agrosciences Co. Esfenvalerate (SumiGold 20%EC) was supplied by Sumitomo Chemical Co. Ltd. Methomyl (Lannate 90% SL) was supplied by E. I. du Pont de Nemours & Co.

Ovicidal activity: Ovicidal activity of the formulated spinosad, spinetoram, chlorpyrifos, emamectin benzoate, methomyl, and lufenuron against the laboratory strain of *S. littoralis* egg masses was determined. The upper layers of each egg mass (0-24 hr old) were removed gently with a fine hair brush. The lower layer in each egg mass was counted by the binocular. The counted egg samples were dipped (5 seconds) in different concentrations of the tested compounds, while the control was dipped in water according to Dittrich (1967). Each treatment was replicated three times. Treatments and control were held in a plastic cups (9x4 cm) at 27 ± 2 °C, 65-75% RH and observed until hatching. The number of un-hatched eggs, dead neonates and live larvae were counted, and the mortality percentages were calculated.

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 the appropriate number and weight of starved larvae were added. Each concentration was replicated four times. Mortality percentages were recorded after 24 hrs of treatment for chlorpyrifos, spinosad, spinetoram, esfenvalerate & methomyl and after 72 hrs. for emamectin benzoate, lufenuron and methoxyfenozide. Mortality percentages were corrected according to Abbott equation (Abbott, 1925) and subjected to probit analysis (Finney, 1971).

Assay of GST activity of the 2nd and 4th instar larvae (Lab. and field strains): Preparation of GST: Total larvae of the 2nd instar and the collected midguts of the 4th instar larvae were rinsed in ice-cold 100 mM phosphate buffer pH 7 and homogenized in glass homogenizer (1: 10 w/v) in the same buffer. The homogenate was centrifuged at 14,000 rpm for 30 min at 4°C using Cryofuge 20-3, Heraeus Christ centrifuge. The supernatant was served as the enzyme source.

Enzyme assay: Glutathione S-transferases activity was measured according to the method of Asaoka and Takahashi (1983) using ethanolic solution of *O*-dinitrobenzene (DNB) as a substrate with slight modification (El-Shahawi and Al-Rajhi, 2000). The standard assay mixture (1 ml) contained: 1.5 mM reduced glutathione (GSH), 100 mM phosphate buffer pH 7, 20 µl of enzyme source, and the reaction was started by the addition of 0.5 mM DNB. After incubation at 37°C for 20 min, the reaction was terminated by the addition of 0.1 ml acetic anhydride. The mixture 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 spectrophotometer (Milton Roy Spectronic 601). An assay mixture without enzyme was used as the blank. GST specific activity was calculated as OD/mg protein/min.

Assay of ALP activity of the 2nd and 4th instar larvae (Lab. and field strains): Preparation of ALP: Total larvae of the 2nd instar and the collected midguts of the 4th instar larvae were homogenized (1: 10 w/v) in 100 mM phosphate buffer pH 9.8, using glass homogenizer. The homogenate was centrifuged at 5000 rpm for 30 min

at 4°C using IEC-CRU 5000 cooling centrifuge. The supernatant was used for ALP activity estimation.

Enzyme assay: Activity of ALP was determined according to the method of Dgkc (1972), using Diamond Diagnostic kit (Diamond Co. Egypt). In this method 20 µl 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 phosphate, 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.

Protein measurements: Protein estimation has been carried out according to Lowery *et al.*, (1951).

Table 1. Ovicidal and residual toxicity of some insecticides against *Spodoptera* (Lab. strain) egg masses

Insecticide	Conc. (ppm)	No. treated eggs	%Mortality 24 hrs after hatching ± SD		
			eggs	neonates	total
Spinosad	0.5	384	8.1 ± 1.2	20.1 ± 2.7	26.6 ± 3.1
	1.0	411	14.7 ± 2.5	34.6 ± 2.4	44.3 ± 5.3
	2.0	364	17.9 ± 1.9	52.8 ± 5.6	61.3 ± 3.6
	5.0	344	19.7 ± 2.1	66.7 ± 4.8	73.8 ± 6.4
	10.0	362	18.9 ± 2.6	92.5 ± 3.7	93.8 ± 3.8
Spinetoram	0.5	350	4.8 ± 0.8	19.5 ± 2.5	23.5 ± 2.6
	1.0	372	6.7 ± 1.0	45.2 ± 5.0	48.9 ± 5.2
	2.0	324	10.1 ± 1.4	69.1 ± 5.3	72.3 ± 6.4
	5.0	356	16.6 ± 1.7	89.9 ± 4.0	91.7 ± 4.1
	10.0	342	19.4 ± 2.0	100.0 ± 0.0	100.0 ± 0.0
Emamectin benzoate	0.5	366	3.5 ± 0.4	28.9 ± 3.2	31.4 ± 3.3
	1.0	402	12.7 ± 1.1	40.7 ± 3.7	48.3 ± 5.0
	2.0	335	15.2 ± 1.7	66.5 ± 6.3	71.5 ± 5.7
	5.0	337	20.9 ± 2.1	100.0 ± 0.0	100.0 ± 0.0
Methomyl	1.0	342	33.6 ± 2.7	11.0 ± 1.8	40.9 ± 4.5
	5.0	351	54.1 ± 3.7	28.6 ± 2.6	67.3 ± 6.3
	10.0	354	83.6 ± 2.4	86.2 ± 5.1	97.7 ± 2.0
	15.0	327	95.4 ± 1.8	80.0 ± 6.7	99.1 ± 0.2
	20.0	352	100.0 ± 0.0	-	100.0 ± 0.0
Chlorpyrifos	1.0	390	20.2 ± 2.4	24.7 ± 3.2	40.0 ± 4.3
	2.0	352	31.6 ± 3.2	52.9 ± 4.3	67.9 ± 6.2
	5.0	341	54.8 ± 3.9	84.8 ± 4.9	93.3 ± 4.7
	10.0	382	80.4 ± 3.5	100.0 ± 0.0	100.0 ± 0.0
	15.0	348	89.6 ± 4.1	100.0 ± 0.0	100.0 ± 0.0
Lufenuron	1.0	344	17.7 ± 2.3	2.5 ± 0.2	19.8 ± 2.1
	5.0	356	37.6 ± 4.2	3.6 ± 0.0	39.9 ± 4.8
	25.0	370	54.9 ± 4.8	5.1 ± 0.7	57.3 ± 4.6
	50.0	360	83.6 ± 4.4	4.8 ± 0.7	84.4 ± 5.8
	100.0	347	100.0 ± 0.0	-	100.0 ± 0.0

RESULTS AND DISCUSSION

Ovicidal activity of tested insecticides against CLW egg masses:

One of our objectives in this study was to evaluate the ovicidal activity of some insecticides against 0-1 day old egg masses of CLW. Some insecticides may be more efficient as ovicides than larvicides. The residual toxicity of these insecticides to the new hatched neonates was also determined. Data in Table(1) showed that, spinosad, spinetoram, emamectin benzoate and lufenuron have low ovicidal activity compared with both chlorpyrifos and methomyl. Chlorpyrifos and methomyl at 10 ppm achieved 80.4 and 83.6% mortality of treated egg masses, respectively. On the other hand, spinosad, spinetoram and emamectin benzoate at the same

concentration caused 18.9, 19.4 and 28.1% mortality of treated egg masses, respectively. Lufenuron at 25 ppm caused 54.9% mortality of treated egg masses, while, mortality of treated egg masses. Although spinosad, spinetoram and emamectin benzoate have low ovicidal activity compared with methomyl and chlorpyrifos, they have a comparable residual toxicity with methomyl and chlorpyrifos against the neonates. The residual toxicity of lufenuron against neonates is low at all tested concentrations.

In previous studies, Pinela *et al.* (2000) stated that treatment of *S. littoralis* 0-24 h eggs by spinosad at 10 mg a.i / litre or above caused 100% mortality of newly emerged larvae after the first day of hatching. When tobacco budworm eggs were treated with spinosad, only 49% egg mortality was observed. On the other hand, none of the tobacco budworm larvae that emerged

survived (Bret *et al.*, 1997). Also, Sherby *et al.*, (2010) recorded 95.8 and 82.6% mortality of eggs and new hatched larvae by spinosad and chlorpyrifos at 10 ppm, respectively. Regarding lufenuron, although it is known that among the diverse actions of IGRs on the life cycles of insects are ovicidal and larvicidal effects (Ascher *et al.*, 1987), it has a low ovicidal activity compared with methomyl and chlorpyrifos. In respect with emamectin benzoate (semi-synthetic of abamectin), Bueno and Freitas (2004) reported that abamectin has no effect on the *Chrysoperla externa* egg viability.

Toxicity of tested insecticides to 2nd and 4th instar larvae of field and laboratory strains of CLW:

Susceptibility of 2nd and 4th-larval instars from the laboratory and field strains, collected during the cotton season of 2008 and 2009 to selected insecticides is presented in Tables (2 and 3).

Table 2. Median lethal concentrations of some insecticides against laboratory and field strains of *Spodoptera littoralis* 2nd instar larvae

Insecticide	Year	Strain	LC ₅₀ (ppm)	Confidence limits	Slope ± SE	RR [†]
Spinosad	2008	Lab.	37.2	28.7 - 47.5	2.3 ± 0.20	1
		Field	63.7	52.1 - 78.8	1.9 ± 0.17	1.7
	2009	Lab.	35.8	29.6 - 43.9	2.1 ± 0.23	1
		Field	68.4	53.0 - 88.8	1.8 ± 0.19	1.9
Spinetoram	2008	Lab.	12.3	8.5 - 17.2	2.5 ± 0.26	1
		Field	28.7	23.9 - 35.1	1.7 ± 0.18	2.3
	2009	Lab.	11.9	8.0 - 16.3	2.0 ± 0.18	1
		Field	26.4	20.8 - 33.2	1.8 ± 0.16	2.2
Emamectin benzoate	2008	Lab.	0.002	0.0015 - 0.0027	1.8 ± 0.17	1
		Field	0.007	0.006 - 0.009	1.8 ± 0.18	3.5
	2009	Lab.	0.002	0.0014 - 0.0026	1.9 ± 0.17	1
		Field	0.008	0.006 - 0.011	1.8 ± 0.20	4.0
Methomyl	2008	Lab.	3.9	2.8 - 5.4	2.6 ± 0.27	1
		Field	171.3	147.8 - 203.5	1.6 ± 0.18	43.9
	2009	Lab.	3.6	3.0 - 4.8	2.9 ± 0.22	1
		Field	182.8	146.9 - 210.6	1.8 ± 0.19	50.8
Chlorpyrifos	2008	Lab.	2.7	2.4 - 3.2	1.8 ± 0.16	1
		Field	74.6	58.1 - 94.9	1.9 ± 0.16	27.6
	2009	Lab.	2.9	2.4 - 3.6	2.0 ± 0.20	1
		Field	71.7	63.2 - 81.5	1.8 ± 0.21	24.7
Esfenvalerate	2008	Lab.	0.5	0.44 - 0.58	1.6 ± 0.14	1
		Field	38.2	30.6 - 48.5	1.5 ± 0.16	76.4
	2009	Lab.	0.5	0.44 - 0.58	1.6 ± 0.17	1
		Field	39.6	31.9 - 51.8	1.5 ± 0.14	79.2
Lufenuron	2008	Lab.	2.3	1.8 - 3.0	1.9 ± 0.21	1
		Field	15.6	11.6 - 21.4	1.8 ± 0.20	6.8
	2009	Lab.	2.0	1.6 - 2.6	1.8 ± 0.21	1
		Field	17.3	12.8 - 23.6	1.8 ± 0.19	8.7
Methoxyfenozide	2008	Lab.	1.8	1.4 - 2.6	2.4 ± 0.25	1
		Field	12.7	9.3 - 16.3	2.3 ± 0.22	7.1
	2009	Lab.	1.9	1.6 - 2.4	2.5 ± 0.23	1
		Field	11.8	8.4 - 16.4	2.2 ± 0.20	6.2

Table 3. Median lethal concentrations of some insecticides against laboratory and field strains of *Spodoptera littoralis* 4th instar larvae

Insecticide	Year	Strain	LC ₅₀ (ppm)	Confidence limits	Slope ± SE	RR ^a
Spinosad	2008	Lab.	162.4	139.8 – 193.2	1.9 ± 0.21	1
		Field	478.3	414.7 – 552.9	1.6 ± 0.18	2.9
	2009	Lab.	173.2	142.5 – 211.9	1.9 ± 0.22	1
		Field	548.5	476.3 – 638.7	1.6 ± 0.19	3.2
Spinetoram	2008	Lab.	74.8	58.4 – 95.2	2.0 ± 0.21	1
		Field	175.4	154.8 – 204.4	1.8 ± 0.20	2.3
	2009	Lab.	66.7	51.3 – 84.6	1.9 ± 0.20	1
		Field	176.3	160.5 – 203.2	1.9 ± 0.17	2.6
Emamectin benzoate	2008	Lab.	0.023	0.018 – 0.030	1.6 ± 0.17	1
		Field	0.055	0.047 – 0.065	1.7 ± 0.19	2.4
	2009	Lab.	0.021	0.018 – 0.025	1.7 ± 0.18	1
		Field	0.056	0.047 – 0.067	1.6 ± 0.17	2.7
Methomyl	2008	Lab.	34.7	30.3 – 42.6	2.0 ± 0.23	1
		Field	2180.7	1860.8 – 2684.7	1.5 ± 0.16	62.8
	2009	Lab.	38.4	31.0 – 47.8	2.1 ± 0.23	1
		Field	2440.4	1820.9 – 3150.6	1.6 ± 0.19	63.6
Chlorpyrifos	2008	Lab.	19.6	16.2 – 24.8	1.9 ± 0.21	1
		Field	859.9	635.6 – 1214.2	1.6 ± 0.18	43.9
	2009	Lab.	17.3	14.2 – 22.5	1.9 ± 0.20	1
		Field	851.9	612.2 – 1211.6	1.7 ± 0.18	49.2
Esfenvalerate	2008	Lab.	3.4	2.5 – 4.5	1.7 ± 0.16	1
		Field	382.3	332.8 – 446.8	1.8 ± 0.19	112.4
	2009	Lab.	3.2	2.5 – 4.1	1.7 ± 0.19	1
		Field	367.3	311.9 – 437.3	1.6 ± 0.16	114.8
Lufenuron	2008	Lab.	4.9	3.8 – 6.4	2.0 ± 0.22	1
		Field	40.2	34.4 – 48.0	1.9 ± 0.20	8.2
	2009	Lab.	5.4	4.1 – 7.3	1.9 ± 0.21	1
		Field	45.8	38.4 – 55.2	1.9 ± 0.21	8.5
Methoxyfenozide	2008	Lab.	3.7	3.1 – 4.5	2.2 ± 0.23	1
		Field	19.0	14.6 – 25.6	2.3 ± 0.24	5.1
	2009	Lab.	4.2	3.6 – 5.0	2.1 ± 0.20	1
		Field	20.1	15.3 – 27.5	2.2 ± 0.23	4.8

Data in Table (2) showed that, the 2nd instar larvae of the field strain demonstrated varied levels of resistance to methomyl, chlorpyrifos and esfenvalerate. The resistance towards methomyl appeared to increase from 43.9-fold at 2008 to 50.8-fold at 2009. Toxicity tests against the 2nd larval instar with chlorpyrifos showed that resistance levels in 2008 and 2009 cotton seasons were 27.6 and 24.7-fold. The 2nd instar larvae of the field strain showed the highest resistance levels against esfenvalerate with resistance ratios 76.4 and 79.2. On the other hand, the 2nd instar larvae of field strain showed no resistance to spinosad, spinetoram and emamectin benzoate. Regarding the two IGR compounds the 2nd instar larvae of field strain showed tolerance ratios 6.8 and 8.7 to lufenuron and 7.1 and 6.2 to methoxyfenozide at 2008 and 2009 cotton seasons, respectively.

Regarding the 4th instar larvae (Table 3), the field strain exerts high resistance levels towards methomyl, chlorpyrifos and esfenvalerate. Resistance ratios in 2008 and 2009 cotton seasons were 62.8 and 63.6 towards methomyl, 43.9 and 49.2 towards chlorpyrifos and 112.4 and 114.8 towards esfenvalerate. The 4th instar larvae of the field strain showed no resistance to spinosad, spinetoram, emamectin benzoate & methoxyfenozide, and tolerance ratios 8.2 and 8.5 towards lufenuron in 2008 and 2009 cotton seasons.

Extensive indiscriminate use of organophosphate, pyrethroid and carbamate insecticides has led to development of resistance in CLW to all these insecticides (Attia, 1999; Abo Elghar *et al.*, 2005). The present study revealed high levels of resistance to esfenvalerate, methomyl and chlorpyrifos in the tested field population.

Table 4. Activity of glutathione S-transferases in the field and laboratory strains of cotton leafworm

Year	Larval instar	S.A (OD/hr/mg protein) \pm SD		Lab./field ratio
		Lab. strain	Field strain	
2008	2 nd	1.061 \pm 0.082	1.942 \pm 0.102	1.83
	4 th	1.712 \pm 0.100	3.716 \pm 0.146	2.17
2009	2 nd	1.172 \pm 0.092	2.051 \pm 0.173	1.75
	4 th	1.547 \pm 0.056	3.403 \pm 0.089	2.20

Table 5. Activity of alkaline phosphatases in the field and laboratory strains of cotton leafworm

Year	Larval instar	S.A (IU / hr / mg protein) \pm SD		Lab./field ratio
		Lab. strain	Field strain	
2008	2 nd	274.8 \pm 17.3	695.2 \pm 38.5	2.53
	4 th	729.4 \pm 27.6	1283.7 \pm 61.6	1.76
2009	2 nd	257.2 \pm 12.9	635.3 \pm 23.4	2.47
	4 th	688.1 \pm 31.4	1121.6 \pm 73.2	1.63

Therefore, there is a need to test different insecticides having different modes of action. In the present study spinosad, spinetoram, emamectin benzoate and methoxyfenozide are between these insecticides. *Spodoptera littoralis* showed low levels of tolerance to these insecticides. Regarding spinosad, these results are in variance with Temerak (2003), who reported that, the field strain of the CLW larvae proved to be more susceptible to spinosad than the laboratory strain (resistance ratios were 0.11 and 0.04 for the 2nd and 4th instars, respectively). In respect with emamectin benzoate, results of this study are in accordance with Abou-Taleb *et al.*, (2009). They reported that, the field strain showed tolerance ratios to emamectin benzoate about 3.5 for the 2nd instar and 1.6 for the 4th instar.

Activity of GST and ALP in the laboratory and field strains of CLW:

Observations that resistance to insecticides in insects may be attributable to enhanced GSH-dependent detoxification (Oppenoorth *et al.*, 1979; Motoyama, 1978) have focused interest on these enzymes in insect pest strains. In the present study, the field strain showed higher GST activity. In respect with the 2nd larval instar, the GST activities in the field strain were 1.83 and 1.75-fold compared with that of lab. strain in 2008 and 2009, respectively. Regarding the 4th larval instar, the GST activities in the field strain were 2.17 and 2.20-fold compared with that of lab. strain in 2008 and 2009, respectively (Table 4). These results are in agreement with that reported by Attia (1999), who reported higher GST activity in the CLW field strain compared with that of lab. strain. Also, Yu *et al.*, (2003) reported that, detoxification enzyme activities of microsomal oxidases,

GST, and hydrolases were higher in field strains of *Spodoptera frugiperda* (has high resistance levels to carbamate, organophosphate and pyrethroid insecticides) than in the susceptible strain.

Phosphatases (APs) are classically described as homodimeric nonspecific metalloenzymes which catalyze phosphomonoesterase reactions (Trowsdale *et al.*, 1990). Table (5) shows that, the activities of ALP in the 2nd instar of field strain were 2.53 and 2.47-fold of the lab. strain in 2008 and 2009, respectively. Regarding 4th instar, ALP activities in the field strain were 1.76 and 1.63-fold its activity in the lab. strain in 2008 and 2009, respectively. Phosphatases have been included in the list 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 paraxon hydrolase compared with susceptible larvae (Srinivas *et al.*, 2003).

Finally, spinosad, spinetoram and emamectin benzoate, methoxyfenozide and lufenuron can alternate with the organophosphate, carbamate and pyrethroid insecticides to avoid increasing selection pressure of CLW populations to these insecticides.

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الملخص العربي

السمية التفاضلية لبعض المبيدات الحشرية على كتل البيض ويرقات دودة ورق القطن مع تقدير نشاط إنزيمين من الإنزيمات المسؤولة عن إزالة السمية

حمدي قطب أبوطالب

القطن مستويات عالية من المقاومة للكلوربيريفوس والميثوميل والإسفيناليرات مقارنة بالسلالة المعملية. بخصوص العمر الثان كانت مستويات المقاومة التي أظهرها للميثوميل هي ٤٣,٩ و ٥٠,٨ في سنة ٢٠٠٨ و ٢٠٠٩، للكلوربيريفوس كانت ٢٧,٦ و ٢٤,٧، للإسفيناليرات كانت ٧٦,٤ و ٧٩,٢. أما العمر الرابع فقد أظهر مستويات من المقاومة ٦٢,٨ و ٦٣,٦ للميثوميل في سنة ٢٠٠٨ و ٢٠٠٩، ٤٣,٩ و ٤٩,٢ للكلوربيريفوس، ١١٢,٤ و ١١٤,٨ للإسفيناليرات. من ناحية أخرى لم تظهر السلالة المعملية مستويات من المقاومة أو أظهرت مستويات منخفضة من المقاومة للـ الإسبينوساد، الإسبينيتورام، الایمامکتين بتروات واللوفينيترون والميثوكسيفينوزايد. أظهرت النتائج أيضا أن نشاط إنزيمي الـ الجلوتاثيون اس ترانسفيريز والالکالين فوسفاتيز كان أعلى في السلالة الحقلية مقارنة بالسلالة المعملية. أخيرا يمكن التلخيص بأنه عند استخدام المبيدات السابقة بالتبادل فيما بينها في مكافحة دودة ورق القطن فإنه يمكن تفادي زيادة مقاومة هذه الآفة لفعل هذه المبيدات.

تم تقدير سمية بعض المبيدات الحشرية المختارة على كتل البيض و عمرين يرقيين لسلالتين من دودة ورق القطن احدهما حقلية والاخرى معملية ومقارنة نشاط انزيمي الجلوتاثيون اس ترانسفيريز والالکالين فوسفاتيز في كلتا السلالتين. اوضحت النتائج ان مبيدات الإسبينوساد، الإسبينيتورام، الایمامکتين بتروات واللوفينيترون لهما سمية ضعيفة على البيض مقارنة بمبيدات الكلوربيريفوس والميثوميل. حيث اعطى المركبين الأخيران بتركيز ١٠ جزء في المليون نسبة موت وصلت الى ٨٠,٤ و ٨٣,٦% على التوالي. من ناحية أخرى وعند نفس التركيز اعطت مركبات الإسبينوساد، الإسبينيتورام، الایمامکتين بتروات ١٨,٩، ١٩,٤، ٢٨,١% موت لكلل البيض المعاملة على التوالي. بينما أعطى اللوفينيترون عند تركيز ٢٥ جزء في المليون ٥٤,٩% موت لكلل البيض المعامل، أعطى ١٠٠% موت عند تركيز ١٠٠ جزء في المليون. على الرغم من أن الإسبينوساد، الإسبينيتورام، الایمامکتين بتروات لها سمية منخفضة على البيض إلا أن النشاط الإبادي للمتبقى على اليرقات حديثة الفقس كان كبيرا. أظهرت يرقات العمر الثان والعمر الرابع للسلالة الحقلية لدودة ورق