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# Comparative toxicity and biochemistry of organophosphates and pyrethroid compounds on both laboratory and field strain of the Cotton Leafworm *Spodoptera littoralis* (Boisd.)

**El- Sheikh, T. A. A.**
**Plant Prot. Res. Inst., Agric. Res. Cent., Dokki, Giza, Egypt.**

## ABSTRACT

Bioassay was carried out for monitoring resistance spectrum toward insecticides include organophosphates, Dursban H-48% EC (Chlorpyrifos) and pyrethroids Cyper (Cypermethrin 10% EC) in three different field populations (Gharbia , Kalubia and Menofeya Governorates) of the Cotton Leafworm, *Spodoptera littoralis* (Boisd.). Toxicity data based on $LC_{50}$ values indicated that the pyrethroid Cyper (Cypermethrin 10% EC) is more toxic insecticide against the laboratory strain, while, the OP Dursban H- 48% EC (Chlorpyrifos) was less toxic. The development of resistance to organophosphates, Dursban H- 48% EC (Chlorpyrifos) and pyrethroids Cyper (Cypermethrin 10% EC) in field strains of the cotton leafworm, in relation to esterases, glutathione s- transferase, and phosphatases was studied. Field strains exhibited moderately high level of resistance to the tested insecticides. A significant increase in the activity of esterases, glutathione s-transferase suggests a good relation to resistance was detected. A high level of acid phosphatase and low level of alkaline phosphatase accompanied the level of resistance to pyrethroid and OP.

**Keywords:** *Spodoptera littoralis,* insecticidal, biological, biochemical effects, organophosphorus, pyrethroid compound and resistance.

## INTRODUCTION

The Cotton Leafworm, *Spodoptera littoralis* (Boisd.) is the most economic pest infesting cotton plant in Egypt causing serious decrease in cotton yield. The use of insecticides in controlling cotton pest has led to serious number of problems among them insect resistance.

Cotton pests possesses the ability to develop resistance to different groups of insecticides in the field (XianChun *et al.,* 1997). Since resistance to insecticides is accumulating one year after another, it is important to evaluate the potency of the old as well as the newly emerged insecticides against the cotton Leafworm, in each cotton season. The occurrence of resistance to an insecticide in insects is mainly due to the action of enzymes, which are either insensitive to the insecticide or able to degrade it to non-toxic metabolites. Armes *et al*. (1997) found that resistance of *Spodoptera litura* (Fab.) to pyrethroids may be due to the enhanced detoxification enzymes and those esterase's were contributing to organophosphate resistance. Gunning *et al.* (1996) found that resistant strains increased levels of esterase activity that detoxified significant quantities of esfenvalerate. Moreover, Gunning *et al.* (1999) reported that the inhibition of esterase was associated with pyrethroid resistance. It is believed that such information would help in developing chemical control programs valid for future use against the existing field populations of the pest.

The present study was conducted to investigate two main groups of insecticides commonly applied on cotton for controlling different insect species. These insecticides include organophosphates, Dursban H-48% EC (Chlorpyrifos) and pyrethroids Cyper

(Cypermethrin 10% EC) against cotton Leafworm, *Spodoptera littoralis* laboratory and field strains.

## MATERIALS AND METHODS

**Tested insect**

Laboratory reference strain of the cotton Leafworm, *Spodoptera littoralis* used as base line for the present study was obtained from laboratory colony continuously reared free from insecticides in Cotton Research Department, Plant Protection Research Institute, Agriculture Research Center, Dokki, Egypt. Field strain was obtained by collecting the egg masses from three Governorates (Gharbia, Kalubia and Menofeya) before and after treatments (early and late season). All cultures were maintained under optimum conditions (25°C ± 2 and 70 ± 5 % R.H) and reared on castor-bean leaves until larvae reached to the 4th instar which were used for the present study.

**Insecticides tested**

Commercial formulations of insecticides were used in this study representing 2 main groups of insecticides commonly applied on cotton for controlling different insect species. These insecticides include organophosphates, Dursban H- 48% EC (Chlorpyrifos) obtained from Daw Agrosciences and pyrethroids Cyper (Cypermethrin 10% EC) obtained from Agrochina group.

**Bioassay**

The leaf-dipping bioassay method was used to determine the median lethal concentration ($LC_{50}$) values. Series of concentrations (in water) of the tested formulated compounds were prepared. Castor-bean leaves were dipped for 30 seconds in each concentration then left for one hour to dry. The treated leaves were offered to newly moulted 4th instar larvae of each strain. Mortality percentages were recorded after 24 hrs. and corrected according to (Abbott, 1925). To estimate the $LC_{50}$ values, the corrected mortality percentages were subjected to probit analysis according to Finney (1952).

**Statistical analysis:**

The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range tests ($p< 0.05$). All analyses was made using a software package "Costat", a product of cohort software Ine., Berkley, California. (Duncan, 1955).

Resistance Ratio (R.R.) values in fold were calculated for each insecticide as follow:

$$R.R. = \frac{LC_{50} \text{ of the field strain}}{LC_{50} \text{ of the laboratory strain}}$$

**Preparation of samples for biochemical studies:**

After the detection of the median lethal concentration ($LC_{50}$) values using the 4th instar larvae lab strain and field population were fed to reach the 6th instar. The collected 6th larval instar starved for 4 hours. Samples were collected after homogenizing the 6th instar larvae representing 1 gm larval body weight, in 5 ml distilled water by using chilled glass Teflon grinder. The homogenate was centrifuged for 10 min. at 5000 rpm and 5 $^{O}$C, the supernatant fraction being used for the enzyme assay Farag (2001).

**Determination of non-specific esterases activities:**

Alpha- and beta-esterases (α-E, β -E) activities were determined according to the method of Van Asperen (1962) using α-naphthyl acetate and β-naphthyl acetate as substrates, respectively.

**Determination of acetylcholine esterase activity:**

The activity of acetylcholine esterase (AChE) was measured according to the method described by Simpson *et al.* (1964) using acetylcholine bromide (AChBr) as substrate.

**Determination of acid and alkaline phosphatase activities:**

Acid phosphatase (AcP) and alkaline phosphatase (AlkP) activities were determined according to the method described by Powell and Smith (1954).

**Determination of Glutatione S-transferase activity:**

The determination of Glutatione S-transferase activity (GST) was preceded in the presence of 1, 2-Dichloro-nitrobenzen as a substrate according to the method of Habig *et al.* (1974).

## RESULTS AND DISCUSSION

**Susceptibility of *S. littoralis* larvae to different insecticides**

The $LC_{50}$, slope (b) values and the calculated RR of different insecticides tested against the Laboratory and field collected strains before and after spraying season in the three Governorates during 2010 and 2011 cotton growing seasons were recorded in Tables (1: 4). In the present studies table(1, 2, 3 and 4) show that the susceptibility of the two tested compounds used in the present work caused variable toxic effects against the 4$^{th}$ larvae of *Spodoptera littoralis* for both laboratory and field strains.

The $LC_{50}$ were 17.5, 18.43 and 14.7, 13.47 ppm for Chlorpyrifos and cypermethrin in laboratory strain during seasons 2010 and 2011, respectively. Cypermethrin is more toxic to *S. littoralis* than chlorpyrifos for lab strain. These results were match with Abd El-Mageed *et al.,* (2011) who demonstrated that The efficiency and residual effects of five new insecticide mixtures chlorosan, feroban, cygron, engeo, and kingbo were studied in the 2$^{nd}$ and 4$^{th}$ instar larvae against *S. littoralis* under field conditions and the obtained results revealed that feroban was the most effective compared with the other toxicants, while engeo was the least toxic insecticide in both instars after 2 and 5 days from treatment.

**Chlorpyrifos**

In the early cotton season the data in (Table 1 and 2) show in general, that pronounced levels of resistance to the organophosphate Chlorpyrifos in all field strains representing different Governorates. This is indicated by the values of the calculated RR which were 7.65, 8.04, 14.64 and 6.76, 8.79, 11.58 for gharbia, kalubia and menofeya Governorates, respectively during 2010 and 2011 cotton growing seasons.

Table 1: Toxicity data of chlorpyrifos against 4$^{th}$ instar larvae of laboratory and field strains of *S. littoralis* at early and late season 2010.

| Strain | Toxicity data early 2010 | | | Toxicity data late 2010 | | |
|---|---|---|---|---|---|---|
| | $LC_{50}$ ppm. | Slope | RR | $LC_{50}$ ppm. | Slope | RR |
| Lab. | 17.50 | 1.92 | - | 17.50 | 1.92 | - |
| Gharbia | 133.93 | 2.72 | 7.65 | 170.58 | 2.48 | 9.77 |
| Kalubia | 140.80 | 2.14 | 8.04 | 170.14 | 2.38 | 9.27 |
| Menofeya | 256.28 | 2.31 | 14.64 | 261.48 | 1.41 | 16.21 |

Table 2: Toxicity data of chlorpyrifos against 4th instar larvae of laboratory and field strains of *S. littoralis* at early and late season 201.

| Strain | Toxicity data early 2011 | | | Toxicity data late 2011 | | |
|---|---|---|---|---|---|---|
| | $LC_{50}$ ppm. | Slope | RR | $LC_{50}$ ppm. | Slope | RR |
| Lab. | 18.43 | 1.31 | - | 18.43 | 1.31 | - |
| Gharbia | 124.62 | 1.84 | 6.76 | 170.58 | 1.62 | 9.25 |
| Kalubia | 162.17 | 1.35 | 8.79 | 170.14 | 1.39 | 9.23 |
| Menofeya | 213.58 | 2.79 | 11.58 | 261.48 | 1.82 | 14.18 |

In the late cotton season the resistance spectrum of field population in the same governorates reveal that the calculated RR were 9.77,9.27,16.21 and 9.25,9.23,14.18 for gharbia, kalubia and menofeya governorates, respectively, during 2010 and 2011 cotton growing seasons. Obviously, these RR values indicated that the tested field menofeya exhibited relatively remarkable, higher levels of resistance than the other two governorates at which the RR values were recorded 16.21 and 14.18 fold for 2010 and 2011 late seasons, respectively.

The development of considerable levels of resistance for OP's representing different governorates of Egypt was previously demonstrated by several authors (Hassan *et al*., 1970, El-Sayed 1973, Kansouh *et al.* 1979).

El-Sayed *et al.* (1984) found that resistance to organo-phosphates in the cotton leafworm, was much higher to most of the methyl esters than to ethyl esters. Later on, (Rashwan *et al*. 1991-1992) found that several field strains of cotton leafworm representing different governorates exhibited high resistance to organophosphates and that a strongly pronounced increase in resistance level was detected at the end of the cotton season than the early in season.

**Cypermethrin**

In general the results of early season (Tables 3 and 4) indicate that resistance to the tested synthetic pyrethroid (cypermethrin) is wide spread and sever in the field-collected population of the cotton leafworm, (although such compound is one of the insecticides in chemical control program). The estimated resistance factor demonstrated that the highest resistance rate was recorded for 20.05 , 21.01 and 23.1,22.06 fold for kalubia and menofeya during 2010 and 2011 cotton growing seasons, respectively. On the other hand, gharbia exhibited the lowest resistance rate at which the RR was 6.39 and 6.84 fold, respectively.

As for the late season (Tables 3 and 4) the data indicate that the cotton leafworm, field populations of different governorates became more resistance to the tested insecticide compared with late spraying season. In this respect, the populations in menofeya followed by kalubia governorates revealed the highest resistance ratios, reached 21.75, 25.33 and 23.67, 27.89 fold during 2010 and 2011 cotton growing seasons, respectively.Whereas, the least resistance ratios of 7.63 and 9.84 fold were recorded in Gharbia at late season 2010 and 2011, respectively.

The resistance to pyrethroid had been established in field strains of *S. littoralis* in most of the Egyptian governorates (El-Guindy *et al.* 1982) which agree with the obtained results in the present study, the same authors added that the different levels of significant resistance to pyrethroids exhibited by the field strains are resulted from cross resistance with other groups of insecticides and not due to intensive or prolonged applications of the used compounds. In agreement, increased levels of methyl parathion resistance were accompanied by pyrethroid cross-

tolerance before the wide spread introduction of the pyrethroid into the cotton fields of Arizona and California (Crowder *et al.,* 1979 and Twin and Reynolds, 1980).

Table 3: Toxicity data of cypermethrin against 4th instar larvae of laboratory and field strains of *S. littoralis* at early and late season 2010.

| Strain | Toxicity data early 2010 | | | Toxicity data late 2010 | | |
|---|---|---|---|---|---|---|
| | $LC_{50}$ ppm. | Slope | RR | $LC_{50}$ ppm. | Slope | RR |
| Lab. | 14.72 | 1.25 | - | 14.72 | 1.25 | - |
| Gharbia | 94.19 | 1.98 | 6.39 | 112.34 | 2.13 | 7.63 |
| Kalubia | 295.14 | 1.34 | 20.05 | 348.65 | 1.57 | 23.67 |
| Menofeya | 309.28 | 1.99 | 21.01 | 320.16 | 1.68 | 21.75 |

Table 4: Toxicity data of cypermethrin against 4th instar larvae of laboratory and field strains of *S. littoralis* at early and late season 2011.

| Strain | Toxicity data early 2011 | | | Toxicity data late 2011 | | |
|---|---|---|---|---|---|---|
| | $LC_{50}$ppm. | Slope | RR | $LC_{50}$ppm. | Slope | RR |
| Lab. | 13.47 | 1. 58 | - | 13.47 | 1. 58 | - |
| Gharbia | 92.14 | 1.92 | 6.84 | 132.58 | 1.72 | 9.84 |
| Kalubia | 311.25 | 2.11 | 23.10 | 376.98 | 1.66 | 27.98 |
| Menofeya | 297.28 | 1.59 | 22.06 | 341.21 | 1.74 | 25.33 |

However, monitoring for resistance carried out by El-Bermawy *et al.* (1992) indicated that most of the tested field strains developed high levels of resistance averaged 50.9-6667.7 fold early in the cotton season versus 142.5-1738.8 fold late in the season for some synthetic pyrethroids. Similar findings were obtained by Kim *et al.* (1998) who reported that field populations of *S. littoralis* showed resistance to commonly used insecticides ranged from 100 - to 2700-fold for pyrethroids and ranged from 2- to 32-fold for organophosphates. They concluded that the broad spectrum of insecticide resistance observed was due to multiple resistance mechanisms, including increased detoxification of insecticides and insensitive acetylcholine esterase.

**Biochemical studies**

The biochemical parameters of the resistance phenomenon in field populations of cotton leafworm collected from Gharbia, kalubia and menofeya governorates were evaluated.

Homogenate samples of 6th instar larvae were used throughout the study.

**Esterases enzyme activities:**

**Non-specific Esterases (α-E & β-E):**

Tables (5, 6, 7 and 8) refer the changes in non-specific esterases activities, alpha esterase (α- E) and beta esterase (β-E), of *S. littoralis* laboratory strain (L-strain) and fields strains (F-strain) .The data represented here are expressed as percentages increase or decrease in the activity relative to the L-strain. The data obtained showed that, in general the activity of α-esterase was greater in case of field strain early and late season at all tested insecticides during 2010 and 2011 cotton growing seasons. In case of Chlorpyrifos enzyme activity recorded (207.6, 284.3, 327.7) and (22.5, 302.3, 292.8) for gharbia, kalubia and menofeya, respectively, in season 2010.

Table 5: biochemical changes of chlorpyrifos against 4th instar larvae of laboratory and field strains of *S. littoralis* at early and late season 2010.

| | Acetyle choline Esterase µg Acetylcholine-bromide/min/ml | Changes % | α- Esterase µg α-Naphthol/ min/ml | Changes % | **β-Esterase µg β-Naphthol/** min/ml | Changes % | Acid phosphatese µg phenol/min/ ml | Changes % | Alkalinee phosphatese µg phenol/min/ ml | Changes % | Glutathion s-transferase activity mol / min/ml | Changes % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Early season 2010** | | | | | | | | | | | | |
| Laboratory | 87. 2 ±2.3 | | 52.8±3.2 | | 32.7±8 | | 4.4±0.9 | | 2.6±0.2 | | 12.41±1.2 | |
| Gharbia | 122.7±7.3 | 40.7 | 162.4±7.2 | 207.6 | 59.2±5.3 | 81.0 | 6.8±0.8 a | 54.5 | 0.92±0.1 | -64.6 | 15.21±0.4 | 22.6 |
| Kalubia | 141.7±6.3 | 62.5 | 202.9±11.2 | 284.3 | 71.8±6. 1 a | 119.6 | 7.3±1.0 a | 65.9 | 1.1 ±0.3 | -57.7 | 14.2±0.6 | 14.4 |
| Menofeya | 212.3±9.4 | 143.5 | 225.8±10.2 | 327.7 | 77.9±6.8 a | 138.2 | 8.4±0.3 | 90.9 | 1.4±0.3 | -46.2 | 12.91±0.7 | 4.0 |
| LSD | 10.8 | | 11.3 | | 9.3 | | 0.6 | | 0.1 | | 0.3 | |
| **Late season 2010** | | | | | | | | | | | | |
| Gharbia | 131.2±4.8 | 50.5 | 170.3±4.7 | 222.5 | 57.6±91 | 76.1 | 6.9±1.2 a | 56.8 | 1.31±0.1 a | -49.6 | 17.3±3.1 | 39.4 |
| Kalubia | 118.9±5.6 | 36.4 | 212.4±6.5 a | 302.3 | 79.4±4.3 | 142.8 | 7.1±0.7 a | 61.4 | 1.02±0.12 | -60.8 | 15.8±2.1 | 27.3 |
| Menofeya | 162.7±7.5 | 86.6 | 207.4±5.9 | 292.8 | 96.2±7.9 | 194.2 | 8.7±0.8 | 97.7 | 1.41±0..3 a | -45.8 | 13.61±1.9 | 9.7 |
| LSD | 6.4 | | 9.4 | | 8.4 | | 0.3 | | 0.2 | | 1.3 | |

Table 6: biochemical changes of chlorpyrifos against 4th instar larvae of laboratory and field strains of *S. littoralis* at early and late season 2011.

| | Acetyle choline Esterase µg Acetyl-choline-bromide/min/ml | Changes % | α- Esterase µg α-Naphthol/ min/ml | Changes % | **β-Esterase µg β-Naphthol/** min/ml | Changes % | Acid phosphatese µg phenol/min/ ml | Changes % | Alkalinee phosphatese µg phenol/min/ ml | Changes % | Glutathion s-transferase activity mol / min/ml | Changes % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Early season 2011** | | | | | | | | | | | | |
| Laboratory | 58.2± 4.8 | | 37.7±6.3 | | 4.2±0.6 | | 2.3±0.3 a | | 14.1± 1.9 | | 332± 9.8 | |
| Gharbia | 121.8±6.8 | 109.3 | 82.3±3.4 | 118.3 | 5.7±1.1 | 35.7 | 2.2±0.1 a | -4.3 | 10.3±1.1 a | -27.0 | 242±8.9 | -27.1 |
| Kalubia | 96.7±3.2 a | 66.2 | 88.4±5.1 | 134.5 | 6.6±1.3 | 57.1 | 1.4±0.3 b | -39.1 | 11.4±1.3 a | -19.1 | 270±14.3 | -18.7 |
| Menofeya | 92.3±4.8 a | 58.6 | 107.2±8.9 | 184.4 | 9.81±2.1 | 133.3 | 1.2±0.1 b | -47.8 | 19.3±2.7 | 36.9 | 298±8.3 | -10.2 |
| LSD | 6.9 | | 4.2 | | 0.2 | | 0.3 | | 2.1 | | 9.7 | |
| **Late season 2011** | | | | | | | | | | | | |
| Gharbia | 94.7±6.1 | 62.7 | 77.1±3.2 | 104.5 | 6.4±0.9 a | 45.2 | 1.8±0.2 a | -21.7 | 14.2±1.5 a | 0.7 | 203±4.3 a | -38.9 |
| Kalubia | 80.4±6.6 | 38.1 | 98.3±6.1 | 160.7 | 6.3±1.1 a | 50.0 | 1.6±0.3 a | -30.4 | 12.9±1.6 a | -8.5 | 170±3.6 a | -48.8 |
| Menofeya | 99.8±7.9 | 71.5 | 66.2±5.4 | 75.6 | 7.4±0.3 | 76.2 | 1.4±0.1 a | -39.1 | 17.4±3.5 | 23.4 | 198±3.8 | -40.4 |
| LSD | 4.1 | | 3.9 | | 0.3 | | 0.3 | | 2.3 | | 10.8 | |

Table 7: biochemical changes of cypermethrin against 4th instar larvae of laboratory and field strains of *S. littoralis* at early and late season 2010.

| | Acetyle choline Esterase µg Acetylcholine-bromide/min/ml | Changes % | α- Esterase µg α- Naphthol/ min/ml | Changes % | **β-Esterase µg β-Naphthol/** min/ml | Changes % | Acid phosphatese µg phenol/min/ ml | Changes % | Alkalinee phosphatese µg phenol/min/ ml | Changes % | Glutathion s-transferase activity mol / min/ml | Changes % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Early season 2010** | | | | | | | | | | | | |
| Lab. | 48.8±4.1 | | 37.5±4.2 | | 5.3±0.6 | | 2.8±0.3 | | 10.8±1.2 | | 342±4.3 | |
| Gharbia | 52.9±2.2 | 8.4 | 59.8±6.4 | 59.5 | 6.2±0.6 a | 17.0 | 1.2±0.1 | -57.1 | 13.9±1.4 a | 28.7 | 231±6.2 a | -32.5 |
| Kalubia | 60.4±3.2 | 23.8 | 47.9±2.3 | 27.7 | 6.7±1.1 b | 26.4 | 1.6 ±0.4 a | -42.9 | 14.8±2.6 a | 37.0 | 229±7.3 a | -33.0 |
| Menofeya | 71.2±3.5 | 45.9 | 52.3±4.8 | 39.5 | 6.4±0.4 a b | 20.8 | 18±0.2 a | -35.7 | 12.1±0.7 | 12.0 | 211±7.2 | -38.3 |
| LSD | 2.4 | | 3.1 | | 0.4 | | 0.3 | | 1.1 | | 8.4 | |
| **Late season 2010** | | | | | | | | | | | | |
| Gharbia | 66.7±3.6 | 36.7 | 70.4±2.7 a | 87.7 | 7.3±1.3 | 37.7 | 1.1±0.1 a | -60.7 | 17.4±2.1 | 61.1 | 201±4.9 a | -41.2 |
| Kalubia | 74.8±4.2 | 53.3 | 62.3±4.3 | 66.1 | 9.1±0.6 | 71.7 | 1.3±0.1 a | -53.6 | 16.2±2.7 | 50.0 | 202±4.1 a | -40.9 |
| Menofeya | 89.3±4.8 | 83.0 | 68.9±2.9 a | 83.7 | 8.1±0.9 | 52.8 | 1. 4±0.2 a | -50.0 | 15.1±0.1 | 39.8 | 214±3.4 | -37.4 |
| LSD | 3.9 | | 2.1 | | 0.4 | | 0.3 | | 1.1 | | 8.4 | |

Table 8: biochemical changes of cypermethrin against 4th instar larvae of laboratory and field strains of *S. littoralis* at early and late season 2011.

| | Acetyle choline Esterase µg Acetylcholine-bromide/min/ml | Changes % | α- Esterase µg α-Naphthol/ min/ml | Changes % | **β-Esterase µg β-Naphthol/** min/ml | Changes % | Acid phosphatese µg phenol/min/ ml | Changes % | Alkalinee phosphatese µg phenol/min/ ml | Changes % | Glutathion s-transferase activity mol / min/ml | Changes % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Early season 2011** | | | | | | | | | | | | |
| Laboratory | 41.9±3.6 | | 35.8±3.1 | | 6.3±1.1 | | 2.9.±0.1 a | | 11.9±1.3 a | | 376±12.3 a | |
| Gharbia | 64.2±5.7 a | 53.2 | 67.2±4.2 a | 87.7 | 8.4±0.9 | 33.3 | 2.9±0.3 a | 0.0 | 17.3±1.4 | 45.4 | 251±11.4 | -33.2 |
| Kalubia | 67.8±6.3 a | 61.8 | 70.4±6.7 a | 96.6 | 9.7±1.3 | 54.0 | 2.9±0.4 a | 0.0 | 12.4±2.1 a | 4.2 | 262±7.3 | -30.3 |
| Menofeya | 90.3±4.9 | 115.5 | 59.3±2.8 | 65.6 | 7.7±0.6 | 22.2 | 2.7±0.5 | -6.9 | 15.6±2.3 | 31.1 | 270±4.9 a | -28.2 |
| LSD | 4.5 | | 4.1 | | 0.4 | | 0.1 | | 1.1 | | 6.2 | |
| **Late season 2011** | | | | | | | | | | | | |
| Gharbia | 80.4±4.9 | 91.9 | 72.9±4.9 | 103.6 | 9.2±1.4 a | 46.0 | 1.8±0.1 | -37.9 | 19.7±1 | 65.5 | 198±2.3 | -47.3 |
| Kalubia | 92.3±6.7 | 120.3 | 89.3±6.1 | 149.4 | 10.8±2.3 | 71.4 | 1.1±0.1 a | -62.1 | 18.4±2.4 a | 54.6 | 214±8.1 | -43.1 |
| Menofeya | 111.8±4.6 | 166.8 | 78.4±4.8 | 119.0 | 9.4±1.4 a | 49.2 | 1.3±0.1 a | -55.2 | 17.8±1.9 a | 49.6 | 231±4.6 | -38.6 |
| LSD | 4.6 | | 4.1 | | 0.4 | | 0.3 | | 1.1 | | 6.2 | |

Values in a column followed by the same small letter are not significantly different ($p< 0.05$ ; Duncan's multiple rang test).

In season 2011 the changes in enzyme activity was recorded (109.3, 66.2, 58.6) and (62.7, 38.1, 71.5) for gharbia, kalubia and menofeya, respectively. The enzyme activity in case of cypermethrin treatment exhibited the same trend in the elevation in late season than in early season which record (8.8, 23.8, 45.9) and (36.7, 53.3, 83) for gharbia, kalubia and menofeya, respectively, in season 2010. In season 2011 the changes in enzyme activity was recorded (53.2, 61.8, 115.5) and (91.9, 120.3, 166.8) for gharbia, kalubia and menofeya, respectively.

The data obtained from beta - esterase were similar to that obtained from –alpha-esterase, in general the activity of field strain early and late season at all tested insecticides during 2010 and 2011 cotton growing seasons. In case of Chlorpyrifos enzyme activity recorded (81, 119.6, 138.2) and (76.1,142.8,198.4) for gharbia, kalubia and menofeya, respectively, in season 2010. In season 2011 the changes in enzyme activity was recorded (118.3, 34.5, 184.4) and (104.5, 160.7, 75.) for gharbia, kalubia and menofeya, respectively. In case of cypermethrin treatment changes in activity were (59.5, 27.7, 39.5) and (87.7, 66.1, 83.7) for gharbia, kalubia and menofeya, respectively, in season 2010. In season 2011 the changes in enzyme activity was recorded (87.7, 96.6, 65.6) and (103.6, 149.4, 119) for gharbia, kalubia and menofeya, respectively.

**Acetylcholinesterase (AChE)**

Tables (5, 6, 7 and 8) refer the changes in AChE F-strains had high level of AChE than L-strain, before spraying season and the greatest level of enzyme activity was recorded for menofeya strain (143.5) in case of chlorpyrifos ,while, the lowest level was found in case of cypermethrin (13.9). After spraying season, the highest level of the enzyme activity was found in menofeya strain (86.8) in case of chlorpyrifos, while, the lowest enzyme activity was recorded for kalubia strain (22.6) in season 2010. Generally, menofeya strain showed the highest level of AChE activity, while, the lowest level of the enzyme activity was recorded for kalubia strain in season 2010.

As for 2011 season, the highest level of AChE was found in gharbia strain before spraying season (109.3) in case of chlorpyrifos and the lowest level was found in kalubia (19.1) in case of cypermethrin. After spraying season however, the highest
level of enzyme activity was recorded for menofeya strain (71.5 and 83.4) for chlorpyrifos and cypermethrin ,respectively, in season 2011. Generally, the obtained data of esterases activities showed that all of the tested field strains showed higher levels of esterase than lab. strain. Therefore, it was concluded that the increase in esterase activity plays an important role in resistance development. Elevated esterase activity in field population was also detected by Srinivas *et al.* (2003) who found that field strain of *Helicoverpa armigera* larvae was characterized by high activities of esterases and phosphatases when compared with susceptible larvae. Abd El-Mageed *et al.,* (2011) demonstrated that The efficiency and residual effects of five new insecticide mixtures chlorosan, feroban, cygron, engeo, and kingbo were affect the biochemical analysis with pronounced changes in acetyl cholinesterase and phenol oxidase . Moreover, Tiancai *et al,.* (2011) reported that Beet armyworm, *Spodoptera exigua* develop resistance to many broad-spectrum insecticides at which moderate resistant level was discovered in 8 of 18 field populations, field population might have specific biochemical mechanisms for tolerance. Artificial selection in laboratory with chlorantraniliprole was carried out, 23 generations of continuous selections resulted in 11.8-fold increase in resistance to chlorantraniliprole, and 3.0-fold and 3.7-fold increases in mixed function oxidase and esterase, respectively. Compared with the susceptible strain kept in laboratory the selection strain had developed 128.6-fold resistance to this insecticide.

**Phosphatases enzyme activities**

**Acid phosphatase (AcP) activity**

Tables (5, 6, 7 and 8) refer the changes in acid phosphatase (AcP) activity. In menofeya strain at early cotton season 2010 showed high level of AcP activity (90.9) while the lowest level was found in Gharbia (54.5). At the end of cotton season, the highest level was recorded in menofeya strain (97.7) and the lowest level was recorded for Gharbia strain (56.8) in case of chlorpyrifos.While, in case of cypermethrin the lowest level of activity was recorded in gharbia (17) early season and the highest level was recorded for kalubia (71.7) for late season. Enzyme activity exhibited the same trend in early and late season 2011 in case of chlorpyrifos, while, kalubia governrate recorded the highest level of activity (54 and 71.4) at early and late season 2011, respectively. Farag (1978) indicated that acid phosphatase increased with the development of resistance in OP-resistant strain, while, alkaline phosphatase showed a slight decrease in resistant rather than the susceptible strain. The data for phosphatases studies indicated that, field population of cotton leafworm, had high level of AcP activity and low level of AlkP activity comparing L-strain. The same findings were obtained by Shakoori *et al.* (1994) who showed (in laboratory studies) that the adults of a malathion-resistant Pakistani (PAK) strain of *Tribolium castaneum* had more active acid phosphatase as compared with those of an organophosphorus-susceptible (FSS-II) strain. Srinivas *et al.* (2003) mentioned that, the high level of resistance detected in the field pests could be due to higher levels of both esterases and phosphatases.

**Alkaline phosphatase (AlkP) activity**

On contrarily, sever decrease in activity was noticed in the alkaline phosphatase activity (AlkP) in case of chlorpyrifos early season 2010 showed that the highest decrease in activity was recorded by gharbia (- 64.6) and the

highest decrease happened in late season was recorded by kalubia (- 60.8) , on the other hand, menofeya recorded the highest decrease in such activity by (- 47.8 and – 39.1) early and late season 2011,respectively. In case of cypermethrin gharbia recorded the highest decrease in activity (-57.1 and - 60.7) in early and late 2010 season, respectively, whereas, kalubia recorded the most decrease in activity by about (-62.1) in late 2011 season.

**Activity of Glutathione S-transferase (GST's)**

Tables (5, 6, 7 and 8) refer the changes in glutathione S-transferase (GST's) of *S. littoralis* L-strain and F-strains. The data showed that, the activity of GST's was greater in field strains than in L-strain with exception gharbia and kalubia early spraying season and kalubia late spreaing season 2011. Also the enzyme activity was greater after spraying season than before spraying season except in menofeya governorate at which the activity were (19.3 and 17.4) for early and late season 2011, respectively, in case of chlorpyrifos. Moreover, (Clark, 1990) reported that detoxifying enzyme assays revealed that activities of Estrases and Glutathion s-transferases were high in resistant field populations. Then, the high resistance to pyrethroids and OP should be explained by high activity of GST. Kim *et al.,* (1998) reported that field populations of the tobacco cutworm, *Spodoptera litura* (Fabricius), showed resistance to cypermethrin and to organophosphorus insecticides chlorpyrifos, Detoxifying enzyme assays revealed that esterase and glutathione S-transferase activities were varied from 2- to 6-fold among the field populations. These results indicated that the broad-spectrum of insecticide resistance observed in the field populations may due to some resistance mechanisms, including increased detoxification of these insecticides. Shankarganesh *et al.,* (2012) reported that resistance in *Spodoptera litura* (Fabricius) has been attributed to enhanced detoxification of insecticides by increased levels of esterases, oxidases and/or glutathione S-transferases.

It was clear that there was a difference in Estrases, phosphatases and Glutathion s- transferases sensitivity to insecticides depending on resistance level of the tested insect through the different governorates during the 2010 and 2011 cotton seasons. These results clearly demonstrated that the field populations of *S. littoralis* developed resistance to pyrethroid and organophosphates. It also showed that there were variations in insecticide resistance among field populations according to the insecticides. Thus, populations from different governorates displayed varied tolerance or resistance level to the tested OP and pyrethroid compounds.

## REFERENCE

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265 - 267.

Abd El-Mageed A. E. M. and Shalaby S. E .M. (2011). Toxicity and biochemical impacts of some new insecticide mixtures on cotton leafworm, *Spodoptera littoralis* (Boisd.). Plant Protect. Sci., 47: 166-175.

Armes, N. J.; J. A. Wightman; D. R. Jadhav and G. V. R. Rao (1997). Status of insecticide resistance in *Spodoptera litura* in Andhra Pradesh India. Pesticide-Science., 50: 3, 240-248.

Clark, AG. (1990). The glutathione S-transferases and resistance to insecticides. pp. 369-378, In Glutathione S- ransferases and drug resistance. Eds. J.D. Hayes, CB. Pickett and T.J. Mantle. Taylor and Francis, London.

Crowder, L. A.; M. J. Tolleston and T. F. Watson (1979).Dosage mortality

studies of synthetic pyrethroids and methyl parathion on the tobacco budworm, in Central Arizona.J. Econ. Entomol., 72: 1- 3.

Duncan, D. B. (1955). Multiple range and multiple F-test. Boimetrics, 11 (1): 1-24.

El-Bermawy, Z. A.; A. A. El-Sheikh; M. H. Rashwan and H. S. A. Radwan (1992). Pyrethroids resistance in *Spodoptera littoralis* (boisd.) (Lepidoptera: Noctuidae) in lower Egypt.Bull. Entomol. Soc. Egypt, Econ. Ser., 19: 41- 51.

El-Guindy, M. A.; S. M. Madi; M. E. Keddis; Y. H. Issa and M. M. Abdel-Sattar (1982). Development of resistance to pyrethroids in field populations of the Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.). Int. Pest. Control, 24: 6-10.

El-Sayed, G. N. (1973). Rate of development of resistance in the Egyptian cotton leafworm, against insecticides. Ph.D. Thesis, Vac. Agric. Cairo Univ. Egypt.

El-Sayed, G. N.; El-Guindy, M. A.; Madi, S. M.; Dogheim, S. M. A. and Moawad, G. M.; (1984). Geographical distribution of organophosphorus insecticides- resistant strains of the cotton leafworm, Spodoptera littoralis (Boisd.), in the Nile-Delta. Bull. ent. Soc. Egypt,.Ser., 12: 71-82.

Farag, M. (1978). Development of resistance in the Egyptian cotton leafworm, *Spodoptera littoralis* and its relation to some biochemical changes in insect body. M. Sc. Thesis, Fac. Agric., Al-Azhar Univ., Egypt.

Farag, M. A. (2001). Biochemical studies on the effect of some insect growth regulator on the cotton leafworm. M.Sc.Thesis,Fac.Agric.,Cairo Univ., Egypt(155Pp).

Finney, D.J. (1971). Probit analysis. A Statistical Treatment of the Sigmoid Response Curve. 7th Ed., Cambridge Univ. Press, England.

Gunning, R. V.; G. Moores and A. L. Devonshire (1999). Esterase inhibitors synergise the toxicity of pyrethroids in Australian *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). Pest. Biochem. Physiol., 63(1): 50-62

Gunning, R. V.; G. Moores and A. L. Devonshire (1996). Insensitive acetylcholinesterase and resistance to thiodicarb in Australian *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae). Pest. Biochem. Physiol., 55: 1, 21-28.

Habig, W. H.; M. J. Pabst and W. B. Jakoby (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biolo. Chem., 249 (22): 7130- 7139.

Hassan, M. H; Maher Ali, A. and El-Sayed, M. S. (1970). Development of tolerance of the Egyptian cotton leafworm *Spodoptera littoralis* to currently applied insecticides. Agric. Res. Cairo 48: 1-11.

Kansouh, A. S.; Shaaban, A. M. and Sobeiha, A. M. K. (1978-1979). Penetration of Nuvacron and Phosvel in larvae of different strains of *Spodoptera littoralis* (Boisd.). Bull. Ent. Soc. Egypt, Econ. Ser., 11: 7-9.

Kim, Y. G.; J. R. Cho; J. N. Lee; S. Y. Kang; S. C. Han; K. J. Hong; H. S. Kim; J. K. Yoo and J. O. Lee (1998). Insecticide resistance in the tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). J. Asia Pacific Entomol., 1 (1): 115-122.

Powell, M. E. A. and M. J. H. Smith (1954). The determination of serum acid and alkaline phosphatases activity with 4-amino antipyrine. J. Clin. Pathol., 7: 245- 248.

Rashwan, M. H.; El-Bermawy, Z. A.; El-Sheikh, A. A. and Radwan, H. S. A. (1991-1992). The onset of organophosphate and carbamate resistance among lower Egypt populations of the cotton leafworm, *Spodoptera littoralis* (boisd.). Bull.

ent. Soc. Egypt, Econ. Ser., 19: 211- 220

Shakoori, A. R.; M. Z. Malik and M. A. Saleem (1994). Toxicity of cyhalothrin (Karate) to sixth instar larvae of Pakistani strain of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Pakistan J. Zool., 26 (1): 57- 64.

Shankarganesh, K., Suresh Walia, Swaran Dhingra, B. Subrahmanyam, S. Ramesh Babu (2012). Effect of dihydrodillapiole on pyrethroid resistance associated esterase inhibitionin an Indian population of *Spodoptera litura* (Fabricius) Pesticide Biochemistry and Physiology 102: 86–90

Simpson, D. R.; D. L. Bull and D. A. Lindquist (1964). A semimicro technique for the estimation of cholinesterase activity in bull weevil. Ann. Ent. Soc. Am., 57 (3): 367- 377.

Srinivas, R.; S. S. Udikeri; S. K. Jayalakshmi and K. Sreeramulu (2003). Identification of factors responsible for insecticide resistance in *Helicoverpa armigera* (Hubner). Resistant Pest Management Newsletter. 13 (1): 59- 65.

Tiancai, Jia Li, Jianya Su (2011). Monitoring of beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae) resistance to chlorantraniliprole in China Pesticide Biochemistry and Physiology 101:198–205.

Twin, P. H. and H. T. Reynolds (1980). Relative susceptibility and resistance of the tobacco budworm to methyl parathion and synthetic pyrethroids in Southern California.J. Econ. Entomol., 73: 239- 242.

Van Asperen, K. (1962). A study of housefly esterase by means of sensitive colourimetric method. J. Insect Physiol., 8: 401- 416.

XianChun Li; W. YinCbang; Z. QianSong; Y. GanJun;Z. DinYang; Y. VanTao; Z. Zhi; Z. JianPing; L. ShouShan; C. CaiXia and D. ShiYin (1997). Insecticide resistance in field strain of *Pictinophora gossypilla* (Saun.) in china and effect of synergists on deltamethrin and parathion methyl activity. Pestic. Sci., 50(3): 183-186.

## ARABIC SUMMARY

**مقارنة السمية والتأثيرات البيوكيميائية للمركبات الفوسفات العضوية والبيروثرويدات على كل من السلالة المعملية والحقلية لدودة ورق القطن سبودبترا ليتورالس.**

**طارق الشيخ**

معهد بحوث وقاية النباتات – مركز البحوث الزراعية

اجريت الدراسة الحالية لتقصي صفة المقاومة لمجموعتين من المبيدات الحشرية وهي مجموعة المركبات الفوسفورية ومنها مركب الدورسبان (الكلوربيريفوس) ومجموعة المركبات البيريثرودية ومنها مركب السيبر (سايبرميثرين) تجاة دودة ورق القطن في السلالة المعملية والسلالة الحقلية وذلك في ثلاث محافظات هي الغربية والقليوبية والمنوفية في موسم القطن قبل الرش وبعد الرش هذا ومن خلال تحديد التركيز القاتل للنصف لتلك المركبات تبين ان المركب البيريثرويدي سيبر اكثر سمية من المركب الفسفوري الدورسبان . كذلك تمت دراسة تطور صفة المقاومة لتلك المركبات وذلك عن طريق قياس مستوي نشاط انزيمات الاستيريزيس والجلوتاسيون-اس- ترانسفيراز والفوسفاتيزيز . اوضحت النتائج زيادة معنوية في مستوي صفة المقاومة في السلالات الحقلية عنها في السلالة المعملية وذلك من خلال وجود زيادة معنوية في نشاط انزيمات الاستيريزس والجلوتاسيون-اس- ترانسفيراز هذا فضلا عن وجود زيادة معنوية في انزيم الفوسفاتيز الحامضي ونقص معنوي في انزيم الفوسفاتيز القلوي موازي لنشوء صفة المقاومة لدودة ورق القطن تجاة المركبات المستخدمة.