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# SUSCEPTIBILITY OF *SPODOPTERA LITTORALIS* (BOISD) TO SOME ANTICHOLINESTERASE INSECTICIDES IN RELATION TO SOME ENZYME ACTIVITES

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## ABSTRACT

Fourth instar larvae of *Spodoptera littoralis* (Boisd) of susceptible, Behera, Dokahlia, Gharbeia, Kafr-El-Sheikh, Sharkeia and Fayoum, Governorates strains were tested in the laboratory. Tests were carried out using dipping technique with three organophosphate and three carbamate insecticides. Treatments were done before and after spraying programs in the fields of these areas. Summerized data in general reported that the results of $LC_{50}$ levels after spraying programs were higher than those before spraying.

With regard to the activity of esterases of $4^{th}$ instar larvae of *S. littoralis* for each Governorate at Mg/min/larva or Mg/min/gram, it was clear that the activity increased gradually with increasing the levels of resistance. On the other hand, a positive correlation was obvious between resistance values and esterases activity.

## INTRODUCTION

The Egyptian cotton plant is liable to be attack by many pests, causing great loss in the crop. The cotton leafworm is one of the most important insect pests in Egypt. The dense use of insecticides had led to the development of resistance against their toxic action. (Keddis *et al.,* 1988).

The toxic effects of organophosphates and carbamates to mammals and insects are due to inhibition of acetylcholinesterase and enzyme vital for nerve function (Casida 1963). Carboxylesterases (alpha and beta esterasese) are ubiquitous nonspecific enzymes that hydrolyze a diversity of esters of carboxylic acids. These enzymes are implicated in many indogenous functions in insects, one of them is the metabolism of insecticides (Ahmed and Forgash, 1976).The presence of an aliphatic esterase (AliE) in the thorax of house flies, which was distinct from the ChE found in the heads. Although the Ali was inhibited *in vivo* by DDVP at the same concentration as ChE. I*n vivo* studies showed that the thoraxic Ali was found to be inhibited faster and to agreeter degree than ChE by the same agent. (Van Asperen, 1958).

The present investigation aims to study the effect of three OP's and 3 carbamates commercial insecticides on $4^{th}$ instar larvae of different Governorates and some esterases enzyme activities.

## MATERIALS AND METHODS

**Strains of *Spodoptera littoralis* (Boisd)**

The susceptible strain supplied by the Central Agric. Pesticides Lab., A. R. C., which was previously collected from cotton fields of Fayoum

Governorate in 1968. Other six strains were collected from Behera, Dakahlia, Gharbia, Kafr-El-Sheikh, Sharkeia and Fayoum Governorates. Egg masses samples were collected from the fields before and after spraying programs, during the cotton season's of (1998). All strains were reared as described by El Defrawi *et al.* (1964) in conditioned room (25±2 ºC and 65±5 % R.H.)

Insecticides treatment

Six formulated insecticides, three organophosphorus compound namely; Chlorpyrifos, Fenitrothion and profenofos and three carbamate compounds methomyl, alanycarb and thiodicarb were used in the present study.

**Toxicological tests**

Toxicological studies of the tested insecticides against 4th instar larvae of *Spodoptera littoralis* (Boisd) were carried out using leaf-dipping technique. Several concentration dissolved in water were prepared for each insecticide. Castor bean leaves were dipped for 30 seconds in each concentration then left for 2 hr. under room temperature to dry before being offered to the 4th instar larvae. Five replicates, ten larvae for each were used for each concentration. Larvae were fed for 24 hr. on the treated leaves. Survived larvae were transferred to fresh untreated leaves to determine the activity of esterases enzyme. The untreated larvae (control) were maintained under similar conditions. Mortality counts were determined after 24 hr. and dead larvae were removed. The observed percentage mortalities were estimated and corrected by Abbott's formula (Abbott 1925), while the $LC_{50}$ and slope values estimated according to the method of Busvine (1957). The rate of development of resistance was estimated as resistant ratios (RR), based on $LC_{50}$ of the field strain compared with their corresponding values of the susceptible strain.

**Biochemical study**

Larvae of field strain which collected as egg-masses after spraying program and susceptible strains were starved for Ca 4 hr. before homogenization in distilled water (5 larvae/ml). The homogenate of each sample was centrifuged for 15 minutes at 12.000 r.p.m. at 2 ºC and the supernatent was used for enzyme assay.

The activity of alpha-esterase (alph-E) and beta-esterase (beta-E) was determined according to the method described by Van Asperen (1962) using alpha- or beta naphthyl acetate as substrates. Diaroblue sodium Lauryl sulphate reagent was used for the estimation of the naphthols produced. With this reagent alpha-naphthol gives a strong blue colour (maximum absorption at 600 mu.) and beta-naphthol gives a strong red colour (maximum absorption at 555 mu).

The reagents used for colourimetric assay of cholinesterase (ChE) and aliphatic esterases (AliE) activity were the same as those described by Simpson *et al.* (1964) except that 0.5 ml. of acetylcholine bromide at $2.5 \times 10^{-3}$ M and 0.5 ml. of methyl n-butyrate( Meb)at $4 \times 10^{-3}$ M. were used as substrates, respectively. The hydroxylamine, sodium hydroxide, hydrochloric acid, and ferric chloride reagents were used at the same molar concentrations. All colourimetric determinations were based on a minimum of 4 replications for each sample, and all homogenates were incubated with the substrates at 37 ºC.

# RESULTS AND DISCUSION

**Toxicological study**

The purpose of the present investigation was to estimate the potency of three OP's and three carbamates insecticides in relation to the management of resistance developmenmt in *Spodoptera littoralis* (Boisd) of field population. Toxicological data on 4th instar larvae obtained as egg-masses from six governorates before and after spraying programs are summarized in Table (1). It is clear that chlorpyrifos was the most potent compound before spraying program in Sharkeia (RR 15.81 fold), followed by Dakahleia (RR 23.49 fold), Fayoum (25.90 fold), and Behera (26.80 fold). The lowest effect was recorded at Gharbeia (51.60 fold) and Kaftr-El-Sheikh (32.80 fold). On the other hand, the same behaviour of chlorpyrifos was observed after spraying programs. The two exceptions which rised up suddenly to become 14.60 and 16.2 fold were recorded at Fayoum and Kafr-El-Sheikh, respectively. In other word, chlorpyrifos was more active in sharkeia and Dakahleia before spraying programs and also in Fayoum and Kafr-El-Sheikh after spraying programs of the cotton fields. Resistance strains of *Spodoptera littoralis* (Boisd) was recorded in Gharbeia Governorate before and after spraying programs. Resistance ratios were 51.60 fold before and 90.10 fold after spraying programs of the cotton fields.

The other OP's insecticide fenitrothion was investigated also on the same assays in laboratory. Fenitrothion was the most potent insecticide in Gharbeia (10.95 fold), followed by Fayoum (11.10 fold) Moderate levels were recorded at Kafr-El-Sheikh (14.5 fold), Behera (26.03 fold) and Sharkeia (18.30 fold). The poor effect was recorded at Dakahleia strain (26.30 fold) before spraying program in all tested cases. On the other hand, and after spraying programs in cotton fields, Kafr-El-Sheikh alternate its position to the top, followed by Fayoum Gharbeia, Dakahleia and Behera,recording resistance ratios of 3.60, followed by 10.70, 13.10, 15.70 and 17.60 folds, respectively. Moderate level of resistance was observed in Sharkeia strain with a level of 31.10 fold against fenitrothion after spraying programs. In conclusion, Gharbeia, Kafr-El-Sheikh and Fayoum strains were more affected by fenitrothion in both treatments. The other governorates strains showed low fitness against fenitrothion as insecticide.

As for profenofos a slight effect was recorded in all tests during cotton growing season.Larvae of Dakahleia strain was the only larvae that tolerate this compound,recording 21.87 fold before and 39.35 fold after spraying programs, respectively. Moderate levels of resistance were estimated in all tests areas, ranged between 35.70 – 94.40 fold of resistance before and after spraying program.

Data in Table (1) indicate that fenitrothion showed the most potent effect followed by chlorpyrifos while profenfos came at the end with the lowest efficacy among the three OP's tested compounds..

**Table (1) : Cross-resistance pattern to insecticides in feild strains of *S littoralis* collected from some Governorate during 1998.**

| | Early Season | | | Late Season | | |
|---|---|---|---|---|---|---|
| | **Slope** | **LC50** | **R.R.** | **Slope** | **LC50** | **R.R.** |
| Chlorpyrifos | | | | | | |
| S.Strain | 4.06 | 2.65 | ------- | 4.06 | 2.65 | ------- |
| Behera | 2.19 | 71.05 | 26.80 | 3.23 | 140.10 | 52.90 |
| Dakahleia | 3.45 | 62.00 | 23.49 | 2.87 | 91.93 | 34.70 |
| Gharbia | 1.49 | 136.82 | 51.60 | 2.48 | 238.78 | 90.10 |
| Kafr El-Shekh | 2.86 | 86.95 | 32.80 | 1.93 | 42.82 | 16.20 |
| SHarkeia | 2.34 | 41.90 | 15.81 | 2.92 | 96.46 | 33.73 |
| Fayoum | 2.11 | 68.62 | 25.90 | 1.67 | 38.60 | 14.60 |
| **Fenitrothion** | | | | | | |
| S.Strain | 3.09 | 155.25 | ------- | 3.09 | 155.25 | ------- |
| Behera | 3.84 | 2489.31 | 16.03 | 2.33 | 2728.13 | 17.60 |
| Dakahleia | 2.62 | 4078.63 | 26.30 | 2.38 | 2433.01 | 15.70 |
| Gharbia | 2.58 | 1700.72 | 10.95 | 2.58 | 2024.78 | 13.10 |
| Kafr El-Shekh | 1.84 | 2251.65 | 14.50 | 2.66 | 552.58 | 3.60 |
| Sharkeia | 3.19 | 2843.90 | 18.30 | 3.08 | 4827.93 | 31.10 |
| Fayoum | 2.46 | 1723.27 | 11.10 | 2.56 | 1659.84 | 10.70 |
| **Profenofos** | | | | | | |
| S.Strain | 5.29 | 10.55 | ------- | 5.29 | 10.55 | ------- |
| Behera | 2.77 | 543.03 | 51.41 | 2.69 | 918.65 | 87.08 |
| Dakahleia | 2.40 | 230.70 | 21.87 | 2.30 | 415.19 | 39.35 |
| Gharbia | 1.78 | 693.28 | 65.70 | 2.94 | 995.54 | 94.40 |
| Kafr El-Shekh | 2.22 | 694.33 | 65.80 | 2.85 | 901.14 | 85.40 |
| SHarkeia | 2.11 | 411.68 | 39.00 | 1.89 | 820.25 | 77.70 |
| Fayoum | 2.54 | 376.37 | 35.70 | 2.73 | 743.12 | 70.40 |
| **Methomyle** | | | | | | |
| S.Strain | 3.03 | 11.41 | ------ | 3.03 | 11.41 | ------ |
| Behera | 2.58 | 253.05 | 22.20 | 3.77 | 1183.93 | 103.80 |
| Dakahleia | 2.08 | 892.88 | 78.30 | 1.75 | 954.40 | 83.64 |
| Gharbia | 1.80 | 372.45 | 32.60 | 2.28 | 677.53 | 59.04 |
| Kafr El-Shekh | 1.42 | 146.64 | 12.90 | 2.06 | 291.76 | 25.60 |
| SHarkeia | 1.90 | 411.78 | 36.10 | 4.00 | 988.35 | 86.60 |
| Fayoum | 2.73 | 456.71 | 40.00 | 2.77 | 639.18 | 56.00 |
| **Alanycarb** | | | | | | |
| S.Strain | 3.48 | 42.39 | ------ | 3.48 | 42.39 | ------ |
| Behera | 1.05 | 4910.37 | 115.80 | 3.11 | 8431.23 | 198.90 |
| Dakahleia | 1.86 | 2526.12 | 59.60 | 1.86 | 3368.57 | 79.50 |
| Gharbia | 1.27 | 3643.14 | 85.90 | 3.19 | 4814.66 | 113.60 |
| Kafr El-Shekh | 1.14 | 5856.75 | 138.40 | 1.94 | 6822.34 | 160.90 |
| SHarkeia | 2.16 | 1270.30 | 30.00 | 2.82 | 2788.92 | 65.80 |
| Fayoum | 2.12 | 520.36 | 12.30 | 3.15 | 3747.39 | 88.40 |
| **Thiodicarb** | | | | | | |
| S.Strain | 3.67 | 24.85 | ------- | 3.67 | 24.85 | ------- |
| Behera | 1.13 | 1451.49 | 62.00 | 2.14 | 1382.88 | 55.60 |
| Dakahleia | 2.23 | 1023.57 | 41.20 | 2.20 | 1169.52 | 47.10 |
| Gharbia | 1.50 | 302.13 | 12.20 | 2.53 | 1439.06 | 57.90 |
| Kafr El-Shekh | 1.65 | 502.26 | 20.20 | 1.80 | 1148.52 | 46.20 |
| Sharkeia | 1.08 | 288.65 | 11.60 | 3.49 | 1778.21 | 71.60 |
| Fayoum | 1.94 | 702.54 | 28.30 | 2.98 | 1179.25 | 47.50 |

Toxicological studies of the tested carbamate insecticides against 4th instar larvae of *S. littoralis* were carried out also using leaf dipping technique Clear effect was recorded by thiodicarb in Sharkeia, Gharbeia, Kafr-El-Sheikh and Fayoum before field treatments. Resistance ratios were 11.60, 12.20, 20.20 and 28.30 folds, respectively. Resistance ratios rose up to become 62 and 41.20 folds for Behera and Dakahleia governorates, respectively.

On the other hand, thiodicarb turned its position from the top before spraying to the bottom after spraying program in Sharkeia strain. Resistance ratio in Sharkeia strain before and after spraying programs was 11.60 and 71.60 fold respectively. The potent effect of thiodicarb was observed in Kafr-El-Sheikh (46.20 fold) post treatments. Moderately levels of resistance were cleare in the other tested governorates. RR levels ranged between 47.10 – 71.6 folds for Dakahleia and Sharkeia, respectively.

The performance of the carbamate methomyl agreed with thiodicarb in the insecticidal activity in Kafr El-Sheikh pre and post spraying program. Resistance ratio of methomyl pre and post spraying program was 12.9 and 25.60 fold for Kafrt El-Sheikh strain. Methomyl was moderately in Behera early in the season (22.20 fold), but lost its capacity as insecticide when tested after spraying programs (103.80 fold).

Moderately levels of resistance to methomyl, ranged between 32.60 to 78.30 fold for Gharbeia and Dakahleia before spray program. The same behaviour was done after spraying program with a level ranging between 56 to 103.80 folds for Fayoum and Behera. In this respect Methomyl agreed with thiodicarb for its recommendations. At least the carbamate alanycarb exhibited slightly to lowest effect in all treatments. Before spraying program Fayoum strain exhibited a moderate levels (12.30 fold) of resistance. Moderate to high levels of resistance were observed in the results of this insecticides. Generally, before spraying program, results of resistance ratios ranged between 12.30 to 138.40 folds for fayoum and Kafr-El-Sheikh. On the other hand, and after spraying program results as resistance ratios ranged between 65.8 to 198.90 folds for Sharkeia to Behera.Acordingly, Sharkeia was the only governorate in which may be using this compound in recommendation.

These findings are in agreement with, Watve *et al.* (1977) who reported that the Banded wing whitefly. *Trialeurodes abutilonea* had developed up to 78 fold and 6 fold resistance to methyl parathion and monochrotophos in certain areas of Louisiana. A high level of resistance to permethrin (>4000 fold) had been induced in larvae of Southern house mosquito (Priester & Georghiou, 1978). Kansouh *et al.* (1978-1979) reported a 102 fold resistance to novacron in *S. littoralis* field strain collected from Menia Governorate. Mohanna *et al.*(1996) mentioned that methomyl exhibited 4.2 fold of tolerance in *S. littoralis* , 4th instar larvae of Dakahleia strain. Mohanna and Allam (1999) found that fenvalerate in Kafr-El-Sheikh Governorate exhibited 4.2 fold of *S. littoralis*. Also Qalyubia strtain of *S. littoralis* exhibited 2.8 fold against profenfos (Mohanna, 1998a) Cypermethrin exhibited 4 fold of tolerance in Dakahleia strain when experimented on 4th instar larvae of *S. littoralis*, (Mohanna, 1998b).

**Biochemical study**

Enzyme activities in 4th instar larvae of *Spodoptera littoralis* (Boisd) different strains having various types of susceptibility are summarized in Table (2). The changes in level and truly esterases activity were obvious as a result of continuous selection with insecticides in all Governorates. Regarding to the total protein in Mg/larva, susceptible strain showed a moderate level followed by Kafr-El-Sheikh , Sharkeia, and Behera. The values in Mg/larvae were 0.636, 0.487, 0.486 and 0.407 respectively. Fayoum, Gharbeia and Dakahleia exhibited slight changes ranged between 0.396-0.290 Mg/larvae. The determination of alpha esterase in susceptible 4th instar larvae of *S. littoralis* exhibited low level of (12.653 Mg/min/larvae) follwed by Dakahleia (14.286), Kafr-El-Sheikh (16.803) and Gharbeia (16.961) Mg/min/larva respectively. On the other hand, Sharkeia exhibited a moderate level of alpha-esterase and Fayoum and Behera came next, alpha-values were, 24.104, 23.174 and 22.834 Mg/min/larvae respectively. Regarding Beta esterase, Behera recorded a moderate level of (37.236 Mg/min/larva), Fayoum, Kafr-El-Sheikh and Sharkeia exhibited slight change in the level of B-Es, ranged between 26.742-24.065 Mg/min/larva.

Specific cholenesterase enzyme was clear in the population of Kafr-El-Sheikh, Fayoum Behera and Sharkeia. Activity of Ch-E as Mg/min/larvae were 2.173, 2.109, 2.073, and 2.033 respectively. Susceptible strain was higher in Ch-E activity than Gharbeia and Dakahleia where activity values were 1.852, 1.668 and 1.6126 Mg/min/larva, respectively. The same behaviour was almostly recorded for Ali esterases. The values of Ali-E.activity in ascending order were 2.001, 1.912, 1.445, 1.178, 1.089, 0.567 and 0.502 Mg/min/larvae for Sharkeia, Kafr-El-Sheikh, Fayioum, Behera Gharbeia, S.strain and Dakahleia, respectively. Results in general conception refers to that , a positive correlation between resistance ratio and activity of enzymes. These reaults are in agree with the data which obtained by Abo El Ghar *et al.* (1984) who reported that esterases play an important role in pyrethoid resistance as well as organophosphaste resistant strain of *S. littoralis* Green *et al.* (1990) reported that esterases play a major role in resistance to organophospotas, and certain cases might also contribute resistance toward carbamates and certain pyrethroids. Rafael (1990) mentioned that profenfos resistant strain had a higher esterase activity than the susceptible one. $LD_{50}$'s for organophostaes were positively correlated with mean esterase activity. Also, our results supports those of Mohanna *et al.* (1996) and Mohanna (1999, and 1999b), Mohanna & Alam (1999).

**Table (2) :The activities of alfa-esterase , beta- esterase, choline esteras, ali-esterase and total protein in the haemolymph of 4*th* larval instar of susceptible and field strains of *S. littoralis* collected from some Governorate during 1998 in late season.**

| Activity | | S.strain | Behera | Dakahleia | Garbia | Kafr El-Shekh | Shar-Keia | Fayoum |
|---|---|---|---|---|---|---|---|---|
| Total Protein | Mg/larva | 0.636 | 0.407 | 0.290 | 0.345 | 0.487 | 0.486 | 0.396 |
| | Mg/min/larva | 27.691 | 13.432 | 14.867 | 13.710 | 13.379 | 13.028 | 13.703 |
| α- Esterase | Mg/larva | 12.653 | 22.834 | 14.286 | 16.961 | 16.803 | 24.104 | 23.174 |
| | Mg/min/larva | 551.087 | 754.403 | 731.844 | 674.245 | 461.736 | 646.258 | 801.717 |
| β -Esterase | Mg/larva | 9.032 | 37.236 | 14.864 | 16.695 | 25.173 | 24.065 | 26.742 |
| | Mg/min/larva | 393.379 | 1230.201 | 761.451 | 663.640 | 691.745 | 645.202 | 925.138 |
| Choline-Esterase | Mg/larva | 1.852 | 2.073 | 1.616 | 1.668 | 2.173 | 2.033 | 2.109 |
| | Mg/min/larva | 80.677 | 68.483 | 82.776 | 66.303 | 59.717 | 54.501 | 72.959 |
| Ali-Esterase | Mg/larva | 0.567 | 1.178 | 0.502 | 1.089 | 1.912 | 2.001 | 1.445 |
| | Mg/min/larva | 25.558 | 38.921 | 25.735 | 43.296 | 52.531 | 53.636 | 49.983 |

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## حساسية دودة ورق القطن لبعض المبيدات المستعملة كمضادات للكولين استيريز مع تقدير بعض الأنشطة الإنزيمية

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تم إختبار العمر اليرقى الرابع فى دودة ورق القطن لكل من السلالة الحساسة كمقارنة وسلالة كل محافظة من المحافظات التالية البحيرة ، الدقهلية ، الغربية ، كفر الشيخ ، الشرقية والفيوم فالمعمل بطريقة الغمر . أستخدمت فى الدراسة ثلاث مبيدات فوسفورية هى الكلوربيريفوس والفينتروثيون والبروفينوفوس وأيضا ثلاث مبيدات كرباميتية هى الميثومايل ، الالانيكارب والثيوكارب . تم إجراء الإختبارات قبل بداية موسم الرش الحقلى وبعده فى المناطق المذكورة . اوضحت النتائج فى مجملها أن قيم الجرعة النصف مميتة LC50 المقدرة بعد الرش كانت اكبر منها قبل الرش لنفس الموسم .

أما بالنسبة للانشطة الانزيمية المقدرة فقد أظهرت الإستريزات أنه كلما زادت قيمة الجرعة النصف مميتة بصفة عامة زاد النشاط الإنزيمى لها .كما أظهرت النتائج علاقة موجبة بين نشاط الإنزيمات والمقاومة للمبيدات موضع الدراسة.