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# STUDIES ON CHANGES IN RESPONSE OF *SPODOPTERA LITTORALIS* (BOISD) FOR SOME INSECTICIDES AND THEIR RELATION WITH SOME ENZYMES ACTIVITIES

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## ABSTRACT

The present work was carried out to study the change in response of *S.littoralis* collected at early and late season for some conventional insecticides e.g Organophosphorous (profenofos and chlorpyrifos), and synthetic pyrethriods (fenvalerate and cypermethrin), and some non traditional insecticides e.g cutabroon and empire. Also correlation values of enzyme activities and their relation with means of lethal concentrations in late season was studied.

The obtained results showed inseceticidal correlation in their toxicity against field strain of *S.littoralis*.

Highly significant positive correlation was recorded between profenofos and empire and also between chlorpyrifos and cutabroon, besides empire.

Also obtained data indicated that elevation of lethal concentrations after spray season for cutabroon, chlorpyrifos, fenvalerate and cypermethrin, in general were either positively correlated with AIK-P or negatively correlated with the tramsaminases. Empire and profenofos gave positive and negative correlation with determined enzymes. No relation was observed between Acid-E or Alpha-E and elevation of lethal concentrations, and no reported have been found, that transaminases (GPT and GOT) play any fixed role in pesticides tolerance or resistance.

## INTRODUCTION

The cotton leaf worm, *spodoptera littoralis* (Boisd) is considered one of the most serious and destructive phytophagous. A lepidopterous insect pest in Egypt not only for cotton plants but also other field crops and vegetables.

Attention was therefore paid to control insects using different traditional insecticides e.g Organophosphorous and Pyrethroides and non traditional insecticides e.g IGR compounds which are considered nowadays one of the mainly component of IPM program in order to minimize the usage of conventional insecticides, hence reduced the environmental Pollution and the hazard to both man and domestic animals.

The mode of action of conventional and non traditional pesticides were studied by many investigator Flint and Smith (1977), Grosscurt and Anderson (1980), Ascher and Eliyahu (1981).

Resistance has been defined as the developed ability of strain of insects to tolerate doses of toxicants which would prove lethal to majority of individuals in a normal population of the same species. This developed ability in the result selecting individuals with a heritable capacity to withstand the toxicant, and is not due to the action of an insceticide on the individual insect, Oppenoorth (1985).

Insecticides resistance has become a major obstacle to successful chemical control. It has been shown that a number of enzymes is involved in the detoxication of insecticides and there by responsible for resistance. These enzymes are the microsomal monooxygenase (Wikinson 1983), phosphorotriester hydrolases, glutathion – S - Transferase*,* Carboxylesterase and DDT – dehydrochlorinase Dauterman (1985). Generally this present work aimed to study the effect of some conventional and non traditional insecticides, and also study the change of response of these compounds between early and late season and their relation with some enzymes in *S.littoralis.*

## MATERIALS AND METHODS

**A. Rearing technique:**

A field strain of *S.littoralis* was collected as egg-masses from Dakhalia Governorates in May 1998 before pesticidal application. Another samples of egg – masses were collected from the same locality at the end of August after the last spray against bollworm.

The obtained cotton leafworm strain were reared in the laboratory according to El-Defrawi et al. (1964).

**B. The tested insecticides:**

**1- Binary mixture compounds:**

- Cutabroon 74% EC (profenfos / chlorfluazuron combination 72%: 2%)
- • Profenofos (curacron): O – (4 – bromo – 2 – chlorophenyl ) O – ethyl – S – propyl phosphorothioate.
- • Chlorfluazuon: 1- (2, 6 – difluorobenzoyl) – 3 – [4 (chloro – 5 – trifluoromethyl – 2 – pyridy loxy) 3, 5 – dichlorophenyl] urea.
- Empire 50%: FL. (chlorpyrifos / diflubenzuron combination 48%:3%):
- • Chlorpyrifos: (0,0 diethyl 1-0) 3, 5, 6 – trichloropyridin – 2 – yl) phosphorothioate.
- • Diflubenzuron: 1- (2,6 – diflubenzoyl) –3-4- chlorophenyl) urea.

**2- Organophosphorous:**

- Profenofos (Curocron) 72% EC.
- Chlorpyrifos (Dursban) 48% EC.

**3- The synthetic prethroid:**

- Fenvalevate (Sumicidin) 20% EC. R-S - $\alpha$ - cyano –3 – phenoxybenzyl – (Rs) – 2 – (4-chorophenyl) – 3 – methylbutyrate.
- Cypermethrin (Ripcord) 25% EC. (Rs) - $\alpha$ - cyano – 3 – phenoxybenzyl (1Rs) – cis, trans – 3 – (2,2 – dichlorovinyl) – 2,2 – dimethylcyclopropancarboxy – late.

**C. Toxicological Studies:**

For the determination of the median lethal concentration values (many times and took mean) of various compounds formulation before and after season spraying, a series of insecticide concentrations was prepared based on (ppm) by diluting the formulated water also with distilled water. Castor – bean leaves were dipped for 15 seconds in each concentration then left to dry for one hour. Newly moulted 4th instar larvae of field strains (7 day old) of *S.littoralis* were confined with treated leaves in glass Jars covered with muslin for 48 hrs. (in test of binary mixture compounds, treated leaves were then removed and fresh untreated leaves were provided for one day. Five replicates each of 10 larvae were used for each concentration.

The average mortality percentage was corrected using Abbott's formula (1925) if necessary. The corrected percentage of mortality of each compound was statistically computed according to Finney (1955) from which the corresponding concentration probit lines (Lc-p lines) could be estimated in additional to determine 50% and 90% mortalities.

**D. Enzyme studies:**

**- Sample preparation for assay: -**

Fifty healthy 4th instar larvae of cotton leafworm (before and after spraying season) were picked up and placed in clean Jars and starved for 4 hours. Starved larvae were homogenized with distilled water (50 larvae / 10ml) at 5°C for 3 minutes. Homogenates were centrifuged at 3500 r.p.m. for 10 minutes at 5 °C. Supernatants were used directly to determine the activity of enzymes invertase, amylase, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (ALK-P), acid phosphatase (AC-P), ali esterase (Ali-E), alpha esterase (Alpha-E) and beta esterase (Beta-E).

**- Determination of enzme activites: -**

Invertase and amylase based on the digestion of sucrose and starch, which were determined spectrophotometrically according to the method described by Ishaaya and Swiriski (1970). Transaminase (GPT and GOT were determined colorimetrically according to the method of Reitman and Frankel (1957). Alkaline and acid phosphatases were determined in the homogenate of the 4th instar larvae by the method described by Powell and Smith (1954). In this procedure, the phenol released by enzymatic hydrolysis of disodium phenyl phosphate (Substrate), under defined condition of time, temperature and PH reacts with 4-aminoantipyrine and potassium ferricyanide producing phenol. The activity of both enzyme was calculated as ug phenol released / minute / insect. Non specific estrases were determined by the method described by Van Aspercn (1962). Alpha and Beta napthyl acetate were used as substrates, and enzyme activity is expressed as ug alpha or beta – napthyl acetate released / min / insect. Ali esterase was determined according to method described by Sympthon et al. (1964).

**- Statistical analysis:**

For interrelation among the toxicity of insecticides used in *S.littoralis*, the values of $Lc_{50}$ for each insecticide (not mean) were subjected to the computer according to simple correlation program to illustrate positive and negative correlation between the insecticide used and level of their significance, and also for relation between association the $Lc_{50}$, s and enzymatic activities, calculate numerical measure of the degree of association called a correlation coefficient.

## RESULTS AND DISCUSSION

**- Susceptibility of *Spodoptera littoralis* to some insecticides:**

Samples of *S.littoralis* egg masses were collected twice, at random, from Dakhalia Governorate; early in May before starting of spraying season and secondary during the end of August after finishing of the last spray against bollworm.

The results demonstrated in Table (1) indicate the mean values of $Lc_{50}$, $Lc_{90}$ and slopes of cutabroon, empaire, profenofos, chlorpyrifos, fenvalerate and cypermethrin, in addition to the change in response.

**Table (1): Susceptibity of field strain (Dakhalia Governorate) of 4th instar larave of *S.littoralis* to tested insecticides:**

| Insecticides | Before spring season | | | After spring season | | | Chang in response |
|---|---|---|---|---|---|---|---|
| | **$Lc_{50}$** | **$Lc_{90}$** | **Slope** | **$Lc_{50}$** | **$Lc_{90}$** | **Slope** | |
| Cutabroon | 18.0 | 138 | 0.98 | 68.0 | 572 | 1.10 | 3.77 |
| Empire | 40.0 | 328 | 1.68 | 74.0 | 652 | 1.88 | 1.85 |
| Profenofos | 620.0 | 3200 | 2.90 | 1860.0 | 7300 | 2.98 | 3.00 |
| Chlorpyrifos | 660.0 | 2100 | 2.20 | 2100.0 | 6200 | 2.30 | 3.18 |
| Fenvalerate | 540.0 | 3210 | 3.10 | 3100.0 | 8350 | 3.80 | 5.74 |
| Cypermethrin | 150.0 | 720 | 1.70 | 950.0 | 2330 | 1.80 | 6.36 |

- $Lc_{50}$ & $Lc_{90}$ expressed as ppm.
- Change in response = $\frac{Lc_{50} \text{ of late season}}{Lc_{50} \text{ of early season}}$

Data in table (1) show that, both means of $Lc_{50}$ and $Lc_{90}$ values varied tremendously according to the chemical structure of tested compounds and time of colony collection

Cutabroon ranked superior insecticidal activity followed by empire, the $Lc_{50}$,s reached 18.0 and 40.0 ppm respectively.

Regarding the late collected insect colony, the $Lc_{50}$'s were drastically increased compared with early one, showing 68.0 and 74.0 ppm. cutabron recording the lowest (Highest insecticidal activity) values of $Lc_{90}$ (138, 572 ppm) also the slope value were 0.98 and 1.1 before and after spraying season, indicating the high suseptibility of *S.littoralis*. The change of response reached to 3.77 and 1.85 with two mixtures, cutabroon and empire.

On the other hand, results appeared that, the susceptibility to the Organophosphorous profenofos and chlorpyrifos (which are still in use up to date in controlling *S.littoralis)* have no level of tolerance notice, and data sure that fact, because the range between $Lc_{50}$ and also $Lc_{90}$ before and after the spray season were very high.

The data of tested pyrethroids appeared that cypermethrin proved to be more effective then fenvlerate, the $Lc_{50}$ values were 150 and 540 ppm. Respectively. The change of response were 5.74 and 6.36 for fenvalerate and cypermethrin, respectively.

**- Interrelation between insecticidal activities:**

Table (1) showed the $Lc_{50}$ values of the tested insecticides against field strain of *S.littoralis* before and after spraying season.

These values were utilized to simple correlation program to illustrate positive or negative correlation between insecticidal activities.

Results in table (2) showed highly significant positive correlation between profenofos and empire and also between chlorpyrifos and cutabroon, besides empire. Only positive correlation were found between empire with cutabroon, profenofos with cutabroon, chlorpyritos with profenofos, fenvalerate with profenofos and with chlorpyrifos and also cypermethrin with profenofos and with fenvalerate. On the other side the relation between others tested insecticides were negatively.

**Table (2): Correlation values among insecticides used as mean of $Lc_{50}$,s:**

| Insecticides | Cutabroon | Empire | Profenofos | Chlorpyrifos | Fenvalerate | Cypermethrin |
|---|---|---|---|---|---|---|
| Cutabroon | 1.00 | | | | | |
| Empire | + 0.322 | 1.00 | | | | |
| Profenofos | + 0.194 | ✷+0.0498 | 1.00 | | | |
| Chorpyrifos | ✷+0.892 | ✷+0.662 | + 0.412 | 1.00 | | |
| Fenvalerate | - 0.024 | - 0.190 | + 0.212 | + 0.314 | 1.00 | |
| Cypermethrin | - 0.058 | - 0.186 | + 0.310 | - 0.39 | + 0.264 | 1.00 |

**(1-tail ✷ 0.05 = + or – 0.498)**

This may lead to an interesting conclusion of an applicable importance, that empire, cutabroon, profenfos and chlorpyrifos should not be applied in sequence under field.

**- Correlation between enzymc activites and insecticide in homogenate larvae of *S.littoralis*:**

Table (3) showed means of enzyme activity expressed as ug substrate / insect / minute of $4^{th}$ larval homogenate of *S.littoralis* before and after spraying season.

**Table (3): Means of enzyme activity of 4th larval homogenate of *S.littoralis* before and after spraying season:**

| Enzyme | Enzyme activity expressed as ug substrate hydrolysed/ insect/minute | |
|---|---|---|
| | Before spraying season | After spraying season |
| Invertase | 4.82 | 3.94 |
| Amylase | 1.75 | 0.92 |
| GPT ✷ | 4.22 | 1.82 |
| GOT ✷ | 9.44 | 8.38 |
| AlK-P ✷ | 2.46 | 10.52 |
| AC – P ✷ | 3.14 | 5.14 |
| Ali – E ✷✷ | 3.45 | 2.28 |
| Alpha – E | 3.24 | 2.32 |
| Beta – E | 0.40 | 2.62 |

-GPT: Glutamic pyruric transaminase - Ali-E : Ali estrase
-GOT: Glutamic oxaloacetic transaminase - Alpha-E : Apha estrase
-AlK-P: alkaline phosphatase - Beta-E : Beta estrase
-AC-P: Acid phosphatase - ✷ : activity $10^{-3}$
✷✷ : activity $10^{-1}$

Also correlation coefficient between the enzyme activities and the mean of insecticides $Lc_{50}$ values are recorded in table (4).

Obtained data indicate that elevation of cutabroon $Lc_{50}$ showed significant positive correlation with AlK-P recording 0.499, it can be added that this chemical created significant negative correlation with GPT and GOT.

Also the elevation of the change in response to chlorpyrifos as a result of increase the lethal concentration in late season showed significant positive correlation with AlK-P and Beta-E whereas it significant negative with GPT and GOT showing respective values of – 0.570 and - 0.662.

**Table (4): Correlation values of enzyme activites relation to means of $Lc_{50}$:**

| Insecticides / Enzymes | Cutabroon | Empire | Profenofos | Chlorpyrifos | Fenvalerate | Cypermethrin |
|---|---|---|---|---|---|---|
| Invertase | - 0.426 | - 0.242 | + 0.122 | - 0.248 | - 0.212 | - 0.312 |
| Amylase | - 0.062 | - 0.080 | + 0.260 | - 0.262 | -0.270 | - 0.412 |
| GPT | ✷- 0.558 | - 0.392 | + 0.148 | ✷- 0.570 | + 0.432 | - 0.492 |
| GOT | ✷- 0.522 | - 0.112 | + 0.256 | ✷- 0.662 | - 0.412 | - 0.484 |
| AlK-P | ✷+ 0.499 | + 0.384 | - 0.028 | ✷+ 0.520 | ✷+ 0.540 | ✷0.542 |
| AC – P | - 0.122 | - 0.208 | ✷0.520 | - 0.388 | ✷+ 0.512 | - 0.398 |
| Ali – E | + 0.128 | +0.012 | -0.312 | - 0.332 | +0.212 | +0.182 |
| Alpha – E | + 0.124 | + 0.164 | + 0.243 | + 0.120 | - 0.136 | + 0.226 |
| Beta – E | + 0.468 | + 0.428 | + 0.286 | ✷- 0.580 | + 0.298 | + 0.110 |

**- critical value 1-tail, 0.05 = or – 0.498**

It is note worthy to say that high value of $Lc_{50}$ of cypermthrin showed positive significant and negative near to significant correlation with the respective enzymes AlK-P and GOT by recording 0.542 and 0.492. Fenvalerate revealed significant positive correlation with AlK-P and AC-P and negative near to significant with the respective enzymes GOT and GPT.

The forementioned findings concluded that the elevation of lethal concentration (after spray season) of cutabroon, Chloryrifos, Fenvalerate and Cypermthrin in general, were either positively correlated with AlK-P and / or negatively correlated with GPT and GOT.

Empire and Profenofos gave positive and negative correlation with the determined enzymes. No relation was observed between Acid-E or Alpha-E and elevation of lethal concentration for insecticides tested under this study, also no reports have been found, that transaminases play any role in pesticides tolerance or resistance.

On the other hand O'Brein (1967) mentioned that, Beta-E and minor increase in phosphate activity (AlK-P) may be accompained with resistance to some insecticides.

Conclusion of this work are also agreed with those reached by lewic and Sawiki (1971) who reported that phosphatases activity could not be detected in susceptable high levels of alkaline phosphatase activity in cypermthrin – resistant strain of *S.littoralis*. The same activity was clearly observed at subcellular levels as well as in the whole insect homogenate. Also Riskallah (1982) reported that positive correlation between high esterase activity and resistance to pyrethroids.

On the other hand in tested anti-chitin synthesis compounds and their binary mixture with organophosphorous before and after spraying season appeared increase in AlK-P and AC-P and showed more reduction in GPT and also lower in amount of Ali-E and Alpha-E.

Abdel-Hafez et.al (1988) reported that Diflubenzuron and Triflumuron were drastically inhibited protein synthesis of treated larvae *S.littoralis* El-Kordy et.al (1995) mentioned that pyriproxyfen and Tebufenozid, could be considered as inhibitory agents for protein synthesis in *S.littoralis.*

Recently Mostafa (1998) stated that all tested IGR's increased the GPT and GOT activity in larvae of *A.ipsilon*, and also showed that the activity of Alkaline and acid phosphates increased in IGR's treated larvae at the same insect than untreated larvae.

In conclusion, it is clearly evident that our present study provides a biochemical basis for detecting change of response, tolerance or resistance that shown in field strain of *S.littoralis*, and an increase in an enzyme activity could be used as an indicator for the existence of insect tolerance or resistance to those insecticides that have significant positive correlation between each other. On the basis of these findings, all insecticides used are to be prohibited to be used in a sequence under field condition.

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## دراسات على تغيير استجابة دودة ورق القطن لبعض المبيدات وعلاقته بنشاط بعض الأنزيمات

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أجريت خطوات هذا البحث بغرض معرفة تأثير بعض المبيدات التقليدية المستخدمة ضد دودة القطن باستخدام بعض المركبات الفسفورية (البرفينوفوس والكلوريرفوس) ومركبات البيرثرويد (الفنفاليرات والسيبرمترين) كذلك تم استخدام بعض مخاليط IGR's مع المركبات الفسفورية وهما الكاتابرون والإمبير.

وتم معمليًا إيجاد متوسط التركيز القاتل لـ 50% وكذلك المتوسط لـ 90% والميل لكل مبيد على العمر الرابع لدودة ورق القطن والتي جمع بيضها وربيت في المعمل قبل الرش وبعد انتهاء عملية الرش لديدان اللوز.

- وقد وجد ارتفاع كبير في قيم التركيز القاتل في نهاية الموسم عن بدايته وأيضًا ارتفاع في معـــدل الاستجابة.
- أوضحت النتائج أن هناك علاقة ارتباط إيجابية معنوية بين بعض هذه المبيدات من ناحية تأثيرها على دودة ورق القطن (يفضل عدم استخدامها في تتابع حقليًا) كذلك وجد أن هناك علاقة ارتباط سالبة بين البعض الآخر.
- أظهرت الدراسة التي أجريت لمعرفة معدلات التغيير في كم بعض الأنزيمات قبل الرش وبعد الرش أن هناك ارتباط إيجابي موجب بين ارتفاع التركيز النصفي القاتل وهذه الأنزيمات. وكان أكثرها ملاحظة هو أنزيم AIK-P ـ كذلك هناك ارتباط سلبي معنوي مع البعض الآخر ـ مع وجود ارتفاع وانخفاض دائم في نسب بعض الأنزيمات قد يكون لها علاقة مباشرة بدرجة تحمل الحشرة أو ظهور أفراد مقاومة، كما وجد أن بعض هذه الأنزيمات ليس له أي علاقة بارتفاع قيم التركيزات النصفية القاتلة.