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# SYNTHETIC PYRETHROIDS , IGR and JHM AS CONTROL AGENTS FOR *Spodoptera littoralis* (Boisd) IN RELATION TO SOME ENZYME ACTIVITIES

**Allam A. M.**
**Central Agric. Pesticides Laboratory, Agric. Res. Center, Dokki, Giza**

## ABSTRACT

The efficacy of five synthetic pyrethroids (Fenvalerate, esfenvalerate, cyfluthrin, fenpropathrin and deltamethrin), one insect growth regulator (tebufenozide) and the Juvenile hormone mimic (pyriperoxfen) were tested on 4th instar larvae of *S. littoralis*. Tests were carried out on larvae of susceptible and field strains collected from Behera, Dakahleia, Gharbeia, Kafr-El-Sheikh and Sharkeia governorates using dipping technique. Field strain were collected and tested before and after spraying programs of different Governorates. Obtained data in general, indicated that the activity of the tested compounds in ascending order, were pyriperoxyfen, tebufenazide, fenvalerate, esfenvalerate, cyfluthrin, Fenpropathrin and deltamethrin.

The biochemical studies on the tested strains after spraying program indicated variable activities in phosphatases (AlkP and AcP) and in carbohydrate hydrolyzing enzymes (amylase, trehalase and invertase).

## INTRODUCTION

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. Phosphatases are defined as enzymes hydrolyzing any phosphorus ester or anhydride bond (O'Brien, 1967). Van Asperen (1960) found that resistant strains of the houseflies could degrade organophosphorus compounds by increasing phosphatase activity.

The insect cuticle is known to contain proteins and their derivatives (in the epicuticle, exocuticle and endocuticle). Diflubenzuron alters cuticle composition in insect by reducing the amount of chitin in the housefly larvae without any appreciable effect on the cuticle protein level. The reduced level of chitin in the cuticle seems to result from inhibition of the biochemical processes leading to chitin formation (Mitusi, 1985; Ishaaya and Casida,1974 and Post *et al.,*1974.

Carbohydrates contribute to the structure and function of all insect tissues. They can be found in nuclei, cytoplasm and cell membrane as well as in haemolymph and supporting tissues. Trehalose and Glucose are the common sugar in haemolymph of most insects (Wyatt and Kalf, 1957). Trehalase is an important enzyme which degrades trehalose to gluscose for interval emerge supply (Wyatt, 1967). Invertase and amylase enzymes are active in digestive system of several insects (Wigglesworth, 1953). The aim of this study is to investigate the activity of IGR, JHM and synthetic pyrethroids on *S. littoralis* during growing cotton seasons in relation to some enzymes activities.

## MATERIALS AND METHODS

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

The susceptible strain used was obtained from of the Central Agric. Pesticides Lab., A. R. C., which was previously collected from cotton fields of Fayoum Governorate in 1968. Other five strains were collected from Behera, Dakahleia, Gharbeia, Kafr-El-Sheikh and Sharkia Governorates. Egg masses samples were collected from the fields before (June) and after (September) 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$^{o}$C and 65±5 % R.H.)

**Insecticidal treatments**

Five synthetic pyrethroid insecticides, namely; fenvalerate, esfenvalerate, cyfluthrin, fenpropathrin and deltamethrin in addition to the juvenile hormone mimic pyriperoxyfen and the insect growth regulator tebufenozide were used in this study.

Toxicological studies of these insecticides on 4$^{th}$ 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 to dry for 2 hr. under room temperature before being offered to the 4$^{th}$ instar larvae. Five replicates ten larvae for each were used for each concentration. Larvae were fed for 24 hr. on the treated leaves.

Mortality counts were determined after 24 hrs. and dead larvae were removed. Survived larvae were transferred onto fresh untreated leaves to determine the activity of some enzymes. 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 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 strain were starved for ca 4hr. 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 $^{o}$C and the supernatant was used for enzyme assay.

The method described by Powell and Smith (1954) were used for determining acid phosphatase (AcP) and alkaline phosphatase (AlkP) activity. The procedure was based on the hydrolysis of disodium phenyl phosphate (Substrate) in acid media (pH 4.5) for AcP or alkaline media (pH 10.5) for AlkP to yield phenol. Hydrolyzed phenol after reacting with 4-aminoantipyrine yield red colour which read spectro-photometrically. Enzyme activity was expressed as ug phenol released/min/larva or Mg/Min/gram.

The activity of carbohydrates hydrolyzing enzymes (invertase,

amylase and trehalase) was measured colourimetrically using the method described by Ishaaya and Swirski (1976). Sucrose 4 %, starch 2 % and Trehalose 3 % were used as substrates for the detection of invertase, amylase and trehalose activities, respectively. The activity of the tested enzymes was expressed as ug glucose unrelated larvae/minute.

## RESULTS AND DISCUSSION

The present study aims to investgate the activity of some synthetic pyrethroids, one JHM and one IGR, insecticides to manage resistance the development in *Spodoptera littoralis* (Boisd) of field strains. Summarized data of the toxicological studies on 4$^{th}$ instar larvae of ten field strains collected from 5 Governorate before and after sprayings programs are recorded in Table (1). Resistance ratios of this table revealed that the JHM pyriperoxyfen was a potent compound in Dakahleia, Sharkeia before the beginning of spraying programs,while slight effect was observed in Kafr-El-Sheikh and Behera. Resistance ratios ranged between 0.001 fold for Dakahleia to 7.02 fold in Behera. On the other hand all resistance ratio levels were increased to reach tolerance and vigor tolerance levels after spraying programs. The exception case of this compound was recorded in Sharkia (0.50 fold) at the end of cotton season. The exhibited in IGR compound, tebufenozide exhebited Dakahleia a toderance level of 1.02 and 6.53 tolerance fold respectively. Different levels of resistance observed in Gharbeia, Kafr-El-Sheikh and Sharkeia, were RR of 15.87 51.37 fold were recorded On the other hand, the compound exhibited a high level of resistance in Behera.

In the case of synthetic pyrethroids,the estimated results of fenvalerate proved that the compound have poor effects during cotton growing season. Resistance ratios were recorded 44.10 fold in Sharkeia before sprays and 376.5 fold in Kafr-El-Sheikh after spraying season. Based on the experimented data of esfenvalerate, it is apparent that the use of this compound in such areas became uneffective. Resistance ratios of exfenvalerate in the Governorate ranged between 72.20 fold in Sharkeia before sprays to 545.00 fold in the same area after spraying programs.

Regarding to cyfluthrin, moderate activity was exhibited before spraying program where resistance ratios recorded were 0.54, 0.86, 11.95, 12.03 and 12.05 fold for Sharkeia, Dakahlia, Behera,. Kafr-El-Sheikh and Gharbeia Governorate, respectively. The activity of the compound was dropped in Gharbeia, Kafr-El-Sheikh and Behera after spraying programs,while in Sharkeia and Dakahleia the compound was still effective showing RR of 0.65 and 1.17 fold after spraying, respectively.

No effect was observed or recorded for fenpropathrin either pre or post spraying programs in the five governorates. Resistance ratios ranged between 90.70 fold for Gharbeia to 735.62 fold for Kafr-El-Sheikh were recorded. On the other hand, Deltamethrin was active at early season at Dakahleia with RR of 1.56 fold and 3.91 fold in Sharkeia. While at the late season the ratios became 17.9 and 47.9 4, respectively. In Kafr-El Sheikh the ratio changed from 106.79 to 121.47 at early and late season, respectively.

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

| | Early Season | | | Late Season | | |
|---|---|---|---|---|---|---|
| | Slope | LC50 | R.R. | Slope | LC50 | R.R. |
| **Fenvalerate** | | | | | | |
| S.Strain | 1.31 | 14.49 | ------ | 1.31 | 14.49 | ------ |
| Behera | 1.79 | 2512.46 | 173.40 | 2.02 | 2796.95 | 193.03 |
| Dakahleia | 2.10 | 1177.46 | 81.30 | 2.21 | 2005.87 | 138.40 |
| Gharbia | 0.83 | 763.89 | 52.70 | 1.56 | 1688.79 | 116.30 |
| Kafr El-Shekh | 0.82 | 2193.15 | 151.40 | 1.78 | 5455.06 | 376.50 |
| SHarkeia | 1.86 | 639.53 | 44.10 | 2.14 | 3957.37 | 273.00 |
| **Esfenvalerate** | | | | | | |
| S.Strain | 1.99 | 1.08 | ------ | 1.99 | 1.08 | ------ |
| Behera | 1.20 | 383.78 | 355.40 | 2.31 | 621.15 | 575.10 |
| Dakahleia | 1.74 | 331.08 | 306.60 | 1.83 | 412.24 | 301.70 |
| Gharbia | 1.40 | 384.40 | 355.90 | 1073.00 | 623.40 | 577.20 |
| Kafr El-Shekh | 1.12 | 193.82 | 179.50 | 2.19 | 730.94 | 676.80 |
| SHarkeia | 1.57 | 77.95 | 72.20 | 1.97 | 588.59 | 545.00 |
| **Cyfluthrin** | | | | | | |
| S.Strain | 1.02 | 140.54 | ------ | 1.02 | 140.54 | ------ |
| Behera | 1.94 | 1677.70 | 11.95 | 1.11 | 8232.10 | 58.61 |
| Dakahleia | 1.11 | 120.04 | 0.86 | 0.76 | 240.49 | 1.71 |
| Gharbia | 2.13 | 1756.10 | 12.05 | 1.93 | 2401.50 | 17.10 |
| Kafr El-Shekh | 1.59 | 1688.90 | 12.03 | 1.03 | 5942.70 | 42.31 |
| SHarkeia | 1.04 | 75.88 | 0.54 | 1.56 | 91.36 | 0.65 |
| **Fenpropathrin** | | | | | | |
| S.Strain | 2.80 | 1.15 | ------ | 2.80 | 1.15 | ------ |
| Behera | 2.16 | 238.93 | 207.80 | 2.42 | 206.11 | 179.20 |
| Dakahleia | 1.80 | 187.40 | 163.00 | 3.25 | 209.29 | 181.99 |
| Gharbia | 2.13 | 2.6.44 | 179.50 | 2.33 | 104.36 | 90.70 |
| Kafr El-Shekh | 1.25 | 515.85 | 448.60 | 2.08 | 846.00 | 735.62 |
| SHarkeia | 4.00 | 219.63 | 191.00 | 1.62 | 258.12 | 224.12 |
| **Deltamethrin** | | | | | | |
| S.Strain | 0.37 | 4.15 | ------ | 0.37 | 4.15 | ------ |
| Behera | 0.53 | 316.52 | 76.27 | 0.58 | 429.60 | 103.52 |
| Dakahleia | 0.52 | 6.46 | 1.56 | 0.44 | 74.29 | 17.90 |
| Gharbia | 0.49 | 69.70 | 16.80 | 0.69 | 201.05 | 48.45 |
| Kafr El-Shekh | 1.07 | 443.21 | 106.79 | 0.29 | 504.09 | 121.47 |
| SHarkeia | 0.25 | 16.24 | 3.92 | 0.43 | 198.96 | 47.94 |
| **Tebufenozide** | | | | | | |
| S.Strain | 1.28 | 2.93 | ------- | 1.28 | 2.93 | ------- |
| Behera | 0.74 | 256.08 | 87.40 | 1.12 | 310.11 | 105.85 |
| Dakahleia | 0.66 | 2.99 | 1.02 | 0.65 | 19.13 | 6.53 |
| Gharbia | 0.56 | 50.44 | 17.22 | 0.62 | 150.50 | 51.37 |
| Kafr El-Shekh | 0.63 | 52.54 | 17.93 | 0.37 | 46.50 | 15.87 |
| SHarkeia | 0.74 | 54.98 | 18.77 | 0.77 | 59.45 | 20.29 |
| **Pyriperoxyfen** | | | | | | |
| S.Strain | 0.69 | 70.31 | ------ | 0.69 | 70.31 | ------ |
| Behera | 1.52 | 493.21 | 7.02 | 1.76 | 534.08 | 7.60 |
| Dakahleia | 0.24 | 0.007 | 0.00 | 1.19 | 103.25 | 1.49 |
| Gharbia | 0.81 | 111.86 | 1.59 | 0.43 | 571.36 | 8.13 |
| Kafr El-Shekh | 0.47 | 459.75 | 6.36 | 0.61 | 525.22 | 7.47 |
| SHarkeia | 0.48 | 25.16 | 0.36 | 0.29 | 35.00 | 0.50 |

In conclusion, the tested compounds could be arranged in an ascending order of their potency as follows : the potency were pyriperoxyfen, cyfluthrin, Tebufenozide, deltamethrin, fenvalerate, esfenvalerate and fenpropathrin.

These findings are in agreement with Dittrich *et al.* (1979) showing that *S. littoralis* strain sampled in 5 provinces of the Nile Delta had a uniform resistance towards monochrotophos, and the resistance ratios ranged between 50 to 137 fold. A high level of resistance to permethrin (>4000 fold) had been induced in larvae of southern house mosquito (Priesher & Georghiou, 1978), Mohanna and Allam (1999) found that fenvalerate in Kafr-El-Sheikh Governorate exhibited tolerance of 4.2 fold of tolerance to *S. littoralis*. Cypermethrin was 4 fold of tolerance in Dakahlia strain when experimented on 4$^{th}$ instar of *S. littoralis* (Mohanna, 1998a).

Enzyme activities of phosphatases, acid phosphatase (AcP) and alkaline phosphatase (AlkP) in addition to carbohydrate hydrolyzing enzymes, Amylase, Invertase and Trehalase were estimated. Fourth instar larvae of *S. littoralis* collected from five Governorates were used for these experiments. Values of these enzymes as Mg/min/larvae or Mg/min/gram. are summarized in Table (2). The moderate level of total protein was recorded in susceptible strain (0.636), followed by Kafr-El-Sheikh (0.487), Sharkeia (0.486) and Behera (0.407) Mg/min. larvae. Lower levels of total protein were recorded in Gharbia (0.345) and Dakahleia (0.290) Mg/min/larva.

**Table (2) : The activities of acid and alkaline phosphatase, invertase, trehalase, amylase and total protein in the haemolymph in 4th larval instar in 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 | Sharkeia |
|---|---|---|---|---|---|---|---|
| Total Protein | Mg/larva | 0.636 | 0.407 | 0.290 | 0.345 | 0.487 | 0.486 |
| | Mg/min/larva | 27.691 | 13.432 | 14.867 | 13.710 | 13.379 | 13.028 |
| Acid Phosphatase | Mg/larva | 0.436 | 1.067 | 1.583 | 0.240 | 1.246 | 0.560 |
| | Mg/min/larva | 18.971 | 35.267 | 81.095 | 9.548 | 34.234 | 15.010 |
| Alkaline Phosphatase | Mg/larva | 0.705 | 2.241 | 2.342 | 0.360 | 1.357 | 0.848 |
| | Mg/min/larva | 30.717 | 74.048 | 119.991 | 14.301 | 37.303 | 22.725 |
| Invertase | Mg/larva | 17.403 | 17.058 | 13.781 | 17.384 | 21.045 | 17.116 |
| | Mg/min/larva | 757.985 | 563.576 | 705.985 | 691.054 | 578.316 | 458.894 |
| Trehalase | Mg/larva | 10.062 | 11.423 | 4.715 | 8.663 | 9.315 | 9.583 |
| | Mg/min/larva | 438.262 | 377.406 | 241.547 | 344.384 | 255.977 | 256.940 |
| Amylase | Mg/larva | 0.719 | 0.652 | 2.032 | 1.974 | 1.993 | 1.763 |
| | Mg/min/larva | 31.304 | 21.530 | 104.081 | 78.477 | 54.777 | 47.277 |

Acid phosphatease (AcP) was more active in Dakahleia, Kafr-El-Sheikh and Behera, with a level of 1.583, 1.246 and 1.067 Mg/min/larva, respectively. Sharkeia, susceptible strain and Gharbeia showed lower level of 0.560, 0.436 and 0.240 Mg/min/larvae, respectively.

Similar trend for ALKP activity was observed in Dakahlia (2.342),

Behera (2.241) and Kafr-El-Sheikh (1.357).

Among the second group of enzyme tested in this work carbohydrate hydrolyzing enzymes, invertase was clearly active (21.045 Mg/min/larva) in Kafr-El-Sheikh strain. Moderate levels of activity was observed in susceptible strain, Gharbeia, Sharkeia, Behera and Dakaleia, Activity in Mg/min/larvae were 17.403, 17.384, 17.116, 17.058 and 13.781, respectively. Trehalase was clearly active in all experimented cases with the excption of Dakahlia strain (4.715 Mg/min/larva). The levels of the activity ranged between 11.423 for Behera to 8.663 in Gharbeia strain. On the other hand, the highest level of amylase activity was observed in Dakahleia strain (2.032 Mg/min/larvae). The second order of activity was recorded by Kafr-El-Sheikh (1.993), Gharbeia (1.974) and Sharkeia (1.763) Mg/min/larva. The lowest category in activity was cleared with susceptible strain (0.719) and Behera (0.652) Mg/min/larva.

These findings are in agreement with those of Menzel *et al.* (1963) they suggested that resistance to OP insecticides such as malathion, was related to higher phosphatase activity. However, activity of both types of phosphatase was slightly higher in the haemolymph of the S-strain than in the fenitrothion resistant strain of *S. littoralis* (Saleh, 1981). Also our results support partially those of Mohanna (1998) who reported that both acid phosphatase and alkaline phosphatase activities were fluctuated between strains of *S. littoralis*. AIKP, was activated by 95.66 % on susceptible strains compared to ACP which inhibited by –23.44 %. On contrast, parent strain recorded 157.81 % activity for ACP and –30.10 % for ALKP. Abd El Hafez (1993a). On the other hand(Mohanna (1999) mentioned that the parent strain of *S. littoralis* (Sharkeia strain) exhibited a low level of activity for trehalase (3.5 %) and invertase (9.62 %) Previous result in this study are in Fully or partially agreement with Radwan *et al.* (1984-1985) and Abd-El Hafez (1993b).

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## إستخدام البيريثرينات المصنعة ، منظمات النمو ومشابهات هرمون النمو فى مكافحة دودة ورق القطن وتقدير بعض الأنشطة الإنزيمية

**عبد الحمـــيد مصطفى علام**

**المعمل المركزى للمبيدات ـ مركز البحوث الزراعية ـ الدقى ـ القاهرة**

تم إختبار خمس مبيدات بيريثرينية هى الفينفليرات ، الإسفينفليريت ، الثيفليوثرين ، الفينبروباثرين والدلتامثرين بالإضافة الى منظم النمو التيبيفينوزيد ومشابه هرمون النمو البيربيروكسيفين على العمر اليرقى الرابع لدودة ورق القطن .

اجريت الإختبارات على السلالة الحساسة المعملية بالمقارنة بالسلالات الحقلية لمحافظات البحيرة ، الدقهلية ، الغربية ، كفر الشيخ والشرقية بإستخدام طريقة الغمر بالمعمل . اجريت المعاملات قبل بداية موسم الرش الحقلى وبعده فى المناطق المذكورة . أظهرت النتائج بصفه عامة نشاط للمركبات المستعملة حيث كان مشابه هرمون النمو البيربيروكسيفين انشطها يليه منظم النمو التيبيفينوزيد ثم الفينفليرات ، الإسفينفليريت ، الثيفليوثرين ، الفينبروباثرين وكانت الدلتامثرين اقلهم نشاطا.

أظهرت الدراسات البيوكيميائية على السلالات المختبرة بعد إنتهاء موسم الرش انشطة مختلفة لإنزيمات الفوسفاتيز وايضاالإنزيمات المحللة للكربوهيدرات (الأميليز ، التريهاليز والإنفرتيز).