COMPARATIVE TOXICITY AND BIOCHEMICAL IMPACTS OF SEVERAL CONVENTIONAL INSECTICIDES ON COTTON LEAFWORM

ABD EL-MAGEED , A.E.M.[1]; E.M. ANWAR [1] AND L.R.A. ELGOHARY[2]

1. Plant Protection Research Institute, ARC , Dokki , Giza , Egypt.
2. Pesticide Department , Faculty of Agricultural , Mansoura university, Egypt.

(Manuscript received 12 September 2005)

Abstract

The objective of this research was to evaluate the susceptibility of both the laboratory and the field strain of 2nd and 4th instars larvae of the cotton leafworm *Spodoptera littoralis* (Boisd.) to two organophosphorus (Profenofos and Chlorpyrifos), two carbamates (Carbaryl and Thiodicarb) and two synthetic pyrethroid insecticides (Fenpropathrin and Beta-cyfluthrin). Based on resistance ratio the fold of resistance varied considerably according to the chemical structure of the studied insecticides and the instar of larvae. Data revealed that small differences in LC_{50} values were observed between the laboratory and the field strains as demonstrated by resistance ratio of 0.892, 0.939and 1.757 fold for Profenofos , Fenpropathrin and Chlorpyrifos relating to 2nd instar larvae, respectively. Thiodicarb showed the highest resistance ratio, followed by Beta-cyfluthrin and Carbaryl reached 3.843, 3.516 and 3.414 fold on the same instar larvae , respectively .The LC_{50} values of both the laboratory and the field strains on 4th instar larvae were remarkably higher compared with 2nd instar larvae. Carbaryl gave the highest resistance ratio, followed by Chlorpyrifos, Profenofos being 6.593, 3.721 and 3.633 fold, respectively. There were low level of resistance ratio to Thiodicarb, Beta-cyfluthrin and Fenpropathrin with degree of fold 1.061, 0.528 and 0.327 , respectively . In addition the effect of tested insecticides on the activity of esterases (aliphatic esterase and α- & β-esterase), phosphatases (acid and alkaline phosphatase), transaminase (AST and ALT) and carbohydrate hydrolyzing enzymes (amylase and invertase) were evaluated on the field strain of *Spodoptera littoralis* (Boisd.) compared with the laboratory strain .

INTRODUCTION

Insecticides resistance has become a major obstacle to successful chemical control with conventional insecticides. The evolution of resistance to insecticides is governed by a complex of events and factors; mainly, intense and repeated applications of insecticides which are often from the same chemical group or which employ the same mode of action. The mechanisms of resistance (specific biochemical

changes that occur) and the speed with which resistance develops vary according to the specific insect (*e.g.* the frequency of alleles for resistance inherent in the population), and the class and application rate of the insecticide. Because the costs of developing new insecticides are enormous, emphasis must be placed on developing management practices which will not only prolong the life of current insecticides, but will also prevent the development of resistance to insecticides which have not yet been introduced. Such management practices cannot stand alone; more research is needed in areas of developing new and novel insecticides to maintain diversity of insecticides available for use, determining the mechanisms of resistance, and monitoring its occurrence (Horowitz *et al.*, 1997) . The occurrence of insect resistance to an insecticide is mainly due to the action of enzymes , which either insensitive to the insecticide or able to degrade it to non toxic metabolites (Al-Elimi , 1994), so it is convenient to study the enzymes that play a role in change of response to some compounds on the cotton leafworm, *Spodoptera littoralis* (Boisd.), which has its importance as one of the most destructive phytophagous lepidopterous pests in Egypt for it causes various ravages not only for cotton plants, but also other field crops and vegetables. The objective of this research was to evaluate the susceptibility of both laboratory and field strains of 2^{nd} and 4^{th} instars larvae of the cotton leafworm *S. littoralis* (Boisd.) to two organophosphorus, two carbamates and two synthetic pyrethroid insecticides . In addition assessment the effect of tested insecticides on the activity of esterases (aliphatic esterase and α- & β-esterase), phosphatases (acid and alkaline phosphatase), transaminase (AST and ALT) and carbohydrate hydrolyzing enzymes (amylase and invertase) on the field strain of *S. littoralis* (Boisd.), compared with the laboratory strain .

MATERIALS AND METHODS

I. Toxicological studies:-

With the purpose of studies the susceptibility of both laboratory and field strains of 2^{nd} and 4^{th} instars larvae of the cotton leafworm *S. littoralis* (Boisd.) to six insecticides belongs to different three groups were used in this research .

A. Tested Insecticides:

1. Organophosphorus insecticides:

(a) Profenofos 72%E.C.(Curacron®)

Chemical name : O-(4-bromo-2-chlorophenyl)O-ethyl S-propyl phosphorothioate.

(b) Chlorpyrifos 48%E.C.(Dursban®)

Chemical name: O,O-diethyl O-(3,5,6-tirchloro-2-pyridinyl) phosphorothioate.

2. Carbamate insecticides :

(a) Carbaryl 85%W.P.(Sevein®)

Chemical name : 1-naphthalenyl methylcarbamate.

(b) Thiodicarb 80%W.P.(Larvin®)

Chemical name : Dimethyl N,N-
[thiobis[(methylimino)carbonyloxy]]
bis(ethanimidothioate).

3. Synthetic pyrethroid insecticides:

(a) Fenpropathrin 30% S.C. (Danitol®)

Chemical name : Cyano(3-phenoxyphenyl)methyl2,2,3,3-
tetramethylcyclopropanecarboxylate.

(b) Beta-cyfluthrin 12.5% S.C.(Bulldock®)

Chemical name : Cyano (4-fluoro-3-phenoxyphenyl)
methyl3-(2,2dichloroethenyl) 2,2-
dimethylcyclopropanecarboxylate .

B. Cotton leafworm strains:-

A laboratory strain of the cotton leafworm *S. littoralis* (Boisd.) was maintained under constant conditions of 25°C±1 and 70 ± 5% RH and kept of any contamination with chemicals till the time of study in order to obtain a susceptible and homogenous strain. A field strain was collected as egg-masses from Dakahlia Governorate in June, 2004 . Egg-mass of cotton leafworm were reared in the laboratory as described by El-Defrawi *et al.* (1964).

C. Toxicity tests:-

A series of concentrations (in water) for each insecticide was prepared on the active ingredient (a.i) based on ppm by diluting the commercial formulation. Castor-bean leaves were dipped for 30 seconds in each concentration then left to dry for one hour. The 2^{nd} and 4^{th} instars larvae of each tested strain were confined with treated leaves in glass jars covered with muslin for 24 hrs. Test also included a non treated control in which leaves were dipped in water (as a check). Treated leaves were then removed and fresh untreated leaves provided for another day. Three replicates (each of 20 larvae) were tested for each concentration. Daily inspection was carried out for all treatments and mortality percentages were recorded until the 2^{nd} day after treatment. The average of mortality percentage was corrected using Abbott's formula

(1925). The corrected mortality percentage of each compound was statistically computed according to Finney (1971). From which the corresponding concentration probit lines (lc-p lines) were estimated in addition to determine 50 and 90% mortalities, slope values of tested compounds were also estimated. In addition, the efficiency of different compounds was measured by comparing the tested compound with the most effective compound by using the following equation Toxicity index = LC_{50} of the most effective compound / LC_{50} of the tested compound x 100 (Sun , 1950). Resistance ratio was calculated by dividing LC_{50} of field strain (ppm) by LC_{50} of laboratory strain (ppm) .

II. Biochemical studies:

This part of study was conducted in order to determined of some enzymes activities in 4th instar larvae of both laboratory and field strains of *S. littoralis* after treatment with tested insecticides.

A. Preparing samples for enzyme assays:

Caster-bean leaves were dipped for 30 seconds in an aqueous solution of each of the tested compounds at the LC_{50} level, then left to dry for 1 hour in room temperature before being offered to the 4th instar larvae of each of laboratory and field strains. Larvae were fed for 24 hours on the treated leaves, then transferred to fresh untreated leaves for one day. Haemolymph was obtained by removing one of the prolegs by forceps and applying gentle. Pressure was on the larvae with the fingers and take the haemolymph by syringe . The haemolymph was collected in cold tubes and stored in a refrigerator until the enzyme activities were determined (Sooker *et al.*, 1999 and Abd El-Mageed 2002).

B. Determination of enzyme activities:

Aliphatic or carboxyl esterase (Ali-E) was measured according to the method described by Sympthon *et al.* (1964) . Alpha esterases (α-E) and beta esterases (β-E) were determined according to the method of Van Asperen (1962). Acid phosphatase (AC-P) and alkaline phosphatase (ALK-P) were determined according to the method described by Powell and Smith (1954). Aspartate transferase (AST)[also known as glutamic oxaloacetic transaminase (GOT)] and Alanine transaminase (ALT) [also known as Glutamine pyruvic transaminase (GPT)] were determined colourimetrically according to the method of Reitman and Frankle (1957). 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).

RESULTS AND DISCUSSION

I. Toxicological studies:-

A. Susceptibility of the cotton leafworm to tested insecticides :

1. Susceptibility of the laboratory strain:

Data in Table (1) showed that Chlorpyrifos proved to be the most effective insecticides against 2nd instar larvae of the cotton leafworm *S. littoralis* (Boisd.) of the laboratory strain followed by Profenofos, Beta-cyfluthrin, Thiodicarb, Fenpropathrin and Carbaryl, respectively , showing the LC_{50} values of 0.572, 4.308, 5.083, 23.894, 25.250 and 1341.649 ppm, respectively. However, LC_{90} reached 1.14, 8.144, 44.653, 122.991, 109.612 and 3744.976 ppm, respectively. The toxicity index being 13.28, 11.25, 2.39, 2.27 and 0.04% for Profenofos, Beta-cyfluthrin, Thiodicarb, Fenpropathrin and Carbaryl (Based on LC_{50} of Chlorpyrifos 100.0%), respectively.

Concerning the efficiency of tested insecticides against 4th instar larvae of the laboratory strain, also Chlorpyrifos was the most effective insecticide giving LC_{50} value of 1.706 ppm followed by Profenofos, Beta-cyfluthrin, Fenpropathrin, Thiodicarb, and Carbaryl, respectively., they were 11.521,14.243, 36.780, 46.350 and 910.131 ppm, respectively. The corresponding LC_{90} reached 3.267, 31.069, 77.454, 115.568 , 133.988 and 13188.536 ppm, respectively. The toxicity index being 14.81, 11.98, 4.64, 3.68 and 0.19 % for Profenofos, Beta-cyfluthrin, Fenpropathrin, Thiodicarb and Carbaryl (Based on LC_{50} of Chlorpyrifos 100.0%), respectively.

2. Susceptibility of the field strain:

Data presented in Table (2) showed that Chlorpyrifos was more toxic to2nd instar larvae of the cotton leafworm *S. littoralis* (Boisd.) of the field strain than the other tested insecticides giving LC_{50} value of 1.005 ppm followed by Profenofos giving LC_{50} value of 3.841 ppm while Beta-cyfluthrin, Fenpropathrin, Thiodicarb and Carbaryl were considered to be the less toxic insecticides showing $LC_{50's}$ of 17.874, 23.722, 91.817, 4579.739 ppm , respectively. However, LC_{90} reached 1.606, 13.581, 92.631, 93.532, 788.969 and 20597.244 ppm , respectively. The toxicity index being 26.17, 5.62, 4.24, 1.09 and 0.02 % for Profenofos, Beta-cyfluthrin, Fenpropathrin, Thiodicarb and Carbaryl (Based on LC_{50} of Chlorpyrifos 100.0%), respectively.

Regarding the efficiency of tested insecticides against 4th instar larvae of the field strain, Chlorpyrifos was the most effective insecticide giving LC_{50} value of 6.348 ppm followed by Beta-cyfluthrin giving LC_{50} value of 7.514 ppm with toxicity index of 84.48 % based on LC_{50} of Chlorpyrifos 100.0%. Fenpropathrin showed an intermediate toxicity it gave LC_{50} value of 12.027 ppm with toxicity index of 52.78 %. Profenofos and Thiodicarb were the less toxic insecticides giving LC_{50} values of 41.853 and 49.179 ppm with toxicity index of 15.17 and 12.91 %, respectively. while Carbaryl was still the least toxic insecticides it gave LC_{50} value of 6000.859 ppm with toxicity index of 0.11 %. However, LC_{90} reached 14.954, 80.750, 29.355. 192.837, 135.959 and 20478.271 ppm of previous tested insecticides, respectively.

B. Resistance ratio of the cotton leafworm to tested insecticides:

Based on resistance ratio, the data in Table (3) revealed that the fold of resistance varied considerably according to the chemical structure of the studied insecticides and the instar of larvae. Data revealed that small differences in LC_{50} value were observed between the laboratory and the field strains as demonstrated by resistance ratio of 0.892, 0.939 and 1.757 fold for Profenofos , Fenpropathrin and Chlorpyrifos relating to 2nd instar larvae, respectively. Thiodicarb showed the highest resistance ratio, followed by Beta-cyfluthrin and Carbaryl reached 3.843, 3.516 and 3.414 fold on the same instar larvae , respectively.

The LC_{50} values of both the laboratory and the field strains on 4th instar larvae were remarkably higher compared with 2nd instar larvae. Carbaryl gave the highest resistance ratio, followed by Chlorpyrifos, Profenofos being 6.593, 3.721 and 3.633 fold , respectively. There were low level of resistance ratio to Thiodicarb, Beta-cyfluthrin and Fenpropathrin with degree of fold 1.061, 0.528 and 0.327, respectively.

Reviewing the obtained results, it could be concluded that the efficiency of different tested compounds against the 2nd and 4th instars larvae of the cotton leafworm *S. littoralis* (Boisd.) varied tremendously according to strain, the instar of larvae and the chemical structure of the tested toxicants. It is worth to note that, Chlorpyrifos seemed to be more potent compared with the other toxicants, while Carbaryl was recorded the least toxic insecticides in both tow instars and tow strains . Hereby, the high level of resistance was recorded with Carbaryl on 4th instar larvae (6.593 fold).

It is concluded that the diversity of resistance of the noctuid cotton pest *S. littoralis* to the different groups of pesticides is related to the type of pesticide and its

frequency of application in the field (El-Sayed and Abdallah , 1988) . In this respect, El-Saidy *et al.* (1990) cited that an organophosphorus-multiresistant strain (MR)of *S. littoralis* had 20-fold resistance to Profenofos , with the esterase activity of the susceptible strain was 20 times as susceptible to inhibition as the MR strain . El-Sayed and Sammour (1991) cited that the field population of cotton leafworm *S. littoralis* demonstrated more tolerant or/and less susceptibility to the tested insecticide comparing to laboratory strain. This is may be due to the exposure field population to many insecticides for many years, and most of them gave a wide range of cross-resistance to many other insecticides. While, Saleh (2000) found that Profenofos proved to be more effective than Chlorpyrifos against 4th instar larvae of *S. littoralis* from Dakahlia Governorate, the LC_{50} values were 620 & 1860 and 660 & 2100 ppm before & after spraying season, respectively, and the change of response reached to 3.00 and 3.18 for the same toxicants, respectively. Bernard *et al.* (1991) found that the growth rate of *S. littoralis* treated with Cyfluthrin was reduced and the reduction in weight gain was not compensated until the end of the 4th instar. Lagadic *et al.* (1991 and1993) studied the kinetics and elimination of the label in a pyrethroid-resistant strain and a susceptible strain (as control) of *S. littoralis* treated with a non-lethal dose of lindane 24 h before the application of the labelled Cyfluthrin . They suggested that pyrethroid resistance was partly due to enhanced metabolic capabilities or more likely to be due to a modification of the target site for Cyfluthrin than a pharmacokinetic mechanism . El-Bermawy *et al.* (1992) mentioned that at the end of the season of 1990 in Egypt, resistance in various strains of *S. littoralis*, increased for Deltamethrin and Fenpropathrin and decreased for alphamethrin and cis-cyfluthrin. There was an appreciable late-season decline in tolerance rates of a Behera population. Tolerance of Gharbia, Dakahlia and Sharkia populations increased by 9.7, 4.3- and 4.0-fold for Fenpropathrin, Deltamethrin and Cypermethrin, respectively . Lagadic *et al.* (1994) indicated that the decrease in toxicity of Cyfluthrin in lindane-pretreated larvae of the noctuid *S. littoralis* was not due to enhanced metabolism, but was more likely to be related to events within the nervous system. Mohamed *et al.* (1994) found that Methomyl was the most toxic insecticide, followed by Danitol [fenpropathrin] and Sumicidin [fenvalerate] on 3rd instar larvae of *S. littoralis*.

In Cyprus Charalambous and Iordanou (1997) evaluated the development of resistance in the field strain *S. littoralis* larvae to different classes of conventional insecticides , they found that the levels of resistance were high to Carbaryl (367-fold),

intermediate resistance was found to Fenpropathrin (213-fold), mild resistance was found to Cyfluthrin (45-fold)), Methomyl (14 fold) and Chlorpyrifos (6-fold). It is suggested that the use of Carbamate insecticide Methomyl and the organophosphates Chlorpyrifos could result in manageable levels of resistance to *S. littoralis*. Chlorpyrifos was more toxic than Thiodicarb against 4th instar larvae of *S. littoralis* with LC_{50} 3.50 and 5.25 ppm, respectively (Abd El-Latief, 2001) . Miles and Lysandrou (2002) found that the colony of *S. littoralis* which collected from the field of Lebanon in November 1999 was 6-10 times less sensitive to Chlorpyrifos which was within the range expected compared with the susceptible laboratory strain . Chlorpyrifos-ethyl (Dursban), which was introduced in Egypt in 1970, has had no record of field failure until now. Sebae (personal communication) believes that Dursban has more that one site of action (Temerak, 2002). The world market share of AChE inhibitors, i.e. organophosphates and carbamates , decreased from 71% in 1987 to 51% in 1999 (Nauen and Bretschneider , 2002)

The phenomenon of decrease of the resistance level of cotton leafworm *S. littoralis* to some conventional insecticides at last few years may be due to the ministry of agriculture was decision likely continue to encourage the reduction of pesticides consumption , moreover the widespread and intensive application of insect growth regulators and other new mode of action of classes of pesticides allowance of conventional insecticides in the spray programmers for the management of cotton pests at last decade in Egypt .

II. Biochemical impacts :-

A. Determination of esterases activities:

1. Aliphatic esterase (Ali-E):

Data in Table (4) revealed that all tested compounds gave reduction in aliphatic esterase (Ali-E) activity with ranged between -1.88 and -16.25% in the laboratory strain of *S. littoralis* (Boisd.) with the exception of Profenofos which gave an increase in the Ali-E activity , it was 12.50 % . Also the field strain gave the same pattern of response but with high level of reduction in the enzyme activity (between -28.57 and -43.27 %).

2. Non-specific esterases:

a. Alpha esterase (α-E):

Data in Table (4) indicated that Beta-cyfluthrin gave the highest decrease in alpha esterase (α-E) activity lower than control in the laboratory strain, it was -47.0 %

, while Chlorpyrifos and Carbaryl were recorded the lowest decrease in α-E activity, they gave -22.00 % . In field strain all tested compounds gave variable levels of reduction between -23.30 and -47.57 % lower than control .

b. Beta esterase (β-E):

Data in Table (4) indicated that Profenofos gave the highest increase in the beta esterase (β-E) activity higher than control in both the laboratory and the field strains, they were 328.57 and 388.24 %, respectively while the lowest increase in the β-E activity were recorded by Beta-cyfluthrin in the laboratory strain and Thiodicarb in the field strain, they gave 185.71 and 129.41% higher than control, respectively.

B. Determination of phosphatase activities:

1. Acid phosphatase (AC-P):

Data obtained in Table (5) revealed that three tested compounds (*i.e.,* Chlorpyrifos, Carbaryl and Fenpropathrin) gave an increase in acid phosphatase (AC-P) with values of 18.64, 13.64 and 9.09 % higher than control in the laboratory strain, respectively , in contrast the other three tested compounds (*i.e.,* Thiodicarb, Profenofos and Beta-cyfluthrin) gave decrease in the AC-P activity they gave -2.27, -5.00 and -5.91% lower than control, respectively. While all tested compounds gave the same pattern of changes in AC-P activity on the field strain with values ranged between 6.67 and 36.41 % .

2. Alkaline phosphatase (Alk-P):

All tested compounds gave the same pattern of reduction in alkaline phosphatase (Alk-P) activity on the laboratory strain with ranged between -16.36 and -32.95 % lower than control . On the other hand Profenofos gave the highest increase in Alk-P activity in the field strain, it was 23.08 % higher than control, while Thiodicarb and Fenpropathrin were recorded reduction in enzyme activity reached -12.62 and -3.38 % lower than control , respectively (Table 5) .

C. Determination of transaminases enzymes activities:

1. Aspartate transaminase (AST**):**

Data in Table (6) showed that Beta-cyfluthrin and Thiodicarb gave the highest reduction in aspartate transaminase (AST) activity in the laboratory strain with same value of -28.84 % lower than control , while the previous first compound was caused the lowest reduction in the AST activity in the field strain, it was -1.97 % in addition Profenofos recorded high level in reduction reached -15.27 % lower than control .

2. Alanine transaminase (ALT):

Thiodicarb, Fenpropathrin and Beta-cyfluthrin gave approximately same reaction on alanine transaminase (ALT) in the laboratory strain with values of -34.07, -34.51 and -34.07 %, respectively (Table 6) while Profenofos gave less reduction in ALT activity , it was -20.88 % , also the field strain gave the same trend of response but with high level of reduction in the enzyme activity (between -30.12 and -55.42%).

D. Determination of carbohydrate hydrolyzing enzymes activities:

1. Amylase:

Data in Table (7) indicated that Thiodicarb gave the highest reduction in amylase activity in the laboratory strain , it recorded -23.08 % .Chlorpyrifos, Carbaryl and Beta-cyfluthrin gave the same level of decrease in the amylase activity in previous strain, it was -10.77 % lower than control . In the field strain all tested compounds gave various decrease in amylase activity ranged between -5.26 and -47.37 % .

2. Invertase:

The results obtained in Table (7) noticed that all tested compounds gave reduction in the invertase activity between -29.78 and -35.56% lower than control in the laboratory strain, also the field strain gave the same trend of response but with less level of reduction in the enzyme activity (between -1.73and -9.83%) with exception of Carbaryl which gave a little increase of the enzyme activity reached 1.16 % .

Investigators consider that haemolymph is a good organ for studying the enzymes percentage and also gave about resistance mechanism (Abdel-Samie *et al.*, 1979 and Sookar *et al.*, 1999) . Farag (1981) found that Curacron and Cypermethrin resistant strains of *S. littoralis* showed 37 and 35% higher β-esterase activity than those corresponding values of laboratory strain . Likewise, Saleh (1981) and Abo-Elghar *et al.* (1984) concluded that esterases including Ali-E and α- and β-naphthylacetate esterases play an important role in pyrethroid and organophosphorus resistance in *S. littoralis*, the activities of these enzymes were higher in the resistant strains than in the laboratory one throughout the development of resistance. Salem (1998) revealed that there is no a pronounced effect of Cypermethrin or Profenofos on carboxyesterase activity in *S. littoralis* larvae, carboxyesterase might increase if these pesticides were used sequently and the insect exposed to them for several generations. Saleh (2000) measured the activity of aliesterase and non-specific esterases of 4th instar larval homogenate of *S. littoralis*, and indicated the occurrence

of decrease in both Ali-E and alpha-E, and increase in beta-E in strain collected after spraying season compared with strain collected before spraying season.

The obtained results are in agreement with those obtained by Motoyama and Dauterman (1974), they reported that phosphatases can hydrolyze organophosphorus insecticides by clearing off the leaving groups and results in the nontoxic dialkyl phosphorothioic acid or phosphoric acid. Farag (1978) found that acid phosphatase increased with development of resistance, while on the other hand, alkaline phosphatase showed a slight decrease in OP-resistant strains of *S. littoralis* rather than the laboratory strains. Also, Farag (1981) found that the acid and alkaline phosphatase activities in Cypermethrin and Curacron resistant laboratory strains of *S. littoralis* were higher than those of the laboratory strains. El-Guindy *et al.* (1985) stated that the higher acid phosphatase activity in the laboratory strain of the larvae of *S. littoralis* might explain why that strain was more tolerant than other strains for the tested compounds, Chlorpyrifos and Phosfolan . Saleh (2000) measured the activity of alkaline and acid phosphatase of 4th instar larval homogenate of *S. littoralis*, and indicated the occurrence of considerable increase in both enzymes in strain collected after spraying season compared with strain collected before spraying season.

In general, it was appeared from the aforementioned results that treatment of *S. littoralis* larvae with the six compounds gave great changes in transaminases activities, the changes were pronounced in ALT than in AST. The data also revealed that transaminases may be play an important role in insecticidal poisoning (Al-Elimi, 1994 and Abd El-Mageed , 2002) . The present results are in agreement with those of Farag (1981), who found that the resistant larvae of *S. littoralis* to Curacron and Cypermethrin showed higher transaminases enzymes activity than laboratory strain. . Saleh (2000) measured the activity of GOT (Official name : AST) and GPT (Official name : ALT) of 4th instar larval homogenate of *S. littoralis*, he indicated the occurrence of decrease in both enzymes in strain collected after spraying season compared with strain collected before spraying season.

Metabolism of carbohydrates are controlled mainly by invertase, amylase and trehalase enzymes, which play a principal role in the digestion and utilization of carbohydratases by insects (Wyatt, 1967 and Wigglesworth, 1973). The our data resulted from carbohydrate hydrolyzing enzymes revealed that a pronounced inhibition in the amylase and invertase activities as a result of treatment with tested compounds. About the same results were found by Radwan *et al.* (1985) they studied the effect of

different insecticidal treatments on the activity of carbohydrate hydrolyzing enzymes in the haemolymph of 6th instar larvae of *S. littoralis,* data indicated that repeated selection with insecticides resulted in varying levels of reduction in amylase activity, while invertase and trechalase showed variable levels of increase in their activity. El-Saidy *et al.* (1992) suggested that in 6th instar larvae of *S. littoralis* under laboratory conditions, methomyl and profenofos inhibit amylase activity indirectly, probably by acting on a physiological system, affecting amylase activity or secretion . Saleh (2000) indicated in field strain of 4th instar laraval homogenates of *S. littoralis* that measurements amylase and invertase activity were considerably decreased after spraying season compared with before spraying season.

Review the results, we can conclude that the change of response to tested insecticides could be associated with the increase in β-E and AC-P activities and decrease in Ali-E, α-E, ALK-P, AST, ALT, amylase and invertase activities

The previous studies don't provide complete a biochemical basis for relations between the effect of tested insecticides and the tested strains.Data gave us rather differences, these differential were probably causing haven't the genetic map of cotton leafworm, also not easily and probably impossible to apply the standardization of many physiological elements of the test organisms in all stages of research(Abd El-Mageed , 2002).

REFERENCES

1. Abbott, W.S. (1925): A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18 : 265 - 267.
2. Abd El-Latief, E.M.A. (2001): Integrated pest management for cotton in Dakahlia governorate. Ph.D. Thesis, Fac. of Agric., Mansoura University.
3. Abd EL-Mageed, A. E. M. (2002): Toxicological And Analytical Studies On Some Insecticides.Ph.D. Thesis, Fac. Of Agric., Mansoura University.
4. Abdel-Samie, M.B.; Salem, M.M. and Mohine, M. (1979): The identification and distribution of esterase and phosphatases isoenzyme in various tissues of susceptible and resistant strains to insecticides of cotton leafworm *Spodoptera littoralis* (Boisd.). Proc. 3rd Arab Pesticide Conf., 540-544.

5. Abo-Elghar, M.R.; Shabaan, A.M.; Abdel-Hafez, M.M. and El-Malla, M.A. (1984): Cholinesterase, aliesterase and non-specific esterases activity in relation to insecticide resistance of *Spodoptera littoralis* (Boisd.). J. Plant Diseases and Protection, 91:476-482.

6. Al-Elimi, M.H.A. (1994): Further toxicological and biochemical studies on cotton leafworm. Ph.D.Thesis, Fac. of Agric., Minoufiya Univ., Shibin El-Kom.

7. Bernard, L. ; Lagadic, L. and Echaubard, M. (1991): Effects of cyfluthrin on growth and food consumption in the 4th larval instar of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent, Vol. 56, No. 3b, pp. 1089-1097, 19 ref.

8. Charalambous, P. and Iordanou, N. (1997):Response of susceptible and resistant strains of *Spodoptera littoralis* to conventional insecticides in Cyprus. Technical Bulletin - Cyprus Agricultural Research Institute, No. 179, pp. 7 , 15 ref.

9. El-Bermawy, Z. A. ; El-Shiekh, A. A. ; Rashwan, M. H. and Radwan, H. S. A (1992): Pyrethroids resistance in *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) in Lower Egypt. Bulletin of the Entomological Society of Egypt, Economic Series , No. 19, pp. 41-51, 18 ref.

10. El-Defrawi, M.E.; Toppozada, A.; Mansour, N. and Zeid, M. (1964): Toxicological studies on Egyptian cotton leafworm *Prodenia litura* (F.). I. Suceptibility of different larval instar to insecticides. J. Econ. Entomol.,57(4):591-593.

11. El-Guindy, K.; El-Bakry, A.; Ahmed, N.S.; Moustafa, F.I. and El-Sebae, A.H. (1985): Interaction between toxicity and some biochemical targets for some insecticides against *Spodoptera littorals* larvae.Alex.Sci.Exch.,6(4):1-11.

12. El-Saidy, M. F. ; Auda, M. and Degheele, D. (1990): Esterases and resistance to organophosphorus insecticides in *Spodoptera littoralis* (Boisd.). Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent, Vol. 55, No. 2b, pp. 565-576, 23 ref.

13. El-Saidy, M. F. ; Auda, M. and Degheele, D. (1992): Effect of methomyl and profenofos on larval growth and digestive enzymes in *Spodoptera littoralis* (Boisd.).Mededelingen van de Faculteit

Landbouwwetenschappen, Rijksuniversiteit Gent, Vol. 57, No. 3A, pp. 825-832, 28 ref.

14. El-Sayed, E. I. and Abdallah, E. F. (1988): Resistance of cotton leafworm, *Spodoptera littoralis* (Boisd) populations, from different governorates of Egypt, to some insecticides.Annals of Agricultural Science (Cairo), Vol. 33, No. 2, pp. 1343-1352, 14 ref.

15. El-Sayed,, E.I. and Sammour, E.A. (1991): Insecticide resistance in field population of the Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.). Bull. Ent. Soc. Egypte, Econ. Ser., 17:77-83.

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

17. Farag, M. (1981): Some biochemical studies on cotton leafworm *Spodoptera littoralis*. Ph.D. Thesis, Fac. Agric., Cairo Univ., Egypt.

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

19. Horowitz, A. R.; Weintraub, P.G. and Ishaaya, I. (1997): ENMARIA : Status of Insecticide Resistance in Insect Pests in Israel . Contribution from the Agricultural Research Organization No. 2320-E, 1997 series.

20. Ishaaya, I. and Swirski, E. (1970): Invertase and amylase activity in the armoured scoles *Chrysomphalus aonidium*. J. Insect Physiol., 16:1599-1606.

21. Lagadic, L. ; Leicht, W. ; Ford, M. G. ; Salt, D. W. and Greenwood, R. (1993): Pharmacokinetics of cyfluthrin in *Spodoptera littoralis* (Boisd.) 1. in vivo distribution and elimination of [14C]cyfluthrin in susceptible and pyrethroid-resistant larvae. Pesticide Biochemistry and Physiology, Vol. 45, No. 2, pp. 101-115, 38 ref.

22. Lagadic, L. ; Weile, M. ; Leicht, W. and Echaubard, M. (1991): Pharmacodynamics of 14C-cyfluthrin in resistant and lindane-pretreated susceptible *Spodoptera littoralis* larvae. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent, Vol. 56, No. 3b, pp. 1119-1127, 14 ref.

23. Lagadic, L. ; Weile, M. ; Leicht, W. ; Salt, D. W. ; Greenwood, R. and Ford, M. G. (1994): Pharmacokinetics of cyfluthrin in *Spodoptera littoralis* (Boisd.). II. Effects of lindane pretreatment on the toxicity

and in vivo metabolism of cyfluthrin in susceptible larvae. Pesticide Biochemistry and Physiology, Vol. 48, No. 3, pp. 173-184, 54 ref.

24. Miles, M. and Lysandrou, M. (2002): Evidence for negative cross resistance to insecticides in field collected *Spodoptera littoralis* (Boisd.) from Lebanon in laboratory bioassays. Mededelingen - Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent, Vol. 67, No. 3, pp. 665-669, 3 ref.

25. Mohamed, A. I. ; Bhuyan, K. C. and El-Meslaty, M. (1994): Laboratory and field evaluations of selected chemical insecticides on the third larval stage of the cotton leafworm *Spodoptera littoralis* (Boisduvals) (Lepidoptera: Noctuidae) in Benghazi, Libya. Zoologischer Anzeiger, Vol. 233, No. 1/2, pp. 69-73, 9 ref.

26. Motoyama, N. and Dauterman (1974): The role of non-oxidative metabolism in organophosphorus resistance. J. Agric. Food Chem., 22(3):350-356.

27. Nauen, R. and Bretschneider, T. (2002): New modes of action of insecticides . Pesticide Outlook 12 , 241-245 .

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

29. Radwan, H.S.A.; Assal, O.M. and Samy, M.A. (1985): Ovicidal action potentiation of synthetic pyrethroids by insect growth regulators against the cotton leafworm, *Spodoptera littora;is* (Boisd.). Bull. Ent. Soc. Egypt, Econ. Ser., 14:275-283.

30. Reitman, S.M.D. and Frankel, S. (1957): A colorimetric method for the determination of serum glutamic-oxaloacetic and glutamic-pyruvic transaminase. Ann. J. Clin. Pathol., 28:56-62.

31. Saleh, A.A. (2000): Studies on changes in response of *Spodoptera littoralis* (Boisd.) for some insecticides and their relation with some enzymes activities. J. Agric. Sci. Mansoura Univ., 25(7):4667-4675.

32. Saleh, W.S. (1981): Comparative biochemical studies on resistant and susceptible strains of the Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.). Ph.D. Thesis, Fac. of Agric., Al-Azhar Univ., Egypt.

33. Salem, M.M.I. (1998): Recent approach to minimize pesticide dosages against cotton leafworm using certain adjuvants. M.Sc. Thesis, Fac. of Agric., (Saba Bacha), Alex. Univ.

34. Sookar, A.; Farghali, A.A. and El-Deeb, A.Y. (1999): Biochemical effects of the *Bacillus thuringiensis* (Bactospeine) on the 4th instar larvae cotton leafworm of *Spodoptera littoralis* (Boisd.). J. Agric. Sci. Mansoura Univ., 24(11):6937-6943.

35. Sun, Y.P. (1950): Toxicity index an improved method of comparing the relative toxicity of insecticides. J. Econ. Ent., 43:45-53.

36. Sympthon, D.R.; Bull, D.L. and Linquist, D.A. (1964): A semimicrotechnique for estimation of Ali esterase and cholinesterase activity in boll weevils. Ann. Ent. Soc. Am., 57(3):367-371.

37. Temerak S. A. (2002): Historical Records of Field Cotton Leafworm (*Spodoptera littoralis*) Resistance to Conventional Insecticides as Influenced by the Resistance Programs in Egypt from 1950-2002 . Resistance Pest Management Newsletter.

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

39. Wigglesworth, V.B. (1973): The Principles of Insect Physiology. 7th Ed. Chapman and Hall.

40. Wyatt, G.R. (1967): The biochemistry of sugars and polysaccharides in insects. Adv. Insect. Physiol., 4:287-360.

Table 1. Susceptibility of 2nd and 4th instars larvae of the laboratory strain of cotton leafworm, *Spodoptera littoralis* (Boisd.) to tested insecticides.

Tested compounds	2nd instar larvae				4th instar larvae			
	LC_{50}(ppm) its limits at 95%	LC_{90}(ppm) its limits at 95%	Slope	Toxicity index(%)	LC_{50} (ppm) its limits at 95%	LC_{90}(ppm) its limits at 95%	Slope	Toxicity index(%)
Profenofos	4.308 3.890 4.771	8.144 7.354 9.019	4.633 ± 0.517	13.28	11.521 9.828 13.506	31.069 26.503 36.421	2.975 ± 0.325	14.81
Chlorpyrifos	0.572 0.500 0.655	1.14 0.996 1.305	4.282 ± 0.497	100	1.706 1.502 1.938	3.267 2.876 3.711	4.542 ± 0.495	100
Carbaryl	1341.649 1163.547 1547.014	3744.976 3247.834 4318.215	2.875 ± 0.344	0.04	910.131 661.391 1250.302	13188.536 5193.15 22421E+1	1.104 ± 0.289	0.19
Thiodicarb	23.894 18.369 29.390	122.991 94.102 180.234	1.801 ± 0.204	2.39	46.35 39.492 53.197	133.988 113.424 166.094	2.78 ± 0.254	3.68
Fenpropathrin	25.25 18.930 33.680	109.612 82.177 146.206	2.01 ± 0.196	2.27	36.78 23.200 51.738	115.568 88.934 235.974	2.578 ± 0.237	4.64
Beta-cyfluthrin	5.083 3.529 6.977	44.653 30.738 72.183	1.358 ± 0.129	11.25	14.243 5.694 23.009	77.454 55.976 285.414	1.743 ± 0.177	11.98

Table 2. Susceptibility of 2nd and 4th instars larvae of the field strain of cotton leafworm, *Spodoptera littoralis* (Boisd.) to tested insecticides.

Tested compounds	2nd instar larvae				4th instar larvae			
	LC_{50}(ppm) its limits at 95%	LC_{90}(ppm) its limits at 95%	Slope	Toxicity index(%)	LC_{50}(ppm) its limits at 95%	LC_{90}(ppm) its limits at 95%	Slope	Toxicity index(%)
Profenofos	3.841 2.741 4.741	13.581 11.252 18.129	2.337 ± 0.343	26.17	41.853 34.285 55.748	192.837 120.840 423.433	1.932 ± 0.262	15.17
Chlorpyrifos	1.005 0.917 1.102	1.606 1.465 1.761	6.305 ± 0.704	100	6.348 5.309 8.422	14.954 10.624 27.054	3.444 ± 0.494	100
Carbaryl	4579.739 3717.429 5642.074	20597.24 16719.03 25375.06	1.963 ± 0.326	0.02	6000.859 4919.033 8194.058	20478.271 13262.330 43608.260	2.404 ± 0.343	0.11
Thiodicarb	91.817 71.113 120.393	788.969 452.797 2108.515	1.372 ± 0.207	1.09	49.179 38.998 58.145	135.959 117.067 165.243	2.902 ± 0.335	12.91
Fenpropathrin	23.722 17.306 29.457	93.532 77.728 120.406	2.151 ± 0.262	4.24	12.027 10.425 13.875	29.355 25.445 33.866	3.307 ± 0.388	52.78
Beta-cyfluthrin	17.874 10.870 24.191	92.631 74.769 125.727	1.794 ± 0.259	5.62	7.514 2.344 15.984	80.75 76.920 3385.191	1.243 ± 0.185	84.48

Table 3. Resistance ratio of 2nd and 4th instars larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.) to different tested compounds.

Tested compounds	2nd instar larvae			4th instar larvae		
	LC_{50} (ppm) Laboratory strain	Field strain	Resistance ratio (Fold)	LC_{50} (ppm) Laboratory strain	Field strain	Resistance ratio (Fold)
Profenofos	4.308	3.841	0.892	11.521	41.853	3.633
Chlorpyrifos	0.572	1.005	1.757	1.706	6.348	3.721
Carbaryl	1341.649	4579.739	3.414	910.131	6000.859	6.593
Thiodicarb	23.894	91.817	3.843	46.35	49.179	1.061
Fenpropathrin	25.25	23.722	0.939	36.78	12.027	0.327
Beta-cyfluthrin	5.083	17.874	3.516	14.243	7.514	0.528

Table 4. Esterases activity in haemolymph of the 4th instar larvae of laboratory and field strains of *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each insecticide.

Tested compounds	Aliphatic Esterase				Non-specific Esterase							
					Alpha esterase				Beta esterase			
	Laboratory strain		Field strain		Laboratory strain		Field strain		Laboratory strain		Field strain	
	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control
Profenofos	3.6	12.5	3.18	-35.1	2.86	-28.5	2.18	-47.09	1.2	328.6	1.66	388.24
Chlorpyrifos	3.14	-1.88	3.5	-28.57	3.12	-22	2.9	-29.61	0.98	250	1.2	252.94
Carbaryl	3.12	-2.5	3.16	-35.51	3.12	-22	2.16	-47.57	1.12	300	1.32	288.24
Thiodicarb	3.12	-2.5	3.12	-36.33	2.6	-35	2.38	-42.23	0.92	228.6	0.78	129.41
Fenpropathrin	2.9	-9.38	2.82	-42.45	2.88	-28	3.16	-23.3	1.1	292.9	1.3	282.35
Beta-cyfluthrin	2.68	-16.25	2.78	-43.27	2.12	-47	3.16	-23.3	0.8	185.7	1.12	229.41
Control	3.2		4.9		4		4.12		0.28		0.34	

Table 5. Phosphatase activity in haemolymph of the 4th instar larvae of laboratory and field strains of *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each insecticide.

Tested compounds	Acid phosphatase				Alkaline phosphatase			
	Laboratory strain		Field strain		Laboratory strain		Field strain	
	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control
Profenofos	4.18	-5.00	5.10	30.77	6.36	-27.73	8.00	23.08
Chlorpyrifos	5.22	18.64	5.32	36.41	6.18	-29.77	7.12	9.54
Carbaryl	5.00	13.64	4.20	7.69	7.12	-19.09	7.16	10.15
Thiodicarb	4.30	-2.27	5.22	33.85	7.36	-16.36	5.68	-12.62
Fenpropathrin	4.80	9.09	5.20	33.33	6.60	-25.00	6.28	-3.38
Beta-cyfluthrin	4.14	-5.91	4.16	6.67	5.90	-32.95	6.98	7.38
Control	4.40		3.90		8.80		6.50	

% of control = (Test - Control) / Control x 100

Table 6. Transaminase activity in haemolymph of the 4th instar larvae of laboratory and field strains of *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each insecticide.

Tested compounds	Aspartate transaminase				Alanine transaminase			
	Laboratory strain		Field strain		Laboratory strain		Field strain	
	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control
Profenofos	7.12	-17.21	6.88	-15.27	3.60	-20.88	3.48	-30.12
Chlorpyrifos	7.15	-16.86	6.90	-15.02	3.12	-31.43	3.00	-39.76
Carbaryl	7.14	-16.98	7.00	-13.79	3.22	-29.23	2.22	-55.42
Thiodicarb	6.12	-28.84	6.94	-14.53	3.00	-34.07	2.98	-40.16
Fenpropathrin	7.15	-16.86	7.62	-6.16	2.98	-34.51	2.92	-41.37
Beta-cyfluthrin	6.12	-28.84	7.96	-1.97	3.00	-34.07	3.00	-39.76
Control	8.60		8.12		4.55		4.98	

% of control = (Test - Control) / Control x 100

Table 7. Carbohydrate hydrolyzing enzymes activity in haemolymph of the 4th instar larvae of laboratory and field strains of *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each insecticide.

Tested compounds	Amylase				Invertase			
	Laboratory strain		Field strain		Laboratory strain		Field strain	
	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control
Profenofos	1.18	-9.23	1.10	-42.11	3.16	-29.78	3.18	-8.09
Chlorpyrifos	1.16	-10.77	1.60	-15.79	3.12	-30.67	3.16	-8.38
Carbaryl	1.16	-10.77	1.00	-47.37	2.90	-35.56	3.50	1.16
Thiodicarb	1.00	-23.08	1.80	-5.26	3.12	-30.67	3.40	-1.73
Fenpropathrin	1.14	-12.31	1.16	-38.95	3.10	-31.11	3.36	-2.89
Beta-cyfluthrin	1.16	-10.77	1.14	-40.00	2.98	-33.78	3.12	-9.83
Control	1.30		1.90		4.50		3.46	

% of control = (Test - Control) / Control x 100

مقارنة التأثيرات السامة و البيوكيميائية لبعض المبيدات التقليدية على دودة ورق القطن

أحمد السيد محمود عبد المجيد[1] ، إيهاب محمد انور[1] ، ليلى رجب على الجوهرى[2]

١. *معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي-الجيزة – مصر*

٢ . *كلية الزراعة – جامعة المنصورة*

أجريت هذه الدراسة بهدف دراسة التأثيرات السامة و البيوكيميائية لاثنين من المبيدات الفوسفورية (بروفينوفوس و كلوربيروفوس) و اثنان من المبيدات الكاربامانية (كاربارايل وثيوديكارب) و اثنان من المبيدات البيرثرينية المصنعة(فينبروباثرين و بيتا ثفلوثرين) على العمر اليرقى الثانى والرابع لسلالة معملية وأخرى حقلية (تم جمعها من محافظة الدقهلية) لدودة ورق القطن.أشارت النتائج إلى وجود اختلافات فى درجات مقاومة السلالة الحقلية تبعا للتركيب الكيماوى للمركبات المختبرة و العمر اليرقى وقد أظهرت النتائج إلى وجود اختلافات صغيرة في قيم التركيز النصفى المميت بين السلالتين حيث قدرت قيم معدل المقاومة ٠,٨٩٢ و ٠,٩٣٩ و ١,٧٥٧ ضعف تجاه بروفينوفوس و فينبروباثرين و كلوربيروفوس على العمر اليرقى الثانى على الترتيب في حين أعطى ثيوديكارب اعلي معدل للمقاومة يليه كل من بيتا ثفلوثرين و كاربارايل بقيم ٣,٨٤٣ و ٣,٥١٦ و ٣,٤١٤ ضعف على الترتيب . بينت النتائج أن قيم التركيز النصفى المميت للمركبات المختبرة لكل من السلالتين على العمر اليرقى الرابع كانت أعلى مقارنة بالعمر اليرقى الثاني حيث سجل كاربارايل أعلى معدل للمقاومة يليه كلوربيروفوس و بروفينوفوس بقيم ٦,٥٩٣ و ٣,٧٢١ و ٣,٦٣٣ ضعف على الترتيب وكان هناك مستوى اقل من المقاومة لكل من ثيوديكارب و بيتا ثفلوثرين و فينبروباثرين بقيم ١,٠٦١ و ٠,٥٢٨ و ٠,٣٢٧ على الترتيب. وكان الهدف الثانى من الدراسة تقدير مدى تأثير المركبات الستة المختبرة على نشاط إنزيمات الإستيريزات (الإستيريز الاليفاتى و الفا و بيتا إستيريز) و إنزيمات الفوسفاتيزات (الفوسفاتيز الحامضى و القاعدى) و الانزيمات الناقلة لمجاميع الامين (الاسبرتات و الالانين) و الإنزيمات المحللة للكربوهيدرات على كل من السلالتين