TOXICITY IMPACTS OF CERTAIN INSECT GROWTH REGULATORS ON SOME BIOCHEMICAL ACTIVITIES OF THE COTTON LEAFWORM

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Abstract

The present work was conducted to study the susceptibility of both laboratory and field strain of 2nd and 4th instars larvae of the cotton leafworm *Spodoptera littoralis* (Boisd.) to six insect growth regulators (Diflubenzuron, Tebufenozide, Hexaflumuron, Flufenoxuron, Chlorfluazuron and Lufenuron) . The obtained data revealed that based on resistance ratio, the fold of resistance varied considerably according to the chemical structure of the studied IGRs and the instar of larvae . The high differences in LC_{50} values were observed between the laboratory and the field strains as demonstrated by resistance ratio of 162850, 17680 and 2145 fold for Flufenoxuron , Lufenuron and Chlorfluazuron relating to 2nd instar larvae, respectively. Resistance ratio was recorded 217.60 and 20.40 for Hexaflumuron and Diflubenzuron , respectively. Tebufenozide gave low resistance ratio with degree of fold ٢,٨١ . Moreover data exposed that the LC_{50} values of both the laboratory and the field strains on 4th instar larvae were remarkably higher compared with 2nd instar larvae and gave the same arrangement between tested compounds . Flufenoxuron showed the highest resistance ratio, followed by Lufenuron reached ٨٨٦٢٧١and 8198.804 fold on the 4th instar larvae , respectively. Chlorfluazuron and Hexaflumuron gave the moderately levels of resistance ratio with degree of fold ٥٢٢,٩٥٠ and 181.131. In addition there were low level of resistance ratio to Diflubenzuron and Tebufenozide with degree of fold 17.51 and 5.390, respectively . In addition the effect of tested IGRs 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

Insect Growth Regulators (IGRs), also called third-generation insecticides, are pesticides that disrupt the normal activity of the endocrine or hormone system of insects, affecting the development, reproduction, or metamorphosis of the target insect. They have a much slower mode of action than synthetic chemical insecticides. IGRs include juvenile hormone (JH) mimics and chitin synthesis inhibitors (CSIs).CSIs,

such as hexaflumuron, lufenuron and diflubenzuron, inhibit the production of chitin, a major component of the insect exoskeleton. Insects treated with CSIs become unable to synthesize new cuticle, and therefore unable to successfully molt into the next stage. CSIs may be toxic to other arthropods, and IGR metabolites may have adverse effects on vertebrates due to their ability to bind to certain members of the nuclear hormone receptor family. It was originally thought that insects would be unable to develop resistance to molecules that mimic their own hormones, but there is already evidence of developing resistance to several IGRs, including kinoprene, pyriproxifen, and diflubenzuron. Resistance seems to result from decreased penetration and increased metabolism of the compound (Hoffman and Lorenz, 1998) .The benzoylphenyl ureas constitute a class of the IGRs that interfere with insect growth and development by inhibiting chitin synthesis in insects (Post and Vincent, 1973). The cotton leafworm *Spodoptera littoralis* (Boisd.) is a major polyphagous key pest in Egypt. It is active all year round without hibernation period and attacking cotton as well as more than 29 hosts from other crops and vegetables. In last few year, Ministry of Agriculture in Egypt doesn't recommend using conventional insecticide applications during the egg masses period so as to conserve the natural enemy populations, meanwhile using insect growth regulators (IGR_S) is considered as the possible alternative way for controlling the newly hatched larvae (Raslan, 2002). 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 six insect growth regulators. In addition assessment the effect of tested IGRs 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:-

The present work was conducted to study 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 insect growth regulators .

A. Tested insect growth regulators:-

1. Diflubenzuron 25% W.P. (Dimilin®)

Chemical name : *N*–[[(4-chlorophenyl) amino]carbonyl]-2,6-difluorobenzamide.

2. Tebufenozide 24% F.L. (Mimic®)
Chemical name: 3,5-dimethylbenzoic acid 1-(1,1-dimethylethyl)-2-(4-ethylbenzoyl)hydrazide.

3. Hexaflumuron 10 % E.C. (Consult®)
Chemical name : *N* –[[[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy) phenyl] amino] carbonyl]-2,6-difluorobenzamide.

4. Flufenoxuron 10 % E.C. (Cascade®)
Chemical name : *N*–[[[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl] amino] carbonyl]-2,6-difluorobenzamide.

5. Chlorfluazuron 5% E.C. (Atabron®)
Chemical name :*N*–[[[3,5-dichloro-4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy] phenyl] amino] carbonyl]-2,6-difluorobenzamide.

6. Lufenuron 5 % E.C. (Sorba®)
Chemical name : *N* –[[[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoroethoxy) phenyl] amino] carbonyl]-2,6-difluorobenzamide

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 IGR 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 three days. 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 4th 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 insect growth regulators .

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 three days. 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 IGRs :

1. Susceptibility of the laboratory strain:

The present data in Table (1) showed that Flufenoxuron proved to be the most effective IGR against 2nd instar larvae of the cotton leafworm *S. littoralis* (Boisd.) of the laboratory strain followed by Lufenuron, Chlorfluazuron, Hexaflumuron, Diflubenzuron and Tebufenozide , respectively , showing the LC_{50} values of 0.00002, 0.000025, 0.0002, 0.040, 15.415 and 25.098 ppm, respectively. However, LC_{90} reached 0.201, 0.041, 0.260, 709.695, 477.788 and 193.668 ppm, respectively. The toxicity index being 80.00, 10.00, 0.05, 1.30E-4 and 7.97E-5% for Lufenuron, Chlorfluazuron, Hexaflumuron, Diflubenzuron and Tebufenozide (Based on LC_{50} of Flufenoxuron 100.0%), respectively.

In relation to the efficiency of tested IGRs against 4th instar larvae of the laboratory strain, as well Flufenoxuron was the most effective IGR giving LC_{50} value of 0.016 ppm followed by Lufenuron, Hexaflumuron, Chlorfluazuron, Tebufenozide , and Diflubenzuron, respectively., they were 0.281 , 3.820, 10.686, 147.491 and 162.398 ppm, respectively. The corresponding LC_{90} reached 12592.756, 1451.342, 22019.071, 1573.332 , 2575.096 and 1351.777 ppm, respectively. The toxicity index being 56.94, 0.42, 0.15, 0.01 and 0.01% for Lufenuron, Hexaflumuron, Chlorfluazuron, Tebufenozide , and Diflubenzuron (Based on LC_{50} of Flufenoxuron 100.0%), respectively .

2. Susceptibility of the field strain:

Chlorfluazuron and Lufenuron were recorded high toxic to 2nd instar larvae of the cotton leafworm *S. littoralis* (Boisd.) of the field strain than the other tested IGRs (Table 2) giving LC_{50} value of 0.429 and 0.442 ppm , respectively followed by Flufenoxuron and Hexaflumuron giving LC_{50} value of 3.257 and 8.704 ppm, respectively while Tebufenozide and Diflubenzuron were considered to be the less toxic insecticides showing $LC_{50's}$ of 70.432 and 314.441 ppm , respectively. However, LC_{90} reached 4765.037, 2252.464, 7789.813, 14598.921, 497.290 and 3463.325 ppm , respectively. The toxicity index being 97.06, 13.17, 4.93, 0.61and 0.14% for

Lufenuron, Flufenoxuron, Hexaflumuron, Tebufenozide and Diflubenzuron (Based on LC_{50} of Chlorfluazuron 100.0%), respectively.

In a different trend the efficiency of tested IGRs against 4th instar larvae of the field strain, Hexaflumuron was the most effective IGR giving LC_{50} value of 691.919 ppm followed by Tebufenozide giving LC_{50} value of 794.928 ppm with toxicity index of 87.04% based on LC_{50} of Hexaflumuron 100.0%. Lufenuron and Diflubenzuron showed an intermediate toxicity they gave LC_{50} value of 2303.864 and 2843.771 ppm with toxicity index of 30.03 and 24.33%, respectively . Chlorfluazuron was the less toxic IGRs giving LC_{50} values of 5705.758 ppm with toxicity index of 12.13 %, while Flufenoxuron recorded the least toxic IGR it gave LC_{50} value of 14181.936 ppm with toxicity index of 4.88 %. However, LC_{90} reached 43346.998, 10964.449, 5272400, 18108.001, 24721E+2 and 52737E+4 ppm of previous tested IGRs, respectively.

B. Resistance ratio of the cotton leafworm to tested IGRs:-

The obtained data in Table (3) revealed that based on resistance ratio, the fold of resistance varied considerably according to the chemical structure of the studied IGRs and the instar of larvae. The high differences in LC_{50} value were observed between the laboratory and the field strains as demonstrated by resistance ratio of 162850, 17680 and 2145 fold for Flufenoxuron , Lufenuron and Chlorfluazuron relating to 2nd instar larvae, respectively. Resistance ratio was recorded 217.60 and 20.٤٠ for Hexaflumuron and Diflubenzuron , respectively. Tebufenozide gave low resistance ratio with degree of fold ٢,٨١.

Moreover data exposed that the LC_{50} values of both the laboratory and the field strains on 4th instar larvae were remarkably higher compared with 2nd instar larvae and gave the same arrangement between tested compound . Flufenoxuron showed the highest resistance ratio, followed by Lufenuron reached ٨٨٦٢٧١and 8198.804 fold on the 4th instar larvae , respectively. Chlorfluazuron and Hexaflumuron gave the moderately levels of resistance ratio with degree of fold ٥٢٢,٩٥٠and 181.131. In addition there were low level of resistance ratio to Diflubenzuron and Tebufenozide with degree of fold 17.51 and 5.390, respectively .

Generally, 2nd instar larvae was more sensitive to tested IGRs than 4th instar larvae and tested IGRs effectiveness on the laboratory strain was remarkably higher compared with the field strain of *S. littoralis* (Boisd.) .

In this respect , the high potency of Chlorfluazuron against *S. littoralis* and various insects, together with its low toxicity to man and the environment, renders this

compound a potential control agent for important agricultural pests (Ishaaya et al., 1986). El-Ghareeb et al. (1988) found that the LC_{50} for Chlorfluazuron for 3rd instar larvae of *S. littoralis* fed on treated leaves was 0.0085 ppm and toxicity decreased with larval age. Emam and Degheele (1988) stated that Diflubenzuron was less toxic to 1st instar than to 3rd instar larvae of both strains of *S. littoralis*, LC_{50}s being 31.9 and 19.5 ppm , respectively, for the susceptible strain and 45.2 and 34.9 ppm for the resistant strain. With Chlorfluazuron, LC_{50}s of 0.13 and 0.36 ppm were recorded for 1st and 3rd instars susceptible larvae, and 0.03 and 0.04 ppm for resistant larvae, respectively. Guyer and Neumann(1988) cited that when injected in the larvae of *S. littoralis*, Chlorfluazuron was more toxic than Diflubenzuron by 2 orders of magnitude. The enhanced toxicity of Flufenoxuron to *S. littoralis* compared with Diflubenzuron can probably be attributed to its slower metabolism and reduced excretion (Clarke and Jewess 1990). Emam and Degheele (1992) cited that the compounds could be classified into 2 groups using mortality curves and LC_{50}s. The first, with low LC_{50}s and high toxicity to the larval stage of *S. littoralis*, includes Hexaflumuron more than or equal to Chlorfluazuron more than or equal to Teflubenzuron. The second, with a high LC_{50}-value and a lower toxicity to the larval stage, includes Diflubenzuron in laboratory assays. El-Ghareeb (1992) found that Chlorfluazuorn was more toxic than Diflubenzuron against 3rd and 5th instars larvae of *S. littoralis* in the laboratory. Ishaaya et al. (1995) indicate that Tebufenozide is potentially potent insecticide for controlling larvae of *S. littoralis* . Smagghe and Degheele (1997) observed that intoxicated larvae of *S.littoralis* showed signs of premature and lethal moulting within 24 h of treatment with Tebufenozide . Bayoumi et al. (1998) found that that 3rd instar were more sensitive to Chlorfluazuron and Flufenoxuron, compared with 5th instar of *S. littoralis*, regardless of the strain used. Percentage accumulative mortality varied according to the compound, concentration, larval instar and/or strain studied. Gobbi et al. (2000) found that the weight of treated larvae of *S. littoralis* with Tebufenozide was significantly reduced compared to that of untreated larvae. Rao and Subbaratnam (2000) cited that Flufenoxuron was relatively more toxic than Diflubenzuron and Lufenuron on the third instar larvae of S. exigua. Özmen and Kilincer (2002) mentioned that Diflubenzuron applied to 5-day-old larvae of *S. littoralis* resulted in LC_{50} values of 51.15 ppm for the 3rd days. Diflubenzuron applied to 10-day-old larvae recorded LC_{50} values of 442.75 ppm, for the 3rd days . Hexaflumuron was applied to 5- and 10-day-old larvae, by feeding method recorded LC_{50} values of 5.43 and 3.36 ppm,

respectively, for the 2nd day.Decombel et al.(2004) found that toxicity bioassays Lufenuron was the most toxic (LC_{50}=0.098 ppm) to *S. exigua* compared with different groups of insecticides with different modes of actions.

The resistance level of *S. littoralis* to the urea derivatives alone fluctuated from one year to another . The emergence of resistance to urea derivatives alone or in mixtures with insecticide was expected to occur (Keddis et al.,1986). It was originally thought that insects would be unable to develop resistance to molecules that mimic their own hormones, but there is already evidence of developing resistance to several IGRs, including kinoprene, Pyriproxifen and Diflubenzuron. Resistance seems to result from decreased penetration and increased metabolism of the compound (Hoffman and Lorenz, 1998) . The remaining part of Diflubenzuron was mostly hydrolysed to 4-chlorophenyl urea and 4-chloroaniline (62% of metabolites) which (Laecke and Degheele 1991).

However it is emphasized that the resistance levels cannot be considered serious, and insecticides in this class should be used rationally to maintain their efficacy for as long as possible. It is concluded that the high levels of resistance to some compounds (i.e., Flufenoxuron) may be is related to the type of pesticide and its widespread and intensive application of this class of pesticides(IGRs) at last decade in the spray programmers for the management of cotton pests in Egypt .

II. Biochemical impacts :-

A. Determination of esterases activities:

1. Aliphatic esterase (Ali-E):

The effect of tested IGRs on aliphatic esterase (Ali-E) activity in the laboratory strain of *S. littoralis* (Boisd.) (Table 4) revealed that Flufenoxuron gave the highest increase , it reached to a maximum level of 18.75%, while Chlorfluazuron, Tebufenozide and Diflubenzuron gave 16.25, 7.50 and 3.75% higher than control, respectively. In contrast, Hexaflumuron and Lufenuron gave a little decrease in Ali-E activity, they were -1.25 and -3.12% lower than control, respectively. Considering the field strain, the data indicate that all the tested IGRs gave the same pattern of changes in Ali-E activity, they gave a decrease in activity between -20.00 and -36.73 % lower than control.

2. Non-specific esterases:

a. Alpha esterase (α-E):

Lufenuron recorded the highest reduction in alpha esterase (α-E) activity on the laboratory strain , it was -45.50% lower than control . Regard to the field strain, Flufenoxuron and Hexaflumuron gave the highest and same pattern of changes in α-E activity, they gave -48.54 and -48.06%, but the same compounds gave less reduction in α-E activity on the laboratory strain, they were -22.00 and -21.00 % lower than control, respectively (Table 4).

b. Beta esterase (β-E):

The data obtained in Table (4) revealed that Lufenuron gave the highest increase in the beta esterase (β-E) activity higher than control in the laboratory strain, it was 400.00 %, while Tebufenozide was recorded the highest increase in the field strain reached 464.71 % higher than control , at the same time as Flufenoxuron was recorded the lowest increase in the β-E activity at both two strains with values 214.29 and 117.65%, respectively.

B. Determination of phosphatase activities:

1. Acid phosphatase (AC-P):

The data in Table (5) revealed that all the tested IGRs caused increase in acid phosphatase (AC-P) activity in the laboratory strain between 11.36 and 18.64 % . In contrary, Tebufenozide gave a little decrease in AC-P activity , it was -4.09 % lower than control. In the field strain Flufenoxuron gave the highest increase in AC-P activity followed by Hexaflumuron, Chlorfluazuron and Lufenuron with values 32.31, 31.79, 31.28 and 28.21% higher than control , respectively.

2. Alkaline phosphatase (Alk-P):

Data showed in Table (5) revealed that all tested IGRs gave reduction in alkaline phosphatase (Alk-P) activity in the laboratory strain between -19.09 and -22.73 % . In the field strain , Lufenuron and Diflubenzuron gave increase in Alk-P activity with values 9.23 and 6.46 %, respectively while Hexaflumuron, Chlorfluazuron and Flufenoxuron gave decrease in the Alk-P activity its reached to -8.00, -4.92 and -4.31 % lower than control, respectively. Regards to Tebufenozide, no changes compared to control was observed.

C. Determination of transaminases enzymes activities:

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

The data in Table(6) indicate that all the tested IGRs gave the same pattern of changes in aspartate transaminase (AST) activity of the laboratory strain, they gave a decrease in activity between -9.07and -28.14% lower than control . Studying the effect of tested IGRs on AST activity in the field strain revealed that Chlorfluazuron gave the highest reduction, it reached to a maximum level of -19.21%, while Hexaflumuron, Lufenuron, Flufenoxuron and Diflubenzuron gave a moderate reduction in AST activity of -13.79, -13.79, -12.56 and -11.95%, respectively. In addition, Tebufenozide gave the lowest reduction in AST activity, it was -3.69% lower than control.

2. Alanine transaminase (ALT):

Data in Table (6) indicate that Flufenoxuron gave the highest decrease in the alanine transaminase (ALT) activity lower than control in both the laboratory and the field strains, they were -34.51 and -42.17 %, respectively while Lufenuron and Diflubenzuron was recorded the lowest decrease, they gave (-13.85 and -23.29 %) and (-20.44 and -21.67 %) in the same strains, respectively.

D. Determination of carbohydrate hydrolyzing enzymes activities:

1. Amylase:

From the results obtained in Table (7) it could be noticed that all tested IGRs gave reduction in the amylase activity between -7.69 and -13.85% lower than control in the laboratory strain, also the field strain gave the same trend of response but with high level of reduction in the enzyme activity (between-36.84 and -47.37%).

2. Invertase:

Data in Table (7) indicate that Hexaflumuron and Lufenuron gave the same level of decrease in the invertase activity lower than control in the laboratory strain, it was -30.67 % while Chlorfluazuron was recorded the lowest decrease, it reached -20.00%, but the same compound gave the highest reduction in enzyme activity on the field strain with value -13.29 %, at the same time as Flufenoxuron was recorded the lowest decrease in the enzyme activity at previous strain with value -4.62 % .

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).Esterase played important roles in the insecticide resistance of the pest (Wu ShiChang et al., 1995). Abdel-Megeed, et al. (2000) found

that the increase in the α- and β-esterase activity in the Menofia field strain of *S. littoralis* was higher than that of the laboratory strain. Chlorfluazuron and Flufenoxuron increased the activity of β-esterase. Hamdy and Azab (2002) found that the enzyme activity of alphaesterase in *S. littoralis* after treatment with IGRs was increased with Chlorfluazuron and Hexaflumuron , except in El-Menia field strain treated with Hexaflumuron was decreased. The levels of beta-esterase enzyme were decreased in field strain with all tested compounds. Except in susceptible strain treated with Chlorfluazuron, the reduction was more obvious in El-Menia field strain than Bany Sweif strain. YuXian et al.(2003) mentioned that inhibition ratios of 60.56 % for carboxylesterase activity after the second-instar larvae of *S. exigua* was fed with leaves treated with Chlorfluazuron (50 mg/litre).

Abdel-Hafez et al. (1993) cited that the IGR/insecticide mixtures or their residual gave variable decrease in the activity of alkaline phosphatase much lower than control, while acid phosphatase enzyme gave higher increase in its activity in the field strain larvae of *S. littoralis.* As a general trend acid phosphatase activity appeared to be lower in field strains of *S. littoralis* than susceptible strain with IGRs and binary mixtures.The activity of alkaline phosphatase increased significantly in Bany Sweif strain (Hamdy and Azab 2002).

The effect of insecticides on enzymes catalyzing amino acids metabolism in insect might be important (Kamin and Handler, 1957) . 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 GPT (Official name : ALT) than in GOT (Official name : AST). The results also showed that the effect of each compound on AST activity was the same that obtained from ALT activity. The data also revealed that transaminases may be play an important role in insecticidal poisoning (Al-Elimi, 1994 and Abd El-Mageed , 2002). Abdel-Hafez et al. (1988) found that there was a reduction in the level of protein and free amino acids in laboratory and resistant strains of *S. littoralis* as a result of IGRs (Diflubenzuron and Triflumuron) treatments, also they found that the changes in GOT and GPT activities were in harmony with the changes in protein and free amino acids. Abdel Hafez et al. (1993) indicated that the treatment laboratory strain larvae of *S. littoralis* with two insecticides (Cyanophos and Methomyl), two IGRs (Diflubenzuron and Flufenoxuron) and their combined mixtures caused variable reduction in GOT, while GPT enzyme exhibited much increase in its activity in relation

to control. El-Kordy et al. (1995) found that GOT enzyme was significantly increased, while there was a significant reduction in the level of GPT on the 4th and 6th instars larvae of *S. littoralis* after the treatment with IGR compounds (Pyriproxfen, Flufenoxuron and Teflubenzuron) .

The data resulted from carbohydrate hydrolyzing enzymes revealed that a pronounced inhibition in the amylase and invertase activities as a result of treatment with tested IGRs. About the same results were found by Radwan et al. (1984), they measured the activity of amylase, invertase and trehalase in the haemolymph of *S. littoralis* with insecticide-growth regulator mixtures, and found that treatment with the Diflubenzuron-Chlorpyrifos alone or in a sequential system with other chemicals led to a reduction in the activity of all three enzymes. Diflubenzuron reduced amylase activity *in vivo* in *S. littoralis*, reduction in activity being positively correlated with concentration, but invertase, trehalase and protease [proteinase] were not affected. In 6th instar larvae, Diflubenzuron probably inhibits amylase indirectly by acting on a physiological system affecting amylase activity or secretion (El-Saidy and Degheele 1990).

Review the results, we can conclude that the change of response to tested IGRs 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).

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

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(%)
Diflubenzuron	15.415 8.321 23.694	477.788 263.326 1250.267	0.859 ± 0.120	1.30E-04	162.398 104.991 209.311	1351.777 852.281 3512.432	1.393 ± 0.264	0.01
Tebufenozide	25.098 18.533 32.513	193.668 133.087 332.839	1.444 ± 0.163	7.97E-05	147.491 99.941 198.621	2575.096 1252.102 11264.915	1.032 ± 0.189	0.01
Hexaflumuron	0.04 0.0011 0.447	709.695 177.138 44435E+2	0.301 ± 0.037	0.05	3.82 0.682 9.701	22019.07 2045.735 10818E+3	0.341 ±0.080	0.42
Flufenoxuron	0.00002 10564E- 11 0.0002	0.201 0.049 2.732	0.32 ± 0.070	100	0.016 0.0001 0.130	12592.76 406.893 22433E+4	0.217 ±0.056	100
Chlorfluazuron	0.0002 11991E- 10 0.0017	0.26 0.070 1.203	0.405 ± 0.085	10	10.686 2.463 22.312	1573.332 389.988 73096.209	0.591 ±0.148	0.15
Lufenuron	0.000025 31902E- 10 19591E-8	0.041 52319E-7 0.321	0.398 ±0.155	80	0.281 0.017 1.067	1451.342 127.185 1717600	0.345 ±0.090	56.94

Toxicity index = LC_{50} of the most effective compound / LC_{50} of the tested compound x 100

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

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(%)
Diflubenzuron	314.441 225.408 438.640	3463.33 2482.699 4831.282	1.23 ± 0.176	0.14	2843.77 1365.597 145630	18108 4149.832 65539000	1.594 ±0.568	24.33
Tebufenozide	70.432 53.812 89.031	497.29 332.059 940.882	1.51 ± 0.195	0.61	794.928 552.335 1144.071	10964.45 7618.360 15780.186	1.125 ±0.262	87.04
Hexaflumuron	8.704 3.534 21.885	14598.9 2398.808 305270	0.397 ± 0.056	4.93	691.919 389.707 1228.492	43347 24414.162 76961.979	0.713 ±0.296	100
Flufenoxuron	3.257 0.128 469.564	7789.81 14375.469 34797E+6	0.379 ±0.044	13.17	14181.9 840.899 11466E+3	5.27E+08 15420E+2 16699E+11	0.28 ±0.064	4.88
Chlorfluazuron	0.429 0.017 11.573	4765.04 3852.040 11588E+5	0.317 ±0.040	100	5705.76 1733.531 18779.984	2.47E+06 75107E+1 81366E+2	0.486 ±0.218	12.13
Lufenuron	0.442 0.113 1.731	2252.46 575.028 8823.207	0.346 ± 0.045	97.06	2303.86 399.538 143390	5272400 99314.522 16233E+7	0.381 ±0.088	30.03

Toxicity index = LC_{50} of the most effective compound / LC_{50} of the tested compound x 100

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

Resistance ratio = LC_{50} of field strain / LC_{50} of laboratory strain

Tested compounds	2nd instar larvae			4th instar larvae		
	LC_{50} (ppm)		Resistance ratio (Fold)	LC_{50} (ppm)		Resistance ratio (Fold)
	Laboratory strain	Field strain		Laboratory strain	Field strain	
Diflubenzuron	15.415	314.441	20.40	162.398	2843.77	17.51
Tebufenozide	25.098	70.432	2.81	147.49	794.928	5.390
Hexaflumuron	0.040	8.704	217.60	3.820	691.919	181.131
Flufenoxuron	0.00002	3.257	162850	0.016	14181.9	886.371
Chlorfluazuron	0.0002	0.429	2145.000	10.686	5705.75	533.95
Lufenuron	0.000025	0.442	17680	0.281	2303.86	8198.804

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 compound.

Tested compounds	Aliphatic Esterase				Non-specific Esterases							
					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
Diflubenzuron	3.32	3.75	3.92	-20	2.68	-33	3.1	-24.76	1.2	329	0.88	158.82
Tebufenozide	3.44	7.5	3.14	-35.92	2.9	-27.5	3	-27.18	1.3	364	1.92	464.71
Hexaflumuron	3.16	-1.25	3.16	-35.51	3.16	-21	2.14	-48.06	1.2	329	1.22	258.82
Flufenoxuron	3.8	18.75	3.12	-36.33	3.12	-22	2.12	-48.54	0.88	214	0.74	117.65
Chlorfluazuron	3.72	16.25	3.1	-36.73	3	-25	2.18	-47.09	1.12	300	1.1	223.53
Lufenuron	3.1	-3.12	3.7	-24.49	2.18	-45.5	2.36	-42.72	1.4	400	1.12	229.41
Control	3.2		4.9		4		4.12		0.28		0.34	

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

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 compound.

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
Diflubenzuron	4.90	11.36	3.98	2.05	6.80	-22.73	6.92	6.46
Tebufenozide	4.22	-4.09	4.22	8.21	7.10	-19.32	6.44	-0.92
Hexaflumuron	5.00	13.64	5.14	31.79	6.90	-21.59	5.98	-8.00
Flufenoxuron	4.92	11.82	5.16	32.31	6.96	-20.91	6.22	-4.31
Chlorfluazuron	5.22	18.64	5.12	31.28	7.12	-19.09	6.18	-4.92
Lufenuron	5.16	17.27	5.00	28.21	7.10	-19.32	7.10	9.23
Control	4.40		3.90		8.80		6.50	

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 compound.

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
Diflubenzuron	7.22	-16.05	7.15	-11.95	3.62	-20.44	3.90	-21.67
Tebufenozide	7.82	-9.07	7.82	-3.69	3.16	-30.55	3.12	-37.35
Hexaflumuron	6.90	-19.77	7.00	-13.79	3.22	-29.23	3.12	-37.35
Flufenoxuron	6.66	-22.56	7.10	-12.56	2.98	-34.51	2.88	-42.17
Chlorfluazuron	6.18	-28.14	6.56	-19.21	3.10	-31.87	3.08	-38.15
Lufenuron	7.62	-11.40	7.00	-13.79	3.92	-13.85	3.82	-23.29
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 compound.

Tested compounds	Amylase				Invertase			
	Laboratory strain		Field strain		Laboratory strain		Field strain	
	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control
Diflubenzuron	1.12	-13.85	1.14	-40.00	3.30	-26.67	3.12	-9.83
Tebufenozide	1.20	-7.69	1.16	-38.95	3.20	-28.89	3.10	-10.40
Hexaflumuron	1.18	-9.23	1.12	-41.05	3.12	-30.67	3.10	-10.40
Flufenoxuron	1.20	-7.69	1.20	-36.84	3.20	-28.89	3.30	-4.62
Chlorfluazuron	1.14	-12.31	1.00	-47.37	3.60	-20.00	3.00	-13.29
Lufenuron	1.16	-10.77	1.12	-41.05	3.12	-30.67	3.16	-8.67
Control	1.30		1.90		4.50		3.46	

التأثيرات السامة لبعض منظمات النمو الحشرية على بعض الأنشطة البيوكيميائية على دودة ورق القطن

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أجريت هذه الدراسة بهدف تقدير مستوى حساسية كل من العمر اليرقى الثـانى و الرابـع لسلالة معملية وأخرى حقلية(تم جمعها من محافظة الدقهلية) لدودة ورق القطن تجاه ستة مركبـات تابعة لمجموعة منظمات النمو الحشرية وهـى دايفلـوبنزيرون ، تيبوفينوزيـد ، هكسـافلوميرون ، فلوفينوكسيرون ، كلورفليوزيرون ، ليوفينرون . أشارت النتائج وفقا لمقياس معدل المقاومة وجـود اختلافات فى درجات المقاومة تبعا للتركيب الكيماوى للمركبات المختبرة و العمر اليرقى حيث سجلت قيم معدل المقاومة ١٦٢٨٥٠ و١٧٦٨٠ و٢١٤٥ و ٢١٧,٦ و ٢٠,٤٠ و٢,٨١ ضعف لكـل مـن فلوفينوكسيرون و ليوفينرون و كلورفليوزيرون و هكسافلوميرون و دايفلوبنزيرون و تيبوفينوزيد على العمر اليرقى الثانى مقارنة بالسلالة المعملية على الترتيب . اظهرت النتائج ان قيم التركيز النصـفى المميت للمركبات المختبرة لكل من السلالتين على العمر اليرقى الرابع كانت اعلى مقارنـة بـالعمر اليرقى الثانى حيث أعطى فلوفينوكسيرون أعلى معدل للمقاومة يليه ليوفينرون بقـيم ٨٨٦٣٧١ و ٨١٩٨,٨ ضعف على العمر اليرقى الرابع في حين أعطى كل من كلورفليوزيرون و هكسافلوميرون مستوى متوسط بلغ ٥٣٣,٩٥ و ١٨١,١٣ ضعف بينما أعطى كل من دايفلوبنزيرون و تيبوفينوزيـد تأثير منخفض بلغ ١٧,٥١ و ٥,٣٩ ضعف على الترتيب . وكان الهدف الثانى من الدراسـة تقـدير مدى تأثير المركبات الستة المختبرة على نشاط إنزيمات الاستيريزات (الاستيريز الاليفاتى و الفـا و بيتا استيريز) و إنزيمات الفوسفاتيزات (الفوسفاتيز الحامضى و القاعدى) و الانزيمات الناقلة لمجاميع الامين (الاسبرتات و الالانين) و الانزيمات المحللة للكربوهيدرات على كل من السلالتين .