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The biochemical influences of some chitin synthesis inhibitors against the cotton leafworm *Spodoptera littoralis* (Boisd.)

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The biochemical action of three chitin synthesis inhibitors insecticides (chlorfluazuron, teflubenzuron and flufenoxuron) on the enzymatic activity of chitinase and phenoloxidase in both haemolymph and cuticle of the 3rd and 5th instar larvae of both susceptible and field strains of cotton leafworm *Spodoptera littoralis,* were carried out in order to investigate the biochemical influences of these compounds. The activities of such enzymes, were determined after 24, 72 and 120 h of treatment at the estimated LC_{50} values given in the diet for 24 h. The results showed that the tested compounds varied in their rate of influences on chitinase and phenoloxidase activity according to enzyme source in haemolymph or cuticle, tested compound and period of investigation after treatment.

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Palabras clave: Chitin Synthesis Inhibitors, Chitinase; Phenoloxidase, *Spodoptera littoralis,* Field Strain, Susceptible Strain.

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is considered the most serious pest of Egyptian cotton plant. Few past decades, the potency of benzoyl phenyl urea (chitin synthesis inhibitors) compounds has been looked at as selective agents to suppress both growth and development of larvae. Since then, several studies have been directed to elucidate the biological as well as biochemical effects of such group of chemicals. The literature cited on the several characteristics of these compounds suggested their potential successful use as alternatives to classical insecticides. Those compounds are mostly used as inhibitors of chitin deposition in the cuticle of arthropods. However the mode of action of such promising type of insecticides, the Chitin Synthesis Inhibitors has been the target of several investigators. It was as early as 1974, when Ishaaya and Casida reported that house fly larvae showed an increase of both the cuticle chitinase and phenoloxidase activities up to about 180 and 155%, respectively when treated with 1 ppm of the compound TH-6040. There are also another reports concerning the biochemical effects of similar compounds (e.g. diflubenzuron, Du-l9111 and/or polyoxin D) which showed that such compounds has the inhibition action on the chitin biosynthesis or cuticle production through the blocking action of the terminal polymerization step in chitin synthesis against another species of insects such as *Musca domestica*

(VAN ECK, 1979) *Manduca sexta* (MITSUI *et al.*, 1980), and *Mamestra Brassica* (MITSUI *et al.*, 1981).

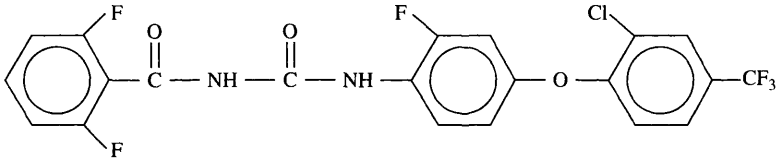
In the present study it was evaluated the biochemical effects of the estimated LC_{50} values of a series of chitin synthesis inhibitors (figure 1) in the form of formulation on the activity of both chitinase and phenoloxidase in both haemolymph and cuticle on susceptible and field strains of the cotton leafworm *S. littoralis* specially against the most dangerous larval instar, 3rd and 5th using feeding larvae on sprayed leaves technique, in order to provide similar spraying process that is using in the field. So that, the aim of such investigation is to reaching a more understanding about the nature of the mode of action of such promising insecticides.

MATERIALS AND METHODS

Insect strains and rearing

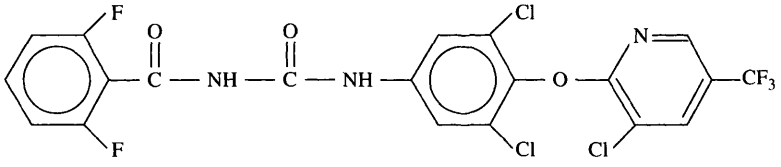
The studies on the larvicidal effects of the chitin synthesis inhibitors, were carried out under laboratory conditions of 27 ± 2 °C and 65 ± 5% R.H. The cotton leafworm, *S. littoralis* susceptible strain and the field-collecting specimen were compared in the present work. The first strain was generously provided, as egg-masses by Ciba-Geigy Co. (Kaliobia, Egypt) while the latter was field-collected as egg masses from the Faculty of Agriculture farm, University of Ain Shams (Shalakan, Kaliobia, Egypt). Both susceptible and field-collected egg-masses were separately confined in sterilized jars, tapped with muslin covers. Upon larval hatching,

Flufenoxuron: (Cascade), (SH 777) 5% EC:



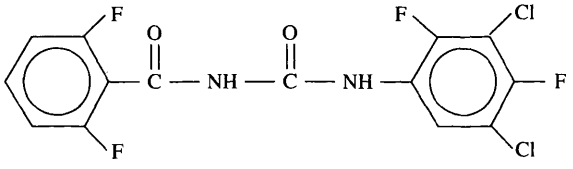
1-(4-(2-chloro-4-(trifluoromethyl)phenoxy)2-fluorophenyl)-3-(2,6-difluorobenzoyl) urea

Chlorfluazuron: (IKI-7899), 5% EC:



1-(3,5-dichloro-4-(3-chloro-5-trifluoromethyl-2-pyridyloxy)phenyl)-3-(2,6-difluorobenzoyl) urea

Teflubenzuron: (CME-12406), 5% FC:



1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl) urea

Fig. 1.–Chemical structure, chemical name and commercial name of the chitin synthesis inhibitors used in present study

fresh and clean castor-bean leaves were provided as food. Daily clean jars were substituted for the used ones. At pupation, the pupae were sexed and then confined, 12 in each jar, at a sex - ratio of 2 females to one male, for moth emergence. Adult moths were supplied by 10% sugar solution in which a cotton wick was immersed for feeding through. In addition two leaves of *Nerium oleander* were provided as ovipositor site. Deposited egg-masses were daily collected for further experimentation.

Whenever it was necessary field-collecting egg-masses were picked up from the previously mentioned faculty farm, reared under the laboratory conditions, for only one generation, after which time egg-masses for the present work were taken.

Establishment of toxicity lines

Each emulsifiable concentrate (EC) or flowable concentrate (FC) formulated insecticides were prepared in water to obtain the proper concentration. Series of seven concentration of each chemical were used to calculate the LC_{50} value. The concentrations studied were prepared from stock solution which were further diluted, with water, to desired concentrations. 3rd and 5th instar larvae of susceptible and field strains weighing 20 to 30 mg for the former and 140 to 180 mg for the latter instar, respectively, were used throughout the present investigation. Castor bean leaves were sprayed with aqueous concentrations of each compound, at rate of 0.7 mL/150 cm^2. After spraying of each surface, leaves were dried using an electric fan. The dried treated leaves for each concentration and/or compound were put in plastic cups of 20 cm in diameter.

Ten of 3rd and five of 5th instar larvae were then transferred to each cup, which were replicated five times for the former and ten replicated for the latter instar larvae, respectively. Those cups were incubated at 27 °C ± 2 °C and 65 ± 5% R.H. The larvae were allowed to continue their feeding on treated castor bean leaves for two days and for fresh untreated leaves for the following three days. Larvae of the same instar were kept, as control, on untreated castor bean leaves. Thereafter mortality counts were assessed and corrected ABBOTT (1925). Concentration mortality regression lines were corrected according to FINNEY (1952).

Chemicals

The chemical names, structures and formulations used in this investigation are shown in figure 1. All other chemicals and/or reagents used were of standard laboratory quality.

Determination of the enzymatic activity

Both of chitinase and phenoloxidase enzymes in addition to total protein were assayed in haemolymph and cuticle extraction of treated and untreated larvae of the 3rd and 5th instars of both strains.

The larvae were fed for 24 h on LC_{50} y for each of the compounds used as tabulated below:

Compounds	Values of LC_{50}'s (% formulation)			
	S-strain		F-strain	
	3rd	5th	3rd	5th
flufenoxuron (Cascade)	0.00048	0.00717	0.00118	0.028
chlorfluazuron (IKI-7899)	0.000465	0.00232	0.00158	0.0102
teflubenzuron (CME-13406)	0.00084	0.0057	0.00099	0.014

S-strain: Susceptible strain. F-strain: Field strain.

Sampling techniques

The enzyme activity was determined after 24, 72 and l 20 h post-treatment. A batch of 25 and 10 of the affected larvae of 3rd and 5th instars, respectively, were washed twice with distilled water and dried with filter paper. The larvae were bled by cutting one of their abdominal legs and haemolymph was collected in a chilled centrifuge tube, diluted with a ten fold quantity of cold distilled water then the samples in triplicate were centrifuged for 25 min at 20.000 × g in a refrigerator centrifuge at 2 °C. The supernatant was then frozen dry, resulting in a coloured powder which was used for determination of enzyme activity and percentage of activity in relation to its specific control.

Cuticle of the larvae used for the collection of haemolymph were used for further experirnentation by cutting off the head capsules with a sharp razor blade and the alimentary canals together with the body fluids were then removed by making longitudinal cutting to free the cuticle. The total body cuticles were rinsed with cold distilled water, macerated and homogenized with a three fold of cold distilled water in a homogenizer for 3 min in ice-bath. The homogenates were centrifuged at 20.000 × g for 25 min in a refrigerator centrifuge at 2 °C. The supernatant was then filtered through Whatman n° 1 paper on and frozen dry.

Determination of phenoloxidase activity

Determination of phenoloxidase activity was based on a method described by Ishaaya (1972). Catechol (Sigma Chemical Co.) at 0.25% was used as a substrate. The frozen dried enzyme powder was dissolved in a phosphate buffer. The enzyme solution together with the substrate were then incubated for 3 min at 25 °C. The developing colour was measured 2 min later using a spectrophotometer model Spekol 11 at 470 nm. The enzyme activity was calculated as extinction units per mg protein.

Determination of chitinase activity

Chitinase activity was assayed according to Ishaaya and Casida (1974) who used the 3,5-dinitrosalicylic acid (DNSA) reagent, originally developed for glucose analysis to determine the free aldehyde groups of hexosamine liberated on chitin digestion. The time of incubation was 60 min at 37 °C. Centrifugation took place for 15 min at 20.000 × g, to eliminate the undigested chitin by filtration which followed. The absorbance of the colour of the supernatant was measured at 550 nm. The standard curve was prepared by using N-acetylglucosamine (NAGA) (Sigma Chemical Co.). Aliquots from 0.01% (NAGA) solution were prepared and reacted directly with (DNSA) reagent, the colours were developed and the optical densities were measured using a spectrophotometer model Spekol 11 at 550 nm. Chitinase activity was expressed as mg (NAGA) released per mg protein per hour and per mg protein.

Total protein determination

The method of Lowry et al (1951) was used to determine the total protein in haemolymph and cuticle preparations which were used as criteria for enzyme activity.

RESULTS

A-Chitinase activity

The results of the specific activity of chitinase are tabulated in table 1. Data clearly showed that at 24 h post-treatment chitinase activity increased, in haemolymph, irrespective of compound tested. Based on comparison with the different treatments flufenoxuron proved to initiate chitinase activity in 3rd instar larvae, 5-fold that of the control. However, teflubenzuron although being 1.3 times as much effective as chlorfluazuron, yet it came second in its potentiation for

Table 1.–**Effect of chitin synthesis inhibitor compounds on chitinase activity in 3rd and 5th instar larvae of both susceptible and field strains of *Spodoptera littoralis* (Boisd.)**

Compounds	Larval instar	% Enzyme activity											
		24 h				72 h				120 h			
		S-strain		F-strain		S-strain		F-strain		S-strain		F-strain	
		Haemol-ymph	Cuticle	Haemol-ymph	Cuticle	Haemol-ymph	Cuticle	Haemol-ymph	Cuticle	Haemol-ymph	Cuticle	Haemol-ymph	Cuticle
Flufenoxuron	3rd	521.6	108.9	154.8	45	116	62.6	144.3	69.8	129.4	71.2	258.9	34.7
	5th	208.1	96.9	66.5	66.1	26.8	98.7	43.1	62.9	268.1	143.4	251.4	68.9
Chlorfluazuron	3rd	346.4	85.8	143.9	112.9	67.9	66.8	241.4	85.3	170.1	83.1	100.6	31.4
	5th	270.5	67.4	88.8	49.1	44.8	64.2	27.7	66.5	205.8	202.5	70.4	23.8
Teflubenzuron	3rd	466.2	113.2	174.9	69	124.1	103.5	139.8	37.8	209.4	104.4	176.2	12.2
	5th	302.4	73.2	14.3	155.9	30.6	83.9	99.5	32.2	308.5	140.6	28.8	32.2
Control	3rd	100	100	100	100	100	100	100	100	100	100	100	100
	5th	100	100	100	100	100	100	100	100	100	100	100	100

* Each value in the table represents a mean of 3 replicates.
S. strain: Susceptible strain.
F. strain: Field strain.

chitinase activity following that of flufenoxuron.

On the other hand, the potency of the enzyme was generally, reduced when tests were conducted using the 5th instar larvae. Reduction in values for such efficacy ranged between 2.5 - 1.3 fold. In a descending order of the tested compounds according to chitinase activity was teflubenzuron, chlorfluazuron and flufenoxuron, respectively.

In case of the cuticle chitinase, the experiments carried out with cuticle of 3rd instar larvae, it seems clear that both teflubenzuron and flufenoxuron were almost similar in their effects, on the enzyme activity, as that of the control. The former had 13% more effect over that of the control, while the latter presented only 8.9%. On the contrary, the effect of chlorfluazuron was 14.2% lower than the control.

The percentage of enzyme activity was much lower in the 5th instar larvae, compared with that of the 3rd. Such activity was reduced by 11-35% compared to that of the 3rd instar larvae for the same compounds tested. The enzyme activity was not much affected as a result of flufenoxuron treatment compared to that of the control. The reduction in such activity was only 3.1%. On the contrary, chlorfluazuron detrimentally affected the enzyme activity by 32.6%, whereas teflubenzuron, caused 26.8% reduction in activity. The enzyme activity values were in a descending order, for control, flufenoxuron, teflubenzuron, and chlorfluazuron, respectively.

In the field strain, the 3rd instar larvae the haemolymph enzyme activity in the treatment with flufenoxuron and chlorfluazuron were almost 5-fold as much as active as the corresponding activity in haemolymph of the control, while teflubenzuron was 1.75%. On the contrary, the enzyme activity in haemolymph of the 5th instar larvae, was decreased in all treatments. The percent reduction were 33.5, 11.5 and 85.7% caused by flufenoxuron, chlorfluazuron, and teflubenzuron, respectively.

Results of cuticle chitinase activity in the field strain was reduced in the 3rd instar larvae compared to that of the 5th, except that caused by chlorfluazuron which had a higher activity even over that of the control. The percent enzyme activities were 45, 112.9 and 69% of flufenoxuron, chlorfluazuron, and teflubenzuron, respectively. In this respect, chlorfluazuron proved to be a weaker enzyme inhibitor than the other two compounds. On contrary, in case of the 5th instar larvae, chlorfluazuron showed greater potency as an inhibitor of such enzyme, while teflubenzuron caused 50% increase in chitinase activity more than that of control.

Generally, the percentage of chitinase activity recorded 24 h post-treatment was reduced in haemolymph and cuticle of field strain compared to that of the susceptible strain, regardless of larval age and/or compound used.

Activity of haemolymph chitinase enzyme at 72 h post-treartment in the 3rd instar larvae of the susceptible strain was greatly reduced by 32.1% when the larvae were fed on LC_{50} of chlofluazuron, while the other two compounds showed little enzyme activation ranging between 16-24% more than that of the control. Comparison between such activity, with the corresponding values recorded one day post-treatment clearly showed that the percent enzyme activity was reduced by 77.7, 80.0 and 73.0% for flufenoxuron, chlorfluazuron, and teflubenzuron, respectively.

Also, the enzyme activity was greatly reduced in 5th instar larvae. Values for chitinase activity were in an ascending order, 26.8, 30.6, 44.8 and 100% for flufenoxuron, teflubenzuron, chlorfluazuron and control, respectively.

In case of cuticle chitinase of the susceptible strain at 72 h post-treatment, data recorded clearly show that the percent of enzyme activity in cuticle of 3rd instar larvae not much affected with teflubenzuron treatment comparing with that of the sarne instar-age at one day post-treatment yet that of flufenoxuron and chlorfluazuron was greatly reduced by about 43% at 72 h post-treatment. The activity in 5th instar larvae showed only

slight variation by time elapse with that of the control.

Enzyme activity in haemolymph chitinase of 3rd instar larvae of the field strain showed higher activation over that of susceptible strain at the sametime yet a reduction in such activity was caused by flufenoxuron and teflubenzuron after 72 h post-treatment compared with that at 24 h after treatment. Conversely an increase reaching 68% was recorded for chlorfluazuron. The reduction in chitinase activity in haemolymph of 5th instar larvae was inhibited by almost 57 and 72% by flufenoxuron and chlorfluazuron, respectively, when compared with that of the control.

Results of cuticle chitinase of the field strain, clearly indicate that the most potent compound causing drastic enzyme inhibition was teflubenzuron. The percent of enzyme activity 37.8 and 32.2 in the 3rd and 5th instar larvae, respectively, compared with that of the control.

Chitinase activity at 120 h post-treatment was generally reduced in both the haemolymph and cuticle of 3rd instar larvae of susceptible strain regardless of compound tested. Conversely a slight increase of activity was noticed in treatments with the 5th instar larvae. However, chitinase inhibition in haemolymph of the 3rd instar larvae was highest with flufenoxuron treatment followed with teflubenzuron while chlofluazuron was the least effective when compared with those values at 24 h. The percent values of activity recorded were 129.4 and 209.4 and 170.1% for flufenoxuron, teflubenzuron and chlorfluazuron, respectively.

When the field strains was taken in consideration, the percent activity of chitinase in haemolymph of the 3rd instar larvae was decreased compared with that at 24 h after treatment regardless of compound studied. As for such activity determined in 5th instar larvae chlofluazuron and teflubenzuron had caused pronounced reduction in the enzyme activity, the recorded values after 120 h were only 70.4 and 28.8%.

Percent chitinase activity determined in cuticle of 3rd and 5th instar larvae of the field strain was reduced greatly as compared with results from 24 h post-treatment. Such reduction in activity ranged between 23-82%. However, teflubenzuron caused the highest inhibition of chitinase activity regardless of instar under test. All values determined in cuticle were less that ofthe control.

B-Phenoloxidase activity

Results concerning the effect of treatment by LC_{50} values of tested compounds on the percentage of phenoloxidase activity at 24, 72 and 120 h post-treatment are shown in table 2. Generally, it is evident that the percentage of phenoloxidase activity determined 24 h post-treatment was highest in haemolymph of the 3rd and 5th instar larvae of susceptible strain, regardless of chemical tested. Activity of the enzyme by the different treatments ranged between 1.5- to 6-fold that of the control. However, such activity was not as much pronounced in the haemolymph of 5th instar larvae as that of the 3rd the percentages of phenoloxidase activity were 894.1, 452 and 622.6% for flufenoxuron, chlorfluazuron and teflubenzuron, respectively, compared to that 100 of the control. The corresponding values recorded for the 5th instar larvae were 328.6, 192.9 and 136.7%.

Determination of the percentage of enzyme activity in haemolymph of susceptible 3rd and 5th instar larvae at 72 h after treatment with any of the tested compounds showed a detrimental drop, the values recorded for flufenoxuron, chlorfluazuron and teflubenzuron were 26.0, 40.3 and 51.9% for the former instar whereas the corresponding activities were 33.3, 44.4, and 33.3 for the latter older instar.

Activity of phenoloxidase estimated in haemolymph at 120 h post-treatment clearly reveal that the per cent of such activity in the 3rd and 5th instar larvae of susceptible strain was comparably lower than that at 24 h but much more higher than the correspon-

Table 2.–**Effect of chitin synthesis inhibitor compounds on phenoloxidase activity in 3rd and 5th instar larvae of both susceptible and field Strains of *Spodoptera littoralis* (Boisd.)**

Compounds	Larval instar	Specific Enzyme Activity (units/mg protein)											
		24 h				72 h				120 h			
		S-strain		F-strain		S-strain		F-strain		S-strain		F-strain	
		Haemol-ymph	Cuticle	Haemol-ymph	Cuticle	Haemol-ymph	Cuticle	Haemol-ymph	Cuticle	Haemol-ymph	Cuticle	Haemol-ymph	Cuticle
Flufenoxuron	3rd	849.1	49.1	25.3	133.9	25.9	49.1	66.7	30.9	121.9	45.0	57.1	37.5
	5th	328.6	50.0	72.7	131.8	33.3	28.0	176.0	70.2	320.0	90.9	95.2	60.0
Chlorfluazuron	3rd	452.8	45.5	50.5	157.1	40.3	63.6	33.3	304.8	173.2	69.0	88.6	90.0
	5th	192.9	50.0	118.2	100.0	44.4	36.0	132	24.6	60.0	181.8	60.3	36.4
Teflubenzuron	3rd	622.6	80.0	70.7	150.0	51.9	30.9	66.7	142.9	200.0	160.0	128.6	72.5
	5th	136.7	40.0	36.4	439.4	33.3	66.0	300.0	26.3	180.0	51.1	46.0	27.3
Control	3rd	100	100	100	100	100	100	100	100	100	100	100	100
	5th	100	100	100	100	100	100	100	100	100	100	100	100

* Each value in the table represents a mean of 3 replicates.
S. strain: Susceptible strain.
F. strain: Field strain.

ding values recorded after 72 h of treatment with all the compounds tested. With the exception of chlorfluazuron for the 5th instar larvae, the haemolymph phenoloxidase activity was higher than the control.

Taking the activity of cuticle phenoloxidase of susceptible strain in consideration, data shown in table 2 proved that reduction in activity took place with all the tested chitin synthesis inhibitors, irrespective of instar tested. No differences in per cent activity were noticed by time. At 24 and 72 h post-treatments the per cent enzyme activity was almost the same. Generally all values were less than that recorded in the control except chlorfluazuron at 5th instar larvae and teflubenzuron at 3rd instar which showed higher activity than the control, at 120 h posttreatment.

The activity of phenoloxidase in the field strain was generally low in haemolymph of 3rd instar larvae after 24 h of treatment regardless of compounds used. However, values recorded for the 5th instar larvae were in most cases higher than those in the 3rd instar the highest percentage of enzyme activity was recorded with chlorfluazuron of 5th instar larvae whereas the lowest was recorded with flufenoxuron at 3rd instar. Such activities were 118.2% for former but only 25.3% for the latter. However, teflubenzuron caused high enzyme activity reaching 300% in haemolymph of 5th instar larvae after 72 h while in haemolymph of 3rd instar was 128.6% after 120 h.

On the contrary, the percentage of phenoloxidase activity in cuticle of both instars was generally higher compared to that in the haemolymph at 24 h post-treatments with all the three chitin synthesis inhibitors. As for the activity after 72 and 120 h of treatment, it showed less activity in most cases.

Only chlorfluazuron and teflubenzuron in the 3rd instar larvae presented high per cent phenoloxidase activity. The percentages recorded were 304.8% out 142.9% for the former and latter, respectively, after 72 h of treatment.

DISCUSSION

Evidence obtained from other workers in the field, i e., corroborates results of the present investigation. However, the activity of chitinase and phenoloxidase as well as protein in haemolymph and cuticle were determined at the LC_{50} value given in diet for one day to 3rd and 5th instar larvae of susceptible and field strains. The enzyme activities were always related to the enzyme activity of the control samples.

Generally, it was found that the percentage of enzyme activity was reduced by time lapse post-treatment. Such drop was either slightly or drastically recorded by the different command treatments strain and/or instar tested.

At 72 h post-treatment, the percentage of chitinase activity in haemolymph of 3rd instar larvae of susceptible strain showed a great reduction reaching 32.1% over that of the control. Flufenoxuron caused 77.7% reduction over those values recorded 24 h post-treatment. The percentage of chitinase activity in the 5th instar larvae, however, decreased drastically by flufenoxuron and teflubenzuron. The reason behind the reduction in the enzyme of activity may be due to blockage and inhibition of the enzyme.

It was also evident that the percentage of phenoloxidase activity determined 24 h posttreatment was highest in haemolymph of the 3rd and 5th instar larvae of susceptible strain, regardless of compound tested. While such percentage after 72 h was detrimentally decreased. This finding is in accordance with other findings reported by many authors in the field, (STEVENSON and ADOMAKO, 1967).

However, in certain cases activation of the enzymes was noticed. Based on comparison with the different treatments, flufenoxuron proved to initiate chitinase activity in 3rd instar larvae 5-fold that of the control. It is possible that distribution of the different compounds tested might had been variable. Accordingly, the rate by which such compounds was exerted might have affected the

percentage of enzymatic activity recorded in the present investigation. This result coincides greatly with similar work carried out by DECOCK *et al.* (1989) who reported that distribution of chlorfiuazuron in the body of 4th instar larvae of the Colorado potato beetle varied by time and that the excrements contained 36.1% and 65.3% of the amount of chlorfluazuron 24 and 72 h after application, respectively.

In addition, the obtained changes in the enzyrne activities may due to the variation in the protein synthesis as a response to the different treatments. Electrophoretic protein analysis carried out by CHEN and LEVENBOOK (1966), revealed variability in the haemolymph protein in the 3rd and 5th instar larvae of the blow flies. The authors in showed that protein synthesis decreased distinctly from the larvae to the white pupae. They further stated that during metamorphosis haemolymph protein concentration fell distinctly.

Moreover, inhibition rate of cuticle protein was reported by HEGAZY *et al.* (1989), who stated that protein dropped to 14% at the first day and proceeded to decrease reaching a per cent of 39% at one week later when treating larvae of Colorado potato beetle with diflubenzuron, chlorfluazuron and teflubenzuron. This finding confirms the results recorded in the present work.

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RESUMEN

BAYOUMI, E.; BALAÑA-FOUCE, R.; SOBEIHA, A. K. y HUSSEIN, E. M. K., 1997: Efectos bioquímicos de algunos inhibidores de la síntesis de quitina frente al gusano del algodón *Spodoptera littoralis* (Boisd.). *Bol. San. Veg. Plagas,* **23**(4): 583-593

Se ha estudiado el modo de acción de tres insecticidas inhibidores de la síntesis de quitina (clorfluazuron, teflubenzuron, flufenoxuron) sobre la actividad enzimática quitinasa y fenol oxidasa en la hemolinfa y la cutícula de larvas de tercer y quinto estadío de cepas susceptibles y de campo del gusano del algodón *Spodoptera littoralis.* La actividad de dichas enzimas se determinó después de tratar a los insectos con cada insecticida 24, 72 y 120 h con una concentración estimada en la LC_{50} a las 24 h. Los resultados muestran que la actividad de dichos compuestos sobre las dos enzimas varió en función del tejido de procedencia y del momento en que se realizó el análisis.

Palabras clave: *Spodoptera littoralis,* inhibidores de la síntesis de quitina, gusano del algodón.

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