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The biological activity of some chitin synthesis inhibitors against the cotton leafworm *Spodoptera littoralis* (Boisduval), (Lepidoptera: *Noctuidae)*

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The action of three chitin synthesis inhibitors insecticides (chlorfluazuron, teflubenzuron and flufenoxuron) against third and fifth instars of both susceptible and field strains of cotton leafworm *Spodoptera littoralis,* (Lepidoptera: Noctuidae), were carried out in order to investigate the biological activity of these compounds, in their formulation form, under controlled laboratory conditions. The estimated LC_{50} values, clearly showed that the third instars were more sensitive to the compounds tested, compared with those of the fifth instars, regardless of strain used. Data from larval dietary bioassay showed an up to 4-fold in LC_{50} values among the strains tested. Such values, expressed as tolerance level depended largely on the chemical tested and/or the instar used. The tolerance level calculated for the third instars was generally less than that corresponding for the fifth instars, irrespective of compounds tested. The highest tolerance level recorded was observed for chlorfluazuron, whereas the lowest pertained to teflubenzuron. The percentage of accumulative mortality varied according to the compound, concentration, the instar larvae and/or strain studied.

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Key words: Accumulation mortality; Chitin Synthesis Inhibitors; *Spodoptera littoralis;* Field Strain; Susceptible Strain; Tolerance Level.

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisduval), *(Lepidoptera: Noctuidae),* is considered the most serious pest of Egyptian cotton. Larvae feed not only on cotton leaves, but also they attack other economically important crops such as; cucumber, potato, okra and egg plants and. Control measures have been directed, to combat this insect. The most widely used method of control, the chemical insecticides, together with other methods failed to achieve complete protection of the cotton crop against this pest. Few past decades, the potency of benzoyl phenyl urea compounds (chitin synthesis inhibitors) 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 and biochemical effects of such group of chemicals on the insects in laboratory and field conditions. Several reports, suggest these compounds use as alternatives to classical insecticides. Some important characteristics of chitin synthesis inhibitors are, the apparent selectivity of some of them, relatively short environmental persistence, low mammalian toxicity, and the easy

use of their formulated application (QUISTAD *et al.*, 1974).

These compounds are mostly used as inhibitors of chitin deposition in arthropods cuticle. The evaluation of insecticide activities of chitin synthesis inhibitors has attracted the attention of many workers in this field. Several authors have evaluated insecticide activity of some chitin synthesis inhibitors in the form of active ingrediente (AI), against different stages of the cotton leafworm *S. littoralis* using different application techniques, (ASCHER and NEMNY, 1976b; RADWAN *et al.*, 1978; ASCHER and ELIYAHU, 1981; EL-GUINDY *et al.*, 1982; SCHEURER *et al.*, 1983; ASCHER and NEMNY, 1984; ISHAAYA *et al.*, 1984; EL-SAYED, 1984; and ALDEBIS *et al.*, 1988). There are also other reports concerning the evaluation of these compounds against other species of insects such as *Mamestra brassica* (GROSSCURT, 1978), *Pseudoplusia includens* (REED and BASS, 1980), *Heliothes virescens* (SCHEURER *et al.*, 1983), *Plutella xylostella* (KOHYAMA, 1986) and *Leptinotarsa decemlineata* (TUTTLE and FERRO, 1988).

In the present study we have evaluated the biological activity of a list of formulated chitin synthesis inhibitors (Fig. 1) against insecticide susceptible laboratory and field strains of 3rd and 5th instar cotton leafworm *S. littoralis* using feeding larvae on sprayed leaves technique.

MATERIALS AND METHODS

Insect Strains and Rearing

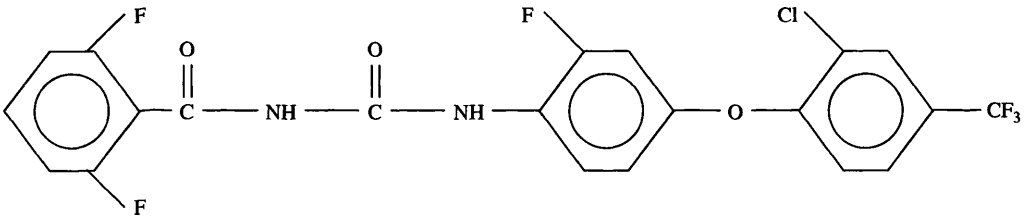
The studies on the larvicidal effects of the chitin synthesis inhibitors, were carried out under laboratory conditions of 27 ± 2 °C and 65 ± 5% R.H. The cotton leafworm, *S. littoralis* susceptible strain and the field-collecting specimen were compared in the present work. The first strain was generously 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, 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

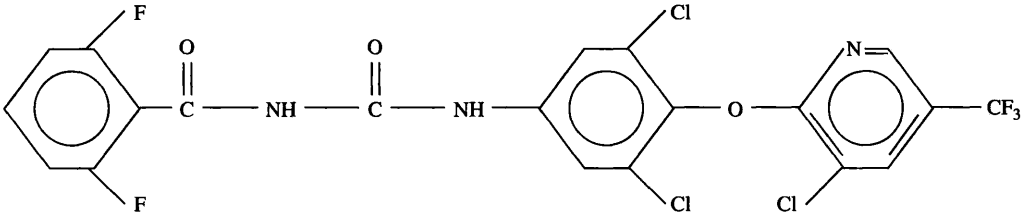
Both emulsifable concentrate (EC) (5% v/v), or flowable concentrate (FC) (5% w/v), formulated insecticides were prepared in water to obtain the proper concentration. Series of seven concentrations of each chemical were used to calculate the LC_{50} value. The concentrations studied were prepared from 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 and electric fan. The dried treated leaves for each concentration and/or compound were put in plastic cups of 20 cm in diameter.

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



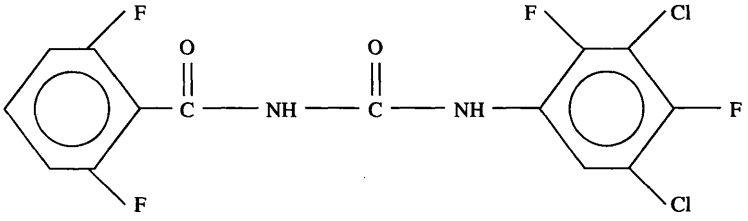
1-(4-(2-chloro-4-(trifluoromethyl)phenoxy)2-fluorophenyl)-3-(2,6-difluorobenzoyl)urea.

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



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

Teflubenzuron: (CME-13406), 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.

Ten of third and five of fifth instars 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 using ABBOT formula (1925). Concentration mortality regression lines were corrected using the method adopted by FINNEY (1952).

Assessment of accumulative mortality

To assess the accumulative mortality, the experimental design consisted of the same steps of the toxicity lines test. The difference between the two experiments was according to the larval number which were one hundred of third and/or fifth instars from susceptible and/or field strain were used. Furthermore, the selected larvae were transferred to separated glass sterile jars of 60 cm in diameter. Series of seven aqueous concentration of each compound were sprayed on seven separated castor bean leaves at the same rate (0.7 ml/150 cm^2). As mentioned in Table 2, for the third instars the concentration used was ranged from 0.000048 to 0.1% as formulation form. Larvae of the same instars were kept, as control, on untreated leaves. The larvae were allowed to continue their feeding for the same period (5 days), two days on treated leaves and three days on untreated leaves. Accumulative mortality counts were assessed by daily counting of the dead larvae, during five days.

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.

RESULTS AND DISCUSSION

a) *Dose-response relationships*

Results on toxicity of the chitin synthesis inhibitors flufenoxuron, chlorfluazuron and teflubenzuron against both third and fifth instars for the susceptible as well as the field strains of the cotton leafworm are tabulated in Table 1.

Susceptible strain

Based on the LC_{50} values, it is clear from Table 1 that flufenoxuron and chlorfluazuron were almost equal in their toxicity at the LC_{50} values against the 3rd instar larvae as presented by 0.24 and 0.2325 ppm (AI) equivalent to 0.00048% and 0.00046% of formulation, respectively. However, teflubenzuron was almost twice as less toxic than the mentioned two chemicals against the same instar larvae. Such LC_{50} value was calculated on 0.42 ppm (AI) or 0.00084% of formulation, respectively.

When the LC_{50} values of the same compounds were tested against the fifth instars they showed more tolerance to all of them. Chlorfluazuron was found to be the most potent compound, followed by teflubenzuron being flufenoxuron the least in its toxic

Table 1.–**LC_{50} values and tolerance level of the chitin synthesis inhibitors on third and fifth instars of susceptible and field strains of *Spodoptera littoralis***

Compound		Flufenoxuron		Chlorfluazuron		Teflubenzuron	
Instar		3rd	5th	3rd	5th	3rd	5th
Susceptible	LC50	0.24 ± 0.07	3.58 ± 1.90	0.23 ± 0.08	1.17 ± 1.10	0.42 ± 0.14	2.85 ± 1.75
Strain	Slope	0.0019	0.733	1.079	0.494	1.19	0.638
Field	LC50	0.59 ± 0.33	14.00 ± 8.00	0.79 ± 0.33	5.10 ± 2.32	4.95 ± 0.33	7.00 ± 4.05
Strain	Slope	0.714	0.644	0.855	0.618	0.529	0.629
Tolerance*		2.5	3.9	3.4	4.3	12.0	2.5

(*) Tolerance level = LC_{50} of field strain/LC_{50} of susceptible strain.

Table 2.–**Response of third and fifth instars of susceptible and field strains of the cotton leafworm, to selected insecticides 5 days after treatment**

Concentration			% Accumulative mortality											
			Susceptible strain						Field strain					
			3rd			5th			3rd			5th		
ppm[a] (a.i.)	ppm[b] Form.	%[c] Form.	Fluf.	Chlor.	Teflu.	Fluf.	Chlor.	Teflu.	Fluf.	Chlor.	Teflu.	Fluf.	Chlor.	Teflu.
500	10000	1	–	–	–	98	92	92	–	–	–	88	90	88
250	5000	0.5	–	–	–	90	82	84	–	–	–	78	80	80
50	1000	0.1	100	100	98	82	80	80	94	98	92	92	72	72
12.5	250	0.025	–	–	–	66	72	76	78	82	70	42	64	60
6.25	125	0.0125	98	96	94	–	–	–	–	–	–	–	–	–
3.125	62.5	0.00625	–	–	–	48	72	52	66	76	66	38	40	42
1.55	31	0.0031	94	84	78	–	–	–	–	–	–	–	–	–
0.78	15.6	0.00156	88	70	66	34	48	30	58	52	64	22	38	30
0.39	7.8	0.00078	60	64	46	–	–	–	–	–	–	–	–	–
0.195	3.9	0.00039	–	–	–	16	24	22	42	20	40	10	20	10
0.095	1.9	0.00019	24	24	20	–	–	–	–	–	–	–	–	–
0.048	0.97	0.000097	–	–	–	–	–	–	18	20	36	2	6	6
0.024	0.48	0.000048	10	20	–	–	–	–	–	–	–	–	–	–
0.012	0.24	0.000024	–	–	–	–	–	–	–	–	18	–	–	–
Control			0	0	0	0	0	0	0	0	0	0	0	0

a: Concentration as part per million corresponding to the concentration of the active ingredient (a.i.) included in the formulation form.
b: Concentration as part per million corresponding to the formulation form.
c: Concentration as hundred percent of the formulation form.

effect against such larvae. Comparison between the tested chemicals further exhibited that chlorfluazuron was 3-fold as much toxic as that of flufenoxuron while only twice as potent as teflubenzuron against the older larvae.

Field strain

Results tabulated in Table 1 show that the third instars were most sensitive to LC_{50} of flufenoxuron compared with the same values of the other two compounds tested in a descending order of toxicity flufenoxuron surpassed chlorfluazuron, whereas teflubenzuron was the least toxic. The LC_{50} values were 0.59, 0.79 and 4.95 ppm (AI) or 0.00118, 0.00158 and 0.0099% of formulation, respectively.

Similar but less pronounced toxic effects were recorded in studies carried out with the 5th instar larvae. Data represented in the Table 1 reveal that chlorfluazuron had most insecticide activity as expressed by the lowest LC_{50} while flufenoxuron presented the highest LC_{50}. The LC_{50} values in a descending order of potency were 5.1, 7.0 and 14.0 ppm (AI) or 0.0102, 0.014 and 0.028 of formulation for chlorfluazuron, teflubenzuron and flufenoxuron, respectively.

Age of treated larvae had a detrimental effect on the response to the compounds tested. Its noticed that the younger larvae were much more sensitive to the three compounds compared with the older ones. The

fifth instars were 5 almost 7 and 15 times as much tolerant as those of the 3rd instar to chlorfluazuron, teflubenzuron, and flufenoxuron, respectively.

b) *Tolerance level*

Data from larval dietary bioassays, recorded in Table 1 show an up to a 4-fold ratio in LC_{50} values among the strains tested. Such values, expressed as tolerance level depended on the chemical tested and/or the instar used. The tolerance level for the 3rd instar larvae was generally less than that corresponding for the fifth instars, regardless of the compounds tested. Such ratios for the former were 3.9, 4.3 and 2.5 whereas those for the latter instar larvae were 2.5, 3.4 and 1.2 for flufenoxuron, chlorfuazuron and teflubenzuron respectively. The highest tolerance level recorded for chlorfluazuron, whereas the lowest pertained of teflubenzuron. Those levels are tabulated as 3.4 and 1.2,m respectively. However, flufenoxuron offered a ratio of 2.5.

c) *Accumulative mortality*

When a series of concentrations of each compound were tested to evaluate the accumulative mortality, this parameter varied according to the compound, concentration, instar and strain studied (Table 2).

It was noticed that the percentage accumulative mortality of the susceptible strain was markedly affected by the treatment, compared with the field strain, regardless to concentration and/or compound tested. Moreover, the third instars exhibited high-response to all compounds more than those of the fifth instars of the same strain. The data also reveal that as higher concentration as higher percentage of accumulative mortality obtained.

The results also show that, when the post-treatment time elapsed, the percentage of the accumulative mortality increased. However, flufenoxuron showed superiority over the other two compounds, when tested against third and fifth instars of the susceptible strain, although both chlorfluazuron and teflubenzuron were less toxic to the susceptible third instars.

Conversely, the response of the field strain to the three compounds was different from that found of the susceptible one. Teflubenzuron was the most toxic compound to the third instars compared with those of the other two compounds. At the same level of concentration, 0.0001% of formulation, it caused 36% accumulative mortality while chlorfluazuron and flufenoxuron presented only 20 and 18%, respectively at 120 hr postreatment. However, 0.000025% (formulation) of teflubenzuron caused similar percentage of accumulative mortality than that caused by flufenoxuron at 0.0001% (formulation).

When larvae got older, they showed higher tolerance to all compounds. At 120 hr post-treatment with the highest concentration used a dosage of 10-fold that used against the 3rd instar was needed to cause a range of 84-92% accumulative mortality in the fifth instars of both susceptible and field strain, regardless to the compound tested.

Results on larvicidal effects of chlorfluazuron flufenoxuron and teflubenzuron revealed that chlorfluazuron was the most potent compound against the fifth instars of the susceptible strain. In addition, the bioassay of the chitin synthesis inhibitor compounds tested revealed that the percentage of accumulative mortality of the susceptible strain was greatly affected by the treatment, irrespective of concentration and/or compound tested. Again, the third instars exhibited high response to all compounds more than those of the fifth instars of the same strain. Such results coincided greatly with results on similar work of (NEUMAN and GUYER, 1983; GUYER and NEUMAN, 1988). However, the lower metabolism of chlorfluazuron, as reported by the above mentioned authors, may explain the great potency it exerted as a result of its long lasting stabi-

lity and persistence. Tefluenzuron caused the highest accumulative mortality as evidenced by 36%. However none of the remaining larvae were able to transform to pupae since they either died at pupation or they remained completely deformed. It seem possible that teflubenzuron had long persistence and latent effect causing the inability of the larvae to continue development. Such result corroborates other results recorded by ASCHER *et al.* (1987) and MARSHALL *et al.* (1988).

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ABSTRACT

BAYOUMI, ALAA E.; BALAÑA-FOUCE, R.; SOBEIHA, A. K. y HUSSEIN, E. M. K.: Actividad biológica de algunos inhibidores de la síntesis de quitina contra el gusano del algodón *Spodoptora littoralis* (Boisd). *Bol. San. Veg. Plagas,* **24**(3): 499-506.

Se ha estudiado el efecto de tres insecticidas inhibidores de la síntesis de quitina (clorfluazuron, teflubenzuron y flufenoxuron) sobre larvas de tercer y quinto estado (cepas susceptibles y de campo), del gusano del algodón (Spodoptera littoralis) (Lepidoptera, Noctuidae). Los valores de CL50 mostraron que los individuos pertenecientes al 3rd estado larvario eran más sensibles que las del quinto, cualquiera que fuera la cepa utilizada. Los datos obtenidos en los bioensayos muestran una resistencia cuatro veces mayor entre la cepa de campo y la cepa sensible. Dichos valores expresados como nivel de tolerancia, dependieron estrechamente del compuesto analizado y/o del estado larvario estudiado. El mayor grado de tolerancia registrado se observó para el clorfluazuron, mientras que el menor grado fue para el teflubenzuron. El porcentaje de mortalidad acumulativa era dependiente del compuesto, su concentración, el estado larvario y/o la cepa estudiada.

Palabras clave: Mortalidad acumulativa, inhibidores de la síntesis de quitina, *Spodoptera littoralis,* cepa de campo, cepa susceptible, nivel de tolerancia.

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