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The Reproductive Potential and Fate of Chlorfluazuron and Leufenuron Against Cotton Leafworm *Spodoptera littoralis* (Boisd.)

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Abstract: The Egyptian cotton leafworm is one of the most destructive insect pests which attacks many field crops.So the the present work aims to investigate the potential and fate of two new acylurea compounds, chlorfluazuron and leufenuron to reduce the reproductive capability of the *Spodoptera littoralis*.The results indicated that all treatments succeeded to decrease the percent pupation, pupal weight and adult emergence. A significant reduction in longevity and mating frequency was found. All reciprocal crosses of chlorfluazuron and leufenuron treatments resuted in a very low percentage of fecundity which ranged between (33.3 to 53.9 %). Egg hatchability was also significantily reduce, it ranged between (44.5 to 61.7%) for chlorfluazuron and (59.7 to 73.2%) for leufenuron compared to (94.7%)for the control. In conparison with fertility of control chlorfluazuron caused (62.2 to 80.4 %) sterility whereas leufenuron caused (52.1 to 74.9 %).So treatments with chlorfluazuron appeared to be more efficient than leufenuron. In order to elucidate the observed differences between the activities of chlorfluazuron and leufenuron, the amount of residues as parent compound existed in differential developmental stages of *S. littoralis* were determined. The results indicated that after 3 days of treatment 58.9 % chlorfluazuron and 36% leufenuron were recovered. Analysis of extract of emerged moths and eggs showed that sufficient quantities of the tested compounds were retairned by treated insects to cause the previously profound reduction in fecundity and egg hatchability. The results also indicated that leufenuron was eliminated two times faster than chlorfluazuron.

Key words: Reproduction · Fate · Chlorfluazuron · Leufenuron · *Spodoptera littoralis*

INTRODUCTION

Chemical control is considered the main method in the policy of pest control, so it is important to develop and use selective insect control agents that are effective and not pose hazards for the man or the environment [1] and have low toxicity to parasitoids [2]. Acylureas derivatives are selective insecticides acting on insects of various orders by inhibiting chitin formation [3], thereby causing abnormal endocuticular deposition and abortive moulting.They do not exercise a knock-down effects on insects but influence a physiological process, such as, ecdyson and pupation and its full effect can be evaluated for longer time. So these compounds may influence the fecundity, fertility and longevity of insects. Some authors have described the sterilizing activity of these compounds [4, 5]. This latent effect of acylureas compounds is related to the amount of substance accumulating at the biochemical site of action [6]. The search for more potent acylureas has led to develop new compounds such as Chlorfluazuron, Teflubenzuron and Leufenuron which are considerabally more potent than diflubenzuron on various agricultural pests [7].

Therefore, in this study the investigation was directed to explore the reproductive potential and fate of two new acylurea compounds, Chlorfluazuron and Leufenuron on the most notorious and destructive phytophagus insect pest *Spodoptera littoralis*.

MATERIALS AND METHODS

Test insect: A laboratory strain of *Spodoptera littolaris* (Boisd) was reared on caster oil bean leaves at (27±2°C) and (65±5%RH). The bioassay tests were carried out using newly moulted 5th instar (50±3mg /larvae).

Treatment: The two acylurea compounds - Chlorfluazuron (IKI - 7899 - Atabron): 1-[3,5dichloro-4-(3-chlor-5-trifluoromethy l-2-Pyridyloxy)phenyl]-3(2,6diflourobenzoyl) urea (97%a.i) and Leufenuron

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(Match-CGA-184699):N-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy) -phenylaminocarbonyl]-2,6diflurobenzamide (99.7% a.i) were used.

Methods of testing: Serial concentrations of the technical material which dissolved in acetone were prepared. fifth instar larvae were topically treated with one ul. Five replicates (each of 10 larvae) were used for each concentration.Untreated control larvae were treated with acetone alone in the same manner. The insects were examined daily for injury and mortality. The effect of the tested compounds were scored until adult emergence and recorded on basis of some categories. All mortality data were corrected for natural mortality using [8]. Regression lines were statistically calculated according to Finney [9] The effective dose (ED_{50}) as cumulative mortality till adult emergence was extracted from the regression lines.

A- Biological activity: The effect of chlorfluazuron and leufenuron on some biological aspects of *Spodoptera littoralis* were tested. The newly ecdysis 5th instar larvae were treated topically with 1 ul of an acetone solution containing 0.03 and 0.05 ug/g.b.w. of chlorfluazuron and leufenuron respectively (ED5o values of cumulative mortality till adult emergence). Data regarding pupation, pupal weight and adult emergence were also recorded. Adult obtained from tested larvae were crossed with those obtained from untreated larvae as follow: (treated ♂x treated ♀), (treated ♂x untreated ♀), (untreated ♂x treated ♀) and (untreated ♂x untreated ♀). Each pair of adult was kept in 2 kg glass jar and fed on 20 % sugar solution. The effect of each compound on the number of eggs deposited by each female (fecundity), percent egg hatch (fertility) and longevity of each sex were determined.Dead females were dissected and the number of spermatophores in bursa copulatrix were counted. The data were analyzed using analysis of varience (ANOVA) with Duncan's new multiple range test [10] to separate treatment means at the $p< 0.05$ level.

B: Residue analysis

1- Treatment and sampling procedure: The newly ecdysis 5th instare larvae were tpoically treated with the ED50 values (0.03 ug /g. b.w.) for chlorflazuron and (0.05 ug /g.b.w.) for leufenuron. Samples of the treated insects were taken initially (3 hours) after the treatment and other consecutive samples were collected at 1,2,3,5,7,9,11,18,19 and 21 days later. These periods of sampling represent the developmental stages of the insect (larvae - pupae - adults and eggs). The samples were stored in the freezer till analysis. The following analytical method used in this study was essentially that described by Spates and Wright [11] with some modifications.

2-Extraction: About 2 grams of each sample were homogenized for 2 min. with 10 ml acetonitrile using homogenizer. The acetonitril was filtered through a plug of glass wool and sodium sulphate unhydrus which was packed in a 30 ml glass funnel. The homogenizer and funnel were rinsed 3 times with 10 ml of acetonitrile each. The combined filtrate was collected in boiling flask and the contents were reduced to near dryness using a Buchi rotavapor at 50°C.About 10 ml of acetonitrile were added to the flask and filtered through millipore glass filtrate 47 m.m.. The filtrate was again evaporated to near dryness. The residues were then picked up using 2 ml acetonitrile for leufenuron determination or 2 ml methanol for chlorflazuron determination.

3 - Determination: The HPLC system consisted of Hewlett packard (HP series 1100), Quaternary pump model (G 1311 A), UV variable wavelength model (G1314 A) monitored at 254 nm. An ODS- Hypersil 5um (20cmx 4.6 m.m.d) was used and the column temperature was 50°C. Chlorflazuron and leufenuron were eluted isocratically with methanol-water (90:10 v/v). A20ul ingector was used at a flow rate 1.0 ml/min Under these conditions, the retention time (Rt) for chlorflazuron and leufenuron were 4.11 and 3.9 respectively.

The percent recovery of chlorflazuron was (97.8%) and of leufenuron was (92.5%).

RESULTS AND DISCUSSION

A- Effect of the tested compounds on the reproductive potential of *Spodoptera littoralis*: The following study examined the potential of acylurea compounds chlorfluazuron and leufenuron to reduce the reproductive capability of the Egyptian cotton leaf worm which have been exposed topically in 5th instar larvae to sub lethal doses (0.03 ug /g.b.w) for chlorfluazuron and (0.05 ug /g.b.w.) for leufenuron.. It is obvious that treatments with these compounds resulted in decrease in percent pupation which was more pronounced in chlorfluazuron (55.7 %) than in leufenuron (68.8 %). (Table 1). The results also indicated that all treatments succeeded to supress the pupal weight to 41.4% and 35.9 % reduction, for chlorfluazuron and leufenuron, respectively. The percentage of Adult emergence was

Table 1: Latent effects of the development of 5th instar larvae of *S. littoralis* treated with chlorfluazuron and leufenuron

Treatment	No. of treated larvae	% Pupation	Pupal Weight (mg)	% reduction in Pupal Weight	% Adult emergence
Chlorfluazuron	210	55.7	340±14.1	41.4	43.3
Leufenuron	180	68.8	372±19.2	35.9	55.5
Control	210	94.7	580±17.3	-	84.3

Table 2: Average longevity, mating frequency and number of eggs from adults resulted from larvae treated topically with chlorfluazuron and leufenuron

	Longevity (days)		No. of	Egges
	Male	Females	mortality	per female
	chlorfluazuron			
Ch♂ X ch♀	5.0 e	5.5 e*	1.4 bcdef	617.0 bcdef
Ch♂ X u♀	7.0 be	7.4 bc	1.5 bcde	879.5 b c
U♂ X ch♀	5.3 ef	5.7 de	1.7 bc	729.0 bcdef
	Leufenuron			
L♂XL♀	6.0 de	6.2 d	1.5 bcde	772.5 bcde
L♂ X U♀	7.4 ab	7.8 ab	1.6 bcd	1006.7 b
U♂"XL♀	6.7 bcd	7.0 c	1.8 ab	855.3 bcd
U♂X U♀	7.9 a	8.5 a	2.3 a	1865.5 a

- Means followed by the same letter in the same column are not significantly different (P< 0.05 Duncan's new multiple rang test).
- Ch = chlorfluazuron L= leufenuron u = untreated control
- Number of pairs = 15

Table 3: The fertility of *S. littoralis* treated with chlorfluazuron and leufenuron during the 5th instar larvae

Pairing	Fecundity (% of control)	% Egg hatchability	Sterility index
	chlorfluazuron		
ch♂x ch♀	33.3	40.6 f*	81.4
Ch♂X U♀	41.7	61.7 bcd	62.2
U ♂ X ch♀	39.0	44.5 ef	72.3
	Leufenuron		
L♂XL♀	41.4	59.7 bcde	74.9
L♂X U♀	53.9	73.2 b	52.1
U♂x L♀	45.8	68.9 b c	63.2
U♂ x U♀	100	94.7 a	-

- Means followed by the same letter in the same column are not significantly different (P< 0.05) Duncan's new multiple rang test).
- Number of pairs = 15
- Ch = chlorfluazuron L= leufenuron u = untreated control

Percent fecundity was calculated according to [22]

$$\%\,\text{Fecundity} = \frac{\text{No.of eggs / treated ♀}}{\text{No.of eggs /untreated ♀}} \times 100$$

Sterility index (S.I.) was calculated according to [23]

$$\text{Sterility index (S.I.)} = 100 - \left[\frac{\text{treatment egg hatch}}{\text{untreatment egg hatch}}\right] \times 100$$

also affected since it ranged between 43.3 to 55.5% compared to 84.3% in untreated control.A significant reduction of imaginal longevity was detected in both sexes (Table 2). The treated adults lived an average of 6.3 days for males and 5.7 days for females, whereas the untreated adults survived (7.9, 8.5 days for ♂ and ♀). The number of mating terminated by spermatophore deposited in bursa copulatrix varied in most experimental series and ranged between (1.4 to 1.8/♀) compared to (2.3/♀) for control. This reduction in number of formed spermatophores transferred by treated males may postulate the activity of BPU as chitin inhibitor on the ectodermal accessory glands which contribute in spermatophore formation in male moths [12].

A clear differences between treated insects and the untreated control was found in the number of deposited eggs.As shown in (Table 2) females produced from treated larvae deposited an average of (743.5 eggs/♀), which represents about (60%) reduction compared to control insects (1865.5 eggs/♀).

All reciprocal crosses of chlorfluazuron and leufenuron treatments resulted in very low percentage of fecundity which ranged between 33.3 to 53.9 % (Table 3).

These results are in agreement with Moursy and Salem [12], Macro and Vinuela [13] and Lyra *et al.* [14]. They attributed the fecundity reduction to, the morphological alternations of ovipositor, inhibition of the ovarian growth, reduction in testicular size and inability from sperm transfer and the toxic effects on the synthesis and metabolism of proteinaceous constituents during the oogensis.

Egg hatchability was also significantly reduced when at least one of the parents was derived from treated larvae, it ranged between (44.5 to 61.7%) for chlorfluazuron and (59.7 to 73.2%) for leufenuron compared to(94.7%)for the control. So it should be mentioned that chlorfluazuron was more effective than leufenuron in reducing egg hatchability. This Preventation of egg hatchability may be due to the penetration of these compounds into the eggs and prevents hatching by interfering with embryonic cuticle synthesis, So the new hatch probably cannot use

Table 4: Residues of Chlorfluazuron and Leufenuron in different developmental stages of the Egyptian cotton leaf worm

Sample analysis		Chlorfluazuron ED50 value=(0.03 ug / g.b.w.),			Leufenuron ED50 value= (0.05 ug / g.b.w.),		
Stage	Days post treatment	Residue* (ppm)	% Elimination	Rate of decomposition	Residue* (ppm)	% Elimination	Rate of decomposition
5th instar	Initial&	0.028	6.7	0.0	0.044	12	0.000
5th instar	1	0.023	23.3	0.196	0.026	48.0	0.526
5th instar	2	0.017	43.3	0.249	0.019	62.0	0.419
5th instar	3	0.013	56.6	0.225	0.009	82.0	0.528
6th instar	5	0.009	70.0	0.226	0.006	88.0	0.398
6th instar	7	0.007	75.6	0.192	0.004	91.8	0.339
Pupae	9	0.0065	78.3	0.162	0.003	94.8	0.314
Pupae	11	0.0062	79.3	0.137	0.002	96.0	0.281
Adult ♀	18	0.006	80.0	0.085	0.0018	96.4	0.177
Adult ♂	19	0.0055	81.6	0.086	0.0015	97.0	0.178
Eggs	21	0.002	93.3	0.125	0.001	98.0	0.180

& = 3 hours

* = Amount as parent compound and average of two replicates

its muscles to free itself from egg wall [13,15] In addition, it is possible that reduced hatchability in *S. littoralis* is caused by defects in the differentiation of oocytes and sperms [16,17].

In comparison with the fertility of the control, Treatments with chlorfluazuron caused(62.2 to 74.9 %) sterility whereas leufenuron caused (52.1 to 74.9 %) sterility. So, treatments with chlorfluazuron appeared in this final evaluation was more efficient than those with leufenuron.

B- Residual behavior of the tested compounds: In order to elucidate the observed differences between the activities of chlorfluazuron and leufenuron, the amount of residues as parent compound existed in differential developmental stages of *S. littoralis* were studied (Table 4). The amount of chlorfluazuron residues detected in the initial sample after 3 hours of treatment was 0.028 ppm which represents 6.7 % loss of the actual applied ED50 value (0.03ug g.b.w.), whereas 12 % loss of leufenuron residues was obtained when compared to the respective ED50 value (0.05 ug/g.b.w.). Such high initial loss of leufenuron residues may be due to low penetration and diffusion of this compound into larval cuticle. Similar initial loss of diflubenzuron residues was determined by Soltani *et al* [18] and Sammour [19].

Tracking the rate of elimination of parent compounds after 24 hours of treatment, the results indicated that leufenuron was eliminated two times higher than chlorfluazuron (the elimination rate was 48 % and 23.3 %, respectively). This fast elimination of leufenuron from the larvae was accomplished with its fast degradation compared to chlorfluazuron, since the decomposition rate was 0.526 and 0.196 respectively. Similar findings by Neumann [6] that chlorfluazuron was about 100 times more toxic than diflubenzuron which was due mainly to a much faster metabolism of diflubenzuron.

Because of the differences in larvicidal activity of chlorfluazuron and leufenuron, the amount of residues of each compound retained in the larvae was of determined. The average of percent compounds recovered during the first 3 days of treatment were 58.9 % chlorfluazuron and 36% leufenuron. This can explain our previously found that chlorfluazuron was more active than leufenuron. However, the remaining residues during this period were sufficient to display that malformations that occurred during the moulting to 6th instar (larval mortality) which represent the main effect of these acylurea compounds.The same finding with El – Saidy *et al.* [20] who indicated that 58 % of teflubenzuron and 38 % of diflubenzuron was found as parent compounds in larval body of *S. littoralis* and caused the effects.

On the other hand the amount of parent compounds underwent a rapid degradation with the time elapsing after the treatment. Since, the percent of elimination reached to 79.3 % and 96 % during the pupal stage for chlorfluazuron and leufenuron respectively.

It is also obvious that the emerged moths still retained a sufficient amounts of chlorfluazuron (18.3 to 20%) and leufenuron (3 to 3.6%) as parent compound in

males and females respectively. This can describe the previously profound effect of these compounds for reducing the fecundity of the treated reciprocal crosses which was more pronounced for chlorfluazuron than leufenuron.

Analysis of extract from eggs indicated that sufficient quantities of the tested compounds were retained by specimens to cause a definite reduction in egg hatchability even 21 day postreatment. The amount of chlorfluazuron recovered from eggs was 6.7 % compared to 2 % for leufenuron. These results reflect the differences between the two compounds in egg hatchability which reached to 40 % and 60 % respectively. This explains the profound effect of these compounds on the adult sterility as an outcome of the reduction in fecundity and in egg hatchability. However, the secretion of unmetabolized acylurea compounds into the eggs and subsequent toxicity to the developing embryos has previously been reported by Ishaaya [7], Spates and Wright [11] and Ive and Wright [21]. So, the enhanced toxicity of chlorfluazuron can be attributed to its lower degradation and high retention as parent compound in the insect as a result of rapid transport from the cuticle into the larval tissues.

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