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TOXICITY AND ACTION OF SOME RELATIVELY SAFE COMPOUNDS ON THE COTTON LEAFWORM *Spodoptera littoralis* (BOISD.)

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ABSTRACT

The insecticidal activity of lufenuron, chromafenozide and ecogen against the cotton leafworm *Spodoptera littoralis* was investigated. The results showed that the treated larvae exhibited different grades of morphological abnormalities which were positively correlated with the age of larvae and tested concentration.

For all treatmetns, third instar larvae were about 4.0, 22.2 and 2.8 times more susceptible than fifth instar ones to lufenuron, chromafenozide and ecogen, respectively. Moreover, lufenuron is the most potent compound followed by chromafenozide and then ecogen.

The three compounds were efficient particularly against earlier larval stages of the insect, and their relative safety may make them useful elements if used properly on cotton and vegetable crops.

The histological changes induced by lufenuron and chromafenozide in the larval body wall were also investigated. The microscopic examination revealed distinct symptoms which were closely related to the nature of the test compound.

INTRODUCTION

The widespread use of traditional pesticides during the last forty years helped in the protection of agricultural production in both North and South countries.

Yet this positive economic contribution was at the cost of a number of adverse effects including disruption of the biological balance,pest resistance, environmental pollution and health hazards. Therefore, a growing concern to develop alternative compounds having selective modes of action and low impact on man and his environment, was adopted.

The insect growth regulators (IGRs), were among this promising alternatives. One group of IGRs is the chitin synthesis inhibitors (Ishaaya,1990). A second group of IGRs called the ecdysone agonists appeared and recently introduced to the markets (Dhadialla *et al.* 1998). Also, the endotoxin of the bacterium *Bacillus thuringiensis (B.t.)* based products, have found widespread use in controlling vast variety of economic pests (Hornby and Gardner, 1987). Another advantage of *B.t*. products was added when the protein toxin in the product is formulated using encapsulation system against the environmental degradation (Zidan *et al*. 1998) .

The present study aims to characterize the insecticidal activities of three rather safe compounds belong to three chemical groups differs in the structure and function against larval instars of the cotton leafworm *Spodoptera littoralis* (Boisd.).

The histological changes induced by these compounds in the structure of integument, were also investigated.

MATERIALS AND METHODS

Tested compounds :

1- Lufenuron (Match): N-[2,5,dichloro-4- (1,1,2,3,3,3-hexafluoropropoxyl)- phenyl amino carbonyl]-2,6 diflurobenzamide, 5% E.C.

2- Chromafenozide (Acro): N-tert-Butyl.-N-(3,5-dimethylbenzoyl) –5-methyl-6-chromacarbohydrazide. 5% E.C.

3- Ecogen: The endotoxin of the entomogenous bacteria *Bacillus thuringiensis* variety Kurstaki encapsulated in killed *Pseudomonas fluorescens.* 10% aqueous flowable.

A laboratory colony of *S. littoralis,* reared as described by El-Defrawi *et al.* (1964), was used in the present study.

Evaluation of the tested compounds:

A series of concentrations of each insect growth regulators, were prepared in water based on percentage of active ingredient. Strips of fresh castor leaves were immersed, for 5 seconds, in a given concentration. They were left, for one hour, to dry before offering to the larvae. The following tests were performed using two different larval instars:

Morphogenetic activity test :

Newly moulted 3rd and/or 5th instar larvae were allowed to feed, for 48 hrs, on the treated castor leaves. Thenafter, the treated leaves were replaced by fresh untreated ones till the treated larvae reached the next molt (4th or 6th instars), respectively. The morphogenetic activity of the molt inhibiting compound Lufenuron, was examined according to Abdalla and Sammour (1992). The intensity of the symptoms were as follows :

Score I: The treated larvae ecdysed with some delay to morphologically normal ones (4th or 6th instars) and survived.

Score II: The treated larvae edysed as in the score I and having pale areas scattered along the dorsal surface of the thorax (Mosaic form), but survived.

Score III: Ecdysis was imperfect, the produced 4th or 6th instars were bloated and the gut was extruded, they died later.

Score IV: The treated larvae failed to moult properly, only a part of the instar was able to leave the old cuticle, consequently, the larvae died trapped in their exuviae.

Score V: Shrinken and blackish 3rd or 5th instar larvae remained unecdysed and eventually died.

To assess the morphogenetic disturbances of the ecdysteroid agonist chromafenozide, another scoring system was developed as follows :

Score I: Partial slippage of the head capsule occurred (premature apolysis) within 8 hrs post feeding. The larvae hardly ecdysed to the next instar but some of them ecdyced after some delay, relative to control.

Score II: The thorasic legs and prolegs are sometimes absent or not fully developed (reduced) or non sclarotized and thus, the ecdysed larvae were unable to adhere to leaves and they seem as drunken creatures.

Score III: The treated larvae underwent partial moulting where adhesion of old cuticle remnants was noticed and an imperfect new cuticle was formed.

The survivors were unable to continue their development .

Score IV: Some of the affected larvae seem to have double head capsules occulude the new mouth parts, which were non-sclerotised and thus, prevent further feeding before dying.

Score V: The affected larvae diminished, darkened, gradually dissociated and died while still within their old cuticle, showing inhibition of ecdysis.

The direct effect of both insect growth regulators, was assessed by examining the treatments daily with the aid of a binocular microscope till ecdysis. The percentage of morphological changes (P-value) was calculated using the Bransby and Williams equation (1971) as follows:

$$P\text{-value} = \frac{1a + 2b + 3c + 4d + 5d}{n \times 5}$$

Where : n= Total number of treated insects, and a, b, c, d and e are number of individuals in scores, I, II, III, IV, and V, respectively.

Toxicity test:

Strips of castor leaves dipped in a series of concentrations of the bacterial endotoxin (ecogen) were prepared as mentioned before. Newly moulted 3rd and /or 5th instar larvae were allowed to feed, for 48 hrs, on the treated leaves. They were provided afterwards by untreated ones till mortality was recorded (4-5 days later).

n all treatments, five replicates of ten identical larvae each were used at each concentration level and control group was fed on castor leaves treated with water.

For all bioassay tests, the data were corrected by using Abbott's formula (1925) whenever necessary. The probit regression lines were drawn and analyzed according to the method of Litchfield and Wilcoxon (1949) . The EC_{50}, EC_{90}, confidence limits and slope values of each compound were deduced.

The relative efficiency of the tested compounds was then calculated using the index proposed by Sun (1950) .

Histological preparations :

Newly moulted 5th instar larvae treated with the respective LC_{50} values of the tested IGRs were used for these experiments. The larvae were allowed to feed on the treated leaves as mentioned before. The larvae were dissected out in the saline solution with the aid of dissecting binocular. Specimens of integument (methothorax) were excised from the larvae, during moulting. At least 5 surviving larvae were picked up, from each treatment as well as the untreated control group.

Each specimen was carefully cleaned, handled and marked; then fixed in Bouin fluid for 24hrs. They processed by the usual manner till sections of 6 μm thickness were obtained and stained by heamatoxylin and eosin. The prepared slides were examined for the pathological symptoms using a Carlazeisis microscope , Germany.

RESULTS AND DISCUSSION

Morphogenetic and toxic action against different larval instars :

The treated larvae exhibited different degrees of morphologi-cal abnormalities, throughout moulting cycle, which was positively correlated with the concentrations used of all compounds. Third instar larvae ingested 0.01 ppm lufenuron, for instance, underwent slight damage (restricted mainly in scores II and I) where, P. value equals 29.2%, table 1. Severe morphological disturbances were dominated in the larvae that were treated with the high concentra-tion, 0.05-ppm lufenuron. These larvae. (presented 19.4, 14.3 and 44.7% deformations in scores III, IV and V, respectively, table 1 and figure 1.

The syndrome, however, became more pronounced with 5th instar larvae. At 0.05 ppm lufenuron, the morphological change percentages (P-value) amounted to 37.67% mainly comprised of 19.0 and 8.74% of scores I and II (table 2). By elevating the tested concentration, the effect gradually shifted to more serious scores. Thus, the majority of fifth instar larvae ingested high concentration (0.3 ppm) suffered great malformations (85.93%); 37.32% of them in score IV and the rest in score V, respectively. (table 2). All of them almost died in few days.

Most of these symptoms usually appear a bit late within the instar; at apolytic phase of the treated larvae. Thus, the proper evaluation should be done 2-3 days later (where the untreated control insects have already molted).

Table (1) : Morphogenetic effects of lufenuron against 3 rd instar larvae of *S. littoralis* fed on treated castor leaves.

Conc. Ppm	Avg. % of affected larvae as indicated in scores					P.value
	I	II	III	IV	V	
0.007	18.94	4.92	0.74	0.0	0.0	24.60
0.01	19.56	6.72	2.04	0.88	0.0	29.20
0.02	18.87	8.18	6.05	2.49	0.0	35.59
0.03	18.87	8.67	8.67	8.67	6.63	51.51
0.04	15.36	2.00	6.68	26.72	15.36	66.12
0.05	5.91	0.0	19.41	14.35	44.73	84.39

Table (2) :Morphogenetic effects of lufenuron against 5 th instar larvae of *S. littoralis* fed on treated castor leaves.

Conc. Ppm	Avg. % of affected larvae as indicated in scores					P.value
	I	II	III	IV	V	
0.03	19.56	6.72	2.04	0.88	0.0	29.20
0.05	19.00	8.74	4.99	3.80	1.14	37.67
0.08	19.54	6.86	8.98	8.98	8.98	53.40
0.1	15.73	2.05	6.84	25.31	18.47	68.40
0.2	5.56	7.94	5.56	29.38	31.76	80.20
0.3	0.0	2.60	6.08	37.32	39.93	85.93

P.value = Percentage of morphological changes.

The disturbing of moulting process beside inhibiting of chitin biosynthesis caused by benzoylphenylurea compounds has been

demonstrated in several lepidopterous species; *Earias insulana* larvae (Abd-El-Ghaffar,1994), *Adoxophyes orane* and *pandemis heparana* (Charmillot and Pasquier, 1995**)**, and *S. littoralis* larvae (Sammour and Abdalla, 2001).

On the contrary, the morphogenetic disorders caused by chromafenozide are greatly varied. The intoxicated larvae showed signs of premature and lethal moulting within one day of feeding on the treated leaves. Therefore, a different scoring system for evaluation of such compounds was developed.

Table 3 and figure 2 explain, to what extent, a wide range of chromafenozide concentrations (0.008-0.1 ppm) could induce five distinct scores. At the lower concentration (0.008 ppm), a small proportion (24.7%) of the treated 3rd instar larvae had slippaged down their head capsules and fragile and non-sclerotized new ones were observed, as commence premature ecdysis (score 1). However, many larvae of this score normally ecdysed to the 4th instar after some delay (larval duration elongated). About 94.1% of the individuals ingested 0.1 ppm chromafenozide were distorted; where 72.1 of them were shrived and showed false double head capsules which may occulude the mouthparts and died (score IV). The rest (15.7%) diminished, gradually desiccated, the color turned to dark and eventually died too (score V), table 3 and figure 2.

Table (3) : Morphogenetic effects of chromafenozide against 3 rd instar larvae of *S. littoralis* fed on treated castor leaves.

Conc.ppm	Avg. % of affected larvae as indicated in scores					P.value
	I	II	III	IV	V	
0.008	19.76	4.12	0.82	0.0	0.0	24.66
0.01	18.96	6.98	3.98	0.0	0.0	29.92
0.03	23.47	1.45	8.81	7.35	2.95	44.03
0.05	25.98	0.0	4.02	7.98	22.02	60.02
0.08	2.84	0.0	8.60	31.56	43.01	86.02
0.1	0.0	0.0	6.30	15.71	72.16	94.17

Similarly, only 23.32% of the 5th instar the larvae that received low concentration of chlorofenozide (0.125 ppm) were affected (table 4). They were morphologically normal, and may suffer in the subsequent developmental stages. Increasing the concentration to 10 times (1.25 ppm), the affected larvae presented 12.06, 18.14 and 60.5% disorder identified by more intensive scores III, IV and V, respectively. Some of these larvae had new and untanned mandibles and prevent further feeding, other diminished and gradually desiccated. Therefore, both of them cannot develop and eventually died.

The morphogenetic disturbances caused by such accelerating molt compounds have been confirmed by many investigators. The non-steroid agonist, tebufenozide induced a premature, abnormal and lethal larval molt to *Lcanobia oleracea* larvae, Blackford and Dinan (1997). Similar effects of many other ecdysteroid agonists were reported on *Cydia pomonella* (Pons *et al*. 1999), and *Diatraea saccharalis* (Rodriguez *et al.* 2001).

Table (4) : Morphogenetic effects of chromafenozide against 5 th instar larvae of *S. littoralis* fed on treated castor leaves.

Conc. ppm	Avg. % of affected larvae as indicated in scores					P.value
	I	II	III	IV	V	
0.125	20.22	2.33	0.77	0.0	0.0	23.32
0.25	21.46	2.80	2.80	0.92	0.0	27.98
0.50	21.53	3.40	6.80	2.28	0.0	34.01
0.75	22.28	1.84	7.41	12.81	11.14	55.70
1.0	19.04	0.0	4.78	16.61	30.87	71.30
1.25	0.0	0.0	12.06	18.14	60.48	90.68

P.value = Percentage of morphological changes.

Unlike the insect growth regulators, the bacterial endotoxin of *B. thuringeneisis* was classified by FAO as biochemical insecticide and its toxic protein can destroy many lepidopterous larvae. The toxic effects of ecogne as an example of this group are presented in table 5. A wide range of concentrations (110-600 ppm) was needed to obtain 15-95% mortality of the third instar with LC_{50} value 220 ppm. Similarly., concentrations of 400-1100 ppm induced 20.9-86% mortality of the fifth instar with LC_{50} value 610 ppm . The death taked place, here as septicemia during moribuned stage, without any morphological affects on the affected larvae. The activity of toxin protein from *B. thuringeneisis* has been demonstrated on growth, survival and feeding behavior of *P. gossypiella and E. insulana* (Zidan *et al.*, 1998) and *S. exigua* (Xiaohui *et al,* 1999).

The obtained results also indicated that the susceptibility of the larvae decreases, in general, by increasing the larval growth (table 6). On the EC_{50} basis, third instar larvae were about 4.0, 22.2 and 2.8 times more susceptible than fifth instar to lufenuron, chromafenozide and ecogen, respectively (table 6).

The increased susceptibility of the young (third) instar larvae may be attributed to the cylindrical shape of caterpillars; the average surface of the body as related to the average body weight is bigger in the 3rd instar compared with the 5th instar, taking into consideration that the larvae move and contact freely during feeding on the treated castor leaves. The increased metabolic rate, in general , in the older larvae than the younger ones my be another explanation for the increased tolerance of the 5th instar larvae to the tested compounds.

Keller *et al.,* (1996) observed an increase in toxic degradation with the loss of sensitivity of 5th instar *S. littoralis* larvae to δ-endotoxins, as detected by profiles change of gut juice proteinase, with larval age. Waldstein and Reissig (2001) also stated that late instars (fourth and fifth) of *Choristoneura resaceana* were three times more tolerant than the neonates to tebufenozide.

The potency of the tested compounds were compared and showed great variation according to the chemical nature of each compound and its mechanism involved (table 6).

T5.6

Thus, lufenuron as antimoulting agent is the most potent ones against both tested instar larvae. Chromafenozide as non-steroid ecdysteroid mimic comes next and presented good efficiency, particularly at the third instar (toxicity index = 86.9). Ecogen, however, as biotoxin cannot be compared with the tested IGR_s (toxicity index <1), but displayed good toxicity against young instar larvae. However, the steepness of the regression lines indicate rather good degree of homogeneity in the test colony.

Histological changes of insect integument

The transversal sections of the integument were prepared from untreated and treated larvae, examined and presented as shown in Figs. 3 and 4.

Fig. 3 presents some sections obtained at different stages throughout normal moulting cycle of the 5^{th} instar larvae. The main components of culticular layers (exo and endo) are clearly visible (Fig. 3A) . the cuticle is, as usually, firmly attached to the epidermal layer at the beginning of the instar. When the larvae enter the apolytic stage, cease to feed and the cuticle detaches from the epidermal layer leaving an ecysial space (fig. 3B). In more advanced stage, the old cuticle undergoes digestion, while build up of new endocuticle matrix evolve underneath as seen in Fig. 3C and so forth.

In sections treated with EC_{50} value of lufenuron, the symptoms appear often late in the larval stadia. The microscopic examination of these sections revealed different grades of endocuticlar lesions. The new cuticle has just begun to evolve while the old one was badly digested; both of them were disturbed in their lay out (Fig. 4A). In some sections, the new cuticle completely thrown into minute tubercles with the persisting old cuticle undigested (Fig. 4 B). These symptoms may trap the larvae into their exuviae and died during moutling. In some other sections, the endocuticle layer was thinner than those of the control ones (Fig. 4C), probably due to deficiency of chitin content (Abd-El-Ghaffar, 1994). The appearance of the new endocuticle, in turn, was soft, irregular and may rupture at certain weak points. Sammour and Abdalla (2001) mentioned that both neem seed extract and diflubenzuron were able to disrupt the endocuticle deposition of *S. littoralis* larvae.

The symptoms appeared in chromafenozide-treated sections were different. At the beginning of moulting , the cuticle features seem to be somewhat normal (Fig. 4D), but such induction of premature moult, would, in turn, lead to form imperfect cuticle structure. However, two distinct features could recognize such –treated sections. The endocuticle was unsclerotized or badly tanned at various regious, thus, disorganized and corrugated fragments of the cuticle are frequently seen (Fig. 4E). Moreover, the epidermal cells showing increasing signs of disintegration or degeneration (Fig. 4F), probably concomitant with untimely apolysis. Retnakaran *et al* (1997) stated that chromafenozide as an ecdysteroid agonist competitively displace the action of natural ecdysone, by arrest growth of the epidermal cells at inappropriate time. The disruptive effects of tebufenozide on cuticle formation have been demonstrated at the ultrastrucural level in the beet armyworm *S. exigua* (Smagghe *et al*. 1996).

FIG 3

FIG 4

It could be concluded that the three compounds are efficient particularly against earlier larval stages of the cotton leafworm and able to contain the early infestation before outbreak resurgence. The relative safety of the compounds may make them rational elements if used properly in regular rotations on cotton and vegetable crops.

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سمية وفعل بعض المركبات الآمنة نسبياً على دودة ورق القطن المصرية

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تم متابعة النشاط الإبادى لكل من اللوفينورون والكرومافينوزايد والايكوجين على يرقات دودة ورق القطن من العمرين الثالث والخامس. وقد أظهرت النتائج درجات متباينة من التشوهات المورفولوجية تناسبت طردياً مع التركيزات المختبرة. كان العمر اليرقى الثالث أكثر حساسية من العمر الخامس فى كل المعاملات، كذلك كان مركب اللوفينيورون أكثر المواد المستخدمة فعالية ضد العمرين المختبرين.

كما درست التغيرات الهستولوجية لمركبى اللوفينروين والكرومافينوزايد على كيوتيكل اليرقات المعاملة وأظهر الفحص الميكروسكوبى تأثيرات جوهرية وأعراض مميزة فى أنسجة الكيوتيكل المختلفة طبقا لطبيعة المركب المختبر.

Table (5): Effectiveness of the bacterial endotoxin *B.thuringiensis* (Ecogen) against 3 rd and 5 th instar larvae of *S.littoralis* fed on treated castor leaves.

TestedInstar	% Corrected mortality at indicated concentrations (ppm)											LC_{50}	Confidence Limits	LC_{90}	Slope
	110	150	200	300	400	500	600	700	800	900	1100				
Third	15.0	27.0	45	70	79.5	89.6	95.0	-	-	-	-	220	(286-196.2)	500	1.92
Fifth	-	-	-	-	20.9	34.8	48.5	62.0	69.0	78.5	86.0	610	(780.8-476.8)	1250	1.72

Table (6): Susceptibility of 3 rd and 5 th instar larvae of *S. littoralis* to the tested pesticides under laboratory conditions

Tested Compound	3 rd instar					5 th instar				
	EC_{50} or LC_{50} (ppm)	Confidence Limits	EC_{90} or LC_{90} (ppm)	Slope	Toxicity Index	EC_{50} or LC_{50} (ppm)	Confidence Limits	EC_{90} or LC_{90} (ppm)	Slope	Toxicity index
Lufenuron	0.02	(0.01-0.03)	0.08	2.78	100	0.08	(0.03-0.11)	0.33	3.2	100
Chromafenozide	0.023	(0.016-.033)	0.088	2.77	86.9	0.51	(0.336-0.775)	2.3	3.2	15.7
Ecogen	220	(169.2-286)	500	1.92	0.009	610	(476.6-780.8)	1025	1.72	0.0001