Minufiya J. Agric. Res. Vol. 33 No.2: - (2008)

BIOCHEMICAL AND HISTOLOGICAL EFFECTS OF FLUFENOXURON , BUXUS CHINENSIS AND BACILLU THURINGIENSIS ON THE SESAMIA CRETICU (Led.)

Smaya Z. Sayed and Magda Khtab
Plant Prot. Res. Inst., Agric. Res. Cent., Dokki, Giza, Egypt.

(Received: Feb. 12, 2008)

ABSTRACT: ***The activity of amino transferases and the histological structure of the integument were studied in the larvae of S. creticu treated with the LC_{50} of chitin synthesis inhibitor, Flufenoxuron;Buxus chinensis and Bacillus thuringiensis var. kurstaki. Aspartate amino transferase (AST) and alanine amino transferase (ALT) were significantly decreased in the treated larvae than those of the control . The decrease in the level of mino transferases was more pronounced in flufenoxuron treatment than that of Buxus chinensis and or Bacillus thuringiensis. AST was more active than ALT in both non-treated and treated larvae.The formation of epicuticle and most of the endocuticular deposition of larvae treated with flufenoxuron were completely disrupted. Treatments with Buxus chinensis and Bacillus thuringiensis slightly affected the three layers as compared to flufenoxuron.***

Key Words: ***S. creticu, Chitin synthesis inhibitor,Buxus chinensis and Bacillus thuringiensis, Amino transferases, Histology of integument.***

INTRODUCTION:

S. cretica is a highly polyphagous insect pest infesting host plants including important field crops such as maize,. The extensive use of insecticides to control S. littoralis larvae has led to its resistance to various classes of insecticides (Tabashink *et al*., 1987), residual toxicity and environmental pollution (Frank *et al*., 1990) and negative effects on non-target organisms (Franz, 1974).

The foregoing problems and hazards that have arisen as a result of using conventional insecticides are incentives for researching alternative control agents with new modes of action. Among these agents are the chitin synthesis inhibitors (CSIs) and microbial control agents.

The present study evaluated the three tested compounds to explore their mode of action and their effects on specific activity of amino transferases as well as the histological changes in the integument.

MATERIALS AND METHODS

Compounds used:

A chitin synthesis inhibitor and two microbial control agents were used in this study. Flufenoxuron 10 % was obtained from Sumitomo Chemical Co.,

Ltd., Buxus chinensis and Dipel 2X (Bacilllus thuringensis) var. kurstaki (Valent Berlner Chemical Co.). The testing and application of the microbial agent was carried out according to Mc-kinley (1985).

1 - Susceptibility of S. cretica larvae to the tested compounds was carried out with the use of leaf-dipping technique (Abo El-Ghar *et al*., 1994). Different aqueous concentrations of flufenoxuron, B. thuringiensis and Buxus chinensis were, used R. communis, leaves were dipped in each concentration of (6, 4, 2, and 1 and 0.5 ppm) for Flufenoxuron. (4, 2, 1, 0.5) for Buxus chinensis and (8, 6, 4, 2 and 1(IU) for B. thuringiensis and then left to dry at room temperature and these were offered to the newly moulted 4th instar larvae. Four replicates (20 larvae/each replicate) were used. Larvae that fed on untreated castor bean leaves were used as control. Larvae were allowed to feed for 24 hrs. then, they were provided with fresh, clean and untreated castor bean leaves until pupation. The percent mortality of treated larvae was corrected against those of the control by using Abbott's formula (Abbott, 1925).

The data were then subjected to probit analysis (Finney, 1971) to obtain the LC_{50} values.

Preparation of samples for biochemical analysis:

Samples were collected according to the method described by Ishaaya *et al*. (1971). The enzyme solutions (samples) were obtained by homogenizing the 6th instar larvae representing 1 gm larval body weight, in 5 ml distilled water by using chilled glass Teflon grinder. The homogenate was centrifuged for 10 min. at 5000 rpm and 5 ^{O}C, the supernatant fraction being used for the enzyme assay.

Determination of homogenate amino transferases activity:

The level of amino transferases activity was determined with the colorimetric method of Reitman and Frankel (1957). The principle of method is based on the advantage derived from the greater differences in the absorption of an alkaline solution of the 2, 4-dinitrophenylhydrazones of α-ketoglutarate, oxaloacetate and pyruvate at 505 nm.

Histological studies:

The tested compounds were used for a comparative study to determine their effects on the histology of integument of late surviving 6th larval instars treated as 4th instars with the LC_{50} values of the respective compounds. The tested tissue was fixed in aqueous Bouin's solution for 24 hr. The normal paraffin wax embedding procedure was followed. The sections were cut 6µ thick and stained with heamatoxylin and eosin for microscopic examination. Control sections of non-treated larvae were also studied.

RESULTS AND DISCUSSION:

Toxicological effects:

Table (1) reveals the LC_{50} values of the tested compounds against the newly molted 4th instar larvae recording 0.303 ppm, 6.031 (IU) gm / L and 4 ppm for flufenoxuron, B. thuringiensis and Buxus chinensis, respectively.

Biochemical studies:

Amino transferases activity:

Data in Table (2) show the effect of flufenoxuron, Buxus chinensis and B. thuringiensis on the activity of amino transferases in the homogenate of late 4th instars of S. cretica treated as 3rd instars. The results indicated that the activities of AST (Aspartate amino transferase) and ALT (alanine amino transferase) were significantly decreased in the caseof the three tested compounds as compared to the control. The data obtained revealed that AST was more active than ALT in both non-treated and treated larvae. The decrease in AST and ALT activities due to treatment with flufenoxuron is similar to the data obtained by El-Sheikh (2002) who found that treatment of Agrotis ipsilon larvae with flufenoxuron decreased amino transferases activity. Furthermore, the 4th instar larvae treated with chlorfluazuron and flufenoxuron show a decrease in amino transferases activity (Abdel-Aal, 2003). Moreover, the results obtained for B. thuringiensis treatment coincide with those of Afifi (2001) who found that, LC_{50} of three commercial products of B. thuringiensis significantly decreased the activity of AST and ALT of the pink bollworm, Pectinophora gossypiella. In insects, the amino transferases are the key enzymes in the formation of non-essential amino acids, in the metabolism of waste nitrogen products and in gluconeogenesis (Mordue and Goldsworthy, 1973; Pant and Kumar, 1980; Kaur *et al*., 1985). Changes in amino transferases levels have been correlated with protein anabolism and catabolism (Knox and Greengard, 1965 and Kaur *et al*., 1985). The decrease in AST and ALT in the present study may be attributed to the binding of the three tested compounds with protein that leads to inhibition in amino transferases activity which is known to be intimately related to protein synthesis

Histological studies:

The integument of normal 4h instars of S. cretica, from outward to inward consists of cutiula, hypodermis and basement membrane (Plate1). The cuticula is divided into three layers: one outer thin layer called the epicuticle followed by two inner ones, the exocuticle and endocuticle. The hypodermis consists of a single layer of more or less cuboidal or columnar cells. Each cell contains a large central nucleus. The basement membrane is so closely attached to the basal surface of the hypodermal cells that it is very difficult to distinguish.

Treatment of S. cretica 4th instars with the LC_{50} of flufenoxuron completely

disrupted the formation of endocuticle of the resulting late 4th instars (Plate2). Treatment with the LC_{50} of both Buxus chinensis and Bacillus thuringiensis (Plates 3 and 4) slightly affected the three layers as compared to control.

Histological changes due to the effect of various toxicants have been a subject of considerable discussion among various authors as the primary cause of insect's inactivity and consequent death. Many of the histological changes of the integument observed in the present study for S. cretica late 4th instars due to treatment of 4th instar ones with the chitin synthesis inhibitor, flufenoxuron have been reported by Hegazy (1990) and (Abdel-Aal, 2003) using different chitin synthesis inhibitors against the same insect species

Yu and Terriere (1975) postulated that the chitin synthesis inhibitor, diflubenzuron causes an accumulation of the molting hormone, beta-ecdysone, by reducing the activity of ecdysone metabolizing enzyme. The increased beta-ecdysone level stimulates the enzyme, chitinase, which degrades chitin in preparation for ecdysis. Beta-ecdysone, causes juvenile hormone deficiency, and stimulates decarboxylase and phenoloxidase (enzymes involved in tanning processes) which would further disrupt the normal moulting pattern. Furthermore, Avignone and Mignone (1995) reported that B. thuringiensis produced lethal active endotoxin that interacts with insect cell membrane and causes septicemia. Increase in Buxus chinensis and B. thuringiensis count in the haemolymph may lead to lack in main haemolymph contents (total protein and carbohydrate) which is necessary for building up of a cuticle. The presence of all these factors at the critical time of insect development probably leads to abnormal endocuticular depositionor, vacuolization of the hypoderms and abortive molting. This hypothesis may explain the failure of endocuticular deposition in S. cretica larvae treated with the LC_{50} of chitin synthesis inhibitor, Flufenoxuron; Buxus chinensis and B. thuringiensis as obtained in the present investigation.

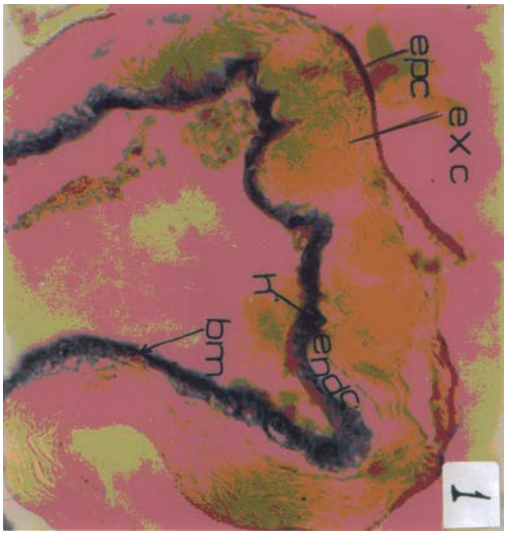

Plate 1: Photomicrograph of T. S. in the integument of normal late 4th larval instar of *S. cretica*

bm : basement membrane.
endc: endocuticle.
epc : epicuticle.
exc : exocuticle.
h : hypodermis.

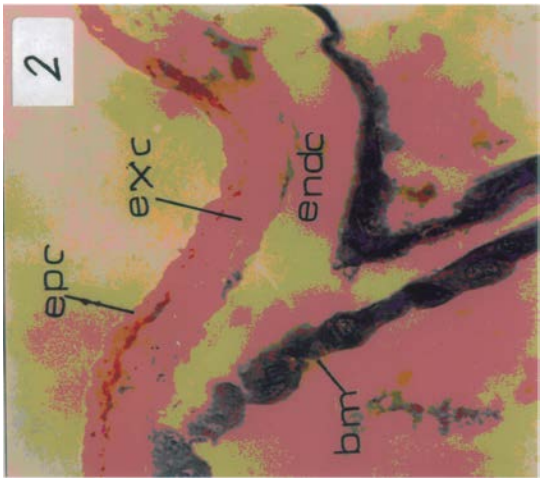

Plate 2: Photomicrograph of T. S. in the integument of late 4th larval instar of *S.*

cretica **treated with LC_{50} of flufenoxuron.**

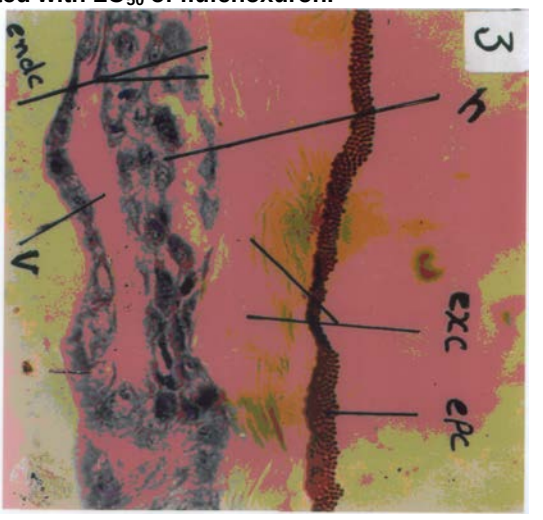

Plate 3: Photomicrograph of T. S. in the integument of late 4th larval instar of *S. cretica* treated with LC_{50} of Nuclear Polyhydrosis Virus
bm : basement membrane.
endc : endocuticle.
epc : epicuticle.
exc : exocuticle.
h :hypodermis.
V : vacuole

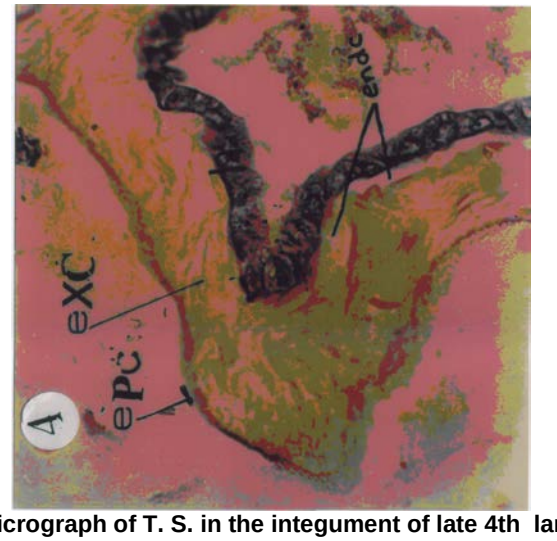

Plate 4: Photomicrograph of T. S. in the integument of late 4th larval instar of *S.*

cretica* treated with LC_{50} of *Bacillus thuringiensis

Table (1): Susceptibility Of S. cretica 4th Instar Larvae To Flufenoxuron, Buxus Chinensis And B. Thuringiensis.

Treatment	LC_{50}	95% Fiducial limits		Slope ± SE	X^2
		Lower	Upper		
Flufenoxuron	0.303 (ppm)	0.166	0.601	1.22 ± 0.270	0.206
Buxus Chinensis	4ppm	0.13	2.7	1.3 ± 0.147	0.877
B. thuringiensis	6.03 (IU)	6.7	17.8	2.00 ± 0.287	13.01

Table (2): Effect of LC_{50} of flufenoxuron, Buxus chinensis and B. thuringiensis on the amino transferases activity of late 4th instars of S. cretica.

Treatments	AST mean activity (µg oxaloacetate/min/ml) ± SE	ALT mean activity (µg pyruvate/min/ml) ± SE
Flufenoxuron	12.48** ± 1.23	7.38** ± 1.37
Buxus Chinensis	30.92* ± 3.66	12.77* ± 0.99
B. thuringiensis	29.71* ± 4.52	13.28* ± 1.00
Control	40.43 ± 2.38	18.42 ± 3.

**: highly significant at P> 0.001. *: Significant at P< 0.05

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التأثيرات الكيميائية الحيوية والهستولوجية للفلوفينو كسيورون والهوهوبا والباسللس ثيورينجينسيز لدودة القصب الكبرى

سامية زين سيد – ماجدة خطاب

معهد بحوث وقاية النبات – مركز البحوث الزراعية – الدقي ، الجيزة.

الملخص العربي

اشتملت الدراسة على تقدير تأثير التركيز قاتل النصف (LC_{50}) لمركب الفلوفينو كسيورون (مثبط لتركيب الكيتين) والهوهوبا والباسلليس ثيورينجينسيز على نشاط الأنزيمات الناقلة لمجاميع الأمين والتركيب الهستولوجي لجليد يرقات دودة القصب الكبرى وقد وجد أن المعاملة بمركب الفلوفينوكسيورون قد أدت إلى نقص معنوي شديد في نشاط الأنزيمات الناقلة لمجاميع الأمين بينما أدت المعاملة بالهوهوبا والباسلليس ثيورينجينسيز إلى نقص أقل معنوية بالمقارنة بالفلوفينوكسيورون وبغير العاملة (المقارنة).

من ناحية أخرى وجد أن أنزيم الأسبرتات أمينو ترانسفيريز أكثر نشاطاً من أنزيم الألنين أمينو ترانسفيريز في اليرقات المعاملة وغير المعاملة (المقارنة).

أحدثت كذلك معاملة الطور اليرقي الرابع لدودة القصب الكبرى بمركب الفلوفينوكسيورون تغيرات هستولوجية شديدة في جليد يرقات الطور الرابع المتأخر الناتج من تلك المعاملة حيث تسببت في تلف لتكوين الجليد الداخلي بأكملة بينما كان هذا التأثير أقل وضوحاً في المعاملة بالهوهوبا والباسلليس ثيورينجينسيز.