J. Plant Prot. and Path., Mansoura Univ., Vol. 4 (10): 837 - 844, 2013

biochemical effects of some insect growth regulators on field strains of the cotton leafworm, *spodoptera littoralis*

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ABSTRACT

Different strains of cotton leafworm, *Spodoptera littoralis* larvae were collected from heavily sprayed fields or recently cultivated fields in different areas in Alexandria, and El-Boheira, proviance governorate. Chitinase activity was determined in laboratory susceptible strain of cotton leafworm and compared with enzyme activity for other collected strains which cleared that highest level of chitinase activity was found in Alexandria strains. The sensitivity of chitinase activity to chlorfluazuron and teflubenzuron was measured by I_{50} values, values of I_{50} in the case of chlorfluazuron were 0.23, 0.34, 0.41, 0.60, and 0.69 µM for lab strain; Borg El-Arab; West of Nobaria; Abou El-Matamir, and Edko strains of *Spodoptera* 2^{nd} larvae respectively, while I_{50} values were 0.31, 0.40, 0.46, 0.66, and 0.74 µM for lab strain and four field strains of *Spodoptera* 4^{th} larvae respectively, similarly, the teflubenzuron were 0.40, 0.50, 0.57, 0.74, and 0.82 µM for lab strain and four field strains of *Spodoptera* 2^{nd} larvae respectively, the I_{50} values were 0.47, 0.59, 0.65, 0.88, and 0.93 µM for lab strain and four field strains of *Spodoptera* 4^{th} larvae respectively. Also, the inhibition constant (K_i) values were determined, the obtained data proved that compounds competitive inhibition of chitinase activity. The significant high mortality percentages were observed at all tested concentrations with chlorfluazuron than teflubenzuron and the result clearly showed that the 2^{nd} instar larvae were more sensitive to the compounds tested, compared to those of the 4^{th} instar, so when IGRs used for *S. littoralis* larvae control, dosage and timing of application should be carefully considered. The results of the present study may add some forward steps to use IGRs as alternative to conventional insecticides especially against this insect, so, the IGRs can be involved in important steps necessary for successful IPM programs applied against *S. littoralis.*

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* is a very destructive pest, it attacks a wide variety of field crops and causing great economic losses, this pest rapidly acquires resistance to nearly all classes of applied insecticides (Abo Elghar *et al.* 2005; Saleem *et al*. 2008, and Ahmed *et al*. 2009), so need to develop novel alternatives or functional combinations of pest control techniques is emphatically a product of this decade, attention was therefore paid to control insects using different non traditional insecticides, e.g., insect growth regulators (IGR). These compounds are less toxic and compatible with insect pest management that were developed to reduce the pollution in food and environment, and have a specific mod of action on insects and a lower toxicity against vertebrates than conventional insecticides, also these compounds are effective suppressors of development for the entire life cycle on insects (Smagghe *et al.* 2004, and Nasr *et al*. 2010). These compounds which are considered nowadays one of the mainly component of IPM program term IGRs describe a new class of bio-rational compounds (Schneider *et al.* 2003).

The purpose of this investigation, describe the development of biochemical assay system for measuring the sensitivity of chitinase to chlorfluazuron, and teflubenzuron, also provide enzyme kinetic data for the chitinase in this four field strains Borg El-Arab, and West of Nobaria (Alexandria, Governorate), Abou El-Matamir, and Edko (El-Boheira, Governorate), and compared them with data obtained of laboratory strain.

MATERIALS AND METHODS

1. Test insects:

Susceptible laboratory strain of cotton leafworm, *Spodoptera littoralis* was provided from central lab of pesticides, Agricultural Research Center (ARC) Cairo, Egypt which was reared for several years.

Field strains of cotton leafworm, *Spodoptera littoralis* egg masses were collected from cotton fields at Borg El-Arab, and West of Nobaria (Alexandria, Governorate), Abou El-Matamir, and Edko (El-Boheira, Governorate), the 2^{nd} and 4^{th} larval instars chosen for bioassay and biochemical assessment.

2. Test insecticides:

Chlorfluazuron (atabron, 5% EC), and teflubenzuron (Nomolt, 15% SC) IGRs insecticides were obtained from Shell Angro, FRG;

3. Bioassay tests:

3.1. Toxicity of the tested insecticides against *S. littoralis*:

Chlorfluazuron and teflubenzuron were bioassayed against the 2^{nd} and 4^{th} larvae of *S. littoralis*. The castor leaves were dipped in different concentrations of the tested insecticides. Chlorfluazuron and teflubenzuron concentrations were prepared in distilled water. Treated and control plants were air-dried for 3 hrs, the treated leaves were placed in clean glass container at the laboratory conditions of 27 ± 2 ^{o}C and 65-70 % RH, ten larvae (Field strains) were used for each test with three replicate at least. Number of alive and dead larvae per replicate was counted 24; 48, and 72 hr, after treatment. Concentrations–mortality percentages were calculated and corrected for natural mortality according to Abbott equation (Abbott, 1925). LC_{50} values were calculated by using the of probit-analysis method of Finney (1971).

4. Biochemical studies:

4.1. Chitinase preparation and activity assay:

Chitinase was prepared from *Spodoptera littoralis* 2^{nd} and 4^{th} instars larvae (lab and field strains) according to the method of Deul *et al*. (1978), homogenized was prepared in 10^{-3} M Cleland,s reagent (dithiotheritol, DTT) (v/w=2), the homogenate was centrifuged at 12,000 g for 15 min, an equal volume of saturated ammonium sulfate solution was slowly added to the supernatant, after stirring for 1 hr, the suspension was centrifuged at 10,000 g for 10 min, the precipitate was washed with half-saturated ammonium sulfate solution and was recentrifuged, after which it was suspended in a small volume of water, followed by dialysis 20 hr, an occasional precipitate was removed by centrifugation and was discarded as it proved to be enzymatically

inactive after dialysis water was added to the original ratio (v/w=2). All manipulations were carried out at 0-2 ^{o}C.

The chitinase activity measurements were done according to method reported by Reissig *et al*. (1955), which modified by Andrew *et al*. (1982), using sodium acetate buffer instead of tris-HCl buffer and wave-leangth 416 nm was used instead of 544 nm. 25 µl of chitin (20 mg/ml), 100 µl of enzyme prep and 225 µl of sodium acetate buffer, (pH 4.5) in total volume 350 µl. The enzyme substrate mixture was incubated at 35 ^{o}C for 60 min, then the reaction was stopped by adding 100 µl of 0.8 M borate buffer (pH 10.0) followed by determination of n-acetylglucoseamine by method of Reissig *et al*. (1955), by adding 1.5 ml of p-dimethyl amino benzaldhyde (DMAB, reagent). The samples were incubated in shaker water bath at 35 ^{o}C for 20 min, the samples were measured spectrophotometrically at λ 412 nm.

The protein content in prepared homogenates of *S. littoralis* was assayed spectrophotometrically by the method of Lowery *et al*. (1951) at λ750 nm using Bovine Serum Albumin (BSA) as a standard protein.

4.2. *In vivo* inhibition of chitinase activity:

The inhibition of chitinase activity was determined in 2nd and 4th instars larvae using the LC_{50} values of each of the tested insecticides (chlorfluazuron and teflubenzuron). In the inhibition studies, of chitinase activity 10 µl of the enzyme preparation was incubated with of the inhibitor for 30min, the enzyme-inhibitor mixture was used to measure the remaining activity. The percent inhibition was calculated using the following formula:-

$$\%\text{Inhibition} = \frac{V - Vi \times 100}{V}$$

Where:- (V) is the specific activity without inhibitor.

(Vi) is the specific activity presence inhibitor

4.3. *In vitro* inhibition and kinetics of chitinase activity:

The inhibitor of chitinase activity was evaluated to determine enzyme kinetic parameters, the method of Dixon and Webb (1964) was adopted to draw the Dixon-plots by plotting 1/V versus concentrations of the inhibitor (chlorfluazuron and teflubenzuron) at two concentrations of the substrate, chitin (the substrate of chitinase) concentrations were 3.0 and 5.0 mM.

Estimation of I_{50} value was carried out by preincubating the enzyme with the inhibitor for 30 min, using the following concentrations 0.1; 1; 5; 10; 50, and 100 µM, K_i (the inhibition constant) values for each inhibitor were estimated from Dixon-plot.

Michaelis-Menten Kinetics (K_m and V_{max}) values were calculated by a linear regression of 6 point on each Lineweaver and Burk Plot (1934).

RESULTS AND DISCUSSION

Toxicity of tested insecticides against *S. littoralis* larvae:

Table (1) shows the LC_{50} values of tested insecticides, chlorfluazuron and teflubenzuron after 24hrs and 48hrs of treatment for 2nd and 4th instar larvae of *S. littoralis* collected from different areas in Alexandria proviance (Borg El-Arab, and West of Nobaria) and El-Boheira, proviance (Abou El-Matamir, and

Edko). The data are compared with the same effect on laboratory strain of 2nd and 4th instar larvae of cotton leaf worm.

It is clear that the toxicity was higher with the chlorfluazuron and teflubenzuron for Borg El-Arab and West of Nobaria, while toxicity was low for Abou El-Matamir, and Edko, also chlorfluazuron was more toxic than teflubenzuron in controlling of *S. littoralis* larvae, in general the treatments provided higher toxic effect to the second than the fourth larval instar. The present results emphasize that during many years of selection pressure in the field, the resistance and/ or tolerance levels to the conventional insecticides had increased due to the intensive application of such conventional insecticides for controlling of *S. littoralis* in cotton fields. IGRs effects depending on species and studied developmental stage and the larvae died during pharate conditions after initiation of molting, without completion of morphogenesis. These results fully agreeded with (Mesbah *et al*. 1982; Abd El-Naby *et al*. 1990; Toscano *et al*. 2001; Dalia, and Badawy 2006).

Table (1): Toxicity of IGRs on *S. littoralis* larvae.

***Spodoptera* strain locations**	**LC_{50} (ppm)**							
	chlorfluazuron				**teflubenzuron**			
	24hr		**48hr**		**24hr**		**48hr**	
	2nd instar	**4th instar**	**2nd instar**	**4th instar**	**2nd instar**	**4th instar**	**2nd instar**	**4th instar**
Laboratory	0.14	0.34	0.024	0.041	0.25	0.46	0.038	0.053
El-ArabBorg	0.33	0.43	0.043	0.054	0.41	0.52	0.049	0.060
West of Nobaria	0.40	0.54	0.052	0.060	0.53	0.63	0.058	0.074
Abou El-Matamir	0.64	0.75	0.073	0.081	0.74	0.86	0.077	0.090
Edko	0.71	0.82	0.080	0.087	0.82	0.91	0.090	0.096

The *in vivo* inhibition of *S. Littoralis* chitinase activity:

The *in vivo* inhibitory effect of the LC_{50} values of tested IGRs against to the *Spodoptera* 2nd and 4th instars lab and Field strains larval chitinase are shown in the data given in Table (2). The data declared that chlorfluazuron exhibited the highest percentages of reduction of chitinase activity, percentages of chitinase inhibition were 87.3, 84.2, 74.1, 68.4, and 61.5 % for lab; Borg El-Arab; West of Nobaria; Abou El-Matamir, and Edko of *Spodoptera* 2nd larvae strains respectively, while values were 82.5, 77.4, 73.6, 65.1, and 56.3 % for lab strain and four field strains of *Spodoptera* 4th larvae respectively, also in case of teflubenzuron percentages of chitinase inhibition were 74.0, 70.4, 66.3, 57.7, and 54.5 % for lab strain and four field strains of *Spodoptera* 2nd larvae strains respectively, while values were 70.5, 68.3, 60.1, 53.4, and 52.2 % for lab strain and four field strains of *Spodoptera* 4th larvae respectively. It is clear that the chlorfluazuron and teflubenzuron active as inhibitor on chitinase activity. Properties of the IGRs were originally recognized through their ability to initiate inappropriately timed and poorly coordinated moulting processes, the resulting perturbation of moulting and metamorphosis leads to death, usually because the insects cannot escape from the exuvia, although there are additional related morphological problems

(Aller and Ramsay, 1988). Nasr *et al*. (2010) who reported that the high toxic effect of buprofezin and pyriproxyfen on chitinase.

Table (2): *In vivo* inhibition of *Spodoptera* larvae chitinase activity by two IGRs (LC_{50}).

Spodoptera strain locations	%Inhibition			
	chlorfluazuron		teflubenzuron	
	2nd instar	4th instar	2nd instar	4th instar
Laboratory	87.3	82.5	74.0	70.5
El-ArabBorg	84.2	77.4	70.4	68.3
West of Nobaria	74.1	73.6	66.3	60.1
Abou El-Matamir	68.4	65.1	57.7	53.4
Edko	61.5	56.3	54.3	52.2

Kinetic parameters of chitinase inhibition:

The kinetic studies were conducted to evaluate the effects of chlorfluazuron and teflubenzuron on chitinase activity in both tested strains of *S. littoralis* 2nd and 4th larvae, Table (3) shows the obtained Lineweaver-Burk (L-B) plots for chitinase in lab strain and four tested field strains and the statistical analysis of the obtained values of K_m (Michaelis-Menten Kinetics, constant) and V_{max} (maximum velocity) of the chitinase. The K_m values for chitinase were generally higher in all four tested field strains than lab strain, the changes in K_m values of chitinase between the four tested field strains indicate changes in the affinities.

The present results show that the V_{max} values of chitinase are obviously higher, this points of the higher substrat turnover which may reflect the physiological importance of the chitinase in the function of the moulting of the *S. littoralis* larvae. The V_{max} values were generally higher in all tested field strains than lab strain, this indicated that the number of active sites on the chitinase of the larvae was increased in the field strains, such change may be followed by decrease in the insect susceptibility which could be altered by field application of the insecticides.

Table (3): Michaelis-Menten Kinetics of the chitinase of larval of *S. littoralis* of collected from different locations.

Spodoptera strain locations	chlorfluazuron				teflubenzuron			
	K_m mM		V_{max} mM		K_m mM		V_{max} mM	
	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar
Laboratory	0.14	0.33	8.6	7.3	0.26	0.38	6.0	5.3
El-ArabBorg	0.28	0.42	6.5	6.3	0.43	0.49	5.1	4.2
West of Nobaria	0.34	0.49	5.7	5.4	0.51	0.56	4.8	3.1
Abou El-Matamir	0.53	0.66	4.3	3.1	0.63	0.70	3.4	2.4
Edko	0.61	0.70	3.2	2.2	0.74	0.78	2.5	1.8

The *in vitro* inhibition of *S. littoralis* chitinase activity:

To characterize more details about the *in vitro* inhibition of chitinase by the inhibitors, the K_i value of each inhibitor was estimated from the graphical method of Dixon and Webb (1964), Table (4). The K_i values were 5, 18, 20, 32, and 44 µM for lab; Borg El-Arab; West of Nobaria; Abou El-Matamir, and Edko of *Spodoptera* 2nd larvae strains respectively in case of chlorfluazuron,

while values were 14, 28, 33, 45, and 52 µM, for lab strain and four field strains of *Spodoptera* 4th larvae respectively, also in case of teflubenzuron percentages of chitinase inhibition were 12, 24, 34, 42, and 53 µM for lab strain and four field strains of *Spodoptera* 2nd larvae strains respectively, while values were 22, 35, 40, 57, and 63 µM for lab strain and four field strains of *Spodoptera* 4th larvae respectively. The obtained data proved that each of chlorfluazuron and teflubenzuron showed competitive inhibition on chitinase, and the present study showed that the larval mortality was clearly caused by moulting failure, this effect is mainly induced by inhibiting chitin formation according to Ishaaya and Casida (1974), thereby causing abnormal endocuticular deposition and abortive moulting, also the actual cause of insect death by chitin inhibitors may be attributed to either a rupture of the newly formed cuticle. (Mitsui *et al*. 1981; Uchida *et al.* 1985; Clarke, and Jewess, 1990; Merzendorfer, and Zimoch, 2003; Salama *et al*. 2008).

Table (4): *In vitro* inhibition of *Spodoptera* larvae chitinase activity by two IGRs (LC_{50}).

Spodoptera strain locations	chlorfluazuron				teflubenzuron			
	I_{50} µM		K_i µM		I_{50} µM		K_i µM	
	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar
Laboratory	0.23	0.31	5	14	0.40	0.47	12	22
El-ArabBorg	0.34	0.40	18	28	0.50	0.59	24	35
West of Nobaria	0.41	0.46	20	33	0.57	0.65	34	40
Abou El-Matamir	0.60	0.66	32	45	0.74	0.88	42	57
Edko	0.69	0.74	44	52	0.82	0.93	53	63

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التأثيرات السامة الكيموحيوية لبعض منظمات النموعلى سلالات حقلية لدودة ورق القطن (سبدوبترا ليتولارس).

سهام منصور إسماعيل

المعمل المركزى للمبيدات – الصبحية – الإسكندرية

الهدف من البحث هو دراسة الأختلافات فى نشاط أنزيم من أهم الأهداف البيولوجية والحيوية فى الحشرة وهو أنزيم الكيتينيز وأيضاً دراسة مستوى حساسية يرقات العمر الثانى والرابع لدودة ورق القطن لمبيدين من منظمات النمو (كلورفلوزيرون و تيفلوبينزيرون) حيث تم أستخلاص الأنزيم من يرقات العمر الثانى والرابع لدودة ورق القطن وذلك مابين أربع عشائر مختلفة جمعت من الحقول المصرية وتمت مقارنتها بعشيرة معملية حساسة، تركزت الدراسة على العشائر المنتشرة فى المناطق التى ترش بمعدل كثيف من المبيدات (أبو المطامير- أدكو) وأيضاً فى المناطق الصحراوية المنزرعة حديثاً (برج العرب – غرب النوبارية) والتى تنتشر فيها زراعة القطن. ولقد أوضحت النتائج أن قيم التركيزات النصف مميتة (LC_{50}) أظهرت أختلافاً محسوساً حيث كانت سلالة برج العرب أكثر السلالات حساسية يليها سلالة غرب النوبارية بينما أبو المطامير وأدكو كانت أكثر تحملاً، وقد أوضحت النتائج أن يرقات الطور الثانى أكثر حساسية للمركبات المختبرة مقارنة بيرقات الطور الرابع مع الأخذ فى الأعتبار الجرعات وأوقات التطبيق (الطور اليرقى المناسب). وكذلك تم دراسة المقدرة التثبيطية للمبيدات المختبرة على النشاط الأنزيمى لأنزيم الكيتينيز وكذلك فقد تم تقدير قيم الـ I_{50} فوجد بالنسبة لتأثير كلورفلوزيرون وقد وجد أن هذه القيم هى ٠,٢٣، ٠,٣٤، ٠,٤١، ٠,٦٠ و ٠,٦٩ ميكرومولر وذلك بالنسبة ليرقات العمر الثانى للسلالة الحساسة، برج العرب، غرب النوبارية، أبو المطامير وأدكو على التوالى بينما على يرقات العمر الرابع كانت ٠,٣١، ٠,٤٠، ٠,٤٦، ٠,٦٦ و ٠,٧٤ ميكرومولر وذلك بالنسبة للسلالة الحساسة والأربع سلالات الحقلية المختبرة على التوالى، وأيضاً كانت قيم الـ I_{50} لمبيد تيفلوبينزيرون ٠,٤٠، ٠,٥٠، ٠,٥٧، ٠,٧٤ و ٠,٨٢ ميكرومولر وذلك بالنسبة ليرقات العمر الثانى للسلالة الحساسة والأربع سلالات الحقلية المختبرة على التوالى، بينما على يرقات العمر الرابع كانت ٠,٤٧، ٠,٥٩، ٠,٦٥، ٠,٨٨ و ٠,٩٣ ميكرومولر وذلك بالنسبة للسلالة الحساسة والأربع سلالات الحقلية المختبرة على التوالى. وأيضاً تم تقدير ثابت التثبيط K_i وقد وجد أن هذه المركبات أظهرت تثبيط تنافسى على نشاط أنزيم الكيتينيز. وبذلك يمكن أستخدام هذه المبيدات المختبرة (منظمات النمو) فى برامج المكافحة المتكاملة لدودة ورق القطن وذلك من قيم النشاط الأنزيمى لها وذلك بهدف تلاشى تأثير المبيدات التقليدية الضار على البيئة.

قام بتحكيم البحث

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