RESPONSE OF CHITINASE AND PHENOLOXIDASE TO INSECT GROWTH REGULATORS AND BIOINSECTICIDES IN *SPODOPTERA LITTOLARIS*

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ABSTRACT

The present study was carried to evaluate the effects of the lethal dose (LC_{50}) of abamectin and two IGRs on cotton leafworm Spodoptera littoralis (Boisd.) from the point of biochemical aspects. Also, is essential to know more about the mode of action of abamectin and to which related to, the chitin synthesis inhibitors or to the juvenoid compounds. The relative susceptibility of two strains (laboratory and field strains) of the strains to three insecticides: iflubenzuron, yriproxyfen nd bamectin, as ested sing ipping echnique. alues of the LC_{50} were calculated for each compound. The tolerance ratio was determined on basis of the LC_{50} values of the two tested strains. Obtained esults evealed hat he ield train xhibited ifferent egrees f tolerance owards he hree ested insecticides which can be arranged as follows: pyriproxyfen (10.5 folds), diflubenzuron (5.5 folds) and abamectin (5.3 folds) as compared to the laboratory strain. Chitinase activity decreased markedly after treating the insects with the LC_{50} of the three tested insecticides when compared to untreated insect. The reduction in the activity continued in case of diflubenzuron and abamectin. At the end of experiment the three tested compounds caused great elevations in chitinase activity. The three tested co - pounds gave the same trend of reducing phenoloxidase activity during the tested time intervals when compared to control.

Key Words: Chitinase, phenoloxidase, IGRs, biopesticides, *Spodoptera littoralis*.

INTRODUCTION

Insect growth regulators (IGRs) received great attention as a hope for the future of insect control. Among these IGRs are the chitin synthesis inhibitors (CSIs) such as Dimilin which interfere with the chitin deposition (Mulder and Gijswijt, 1973). Other groups of insect growth regulators (IGRs) are the insect hormones like juvenile hormone mimics (JHMs); such as pyriproxyfen (admiral). These compounds interfere with cuticle deposition, by the inhibition of chitin synthesis. (Riddiford and Truman, 1978). Pyriproxifen acts by altering normal insect growth patterns, eventually and indirectly resulting in death of the treated insect (Chandler *et al.*, 1992).

Bioinsecticides such as abamectin are a complex of chemically related, naturally occurring macrocyclic lactones which were first isolated from a novel species of actinomycete *Streptomyces avermitilis* (Burg *et al.*, 1979). Physiologically, abamectin blocks postsynaptic potentials of neuromuscular junctions (Fritz *et al.*, 1979). However, there is no information to date on the effect of abamectin on biochemical processes in insects.

The aim of the present work is to study the toxicological effects of the IGRs (diflubenzuron and pyriproxifen) and the bacterial product (abamectin) on the field and laboratory strains of *Spodoptera littoralis* larvae and their effect on both phenoloxidase and chitinase activities.

MATERIALS AND METHODS

1. Insects, collecting and rearing

A susceptible laboratory strain (L-strain) of the cotton leafworm *S. littoralis* was established since 1970 from Menofia Governorate cotton fields and was maintained under constant conditions of 25°C±1 and 70±5 % R.H and out of any contam - nation with chemicals till the time of the study. A field strain (F-strain) was collected as egg-masses from Dakahlia Governorate in 1998, and maintained under the same conditions until the 4th instar larvae following the technique of (El-Defrawi *et al.*, 1964).

2. Insecticides and insect growth regulators

Commercial formulations of insecticides were used in this study represented three main groups of insecticides commonly applied on cotton for controlling different insect species. These insecticides were diflubenzuron (25 % WP), pyriproxyfen (EC 10 %) and abamectin (1.8 % EC).

3. Toxicological studies:

A series of concentrations (in water) for each compound was prepared using the commercial formulation of each. Castor-bean leaves were dipped for 15 seconds in each concentration then left for one hour to dry. Newly moulted 4th instar larvae were fed on treated leaves for 48 hours. Five replicates (20 larvae each) were tested for each concentration. Mortality percentages were recorded on the 3rd day after transferring the treated insects onto untreated leaves and corrected according to natural mortality (Abbott, 1925). To estimate the LC_{50} values, the corrected mortality percentages were subjected to probit analysis according to Finney (1952).

4. Preparation of samples for biochemical studies

Larvae were fed for 48 hours on the LC_{50} treated leaves, and then transferred to fresh untreated leaves. Larval samples for biochemical assays were collected at 24-and 48-hr. during treatment periods and at 24-, 48-, 72-, and 96-hr. after transferring the treated larvae onto untreated leaves.

The collected larvae were starved for 4 hours. The starved larvae were homogenized in distilled water using a Teflon homogenizer -surrounded with a jacket of crushed ice for 3 minutes. Homogenates were centrifuged at 6000 r.p.m. for 10 minutes at 5°C and the supernatants were used directly for enzyme assays.

5. Enzyme assays

Determinations of chitinase and phenoloxidase activities were carried out according to the method described by Ishaaya and Casida (1974).

RESULTS AND DISCUSSION

1. Toxicological studies

Table (1) shows the levels of sensitivity of laboratory (L) and field (F) strains of *S. littoralis* to the tested compounds. As shown Table (1), it is clear that, diflubenzuron was more toxic to *S. littoralis* than the other IGR (pyriproxyfen) and abamectin, the LC_{50} values were 7.86, 120.54 and 119.95 ppm, respectively. On the other hand, the same trend of toxicity was obtained from F-strain, the LC_{50} values were 42.98, 1264.93 and 640.58 ppm, and resistance ratios were. 5.47, 10.5 and 5.34 fold respectively. These results could be interpreted in the light of previous report of Guyer and Neumann (1988), who reported that different rates of detoxification could account for the different persistence of insect growth regulators and in turn for the toxicological differences in insects.

2. Biochemical studies:

2.1. Chitinase activity:

Table (2) indicates the changes in chitinase activity of *S. littoralis* L- and F-strains at different time intervals after feeding the 4th instar larvae on leaves treated with the LC_{50} of each of diflubenzuron, pyriproxyfen, and abamectin. The data revealed that the activity of the enzyme increased gradually during larval stage and reached its maximum at 4-day time interval then a decrease in the enzyme activity took place until the end of larval life.

Table (1): Sensitivity of laboratory and field strains of *Spodoptera littoralis* (Boisd.) to different insecticides.

Insecticides	Laboratory strain			Field strain			RR**
	LC_{50}*	LC_{90}*	Slope	LC_{50}	LC_{90}	Slope	
Diflubebzuron	7.86	92.12	1.199	42.98	1904.90	0.778	5.47
Pyriproxyfen	120.54	5899.70	0.759	1264.90	8027.40	1.597	10.5
Abamectin	119.95	30716.00	0.532	640.58	6549.90	1.269	5.34

*LC_{50} and LC_{90} = ppm **RR = Relative resistance ratio

Regarding diflubenzuron and abamectin treated L-strain larvae, the data revealed a significant decrease in chitinase activity. The decrease was gradual during the first four days reaching the maximum level of reduction (37.24% of control) on 3-day in the case of diflubenzuron, and (7.71%) on 4-day in the case of abamectin. A significant increase in the activity was observed

during the last two time intervals for both insecticides.

On the other hand, the treatment of L-strain larvae with pyriproxyfen caused a significant increase in the enzyme activity on the first day, then a sharp drop in the activity was observed on 2-day time interval, then, a gradual increase in the enzyme activity was took place during the next three time intervals (3-, 4-, and 5-day). The maximum level (1056.1% relative to control) was observed on 5-day time interval. While, on 6-day the activity of the enzyme decreased again but still much higher than control.

In the case of field-strain as shown in Table (2), the three tested insecticides gave a significant increase in chitinase activity in larvae during the all time intervals with exception to 4-day time interval. At that time the three tested compounds caused a reduction in the enzyme activity especially in abamectin treatment.

The elevation of chitinase activity as a result of benzophenyl urea (diflubenzuron) treatment was also observed by Ishaaya and Casida (1974) on the housefly. Also, Lee *et al.* (1994) found that the treatment of *Hyphantria cunea* with the LC_{50} of chlorfluazuron, tebufenozide, pyriproxyfen and diflubenzuron caused increases in chitinase activity compared to untreated larvae. On the other hand, Yu and Terriere (1975 & 1977) explained the increased chitinase activity by the reduced activity of β-ecdysone metabolizes enzymes, consequently β-ecdysone accumulation timulated hyperchitinase activity.

On the other hand, Deul *et al.* (1978), and Lee *et al.* (1990) found that chitinase was inhibited after treatment with CSIs, but Deul *et al.* (1978) did not record any increase in chitinase activity (than control larvae) of *P. brassicae* L. Also, Wu *et al.* (1998) found that the activities of cuticle chitinase and phenoloxidase [monophenol monooxygenase] decreased by 48 and 60%, respectively, in chlorfluazuron resistant strain and field strain compared with the susceptible strain.

Table (2): Changes in chitinase activity in laboratory and field strains of *Spodoptera littoralis* (Boisd.) during different time intervals after treatment with the LC_{50} of tested compounds.

Strain	Time (day)	Mean ± SE of enzyme activity*						
		Control	Diflubenzuron	%**	Abamectin	%	Pyriproxyfen	%
Lab.	1	0.148±0.01 Be	0.133±0.02 Bd	90.64	0.122±0.01 Bc	83.63	0.284±0.01 Ac	194.15
	2	0.53±0.04 Ac	0.268±0.01 Cc	50.04	0.153±0.01 Dc	28.53	0.35±0.02 Bc	66.14
	3	0.83±0.07 Bb	0.312±0.01 Cc	37.24	0.107±0.01 Dc	12.76	1.022±0.07 Ad	121.94
	4	2.27±0.06 Ba	1.098±0.07 Cb	48.31	0.175±0.04 Dc	7.71	4.350± 0.10 Aa	191.35
	5	0.350±0.07 Dd	2.008±0.15 Ba	573.17	0.479±0.03 Cb	136.59	3.701±0.08 Ab	1056.1
	6	0.18±0.11 De	1.966± 0.11 Aa	1045.5	0.667±0.02 Ca	354.55	1.197±0.09 Bc	636.36
Field	1	0.23±0.01 Ad	0.260±0.01 Ae	109.75	0.253±0.01 Ae	106.86	0.244±0.01 Ad	103.25
	2	0.402±0.03 Ccd	0.756±0.02 Ac	188.3	0.496±0.02 Bd	123.4	0.720±0.02 Ac	179.26
	3	0.57 ±0.06 Bb	0.560±0.02 Bd	97.04	0.731±0.01 Ac	126.67	0.620±0.02 Bc	107.41
	4	2.57 ±0.15 Aa	2.282±0.14 Bb	88.07	0.694±0.02 Dc	26.99	1.359±0.08 Ca	52.82
	5	0.453 ± 0.2 Cc	3.008±0.09 Aa	664.15	0.842±0.04 Bb	185.85	0.812±0.08 Bbc	179.25
	6	0.321±0.06 Cd	0.286±0.09 Ce	89.33	3.812±0.09 Aa	1189.3	0.902±0.08 Bb	281.33

* Activity = mg NAGA/hr./larva; ** % = percentage relative to control.

Values in a row followed by the same capital letter and values in a column followed by the same small letter are not significantly different ($P<0.05$; Duncan's multiple range test).

L.S.D. of treatment compounds of L-strain = 0.08 and of F-strain = 0.09.

L.S.D. of treatment time of L-strain = 0.09 and of treatment time of F-strain = 0.09.

2.2. Phenoloxidase activity

Table (3) shows the changes in phenoloxidase (PhO) activity of *S. littoralis* L- and F-strain larvae at different time intervals (1, 2, 3, 4, 5, and 6 days) after feeding the 4th instar larvae on leaves treated with LC_{50} of each of the tested compounds. Diflubenzuron and abamectin caused significant increases in the enzyme activity of L-strain 221.01% and 201.81% relative to control -day ime nterval, respectively. Thereafter, a sharp drop in the enzyme activity was happened during the next three time interval (2-, 3-, and 4-day). The minimum level in the enzyme activity resulted from diflubenzuron and abamectin treatment was recorded at 3-day time interval (12.38% and 27.08% relative to control for both insecticides, respectively). On the other hand, pyriproxyfen treatment of the L-strain caused a significant redu - tion in the PhO activity during the first three time intervals specially at 3-day (56.52% lower than control.

Regarding F-strain, the three tested compounds caused a significant increase in PhO activity during the first two time intervals (1-, and 2-day), then a sharp drop in the activity was observed at 3-day time interval especially in the case of diflubenzuron and pyriproxyfen. The reduction in the enzyme activity continued during the next time interval (4-day) for diflubenzuron and abamectin, while in the case of pyriproxyfen, the activity became normal. At 5-day time interval diflubenzuron and pyriproxyfen gave an increase in PhO activity (higher than control), while a reduction effect of abamectin continued during this time interval.

Table (3): Changes in phenoloxidase activity in laboratory and field strains of *Spodoptera littoralis* (Boisd.) at different time intervals after treatment with the LC_{50} of tested compounds

Strain	Time (day)	Mean ± SE of enzyme activity*						
		Control	Diflubenzuron	%**	Abamectin	%	Pyriproxyfen	%
Lab.	1	0.031±0.00 Bf	0.068±0.00 Ad	221.01	0.062±0.00 Ad	201.81	0.025±0.00 Cf	82.24
	2	0.105±0.01 Ae	0.034±0.00 Ce	32.38	0.068±0.00 Bd	64.97	0.102±0.00 Ae	97.09
	3	0.268±0.01 Ac	0.033±0.00 De	12.38	0.073±0.00 Cd	27.08	0.152±0.00 Bd	56.52
	4	0.536±0.01 Aa	0.221±0.01 Bb	41.29	0.181±0.00 Cc	33.87	0.537±0.01 Aa	100.21
	5	0.161±0.02 Cd	0.399±0.01 Aa	247.59	0.345±0.01 Ba	213.79	0.348±0.02 Bb	215.86
	6	0.341± 0.02 Ab	0.089±0.02 Dc	26.06	0.303±0.01 Bb	88.76	0.248±0.02 Cc	72.64
Field	1	0.008±0.00 Bf	0.008±0.00 Bf	106.94	0.016±0.01 Ae	198.61	0.016±0.00 Af	195.83
	2	0.033±0.00 De	0.057±0.00 Cd	174.58	0.091±0.00 Ac	278.81	0.065±0.00 Bd	138.98
	3	0.068±0.01 Ad	0.021±0.00 De	30.08	0.058±0.00 Bd	84.55	0.034±0.00 Ce	50.41
	4	0.160±0.01 Ab	0.073±0.02 Bc	45.83	0.052±0.00 Cd	32.64	0.160±0.02 Ab	100
	5	0.173±0.01 Ca	0.271±0.01 Aa	156.41	0.165±0.01 Db	95.51	0.194±0.01 Ba	112.18
	6	0.124±0.01 Bc	0.117±0.01 Bb	94.2	0.213±0.01 Aa	171.43	0.101±0.00 Cc	81.25

* Activity =.extinction unit/min/larvae

** % = percentage relative to control

Values in a row followed by the same capital letter and values in a column followed by the same small letter are not significantly different ($P<0.05$; Duncan's multiple range test).

L.S.D. of treatment compounds of L-strain = 0.01 and of F-strain = 0.01

L.S.D. of treatment time of L-strain = 0.02 and of F-strain = 0.01

The data obtained in the present study led us to suggest that the lethal effect of IGRs may be occurred through the alteration of enzyme balance of both chitinase and phenoloxidase, since these

two enzymes played a known role in ecdysis and metamorphosis. This suggestion could be supported by many reports, Elewa *et al.* (1984) studied the inhibition of phenoloxidase activity in *S. littoralis* by deltamethrin and dialifos and found that the inhibitory effect was found to be 5 times as great for dialifos as for deltamethrin

Wu *et al.* (1998) found that the activities of cuticle chitinase and phenol oxidase in resistant strain and field strain was decreased by 48 and 60%, respectively, compared with the laboratory strain.

Although the data obtained in the present work could threw some light on the relation between toxicity of IGRs and the two tested enzymes, there is a need for further studies on this point to the role of these enzymes in the mode of action of IGRs.

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