ROLE OF CARBOHYDRATE HYDROLYZING ENZYMES IN THE MODE OF ACTION OF IGR AND BIOINSECTICIDES

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ABSTRACT

The present study was carried out to evaluate the effects of the lethal dose (LC_{50}) of abamectin and two IGRs on cotton leafworm Spodoptera littoralis from the point of biochemical aspects. The relative susceptibility of two strains of the pest (laboratory and field strains) to three insecticides; i.e., diflubenzuron, pyriproxyfen and abamectin, was tested using dipping technique. Values 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 results revealed that the field strain exhibited different degrees of tolerance towards the three tested insecticides which can be arranged as follows: pyriproxyfen (10.5 folds), diflubenzuron (5.5 folds) and abamectin (5.3 folds). The three tested compounds caused inhibitions of trehalase and invertase activities in treated larvae during all time intervals when compared to untreated insects with exception to invertase. An increase in its activity was obtained at the last time intervals after treatment with diflubenzuron and pyriproxyfen. Both diflubenzuron and pyriproxyfen caused an increase in the amylase activity, while abamectin caused a decrease in the enzyme activity.

Key Words: Carbohydrate hydrolyzing enzymes, IGR, bioinsecticides, *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). 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 abamectins are a complex of chemically related, naturally occurring macrocyclic lactones which were first isolated from a novel species of actinomycele *Streptomyces avermitilis*, Burg *et al.*, (1979). Physiologically, avermectin blocks postsynaptic potentials of nuromuscular junctions (Fritz *et al.*, 1978). 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 (dimilin and pyriproxifen) and the bacterial product (abamectin) on field and laboratory strains of *Spodoptera littoralis* (Boisd.) larvae and their effect on both carbohydrate hydrolyzing enzymes 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 laboratory conditions of 25°C±1 and 70±5 % R.H and out of any contamination 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 include diflubenzuron (25% WP), pyriproxyfen (EC 10%) and abamectin (1.8% EC).

3. Toxicological studies:

A series of concentrations (in water) for each compound were prepared using the commercial formulations. Castor-bean leaves were dipped for 15 seconds in each concentration then left for one

hour to dry. Newly molted 4th instar larvae were fed on treated leaves for 48 hr. 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 the 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 rpm for 10 minutes at 5°C and the supernatants were used directly for enzyme assays.

5. Enzyme assays

The method was based on the digestion of trehalose, starch, and sucrose by trehalase, amylase, and invertase, respectively (Ishaaya and Swiriski, 1976). The free aldehydic group of glucose formed after trehalose, starch, and sucrose digestion were determined using 3, 5 dinitrosalicylic acid reagent.

6. Statistical analysis

Significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($P<0.05$) (Duncan, 1955).

RESULTS AND DISCUSSION

I. 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 in the table, it is clear that, diflubenzuron was more toxic to the 4th instar larvae of *S. littoralis* than the other compounds (pyriproxyfen and the 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 folds, respectively). These results could be interpreted in the light of previous reports 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.

It was observed that the field strain was more tolerant to all tested insecticides than the laboratory strain. This may be due to the continuous exposure of field strains to different insecticides. This phenomenon was previously given by Dittrich *et al.* (1979), who showed that *S. littoralis* strains sampled in 5 provinces of the Nile Delta had a uniform resistance towards monocrotophos and the resistance ratios ranged between 50-137 folds.

2. Biochemical studies

2.1. Carbohydrate hydrolyzing enzymes

Carbohydrates, proteins and lipids are very efficiently utilized by insects and most species derive the main part of their nourishment from these nutrients. The utilization of these nutrients depends on the digestive enzymes; amylase, trehalase, invertase, protease and lipase. The changes invertase, trehalase and amylase activities of *S. littoralis* L- and F-strains larvae at different time intervals after exposure the 4th instar larvae to the LC_{50} of each of the tested compounds are shown in Tables (2, 3 and 4).

2.1.1. Invertase

Data in Table (2) indicated that the tested insecticides caused significant increases in invertase activity of the laboratory strain larvae just after treatment (114.51, 112.53 and 112.77 % higher than control for diflubenzuron, pyriproxyfen and abamectin, respectively). A great reduction in invertase activity lower than control was recorded during the following three time intervals (2, 3, and 4 days) the reduction was pronounced in abamectin and diflubenzuron treatments. The reduction in the activity continued by abamectin treatment larvae till the end of experiment, while a significant increase in the activity were observed in diflubenzuron and pyriproxyfen treatments.

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

Table (2): Changes in invertase 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	Diflubenzuon	%**	Abamectin	%	Pyriproxyfen	%
Lab.	1	15.754 ± 0.43 f	18.041 ± 0.38 Ad	114.51	17.768 ± 0.06 Acd	112.77	17.729 ± 0.05 Af	112.53
	2	46.096 ± 0.13 Ae	17.020 ± 0.07 Bd	36.92	15.506 ± 0.65 Bd	33.64	46.527 ± 0.17 Ae	100.94
	3	84.717 ± 1.83 Ad	21.908 ± 0.22 Cd	25.86	14.720 ± 0.15 Dd	17.38	64.208 ± 4.65 Bd	75.79
	4	207.958 ± 0.19 Aa	77.526 ± 1.09 Cc	37.33	23.527 ± 0.75 Dc	11.31	184.000± 19.28 Bc	88.48
	5	170.008 ± 4.33 Cc	212.367 ± 3.09 Ba	124.91	69.671 ± 2.70 Da	40.98	222.142 ± 1.34 Aa	130.66
	6	178.058 ± 1.17 Cb	198.183 ± 4.33 Bb	111.3	44.179 ± 0.69 Db	24.81	210.833 ± 0.19 Ab	118.41
Field	1	20.068 ± 0.27 BCf	21.122 ± 0.28 ABf	105.25	19.301 ± 0.07 Ce	96.18	22.310 ± 0.03 Ae	111.17
	2	57.835 ± 0.29 Ac	54.337 ± 0.3 Bd	93.95	40.580 ± 1.32 Cd	69.26	52.996 ± 0.13 Bc	91.63
	3	102.542 ± 1.43 Ac	51.223 ± 0.73 Bc	49.95	50.025 ± 0.08 Bc	48.75	50.456 ± 0.08 Bd	49.21
	4	182.467 ± 0.84 Cb	191.283 ± 2.78 Bb	104.83	50.840 ± 0.39 Dc	27.86	199.333 ± 1.89 Aa	109.24
	5	194.733 ± 0.22 Ca	213.517 ± 3.39 Aa	109.65	72.450 ± 1.73 Db	37.21	200.867 ± 0.69 Ba	103.15
	6	92.650 ± 1.27 Dd	98.613 ± 0.17 Cc	110.41	205.275 ± 3.27 Aa	229.83	114.521 ± 0.58 Bb	128.219

* Activity = µg glucose/min./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 = 5.07 and of treatment compounds of F-strain = 1.71 L.S.D. of treatment time of L-strain = 6.21 and of treatment time of F-strain = 2.1

Regarding to F-strain treatment both diflubenzuron and pyriproxyfen gave a significant increase in their invertase activity in larvae just after treatment, then a gradual decrease in the activity took place during the next two time intervals reaching the minimum of 49.95 and 49.21% lower than control for diflubenzuron and pyriproxyfen, respectively at (3-day time interval). During the last three time intervals a significant increase in the enzyme activity was observed. On the other hand, treatment F-strain larvae with abamectin gave gradual decreases in invertase activity during all time intervals reaching the least level of 27.86 and 37.21% (lower than control) at 4- and 5-day respectively.

In general it could be concluded that treatment of *S. littoralis* larvae with the three insecticides gave great reductions in invertase activity and the reduction was pronounced with abamectin.

2.1.2. Trehalase

Data in Table (3) showed the changes in trehalase activity of *S. littoralis* larvae post treatment with

different insecticides. The results indicated that trehalase activity showed different behaviours in the normal larvae of cotton leafworm in the two strains during the larval stage. The level of the enzyme in L-strain increased gradually throughout the larval life reaching the maximum (165.938 µg glucose/min./larva) at the end of larval life (6-day time interval). While, in F-strain the changes in trehalase activity took a bell-shape pattern in relation to time, a gradual increase in the enzyme activity reaching the maximum (156.017 µg glucose/min./larva) at 4-day time interval, then the enzyme exhibited a gradual decrease in its activity.

Table (3): Changes in trehalase 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	10.580 ± 0.2 Af	10.134 ± 0.08 Ae	95.79	3.009 ± 0.12 Cb	28.44	6.037 ± 0.07 Bc	57.07
	2	36.129 ± 0.13 Ae	12.727 ± 0.38 Ce	35.23	4.792 ± 0.04 Db	13.26	16.100 ± 0.08 Bc	44.56
	3	46.288 ± 1.73 Ad	18.228 ± 0.34 Bd	39.38	4.140 ± 0.28 Db	8.94	12.554 ± 0.91 Cd	27.12
	4	131.675 ± 2.39 Ac	42.742 ± 2.36 Bc	32.46	5.223 ± 0.46 Db	3.97	44.467 ± 2.16 Bb	33.77
	5	141.258 ± 1.34 Ab	111.742 ± 1.83 Bb	79.10	13.608 ± 0.75 Da	9.63	86.058 ± 2.49 Ca	57.07
	6	165.983 ± 1.01 Aa	143.558 ± 2.13 Ba	86.49	14.184 ± 1.12 Da	8.55	87.208 ± 1.57 Ca	52.54
Field	1	9.457 ± 0.14 Bf	15.544 ± 0.53 Af	170.38	14.605 ± 0.19 Ae	160.08	15.656 ± 0.64 Ae	171.64
	2	21.419 ± 1.04 Ce	32.392 ± 0.62 Ae	151.23	24.581 ± 0.5 Bd	114.76	24.007 ± 0.74 Bd	112.08
	3	87.496 ± 0.94 Ac	45.521 ± 0.98 Bd	52.03	38.621 ± 0.37 Cc	44.14	39.771 ± 0.77 Cc	45.45
	4	156.017 ± 4.7 Aa	158.125 ± 0.66 Aa	101.35	38.382 ± 0.36 Cc	24.6	135.892 ± 3.85 Ba	87.10
	5	105.225 ± 0.66 Bb	111.742 ± 3.55 Ab	106.19	51.367 ± 2.33 Db	48.82	75.517 ± 4.77 Cb	71.77
	6	79.445 ± 0.97 Cd	88.742 ± 0.35 Bc	111.7	105.225 ± 1.52 Aa	132.45	40.825 ± 1.16 Dc	51.39

Regarding to insecticides treatment, the three tested insecticides gave a significant decrease in the enzyme activity of L-strain larvae during all time intervals when compared to control. The changes in trehalase activity resulting from diflubenzuron and pyriproxyfen took the same pattern during different time intervals after treatment. During the first three time intervals, a steady decrease in the activity was observed, then an increase in the enzyme activity took place during the last two time intervals but still lower than control. On the other hand, treatment L-strain with abamectin caused a severe reduction in trehalase activity during all time intervals.

In the field strain, the treatment with the tested insecticides revealed great differences in trehalase activity than that obtained from L-strain. The three insecticides gave a significant increase in the enzyme activity during the first two time intervals. The increase in the activity was 170.38, 171.64 and 160.08% relative to control for diflubenzuron, pyriproxyfen, and abamectin, respectively. During 3- and 4-day time intervals, a significant decrease in the activity (but still lower than control) took place in diflubenzuron- and pyriproxyfen-treated larvae, reaching 101.35 and 87.1% 4-day, respectively. Then after significant decrease in the activity was observed during the last two time intervals, however, the reduction in the activity resulted from diflubenzuron still higher

than control. In contrast, treated-larvae with abamectin showed a significant decrease in trehalase activity compared to the other insecticides during the same time intervals. At the 6-day time interval abamectin caused a great increase in the enzyme activity (than control) more than the other tested insecticides

Table (4): Changes in amylase 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	0.249 ± 0.09 Cf	1.713 ± 0.06 Bd	678.50	0.786 ± 0.05 BCb	315.39	4.849 ± 0.05 Af	1946.16
	2	6.205 ± 0.21 Bd	1.629 ± 0.17 Cd	26.26	0.824 ± 0.07 Cb	13.28	10.829 ± 0.21 Ae	174.52
	3	4.265 ± 0.25 Be	2.530 ± 0.09 Cd	59.33	1.457 ± 0.05 Db	34.16	18.400 ± 0.33 Ad	431.46
	4	7.954 ± 0.53 Cc	12.842 ± 0.42 Bc	161.47	0.910 ± 0.21 Db	11.45	32.775 ± 1.52 Ab	412.05
	5	15.717 ± 1.22 Ca	34.500 ± 3.45 Aa	219.51	10.063 ± 0.17 Da	64.02	24.917 ± 0.19 Bc	158.54
	6	12.650 ± 0.6 Cb	16.388 ± 1.16 Bb	129.55	9.967 ± 0.67 Da	78.79	58.650 ± 1.33 Aa	463.64
Field	1	1.658 ± 0.14 Ab	6.402 ± 0.46 Ad	386.13	3.814 ± 0.12 Ac	230.06	4.907 ± 0.25 Ac	295.95
	2	21.419 ± 1.04 Bab	12.938 ± 0.51 Acd	220.41	10.638 ± 0.9 ABbc	181.22	13.992 ± 0.63 Ab	238.37
	3	87.496 ± 0.94 Bab	14.567 ± 0.21 Ac	394.81	11.308 ± 0.51 Ab	306.49	12.602 ± 0.25 Ab	341.56
	4	156.017 ± 4.7 Ca	58.075 ± 2.64 Ba	582.69	9.008 ± 0.21 Cbc	90.39	69.575 ± 1.45 Aa	698.08
	5	105.225 ± 0.66 BCa	40.250 ± 3.27 Ab	411.77	14.759 ± 0.42 Bb	150.98	7.571 ± 1.45 Cbc	77.45
	6	79.445 ± 0.97 Cab	14.663 ± 1.36 Bc	668.91	40.825 ± 1.52 Aa	715.97	3.450 ± 0.63 Cc	60.50

* Activity =.µg glucose/min/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 = 1.07 and of F-strain = 6.05
L.S.D. of treatment time of L-strain = 1.31 and F-strain = 7.4.

2.1.3. Amylase

The changes in amylase activity of laboratory and field strains in the normal status and after insecticidal treatment were given in Table (4). Results showed that larvae in the normal status revealed the same pattern of changes in amylase activity in both tested strains throughout the larval life. There was an increase in the activity during the 4th larval instar (1- and 2-day). During the 5th larval instar, a decrease in the enzyme activity was observed and reached the minimum (4.256 and 3.69

µg glucose/min./larva for L- and F-strains, respectively) on the 3rd day. The enzyme activity increased again at the end of 5th larval instar and the beginning of 6th larval instar. The level of enzyme activity continued to increase during the 6th larval instar reaching the maximum of 15.717 and 9.775 of 156.017 µg glucose/min./larva for L- and F-strains, respectively at 5th day. At the end of larval life (6-day) the enzyme activity decreased again.

The results obtained from L-strain after treatment with the tested IGRs showed that pyriproxyfen caused a significant increase in amylase activity when compared to control during all time intervals reaching the maximum of 463.636% (relative to control) at the end of experiment (6-day). While diflubenzuron caused a significant increase in amylase activity just after treatment (1-day), then a great decrease in the activity was observed during the next two time intervals (2- and 3-day). During the last three time interval (4-, 5- and 6-day) a significant increase in the enzyme activity was observed. In contrast, the treatment of L-strain larvae with abamectin caused variable degrees of inhibition in amylase activity (lower than control) during all time intervals with the exception of 1-day abamectin treatment which gave a significant increase in the enzyme activity of 315.385% higher than control.

Table (4) showed the effect of the two IGRs and the bioinsecticide on amylase activity of F-strain. It was observed that, the three insecticides gave a significant increase in the enzyme activity during all tested time intervals after 5- and 6-day. In this period, pyriproxyfen gave a significant decrease in the activity of 77.451 and 60.405%, respectively. At 4-day, abamectin reduced amylase activity to 90.385% (lower than control). The maximum increase in amylase activity was recorded at 4-day for diflubenzuron and pyriproxyfen (582.692 and 698.077% relative to control, respectively), while the maximum increase in the enzyme activity as a result of abamectin treatment was recorded at 6-day (715.966% relative to control).

The data resulted from carbohydrate hydrolyzing enzymes revealed that a pronounced increase in amylase activity was observed as a result of treatment the cotton leafworm larvae with IGRs and abamectin compound. However, the three tested compounds caused a reduction in both invertase and trehalase activities. The same results were found by Saleem and Shakoori (1987) and Ishaaya and Ascher (1977).

Also, amylase and beta-fructofuranosidase activities were lower in *Hyphantria cunea* after treatment with the LC_{50} of the insect growth regulators chlorfluazuron, tebufenozide, pyriproxyfen and diflubenzuron became lower than those in untreated larvae (Lee *et al.* 1994)

It is well known that in insects, trehalase degrades the disaccharide trehalose to glucose for internal energy supply and generates, (during molting) glucose needed for chitin build-up. So the inhibition of trehalase observed in the present work might affect this process. Study the mode of action of Dimilin revealed that this compound alter the cuticle composition of insect, especially that of chitin (Ishaaya and Casida, 1974). Also, Post and Vincent (1973) found that the reduced level of chitin in the cuticle is due to the inhibition of biochemical processes leading to chitin formation. In addition, trehalase played a significant role in the supply of energy to the insect and the activity of trehalase might serve as an indicator of energy reserves resulting from availability of carbohydrate nutrients (Wyatt 1967). During molting cycles, the trehalose-trehalase system is activated to generate glucose needed, probably, for chitin build-up in the newly synthesized cuticle (Candy and Kilby, 1962).

In this concern Ishaaya and Ascher (1977) concluded that carbohydrates might be affected due to the reduced levels of amylase, trehalase and invertase of the 4th larval instar of *T. castaneum* treated with diflubenzuron. Also, Saleem and Shakoori (1987) recorded a reduction in trehalase and elevated amylase activity in the 6th larval instar of the same insect treated also with diflubenzuron. Similar to our findings, El-Saidy and Degheele (1990) found amylase activity was reduced, but neither invertase nor trehalase activity after treatment with diflubenzuron was affected. But we found that trehalase was affected only by CFA.

On the other hand, the present data disagreed with that obtained by Abdel Hafez *et al.* (1993) and Radwan *et al.* (1985), who found that repeated selection of the cotton leafworm larvae with deenate (dimilin + nudrin) and DC-702 (dimilin + dursban) increased the invertase activity and decreased the amylase and trehalase activity.

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