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Toxic and Biochemical Effects of Some Bioinsecticides and Igrs on American Bollworm, *Helicoverpa armigera* (h ü b.) (noctuidae: lepidoptera) in Cotton Fields

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Abstract

Field experiments were carried out to evaluate efficiency of three bioinsecticides (Dipel DF, Protecto and Bioranza) and two insect growth regulators (IGRs) (chlorfluazuron and pyriproxyfen) against larvae of American bollworm, *Helicoverpa armigera* (ABW) and their side effects on some common predators in Egyptian cotton fields during 2009 and 2010 at Aga region, Dakahlia Governorate, Egypt. Results indicated that chlorfluazuron showed the highest initial reduction (75.00 and 80.6%); residual mean (83.75 and 79.45%) and annual mean (80.83 and 79.83%) on *H. armigera* during the two successive seasons, respectively. Moreover, chlorfluazuron was the most toxic and gave the highest reduction in predator numbers recorded (20.70, 23.20 and 22.37%) in the 2009 season and (23.30, 20.90 and 21.70%) in the 2010 season at the initial, residual and annual means, respectively.

Chlorfluazuron, pyriproxyfen and Dipel DF gave the lowest significant decrease in the activity of amylase enzyme (61.86 and 59.86% relative to control), invertase enzyme (75.28 and 80.13%) and trehalase enzyme (73.64 and 83.74%), respectively after 3 and 7 days post treatment. Insect growth regulators (chlorfluazuron and pyriproxyfen) caused highly significant increases in the activity of chitinase enzyme (130 and 122.6% 141.89 and 131.64%, respectively) at both interval times, respectively.

Keywords: *Helicoverpa armigera*; biopesticides; insect growth regulators (IGRs); enzyme

Introduction

The American bollworm (ABW), *Helicoverpa armigera* (Hüb.), Lepidoptera: Noctuidae, is one of the most important economic insect pests in Egypt [1]. The larvae of this pest feed on a wide range of the economically important crops including cotton, corn, tomato, sunflower, legumes, tobacco, and several cucurbitous and citrus crops [2]. In India, where *H. armigera* commonly destroys more than half the yield crop, losses were estimated at over $300 million per annum [3]. Field failure resulting from *H. armigera* resistance to pyrethroids has been reported worldwide by many authors [4]. Because of their economic advantages and low toxicity to mammals and to some predators [5] much effort has been directed towards developing management aimed at using biopesticides and insect growth regulators in control programs.

Metabolism of carbohydrate hydrolyzing enzymes that play a principal role in digestion and utilization of carbohydrate by insect [6] is controlled mainly by amylase, invertase and trehalase enzymes. Trehalose is one of the most important storage carbohydrates that is present in almost all forms of life except mammals. Trehalose is split into glucose units by trehalase enzyme. Amylase enzyme is required to digest carbohydrates (polysaccharides) into smaller units (disaccharides), and eventually into even smaller units (monosaccharides) such as glucose. Amylase enzyme plays a key role in plant defense toward pests and pathogens [7] which cause severe damage to field crops and stored grains [8]. Invertase enzyme hydrolyzes sucrose, forming fructose and glucose.

Chitin is a structural component of the cuticle and peritrophic membrane in the mid-gut of insects, and strict regulation of its metabolism is essential for the normal growth of insects. Chitenase is among a group of proteins that insects use to digest this structural polysaccharide in their exoskeleton ad gut linings during the molting process [9].

The present study was proposed to evaluate the effect of three bioinsecticides and two IGRs against *H. armigera* as well as their side effects to some common predators. Additionally, this research was proposed to elucidate some biochemical relationships among treatments and activities of some enzymes in *H. armigera*.

Materials and Methods

Tested compounds

Insect growth regulators (IGRs)

-Benzoylurea, Chlorfluazuron (Atabron® 5 % EC) 1- (2, 6, -difluorobenzoyl 3 - [4 (chloro – 5- trifluoromathyl-2-pyridyloxy) 3, 5,-dichlorophenyl] urea. used at rate of 400 ml/ feddan. Basic product of Syngenta Agro, Switzerland (local manufacture: Syngenta Agro, Dokki, Giza, Egypt)

-Juvenile hormone mimic, pyriproxyfen (Admiral®) 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine, used at rate of 200 ml/ feddan. Basic product of Sumitomo chemical Co., Tokyo, Japan.

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Bio-insecticides compounds

Bacterial insecticides: Dipel DF®, *Bacillus thuringiensis* subsp. *Kurstaki* (32, 258 Potency I.U. / mg) WP used at rate of 200 g/feddan (feddan = 4200 m^2). Basic product: Valent Biosciences Corporation, Libertyville, USA.

-Protecto®, *Bacillus thuringiensis* var. *Kurstaki*, (32000 I.U. /mg) WP used at rate of 300 g/feddan. Basic product: Insect pathogens unite, Plant Protection Research Institute, Agriculture Research Centre, Dokki, Giza, Egypt.

Fungus: Bioranza®, *Metarehizum aneasopliea* Sorok. Bioranza 10% WP ($32x10^6$ spores /ml) at rate of 200 g/feddan. Insect pathogens unite, Plant Protection Research Institute, Agriculture Research Centre, Dokki, Giza, Egypt.

Field trial

Field experiments were carried out at Aga region, Dakahlia Governorate, Egypt during two consecutive cotton growing seasons of 2009 and 2010. The experiment area of one feddan (4200 m^2) was divided into 6 equal randomized blocks (one for either treatment plus one for control). Each block was divided 4 experimental plots as replicates (175 m^2 for each) cultivated with the Egyptian cotton variety, Giza 86. Cotton plants were treated once with each treatment at 18th and 21st June during the both seasons, respectively. A plastic curtains used as borders between treatments during spray to avoid drift.

Samples

Weekly 20 cotton plants were investigated from the first of June until the mean numbers of ABW larvae reached 3 larvae / sample (0.15 larvae/ plant) [10] and then cotton plants were treated. The tested insecticides were applied at the recommended field rate, while control plots were sprayed with water only. Treatment plots were sprayed using a knapsack motor sprayer, 20 liters in capacity and using 200 -liter volume of insecticidal solution (insecticide + water as solvent) per feddan.

Directly pre treatment count was made visually on cotton plant (including leaves, stem, squares and bolls) for each treatment. Post treatment counts were recorded after 3, 7 and 10 days. The common predators: green lacewing, *Chrysoperla carnea*; lady beetles, *Coccinella* spp.; anthocoride bugs, *Orious* spp.; staphylinid beetle, *Peaderus alfierii* and True spiders found on cotton plants were counted and recorded at the same time. The efficiency of tested treatments was measured as a percentage of reduction in population density of American bollworm and some common predators using the Henderson and Tilton equation [11].

Biochemical assay

Preparation of samples: The present experiment was designed to study the changes in the activities of carbohydrate hydrolyzing enzymes and chitinase enzyme after treatment the field strain larvae of *H. armigera* with the tested biopesticides and insect growth regulators as compared to untreated larvae (control). The samples in the last season (2010) were studied only because this study not concerned on the inheritance of enzymes or follow the recipe in insect resistance. The biochemical analysis processes were carried out after 10 days because chlorfluazuron (IGR) has a latent effect and it was distinct after 10 days.

The preparation of samples involved the use of four healthy American bollworms larvae from each replicates (16 larvae/ each treatment and control) after 3 and 7 days of treatment with all tested compounds and control. The field populations of ABW were collected from cotton bolls during growing season 2010 and transferred to the laboratory in paper bags then placed in clean jars. Larvae were homogenized in distilled water (1 larvae/1 ml) using a Teflon homogenizer surrounded with jacket of crushed ice for 3 minutes. The homogenate was centrifuged at 3500 r.p.m for 10 minutes at 5°C to remove the haemocytes. The samples were divided into small portions and kept in a deep freezer at (- 20°C) until required [12]

Enzymes measurements

The methods used to determine the activities of carbohydrates hydrolyzing enzymes (amylase, trehalase and invertase) in digesting sucrose, trehalose and starch, respectively, were determined according to Ishaaya and Swiriski [13]. The free aldehedic group of glucose after starch, trehalose and sucrose digestion was determined using 3,5 dinitrosalicylic acid reagent.

Chitinase activity was determined using 3, 5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexoamines liberated on chitin digestion according to the method described by Ishaaya and Casida [14]

Statistical analysis

The significance of enzyme activities was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($p < 0.05$) [15]. Data were subjected to statistical analyses using a software package CoStat® Statistical Software [16] a product of Cohort Software, Monterey, California.

Results

Effect of different compounds on American bollworm larvae

Data in Table 1 indicate that the initial reduction percentages of the ABW Larvae in 2009 were 75.0, 17.5, 17.5, 11.4 and 10.0% after three days of treatment with chlorfluazuron, pyriproxyfen, Protecto, Dipel DF and Bioranza, respectively. After 7 days of application residual mean of reduction percentages were 83.3, 20.0, 13.3, 20.0 and 9.1% with chlorfluazuron, pyriproxyfen, Bioranza, Protecto and Dipel DF, respectively. In addition, chlorfluazuron caused the highest annual mean of reduction percentage 80.8%, while Dipel DF recorded the lowest one, 11.5%. During the 2010 season, the initial reduction percentage of ABW larvae after three days of treatment were 80.6, 15.8, 15.8, 10.2, and 10.2% for chlorfluazuron, pyriproxyfen, Dipel DF, Protecto and Bioranza in Table 1, respectively. The highest residual mean was 79.8% recorded with the chlorfluazuron followed descendingly by 17.9, 16.6, 13.9 and 12.0% recorded with Bioranza, pyriproxyfen, Protecto and Dipel DF, respectively. Chlorfluazuron caused the highest annual mean of reduction percentage (79.8%) against the ABW larvae, whereas Dipel DF recorded the lowest one (12.0%).

The statistical analysis shows that there are significant differences between chlorfluazuron and other pesticides, while no significant differences among Dipel DF, Protecto, Bioranza and pyriproxyfen. The LSD value is 10.5.

Effect of different compounds on the common predators

Data in Table 2 show that, the side effects of the compounds on the common predators found on cotton plants during both seasons in cotton field. The initial reduction percentages of common predators were 20.7 and 23.3% 10.2 and 11.5% 10.2 and 11.5% 4.6 and 11.1% and 2.8 and 5.6% for chlorfluazuron, pyriproxyfen, Protecto, Dipel DF and

Treatments	2009 Season								2010 Season							
	Pre count ±SE*	Mean number of *H. armigera* larvae ± SE			Reduction %				Pre count ±SE	Mean number of *H. armigera* larvae ± SE			Reduction %			
					Initial	Residual							Initial	Residual		
		3 days	7 days	10 days	3 days	7 days	10 days	Mean	Annual Mean		3 days	7 days	10 days	3 days	7 days	10 days	Mean	Annual Mean
Dipel DF	11.0±0.4	13.0±0.4	15.0±.08	15.0±0.8	11.4	9.1	13.9	11.5	11.5^{b}	14.0±0.8	14.0±0.8	17.0±0.8	16.0±0.9	15.8	11.7	8.6	10.2	12.0^{b}
Protecto	10.0±0.9	11.0±0.9	12.0±0.9	13.0±0.9	17.5	20.0	17.90	18.9	18.5^{b}	15.0±0.9	16.0±0.4	16.0±0.9	17.0±0.9	10.2	22.4	9.3	15.9	13.9^{b}
Bioranza	10.0±0.4	12.0±0.8	13.0±0.8	11.0±0.8	10.0	13.3	30.50	21.9	17.9^{b}	15.0±0.8	16.0±0.8	18.0±0.8	13.0±0.8	10.2	12.7	30.7	21.7	17.9^{b}
Chlorfluazuron	12.0± 0.8	4.0±0.4	3.0±0.4	3.0±0.4	75.0	83.3	84.20	83.7	80.8^{a}	13.0±0.9	3.0±0.4	4.0±0.9	3.0±0.8	80.6	77.4	81.5	79.5	79.8^{a}
Pyriproxyfen	10.0±0.4	11.0±0.9	12.0±0.8	12.0±0.8	17.5	20.0	24.20	22.1	20.6^{b}	17.0±0.8	17.0±0.8	19.0±0.8	18.0±0.8	15.8	18.7	15.3	17.0	16.6^{b}
Control	12.0±8	16.0±0.8	18.0±0.9	19.0±0.9	----	----	----	----	----	16.0±0.9	19.0±0.9	22.0±0.8	20.0±0.4	----	----	----	----	----
$LSD_{0.05}$									10.48									11.49
P values									000***									000***

*SE = Standard Error
**Means under each variety sharing the same letter in a column are not significantly different at P<0.05.

Table 1: Reduction percentage of the tested insecticides against *Helicoverpa armigera* larvae in cotton field during two successive seasons, 2009 and 2010 seasons.

Bioranza in 2009 and 2010, respectively. The highest residual mean of reduction percentages were 23.2 and 20.9 recorded with chlorfluazuron in 2009 and 2010 followed by 9.6, 8.6, 8.5 and 8.5% for Bioranza, Dipel DF, Pyriproxyfen and Protecto in the first season. In the second season, the tested compounds showed lowest influence reduction were 9.9, 9.6, 6.8 and 4.1% with Bioranza, pyriproxyfen, Protecto and Dipel DF compared to the highest residual mean of reduction (20.9%) with chlorfluazuron. The highest annual mean of reduction percentages were 22.4 and 21.7% recorded with chlorfluazuron compound in 2009 and 2010 seasons. The lowest annual reduction takes place with Dipel DF treatments in both seasons (2009 and 2010). It was 7.3 and 6.4%, respectively.

The statistical analysis shows that there are significant differences between chlorfluazuron and other pesticides, while no significant differences among Dipel DF, Protecto, Bioranza and pyriproxyfen. The LSD value is 5.32.

Biochemical responses

The changes in the activity of carbohydrate hydrolyzing enzymes (amylase, invertase and trehalase) and chitenase enzymes in the supernatant of the homogenated larvae of field strain of *H. armigera* were measured at two different time intervals (3 and 7 days).

Carbohydrate Hydrolyzing Enzymes

Amylase enzyme

Data tabulated in Table 3 show that, in all treatments the level of amylase activity in the supernatant of the homogenated larvae was lower than that obtained with the untreated larvae at all inspected times. The activity of amylase was decreased greatly with chlorfluazuron treatments followed by pyriproxyfen, Dipel DF, Bioranza and Protecto. It was 61.9, 73.8, 88.8, 93.9 and 95.9% compared with control.

The statistical analysis shows that a significant difference between chlorfluazuron and other pesticides after 3 and 7 days. The LSD values are 46.199 and 49.789, respectively (Table 3). The same result was take place after 7 days. Chlorfluazuron and pyriproxyfen (IGRs) caused the highest decrease at the last inspected time (59.9 and 69.9% relative to control, respectively.

Invertase enzyme

Regarding to invertase enzyme, there were decrease in the activity in *H. armigera* resulted from all treatments during all tested periods as compared to control (Table 3).

The activity of invertase enzyme tended to give the highest decrease at the first inspected time as affected by all treatments with the exception of chlorfluazuron that gave the highest reduction at last time (83.9% relative to control). Pyriproxyfen only gave the highest decrease in the activity after 3 days that recorded (75.3) comparing to other treatments. Whereas, the reduction in the invertase activity after 7 days of treatment ranged between a minimum value of 96.1% for Bioranza to a maximum value of 80.1% for pyriproxyfen.

The statistical analysis shows that a significant difference between pyriproxyfen and other treatments after 3 days, while no significant difference among all treatments after 7 days. The LDS values are 380.1 and 359.755, respectively.

Trehalase enzyme

Data presented in Table 3, indicate that the activity of trehalase

Treatments	2009 Season									2010 Season								
	Pre count ±SE*	Mean number of natural enemies ± SE			Reduction %			Annual Mean	Pre count ± SE	Mean number of natural enemies			Reduction %				Annual Mean
		3 days	7 days	10 days	Initial	Residual				3 days	7 days	10 days	Initial	Residual			
					3 days	7 days	10 days	Mean					3 days	7 days	10 days	Mean	
Dipel DF	15.0±0.8	17.0±0.8	20.0±0.9	15.0±0.9	4.6	11.1	6.1	8.6	7.3[b]	17.0±0.9	16.0±0.9	22.0±0.9	15.0±0.8	11.1	4.4	3.9	4.1	6.4[b]
Protecto	15.0±0.9	16.0±0.8	21.0±0.8	15.0±0.9	10.2	6.7	10.4	8.5	9.1[b]	16.0±0.9	15.0±0.8	20.0±0.4	13.0±0.9	11.5	7.6	6.0	6.8	8.4[b]
Bioranza	13.0±0.9	15.0±0.9	17.0±0.8	19.0±0.4	2.8	12.8	6.5	9.6	7.4[b]	15.0±0.8	15.0±0.4	18.0±0.8	11.0±0.4	5.6	11.3	8.5	9.9	8.4[b]
Chlorflua-zuron	17.0±0.8	16.0±0.9	19.0±0.9	21.0±0.4	20.7	25.5	20.9	23.2	22.4[a]	16.0±0.9	13.0±0.9	17.0±0.9	12.0±0.9	23.3	21.5	20.3	20.9	21.7[a]
Pyriproxyfen	15.0±0.8	16.0±0.8	21.0±0.8	19.0±0.8	10.2	6.7	10.4	8.5	9.1[b]	16.0±0.9	15.0±0.9	19.0±0.4	14.0±0.4	11.5	12.2	7.0	9.6	10.2[b]
Control	16.0±0.8	19.0±0.9	24.0±0.9	25.0±0.9	----	----	----	----	----	17.0±0.8	18.0±0.4	23.0±0.9	16.0±0.9	----	----	----	----	----
$LSD_{0.05}$									5.93									5.32
P values									0.0009***									0.0005***

*SE = Standard Error
**Means under each variety sharing the same letter in a column are not significantly different at P<0.05.

Table 2: Reduction percentage of the tested insecticides against common predators in cotton field during two successive seasons, 2009 and 2010 seasons.

enzyme in the larvae of *H. armigera* was generally decreased in Dipel DF compared to other treatments in both times. It was 73.6 and 83.7% after 3 and 7 days, respectively, followed by chlorfluazuron (84.1 and 86.4%). The activity of trehalose enzyme increased in Bioranza treatment. It was 101.1% compared to control.

The statistical analysis shows that a significant difference between Dipel DF and other treatments after 3 days, while no significant difference among all treatments after 7 days. The LDS values are 78.462 and 249.822, respectively.

Chitinase enzyme

Results obtained in Table 3 show a remarkable significant increase in the enzyme activity in *H. armigera* using pyriproxyfen (141.9 and 131.6% relative to control) and chlorfluazuron (130.0 and 122.6%) after 3 and 7 days, respectively. On the other hand, Protecto caused decrease in the activity (92.5 and 98.1%, respectively).

The statistical analysis shows that a significant difference among chlorfluazuron and pyriproxyfen, and other treatments after 3 and 7 days. The LDS values are 9.403 and 10.799, respectively.

Discussion

Generally, chlorfluazuron was the most potent insecticide against ABW, *H. armigera* in initial, residual mean and annual mean causing highly reduction percentage comparing to other treatments during the 2009 and 2010 seasons. The same results were found by other authors. Methoxyfenozide and triflumuron significantly reduced the damage caused by *H. zea* [17]. Similarly, applying lufenuron at 37 and 49 g ha effectively suppressed *H. armigera* populations and resulted significant reduction in crop damage at lower doses, while in buprofezin was not effective at any tested dose for any time of treatment [18]. Dipel DF (*B. thuringiensis*) was the least effective in the residual and annual means, whereas Bioranza (Bt) was the lowest one in the initial activity at the two successive seasons. Other researchers found that the biopesticide Agerin (Bt) which similar to Dipel DF formulation (both of them *Bacillus thuringiensis* subsp. *Kurstaki*) caused the least effective treatment against bollworms than the conventional pesticides [19]. Additionally, spinosad caused significantly less dead moths of *H. armigera, H. punctigera* and other Noctuid compared to methomyl and could be used where quick action is not needed [20].

On contrary, during 1992 – 1993 seasons, MVP (Bt) resulted in 100% mortality of larvae at 10 days after the first application. Likewise 10 days after the second application, MVP product and Karate (lambda-cyhalothrin) gave 100% mortality of *Heliothis* larvae. While, during 1993-94 cotton seasons all the strains gave better control than the pesticide Karate [21]. Also, using Dipel 2X recorded 63.04 and 44.46% against spiny and American boll worms infesting cotton bolls during 2004 and 2005 seasons, respectively; whereas, application of Dipel 2X followed by conventional insecticides gave (40.40 and 50.85%), respectively [22].

Populations of predator insects found in all treated areas with tested insecticides were reduced comparing to predator numbers registered in untreated areas during the two successive seasons. However, the highest initial, residual and annual means of reduction percentages recorded with chlorfluazuron treatment in both tested seasons did not exceed 23.3%, whereas the least initial and annual means were given by Bioranza and Dipel DF, respectively at both seasons. The same symmetry was ordered by other authors. They found that spraying with biological insecticides, chemical insecticides and Bt transgenic cotton plants

Treatments	Amylase				Invertase				Trehalase				Chitinase			
	Enzyme activity (Mean± S. E**) at the indicated tested times				Enzyme activity (Mean± S. E) at the indicated tested times				Enzyme activity (Mean± S. E) at the indicated tested times				Enzyme activity (Mean± S. E) at the indicated tested times			
	3days		7days		3days		7days		3days		7days		3days		7days	
	µg/glucose/ min./g b.wt	*%	µg glucose/ min./g b.wt	*%	µg glucose/ min./g b.wt	*%	µg glucose/ min./g b.wt	*%	µg glucose/ min./g .wt	*%	µg glucose/ min./g b.wt	*%	µg AChBr/ min./g b.wt	*%	µg AChBr/ min./g b.wt	*%
Dipel DF	578.8±11.4^{b}	88.8	784.0±15.5^{b}	92.0	1398.9±200.3^{a}	81.87	1302.1±156.4^{a}	84.9	654.4±39.1^{c}	73.6	910.4±21.0^{a}	83.7	56.3±4^{b}	108.6	65.3±2.7^{b}	92.9
Protecto	624.1±11.1^{ab}	95.9	833.2±18.7^{ab}	97.8	1435.6±178.5^{a}	84.02	1319.4±184.5^{a}	86	860.3±22.7^{a}	96.8	1058.7±82.3^{a}	97.4	47.9±3.2^{b}	92.5	68.9±3.3^{b}	98.1
Bioranza	610.5±11.6^{ab}	93.9	807.6±16.0^{ab}	94.8	1584.2±214.8^{a}	92.71	1473.8±134.6^{a}	96.1	873.3±12.9^{a}	98.3	1099.5±77.7^{a}	101.1	54.5±3.4^{b}	105.1	74.3±3.1^{b}	105.7
Chlorfluazuron	402.3±9.8^{d}	61.9	510.1±13.4^{d}	59.9	1507.7±191.1^{a}	88.23	1286.7±150.6^{a}	83.9	747.4±27.0^{b}	84.1	939.4±19.6^{a}	86.4	67.4±2.6^{a}	130.0	86.2±4^{a}	122.6
Pyriproxyfen	480.6±15.7^{c}	73.8	595.5±12.7^{c}	69.9	1286.3±176.4^{b}	75.28	1229.4±97.7^{a}	80.1	858.6±20.9^{a}	96.6	1037.2±55.6^{a}	95.4	73.5±2.5^{a}	141.9	92.50±4.1^{a}	131.6
Control	650.4±24.9^{a}	100	852.2±19.4^{a}	100	1708.7±163.9^{a}	100	1534.2±157.3^{a}	100	888.6±22.7^{a}	100	1087.1±62.1^{a}	100	51.8±2.3^{b}	100	70.3±3.6^{b}	100
L.S.D$_{0.05}$	46.199**		49.789**		380.100**		359.755^{ns}		78.462**		249.822^{ns}		9.403**		10.799**	
P values	0.0000		0.0000		0.0095		0.6772		0.0002		0.4863		0.0005		0.0008	

*Activity %= percentage relative to control.
**SE = Standard Error
***Means under each variety sharing the same letter in a column are not significantly different at P<0.05.

Table 3: Changes in the activity of some enzymes in the supernatant of the homogenated *H. armigera* larvae as affected by tested insecticides.

reduced predator population by 2.64-14.2% [23]. Bt cotton efficiently controls cotton bollworms, while the decrease of pesticide applications allows the buildup of high populations of predators, such as lady beetles, *Coccinella septempunctata*, lacewings, *Chrysopa sinica*, spiders and others in mid-season [24]. Buprofezin and lufenuron (IGR,s) at lower doses, appeared safe to predator populations, which did not differ significantly in IGR-treated versus untreated control plots. Population densities of coccinellids were significantly lower at high concentrations of both IGRs in treatment plots, possibly as a result of reduced prey availability [18]. Side effect of seven pesticides on beneficial arthropods was highly influence compared with Agerin (Bt) treatment which had the minimum side effect on beneficial arthropods [19].

Carbohydrates are of vital importance since they can be utilized by the insects' body for production of energy or conversion to lipids or proteins. Metabolism of carbohydrates is controlled mainly by carbohydrate hydrolyzing enzymes. The final product of carbohydrates metabolism is glucose, the increase of these enzymes during the larval stage suggested that these enzymes degrade carbohydrates to glucose for chitin build-up [6]. This clears in Table 3, chlorfluazuron decreased the activity of amylase. So, degradation of carbohydrates also decreases. This leads to disturbance in chitin building and failure of molting process.

Therefore, the inhibition of carbohydrate hydrolyzing enzymes recorded in the present study might affect the molting process and subsequently may explained the reason of mortality occurred in *H. armigera* larvae as illustrated previously in the toxicological experiments. These results are in agreement with previous research who observed pronounced decrease in the carbohydrate hydrolyzing enzymes especially amylase and invertase was observed after treated 5th instar larvae of cotton leafworm, *S. littoralis* (Lepidoptera: Noctuidae) with sub-lethal concentrations of thuringeinsin (beta-exotoxin of *B. thuringiensis*) [25]. Consult and Mimic (IGRs) decreased the invertase activity after 5 days of treatment, whereas Consult, Atabron and Cascade exhibited reduction in trehalase and invertase activities in *S. littoralis* [26]. Additionally, the activities of trehalase, invertase and amylase enzymes in *S. littoralis* larvae treated with Tracer (spinosad) and triflumuron were generally decreased than untreated larvae during different tested times [27].

Ecdysis is initiated by apolysis the process that separates epidermal cells from the old cuticle by molting fluid secretion and ecdysal membrane formation. The molting fluid contains proteases and chitinases, enzymes that digest the main constitution of the old endocuticle [28]. The insect growth regulator, diflubenzuron interferes with the development of the cuticle, to which insect skeletal muscle is attached. The effect of diflubenzuron on the ultra structure of the muscle attachment to the cuticle in larvae of Noctuid *S. littoralis* is described, and it is concluded that there is no digestion of the affected old cuticle, and no digestion of the tonofibrillae (microtubules passing through the pore canals and attached to the cuticulin layer) [29].The fluctuation in the chitinase activity in the homogenated larvae was observed by many authors. Markedly increase in chitinase activity occurred when treated 4th instar larvae of *S. littoralis* were treated with diflubenzuron [30]. Chlorfluazuron caused a significant increase in chitinase activity of *S. littoralis* [31].

These results confirmed that the insect growth regulators (chlorfluazuron and pyriproxyfen) were more effective than the biopesticides against *H. armigera*, but these pesticides have a side effect on the natural enemies compared with the biopesticides. So, the biopesticides is more suitable to integrated pest management program.

On the other hand, the activity of amylase, invertase and trehalase was clearly decreased in chlorfluazuron and pyriproxyfen (IGR,s) especially after 7 days of treatment. While, the activity of chitinase was increased in chlorfluazuron and pyriproxyfen compared to the biopesticides. This mean that chitinase play an important role in *H. armigera* resistant to insect growth regulators.

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