Efficacy of Three Insect Growth Regulators on Growth and Development of the Egyptian Cotton Leafworm *Spodoptera littoralis* (Boisd.)

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ABSTRACT

The direct and latent effects of the three commercial insect growth regulators lufenuron, methoxyfenozide and teflubenzuron on the 2nd and 4th instar larvae of susceptible strain of cotton leafworm *Spodoptera littoralis* were studied. Different concentrations of each insecticide (0.01, 0.1, 1 and 2.5 mg (a.i)/Kg diet) were incorporated into the artificial diet. The newly moulted 2nd and 4th larval instars were fed for 6 days on the treated diet in order to investigate the biological activity of these compounds under controlled laboratory conditions. Activity of chitinase and polyphenol oxidase (PPO) in surviving larvae after treatment was carried out in order to investigate the biochemical influences of these compounds. The insecticides proved to be high toxic to the test insect larvae and they showed adverse effects on pupae, and emergence of adults. The larvae suffered from more mortality when they were fed for a longer period on the diet that treated with concentrations of 1 and 2.5 mg (a.i)/Kg diet. The significant high mortality percentages were observed at all tested concentrations with teflubenzuron followed by lufenuron and then methoxyfenozide and the result clearly showed that the 2nd instar larvae were more sensitive to the compounds tested, compared to those of the 4th instar. Larval weight gain was considerably decreased as concentration increased and the reduction in weigh was highly effective in case of teflubenzuron followed by lufenuron and then methoxyfenozide. The affected larvae ceased feeding within 48h and most deaths occurred during moulting to the next instar. Some larvae failed to pupae successively. The insecticides varied in their influences on chitinase and PPO activity, and these enzymes could have relation with their toxicity against *S. littoralis* larvae. The susceptibility of the fourth larval-stage *S. littoralis* to these insecticides evidenced a similar pattern however; the activity was lower than that obtained with the second larval-stage. So, when these insecticides should be used for *S. littoralis* larvae control, dosage and timing of application should be carefully considered.

Keywords: **lufenuron, methoxyfenozide, teflubenzuron, *Spodoptera littoralis*.**

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is a commonly pest and it is found in different areas of the world. This caterpillar is very polyphagous, causing important economic losses in both greenhouses and open fields on a broad range of ornamental, industrial and vegetable crops (Brown and Dewhurst, 1975; Holloway, 1990). Besides, many populations of *S. littoralis* have acquired resistance towards most insecticide groups (Ishaaya *et al.*, 1995; Abo Elghar *et al.*, 2005; Saleem *et al.*,2008). Nowadays, the scientists of pest control and environmental protection oriented their activities to limit the environmental pollution. The efficiency of broad-spectrum neurotoxic insecticides and their mixture with insect growth regulators (IGRs) against the cotton leafworm attracted several investigators (Smagghe and Degheele, 1994; Darvas and Polgar, 1998; Smagghe *et al.*, 1999; Abo Elghar *et al.*, 2005; Saleem *et al.*, 2008).

Insect growth regulators (IGRs) are third-generation insecticides and they have less toxic and compatible with insect pest management that were developed to reduce the pollution in food and environment. These compounds have a specific mode of action on insects and a lower toxicity against vertebrates than conventional insecticides. These compounds are effective suppressors of development for the entire life cycle on insects (Van Eck, 1979; Smagghe and Degheele, 1994; Smagghe *et al.*, 1999; Nasr *et al.*, 2010). However, these compounds, also, affect the hormonal balance in insects, thereby resulting in physiological disturbances, such as inhibition of DNA synthesis (Dhadialla *et al.*, 1998); alteration of carbohydrates (Ishaaya and Ascher, 1977); cuticular lipids (Salama *et al.*, 1976) and microsomal oxidase (Yu and Terriere, 1975).

Some IGR compounds such as teflubenzuron and hexaflumuron act preferentially by interfering with the chitin synthesis metabolism (chitin synthesis inhibitors). They appear to have high target pest specificity and their effects can differ significantly among insect species (Dhadialla *et al.*, 1998). The most important group of chitin synthesis inhibitors is benzoylphenyl ureas which have been subjected to intensive investigation because of their

commercial importance and their interference with the moulting and other physiological processes in several insect species (Ishaaya, 1990; Soltani *et al.*, 1993; Casida and Quistad, 1998, Tassou and Schulz, 2011). The principal effect of IGRs is to disturb development and metamorphosis and their action is therefore, much slower than that of conventional insecticides. These compounds claim to be safe for natural enemies and provide an alternative to conventional insecticides because lethal and sublethal effects of the latter are usually considered highly risky to beneficial species (Marks, 1980).

Lufenuron (Fluphenacur or Match or CGA-184699) is a chitin synthesis inhibitor, in general, manufactured by Ciba Gaigi, Basel, Switzerland. It was assessed against several insect pests, such as summer fruit tortix, *Adoxophyes orana*; fleas and it inhibited chitin synthesis and influenced the development of eggs and larvae (Charmillot *et al.*, 1991; Hink *et al.*, 1991; Ioriatti *et al.*, 1993; Dean *et al.*, 1999). Methoxyfenozide was discovered in 1990 and is the most effective member of the diacylhydrazine class, has very high affinity for ecdyseteroid receptors in Lepidoptera, where it mimics the insect molting hormone, 20-hydroxyecdysone (Ishaaya *et al.*, 1995; Carlson *et al.*, 2001). Teflubenzuron is an insecticide from the functionally-derived group of acylureas. It is a chitinase inhibitor insecticide, interfering with the deposition of chitin in the insect cuticle. It was found to be high toxic and selective towards larval Lepidoptera and it is now widely used in several crops throughout the world (Clarke and Jewess, 1990; Dhadialla *et al.*, 1998).

Therefore, the objectives of the current study were to investigate the effects of luvenuron methoxyfenozide and teflubenzuron as IGRs on larval development, pupation and the emergence of adults of the cotton leafworm *S. littoralis* (Boisd.). In addition, these insecticides may manipulate physical and biochemical process of insect by influencing relative enzymes. Therefore, the activities of chitinase and polyphenol oxidase (PPO) were determined in surviving larvae after treatment to elucidate the insecticidal mechanism of these insecticides.

MATERIALS AND METHODS

1. Chemicals and test insecticides

Lufenuron (Match, 5% EC, Ciba Gaigi) *N* {[2,5-dichloro-4-(1,1,2,3,3,3- hexafluorapropoxyl) phenyl]amino] carboryl}-2,6-difluoro benzamide, methoxyfenozide (Runner, 24% SC, Dow Agro Sciences) 3-methoxy-2-methylbenzoic acid 2-(3,5 dimethylbenzoyl)-2 - (1,1 dimethylethyl) hydrazide, and teflubenzuron (Nomolt, 15% SC, Shell-Agro, FRG)1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl) urea were obtained in the form of their commercial formulations from the respective manufacturers. Bovine serum albumin (BSA), Folin-Ciocalteu phenol regent, 3,5-dinitrosalicylic acid and pyrocatechol were purchased from Sigma-Aldrich Chemical Co., USA. All chemicals were used without further purification. The stock solutions of each compound were made in water on the day of experiments and were used immediately. Concentrations of 0.01, 0.1,1 and 2.5 mg (a.i)/Kg fresh diet were prepared.

2. Test insects

Caterpillars of a laboratory strain of Egyptian cotton leafworm *Spodoptera littoralis* Boisd. (Lipidoptera: Noctuidae) were obtained from the Department of Entomology, Faculty of Agriculture, Alexandria University. The colony was reared on artificial diet under controlled conditions at 25±2°C, 70±5% relative humidity and a 16h light photoperiod (Hegazi *et al.*, 1977a, b).

3. Insecticidal and growth - inhibitory assay against *S. littoralis*

Newly moulted second and fourth instars larvae of *S. littoralis* were continuously fed the artificial diet containing different concentrations of lufenuron, methoxyfenozide and teflubenzuron for 6 days. An artificial diet containing 2600 ml of sterile water, 38.0g of agar, 300 g of corn meal, 120g wheaten flour, 100.0g of yeast extract, 20.0g casein, 2.0 g of sorbic acid, 8.0g of ascorbic acid, 14.0g of Wesson salt mixture, 4.0g nipagine, 2.5ml of formaldehyde, 600 mg of streptomycin and 15.0ml of Vanderzant vitamin mixture for insects, were used for the bioassay, which was prepared by the procedure described earlier (Hegazi *et al.*, 1977a,b). Concentrations of 0.01, 0.1, 1 and 2.5mg (a.i)/Kg fresh diet were tested. These concentrations were prepared in water and mixed with the diet during its preparation. Each diet in a cup (a treatment) was divided into three portions and seeded in Petri dishes (9cm in diameter). Untreated diet was provided to control. Newly moulted second and fourth instars larvae (30 per each concentration) were placed in Petri dishes. For each compound, the test larvae were left to feed for 6 days. When the medium was finished in the control and low concentrations, the larvae were transferred to untreated diet until the achievement of mortality counts or moulted to pupae. Larval mortality and weight gain were assayed at days 2, 4 and 6 of feeding. The experiment was stopped when larvae entered the prepupal stage and emerged to the adult stage. The larvae that not formed pupae were counted as died larvae. Pupal mortalities were observed during the pupal period and pupation % was scored. Pupae were transferred and kept in plastic cups (7×5.8 cm) covered with muslin. After emergence, the adults were fed on 10% sugar solution contained in suspended cotton plugs and the emergence of adults was calculated as

percentage basing on the successfully emerged individuals. All treatments were carried out in a controlled environmental chamber with a 16 h light photoperiod, at 25 ± 2°C, and a relative humidity of 70±5%. There were three replications for each assay.

4. Biochemical studies

4.1. Preparation of samples for biochemical studies

The second and fourth instars larvae of *S. littoralis* that were continuously fed the artificial diet containing 0.01, 0.1, 1 and 2.5mg (a.i)/Kg fresh diet of lufenuron, methoxyfenozide and teflubenzuron were used to study the effect of these IGRs on chitinase and PPO enzymes in the whole larval body extracts. Larvae fed on media with water were used as control. Definite weight of larvae was homogenized in 3 mL of potassium phosphate buffer (pH 7.0) with a tissue Tearor on ice. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was used in protein determination and as the crude enzyme extract.

4.2. Total protein assay

The Lowry *et al.* (1951) method was used to determine protein content in surviving larvae obtained after continuous feeding for 2, 4 and 6 days. 10 µL of protein extract was added to 2 mL of alkaline copper reagent [48 mL of 2% (w/v) sodium carbonate in 0.1N sodium hydroxide + 1 mL of 1% (w/v) sodium-potassium tartrate + 1 mL of 0.5% (w/v) copper sulphate] and immediately mixed. After 10 min, 0.2 mL of Folin-Ciocalteu phenol reagent was added and the samples were thoroughly mixed. The absorbance of the developed blue color was measured at 600 nm using a Unico 1200-Spectrophotometer. The sample protein content was determined by comparing to the standard curve of BSA.

4.3. Chitinase activity assay

Chitinase (EC 3.2.1.14) is specific hydrolyze enzyme which hydrolyse chitin (chitobiose polymer) to *N*-acetyl-D-glucosamine (reduced sugar monomer). The specific activity was determined in the surviving larvae after 2, 4 and 6 days of feeding on diet treated with the tested insecticides. The larvae were homogenized in 0.1M phosphate buffer (pH 7.0) with a tissue Tearor on ice. The homogenates were then centrifuged at 5000 rpm for 20 min at 0°C. The supernatants were used as enzyme source for chitinase activity assay. Enzyme activity was measured according to Monreal and Reese (1969) method. One ml of colloidal chitin, as a substrate, in 0.05 M citrate phosphate buffer (pH 6.6) was mixed with 1mL of enzyme extract. Colloidal chitin was prepared by the method that described by Shimahara and Takiguchi (1988). A suspension containing 1% (w/v) of moist colloidal chitin is prepared in appropriate buffer and pH. The vials were placed on their sides on a rotary platform at a speed sufficient to keep the chitin in suspension. The vials were incubated for 2h at 25°C. Subsequently the vials were placed into a boiling water bath for 5 min then were cooled to room temperature by placing the vials in a cold water bath. The reaction mixtures were centrifuged at 5000 rpm for 10 min at 0°C and the supernatant was retained. Enzyme activity was assayed by measuring the amount of reducing sugar that produced by enzyme reaction (Miller, 1959). Reducing sugar was determined by mixing of 1 mL of the supernatant with 2 mL phosphate buffer (pH 6.8) and 1.5 mL of 3,5-dinitrosalicylic acid (96 mM, 438 mg of 3,5-dinitrosalicylic acid in 20 mL of deionized water and heat in a boiling water bath to dissolve). The tubes were boiled for 5 min. After cooling the tubes, the optical density (OD) was measured at 450 nm using a Unico 1200 - Spectrophotometer. The specific activity of chitinase was calculated as $OD_{450}.mg^{-1}$ protein. $1h^{-1}$. Blank sample was determined in the manner described above without enzyme solution.

4. 4. Polyphenol oxidase (PPO) activity assay

The activity of PPO (EC 1.10.3.2) was determined according to Zhi-qing *et al.*, (2008) by mixing of 1.5mL of 0.2 mol/L pyrocatechol, 1.4 mL of 0.05 mol/L phosphate buffer (pH 6.8) and 0.1 mL enzyme extract, respectively. The mixture was incubated at 25°C for 30 min and the absorbance was measured at 420 nm using a Unico 1200-Spectrophotometer. The specific activity of PPO was calculated as OD_{420}. $30\ min^{-1}$. mg^{-1} protein.

5. Statistical analysis

All the quantitative estimations of toxicity and biochemical parameters were based on three replicates and the values are expressed as mean ± standard error. The data were statistically analyzed separately for each experiment and were subjected to analysis of variance (ANOVA) using SPSS 12.0 software (Statistical Package for Social Sciences, USA). Results are given in the text as probability values, with $p \leq 0.05$ adopted as the criterion of significance. Differences between treatment means were established with a Student-Newman-Keuls (SNK) test according to Snedecor and Cochran (1989). When F-test was significant, means were separated using SNK test at the 0.05 level of significance.

RESULTS AND DISCUSSION

1. Insecticidal activity of lufenuron, methoxyfenozide and teflubenzuron on growth and development of *S. littoralis* larvae

Data presented in Tables 1 and 2 indicate the insecticidal activity of three IGRs lufenuron, methoxyfenozide and teflubenzuron on development

of the second and fourth larval-stages *S. littoralis*. Generally, the results indicate high toxicity on both larval instars with latent effects on larval growth, deformation of pupae and adults emergence. When second instar larvae were fed on artificial diet containing varying concentrations (0.01, 0.1, 1 and 2.5 mg (a.i)/Kg diet) of lufenuron, methoxyfenozide and teflubenzuron, weight gain of the larvae was dramatically decreased with the increase of the concentration (Table 1). After 2 days of feeding on treated diet, most of the larvae showed clear symptoms of a double head capsule and died shortly afterwards. With 1 and 2.5 mg (a.i)/Kg diet, 100% of larval mortality occurred within 4 days and larval weight gain was halted as intoxicated larvae had stopped feeding within the first three days. The effects of the treatments were significant comparing to the untreated larvae and the reduction in weigh was highly effective in case of teflubenzuron followed by lufenuron and then methoxyfenozide., i.e. 2 days after treatment (dF = 12, 26; $F = 41.62$; $p < 0.0001$), 4 days (df = 6, 14; $F = 1799.22$; $p < 0.0001$) and 6 days (df = 4, 10; $F = 5540.50$; $p < 0.0001$). There is no larva survived at day 4 of the treatment with the highest concentrations (1 and 2.5 mg (a.i)/Kg diet) among the insecticides therefore, the mean weight gained was not calculated. However, only the lowest concentration (0.01 mg (a.i)/Kg diet) allowed treated larvae to survive thought the experiment in case of lufenuron and teflubenzuron and the mean weight gained at day 6 were 507.62 and 85.67 mg/larva, respectively compared with 958.33 mg/larva in the control. Treatments with 0.01 and 0.1 mg (a.i) of methoxyfenozide/Kg diet allowed the treated larvae to survive thought the experiment and were significantly effective on the growth at day 6 (668.31 and 308.80 mg/larva, respectively compared with 958.33 mg/larva in the control). At the low concentration of 0.01 mg (a.i)/Kg diet, there was no clear moulting disturbing effect with methoxyfenozide as most of the tested larvae survived after treatment. However, the other concentrations of methoxyfenozide and all concentrations of lufenuron and teflubenzuron induced serious effects within the first two days after treatments in both larval stages and many larvae suffered from moulting failure. Furthermore, the larvae fed on the treated diet showed little growth and were very small compared to the control during the observation times. In intoxicated larvae, the decrease in weight gain and feeding in larval Lepidoptera treated with these insecticides may result from an ecdysonergic action. It is well known that cessation of feeding and weight gain occurs prior to ecdysis as a result of ecdysteroid secretion.

The susceptibility of the fourth larval-stage *S. littoralis* to these insecticides evidenced a similar pattern as shown in Table 2 however; the activity was lower than that obtained with the second larval-stage. The reduction in weigh was also highly effective in case of teflubenzuron followed by lufenuron and then methoxyfenozide., i.e. 2 days after treatment (df = 12, 26; $F = 246.23$; $p < 0.0001$), 4 days (df = 7, 16; $F = 1242.06$; $p < 0.0001$) and 6 days (df = 4, 10; $F = 1349$; $p < 0.0001$). There is no larva survived at day 4 with 0.1, 1 and 2.5 mg (a.i) /Kg diet of teflubenzuron. However, there is no larva survived with 2.5 mg (a.i)/Kg diet of lufenuron and methoxyfenozide at the ssame time therefore, the mean weight gained was not calculate. At day 6, treatments with 0.01 and 0.1mg (a.i) of methoxyfenozide / Kg diet allowed the larvae to survive and the growth was significantly affected (753.50 and 314.57 mg/larva, respectively compared with 1046.39 mg/larva in the control). However, only the lowest concentration (0.01) of lufenuron and teflubenzuron allowed the larvae to survive and the mean weight gained were 520.41 and 240.78 mg/larva, respectively compared with 1046.39 mg/ larva in the control.

The results (Tables 1 and 2) showed that mean percent of mortality of the second and fourth instar larvae varied significantly between the treatments and control. The significant high mortalities were observed at all tested concentrations of teflubenzuron followed by lufenuron and then methoxyfenozide., i.e. 2 days after treatment (df = 12, 26; $F = 108.92$; $p < 0.0001$), 4 days (df = 12, 26; $F = 290.69$; $p < 0.0001$) and 6 days (df = 12, 26; $F = 431.46$; $p < 0.0001$). It was evident that the three insecticides caused 100% mortality at day 4 when the second larval stage was treated with 1 and 2.5 mg (a.i)/Kg diet. The susceptibility of the fourth larval-stage to these insecticides evidenced a similar pattern however; the activity was lower than that obtained with the second larval-stage where there is no mortality at day 2 among all treatments except 6.67% mortality that scored with the concentration of 2.5 mg (a.i)/Kg diet of teflubenzuron (Table 2).

When second instar larvae of *S. littoralis* were fed on artificial diet containing varying concentrations of lufenuron, methoxyfenozide and teflubenzuron, those insects exposed to all concentrations tested experienced increasing disruption of moulting, so that the percentage successfully emerging as adults fell from 96.30% in the control group to only 53.33% and 16.67% at 0.01 and 0.1 mg (a.i) / Kg diet, respectively of methoxyfenozide (Table 1). However, there were no adults emerged in the treatment of 0.01 mg (a.i) lufenuron/Kg diet.

The lowest dose (0.01 mg (a.i) / Kg diet) of lufenuron and methoxyfenozide allowed treated larvae of the second and fourth instars to survive and produce pupae while the surviving pupae reduced in size compared with untreated counterparts. However, exposure to 0.01 mg (a.i) /Kg diet of teflubenzuron failed to produce

Table 1: Effect of lufenuron, methoxyfenozide and teflubenzuron on larval growth, mortality, pupation and adult emergence of second-instar larvae of *S. littoralis*.

Treatment	Conc. (mg/Kg diet)	Mean weight gained (mg/larva) ± SE			Larval mortality (%) ± SE			Pupation (%) ± SE	Adult emergence (%) ± SE
		2 day	4 day	6 day	2 day	4 day	6 day		
Control	0.00	39.8^{a}±1.9	387.9^{a}±4.1	958.3^{a}±5.8	0.0^{d}±0.0	0.0^{e}±0.0	0.0^{c}±0.0	100.0^{a}±0.0	96.3^{a}±3.7
Lufenuron	0.01	34.3^{ab}±2.8	247.1^{c}±5.2	507.6^{c}±6.6	0.0^{d}±0.0	20.0^{c}±5.7	26.7^{c}±3.3	31.8^{d}±1.6	0.0^{c}±0.0
	0.1	16.9^{c}±2.3	44.7^{e}±2.9	-	13.3^{d}±3.3	93.3^{a}±3.3	100.0^{a}±0.0	-	-
	1	11.7^{cd}±3.3	-	-	76.7^{b}±6.7	100.0^{a}±0.0	100.0^{a}±0.0	-	-
	2.5	6.3^{de}±1.9	-	-	93.3^{a}±3.3	100.0^{a}±0.0	100.0^{a}±0.0	-	-
Methoxyfenozide	0.01	34.9^{ab}±1.3	341.6^{b}±5.7	668.3^{b}±3.7	3.3^{d}±3.3	10.00^{d}±0.0	16.7^{d}±3.3	77.9^{b}±7.7	53.3^{b}±3.3
	0.1	30.1^{b}±1.4	158.5^{d}±2.1	308.8^{d}±1.6	6.7^{d}±3.3	13.3^{cd}±3.3	43.3^{b}±3.3	50.0^{c}±0.0	16.7^{c}±16.7
	1	14.5^{c}±0.7	-	-	50.0^{c}±5.7	100.0^{a}±0.0	100.0^{a}±0.0	-	-
	2.5	10.7^{cd}±1.9	-	-	53.3^{c}±5.7	100.0^{a}±0.0	100.0^{a}±0.0	-	-
Teflubenzuron	0.01	15.1^{c}±0.8	34.4^{e}±1.5	85.7^{c}±2.9	0.0^{d}±0.0	66.7^{b}±3.3	93.3^{a}±3.3	0.0^{e}±0.0	-
	0.1	11.2^{cd}±2.9	11.7^{f}±1.2	-	86.7^{ab}±3.3	96.7^{a}±3.3	100.0^{a}±0.0	-	-
	1	5.0^{de}±0.6	-	-	93.3^{a}±6.7	100.0^{a}±0.0	100.0^{a}±0.0	-	-
	2.5	2.3^{e}±0.3	-	-	93.3^{a}±3.3	100.0^{a}±0.0	100.0^{a}±0.0	-	-
F		41.62	1799.22	5540.50	108.92	290.69	431.46	122.19	24.15
df		12, 26	6, 14	4, 10	12, 26	12, 26	12, 26	4, 10	3, 8
P		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

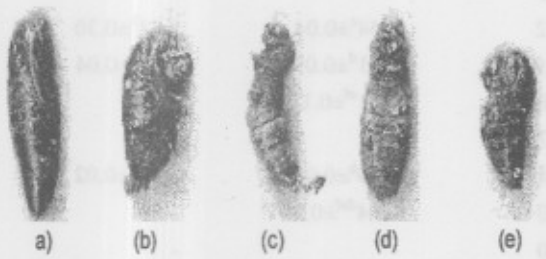
Data are averages ± SE of three replicates. Values followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls (SNK) test. *F*: F ratio, df: degree of freedom, *P*: p value (significance of the F ratio).

Table 2: Effect of lufenuron, methoxyfenozide and teflubenzuron on larval growth, mortality, pupation and adult emergence of fourth-instar larvae of *S. littoralis*.

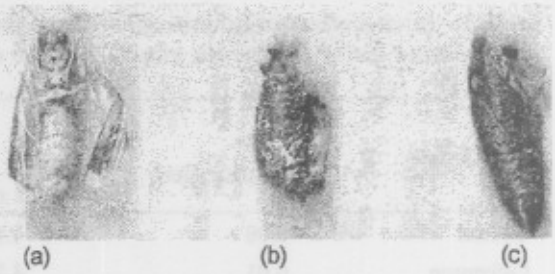
Treatment	Conc. (mg/Kg diet)	Mean weight gained (mg/larva) ± SE			Larval mortality (%) ± SE			Pupation (%) ± SE	Adult emergence (%) ± SE
		2 day	4 day	6 day	2 day	4 day	6 day		
Control	0.00	124.5±3.4	403.2^{a}±5.1	1046.4^{a}±6.4	0.0^{b}±0.0	0.0^{e}±0.0	0.0^{d}±0.0	100.0^{a}±0.0	92.1^{a}±3.9
Lufenuron	0.01	113.2±0.1	259.2^{c}±6.7	520.4^{c}±7.2	0.0^{b}±0.0	13.3^{d}±3.3	20.0^{c}±5.8	16.7^{d}±4.2	0.0^{c}±0.0
	0.1	71.9^{e}±0.3	63.5^{f}±5.1	-	0.0^{b}±0.0	83.3^{b}±3.3	100.0^{a}±0.0	-	-
	1	44.5^{g}±2.6	29.0^{g}±3.5	-	0.0^{b}±0.0	90.0^{ab}±5.8	100.0^{a}±0.0	-	-
	2.5	42.3^{g}±2.9	-	-	0.0^{b}±0.0	100.0^{a}±0.0	100.0^{a}±0.0	-	-
Methoxyfenozide	0.01	115.1^{b}±2.2	376.9^{b}±0.9	753.5^{b}±1.8	0.0^{b}±0.0	10.0^{de}±0.0	20.0^{c}±5.8	76.7^{b}±1.7	38.9^{b}±5.6
	0.1	92.3^{c}±0.2	210.3^{d}±0.2	314.6^{d}±0.2	0.0^{b}±0.0	16.7^{d}±6.7	30.0^{c}±5.8	50.0^{c}±0.0	0.0^{c}±0.0
	1	44.6^{g}±2.4	59.2^{f}±5.6	-	0.0^{b}±0.0	83.3^{b}±3.3	100.0^{a}±0.0	-	-
	2.5	42.9^{g}±3.0	-	-	0.0^{b}±0.0	100.0^{a}±0.0	100.0^{a}±0.0	-	-
Teflubenzuron	0.01	80.5^{d}±0.3	99.4^{e}±0.2	240.8^{e}±0.4	0.0^{b}±0.0	40.0^{c}±5.8	70.0^{b}±5.8	0.0^{e}±0.0	-
	0.1	52.3^{f}±1.3	-	-	0.0^{b}±0.0	86.7^{ab}±3.3	100.0^{a}±0.0	-	-
	1	42.3^{g}±2.0	-	-	0.0^{b}±0.0	100.0^{a}±0.0	100.0^{a}±0.0	-	-
	2.5	39.9^{g}±1.1	-	-	6.7^{a}±3.33	100.0^{a}±0.0	100.0^{a}±0.0	-	-
F		246.23	1242.06	1349	4.00	135.88	151.38	396.35	163.62
df		12, 26	7, 16	4, 10	12, 26	12, 26	12, 26	4, 10	3, 8
P		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Data are averages ± SE of three replicates. Values followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls (SNK) test. *F*: F ratio, df: degree of freedom, *P*: p value (significance of the F ratio).

pupae and malformation was observed. On reaching the pupal stage, some larvae suffered from partial moult inhibition and died during their attempt to shed the old cuticle. Moreover, the pupae resulting from the treated larvae were apparently morphologically affected. The majority of pupal deformities produced by the action of these insecticides can easily be observed in Figure 1. These deformities varied between charred body, collapsed appendages and atrophied elytron pads. All treatments resulted in similar degrees of pupal deformation in addition to dorso-ventrally compressed body, failure of complete escape from the prepupal skin, tubercle thorax and dwarf wing pad. There were no observed differences between the pupal periods of all resulting pupae including those of control. However, delayed effects were observed among some of the treated larvae. Some of the latter developed into malformed pupae and some failed to pupate successfully instead they formed larval pupal intermediate. All these larvae died while attempting to moult. A complete pupation (100%) resulted to the control, whereas 31.75%, 77.98% and 0% pupation were achieved at 0.01 mg (a.i)/Kg diet of lufenuron, methoxyfenozide and teflubenzuron, respectively (df = 4, 10; $F = 122.19$; $p < 0.0001$). The emerged adults occurred with the lowest treatments of methoxyfenozide (a.i)/Kg diet and serious effects that faced some adults resulting from these treatments were observed. This phenomenon was noted when the affected adults attempted to extricate themselves from the pupal skin. In other cases, some adults freed the abdomen successfully from their pupal exuvia, but the thorax and head remained bound to the pupal skin and others having vestigial wings (Figure 2). In general the treatments provided higher toxic effect to the second than the fourth larval instar, although all insecticides tested at all concentrations showed high efficacy against the fourth larval stage of *S. littoralis*.



Fig, 1: Malformation of pupae that treated with lufenuron and methoxyfenozide compared to the untreated control (a). b and c resulted from the treatment of 0.01 mg lufenuron (a.i)/Kg diet on the second and fourth instar larvae of *S. littoralis*, respectively; d and e resulted from the treatment of 0.01 mg methoxyfenozide (a.i)/Kg diet on the second and fourth instar larvae, respectively.



Fig, 2: Application of 0.01 mg methoxyfenozide (a.i)/Kg diet on the second and fourth instar larvae of *S. littoralis* resulted in adults deformation (b and c, respectively) compared to the control (a), ventral side of deformed adult with collapsed body as well as remains of pupal exuvium.

2.Inhibitory effect of lufenuron, methoxyfenozide and teflubenzuron on chitinase activity in the larvae of *S. littoralis*

The *in vivo* inhibitory effects on chitinase isolated from surviving larvae fed on 0.01, 0.1, 1 and 2.5 mg (a.i)/Kg diet of lufenuron, methoxyfenozide and teflubenzuron in second and fourth larval stages of *S. littoralis* are presented in Tables 3 and 4. Determination of the specific activity of the enzyme in susceptible larvae after 2, 4 and 6 days of all treatments showed a detrimental drop. The inhibitory effects of different rates of the recommended doses were significant at day 4 (df = 6, 14; $F = 1190.29$; $p < 0.0001$) and day 6 (df = 4, 10; $F = 397.62$; $p < 0.0001$) compared with the untreated larvae, whereas the treatments at day 2 not significantly differ compared with the control (df = 12, 26; $F = 1.96$; $p = 0.073$) (Table 3). However, there are considerable differences between the treatments and the control at day 2 when the fourth instar larvae were fed on diet containing varying concentrations of the insecticides (Table 4). The result revealed that the specific activity of chitinase was increased with increasing in the larval stage where that 4.44, 5.03 and 5.86 were found in untreated second instar larvae after 2, 4 and 6 days of feeding, respectively whereas the specific activities of 4.59, 5.11 and 6.03 were found in the case of fourth instar larvae at days 2, 4 and 6, respectively. It can be noticed that the specific activity of chitinase estimated at day 4 post-treatment clearly reveal that these activities in the second and fourth instar larvae were comparably lower than that at days 2 and 6. The values of the specific activity recorded with lufenuron, methoxyfenozide and teflubenzuron were 0.05, 0.11 and 0.00 for the former instar, whereas the corresponding activities were 1.01, 1.11 and 0.94 for the latter older instar, respectively at 0.1 mg (a.i)/Kg diet.

Table 3: *In vivo* effect of lufenuron, methoxyfenozide and teflubenzuron on chitinase activity in larvae of second instar *S. littoralis* after 2, 4 and 6 days of feeding.

Treatment	Conc. (mg/Kg diet)	Specific activity (OD_{450}.mg protein^{-1}. 1h^{-1})		
		2 day	4 day	6 day
Control	0.00	$4.44^{a}\pm0.04$	$5.03^{a}\pm0.03$	$5.86^{a}\pm0.03$
Lufenuron	0.01	$4.20^{ab}\pm0.08$	$0.10^{b}\pm0.05$	$1.57^{c}\pm0.08$
	0.1	$4.09^{ab}\pm0.19$	$0.05^{b}\pm0.05$	-
	1	$4.00^{ab}\pm0.07$	-	-
	2.5	$3.87^{ab}\pm0.06$	-	-
Methoxyfenozide	0.01	$4.30^{ab}\pm0.05$	$0.18^{b}\pm0.09$	$2.23^{b}\pm0.08$
	0.1	$4.13^{ab}\pm0.12$	$0.11^{b}\pm0.07$	$1.06^{d}\pm0.18$
	1	$4.12^{ab}\pm0.09$	-	-
	2.5	$3.87^{ab}\pm0.24$	-	-
Teflubenzuron	0.01	$4.07^{ab}\pm0.23$	$0.07^{b}\pm0.04$	$1.26^{cd}\pm0.04$
	0.1	$3.92^{ab}\pm0.27$	$0.00^{b}\pm0.00$	-
	1	$3.79^{ab}\pm0.12$	-	-
	2.5	$3.65^{b}\pm0.18$	-	-
F		1.96	1190.29	397.62
df		12, 26	6, 14	4, 10
P		0.073	< 0.0001	< 0.0001

Data are averages ± SE of three replicates. Values followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls (SNK) test. *F*: F ratio, df: degree of freedom, *P*: p value (significance of the F ratio).

Table 4: *In vivo* effect of lufenuron, methoxyfenozide and teflubenzuron on chitinase activity in larvae of fourth instar *S. littoralis* after 2, 4 and 6 days of feeding.

Treatment	Conc. (mg/Kg diet)	Specific activity (OD_{450}.mg protein^{-1}. 1h^{-1})		
		2 day	4 day	6 day
Control	0.00	$4.59^{a}\pm0.02$	$5.11^{a}\pm0.06$	$6.03^{a}\pm0.25$
Lufenuron	0.01	$2.86^{bc}\pm0.11$	$1.54^{c}\pm0.06$	$3.75^{b}\pm0.03$
	0.1	$2.83^{bc}\pm0.12$	$1.01^{de}\pm0.07$	-
	1	$2.50^{d}\pm0.12$	$0.62^{f}\pm0.08$	-
	2.5	$1.04^{f}\pm0.01$	-	-
Methoxyfenozide	0.01	$2.90^{b}\pm0.12$	$1.54^{c}\pm0.04$	$3.84^{b}\pm0.30$
	0.1	$2.78^{bcd}\pm0.05$	$1.11^{d}\pm0.09$	$2.87^{c}\pm0.04$
	1	$2.55^{cd}\pm0.11$	$0.71^{ef}\pm0.11$	-
	2.5	$1.06^{f}\pm0.07$	-	-
Teflubenzuron	0.01	$2.11^{e}\pm0.08$	$3.49^{b}\pm0.09$	$3.55^{b}\pm0.02$
	0.1	$1.99^{e}\pm0.03$	$0.94^{def}\pm0.19$	-
	1	$1.27^{f}\pm0.10$	-	-
	2.5	$1.03^{f}\pm0.04$	-	-
F		148.48	247.80	46.04
df		12, 26	8, 18	4, 10
P		< 0.0001	< 0.0001	< 0.0001

Data are averages ± SE of three replicates. Values followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls (SNK) test. *F*: F ratio, df: degree of freedom, *P*: p value (significance of the F ratio).

Table 5: *In vivo* effect of lufenuron, methoxyfenozide and teflubenzuron on PPO activity in larvae of second instar *S. littoralis* after 2, 4 and 6 days of feeding.

Treatment	Conc. (mg/Kg diet)	Specific activity (OD_{420}.mg protein^{-1}. 30 min^{-1})		
		2 day	4 day	6 day
Control	0.00	$5.45^{a} \pm 0.10$	$7.46^{a} \pm 0.05$	$8.57^{a} \pm 0.13$
Lufenuron	0.01	$3.22^{bc} \pm 0.15$	$2.80^{b} \pm 0.17$	$5.03^{c} \pm 0.09$
	0.1	$2.99^{bcd} \pm 0.07$	$1.78^{c} \pm 0.17$	-
	1	$2.61^{def} \pm 0.11$	-	-
	2.5	$2.23^{efg} \pm 0.13$	-	-
Methoxyfenozide	0.01	$3.29^{b} \pm 0.05$	$3.21^{b} \pm 0.05$	$5.65^{b} \pm 0.11$
	0.1	$2.98^{bcd} \pm 0.16$	$2.48^{b} \pm 0.08$	$3.73^{e} \pm 0.07$
	1	$2.70^{cde} \pm 0.16$	-	-
	2.5	$2.51^{defg} \pm 0.15$	-	-
Teflubenzuron	0.01	$3.15^{bc} \pm 0.08$	$2.54^{b} \pm 0.45$	$4.29^{d} \pm 0.14$
	0.1	$2.74^{cde} \pm 0.13$	$0.39^{d} \pm 0.07$	-
	1	$2.18^{fg} \pm 0.11$	-	-
	2.5	$2.09^{g} \pm 0.14$	-	-
F		47.75	120.22	283.48
df		12, 26	6, 14	4, 10
P		< 0.0001	< 0.0001	< 0.0001

Data are averages ± SE of three replicates. Values followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls (SNK) test. *F*: F ratio, df: degree of freedom, *P*: p value (significance of the F ratio).

Table 6: *In vivo* effect of lufenuron, methoxyfenozide and teflubenzuron on PPO activity in larvae of fourth instar *S. littoralis* after 2, 4 and 6 days of feeding.

Treatment	Conc. (mg/Kg diet)	Specific activity (OD_{420}.mg protein^{-1}. 30 min^{-1})		
		2 day	4 day	6 day
Control	0.00	$6.93^{a} \pm 0.05$	$7.53^{a} \pm 0.05$	$11.29^{a} \pm 0.15$
Lufenuron	0.01	$4.35^{c} \pm 0.11$	$3.22^{b} \pm 0.08$	$7.01^{b} \pm 0.11$
	0.1	$4.11^{cd} \pm 0.16$	$2.94^{b} \pm 0.13$	-
	1	$3.59^{de} \pm 0.21$	$2.14^{c} \pm 0.18$	-
	2.5	$2.94^{f} \pm 0.29$	-	-
Methoxyfenozide	0.01	$4.94^{b} \pm 0.08$	$3.24^{b} \pm 0.02$	$7.14^{b} \pm 0.38$
	0.1	$4.45^{c} \pm 0.17$	$3.04^{b} \pm 0.16$	$5.78^{c} \pm 0.03$
	1	$4.36^{c} \pm 0.16$	$2.28^{c} \pm 0.18$	-
	2.5	$3.93^{cd} \pm 0.11$	-	-
Teflubenzuron	0.01	$4.27^{c} \pm 0.09$	$2.10^{c} \pm 0.04$	$5.54^{c} \pm 0.04$
	0.1	$4.08^{cd} \pm 0.09$	$1.08^{d} \pm 0.32$	-
	1	$3.26^{ef} \pm 0.00$	-	-
	2.5	$2.51^{g} \pm 0.00$	-	-
F		58.06	131.00	151.07
df		12, 26	8, 18	4, 10
P		< 0.0001	< 0.0001	< 0.0001

Data are averages ± SE of three replicates. Values followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls (SNK) test. *F*: F ratio, df: degree of freedom, *P*: p value (significance of the F ratio).

3.Inhibitory effect of lufenuron, methoxyfenozide and teflubenzuron on Polyphenol oxidase activity in the larvae of *S. littoralis*

The *in vivo* inhibitory effects of lufenuron, methoxyfenozide and teflubenzuron on PPO in surviving larvae of *S. littoralis* treated in second and fourth instars after 2, 4 and 6 days of feeding on 0.01, 0.1, 1 and 2.5 mg (a.i)/Kg diet are shown in Tables 5 and 6. The highest values of the enzyme activity were found in the untreated larvae and the specific activity was gradually increased as the increase of the larval age where that 5.34, 7.33 and 8.12 were found in control at 2, 4 and 6 days of feeding, respectively. Enzyme activity in all treatments was concentration dependant and it was significantly declined to the lowest value with the highest concentration (2.5 mg (a.i)/Kg diet). The results showed that the inhibitory effects when the second and fourth instar larvae varied significantly between the treatments and control and it can be noted that the treatment of the second larval stage was more effective than the fourth instar. The significant high inhibitions were observed at all concentrations of teflubenzuron followed by lufenuron and then methoxyfenozide (Table 5), i.e., 2 days after treatment (df = 12, 26; $F = 47.57$; $p < 0.0001$), 4 days (df = 6, 14; $F = 120.22$; $p < 0.0001$) and 6 days (df = 4, 10; $F = 283.48$; $p < 0.0001$).

The susceptibility of the fourth larval-stage to these insecticides evidenced a similar pattern however, the activity was lower than that obtained with the second larval-stage where that there is no larval mortality at day 2 among all treatments except 6.67% mortality scored with 2.5 mg (a.i)/Kg diet of teflubenzuron (Table 2). As found with chitinase, it can be noticed that the high inhibition of PPO was achieved at day 4 of feeding if the treatments started with the second or fourth larval instar and the specific activity was declined from 7.46 (control) to 0.39 with the treatment of 0.1 mg (a.i) teflubenzuron / Kg diet in the case of the second larval instar (Table 5) and from 7.53 to 1.08 in the case of the fourth larval instar (Table 6).

In this paper, we report that larval exposure to lethal and sublethal doses of the IGRs lufenuron, methoxyfenozide and teflubenzuron significantly impairs development and moulting in *S. littoralis*. Second and fourth instars larvae are susceptible to the insecticides incorporated into the diet up to 0.01 mg (a.i)/Kg diet. The well documented insecticidal 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. This mode of action has led these compounds to be classified as insect growth regulators (Aller and Ramsay, 1988; Oberlander *et al.*, 1995).

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 (Mulder and Gijswigi, 1973). Also, the actual cause of insect death by chitin inhibitors may be attributed to either a rupture of the newly formed cuticle (Salama *et al.*, 1976; Radwan *et al.*, 1986).

Other effects of the chitin inhibitor-compounds were reported. They are known to act on the peritrophic membrane by affecting its chitin-protein structure, hindering its role in protecting the secreting cells from any damage (Clark *et al.*, 1977). These effects were found in the present study wherever the larvae fed on doses of 0.01,0.1, 1 and 2.5 mg (a.i)/Kg diet from lufenuron, methoxyfenozide and teflubenzuron failed to convert to pupal stage and some pupae did not emerged adults. This indicates that the use of these insecticides at the range of concentrations we examined (bioassay rates were lower than field recommended label rates) under laboratory conditions in Petri dishes significantly affect the development of *S. littoralis*.

There is another appreciated suggestion for explicating the death or mortality of different insect stages by the action of IGRs. According to this suggestion, mortalities are not directly related to inhibit chitin formation or the hormonal activity of the IGR, but to other factors or causes, such as: suffocation, bleeding and desiccation due to imperfect exuvation, starvation due to morphological defects, and/or failure of vital homeostatic mechanisms (Sehnal, 1983; Smagghe and Degheele, 1994). The latter suggestion is, at least, partially conceived in the present study upon *S. littoralis* since water loss of larvae increased parallel to the increasing mortality with ascending dose-level of insecticides which indicated an adverse condition of the water body content.

Methoxyfenozide is a potent ecdysone agonist against Lepidoptera. By ingestion, this compound is highly active, and to a lesser degree, by contact or topical application (Moulton *et al.*, 2002). Pineda *et al.*, (2004 and 2007) reported that a LC_{50} value of 5.17 mg (a.i)/kg diet was found, which is very similar to the value (3.98 mg (a.i)/kg diet) previously estimated for third instars of *S. littoralis*. In another study, Smagghe *et al.*, (2004) reported a LC_{50} value of 3.44 (a.i)/kg diet for last instars of *S. littoralis;* but in this case, larval mortality was recorded at day 7 after treatment. This result is in agreement with that obtained in the present work which indicates that methoxyfenozide did not show

significant mortality after two days of feeding in case of fourth instar larvae treatment.

The suppression of weight gain caused by IGRs has been reported in several caterpillars. In this study, it was observed that the means of weight gain of second and fourth instars of *S. littoralis* continuously fed with artificial diet containing different concentrations of lufenuron, methoxyfenozide and teflubenzuron decreased significantly compared to the control. Similarly, studies with methoxyfenozide and tebufenozide administered in artificial diet drastically reduced weight gain in fourth instars of *D. grandiosella* (Trisyono and Chippendale, 1998). Smagghe and Degheele (1994) reported a significant suppression of larval weight on last instars of *S. exempta* (Walker) and *S. exigua* treated with tebufenozide. As reported for other ecdysone agonists, the suppression of weight gain could be caused by an ecdysonergic activity of methoxyfenozide or by the continuous binding to the ecdysteroid receptors to lepidopterous larvae (Smagghe *et al.*, 2004). In addition, Smagghe *et al.*, (1997) reported that larvae treated with tebufenozide could suffer gut alterations, suggesting that such larvae stopped feeding and as consequence lost weight. Recently, lethal and sublethal effects of two IGRs buprofezin and pyriproxyfen were evaluated in our laboratory on the larvae of *S. littoralis* (Nasr *et al.*, 2010). The data demonstrated that the larval weight gain was considerably decreased as concentration increased and high doses of both compounds showed adverse effects on pupae, and adult emergence.

Dealing with the action of the tested insecticides on the adult emergence in the present study, the treatments reduced it in an effect reversibly correlated with the dose-level. The high dose-levels of lufenuron, methoxyfenozide and teflubenzuron completely prevented the adult eclosion. Similar effect was reported for various insects by Dimilin and Dimiloids (Salama *et al.*, 1976; Abo El-Gar *et al.*, 1978). The inhibition of pupation and blockage of adult eclosion, as distinctly found in the present study by the action of lufenuron, methoxyfenozide and teflubenzuron, may be considered as a result either to the haemolymph ecdysteroids or to a delay in the appearance of the last ecdysteroid peak as reported by Handler (1982). In other words, inhibition of pupation and blockage of adult emergence may be explained by the reduction of eclosion hormone production release, since this hormone is responsible for some prerequisite processes of the completion of moulting (Ghoneim *et al.*, 1998).

To clarify the possible morphogenic action of lufenuron, methoxyfenozide and teflubenzuron on pupation and adult eclosion programs, available data in the present study unambiguously prevailed increased pupal deformity, approximately, by the increasing dose-level of these insecticides. The pupal malformation varied between charred body color, collapsed appendages, dorso-ventrally compressed body and presence of some prepupal skin remains, irrespective of the used IGR (Figure 1).

IGRs have been looked at as selective agents to suppress both growth and development of larvae. Since then, several studies have been directed to elucidate the biological as well as biochemical effects of such group of chemicals. In the present work, the biochemical effects of lufenuron, methoxyfenozide and teflubenzuron on the activity of chitinase and PPO as biochemical indicators in the surviving larvae of *S. littoralis* after feeding with different concentrations were also illustrated. These enzymes mainly participate in metabolizing the toxicants and the metabolic processes include hydrolyzing, oxidizing, deoxidizing, and conjugating. Therefore, metabolic enzymes of insects are relative to insecticidal function and insect resistance. In addition, the usefulness of measuring such biochemical parameters is in determining the probable cause of lethal and sublethal effects or monitoring for the adverse effects of long-term exposures to such toxicants.

Chitinase plays an essential role during ecdysis chitin. This enzyme is vital to moult in insects, and may also affect gut physiology through their involvement in peritrophic membrane turnover (Merzendorfer and Zimoch, 2003). The exoskeleton of insects might constitute a useful target site for insecticidal chemicals. In the present study, the statistical tests performed on the data sets for lufenuron, methoxyfenozide and teflubenzuron showed significant differences in specific activity of chitinase attributable to treatments compared to the control. The inhibition was relative to insecticidal function, concentration and the larval stage. It was clear that the specific activities in the larvae fed on teflubenzuron were lower than those found with lufenuron and methoxyfenozide.

Based on comparison with the different treatments, teflubenzuron proved to be more inhibitor followed by lufenuron and methoxyfenozide. The obtained changes in the enzymes activity may due to the variation in the protein synthesis as a response to the different treatments. Electrophoretic protein analysis carried out by Chen and Levenbook (1966), revealed variability in the haemolymph protein in the 3rd and 5th instar larvae of the blow flies. The authors showed that protein synthesis decreased distinctly from the larvae to the white pupae. They further stated that during metamorphosis haemolymph protein concentration fell distinctly. Moreover, inhibition rate of cuticle protein was reported by Hegazy *et al.*, (1989), who stated that protein dropped to 14% at the first day and proceeded to

decrease reaching a per cent of 39 % at one week later when treating larvae of Colorado potato beetle with diflubenzuron, chlorfluazuron and teflubenzuron. This finding confirms the results recorded in the present work. It was also evident that the inhibition of chitinase and PPO activities determined 4 days after treatment was highest in second and fourth instar larvae, regardless of compound tested. However, such activity after 4 days was detrimentally decreased. This finding is in accordance with previous findings reported by Nasr *et al.*, (2010) who reported that the high toxic effect of buprofezin and pyriproxyfen on chitinase and PPO was found at day 4 of treatment compared to the other times.

According to the results presented, lufenuron, methoxyfenozide and teflubenzuron are potentially potent insecticides for controlling *S. littoralis*. Their high activity both through second and fourth instar larvae may indicate that these insecticides represent an important choice for use in integrated pest management programs in agricultural crops. The information based on these results will help in precise calculation of the dosage of candidate insecticides for effective control of *S. littoralis*, and consequently help in avoiding economic losses due to miscalculations of insecticide dosage. The use of these insecticides with low doses may introduce good control results. Such treatments will minimize the environmental pollution. Further- more, these data would facilitate better integration of candidate insecticides for the control of target pests in Egypt.

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الملخص العربى

فاعلية ثلاث من منظمات نمو الحشرات على نمو وتطور دودة ورق القطن المصرية (سبدوبترا ليتولاريس)

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تمت دراسة التأثيرات المباشرة والكامنة لثلاثة من منظمات نمو الحشرات التجارية ميثوكسيفينوزايد وليفيرون وتيفلوبينزورون على يرقات العمر الثانى والرابع من السلالة الحساسة ليرقات دودة ورق القطن. تم خلط تركيزات مختلفة (٠,٠١، ٠,١، ١، ٢,٥ مجم/كجم بيئة) من كل مبيد حشرى مع البيئة الغذائية. وتم تغذية الطور اليرقي الثانى والرابع لمدة ٦ أيام من أجل التحقق من النشاط البيولوجي لهذه المركبات تحت الظروف المعملية. تم تقدير نشاط إنزيمى الكيتينز والبولي فينول أوكسيديز في اليرقات الحية بعد المعاملة من أجل التحقق من التأثيرات البيوكيميائية لهذه المركبات. أظهرت هذه المبيدات الحشرية سمية عالية على اليرقات المختبرة وكذلك تأثيرات سلبية على العذارى وخروج الحشرات الكاملة. عانت اليرقات من نسبة موت أكثر عندما كانت تتغذى لفترة أطول على البيئة التي عوملت بتركيزات ١ و ٢,٥. ولوحظت أعلى نسبة موت معنوية على كل التركيزات المختبرة للتيفلوبينزورون متبوعا بالليفيرون ثم الميثوكسيفينوزايد، وأوضحت النتائج أن يرقات الطور الثانى أكثر حساسية للمركبات المختبرة مقارنة بيرقات الطور الرابع. وأن زيادة الوزن اليرقى يقل مع زيادة التركيز، والإنخفاض فى الوزن تأثر بدرجة عالية فى حالة التيفلوبينزورون متبوعا بالليفيرون ثم الميثوكسيفينوزايد. توقفت اليرقات المتضررة عن التغذية بعد ٤٨ ساعة ومعظم نسب الموت حدثت أثناء الإنسلاخ إلى الطور التالى. بعض اليرقات أيضا فشلت فى التحول إلى عذارى بنجاح. ولقد إختلفت المركبات في تأثيرها على نشاط إنزيمى الكيتينيز والبولي فينول أوكسيديز، وهذه الإنزيمات قد يكون لها علاقة بسمية كل من هذة المبيدات ضد دودة ورق القطن. وقد وجد أن حساسية يرقات الطور الرابع لهذه المبيدات الحشرية متماثلة ولكن النشاط كان أقل من الذى تم الحصول عليه مع يرقات الطور الثاني. لذلك، عند استخدام هذه المبيدات الحشرية لمكافحة يرقات سبدوبترا ليتولارس، ينبغي النظر بعناية للجرعات وأوقات التطبيق.