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Insect growth regulator insecticides toxicity evaluation in relation to catalase, pyroxidase, lactate-dehydrogenase and total antioxidant activities in field population of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae)

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Abstract

The pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is a major pest of cotton in Egypt. The insect causes high economic damage to cotton crop and resistance to insecticides. Insect growth regulator insecticides (IGRs) are used in many pest control programs as an active and safe control. In this study, the toxic effect of chitin synthesis inhibitors; diflubenzuron, hexaflumeron, lufenuron and teflubenzuron on 3rd instar larvae of the field pink bollworm was evaluated in the laboratory. The LC_{50} values were 0.81, 1.3, 1.66 and 1.7 Ppm for lufenuron, hexaflumeron, teflubenzuron and diflubenzuron, respectively. The antioxidant response of *P. gossypiella* larvae to LC_{50} treatment of four tested IGRs was estimated as the activity of peroxidase (POX), Lactate dehydrogenase (LDH) and catalase (CAT) enzymes and total antioxidant capacity (TAC) and protein contents. Results showed significant discrepancies in the antioxidant system of *P. gossypiella* body tissues after IGRs treatments. An insignificant increase in activity of POX was detected in larvae treated with all tested IGRs compared with untreated larvae. A moderate increase (23% and 28.4%) in CAT activity and TAC was recorded in teflubenzuron treated larvae. A significant increase (44.4%) in TAC was observed in hexaflumeron treated larvae. The high significant induction of LDH activity produced by lufenuron, diflubenzuron and teflubenzuron treatments (73, 56 and 47%, respectively) compared with control. A moderate depletion (27%) in CAT activity was presented in lufenuron larvae only and a significant reduction (44.7 and 40.5 %) of total protein was found in hexaflumeron and diflubenzuron larvae. Generally, these data approved that the pink bollworm exhibits susceptibility to insect growth regulator insecticides. The use of IGRs for controlling of this pest seems to be promising via an integrated pest management program and safer for environment.

Introduction

The cotton crop suffers from seasonal infestation by many agricultural pests. This crop is attacked by 1326 species of insect pests throughout the world, of which about 130 different species of insects and mites found to devour cotton at different stages of crop growth (Parmar and Patel, 2016). The lepidopterous bollworm complex worldwide spread are *Pectinophora gossypiella* (Saunders) besides *Helicoverpa armigera* (Hubner), *Earias vittella* (Fab.), *E. insulana* (Boisduval), *Spodoptera litura* (Fab.) and other arthropods (Khidr *et al*., 1996; Sandhya *et al*., 2010 and Sarwar, 2017). In Egypt, the pink bollworm *Pectinophora* gossypiella (Saunders) (Lepidoptera: Gelechiidae) is a serious mid-and late-season cotton pest that causes severe damage where its larvae feed on buds causing fruit shedding and seed loss. This insect represents a commercial problem because its larval stage frequently enters diapause in cotton seed capsules for many months under different climate conditions (Abd-Elhady and Abd El-Aal, 2011 and Darwish *et al*., 2017). Insecticide application can control only the eggs and first larval instar that reside outside cotton bolls but other larval instars enter bolls and remain inside till pupal emergence in addition to the development of resistance toward most classes of insecticides (Tanani and Ghoneim, 2018 and Salama and Abd El-Baki, 2013).

Insect growth regulators are mostly used in pest management since they are nontoxic to other organisms and have a short half-life in the environment. Insect growth regulators (IGR) act as hormone analogues or anti-hormones and induce a variety of morphogenetic, developmental and reproductive effects in insects by preventing egg-hatch and molting from stage to the next and not directly toxic (Dhadialla *et al*., 2005 and Tanani and Ghoneim, 2018). Insecticides with growth regulating properties may adversely affect insects by regulating or inhibiting specific biochemical pathways or processes essential for insect growth and development (Tunaz and Uygun, 2004).

Modifications of normal conditions are biological factors in invertebrates that can alter the physiological oxidative stress that causes disturbance in reactive oxygen species (ROS) production that is important for the cellular functions and the antioxidant defenses balance and induce cellular sense, apoptosis, and cell growth regulatory pathways. Reactive oxygen species are naturally produced in all cells and organisms (Koprucu *et al*., 2008).

Many pesticide classes can enhancement of oxidative stress by the formation of reactive oxygen species which may start oxidation of lipids and cause variations in antioxidant products of free oxygen radicals (highly active) in some organisms (Livingstone, 2001). Some literature talked about IGR insecticides toxicity and biochemical investigation in relation to oxidative stress effect on insect pests like Fahmy (2012).

Oxidative stress is defined as an imbalance between higher cellular levels of ROS and the cellular antioxidant defense (Ilhan *et al*., 2005). Oxidative stress may result in the degradation of proteins, lipids, and nucleic acids that are necessary for an organism's normal function. Typically, the internal antioxidant machinery is capable of minimizing the toxic effects of ROS by either removing them or by transforming them into less harmful compounds: this defense system usually includes both enzymatic and non-enzymatic reactions.

The antioxidant defense system is found in different organisms to prevent cellular damage from ROS. Both antioxidant enzymes such as catalases (CAT), peroxidases (POX) superoxide dismutases (SOD) and glutathione-S-transferase (GST) and nonenzyme antioxidants such as glutathione reduced (GSH) and ascorbic acid

(vitamin C) counteract the deleterious action of ROS and capable of scavenging them (Kale *et al*.,1999) to prevent cellular and molecular damage (Livingstone, 2001).

In insects antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX)] provide defenses against pathogens and insecticides (Felton and Summers, 1995). Previous studies showed that these enzymes can be quickly upregulated in response to xenobiotic threats and that an increase in the activities of these enzymes is related to pesticide resistance and melanization in insects (Campa-Córdova *et al*., 2002; Müller *et al*., 2007; Gopalakrishnan *et al*., 2011 and Wu *et al*., 2011).

Catalase (CAT) is one of the antioxidant enzymes which catalyzes the degradation of hydrogen peroxide (H_2O_2) to water and oxygen (Switala and Loewen, 2002), CAT is recognized as the scavenger of ROS in insects. Peroxidase (POX) is found in the mitochondria and eliminates hydrogen peroxide (Helen *et al*., 2018).

Lactate dehydrogenase enzyme (LDH) is responsible for pyruvate formation in anaerobic glycolysis and NADH to NAD^+ during glycolysis under hypoxia conditions and changes in its levels occur in association with a range of chemical stresses as pesticide exposures, become very important tools to investigate insect metabolic activities in toxicology and in clinical chemistry to cell, tissue, diagnose, and organ damage (Nathana *et al*., 2006).

This study aims to examine the biochemical alterations in activities of catalase, lactate dehydrogenase, and peroxidase enzymes and total antioxidant capacity in *P. gossypiella* larvae exposed to LC_{50} of Diflubenzuron, Hexaflumeron, Lufenuron and Teflubenzuron (IGR insecticides) as biomarkers to monitoring the resistance of field insect population to IGR and other insecticides.

Materials and methods

1.Insects:

A lot of cotton bolls infested by *P. gossypiella* were collected in cloth bags from the cotton fields of Dakahlia governorate in September 2022 and transferred to Central Agricultural Pesticides Laboratory, Agricultural research center. The cotton bolls were inspected and dissected in the laboratory to bring out the fully grown larvae then put in petri dishes provided with pieces of cotton and kept under constant laboratory conditions (25±2ºC, 75% relative humidity and 16h light: 8hrs. dark photoperiod till pupation.

Pupae were separated and kept in glass tubes until the moth's emergence. Groups of 5 pairs of emerged moths were placed in a glass jars used as cages provided with 10% sugar solution for adult nutrition. Eggs were obtained and kept in glass jar till hatching. Larvae were reared for one generation on an artificial diet according to Rashed and Ammar (1985).

2.Tested insect growth regulators :

2.1. Teflubenzuron; Trade name**:** (Nomolt 10%EC), Molecular Formula: $C_{14}H_6CL_2F_4N_2O_2$ and IUPAC name: N-[(3,5-dichloro-2,4-difluorophenyl)carbamoyl]-2,6-difluorobenzamide. The Producing company:Basf Se, Germany.

2.2. Hexaflumeron; Trade name: (Dimeuron % 95 Wp), Molecular Formula: $C_{16}H_8CL_2F_6N_2O_3$ and IUPAC name**:** N-[[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy) phenyl]carbamoyl]-2,6-difluorobenzamide. The Producing company: Agrochem for Fertilizers & Chemicals, Egypt.

2.3. Diflubenzuron; Trade name**:** (Newbenzuron 10% SC), Molecular Formula: $C_{14}H_9CL_2F_2N_2O_2$ and IUPAC name**:** N-[(4-chlorophenyl)carbamoyl]-2,6-difluorobenzamide. The producing company: Cam for Agrochemicals, Egypt.

2.4. Lufenuron; Trade name**:** (Match 5% EC), Molecular Formula: $C_{17}H_8CL_2F_8N_2O_3$ and IUPAC name**:**N-[[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]carbamoyl]-2,6-difluorobenzamide. The Producing company:Syngenta, China.

3. Chemicals and reagents:

3.1. Bovine albumin standard was purchased from Stanbio laboratory (Texas, USA).

3.2. Commasie brilliant blue G-250 was from (Sigma chemical co.).

3.3. P-nitroanisole (purity 97%) was obtained from Ubichem Ltd. (Ham pshire).

3.4. Nicotinamide adenine dinucleotide phosphate (reduced form, NADPH) was from BDH chemicals Ltd. (Poole, England).

3.5. The rest of chemicals were of high quality and purchased from commercial local companies.

4. Bioassay:

A toxicological test was completed according to Paramasivam and Selvi (2017) by using Watmann filter paper saturated with insecticides. Formulations of four IGR insecticides were dissolved in acetone to give seven serial concentrations of each one, 5 ml of each concentration were applied on the filter paper and then left to dry. Every filter paper was placed on petri dish and provided with ten 3rd instar larvae for insecticide exposure.

Four replications were performed for each insecticide concentration and control (Treated with acetone only). Dishes are maintained under laboratory conditions. Mortality was counted after 24 hrs. of treatments and data was statistically analyzed using Polo software (LeOra Software, 1987) to calculate LC_{50}, LC_{90} and slope of toxicity line according to Finney (1971). The surviving larvae from insecticides LC_{50} treatment was collected and kept in deep freezer (-20 ºC) till biochemical investigations.

5. Preparation of insects for biochemical analysis:

The insects were prepared as described by Amin (1998) and homogenized in distilled water (50 mg /1 ml), by chilled glass Teflon homogenizer then centrifuged at 8000 r.p.m. for 15 min at 2 ºC in a refrigerated centrifuge. The supernatants were kept in a deep freezer at -20ºC till used for biochemical assays. A double beam ultraviolet/visible spectrophotometer (Spintronic 1201, Milton Roy Co.,USA) was used to measure the absorbance of colored substances or metabolic compounds.

6. Biochemical analysis:

6.1. Activity of peroxidase (POX) was determined according to Hammer-Schmidt *et al*. (1982). Where 1.5 ml of pyrogallol (0.05 M) and 100 µl enzyme extract were added to sample cuvette and 100 µl of hydrogen peroxide (1%) were added to initiate the reaction. The enzyme activity as change in absorbance /min/ g sample was read at 420 nm.

6.2. Lactate dehydrogenase activity (LDH) was conducted using the procedure developed according to the German Society of clinical chemistry (DGCK, 1972). LDH catalyzes the conversion of pyruvate to lactate, NADH is oxidized to NAD in the process. The rate of decrease in NADH is directly proportional to the LDH activity and was determined photometrically. The reaction mixture consisted of phosphate buffer; 68mmol/L, pH 7.5, pyruvate 0.73 mmol/L, and 1.1 mmol/L NADH. Hundred ml samples were mixed with 2.5 ml of the reaction mixture that pre-incubated at 37˚C and poured into cuvette, and the initial absorbance was read.

Timer was started simultaneously, and the absorbance was read again after 1, 2 and 3 min. Zero adjustments was against air. LDH activity was calculated according to the following equation: LDH activity = Factor × Δ A at 340 nm/min Where: Factor = 4468

(As recommended by kit; Randox, United Kingdom). And Δ A = the change in absorbance / min. three replicates were used.

6.3. Catalase activity (CAT) was measured using bio diagnostic kit No. CA 2517 is based on the method described by Aebi (1984). Catalase reacts with a known quantity of hydrogen peroxide and stops the reaction after 1 min by catalase inhibitor. In the presence of peroxidase, the remaining H_2O_2 reacts with 3, 5-Dichloro-2-hydoxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample. The absorbance was measured at 510 nm.

6.4. The total antioxidant capacity was assayed according to method of Prieto *et al*. (1999). Each sample were deposited in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybedate). The tubes were capped and incubated in a thermal block at 90ºC for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against blank.

6.5. Measurement of total protein content was done according to Bradford (1976), where Protein reagent was prepared by dissolving 100mg of Coomassie Brilliant blue G-250 in 50ml 95% ethanol and 100 ml 85% (W/V) phosphoric acid added. The resulting solution was diluted to a final volume of 1 liter. Sample solution (50µl) or for preparation of standard curve 50µl of serial concentrations containing 10 to 100µg bovine serum albumin were pipetted into test tubes.

The volume was adjusted to 1 ml with phosphate buffer (0.1M, pH 6.6). Five ml of protein reagent were added and the contents were mixed either by inversion or vortexing. The absorbance at 595 nm was measured after 2 min and before 1 hr against a blank prepared from 1 ml of phosphate buffer and 5 ml protein reagent.

7. Statistical analysis:

All results of enzyme activities were analyzed for significant differences using one way ANOVA between and within insecticide as response and enzymes as a factor, with Duncan's Multiple Range test, t test and P value (Level of significance) at < 0.05 using SPSS software V.20. Multiple linear regression and t test were performed to evaluate the differences between insecticide efficiency against *P.gossyoiella* treated samples using Fast statistic software.

Results and discussion

1. Bioassay:

Toxicity results of the tested insecticide (Slop, LC_{50}, LC_{90}, intercept and chi-squared) against the pink boll worm, *P. gossypiella* larvae were shown in Table (1). The data revealed that the LC_{50} and LC_{90} values of all IGR insecticides under investigation, closer to each other, refer to their similar effect of them on these larvae. Whereas LC_{50} values were 0.81, 1.3, 1.66 and 1.7 Ppm, and LC_{90} were 7.8, 6.6, 7.2 and 8.7 Ppm for Lufenuron, Hexaflumeron, Teflubenzuron and Diflubenzuron, respectively.

Generally, these data approved that the pink bollworm larvae may be exhibit susceptibility to IGR insecticides, and considered proofing that many fields of Egyptian cultivated cotton didn't expose to this class of insecticides and subsequently the insect is very susceptible and easily controlled by these compounds. Many works of literatures proved and agree with these results as El-Barkey *et al*. (2009), who said that the laboratory treatment of Hexaflumuron against eggs of *P. gossypiella* revealed that LC_{50} was 3.754 Ppm and sub lethal treatment show a prolongation in larval and pupal developments reached 22.3 days for larvae and 10.8 days for pupae.

Results also indicated too high reduction in total eggs laid, percentage of hatchability and longevity of adults. Kandil *et al.* (2012) studied the toxicity of LC_{50} treatment of lufenuron chlorfluazuron and chromafenozide (IGR insecticides) on one day old eggs of *P. gossypiella* laboratory colony and their effects on the percentage of hatchability and duration of immature stages and longevity, fecundity and fertility of resulted adults which reached to 0.5 percentage of the control.

Salama and Abd El-Baki (2013) found that field treatments of *P.gossypiella* by IGRs applied in arrangement of days intervals with other compounds caused a good reduction in larval population reaching 57%. While the sequence of methomyl followed by oxymatrine + prosuler and lambdacyhalothrin induced the least reduction (38.02%). Dose-response bioassay of Khorshidi *et al.* (2019) showed that following 72 hrs. of exposure *H. armigera* 3rd larval instar, to hexaflumuron, lufenuron and chlorfluazuron the LC_{50} values were 31.75, 6.16 and 61.31 mg a. i./L, respectively.

The results revealed that lufenuron had the highest toxicity against *H. armigera* compared to other insecticides. Subsequently, sublethal effects of hexaflumuron, lufenuron and chlorfluazuron were assessed using LC_{10} concentration to find their possible effects on the biological and physiological parameters of treated larvae. The results showed significant changes in biological parameters, where the mean larval and pupal weights significantly decreased following treatment, while their developmental time significantly increased compared to the control.

Table (1): Toxicity of tested IGR insecticides against *Pectinophora gossypiella* larvae.

Pesticide	Slope	LC50 (95 %CI)	LC90 (95 % CI)	Intercept	$\chi 2$
Lufenuron	1.3±0.153	0.81(0.41-1.6)	7.8(3.9-15.7)	5.1	0.88
Hexaflumeron	1.8±0.13	1.3(0.73-2.3)	6.6(3.6-11.8)	4.79	0.97
Teflubenzuron	2.0±0.116	1.66(0.98-2.8)	7.2(4.2-12.18)	4.56	0.91
Diflubenzuron	1.8±0.129	1.7(0.94-3.0)	8.7(4.9-15.6)	4.59	0.82

2. Biochemical analysis:

Enzymes activities were measured in *P.gossypiella* after 24 hrs. of IGR insecticides LC_{50} exposure (Table 2). The difference in these enzymes of treated larvae as defined by the percentage compared to those of control larvae was presented in Table 3. Data of peroxidase (POX) showed a slight increase in enzyme activity in tissues of larvae treated with four tested IGRs when compared with control. These values reached 27.2, 28.1, 28.7, 30.6 and 30.1 (U/g b.wt.) for control, diflubenzuron, lufenuron, hexaflumeron and teflubenzuron, respectively.

Also, Wu *et al.* (2020) mentioned that the exposure of the fourth instar larvae of *Spodoptera litura* to the treated diet with matrine (Naturally occurring heterocyclic compound) and entomopathogenic fungus *Beauveria brongniartii* caused varied values of POX in larvae fat body and hemolymph. In the fat body, the highest activity was found on the 5th day after treatment by matrine and the lowest activity was noted on the 7th day after combined treatment by matrine and *B. brongniartii*. Munir *et al.* (2021) found that the POX level was significantly increased in tissues of the fish *Channa striata* exposed to LC_{50} concentration ($1.374 \mu g L^{-1}$) deltamethrin+ endosulfan (DM+ES) mixture for 24 hrs. period in relation to control.

Data in Tables (2 and 3) revealed insignificant increase (9.6%) in activity of lactate dehydrogenase enzyme (LDH) in pink bollworm larvae treated with hexaflumeron but lufenuron, diflubenzuron and teflubenzuron treatments produced a highly significant induction of LDH (73, 56, 47%, resp.) compared with control. The same result was obtained by Mirhaghparast *et al. (*2015) who mentioned that enhanced activity

of lactate dehydrogenase in rice striped stem borer, *Chilo suppressalis* (Walker) was only obtained by treating 180 and 470 µg/ml concentrations of hexaflumuron. Khorshidi *et al. (*2019) found that the LC_{10} concentration of hexaflumuron, lufenuron and chlorfluazuron increased the activity of lactate dehydrogenase enzyme in *H. armigera* larvae.

Data of catalase enzyme activity (CAT) of *P.gossypiella* larvae was increased in teflubenzuron (25.2%) and hexaflumeron (10.4%) treatments. Diflubenzuron treatment caused a slight decrease (4%) but lufenuron produced a moderate depletion (27%) in catalase activity than the control (Tables 2 and 3). Many researches found this phenomenon and interpret the decrease in activity by individual insecticide products while others are not Fahmy (2012) said that buprofezin and pyriproxyfen showed catalase significant increase in its activity, for only one day post treatment of *S. littoralis* 4th instar larvae then it was inhibited to be very close to that level in normal untreated larvae. This may be due to its consumption in scavenging reactive oxygen species produced due to significant accumulation of Malonaldehyde used as substrate.

Also, Sezer and Ozalp (2015) found that the effects of the juvenile hormone analogue, pyriproxyfen on catalase of *Galleria mellonella* after treatment, a significant increase of catalase activity in larvae exposed to increased concentrations of pyriproxyfen that induced oxidative stress. Pyriproxyfen treatments may stimulate SOD and CAT to defend the body against the damage caused by an excessive amounts of oxygen free radicals. Wu *et al*. (2020) reported that feeding *S. litura* larvae on entomopathogenic fungus *B. brongniartii* and matrine could significantly affect the activity of CAT. On the 3rd and 5th days after treatment, CAT activity in the fat body showed an increasing trend and was significantly higher than the control. On the 7th day after treatment, CAT activity showed a decrease both in the fat body and in hemolymph.

The same trend was detected in the total antioxidant capacity (TAC) of *P.gossypiella* larvae which significantly increased in hexaflumeron (44.4%) and moderate in teflubenzuron (28.4%) treatments. Also, lufenuron treatment produced an insignificant reduction (13.1%) in treated larvae total antioxidant, while Diflubenzuron caused a negligible decrease (0.4%) of it than the control. This result agrees with that of Słowińska *et al*. (2015), the exposure to imidacloprid (IMD) affected TAC of haemolymph of 1-day-old honeybees.

TAC in haemolymph of 1-day-old bees was lower in treatments with the addition of 5 and 200 ppb IMD. Exposure to IMD decreased TAC of haemolymph of 1-day-old bees but not 30-day-old. Quantitative measures of TAC appear to be a good indicator to evaluate the changes in oxidation status in honeybee body fluids caused by ageing and exposure to pesticides (Johnson and Carey, 2014).

The total protein content value of hexaflumeron was 34.7 followed by diflubenzuron 37.3, lufenuron 61, teflubenzuron 61.7 and control 62.7 (mg/g.b.wt.). The high significant depletion (44.7 and 40.5%) was presented in total protein contents of hexaflumeron and Diflubenzuron treated larvae and a negligible decrease (2.7 and 1.6%) was found in lufenuron and teflubenzuron larvae when compared with control ones. Results of Zibaee *et al*. (2011) were detected a significant discrepancies in amount of biochemical components of the hemolymph and the reserved macromolecules in the Sunn pest, *Eurygaster integriceps* Puton after pyriproxifen treatment.The amount of protein in hemolymph was lower than control by

using 0.74 and 1.49 µg /mg insect concentrations of pyriproxifen after 24 and 48 hrs. but it was compensated after 120 hrs. and reached to amount of control.

Table (2): Activity of antioxidant enzymes (U/g b.wt.) in *Pectinophora gossypiella* 3rd instar larvae untreated (Control) and treated with LC_{50} of four IGR insecticides.

Enzyme	Teflubenzuron	Hexaflumeron	Diflubenzuron	Lufenuron	Control
Peroxidase	30.1±0.24	30.6±0.42	28.1±0.088	28.7±0.233	27.2±0.12
Lactat Dehydrogenase	65.2±0.789	45.8±0.37	61.6±1.13	72.3±0.55	41.8±0.48
Catalase	188.6±1.77	166.2±1.82	144.6±1.62	109.9±1.17	150.6±1.85
Total Anti-Oxidant	331±2.85	372±3.5	256.7±1.03	224±1.45	257.7±2.1
Total Protein Content (mg/g b.wt.)	61.7±0.67	34.7±1.48	37.3±0.86	61±0.76	62.7±2.0

Values represent mean±S.E.

Table (3): Difference percentages of antioxidant enzymes activity in *Pectinophora gossypiella* 3rd instar larvae treated with LC_{50} of four IGR insecticides compared with control.

Enzyme	Difference percentage (%)			
	Teflubenzuron	Hexaflumeron	Diflubenzuron	Lufenuron
Peroxidase	+10.7	+12.5	+3.3	+5.5
Lactat Dehydrogenase	+47	+9.6	+56	+73
Catalase	+23.2	+20.4	+4	-27
Total Anti-Oxidant	+28.4	+44.4	-0.4	-13.1
Total Protein Content	-1.6	-44.7	-40.5	-2.7

% difference = (treated mean–control mean/control mean) ×100, (-) =decrease, (+) =increase

Briefly the regression coefficients describe the relationship between the independent and dependent variables insecticide versus control and enzyme activity. The coefficient value represents the mean change of the control variable given a one-unit shift in insecticide variable. Thus, the most important variable was lufenuron have larger coefficient signifies a greater change in mean of the insecticide variable. Linear regression analysis, t and f test analysis of *P.gossypiella* treated with insecticides were provided in Table (4). Correlation coefficient analysis and t test plus fiducial limits and p values were completed can measure the relationship between enzyme test scores and insecticide performance and to make knowledge of the various qualities of insecticides that affect the antioxidant enzymes levels in treated *P. gossypiella* (Table 5) (Helen *et al*., 2018; Sezer and Ozalp, 2015 and Munir *et al.*, 2021).

Table (4) : Linear regression analysis, t and f test analysis of *Pectinophora gossypiella* insecticide treatments.

Variable	Coefficient	Std.Er.	t-Statistic	P-value	Lower95%	Upper95%	VIF	Std. Coeff.
Constant	18.117	2.480	7.305	0.000	12.507	23.727	0.000	0.000
Diflubenzuron	0.003946	0.373	0.011	0.992	-0.839	0.847	6288.792	0.003931
Hexaflumeron	-0.789	0.303	-2.604	0.029	-1.475	-0.103	9767.880	-1.204
Lufenuron	0.502	0.220	2.282	0.048	0.004372	1.000	1358.520	0.394
Teflubenzuron	1.327	0.089	14.830	0.000	1.124	1.529	621.093	1.730

Table (5): Correlation coefficient of the insecticide treatments versus *Pectinophora gossypiella* antioxidant enzymes.

Variable	Control					
Control	1.000	Diflubenzuron				
Diflubenzuron	0.986	1.000	Hexaflumeron			
Hexaflumeron	0.985	0.992	1.000	Lufenuron		
Lufenuron	0.972	0.980	0.980	1.000	Replicate	
Replicate	-0.419	-0.367	-0.463	-0.321	1.000	Teflubenzuron
Teflubenzuron	0.996	0.997	0.991	0.981	-0.385	1.000

Generally, the treatment of *P. gossypiella* larvae with Lufenuron, Hexaflumeron, Teflubenzuron and Diflubenzuron in this study may represent a model for induction of lipid peroxidation and an enhancement of the insect antioxidant system for scavenging ROS resulted due to oxidative stress. These data approved that the pink bollworm exhibits susceptibility to insect growth regulator insecticides and the use of IGRs for control this pest seems to be promising via integrated pest management program and safer to environment.

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