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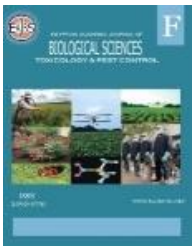

Biochemical Effects of Some Chitin Synthesis Inhibitors Against Red Palm Weevil, *Rhynchophorus ferrugineus* Insect

A. E. A. M. El-Sobki and A. A. I. Ali
Department of Plant Protection, Faculty of Agriculture, Zagazig University, Egypt
*Email: aeelsobki@zu.edu.eg

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ABSTRACT

The widespread use of the insecticides for controlling the destructive pest, red palm weevil (*Rhynchophorus ferrugineus*), raises the question about efficacy the chitin synthesis inhibitors (CSIs). Therefore, this study aims to explore the tested CSIs acute toxicity on the field strain of red palm weevil. Also, some biochemical changed in the 6th instar larvae after treatment with tested LC_{50s} were measured. The LC_{50} values of chlorfluazuron, hexaflumuron, and lufenuron were determined at 454.01,1293.02 and 919.13 µg/ml, respectively for the 6th larval instar exposed using the food dipping method. The lufenuron showed the lowest significant reduction in total protein (7.29%) accompanied with activation GST, ß-esterase, carboxylesterase, phenoloxidase, chitinase, and α-Esterase recorded 58.50, 58.54, 26.09, 22.56, 11.52 and 7.77% increasing respectively. The chlorfluazuron showed mediated significant reduction in total protein (23.54%) accompanied with activation GST, ß-esterase and carboxylesterase and chitinase recorded 24.90, 23.94, 4.89 and 2.28 % increasing respectively. The highest significant reduction in total protein (45.96%) with hexaflumuron treatment accompanied with activation GST, carboxylesterase and chitinase recorded 48.02, 38.86 and 25.22% increase respectively.

INTRODUCTION

Date palm trees, *Phoenix dactylifera* L. is widely cultivated across northern Africa, the Middle East, the horn of Africa and South Asia, and is naturalized in many tropical and subtropical regions worldwide ("World Checklist of Selected Plant Families: Royal Botanic Gardens, Kew," n.d.). The date palm is an important economic crop for many people. The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) belongs to the family of Curculionidae (Coleoptera) is host-specific pest targets the date palm trees. *R. ferrugineus* is a major economic pest of different species of palm (Dhouibi *et al.*, 2017). The pest was recorded in the Emirates and Oman in 1986 (El-Ezaby *et al.*, 1998), then recorded for the first time in Egypt in 1992 (Cox, 1993). Now, *R. ferrugineus* is known as the most destructive pest to date palms in the Arabic region and south East Asia (El-Juhany, 2010). Difficulty controlling due to the shortage of early detection methods and the hidden behavior, which makes it hard to detect the injury in its early stages (Sayed *et al.*, 2016). The larval stages fed in the core palm trunk close to the terminal buds

considered the most dangerous stage which kills it in the final (Kaakeh, 2006). The insect spends most of life inside the trunk and leaves the infested tree for mating and egg deposit in the new host plant, which increases the control difficult. So, Early detection of the injury is considered to be the success of any *R. ferrugineus* -IPM programs(Al-Dosary *et al.,* 2016). The insect has caused up to 20% loss of these plantations in Asia and the Middle East(Hussein *et al.,* 2010) with 3 or 4 generations per year approximately. Larvae is the harmful stage and the red palm weevil has 10 to 13 larval instars (Abe *et al.,* 2009). During the last decade, several control methods used focused on the use of insecticides, pheromone traps, biological control agents in the management of insect pests has increased in recent years. Chemical control considered the main control method because of the efficiency and rapid effect on infected palm trees (Abdel-Salam *et al.,* 2014). Among the different classes of chemical insecticides, insect growth regulators (IGRs) class causes an adverse effect on insect growth and development by interfering with specific biochemical pathways or processes. After exposed insects to IGRs results in mortality due to abnormalities during organ development. Insects mortality required prolonged exposure during susceptible development periods e.g. molting period and pupation (Tunaz and Uygun, 2004). The IGR activities applied for insect control categories to chitin synthesis inhibitor, juvenile hormone mimic/ analog, molting hormone agonist /analog and molting inhibitor. The chitin synthesis inhibitors act as an anti-molting agent, inhibits biosynthesis of chitin in insect cuticle causing loss of cuticle elasticity and firmness, and results in abortive molting.

The present work was carried out to evaluate the biochemical effects of LC_{50} of three chitin synthesis inhibitors included chlorfluazuron, hexaflumuron, and lufenuron against the 6th instar larvae of *R. ferruginous*.

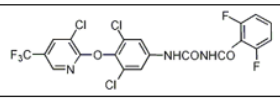
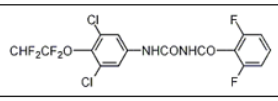
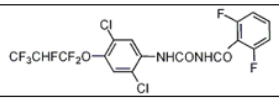
MATERIALS AND METHODS

Insect Culturing:

A field strain of the red palm weevil, *R. ferrugineus*, was collected as pupae from infested date palm fields preexposed to pesticides from El-kasasin district, Ismailia Governorate, Egypt during 2018-2019. The weevil was reread on sugarcane (*Saccharum officinarum* L.) stems under constant laboratory conditions (29± 2 °C and 60-70% RH) with light intensity equal 30 FC provided by fluorescent tube (Rananavare *et al.,* 1975; Salama and Abdel-Razek, 2002; Mahmoud, 2013).

Insecticides Used:

Table 1 : List of benzoylphenylurea derivatives pesticides inhibited chitin synthesis used in the study.

Active ingredient	chlorfluazuron	hexaflumuron	lufenuron
Chemical structure			
Formulation	Caprice™ 5 % EC	Dieuron™ 10 % EC	Match™ 5 % EC

The pesticide formulations use in the study were supplied by the Plant Protection Research Institute, Giza, Egypt.

Treatments:

The 6th larval instar (25 days) of the red palm weevil, was chosen for bioassay using food dipping method. Based on preliminary experiments, serial dilutions of each

test insecticides (20-85 % mortality) were freshly prepared in distilled water. Each treatment replicated three times, with 10 larvae/replicate. After dipping the fresh pieces of sugarcane stalks (≈ 15 cm length, 1.5 cm diameter) for 30 s in the tested concentration solutions, the treated pieces were air-dried before offering to the starved larvae in glasses jars (1000 cm^3) with a perforated cover, while, the pieces of sugarcane stalks in untreated control were dipped in distilled water. The mortalities were recorded daily and the experimental endpoint was 72 h after exposure. After calculating the median lethal concentration (LC_{50}), the 6^{th} larval instar (25 day) of the red palm weevil, was treated the calculated LC_{50} values for chlorfluazuron, hexaflumuron and lufenuron were454.01, 1293.02 and 919.13 µg a.i /ml, respectively in three replicates each one contained ten larvae. The survival larvae were transferred into a 50 ml centrifuge tube then frozen directly for 24 h, at -20 ºC and then homogenized to subject for biochemical analysis.

Enzyme Measurement Procedures:

Chemicals:

Bovine albumin standard was purchased from Stanbio laboratory (Texas, USA). Coomassie brilliant blue G-250 was from sigma (sigma chemical co.). P-nitroanisole (purity 97%) was obtained from Ubichem Ltd. (Ham pshire), while nicotinamide adenine dinucleotide phosphate (reduced form, NADPH) was from BDH chemicals Ltd. (Poole, England). The rest of the chemicals were of high quality and purchased from commercial local companies.

Apparatus:

Insects were homogenized for biochemical analysis in a chilled glass Teflon tissue homogenizer (ST-2 Mechanic-Preczyina, Poland). After homogenization, supernatants were kept in a deep freezer at -20ºc till used for biochemical assays. Spectrophotometer (Spectronic 1201, Milton Roy Co., USA) emitted beam ultraviolet/ visible was used to measure the optical density.

Preparation of Insects for Analysis:

The insects were prepared according to (Amin, 1998) by homogenizing insects in distilled water (50 mg/ml). The homogenate was subjected for centrifugation at 8000 r.p.m. for 15 min at 2 ºC in a refrigerated centrifuge. The precipitate was discarded by transferring the supernatant's new tube to subjected for biochemical determinations after storing at -5 °C. The supernatant of different treatments subjected determinations included:

1. Total proteins were determined according to (Bradford, 1976).
2. Total carbohydrates were determined according to (Dubois *et al.,* 1956).
3. Invertase and trehalase activity was determined according to (Ishaaya and Swirski, 1976).
4. Chitinase activity was determined according to (Bade and Stinson, 1981)
5. Carboxylesterase activity was determined according to (Cao *et al.,* 2008)
6. Glutathione S-transferase activity was determined according to (Habig *et al.,* 1974)
7. α- and β-esterases were determined according to (Asperen, 1962).
8. Phenoloxidase activity was determined according to (Ishaaya, 1971).

Statistics:

The corrected mortality percentage was calculated using Abbott's formula (Abbott, 1925) based on control treatments. The dose-response relationship curve was statistically computed according to (Finney, 1971) to calculate the median lethal concentration (LC_{50}) and regression equation components of each insecticide toxicity line using Analystsoft Biostat Pro V 5.8.4.3 Software.

The significance of the main differences in biochemical parameters was determined by analysis of variance (ANOVA) using Costat Statistical Software (Cohort software,

Berkeley). When the ANOVA statistics were significant (P<0.01), the means were compared by Duncan's multiple range test. The changes % in biochemical parameter calculated using the equation:

The reduction percentage (%) = (Control value-Treatment value)/ (Control value) ×100

RESULTS AND DISCUSSION

Bioassay Experiments:

The Median Lethal Concentration of Tested CSIs Against *R. ferrugineus*:

A bioassay test was performed to determine the lethal effect of chlorfluazuron, hexaflumuron and lufenuron on exposed the 6th larval inter of the red palm weevil, *R. ferrugineus* treated using food dipping method. The primary experiment conducted to define the suitable concentration range and starvation period. The median lethal concentration (LC_{50}) of tested CSIs was calculated based on the mortality ratios shown in Table (2). The calculated LC_{50} for Chlorfluazuron was 454.01 µg/ml with difference 65.28 between UCL and LCL. The exposed larvae of red palm weevil showed a high homogeneity recorded 3.51± 0.32. Concerning lufenuron caused mediated toxicity recorded 919.13 µg/ml with LC_{50}value, with difference 67.94 between UCL and LCL. The exposed larvae showed a high heterogeneity recorded 6.82± 0.62. In the last rank, hexaflumuron showed the lowest acute toxicity to the 6th larval instar of red palm weevil. The LC_{50}value was 1293.02 µg/ml for, with difference 113.16 between UCL and LCL. While the slope recorded 5.76± 0.52 with mediated heterogeneity closer to lufenuron. The represented log dos-probit lines are showing Figure (1).

Table 2. Toxicity of chlorfluazuron, hexaflumuron, and lufenuron on instar larvae of the red palm weevil, *Rhyncophorus ferrugineus*

Treatment	LC_{50} (µg a.i/ml)	LC_{50}		Slope ± SE
		UCL	LCL	
Chlorfluazuron	454.01	422.48	487.76	3.51± 0.32
Hexaflumuron	1293.02	1350.54	1237.38	5.76± 0.52
Lufenuron	919.13	953.65	885.71	6.82± 0.62

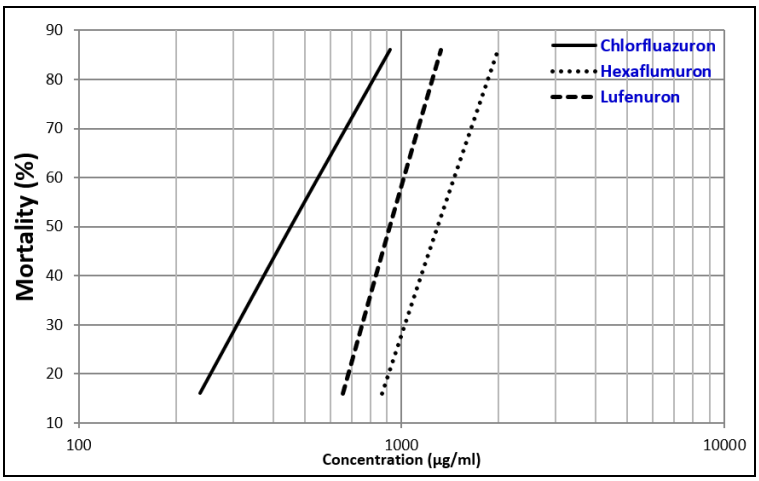


Fig. 1 Toxicity lines of chlorfluazuron, hexaflumuron and lufenuron on exposed the 6th larval inter of the red palm weevil, *Rhyncophorus ferrugineus* treated using food dipping method after 96 h.

The tested pesticides are mainly chitin biosynthesis inhibitors. Insect growth regulator which acts as an anti-molting agent, leading to the death of the larvae and pupae and acts through ingestion. Chlorfluazuron and lufenuron are contact insecticides while hexaflumuron is a systemic insecticide. The selected IGRs mainly target lepidopteran larvae but it showed efficiency against coleopteran larvae. Not only the anti-molting effect of lufenuron on treated larvae but also cease feeding and sterilant effect e.g. reducing fecundity and egg hatch(MacBean, 2012).

Data in Table (3) show some physiological parameters in larval homogenate exposed to LC_{50} of the tested CSIs illustrated in Table (2). On the response of total carbohydrate level to exposure to CSIs, hexaflumuron caused the highest significant reduction in total carbohydrate recorded 41.78% followed by Chlorfluazuron occupied the second significant rank recorded 8.27% reduction and in contrast with lufenuron showed a significant increase in total carbohydrate with 8.80 % increasing comparing with untreated larvae, as shown in Figure (2,b).

β-fructofuranosidases or invertases (EC 3.2.1.26) are glycosidehydrolases that catalyze the hydrolysis of sucrose (β-Dglucopyranosyl-S-D-fructofuranoside) into the monosaccharides glucose and fructose (Pedezzi *et al.,* 2014; Zibaee *et al.,* 2008). Invertase activity showed the highest influence with chlorfluazuron recorded 51.19 % reduction, lufenuron caused a limited significant reduction reached to 35.95 %, in contrast with hexaflumuron showed significant increasing in invertase activity raising to 6.9 % increasing, as shown in Figure (2,c).

Trehalase (EC 3.2. 1.28) hydrolyzes only α, α′-trehalose (two molecules of glucose) primary blood sugar in insects. Trehalase is highly specific to trehalose and presents in a variety of organisms, but is most important in insects serves as blood-sugar (Silva *et al.,* 2010). Also, (Benoit *et al.* 2009) indicate to trehalose accumulates with higher concentrations in response drought. Desiccation tolerance has been attributed to the stress protectant ability of trehalose in other insects like the fruit fly and mosquito (Juliano *et al.* 2002; Thorat *et al.* 2012). Trehalase plays a fundamental role in insects forming the major link between trehalose metabolism and glucose transport and glycolysis in insects. Trehalase not only acts as a stress recovery factor but also plays an essential role in insect glycometabolism and homeostasis. Therefore, trehalase has been considered as a possible target for insect pest control (Shukla et al., 2015). Also, trehalase activity showed a fluctuated response to CSIs exposure, to raise activity significantly after exposure lufenuron to 1.64 %. Whereas, trehalase activity showed non-significant decreasing recorded 14.1 and 15.08 % reduction after exposure to hexaflumuron and chlorfluazuron, respectively comparing check treatment, as shown in Figure (2,d). The high reduction in carbohydrate resulted from hexaflumuron treatment (41.78%) in agreement with (Tan *et al.,* 2014) elucidated that the hexaflumuron sublethal concentrations could interfere the normal carbohydrate metabolism by depressing the expression of soluble trehalase genes in *Apolygus lucorum* nymphs. Although, increasing trehalose content in 1st instar nymphs significantly following hexaflumuron treatment while the glucose content and soluble trehalase activity decreased significantly. This indictor ascertains trehalase inhibition recorded 14.10 % accompanied with invertase activation (-6.9%) subsequently highest significant reduction in carbohydrate content following hexaflumuron treatment, therefor, Trehalose content increased after hexaflumuron treatment accompanied with the glucose content, and soluble trehalase activity decreased significantly (Tan *et al.,* 2014). While, chlorfluazuron showed a limited significant reduction in carbohydrate content (8.27%) accompanied with high inhibition in invertase activity (51.19%) and limited reduction trehalase activity (15.08 %) in contrast with Lufenuron treatment showed a highest carbohydrate content (8.8%)

resulted from raising trehalase activity (1.64) and inhiation invertase activity (35.95 %) but α, α′-trehalose (two molecules of glucose) a primary blood sugar in insects subsequently trehalase activity more vitality than invertase activity, also, the total carbohydrate is high negative correlated with trehalose amount.

Table 3:Changes in carbohydrates concentration and carbohydrate related enzymes in the larval stage homogenates of the red palm weevil, *Rhyncophorus ferrugineus* treated with the tested insecticides

Treatment	The concentration (mg/g b. wt.) of	The activity (µg glucose /min /g b.wt.) of	
	Total carbohydrates	Invertase	Trehalase
Chlorfluazuron	58.03±2.58^{c} (8.27)	68.33±4.72^{c} (51.19)	86.33±5.68^{b} (15.08)
Hexaflumouron	36.83±1.75^{d} (41.78)	149.66±5.68^{a} (-6.9)	87.33±3.05^{b} (14.10)
Lufenuron	68.83±2.75^{a} (-8.80)	89.66±5.50^{b} (35.95)	103.33±1.52^{a} (-1.64)
Control	63.26±1.02^{b}	140.00±9.53^{a}	101.66±5.50^{a}

Means standard error (±SEM) followed by the same letter within columns indicate no significant difference ($P \leq 0.05$); the number between parentheses refers to the reduction percentage (%) resulted from treatment.

Data in Table (4) continued to display the enzyme activity influence after expose red palm weevil larvae to LC_{50} of tested insecticide's effect on chitin biosynthesis. The total protein showed a highly significant reduction recorded 45.96% with larvae of red palm weevil exposed to hexaflumuron followed by chlorfluazuron causing significant reduction recorded 23.54% reduction and finally lufenuron showed 7.29 % reduction represented the lowest significant reduction among the tested CSIs and the closer to control treatment, as shown in Figure (2,a).

Chitin is a long-chain polymer of N-acetylglucosamine, the derivative of glucose presences in insoluble structural polysaccharide in the exoskeletal and gut linings of insects. Chitin biosynthesis is a metabolic target with selectivity for pest control. This potential target effects insect molting enzyme chitinase which degrades chitin polymers to low molecular weight during the molting process in the exoskeleton (Kramer and Muthukrishnan, 1997). Chitinases (EC 3.2.1.14) are glycosyl hydrolases that catalyze the hydrolysis of β-(1, 4)-glycosidic bonds in chitin, the major structural polysaccharide present in the cuticle and gut peritrophic matrix of insects (Lu *et al.,* 2002). Increasing of chitinase before the molt resulted in decreased cuticle thickness (Fitches *et al.,* 2004). The chitinase considers a secondary target for some CSIs. Chitinase activity showed a high activity raised significantly to 25.22 % increasing after exposer larvae to hexaflumuron, followed by lufenuron treatment in the second significant rank recorded increasing 11.52 %, then, chlorfluazuron showed the lowest increase in chitinase activity with increasing 2.28 % comparing untreated control treatment.

Glutathione S-tranferase (GST; EC 2.5.1.18) is a family of detoxifying enzymes that catalyzed the conjugation of reduced glutathione with a group of compounds having electrophilic centers e.g., nitrocompounds, organophosphates and organochlorides (Clark *et al.,* 1986). GST offers passive protection towards pyrethroid insecticides by binding to their molecule in a sequestering mechanism (Kostaropoulos *et al.,* 2001). Insecticide-resistant strain showed GST high activity correlated with a high level of GST1 transcript

(Fournier *et al.,* 1992). Also, GST has a similar role in the regulation of energetics in mitochondria as that in diapause and metamorphosis (Jovanović-Galović *et al.*, 2007). Glutathione S-transferase activity showed a surpass activity to record 48.02 and 58.50 % increasing in hexaflumuron and lufenuron respectively with a significant difference, then, chlorfluazuron treatment showed significant increasing 24.90 %.

Phenol oxidase (PO) plays an important role in defense mechanisms in insect immunity (Jiang *et al.,* 1997). Insects employ phenoloxidase for melanin biosynthesis and clotting (Sugumaran, 2002). Also, Insect POs generate quinones and other reactive intermediates to immobilize and kill invading pathogens and parasites (Eleftherianos *et al.*, 2011; Lu and Jiang, 2007). The phenoloxidase system is responsible for the sclerotization of cockroach ootheca (Sugumaran and Nellaiappan, 1990). Phenoloxidase activity in lufenuron treatment surpassed control treatment recorded 22.56% increasing with a significant difference, while, hexaflumuron and chlorfluazuron treatments showed phenoloxidase activity reduction was limited in exposure to chlorfluazuron and the highest reduction in hexaflumuron recorded 46.72%.

The esterase reaction, defined as the hydrolysis of an ester to its component alcohol and acid in a chemical reaction with water called hydrolysis, encompasses hydrolysis of a diverse range of carboxylic, thio-, phospho-, and other ester substrates. It sits within a broader set of hydrolase reactions that also include glycosylases, proteases, amidases, and many others (Webb, 1992). The esterases categorized as alpha and/or beta esterases according to the enzyme's ability to hydrolyze either alpha- or beta-naphthyl acetate. These esterases were further divided into acetylesterases, arylesterases, carboxylesterases and acetylcholinesterase (Dahan-Moss and Koekemoer, 2016). Esterase activity both types α and ß-Esterase surpassed the control treatments recorded 7.77 and 58.54% significant increasing respectively in lufenuron treatment, while, chlorfluazuron treatment occupied the second significant rank recorded 23.94 % increasing with ß-Esterase and 12.91 % reduction with α-Esterase. Finally, hexaflumuron caused inhibition activity of α and ß-Esterase recorded 27.28 and 11.24 % significant reduction compared with control treatment.

Carboxylesterase (EC 3.1.1.1) provides key mechanisms of resistance to insecticides, particularly organophosphates, in insects. The enzyme is responsible for cross-resistance to a wide range of insecticides (Devonshire *et al.,* 1986; Feng *et al.,* 2018). Carboxylesterase data exhibited the same trend, where, hexaflumuron treatment showed raising carboxylesterase activity with 38.86 % increasing followed by lufenuron record 26.09 % increasing then chlorfluazuron showed the lowest raising activity recorded 4.89 % increasing with a significant difference among treatments, as shown in Figures (2, e and f).

Table 4 Changes in total protein, enzymes activity in the larval homogenates of the red palm weevil, *Rhyncophorus ferrugineus* treated with the tested insecticides.

Treatment	Total protein	Chitinase	GST	Phenoloxidase	Esterase activity		
					α-Esterase	ß-Esterase	Carboxylesterase
Chlorfluazuron	31.83±1.04^{c} (23.54)	313.66±12.05^{c} (-2.28)	21.06±0.92^{c} (-24.90)	39.70±0.88^{b} (4.72)	49.70±2.56^{b} (12.91)	94.06±5.25^{b} (-23.94)	128.66±5.50^{c} (-4.89)
Hexaflumuron	22.50±1.50^{d} (45.96)	384.00±2.64^{a} (-25.22)	24.96±1.05^{b} (-48.02)	22.20±1.47^{c} (46.72)	41.50±1.32^{c} (27.28)	67.36±3.00^{c} (11.24)	170.33±10.21^{a} (-38.86)
Lufenuron	38.60±1.24^{b} (7.29)	342.00±10.58^{b} (-11.52)	26.73±1.07^{a} (-58.50)	51.06±3.61^{a} (-22.56)	61.50±3.96^{a} (-7.77)	120.33±9.50^{a} (-58.54)	154.66±4.50^{b} (-26.09)
Control	41.63±1.305^{a}	306.66±15.27^{c}	16.86±1.00^{d}	41.66±1.52^{b}	57.06±2.58^{a}	75.90±2.28^{c}	122.66±6.429^{c}

Means standard error (±SEM) followed by the same letter within columns indicate no significant difference ($P \leq 0.05$); the number between parentheses refers to the reduction percentage (%) resulted from treatment.

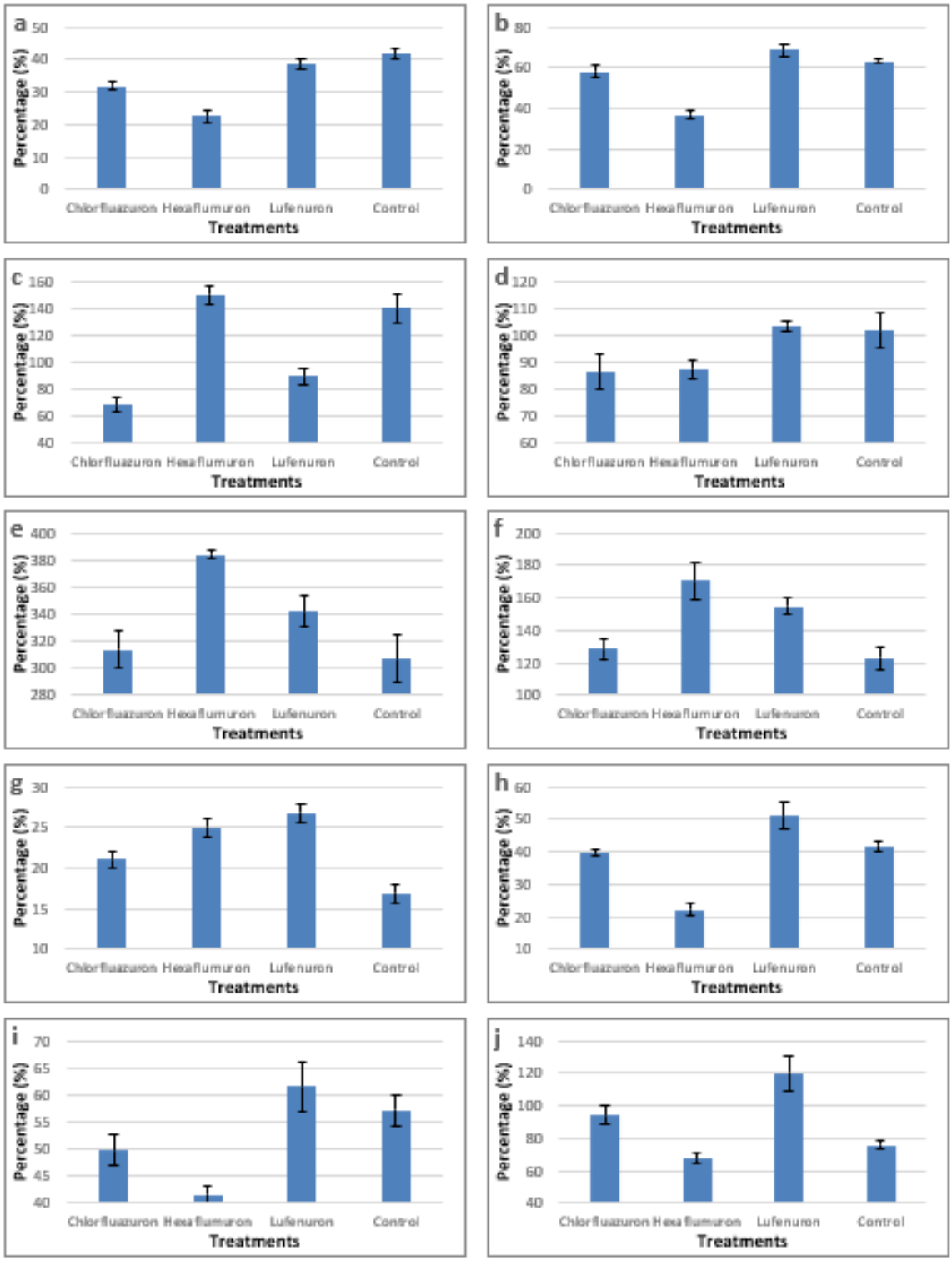


Fig.2: Different biochemical parameters of the red palm weevil, *Rhyncophorus ferrugineus* treated with the tested insecticides: a) Total protein; b) Total carbohydrates; c) Invertase activity; d) Trehalase; e) Chitinase; f) Carboxylesterase; g) GST; h) Phenoloxidase; i) α-Esterase activity and j) β- Esterase activity. Values are presented as mean ± $CI_{95\%}$.

The lufenuron showed the lowest significant reduction in total protein (7.29%) accompanied with activation GST, ß-esterase, carboxylesterase, phenoloxidase chitinase, and α-Esterase recorded 58.50, 58.54, 26.09, 22.56, 11.52 and 7.77% increasing respectively. The chlorfluazuron showed mediated significant reduction in total protein (23.54%) accompanied with activation GST, ß-esterase and carboxylesterase and chitinase recorded 24.90, 23.94, 4.89 and 2.28 % increasing respectively. The highest significant reduction in total protein (45.96%) in hexaflumuron treatment accompanied with activation GST, carboxylesterase and chitinase recorded 48.02, 38.86 and 25.22% increase respectively. The main enzymes increase activity with treatment was glutathione *S*-transferases then esterases according to (Devonshire and Field, 1991; Oppenoorth, 1985) showed that insecticides resistance associated with molecular target modifications and increased levels of detoxification enzymes e.g. glutathione *S*-transferases, cytochrome P450s, and carboxylesterases in many insect species. Carboxylesterase enzymes were found to differ in their sensitivity to the inhibitors employed, and some compounds caused dramatic activation of the hydrolysis. According to this, GST may offer protection towards CSIs (Kostaropoulos *et al.,* 2001).

Lufenuron topically applied onto pupae showed mortality with a lower concentration and increase, increasing water loss, depleting, lowering body weights, shortened pupal duration and adult morphogenesis (Ghoneim *et al.,* 2007). Hexaflumuron and chlorfluazuron at a concentration of LC_{50} values effect on fecundity and fertility of pink bollworm, *Pectinophora gossypiella*. Hexaflumuron was more potent than chlorfluazuron in total protein reduction (Kandil *et al.,* 2013). prepupae of the *R. ferrugineus* treated with 1.0, 0.1 or 0.01 µg lufenuron/insect using topical application method showed that the lowest dose of Lufenuron caused a gradual decrease in carbohydrate content, but the highest and medium doses induced a reciprocal V-shaped trend in this metabolite throughout the pupal life. Also, all dose-levels of lufenuron, induced such a trend in protein content of pupae with greater reduction action on the protein content of lufenuron (Ghoneim *et al.,* 2003). The growth of Lufenuron-treated nymphs of desert locust *Schistocerca gregaria* was profoundly inhibited because their weight gain was drastically reduced with the same response mentioned before (Bakr and Ghoneim, 2008). But overall all CSIs seriously suppressed the protein content, regardless of the age (Basiouny *et al.,* 2016) subsequently all protein structures affected.

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ARABIC SUMMARY

التأثيرات البيوكيميائية لبعض مثبطات تخليق الكيتين ضد حشرة سوسة النخيل الحمراء، *Rhynchophorus ferrugineus*

أحمد السيد أحمد محمد السبكى[١] وعبدالهادى عبد الحميد إبراهيم على [١]
[١]قسم وقاية النبات– كلية الزراعة– جامعة الزقازيق– مصر

الاستخدام الواسع النطاق للمبيدات الحشرية لمكافحة آفة سوسة النخيل الحمراء (*Rhynchophorus ferrugineus*)، المدمرة يثير السؤال حول فعالية مثبطات تخليق الكيتين. لذلك تهدف الدراسة لاستكشاف السمية الحادة المختبرة لمثبطات تخليق الكيتين على سوسة النخيل الحمراء. بالإضافة لبعض التغيرات البيوكيميائية على يرقات العمر السادس بعد المعاملة بالتركيز النصفي القاتل لمبيدات كلوروفلورزورون وهكسافلومورون ولوفينورون وكانت ٤٥٤,٠١ و١٢٩٣,٠٢ و٩١٩,١٣ ميكروجرام/ ملليتر على التوالي، وذلك عند معاملة يرقات العمر اليرقي السادس بطريقة غمر الطعام في محلول المبيد. أظهر مبيد اللوفينورون أدنى انخفاض معنوي في محتوي البروتين (٧,٢٩٪) مصحوبًا بتنشيط إنزيمات GST وß-esterase وcarboxylesterase وphenoloxidase وchitinase وα-Esterase وسجل ٥٨,٥٠ و٥٨,٥٤ و٢٦,٠٩ و٢٢,٥٦ و١١,٥٢ و٧,٧٧٪ على التوالي. كما أظهر مبيد كلوروفلورزورون انخفاضًا معنوي أكبر علي المحتوي الكلي للبروتين (٢٣,٥٤٪) مصحوبًا بتنشيط إنزيمات GST وß-esterase وcarboxylesterase وchitinase مسجلين ٢٤,٩٠ و٢٣,٩٤ و٤,٨٩ و٢,٢٨٪ على التوالي. أما أعلى نسبة خفض معنوي لمحتوي البروتين الكلي في معاملة هكسافلومورون (٤٥,٩٦٪) مصحوبة بتنشيط GST وcarboxylesterase وchitinase بزيادة ٤٨,٠٢ و٣٨,٨٦ و٢٥,٢٢٪ على التوالي.

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