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Biochemical and Histological Responses of Red Palm Weevil, *Rhynchophorus ferrugineus* Exposed to Sub-lethal Levels of Different Insecticide Classes

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ABSTRACT

By introducing the 9th larval instar of the red palm weevil (RPW), *Rhynchophorus ferrugineus*, to feed on sugarcane stem dipped in insecticide solutions of chlorpyrifos, methomyl, and spinosad to monitor the biochemical and histological changes in larvae after 24 h exposure. The estimated LC₅₀ values for chlorpyrifos, methomyl and spinosad were 109.73, 589.55 and 112.09 µg a.i./ml, respectively. Exposure of RPW larvae to LC₅₀s of the tested insecticides causes a significant inhibition in Acetylcholinesterase activity associated with increasing total protein in all treatments but alanine aminotransferase and aspartate aminotransferase activities enhanced with methomyl but decreased in spinosad-larvae treated. Chlorpyrifos was only significantly induced oxidative stress (increasing protein carbonyl indicator) followed by increasing antioxidant enzymes (catalase and glutathione peroxidase). While superoxide dismutase and glutathione peroxidase were reduced significantly in methomyl and spinosad treatments. Catalase activity raised significantly with chlorpyrifos followed spinosad. All the treatments of insecticides exhibited lower glutathione S-transferases activities especially in spinosad than chlorpyrifos treatments. The histological damage in the midgut of larvae treated included vacuolar degeneration, proliferation and necrosis of epithelial lining with destruction and separation of basement membrane and with desquamation in its lumen. In conclusion, spinosad insecticide was more effect on biochemical and histopathological responses of RPW than both methomyl and chlorpyrifos insecticides.

INTRODUCTION

Date palm *Phoenix dactylifera* L. is cultivated well especially in tropical and semi-tropical climates. It is considered an economic fruit crop in Egypt and the Middle East. Date palm fruits are a very good source of food with several medicinal qualities (Al-Dosary *et al.*, 2016). Palms are infested by many harmful pests, but the red palm weevil is the most important of them at all (Salman *et al.*, 2020). The red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is the main borer attacking palm trees (Milosavljević *et al.*, 2019). Most of the popular date palm cultivars are also suitable for RPW infestation (Sallam *et al.*, 2012).

The widespread RPW enabled it to expand its geographical range to extend to Asian, African, European and American countries, causing catastrophic damage to palm production

(Giblin-Davis *et al.*, 2013). RPW was an invasive pest reported in 1992 first recorded in date palm plantations of Sharqia and Ismailia Governorates in Egypt (Saleh, 1992) and converted to a major pest of date palm (Arafa, 2020). Because the high risk of RPW led to a lower economic threshold required implementation the effective control methods after date palms infestation reached 1% (Faleiro *et al.*, 2010). So monitoring the population density of *R. ferrugineus* is essential to protect palms (Vacas *et al.*, 2013) which was conducted using pheromones and other semi chemicals in traps (Arafa, 2020) to determine the most appropriate time to apply the means of control.

Due to the difficulty of delivering biopesticides to the inside of the palm, lack of their efficiency and the appropriate conditions to ensure their sustainability in microhabitat besides their long incubation period compared with chemical insecticide (Mampallil *et al.*, 2017). So, current control methods recommended for management against *R. ferrugineus* have focused on integrated pest management involving, chemical insecticide treatments (Salem *et al.*, 2018).

When the RPW is detected, attention turns to chemical insecticides because of their quick action and effectiveness in pest control. Among the different chemical insecticides, organophosphate and carbamate insecticides were recommended for controlling *R. ferrugineus* (Faleiro, 2006; Murphy and Briscoe, 1999). Most of the insecticides used are neurotoxic in their main target i.e. Organophosphates and carbamates are anti-cholinesterase compounds (Gupta, 2011). But RPW developed resistance against used insecticides and phosphine fumigants which is considered a serious concern (Wakil *et al.*, 2018). So lately, biologically based synthetic insecticides are applied as a curative insecticide against *R. ferrugineus* in date palm (Abdel-Salam *et al.*, 2014; Mashal and Obeidat, 2019). The spinosad (spinosyns) is a natural and active insecticide derived from the rare soil-dwelling actinomycete bacterium called *Saccharopolyspora spinosad* (Mertz and Yao, 1990). Spinosad is a neurotoxin with a novel mechanism of action by activation of the nicotinic acetylcholine receptor and gamma-aminobutyric acid (GABA) receptor but at a unique site of action from imidacloprid and nicotine (Salgado, 1998).

After insecticides application under field conditions, the initial concentration deposited highly on the application site but decreased to lose activity and presence with sublethal concentration. The sub-lethal effect targets the activities of the enzymes in insects (Sabri *et al.*, 2017) may be leading to mortality or disturbing the biological system (Yin *et al.*, 2008). Depending on the stage of the insect, instar, enzyme type, and enzyme concentration, insecticidal chemicals had different effects on enzymatic activity. But the cells have evolved a complicated antioxidation system that comprises both exogenous antioxidants and endogenous antioxidant enzymes to resist the harmful effects of free radicals e.g., as catalase, glutathione S-transferase and superoxide dismutase (Samarghandian *et al.*, 2014).

Therefore, the present study was constructed to determine a possible mechanism of the fat body injury after *R. ferrugineus* exposure to the sublethal short-term of insecticides (chlorpyrifos, methomyl and spinosad) to measure biochemical aspects in larvae homogenate. Likewise, oxidative stress indices and histopathological changes were determined, to contribute in understand the toxic sub-lethal effect of the tested insecticides and warn early of the possibility of developing resistance to insecticides applied to one of the study criteria.

MATERIALS AND METHODS

Founding the Colony:

The pupae of the red palm weevil, *R. ferrugineus* collecting from the infested date palm field from El-Kasasin district, Ismailia Governorate, Egypt. To start the insect colony, the rearing of RPW was carried out in a controlled rearing incubator (29 ± 2 °C and 60-70% RH, with photoperiod (12:12 h L:D). the light intensity of about 30 FC is provided with fluorescent tubes according to (Hamadah, 2019; Salama and Abdel-Razek, 2002). The RPW was maintained on the stems of sugarcane (Kaakeh *et al.*, 2001).

Insecticides Used:

Three commercial insecticides included organophosphate “chlorpyrifos (Pyriban A[®] 48 % EC)”, carbamate “methomyl (Neomyl[®] 90 % SP)” and biologically based synthetic “spinosad (Tracer[®] 24 % SC)” were obtained from Central Agricultural of Pesticides Laboratory (CAPL), Agricultural Research Centre (ARC), Dokki, Giza, Egypt.

Bioassay Experiment:

The ninth larval instar of RPW was exposed to the tested insecticides using the dipping food technique (Salman *et al.*, 2020; Shawir *et al.*, 2014). The fresh sugarcane stalks were cut to pieces (\approx 15 cm in length, 1.5 cm diameter). These fresh-cut pieces were immersed in the prepared concentration solutions for 30 minutes. After the immersion period has passed, the treated pieces were air-dried then introduced to the starved larvae for nearly 6 h in glasses jars (1 L) with a perforated cover. In conjunction with these treatments, the untreated control consisted of the immersed pieces of sugarcane stalks in distilled water.

Based on preliminary experiments, different serial dilutions of each test insecticide were freshly prepared in distilled water to define the concentration range causing 20-85% mortality. Each treatment consisted of replicated three with 10 larvae/replicate. The larval mortalities were recorded after 24 h exposure.

After calculating the median lethal concentration (LC₅₀), the 9th larval instar of the RPW was treated with the estimated LC₅₀ values for the tested insecticides. The treatment was triplicated, each one contained ten larvae. The survival larvae were transferred into a 50 ml centrifuge tube then frozen directly at -20 °C until the subject for biochemical assessments.

Preparation of Larvae for Analysis:

For determination enzymatic activities, larvae of the RPW were homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.4) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA) yielding 5 % (W/V) homogenate. The homogenates were centrifuged for 30 minutes at 4 °C at 12.000 g. The supernatant was used to study enzyme activity and other biochemical parameters.

Biochemical Assay:

The supernatant of different treatments subjected determinations included:

1. Acetylcholinesterase (AChE) activity in *R. ferrugineus*, was obtained by adapting the technique described by (Ellman *et al.*, 1961) by the addition of 10 μ l acetylthiocholine iodide (3 mM/l) and absorbance at 412 nm was read by spectrophotometer.
2. The activities of transaminases Aspartate aminotransferase (AST) and Alanine Aminotransferase (ALT) were determined according to (Reitman and Frankel, 1957).
3. Total protein (TP) level was quantified by the procedure of (Bradford, 1976).
4. Biomarkers of oxidative stress include:
 - a. Protein carbonyl (PC) content was assayed by the described method (Yan *et al.*, 1995).
 - b. The activity of superoxide dismutase (SOD) was measured with the method of (Marklund and Marklund, 1974).

- c. The activity of glutathione-S-transferases (GST) was determined at 340 nm in the reaction medium containing phosphate buffer (0.1M, pH 6.5), reduced glutathione, GSH (1.0 mM), 1-chloro-2, 4-dinitrobenzoic acid (1.0mM) and a sample (Habig *et al.*, 1974). Also, Glutathione peroxidase (GPx) activity was measured by (Beutler *et al.*, 1963) method.
- d. Catalase activity (CAT) was measured by the method of (Aebi, 1984). The reaction started by adding H₂O₂ (30mM) to an appropriate volume of homogenate in 50 mM sodium phosphate buffer with pH 7. Then, the absorbance was read at a wavelength of 240 nm within 3-min.

The percentage of changes of biochemical parameters was calculated according to (Söğüt *et al.*, 2021) as Δ parameter (%) = (control value – treatment value) / (control value) × 100.

Histopathological Changes:

Treated and untreated larvae of the RPW were dissected in Ringer's solution. The midgut was isolated and fixed in Bouin's solution then embedded in paraffin. Many sections 5 µm thickness were obtained and stained with hematoxylin and eosin (H & E stain) according to the method of (Suvarna *et al.*, 2018). The sections were examined by microscope under 400X.

Statistical Analysis:

Bioassay Experiments: Abbott's formula (Abbott, 1925) was used to compute the corrected mortality percentage based on control treatments. The dose-response relationship curve was statistically computed according to (Finney, 1971) to calculate the median lethal concentration (LC₅₀) and regression line equation components of each insecticide using Analystsoft Biostat Pro V 5.8.4.3 Software. Also, **Toxicity index** = (lowest LC₅₀ value) / (LC₅₀ value of insecticide) × 100, was calculated according to (Sun, 1950).

Biochemical Assays: Data were expressed as the mean ± SE of five replicates. The data were subjected to statistical analysis using one-way Analysis of Variance (ANOVA) and complemented with the least significant difference (LSD) by using SPSS program (Statistical Package for Social Science) version 25 for Windows (IBM SPSS Inc, USA). The statistical difference between the group means compared to the control group is indicated as follows: * (p ≤ 0.05), and ** (p ≤ 0.01).

RESULTS AND DISCUSSION

The RPW is one of the insects that were able to successfully invade new areas continuously causing to inflict heavy economic losses. Perhaps the reason for this success is the insect's strength and its great survival potential besides high adaptability and reproductive potential in the newly infested area. All these reasons make the RPW a serious insect pest that deserves careful study in terms of its response to the chemical pesticides applied to it, as well as the changes that occur internally after exposure to different pesticides, and the possibility of recovery from them (Haynes, 1988).

Therefore, the study concerns the diagnosing the current situation of the pest towards the widely used insecticides in controlling. Based on the obtained data in Table (1) and Figure (1) shows the results of bioassay experiments of the tested insecticides on the 9th larval instar of RPW fed on sugarcane stalk dipped in the solutions of the tested insecticides. Chlorpyrifos showed the lowest LC₅₀ recorded 109.73 µg/ml with the lowest range of fiducial limit (102.1569 and 117.7931) and toxicity index (Ti) attained 100%. While spinosad occupied the second rank in potency with LC₅₀ value (112.09 µg/ml) with fiducial limit (103.6774 and 121.1984) and Ti = 97.89%. Methomyl achieved the lowest potency (LC₅₀ = 589.55 µg/ml, and FL=538.2336-643.8902) recorded 18.61 % with Ti. As for the

homogeneity of the tested strain of the RPW, it was found that it was more homogeneous with methomyl (2.91 ± 0.23), compared to the rest of the tested pesticides.

Table 1: Responses of the 9th larval instar of the red palm weevil, *Rhynchophorus ferrugineus*, fed on sugarcane stalk dipped in the solutions of the tested insecticides.

Insecticide	LC ₅₀ (µg/ml)	LC ₅₀ Fiducial Limit (95%)		Slope ± SE	Intercept	Ti
		Lower	Upper			
Chlorpyrifos	109.73	102.1569	117.7931	3.67 ± 0.29	-2.48	100
Methomyl	589.55	538.2336	643.8902	2.91 ± 0.23	-3.07	18.61
Spinosad	112.09	103.6774	121.1984	3.46 ± 0.25	-2.09	97.89

LC₅₀: Median lethal concentration; SE: standard error; Ti: Toxicity index

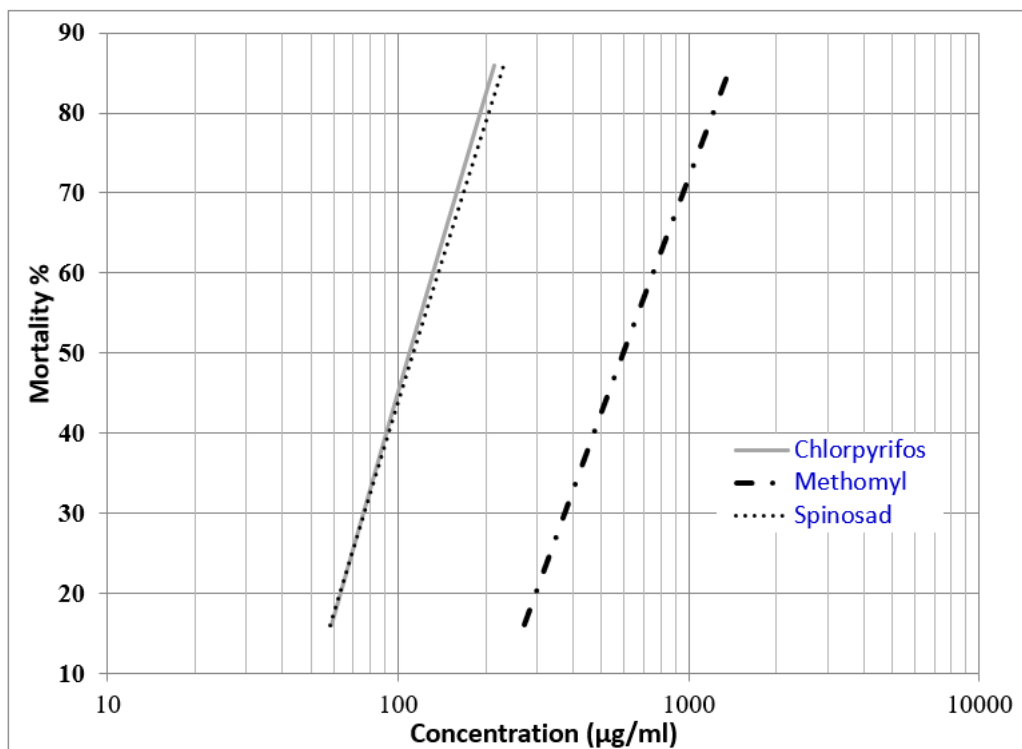


Fig. 1. Toxicity lines of chlorpyrifos, methomyl and spinosad on exposed the 9th larval instar of the red palm weevil, *Rhynchophorus ferrugineus*, treated using the food dipping method

Chlorpyrifos estimated LC₅₀ = 2597 µg/ml, against larvae (20 days old) using dipping food technique (Shawir *et al.*, 2014). Chlorpyrifos was an effective insecticide against the egg stage of RPW, and methomyl recorded the highest effect against the adult stages (♀, ♂) (Elgohary *et al.*, 2015). The LC₅₀ of females treated with spinosad was calculated at 44.3 µg/ml. The larval, pupa and adult instars of RPW are characterized by large and robust structures compared to other insect pests. The larval stage has a 13 larval instars (Dembilio and Jaques, 2012). Using dipping technique for sugarcane stalk in insecticide solution uptake a little amount on the outer surface. When taking the nutritional behavior of the insect inside the sugarcane stems, also the weight of the treated larvae “9th larval instar”, requires a higher concentration reaching the median lethal concentration compared to other treatment methods such as the insect immersion method. Perhaps these variations explain the great discrepancy in the results of the bioassay of the RPW, not only at the level of the same phase but also according to the different gender tested as reported (Elgohary *et al.*, 2015).

Acetylcholinesterase (AChE) is an enzyme that catalyzes the breakdown of the neurotransmitter acetylcholine at peripheral and central synapses in the nervous system of insects (Bourne *et al.*, 2016). AChE is also an important target site for insecticide action (organophosphorus and carbamates) in the central nervous system of insects (Naqvi *et al.*, 1994; Nathan *et al.*, 2008). During this investigation, AChE activities of RPW larvae decreased throughout the experiment following treatment with methomyl and spinosad. The decrease in AChE activities can be related to the mode of action of most pesticides.

Chlorpyrifos and methomyl are cholinesterase inhibitors, acetylcholinesterase (AChE) is a primary target site for carbamate and phosphorous insecticides. While spinosad is mainly binding the nicotinic acetylcholine receptor to work as an agonist but at a site distinct from that of nicotine or the neonicotinoids (Tomlin, 2009). The obtained data in Table (2) showed that methomyl and spinosad treatment provide significant inhibition of AChE activity in RPW larvae at $p \leq 0.01$ but non-significant inhibition was observed with chlorpyrifos. The % changes of AChE activities were 31.18 and 41.75 % in larvae treated with methomyl and spinosad respectively. The surpassed Spinosad potency as AChE inhibitor was reported on different insects. Spinosad decreased AChE activity in different organs of honeybee workers at varying concentrations, according (Rabea *et al.*, 2010). Also, Spinosad had anticholinesterase action against one *Electrophorus electricus* acetylcholinesterase and two *Drosophila melanogaster* acetylcholinesterases (Bucur *et al.*, 2014).

Although the insecticide spinosad is not specific to inhibit the AChE it considers a secondary target compared with chlorpyrifos and methomyl which targets mainly AChE. Therefore Spinosad has a potent insecticidal effect on RPW because of aiming two vital targets in insects (Müller, 2018). While surpassing the AChE inhibition of chlorpyrifos compared with methomyl due to the aging of organophosphorus insecticides with AChE causing irreversible binding. In contrast with the carbamate insecticide methomyl, the binding with AChE is reversible (Vale and Lotti, 2015). Besides, the high hydrophilicity of methomyl other insecticides reduced the uptake amount and reduced the permeability of parent compounds through the hydrophobic barriers e.g., insect and plant cuticle and internal barriers surrounding neurons reaching the target site (Durlin *et al.*, 1987).

Transaminases (ALT and AST) help in the production of energy (Azmi *et al.*, 1998), and serve as a strategic link between carbohydrates and protein metabolism and are known to be altered during various physiological and pathological conditions. The activity of both ALT and AST increased in larvae treated with methomyl this may be attributed to the occurrence of reversible binding between insecticide and enzymatic site of action on the enzyme surface. This may be because the relationships between protein synthesis and transaminase levels were affected by the hormonal control of protein synthesis and neurosecretory hormones which are involved in the regulation of transaminase levels (Etebari *et al.*, 2005).

ALT and AST activities were affected highly significantly after exposure to insecticides, decrease spinosad treatment (65.01 and 51.29 %) and an increase in methomyl treatment (81.40 and 105.55%) compared to check treatment which did not show a significant difference from the chlorpyrifos treatment, as illustrated in Figure (2). Conversely, the higher activities of ALT and AST in spinosad may relieve the biochemical impact on total protein content causing a significant increase (4.81) compared to methomyl treatment that showed a highly significant positive effect on ALT and AST activities reflected highly significantly adversely on total protein amount. On the contrary, chlorpyrifos was not a significant difference in the control treatment.

Table 2 : Changes in Acetylcholinesterase activity of the red palm weevil, *Rhynchophorus ferrugineus*, hemolymph exposed to median lethal concentrations of different insecticide classes.

Parameters	Treatments			
	Control	Chlorpyrifos	Methomyl	Spinosad
Acetylcholinesterase (µM/min)	500.87 ± 13.724	469.86 ± 30.293 (6.19)	344.68 ± 8.615** (31.18)	291.75 ± 12.617** (41.75)

Values are expressed as Mean ± SE (n = 5); Numbers between parentheses refer to "change percentage (%)" ascribed to control; * and ** significant versus control at P ≤ 0.05, and 0.01 respectively.

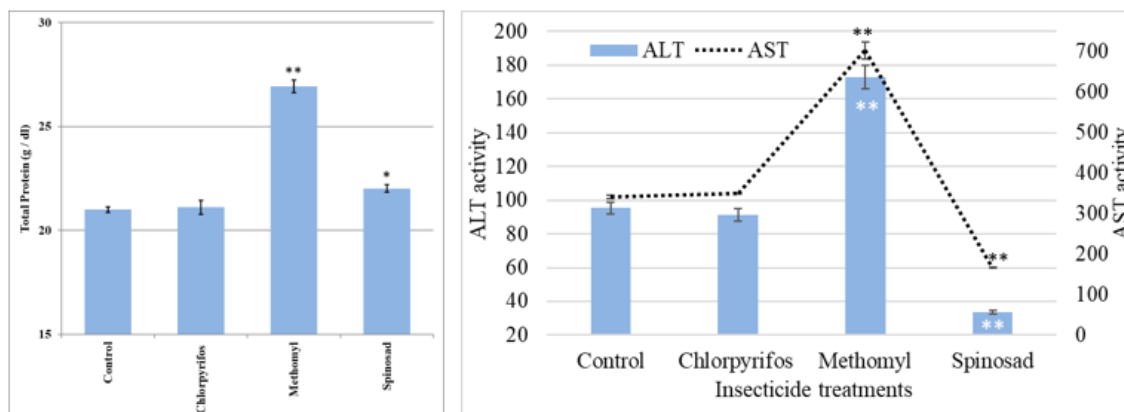


Fig. 2: Changes in protein content and enzymes (ALT and AST) in the red palm weevil, *Rhynchophorus ferrugineus* hemolymph exposed to median lethal concentrations of different insecticide classes. *, and ** significant versus control at P ≤ 0.05, and 0.01 respectively

The lower activities of ALT and AST after exposure to spinosad in our result were documented by (Abdel-Razik and Mahmoud, 2017) with Jojoba, *Simmondsia chinensis* extract on cotton leafworm, *Spodoptera littoralis* (Boisd.). Also, (Gaaboub *et al.*, 2012) reported that Neemix, caused a significant decrease in the activity of GPT of the treated 4th instar larvae of *S. littoralis*. The methomyl trend in insects was also the same in the *Spodoptera littoralis* larvae with emamectin benzoate, abamectin and spinosad (Megahed *et al.*, 2013). Chlorpyrifos decreased (ALT) levels insignificantly of *Spodoptera littoralis* (Magdy *et al.*, 2019). On the other hand, spinosad treatments induced a significant inhibitory effect on transaminases (ALT and AST) activities. These results agree with (Assar *et al.*, 2016) by emamectin and spinetoram, and hexaflumuron against the 4th instar larvae of *Spodoptera littoralis* and with (Abd-El-Aziz, 2014; Amin and Fahmy, 2011) using spinetoram reduced both GOT and GPT of *Spodoptera littoralis*. Also, Spinosad and lufenuron significantly increased the ALT of *Spodoptera littoralis* (Magdy *et al.*, 2019).

Oxidative stress, an imbalance toward the pro-oxidant side of the pro-oxidant/antioxidant homeostasis. Because of the relatively early synthesis and relative stability of carbonylated proteins, using them as biomarkers of oxidative stress is favored. (Dalle-Donne *et al.*, 2003). Influence of antioxidant and oxidative stress parameters in the RPW, exposed to sub-lethal concentrations of the tested pesticides were included in Table (3). The results revealed that protein carbonyl (PC) level was highly raised significantly in chlorpyrifos-treated larvae recorded a 12.48 % increase (p ≤ 0.01). Long-term exposure to chlorpyrifos increased the production of reactive oxygen species (ROS), resulting in cell oxidative damage and a reduction in antioxidant capacity. while protein carbonyl reduced significantly in spinosad treatment (43.27%) compared with unexposed larvae and methomyl treatment.

Table 3 :Changes in protein carbonyl content and activities of antioxidant enzymes in the red palm weevil, *Rhynchophorus ferrugineus* exposed to sub-lethal concentrations of different insecticide classes.

Parameters	Treatments			
	Control	Chlorpyrifos	Methomyl	Spinosad
Protein carbonyl (nM/ml)	72.69 ± 0.976	81.76 ± 1.101** (- 12.48)	69.63 ± 2.52 (4.21)	41.24 ± 1.512** (43.27)
Superoxide dismutase (U/ml)	6.63 ± 0.041	6.83 ± 0.095 (- 3.02)	4.77 ± 0.006** (28.05)	6.07 ± 0.084** (8.45)
Catalase (U/mg)	2.07 ± 0.063	5.33 ± 0.210** (- 157.49)	1.91 ± 0.096 (7.73)	10.73 ± 0.255** (- 418.36)
Glutathione peroxidase (nM/ml)	209.43 ± 4.074	227.96 ± 7.684* (- 8.85)	161.21 ± 2.420** (23.02)	143.68 ± 2.645** (31.39)
glutathione-S-transferases (µmol/min)	19.25 ± 0.995	16.50 ± 0.473* (14.29)	17.57 ± 0.845 (8.73)	13.57 ± 0.649** (29.51)

Values are expressed as Mean ± SE (n = 5); Numbers between parentheses refer to "change percentage (%)" ascribed to control; *and ** significant versus control at P ≤ 0.05, and 0.01 respectively.

Antioxidant enzymes (SOD, CAT, and GPx) are also called enzymes of the protective cellular system as superoxide dismutase can enhance H₂O₂ production from O₂ through dismutation while catalase and peroxidase are known to catalyze H₂O production from H₂O₂. These reactions can result in the elimination of bio-membrane damage by reactive oxygen species, ROS, (Shuang-yang *et al.*, 2001). During this trial, the activities of antioxidant enzymes (SOD and GPx) dropped whereas the activity of CAT increased. Reduced antioxidant enzyme activity (SOD and GPx) can lead to a reduction in ROS clearance, which can denature several biomolecules in the insect body. The denaturation of biomolecules can stop all the cellular processes, so leading to the death of the insect (Felton, 1995). Superoxide dismutase (SOD) activity was decreased significantly at both methomyl and spinosad treatments may be due to the absence of oxidative stress in both treatments as shown in Table (3).

Catalase function is mainly destructing of hydrogen peroxide to water and oxygen. Hydrogen peroxide can oxidize a variety of substrates also inflicting biological harm. The Fenton reaction is a complicated reaction that can produce both hydroxyl radicals and higher oxidation states of iron. Catalase activities were raised significantly vigorously in spinosad (418.36 %) and Chlorpyrifos (157.49%) treatments comparing control.

In insects, detoxification of insecticides is mainly accomplished by glutathione-S-transferase, GSTs, (Claudianos *et al.*, 2006). Our findings showed reduce in GSTs activities following chlorpyrifos and spinosad treatments which are in line with the findings of (Ali *et al.*, 2017) by the insecticide matrine against *Bemisia tabaci* and (Jia *et al.*, 2016) who found a comparable decrease in enzyme activity in *Locusta migratoria* after using chlorantraniliprole and *Metarhizium anisopliae* together. Although there are few reports on changes in GST activity as a result of the studied pesticides, the magnitude of changes can vary according to the insect species targeted, the type of insecticide employed, and the dosage of insecticide utilized. Also, the reduction in GST activities in response to different classes of insecticide throughout the experimental period can be related to the sequence of chemical's/pathogen's action against *R. ferrugineus* (Ali *et al.*, 2017).

The cytosolic enzyme glutathione peroxidase (GPx) catalyzes the conversion of hydrogen peroxide to water and oxygen (Fanucchi, 2004). A reduction was recorded on GPx activity in all experimental exposure to the tested insecticides. In addition, exposure of RPW larvae to the sublethal level of chlorpyrifos and spinosad caused significant inhibition of glutathione-s-transferase and elevation of catalase activity compared to the untreated group.

In insects, detoxification of insecticides is mainly accomplished by glutathione-S-transferase, GSTs, (Claudianos *et al.*, 2006; Simon, 1996), also, GSTs may contribute to the antioxidant defense in insecticide resistance (Che-Mendoza *et al.*, 2009; Vontas *et al.*, 2001). GST was reduced significantly in spinosad (29.50%) followed by chlorpyrifos (14.29) from the control treatment.

The activity of the enzymes varies according to the organ, route of administration, the dosage, gender in which it is estimated. As observed on *R. ferrugineus* treated with LC₅₀ of spinosad to study antioxidative responses showed inhibition of the activities of SOD, and GST in the midgut. However, in the testes, no significant changes in the activity of these enzymes were identified. (Abdelsalam *et al.*, 2016). On the other hand, in adult females a significant increase in CAT activity only in response to higher concentrations (Abdelsalam *et al.*, 2020).

A longitudinal muscle layer occurs outwardly followed by an interior circular muscle layer midgut in *R. ferrugineus* (Olivier) larvae, as shown in a cross-section (CS) of the typical midgut (Figs. 3a & b). While CS of the midgut treated with chlorpyrifos revealed that vacuolar degeneration, thinning, and desquamation of the epithelial lining also, necrosis with separation of the basement membrane (Figs. 4a, b & c). Where destruction, degeneration, and necrosis with separation of the basement membrane of the epithelial lining and with desquamation in its lumen were found in the midgut of larvae treated with methomyl (Figs. 5a, b & c). On the other hand, the histopathological damages were severity in spinosad-larvae treated as shown in (Figs. 6a-c). The pathological changes were severed vacuolar degeneration, necrosis, and proliferation of epithelial lining with separation and destruction of the basement membrane.

Alterations in the cellular organelles of the midgut, Malpighian tubules, and testes of *R. ferrugineus* were evaluated for spinosad histopathological changes. (Abdelsalam *et al.*, 2016). Also, varying intensity changes in cell organelles (Abdelsalam *et al.*, 2020). In *Culex pipiens*, exposure resulted in epithelial cell degeneration, the disintegration of plasma and basement membranes, and severe damage of the cellular microvillar system in the midgut. (El *et al.*, 2020). Exposure of *Partamona helleri* to spinosad caused morphological alterations in the midgut and intensified the processes of apoptosis, autophagy and oxidative stress in this organ (Araujo *et al.*, 2019). While The spinosad LC₅₀ caused disorganization of the epithelia of tested organs and induced oxidative stress and cell death on *A. mellifera*. (Lopes *et al.*, 2018).

The midgut of the red palm larvae contains epithelial cells, and the distinctive features of the midgut were the villi. The interior surface of the epithelial cells is provided with borders. The basement membrane supports epithelial cells. (Abd El-Fattah *et al.*, 2020). The obtained results for pathological damage of treated RPW larvae, *R. ferrugineus*, revealed that midgut was sensitive to the tested insecticides. A similar result was obtained by (Abd El-Fattah *et al.*, 2020) who reported that the damage included vacuolation of cytoplasm, analyzes and destroyed nuclei of the epithelial cells of RPW larvae treated with imidacloprid and chlorpyrifos and their nano form as well as by (Abdel-Ghaffar, 2004) from different insecticides against different insects. Also, (Ahmed, 1995) reported that the histopathological action of some pesticides was observed to cause epithelial cell expansion and the formation of vacuoles. On the other hand, (Hussein *et al.*, 1994) mentioned that the effect on midgut may be due to digestion and absorption of insecticides.

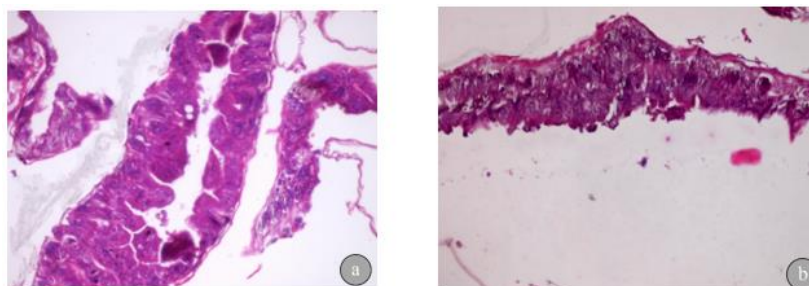


Fig. 3: Cross-sections of the midgut of *Rhynchophorus ferrugineus* larva in control treatment showed:

- a: Normal structure of lining epithelial. (H & E \times 400)
 b: Normal Columnar epithelial lining. (H & E \times 400)

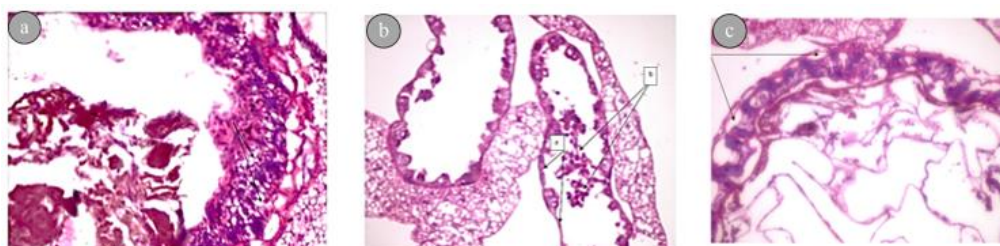


Fig. 4: Cross-section of the midgut of *Rhynchophorus ferrugineus* larva treated with chlorpyrifos showed:

- a: Vacuolar degeneration of the epithelial lining. (H & E \times 400)
 b: Thinning (a) and desquamation (b) of epithelial lining in the lumen. (H & E \times 400)
 c: Necrosis of epithelial cells lining with separation of the basement membrane. (H & E \times 400)

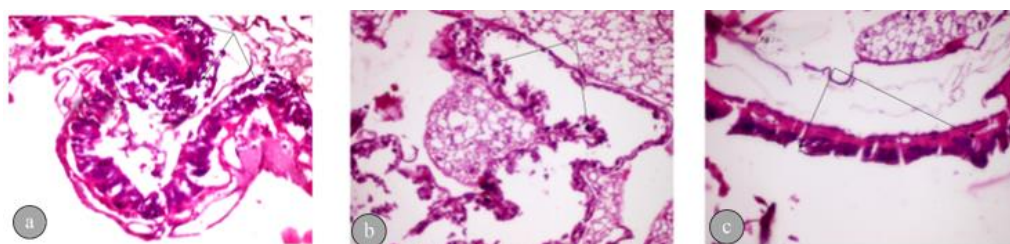


Fig. 5: Cross-section of the midgut of *Rhynchophorus ferrugineus* larva treated with methomyl showed:

- a: Sever destruction and necrosis of epithelial lining with separation of the basement membrane. (H & E \times 400)
 b: Sever degeneration and necrosis of epithelial lining with desquamation in its lumen. (H & E \times 400)
 c: Thinning and necrosis of the epithelial lining. (H & E \times 400)



Fig. 6: Cross-section of the midgut of *Rhynchophorus ferrugineus* larva treated with spinosad showed:

- a: Sever vacuolar degeneration and necrosis of epithelial lining with separation of the basement membrane. (H & E \times 400)
 b: Sever destruction and necrosis of epithelial lining. (H & E \times 400)
 c: Sever necrosis (a) and proliferation of epithelia lining (b) with destruction and separation of basement membrane (c). (H & E \times 400)

CONCLUSION

The biochemical and histopathological responses of *R. ferrugineus* to sub-lethal levels (LC₅₀ values) of the tested insecticides displays changes in the enzymatic activities but it's agreed in its effectiveness on AChE inhibition and increasing total protein content and transaminases activity however methomyl showed a constant trend in increasing ALT and AST activity in contrast to spinosad. Chlorpyrifos was only induced by increasing protein carbonyl (oxidative stress indicator) followed by increasing antioxidant enzymes (Catalase). Exposure RPW accompanied with lower GST and GPx activities especially in spinosad then chlorpyrifos treatments. Cross-section of the midgut of RPW larva treated with the tested insecticides show various histopathological responses was spinosad > methomyl > chlorpyrifos.

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ARABIC SUMMARY

الاستجابات الكيموحيوية والنسجية لسوسة النخيل الحمراء *Rhynchophorus ferrugineus* المعرضة لمستويات شبيهة المميتة من مبيدات حشرية تابعة لمجموعات مختلفة

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عند تقديم العمر اليرقي التاسع لسوسة النخيل الحمراء *Rhynchophorus ferrugineus* للتغذية على سوق قصب السكر المنغمسة في محاليل مبيدات الكلوربيريفوس والميثوميل والإسبينوساد ومراقبة التغيرات البيوكيميائية والنسجية في اليرقات المعرضة بعد 24 ساعة. فكانت قيم التركيز المميت النصفية المقدر للكلوربيريفوس والميثوميل والإسبينوساد 109.73 و 589.55 و 112.09 ميكروجرام مادة فعالة/مليلتر على التوالي. كما أدى تعرض يرقات سوسة النخيل الحمراء إلى التركيزات النصفية القاتلة للمبيدات الحشرية المختبرة إلى تثبيط معنوي في نشاط أسيتيل كولينستريز وزيادة البروتين الكلي في جميع المعاملات، في حين زاد نشاط إنزيمات ألانين والأسبارتات امينوترانسفيريز في اليرقات المعاملة بالميثوميل وانخفضت في اليرقات المعاملة بالإسبينوساد. كما أدت المعاملة بالكلوربيريفوس فقط لتحفيز على الإجهاد التأكسدي بشكل كبير (زيادة معيار البروتين الكربوني) متبوعاً بزيادة الإنزيمات المضادة للأكسدة (الكاتاليز والجلوتاثيون بيروكسيداز). بينما انخفض إنزيم سوبر أكسيد ديسميوتاز الجلوتاثيون بيروكسيداز بشكل ملحوظ في معاملات الميثوميل والإسبينوساد. وأيضاً ارتفع نشاط الكاتاليز بدرجة معنوية مع معاملات الكلوربيريفوس تلاها الإسبينوساد. كما أبدت جميع معاملات المبيدات الحشرية انخفاض نشاط جلوتاثيون أس-ترانسفيراز خصوصاً بمعاملة الإسبينوساد فضلاً عن الكلوربيريفوس. أما بالنسبة للأضرار النسجية بالمعنى الواسع لليرقات المعاملة فتضمنت تنكس فجوي، تكاثر وموت في البطانة الظهارية (الخلايا الطلائية) مع تدمير وفصل الغشاء القاعدي وتقسر في تجويفه. وختاماً يمكن القول بأن مبيد الإسبينوساد كان الأكثر تأثيراً على الاستجابات الكيموحيوية والنسجية لسوسة النخيل الحمراء عن مبيد الميثوميل أو الكلوربيريفوس.