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TOXICOLOGICAL AND BIOCHEMICAL IMPACT OF SOME BIOINSECTICIDES AGAINST THE LARVAE OF THE RED PALM WEEVIL, *Rhynchophorus ferrugineus* (OLIVIER) UNDER LABORATORY CONDITIONS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), is one of the most important and destructive pests for date palms causing high economic losses. Several control methods have been applied to manage this pest. Intensive use of conventional insecticides to control RPW successfully minimized the weevil number, but they are still harmful for the environment as they cause pollution and damage other useful creatures. The present study aimed to find suitable, effective, and safe alternative control means. In addition, the impact of tested compounds on the enzymatic activity of the third instar larvae were assayed spectrophotometrically. Four commercial insecticides were applied against the 3rd instar larvae of RPW under laboratory conditions and the LC_{50} values were estimated. Larvae that survived treatment were collected 24h post treatment and were prepared for further enzymatic activities analysis. All experimentations were carried out at Wood and tree scavenger research department, Plant protection research institute, Agricultural research center. Results showed that Dr. Sure® was the most toxic compound according to low LC_{50} value obtained. In addition, results revealed that BIO-MAGIC® was the least toxic as the high LC_{50} value compared to the other compounds. In addition, results revealed significant impacts on the detoxifying enzymes in the 3rd instar larvae treated with LC_{50} of tested compounds as a defensive response against those compounds. These results reveal the suitability of the non-conventional insecticides to control the youngest larval instars effectively.

Keywords: Red palm weevil; *Rhynchophorus ferrugineus*; detoxifying enzyme; esterases; acetyl cholinesterase; acid phosphatase; alkaline phosphatase; glutathione-s-transferase; cytochrome P450.

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1. INTRODUCTION

Date palms, *Phoenix dactylifera*, are one of the most economically important crops in the Middle East and Egypt and they are threatened strongly by the red palm weevil. The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), is one of the most important and destructive pests for date palms causing high economic losses [1,2]. It develops within the stipe of the date palm and subsequently destroys the vascular system causing collapse tree death of the plant. *R. ferrugineus* spreads in Europe Oceania, Africa, and Asia [3]. The first record of this pest in the middle east was in the 1980s and has heavily damaged date production by destroying many thousands of date palms [4,3]. It was first recorded in Egypt in 1992 in date palm farm in Sharkia and Ismailia Governorates [5]. Several control methods have been applied to manage this pest, including plant quarantine treatments, improved farming practices, insecticides, and pheromone traps [6]. Although the application of insecticides are the most effective means for minimizing the weevil numbers, they still harmful for the environment as they cause pollution and damage other useful creatures [6,**7]**. Consequently, it is vitally important to find safe alternative control means with new and unique mode of action. Of these alternatives, the entomopathogenic fungi [8, 9,10], nematodes [8], bacteria 11,12], and plant-based products [13, 14, 15, 16]. Implementation of entomopathogenic fungi in pest management program of RPW proved their efficiency and compatibility with other control means [17, 18, 19, 20, 21]. On the other hand, using plant-based insecticides showed some progress in controlling the RPW infestation [13,22,23]. The extracted products of the neem tree, *Azadirachta indica* A. Juss (Sapindales: Meliaceae) are promising compounds that proved their potential and environmentally safe to vertebrates, plant species and useful invertebrates in addition to their wide use in control many insect pests including wood borers as promising agents [24,25,26, 27]. In this context, the present study was carried out in order to evaluate the efficiency of different groups of eco-friendly insecticides compared to conventional means considering the management of the red palm weevil larvae. In addition, the impact of tested compounds on the enzymatic activity of the fifth instar larvae.

2. MATERIALS AND METHODS

2.1 Insect Rearing

Larval stage of RPW was collected from heavily infested and untreated date palm trees in Sharkia governorate, Egypt. Collected larvae were reared on clean sugar cane's cuts under laboratory conditions of 25±2ºC and R.H. of 65±5% [28] in Wood and tree scavenger research department, Plant protection research institute, Agricultural research center, Dokki, Giza, Egypt.

2.2 Tested Compounds

Four commercial insecticides were applied against the 3rd instar larvae of RPW. These compounds were as follow; Pyrifos® 480 g/L EC (Chloropyrifos 48%) was obtained from El-Nasr Co. for intermediate chemicals, Egypt, a bioinsecticides BIO-MAGIC® (*Metarhizium anisopliae* (Metchnikoff) Sorokin) as 1.75% WP and was provided from Gaara Establishment (Import and Export), two botanical-based insecticides; Dr. Sure® 2ml/L and was supplied from Krishna Valley Agrotech LLP, India, and Achook® 0.15% EC (azadirachtin) and it was supplied from Godrej Agrovet Ltd., India.

2.3 Toxicity Assays

Five concentrations of each tested compounds were tested against the 3rd instar larvae of RPW. Four replicates each containing ten larvae were treated by offering treated sugar cane cuts to them. Fresh clean cuts of sugar cane were dipped in prepared suspension for 10s and then were left to dry at room temperature to be offered later to RPW larvae. For the control experiment, the same numbers of larvae were offered fresh clean sugar cane cuts dipped in distilled water. Larval mortality was recorded daily and the mortality percentage was corrected according to Abbott's formula [29]. The LC_{50} values of tested compounds were estimated according to Finney [30] using "LdPLine®" software.

2.4 Biochemical Assays

2.4.1 Sample preparation

The 3rd instar larvae were treated with the determined LC_{50} of tested compounds. Larvae that were survived treatment of tested compounds after 24h. were collected for further biochemical analysis. One gram of treated and untreated larvae was weighed. Larvae were then homogenized with 1.5-4.5 ml of 0.01MTris buffer (pH 7.8) and physiological saline solution (NaCl 8.8 gm, KCl 0.2 gm, and CaCl2 0.3 gm/Liter, pH 6.7-6.8) with traces of phenylthiourea crystals in an ice bath for three minutes. The homogenate was centrifuged for 20 minutes at 4°C at 10,000 g and the supernatant was filtered through an l-µm glass wool membrane syringe pre-filter and was used as the enzyme source for further enzyme activity assays. Control specimens were obtained by

homogenizing healthy larvae through the same technique.

2.4.2 Determination of α- and β- esterases activities

α- and β- esterases activities were detected by Gomori's colorimetric method (van Asperen, 1962) using α- and β-naphthyl acetate as substrates. Absorbance was read 15 min later at 600 and 555 nm for the produced α-and β-naphthol, respectively, against blank that lacked enzyme.

2.4.3 Determination of acid and alkaline phosphatase activities

The activity of acid phosphatase was determined according to Tietz's procedure (Rifai et al., 2018). The enzyme activity was measured calorimetrically at 405 nm using spectrophotometer. On the other hand, the activity of alkaline phosphatase was assayed according to Klein et al. [31] using sodium phenolphthalein phosphate as substrate. The enzyme activity was measured calorimetrically at 550 nm using spectrophotometer.

2.4.4 Determination of acetyl cholinesterase activity

The activity of acetyl cholinesterase was estimated using acetylcholine I (AcSChI) as substrate according to Ellman et al. [32] and the activity were estimated at 412 nm calorimetrically using spectrophotometer.

2.4.5 Determination of glutathione-s-transferase (GST) activity

The activity of GST was estimated using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate [33]. The enzyme activity was assessed calorimetrically using spectrophotometer at 340 nm.

2.4.6 Determination of cytochrome P450 activity

The activity of cytochrome p450 was determined using means of the difference spectrum of dithionite-reduced carbon monoxide (CO) [34,35]. The activity was estimated calorimetrically using spectrophotometer at 400-500 nm.

2.5 Statistical Analysis

Obtained results were presented as mean ± S.D. and the data were statistically analyzed using one-way analysis (ANOVA) followed by Duncan's New Multiple Test where appropriate [36] at $P<0.05$ using SPSS statistics 17.0 release 17.0.0 software.

3. RESULTS AND DISCUSSION

3.1 Toxicity Assay

Results presented in Table 1 showed the LC_{50} of tested compounds against the 3rd instar larvae of red palm weevil, *R. ferrugineus*. Results showed that Dr. Sure® was the most toxic compound according to low LC_{50} value obtained. In addition, results revealed that BIO-MAGIC® was the least toxic as the high LC_{50} value compared to the other compounds. These results reveal the suitability of the non-conventional insecticides to control the youngest larval instars effectively. This agreed to Hussain et al. [10,37,20,38,39].

3.2 Biochemical Assay

Data presented in Table 2 showed the effect of tested compounds on α- and β- esterases activities in the RPW 3rd instar larvae. Results revealed that the activity of the enzymes increased significantly compared to control. This demonstrate that nevertheless the tested compound α-esterase activity exhibited higher activity for detoxification than β-esterase. The same results were obtained by Ragheb et al. [40,41,42,43]. The esterase enzymes belong to the detoxifying enzymes which are responsible for the detoxification of any foreign substance in insect's body. Moreover, esterase is an important detoxifying enzyme which hydrolyzes the esteric bond in any toxicant. Also, esterase is one of the enzymes showing the strongest reaction to environmental stimulation [44]. Their high activity may be an indication of the insect's response to body intoxication and may be consider as a remark of resistance development [45, 46, and 21]. Furthermore, it is well known that any infectious disease for insect regardless the infection-causing factor leads to increased activity of detoxifying enzymes in general, and the esterases in particular [47,48]. Our results were in accordance with Dubovskiy et al. [49,50,51] who determined high esterase activity when treated different insects with entomopathogenic fungi.

The effect of LC_{50} of Pyrifos®, Achook®, BIO-MAGIC®, and Dr. Sure® on AChE, GST, and Cytochrome P450 activities. Treatment with tested compounds declined the activity of acetyl cholinesterase compared to control. Furthermore, treatment of the 3rd instar larvae with LC_{50} of tested compounds caused fluctuations in GST activity as no particular pattern was recognized. Moreover, results showed no significant difference in the cytochrome P450 activity compared to control. Insects use detoxification enzymes such as acetylcholinesterase, glutathione S-transferases and cytochrome P450 for

their defense against xenobiotics [47]. These enzymes degrade the toxic chemicals in insects before reaching the target sites [52]. Of the detoxifying enzymes, AChE that catalyzes the hydrolysis of acetylcholine, a neurotransmitter [53, 54]. The inhibition of AChE activity causes insect death [54]. Many studies were conducted to show the inhibitory activity of many plant-based products against many insects [55,56,57,54]. GST gain its importance from its role in the degradation of insecticides and toxic substances. Besides degradation of xenobiotics, GST takes part in metabolite removal and protection of tissues from damage by free radicals [58]. Moreover, GST may play a role in protecting insects from pathogen infection [59]. The suppression of GST activity in insect cause eventually death to insect [60, 61].

Table 1. Susceptibility of the red palm weevil, *Rhynchophorus ferrugineus*, to tested compounds

Tested compounds	LC_{50} (g/L)	Fiducial limits (95% C. I.)		Slope
		Lower	Upper	
Pyrifos®	2.14	1.40	2.81	3.16±0.27
Achook®	3.87	2.99	5.12	2.30±0.21
BIO-MAGIC®	4.48	3.99	5.02	2.70±0.31
Dr. Sure®	0.89	0.52	1.69	2.13±0.23

Table 2. α- and β- esterase activities in the 3rd instar larvae of *Rh. ferrugineus* after 48-h treatment with LC_{50} of the tested insecticidal compounds

Tested Compounds	α -esterase activity (µg α-naphthol /min/ gm. b.w.) (Mean ±S.D)	β-esterase activity (µg β -naphthol /min/ gm. b.w.) (Mean ±S. D)
Pyrifos®	466.5±2.5 ^{b}	290.3±3.3 ^{b}
Achook®	482.6 ± 7.5 ^{b}	229.4 ± 4 ^{b}
BIO-MAGIC®	479.33 ± 24.2 ^{b}	266.3 ± 6.02 ^{c}
Dr. Sure®	536.6 ± 3.5 ^{c}	212.3 ± 2.5 ^{b}
Control	195 ± 2.9 ^{a}	139.3 ± 6.02 ^{a}

-Means of the same column followed by different letters are significantly different, P≤0.05, b.w. = body weight.

Table 3. AChE, GST and P450 activity in fat body of the early 4th instar larvae of *Rh. ferrugineus* after 48-h treatment with LC_{50} of the tested insecticidal compounds

Tested compounds	AChE activity (µg ACh Br/min/ml) (Mean ±SD)	GST activity (µmole/min/ml) (Mean ±S. D)	P450 activity (µ mole/min/ml) (Mean ±S. D)
Pyrifos®	203.3 ± 3.6^{a}	55.2 ± 2.8^{a}	90.1 ± 3.87^{a}
Achook®	224.7 ± 7.2^{b}	25.2 ± 1.6^{c}	85.9 ± 3.19^{a}
BIO-MAGIC®	312.6 ± 13.79^{c}	47.2 ± 2.9^{b}	89.1 ± 3.87^{a}
Dr. Sure®	196.4 ± 6.33^{a}	42.1 ± 1.9^{b}	92.7 ± 3.03^{a}
Control	333.1 ± 8.5^{c}	46.5 ± 2.87^{b}	91.2 ± 5.05^{a}

-Means of the same column followed by different letters are significantly different, P≤0.05.

Table 4. ALP and ACP activity in fat body of the early 4th instar larvae of *Rh. ferrugineus* after 48-h treatment with LC_{50} of the tested insecticidal compounds

Tested Compounds	Alkaline phosphatase activity (U x10^3 / gm. b.w.) (Mean ± S.D.)	Acid phosphatase activity (U x10^3 / gm. b.w.) (Mean ± S.D.)
Pyrifos®	186.66 ± 4.50 ^{b}	128.0 ± 6.08 ^{a}
Achook®	190.33 ± 14.57 ^{a}	130.6 ±7.09 ^{a}
BIO-MAGIC®	177.3 ± 14.57 ^{c}	122.9 ± 6.03 ^{b}
Dr. Sure®	169.9 ± 6.32 ^{a}	119.9 ± 11.3 ^{a}
Control	197.66 ± 12.34 ^{a}	130.3 ± 6.42 ^{a}

-Means of the same column followed by different letters are significantly different,P≤0.05, b.w. = body weight.

The effect of LC_{50} of Pyrifos®, Achook®, BIO-MAGIC®, and Dr. Sure® on acid and alkaline phosphatase activities were shown in Table 4. Results showed insignificant decrease in both ACP and ALP compared to control. Results also showed that Bio-Magic and Dr.Sure were the most effective compounds compared to the rest compounds. Acid and Alkaline phosphatases are hydrolytic enzymes that are responsible for hydrolyzing the phosphomonoesters under acid or alkaline conditions, respectively [62]. Acid and Alkaline phosphatase are involved in insect development, nutrition and egg maturation [63]. Acid phosphatase is extensively studied due to its association with histolysis. ACP hydrolyzes a variety of orthophosphorylation reactions [64]. This could be due to decreasing the in acid-soluble phosphorus content [65]. These results agreed with El-Banna & Abd El-Kareem, [66,67,68] when different insects were treated with conventional and non-conventional insecticides.

4. CONCLUSION

Finally, we can conclude that using biological based insecticides against the red palm weevil can provide valuable substitutes for conventional chemical insecticides. In addition, the application of plant extracted product demonstrate the efficacy of this group of pesticides against the red palm weevil larvae not only as killing effect but also as latent effect of these compounds.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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