

**LARVICIDAL, ANTIOXIDANT ACTIVITIES AND PERTURBATION OF
TRANSMINASES ACTIVITIES OF TITANIUM DIOXIDE NANOPARTICLES
SYNTHESIZED USING *MORINGA OLEIFERA* LEAVES EXTRACT AGAINST THE RED
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ABSTRACT

The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) is one of the major pests of palms. The larvae bore into the palm trunk and feed on the palm tender tissues and sap, leading the host tree to death. The aim of this study is to assess the larvicidal activity of titanium dioxide nanoparticles (TiO_2NP_s) synthesized from the aqueous extract of *Moringa oleifera* against the *Rhynchophorus ferrugineus*. The present study also aimed to investigate the effects of *Moringa oleifera* on the glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities in tissue homogenates of *Rhynchophorus ferrugineus* larvae beside measuring antioxidant enzyme activities of SOD and MDA. The gut microbiota of insects also plays a remarkable role in the host life and understanding the relationship dynamics between insects and their biological control of insect pests so we also measured transaminase enzyme activities in the gut of the insects. Results showed that mortality increased in the larvae stage by using this prepared solution of natural extract with titanium dioxide nanoparticles at concentration 75 mg/L and this was the best concentration used. **Conclusions:** TiO_2NP_s could be used along with *Moringa oleifera* extract against *Rhynchophorus ferrugineus* larvae.

KEYWORDS: *Moringa oleifera* extract, Titanium dioxide nanoparticles, *Rhynchophorus ferrugineus*, antioxidant activities.

ABBREVIATIONS

Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), SOD (Superoxide dismutase), MDA (Malondialdehyde).

INTRODUCTION

The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) is widely considered the most damaging insect pest of palms in the world, even in all the countries where it has been accidentally introduced. RPW larvae feed within the apical growing point of the palms, producing a wet fermenting frass inside the tunnels, creating extensive damage to palm tissues and weakening the structure of the palm trunk; the resulting damage is often only visible long after infestation, when palms are close to death (Saleh, 1992).

The red palm weevil *Rhynchophorus ferrugineus* is a serious pest of coconut causing damage and often killing the palm trees in its prime of life. The hatched grubs burrow into the trunk and feed on tissue of the stem. The larvae and adult emergence within the same stem allow successive generations. *R. ferrugineus* (oliver) is a

devastating insect pest of date palm in the Arabian Gulf region. It was reported on date palm, for the first time, from the United Arab Emirates in the mid-1980s, then its reported distributed expanded its range westwards until it reached Egypt in 1992 (Cox, 1993).

In late years, nanoparticle/polymer composites have become important owing to their diminished size and large surface area and because they display unique properties not considered in bulk materials. As a result, nanoparticles have useful applications and biological sensors, conductive materials, and coating formulations (Templeton et al., 2000). The plant-mediated biosynthesis of nanoparticles is advantageous over chemical and physical methods because it is a cost-effective and environmentally friendly method, where it is not necessary to use high pressure, energy, temperature, and toxic chemicals (Goodsell, 2004).

The titanium dioxide nanoparticles (TiO_2NP_s) were synthesized from *Bacillus subtilis* possess antibacterial activity and antiparasitic activity (Kirthi et al., 2011).

Moringa oleifera is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. English common names include: moringa, drumstick tree. It is a fast-growing, drought-resistant tree, native to the southern foothills of the Himalayas in northwestern India, and widely cultivated in tropical and subtropical areas where its young seed pods and leaves are used as vegetables. It can also be used for water purification and hand washing, and is sometimes used in herbal medicine (Roloff *et al.*, 2009).

The conventional pesticides are costly and result in problems of residues, resistance, pollution and health hazards. Therefore, the biologist diverted their attention to investigate the feasibility of new generation more safety of biopesticides like natural oils, pathogenic bacteria, IGRs, pheromones, nematode and marine toxins. Many investigations have been conducted on the antifeedant effects, growth inhibition and abnormal development in various insects caused by using natural insecticides (Bream *et al.*, 2001) like oil and as well as natural extracts which are powerful insect antifeedant and repellents. They may also disrupt growth, inhibit moulting (Dorn *et al.*, 1986) and oogenesis (Senthilnathan and Sehoon, K. (2005).

Despite the economic and environmental damages caused by the RPW in all the areas where it is endemic and where it has been accidentally introduced, little is known about its gut microbiota. Many natural products are known to have a range of useful biological properties against insect pests (Isman, 2006). Over the past two decades, botanical insecticides threw attention as an approach for the control of insect pests.

The present laboratory study was undertaken to evaluate the efficacy, in the laboratory, of titanium dioxide nanoparticles prepared in *Moringa oleifera* leaves extract as a control measure for disrupting growth and development of *red palm weevil* larvae. Furthermore, SOD and MDA in larvae gut exposed to titanium dioxide nanoparticles synthesized in *Moringa oleifera* aqueous extract.

2. MATERIALS AND METHODS

2.1. The experimental insect.

In the present study the larvae, pupae and adults were collected from large cavities of infested date trees in the valley region of Egypt, these trees received no chemicals such as insecticides. Using sugarcane as food, laboratory culture of *Rh. Ferrugineus* (Figure 1) and *Rh. ferrugineus* was obtained from this young palm (Figure 2) and this method established according to methods of Nassar and Abdullah (2001).

2.2. Bioassay and administration of chemicals

Titanium dioxide nanoparticles with *Moringa* leaves were bioassayed against *Rh. ferrugineus*. The Purified titanium dioxide nanoparticles (dissolved in acetone)

were obtained from "Sigma Chemical Company" while *Moringa oleifera* was obtained from botanical garden of, Sudan in month of July 2015 with high purity and organic farming. The freshly collected leaves (1 kg) of *Moringa oleifera* were first washed with distilled water dried in tray dryer under controlled conditions and powdered. The powdered plant materials (250g) was macerated with distilled water. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure. The yield of dried extract obtained was 25.0 g (2.5 % w/w). The 50% water extracts obtained.

Titanium dioxide of 1.5 cm was sprayed with the prepared *Moringa oleifera* extract. The treated *Moringa oleifera* with titanium dioxide were left to dry under laboratory conditions.

Five concentrations were prepared from Titanium dioxide nanoparticles and *Moringa oleifera* extract: 50, 75, 100, 150 and 200 mg/L after preliminary tests against of *Rh. Ferrugineus* larvae. Three replicates contain 15 larvae (10 days old) each was topically treated with the different concentrations. Parallel to treated replicates the control replicates were prepared without any treatment. All treated and control insects were kept at $37 \pm 2^\circ\text{C}$ and $50 \pm 60\%$ RH. The number of dead larvae was recorded every 48 hours to calculate the mortality percentage.

2.3. Synthesis of TiO_2NPS

The aqueous solution of $\text{TiO}(\text{OH})_2$ (5 mmol/L) was prepared and used for the synthesis of TiO_2NPS . About 20 mL of boiled *Moringa oleifera* extract was added into 80 mL of aqueous solution of 5 mmol/L $\text{TiO}(\text{OH})_2$ for the reduction at 50°C for 4 h with continuous stirring.

2.4. Characterization of nanoparticles

TiO_2NPS reaction mixture was centrifuged at 60 000 r/min for 40 min and the resulting pellet was dissolved in deionized water and filtered through Whatmann filters (0.45 μm). An aliquot of this filtrate containing TiO_2NPS was used for X-ray diffraction method (XRD) and energy dispersive X-ray spectroscopy (EDX). The surface morphology and composition of TiO_2NPS were analyzed by FE-SEM performed on a Philips instrument equipped with an EDX attachment, and for transmission electron microscopy (TEM) analysis TiO_2NPS were prepared on carbon-coated copper TEM grids. TEM measurements were performed on a JEOL model 1200EX instrument operated at an accelerating voltage of 120 kV and later with an XDL 3000 powder.

2.5. Larvicidal bioassay

One gram of aqueous *Moringa oleifera* extract was first dissolved in 100 mL of distilled water (stock solution). From the stock solution, 200, 150, 100, 75 and 50 mg/L was prepared with dechlorinated tap water for a bioassay using *Moringa oleifera* extract. The larvicidal activity was assessed following WHO (1996) and as per the

method of (Rahuman et al.,). For the bioassay test, larvae of *Rhynchophorus ferrugineus* was taken in five batches of 20 in 249 mL of water and 1 mL of aqueous plant extract (200, 150, 100, 75 and 50 mg/L). Control was set up with dechlorinated tap water. The number of dead larvae was counted after 24 h of exposure, and the percentage of mortality was reported from the average of three replicates.

Synthesized TiO₂NP_s toxicity tests were conducted using a multi-concentrations test, consisting of a control and different concentrations of nanoparticles. Each test was performed by placing 20 *Rhynchophorus ferrugineus* larvae into 200 mL of sterilized double distilled water with nanoparticles into a 250-mL beaker.

2.6. Biochemical analysis.

2.6. 1. Preparation of insect gut tissue homogenates

Red palm weevil larvae fed on titanium dioxide synthesized with *Moringa oleifera* for 20 days were collected, and their mid piece of their body were homogenized by a Heidolph Silent Crusher M at 4 °C for 10 seconds in homogenization buffer 0.1M sodium phosphate. Subsequently the homogenates were centrifuged by a Minispin Plus Eppendorf at 10,000 g for 15 min at 4 °C. The obtained extract was collected for biochemical analysis of GOT, GPT, SOD and MDA activities.

The activities were determined by measuring the absorbance of the samples in a dual beam spectrophotometer (Shimadzu-1700, UV/vis, Kyoto, Japan). All chemicals used were analytical grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.6.2. Estimation of Superoxide level (SOD)

The total SOD (EC 1.15.1.1) activity was determined according to (Marklund and Marklund, 1974) assaying the autooxidation and illumination of pyrogallol at 440 nm for 3 min. One unit total SOD activity was calculated as the amount of protein causing 50% inhibition of pyrogallol autooxidation. The total SOD activity was expressed as units per milligram of protein (U mg⁻¹). A blank without homogenate was used as a control for non-enzymatic oxidation of pyrogallol in Tris-EDTA buffer (50 mM Tris, 10 mM EDTA, pH 8.2).

2.6.3. Estimation of Malondialdehyde (MDA)

MDA is secondary product of lipid peroxidation (LPO). *Sitophilus oryzae* tissues were incubated at 95 °C with thiobarbituric acid under aerobic conditions (pH 3.4). The pink colour produced by these reactions was measured spectrophotometrically at 532 nm to measure MDA levels (Ohkawa et al., 1979). MDA levels were defined as nanomole per gram tissue.

2.6.4. Transaminase Measurements

The GOT and GPT activities were determined according to the method of Harold (1975) using a kit of Bioadwic. The enzyme was measured at wave length 546 nm by spectrophotometer.

2.7. Statistical analysis

Statistical analysis of data was based on SAS's program. The data were subject to analysis of variance (ANOVA). Means were compared by Duncan's multiple range test (Duncan, 1955) at P < 0.05. Percentages of the mortalities were corrected according to Abbott's formula (Abbott, 1925) as follows: % Corrected mortality = $(T - C)/(100 - C)$

Where: T: % mortality in treatment, C: % mortality in check (control).

3- RESULTS AND DISCUSSION

3.1. XRD images of biosynthesized TiO₂NP_s.

The XRD of TiO₂NP_s synthesized using *Moringa oleifera* extract showed the presence of broad peaks at 25.25, 37.79, 48.03, 55.06, 62.10, 68.75 and 70.28 degrees (Figure 3). The surface of nanoparticles was investigated using FE-SEM (Figure 5). The observed micrograph showed synthesized nanoparticles aggregates and spherical form. TEM revealed that the sizes of the TiO₂NP_s were 20.46-39.20 nm (Figure 4).

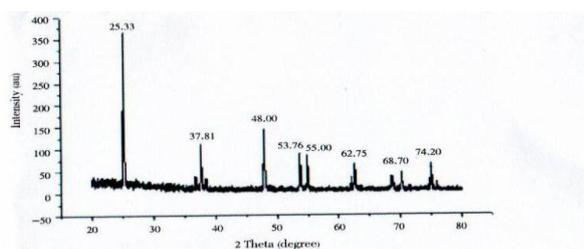
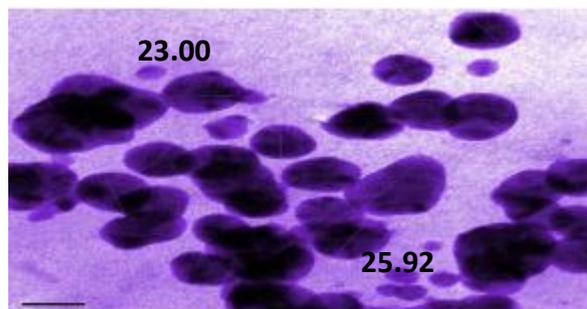
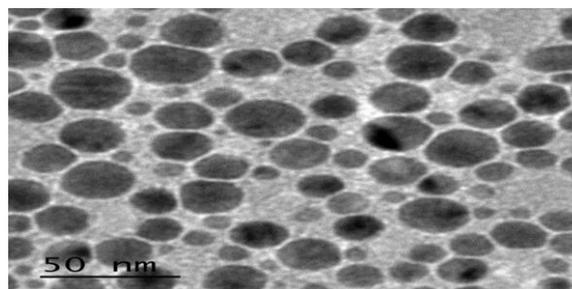
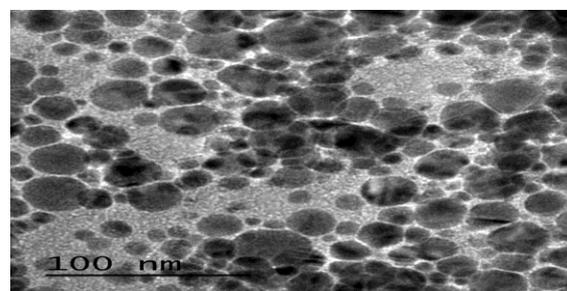
3.2. Percent Mortality of *Rhynchophorus ferrugineus* larvae treated with *Moringa oleifera* synthesized with titanium dioxide nanoparticles (TiO₂NPs)

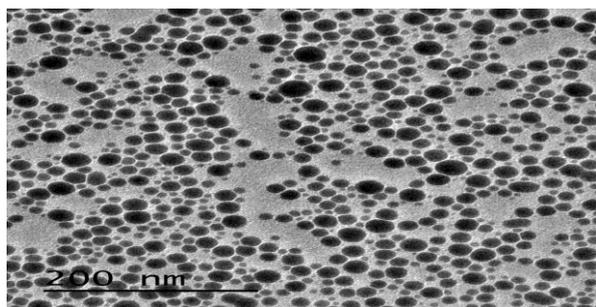
The result clearly indicated that the synthesized TiO₂NP_s at very low concentration was toxic against *Rhynchophorus ferrugineus* larvae when compared with the aqueous extract of *Moringa oleifera*. As shown in Table 1 and Figure 4, the potential effect of *Moringa oleifera* synthesized with titanium dioxide nanoparticles as a control measure against red palm weevil was concentration dependent and time lapse after treatment. Mortality of larvae was increased after treatment with the prepared concentration of titanium dioxide with *Moringa oleifera* water extract. Statistical analysis indicated that red palm weevil larvae treated with the prepared concentration of titanium dioxide with *Moringa oleifera* extract caused 70.25 % mortality to larvae after 8 days in the concentration 75 mg/L of *Moringa oleifera* extract synthesized with titanium dioxide nanoparticles (TiO₂NPs). Similarly, 100% mortality was also recorded in red palm weevil larvae at a later time of 10 days at the same concentration and this was the best recorded result between the five concentrations.

Table 1. Mortality of *Rhynchophorus ferrugineus* larvae offered to five concentrations of *Moringa oleifera* synthesized with titanium dioxide nanoparticles (TiO₂NPs)

Time following exposure (days)		Concentrations of <i>Moringa oleifera</i> extract (mg/L)					
		50	75	100	150	200	Control
2	Mean No of dead larvae \pm SE	2.52 \pm 0.25 ^c	4.68 \pm 1.02 ^{ab}	2.01 \pm 0.26 ^d	3.25 \pm 0.25 ^b	4.12 \pm 0.63 ^b	0 ^d
	% Corrected mortality	18.52	40	15.20	22.55	30.52	0
4	Mean No of dead larvae \pm SE	4.25 \pm 1.02 ^b	6.35 \pm 2.01 ^a	3.97 \pm 1.52 ^c	2.35 \pm 1.40 ^{de}	2.10 \pm 0.84 ^e	0 ^d
	% Corrected mortality	42.30	62.25	38.25	35.25	33.62	0
6	Mean No of dead larvae \pm SE	4.02 \pm 1.02 ^d	7.02 \pm 2.02 ^a	4.36 \pm 1.36 ^c	5.02 \pm 0.36 ^b	3.25 \pm 1.02 ^e	0.2 \pm 0.31 ^c
	% Corrected mortality	64.15	82.25	65.25	70.25	55.41	2.1
8	Mean No of dead larvae \pm SE	6.24 \pm 1.25 ^b	8.25 \pm 2.25 ^a	5.36 \pm 2.02 ^d	6.02 \pm 1.25 ^c	4.12 \pm 1.02 ^e	0.3 \pm 0.11 ^b
	% Corrected mortality	70.25	92.25	81.23	77.25	65.25	2.3
10	Mean No of dead larvae \pm SE	8.02 \pm 3.20 ^d	10 \pm 1.05 ^a	8.26 \pm 1.02 ^c	8.65 \pm 1.63 ^b	7.36 \pm 1.25 ^e	1.2 \pm 0.31 ^a
	% Corrected mortality	92.25	100	94.54	90.25	85.25	4

Means within the same rows in each category refers to different letters are significant at ($P \leq 0.05$) using Duncan's multiple range test (Duncan 1955).

**Fig(1): *Rhynchophorus ferrugineus* larvae****Fig (2): Young Palm infected near the base.****Fig(3): XRD images of biosynthesized TiO₂NPs.****Fig (4): TEM images of biosynthesized TiO₂NPs**



Fig(5): SEM images of biosynthesized TiO₂NPS

Table 2: Transaminases levels in *Rhynchophorus ferrugineus* larvae gut homogenates offered milled concentration of *Moringa oleifera* synthesized with titanium dioxide nanoparticles.

Enzyme	Concentrations of <i>Moringa oleifera</i> extract synthesized in titanium dioxide nanoparticles (mg/L)± se					
	Control	50	75	100	150	200
GOT (IU/ml)	146.25±9.41 ^f	155.39±7.58 ^e	197.62±9.41 ^a	161.35±9.24 ^d	178.25±4.25 ^b	170.84±9.41 ^{cd}
GPT (IU/ml)	161.25±7.21 ^f	171.34±6.25 ^c	215.26±7.41 ^a	174.25±4.25 ^b	169.25±9.27 ^d	164.24±3.54 ^e

Means within the same rows in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range tests. (Duncan 1955).

Table 3: SOD and MDA levels in *Rhynchophorus ferrugineus* larvae gut homogenates offered milled concentration of *Moringa oleifera* synthesized with titanium dioxide nanoparticles.

Enzyme	Concentrations of <i>Moringa oleifera</i> extract synthesized in titanium dioxide nanoparticles (mg/L)± se					
	Control	50	75	100	150	200
SODU/mg protein)	4.41±0.23 ^b	3.25±1.02 ^c	2.74±1.52 ^f	4.74±2.12 ^a	3.96±1.02 ^d	4.25±1.63 ^c
MDA (nmol/g)	6.25±1.02 ^f	15.27±2.36 ^d	28.94±3.45 ^a	16.27±4.25 ^c	14.53±3.25 ^e	17.24±3.22 ^b

Means within the same rows in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range tests. (Duncan 1955).

3.3.1. Transminase levels in *Rhynchophorus ferrugineus* larvae homogenate

Statistical analysis showed highly significant differences between the levels of transminases enzymes in the larvae, while it decreased in *Rhynchophorus ferrugineus* larvae offered titanium dioxide nanoparticles treated with *Moringa oleifera* extract 50, 75, 100,150 or 200 mg/L being 144.25,155.39,197.62,161.35,178.25 and 170.84 respectively, as compared to 146.25 in untreated larvae and this for GOT , but for GPT , the high level was recorded in concentration 75 mg/L of *Moringa oleifera* extract synthesized with titanium dioxide nanoparticles (Table 2).

And these findings support Tanani *et al.*, 2009 who reported that contradictory results of disturbed GOT activity in several insects by various botanicals had been reported in the available literature. Enhancement or prohibition of the enzyme activity usually depends not only on the insect species but also on its developmental stage, age, tissue, nature of the botanical and method of treatment.

3.3.2. Antioxidant enzyme SOD level in *Rhynchophorus ferrugineus* larvae homogenate

As shown in Table 3, Exposure of *Rhynchophorus ferrugineus* larvae to *Moringa oleifera* extract treated

with titanium dioxide nanoparticles at a concentration of 50 or 75 mg/L decreased superoxide dismutase enzyme level to 3.25 and 2.74 respectively, as compared to its level 4.41 in the control. These results supported with statistical analysis which appear highly significant differences between two concentrations.

3.3.3. Final lipid peroxidation marker (MDA) level in *Rhynchophorus ferrugineus* larvae homogenate

Table 3 revealed that the exposure of *Rhynchophorus ferrugineus* larvae to *Moringa oleifera* synthesized with titanium dioxide nanoparticles elevation of Malondialdehyde level (The final lipid peroxidation marker) in the concentration 75 mg/L to 28.94 as compared to control group 6.25 and this revealed the success of the preped solution in induction of free radicl flux ad thus elevating the lipid peroxidation level and increasing mortality rate in the larve Hamza *et al.*, (2015).

REFERENCES

1. Saleh, M.R.A. 1992. Red palm weevil, *Rhynchophorus ferrugineus* Oliver is first recorded in Egypt and introduced in African continent. List No. 10634 Africa: Collection No. 22563. Int. Institute of Entomol., London.

2. Cox, M.L. Red palm weevil, *Rhynchophorus ferrugineus*, in Egypt. FAO Plant, Prot. Bull., 1993; 41(1): 30-31.
3. Templeton A.C., Wuelfing W.P. and Murray R.W. Monolayer protected cluster molecules. *Acc Chem Res.*, 2000; 33: 27-36.
4. Goodsell, D.S. 2004. *Bionanotechnology: lessons from nature*. Hoboken: Wiley.
5. Kirthi, A.V., Rahuman, A.A., Rajakumar, G., Marimuthu, S., Santhoshkumar, T., Jayaseelan, C. Biosynthesis of titanium dioxide nanoparticles using bacterium *Bacillus subtilis*. *Mater Lett.*, 2011; 65: 2745-2747.
6. Roloff, A., Weisgerber, H., Lang, U.M. "Moringa oleifera". *Enzyklopädie der Holzgewächse (PDF)*, 2009; 978-3.
7. Bream, A.S. 2003. Effect of azadirachtin on phosphatases and transaminases activities in pupae of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *Proc. Inter. Conf. Date palm*, King Saud Univ., Saudi Arabia.
8. Senthilnathan, S. and Sehoon, K. Effects of *Melia azedarach L.* extract on the teak defoliator *Hyblaea puera* (Lepidoptera: hyblaeidae). *Crop Prot.*, 2005; 10: 1-5.
9. Isman, M.B. and Machial, C.M. Pesticides based on plant essential oils: from traditional practice to commercialization. In: Rai, M. and M.C. Carpinella, (eds.), naturally occurring bioactive compounds. *Advan. Phytomedi.*, 2006; 3: 29-44.
10. World Health Organization. Report of the WHO informal consultation on the evaluation and testing of insecticides. Geneva: World Health Organization; 1996. [Online] Available from: http://whqlibdoc.who.int/hq/1996/CTD_WHOPES_ [Accessed on 8th March, 2014].
11. Rahuman, A.A., Gopalakrishnan, G., Ghose, B.S., Arumugam, S.H. Effect of *Feronia limonia* on mosquito larvae. *Fitoterapia.*, 2000; 71: 553-555.
12. Marklund, S. and Marklund, G., Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, *European Journal of Biochemistry.*, 1974; 47: 469-474.
13. Ohkawa, H., Ohishi, N., Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.*, 1979; 95: 351-8.
14. Harold, V. *Practical Clinical Biochemistry*. 4th ed., Arnold-Heinemann Publishers (India), Private Limited, India., 1975; 294.
15. Abbott, W.S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 1925; 18: 265-267.
16. Tanani, M.A. Ghoneim, K.S. and Basiouny, A.L. Impact of the wild plant, *Fagonia bruguieri*, extracts on the transaminase activities in some tissues of *Schistocerca gregaria* (Orthoptera: Acrididae). *Egypt. Acad. J. Biol. Sci.*, 2009; 1(1): 45-55.
17. Hamza, R.Z., El-Shenawy, N.S. and Ismail, H.A. Protective effects of blackberry and quercetin on sodium fluoride-induced oxidative stress and histological changes in the hepatic, renal, testis and brain tissue of male rat. *J Basic Clin Physiol Pharmacol.*, 2015; 26(3): 237-251.