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TESTING NANO-PESTICIDES TOXICITY AGAINST RED PALM WEEVIL *RHYNCHOPHORUS FERRUGINEUS* (OLIVIER) IN EGYPT

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Abstract

The toxicity of four compounds belong to different groups of insecticides; chlorpyrifos, imidacloprid and their nano derived were evaluated in the laboratory against larvae and adults of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier), by using dipping food technique. The results revealed that nanochlorpyrifos was the most toxic insecticide among the tested compounds against the larvae, followed bychlorpyrifos, imidacloprid and nanoimidacloprid; LC_{50} values were 52.0, 126.1, 28.4 and 123.3 ppm for chlorpyrifos, imidacloprid, nanochlorpyrifos and nanoimidacloprid, respectively. The larvae were more susceptible than the adults to most of the tested insecticides. Moreover, increasing time of exposure, the toxicity of imidacloprid to both larvae and adults increased and also was more effective than nanoimidacloprid while the opposite situation was obtained with nanochlorpyrifos. Determination of the shape and structure of chlorpyrifos and imidaclopridnano particles was characterized by transmission electron microscopic (TEM). The activity of pesticides was determined through Fourier Transform Infrared Spectrometry (FTIR) to obtain number of active terminals. Then field studies was done, it was carried out in the orchard of Research Station of the Agricultural Research Center Giza Governorate, Egypt to evaluate the efficiency of the field-based nano-chlorpyrifos pesticide compared to chlorpyrifos on both larvae and adults according to the highest obtained laboratory results at a concentration of 500 ppm. Four infested palm trees with red palm weevil for each treatment have been identified by using RPW detection device as well as visual detection. The pesticide solution was injected into 3 holes against larvae and adults of red palm weevil. Chlorpyrifos and their nano derived were applied at a rate of 1 liter per palm the treated palm trees were monitored weekly to determine the date palm that was recovery, which recorded after 21-30 days. The results showed that the efficacy of nanochlorpyrifos in the recovery of infected palms by stopping oozing brown fluid.

Key words *:* Chlorpyrifos, imidacloprid, nanopesticide, FTIR, TEM. *Rhynchophorus ferrugineus*, RPW detection device.

Introduction

The date palm *Phoenix dactylifera* L. is one of the most and common important fruit crops in the Middle East and North Africa, which is rich in carbohydrates (Abbas, 2013). It is the host of many insects that cause great economic losses (Abdel-Salam., *et al.,* 2014). The red palm weevil *Rhynchophorus ferrugineus* (RPW) (Olivier) (Coleoptera: Curculionidae) is one of the most devastating pests of different species of palm (Habib *et al*., 2017; Rabel and Solaiman, 2011). It attacks nearly all species of palm around the world (Faleiro, 2006a & b). It has become a major threat since the invasion of the Gulf region in the mid 1980's for first time in United Arab Emirates It was then discovered in Saudi Arabia in 1987 and it was first recorded in Egypt in 1993 (Cox, 1993). Recently it was recorded in North America 2009 (Fiaboe *et al*., 2012). Now the weevil has spread all over the palm cultivated area (Murphy and Briscoe, 1999). This pest attacks 40 palm species in the world (Al-Dosary *et al*., 2016: FAO stat. 2013). The annual losses are estimated at between US $ 1.7 to 8.7 million in the Gulf region due to the disposal of 1-5% of infected palm trees (El-Sabea *et al*., 2009). Infestation by this pest, is not detectable early enough to avoid damage to the tree as it completes its life cycle hidden inside the palm (Salem *et al*., 2012; El-Shafie *et al*., 2013). Symptoms of infection are as follows: oozing of brown fluid from larval feeding,

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view the tunnels on the trunk, the appearance of plant tissues with a fermented smell around the tunnel openings, drying the offshoots infected, breaking the trunk or the crown in cases of severe injury (Abraham *et al.,* 1998; Faleiro 2006; Sharaby *et al*., 2013). The aim of the present work is to evaluate the insecticidal of efficiency in laboratory tests by transforming recommended pesticides into nano particles against different stages of the red palm weevil and further to evaluate the efficiency of the highest laboratory concentration of chlorpyrifos and their nano form under field conditions.

Materials and Methods

Test insect

The different stages of RPW, *R. ferrugineus* were collected from infested date palms from three governorates in Egypt (Giza, Sharqeiaa and Ismailia). Adults were transferred to the laboratory and laid eggs were collected and observed for newly hatched larvae that were transferred to artificial diet. Several generations were obtained and mass rearing was maintained for laboratory tests.

The main culture of *R. ferrugineus* was reared on semi-artificial diet by Kaakeh (2001) which contained oats (57 %),sugar (22 %), brewer's yeast (9 %), molasses (11%), agar (30g), sorbic acid (4.5g), ascorbic acid (15g), in 1of water and 2ml formalin 40%. These ingredients were mixed together. Neonate larvae were maintained on the diet while the last three larval in stars were given sugarcane stems to form cocoons. The culture was kept at 25±1°C, 70-75% R.H. Eggs were kept under similar conditions in 9 cm diameter Petri-dishes, with wet filter paper, until hatching, then provided with semi-artificial diet. Larvae were placed individually in small plastic vials.

Preparation of Nano Pesticides

Nano chlorpyrifos and nanoimidacloprid were both prepared at Nano Tech Company, Dreamland, 6 October City, Egypt according to the method described byHuanan *et al.,* (2008).

Tested pesticides in field

The palm trees were treated with chlorpyrifos injection and their nano with four palms per pesticide treatment steps have been implemented as follows: - The infected palm was identified using a red palm weevil detection device as well as visual detection for infection purposes.

- Four replicates were identified for each treatment (4 palm trees). The variety of cultivar (Zaghlul) (Sharshir, 2006) was randomly selected from the palm farms of Research Station of the Agricultural Research Center Giza Governorate.
- Pesticide concentrations were prepared after conversion of the nanoparticle using a concentration of 500 ppm according to a laboratory result.
- Using the injection device, 3 holes were made around the injury area 10 cm away from the injury area. (El Ezaby *et al*., 1997)
- Each palm was injected at a rate of 1 liter of the concentration of the pesticide used. (Abdel-salam *et at*., 2014)
- The injection holes were filled with a piece of fiber and clay. (Abbas, 2013)
- The treated palm was examined after 21 & 30 days according to Abbas, 2013; Kaakeh, 2006 to evaluate the pesticide used according to the disappearance of the obvious symptoms of infection:

1. The fluid oozed was dry and stopped (Abbas, 2013; Abdel-salam *et al*., 2014)
2. Dry tunnels.
3. The control was treated with 1 liter of water only (Abdel-salam *et at*., 2014). The treatments were performed on Zaghloul cultivar, ranging from 4-15 years and lengths ranging from (1, 5- 4) meters and width 30-60 cm. In addition, the height of the injuries from the ground was between 20-140 cm. The injected pesticides were at 90° degrees using 1 liter per palm, over the 10 cm area using the injection device and was to determine the efficiency of pesticides after 21-30 days.

The Fourier Transform Technique (for Advanced Readers)

Fourier Transform type (Mac Company) was used; the technique was according to Griffiths & Fuller. (1982).

TEM analysis

The shape and size of both nanochlorpyrifos and nanoimidacloprid were analyzed and observed under Transmission Electron Microscope (TEM), JEOL model 3010 Philips CM-200, Japan operated at 120 kv, They were prepared by drop coating the pesticide particles onto carbon coated copper grid. The nano pesticides films were allowed to stay for 10 min, then the extra solution was removed with blotting paper and finally grids were allowed to dry then analyzed, The shape and size were manually interpreted by counting 100 particles randomly of each pesticide and calculated the mean according to Parveen *et al.,* (2014).

Statistical analysis

Regression equations of normal equivalent deviates (y) *versus* log dose (x), and LC_{50} values and their 95%

fiducially limits, were estimated according to Finney (1971). Relative toxicity of each insecticide at the LC_{50} & LT_{50} levels was also estimated.

Results and Discussion

Bioassay

Adult response to pesticide treatments

Tested pesticides were applied to adults at five concentrations 100, 200, 300, 400 and 500 ppm in either their original or nano form. Results were taken till death of adults; LC_{50} was measured and found to be 52.04, 126.129, 28.420 and 123.337 ppm for chlorpyrifos, imidacloprid, nanochlorpyrifos and nanoimidacloprid, respectively. Fig. 4 and Table 1 showed that lower concentrations of imidacloprid were more effective than higher ones where gave 15 days post application tested concentrations 93.3, 100.0, 100.0, 73.3 and 60.0 % mortality with 100, 200, 300, 400 and 500 ppm, respectively. Also imidacloprid was more effective than its nano form, where 9 days post application it caused 100 % with 200 and 300 ppm imidacloprid; while it gave only 6.7 % mortality with 500 ppm nanoimidacloprid (Fig. 6, 7 and 8) While; both chlorpyrifos and nanochlorpyrifos, 15 days post application chlorpyrifos and nanochlorpyrifos, 15 days post application gave 100% mortality with all tested concentration. However, in both chlorpyrifos and nanochlorpyrifos 6 days post application the lower concentrations gave the higher% mortalities (Table 2 & Fig. 5).

Larval response to pesticide treatments

Susceptibility of the 3rd instar larvae

Nano chlorpyrifos was the most effective pesticide with both 200 and 500 ppm concentrations (Fig. 9a,b), where 4 days post application it caused total deaths recorded 00.0, 22.2, 66.7 and 100.0 % mortality with 200 ppm and 00.0, 33.3, 66.6 and 100.0 % mortality with 500 ppm, respectively. Chlorpyrifos came second with 00.0, 22.2, 55.6 and 55.6 with 200 ppm and 00.0, 00.0, 66.7 and 88.9 death percentage with 500 ppm. Imidacloprid gave lower death

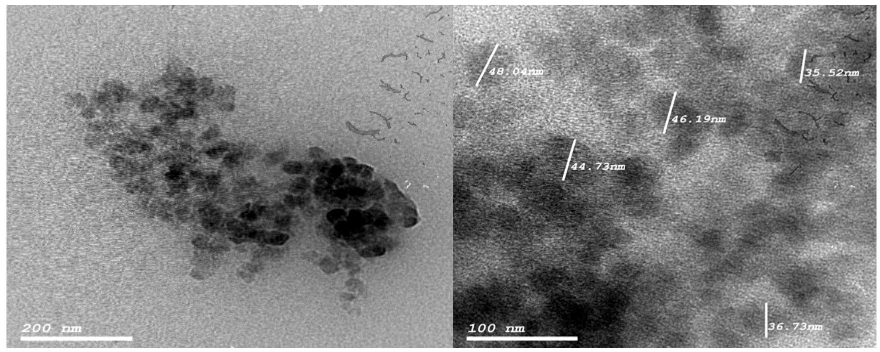

Fig. 1: A-TEM image showing synthesised imidaclopridnano particles, B-TEM image showing synthesised chlorpyrifosnano particles.

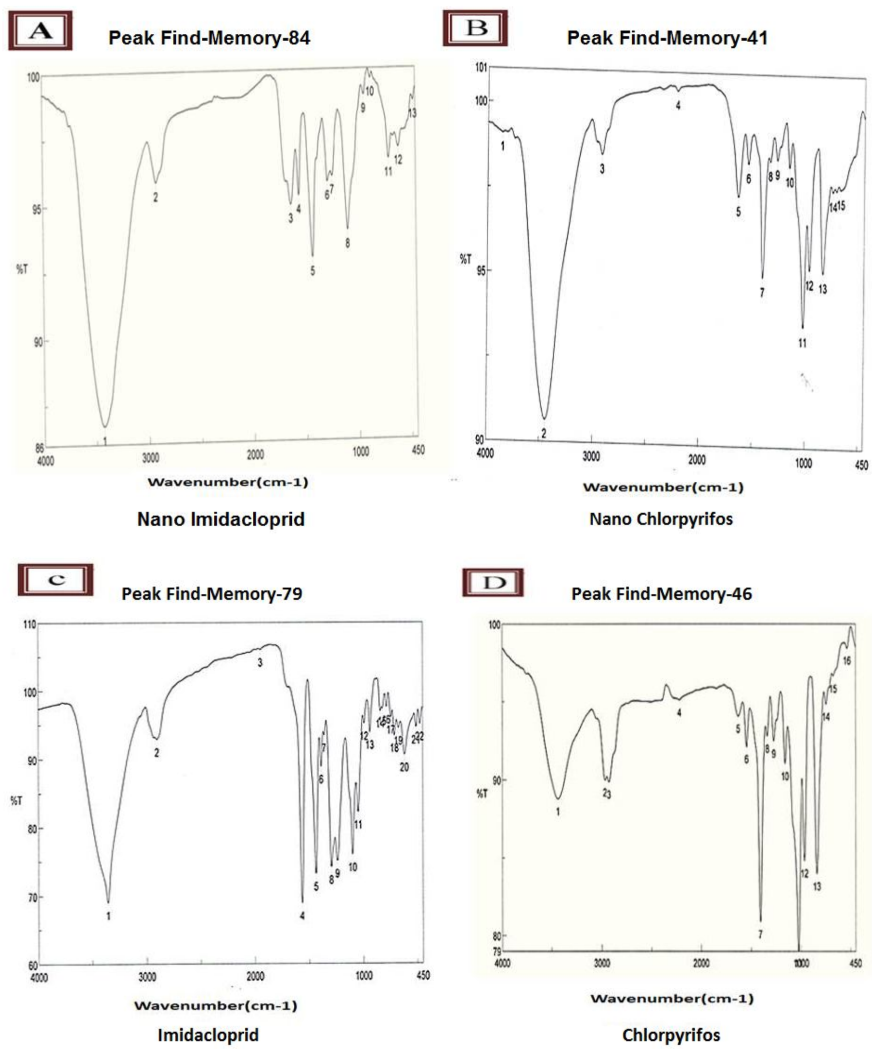

Fig. 2: The Fourier Transform Technique (FTIR)absorption curves **(A)**nanoimidacloprid, **(B)**nanochlorpyrifos, **(C)**imidacloprid and **(D)**chlorpyrifos.

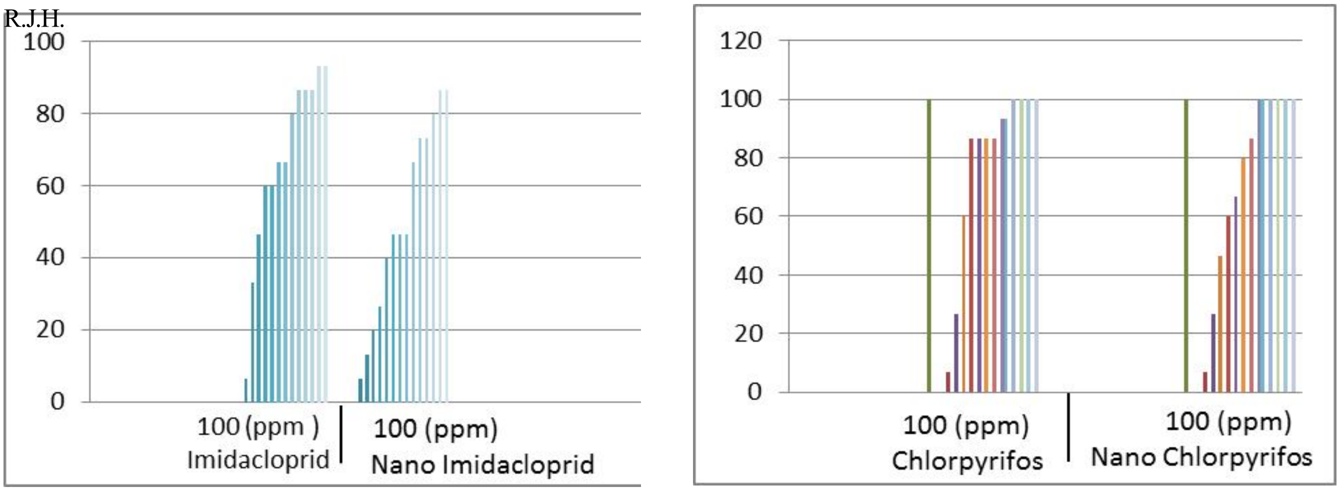

Fig. 3: Mortality % in adults of *R. ferrugineus* caused by (**A**) imidacloprid and nanoimidacloprid treatments; (**B**),chlorpyrifos and nanochlorpyrifos Treatments with 100 ppm concentration.

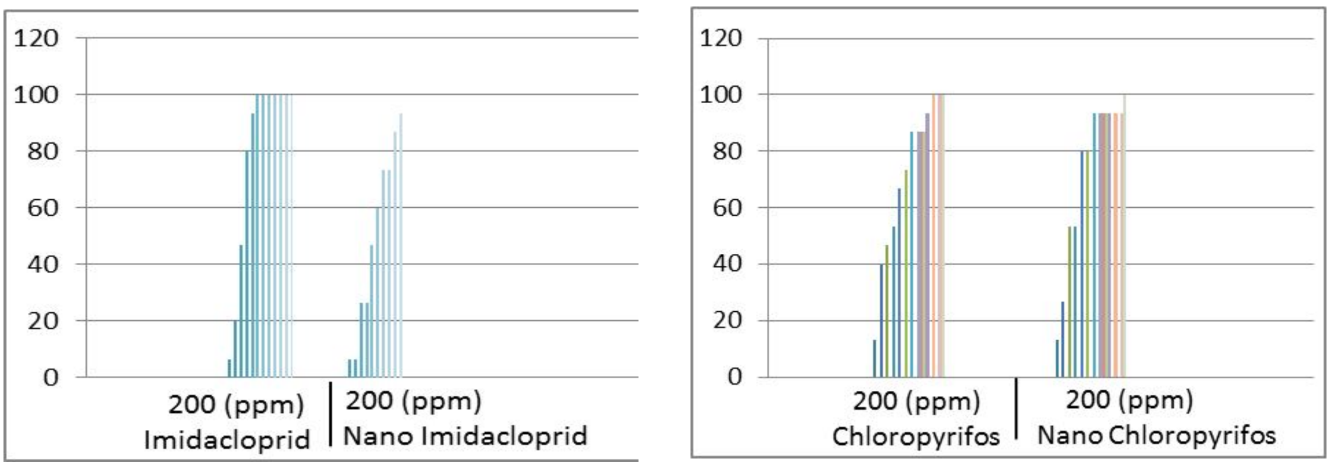

Fig. 4: Mortality % in adults of *R. ferrugineus* caused by (**A**) imidacloprid and nanoimidacloprid treatments; (**B**) chlorpyrifos and nanochlorpyrifos treatments with 200 ppm concentration.

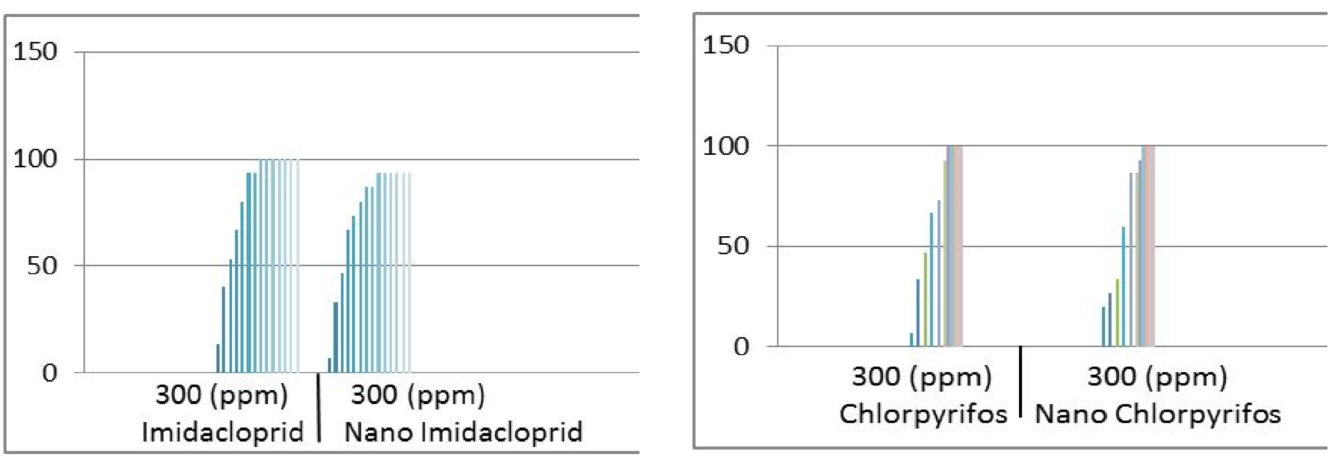

Fig. 5: Mortality % in adults of *R. ferrugineus* caused by (**A**) imidacloprid and nanoimidacloprid treatments, (**B**) chlorpyrifos and nanochlorpyrifos treatments with 300 ppm concentration.

% where it was only 33.3 and 77.8 % and 33.3% and 44.4 % with nanoimidacloprid at both 200 and 500ppm, respectively.

Susceptibility of the 6th instar larvae

Nano chlorpyrifos was the most effective pesticide with both 200 and 500 ppm concentrations (Fig. 10a,b), where 4 days post application it caused total deaths recorded 00.0, 22.2, 100.0 and 100.0 % mortality with

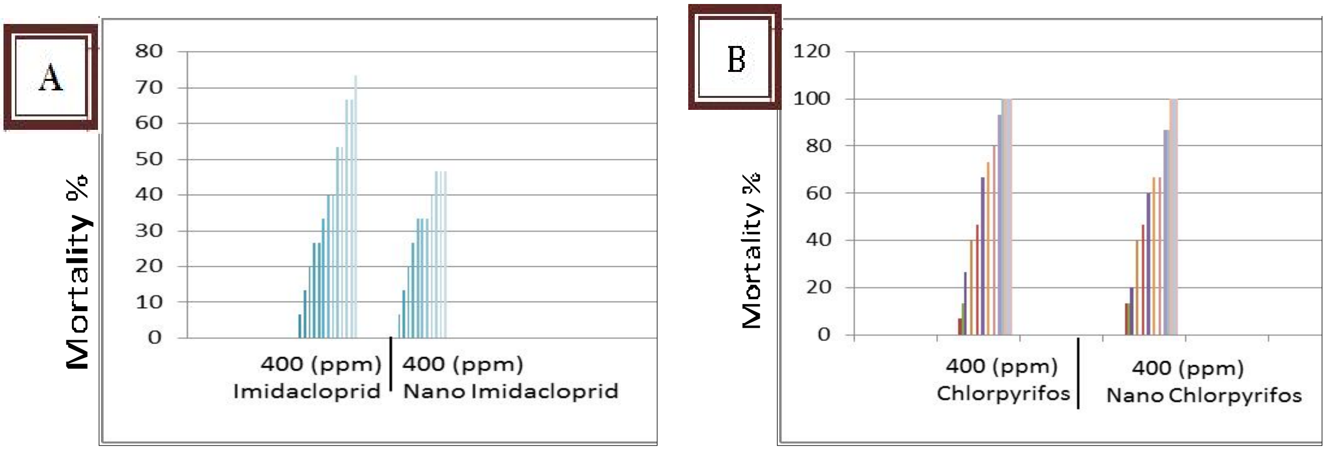

Fig. 6: Mortality % in adults of *R. ferrugineus* caused by (**A**) imidacloprid and nanoimidacloprid treatments, (**B**) chlorpyrifos and nanochlorpyrifos treatments with 400 ppm concentration.

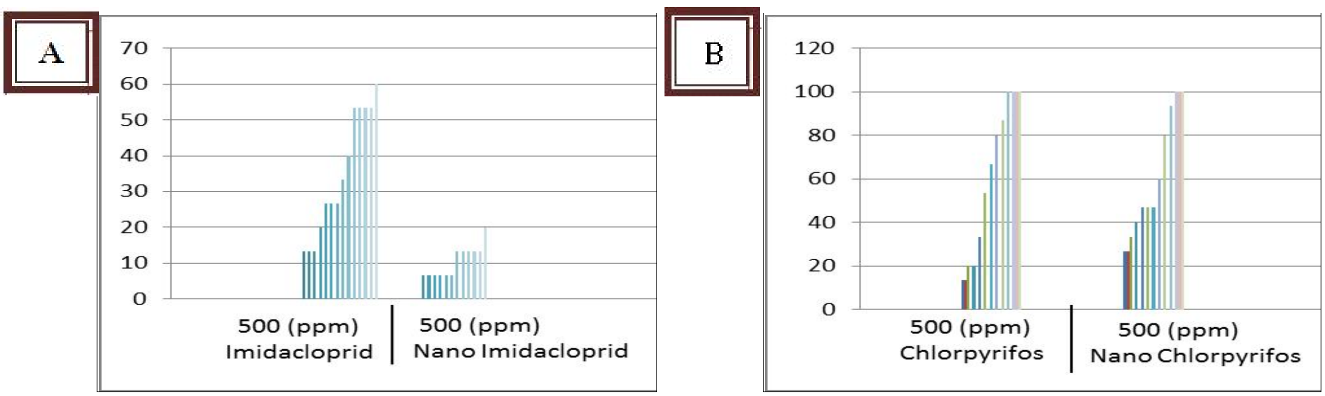

Fig. 7: Mortality % in adults of *R. ferrugineus* caused by (**A**) imidacloprid and nanoimidacloprid treatments, (**B**) chlorpyrifos and nanochlorpyrifos treatments with 500 ppm concentration.

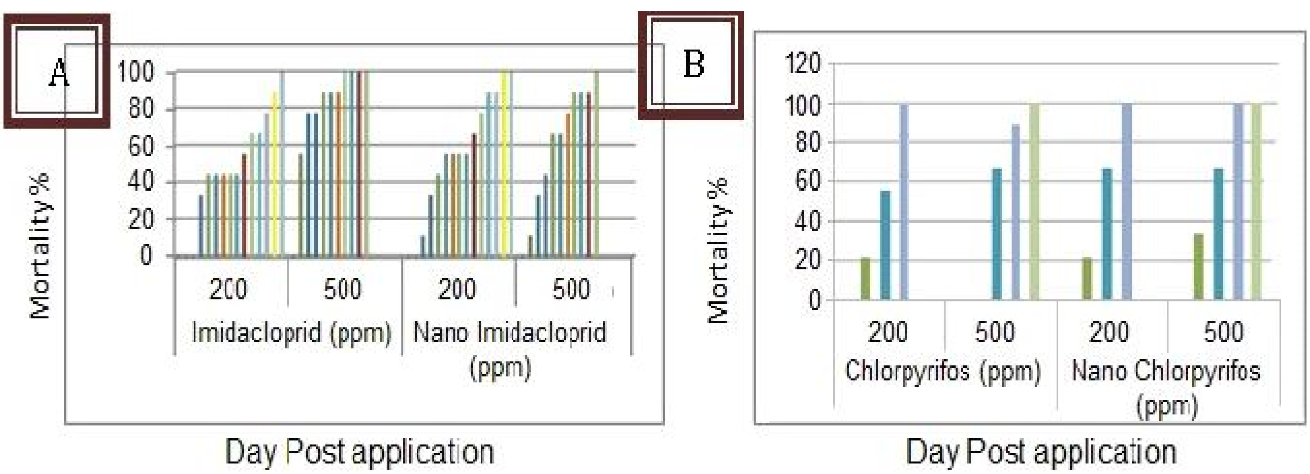

Fig. 8: Mortality in 3rd instar larvae of *Rhynchophorusferrugineus* caused by (**A**) imidacloprid and nanoimidacloprid and (**B**) chlorpyrifos and nanochlorpyrifos.

200 ppm and 00.00, 11.1, 66.7 and 100.00 % with 500 ppm, respectively. Chlorpyrifos came second in this order recorded 00.0, 00.0, 66.7 and 100.0% mortality with 200 ppm and 00.0, 00.0, 55.6 and 100.0% mortality with 500 ppm. Imidacloprid followed with 44.4 % mortality in both concentrations 200 and 500 ppm. Nano imidacloprid gave 44.4% mortality at 200 ppm while 33.3 % at 500 ppm four days post application.

Susceptibility of the9th instar larvae

Nano chlorpyrifos was singled out again with both 200 and 500 ppm concentrations (Fig. 11a,b), where 5 days post application it caused total deaths recorded 00.0, 22.2, 55.6, 77.8 and 100.0 % mortality with 200 ppm and 00.0, 11.1, 55.6, 100.0% and 100.0 % mortality with 500

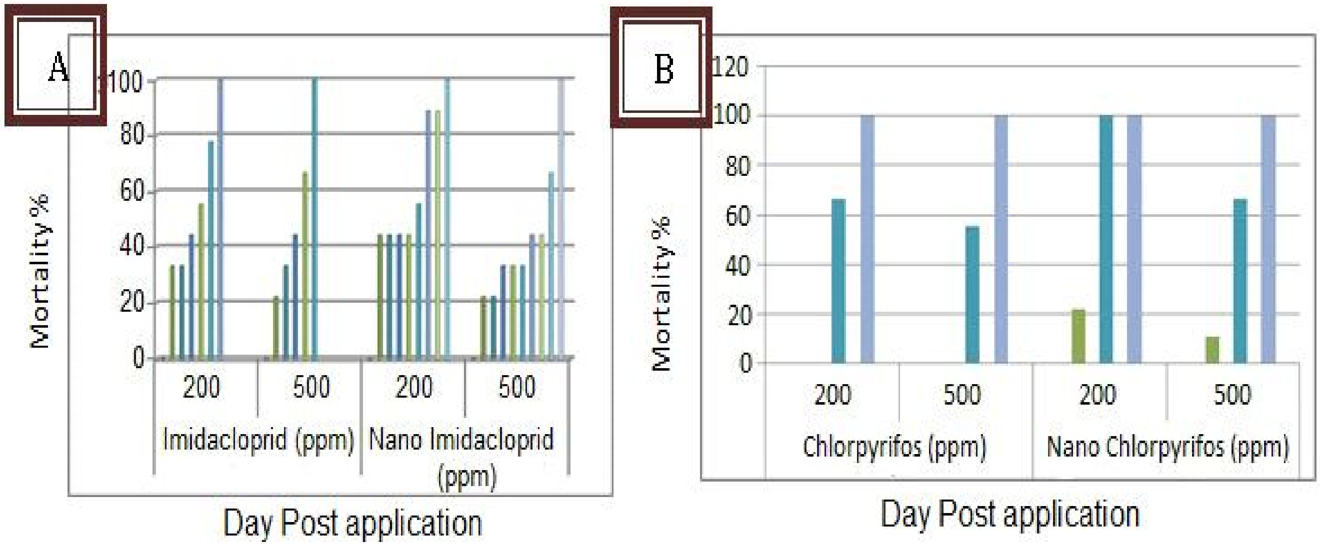

Fig. 9 : Mortality in 6th instars larvae of *Rhynchophorusferrugineus* caused by (**A**) imidacloprid and nanoimidacloprid and (**B**) chlorpyrifos and nanochlorpyrifos.

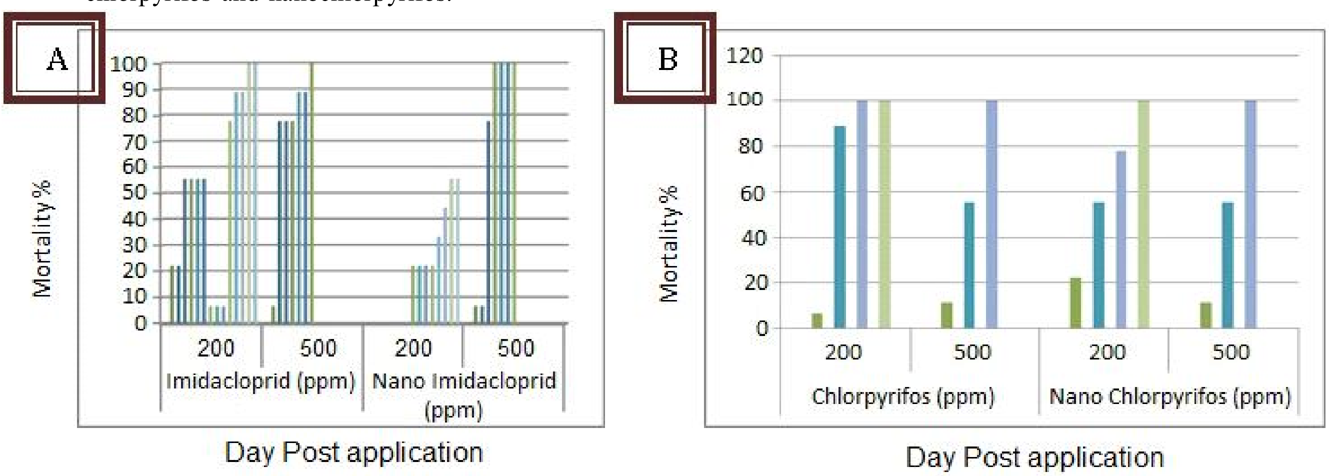

Fig. 10: Mortality in 9th instars larvae of *Rhynchophorusferrugineus* caused by (**A**) imidacloprid and nanoimidacloprid and (**B**) chlorpyrifos and nanochlorpyrifos.

Fig. 11: (A) Early infection with red palm weevil (oozing of brownish Fluid). (B)Filling the injection hole with a piece of fiber and mud after the injection.

ppm, respectively. Chlorpyrifos came second recorded 00.0, 66.7, 88.9, 100.0 and 100.0% mortality with 200 ppm and 00.0, 11.1, 55.6, 100.0% and 100.0% mortality with 500 ppm. At five days post treatments, imidacloprid gave lower deaths % of only 55.6 and 77.8 % and only 00.0% which was increased to 100.0 % with

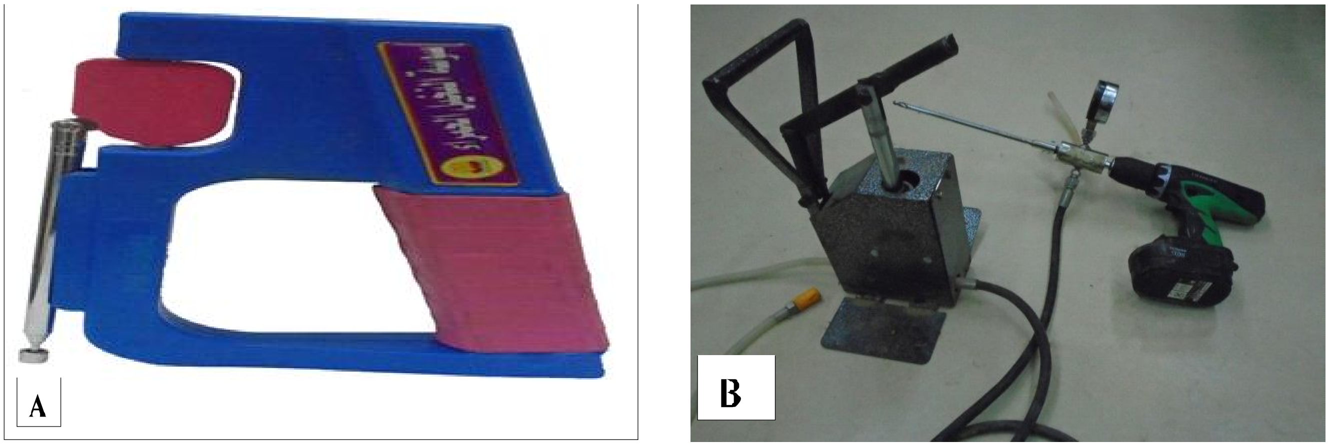

Fig.12. (A) Red palm weevil detection device. (B) Palm injection device.

Table 1: Mean % mortality of *Rhynchophorus ferrugineus* adults caused by imidacloprid and nanoimidacloprid treatments*.

Days post treatment	Imidacloprid (ppm)					Nano imidacloprid (ppm)				
	100	200	300	400	500	100	200	300	400	500
1st	00.0 **(0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)
5th	46.7 (7/15)	20.0 (3/15)	66.7 (10/15)	20.0 (3/15)	20.0 (3/15)	26.6 (4/15)	00.0 (0/15)	66.7 (10/15)	6.7 (1/15)	6.7 (1/15)
10th	80.0 (12/15)	100.0 (15/15)	100.0 (15/15)	40.0 (6/15)	40.0 (6/15)	66.7 (10/15)	46.7 (7/15)	93.3 (14/15)	33.3 (5/15)	13.3 (2/15)
15th	93.3 (14/15)	100.0 (15/15)	100.0 (15/15)	73.3 (11/15)	60.0 (9/15)	86.7 (13/15)	93.3 (14/15)	93.3 (14/15)	46.7 (7/15)	20.0 (3/15)

*All control treatments were alive until end of tests.

**Numbers in brackets are no. of dead /total no. of tested insects.

Table 2: Mean % mortality of *Rhynchophorus ferrugineus* adults caused by chlorpyrifos and nanochlorpyrifos treatments*.

Days post treatment	Chlorpyrifos (ppm)					Nano chlorpyrifos (ppm)				
	100	200	300	400	500	100	200	300	400	500
1st	00.0** (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)	00.0 (0/15)
5th	60.0 (9/15)	53.3 (8/15)	6.7 (1/15)	26.7 (4/15)	20.0 (3/15)	46.7 (7/15)	53.3) (8/15)	20.0 (3/15)	20.0 (3/15)	33.3 (5/15)
10th	93.3 (14/15)	86.7 (13/15)	93.3 (14/15)	80.0 (12/15)	80.0 (12/15)	100.0 (15/15)	93.3 (14/15)	86.7 (13/15)	66.7 (10/15)	60.0 (9/15)
15th	100.0 (15/15)	100.0 (15/15)	100.0 (15/15)	100.0 (15/15)	100.0 (15/15)	100.0 (15/15)	100.0 (15/15)	100.0 (15/15)	100.0 (15/15)	100.0 (15/15)

*All control treatments were alive until end of tests.

** Numbers in brackets are no. of dead /total no. of tested insects.

nanoimidacloprid treatment at both 200 and 500ppm, respectively.

Effect of exposure time on the toxicity of nanochlorpyrifos as one of the highly toxic insecticide against both adults and larvae revealed that toxicity increased with increasing exposure time from 24 hr. to 96 hr. In the meantime, the data showed that the susceptibility of *R. ferrrugineus* larvae was higher than that of adults to this insecticide. In general, the results showed that nanochlorpyrifos and chlorpyrifos had a

Table 3. Comparison between chlorpyrifos and their nano forms on controlling RPW in infested palm tree.

		Dry tunnels	**Dry heart**	**Dry off shoots**	**Death rate**	**Fruits**	**Dry brownish Fluid**	**Duration of the examination**
Control	R1	-	-	-	-	-	-	21 & 30days
	R2	-	-	-	-	-	-	
	R3	-	-	-	-	-	-	
	R4	-	-	-	-	-	-	
Chlorpyrifos	R1	"	×	×	-	×	-	
	R2	"	×	×	-	×	-	
	R3	"	×	×	-	×	-	
	R4	"	×	×	-	×	-	
Nano Chlorpyrifos	R1	"	×	×	-	×	"	
	R2	"	×	×	-	"	"	
	R3	"	×	×	-	"	"	
	R4	"	×	×	-	×	-	

remarkable effect on all tested larval instars. By increasing time of exposure, the toxicity of imidacloprid increased which might relate to the dose of insecticide injested by insects sufficient to suppress biochemical targets. Obtained data revealed that imidacloprid was not effective at all when transferred into nano form. The extra usefulness could achieved if this insecticide implemented in the integrated pest management programs IPM's that delivered to control the palm insect pests. The advantages of nanochlorpyrifos are its systemic properties, broad spectrum activity with relative low rate of application, long lasting efficacy and mode of action, (Cox 2001 & Kaakeh 2006). Furthermore, application technique could add another advantage, so drenched into the root zones of palm may offer a practical solution for controlling some palm pests (Howard & Stopek, 1999). Innovative soil application makes nanochlorpyrifos beneficially for IPM programs (Zillekens 2006). Lastly, current efforts are examining the potential developing of nano pesticides and focus on IPM (Abraham *et al*., 1989; Moura *et al*., 1995; Abraham *et al*., 1998; Kaakeh 2006).

Thus, nanochlorpyrifos could break down the life cycle of *R. ferrugineus* and might be suitable to incorporate in the control programs of the pest as a curative agent. Data in Table (2) showed the efficacy of the pesticides in recovery the infested palm trees. RPW detection device and visual detection. So far there is no a guaranteed effective method of control for the pest. Current tactics to control the weevil are largely based on insecticides. Abuzuhairah *et al.,* (1996); Abraham &Vidyasagar (1992) who reported that chlorpyriphos is recommended for R. ferrugineus control. Cabello *et al.,* (1997) and Kaakeh (2006) concluded that imidacloprid (Confidor) may be used to control all ages of *R. ferrugineus* larvae. Nanotechnology is one of the most important fields (Ragaei, and Sabry, 2014; Dimetry and Hussein, 2016). Several studies have pointed to the many uses in agriculture such as application of agricultural fertilizers, pest control. El-bendary and El Helaly, (2013) refer to controlling *Spodoptera littoralis* insect using nano-silica has been given high toxicity in all concentrations used. *Bacterica dorsalis* could be controlled by loading the pheromones in the form of nanogel and had a high survival period (Bhagat *et al*., 2013). Nano imidacloprid has been used to control flour beetle *Tribolium confusum* acquelin (Coleoptera: Tenebrionidae) and *Myzus persicae* (Sulzer) (Homoptera: Aphididae), (Sabbour 2015; Assemi *et al*., 2014). Four replicates were chosen from the palm farms in Giza governorate using concentration 500ppm of the pesticide and their nano from each palm. The efficiency of pesticides ware evaluated after 21 and 30 days on outer symptoms through dryness tunnels and the heart of tree, growth new off shoots, stopping oozing brown fluid, this study carried out to evaluate the toxicity of nano-pesticides against red palm weevil. The results showed that chlorpyrifos and nanochlorpyrifos were the most efficient to control RPW.

The Fourier Transform Technique

Some of the major advantages of FT-IR over the dispersive technique include: Speed- because all of the frequencies are measured simultaneously, Sensitivity- the detectors employed are much more sensitive, the optical throughput is much and the fast scans enable the condition of several scans in order to reduce the random measurement noise to any desired level and mechanical simplicity the moving mirror in the interferometer is the only continuously moving part in the instrument. Thus, there is very little possibility of mechanical breakdown finally these instruments are self-calibrating, (Griffiths & Fuller 1982; Griffiths & De Haseth 1986; Calvert *et al*., 1993; Reuter *et al*., 1998; Skoog *et al*., 1998; Johnson *et al*., 2000). Data given in Fig.3 revealed that nanochlorpyrifos gave 15 peaks as compared to 16 peaks with chlorpyrifos while nanoimidacloprid gave only 13 peaks compared to 22 active peaks with imidacloprid.

TEM analysis

Both imidacloprid and chlorpyrifos nanoparticles revealed nano size where the mean of counted particles were less than 100nm for nanochlorpyrifos (with median range 44.5) and nanoimidacloprid (with median range 88.7) (Fig. s1 & 2).

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