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Joint Toxicity of Insecticides and some Entomopathogenic Nematode Species against *Rhynchophorus ferrugineus* (Olivier) Insect *In Vitro*

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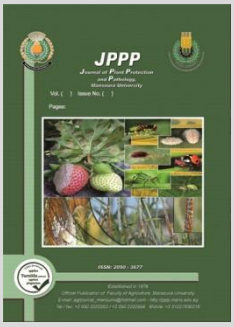

ABSTRACT

Efficacy of imported and local entomopathogenic nematodes (EPNs) alone or in combinations with the insecticides, imidacloprid, zeta-cypermethrin, profenfos and emmectin benzoate against red palm weevil (RPW), *Rhynchophorus ferrugineus* differed greatly according to nematode species and instar larvae under laboratory conditions.Both *Heterorhabditis bacteriophora* (HP88 strain) and local species *H.bacteriophora* (Ar-4 strain) showed promising results by killing 92.20 and 82.13 % of the 4th instar larvae after 9 days of exposure, respectively. Whereas, *S. feltiae* and *H. bacteriophora* (Ht strain) showed less insecticidal activity against 4th, 9th and 11th instar larvae. On the other hand, Egyptian *H. bacteriophora* (Ar-4 strain) was more effective than *H.bacteriophora* (Ht strain). Adults of *R. ferrugineus* were less susceptible to nematode infection than instar larvae. According to LD_{50} with LT_{50} values, *H. bacteriophora* (HP88 strain) was more aggressive killer to RPW than native strains and LT_{50} values were 5.693, 4.319 and 2.943 days at concentrations of 500, 1500 and 2500IJs/ml, respectively. At the LC_{50} and LC_{90} values, imidacloprid was the most toxic for 9th and 11th Instars of *R. ferrugineus*, whereas, profenfos was the least effective one after 24 hr of exposure. The joint action of chemical pesticides with IJs of EPNs in controlling the 9th and 11th instar larvae of *R. ferrugineus* showed an additive or potentiation reaction with no evidence of antagonistic action.Overall, results indicate the feasibility of an integrated use of these nematode species and chemical pesticides in controlling red palm weevil under field conditions.

Keywords: EPNs, Insecticides, *Rhynchophorus ferrugineus*, Control, Lethal toxicity, Compatibility, Joint action.

INTRODUCTION

Red palm weevil, *Rhynchophorus ferrugineus*, (Olivier) (Family: Curculionidae, Order: Coleoptera) was recorded for first time in Egypt in date palm plantations at Sharkia and Ismailia Governorates (Saleh, 1992; and Saleh and Gouhar, 1993). Red palm weevil, *R. ferrugineus* is observed as extremely dangerous insect pests of 25 species of palm trees in Africa, South East part of the Asia and Middle East (El- Sabea *et al.*, 2009 & Falerio , 2006 and Falerio *et al.*, 2016). Many methods used for control red palm weevil (RPW) including entomopathogenic fungi (EPFs) and nematodes (EPNs) showed great potential against different insect pests (Thurston *et al.*,1993; Koppenhöfer *et al.*, 1999 ;Yasin *et al.*, 2017; Wakil *et al.*, 2017and Abd El-Fattah *et al.*, 2020). Chemical control is considered the main rapid treatment of infected palm trees and one of the most effective methods in pest control (Al Dawood *et al.*, 2013 and Abdel-Salam *et al.*, 2014). Nano-Imidacloprid and chloropyrophos caused damage included vaculation of cytoplasm, analyzes and destroyed nuclei of the epithelial cells in *R. ferrugineus* (Abd El-Fattah *et al.*, 2020). In Egypt, many attempts have examined the efficacy of EPNs in laboratory as well as field conditions against red palm weevil (Shamseldean , 2004 ; Shamseldean and Atwa , 2004; Atwa and Hegazi, 2014). The larger proportion of formulations in the insecticide-acaricide-nematicide group were harmful to juveniles of *Steinernema carpocapsae* and *S. feltiae* (Rovesti and Deseö,1990). EPNs being highly lethal to many important insect-pests, their IJs are tolerate short-term exposure to many chemical and biological insecticides, fungicides, herbicides, fertilizers and growth regulators, hence providing an opportunity of tank-mixing and application together. EPNs are also reported to be compatible with a number of agrochemicals and their use can offer a cost-effective alternative to pest control (Vashisth *et al.*, 2013). To save time and money, pesticides can be tank-mixed or applied simultaneously with EPNs in IPM (Poinar,1990).

Application of EPNs in IPM program ask more information about the joint action with chemical pesticides to predict the EPNs efficiency by simultaneous or sequential application of chemical pesticides and its effect on EPNs viability, so, the aim of combination between nematodes and other control agents is to achieve better control than of a single-use through additive or, preferably, synergistic effects on pest mortality (Nardo and Grewal, 2003; Laznik et *al.*, 2012 ; Laznik and Trdan 2014) However, an ecologically integrated approach to pest management involving nematode-chemical insecticide combinations in tank mixes can be developed for foliar application, but, the compatibility of these nematodes with new and routinely used insecticides needs to be established.

The objectives of the study were to evaluate the effects of different biocontrol method (EPNs) with common chemical pesticides on red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) *in vitro*. The strategy during this study was to use native entomopathogenic nematode previously isolated

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by EL-Ashry *et al*. (2018) and compare its bioefficacy with imported EPNs when they are applied alone or combined with certain pesticides against larval stages of *R. ferrugineus* in addition to suitable local species for future management under field conditions.

MATERIALS AND METHODS

1-Collection and rearing of the red palm weevil, *R. ferrugineus*.

Adults

Adults collected from the field were cleaned and kept as group of at least 10 males and females but not sexed, in rectangular boxes with press-on tight-fitting lids., or kept as individual pairs of males and females in plastic boxes measuring 25x18x12 cm. All adults were provided with freshly and soft shared sugarcane stems tissues for feeding and egg laying. Boxes were staked side by side (or on the top of each other) on working benches. Few holes were made on all lids of boxes and jars for ventilation. Females lay their eggs on freshly and soft shared sugarcane stems tissues (i.e., oviposition site). Association of both sexes for 24 hr ensures fertilization of females and no further mating of females was necessary (Kaakeh, 1998).Adults after emergence from cocoons were sexed and kept separately in small jars(1liters) for mating and egg laying. Sexing the adults was identified according to the presence of a series of black hairs on the dorsa, frontal part of snouts of males and their absence in the females.

Eggs

Freshly and soft shared sugarcane stems tissues holding eggs were removed from the oviposition sites (i.e., large plastic boxes) and placed in separate boxes. Shared sugarcane stems tissues were wet with water to avoid drying. Other eggs were transferred daily with the camel soft hair brush and placed on wet filter papers inside the petri dishes for further studies. New shared sugarcane tissues saturated with others, were placed in all containers holding the adult stages. After 2 to 3 days, larvae from hatched eggs were removed to separate containers and provided with pieces of sugarcane stems for feeding.

Larvae

Newly hatched larvae on sugarcane stems were transferred with a camel's hair brush to pieces of sugarcane stems (at least 15 mm in diameter). A small hole was made at the end of each piece of sugarcane stem using a cork borer. One week after feeding, larger larvae were transferred to larger fresh pieces of sugarcane stems (10–40 mm in diameter; this was based on the size of larvae at different developmental stages). Last larval instars made cocoons from the fibers inside the sugarcane stems. When sugarcane was infested with *Drosophilla* flies, yellow sticky traps were placed above the rearing containers as a control tool. Also, larvae were reared individually in sugarcane stems to avoid cannibalism.

New sugarcane pieces were used to feed the neonate larvae until pupation, next they were placed in adult jars for mating and purposes of feeding, oviposition again. The new RPW colony was established in boxes sized 30×60×60 cm with a mesh gauze (60 cm) covered lid in the middle portion (10 cm) used for aeration purpose. After 3 days, the diet was replaced with the pieces of sugarcane.

Pupae

After 10-14 days of feeding of last larval instars, the sugarcane stems were split open and cocoons were collected. Cocoons were placed in a plastic container's plastic boxes sized 60x60x30 cm, wet with water as needed, and closed with lids. Two weeks after collecting the cocoons, they were checked daily for adult emergence. Adults were collected by hand and placed in plastic containers (as unsexed groups of adults) or placed individually in glass jars (as sexed, paired males and females). Such a method provided enough insects in all developmental stages required for the experimental work (Mahmoud *et al.*, 2015).

2-Source and culturing of entomopathogenic nematodes (EPNs)

Infective juveniles (IJs) of the tested nematode species were *Heterorhabditis bacteriophora* (HP88), *H.bacteriophora* (Ht strain) isolated from Giza, *H.bacteriophora* (Ar-4 strain) isolated from EL-Arish, *Steinernema carpocapsae* (All strain) and *S. feltiae* . EPNs were cultured separately in last instar larvae of the greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) according to the technique of Dutkey *et al*. (1964). IJs emerged from cadavers were stored in distilled water at 12 °C for one week until application (Woodring & Kaya, 1988).

Greater wax moth, *Galleria mellonella* (Fabr.):

The greater wax moth, *G. mellonella* were reared under laboratory condition according to Woodring and Kaya (1988) and Kaya and Stock (1997) to mass production of tested entomopathogenic nematodes.

3-Testing EPNs efficacy (virulence) against adults and larval population of red palm weevil (RPW)

Collections of five larvae (3rd, 4th, 9th and 11th larval instars), and adults were used in the current assays. Plastic boxes sized 9x5x5 cm were used and Whatman filter papers were placed on the base of each. Larval instars (10 larvae), were inoculated with 250 IJs of EPNs /ml in separate glass Petri dishes (9 cm diameter) at 24±2°C incubation. While adults (10 adults) were separately inoculated with three concentrations of EPNs i.e. 500, 1500 and 2500IJs/ml. Water was used for control treatment. Observations were recorded after 24 hours and mortality was recorded till 9 days post-treatment. To distinguish between virulence of nematode species, infection and time factor was also measured by measuring the percentage mortality after 24 hours of duration. The bioassays were repeated three times for every treatment. Cadavers were transferred individually to a white trap to determine the emergence of new generation of IJs.

4-Toxicity of insecticides on larvae of *R. ferrugineus*

Test Insecticides

Four commercial insecticides namely imidacloprid (Best 25 % EC), zeta-cypermethrin (fury 10 % EC), profenfos (Sylian 72 % EC) and emmectin benzoate (Pasha 1.9 % EC) were supplied by Plant Protection Research Institute, Giza Governorate, Egypt and used in current study.

Treatments

Toxicity bioassays were conducted with larval of *R. ferrugineus* populations exposed to insecticides using dipping food bioassay technique (Ajlan *et al.*, 2000 and Shawir *et al*., 2014). Serial dilutions of each test insecticides (ug a.i./ml) of imidacloprid, zeta-cypermethrin, profenfos and emmectin benzoate were prepared besides the control that comprised only water. The 9th and 11th of *R. Ferrugineus* larvae were chosen for bioassay assessment. Three replicates were used

for each insecticide concentration and bioassays carried out following a completely randomized design. Ten individuals of larvae per each were used for each concentration. Fresh sugarcane stem pieces were dipped in insecticides dilutions for one minute, then treated pieces were allowed to dry before offering to the larvae. This trial was carried out in the incubator at 26±1ºC and 65±5% RH. Mortalities were recorded in the treated and control treatments after 24 hr of exposure. Larval mortality was scored: if no movement was observed, larvae were considered as dead. Natural mortality was corrected according to Abbott's formula (Abbott, 1925). Concentration-mortality results were subjected to a Probit analysis correcting the data for natural mortality (BIOSTAT PROBIT). The 25, 50, 75 and 90% lethal concentrations (LC_S) were recorded from concentration mortality curves .Toxicity index (T.I.) was determined by using Sun's equation (1950) as follows: LC_{50} or LC_{90} of the highest efficient compound/ LC_{50}or LC_{90} of the other compound×100.

5-Joint action technique

Spray solution of the tested entomopathogenic nematodes at the concentration that kill 25% of 9th and 11th of *R. Ferrugineus* larvae was prepared separately. Toxicity lines of the insecticide, imidacloprid, zuta-cypermethrin, profenfos and emmectin benzoate were done. Spray solutions of the tested insecticide at the concentration that kill 25 and 50% of laboratory strain were prepared separately. Hundred milliliters of the tested solution of entomopathogenic nematodes was mixed with an equal quantity of any of the two prepared solutions of the insecticide. The efficacy of these mixtures was evaluated as described previously. However, fenamiphos was used for its potency against nematodes as positive control. The joint action data of the tested mixtures in terms of Co-toxicity Factor (C.F.) was estimated according to Mansour *et al.* (1966) using the following equation:

$$C.F = \frac{\%\,\text{Observed mortality} - \%\,\text{Expected mortality}}{\%\,\text{Expected mortality}} \times 100$$

This factor was used to classify results into three categories. A positive factor 20 or more is considered potentiation, a negative factor 20 or more means antagonism and intermediate values between–20 and + 20 indicated additive effect.

RESULTS AND DISCUSSION

Percentage mortality obtained from entomopathogenic nematodes (EPNs) used against different larval stages of red palm weevil larvae greatly differed according to nematode species and larval stages. Both *Heterorhabditis bacteriophora* (HP88 strain) and *H.bacteriophora* (Ar-4 strain) showed promising results by killing 92.10 and 82.13 % of the 4th instar larvae after 9 days of exposure, respectively. (Table 1). Whereas, *S. feltiae* and *H. bacteriophora* (Ht strain), showed less effectiveness as insecticidal activity against 4th, 9th and 11th instar larvae of red palm weevil. Efficacy of tested imported and local nematode species decreased gradually with 9th and 11th instar larvae of red palm weevil after 14 days of application. Efficacy of three imported nematode species, *H. bacteriophora* (HP88 strain), *S. carpocapsae* (All strain) and *S.feltiae* compared to Egyptian *Heterorhabditis* strains , *H. bacteriophora* (Ar-4 strain and Ht strain as insecticidal activity against red palm weevil larvae under laboratory conditions clearly showed that Egyptian *H. bacteriophora* (Ar-4 strain) was more effective than *H.bacteriophora* (Ht strain) and the two imported species *S. carpocapsae* (All strain) and *S.feltiae* with percentage mortalities reached 63.42, 25.44 ; 42.44, 21.10 ; 41.44, 30.51 and 32.71,16.54, respectively.

Table 1. Accumulative mortality of larval stage of red palm weevil, *Rhynchophorus ferrugineus* using the entomopathogenic nematodes after 9 days of exposure.

Nematode species	% Mortality of different larval instars			
(250 IJs/10 larvae)	3rd instar larvae(4.61)	4th instar larvae(5.04)	9th instar larvae(16.79)	11th instar larvae(20.63)
Heterorhabditis bacteriophora (HP88)	100 a	92.20 a	74.20 a	40.85 a
H.bacteriophora (Ht strain)	100 a	75.10 c	42.44 c	21.10 c
H.bacteriophora (Ar-4 strain)	100 a	82.13 b	63.42 b	25.44 c
Steinernema carpocapsae (All strain)	100 a	73.42 c	41.44 c	30.53 b
S. feltiae	100 a	45.53 d	32.71 d	16.54 d

Each value is a mean of five replicates with 10 larvae in each replicate.
Tested larvae were observed daily for mortality but table contains data after 9 days.
The same letter (s) in columns indicates no significant differences at P≤ 0.05according to Duncan's multiple range test.

Adults of *R. ferrugineus* were less susceptible to nematode infection than instar larvae. Data in Table (2) showed accumulative mortality of adult stage of red palm weevil, *R. ferrugineus* using three concentrations of EPNs after 9 days of exposure. Percentage moralities ranged from 3.33 to 9.33% with *S.feltiae* and *H. bacteriophora* (HP88 strain) at concentration 2500IJs/adult. Likewise, imported nematode species *H. bacteriophora* (HP88 strain) was more effective one followed by local nematode species *H. bacteriophora* (Ar-4 strain) at the three concentrations.

Results in Table (3) indicated the LD_{50} and LT_{50} values for the adult stage of red palm weevil, *R. ferrugineus* after 9 days of exposure to EPNs. *H. bacteriophora* (HP88 strain) was the most aggressive killer to red palm weevil, *R. ferrugineus* than native strains of *H. bacteriophora* and LT_{50} values were 5.693, 4.319 and 2.943 days at concentrations of 500, 1500 and 2500 IJs/ml, respectively. Whereas, LT_{50} with *H. bacteriophora* (Ar-4 strain), *H. bacteriophora* (Ht strain), *S. carpocapsae* (All strain) and *S.feltiae* were 5.522, 11.034, 5.971 and 13.034 days at concentrations of 2500 IJs/ml, respectively.

The red palm weevil *R.ferrugineus* (Olivier, 1790) is one of the major pest affecting palm trees in Egypt, Arabian Gulf, Middle East and other parts of world (Malumphy and Moran, 2007 & El-Sabea *et al*., 2009). Beside chemical control, application of *Steinernema* and *Heterorhabditis* species does not require masks or other safety equipment as chemicals.

Table 2. Accumulative mortality of adult stage of red palm weevil, *Rhynchophorus ferrugineus* using three concentrations of EPNs after 9 days of exposure.

Concentrations (IJs/ml)/ adult	Nematode species mortality %				
	Heterorhabditis bacteriophora (HP88)	*H.bacteriophora* (Ht strain)	*H.bacteriophora* (Ar-4 strain)	*Steinernema carpocapsae* (All strain)	*S. feltiae*
500 IJs/ 10 adults	6.67 a	3.33 cd	5.33 ab	4.33 bc	2.00 d
1500 IJs/10 adults	8.33 a	5.00 c	6.33 b	6.33 b	2.67 d
2500 IJs/10 adults	9.33 a	6.33 b	7.33 b	7.33 b	3.33 c

Each value is a mean of three replicates with 10 adults in each replicate.
Tested adult stages were observed daily for mortality but table contains data after 9 days.
The same letter (s) in columns indicates no significant differences at P≤ 0.05according to Duncan's multiple range test.

Table 3. LD $_{50}$ and LT $_{50}$ for adult stage of the red palm weevil, *Rhynchophorus ferrugineus* after 9 days of exposure to three concentrations of EPNs.

Concentrations (IJs/ml)/ adult	LD $_{50}$ & LT $_{50}$ (After 9 days) for used nematodes									
	Heterorhabditis bacteriophora (HP88)		*H.bacteriophora* (Ht strain)		*H.bacteriophora* (Ar-4 strain)		*Steinernema carpocapsae* (All strain)		*S. feltiae*	
	LD $_{50}$	LT $_{50}$	LD $_{50}$	LT $_{50}$	LD $_{50}$	LT $_{50}$	LD $_{50}$	LT $_{50}$	LD $_{50}$	LT $_{50}$
500 IJs/10 adults	257.1087	5.693	15,683.580	17.696	410.0843	7.339	715.0215	9.912	15,683.580	17.696
1500 IJs/10 adults		4.319		15.3801		6.1195		6.9623		15.3801
2500 IJs/10 adults		2.943		11.0347		5.522		5.9711		13.0347

Our results are related with Gözel *et al.* (2015), Triggiani and Tarasco (2011) in respect to the applications of steinernematid and heterorhabditid nematodes against the *R. ferrugineus* larvae. Clausi and Vinciguerra (2009) reported higher susceptibility of small larvae (the 4th stage larvae) rather than the large larvae (9th and 11th) to the tested nematode species. As well as, the minimum infestation of EPNs at adult stage of *R.ferrugineus* population were also reported by Atwa and Hegazi (2014) who defined that *H.bacteriophora*, *S. carpocapsae* and *S. feltiae* undergo minimum% age infestation of 15.3, 8.0 and 2.0%, respectively.

Results revealed that, in most cases imported EPNs (*S.carpocapsae* All strain and *H. bacteriophora* (H88 strain) belonging to steinernematids and heterorhabditids were more effective than local isolates of *H. bacteriophora* (Ar-4 strain and Ht strain) against larvae and adult stages of *R. ferrugineus* at high (2500 IJs/ml) or low concentrations (500 IJs/ml).

Toxic effects of the tested insecticides against the red palm weevil, *Rhynchophorus ferrugineus* (Olivier)

Four insecticides belonging to different groups were selected to study their toxic effects against 9th and 11th instars larvae of *R. ferrugineus* as follows: imidacloprid, zeta-cypermethrin, profenfos and emmectin benzoate using dipping food technique. The toxic patterns of such insecticides against the 9th and 11th instars larvae of *R. ferrugineus* and LC values obtained from probit analysis after 24 hours are given in Table (4) and Fig.(1). At the LC_{50} and LC_{90}, imidacloprid was the most toxic one against the two tested instars larvae of *R. ferrugineus* (9th and 11th instars) whereas, profenfos was the least effective one. Hence, LC25, LC50, LC75 and LC 90 of imidacloprid against the 9th instars larvae under study were 49.22, 71.51, 103.90 and 145.48 ug a.i/ml after 24 hr of exposure, respectively (Table 4). Meanwhile, previous lethal concentrations recorded 75.59, 100.44, 133.47and 172.43 ug a.i/ml against the 11th instars larvae of *R. ferrugineus* after 24 hours of treatment, respectively. In laboratory and semi-field conditions, imidacloprid successfully controlled *R. ferrugineus* (Kaakeh, 2006, Abd El-Fattah *et al*., 2020). Emmectin benzoate and zeta-cypermethrin were moderately toxic against the both two larval instars. Biopesticide, emmectin benzoate came in the second position after imidacloprid recording LC25 = 128.20; LC50= 158.92; LC75=197.00 and LC90 =239.07 ug a.i/ml against the 9th instar larvae of *R. ferrugineus* after 24 hr of exposure. Meanwhile, the previous lethal concentrations recorded 193.54, 230.69, 274.96 and 322.10 ug a.i/ml against the 11th instars larvae after 24 hr of treatment, respectively (Table 4). Avermectins, are recommended pesticides for controlling *R. ferrugineus* (MARM, 2014) .

On the other hand, LC values of pyrethroid insecticide, zeta-cypermethrin against the 9th instar larvae of *R. ferrugineus* reached 243.13, 324.08, 433.00 and 559.72 ug a.i/ml for LC25, LC50, LC_{75} and LC_{90}, respectively after 24hr of treatment. However, the previous LC values recorded 257.48, 393.23, 600.55 and 879.57 ug a.i/ml against the 11th instar larvae, respectively (Table 4). Whereas, the organophosphate insecticide, profenfos recorded LC values equal 695.44 (LC25) ,920.29(LC50) ,1217.85 (LC75) and 1567.60 (LC90) ug a.i/ml against the 9th instar larvae. Meanwhile, such lethal concentrations recorded 1096.36, 1379. 65,1736.14 and 2135.63 ug a.i/ml against the 11th instars larvae of *R. ferrugineus*, respectively(Table 4).

Regression line analysis showed high slope values in zeta-cypermethrin (3.11) and zeta-cypermethrin (2.47) against the 11th instar larvae of *R. ferrugineus* followed by imidacloprid (3.07) and Zeta-Cypermethrin (2.50) against the 9th instar larvae of *R. ferrugineus* which indicating homogeneity among individiuals (Table 4 and Fig.1).

However, low slope values were recorded for profenfos and emmectin benzoate.

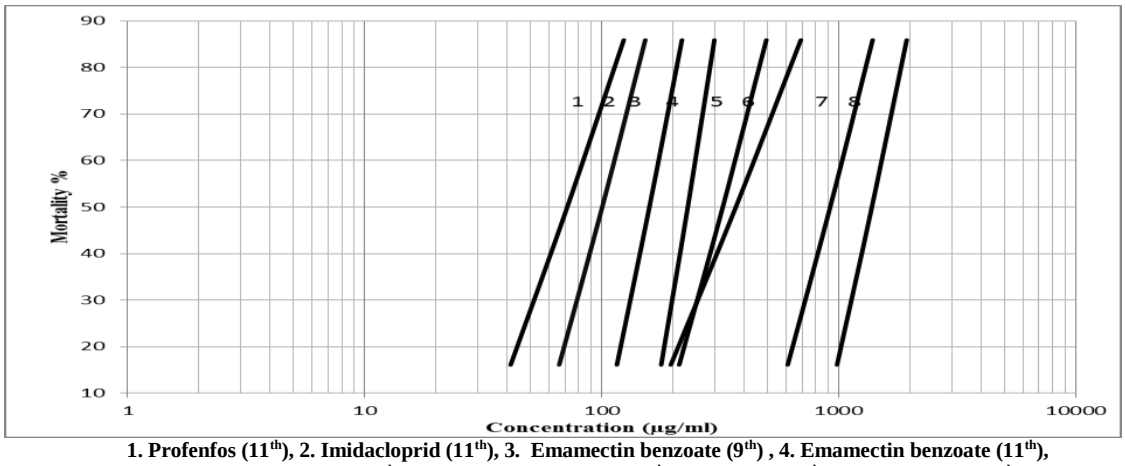
Toxicity index (TI) was obtained by comparing the efficiency of the tested insecticides, at certain level, with a highly potent compound. Therefore, imidacloprid was considered the standard chemical for calculating the toxicity index. The toxicity index values ranged from 0.072 to 100.00for the tested toxicants. The obtained results revealed the general similarity in the trend of the toxicity index at both LC_{50} and LC_{90} (Table, 4 and Fig.1).

Control programs of *R. ferrugineus* in Egypt mostly depend on the use of various conventional insecticides. Current efforts are focused on IPM for the management of *R. ferrugineus* so several studies have proved the effectiveness of insecticides against the red palm weevil. For example (Abraham *et al*., 1992; Moura *et al*. 1995; Abraham and Vidyasagar 1998; Kaakh, 2010; Hamadah and Tanani, 2013; Shawir *et al*., 2014; El-Deeb *et al*., 2015, Al-Saroj *et al*., 2017, Milosavljević *et al*., 2018 and Gonzalez *et al*., 2019).

Table 4. Acute toxicity of imidacloprid, zeta-cypermethrin, profenfos and emmectin benzoate against 9th and 11th instars larvae of the red palm weevil, *Rhynchophorus ferrugineus in vitro* .

Treatment	The 9th instar larvae of red palm weevil, *R. ferrugineus*					
	LC 25 (ug a.i/ml)	LC 50 (ug a.i/ml)	LC 75 (ug a.i/ml)	LC 90 (ug a.i/ml)	Slope (± SE)	TI* (LC50)
Imidacloprid	49.22	71.51	103.90	145.48	3.07±2.51	100.00
Zeta-Cypermethrin	243.13	324.08	433.00	559.72	2.50±8.74	0.220
Profenfos	695.44	920.29	1217.85	1567.60	2.43±24.19	0.077
Emmectin benzoate	128.20	158.92	197.00	239.07	1.71±3.20	0.449
	The 11th instar larvae of red palm weevil, *R. ferrugineus*					
Imidacloprid	75.59	100.44	133.47	172.43	2.47±2.68	100.00
Zeta-Cypermethrin	257.48	393.23	600.55	879.57	3.11±15.58	0.255
Profenfos	1096.36	1379.65	1736.14	2135.63	1.96±29.84	0.072
Emmectin benzoate	193.54	230.69	274.96	322.10	1.22±3.83	0.435

TI* Toxicity index



1. Profenfos (11th), 2. Imidacloprid (11th), 3. Emamectin benzoate (9th) , 4. Emamectin benzoate (11th), 5. Zeta- Cypermethrin (9th), 6. Zeta- Cypermethrin (11th),7. profenfos (9th), 8. Imidacloprid (9th)

Fig. 1. Toxicity lines of the tested insecticides against the 9th and 11th instars larvae of the red palm weevil, *R. ferrugineus*.

Interactions between the tested insecticides and entomopathogenic nematodes

Interactions between chemical pesticides and different species or strains of entomopathogenic nematodes in controlling the 9th and 11th instar larvae of *R. ferrugineus in vitro* were demonstrated in Tables (5&6). LC_{25} and LC_{50} of imidacloprid mixed with different strains of the tested EPNs against the 9th instar larvae of *R. ferrugineus* showed potentiation interaction recording Co-toxicity factor + 36.80 and +23.73, respectively, whereas, LC_{25} and LC_{50} of Zuta-Cypermethrin mixtures showed additive interaction recording +16.40 and +3.46, respectively. On the other hand, LC_{25} of Profenfos presented potentiation interaction recording Co-toxicity factor + 21.60 whereas, LC_{50} of Profenfos displayed additive interaction recording Co-toxicity factor +7.46. As well , LC_{25} and LC_{50} of Emmectin benzoate showed additive effect recording +13.20 and 1.60, respectively. It is important to mention that, *S. feltiae* was the least effective nematode against 9th larvae of *R. ferrugineus* and its infectivity was significantly reduced following exposure to tested pesticides except imidacloprid and these findings were in agreement with Head *et al*.(2000).

Regarding 11th instar larvae of *R. ferrugineus*, only LC_{25} and LC_{50} values of imidacloprid mixed with EPNs showed potentiation interaction recording Co-toxicity factor +33.60 and +20.26, respectively. While, LC_{25} and LC_{50} of Zuta-Cypermethrin , Profenfos and Emmectin benzoate mixed with the tested nematode species showed additive effect and recorded +11.20,+0.80; +18.80,+4.80 and+4.40,-5.06 ,respectively.

On the other hand, fenamiphos as positive control exhibited high toxicity and antagonistic effect against *S. carpocapsae, S. glaseri, H. bacteriophora* (HP88), *S. feltiae* and *H. bacteriophora* (Ba-1) and recorded -26.83, -29.73, -38.39, -40.42 and-42.88, respectively.

Dembilio *et al*. (2010) assessed that combination between imidacloprid and *S. carpocapsae* in a chitosan formulation proved highly effective against *R. ferrugineus* under field conditions, and their efficacies did not significantly change. The synergistic interaction of imidacloprid and both steinernematid and heterorhabditid nematodes was confirmed in a greenhouse experiment on eastern subterranean termite, *Reticulitermes flavipes* **(**Manzoor, 2012) but interaction type depends on abamectin concentration. Also, studies revealed that fenamiphos showed a strong sub-lethal effect on the *S.carpocapsae* and *Heterorhabditis indica* nematode reproductive potential, limiting seriously their possible recycling in the field (Devindrappa *et al.*, 2017). Overall, results indicate the feasibility of an integrated use of these nematode species and chemical pesticides in crop protection (Rovesti and Deseö, 1990). These results are useful to optimize EPN dosages and to estimate their field recycling (Campos-Herrera *et al*., 2008).

Table 5. Joint action of entomopathogenic nematodes mixed with imidacloprid, zeta-cypermethrin, profenfos and emmectin benzoate against the 9th of red palm weevil, *Rhynchophorus ferrugineus* larvae *in vitro*.

Mixtures	% Mortality		Co-toxicity factor (C.F.*)	Response
	Expected	Observed		
Steinernematid and Heterorhabditid species (LC_{25}) + Imidacloprid (LC_{25})	50	68.40	+ 36.80	Potentiation
Steinernematid and Heterorhabditid species (LC_{25}) + Imidacloprid (LC_{50})	75	92.80	+ 23.73	Potentiation
Steinernematid and Heterorhabditid species (LC_{25}) + Zuta-Cypermethrin (LC_{25})	50	58.20	+ 16.40	Additive
Steinernematid and Heterorhabditid species (LC_{25}) + Zuta-Cypermethrin (LC_{50})	75	77.60	+ 3.46	Additive
Steinernematid and Heterorhabditid species (LC_{25}) + Profenfos (LC_{25})	50	60.80	+ 21.60	Potentiation
Steinernematid and Heterorhabditid species (LC_{25}) + Profenfos (LC_{50})	75	80.60	+ 7.46	Additive
Steinernematid and Heterorhabditid species (LC_{25}) + Emmectin benzoate (LC_{25})	50	56.60	+ 13.20	Additive
Steinernematid and Heterorhabditid species (LC_{25}) + Emmectin benzoate (LC_{50})	75	76.20	+ 1.60	Additive

C.F.* : Co-Toxicity Factor

Table 6. Joint action of entomopathogenic nematodes mixed with imidacloprid, zeta-cypermethrin, profenfos and emmectin benzoate against the 11th of red palm weevil, *Rhynchophorus ferrugineus* larvae *in vitro* laboratory.

Mixtures	% Mortality		C.F.*	Joint action
	Expected	Observed		
Steinernematid and Heterorhabditid species (LC_{25}) + Imidacloprid (LC_{25})	50	66.80	+ 33.60	Potentiation
Steinernematid and Heterorhabditid species (LC_{25}) + Imidacloprid (LC_{50})	75	90.20	+ 20.26	Potentiation
Steinernematid and Heterorhabditid species (LC_{25}) + Zuta-Cypermethrin (LC_{25})	50	55.60	+ 11.20	Additive
Steinernematid and Heterorhabditid species (LC_{25}) + Zuta-Cypermethrin (LC_{50})	75	75.60	+ 0.80	Additive
Steinernematid and Heterorhabditid species (LC_{25}) + Profenfos (LC_{25})	50	59.40	+ 18.80	Additive
Steinernematid and Heterorhabditid species (LC_{25}) + Profenfos (LC_{50})	75	78.60	+ 4.80	Additive
Steinernematid and Heterorhabditid species (LC_{25}) + Emmectin benzoate (LC_{25})	50	52.20	+ 4.40	Additive
Steinernematid and Heterorhabditid species (LC_{25}) + Emmectin benzoate (LC_{50})	75	71.20	- 5.06	Additive

C.F.* : Co-Toxicity Factor

CONCLUSION

Based on this research work, it could be concluded that compatibility is not only a species-specific, but also a strain-specific characteristic. Management options to reduce *R. ferrugineus* populations in palms are limited because of the cryptic nature of the pest, so current data revealed that both entomopathogenic nematodes and imidacloprid offer an efficient alternative for its control (potentiation effect) and somewhat with profenfos. Also, results approve that *S. carpocapsae* , *Heterorhabditis bacteriophora* local and imported strains do not waiting for its host but, actively looking for and infecting *R. ferrugineus* larvae. Moreover, steinernematid species were more sensitive than heterorhabditid species. Most tested strains showed additive effect after mixing with the tested insecticides, therefore, as a precaution, mixing can be included when necessary, but it is preferable to use EPN after applying pesticides to avoid adverse effects and also ensure sustainability.

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السمية المشتركة للمبيدات الحشرية و بعض أنواع النيماتودا الممرضة للحشرات على حشرة سوسة النخيل الحمراء تحت ظروف المعمل

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اختلفت كفاءة النيماتودا المتطفلة على الحشرات المعزولة محليا والمستوردة منفردة أو المخلوطة مع المبيدات الحشرية, ايميداكلوبريد, برفينوفوس, زيتا – سيبرمثرين وايماكتين بنزوات ضد الاعمار اليرقية لحشرة سوسة النخيل الحمراء *Rhynchophorus ferrugineus* تبعا لنوع النيماتودا والاعمار اليرقية تحت ظروف المعمل. أظهرت كل من النيماتودا المستوردة *Heterorhabditis bacteriophora* (HP88 strain) والمعزولة محليا *H.bacteriophora* (Ar-4 strain) منفردة نتائج واعدة فى مكافحة يرقات العمر اليرقى الرابع بنسبة موت مقدارها ٩٢,١٠% و ٨٢,١٣ % على التوالى بعد تسعة ايام من المعاملة. بينما كانت نيماتودا *S. feltiae* و النوع المحلى *H. bacteriophora* (Ht strain) أقل كفاءة كمبيدات حيوية ضد يرقات العمر اليرقى الرابع والتاسع والحادى عشر ، ومن ناحية اخرى السلالة المحلية من نيماتودا *H.bacteriophora* (Ar-4 strain) أكثر فاعلية من السلالة المحلية *H.bacteriophora* (Ht strain) . الأطوار الكاملة لسوسة النخيل أقل قابلية للاصابة من الأطوار اليرقية. ولقد أظهر تحليل LT 50 و LD 50 أن النيماتودا المستوردة *H. bacteriophora* (HP88 strain) كانت قاتلا أكثر عدائية لسوسة النخيل الحمراء من الأنواع المعزولة محليا وكان الوقت اللازم لقتل ٥٠% (LT_{50}) من اليرقات المعاملة ٥,٦٩٣ و ٤,٣١٩ و ٢,٩٤٣ يوم عند استخدام تركيز ٥٠٠ و ١٥٠٠ و ٢٥٠٠ طور معدى على التوالى . عند مستوى LC_{50} و LC_{90} ، سجل مبيد إيميداكلوبريد أكثر سمية ضد العمريين المختبرين (العمر اليرقى التاسع و العمر الحادى عشر لحشرة سوسة النخيل الحمراء *R. ferrugineus* ، في حين سجل مبيد بروفينوفوس أقل فعالية ضد العمرين بعد ٢٤ ساعة من التعرض. كما أظهر الفعل المشترك لمبيدات الحشرية التي تم اختبارها مع الأطوار المعدية للنيماتودا المتطفلة على الحشرات والمستخدمة فى مكافحة العمر اليرقى التاسع والحادى عشر لسوسة النخيل الحمراء تفاعلًا إضافيًا أو تنشيطيا مع عدم وجود دليل على الفعل التثبيطى. وعلى كل حال توضح النتائج المتحصل عليها امكانية الجمع بين انواع النيماتودا والمبيدات الكيماوية لمكافحة سوسة النخيل الحمراء تحت ظروف الحقل.