EVALUATION OF SOME ENTOMOPATHOGENS ON THE RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* UNDER LABORATORY AND FIELD CONDITIONS

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Abstract

The present study was carried out at El-Kassasin district Ismailia Governorate, Egypt to evaluate the efficiency of three entomopathogens (*Heterorhabditis. bacteriophora*-HP-88, *Metarhizium anisopliae* and *Bacillus thuringiensis* subsp. *Kurstaki*) against of the red palm weevil, *Rhynchophorus ferrugineus* under laboratory and field conditions. The results revealed that *Heterorhabditis bacteriophora*-HP-88 was most virulent to the red palm weevil, *Rhynchophorus ferrugineus* under laboratory conditions. On the other hand, the activity of *M. anisopliae* and *B thuringiensis* subsp. *Kurstaki* against red palm weevil were low. However, field experiments indicated that *H. bacteriophora*-HP-88, *M. anisopliae* and *B. thuringiensis* subsp. *kurstaki* had no external sign of recovery from the infestation levels.

Key words: *Rhynchophorus ferrugineus*, bioassay, entomopathogens, *Metarhizium anisopliae, Heterorhabditis bacteriophora, Bacillus thuringiensis*.

INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* is known as the most destructive pest to date, coconut and oil palms in Arabic region and south East Asia (Hanounik, 1998). It has been known first in south east Asia until it appeared in United Arab Emirates in 1985, Kingdom of Saudi Arabic in 1987 and Egypt in 1992.

The use of biological control agents in the management of insect pests has increased in recent years. However, there are now deep concerns about environmental pollution and health risks associated with use of chemical insecticides. Among biological control measures are the entomopathogenic fungi which there are potential and environmentally safe to invertebrates and plant species in addition to their wide use in control of many insect pests. Gindin *et al*., (2006) and El-Sufty *et al*., (2007) recommended using the fungi in the management programs of *R. ferrugineus*.

Bacillus thuringiensis is among the most successful biological control agents for the suppression of agriculturally and medically important insect pests being toxic to the larvae of lepidopteron, dipteran and coleopteran insects**.** The efficacy of the

entomopathogenic bacterium *B. thuringiensis* to control red palm weevil, *R. ferrugineus* has been tested in laboratory conditions (Manachini *et al.,* 2011). Although there were evidences indicating midgut damage and feeding inhibition among larvae that survived the treatments.

Entomopathogenic nematodes are effective in controlling a variety of economically important pests including the larvae of several weevil species (Coleoptera: Curculionidae) such as the black vine weevil, *Otiorhynchus sulcatus* (F.), and the Diaprepes root weevil, *Diaprepes abbreviatus* (L.) (Shapiro-Ilan *et al*., 2002). When applied in the laboratory, nematodes are capable of causing host death within 24–48 h; however, under field conditions pest control can be somewhat more unpredictable.

The aim of the present study was to test the susceptibility of the red palm weevil to the entomopathogenic fungus (*M. anisopliae*), entomopathogenic bacteria (*B. thuringiensis* subsp. *kurstaki*) and entomopathogenic nematode (*H. bacteriophora*-HP-88) to provide information help in integrated control program for the red palm weevil.

MATERIALS AND METHODS

1. Red Palm Weevil, *Rhynchophorus ferrugineus*

The red palm weevil, *R. ferrugineus* were reared under laboratory conditions at 28±1ºC and 60-70% R.H. Adult weevils were collected from infested date palm trees at a plantation located at El-Kassasin district, Ismailia Governorate, Egypt. Rearing of the red palm weevil maintained on the stems of sugarcane as described by Kaakeh *et al*. (2001).

2. Entomopathogens:

2.1. *Metarhizium anisopliae* (Metchnikoff).

Fresh slant culture *M. anisopliae* Sorokin was obtained from Insect pathogen Unit at Plant Protection, Research Institute, Agricultural Research Center, Dokky, Giza, Egypt.

The conidia fungus were cultured on agar Petri dishes of Dox medium (Thom and Raper., 1945). The medium consists of Sucrose 20 g/L, $KHPO_4$ 1 g/L, KNO_3 2 g/L, KCl 0.5 g/L , Yeast extract 2g/L, $MgSO_4.7H_2O$ 0.5 g/L , $FeSO_4.7H_2O$ 0.002 g/L and agar 20 g/L and incubated at 25±2 ºC and 60-70% R.H for two weeks. At the end of incubation period the conidia were harvested from the surface of the culture directly by scraping with sterile solution of 0.02% Tween-80. The conidia were separated by filtration through sterilized glass-wool. For experimental work, conidia of *B. bassiana*-9894 were suspended in 50 ml sterile

distilled water with 0.02% Tween-80 and vortexes for 1 minute to break spore chains into individual spores and assure uniform mixing. Spores suspended in sterilized water were counted using haemocytometer counts technique**.**

2.2. Entomopathogenic bacteria *Bacillus thuringiensis* subsp. *Kurstaki*.

Fresh slant culture *B. thuringiensis* subsp. *kurstaki* was obtained from Insect pathogen Unit at Plant Protection Research Institute, Agricultural Research Center, Dokky, Giza, Egypt**.**

Culture condition of *B.t* subsp. *kurstaki* were carried out according to Attathom *et al.* (1995). T3 agar medium was prepared which composed of tryptone 3.0 g; tryptose 2.0 g; yeast extract 1.5 g; $MnCl_2$ 0.005 g; $NaH_2PO_4H_2O$ 9 g and agar 15 g, adjust pH To 6.8. The final volume was made up to 1 liter with distilled water and poured in test tubes and Petri dishes (7-9 ml/tube or dish). The tubes and the Petri dishes were sterilized at 121 ºC for 20 minutes in the autoclave. Tubes and Petri dishes were inoculated with *B. thuringiensis* subsp. *kurstaki* and incubated for 72 h at 30 ºC ±1 and 50-60% R.H.

At the end of the incubation period, the resulted spores were harvested from the surface of the culture directly by scraping with sterile solution. Then, resulting spores in a suspension were counted using the haemocytometer counts technique**.** Five concentrations were prepared i.e., $3x10^9$, $3x10^8$, $3x10^7$, $3x10^6$ and $3x10^5$ spores / ml using distilled water and 0.01% tween-80.

2.3. Entomopathogenic nematode *Heterorhabditis bacteriophora*-HP-88.

The entomopathogenic nematode, *H. bacteriophora*-HP-88 was obtained from stock culture maintained for several generations in the laboratory of the Department of Pest Physiology, Plant Protection Research Institute, Agricultural Research Center, Dokky, Giza, Egypt**.**

The last instars larvae of *G. mellonella* Fabr. (Lepidoptera: Pyralidae) were used to multiply the IJs nematodes, following the methods described by **Kaya and Stock (1997).** Add to the larvae were topically inoculated with IJs using a micropipette, and subsequently incubated at 24 ± 2ºC in a dry chamber until they presented symptoms of infection. *H. bacteriophora*-HP-88 was left for 10-13 days before being put onto a White trap**.** The larvae were then placed in "White traps" to stimulate IJs emergence. When IJs emerged they were harvested every two days and washed three times in 500 ml of tap water. Suspensions were stored in 25 - 30 ml of sterilized distilled water at a concentration of 2000 IJs/ml and stored at 9˚C for no more than two weeks before they were used**.** The considered concentrations were 250, 500, 1000, 1500 and 2000 IJ/ml.

4. Virulence assays.

4.1. Laboratory evaluations of effectiveness of entomopathogens against the red palm weevil.

The effectiveness of *M. anisopliae* against the eggs, larval instars, pupae and adults of the red palm weevil was evaluated. Whereas, the larval instars were exposed to the bacteria, *B.t* subsp. *Kurstaki*. While the nematode, *H. bacteriophora*-HP-88 was tested against the larval and adult stages.

4.1.1. Pathogenicity of *M. anisopliae*.

A serial of concentrations of the two tested fungi were prepared from stock solution in distilled water and 0.02% Tween-80. The considered concentrations were $3x10^5$, $3x10^6$, $3x10^7$, $3x10^8$ and $3x10^9$ spores/ml. The experiments were conducted in plastic cups (9x3cm) as described by Dembilio *et al. (*2010).

4.1.1.1. Eggs exposure.

For bioassay, eggs (1-4 days old) were obtained from egg-laying cages. Ten eggs were placed in each plastic boxes containing 5g sugar cane sawdust. The sawdust was sprayed with fungal spore suspensions ($3x10^5$, $3x10^7$ and $3x10^9$ spores/ml) or an aqueous solution of 0.02% Tween-80 as control (both at a rate of 0.2ml per gram of sawdust) and well mixed. The boxes with eggs were incubated for 7 days at 28^0C in darkness and inspected every day for egg hatching. The eggs bioassay was repeated four times.

4.1.1.2. Larvae exposure.

Groups of five larvae fourth, eighth and eleventh larval instars were directly immersed for 60s in a conidia aqueous suspension of either $3x10^9$, $3x10^8$, $3x10^7$, $3x10^6$ and $3x10^5$ spores/ml, and transferred onto plastic boxes (5 larvae per box) containing filter paper for 24h then transferred onto plastic boxes containing 50g of moist sugar cane sawdust and succulent pieces of sugarcane stems and incubated at 28˚C. Both treated and control solution contained 0.02% Tween-80. Four replicates of 5 larvae per concentration were treated. The larvae were examined after 2, 4, 6, 8, 10, 12, 14 days and dead ones were recorded and removed. Values of LC_{50} were computed at 14 days exposure period by Probit analyses (Finney, 1971). Furthermore, median lethal time (LT_{50}) values were calculated for each treated concentration using log time/Probit relationship.

4.1.1.3. Pupae and Adult exposure.

Groups of five pupae (7 days post pupation) or adults (full grown adult, approximately one week post emergence) were immersed for 60s in 50 ml of a conidia aqueous suspension of either $3x10^9$, $3x10^8$, $3x10^7$, $3x10^6$ and $3x10^5$ spores/ml or in a control aqueous solutions. Both control and treated solutions contained 0.02%

Tween-80. Four replicates per concentration were considered. Subsequently each treated or control weevils was placed in a plastic box with 50g of moist sugar cane sawdust, and incubated at 28°C under a 12:12 L:D regime for 2-3weeks. Fresh substrate was added to the boxes every week. The adults were examined after 2, 4, 6, 8, 10, 12, 14 days and dead ones were recorded and removed. In case of pupae, were inspected at the same time for emergence adults. Values of LC_{50} were computed at 14 days exposure period by Probit analyses (Finney, 1971). Furthermore, median lethal time (LT_{50}) values were calculated for each treated concentration using log time/Probit relationship.

4.1.2. Effectiveness of bacteria *Bacillus thuringiensis* subsp. *kurstaki*.

The experiments were conducted in plastic cups as mentioned before (4.1.1). The shreds of sugarcane were soaked for 30s in a suspension of known concentrations of the tested isolate and then left to dry singly in plastic cups. Groups of instars larvae (4^{th}, 8^{th} and 11^{th}) were confined with treated pieces of sugarcane and incubated at 28 °C. Four replicates of 5 larvae per concentration were treated. Observations on larval mortality were recorded after 7 days of treatment. Larvae fed on untreated sugarcane shreds were used as control. Mortality percentages were recorded and the $LC_{50's}$ were calculated at 7 days post-treatment by Probit analyses (Finney, 1971).

4.1.3. Effectiveness of the nematode, *H. bacteriophora*-HP-88 treatment.

A virulence assay based on the methods described by Shapiro-Ilan *et al*. (2004) was carried out. After washing with distilled water 4^{th}, 8^{th}, 11^{th} instars larvae and adults were expose to 50ml water suspension of entomopathogenic nematode at 250, 500, 1000, 1500 and 2000 IJs/ml in plastic cup (9x3cm) lined with moist Whatman No. 1 filter paper. Each cup contained 5 larvae or adults replicated 4 times for every treatment. Water was used for control treatment. Cups were covered with an aerated lid and incubated at 28°C. Observations were recorded after 24hour till 9 days post-treatment. Cadavers were transferred individually to a white trap to determine the emergence of new generation of IJs. Values of LC_{50} were computed at 9 days exposure period by Probit analyses (Finney, 1971). Furthermore, median lethal time (LT_{50}) values were calculated for each treated concentration using log time/Probit relationship.

5. Field evaluations of the effectiveness of the entomopathogens against the red palm weevil, *R. ferrugineus*.

Field applications were conducted on 17 Fadden's containing 1134 date palm, *P. dactylifera*. The field plantation included naturally RPW infested and non-infested

palm trees ranging in age between 7-12 years (5x6m between palms) at El-Kassasin area, Ismailia Governorate, Egypt. Infested date palms were marked at the start of the experiments.

5.1. Injection of fungus, *M. anisopliae* suspension in palm trees.

A stock solution of the fungal formulation was prepared (2 g *M. anisopliae* spores suspended in 100ml sunflower oil and 0.02 % Tween-80 and then sterilized distilled water was added to make 1liter and well mixed). The trees infested of the surface were chosen to be injected with suspension of fungus, *M. anisopliae*. Seven trees were treated with water suspension of tested fungus at a concentration of 10^8/ml. Each tree infestation received approximately 2 liter of *M. anisopliae* preparation. Infestation sites were observed weekly for recovery (complete drying).

5.2. *B. thuringiensis* subsp. *kurstaki* injection.

The surface-infested trees were injected with suspension of bacterium. Five trees treated with water suspension of tested bacteria at a concentration of 10^8/ml. Each tree infestation received approximately 2 liter of bacterium preparation. Infestation sites were observed weekly till recovery (complete drying).

5.3. Nematode injection.

Infested trees on the surface were injected with infected juveniles. Five trees treated with water suspension of tested nematode at a concentration of 2000 IJs/ml. Each tree infestation received approximately 2 liter of nematode preparation. Infestation sites were observed weekly till recovery (complete drying).

6. Analysis of results.

Insect mortality was recorded daily and corrected by Abbott's formula (Abbott, 1925). Mortality regression lines were estimated according to Finney (1971) using "LdPLine®" software to determine LC_{50}, as well as the slope and regression values. In addition, the time required to kill 50% of the target insect (LT_{50}) was also calculated for each treated developmental stage using log time/Probit relationship.

RESULTS AND DISCUSSIONS

1. Laboratory evaluations of the effectiveness of the entomopathogens against the red palm weevil.

The effectiveness of *M. anisopliae* against the eggs, larval instars, pupae and adults of the red palm weevil was evaluated. Whereas, the larval instars were exposed to the bacteria, *B.t* subsp. *Kurstaki*. While the nematode, *H. bacteriophora*-HP-88 was tested against the larval and adult stages.

1.1. Pathogenicity of the entomopathogenic fungus, *M. anisopliae* isolate to the red palm weevil, *R. ferrugineus.*

Data in Table (1) represent the effect of *M. anisopliae* on different developmental stages of the red palm weevil. The newly laid eggs were treated with different concentrations of *M. anisopliae* ($3x10^9$, $3x10^8$, $3x10^7$, $3x10^6$ and $3x10^5$ spores/ml). which gave the mortality percent 46.64, 35.40, 25.32, 16.97 and 10.65% resectivily, at the corresponding concentrations after 7-9 days post-treatment (Table 1).

Meanwhile, Gindin *et al.* (2006) found that 80% mortality of the tested eggs of *R. ferrugineus* with the entomopathogenic fungus, *M. anisopliae* using $1x10^8$ spores/ml. Efficacy of *M. anisopliae* concentrations against RPW larval, pupal and adult stages is represented in Table (1). The different stages of the red palm weevil showed less susceptibility to *M. anisopliae* isolate.

Data in Table (1) showed that mortality of treated 4th instar larvae ranged from 0.13 to 34.77% after 14 days post treatment when treated with the lowest and highest concentrations, respectively.

On the other hand, mortality did not occur in the 8th, 11th instar larvae and pupa stages within any of the tested concentrations of fungal isolate.

As for adults, the bioassay experiments indicated that the mortality of *R. ferrugineus* was differed according to the fungus concentrations. Mortality percentages was ranged between 0.03 and 20.58% when treated with the lowest and highest concentration of *M. anisopliae*. According to results in Table (2) the LC_{50} values for 4th instar larvae and adults were $1.3x10^{10}$ and $3.1x\ 10^{10}$ spores/ml, respectively.

Table 1. Accumulative mortality of different stages of red palm weevil using *M. anisopliae*.

Concentration Spores/ml	% mortality of developmental stage*					
	Eggs	4th instar larvae	8th instar larvae	11th instar larvae	Pupae	Adults
$3x10^9$	46.64	34.77	0.00	0.00	0.00	20.58
$3x10^8$	35.40	14.79	0.00	0.00	0.00	7.01
$3x10^7$	25.31	4.46	0.00	0.00	0.00	1.66
$3x10^6$	16.97	0.93	0.00	0.00	0.00	0.27
$3x10^5$	10.65	0.13	0.00	0.00	0.00	0.03

* Data were recorded after 14 days post treatment.

Table 2. LC_{50} values of *M. anisopliae* against instar larvae and adults of the red palm weevil.

Developmental stages	LC_{50} (spores/ml)	95% fiducial limits		Intercept	Slope
		Lower	Upper		
Eggs	$5.8x10^9$	-	-	-	0.290± 0.102
4th instar larvae	$1.3x10^{10}$	$2.3x10^9$	$6.3x10^{12}$	1.763± 0.749	0.631± 0.200
Adults	$3.1x10^{10}$	$4.3x10^9$	$1.1x10^{16}$	0.854± 1.306	0.756± 0.326

Merghem (2011) found that the fungus effectively killed the red palm weevil stages. Gindin *et al.* (2006) reported that *M. anisopliae* strains caused mortality up to 80-100% of the larvae and adult weevils under laboratory conditions. Positive results in the biological control of red palm weevil were also obtained by Vitale *et al*. (2009) using a commercial product of *M. anisopliae* mixed with *B. bassiana*, whereas the use of *B. bassiana* only, isolated from a dead RPW, was not successful in killing the adults.

1.2. Pathogenicity of entomopathogenic bacterium, *B. thuringiensis* subsp. *kurstaki* isolate to the red palm weevil, *R. ferrugineus.*

The 4th, 8th and 11th were used to evaluate the biological activity of *B. thuringiensis* subsp. *kurstaki* isolate. The larvae and adults were exposed to the bacterial-treated shreds sugarcane for 2 days then to untreated shreds sugarcane for 5 days. The bioassay data for the bacterium isolate against red palm weevil were recorded after 1 to 7days. Data in Table (3) showed that the accumulative mortality percentage in the 4th instar larvae caused by *B.t* subsp. *kurstaki* ranged from 0.01 to 22.29% after 7 days post treatment. Meanwhile, mortality did not occur in the 8th and 11th instar larvae within any of the tested concentrations of *B.t* isolate. The LC_{50} value for 4th instar larvae was $3.1x10^{10}$ spores/ml (Table, 4).

This finding is matching with Manachini *et al.,* 2009 which tested the efficacy of the entomopathogenic bacterium *B. thuringiensis* to control the red palm weevil, *R. ferrugineus* in laboratory. They stated that although there were evidences indicating midgut damage and feeding inhibition among larvae that survived the treatments, results showed that the activity of *B. thuringiensis* against *R. ferrugineus* immature stages was low. On the contrary, Sivasupramaniam *et al*., 2007 found differences in susceptibility of the RPW to *B. thuringiensis*. However, a larval growth inhibition was observed for the red palm weevil and an interaction with hemocytes was primarily

described, showing that the bacterium was able to grow in the hemolymph when ingested by the larvae (Manachini *et al.,* 2011).

The growth of the gram-positive *B. subtilis* and *B. thuringiensis* is inhibited by the polar extracts of red palm weevil adults and large larvae (El-Sufty *et al.*, 2007).

Table 3. Accumulative mortality of different instar larvae of red palm weevil by using *B. thuringiensis* subsp. *Kurstaki* after 7days.

Concentration	% mortality of developmental stage		
Spores/ml	4th instar larvae	8th instar larvae	11th instar larvae
$3x10^9$	22.29	0.00	0.00
$3x10^8$	6.45	0.00	0.00
$3x10^7$	1.15	0.00	0.00
$3x10^6$	0.12	0.00	0.00
$3x10^5$	0.01	0.00	0.00

Table 4. LC_{50} values of *B. thuringiensis* subsp. *Kurstaki* against 4th instar larvae of the RPW.

Treated stage	LC_{50} (spores/ml)	95% fiducial limits		Intercept	Slope
		Lower	Upper		
4th instar larvae	$3.1x10^{10}$	$5.2x10^9$	$9.4x10^{12}$	0.854± 0.939	0.756± 0.230

1.5. Pathogenicity of entomopathogenic nematode, *H. bacteriophora*-HP-88 to the red palm weevil.

The effects of *H. bacteriophora*-HP-88 on mortality of *R. ferrugineus* instar larvae and adults were investigated. The different instar larvae and adults were expose to 50ml water suspension of entomopathogenic nematode at 250, 500, 1000, 1500 and 2000 IJs/ml. Observations were recorded after 24 hour till 9 days post-treatment. Data in Table (5) indicated that high virulence of tested nematode to 4th instar larvae and adults after 9 days of treatments. *H. bacteriophora*-HP-88 looked the most effective nematode against the 4th instar larvae because it achieved 100% mortality at all tested concentrations.

Meanwhile, the 8th and 11th instar larvae were less susceptible to nematode infection than 4th instar larvae and adults. The nematode was more effective on the 8th instar larvae than the 11th instar larvae. Mortality in 8th and 11th instar larvae ranged from 34.71 to 85.20% and 16.54 to 67.94% due to nematode, *H. bacteriophora*-HP-88 treatment after 9 days, respectively.

The third stage of nematode juveniles of infect and kill larvae (Faleiro, 2006), due to the gram- negative bacterium, *Xenorhabdus nematophila* (Poinar and Thomas) (Enterobacteraceae), carried in its gut. Indeed, the adults and larvae of the red palm weevil have no antimicrobial activities against gram-negative bacteria. However, limitation in the use of *S. carpocapsae* against red palm weevil for the inability to complete its life cycle and to reproduce inside the weevil, possibly due to some unknown inhibitory substances produced by the red palm weevil.

The higher susceptibility to the nematode by the small larvae rather than the large larvae also supports our findings. Confirming Aronson and Shai (2001) also, stated that methanol extracts of the small larvae do not show any antimicrobial activity, thus suggesting that they are the most susceptible stages to diseases.

Table 5. Accumulative mortality of different stages of red palm weevil by using *H. bacteriophora*-HP-88 after 9 days.

Concentration IJs/ml	% mortality of the different stages			
	4th instar larvae	8th instar larvae	11th instar larvae	Adults
250	100	34.71	16.54	72.43
500	100	53.44	31.10	85.88
1000	100	71.42	49.46	94.00
1500	100	80.13	60.53	96.68
2000	100	85.20	67.94	97.90

Table 6. LC_{50} values of *H. bacteriophora*-HP-88 against instar larvae and adults of the red palm weevil.

Developmental stages	LC_{50} (IJs/ml)	95%fiducial limits		Intercept	Slope
		Lower	Upper		
8th instar larvae	435.16	192.09	649.87	0.906± 1.204	1.552± 0.416
11th instar larvae	1045.34	662.39	205.74	0.844± 1.221	1.376± 0.413
Adults	167.90	38.15	273.24	0.065± 1.786	2.276± 0.666

Table 7. LT_{50} values of the red palm weevil stages treated with different concentrations of *H. bacteriophora*-HP-88 after 9 days.

Concentration IJs/ml	LT_{50} values (days)			
	4th instar larvae	8th instar larvae	11th instar larvae	Adults
250	4.32	11.01	16.02	6.39
500	3.84	8.55	11.02	5.76
1000	3.32	6.61	9.38	4.06
1500	2.84	5.03	7.89	3.31
2000	2.03	4.07	5.75	2.99

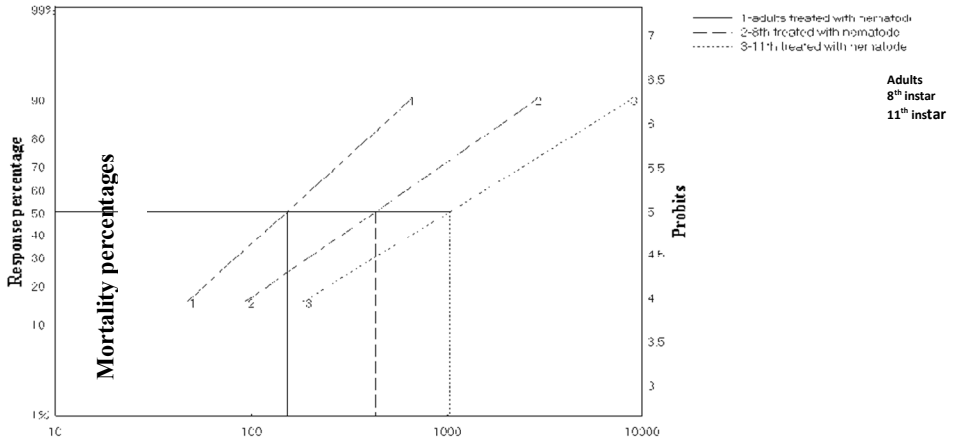

Fig. 1. Mortality regression lines of the red palm weevil (8th, 11th larvae and adults) treated with different concentration of *H.*

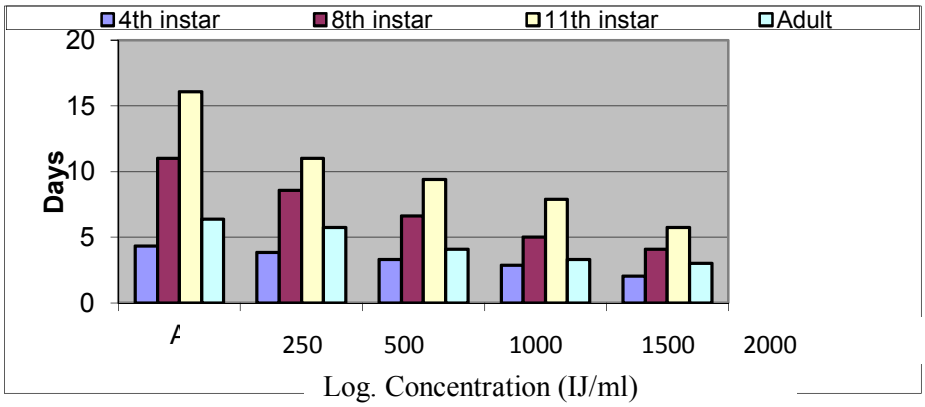

Fig. 2. LT_{50} values of RPW developmental stages treated with different concentrations of nematode.

Adults of *R. ferrugineus* were less susceptible to nematode infection than 4th instar larvae. Mortality induced nematode to the adults ranged between 72.43% and 97.90% for the five tested concentrations (Table 5 and 6).

The LC_{50} value for nematode treatment against the 4th instar larvae was not applicable for computing because this particular stage was very susceptible to tested nematode so that all nematode concentrations induced 100% mortality. However, LC_{50} values for 8th, 11th and adults were 435.16, 1045.34 and 167.90 IJ/ml, respectively (Table, 6). Results in Table (10) and Fig. (10) indicated that the LT_{50} values for the 4th instar larvae were 4.32, 3.84, 3.32, 2.84 and 2.03 days at concentrations of 250, 500, 1000, 1500 and 2000 IJs/ml. The LT_{50} values were 11.01, 8.55, 6.61, 5.03 and 4.07 days in 8th instar larvae at the same concentrations. Meanwhile, in the 11th instar larvae the LT_{50} values were 16.02, 11.02, 9.38, 7.89 and 5.75 days at the same concentrations. Table (10) and Fig. (10) showed that the LT_{50} values were increased with increasing the larval stage. The LT_{50} values (Table, 10) for the experimented adults were 6.39, 5.76, 4.06, 3.31 and 2.99 days at the same concentrations.

Our results agree with Abbas and Hanounik (1999), they reported that *Steinernema riobravis, S. carpocapsae* and *Heterorhabditis sp*. were virulent to both larval and stages of *R. ferrugineus* in laboratory tests. Saleh and Alheji (2003) reported that in the laboratory, *H. indica* from Saudi Arabia caused 100, 70 and 75% mortality in young, grown larvae and adults of *R. ferrugineus*, respectively.

2. Filed evaluations of the effectiveness of the entomopathogens against red palm weevil, *R. ferrugineus*.

Field experiments using that the fungus, *M. anisopliae*; bacterium, *B. thuringiensis* and nematode, *H. bacteriophora*-HP-88 showed no external sign of recovery from the three infestation levels (Table, 14). Results cleared that the fungus, *M. anisopliae*; bacterium, *B. thuringiensis* and nematode, *H. bacteriophora*-HP-88 were not good candidates as a bio-control agent for red palm weevil, *R. ferrugineus*. Besides, these agents showed little effects on the pest in laboratory and no effect due to field applications. Therefore, the fungus, *M. anisopliae*; bacterium, *B. thuringiensis* and nematode, *H. bacteriophora*-HP-88 need more studies as bioagents used against the red palm weevil. Thus, we cannot completely discord its use in biological controls.

Table 14. Injection of *B. thuringiensis*, *M. anisopliae* and *H. bacteriophora*-HP-88.

Type of entomopathogen	Number of treated palms	Mean number of tubes/palm	% percentage of palm recovery after treatment*		
			One week	Two weeks	Three weeks
M. anisopliae (10^8 spores/ml)	7	5-8	0	0	0
B. thuringiensis (10^8 spores/ml)	5	5-8	0	0	0
H. bacteriophora-HP-88 (2000 IJs/ml)	5	5-8	0	0	0

*** Experiments were carried out starting May 2014. The entomopathogens were applied at different times and different trees.**

In conclusion, in this study, we have studied the infection of red palm weevil stages by the fungus, *M. anisopliae* , bacteria, *B.t* subsp. *Kurstaki* and nematode, *H. bacteriophora*-HP-88. Data in this study showed that the fungus, *M. anisopliae* was less virulent to eggs, 4th instar larvae and adults stage of red palm weevil. Meanwhile, 8th, 11th instar larvae and pupae were most tolerant to *M. anisopliae*.

Our results showed that the activity of *B. thuringiensis* against red palm weevil was low.

mortality of red palm weevil stages infected by *H. bacteriophora*-HP-88 was increased with the increasing of concentrations under laboratory conditions.

Field application obtained that *M. anisopliae, B. thuringiensis* subsp**.** *kurstaki* and *H. bacteriophora*-HP-88 showed no external sign of recovery from the three infestation levels. Therefore, the fungus, *M. anisopliae*; bacterium, *B. thuringiensis* and nematode, *H. bacteriophora*-HP-88 need more studies as bio-agents used against the red palm weevil. Thus, we cannot completely discord its use in biological controls.

REFRENCES

1. Abbas, M. S. T. and S. P. Hanonik. 1999. Pathogenicity of entomopathogenic nematodes to red palm weevil, *Rhynchophorus ferrugineus*. Intern. J. Nematol., 9(1): 84-86.
2. Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol 18: 265-267.
3. Aronson, A. and Y. Shai. 2001. Why *Bacillus thuringiensis* insecticidal toxins are so effective: unique features of their mode of action. Fems Microbiology Letters, 195: 1-8.
4. Attathom, T.; W. Chongrattanameteekul; J. Chanpaisang and R. Siriyan. 1995. Morphological diversity and toxicity of delta-endotoxin produced by various strain of *Bacillus thuringiensis*. Bull. Intemol. Res. 5: 167-173.

5. Dembilio, O.; E. Q. Moraga, C. S. Alvarez and J. A. Jacas. 2010. Potential of an indigenous strain of the entomopathogenic fungus *Beauveria bassiana* as a biological control agent against the red palm weevil, *Rhynchophorus ferrugineus*. J. Inverteb. Pathol. 104, 214-221.
6. El-Sufty, R; S. A. Al-Awash; A. M. Al-Amiri ; A. S. Shahdad ; A. H. Al-Bathra and S. A. Musa. 2007. Biological control of red palm weevil, *Rhynchophorus ferrugineus* (Col.:Curculionidae) by the entomopathogenic fungus *Beauveria bassiana* in United Arab Emirates. Acta-Horticulturae. (736): 399-404.
7. Faleiro J.R. 2006. A review of the issues and management of the red palm weevil Rhynchophorus ferrugineus (Coleoptera: Rhynchophoridae) in coconut and date palm during the last one hundred years. Int. J. Trop. Insect Sci., 26:135–154.
8. Finney, D.J. 1971. Probit Analysis, a Statistical Treatment of the Sigmoid Response Curve, 6th Ed., Cambridge Univ. Press, Cambridge, England.
9. Gindin, G; S. Levski; I. Glazer and V. Soroker. 2006. Evaluation of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against the red palm weevil *Rhynchophorus ferrugineus*. Phytoparasitica-. 34(4): 370-379.
10. Hanounik, S. B. 1998. Steinernematides and Heterorhabditides as biological control agents for the red palm weevils (*Rhynchophorus ferrugineus)* Sultan Qabus Univ. J. Scientific Res. Agric. Sci. 3:95-102.
11. Kaakeh, W.; M. Aboui-Anour and A. A.Khamis. 2001. Mass rearing of the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) on sugarcane and artificial diets for laboratory studies. Pp.344-357. proceedings of the Second International Conference on Date Palm (refereed), Al-Ain, UAE.
12. Kaya, H. K. and S. P. Stock. 1997. Techniques in insect nematology. In: Lacey, L. (ed.)
13. Manachini, B.; M. Vazzana; M. Celi and V. Arizza; 2011. *Bacillus thuringiensis* treatment alters larval growth, hemocytes and modulation of Hsp70 in Rhynchophorus ferrugineus. IOBC/WPRS Bulletin 66: 53-57. 328.
14. Manachini, B.; P. L.Bue; E. Peri; S. Colazza; R. U. Ehlers; N. Crickmore; J. Enkerli; I. Glazer; M. Lopez-Ferber and C. Tkaczuk (2009). Potential effects of *Bacillus thuringiensis* against adults and older larvae of *Rhynchophorus ferrugineus*. IOBC/WPRS Bulletin, 45: 239-242.
15. Merghem, A. 2011. Susceptibility of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) for the green muscardine fungus, *Metarhizium anisopliae* (Metsch.) in the laboratory ad in palm trees orchards. Egyptian J. Biol. Pest Control 21(2): 179-183.

16. Saleh, M. M. E and M. Alheji. 2003. Biological control of red palm weevil with entomopathogenic nematodes in the Eastern province of Saudi Arabia. Egyptian Journal of Biological Pest Control. 2003; 13(1/2): 55-59.
17. Shaiju, S.; R. Kumar and C. Gokulapalan. 2003. Occurrence of *Beauveria* sp. on red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) of coconut. Insect Environ. 9(2): 66-67.
18. Shapiro, D. I.; M. Jackson; C. C. Reilly and M. W. Hotchkiss. 2004. Effects of combining an entomopathogenic fungi or bacterium with entomopathogenic nematodes on mortality of *Curculio caryae* (Coleoptera: Curculionidae). Biol. Control 30, 119–126.
19. Shapiro-Ilan, D. I.; D. H. Gouge; A. M. Koppenhofer. 2002. Factors affecting commercial success: case studies in cotton, turf and citrus. In: Gaugler, R. (Ed.), Entomopathogenic Nematology. CABI, New York, pp. 333–356.
20. Sivasupramaniam S.; G. p. Head; L. English; Y. J. Li and T. T. Vaughn 2007. A global approach to resistance monitoring. J. Inverteb. Pathol., 95: 224-226.
21. Thom, C. and K. B Raper. 1945. Amanual of Aspergillus Willamas and Wilkins Co. Batimore, Mol.
22. Vitale, A.; V. Leone; L. Torta; S. Burruano and G. Polizzi. 2009. Prove preliminari di lotta biologica con *Beauveria bassianae Metarhizium anisopliaenei* confronti del punteruolo rosso, pp. 169-172. In: La ricerca scientifica sul punteruolo rosso e gli altri fitofagi delle palme in Sicilia, Vol.1.- Regione Siciliana, Assessorato Agricoltura Foreste Dipartimento Interventi Infrastrutturali, Italy (abstract in English).

تقييم بعض الممرضات الحشرية على سوسة النخيل الحمراء تحت الظروف المعملية والحقلية

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تم إنجاز هذا العمل بمنطقة القصاصين- محافظة الإسماعيلية- مصر، لتقييم كفاءة ثلاثة ممرضات حشرية ضد الأطوار المختلفة لسوسة النخيل الحمراء تحت الظروف المعملية والحقلية. أوضحت النتائج أن النيماتودا ذات قدرة مرضية عالية ضد سوسة النخيل الحمراء تحت الظروف المعملية. وعلى الجانب الاخر كانت فعالية فطر الميتاريزيم انيزوبليا وبكتيريا الباسيلس ثورينجينسيز منخفضة.

ومن ناحية أخرى، أوضحت التحارب الحقلية أن المعاملة بنيماتودا الهيتيروربديتس وفطر الميتاريزيم انيزوبليا وبكتيريا الباسيلس ثورينجينسيز لم تظهر أي نسبة شفاء فى الأشجار المعاملة.