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Efficiency of Two Entomopathogenic Nematodes against the Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae).

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Abstract

In the present study, infective capabilities of two entomopathogenic nematode species including *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* were evaluated against red palm weevil, under laboratory and field conditions. Bioassay experiments took place to estimate theLC_{50}, LC_{90}, LT_{50} and LT_{90} values. By *H. bacteriophora* treatment, theLC_{50}'s against the 2nd, 4th, 8th and 12th larval instars of *R. ferrugineus* were 909.48, 1623.00, 1771.08 and 2897.44 IJs/ml, respectively. The correspondent LC_{90} values were 3389.86, 6586.00, 8096.61 and 22548.21 IJs/ml, respectively after 15 days of treatment. Also, the LT_{50} values after using the concentration 2500 IJs/ml were 7.43, 9.54, 10.83 and 16.28 days, respectively, while those of LT_{90} were 21.81, 41.33, 52.68 and 116.40 days, respectively. By *S. carpocapsae* treatment, theLC_{50}'s were 728.97, 1370.00, 1547.21 and 2771.84 IJs/ml, respectively. LC_{90} values were 3507.36, 6784.74, 9293.00 and 18916.27 IJs/ml, respectively for the 2nd, 4th, 8th and 12th instars after 15 days of treatment. Also, the LT_{50} values after using the concentration 2500 IJs/ml were 7.04, 7.13, 10.16 and 14.62 days, respectively, while those of LT_{90} were 19.05, 22.57, 43.46 and 96.78 days, respectively. Whereas, the two species of EPN (*H. bacteriophora* and *S. carpocapsae*) by 2500 IJs /ml concentration caused 90 & 95% mortality for 2nd instar, 70 & 75% for 4th instar, 65 & 70% for 8th instar and 50 & 50% for 12th instar, respectively, 15 days after treatment. The field study showed that infested date palm trees injected by *H. bacteriophora* and *S. carpocapsae* by concentration 2 x 10^7 IJs/liter at the infestation site by the red palm weevil caused 60 and 80% recovery, respectively, recovery from infestation after 25 days of treatment.
The study recommends using of *S. carpocapsae* to control the red palm weevil.

Keyword: Bioassay, Red palm weevil, *Rhynchophorus ferrugineus*, *Hetrerorhabditis bacteriophora*, *Steinernema carpocapsae*

Introduction

The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Curculionidae) also known as the Asian palm weevil is a key pest of palms in different agro-ecosystems. In 2017 the Food and Agricultural Organization (FAo) of the United Nations (UN) through its "Room Declaration "called for the urgent need to combat RPW by collaborative efforts and commitments at the country, regional and global levels to stop the spread of this devastating pest **(El-Shafie, 2020).**

Red palm weevil (RPW). *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is one of the most common palm pests in Egypt is the red palm weevil **(Abdel-Hameid, 2022).** It was first recorded in India as a serious pest of coconut palm **(Lefroy, 1906)**; later it has been recorded on date palm **(Lal, 1917; Buxton, 1918).** The palm weevil is currently a serious pest in Egypt, as well as in many other countries, for example in Malaysia, RPW is a lethal pest of coconut in Terengganu and sago palm in Sarawak (**Wai *et. al.*, 2015)**.

Laboratory culture and biology of the red palm weevil has been investigated by several authors, e.g. (**Wai *et. al.*, 2015; El-deeb *et. al.*, 2019; Mohanny *et. al.*, 2020; Aldawood *et. al.*, 2022 and Abdel-Hameid, 2022)**.

On the other hand, the entomopathogenic nematodes (EPNs) within the families, Steinernematidae and Heterorhabditidae are obligate insect parasites. Their easy multiplication, broad host range, compatibility with chemical pesticides, and ease in application has attracted interest among research practitioners to work on these beneficial microorganisms. These beneficial EPNs can be easily mass produced using both in vivo, in insect larvae, and in vitro techniques, using solid or liquid fermentation. **(Piedra-Buena, *et. al.* 2015; García del Pino and Morton 2015; Bhat, *et. al.* 2020 and Rehman *et. al*. 2022**).

Due to the high efficacy of EPNs biocontrol agent of *R. ferrugineus* compared to the low efficacy of most of the chemical pesticides, EPNs have been considered one of the principal control methods of this weevil pest **(El Sadawy *et. al.*, 2020; Cappa *et. al.*, 2020; Anes *et. al.*, 2020; Manzoor *et. al.*, 2020; Nurashikin-Khairuddin *et. al.*, 2022; Manochaya *et. al.*, 2022).** The pathogenic effects and symptoms of the entomopathogenic nematodes against RPW have been documented by several authors, including **(Binda-Rossetti *et. al.*, 2016 and Santhi *et. al.*, 2016)**

Materials and Methods:

1-Collection and rearing of red palm weevil (RPW)

Red palm weevil (*R. ferrugineus*) adults were collected from date palm groves in Al Qassaseen, Ismailia Governorate, Egypt during 2021. Collected adults were bred on pieces of sugar cane stems. The females laid eggs below the upper surface of the sugar cane slices. Deposited eggs were separated by a fine brush and transferred into Petri-dishes containing a filter paper wetted with water. Freshly hatched larvae were reared on pieces of sugar cane stems, and the dissection process of sugar cane stems was monitored until pupation inside the cocoon till emergence of insect adults.

Rearing of RPW occurred under 27 ± 2 ºC and 70 ± 2% R.H. in the insectary of Insect Biology, Plant Protection Department., Faculty of Agriculture, Benha University.

2-Nematode production

The Wax Moth, *Galleria mellonella* (Lepidoptera: Pyralidae) developmental stages were supplied from the Plant Protection Research Institute at Dokki, Egypt. The wax moth larvae were reared in the laboratory on an artificial diet as recommended by **(Kulkarni *et. al.*, 2012)**. The diet consisted of Wheat flour 100g, Wheat bran 100g, Milk powder 100g, Maize flour 200g, Dried yeast 50g, Honey 175ml and Glycerine 175ml.

Infection of entomopathogenic nematodes of the two species, *Heterorhabditis bacteriophora* (HP 88) (Fam: Heterorhabditidae) and *Steinernema carpocapsae* (all) (Fam: Steinernematidae) was carried out to multiply the infectious stages of nematode. A paper napkin was placed in a plastic box of 8cm length, 8cm width, and 4cm height, and 2ml of water containing the juvenile infective nematode individuals were placed on the tissue, and ten individuals of the *G. mellonella* 5th larval instar were placed (plate 1 A).

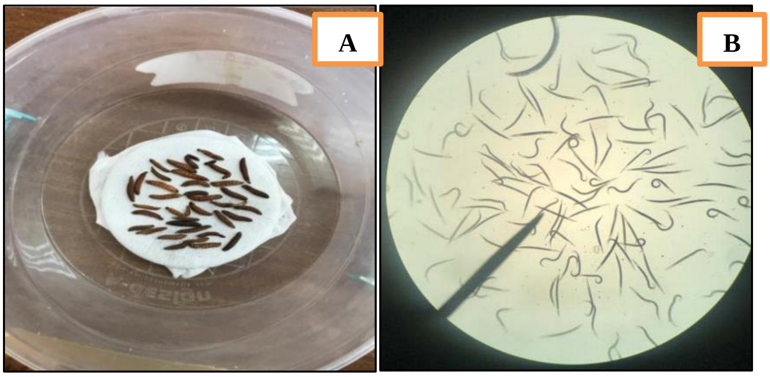

Plate 1: A: Stage of isolation of dead *G. mellonella* larvae to separate nematodes.
B: The stage of examining the counting of nematodes under a light microscope using a dedicated counting slide.

Dead larvae were transferred to another container of the same size, containing water and a plastic cover covered with gauze, on which the dead larvae were placed to attract the juvenile infective nematodes to descend into the water. The number of individuals of juvenile infective nematodes in the water was counted using the slide designated for counting nematodes under the examination microscope (plate, 1 A,B). (**Xuejuan and Hominick, 1991).**

3-Treatment of different RPW stages by two species of entomopathogenic nematodes; *H. bacteriophora* and *S. carpocapsae*

To investigate the efficiency of the two species of entomopathogenic nematodes to control RPW, three red palm weevil larval instars (2nd, 4th and 8th) were treated with five concentrations of 500, 1000, 1500, 2000, and 2500 IJs however, the 12th larval instar was treated with the concentrations of 500, 1000, 1500, 2000, 2500 and 5000 IJs of both nematode species. The 2nd instar was assayed on eight larvae with five replications; thus 40 larvae were treated with each concentration, and another 40 larvae, divided into five replicates, received distilled water treatments as untreated control. Each concentration was assayed on the 4th, 8th and 12th larval instars on four larvae treatment with five replications, thus 20 larvae were treated with each concentration. Another 20 larvae, were divided into five replicates, received distilled water treatments as control.

After treatment, the larvae were, daily, examined for 15 successive days, and the dead larvae were counted, and consequently mortality, percentages were calculated. Concentrations were added to be absorbed in 10g of grated sugar cane infested with the R.P.W 2nd larval instar and 15g of grated sugar cane with the 4th, 8th, 12th instars and control. Treatments were them kept in plastic cans (8cm length, 8cm width, and 4cm height) contain with the treated diet; the lid was perforated for aeriation, and the treatments were incubated at 27 ± 2 °C and 70 ± 5 R.H. The RPW larvae in each treatment were daily examined and considered dead if they were immobile plate 2**.**

Plate 2: Plastic cans used for laboratory treatments for RPW larval instars with *H. bacteriophora* and *S. carpocapsae*.

The obtained LC_{50} values of each tested entomopathogenic nematodes (*H. bacteriophora* and *S. carpocapsae*) were used to design the adults experiment. Three freshly emerged pairs of RPW adult were used for each EPN species and three pairs were used as control. These pairs were subjected to the treatment for 48 hours and then transferred to fresh food in new jars (5cm diameter and 10cm height) incubated under the same temperature and R.H. to determine the pre-oviposition, oviposition, post-oviposition periods, number of eggs, percentage of eggs hatching, incubation period of eggs, larval and pupal duration, longevity of male and female, and sex-ratio. Rearing was continued up to the subsequent generation, and the mortality rate among eggs, larvae, pupae, and adults was estimated for each generation.

4- Field trials of entomopathogenic nematodes against red palm weevil

Two experiments were carried out in December 2022 on RPW infested date palms in the Al-Qassasin district, Ismailia Governorate. Five RPW-infested date palm trees were randomly chosen to represent five replicates. Each of those trees received an injection of the Nematode suspension of *H. bacteriophora* and EPN species *S. carpocapsae* depending on the experiment (5 trees for each) using a large iron pin for making hole around the site of infestation, then injection through plastic piping. The nematode concentration used was 20000000 ($2x10^7$ IJs) per liter of distilled water in every infested tree. Successive field observation on date palm trees was evaluated for injury 15 and 25 days after the date of application by skimming and cleaning the injured places and noting the dead individuals of RPW, then rating the treated trees as still either infested or recovered (plate, 3).

Plate 3: Field injection of entomopathogenic nematodes *H. bacteriophora* and *S. carpocapsae* at $2x10^7$ IJ / liter of distilled water in RPW infested date palm trees.

5- Statistical analysis:

Probit analysis according of **Finney (1971)** was applied for statistical analysis**.** Probit analysis computer programs of Finney (1971) was applied for statistical analysis.Cumulative mortality at the end of the experiment was analyzed by ANOVA. The lethal concentrations causing 50 and 90% mortalities, (LC_{50} and LC_{90}) and time needed for causing 50 and 90% cumulative mortalities (LT_{50} and LT_{90}) were calculated using the probit analysis program LPD-line **(Bakr, 2005).**

Results and Discussion

1- Efficacy of the entomopathogenic nematodes, *H. bacteriophora* against the red palm weevil.

Results presented in Table, 1 and illustrated in Fig.1 show the cumulative RPW larval mortality percentages after 3, 5, 7, 10 and 15 days of treatment. The 2nd instar larvae of RPW proved to be the highest susceptible instar to *H. bacteriophora* treatment. The obtained mortality percentages showed a concentrations-dependent mortality response. The mortality rates were 90, 85, 60, 55 and 40% at 15 days post-treatment for the tested *H. bacteriophora* concentrations of 2500, 2000, 1500, 1000 and 500 IJs/ml distilled water, respectively. On contrary, larvae of the 12 instar showed the lowest correspondent mortality rates, being 65, 50, 40, 30, 25 and 15%, for the same tested concentrations, respectively, 15 days after treatment for the same mentioned *H. bacteriophora* concentrations. The larvae of remaining 4th and 8th instars reacted intermediate values (Table, 1 and Fig, 1) obtained data revealed that the susceptibility of RPW larvae to *H. bacteriophora* treatment decreased by treatment of younger larvae. It can be also observed that the mortality rate is a function of the nematode dose concentration; where the lowest percentage of mortality was recorded at a concentration of 500 IJs/ml in most post treatment periods. Generally, the maximum cumulative mortality was reported for the youngest larval instar after the longest post – treatment period (Table 1 and Fig. 1).

Results of the present study in Table, 1 and Fig.1 showed that RPW proved as highly susceptible to *H. bacteriophora*, especially when *H. bacteriophora* treatment were oriented on young larvae of the second instar where mortality rate was high and reached 90% at 15 days post treatment. In a similar study, **Gözel *et. al.*, (2015**) used several entomopathogenic nematode species at the rate of 500IJs/larva and incubated at 25°C against the RPW; they found that all the used entomopathogenic nematodes caused different mortality rates of the red palm weevil larvae. The study also indicated that the highest mortality (93.5%) occurred by *H. bacteriophora*. Later, (**El Sobki *et. al.*, 2020)** found that both *Heterorhabditis bacteriophora* (HP88 strain) and local species *H. bacteriophora* (Ar-4) exhibited promising results by killing 92.20 and 82.13 % of the 4th instar larvae after 9 days of exposure, whereas, *S. feltiae* and *H. bacteriophora* (HT) showed less insecticidal activity against 4th, 9th and 11th instar larvae. Meanwhile, the authors documented that the Egyptian *H. bacteriophora* (Ar-4) was more effective than *H. bacteriophora* (HT). *S. carpocapsae* followed it with 91.4%. *S. feltiae* and *S. affine* which caused 36.4% and 21.2% mortality on *R. ferrugineus* larvae respectively.

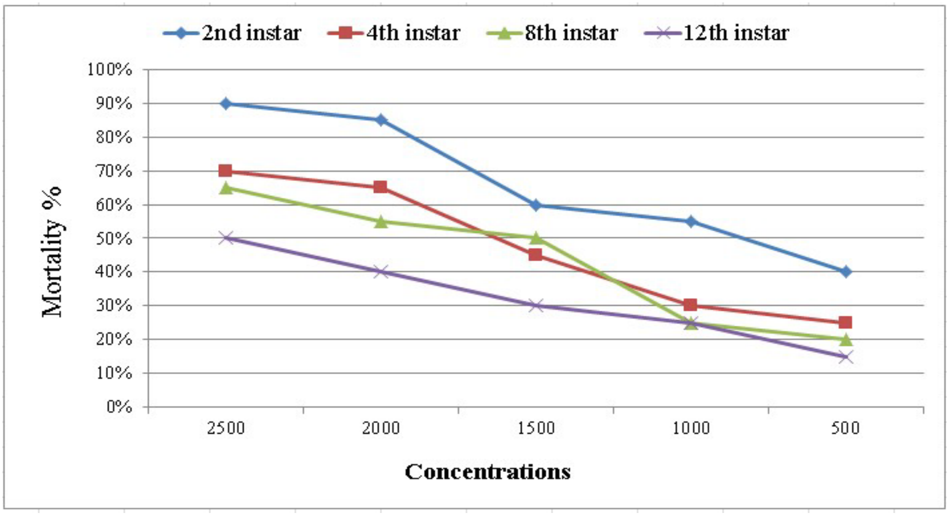

Fig 1: Mortality percentages among *R. ferrugineus* larvae 15 days after treatment with *H. bacteriophora* (at different concentrations).

Table 1. Mortality percentages among larvae of *R. ferrugineus* treated against 4 instars with different concentrations of *H. bacteriophora*. (Total number of 20 larvae / each conc.)

Conc. IJs	periods after application (days)																			
	3 days				5 days				7 days				10 days				15 days			
	2nd	4th	8th	12th	2nd	4th	8th	12th	2nd	4th	8th	12th	2nd	4th	8th	12th	2nd	4th	8th	12th
5000				15%				20%				25%				50%				65%
2500	20%	20%	20%	15%	30%	25%	20%	20%	40%	35%	35%	30%	55%	50%	45%	35%	90%	70%	65%	50%
2000	20%	20%	20%	15%	20%	30%	20%	20%	35%	30%	30%	25%	55%	45%	35%	30%	85%	65%	55%	40%
1500	20%	15%	20%	15%	30%	25%	20%	20%	35%	30%	25%	20%	55%	40%	40%	25%	60%	45%	50%	30%
1000	20%	15%	15%	10%	20%	20%	15%	15%	25%	20%	15%	15%	35%	25%	25%	20%	55%	30%	25%	25%
500	15%	10%	10%	10%	15%	15%	10%	15%	15%	15%	15%	15%	30%	20%	15%	15%	40%	25%	20%	15%
Control	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	10%	10%	0%	0%	10%	10%	0%	0%

1-1- Pathogenicity of *H. bacteriophora* against larvae of RPW:-

The lethal concentrations causing 50% LC_{50} and 90% LC_{90} larval mortalities at the 3, 5, 7, 10 and 15 days after treatment were assessed (Table, 2 and Fig. 2). As previously mentioned, the efficiency of *H. bacteriophora* was concentration mortality dependent; efficiency increased as the applied concentration was increased and vice versa. Considering the 2nd instar larvae, those were the highest susceptible, showing the lowest LC_{50} (909.48 IJs/ml). On contrary, the 12th instar larvae manifested least susceptibility as those showed the highest (LC_{50}, 2897.44 IJs/ml). In this respect, the 4th and 8th instars larvae showed intermediate position in their susceptibility to *H. bacteriophora* treatments between the 2nd and 12th instars showing (LC_{50}, 1623 and 1771.08 respectively) (Table, 2 and Fig. 2). As for the LC_{90}'s, those manifested the same trend of susceptibility, being (3389.86, 6586, 8096.61 and 22584.21 IJs/ml for the tested 2nd, 4th, 8th and 12th instars, respectively. (Table, 2 and Fig. 2).

Table 2. Pathogenicity of *H. bacteriophora* after 15 days of treatments tested (LC_{50} and LC_{90} values), against 4 larval instars of *R. ferrugineus*.

Larval instar	LC_{50} IJs/ml	LC_{90} IJs/ml	Slope ± SE
2nd	909.48 (842.32 - 986.45)	3389.86 (2126.62 - 3847.29)	2.24 ± 0.24
4th	1623.00 (1478 - 1949)	6586.00 (4895 - 9485)	2.11 ± 0.26
8th	1771.08 (1450.18 - 1981.57)	8096.61 (5474.85 - 10644.59)	1.87 ± 0.25
12th	2897.44 (2257.20 - 4565.50)	22548.21 (10744.66 - 41522.19)	1.43 ± 0.25

*Results were calculated 15 days after treatment.

Table 3. LT_{50} and LT_{90} values of *H. bacteriophora* tested against 4 larval instars of *R. ferrugineus*. Results were calculated using concentration 2500 IJs/ml.

Larval instar	LT_{50} (days)	LT_{90} (days)	Slope ± SE
2nd	7.43 (5.49 - 9.02)	21.81 (18.66 - 26.89)	2.74 ± 0.27
4th	9.54 (8.32 - 11.33)	41.33 (28.08 - 65.22)	2.01 ± 0.25
8th	10.83 (9.27 - 13.45)	52.68 (34.02 - 81.22)	1.86 ± 0.24
12th	16.28 (12.63 - 20.71)	116.40 (57.02 - 187.08)	1.50 ± 0.26

The median lethal time LT_{50} (Time until death 50% of the tested population) and lethal time of 90% the tested population (LT_{90}) were calculated after *R. ferrugineus* larval treated with only the *H. bacteriophora* at 2500 IJs/ml of water. As shown in Table, 3 and Fig. 3, the 2nd instar took the shortest period of LT_{50} and LT_{90}, being 7.43 & 21.81 days, respectively, opposed to 9.54 & 41.33, 10.83 and 52,68 and 16,28 & 116.40 days for treatment of the 4th, 8th and 12th instars, respectively.

(**Triggiani and Tarasco 2011)** assayed the pathogenicity of seven *Steinernema* species and four *Heterorhabditis* species pathogenicity against *R. ferrugineus* larvae and adults. The authors documented that 10 days after treatment, the EPNs that yielded the highest larval mortality were *H. bacteriophora* ALG12, CS17 and C3, NEMATOP (93-100%), *Steinernema longicaudum* Shen et Wang (100%), *Steinernema glaseri* (Steiner) (100%), *S. carpocapsae* NEMASTAR (100%) and *Steinernema kraussei* (Steiner) 3D (100%). The same authors concluded that a high rate of mortality caused by *H. bacteriophora* C3 (100%) and CS17 (80%), *S. longicaudum* (96%), and *S. carpocapsae* MR7 (80%) were determined as being the most effective EPNs**.** On the other hand, **El Sobki *et al* (2020)** found that the LTs of RPW infected with *Heterorhabditis bacteriophora* (HP88) ranged from 2.943 to 5.693 days, at concentrations of 500 IJs/10 adults and 2500 IJs/10 adults, respectively.

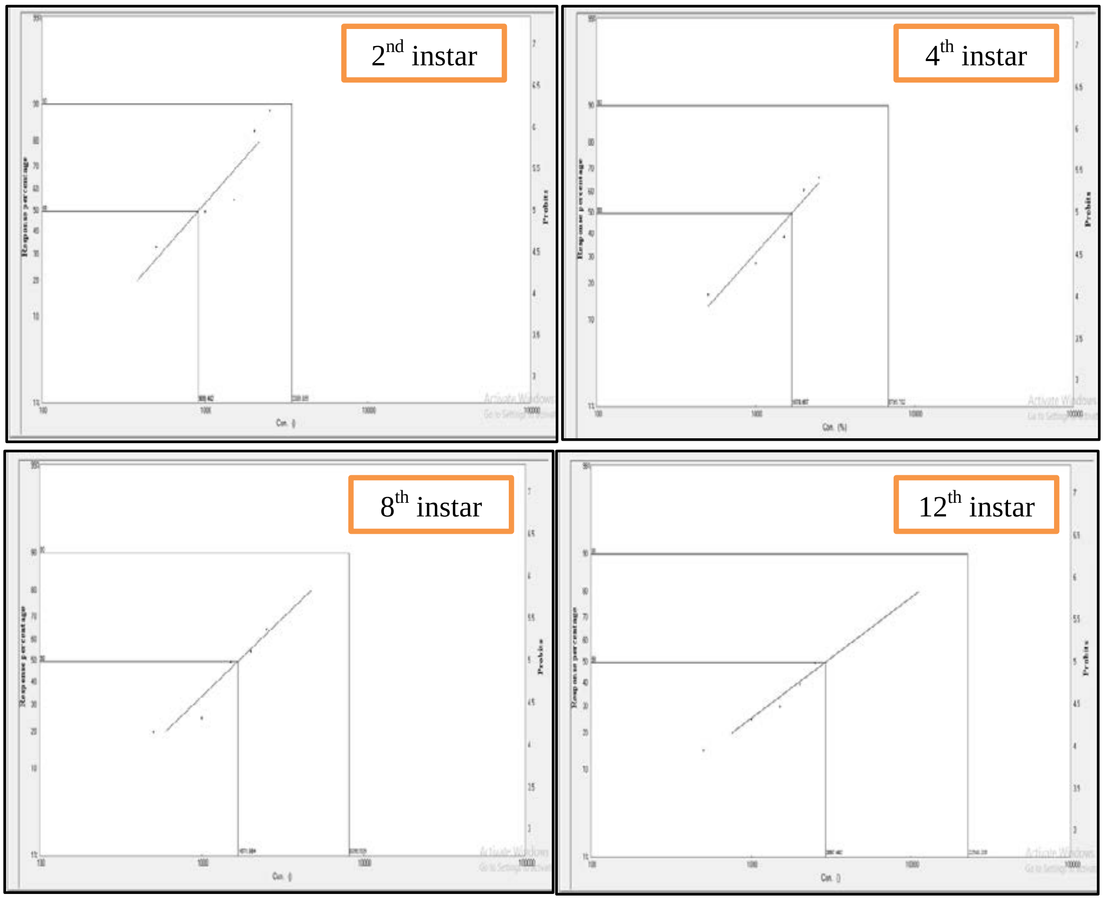

Fig 2: Ldp-line of *H. bacteriophora* used at concentrations against *R. ferrugineus* larval instars (2nd, 4th, 8th and 12th).

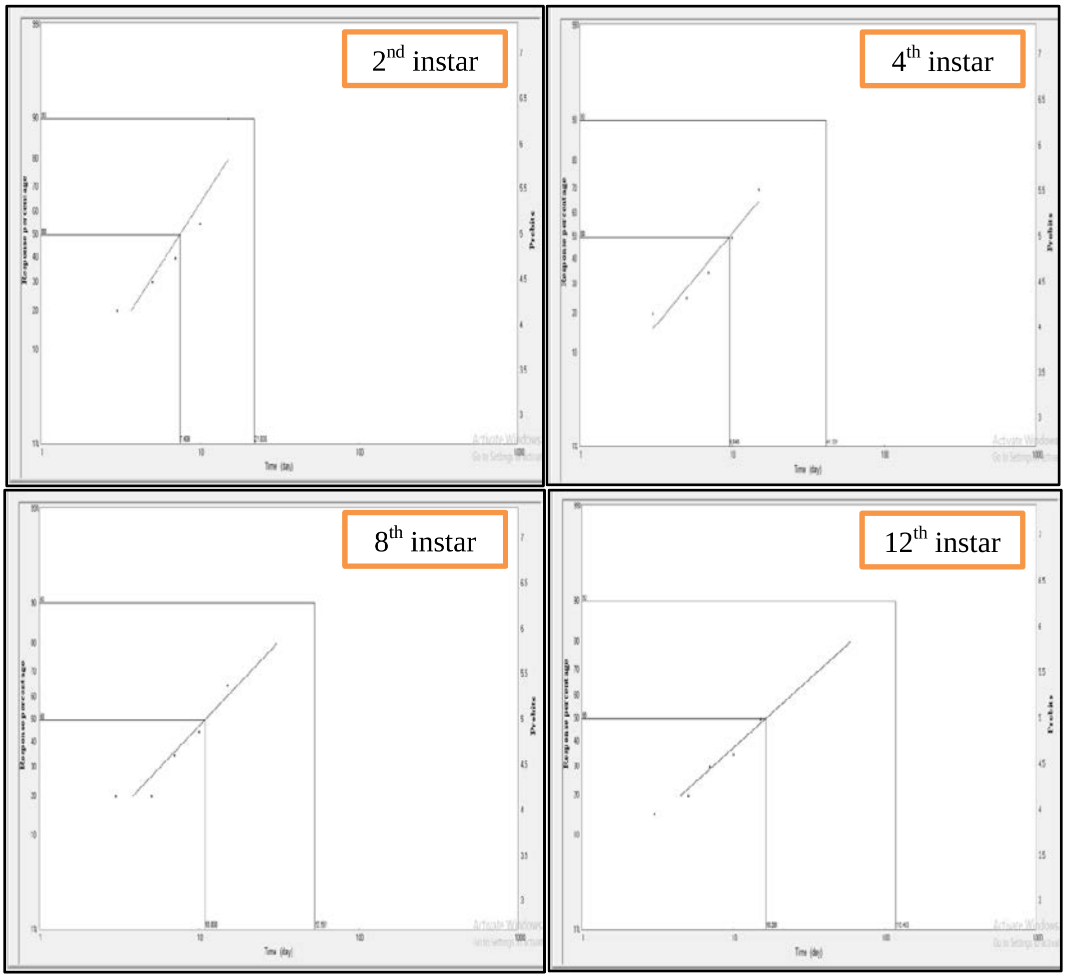

Fig. 3: Period until mortality of *R. ferrugineus* larval instars after treatments by *H. bacteriophora* for indicating the $LT_{50's}$ and $LT_{90's}$.

2-1- Field application of *H. bacteriophora* on palm trees for RPW control:-

The field treatments were performed upon infections in AL-Kasasen region in Ismailia Governorate in December 2022, where five replicates of an infested date palm tree each were, randomly chosen. Infested trees were treated with the entomopathogenic nematodes suspension through a crescent shape hole made around the site of infestation by using a large iron pin. The EPN juveniles were injected by using plastic piping. The concentration of *H. bacteriophora* used was $2x10^7$ IJs/ liter of distilled water. Field observations were photographed and recorded by naked eyes after 10, 15, 20 and 25 days. From data in Table, 4 the infection began to stop 20 days after treatment. Also, the treated date palm trees manifested of 60% recovered after 25 days from treatment. On the same concern, **Saleh *et. al.*, 2010** found that the mortality of RPW adults infesting caged 5-year-old date palm trees reached 90 and 100% after 10 days of *Steinernema* and *Heterorhabditis* injection, respectively on and around the infested date palm trees in the study. The authors also documented that increasing the concentration from $2×10^6$ to $4×10^6$ infective juveniles (IJ)/tree did not result in a significant increase in the pest mortality. On the other hand, (**El Roby *et. al.*, 2018)** determined the efficiency of three entomopathogenic nematodes isolates indigenous in Egypt; they found that the isolates of *Steinernema* sp. (B32) and *Heterorhabditis bacteriophora* (EKB20) were more effective than *Heterorhabiditis* sp. (Kasassien isolate) against *R. ferrugineus* larvae at inoculum levels of 1000 and 2000 infective juveniles (IJs)/ml. Both isolates of B32 and EKB20 were faster killers achieving more than 90% mortality to the 3rd instar larvae of the red palm weevil after 72 hours of treatment

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Table 4. Field treatments of date palm trees with *H. bacteriophora* at (2x10^7 IJs/liter of distilled water) at Al Kasasen region (date of treatment, December 2022).

Post-injection period (days)	Indication for infestation				
	1st Tree	2nd Tree	3rd Tree	4th Tree	5th Tree
10	X	X	X	X	X
15	X	X	X	X	X
20	X	D	X	X	X
25	D	--	D	X	X

N.b: X= still infested & **D=** recovered

2- Efficacy of *S. carbocapsae* against the red palm weevil.

Results in Table, 5 and Fig.4 indicate the cumulative mortality percentages among RPW larvae after 3, 5, 7, 10 and 15 days. The 2nd instar larvae of RPW proved as highest susceptible instar to *S. carpocapsae*, where the recorded mortality rates were 50, 60, 65, 80 and 95% at 15 days post-treatment at the concentrations of 500, 1000, 1500, 2000 and 2500 IJs/ml distilled water, respectively. On contrary, larvae of the last instar (12th) manifested the lowest mortality percentages after treatment with the same concentrations of *S. carpocapsae* juveniles, being 15, 20, 35, 40 and 50% 15 days after treatment. In addition to 70% mortality after treatment by the highest concentration (5000 IJs/ml). The remaining larval instars (4th and 8th) showed intermediate susceptibility response (mortality rates) between 2nd and 12th instars (Table, 5 and Fig. 4). It is clear that the efficiency of *S. carpocapsae* against RPW increased depending upon three factors: a- increasing the applied concentration, b- prolongation of the period after treatment, and c- treatment at earlier instars.

The pathogenic potential of selected nematode species against the rea palm weevil was assessed based on dissection and adult emergence of weevils by **Rehman *et. al.*, (2022).** The authors indicated that *S. carpocapsae* and *H. bacteriophora*, that caused a respective 94.68 and 92.68% infection rate, were the most effective EPN species against red palm weevil larvae. However, on adult emergence, the aforementioned EPNs were comparatively less pathogenic and resulted in 63.60 and 60.20% pupae infection, respectively. They considered the rate of adult emergence was the better option to evaluate the pathogenic potential of EPNs, compared with the dissection of insects.

Our study indicated that *S. carpocapsae* was more effective than *H. bacteriophora*; similarly to **Rehman *et. al.*, (2022)** who documented that *S. carpocapsae* was most effective against the 6th instar larvae of the red palm weevil and caused 100% mortality at 240 h after treatment. *H. bacteriophora* was found to be the most pathogenic by causing 83.60% mortality among the red palm weevil larvae**.**

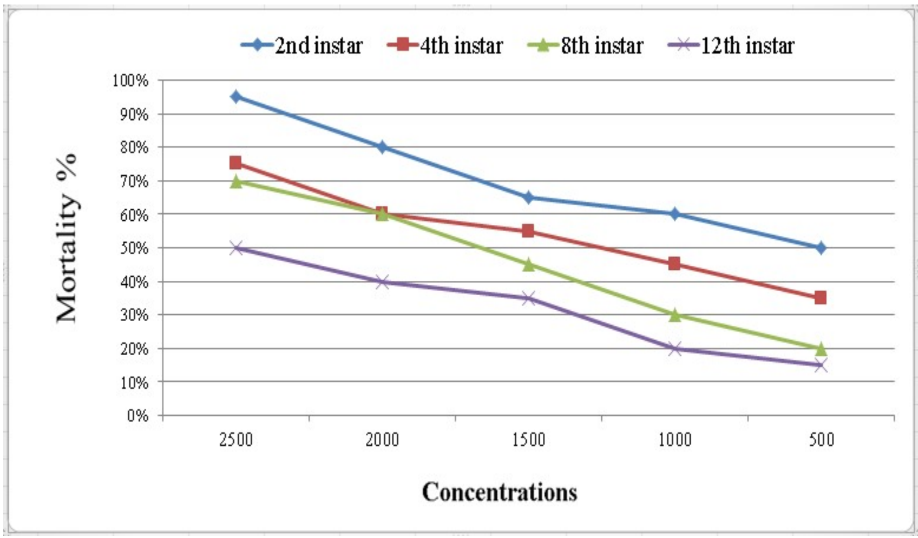

Fig 4: Mortality percentages among *R. ferrugineus* larvae 15 days after treatment with *S. carpocapsae* (at different concentrations).

Table 5. Mortality percentages among larvae of *R. ferrugineus* treated at 4 instars with *S. carpocapsae* (at different concentrations). (data from 20 larvae / each conc.)

Conc. IJs	periods after treatment (days)																			
	3 days				5 days				7 days				10 days				15 days			
	2nd	4th	8th	12th	2nd	4th	8th	12th	2nd	4th	8th	12th	2nd	4th	8th	12th	2nd	4th	8th	12th
5000	--	--	--	10%	--	--	--	10%	--	--	--	25%	--	--	--	60%	--	--	--	70%
2500	20%	20%	20%	15%	30%	20%	20%	20%	45%	60%	35%	30%	55%	70%	45%	35%	95%	75%	70%	50%
2000	20%	15%	20%	15%	25%	15%	20%	20%	40%	25%	30%	20%	55%	50%	40%	30%	80%	60%	60%	40%
1500	15%	10%	20%	15%	20%	15%	20%	15%	30%	25%	20%	15%	45%	45%	30%	20%	65%	55%	45%	35%
1000	20%	10%	10%	5%	25%	10%	15%	15%	30%	20%	15%	15%	40%	30%	20%	15%	60%	45%	30%	20%
500	15%	0%	10%	10%	25%	15%	10%	15%	30%	20%	10%	15%	35%	30%	15%	15%	50%	35%	20%	15%
Control	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	10%	10%	0%	0%	10%	10%	0%	0%

1-1- Pathogenicity of *S. carpocabsae* against larvae of RPW:-

The lethal concentrations causing 50 and 90% larval mortality (LC_{50} and LC_{90}) at the 3, 5, 7, 10 and 15 days after treatment were assessed (Table, 6 and Fig. 5). As a general, the concentration 2500 IJs/ml highest concentration caused the highest mortality rate and vice versa. The four tested larval instars (2nd, 4th, 8th and 12th) behaved differently in their reaction to *S. carpocabsae* treatments. Considering the 2nd instar larvae, those were the highest susceptible, showing the lowest LC_{50} (728.97 IJs/ml). On contrary, the 12th instar larvae manifested least susceptibility as those showed the highest LC_{50} (2771.84 IJs/ml). In this respect, the 4th and 8th instars larvae showed intermediate position in their susceptibility to *S. carpocabsae* treatments between the 2nd and 12th instars (LC_{50} = 1370 and 1547.21 respectively) (Table, 6 and Fig. 5). As for the LC_{90}'s resulted by assaying *S. carpocabsae* concentrations on *R. ferrugineus* 2nd, 4th, 8th and 12th larval instars, those manifested the same trend of susceptibility, as the 2nd instars was the highest susceptible (3507.36 IJs/ml), followed by the 4th instar (6784.74 IJs/ml) followed by the 8th instar (9293 IJs/ml). While, larvae of the 12th instar LC_{90} (18916.27 IJs/ml) (Table, 6 and Fig. 5).

Table 6. Virulence (LC_{50} and LC_{90} values), *S.carpocabsae* against larval instars of *R. ferrugineus* 15 days after treatments.

Larval instar	LC_{50} (IJs/ml)	LC_{90} (IJs/ml)	Slope ± SE
2nd	**728.97 (684.96 - 846.48)**	**3507.36 (4421.78 - 2254.64)**	**1.87 ± 0.24**
4th	**1370.00 (1151 - 1640)**	**6784.74 (5760 - 9377)**	**1.54 ± 0.24**
8th	**1547.21 (1355.02 - 1794.18)**	**9293.00 (4830.42 -11770.96)**	**1.99 ± 0.25**
12th	**2771.84 (2205.13 - 4121.69)**	**18916.27 (9714.54 - 22792.69)**	**1.53 ± 0.26**

*Results were calculated after 15 days of treatment.

Table 7. Time until mortality of 50 and 90% (LT_{50} and LT_{90}) among RPW larval instars after treatments by *S. carpocabsae.*

Larval instar	LT_{50} (days)	LT_{90} (days)	Slope ± SE
2nd	**7.04 (5.12 - 8.86)**	**19.05 (16.25 - 28.44)**	**2.96 ± 0.27**
4th	**7.13 (6.76 - 8.57)**	**22.57 (18.27 - 32.73)**	**2.40 ± 0.26**
8th	**10.16 (8.60 - 12.88)**	**43.46 (32.78 - 76.32)**	**2.03 ± 0.25**
12th	**14.62 (12.96 - 18.32)**	**96.78 (72.46 - 106.25)**	**1.65 ± 0.172**

The days spent till 50 and 90% insect mortality (LT_{50} & LT_{90}) were calculated for the treated larvae at only the concentration of 2500 IJs/ml of water. As shown in Table, 7 and Fig. 6, the 2nd instar took the shortest time to mortality of 50 or 90% of the treated larvae, then the 4th, 8th and 12th instars which took the longest period until mortality. Results indicated that the LT_{50}'s were 7.04, 7.13, 10.16 and 14.62 days, respectively, as opposed to 19.05, 22.57, 43.46 and 96.78 days, for the LT_{90}'s.

Rehman *et. al.*, 2022 investigated the infective power of the entomopathogenic nematodes, *Hetrerorhabditis bacteriophora*, *Steinernema feltiae*, *S. glaseri*, and *S. carpocapsae* on the larval, pupal and adult stages of the red palm weevil. They found that *S. carpocapsae* and *H. bacteriophora* were the most pathogenic, causing 94.68 and 92.68% infection rates. *S. glaseri* and *S. feltiae* found to be the least pathogenic causing 70 and 76% mortality, respectively. Moreover, the four tested nematode species were found to be highly infective under field conditions. However, *S. carpocapsae* was found to be the most pathogenic, causing 83.60% mortality of the red palm weevil. Recently, four species of entomopathogenic nematodes, *H. bacteriophora*, *S. feltiae*, *S. glaseri*, and *S. carpocapsae* have been tested under laboratory and field conditions on the larval, pupal and adult stages of the red palm weevil were found most effective against larvae, followed by adult weevils, but their effect was minimal against the pupae of red palm weevils. Based on these findings, we conclude that the *S. carpocapsae* and *H. bacteriophora* could be used as a sustainable option for the efficient management of the red palm weevil**.**

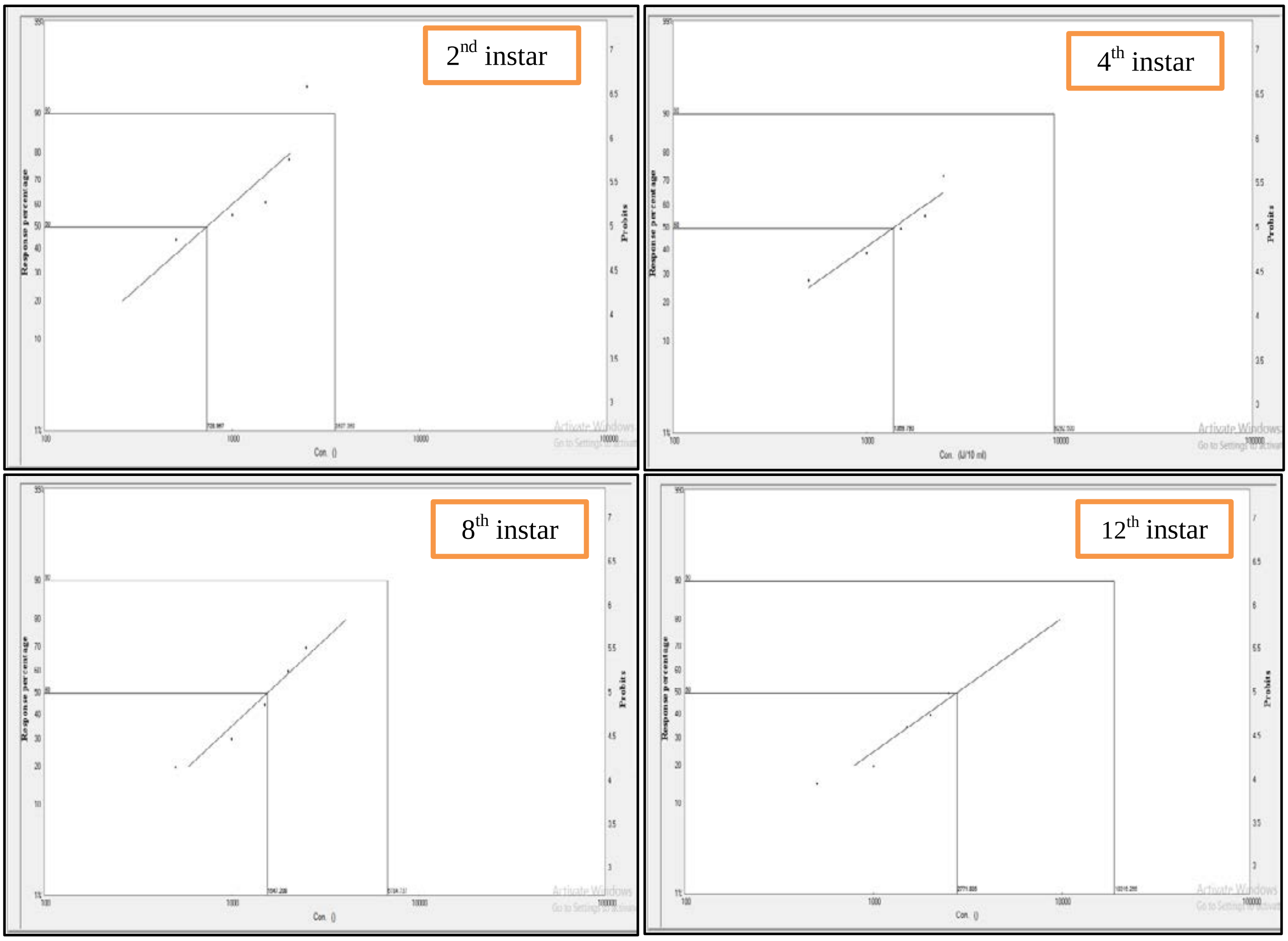

Fig. 5: Ldp-line of *S. carpocapsae* tested against larval instars of *R. ferrugineus*.

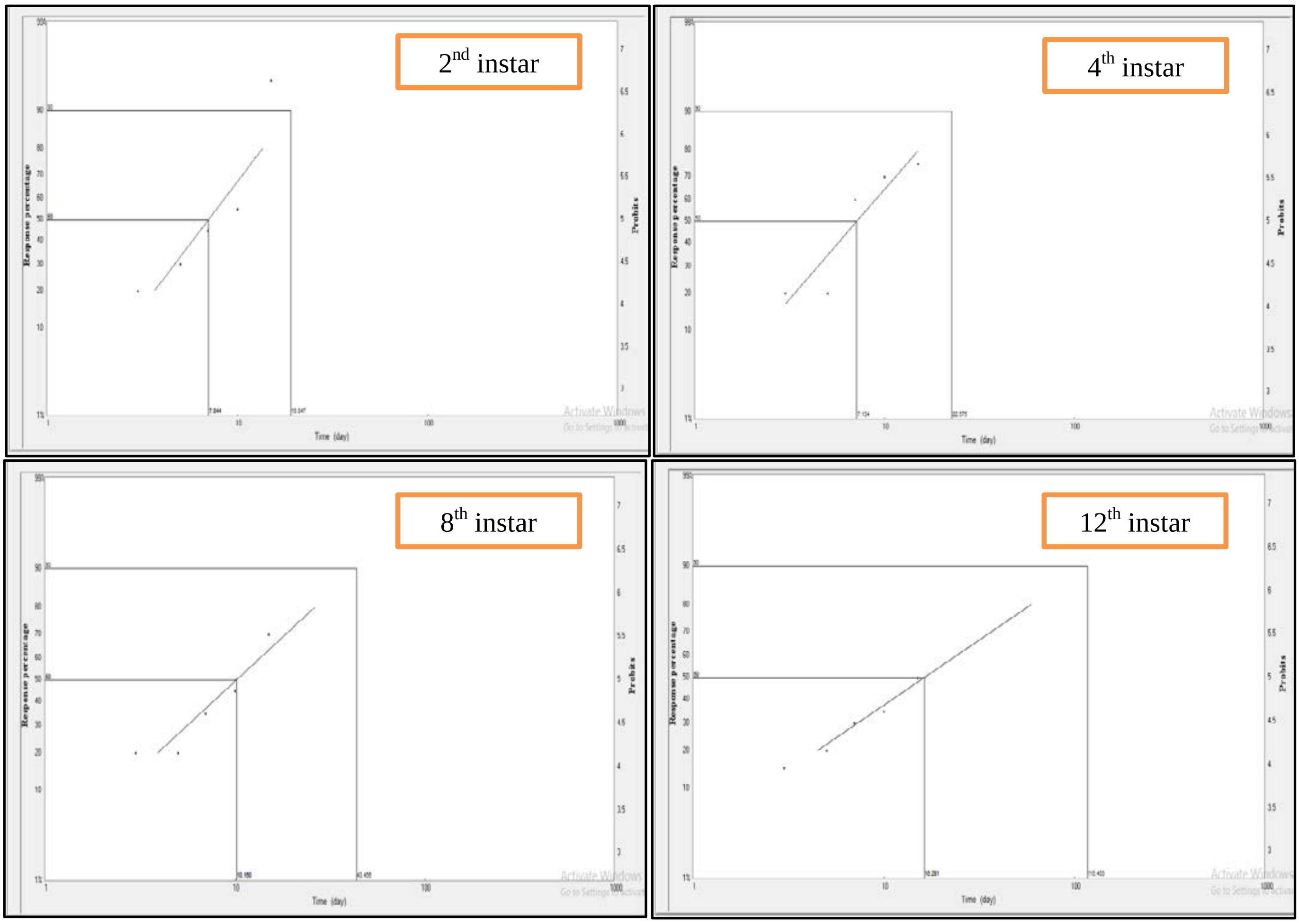

Fig. 6: Lethal times (LT's) until mortality of 50 and 90% of RPW larvae after treatment by *S. carpocapsae* at 2500 IJs/ml.

2-2- Field application of *S. carpocapsae* on palm trees for RPW control:-

These treatments were performed upon infections in AL-Kasasen region Ismailia Governorate in December 2022, where five replicates of an infested date palm tree each was, randomly chosen. Infested trees were treated with the *S. carpocabsae* juveniles suspension through a crescent shape holes which were made around the site of infestation by using a large iron pin. The juveniles were injected by using plastic piping. The concentration of *S. carpocabsae* used was $2x10^7$ IJs/ liter of distilled water. Field observations were photographed and recorded by naked eyes after 10, 15, 20 and 25 days. From data in Table 8, it could be concluded that the infection began to stop 20 days after treatment. Also, the treated date palm trees manifested 80% recovery after 25 days from treatment.

Field assessments using trunk injection resulted in a substantial decline in the population of red palm weevil after two successive applications within 3 weeks. Efficacies ranged between 48 and 88% were achieved in the curative assay resulting in a significant increase in palm survival compared to untreated control. In conclusion, EPNs Steinernema sp. (EGG4), showed a great potential for the control of the red palm weevil when injected in the date palm (**Atwa and Hegazi 2014).**

Saleh *et. al.*, (2010) indicated that the tested EPNs caused high mortality rate in cocoons of the RPW aggregated in leaf petioles of 10-year-old date palm trees. Regardless of the insect stage, *Steinernema* sp. S1 was (60.35%), while the least effective nematode was *Steinernema* sp. S2 (51.17%). These results support the possibility of using EPNs to prevent the emergence of adults from RPW cocoons at the beginning of spring and, in turn suppress the population density of RPW in the surrounding**.**

Table 8. Field treatments of date palm trees with *S. carpocabsea* at concentration (2 x 10^7 IJs/liter of distilled water) at Al Kasasen region in December 2022.

Period after treatment (days)	Indication of infestation				
	1st Tree	2nd Tree	3rd Tree	4th Tree	5th Tree
10	X	X	X	X	X
15	X	X	X	X	X
20	X	X	X	D	D
25	D	D	X		

N.b: X= still infested & D= recovered

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تقدير كفاءة نوعين من النيماتودا الممرضة للحشرات ضد سوسة النخيل الحمراء *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae).

في هذه الدراسة، تم تقييم القدرات المرضية لنوعين من النيماتودا الممرضة للحشرات بما في ذلك *Heterorhabditis bacteriophora* و *Steinernema carpocapsae* ضد سوسة النخيل الحمراء، تحت الظروف المختبرية والحقلية. أجريت تجارب الإختبار الحيوي لتقدير قيم LC_{50}، $_{LC}90$، LT_{50} وLT_{90}. من خلال المعاملة ب *H. bacteriophora*، كانت الـ LC_{50} ضد الأعمار اليرقية الثاني والرابع والثامن والثاني عشر لـ *R. Ferrugineus* 909.48 ، 1623.00، 1771.08 و2897.44 IJ/ml، على التوالي. وكانت قيمة LC_{90} 3389.86، 6586.00، 8096.61 و22548.21 IJs/ml، على التوالي بعد 15 يومًا من العلاج. كما أن قيم LT_{50} بعد المعاملة بالتركيز 2500 IJ/ml كانت 7.43، 9.54، 10.83 و16.28 يوم، على التوالي، بينما قيم LT_{90} كانت 21.81، 41.33، 52.68 و116.40 يوم، على التوالي. وباستخدام *S.carpocapsae*، كانت مستويات LC_{50} هي 728.97، 1370.00، 1547.21 و2771.84 IJ/ml ، على التوالي. وكانت قيم LC_{90} هي 3507.36 و6784.74 و9293.00 و18916.27 IJ/ml ، على التوالي للأعمار اليرقية الثاني والرابع والثامن والثاني عشر بعد 15 يومًا من المعاملة. كما أن قيم LT_{50} بعد المعاملة بالتركيز 2500 IJ/ml كانت 7.04، 7.13، 10.16 و14.62 يوم على التوالي، بينما كانت قيم LT_{90} 19.05، 22.57، 43.46 و96.78 يوم، على التوالي. حيث أن نوعي EPN *(H.bacteriophora* و*S. carpocapsae*) بتركيز 2500 IJ/ml تسببا في موت بنسبة 90 & 95% في العمر اليرقي الثاني، و70 & 75% في العمر اليرقي الرابع، و65 & 70% في العمر اليرقي الثامن و 50 & 70% للعمر اليرقي الثاني عشر، على التوالي، بعد 15 يومًا من العلاج. أظهرت الدراسة الحقلية أن أشجار النخيل المصابة بسوسة النخيل الحمراء بعد حقنها *H. bacteriophora* و*S. carpocapsae* بتركيز 2×10^7 IJ /لتر في موقع الإصابة بسوسة النخيل الحمراء أدت إلى موت الحشرات وانتهاء الاصابة بنسبة 60 و80% على التوالي، والكشف على الإصابة كان بعد 25 يوماً من العلاج.

الكلمات الدالة: Bioassay, Red palm weevil, *Rhynchophorus ferrugineus, Hetrerorhabditis bacteriophora*, *Steinernema carpocapsae*