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Compartive Efficiency of Egyptian Entomopathogenic Nematodes and Imidacloprid on the Red Palm Weevil, *Rhynchophorus ferrugineus* Oliv

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ABSTRACT

The efficiency of three isolates of entomopathogenic nematodes indigenous in Egypt, (i. e. *Heterorhabditis bacteriophora* (EKB20), *Steinernema* sp. (B32) and *Heterorhabiditis* sp. (Kasassien isolate)) were determined in the laboratory and field compared with imidacloprid insecticide on the larvae and adults of the red Palm weevil, *Rhynchophorus ferrugineus*. Results showed that isolates of entomopathogenic nematodes, B32 and EKB20 are more effective than Kasassien 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 hrs. Field evaluation showed that Kasassien and EKB20 achieved higher levels of control against diferent stages of *R. ferrugineus* reaching 100% reduction than the isolate B32 (80.33 % reduction). When the level of palm weevil infestation was moderate (1-3 weevil tunnels/ tree, the reduction percent was 80.33, 100 and 80.66% when the trees were treated with EPN isolates of EKB20, Kasassien and B32 respectively. There was no significant effect when the trees with higher infestation (more than 3 tunnels/palm tree) level were treated with EPN. While as imidacloprid insecticide gave 100% mortality in the three levels of infestation. The current work has indicated that EPN are considered promising biocontrol agents, if correctly applied and released in an integrated control schemes against the red palm weevil.

Keywords: Biological control agents, Entomopathogenic nematodes, *Heterorhabiditis bacteriophora*, *Steinernema* spp., Red palm weevil, *Rhynchophorus ferrugineus*.

INTRODUCTION

The red palm weevil, *Rhynocophorus ferrugineus* (Coleoptera: Curculionidae), is a major pest of palm trees in general and specifically date palm trees *Phoenix dactylifera* L., in Egypt, Middle East and the Mediterranean area (Saleh 1992). Efforts to control this devastative pest have focused primarily on the use of certain cultural practices, limited biological agents, pheromone traps and conventional chemical insecticides. Chemical insecticides have been the principal component of most insect control programs in Egypt and other countries due to their fast impact, relatively low cost, easy to apply and use, persistent, and have a broad spectrum of activity that includes many different types of insects. However, their extensive use has resulted in development of resistance in many pest populations (Hussien *et al*. 1991) the eradication of natural enemies and disruption of natural ecosystems (AL-Daikh *et al*. 2017). The widespread application of none specific chemical control has elevated environmental contamination particularly in food and water specially with the use of persistent and harmful chemicals causing acute and chronic toxicity to both human and animal (EL Roby *et al*. 2015). All these negative impacts have led to the development of alternatives safe control agents and strategies include novel biological control agents. Entomopathogenic nematodes (EPN) belong to both families of Steinernematidae and Heterohabditidae and their associated symbiotic bacteria, *Xenorhabdus* and *Photorhabdus* spp. are excellent biological control agents of many insect pests (EL Roby 2011, Hussaini 2014, De Luca *et al*. 2015, Shapiro-Ilan *et al*. 2015, Subramanian and Muthulakshmi 2016, ABD-ELGAWAD 2017, EL Roby 2018). The present study was performed to evaluate different local entomopathogenic nematodes isolates compared with the specific chemical insecticide, imidachlopride against *R. ferrugineus* through laboratory and field trials.

MATERIALS AND METHODS

1- Laboratory Experiments:

The efficicay of three Egyptian entomopathogenic nematodes (EPNs), was estimated at the laboratory of Plant Protection Dept. Faculty of Agric. Minia University against larval and adult stages of the red palm weevil, *Rhynchophorus ferrugineus.*

Entomopathogenic nematodes:

The three isolates of EPN used in the current experiments, namely, (EL-Kasassien) isolated from the soil cultivated with date palm trees (EL Roby 2011), meanwhile EKB20 and B32 isolates were collected from soils cultivated with clover (Shamseldean *et al*. 1998). All entomopathogenic nematodes were reared on last instar larvae of *Gallaria mellonella* L. (Lepidoptera: Pyralidade) (Dutky 1964). Larvae of *G. mellonella* were reared on old bee wax at 28±2 ºC and relative humidity of 65±5 % in the insect rearing laboratory. The emerging infective juveniles (IJs) were harvested from nematode traps and stored in sterilized water at 10ºC (Woodring and Kaya 1988).

Insect source:

Different stages of the red palm weevil were collected from infected palm trees (*Phoenix dactylifera* cv. Zaghlol) 17-years-old located at El-Kasassien, ELismailia governorate and transported to the laboratory in perforated polyethelene bags.

Pathogenicity of the Egyptian entomopathogenic nematodes on the red palm weevil (RPW):

The pathogenicity of entomopathogenic nematodes on the red palm weevil larvae (3rd and last instars) and adults was evaluated under laboratory condation. The virulence of selected nematode isolates (B32, EKB20 and Kasassien) was evaluated by using two concentrations from each isolate 1000 and 2000 IJs/insect. Two mL. from each concentration of the tested nematode were dispensed on moistened filter paper to keep suitable moisture for the nematode activity inside Petri-dishes containing the 3rd and last instar larvae and adults of RPW (about 10 individuals from each instar/treatment). Check treatment was treated with 2 ml. of distilled water. Numbers of dead insects were recorded after 72 hours post treatments. Virulence of all the tested nematode isolates in the laboratory was investigated and the percentage of pathogenicity was estimated as % mortality. Reisolation for the pathogens has been carried out from the infected RPW individuals.

2-Field experiments:

The tested palm trees (*Phoenix dactylifera* cv. Zaghlol) are 17- years-old trees located at El-Kasassien, in EL-Ismailia governorate. Three levels of the weevil infestation (according to Atwa, 2003) were tested i. e. recent infestation 2-3 tunnels/palm tree, Medium infestation 5-6 tunnel/palm tree and heavy infestation 7 or more tunnels/palm tree. Each treatment consists of 9 trees with the three levels of infestation were tested. Nine infested date palm trees with the three level of infestation were left untreated as a check. Block randomized design was used. Three weeks after application, the numbers of active and inactive tunnels were recorded pre and post treatments. Egyptian nematode isolate of *Steinernema* sp., B32 and *Heterorhabditis* spp., EKB20 and Kasassien were reared on last instar larvae of *G. mellonella* according to the technique by (Dutky 1964) and IJs were harvested from the nematode traps. A stock suspension of the nematode IJs was stored in sterilized distilled water at 10^{o}C until needed. All nematodes were used within 2 weeks of harvest and a new stock was made every 2 weeks if needed.

The application technique for the field evaluation of EPN against the red palm weevil was described by (EL Roby 2011, Atwa and Hegazi 2014). Nematode suspensions were injected through 4 artificial tunnels made in a 45^{o} angle into the core of an infected date palm tree right at the top of the insect infection level. These tunnels were made by an 830 W electrical hammer drill equipped with a 45-cm long and 13-mm thick screw. Electricity was supplied in the field with a portable 1000 W generator. An 11-mm thick and 50 cm long PVC tubes were placed in each artificial tunnel to avoid repeated perforation of the palm trees with the hammer drill which could damage the tree tissues, if reinjection were necessary (Atwa 2003). The nematode infective juveniles (IJs) were applied through two injections of 60 ml of nematode suspension with a 60-ml plastic syringe into each artificial tunnel. The concentration of the nematode suspension was 2000 IJs/ml, which is equal to 960,000 IJs/palm tree injected through a 4 tunnels in each tree (60 ml X 2000 X 4 tunnels X 2 injections). After the nematode injections the external end of the PVC tubes were sealed with tight plastic buttons. The results were recorded three weeks post treatment. The symptoms due to palm weevil infection were obvious and can be summarized in a leaky dark gummy material with a foul odor oozing out of the initial insect penetration spot and the presence of dead lower leaves due to the development and activities of the insects inside the palm tree. The recovery from all the symptoms was followed and recorded during the next month. The experiment was repeated again after 3 months post first application. Number of active and none active tunnels were recorded before and post treatments and the reduction% in the population of *R. ferrugineus* were calculated according to the following formula (Henderson and Telton 1955)

$$\text{Reduction } \% = \left(1 - \left(\frac{Ta*Cb}{Tb*Ca}\right) X100\right)$$

Where:

T_b is number of living larvae (active tunnel) before treatment.

T_a is number of living (active tunnel) larvae after treatment.

C_b is number of living (active tunnel) larvae before treatment for the control.

C_a is number of living larvae (active tunnel) after treatment for the control.

The averages of the two applications were subjected to analysis of variance and means were compared with LSD test, the software of the "Costat" program was used.

RESULTS AND DISCUSSION

1) Pathogenicity of entomopathogenic nematode isolates to larva and adult stages of RPW:

The pathoggencity of each entomopathogenic nematode isolates i. e. *Heterorhabiditis* sp. (EKB20), *Steinernema* sp. (B32) and *Heterorhabiditis* sp. (Kasassien) on the larvae (3rd and last instars) and adults of *R. ferrugineus* was evaluated under laboratory conditions. Data summarized in (Figure, 1) showed the percentages of infection caused by each entomnematode suspension. The percentages of infection caused by EKB20, B32 and Kasassien (with a concentration of 1000 IJs/insect) to the 3rd instar larvae had 96.00, 90.00 and 84.0 % mortality as a result of infection with the three isolates strains respectively, while the control experiment (distilled water) had 2.0, 4 and 0.0 % respectively. In general, the steinernematids were more pathogenic to third instar red palm weevil larvae than was *Heterorhabiditis* sp. Also *Steinernema* sp. (B32) and *Heterorhabditis* sp. (EKB20) were the most pathogenic isolates against adults of *R. ferrugineus* in the filter paper. Mortality caused by *Heterorhabiditis* sp. (Kasassien) was significantly lower and more variable. (80, 68 and 66% for third and last instars and adult respectively%).

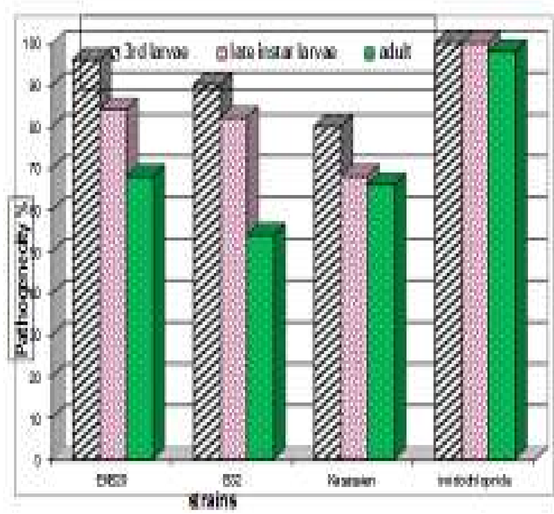

Figure 1. Pathogenety of entomopathogenic nematodes (*Steinernema* sp. (B32), *Heterorhabditis* sp. (EKB20) and *Heterorhabiditis* sp. (Kasassien) at concentration 1000 IJs/insect compared with imidachlopride, to the 3rd and last instar larvae and adult stage of *R. ferrugineus* after 72 hours of exposure on 10-mm filter discs in Petri dishes.

Also data presented in Fig 2 showed that the *Steinernema* sp. (B32), *Heterorhabditis* sp. (EKB20) are more effective against the third instar larvae than *Heterorhabiditis* sp. (Kasassien) when treated with 2000IJs/ insect with no significant references between them.

Entomopathogenic nematodes can kill broad spectrum of insects in the laboratory bioassays performed under optimal conditions where no ecological and behavioral barriers and impacts affecting nematode infections exist. However, the potential to kill an insect in the laboratory assay cannot always be transferred to the fields, particularly when high levels of insect pest control are required (EL Roby 2011, Lacey and Georgis 2012, EL Roby 2018).

Entomopathogenic nematodes have been recognized as excellent biological control agents of soil dwelling insect pests. Recent advances in mass-production and formulation technology have made insecticidal EPN available commercially for large-scale application in Date palm fields, strawberry plantation, cranberry bogs, artichokes, mint, mushrooms, ornamentals, and turf grass (EL Roby 2011, Guo *et al*. 2013, Beck *et al.* 2014).

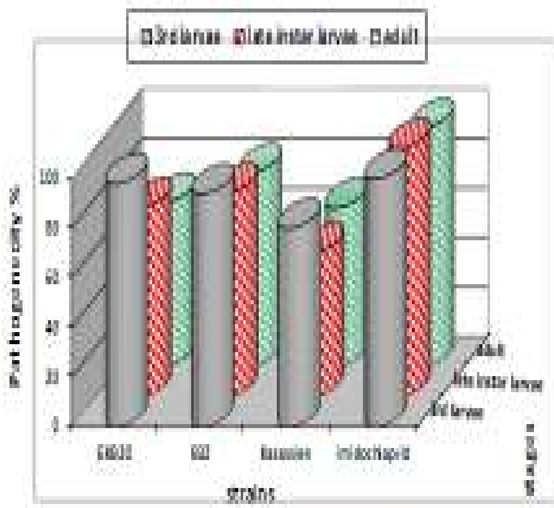

Figure 2. Pathogenety of entomopathogenic nematodes (*Steinernema* sp. (B32), *Heterorhabditis* sp. (EKB20) and *Heterorhabiditis* sp. (Kasassien), at concentration 2000 IJs/insect, to the 3rd and last instar larvae and adult stage of *R. ferrugineus* after 72 hours of exposure on 10-mm filter discs in Petri dishes.

The current data demonstrate variability in the susceptibility of different larval instars, and adult stage of the red palm weevil to entomopathogenic nematode isolates. Also, (Abbas *et al*. 2000) reported that, *Steinernema riobravae*, *Steinernema carpocapsae,* and *Heterorhabditis* sp. proved to be virulent to both larvae and adults of *R. ferrugineus*. They added that high numbers of IJs/adult insect are required to reach 50% mortality of the adult stage (LC_{50} ranged from 900-1416 IJs/adult). Our EPN isolates of B32 and EKB20 strains are more effective than the Kasassien isolate against larvae of *R. ferrugineus* at 1000 IJs and 2000 IJs inoculum levels. Both nematode isolates of B32 and EKB20 were faster to achieve more than 90% mortality of the red palm weevil 3rd instar larvae. Mortality percentages avaible for the other stages have ranged from 58 to 84%. These results are supported by the findings of (Salama and Abd-Elgawad 2001, Shamseldean 2004) who found that *Heterorhabditis* spp. (different geographical isolates) achieved a full mortality of *R. ferrugineus* pupae but mortality rates were lower when the nematodes were used against different larval stages. Insect host larvae were more susceptible to infection by *Steinernema* sp. than *Heterorhabditis* sp. in filter paper bioassays. However, infectivity as a measure of EPN efficacy is subjective. (Fallon *et al*. 2004) suggested that differences in efficacy between the two tested nematode isolates of *S. carpocapsae* were the result of differences in nematode infectivity. However, *Heterorhabditis* sp. often infect at lower rates than *Steinernema sp.* (Wakil *et al*. 2017) but comparative mortality is often similar or higher (Shapiro-Ilan *et al*. 2002). The low infectivity and insect mortality by *Heterorhabiditis* sp. *(*Kasassien strain) may have been attributed to the failure of its symbiotic bacteria to establish in *R. ferrugineus*. However, (Solter *et al*. 2001) demonstrated the efficacy of *H. marelatus* against *A. glabripennis* may be attributed to the longer exposure period used by (Solter *et al*. 2001) in their bioassays, and the greater length of time required to penetrate or kill adult coleopterous species. The 72 hours exposure period used in our study may have been insufficient for heterorhabditids to penetrate the host, accounting, in part, for the low mortality by *Heterorhabiditis* sp. The use of the traditional filter-paper nematode bioassay demonstrated a broad susceptibility of *R. ferrugineus* larvae to nematode infection. Additional bioassays using *R. ferrugineus* from different populations are required to confirm the effects of EPN on different life stages and further assess the potential for the use of EPN under field conditions.

2) Field evaluation of EPN isolates against the red palm weevil:

As showed in Table 1 it was observed that *Heteroharbitits* sp. nematode isolates were more promising in controlling *R. ferrugineus* than *Steinernema sp.* (B32). *Heteroharbitits* sp. (EKB20) and Kasassien nematode strains gave as high as 100% mortality while *Steinernema* sp. (B32) gave 80.66 % insect reduction in red palm weevil populations. However, the Kasassien and EKB20 achieved better control of *R. ferrugineus* stages (100% reduction) than the B32 (80.66) reduction % when the level of infestation was lower, 1-3 tunnels / tree. In contrast, when the palm trees have medium infestation level of (4-7 tunnels/tree) and treated with EPN, the reduction percentages in RPW populations were 80.33, 100 and 80.66% when the infested trees treated with EKB20, Kasassien and B32, respectively.

The high efficacy of *Heterorhabditis* nematodes can be explained by the fact that, the anterior dorsal tooth in the infective juveniles of *Heterorhabditis* sp. facilitates penetration of coleoptrean larvae through the cuticle and/or intersegmental membranes (Atwa 2003). Also, the earlier release of the high virulent bacterium by *Heterorhabditis* sp. than do by *Steinernema* sp. (Wang and Gaugler 1998) may account for the former nematode strain (EKB20) and Kasassien being more infective than the latter (B32) in killing *R. ferrugineus* in the field trails. Similar findings were obtained by (Jansson *et al*. 1990), who found that *Heterorhabditis* was better than *S. carpocapsae* at reducing populations of larvae and pupae of the weevil, *Cylas formicarius* which infects the storage roots of sweet potato in the field. Our current results also agree with (Abbas *et al*. 2000) who reported in a laboratory test that, *Steinernema riobravae, Steinernema carpocapsae,* and *Heterorhabditis sp.* proved to be virulent to both larvae and adults of the red palm weevil. Our results are also supported by the findings of (Salama and Abd-Elgawad 2001, Shapiro-Ilan *et al*. 2002, Rugman-Jones *et al*. 2013, Sun *et al*. 2016). They have found that *Heterorhabditis spp*. (other geographical isolates) achieved a full mortality of *R. ferrugineus* but mortality rates were less in different larval stages. Our data also revealed that applications of EKB20, Kasassien and B32 nematode isolates reduce damage to date palms (over 90%) in case of low and moderate weevil infestation. The chemical insecticide, Imidacloprid also showed high efficiency against all stages of the red palm weevil at the three levels of the palm infestation. Imidacloprid is belonging to the neonicotinoid group and can be used in prophylactic and curative applications against RPW in infested date palm trees (Kaakeh 2006, Al-Shawaf *et al*. 2010). Application of the EPN in field trials proved effective against *R. ferrugineus* with insect mortality of up to 90% in the case of low and medium weevil infestations but if the infestation arrived to a dangerous level of almost killing the palm tree, treatment with the chemical insecticide imidacloprid is the best approach to be applied.

Table 1. Average reduction% in *Rhynchophorus ferrugineus* after 21 days post treatment with different local isolates of EPN and the insecticide, imidacloprid.

Treatments	Concentration	Level of infestation		
		1	2	3
Steinernema sp. B32	960000 IJs/tree	80.66	80.33	10.0
Heteroharbitis sp. (EKB20)	960000 IJs/tree	100	100	40.66
Heterorhabditis sp. Kasassien strain	960000 IJs/tree	100	80.66	40.66
Imidacloprid (Confidor® 240 OD)	1.5 gm a.i./L.	100	100	100
Check	0.0	0.0	0.0	0.0

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مقارنة فاعلية النيماتودا المصرية الممرضة للحشرات ومبيد الاميدا كلوبريد على سوسة النخيل الحمراء ، *Rhynchophorus ferrugineus* **Oliv**

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تعتبر النيماتودا الممرضة للحشرات عائلتي (Steinernematidae و Heterohabiditdae) عامل مكافحة حيوية ناجح للعديد من الآفات الحشرية. تم اختبار حساسية اليرقات والحشرات الكاملة من سوسة النخيل الحمراء *R. ferrogenious*للإصابة لثلاث عزلات لنيماتودا ممرضة محلية وهي *Heterorhabiditis* (B32) (EKB20) *bacteriophora*، *Steinernema sp* (B32) وHeterorhabiditis sp (Kasassien) في المعمل والحقل مقارنة مع المبيد الحشري ألأميداكلوبريد وأظهرت النتائج أن عزلات النيماتودا الممرضة المحلية ، B32 و EKB20 أكثر فعالية من عزلة القصاصين ضد الأعمار اليرقية لسوسة النخيل *R. ferrugineus* عند تركيزات 1000 طور يرقي معدي / مل وكذلك و 2000 طور يرقي معدي / مل وأظهرت عزلات B32 و EKB20 تأثير سريع لتحقيق معدل أكثر من 90 ٪ موت ليرقات العمر الثالث. وأوضح التقييم الحقلي للنيماتودا الممرضة ضد سوسة النخيل عند 3 مستويات من الإصابة أن عزلات القصاصين و EKB20 حققا أفضل مستوي مكافحة 100% نسبة خفض في الإصابة وذلك عندما كانت نسبة الإصابة تتراوح (1-3 نفق/ شجرة) بينما كان الانخفاض٪ 80.33 و 100. 80.66٪ عندما تمت معاملة الأشجار ذات مستوى الإصابة من (5-6 أنفاق / نخلة) بالعزلات EKB20 و القصاصين و B32 على التوالي. واتضح من النتائج أنه إذا تعدت نسبة الإصابة (أكثر من 7 أنفاق/ نخلة) فان المعاملة بالنيماتودا الممرضة يكون تأثيرها ضعيف ويستخدم مبيد الأميداكلوبريد الذي أعطي أفضل النتائج عند هذا المستوي من الإصابة. وخلصت النتائج إلي أن العزلات المحلية للنيماتودا الممرضة من كلا العائلتين تعتبر عناصر جيدة لتصميم برنامج مكافحة متكاملة لحشرة سوسة النخيل الحمراء .

الكلمات المفتاحية : النيماتودا الممرضة للحشرات - *Heterorhabiditis bacteriophora*, *Steinernema* sp. *Rhynchophorus ferrugineus* – سوسة النخيل الحمراء , المكافحة الحيوية