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Efficiency of entomopathogenic nematodes (Rhabditida) against *Saccharococcus sacchari* (Cockerell) (Homoptera: Pseudococcidae) under laboratory conditions

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Abstract

The objective of this study was to study the efficacy of four indigenous and one exotic isolated entomopathogenic nematodes (EPNs) against sugarcane mealybug *Saccharococcus sacchari* under laboratory conditions (27 ± 2 °C, RH 65 ± 10%). The EPNs used in the study are *Steinernema* sp. (strain: AT4), *Steinernema* sp. (strain: EMB), *Heterorhabditis bacteriophora* (strain: EKB20), *Heterorhabditis* sp. (strain: EIK) and *Steinernema glasri* (strain: New Jersey). The bioassays were carried out on Petri dishes containing parts of pumpkin infested with adult females of *S. sacchari*, and were sprayed with EPN juveniles. Results showed that *H. bacteriophora* strain (EKB20) and *Heterorhabditis* sp (EIK) were more efficient with higher pathogenicity and virulence in the laboratory than the other strains and gave the highest corrected mortality percentage in the infestation (from 68.59% to 70.83%). The LC_{50} were 25.42 and 45.5 IJs/ insect for the two strains, respectively.

Keywords: EPNs, sugarcane mealy bug, biological control, *Steinernema* sp., *Heterorhabditis* sp. and *Steinernema glasri*.

The sugarcane mealy bug, *Saccharococcus sacchari*, (Cockerell) (Homoptera: Pseudococcidae), is a common pest to all countries that cultivate sugarcane including Egypt (Cox, 1981; Yakoub, 2012). The body fluid of *S. sacchari* can suppress early growth of sugarcane; heavy infestations, which result in the production of honeydew quantities in the leaf sheath pockets, are implicated in processing difficulties during sugar manufacture (Dick, 1969). Immature and mature mealy bugs are found in clusters on the roots, on the stem buds, underneath of the leaf sheaths and covered with white mealy wax, which makes them difficult to control. Furthermore, the damage caused by the pink sugarcane mealy bug occurs by sucking the plant sap preventing plants from essential nutrients which may lead to leaves yellowing and thin canes. A large amount of honeydew which is considered as a suitable medium for the growth of black sooty mould fungus is found and attracts ants on the leaves and stalks. Thus, reduction of sugar yield and difficulty in filtration and clarification of juices in a factory may be associated with severe mealy bug infestation (Charles *et al*., 2006).

Chemical control of the sugarcane mealy bug is difficult because of the waxy material which covers eggs and adult females (Dean *et al*., 1971) as well as the rising cost of pesticides and their acute and chronic toxicity and their risk ratio on the environment. Recently, biological controls are one of the most elemental in integrated pest management of sugarcane pests. One of the important elements of biological control is using of entomopathogenic nematodes (EPNs) for *S. sacchari* control, because the suitable environment of the mealy bug, especially when infested the roots, is the same to that required by the EPNs (Ehlers, 2001, 2005). Because of these reasons and the fact that

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previous researchers observed that *Heterorhabditis* sp. and *Steinernema* sp. caused high mortality of *Dysmicoccus texensis* females (Pseudococcidae), *Planococcus ficus* (Signoret) (le Vieux & Malan, 2013) under laboratory tests. Moreover, certain strains of *Heterorhabditis* sp. showed high efficiency against mealy bug in laboratory, greenhouse, and field tests. *S. sacchari*, is also associated with the soil, thus the possibility of controlling this pest with EPNs needed to be tested. The aim of this work was to evaluate different entomopathogenic nematodes against *S. sacchari* under laboratory conditions and to determine the effective dose for field application.

Materials and Methods

The experiments were carried out at the laboratories of Plant Protection Dept. Faculty of Agric. Minia University. Initially, the mealy bugs were collected from roots and leaves of sugarcane crop cultivated in Abokorkas region, which were reared in pumpkin fruits (*Cucurbita maxima* Duchesne), at room temperature (26 ± 2 °C) (Walton & Pringle, 2004). Five strains of EPNs were used in the experiments, of which 4 were native i.e., *Steinernema* sp. (AT4 and EMB) isolated from the clover leaf weevil, (Atwa, 2003) and *Heterorhabditis bacteriophora* (EKB20) and *Heterorhabditis* sp. (EIK) isolated from soils (Shamseldean & Abd-Elgwad, 1994) and for comparison purposes, an exotic strain New Jersey (*Steinernema glasri*) were reared on last instar larvae of *Gallaria mellonella* L. (Lepidoptera: Pyralidade) (Dutky *et al*., 1964). Larvae of *G. mellonella* were reared on old bee wax at 28±2 ºC and relative humidity 65±5 % in insect rearing laboratory. The emerging infective juveniles (IJs) were harvested from nematode traps and stored in sterilized water at 10ºC (Woodring & Kaya, 1988). *Steinernema glasri* was stored in a milk cool container modified by Shamseldean (unpublished data) at 18±2 º C for 14 -28 days before use. The experiment aimed to evaluating the virulence of EPNs strains against females of *S. sacchari*. The experiment was conducted in Petri dishes (10 cm diameter, 1.5 cm height). Four concentrations of 100 IJs, 200IJs, 300IJs and 400IJs from each strain were used. A piece of pumpkin fruit (10 cm^2 by 1.5 cm height) was placed on filter paper in Petri dish infested with different live numbers of *S. sacchari*, 2 ml from each concentration of each strain were poured on moistened Whatman No.1filter paper to keep suitable moisture for nematode activity, and the insects covered with treated filter paper; 3 replicates were used for each treatment. Check treatment was treated with 2 ml. distilled water. After that, the dishes were incubated at (26 ± 2 °C, RH 65 ± 5 %) for four days. The numbers of alive and dead insects were recorded. The virulence of all tested nematode strains were assessed as corrected mortality % for each concentration. In all nematode infections were confirmed by collecting emerged IJs from insect cadavers. The corrected % mortality was corrected for control mortality according to Schneider-Orellis formula (Puntener, 1981).

$$\text{Corrected \%} = \frac{(\text{Mortality \% in treatment} - \text{Mortality \% in control}) \times 100}{100 - \text{Mortality \% in control}}$$

Completely randomized design was used. ANOVA test was used and means of mortality were differentiated with the least significant difference LSD. Based on mortality % obtained in the first experiment, the two most virulent strains were selected to determine their lethal concentration (LC_{50}, LC_{90}, and LC_{99}) and their fuidicial limits to control between them (Finney, 1971). The technique similar to the above described, using the following concentrations: 0 (control) 200, 400, 800, 1600 and 2400 IJs per Petri dish was used. The experimental design was randomized, with two treatments (EPNs isolates) and the control (sterilized water). Corrected mortality means were subjected to the probit analysis to calculate the lethal concentrations dose (Finney, 1971). The third experiment was carried out to evaluate the efficiency of *H. bacteriophora* (EKB20) selected as the most virulent nematode strain, to spray pumpkin infested with *S. sacchari.* Infested pieces of pumpkins (8 cm

diameter) were placed in Petri dishes (10 cm diameter, 1.5 cm height) containing wet filter paper. The application of nematodes was done with hand sprayer (1L), with a volume of 3.0 ± 0.1 mL of aqueous suspension per dish, with a concentration of the Upper fuidicial limit of LC_{99} and three replicates were used for each treatment. Percentage of reduction in the mealy bug population was calculated using the formula of (Henderson & Telton, 1955) as follows:

Where:

Tb, and Ta: No. of alive insects in treatment pre and post treatment
Cb, and Ca: No. of a live insect in control before and after treatment
Data were submitted to ANOVA and compared by LSD test at 5% probability.

Results

H. bacteriophora, (EKB20) and *Heterorhabditis* sp. (EIK), caused average corrected mortality 70.83% and 68.59 % of *S. sacchari*, respectively. Un significant differences between them was observed while *Steinernema* sp. (AT4) and (EMB) and *Steinernema glasri* New Jersey showed significant differences in the mortality of *S. sacchari* between them and the two strains 42.92, 48.69 and 35.4 %, respectively. The five EPNs strains were pathogenic to *S. sacchari* in the laboratory, from the five tested strains (F = 9.7; P = 0.001, and LSD values 15.1). Although no significant differences were found between the first two strains EKB20 and EIK (Table 1), we determined the LC_{50} for the most effective strains as shown in Table 2.

H. bacteriophora (EKB20) showed the highest virulence on *S. sacchari*, presenting LC_{50} equal to 25.42 (28.42- 22.42) and LC_{90} equal to 69.71 (57.1- 85.6) IJs / insect. Strain (*Steinernema* sp. (EMB) strain was less virulent 45.5 (34.89-59.32). The third strain (EBM) was the least virulent with significant differences between strains EKB20 and EIK LC_{50} value was 54.96 IJs/insect (71.56-42.2). Based on these results, the strain *H. bacteriophora* (EKB20) and *Heterorhabditis* sp (EIK) were selected for the subsequent bioassay with the concentration of the Upper limit of LC_{99} Results of the third assay confirmed efficiency of the isolated *H. bacteriophora* (EKB20) and *Heterorhabditis* sp (EIK) (77.79% and 65.36%) reduction compared to control with no significant differences between the two strains, indicating that the two strains are promising strains for controlling *S. sacchari* with concentrations equal to the upper limit of LC_{99} in the field.

Table 1. Reduction % of the different strains of entomopathogenic nematodes against *Saccharococcus sacchari* calculated with Schneider-Orellis formula (Puntener, 1981) with the value of LSD.

Strains	Aveg. Corrected M%	F value	LSD 0.05
***H. bacteriophora* (EKB20)**	70.83a		
***H.* sp (EIK)**	68.59a		
***Steinernema* sp (AT4)**	42.92b	9.7**	15.1
***Steinernema* sp (EMB**	48.69b		
Steinernema glasri (New Jersy)	35.4b		

Discussion

In this study, effects of EPNs against *S. sacchari* are similar to that observed by Alves *et al*., 2009a and b, and Shamseldean & Abd-ELgwad, 1994) who demonstrated that the potential of EPNs to control different species of Pseudococcidae mealy bugs at different crops. Also, these results are similar to those conducted to control *Planococcus ficus* with EPN and that conducted against different mealy bug species, specifically on *P. viburni* by (Stokwe, 2009) and on *P. citri* by Van Niekerk & Malan, (2012).

Table 2. Probit analysis to determine the lethal concentration (LC_{50}, LC_{90} and LC_{99}) of *Heterorhabditis bacteriophora*, EKB20, EIK and *Steinernema* sp (EMP) against *S. sacchari* females and their fudicial limits.

Strains	LC_{50}	Fudicial limits		slope	LC_{90}	LC_{99}	Fudicial limits	
		Upper	Lower				Upper	Lower
H.bacteriophora (EKB20)	25.42	28.41	22.41	5.3	69.71	295.12	742.31	101.39
H. sp. (EIK)	45.5	59.32	34.89	2.37	435.7	1318.25	1560.2	1001.3
Steinernema sp. (EMB)	54.96	71.56	42.2	3.32	725.5	1820.2	2132.2	1322.8

In previous studies, *Heterorhabditids* strains were the most pathogenic species against mealy bug strains (Stokwe, 2009, Ferreira & Malan, 2013). However, studies on other insect pests showed similar results concerning *H. bacteriophora*. *H. bacteriophora* was selected as the best candidate for the control of codling moth, *Cydia pomonella* (L.), (EL Roby, 2011).

The performance of the two families is varied, considering that *Heterorhabditis bacteriophora* EKB20 and *Heterorhabditis* sp. (EIK) were the highest potential arguments concerning the best performance of heterorhabditids compared with steinernematids as the heterorhabditids can penetrate through intersegmental membranes by scratching away at these with a special dorsal tooth (Bedding & Molyneux, 1982).

When considering the current study and previous studies, these two strains *Heterorhabditis bacteriophora* EKB20 and *Heterorhabditis* sp. (EIK) clearly displayed highly virulent qualities to a variety of insect pests, including *P. viburni*, and therefore were selected for further tests.

Future studies should be conducted to evaluate the efficiency of *H. bacteriophora* (EKB20) and in the field. Also, biological studies are needed to know if the EPNs can develop and complete their life cycle in *S. sacchari*. If they are able to do so, it would affect the success and persistence of these species as bio-control agent in sugarcane crop and can be effective against other soil insect pests.

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