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Biological Control of Red Palm Weevil with Entomopathogenic Nematodes in the Eastern Province of Saudi Arabia

M.M.E. Saleh* and M. Alheji**

*Pests and Plant Protection Department, National Research Centre, Dokki, Giza, Egypt

** Horticulture Division, Project for Agricultural Development in Qatif, Eastern Province, Kingdom of Saudi Arabia

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ABSTRACT

This work has been conducted through a Project on Biological Control of the Red Palm Weevil in Arab Gulf Countries executed by Arab Organization for Agricultural Development (AOAD) in cooperation with Ministries of Agriculture in Arab Gulf Countries and funded by Islamic Developmental Bank and The International Fund for Agricultural Development (IFAD) and (AOAD). It includes laboratory and field experiments on the utilization of entomopathogenic nematodes for biological control of the red palm weevil in the infestation region in Qatif, Eastern Saudi Arabia. Results indicated that the Saudi strain *Heterorhabditis indicus* (SA) caused 100, 70 and 75% mortality in young, grown larvae and adults of red palm weevil, respectively in the laboratory. In the field 60% of larvae and 46% of adults were killed in infested trees. Nematodes need only 2 hours of contact to penetrate and subsequently kill the host. Best reproduction of *Steinernema carpocapsae* in adults was 218787 IJ/adult. Infection has been completed within 4 hours of contact between the nematode and the host. When nematodes applied in soil under date palm trees, 77.5% of adults in soil were killed. Although the local strain *H. indicus* (SA) is less in its performance against red palm weevil adults than *S. carpocapsae* it has the advantage of tolerance to the local environmental conditions. These results add evidence to the recommendation of introducing entomopathogenic nematodes in IPM of the red palm weevil.

Key Words: Biological control, red palm weevil, entomopathogenic nematodes, Saudi Arabia.

INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* Oliv. is known as the most destructive pest to date, coconut and oil palms in Arabic region and south east Asia (Kalshoven, 1950; Hanounik, 1998). It has been known in south east Asia until its appearance in United Arab Emirates in 1985, Kingdom of Saudi Arabia in 1987, Iran in 1992 and Egypt in 1993 (Murphy and Briscoe, 1999). Primary infestations always escape attention and symptoms may not become evident until extensive damage has already occurred (Griffith, 1987, Sivapragasam *et al.*, 1990). Larvae excavate long tunnels in palm trunks causing great damage along their 12 instars then pupate in cocoons maid of dry tissues near the surface. It may spend more than a generation inside an infested tree. Adults of *R. ferrugineus* were observed between leaf petioles for feeding and egg laying (Kalshoven, 1950, Sadakathulla, 1991). In Arabic gulf region adults of *R. ferrugineus* were frequently observed aggregating on the basal buried part of the trunk in the soil and among leaf axils for feeding, mating and egg laying (Hanounik, *et al.*, 2000). During the last decade efforts to control *R. ferrugenieus* focused on the use of insecticides and pheromone traps (Abraham, *et al.*, 1998). Because of the cryptic feeding habit of larvae, their chemical control is difficult.

Entomopathogenic nematodes of the families Heterorhabditidae and Steinernematidae are commercially produced and used in biological control of insect pests especially in soil and cryptic habitats (Georgis, 2002). Bedding (1990) and Georgis (1992) stated that indigenous nematodes are expected to be suitable for management of local insect pests because of their adaptation to local climate and population regulators. Through a project on biological control of the red palm weevil in Arab Gulf Countries, local entomopathogenic nematodes were isolated, identified and tried against the red palm weevil (Abbas and Hanounik, 1999; Saleh *et al.*, 2001). This work includes virulence evaluation of indigenous and imported entomopathogenic nematodes used against larvae and adults of the red palm weevil and application of the nematodes against larvae and adults in the field.

MATERIALS AND METHODS

This work has been conducted through a project for biological control of the red palm weevil in Arab Gulf Countries sponsored by Arab Organization for Agricultural Development in cooperation with Ministries of Agriculture in Arab Gulf Countries

Virulence of Nematodes to *R. ferrugineus*

Heterorhabditis indicus (SA from Kingdom of Saudi Arabia), *Steinernema abbasi* (from Sultante of Oman), *H. bacteriophora* (HP88 from USA), *Steinernema carpocapsae* (Weiser from Germany) were compared for virulence to 3rd, 8th larval instars, and adults of *R. ferrugineus* in the laboratory as described by Woodring and Kaya (1988). A concentration of 100 IJ/ml/larva was chosen for comparative virulence of tested nematodes to the pest larvae. Adults of the pest however were exposed to serial concentrations of infective juveniles (IJ) between 10 and 100 IJ/cm^2 of soil surface. Experiments took place in plastic Petri dishes 9cm in diameter furnished with filter paper in case of larvae or filled with 50 g fine sand of 15% water content (w/w) in case of adults. Fifteen replicates were specified for each treatment. Individual adult or larva in a Petri dish represented a replicate. Experiments were conducted in the laboratory at 25°C. Insects were observed daily for mortality and dead insects were transferred to White traps (White, 1927) and numbers of reproduced IJ were recorded. Calculations, graphs and statistical analyses, as required, were done using Excel Microsoft Office (2000).

Time Required to Induce Infection in Adults

Adults of *R. ferrugineus* were exposed to IJ of *S. carpocapsae* for 2, 4, 6, 8 and 24 hours to determine optimal duration for infection and nematode reproduction. Pest adults were exposed to the nematode IJ in a plastic tray 15X25 cm filled with fine sand of 15% moisture (w/w) at a concentration of 50 IJ/cm^2 soil. After each assigned exposure time adults were transferred to clean dishes with pieces of soft date palm wood as food and observed for mortality for 5 days. Dead insects were transferred to White traps and numbers of emerging IJ/adult were counted. Each treatment consisted of 10 replicates. Experiments were conducted in the laboratory at 25°C.

Control of Larvae in Infested Trunks

Suspension of *H. indicus* (SA) at 10000 IJ/ml was injected in naturally infested date palm trees to control *R. ferrugineus* larvae. Number of active galleries with fresh exudates and frass were determined. Few small holes were drilled at site of infestation to reach the tunnel network in the trunk. Nematodes were delivered into galleries using an injector with a plastic tube. Then the openings were blocked with soil. Two weeks after treatment, galleries were inspected and numbers of dead and alive larvae or adults were recorded. As many as 20 infested trees with 30 active galleries were inspected for larval and adult mortality after treatment. Other 5 treated trees with 12 galleries were observed and number of dry and wet galleries was recorded weekly for 3 weeks.

Adult Control in Soil

Suspension of *H. indica* SA or *S. capocapsae* was applied in soil around date palm trees under screen cages at 2 million IJ/3 L of water/tree. Trees were artificially infested with adults of *R. ferrugineus* at 10 adults/tree two hours before nematode treatments to insure disappearance of adults in or under the trees. Four replicates represented by four trees were specified for each treatment. Control plots received water without nematodes. Trees were inspected daily for 10 days and numbers of dead and alive adults were recorded. Insect cadavers were transferred to Whit traps for nematode reproduction. The experiment was conducted during February when daily mean temperature ranged between 8 and 20°C.

RESULTS AND DISCUSSION

Nematode Efficacy against Larvae

Data in Table (1) indicated high virulence of tested nematodes to larvae of *R. ferrugineus*. Third instar larvae were susceptible to nematode infection so that a concentration of 100 IJ/ml/larvae of all tested nematodes induced 100% mortality within 2-3 days. Eighth instar larvae were less susceptible, *S. carpocapsae* being the most virulent killed 80% of these larvae in 2-5 days while *S. abbasi*, the least virulent killed 60% of these larvae in 2-4 days. The local *H. indica* SA killed 70% of 8th instar larvae in 2-5 days. Although Abbas and Hanounik (1999) obtained up to 100% larval mortality in the laboratory using concentrations up to 240 IJ/ml/larva of *S. carpocapsae, S. riobravae* and/or *Heterorhabditis* sp. Shamseldean (2002) reported high mortality in larvae when some Egyptian isolates of entomopathogenic nematodes were used against this pest.

Table (1): Mortality in larvae of *R. ferrugineus* after treatment with four entomopathogenic nematodes (100 IJ/ml/larva).

Nematode	*3rd* instar		*8th* instar	
	% Mortality	Duration (days)	% Mortality	Duration (days)
H. indicus SA	100	2.5 (2-3)	70	2.5 (2-3)
H. bacteriophora	100	2.5 (2-3)	70	3.0 (2-4)
S. carpocapsae	100	2.5 (2-3)	80	3.5 (2-5)
S. abbasi	100	2.5 (2-3)	60	3.0 (2-4)

Nematode Efficacy against Adults

Adults of *R. ferrugineus* were less susceptible to nematode infection than larvae. Tested nematodes differed in their virulence to adults and steinernematids looked more virulent to adults than heterorhabditids (Table 2). Concentrations of 10-100 IJ/cm^2 soil of *S. carpocapsae* (the most virulent) caused mortality in adults between 33.33 and 91.67% within 2-5 days with LC_{50} of 6.38 IJ/cm^2 soil. The local *H. indicus* SA caused 16.67-75% mortality with LC_{50} of 49.88 IJ/cm^2 of soil. Values of correlation coefficient (R^2) indicated high correlation between nematode concentration and insect mortality. Abbas and Hanounik (1999) and Shamseldean (2002) agreed with the present results that adults of *R. ferrugineus* were less susceptible to nematode infection than larvae.

Nematode Reproduction in Adults

All tested nematodes reproduced in infected adults with differences in yield of infective juveniles and percentages of successful reproduction (Table 3). steinernematids yielded more IJ from adults than heterorhabditids. *S. carpocapsae* yielded a mean 218787 IJ/adult with a maximum of 900000 IJ/adult. This nematode reproduced successfully in 80% of infected adults. Meanwhile *H. indicus* (SA) yielded a mean of 98210 IJ/adult and a maximum of 435000 IJ/adult with 60% successful reproduction. Wide ranges in yield of new IJ from weevil cadavers were observed. It might be attributed to differences in micro-flora content in insect cadavers. Such microorganisms may compete with nematodes for nutrients or secrete toxins in insect cadavers. Although adults of *R. ferrugineus* looked less susceptible to nematode infection, they were more suitable for nematode development and reproduction. Preliminary experiments recorded poor nematode development and reproduction in young larvae while no reproduction occurred in grown larvae (unpublished data).

Time Required to Induce Infection in Adults

Data in Table (4) indicated that 2 hours were enough for *S. carpocapsae* IJ to infect adults of *R. ferrugineus*. Mortality has ranged from 90 to 100% through exposure time 2-24 hours. Nematode reproduction in adults of *R. ferrugineus* was affected greatly with the exposure time during the infection. Highest yield of 182737 IJ/adult at a total of 14619000 IJ was obtained from 4 hours exposure

Table (2): Mortality in adults of *R. ferrugineus* after treatment with four entomopathogenic nematodes.

Treatment	Concentration (IJ/cm^2 soil)	% Mortality	Correlation coefficient	LC_{50} (IJ/cm^2 soil)	Duration (days)
H. indicus SA	10	16.67	0.88	49.88	3.0 (2-4)
	25	41.67			3.5 (2-5)
	50	58.33			2.5 (2-3)
	100	75.00			2.5 (2-3)
H. bacteriophora	10	25.00	0.92	40.19	3.5 (2-5)
	25	41.66			3.5 (2-5)
	50	66.67			3.0 (2-4)
	100	83.33			2.5 (2-3)
S. carpocapsae	10	33.33	0.63	6.38	3.5 (2-5)
	25	75.00			3.0 (2-4)
	50	83.33			3.5 (2-5)
	100	91.67			2.5 (2-3)
S. abbasi	10	33.33	0.68	32.39	3.5 (2-5)
	25	58.33			2.5 (2-3)
	50	66.67			2.5 (2-3)
	100	75.00			2.5 (2-3)

Table (3): Reproduction of entomopathogenic nematodes in adults of *R. ferrugineus.*

Nematode	% Mortality	% Reproduction	Mean yield IJ/adult	Range yield
H. indicus (SA)	75.00	60	98210	4200-435000
H bacteriophora	83.33	60	61571	2200-230000
S. carpocapsae	91.67	80	218787	1000-900000
S. abbasi	75.00	70	180200	4000-390000

Table (4): Mortality and nematode reproduction in adults *of R. ferrugineus* at different exposure durations to *S. carpocapsae.*

Exposure time to nematodes	% Mortality	% Reproduction	Yield IJ/adult	Total yield
2 hours	90	70	162857	11400000
4 hours	90	80	182737	14619000
6 hours	90	50	230825	11541250
8 hours	90	50	49460	2473000
24 hours	100	50	38400	1945000

to the nematodes. Long exposure times have caused inter-specific competition of nematodes in the host resulted in decreased nematode reproduction. Such finding may encourage field application of entomopathogenic nematodes to control adults of *R. ferrugineus* at lower concentrations.

Control Insect Larvae in Infested Trunks

Water suspension of the local *H. indicus* (SA) was injected in active galleries of *R. ferrugineus* in date palm trunks then galleries were blocked with soil as followed when chemical insecticides were applied. The local nematode caused 46.67% dry galleries, 58.82% mortality in larvae and 43.47% mortality in adults within two weeks (Fig.1). In plots observed for external symptoms of recovery (Fig 2), percent of dry galleries increased from 8.33% in the first week to 40% in the second, then to 50% in the third week as a result of nematode treatment. Shamseldean (2002) treated infested date palm trees with Egyptian *H. indicus* and *H. bacteriophora* at the same method and obtained completely dry galleries throughout a month. Although, injection technique is considered as a time and effort consuming it is commonly used in controlling larvae of *R. ferrugineus* with chemical insecticides.

Adult Control in Soil

Application of *S. carpocapsae* and *H. bacteriophora* in soil under date palm trees artificially infested with adults of *R. ferrugineus* caused 77.5 and 17.5% mortality, respectively in the pest adults within 10 days as shown in Fig. (3). Such finding indicates that during winter *S. carpocapsae* could be suitable for field application than *H. bacteriophora* in Qatif. An isolate of *H. indicus* from Saudi Arabia (HSA-17) achieved 65% in adults of *R. ferrugineus* in soil and in date palm leaf axils near soil surface (Hanounik *et al.*, 2000). Most *Heterorhabditis*

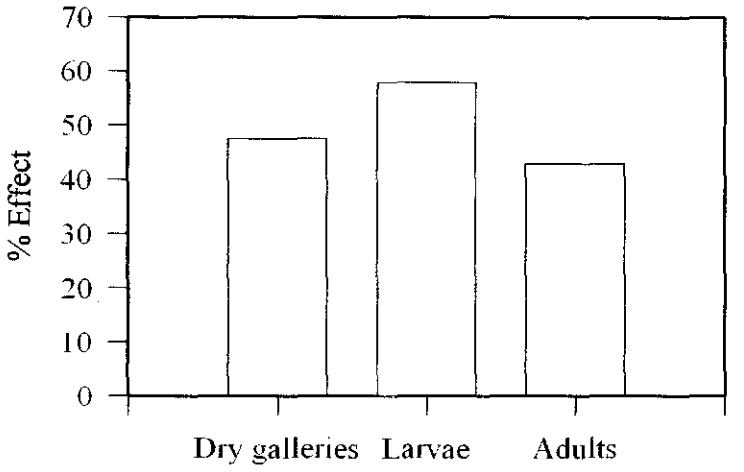

Fig. (1): Percentage of dry galleries, mortality in larvae and in adults of *R. ferrugineous* after injection with *H. indicus* (SA)

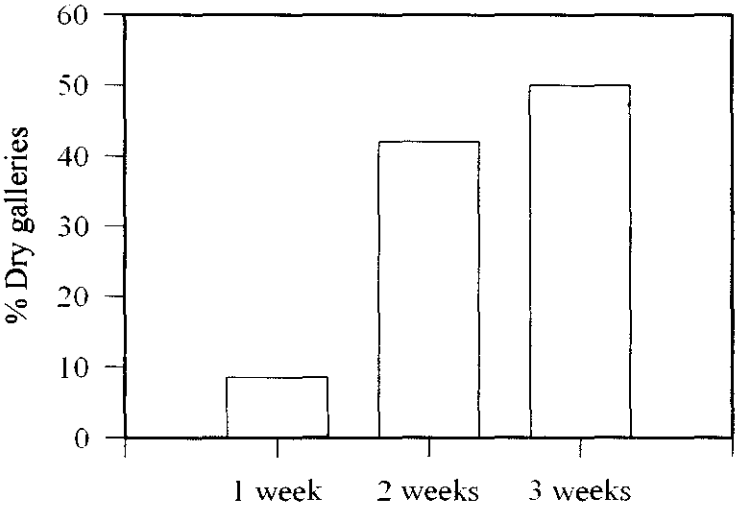

Fig. (2): Percentage of dry galleries in date palm trees injected with *Heterorhabditis indicus* (SA)

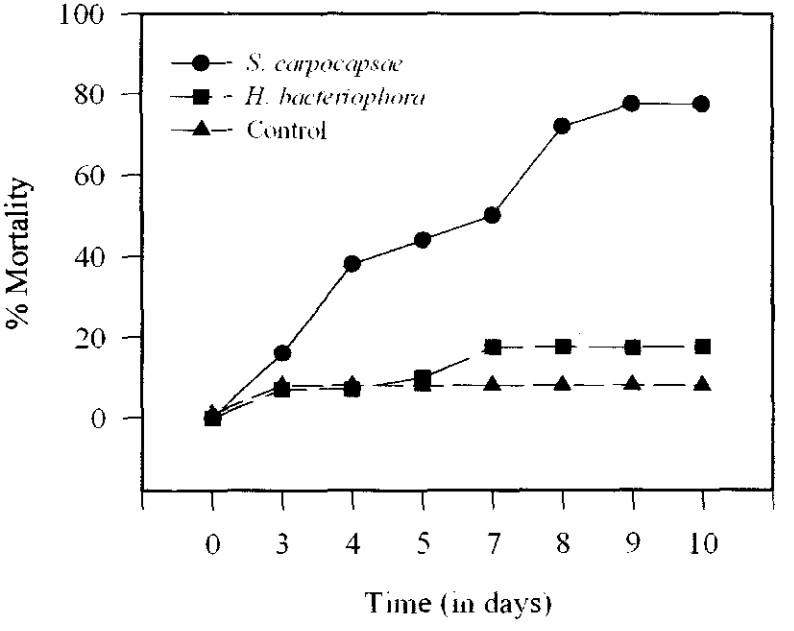

Fig. (3): Mortality in *R. ferrugineous* in soil under palm trees caused by *S. carpocapsae* and *H. bacteriophora*

spp. were isolated from tropical and semi tropical regions (Poinar *et al.*, 1992).

Lower effect of *H. bacteriophora* happened because of low temperature in the field during February. Mean temperature during February was 15°C in Qatif. In contrast, Abbas *et al.* (2000) applied *S. riobravae* in soil around date palm trees that artificially infested with *R. ferrugineus* adults and obtained 100% mortality in the pest adults. Although the local strain *H. indicus* (SA) has less efficacy against red palm weevil adults than *S. carpocapsae* it has the advantage of tolerance to the local environmental conditions. These results add evidence to the recommendation of introducing entomopathogenic nematodes in IPM of the red palm weevil.

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