BIOLOGICAL CONTROL OF THE RED PALM WEEVIL

With entomopathogenic nematodes

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Abstract

This work has been carried out in Saudi Arabia through the project of "Transfer of bio-control techniques to management of the red palm weevil in Middle East" a project conducted by "Arab Organization for Agricultural Development" (AOAD). The overall objective of this work was to contribute to the decision makers a program for the biological control of the red palm weevil (RPW) with entomopathogenic nematodes (EPN). This biological control program is ready to be transferred into IPM program of the pest in our Arabic region. The specific objectives were (1) isolation and identification local entomopathogenic nematodes from Saudi Arabia and Qatar, (2) Laboratory evaluation of the nematodes against adults of the RPW, (3) studying the suitability of new nematodes to mass production, (4) semi field evaluation of the nematodes against adults of RPW in date palm trees, and (5) field application of local nematodes against natural population of RPW in date palm farms.

Tow isolates of EPN of genus Steinernema had been discovered. It was the first record of Steinernema from Saudi Arabia and Qatar. Laboratory studies showed that the two new isolates were highly virulent to the RPW. They had high reproductive potential producing 400000-600000 nematodes/Galleria mellonella larva. The studies showed differences between the two isolates in their values of lethal concentration

and lethal time for RPW adults and reproductive potential in G. mellonella larvae. Semi field studies showed high efficiency of the two strains in controlling adults of RPW and recycling in them. In field studies in Qatif, Saudi Arabia: A single spray of a local steinernematid nematode caused reduction in RPW population of 32.47% in date palm farms within one week. However a double spray of a local heterorhabditid nematode -two weeks between sprays- lasted effectively in the field for 4 weeks causing reduction from 31 to 94% in the population of adults of RPW.

Key words: Rhynchophorus, Steinernema, Heterorhabditis, biological control, red palm weevil, entomopathogenic nematodes.

Introduction

The date palm is a holy tree in Arabic region, where every part of it is useful. Arab countries produce two thirds of the world production of dates (6.7 million tons). Egypt being the first country in date production in the world produces over 1.1 million tons followed by Kingdom of Saudi Arabia (830000 tons) and United Arab Emirates (UAE) (760000 tons). Over 55 insect, arthropod or animal pests besides many fungal and bacterial diseases have been recorded on date palm (Al Ahmadi & Salem 1999). The red palm weevil (RPW), Rhynchophorus ferrugineus (Coleoptera: Curculionidae) is the most destructive pest of the date palm in the region since its invasion to UAE coming from the east. It also attacks other palm species like coconut, oil palms and Washingtonia palms. It invaded UAE in1985, Saudi Arabia 1987, Iran 1990, Egypt 1993 (Murphy and Briscoe 1999). It went west to Spain 1994 (Barranco et al 1995), and Italy 2004. All cases of regional and international invasion were through illegal transportation of infested date palm offshoots.

Adults attack palm trees and deposit eggs individually in wounded and soft tissues. The hatched larvae tunnel into the trunk or the terminal bud leading directly to the death of the tree (Griffith 1987, Sivapragasam et al. 1990). Because of the cryptic feeding habit of larvae their control has been difficult. Primary infestations always escape attention and symptoms may not become evident until extensive damage has already occurred (Hanounik et al. 2000). Larval stage lasts 2-3 months with 12 larval instars. The female my lay 370 eggs during its 2-3 month-life. The insect has 4 generations annually (Al Mohanna et al., 2000). OEPP/EPPO (2008) reported that complete life cycle of the weevil from the egg to adult emergence takes an average of 82 days. Males excrete aggregation pheromone that attract both sexes for food, shelter and egg deposition. The chemical composition of the pheromone is: 4-methyl-5-nonanol and 4-methyl-5-nonanon (Sanchez et al., 1996; Faleiro et al., 2003).

Despite intensive efforts and high costs of controlling RPW, the pest is continuously spreading everywhere and destroying the holy tree. Management programs of RPW depend mainly on chemical insecticides (Girgis et al. 2002). Date palm orchards are sprayed periodically with insecticides for protection against the pest. Infested trees usually injected with chemical insecticides. Chemicals go through our sandy soil to ground water and subsequently to all living organisms causing many environmental and health hazards like cancers, kidney failure and liver failure. Biological control with safe measures is aggressively required for management of this pest. Among promising biological control agents are the entomopathogenic nematodes (EPN). EPN of the families Heterorhabditidae and Steinernematidae are commercially produced and used in biological control of many insect pests in the world. These two nematode families are symbiotically associated with bacteria in genera Xenorhabdus (with Steinernematidae) and Photorhabdus (with Heterorhabditidae). The free-living infective juveniles of these nematodes are motile and have chemo-receptors. They are highly virulent, killing their hosts within 24-48 hours, can be mass produced, have highly reproductive potential, have broad host range, are easily applied in the field and are safe to vertebrates, plants, and other non-target organisms. Development of large-scale mass-production technology and easy-to-use formulations led to expanded use of EPN in several countries. Larvae of the wax moth, Galleria mellonella are the most preferred hosts for mass production of many biological control agents because of their high susceptibility, ease and lower cost of culture. Many EPN of families Steinernematidae and Heterorhabditidae are maintained on these larvae in many countries. Indigenous EPN are expected to be suitable for management of local insect pests because of their adaptation to local climate and population regulators.

Local isolates of EPN were recorded for the first time from Arabic Gulf countries (Saleh et al 2001). Among them was Heterorhabditis

indica SA which has been evaluated against larvae and adults of RPW and gave encouraging results in the laboratory and the field (Saleh and Alheji 2003) Elawad et al (2007). Some research works have been carried out in Egypt including pathogenicity of local EPN to RPW in the laboratory (Shamseldean and AbdelGawad 1994; Shamseldean, 2002; Alfazairy et al., 2003; Abdel-Razek et al. 2004) and efficacy of injection of EPN in reducing larval population of RPW in the field (Abbas et al. 2001, Shamseldean and Atwa 2004).

Adults are aggregating mainly in the leaf axils of palm trees for resting, mating and oviposition. They also aggregate at the basal part of the trunk of young date palm trees, near or below the soil level. Leaf axils of date palm being more shaded and humid compared to other external plant parts, are more suitable for the persistence and activity of entomopathogenic nematodes (Hanounik et al. 2000). A considerable portion of 35% of R. ferrugineus infestation in date palm trees in eastern region in Saudi Arabia were found at or below the soil surface where EPN can work very effectively against RPW (unpublished data).

The overall objective of this work was to contribute to the decision makers a program for the biological control of the red palm weevil (RPW) with entomopathogenic nematodes (EPN). This biological control program will be ready to be transferred into IPM program of the pest in our Arabic region. The specific objectives were (1) isolation and identification of two isolates of local entomopathogenic nematodes from Qatar and Saudi Arabia, (2) Laboratory evaluation of the nematodes against adults of the RPW, (3) studying the suitability of new nematodes to mass production, (4) semi field evaluation of the nematodes against adults of RPW in date palm trees, and (5) field application of the nematodes against natural population of RPW in date palm farms.

The Arab Organization for the Agricultural Development (AOAD) - league of Arab Countries – conducted a project (1997-2007) for the biological control of RPW in the Arabian Gulf region, using entomopathogenic nematodes as a major component. There is a long list of publications of AOAD project. This work is a part of un-published achievements of AOAD project.

MATERIAL AND METHODS

Isolation and identification

1 - The Saudi isolate :

A naturally-infected adult of RPW was collected from Ben-Hammam farm in Qatif. The infected weevil was transferred to the laboratory and placed in a White trap (White 1927) for nematode extraction. The extracted nematodes were reared on larvae of the greater wax moth Galleria mellonella according to Woodring and Kaya (1988). Identification of the extracted nematodes to the genus level depended on symptoms appeared on nematode-infected host larvae and the morphology of nematode developmental stages described by Woodring and Kaya (1988), Poinar (1990), and Kaya and Stock (1997).

2 - The Qatar isolate:

Soil samples were collected from a date palm farm in Qatar and transferred to Qatif laboratory in Saudi Arabia where they inspected for the presence of entomopathogenic nematodes. The nematode was isolated from the soil samples using Galleria-bait technique (Bedding and Akhurst 1975) in which larvae of G. mellonella were placed in soil samples and incubated at 25°C for one week then, the infected larvae were transferred to White traps for nematode extraction. Identification of the extracted nematodes to the genus level depended on symptoms appeared on nematode-infected host larvae and the morphology of nematode developmental stages.

Virulence to adults of RPW

Sand barrier bioassay technique (Woodring and Kaya 1988) was used to determine the virulence of the new isolates Steinernema sp SA & Steinernema sp Q to adults of the red palm weevil. The weevils were individually exposed to serial concentrations (treatments) of each nematode (0,500,1000,2000 and 4000 IJ/ml) in 50cc tubes filled with 9 gm fine sand and wetted with 1ml distilled water. Ten replicates were prepared for each treatment. The experiment consisted of 100 units (2 nematode-isolates X 5 concentrations X 10 replicates). Mortality of the weevils was recorded daily. Data were statistically analyzed by plotting regression lines of concentration vs. mortality and values of LC50 were compared.

Suitability for in vivo mass production:

Larvae of G. mellonella were exposed to nematode suspension at concentrations (treatments) of 0, 25, 50, 100, 200, 400 and 800 infective juveniles/ ml/ 5larvae in Petri dishes furnished with filter paper. Each treatment was replicated 4 times. Each experimental unit was represented by a Petri dish contained 5 larvae and 1ml of nematode suspension. Number of experimental units= 2 isolates x 7 treatments x 4 replicates = 56 units. Dishes were kept at 28oC and numbers of alive and dead larvae were recorded daily. Regression lines of mortality vs. concentrations were plotted and values of half lethal concentrations (LC50) were computed. Regression lines of mortality Vs exposure time were plotted and values of half lethal time (LT50) were also computed. The numbers of nematode

offspring migrated from infected G. mellonella larvae for each treatment were recorded and the rates of nematode reproduction were computed for each tested nematode.

Cage studies:

Efficiency of new steinernematid isolates in controlling RPW on date palm trees under cages.

Date palm trees -5 years old- were transferred individually to halves of polyvinyl barrels, 80cm diameter X 80 cm high, filled with sandy soil. Each tree was covered with a 2 meter high plastic screen cage to prevent escape of weevil adults. The cages were arranged out the laboratory building in Qatif. The experiment consisted of 3 treatments (Steinernema sp SA, Steinernema sp Q and control). Each treatment consisted of 4 replicates. Each plot represented by a tree in a cage. A total of 12 plots were used in this experiment. Firstly, the trees were artificially infested with adults of RPW at a rate of 10 weevils/ tree/ cage. After 24 hours, the water suspension of a specified nematode was sprayed on the basal part of the trees and soil around them at a rate of 4 million infective juveniles/ 5 liters / tree. Control plots received only water. The trees and the soil under cages were inspected after 5 and 8 days and numbers of dead and alive weevils were recorded. Dead weevils were transferred to White traps for detecting nematode development and propagation. Insect mortality was calculated for each treatment at specified inspection days. Migration of nematode offspring out infected weevil was the evidence of successful propagation.

Field studies:

Performance of local isolates of EPN in controlling RPW in date palm farms.

Nematodes:

Steinernema sp SA, Heterorhabditis indica HSA

Trapping system:

Pheromone-kairomone terrestrial traps (AOAD traps) described in Hanounik et al (2000) were used for monitoring the adult population of the red palm weevil in treated and untreated date palm farms. The traps were distributed at 100 meters distance between traps (i.e. 1 trap/ hectare).

Experiments:

A date palm farm of approximately 5 hectors was specified for each treatment. The nematode suspension was sprayed in the field using a 600 liter-spraying motor at a rate of 2 million nematodes/ 5 liters/ tree. The spray was directed to the base of palm trees and soil around the trees. Trap catches were recorded weekly in all experimental plots before and after each treatment. The caught weevils were kept individually with food in cups in the laboratory and observed for 3 days and dead weevils were placed in White traps for detecting nematode infections. After field applications dead insects found out of traps or inside treated trees were collected, transferred to the laboratory and inspected for nematode infections.

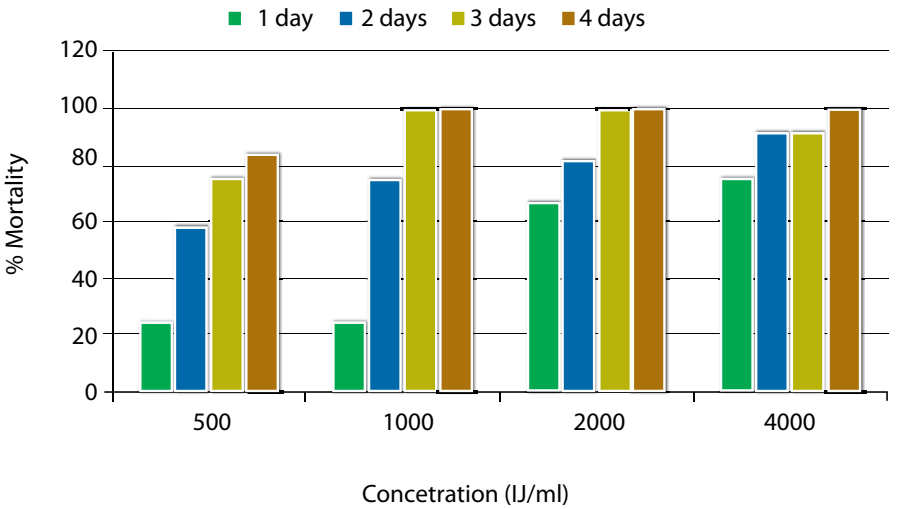

Fig: (1) Mortality in adults of *Rhynchophorus ferrugineus* caused by *Steinernema* sp SA

Statistical analyses:

Population of RPW in studied farms was represented by mean of weevils/trap/week. Means were compared by ANOVA test and SE values were computed and given with their means. Percentages of reduction in the insect population due to different treatments are calculated according the equation of Henderson and Tilton (1955) as follows:

$$R = 100 \times 1 - \left[\frac{Ta \times Cb}{Tb \times Ca} \right]$$

Where: R = Percent of population reduction, Tb = Numbers of insects in treated plots before treatment, Ta = Numbers of insects in treated plots after treatment, Cb = Numbers of insects in control plots before treatment, Ca = Numbers of insects in control plots after treatment.

Results

Isolation and identification

1 - The Saudi isolate :

This nematode was extracted from a naturally-infected RPW adult in Qatif, Eastern Province, Saudi Arabia. The extracted nematodes were reared on larvae of the greater wax moth Galleria mellonella. The nematodes could be identified to the genus level depending on symptoms appeared on infected G. mellonella larvae and the morphology of nematode developmental stages. Evidence certify that the nematode belongs to Steinernema were: (1) the pale yellow color of nematode-infected host larvae, (2) the giant amphemectic females of the first generation found inside host cadavers three days after infection, (3) the identical shape of the tail of steinernematid IJ and (4) the identical appearance of coiled steinernematid IJ. Sample of this new isolate is intended to be sent for identification to the species level by DNA analysis. Until the complete identification, the nematode isolate was given the name Steinernema sp SA. This is the first record of a steinernematid EPN from Saudi Arabia.

2 - The Qatar isolate:

This nematode isolate was extracted from

soil samples from a date palm farm in Qatar. Extracted nematodes were maintained on G. mellonella larvae and identified to the genus level according to symptoms on infected larvae and morphology of developmental stages. Identical morphological characters of steinernematids -mentioned previously- were detected for this isolate. Samples are going to be sent for identification to the species level using DNA analysis. Until the complete identification, the nematode isolate was given the name Steinernema sp Q. This is the first record of a steinernematid EPN from Qatar.

Virulence to adults of RPW

1- Steinernema sp. SA

Data in Fig (1) show that mortality in adults of RPW exposed to serial concentrations of Steinernema sp SA started after 2 days for all used concentrations and reached its maximum (100%) after 4 days for the concentrations 1000 and 2000 IJ/ml and after 5

days for the concentrations 4000 IJ/ml. After 5 days, concentrations above 500 IJ/ml achieved 100% insect mortality. The relative delay in the effect of the highest concentration (4000 IJ/ml) may be due to over-crowding of developing nematodes inside the insect cadaver. From Table (1) a high degree of correlation between the nematode concentration and the insect mortality (R2 = 0.82) was found after 3 days of exposure. The LC50 after three days of exposure was 1373 IJ/ml. Also a high degree of correlation between time of exposure and the insect mortality (R2 = 0.92) was found at the concentration 500 IJ/ml. The LT50 was 1.95 days. This value explained how fast that Steinernema sp. SA can kill the RPW adults.

2 - Steinernema sp Q:

Data in Fig. 2 show that mortality in adults of the red palm weevil exposed to Steinernema sp Q started after 1 day when the concentrations 1000 and 2000 IJ/ml caused 10% RPW mortality. These two medium concentrations caused 100% mortality after 3 days. The lowest and the highest concentrations started their effect and reached their maximum effect one day later. A high degree of correlation between the nematode concentration and the insect mortality (R2 = 0.97) was found (Table 1). The half lethal concentration value (LC50) after 2 days of exposure was as low as 737 IJ/ml/insect. Also a high degree of

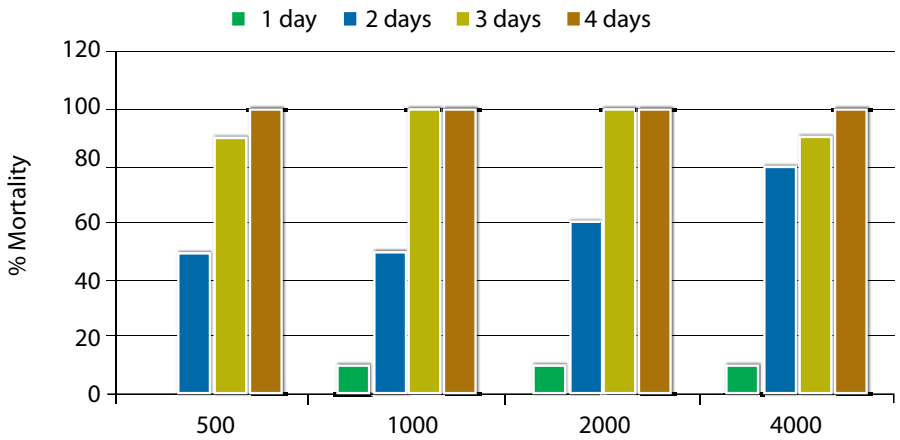

Fig: (2) Mortality in adults of *Rhynchophorus ferrugineus* caused by *Steinernema* sp Q

Nematode	LC_{50} (IJ/ml)	R^2(concentration-mortality)	LT_{50} (days)	R^2(time-mortality)
Steinernema sp SA	1373	0.82	1.95	0.92
Steinernema sp Q	737	0.97	2.2	0.93

Table (1) Statistics of lethal concentration and time of *Steinernema* sp SA and *Steinernema* sp Q against adults of *Rhynchophorus ferrugineus*

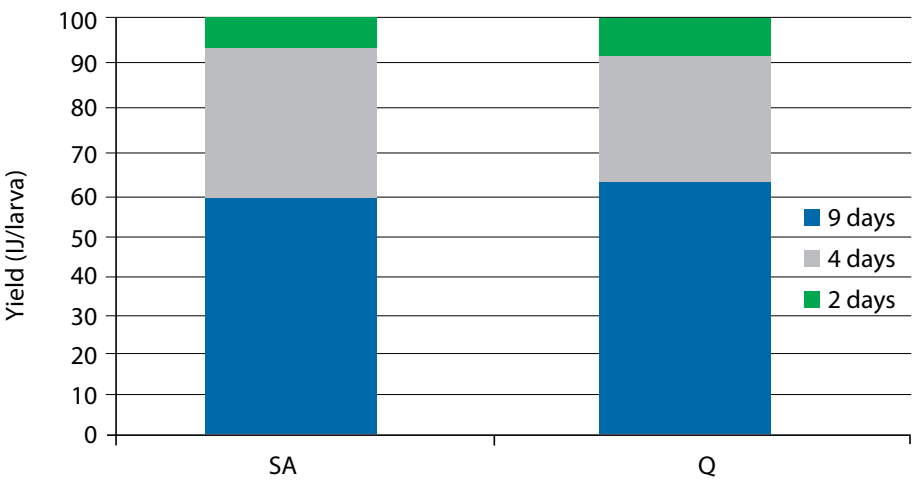

Fig: (3) Distribution of yeild of *Steinernema* sp SA and Steinernema sp Q collected from larvae of *Galleria mellonella*

Inoculum IJ/ml/5larvae	Steinernema sp SA		Steinernema sp Q	
	Yield± SE (IJ/larva)	% larvae producing nematodes	Yield± SE (IJ/larva)	% larvae producing nematodes
25	96875± 46786	55± 9.5	128333± 92071	20± 5.7
50	95625± 34827	50± 12.9	363750± 143837	40± 8.1
100	157300± 46231	90± 5.7	282166± 64638	70± 10
200	282000± 56950	100	495375± 102020	95± 5
400	298300± 10799	100	506375± 222979	95± 5
800	642000± 81425	90± 5.7	246000± 90730	100

Table (2) Reproduction of *Steinernema* sp SA and *Steinernema* sp Q in larvae of *Galleria mellonella* at different inoculum concentrations

correlation between time of exposure and the insect mortality (R2 = 0.93). The half lethal time value (LT50) of the concentration of 500 IJ/ml/ insect was 2.2 days. These results show that both of the new isolates are efficient and fast in killing the adults of the pest with remarkable superiority for the Qatar strain.

Suitability for mass production on *G. mellonella*

1- Steinernema sp SA

Data in Table (2) show that the new steinernematid strains have efficiently reproduced in larvae of G. mellonella. The yield of infective juveniles (IJ) of Steinernema sp SA collected from a single larva reached 642000 nematodes. This yield was obtained when the inoculum concentration was 800 IJ/ml/5larvae. Such a high rate of reproduction may be because the strain still has the power of wildness and still has its high reproductive potential. Lower inoculations resulted in lower yields. Lowest inoculums (25 and 50 IJ/ml/5larvae) produced yields lower than 100000 IJ/larva. Percentages of wax moth larvae produced nematodes have positively correlated with the inoculum concentration. At the concentration 200 IJ/ml/5 larvae or more, Steinernema sp SA successfully reproduced in 100% of host larvae. At concentrations 25-50 IJ/ml/5 larvae, it reproduced in 50-55% of host larvae. From Fig (3) as high as 94 % of the yield of Steinernema sp SA could be collected during the first 4 days after commencement of migration. 56 % of the nematode yield migrated out the host cadavers during the first 2 days. The rest of yield (6%) obtained after 9 days of the commencement of IJ migration. The late nematode yield has always lower quality.

2- Steinernema sp. Q:

From Table (2) Steinernema sp Q gave its maximum yield (506375 IJ/larva) when the inoculum concentration was 400 IJ/ml/5larvae. Unexpectedly the highest inoculum concentration (800 IJ/ml/5larvae) yielded only 246000 IJ/larva. This happened due to probable intra-specific competition resulted from crowded growing nematodes in limited space and food supply. The lowest yield of Steinernema sp Q (128333 IJ/larva) came from the lowest inoculation (25 IJ/ml/5larvae). Percentage of host larvae that produced nematodes correlated positively with the inoculum concentration. At the lowest concentration (25 IJ/ml/5larvae) the nematode reproduced in only 20% of the infected larvae. At a concentration 200 IJ/ml/5larvae or more the nematode successfully reproduced in 95-100% of the host larvae. Fig (3) shows that most of nematode offspring (63 %) migrated out the cadavers of G. mellonella during the first 2 days after commencement of migration. Approximately 90% of the nematode yield could be obtained during the first 4 days after commencement of migration. The rest of nematode yield was collected after 9days of commencement of IJ migration. Inoculation concentrations lower than 200 IJ/ml/5larvae are not advisable for mass rearing on larvae of G. mellonella. The best inoculation concentration is 200-400 IJ/ml/5larvae for the Qatar strain and 800 IJ/ml/5 larvae for the Saudi strain. Over 90% of the yield for both isolates can be harvested during the first four days after commencement of migration out the host cadavers. Waiting for 9 days to obtain less than 10% of the yield is not advisable from economical point of view.

Cage studies:

Efficiency of new steinernematid isolates in controlling RPW on date palm trees under cages

Data in Fig (4) show that both of the new steinernematid isolates (Steinernema sp SA and Steinernema sp Q) were efficient in controlling adults of the red palm weevil under semi-field conditions. Weevil mortality in trees treated with the Qatar isolate, Steinernema sp Q recorded 68.19 % after 5 days of treatment and increased to 93.75% after 8 days of treatment. Weevil mortality due to the Saudi isolate Steinernema sp SA was 84.79 and 97.5% after 5 and 8 days of treatment, respectively. Natural mortality in control cages during the experimental duration was 7.5-10%. Mortality caused by Steinernema sp SA was significantly higher than that caused by Steinernema sp Q after 5 days of treatment. However the difference in mortality caused

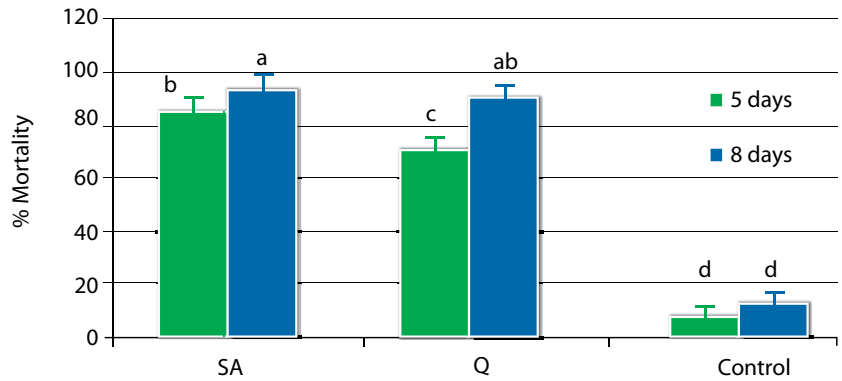

Fig.(4) Mortality in adults of Rhynchophorus ferrugineus in date palm trees under cages after application of Steinernema sp SA and Steinernema sp Q in Qatif, Saudi Arabia. Columns with different letters are significantly different, ANOVA test at P>0.001

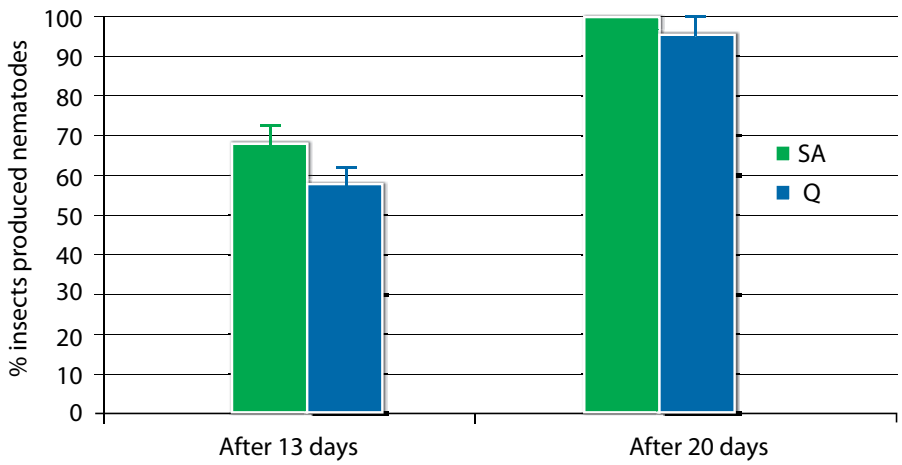

Fig.(5) Propogation of Steinernema sp SA and Steinernema sp Q in adults of Rhynchophorus ferrugineus in date palm trees under cages in Qatif KSA

by both isolates was insignificant after 8 days. Generally, the Saudi isolate gave faster and stronger effect against the pest than the Qatar isolate did. Both of the new nematode isolates were not only efficient in controlling adults of the red palm weevil but also able to propagate in weevil cadavers under semi-field conditions. Propagation of the two isolates in infected weevils differed in speed and level. Percentage weevils produced offspring in case of the Saudi isolate Steinernema sp SA was 66.39% after 13 days of treatment and increased to 100% after 20 days of treatment. In case of the Qatar isolate however, nematode propagation was 57.15% and 94.44% after 13 and 20 of treatment, respectively (Fig 5).

Field studies:

Performance of local isolates of EPN in controlling RPW in date palm farms.

Data in Table (3) show weekly numbers of red palm weevils monitored by pheromone-kairomone traps in date palm farms in Qatif, before and after field application of Steinernema sp SA during April 2007. In the treated farm, a weak Table (3) Population reduction in Rhynchophorus ferrugineus adults after application of Steinernema sp SA in date palm farms in Qatif, Saudi Arabia during April 2007.

ncrease in the pest population from 5.67 to 6 weevils/trap/week has been recorded in the first week after treatment (10-17/4/2007). During the same period, a high increase (from 4 to 6.67 weevils/trap/week) in the natural population of the pest occurred in the control farm. According to Henderson and Tilton's equation a theoretical reduction of 32.87% in the pest population has been registered as a result of Steinernema sp SA application. The effect of this steinernematid isolate was ended in the second week when the traps in treated date palm farm recorded no reduction in the pest population.

In the other field experiment, the local heterorhabditid strain Heterorhabditis indica HSA was sprayed twice – on 27/3/07 and on 10/4/07 – in other date farm in the same region. Table (4) shows the effect of double spray with H. indica HSA against adults of RPW in Qatif date palm farms infested with RPW during April. The effect of the first spray (27/3/07) of this heterorhabditid sustained effectively for two successive weeks. It induced a decrease in the RPW population from 9.66 weevils/trap/week to 5.66 weevils/trap/week in the first week and to 5 weevils/trap/week in the second week of treatment. The natural population during this period was almost stable in the control farm (3.33-3.66 weevils/trap/week). Population reduction as a result of the first spray was 35.5% and 48.27% during the first and the second weeks, respectively. The second spray (10/4/07) with achieved very sharp decrease in RPW population from 5 weevils/trap/week to only 0.33 weevils/trap/week in the first week after application. During this week the natural

Date	Weevils/trap/week ±SE		% Population reduction
	Control	Treated	
10/04/2007	4±0.94	5.67±0.27	
17/04/2007	6.67±0.72	6±0.94	32.47
24/04/2007	3.67±0.27	5.33±0.27	No reduction

Table (3)

Date	Weevils/trap/week ±SE		% Population reduction
	Control	Treated	
27/3/07 (1st treatment)	3.66^{c} ±0.88	9.66^{a}±2.9	
3/4/07	3.33^{c} ±1.76	5.66^{bc}±1.45	35.51
10/4/07 (2nd treatment)	3.66^{c}±1.2	5^{bc} ±2.3	48.27
17/4/07	4.33^{bc}±2.33	0.3 ^{d} ±0.33	97.08
24/4/07	3.66^{c}±0.33	6.66^{b}±1.22	31.03

Table (4) Population reduction in *Rhynchophorus ferrugineus* adults after application of *Heterorhabditis indica* HSA in date palm farms in Qatif, Saudi Arabia during April 2007. Means followed by different letters are significantly different P>0.05, LSD= 2.44

pest population recorded increase from 3.66 to 4.33 weevils/trap/week. The ultimate population reduction obtained until that time was 97.08%. In the second week after the second spray although the pest population recorded an increase in the treated farm, the ultimate effect of the double spray achieved reduction of 31.03% in the pest population. AOVA analysis at P>0.05 showed significant differences between means of weekly trapped weevils in the treated farm before and after treatment for both nematode sprays. The field performance of the heterorhabditid in controlling the pest was better than that of the steinernematid isolate during April. The heterorhabditid sustained effectively in the field for longer time and achieved deeper reduction in the pest population than the steinernematid isolate did. The heterorhabditid nematode looked more tolerant to high temperature in Qatif fields during April than the steinernematid one. High temperatures usually have adverse effects on the activity and persistence of entomopathogenic nematodes. Many references agree that heterorhabditid nematodes are more suitable for high field temperatures than steinernematid ones. These results ensure the importance of choosing the right nematode species and/or strain for field application in a specific environment.

Discussion

The overall objective of this work was to contribute to the decision makers a program for the biological control of the red palm weevil (RPW) with entomopathogenic nematodes (EPN). This biological control program will be ready to be transferred into larger IPM program of the pest in our Arabic region. The specific objectives were (1) isolation and identification of two isolates of local entomopathogenic nematodes from Qatar and Saudi Arabia, (2) Laboratory evaluation of the nematodes against adults of the RPW, (3) studying the suitability of new nematodes to mass production, (4) semi field evaluation of the nematodes against adults of RPW in date palm trees, and (5) field application of the nematodes against natural population of RPW in date palm farms.

In the present work, steinernematid nematodes were isolated for the first time from Saudi Arabia and Qatar. The heterorhabditid H. indica HSA was isolated from Saudi Arabia for the first time by the same author. Qatif Oasis is rich with H. indica HSA so that it could be isolated all the year round from date palm farms (Saleh et al., 2001). Steinernematids are easier for mass production, storage and more preferable to use in moderate temperatures than heterorhabditids. The two nematode genera differ in their life cycles in that the steinernematids contain only amphimictic forms (males and females), whereas the first generation of heterorhabditids (arising from infective juveniles) contain only hermaphrodites (Strauch et al. 1994). They differ in their mutualistic bacteria. Steinernematids are associated with Xenorhabdus spp. and heterorhabditids are associated with Photorhabdus spp. (Poinar 1990).

The present results showed that the two new steinernematid isolates were highly virulent to adults of the RPW. They caused 100% mortality in RPW within 4-5 days using nematode concentration of 500 IJ/ml or more. The two new isolates differed slightly in killing speed, required concentration and rate of reproduction in the pest cadavers. This indicates that they might belong to different species and/or strains. Choosing the right species against a particular pest in a particular environment is very important for successful biological control (Shapiro et al 2002). Some research works have been carried out in Egypt including pathogenicity of local EPN to RPW in the laboratory (Shamseldean and AbdelGawad 1994; Shamseldean, 2002; Alfazairy et al., 2003; Abdel-Razek et al. 2004).

The two isolates were suitable for in vivo mass production in G. mellonella larvae. The Saudi isolate S. carpocapsae SA produced over 600 000 IJ from a single larva. A key factor in the success of EPN as biopesticides is their amenability to mass production (Shapiro and Gaugler 2002). Such a high rate of reproduction may be because the isolates still wild and still have their high reproductive potential. The most common host used for EPN mass production is last instar larvae of G. mellonella because of high susceptibility to most EPN, ease of culture and its ability to produce high yields (Woodring and Kaya 1988). Inoculum concentrations lower than 200 IJ/ml/5larvae are not advisable for mass rearing on larvae of G. mellonella. In vivo production depends on nematode dosage (Boff et al. 2000, Zervos et al. 1991). The best inoculation concentration is 200-400 IJ/ml/5larvae for Steinernema sp Q and 800 IJ/ml/5 larvae for Steinernema sp SA. Over 90% of the yield for both isolates can be harvested during the first four days after commencement of migration out the host cadavers. Waiting for 9 days to obtain less than 10% of the yield is not advisable from economical point of view.

Cage studies showed that both of the new steinernematid isolates were efficient in controlling adults of the red palm weevil under semi-field conditions. The spray was directed to the heart of the tree and soil around it where adults of RPW aggregate. Hanounik et al. 2002 reported that adults of RPW aggregate in leaf axils for mating, feeding and oviposition. Mortality in adults of RPW in date palm trees under cages reached 93.75% and 97.5% after 1 week of a single spray of 2million IJ/5liters/tree of Steinernema

sp Q and Steinernema sp SA, respectively. That was expected result after high virulence shown by both isolates against the adult weevils. H. indica HSA isolated from Qatif achieved 86% mortality in adults of RPW under cages when used with anti-desiccant. The steinernematid nematodes propagated successfully in almost all infected weevils but not in the same time. For Steinernema sp SA for example, it propagated in 66% of infected weevils after 13 days of application and in 100% of infected weevils after 20 days of nematode application. That means that the nematodes do not attack their hosts at the same time. Long field persistence is very important for successful biological control with EPN. Saleh et al 2004 found that S. carpocapsae remained able to kill adults of RPW for 16 days in a date palm farm in Qatif, Saudi Arabia.

Field studies included two experiments. In the first experiment a single spray of Steinernema sp SA was conducted in a date palm farm infested with RPW in Qatif region during April 2007. Under field conditions the steinernematid nematode lasted effectively for one week and caused 32.47% population reduction in RPW adults in the farm. The nematode effect has stopped in the following week. Mean day temperature during April in Qatif is 26.5oC and maximum reaches 35oC. These temperatures seemed unsuitable for longer persistence of the steinernematid isolate in the field. Saleh et al. (2004) recorded active persistence of S. carpocapsae for 16 days in the same region during March. In the second experiment, double spray of 2 weeks separation between them, with H. indica HSA was conducted in the same region and at the same time. These two successive sprays induced population reduction sustained for 4 successive weeks. The first spray persisted actively for two weeks and achieved 35.5 % and 48.27% reduction in the pest population in the two weeks, respectively. Population reduction became 97% and 31% after 1 and 2 weeks of the second spray, respectively. The heterorhabditid isolate sustained effectively in the field for longer time and achieved deeper reduction in the pest population than the steinernematid isolate did. Molyneux (1986) and Grewal et al. (1994) reported that steinernematids were more active at lower temperatures than heterorhabditids of the same origin. The high temperature in Qatif fields during April adversely affected the steinernematid isolate rather than the heterorhabditid one. These results ensure the importance of choosing the right nematode for field application in a specific place and time.

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