Evaluation of Steinernema sp. SA a Native Isolate from Saudi Arabia for Controlling Adults of the Red Palm Weevil. Rhynchophorus ferrugineus (Oliver)

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(Received: October 12, 2011and Accepted: November 17, 2011)

ABSTRACT

Steinernema sp. SA a native isolate of entomopathogenic nematodes (EPNs) was extracted for the first time from naturally infected adult of the red palm weevil (RPW) in Eastern Province in Saudi Arabia. The role of the native isolate in the biological control of RPW adults was evaluated. Laboratory bioassay studies showed high virulence of the nematode to adults of the RPW. Half lethal concentration (LC₅₀) after 3 days was 2077 infective juveniles (IJs)/ml and the half lethal time (LT₅₀) - using the concentration 500 IJ/ml - was 1.95 days. In the Semi-field evaluation Steinernema sp SA caused over 97% mortality in adults of the RPW in date palm trees under screen cages. The nematode propagated successfully in all infected insects. In the field, the nematode was sprayed on the basal parts of palm trunks and soil around them. A single spray of $2x10^6$ infective juveniles (IJs)/tree caused 37.16% reduction in RPW population in date palm farms after one week of application. However, the effect was reduced to 34.75% in the following week.

Key words: Rhynchophorus ferrugineus, Steinernema sp., red palm weevil, entomopathogenic nematodes.

INTRODUCTION

The date palm Phoenix dactylifera L is a strategic food crop in the Arabic region where every part of the tree is useful. According to the UN Food and Agriculture Organization (FAO 2005), Arab countries produce over 90% of world production of dates (16,696.56 million tons). The red palm weevil (RPW), Rhynchophorus ferrugineus (Oliver) (Coleoptera: Curculionidae) is the most destructive pest of the date palm in the region since its invasion to the United Arab Emirates (UAE) in 1985. It also attacks other palm species like coconut, oil palms and ornamental palms. It invaded Saudi Arabia 1987, Iran 1990 (Murphy and Briscoe 1999) and Egypt 1992 Saleh (1992). It went west to Spain 1995 (Barranco et al. 1995), and Italy 2004 (Sacchetti et. al. 2005). Adults of the RPW 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 (Sivapragasam et al. 1990). Because of the cryptic feeding habit of larvae their control is difficult. Primary infestations always escape attention and symptoms may not become evident until extensive damage has already occurred (Hanounik et al. 2000a). Larval stage lasts 2-3 months with 12 larval instars. The female may lay 370 eggs during its 2-3 month-life span. The insect has 4 generations annually in Saudi Arabia (Al Mohanna et al., 2000). OEPP/EPPO (2008) reported that a 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.

Management of RPW depends mainly on chemical insecticides (Girgis et al. 2002). Date palms are sprayed periodically with chemical insecticides for protection and infested trees are injected for cure. Chemicals go through the sandy soil to ground water and subsequently to all living organisms causing many environmental and health hazards.

Biological control, as a safe measure, is strongly recommended for management of this pest. Among promising biological control agents are EPNs. Heterorhabditis indica HSA was recorded for the first time in Saudi Arabia (Saleh et al. 2001) and was evaluated against larvae and adults of RPW (Saleh and Alheji 2003). EPNs proved high virulence against RPW stages UAE (Abbas et al. 2001 a&b) and in Egypt (Shamseldean and Abdelgawad 1994).

Adults of the RPW aggregate in 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, below the soil level (Hanounik et al. 2000a). Abbas et al (2000) justified the presence of adults of the RPW in the soil as: up to 20 to 100% of adults collected from the fields were found to be parasitized with unidentified nematodes, EPNs could be isolated from RPW adults and young date palms (<10 years old) were found to undergo severe infestation below the soil surface. 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 (Alkhazal et al. 2009).

The objective of this work is to evaluate the role of the native isolate of Steinernema sp. SA for biological control of RPW adults through semi-field and field applications in Saudi Arabia.

MATERIALS AND METHODS

Nematode isolation

A naturally nematode-infected adult of RPW was brought from Ben-Hammam farm in Qatif, Eastern Province of Saudi Arabia in October, 2006. This 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 L. according to Woodring and Kaya (1988). Primary 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 Poinar (1990).

Virulence to adults of RPW

Sand barrier bioassay technique (Woodring and Kaya 1988) was used to determine the virulence of the new nematode to adults of the RPW. The weevils were individually exposed to serial concentrations (treatments) of the nematode (0,500,1000,2000 and 4000 IJs/ml) in 50cc tubes filled with 9g fine sand damped with 1ml distilled water. Ten replicates were prepared for each treatment. Mortality of the weevils was recorded daily. Data were statistically analyzed by plotting regression lines of concentration vs. mortality and time vs. mortality and values of LC₅₀ and LT₅₀ were calculated.

Semi-field evaluation

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 adult weevils. The cages were arranged outdoors beside the laboratory building in Qatif. The experiment consisted of 2 treatments (Steinernema sp. SA and control). Each treatment consisted of 4 replicates. Each plot represented by a tree in a cage. The trees were artificially infested with adults of RPW at a rate of 10 weevils/tree/cage. After 24 hours, the water suspension of the nematode was sprayed on the basal part of the trees and soil around them at a rate of 2 million IJs/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 to detect nematode development and propagation.

Field evaluation

Pheromone-kairomone terrestrial traps described by Hanounik et al (2000a) were used for monitoring the adult population of the red palm weevil in treated and untreated date palm farms. Capacity of the trap is 10L with 3 holes in the cover and 6 holes near the upper edge of side walls. A bag containing the aggregation pheromone (Chemtica International S.A. Costa Rica) and a bottle containing kairomone (locally extracted from date fruits) are attached to the cover. The traps were distributed at 100 meters distance (i.e. 1 trap/ hectare).

A date palm farm of approximately 5 hectares 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 IJs/5 liters/tree. The spray was directed to the trunk base and soil around the trees. Trap catches were recorded weekly in both farms before and after each treatment. The caught weevils were kept individually with food in 200cc cups in the laboratory and observed for 3 days and dead weevils were placed in White traps to detect nematode infections. After field applications dead insects found out traps or inside treated trees were collected, transferred to the laboratory and inspected for nematode infections.

Populations of RPW in tested farms were 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 were calculated according the equation of Henderson and Tilton (1955) as follows:

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

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 AND DISCUSSION

Primary nematode identification

Evidence certify that the nematode belongs to Steinernema were: (1) the pale yellow color of nematode-infected host larvae, (2) the giant amphemetric 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 IJs. Sample of this new isolate was sent for identification to the species level by DNA analysis to Kiel University, Germany. Until the complete identification, the nematode isolate was given the name Steinernema sp SA. This was the first record of steinernematid nematode from Saudi Arabia.

Nematode virulence to RPW adult stage

Data in table (1) show that mortality in RPW adults 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 concentration 4000 IJ/ml. After 5 days, concentrations above 500 IJs/ml caused 100% insect mortality. A high degree of correlation between the nematode concentration and the insect mortality ($R^2 = 0.82$) was found after 3 days of exposure. The LC₅₀ after three days of exposure was 1373 IJs/ml. The highest degree of correlation between nematode concentration and insect mortality ($R^2 = 0.82$) was recorded after 3 days of exposure (Fig1). The LT₅₀ measured at the concentration 500 IJs/ml was 1.96 days. The highest degree of correlation between the exposure time and insect mortality ($R^2 = 1.92$) was recorded when the nematode concentration was 500 IJs/ml (Fig 2).

Semi-field evaluation

Steinernema sp. SA was significantly effective for controlling RPW adults under semi-field conditions. Weevil mortality was 84.79 and 97.5% after 5 and 8 days of treatment, respectively while natural mortality in control cages during the experimental duration was 7.5-10%. This native nematode was not only efficient for controlling adults of the RPW, but also able to propagate in the weevil cadavers. Percentage weevils produced offspring was 66.39% after 13 days of treatment and increased to 100% after 20 days of treatment.

Field evaluation

During the first week post treatment the natural RPW population in control farm increased from 4 to 6.67 weevils/trap/week recording (+ 2.67 weevils). In the treated farm the single nematode spray suppressed

Table (1): Mortality in adults of Rhynchophorus ferrugineus after application of Steinernema sp. SA at different concentrations

Nematode concentration (IJ/ml)	% Insect mortality after:				
	1day	2days	3days	4days	5days
500	0	25	58.33	75	83.33
1000	0	25	75	100	100
2000	0	66.67	81.82	100	100
4000	0	75	91.67	91.67	100

Table (2): Population reduction in Rhynchophorus ferrugineus adults after application of Steinernema sp. SA in date palm farms in Qatif, Saudi Arabia during April 2007

Date	Weevils/tra	%	
	Control	Treated	Population reduction
10/04/2007	4±0.94	5.67±0.27	
17/04/2007	6.67±0.72	6±0.94	37.16
24/04/2007	5.67±0.27	5.33±0.27	34.75

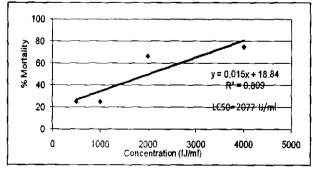


Fig. (1): Concentration-mortality line of Steinernema sp. SA against adults of Rhynchophorus ferrugineus calculated after 3 days of treatment.

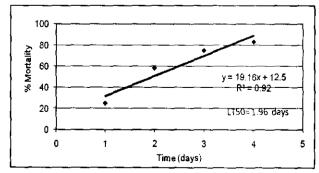


Fig. (2): Time-mortality line of Steinernema sp SA against adults of Rhynchophorus ferrugineus calculated at 500 IJs/ml

the population increase to be only + 0.33 weevils (from 5.67 to 6 weevils/trap/week) in the first week and to - 0.33 weevils (from 5. 67 to 5.33 weevils/trap week) in the second week. This effect was calculated according Henderson and Tilton (1955) equation as 37.16 and 34.75% population reduction in the first and the second weeks, respectively (Table 2).

This work comprised the first record of steinernematid nematodes (Steinernema sp. SA) from Saudi Arabia. The same authors previously recorded heterorhabditid nematodes (H. indica HSA) for the first time from Saudi Arabia (Saleh et al., 2001). The steinernematid nematode was isolated from naturally infected RPW adult in Qatif region. However, Abbas et al. (2001a) could isolate S. abbasi and H. indicus (= indica) from adults of the RPW in UAE. Like most native EPN isolates obtained Steinernema sp. SA was virulent against RPW adults. This result agrees with the results of Abbas et al. (2001b) in UAE, Shamseldean and AbdelGawad (1994) in Egypt, who worked on different native EPN isolates. Shapiro et al. (2002) stated that choosing the right EPN species against a particular pest in a particular environment is very important for successful biological control.

Steinernema sp. SA showed high potential for in vivo reproduction on G. mellonella larvae so that a single larva produced over 600 000 IJs. A key factor in the success of EPN as bio-pesticides is their amenability for mass production (Shapiro and Gaugler 2002).

The semi-field evaluation showed that Steinernema sp. SA was efficient for controlling adults of the RPW under cages. The nematode spray was directed to the base of the tree and soil around it where adults of RPW usually aggregate (Hanounik et al. 2000a). Mortality in adults of RPW in date palm trees under cages reached 97.5% within one week after a single spray of 2×10^6 IJ/tree. Hanounik et al. (2000b) were the first who targeted adults of the RPW in date palm trees and soil around them with H. indica HSA isolated from Qatif. H. indica HSA caused 86% mortality in adults of RPW under cages when used with anti-desiccants. Llacer et al. (2009) in Spain used S. carpocapsae against RPW under cages and obtained mortality around 80% in a curative assay, and up to 98% in a preventative treatment. Successful propagation of Steinernema sp. SA in cadavers of the RPW under the semi-field conditions gives prediction that the nematode will persist and recycle in date palm fields. Saleh et al. (2004) found that S. carpocapsae remained able to kill adults of RPW for 16 days in a date palm field in Qatif.

Field spraying of EPNs against adult stage of RPW was mentioned first by Hanounik et al. (2000a) in Saudi Arabia and Abbas et al. (2000) in UAE through a project of Biological Control of the RPW (1997-2007). This work belongs to the un-published results of the same project. The first field study showed that a single spray of Steinernema sp. SA of $2x10^6$ IJs/tree in Qatif region during April resulted in 37.16 and 34.75% population reduction in RPW adults in the farm. This steinernematid nematode lasted effectively at least for two weeks. Temperature in Qatif during April (around 35°C) seemed unsuitable for stronger effect or longer persistence of the steinernematid nematodes. Saleh et al. (2004) recorded active persistence of S. carpocapsae for 16 days in the same region during March.

The present results prove proved that the potential of native EPNs to control R. ferrugineus. The results give a ready-to-use bio-control program based on EPNs and add evidence to the recommendation of transferring biological control with EPNs to the IPM programs of RPW.

ACKNOWLEDGMENT

This work was 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 research project conducted by "The Arab Organization for Agricultural Development" (AOAD).

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تقييم سلالة النيماتودا Steinernema sp. SA الممرضة للحشرات من المملكة العربية السعودية في مكافحة سوسة النخيل الحمراء

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تم استخلاص سلالة النيماتودا المعرضة للحشرات Steinernema sp. SA في المعلكة العربية السعودية من إحدى مزارع النخيل بالمنطقة الشرقية، وقد استهدف هذا البحث تقييم أداء هذه السلالة في مكافحة سوسة النخيل الحمراء من خلال تجارب معملية ونصف حقلية وحقلية. أظهرت النتائج أن هذا هو أول تسجيل لهذا الجنس من النيماتودا المعرضة للحشرات بالمعلكة العربية السعودية وجاري التعريف إلى مستوى النوع بواسطة تطيل بصمة الحمض النووي DNA. كانت النيماتودا المكتشفة Steinernema sp. SA قعالة في القضاء على الآفة من خلال تجارب المختبرحيث كان التركيز النصفي (CC5) لها 1373 نيماتودا/ مل بعد 3 أيام من المعاملة، وزمن الموت النصفي (LT5) 195 يوما عند التركيز 500 نيماتودا/ مل بعد 3 أيام من المعاملة، وزمن الموت النصفي (64200 نيماتودا، أما في تجارب التقييم نصف الحقلي ملى. قابلة للإنتاج الكمي على يرقات دودة الشمع الكبرى بكفاءة حيث بلغ إنتاج البرقة الولددة 642000 نيماتودا، أما في تجارب التقييم نصف الحقلي المكافة النبوسة 19.5% بعد المعاملة بمعدل 2 مليون نيماتودا أمستخدام المصائد الفرمونية الكيرمونية الأرضية فقد حققت رشة ولحدة من مستحضر النيماتودا المقاملة، على التوالي، وذلك خلال شهر أبريل. يضيف هذا البحث أيضا دليلا على جدوى المكافحة الحيوية بالنيماتودا الممرضة الحشرات بطريقة الرش على مواقع تجمع الحشرات الكاملة بأسفل جنوع النخيل حديث العمر والترية المحيطة بالجنوع حيث تتوفر البيئة المقضلة المناتودا الممرضة المصرفة المكاملة للآفة.

كلمات مفتاحية: النيماتودا الممرضة للحشرات، Steinernema sp. SA، مكافحة حيوية، سوسة النخيل الحمراء، تقييم، المملكة العربية السعودية.