ORIGINAL RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
OPEN ACCESS

EFFICACY OF ENTOMOPATHOGENIC NEMATODES ON THE RED PALM WEEVIL *RHYNCHOPHORUS FERRUGINEUS* (OLIVIER, 1790) (COLEOPTERA: CURCULIONIDAE) LARVAE

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Received for publication: July 31, 2015; **Revised**: August 12, 2015; **Accepted**: September 17, 2015

Abstract: In this study the efficacy of entomopathogenic nematodes; *Steinernema affine* (Bovien), *S. carpocapsae* (Weiser), *S. feltiae* (Filipjev) and *Heterorhabtidis bacteriophora* (Poinar) on the larvae of *Rhynchophorus ferrugineus* (Olivier, 1790) (Red Palm Weevil) were investigated. In the bioassays, 9x5x5 cm sized plastic boxes were used. Base of each box, Whatman filter paper was placed and a last instar larva of red palm weevil was put. The entomopathogenic nematodes were inoculated to the larvae at the rate of 500IJs/larva and were incubated at 25°C. After infection, *R. ferrugineus* larvae were checked daily and mortality on larvae were recorded. The study was ended at the end of 7th day and the results were evaluated. All entomopathogenic nematode species used in this study caused different mortality on red palm weevil larvae. The highest mortality was caused by *H. bacteriophora* with 93.5%, *S. carpocapsae* followed it with 91.4%. *S. feltiae* and *S. affine* caused 36.4% and 21.2% mortality on *R. ferrugineus* larvae respectively. As a result, potential use of EPNs against the red palm weevil should be discussed to control this pest.

Key words: *Rhynchophorus ferrugineus*; *Steinernema affine*; *S. Carpocapsae*; *S. Feltiae*; *Heterorhabditis bacteriophora*

INTRODUCTION

Palms trees are botanical family of perennials lianas, shrubs, and trees, well known palm trees are; date palm and coconut palm. Most palms are distinguished by their large, compound, evergreen leaves arranged at the top of an unbranched stem. They grow in hot climates, most palm trees grow in the tropical and subtropical regions of the world. They occur from about 44° northern latitude to about 44° southern latitude.

The red palm weevil *Rhynchophorus ferrugineus* (Olivier, 1790) is one of the major pest affecting palm trees which is native Southeast Asia and has spread through the Arabian Gulf. Lefroy, 1906 described this pest as a deadly insect pest of coconut palm throughout India for the first time (Lefroy, 1906). The red palm weevil causes damages on palms, coconut, date, oil, and sago palms (Malumphy and Moran, 2007). It is originally from tropical Asia, and has spread to Africa and Europe, reaching the Mediterranean in the 1980s. It was first recorded in Spain in 1994, and in France in 2006 (Ferry and Gomez, 2002). In Asia the red palm weevil has been recorded in Bangladesh, Cambodia, China (Guangdong, Taiwan), Pakistan, India, Indonesia, Japan, Laos, Malaysia (Sabah, Sarawak), Myanmar, Philippines, Singapore, Sri Lanka, Thailand, and Vietnam. In Africa; Algeria, Egypt, Libya, Madagascar, Malta, Morocco. In Middle East: Bahrain, Georgia Palestine, Syria, Iran, Iraq, Israel, Jordan, Kuwait, Oman, Qatar, Saudi Arabia, and the United Arab Emirates. In Europe: Cyprus, France, Greece, Italy, Spain, Portugal and Turkey. In Oceania: Australia, Papua New Guinea, Samoa, and the Solomon Islands. In the Caribbean: Aruba. In the United States: Laguna Beach, Orange County, California (Anonymous, 2015).

The adult female lays approximately 200-250 eggs on new growth in the crown of the palm, at the base of young leaves, or in open lesions on the plant. The egg hatches into a white, legless larvae. The larvae will feed on the soft fibres and terminal buds, tunneling through the internal tissue of the tree for about a month. The larvae can occasionally grow to a length of six to seven centimeters. At pupation, the larvae will leave the tree and form a cocoon built of dry palm fibers in leaf litter at the base of the tree. The total life cycle of *R. ferrugineus* takes about 7-10 weeks. The weevil usually infests palms younger than 20 years. *R. ferrugineus* adults are active mostly during the day and can fly long distances (>900 meters) to search the hosts or breeding sites (Usda-Aphis, 2010).

The currently methods to control red palm weevil are largely based on the application of large quantities of synthetic chemical insecticides (Abuzuhairah *et al.,* 1996). However, other techniques of management such as sanitation, baits and traps of palm weevils have been researched in tropical Asia (particularly India) and the Americas, and when used in combination with chemicals have proved effective in field trials (Abraham *et al.,* 1998; Moura *et al.,* 1995).

EPNs are a group of soil-dwelling organisms that attack soilborne insect pests that live in, on, or near the soil surface and can be used effectively to control important pests. EPNs of the families Steinernematidae and Heterorhabditidae are symbiotically associated with bacteria in the genera *Xenorhabdus* (Thomas and Poinar) and *Photorhabdus* (Boemare, Akhurst and Mourant), respectively (Boemare *et al.,* 1997; Burnell & Stock, 2000). The bacteria kill the host by producing toxins, provide nematodes with nutrition, and prevent secondary invaders from contaminating the host cadaver (Forst & Clarke, 2002). Infective juveniles (IJs) enter the host body mainly through natural openings such as the mouth, spiracles, anus or thin parts of the host cuticle and release their bacteria inside the hemocoel. Nematodes can kill insects usually in 24-48 hours while most biological agents require days or weeks to kill the host.

EPNs have many advantages; they are easy and relatively inexpensive to culture, live from several weeks up to months in the infective stage, are capable of infecting a

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broad range of insect species, occur in soil and have been isolated from most regions of the world except Antarctica (Griffin *et al.,* 1990; Kaya & Gaugler, 1993). Foliar applications of nematodes have been successfully used to control the quarantine leaf eating caterpillars on various crops and have the potential for controlling various other insect pests. Application of EPNs does not require masks or other safety equipment as chemicals. EPNs and their associated bacteria have no detrimental effect to mammals or plants (Poinar *et al.,* 1982; Boemare *et al.,* 1996; Akhurst & Smith, 2002). The aims of the work were to determine the efficacy of EPNs against *Rhynchophorus ferrugineus* and to reduce the use of pesticides.

MATERIALS AND METHODS

Isolation and Identification of the Nematodes

Turkish isolates of the entomopathogenic nematodes; *Steinernema affine*, *S. carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora*, were used in the study. The nematodes were isolated from different cities (Sakarya, Balıkesir and İstanbul) in the western part of Turkey. The species were identified by morphometrics and sequencing of the D2/D3 and ITS regions of rDNA using molecular identification methods (Gözel and Güneş, 2013).

Table 1: Entomopathogenic nematode species and habitat structure

EPN species	Location	Soil type	pH	Habitat
Heterorhabditis bacteriophora	Sakarya	Sand	7.96	Plane tree
Steinernema carpocapsae	Sakarya	Sand	7.87	Poplar tree
Steinernema feltiae	Balıkesir	Sand-clay	7.64	Pinery
Steinernema affine	İstanbul	Sand-clay	6.97	Oakwood

Propagation of Nematodes

The nematodes were propagated in the last instar larvae of the greater wax moth, *Galleria mellonella* (Lep.: Pyralidae), as described by Dutky *et al.,* (1964). The emerged infective juveniles (IJs) were stored in Ringer solution (9.0g NaCl, 0.42g KCI, 0.37g CaCI2, 0.2g NaHCO3 and 1 l distilled water) in culture flasks for 5 days at 4-5°C prior to use in the bioassays (Solomon *et al.,* 1999).

Figure 1: EPN infected *Galleria mellonella* larvae on White trap.

Test Organisms

Last instar larvae of the *Rhynchophorus ferrugineus* were used as the test insect in this study. *R. ferrugineus* were collected from palm trees in South part of Turkey. Field-collected insects were mass produced on palms in a climate controlled room at 25±1°C and 70±5% moisture.

Efficacy of Entomopathogenic Nematodes on Rhynchophorus ferrugineus Larvae

For efficacy assays, Whatman filter papers were placed on the base of 9x5x5 cm sized plastic culture boxes and a last instar larva of *R. ferrugineus* was put on each box. At a rate of 500 IJs/larva was inoculated for each red palm weevil larva and incubated at 25°C. Infected larvae were checked daily, mortality on larvae were recorded and the study were ended at the end of 7th day. 24 *R. ferrugineus* larvae were used for each EPN species, and the bioassays were repeated twice. To be sure that the mortality were caused by EPNs, infected larvae were put on White trap and the emergences of IJs were observed.

RESULTS AND DISCUSSION

Different mortality on last instar larvae of red palm weevil caused by EPNs were observed. Only water were inoculated to the larvae of control groups and no death larva was observed.

Figure 2: Infected *Rhynchophorus ferrugineus* larvae on White trap.

All of four EPN species were caused mortality on red palm weevil larvae in this study. The highest mortality (93.5%) caused by *H. bacteriophora* on *R. ferrugineus* larvae. Followed by *S. carpocapsae* 91.4%, *S. feltiae* 36.4% and *S. affine* 21.2% respectively.

Table 2: Mortality on the *Rhynchophorus ferrugineus* by entomopathogenic nematodes

EPN species	Mortality (%)
Heterorhabditis bacteriophora	93.5
Steinernema affine	21.2
Steinernema carpocapsae	91.4
Steinernema feltiae	36.4

H. bacteriophora species can enter the hemocoel through the insect mouth, anus or spiracles or by penetrating the exoskeleton using a buccal 'tooth'-like structure (Bedding and Molyneux, 1982). This type of effective parasitism helps to cause high mortality on its hosts. Based on the results of this study and according to the mortality, EPNs can be hoping candidates to control the larvae of red palm weevil. The report about natural *H. bacteriophora* infection of *R. ferrugineus* from Mediterranean Region explains this high mortality clearly (Atakan *et al.,* 2009). In other study, *H. bacteriophora* was reported as the most effective species on *R. ferrugineus* larvae. *Steinernema longicaudum*, *S. glaseri*, *S. carpocapsae* and *S. kraussei* were followed it respectively (Triggiani and Tarasco, 2011).

CONCLUSION

In this study among four entomopathogenic nematode species, *H. bacteriophora* caused the highest mortality on *R. ferrugineus* larvae. The second effective species was determined as *S. carpocapsae.* Also *S. feltiae* and *S. affine* caused mortality on *R. ferrugineus* larvae but in the present study it is not enough to control this pest.

Our results demonstrate that larvae of *R. ferrugineus* were highly susceptible to the EPNs tested. All four EPNs tested showed efficacy at different rates against *R. ferrugineus.* In conclusion, it could be suggested that EPNs have a potential to use as biocontrol agents for the management of red palm weevil. Especially the using potential of *H. bacteriophora* and *S. carpocapsae* which are native to Turkey should be investigated for further biological control studies.

REFERENCES

1. Abraham, V.A., M.A. Shuaibi, J.R. Falerio, R.A. Abozuhairah and P.S.P.V. Vidyasagar. An Integrated Management Approach for Red Palm Weevil *Rhynchophorus ferrugineus* Oliv. A Key Pest of Date Palm in the Middle East. *Agricultural Science* 3 (1998): 77-83.

2. Abuzuhairah, R.A., P.S.P.V. Vidyasagar and V.A. Abraham. Integrated Pest Management of Red Palm Weevil *Rhynchophorus* F. in Date Palm Plantations of the Kingdom of Saudi Arabia. *Proceedings, XX International Congress of Entomology, Firenze, Italy,* 25-31 August (1996): 541 pp.

3. Akhurst, R., and K. Smith. Regulation and Safety. In: Gaugler, R. (Ed.), Entomopathogenic Nematology. *CABI, New York, NY* (2002): 311-332.

4. Anonymous. Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleopetera: Curculionidae) Distribution. *Center for Invasive Species Research, University of California Riverside. http://cisr.ucr.edu/red_palm_weevil.html, 2015.*

5. Atakan, E., H. Elekçioğlu, U. Gözel, and O. Yüksel. First Report of *Heterorhabditis bacteriophora* (Poinar, 1975) (Nematoda: Heterorhabditidae) Isolated from *Rhynchophorus ferrugineus* (Olivier, 1970) (Coleoptera: Curculionidae) in Turkey. *Bulletin OEPP/EPPO* 39 (2009): 155-160.

6. Bedding, R.A., and Molyneux, A.S. Penetration of Insect Cuticle by Infective Juveniles of *Heterorhabditis* spp. Heterorhabditidae Nematoda. *Nematologica* 28 (1982): 354-359.

7. Boemare, N.E., C. Laumond, and H. Mauleon. The Entomopathogenic Nematode-bacterium Complex: Biology, Life Cycle and Vertebrate Safety. *Biocontrol Science Technology* 6 (1996): 333-346.

8. Boemare N.E., A. Givandan, M. Brehelin, and C. Laumond. Symbiosis and Pathogenicity of Nematode-Bacterium Complexes. *Symbiosis* 22 (1997): 21-45.

9. Burnell, A.M., and S.P. Stock. *Heterorhabdits*, *Steinernema* and Their Bacterial Symbionts-Lethal Pathogens of Insect. *Nematology* 2 (2000): 31-42.

10. Dutky, S.R., J.V. Thompson, and G.E. Cantwe. A Technique for the Mass Propagation of the DD-136 Nematode. *Journal of Insect Pathology* 6 (1964): 417-422.

11. Ferry, M., and S., Gómez. The Red Palm Weevil in the Mediterranean Palms (formerly Principes), *Journal of the International Palm Society.* linkhttp://www.palms.org/palmsjournal/2002/redweevil.htm link. 46.4 (2002).

12. Forst, S., and D. Clarke. "Bacteria-Nematode Symbiosis, 54-77". In: Entomopathogenic Nematology (Ed: R. Gaugler). *CABI Publishing, Wallingford UK* (2002): 400 pp.

13. Gözel, U., and Ç. Güneş. 2013. Effect of Entomopathogenic Nematode Species on Corn Stalk Borer (*Sesamia cretica* Led. Lepidoptera: Noctuidae) at Different Temperatures. *Turkish Journal of Entomology* 37: 65-72.

14. Griffin, C.T., M.J. Downes, and W. Block. Test of Antarctic Soils for Insect Parasitic Nematodes. *Antarctic Science* 2 (1990): 221-222.

15. Kaya, H.K., and R. Gaugler. Entomopathogenic Nematodes, *Annual Review of Entomology*. 38 (1993): 181-206.

16. Lefroy, H.M. The More Important Insects Injurious to Indian Agriculture. *Government of Indian Printing Press*, Calcuta, India (1906): 151 pp.

17. Malumphy, C., and H. Moran. Red Palm Weevil *Rhynchophorus ferrugineus*. *Plant Pest Notice, Central Science Laboratory* 50 (2007): 1-3.

18. Moura, J.I.L., M.L.B. Resende, and E.F. Vilela. Integrated Pest Management of *Rhynchophorus palmarum* (L). (Coleoptera: Curculionidae) in Oil Palm in Bahia. *Anais da Sociedade Entomoloica do Brazil* 24 (1995): 501-506.

19. Poinar, G.O. Jr., G.M. Thomas, S.B. Presser, and J.L. Hardy. Inoculation of Entomogenous Nematodes, *Neoaplectana* and *Heterorhabditis* and Their Associated Bacteria, *Xenorhabdus* spp., into Chicks and Mice. *Environmental Entomology* 11 (1982): 137-138.

20. Solomon, A., I. Paperna, and I. Glazer. Desiccation Survival of the Entomopathogenic Nematode *Steinernema feltiae*: Induction of an Hydrobiosis. *Nematology* 1 (1999): 61-68.

21. Triggiani, O., and E., Tarasco. Evaluation of the Effects of Autochthonous and Commercial Isolates of Steinernematidae and Heterorhabditidae on *Rhynchophorus ferrugineus*. *Bulletin of Insectology 64.2 (2011): 175-180. http://www.bulletinofinsectology.org/*.

22. Usda-Aphis-Ppq. New Pest Response Guidelines. Red Palm Weevil. *USDA-APHISPPQ-Emergency and Domestic Programs-Emergency Planning, Riverdale, Maryland. http://www.aphis.usda.gov/import_export/plants/manuals/emergency/downloads/nprgredpalmweevil.pdf 2010.*

CITE THIS ARTICLE AS:
Uğur Gözel, Çiğdem Gözel, Çiğdem Yurt and Deniz İnci. Efficacy of Entomopathogenic Nematodes on The Red Palm Weevil *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Curculionidae) Larvae. *International Journal of Bioassays* 4.10 (2015): 4436-4439.

Source of support: Nil
Conflict of interest: None Declared