INTRODUCTION

Palm 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 (Malumpy 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 larva 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 nutrients, and prevent secondary invaders from contaminating the host cadaver (Forst & Clarke, 2002). Infective juveniles (IJ's) 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

Abstract: In this study the efficacy of entomopathogenic nematodes; Steinernema affine (Bovien), S. carpocapsae (Weiser), S. feltiae (Filipjev) and Heterorhabditis 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 500JJs/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

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A 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

<table>
<thead>
<tr>
<th>EPN species</th>
<th>Location</th>
<th>Soil type</th>
<th>pH</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterorhabditis bacteriophora</td>
<td>Sakarya</td>
<td>Sand</td>
<td>7.96</td>
<td>Plane tree</td>
</tr>
<tr>
<td>Steinernema carpocapsae</td>
<td>Sakarya</td>
<td>Sand</td>
<td>7.87</td>
<td>Poplar tree</td>
</tr>
<tr>
<td>Steinernema feltiae</td>
<td>Balıkesir</td>
<td>Sand-clay</td>
<td>7.64</td>
<td>Pinery</td>
</tr>
<tr>
<td>Steinernema affine</td>
<td>İstanbul</td>
<td>Sand-clay</td>
<td>6.97</td>
<td>Oakwood</td>
</tr>
</tbody>
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**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 KCl, 0.37g CaCl2, 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).

**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.

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

<table>
<thead>
<tr>
<th>EPN species</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterorhabditis bacteriophora</td>
<td>93.5</td>
</tr>
<tr>
<td>Steinernema affine</td>
<td>21.2</td>
</tr>
<tr>
<td>Steinernema carpocapsae</td>
<td>91.4</td>
</tr>
<tr>
<td>Steinernema feltiae</td>
<td>36.4</td>
</tr>
</tbody>
</table>

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 hopeful 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 (Trigiani 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


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