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Original article (Özgün makale)

Effectiveness of four Turkish entomopathogenic nematode isolates against *Bactrocera oleae* (Diptera: Tephritidae) pupae at different temperatures

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Dört yerel entomopatojen nematod türünün farklı sıcaklıklarda *Bactrocera oleae* (Diptera: Tephritidae) pupaları üzerindeki etkinliği

Öz: Zeytinin anavatanı olan Türkiye, dünyanın önde gelen zeytin üreticilerinden biridir. Türkiye'de en fazla zeytin üretimi yapan iller arasında İzmir, Aydın, Çanakkale, Balıkesir, Muğla ve Bursa yer almaktadır. Zeytinin ana zararlısı olan *Bactrocera oleae* (Diptera: Tephritidae), ülkemiz zeytin yetiştiriciliğinde önemli bir sorundur. Daha önceki çalışmalarla ülkemizde tespit edilmiş 4 yerel entomopatojen nematod türünün (*Steinernema feltiae*/12, *Steinernema carpocapsae*/1133, *Heterorhabditis bacteriophora*/70, *Heterorhabditis bacteriophora*/91), tek doz ve 5 farklı sıcaklıkta (10, 15, 20, 25 ve 30 ^{o}C) laboratuvar koşullarında *B. oleae* pupaları üzerinde meydana getirdikleri infeksiyon oranları belirlenmiştir. Elde edilen sonuçlara göre en yüksek infeksiyon oranı 25 °C'de *Heterorhabditis bacteriophora*/91 izolatında, en düşük infeksiyon oranı 10 °C'de *Steinernema feltiae*/12 izolatında gözlemlenmiştir.

Anahtar Kelimeler: *Bactrocera oleae*, *Steinernema feltiae*, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, biyolojik mücadele

Abstract: Türkiye, is the country of origin of the olive tree and is among the world's leading olive producers. Most of the olive production in Türkiye is concentrated in Izmir, Aydın, Çanakkale, Balıkesir, Muğla and Bursa Provinces. *Bactrocera oleae* (Diptera: Tephritidae), being one of the main pests of olive trees, is an important problem in olive cultivation in Türkiye. The efficacy of four Turkish populations of entomopathogenic nematodes against *B. oleae* pupae was determined under laboratory conditions. They were *Steinernema feltiae/12*, *S. carpocapsae*/1133, *Heterorhabditis bacteriophora*/70, *H. bacteriophora*/91, which were collected in different regions of Türkiye during our earlier studies. They were tested against the pest by using a single dose at 5 different temperatures (10, 15, 20, 25 and 30 °C). The highest infection rate was observed for*H. bacteriophora*/91 isolate at 25 °C, and the lowest infection rate was observed for *S. feltiae*/12 isolate at 10 °C.

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Introduction

Türkiye is an important olive producer and exporter worldwide. Olive trees are widely grown due to the suitable climate and soil characteristics. Olive production is mostly concentrated in the Aegean, Marmara and Mediterranean Regions. In Türkiye, olives are generally divided into two main categories: olives for oil and table olives. Türkiye's olive oil is well known worldwide for its quality and taste. Olive production fluctuates over the years depending on weather conditionsand farming techniques. Therefore, Türkiye promotes sustainable agricultural practices for the table olive and olive oil industries. Nevertheless, as with all cultivated plants, some diseases and pests negatively affect the yield and quality of the crop. Important pests of olives includes the olive fly (*Bactrocera oleae* Rossi (Diptera: Tephritidae), the olive moth (*Prays oleae* Bern (Lepidoptera: Yponomeutidae), and the olive leaf moth (*Palpita unionalis* Hübn (Lepidoptera: Pyralidae).

The main pest of olives is *B. oleae* which is aninsect that damages olive fruits and is especially common in the Mediterranean Region. *Bactrocera oleae* damages the olive fruit through females laying eggs and the larvae hatching and feeding inside the fruit. This causes deterioration in the internal structure of the olive, loss of quality and decrease in the commercial value. The larvae feed on the fleshy part of the fruit and drop to the soil close to pupation time and pupate in the soil. Various management methods have been used against *B. oleae*. These methods include biotechnical, biological, chemical and cultural methods. Due to the negative effects of chemical pesticides on the environment, biological control has recently gained importance for controlling pests and pathogens (Lacey 2001).

Many nematode species are associated with insects, and so far, 23 families of nematodes that have parasitic relationships with insects have been reported. Seven of which are insect parasites. Nematodes from Steinernematidae Travassos, 1927 and Heterorhabditidae Poinar, 1976 (Nematoda: Rhabditida) are used as microbial insecticides to infect insects with pathogenic microbes, and are commercially produced and marketed by various companies (Koppenhöfer 2007) for biological control studies and are used effectively against many insect pests species (Grewal et al. 2005). EPNs, which are obligate parasites of insects with a part of their life-cycle in the soil, can be effective biological control agents oft insects pests. The host specificity of EPNs is one of the main features that distinguish them from insecticides (Smart 1995). EPNs particularly provide a unique option for controlling a pest such as *B. oleae*; because when applied to plants they actively seek out their hosts, cause no harm to vertebrates, and effectively can kill insects even when applied at very low dose levels. EPNs can kill their hosts within approximately 48 hours by causing septicemia (blood poisoning) in the host through the activity of symbiotic bacteria associated with them (Ünlü & Özer 2003). In host infection, the third larval stage (IJ or dauer juvenile) is the most active larval stage found in the soil seeking its host. The third larval stage (J3) can

survive in the soil without a host for at least a year. TheJ3 of EPNs enters the host's hemocoel through the host's orifices (mouth, anus, stigma) or thinned parts of the cuticle (only in Heterorhabditidae, which have dorsal labial teeth in the mouth) (Bedding & Molyneux 1982; Wang & Gaugler 1998). Nematodes and their symbiotic bacteria which feed on damaged tissues of their hosts, develop and reproduce within the insect's cadaver (Poinar & Grewal 2012). Feeding of IJs continues for approximately 2-3 generations until the infected host is consumed. Nematodes at the J3 stage, which have consumed all the tissues of the insect host, leave the cadaver, move to the soil and begin to look for new hosts (Poinar 1979; Akhurst & Boemare 1990).

Entomopathogenic nematodes can be mass produced in vivo or in vitro in solid or liquid media (Grewal & Georgis 1998). EPNs can be applied against insects pests in the soil, animal manure, aquatic habitats and on leaves. However, the most common of these applications is soil application (Klein 1990). In biological control, EPNs that exist naturally in the soil have a significant advantage in their use on harmful insect species that spend at least one period of their life cycle in the soil. *Bactrocera oleae* is one of the insects that pupate in the soil where it is potentialy vulnerable to EPNs.

The effectiveness and virulence of EPN species have been investigated for many insect species in Türkiye (Ataş et al. 2020; Gözel et al. 2020; Özdemir & Evlice 2020; Şahin & Gözel 2021; Erdoğuş et al. 2023). This study aimed to investigate the effectiveness of four EPN species, which are capable of suppressing many important pests, against the pupal stage of *B. oleae* under laboratory conditions.

Materials and Methods

Obtaining *Bactrocera oleae*

The main material of the study was *B. oleae* pupae and EPN isolates obtained from Edirne, Çanakkale and Sakarya Provinces. Olives damaged by *B. oleae* were collected from Çanakkale center and Geyikli and brought to the Nematology Laboratory of Çanakkale Onsekiz Mart University, Faculty of Agriculture and stored at 9 ^{o}C. In the course of time, pupaes of *B. oleae* were obtained from damaged olives (Figure 1).

Figure 1. Obtaining pupae of *Bactrocera oleae* from damaged olives

Mass production of *Galleria mellonella*

Since the last instar larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae) are very vulnerable to EPNs, it is used to obtain EPNs from soil and in mass production (Bedding & Akhurst 1975). *Galleria mellonella* larvae were reared in an artificial nutrient medium (a mixture of 500 g coarse bran, 50 g honey, 65 ml glycerin and 25 ml pure water) in incubators in glass jars at 27±1 ^{o}C (Kaya & Stock 1997). Some of the grown larvae were returned to the glass jars to continue the culture, and some were used in the mass production of EPNs.

Mass production of entomopathogenic nematodes

EPN isolates obtained from soil in Türkiye were mass produced on the last instar of larvae of *G. mellonella* (Table 1) (Figure 2). Depending on the EPN species, emergence from each *G. mellonella* larva was observed within 2-4 days under optimal conditions (25 ^{o}C, 70% humidity).

Table 1. Entomopathogenic nematode species used against the olive fruit fly, *Bactrocera oleae*

No of the EPN isolate	Name of EPN species	Obtained place
12	*Steinernema feltiae* Filipjev, 1934	Edirne
1133	*Steinernema carpocapsae* Weiser, 1955	Sakarya
70	*Heterorhabditis bacteriophora* Poinar, 1976	Çanakkale
91	*Heterorhabditis bacteriophora* Poinar, 1976	Çanakkale

Figure 2. Production of EPNs obtained from soil in Turkiye on last instar of *Galleria mellonella* larvae

Determining of the effectiveness of entomopathogenic nematodes on *Bactrocera oleae* pupae

In the laboratory, Whatman filter papers were placed in Petri dishes with a diameter of 3 cm. All Petri dishes were labelled according to the treatment. For one

repetition, 20 Petri dishes were prepared and then one *B. oleae* pupa was placed in each Petri dish. In the experiment, 5 different temperatures (10, 15, 20, 25 and 30 ^{o}C) and four different EPN isolates were used. Two hundred IJ/pupae in 200 µl of pure water were placed in each Petri dish (Figure 3). The experiment was set up with 3 replications. After inoculation, the Petri dishes were placed in incubators at one of five different temperatures. The Petri dishes were moistened for 15 days. The pupae were then dissected under a binocular microscope and checked for EPN emergence. The Petri dishes in which emergence was observed were recorded asconfirmation that the deaths of the *B. oleae* pupae were caused by the EPNs.

Figure 3. Infecting pupae of *Bactrocera oleae* with EPNs in pure water

Statistical analysis

The data generated in this study were evaluated with one-way analysis of variance and the differences were grouped with the Tukey test.

Results and Discussion

There weredifferences in efficacy against the the olive fruit fly, *Bactrocera oleae* among four different Turkish EPN isolates and at different temperatures in a laboratory study (Table 2).

Table 2. Levels ofinfection of *Bactrocera oleae* pupae by four entomopathogenic nematode isolates collected in Turkiye

Place	Nematode Species /Isolate Code	Temperature (°C)				
		10	**15**	**20**	**25**	**30**
Edirne	*Steinernema feltiae*/12	10 ±5,77 *ABc	15 ± 2,89 ABbc	25 ± 5,77 Aab	25 ± 2,89 Bab	30± 2,89 Aa
Sakarya	*Steinernema carpocapsae*/1133	15 ± 2,89 ABb	25 ± 5,77 Aab	30 ± 5,77 Aa	35 ± 2,89 ABa	35 ± 0,00 Aa
Çanakkale	*Heterorhabditis bacteriophora*/70	15 ± 5,77 ABc	20 ± 2,89 Abc	30 ± 2,89 Aab	30 ± 0,00 ABab	35 ± 0,00 Aa
Çanakkale	*Heterorhabditis bacteriophora*/91	20 ± 2,89 Ac	25 ± 5,77 Abc	35 ± 2,89 Aab	40 ± 2,89 Aab	35 ± 0,00 Aa**

* Statistical differences between EPN groups at the same temperature are indicated with a capital letter (P<0,05).
** Statistical differences between temperatures in the same EPN group are indicated by lowercase letters (P<0,05).

The highest infection rate was observed for *Heterorhabditis bacteriophora*/91 isolate at 25 °C, and the lowest infection rate was observed for *Steinernema feltiae*/12 isolate at 10 °C. The efficacy rates of the EPNs against *B. oleae* pupae varied significantly, depending on the EPN strain and the temperature applied.

Similar results have been obtained in studies conducted with *Ceratitis capitata* Wied. (Diptera: Tephritidae), *Rhagoletis cerasi* L. (Diptera: Tephritidae), *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) and *Bactrocera dorsalis* Handel (Diptera: Tephritidae), which are in the same family as *B. oleae*. It has been reported that insects in the order Diptera are vulnerable to infection by species in the genera, *Steinernema* and *Heterorhabditis* (Rohde et al. 2012; Kepenekci et al. 2015; Minas et al. 2016; Heve et al. 2017; Godjo et al. 2018).

In studies investigating the effectiveness of EPNss against *B. oleae* larvae and pupae, *S. fetiae* (Sirjani et al. 2009; Torrini et al. 2020) and *S. carpocapsae* (Torrini et al. 2017) were effective at high application rates.

In the present study, the infection rates of *B. oleae* pupae by EPNs varied, depending on the nematode species and strain? and the temperature applied, with the rate of infection increasing with increasing temperature. *Steinernema fetiae*/12 isolate hadthe lowest activity against *B. oleae* pupae. Separately,, *S. carpocapsae*/1133 and *H. bacteriophora*/70 isolates caused similar mortality of *B. oleae* pupae at all temperatures. Overall, *H. bacteriophora*/91 had the highest efficacy against *B. oleae* at almost all temperatures.

The use of biological control methods as an alternative to chemical control against insect pests is increasing. The EPNs used in biological control have a wide

host range, cause high rates of mortality of their hosts, are easy to apply, and are compatible withhuman and environmental health, which facilitates their use.

In this study, the four EPN isolates had similar efficacy rates to those reported in earlier studies of infection levels of*B. oleae* larvae and pupae. *Bactrocera oleae* larvae after feeding on the fleshy part of the olive fruit fall to the soil where they pupate and are vulnerable to EPNs. the isolate *Heterorhabditis bacteriophora*/91 infected *B. oleae* pupae more effectively than the other EPN isolates and it is a potential biological contol agent of this major pest.

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