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Original article (Orijinal araştırma)

Efficacy of entomopathogenic nematodes against neonate larvae of *Capnodis tenebrionis* (L., 1758) (Coleoptera: Buprestidae)[1]

Capnodis tenebrionis (L., 1758) (Coleoptera: Buprestidae)'in ilk dönem larvalarına karşı entomopatojen nematodların etkinliği

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Abstract

Entomopathogenic nematodes (EPN) have a high potential for control of pests living in isolated places such as underground or galleries. In this study, mortality rates of *Capnodis tenebrionis* (L., 1758) (Coleoptera: Buprestidae) larvae from four EPN species *Steinernema affine* Bovien, 1937, *Steinernema carpocapsae* Weiser, 1934, *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) collected from Turkey under controlled conditions were determined. EPN used in the study were cultured on *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae). Adults of *C. tenebrionis* were collected from the orchards of Çanakkale Province and, eggs and larvae were cultured under controlled conditions. Three densities of EPN species, viz. 50, 500 and 1000 infective juveniles/*C. tenebrionis,* were applied in 12-well plates. Cherry saplings were planted into pots with sterilized soil mixture and 10 neonate larvae of *C. tenebrionis* added to each pot. To each pot, 40,000 infective juveniles were applied for each EPN species in 10 ml of water. Mortalities of *C. tenebrionis* larvae were determined 1, 3, 5 and 7 d after application. In the plates, mortality of *C. tenebrionis* larvae increased with time after EPN application. For all application rates, mortality of *C. tenebrionis* larvae was 100% by day 5. Mortality of *C. tenebrionis* larvae ranged between 50 and 90% depending on species and time in pots. Efficacy studies were conducted in 2016 in Çanakkale. Research on the efficacy of EPN species that have a high mortality under controlled conditions is important to determine their potential to control the target pest.

Keywords: *Capnodis tenebrionis, Heterorhabditis bacteriophora*, *Steinernema affine*, *Steinernema carpocapsae, Steinernema feltiae*

Öz

Toprak altı ve galeriler gibi izole alanlarda yaşayan zararlıların mücadelesinde entomopatojen nematodlar (EPN) yüksek bir potansiyele sahiptir. Bu çalışmada Türkiye'den elde edilen dört entomopatojen nematod (EPN) türünün *Steinernema affine* Bovien, 1937, *Steinernema carpocapsae* Weiser, 1955, *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) ve *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) kontrollü koşullarda *Capnodis tenebrionis* (L., 1758) (Coleoptera: Buprestidae) larvalarında meydana getirdikleri ölüm oranları belirlenmiştir. Çalışmada kullanılan EPN'ler laboratuvarda *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) üzerinde üretilmiştir. *Capnodis tenebrionis* erginleri Çanakkale ili meyve bahçelerinden toplanmış ve kontrollü koşullarda yumurta ve larvaları üretilmiştir. EPN türlerinin 50, 500 ve 1000 infektif jüvenil/*C. tenebrionis* olmak üzere 3 farklı yoğunluğu 12 hücreli kuyucuklarda uygulanmıştır. Saksı denemelerinde, sterilize edilmiş toprak karışım içeren saksılara kiraz fidanları dikilmiş ve her saksıya 10'ar adet 1. dönem *C. tenebrionis* larvası bulaştırılmıştır. Kiraz fidanları sterilize toprak karışımı içeren saksılara dikilmiş ve her saksıya 10 adet 1. dönem *C. tenebrionis* larvası aktarılmıştır. Her saksıya her bir EPN türü için 40.000 infektif juvenile 10 ml su içerisinde uygulanmıştır. *C. tenebrionis* larvalarının ölüm oranları uygulamadan 1, 3, 5 ve 7 gün sonra belirlenmiştir. Tüm uygulama oranlarında *C. tenebrionis* larvalarının ölüm oranı 5. günde %100'dür. *Capnodis tenebrionis* larvalarının ölüm oranları türe ve zamana bağlı olarak %50-90 arasında değişiklik göstermiştir. Etkinlik çalışmaları 2016 yılında Çanakkale ilinde gerçekleştirilmiştir. Kontrollü koşullar altında yüksek ölüm oranına sahip olan EPN türlerinin etkinliklerinin araştırılması hedef zararlının kontrolü için potansiyellerini belirlemek için önemlidir.

Anahtar sözcükler: *Capnodis tenebrionis, Heterorhabditis bacteriophora*, *Steinernema affine*, *Steinernema carpocapsae*, *Steinernema feltiae*

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Introduction

Capnodis tenebrionis (L., 1758) (Coleoptera: Buprestidae) is an important pest in stone fruit orchards. Larvae of the pest can cause the death of trees and yield loss by burrowing to form galleries in the trunk of trees. This pest has been reported in Spain, Italy, Turkey, Iran, Syria, North Africa, Israel, France and Palestine (David'yan, 2003, Abu Jbara, 2005**,** Bonsignore et al., 2008; Şahin & Gözel, 2017).

Chemical control is effective only against the adult stage, as neonate larvae of the pest are under the soil and following larval stages feed under the bark of the tree. Consequently, chemical control of the adults is not an effective control method, so alternative methods such as entomopathogenic nematodes (EPN) are needed. EPN kill their hosts with the help of symbiotic bacteria species *Xenorhabdus* spp. Thomas & Poinar, 1979 and *Photorhabdus* spp. (Boemare et al. 1993) (Bacteria: Enterobacteriales) (Akhurst, 1993). Generally, each EPN species is associated with only one bacterial species, except some *Steinernema* spp. (Steinernematidae: Rhabditida), which share the same *Xenorhabdus* bacteria (Akhurst, 1993). EPN reproduce in the cadaver of their insect host under suitable conditions created by the symbiotic bacteria. Several EPN generations can be completed in a single host. Infective juveniles (IJ), the only life stage of EPN that they can move freely in the soil, are produced in the event of food depletion and are released from the cadaver (Grewal et al., 1997).

There are several studies using EPN against *C. tenebrionis*, such as the study of Marannino et al. (2003) in which they reported 100% mortality of *C. tenebrionis* larvae from *Steinernema carpocapsae* Weiser, 1955 and *Heterorhabditis bacteriophora* Poinar, 1976. Also, Garcia del Pino & Morton (2005) has reported that the mortality of *C. tenebrionis* larvae caused by *Steinernema arenarium* Artyukhovsky, 1967 was 90%, which was significantly higher than *Steinernema feltiae* Filipjev, 1934 (76%), *H. bacteriophora* (76%) and *S. carpocapsae* (59%). Other studies have obtained similar results (Hourieh et al., 2008; Martinez de Altube et al., 2008; Morton & Garcia del Pino, 2008, 2009; Yiğit et al., 2015; Şahin et al., 2018a, b).

EPN have an important place in biological control of underground pests due to their ability to survive for long periods and their active behavior in searching for hosts in soil. Also, according to Bedding et al. (1983) and Kaya (1985) EPN can be effective against pests that live in sheltered habitats like galleries.

In this study, four native EPN isolates collected from Turkey were studied at different application rates to determine their efficacy against *C. tenebrionis* in 12-well plates and potted saplings under controlled conditions.

Materials and Methods

EPN mass rearing

In this study, four native species of nematodes; *Steinernema affine* Bovien, 1937 (isolate 47), *S. carpocapsae* (isolate 1133), *S. feltiae* (isolate 96) and *H. bacteriophora* (isolate 1144) were used against neonate larvae of *C. tenebrionis.* All isolates were reared in the last instar of *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae), which is the most commonly used insect host of EPN (Bedding & Akhurst, 1975; Kaya & Stock, 1997). Before using the nematodes, their viability and numbers were checked under the stereomicroscope Leica DM1000.

Rearing of *Capnodis tenebrionis* larvae

Adults of *C. tenebrionis* were collected from neglected cherry nurseries in Çanakkale Province. These adults were transferred to the laboratory in sampling boxes with fresh apricot shoots.

Rearing technique of *C. tenebrionis* was modified from the method developed by Garrido et al. (1987). *Capnodis tenebrionis* adults were placed into insect rearing cages with young apricot shoots from chemically-untreated orchards for adult feeding.

Sterilized (121°C, 12 h) and screened (1 mm) sand was used as the egg laying medium for females by spreading the sand at 2 cm deep on the bottom of cages. Sand was checked daily for eggs and was screened with a sieve. Particles remaining on the sieve were controlled under binocular microscope and

eggs were placed into Petri dishes with a soft brush. These Petri dishes were placed into a climate chamber (25°C, 60-70% RH, 16:8 h L:D photoperiod) for incubation. Hatched neonate larvae were transferred to another Petri dish to be used in EPN experiments with daily controls.

EPN efficacy experiments

EPN inoculation of *Capnodis tenebrionis* in the laboratory

The method of Garcia del Pino and Morton (2005) was used in the experiment. Efficacy of EPN on *C. tenebrionis* was investigated in plates with 12 wells (3 cm diameter). Each well (3 x 4 cm) in the plates was filled with 6 cm^3 sterilized sand with one 2-d-old *C. tenebrionis* neonate larva added. Each EPN isolate was applied in three application rates, 50, 500 and 1000 IJ/*C. tenebrionis*, in 100 µl distilled water with 12 replicates. The experiment was repeated two times on different days. Distilled water was used as a control treatment.

To determine the efficacy of the EPN, mortality rates of the larvae were calculated by examining individuals 1, 3, 5 and 7 d after establishment of the experiment to observe the change in mortality related to time, according to Morton and Garcia del Pino (2009). Dead individuals were transferred to White traps with a soft tipped brush to verify that death of the larvae was caused by EPN (White, 1927). Efficacy tests of EPN were conducted in at 23±2°C in the dark. IJ emerging from cadavers were photographed.

EPN inoculation of *Capnodis tenebrionis* in cherry saplings

Cherry cv. Regina saplings (grafted on cv. Maxima rootstocks) were planted into pots (30 x 30 cm) containing a sterilized (120°C, 12 h) soil mixture. The soil mixture was prepared with sand and soil, and 750 g soil mixture was placed into each pot and the pots were watered. Two d after planting, the saplings, 10 *C. tenebrionis* neonate larvae were transferred to the soil surface near the root collar of each sapling. The saplings were stored in a climate chamber at 23±2°C and 12:12 h L:D photoperiod for a day. Then the EPN were applied at 25 IJ/cm^2, which is a typical rate used for releasing EPN (Shields, 2015), with a total of 40,000 IJ per pot in 10 ml water. This rate was calculated based on the soil quantity in order to achieve homogeneous dispersal of the EPN. Given that too much water can kill *C. tenebrionis* larvae, soil surface was just dampened every 2 d to ensure EPN survival. Saplings were uprooted 1, 3, 5 and 7 d after EPN application and the number of living and dead *C. tenebrionis* larvae in the soil counted.

Dead larvae were transferred to White traps to verify that death of the larvae was caused by EPN. Also, the damage on the roots of the sapling and the number of larvae inside the roots were noted and photographed. Efficacy studies were conducted in 2016 in Çanakkale.

Statistical analysis

Data from the study was analyzed with repeated measures ANOVA using SPSS® 23 software. The Tukey's multiple comparison test ($P < 0.01$) was used to determine the differences between days, rates and species in MSTATC®.

Results

***Capnodis tenebrionis* larval mortality in the laboratory**

Mortality rates of *C. tenebrionis* larvae caused by the different EPN isolates on different days after EPN application are given in Table 1. Dead larvae were not observed until day 7 in all control treatments. By day 5, all EPN isolates at all application rates had killed 100% of the neonate larvae of *C. tenebrionis*. Although larval mortality of *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* reached 100% by day 5, 100% mortality of *S. affine* with 50 IJ had occurred by day 3. With 500 and 1000 IJ, 100% of *C. tenebrionis* neonate larvae of *S. feltiae* were dead by day 1. *Steinernema affine* reached 100% mortality by day 3, but it took 5 d for *S. carpocapsae* and *H. bacteriophora* at both rates.

Table 1. Mortality of *Capnodis tenebrionis* larvae with different entomopathogenic nematodes at different application rates and days after application (mean±se)

Entomopathogenic nematode	Day	50 IJ	500 IJ	1000 IJ
Steinernema feltiae	1	33.3±6.8 B a Ⅲ*	100.0±0.0 A a Ⅰ	87.5±8.0 A a Ⅰ
	3	66.7±6.8 A b Ⅱ	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ
	5	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ
	7	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ
Steinernema carpocapsae	1	20.8±4.2 A ab Ⅲ	20.8±4.2 A c Ⅰ	25.0±4.8 A b Ⅱ
	3	70.8±8.0 A b Ⅱ	83.3±6.8 A a Ⅰ-Ⅱ	83.3±11.8 A ab Ⅰ-Ⅱ
	5	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ
	7	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ
Steinernema affine	1	20.8±4.2 B ab Ⅱ	66.7±11.8 AB b Ⅱ	87.5±8.0 A a Ⅰ
	3	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ
	5	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ
	7	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ
Heterorhabditis bacteriophora	1	8.3±4.8 A b Ⅲ	16.7±0.0 A c Ⅱ	16.7±6.8 A b Ⅲ
	3	66.7±9.6 A b Ⅱ	83.3±6.8 A a Ⅰ-Ⅱ	66.7±15.2 A b Ⅱ
	5	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ
	7	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ	100.0±0.0 A a Ⅰ

* Means followed by the same uppercase letter for the same entomopathogenic nematode (EPN) and day are not significantly different (P≤0.01); means followed by the same lowercase letter for the same EPN application rate and day are not significantly different (P≤0,01); means followed by the same roman letter in the same EPN application rate and eEPN are not significant different (P≤0.01).

After 1 d, the lowest mortality was with 50 IJ of *H. bacteriophora* with 8.3% and the highest mortality was 100% with 500 IJ of *S. feltiae*. Only this application rate of *S. feltiae* was able to kill 100% of the *C. tenebrionis* larvae in 1 d. With 50 IJ, the mortality caused by *S. feltiae* was significantly higher than the other isolates by day 1 (F=24.8, P=0.000, df=9). The difference between the mortalities from *S. carpocapsae*, *S. affine* and *H. bacteriophora* was not statistically significant. By day 3 with this application rate, the highest mortality was with *S. affine,* but with the other isolates there was no statistically significant increase in mortality. By day 5, the differences between mortalities with isolates as they all have reached 100%.

After 1 d with 500 IJ, larval mortality with *S. feltiae* had reached to 100%, while it was 20.8% with *S. carpocapsae*, 66.7% with *S. affine* and 16.7% with *H. bacteriophora*. The mortality caused by *S. feltiae* was

significantly higher than with the other isolates, and mortality with *S. carpocapsae* and *H. bacteriophora* was significantly lower than the others. With 500 IJ, 100% larval mortality was reached with *S. affine* by day 3 and with *S. carpocapsae* and *H. bacteriophora* by day 5 (F=5.81, P=0.000, df=6).

After 1 d with 1000 IJ, the lowest larval mortality was with *H. bacteriophora* at 16.7%, while the highest was with *S. feltiae* and *S. affine*, both at 87.5%. Mortality with *S. carpocapsae* was 25.0%, which was significantly lower than with both *S. feltiae* and *S. affine* (F=13.3, P=0.000, df=6). There was no significant difference between *S. carpocapsae* and *H. bacteriophora*. Larval mortality of 100% was reached by day 3 with *S. feltiae* and *S. affine*, but by day 5 with *S. carpocapsae* and *H. bacteriophora*.

***Capnodis tenebrionis* larval mortality in cherry saplings**

Mortality of *C. tenebrionis* larvae caused by different EPN at different days after application are given in Table 2. After 1 d, larval mortalities were 62.5, 52.5, 70.0, 50.0 and 0.00% with *S. feltiae*, *S. carpocapsae*, *S. affine*, *H. bacteriophora* and control, respectively. There was no significant difference between the EPN species on day 1 (F=1.90, df=3, p=0.271) and 7 (F=0.73, df=3, p=0.584). After 3 d, mortalities with *S. feltiae, S. carpocapsae* and *S. affine* were not significantly different, however, mortality with *H. bacteriophora* was significantly lower than *S. feltiae* and *S. carpocapsae* (F=0.90, df=3, p=0.031). Similarly, there was no significant difference with *S. feltiae, S. carpocapsae* and *S. affine* on day 5, however, mortality with *H. bacteriophora* was significantly lower than *S. carpocapsae* but not from *S. feltiae* and *S. affine* (F=0.44, df=3, p=0.027). Mortality was 5% in control treatment by day 7, with no mortality observed on the other days.

Table 2. Mortality of *Capnodis tenebrionis* 1, 3, 5 and 7 d after inoculation with entomopathogenic nematodes in cherry saplings (mean±se)

Entomopathogenic nematode	Day 1	Day 3	Day 5	Day 7
Steinernema feltiae	62.5±2.5 B a*	75.0±5.0 AB a	80.0±5.0 AB ab	92.5±2.5 A a
Steinernema carpocapsae	52.5±7.5 B a	77.5±2.5 AB a	85.0±5.0 A a	92.5±2.5 A a
Steinernema affine	70.0±10.0 A a	72.50±12.5 A ab	80.0±10.0 A ab	87.5±7.5 A a
Heterorhabditis bacteriophora	50.0±5.0 B a	57.5±2.5 B b	75.0±0.0 A b	82.5±7.5 A a

* Means followed by the same uppercase letter in the same row are not significantly different (P ≤ 0.05); means followed by the same lowercase letter in the same column are not significantly different (P ≤ 0.05).

Discussion

Results of the laboratory study show that, all four EPN isolates were capable of killing neonate larvae of *C. tenebrionis* to varying degrees. Similarly, Garcia del Pino & Morton (2005) reported 95% mortality of *C. tenebrionis* with *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* after 5 d. Marannino et al. (2003) reported that, the mortality of *S. carpocapsae* with *H. bacteriophora* reached 100%.

Mortality of *C. tenebrionis* caused by different EPN changed with time after application. Especially mortality with *S. feltiae* was observed to reach to 100% in 1 or 3 d, while it took 5 d with *H. bacteriophora*. Similar results were recorded in the study by Morton & Garcia del Pino (2009), with 100% mortality after 1 and 3 d with *S. feltiae* and *S. carpocapsae*, and 100% mortality after 5 d with *H. bacteriophora*. Therefore, we concluded that *S. feltiae* is faster at infecting the host than other EPN isolates even at higher application rates.

Generally, the mortality with different application rates of the EPN did not differ significantly between the EPN on the same day of assessment. Only, the mortalities with 50 IJ of *S. feltiae* and *S. affine* on day 1 and *H. bacteriophora* on day 3 were significantly different from the other application rates. Also, all the

EPN were able to kill 100% of the neonate larvae after 5 d. Accordingly, it can be said that application of 50 IJ is potentially enough to kill neonate larvae of *C. tenebrionis* under controlled conditions. Marannino et al. (2003) also reported that 50 IJ of *S. carpocapsae* and *H. bacteriophora* in 2 ml tap water were able to kill 100% of the *C. tenebrionis* neonate larvae in a plate experiment. We also know that all EPN species can be effective in killing their hosts with the help of their symbiotic bacteria in 24-48 h, depending on the temperature and humidity.

In the cherry sapling experiment, mortality of *C. tenebrionis* larvae ranged between 50 to 92.5% over the assessment period and EPN applied. These results were lower than those of Marannino et al. (2003), who reported mortality of *C. tenebrionis* larvae in plants at 100% with *S. carpocapsae* and 98.9% for *H. bacteriophora*.

Results of our study support the idea that EPN are effective in controlling *C. tenebrionis* and the mortality caused by EPN is higher than insecticide treatments. Marannino et al. (2003) and Sanna-Passino & Delrio (2001) report mortality of 67.3 and 83.3% with diazinon (banned in Turkey in 2009 because it causes lung cancer) and chlorpyrifos, respectively. Also, EPN do not have the unwanted effects of insecticides, such as insecticide residues and pest resistance. Ben-Yehuda et al. (2000) tested nine chemical compounds and three application methods to improve the chemical control of *C. tenebrionis* and *Capnodis carbonaria* (Klug, 1829) (Coleoptera: Buprestidae), and have found that *C. tenebrionis* is more resistant insecticides than *C. carbonaria*.

Using local EPN species and biopesticides for the biological control of *C. tenebrionis*, which is an important pest of stone-fruits in Turkey, are important strategies for its successful control. In this study, our local isolates of EPN were effective against *C. tenebrionis* neonate larvae. In the light of these results, we think any application method with water would be highly effective for control of neonate larvae of *C. tenebrionis*, because of the EPN need moisture to remain infective. With the prevalence of drip irrigation in fruit orchards of Turkey, we suggest farmers could use their drip irrigation systems or surface systems to easily applying EPN to soil. Also, efficacy of EPN is generally higher than chemical control against underground pests and the ability of EPN to reproduce on other underground insect species contributes to their survival for the long term, thus increases their persistence in soil. Research on the efficacy of these EPN against *C. tenebrionis* under field conditions would be an important component of future studies.

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