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Orijinal araştırma (Original article)

Determination of the soil persistency of native entomopathogenic nematodes applied with a drip irrigation system in a peach orchard[1]

Çiğdem ŞAHİN [2,3*], Uğur GÖZEL[3]

Şeftali bahçesinde damla sulama yöntemi ile toprağa uygulanan yerel entomopatojen nematodların kalıcılığının belirlenmesi

Öz: Entomopatojen nematodların topraktaki hayatta kalma süreleri biyolojik mücadele çalışmalarının başarısı için önemlidir. Bu çalışmada bir şeftali bahçesinde 4 yerel entomopatojen nematod (EPN) türü*; Steinernema feltiae* Filipjev (Nematoda: Steinernematidae), *S. carpocapsae* Weiser (Nematoda: Steinernematidae), *S. affine* Bovien (Nematoda:Steinernematidae) ve *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae)'nın topraktaki kalıcılığı damla sulama yöntemi ile 2018 ve 2019 yıllarında araştırılmıştır. Entomopatojen nematodlar toprağa 50 IJs/cm^2 dozunda uygulanmıştır. Uygulama sonrası ayda bir toprak örneklemesi yapılarak entomopatojen nematodların topraktaki kalıcılığı belirlenmiştir. Araştırma sonucunda 2018 yılında EPN'lerin toprakta kalıcılıklarının 90 gün, 2019 yılında ise 150 gün sürdüğü belirlenmiştir. Elde edilen bulgular neticesinde EPN'lerin toprağa uygulandıktan sonra çevresel faktörlere bağlı olarak kalıcılık sürelerinin değiştiği belirlenmiştir. Uygulama sonrası EPN'lerin toprakta kalma sürelerinin uzatılması topraktaki zararlılara etkinliklerinin artırılması açısından önemlidir.

Anahtar Kelimeler: *Steinernema feltiae, Steinernema carpocapsae, Steinernema affine,* Entomopatojen nematod, Kalıcılık

Abstract: The period of persistence of entomopathogenic nematodes (EPNs) in soil is an important factor in the success of biological control efforts. In this study, the persistency of four native Turkish entomopathogenic nematode species, *Steinernema feltiae* (Filipjev), *S. carpocapsae* (Weiser), *S. affine* (Bovien) and *Heterorhabditis bacteriophora* (Poinar), was investigated in a peach orchard after their application with a drip irrigation system in Çanakkale Province, Turkiye, in 2018 and 2019. The EPNs were applied at the rate of 50 IJs/cm^2. After application, the persistency of the EPNs was determined with soil sampling on a monthly basis. EPN persistence in the soil was 90 days and 150 days in 2018 and 2019, respectively. Additionally, the persistency of the EPNs in soil after their application varied, depending on environmental factors. The longer survival of EPNs in soil after

[1] Bu çalışma Doktora tezinden özetlenmiştir

[2] Lapseki, Çanakkale, Türkiye
[3] Çanakkale Onsekiz Mart Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Çanakkale,Türkiye
[*] Sorumlu Yazar (Corresponding Author): cigdem.sahinyilmaz@tarimorman.gov.tr
ORCID ID (Yazar sırasıyla): 0000 0002-2019-6704; 0000-0003-1363-1189
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application is important for the increase of the efficiency of EPNs against pests. It was concluded that the local EPN species have potential for use in the biological control of agricultural pests.

Keywords: *Steinernema feltiae*, *Steinernema carpocapsae*, *Steinernema affine*, Entomopathogenic nematode, Persistency

Introduction

Entomopathogenic nematodes (EPNs) have a range of effects on pests, including reducing the number of eggs; growth deficiency and abnormalities in behavior, physiology, and morphology; and death (Koppenhöfer 2000). The management of pests living in difficult to access places such as the soil and galleries in plant tissue is difficult because of the challenge of reaching them with the treatment. Especially the inability of chemical controls to reach confined spaces highlights the usefulness of EPNs, which can live for extended periods in soil (Hazır et al. 2003). The life stage of the EPN that lives in the soil is the infective juvenile (IJ). IJs do not feed and use their stored energy to find and infect new hosts (Noitubtim et al. 2022).

Entomopathogenic nematodes are generally good biocontrol agents for the control of pests living in the soil and plant tissues. The neonate larvae and other pre-adult stages of flatheaded woodborers (*Capnodis tenebrionis* L. (Coleoptera: Buprestidae)) are suitable targets for EPN, because they live in soil and in the tissues of trees.

There have been studies on the management of *C. tenebrionis*, which is an important pest of stone fruit trees, especially the saplings of “apricot, cherry and peach, in Turkiye (Deviren 2011; Karaca 2012; Şahin & Gözel 2017; Zobar & Kıvan 2019).

The survival of EPNs in the soil after release, which is also called soil persistence, depends on factors such as its composition, moisture level, temperature and the presence of other organisms (Khan & Javed 2018). Also, persistency may differ between laboratory and field studies, because of the interaction and influence of these factors (Gray & Johnson 1983). Soil persistence is important for the determination of the timing of supplementary releases, or whether they are even necessary, because some EPN species may successfully establish and reproduce in the new environment. Successful establishment and persistence is termed inoculative release (Shields et al. 2018).

The purpose of this study was to determine the soil persistence of local entomopathogenic nematode species in Çardak town of Lapseki District in Çanakkale Province, Turkiye, in 2018 and 2019.

Materials and methods

Orchard

The study was conducted in an 8-year-old peach orchard (*Prunus persica* L.) (Rosaceae) (40°23'39" N, 26°44'48" E, 8 m). The orchard contained the cultivars Black Hale, Royal Glory and Abdos. Before choosing the orchard for the study, soil samples were taken to confirm that EPN species were not naturally present (Bedding & Akhurst 1975; Griffin et al. 2000).

Production of entomopathogenic nematodes

Steinernema feltiae (96) Bursa, *S. carpocapsae* 1133 (Sakarya), *H. bacteriophora* 1144 (Sakarya) and *S. affine* 47 (Istanbul) were mass produced in the last instar of *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae), which is the most commonly used insect host for EPN rearing (Bedding & Akhurst 1975; Kaya & Stock 1997). Dead infected *G. mellonella* larvae were placed in White traps to collect the emerging infective juveniles (White 1929). These nematodes were stored in a flask in a refrigerator. The EPNs were prepared at a concentration of 140.000 IJ/tree in distilled water in 50 cc Falcon tubes prior to being transported to the orchard in an ice box.

Soil application of entomopathogenic nematodes with a drip irrigation system

An 8-armed drip irrigation system was constructed with t-pipes and plugs. The system was placed on the soil around the trees (Figure 1). A 30 L container was filled with water and the EPN suspension was added. The container was connected to the drip system and the EPNs were applied to the soil at 50 IJs/cm^2. The water in the container was stirred to prevent the EPNs from sinking to the bottom. Before the application, the ability of EPNs to pass through the emitters of the drip irrigation system was tested in the laboratory to determine the rate of EPN release. In addition, the EPNs were confirmed to be alive under a detail of microscope after travelling through the system. The experiments were designed with 3 repetitions and were conducted in September of 2018 and July of 2019.

Figure 1. Drip irrigation system used to apply entomopathogenic nematodes to the soil in a peach orchard in Çanakkale Province, Turkiye

Determination of the persistency of entomopathogenic nematodes in soil under natural conditions in a peach orchard

Soil sampling was conducted on a monthly basis around the peach trees to determine the persistency of EPNs in the soil. Soil samples were collected from 5-30 cm depth where EPNs had been applied (Stock et al. 1999). The collected soil

samples were placed in polyethylene bags and transferred to the laboratory in an ice box (Kaya & Stock 1997). After thorough mixing, the soil samples were placed in 500 mL plastic containers and 6-8 *G. mellonella* larvae were put in the soil in Petri dishes with wire mesh covers (Bedding & Akhurst 1975) (Figure 2). After a four-day period, the larvae were checked and the dead individuals were collected. These cadavers were placed in White traps. The cadavers were checked daily to observe EPN emergence. Soil sampling in the experimental orchard was continued until the end of EPN persistence.

Results and discussion

Entomopathogenic nematode persistency in soil of 2018 and 2019

In 2018, the first EPN application was done on 18 August. Surface soil and air temperature were recorded as 21.2°C and 22°C, respectively. The last EPN's isolated were from soil samples from 17 December. The soil and air temperature on that date were 10.3°C and 9.7°C, respectively. No living EPNs emerged from the soil samples of 17th of January (Table 1).Therefore, EPN persistency in the soil after application was three months. Based on these results, it is thought that the number of EPNs declined after the 1st of January, with soil temperatures falling below 7.9°C.

In 2019, the first EPN application was conducted on 24 July. On that date, the soil and air temperatures were 23.9°C and 23.8°C, respectively. Nematode applications were started 60 days earlier than in 2018. The soil persistency of EPNs was 150 days. The last EPN isolation from the soil samples was on 27 December (Table 1), and the soil and air temperature 6.5°C and 9.9°C, respectively.

Table 1. Native entomopathogenic nematode persistency in soil in a study in a peach orchard in Turkiye in 2018 and 2019

	2018				2019			
Months	**Sf***	**Sc**	**Sa**	**Hb**	**Sf**	**Sc**	**Sa**	**Hb**
January	-	-	-	-	+	+	+	+
February	-	-	-	-	-	-	-	-
March	-	-	-	-	-	-	-	-
April	-	-	-	-	-	-	-	-
May	-	-	-	-	-	-	-	-
June	-	-	-	-	-	-	-	-
July	-	-	-	-	EPNA	EPNA	EPNA	EPNA
August	EPNA**	EPNA	EPNA	EPNA	+	+	+	+
September	+	+	+	+	+	+	+	+
October	+	+	+	+	+	+	+	+
November	+	+	+	+	+	+	+	+
December	+	+	+	+	+	+	+	+
January	-	-	-	-	-	-	-	-

*Sf: *Steinernema feltiae*, Sc*: S. carpocapsae*, Sa: *S. affine*, Hb: *H.bacteriophora*

** EPNA: EPN applied, +: EPN present, -: EPN not present

In a study of the effectiveness of EPNs against *C. tenebrionis* under laboratory conditions, the mortality of *C. tenebrionis* larvae ranged between 50% and 90%, depending on the species and duration of exposure (Şahin & Gözel 2019). In

addition, Garcia del Pino & Morton (2005) reported 95% mortality of *C. tenebrionis* with *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* after 5 days.

In the present study, EPN presence in soil samples was determined with the trap insect method. EPN emergence was observed from infected cadavers of *G. mellonella* larvae. Crimson-colored cadavers caused by the symbiotic bacteria, *Photorhabdus*, revealed the presence of *H. bacteriophora*, and yellow-colored cadavers caused by the symbiotic bacteria, *Xenorhabdus*, revealed the presence of *S. feltiae*, which were photographed under a binocular microscope (Figure 2).

Figure 2. Emergence of infective juvenile nematodes from *Galleria mellonella* (a), *Steinernema feltiae*-infested cadaver (b,c,d) and *Heterorhabditis bacteriophora*-infested cadaver (e, f)

The efficacy of entomopathogenic nematodes under natural conditions applied with different irrigation methods was researched and the mortalities were calculated on the 1st, 3rd, 5th and 7th days of the experiment. The larval mortality of *C. tenebrionis* caused by EPNs was as high as 100% on the fifth day, indicating all the EPN treatments were highly effective (Şahin & Gözel 2021).

Many EPN species are not able to survive in temperatures below 8°C (Griffin 1993; Grewal et al. 1994), to which some *Steinernema* species are an exception, because they are more resistant to lower temperatures. Martinez de Altube et al. (2008) reported that *S. carpocapsae* was able to survive in soil for up to 170 days. In another study, *S. carpocapsae* and *H. bacteriophora* persisted in soil for up to 70 days (Guo et al, 2013). In contrast, Susurluk & Ehlers (2008) reported 22 months of activity in soil under optimum conditions for IJ's of *H. bacteriophora*. Also, Morton & Garcia del Pino (2008) reported shorter periods of soil persistency, namely 2 weeks at the soil surface and 6 weeks at a soil depth of 14-20 cm.

According to Noitubtim et al. (2022), under laboratory conditions, *H. bacteriophora* was able to survive in soil for up to 45 days, with the population decreasing over time.

EPNs can aggregate to form large clumps or stay in an insect cadaver to survive low humidity conditions, which can allow *Heterorhabditis* species to survive for up to 3 weeks (Womersley 1990; Kaya & Gaugler 1993; Glazer 1996; Koppenhöfer et al. 1997). Also, the addition of organic matter can support the persistence of EPNs in soil by providing a more humid environment (Khumalo et al. 2021), which may be the reason for have contributed to the long persistence of EPNs in our study in a peach orchard.

Additionally, other studies have shown the higher infectivity and persistence in soil of EPNs from *in vivo* production than from *in vitro* production (Perez et al. 2003; Shapiro-Ilan et al. 2003). Thus, the *in vivo* production of EPNs in our study may have contributed to the long period of their soil persistency.

Conclusions

In this study in 2018 and 2019, the soil persistency of local Turkish populations of entomopathogenic nematode species was examined for two years under natural conditions in a peach orchard. The soil and air temperatures of the study area were recorded, and the data were evaluated together to enhance the results of the research.

The persistency of EPNs in agricultural soil was determined by soil sampling after EPN application. In the first year, EPNs were isolated from the soil samples up to 90 days post-application, while they were isolated up to 150 days post-application in the second year. The EPNs isolated from the soil samples were re-isolated to test and show their infectivity.

Further studies about the adaptation of EPNs to different ecological conditions and increasing their effectiveness would be beneficial to biocontrol programs. The examination of the effectiveness and persistence of the best adapted strains and isolates of EPNs in different locations in detail will conducted in upcoming studies.

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