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Determination of Entomopathogenic Nematode Persistency with Surface Spraying and Soil Injection Applications in a Peach Orchard

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ABSTRACT

Entomopathogenic nematodes (EPN) are effective biological control agents against underground and cryptic pests. Persistency and survival of EPN in soil after soil application is important for long term success of management programs. In this study, it was aimed to determine the soil persistency of 4 native EPN species after surface spraying and soil injection applications in a peach orchard. In the study, *S. feltiae* (96), *S. carpocapsae* 1133, *H. bacteriophora* 1144 and *S. affine* 47 species were applied in 30 l water with 140.000 IJ/tree dose per tree by surface spraying and soil injection methods. EPN were applied to soil with a watering can in surface spraying method and with a pulverizator into 5-15 cm depth in soil injection method. After the monthly application, soil samples were collected and EPN presence was tested with *G. mellonella* larvae and White traps in the laboratory. The study was conducted for 2 times in 2018 and 2019. At the end of the study, EPN persistency in soil was found to be 90 days in 2018 and 150 days in 2019.

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Spreyleme ve Toprak Enjeksiyonu Yöntemleri ile Toprağa Uygulanan Entomopatojen Nematodların Şeftali Bahçesinde Kalıcılığının Belirlenmesi

ÖZET

Entomopatojen nematodlar (EPN'ler), toprak ve korunaklı habitatlarda yaşayan zararlılara karşı etkili biyolojik mücadele etmenleridir. EPN'lerin toprağa uygulanmasından sonra topraktaki kalıcılıkları ve yaşamlarını sürdürebilmeleri mücadele çalışmalarında uzun süreli başarı için önemlidir. Bu çalışmada 4 yerel EPN türünün spreyleme sulama ve toprak enjeksiyonu yöntemleri ile şeftali bahçesinde uygulanmasından sonra topraktaki kalıcılık sürelerinin belirlenmesi amaçlanmıştır. Çalışmada, *Steinernema feltiae* 96, *S. carpocapsae* 1133, *Heterorhabditis bacteriophora* 1144 ve *S. affine* 47 türleri ağaç başına 30 L su içinde 140.000 IJ/ağaç dozunda spreyleme ve toprak enjeksiyonu yöntemleri ile uygulanmıştır. Spreyleme yönteminde, EPN'ler sulama bidonu ile toprak yüzeyine uygulanırken, toprak enjeksiyonu yönteminde bir pülverizatör ile toprağın 5-15 cm derinliğine uygulanmıştır. Uygulamadan sonra aylık olarak toprak örnekleri alınmış ve laboratuvarda EPN varlığı *G. mellonella* tuzağı yöntemiyle test edilmiştir. Çalışma 2018 ve 2019 yıllarında 2 tekrarlı olarak gerçekleştirilmiştir. Araştırma sonucunda 2018 yılında EPN'lerin toprakta kalıcılıklarının 90 gün, 2019 yılında ise 150 gün sürdüğü belirlenmiştir.

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INTRODUCTION

Entomopathogenic nematodes (EPN) are important biological control agents that can be used to control many agricultural pests. They are especially important as an alternative to chemical control of pests in isolated places like soil or cryptic habitats. Also they can be applied just like insecticides and there are many studies to develop successful EPN preparations (Caamano et al., 2008).

Even though there are approximately 40 nematode families that are associated with insects, only Heterorhabditidae and Steinernematidae families are suitable to use in biological control applications (Gaugler and Kaya, 1990).

When we look at the number of EPN species identified, according to Bhat et al. (2020) from the first identified EPN *Steinernema kraussei* (Steiner, 1923) to *S. riojaense* (Puza et al., 2020), there are 100 steinernematid species. In *Heterorhabditis* genus, after the identification of *Heterorhabditis bacteriophora* (Poinar, 1976) to *H. noenieputensis* (Malan et al., 2014) a total of 16 species were identified.

First heterorhabditid species in Turkey was *H. bacteriophora* from *Aelia rostrata* (Hemiptera: Pentatomidae) by Kepenekçi et al (1999). Other EPN species identified in Turkey are *S. carpocapsae*, *S. feltiae*, *S. affine*, *S. websteri*, *S. anatoliense*, *S. weiseri*, *S. bicornutum*, *S. kraussei, H. marelatus* and *H. megidis* (Kepenekçi and Susurluk, 2000; Kepenekçi, 2002; Hazır et al., 2003; Ünlü et al., 2007; Yılmaz et al., 2009; Ertürk et al., 2014; Gökçe et al., 2015; Canhilal et al., 2016; 2017).

Entomopathogenic nematodes are dependent on their symbiotic bacteria to survive and reproduce. Bacteria not only help the EPN to kill their host, but they also help by killing competing organisms, maintaining a suitable environment for EPN survival, making nutrients from host body digestible for EPN and by directly being food for EPN. In return, bacteria live inside the nematode's body and are protected from environmental elements, also they use the suppression of the host's defenses by EPN to their advantage for reproduction (Akhurst and Boemare, 1990; Forst and Clarke, 2002; Hazır et al., 2003; Stock and Goodrich-Blair, 2008).

EPN enter the host from natural orifices or from wounds on the skin. Also, Heterorhabdits can penetrate the host's skin where it is thinner like the skin between segments by using their dorsal tooth (Bedding and Molyneux, 1982). Bacteria released from EPN guts into the insect haemocoel kill the host insect with septicemia in 24-48 hours. After 2-3 generations of reproduction in the host body, IJ that are more resistant to environmental conditions are generated with the decline of nutrients in the host body. These IJs emerge from the host cadaver and search for new hosts to infect (Kaya and Gaugler, 1993; Koppenhöfer and Gaugler, 2009).

In this study, we aimed to determine the soil persistency of four native EPN species of Turkey, *Steinernema carpocapsae*; *S. feltiae*; *S. affine* (Rhabditida: Steinernematidae), and *H. bacteriophora* (Rhabditida: Heterorhabditidae) in a peach orchard after surface spraying and soil injection application methods.

MATERIALS and METHODS

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) in Lapseki district in Çanakkale province. The orchard consist of Black Hale, Royal Glory and Abdos cultivars. Before choosing the orchard for the study soil samples were taken to eliminate the chance of naturally occurring EPN species in the area (Bedding and Akhurst 1975; Griffin et al. 2000).

Production of Entomopathogenic nematodes

Entomopathogenic nematodes were produced in vivo on mature *G. mellonella* larvae in the laboratory. The species used in the experiment were *Steinernema feltiae* 96 (Bursa), *S. carpocapsae* 1133 (Sakarya), *H. bacteriophora* 1144 (Sakarya) and *S. affine* 47 (İstanbul), which were prepared in the dose of 140.000 IJ/tree in a 50 cc falcon tubes in distilled water before the experiment. Then they were transferred to the experiment orchard in an ice box. The experiments were designed with 3 replications in randomized block design, with the blocks consisting of tree lines. The study was conducted for two years in 2018 and 2019.

Soil Injection Application

In soil injection application, EPN were applied with a 15 L capacity manual pulverizator directly into 5-15 cm depth of soil (Figure 1a, b) by penetrating the soil surface with the tip of the pulverizator. Before the application in the orchard, the ability of EPN to pass through the nozzle of the pulverizator was tested in the laboratory and the EPN were confirmed to be alive under the microscope. Using this pulverizator, 140.000 IJ/tree dose was applied with 30 l of water into the soil around each tree.

Surface Application with Watering Can

In surface application, EPN were applied to the soil with a watering can. Before the application the ability of EPN to pass through the holes of the watering can was tested in the laboratory and the EPN were confirmed to be alive under the microscope. The watering can had a capacity of 10 l and 140.000 IJ/tree dose was applied with 30 l of water per each tree (Figure 1c, d).

Determination of EPN Persistency in Soil in Natural Conditions

Soil sampling was conducted monthly around the trees to determine the persistency of EPN in soil. Soil samples were collected from 5-30 depth where EPN were applied around the trees (Stock et al., 1999). Soil samples were placed into polyethylene bags and transferred to the laboratory in an ice box (Kaya and Stock, 1997). After thoroughly mixing the samples, they were placed into 500 ml volume plastic boxes and 6-8 *G. mellonella* larvae were also put into the soil in petri dishes with wire mesh lids (Bedding and Akhurst, 1975). After four days, larvae were checked to collect the dead ones and the cadavers were placed on White traps. These cadavers were checked daily to observe EPN emergence. Soil sampling in the experimental orchard was continued until EPN persistence in soil has ended.

Şekil 1. Toprak Enjeksiyonu (a, b), Yüzey Sulama Uygulaması (c, d)
Figure 1. Soil injection (a, b), Surface Spraying Application (c, d)

RESULTS and DISCUSSION

In 2018, first EPN application was done on 18th of September. On this date, soil and air temperature were recorded as 21.2°C and 22°C, respectively. EPN persistency in soil after the application was determined as 3 months. Last EPN isolated from the soil samples were on 17th of December. The soil and air temperature on this date were recorded as 10.3°C and 9.7°C, respectively. With this data in mind, it is thought that the number of EPN declined after the 1st of January with soil temperatures falling below 7.9°C. From the soil sample of 17th of January, no living EPN has emerged.

In 2019, the second year of the study, first EPN application was conducted on 24th of July. On this date, soil and air temperature were recorded as

23.9°C and 23.8°C, respectively. Applications have started 60 days before the previous year. Soil persistency of EPN was found to be 150 days. Last EPN isolation from the soil samples was on 27th of December and the soil and air temperature on this date was recorded as 6.5°C and 9.9°C, respectively.

According to several studies, *Steinernema feltiae* is species that is adapted to colder climates and can infect its host between 8-28°C temperatures, while it can reproduce between 8-25°C temperatures (Hazır et al., 2001; Grewal et al., 1996; Umana, 2014). According to Griffin (1993) and Grewal et al. (1994), many EPN species cannot survive temperatures under 8°C. Similar to these studies, in this results confirm that EPN presence and persistency in soil is closely related to climatic conditions. Insects from different orders (Diptera, Orthoptera, Coleoptera) were observed in soil during soil samplings, which may be used as hosts by EPN. This host presence is also known to be closely linked to EPN persistency and survival in soil.

When we look at some studies about EPN persistency in natural conditions, Martinez de Altube et al., (2008) reported that *S. carpocapsae* can live up to 170 days in soil. In another study, Guo et al. (2013) have determined a 70-day persistency from *S. carpocapsae* and *H. bacteriophora*. Under suitable conditions, IJs of *H. bacteriophora* were active for 22 months in soil (Susurluk and Ehlers, 2008). However, Morton and Garcia Del Pino (2008) have reported a much shorter period of persistency, such as 2 weeks on soil surface and 6 weeks in 14-20 cm depth for *H. bacteriophora*. In addition, some studies have reported higher infectivity and persistency in soil from EPN produced in vivo than EPN produced in vitro (Perez et al., 2003; Shapiro-Ilan et al., 2003). Thus, we think that the reason for high EPN persistency in this study may be because of they are produced in vivo.

Ishibashi and Kondo (1986) have examined the persistency of *S. feltiae* and *S. glaseri* in sandy soil and bark compost. At the end of the study, they have determined that the longest persistency was in sterilized soil and compost with 8 weeks. According to their results, the longer persistency in sterilized material was caused by the competition between EPN and other microorganisms in unsterilized materials. Same researchers have also found the persistency of *S. feltiae* in soil as 14 weeks (Ishibashi and Kondo, 1987). In the same study they also reported that EPN infectivity is higher in sandy soil than clay soil, but persistency in clay soil is higher than sandy soil because of the higher water holding capacity of the former. Thus, we think that the high persistency of EPN in the experimental orchard may also be caused by the high-water holding capacity of the soil's loamy clay texture.

CONCLUSIONS

It is predicted that EPN will increase their share on the market for biological control agents because of their high adaptation, effectiveness against multiple hosts, fast host finding ability, mass production in artificial mediums, high reproduction capacity and high potential for the control of agricultural pests, in addition to being harmless to non-target organisms. Thus, it is important to increase the number of studies on EPN to devise better application programs.

In this study, we determined the soil persistency of four native EPN species as 90 days in 2018 and 150 days in 2019. EPN reisolated from the soil samples were tested for their infectivity and found to be still infective.

This study is one of the few studies in Turkey to determine the persistency and reestablishment of native EPN species into the soil. We think that it is important to focus on adaptation to different ecological conditions and increasing the effectiveness of EPN against pests.

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Declaration of researcher contribution

We declare that all researchers have equal amount of contribution

Declaration of Conflict of interest

We declare no conflict of interest

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