Türk. Biyo. Mücadele Derg. 2019, 10 (2):118-126
DOI: 10.31019/tbmd.571191 ISSN 2146-0035-E-ISSN 2548-1002

***Orijinal araştırma* (*Original article*)**

The Efficacy of Native Entomopathogenic Nematodes against the Pine Processionary Moth, *Thaumetopoea pityocampa* Den. & Schiff. (Lepidoptera: Thaumetopoeidae)

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Yerel Entomopatojen Nematodların Çam Kese Böceği *Thaumetopoea pityocampa* Den. & Schiff. (Lepidoptera: Thaumetopoeidae)'ya Karşı Etkinliği

Abstract: The pine processionary moth, *Thaumetopoea pityocampa* Den. & Schiff. (Lepidoptera: Thaumetopoeidae), is one of the most important pests of forest trees. In this study, the efficacy of native entomopathogenic nematodes (EPNs) against the larvae of *T. pityocampa* was investigated under laboratory conditions. Isolates of *Steinernema carpocapsae* Weiser, *S. feltiae* Filipjev (Rhabditida: Steinernematidae), *Heterorhabditis bacteriophora* (91) Poinar and *H. bacteriophora* (200) (Rhabditida: Heterorhabditidae) from İstanbul, Sakarya and Çanakkale Provinces of Turkey were used in the study. *Thaumetopoea pityocampa* larvae were collected from infested trees on the campus of Canakkale Onsekiz Mart University in Canakkale Province. The efficacy assays were conducted with 1 larva and 200 infective juveniles (IJs) nematodes in a plastic petri dish at 10, 15 and 25 ºC. The larvae were checked daily for 7 days after inoculation with the IJs and the mortalities were recorded. No mortality of *T. pityocampa* larvae was observed on the first day. Depending on the nematode species and the temperature, larval mortality ranged from 60% to 100%. Based on the results of this study, there is considerable potential for the use of EPNs for the biological control of *T. pityocampa*.

Key words: Pine processionary moth, larva, entomopathogenic nematode, efficacy

Özet: Çam kese böceği, *Thaumetopoea pityocampa* Den. & Schiff. (Lepidoptera: Thaumetopoeidae) orman ağaçlarının en önemli zararlılarından biridir. Bu çalışmada yerel entomopatojen nematodların (EPN) *T. pityocampa* larvalarına karşı etkinlikleri laboratuvar koşullarında araştırılmıştır. Çalışmada *Steinernema carpocapsae* Weiser, *S. feltiae* Filipjev (Rhabditida: Steinernematidae), *Heterorhabditis bacteriophora* (91) Poinar ve *H. bacteriophora* (200) (Rhabditida: Heterorhabditidae) izolatları kullanılmıştır. EPN'ler İstanbul, Sakarya ve Çanakkale illerinden, *T. pityocampa* larvaları ise Çanakkale Onsekiz Mart Üniversitesi kampüs alanındaki zarar görmüş çam ağaçlarından elde edilmiştir. Etkinlik denemeleri plastik petrilerde 10, 15 ve 25 ºC'de, 200 IJs/*T. pityocampa* larva yoğunluğunda yürütülmüştür. Petrilerdeki larvalar nematod inokulasyonundan sonraki 7 gün boyunca

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Alınış (Received): 28.05.2019 Kabul ediliş (Accepted): 15.11.2019

kontrol edilmiş ve ölüm oranları kaydedilmiştir. İlk gün *T. pityocampa* larvalarında ölüm görülmemiştir. Nematod türüne ve sıcaklığa bağlı olarak larvalardaki ölüm oranı %60-100 oranında değişmiştir. Çalışmadan elde edilen sonuçlara göre, *T. pityocampa*'nın biyolojik mücadelesinde EPN'lerin önemli bir kullanım potansiyeli vardır.

Anahtar sözcükler: Çam kese böceği, larva, entomopatojen nematod, etkinlik

Introduction

Forests provide many benefits such as producing oxygen and absorbing carbon dioxide. For example, a mature tree can produce a day's supply of oxygen for anywhere from two to ten persons. Not only do trees provide fruits, nuts and seeds but they also support a wealth of biodiversity on the forest floor. In addition, timber is used to make everything from paper and houses to furniture and clothing. Forests also provide many natural medicines and increasingly inspire synthetic spin-offs (Anonymous, 2019).

Unfortunately, improper exploitation and fires cause these valuable forest resources to diminish day by day. In addition, pests feeding on forest trees also cause major damage. The pine processionary moth, *Thaumetopoea pityocampa* (Den. and Schiff.) (Lepidoptera: Thaumetopoeidae), is one of the most destructive species of pines and cedars in Central Asia, North Africa, Southern Europe and Turkey (Kerdelhué et al, 2009). *Thaumetopoea pityocampa* prefers to consume Calabrian pine (*Pinus brutia* Ten.), Black pine (*P. nigra* Arnold.), Aleppo pine (*P. halepensis* Mill.) and Scotch pine (*P. sylvestris* L.) foliage in Turkey (Ozkazanc, 2002). The defoliation they inflict and nests they build in trees cause aesthetic problems, in addition to substantial environmental damage and economic losses to *Pinus* spp. Furthermore, the urticating hairs of the last instar larvae cause harmful reactions in humans and other mammals (Démolin et al, 1996).

Many natural enemies, such as ants (Way et al, 1999), Tettigonidae (Ledesma, 1971; Gonzalez-Cano, 1981), parasitoids (Tarasco, 1995; Zamoum et al, 2007), entomopathogenic fungi (Akinci et al, 2017), entomopathogenic nematodes (EPNs) (Triggiani & Tarasco, 2002) and insectivorous birds (Jarry, 1994; Battisti et al, 2000; Barbaro et al, 2008), have the potential to restrict the numbers of *T. pityocampa* in pine forests. EPNs, which belong to the families Steinernematidae and Heterorhabditidae, are widely distributed in soils worldwide (Hominick, 2002; Adams et al, 2006).

The use of EPNs in biological control has many advantages; they are relatively easy and inexpensive to mass produce in vivo and in vitro, live for periods ranging from several weeks up to months, and are capable of infecting a broad range of pests by carrying species-specific symbiotic bacteria, namely the genera *Xenorhabdus* for *Steinernema* and *Photorhabdus* for *Heterorhabditis*, which can kill the host within 48 h (Griffin et al, 1990; Kaya, 1990; Kaya & Gaugler, 1993; Ehlers, 1996; Shapiro-Ilan & Gaugler, 2002; Gozel & Gozel, 2013; Gozel & Kasap, 2015). The effects of EPNs and these bacteria are not detrimental to non-target organisms. Also, masks and other safety equipment are not needed for the application of EPNs, unlike chemicals (Poinar et al, 1982; Boemare et al, 1996; Brown & Gaugler, 1997; Akhurst & Smith, 2002).

The current study is one of the few studies ever conducted on the potential for the biological control of *T. pityocampa* with EPNs, and is the first study of its type in Turkey.

Materials and Methods

Sources of entomopathogenic nematodes

Four Turkish EPN isolates, *Heterorhabditis bacteriophora* (91), *H. bacteriophora* (200), *Steinernema carpocapsae* 1133 and *S. feltiae* 47, were used in the study. These EPNs were collected from İstanbul, Sakarya and Çanakkale Provinces and mass produced by using the last instar larvae of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). A new *G. mellonella* colony was prepared for this study and larvae were obtained from it. During the study, 2-3 days old infective juveniles (IJs) were used in the assays.

Source of *Thaumetopoea pityocampa*

Thaumetopoea pityocampa nests containing larvae were collected from infested pine trees on the campus of Çanakkale Onsekiz Mart University in 2015. Last instar larvae of *T. pityocampa* were taken from these nests and housed in plastic boxes.

Inoculation of the entomopathogenic nematodes

Infective juveniles of the four EPNs were counted and prepared under a stereomicroscope for inoculation of the *T. pityocampa* larvae. A Whatmann filter paper was placed on the bottom of each Petri dish (60 x 15 mm) and one last instar *T. pityocampa* larva was placed on the paper. The efficacy assays were conducted at 10, 15, 25 ºC and 65% RH under dark conditions in Nüve EN 120 incubators with 200 IJs/larva (Kalia et al, 2014). The IJs were placed directly on the larva. For each temperature used in the study, 20 last instar *T. pityocampa* larvae were used. Each treatment was replicated 20 times. The same number of *T. pityocampa* larvae were used in the control Petri dishes but only distilled water was added.

Mortality of *Thaumetopoea pityocampa* larvae after EPN inoculation

The percentage mortality of *T. pityocampa* larvae was recorded every day for 7 days after they were inoculated with the EPNs. To determine whether the dead larvae were infected by EPNs, the larvae were dissected with forceps and a dissecting needle. All of the dead larvae were infested with EPNs and emerging EPNs were observed.

Results and Discussion

The efficacy of four EPN isolates against the larvae of the foliar feeding forest pest *T. pityocampa* was determined as the percentage mortality at three different temperatures under laboratory conditions. In the assays, for all the EPN isolates, the mortality was 100%, except at 10 ºC. The lowest and highest mortalities were 60% and 80%, respectively, at 10 ºC, which was the lowest temperature used, and there was 100% mortality at both 15 ºC and 25 ºC for all isolates. No mortality of *T. pityocampa* larvae was observed on the first day after inoculation for all the isolates. Mortality occurred on the second day and it increased daily but varied with the temperature. No larval mortality occurred in the control Petri dishes at all temperatures.

Figures 1, 2 and 3 show the EPN isolates and time (as days) effects at different temperatures. At 10 ºC, the effects of time (F=109,39; p<0.05) and EPN isolate (F=5,02; p<0.05) were significant. At 15 ºC, the effects of time (F=134,23; p<0.05) and EPN isolate (F=4,95; p<0.05) were again significant. At 25 ºC, the effect of time was significant (F=262,77; p<0.05) but EPN isolate was not significant (F=1,30; p>0.05).

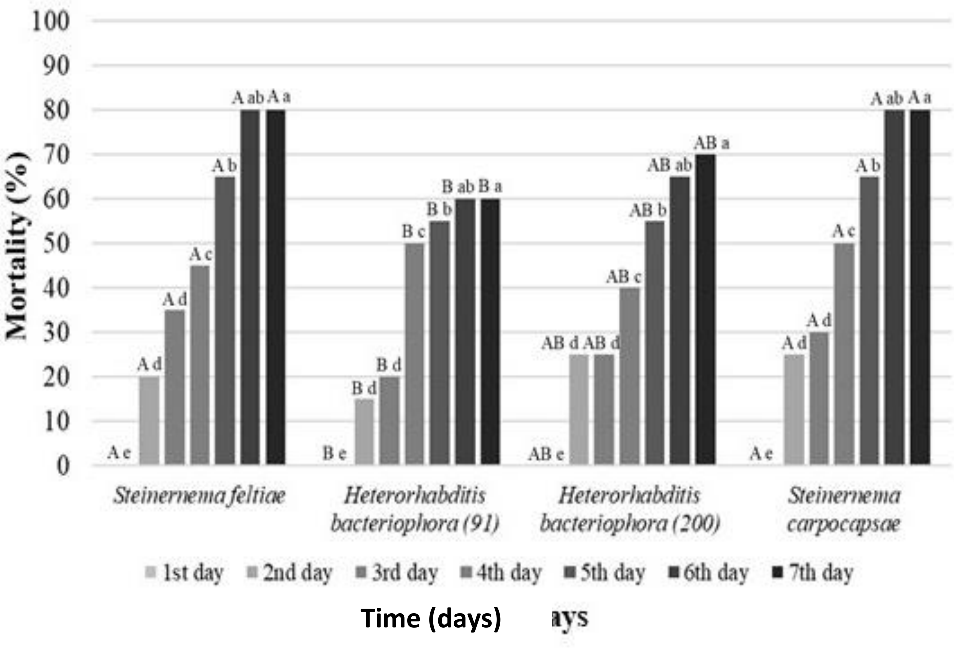

Figure 1. Mortality of *Thaumetopoea pityocampa* larvae caused by the entomopathogenic nematodes at 10 ºC

The efficacy of EPNs can be affected by the location from which they were isolated. For example, *Steinernema bicornutum* from the former republic of Yugoslavia was more effective at low temperatures but *H. indica* LN2 from a tropical region in India was more effective at high temperatures (Griffin, 1993; Glazer, 2002). In the present study, all four of the EPN isolates from Turkey required a relatively high temperature to infect their hosts. The relationship between the optimal temperature for EPN isolates to infect hosts and their geographic origins was also reported by Ulu & Susurluk (2014).

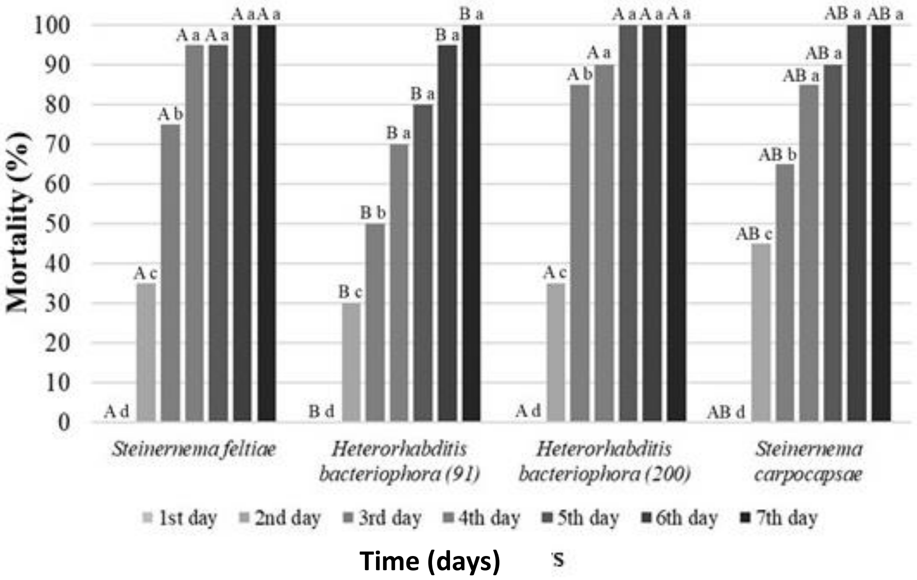

Figure 2. Mortality of *Thaumetopoea pityocampa* larvae caused by the entomopathogenic nematodes at 15 ºC.

Triggiani & Tarasco (2000) conducted a preliminary survey with 3 different strains of Steinernematid nematodes in the nests of pine processionary caterpillars at a *P. halepensis* reforestation site in the Apulia region of southern Italy. A suspension containing 300,000 IJs/20 ml of gel (Idrosorb SR 2002-Nigem®) was inserted in each nest and mortality was recorded every 10 days for 30 days. The application of *S. feltiae* was effective in reducing the overwintering larval populations of *T. pityocampa* and it was also demonstrated that IJs can live in nests for more than 20 days. In addition, there were very low effects on *Phryxe caudata* Rond. (Diptera: Tachinidae), a parasitoid of *T. pityocampa*.

Triggiani & Tarasco (2002) carried out a 3 year study at the same *P. halepensis* reforestation site in southern Italy. They injected IJs of *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* in aqueous and gel suspensions (Idrosorb SR 2002 and Compex, respectively) into the nests of *T. pityocampa* caterpillars. They demonstrated that the gel suspensions did not percolate and that the slow release of water from the gels helped the nematodes to survive and complete their life cycle in the host. In addition, the gel suspension of *S. feltiae* was able to reduce the number of overwintering larvae and there were no negative effects on the endoparasite, *P. caudata*.

Furthermore, in January 2001, gel suspensions of *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* were inoculated into the nests of *T. pityocampa* to determine their effects on overwintering larval populations. The highest efficacy was obtained with *S. feltiae* which reduced the winter populations of the pest by more than 50%. Moreover, the EPN completed its life cycle and reproduced in the cadavers of *T. pityocampa* (Triggiani & Tarasco, 2003).

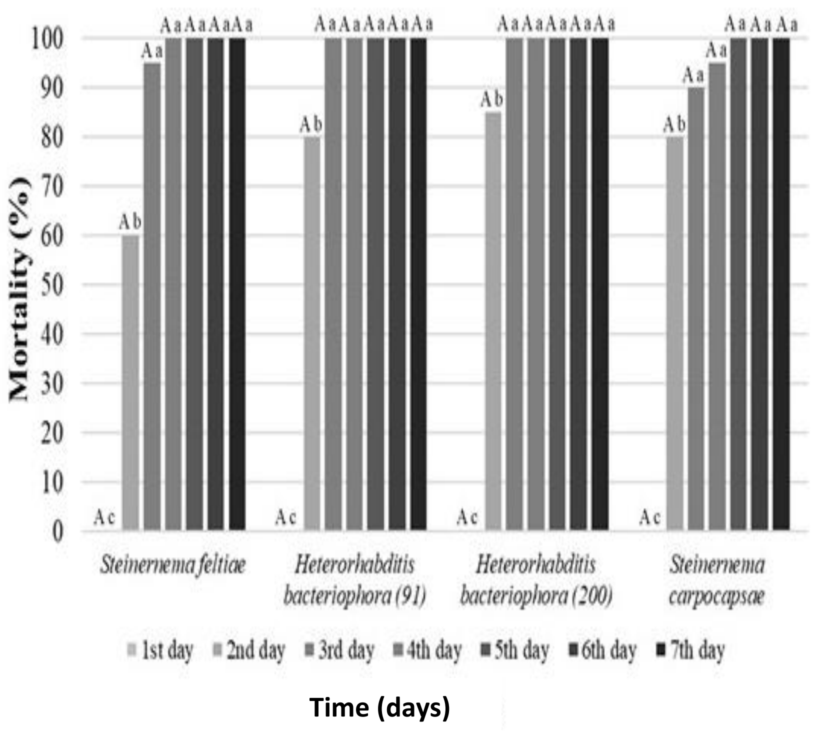

Figure 3. Mortality of *Thaumetopoea pityocampa* larvae caused by the entomopathogenic nematodes at 25 ºC.

The recent development of more effective application techniques and tools for EPNs promises to improve the efficacy of nematodes against pests (Laznik et al, 2010) but it is important to match the right nematode species to the right pest; poor host suitability has been the most common mistake in the application of EPNs (Kaya & Gaugler, 1993; Gaugler, 1999). Application method and correct matching of the EPN to the pest are the main factors determining the chances of success, particularly for pests that are difficult to control, such as *T. pityocampa*.

The native, Turkish EPNs from different regions that were used in the current laboratory study were able to infect the larvae of *T. pityocampa* and cause high mortality. The Italian studies mentioned earlier demonstrated that the insertion of EPNs in gel formulations into the nests of *T. pityocampa* facilitated infection of the larvae under natural conditions. In conclusion, the positive results from the present study further suggest that EPNs have considerable potential for use as biological control agents for the management of *T. pityocampa*, a serious, widespread forest pest.

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