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Biological Control Potential of *Steinernema carpocapsae* (Steinernematidae) on *Monochamus galloprovincialis* (Cerambycidae) Populations in Pine Logs

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Abstract

Aim of study: Pine wilt disease (PWD) is a serious threat to the susceptible pine forests. It is caused by *Bursaphelenchus xylophilus* (Nematoda: Parasitaphelenchidae) (Steiner and Buhrer 1934), Nickle 1970 and transmitted by *Monochamus* Dejean beetles. In the recent study, we assessed the effects of entomopathogenic nematode, *Steinernema carpocapsae* (Nematoda: Steinernematidae) against *Monochamus galloprovincialis* larvae in Anatolian black pine and Scots pine logs.

Area of the study: The experiments were conducted in Duzce University, Faculty of Forestry and in a pine forest at Duzce University campus area.

Material and methods: The mean number of eggs per pine logs, and the productivity of *S. carpocapsae* in *M. galloprovincialis* larvae were compared under laboratory conditions. The nematode experiments were conducted using oviposited pine logs in the field.

Main results: The females of *M. galloprovincialis* oviposited more eggs on Scots pine compared to black pine logs. Both in black pine and in Scots pine, the survival rates of *M. galloprovincialis* after nematode application was significantly lower than control.

Highlihts: As a result of the study, *S. carpocapsae* can be an efficient biological control agent of this wood-boring insect.

Keywords: Entomopathogenic Nematode, *Steinernema*, *Monochamus*, Pine Wilt Disease

Çam Kütüklerindeki *Monochamus galloprovincialis* (Cerambycidae) Populasyonlarına Karşı *Steinernema carpocapsae* (Steinernematidae)'nin Biyolojik Mücadelede Kullanılması

Öz

Çalışmanın amacı: Çam kuruma hastalığı hassas çam ormanları için çok ciddi bir tehditdir. Bu hastalığın sebebi *Monochamus* Dejean cinsi böcekler tarafından taşınan *Bursaphelenchus xylophilus* (Nematoda: Parasitaphelenchidae) (Steiner and Buhrer 1934), Nickle 1970 türü nematodlardır. Bu çalışmada Anadolu karaçamı ve sarıçamında bulunan *Monochamus galloprovincialis* larvalarına karşı entomopatojenik nematod, *Steinernema carpocapsae* (Nematoda: Steinernematidae)'nin etkinliği test edilmiştir.

Çalışma alanı: Deneyler Düzce Üniversitesi Orman Fakültesi ve Düzce Üniversitesi kampüs alanı içerisindeki çam ormanında yürütülmüştür.

Materyal ve Yöntem: Laboratuar deneylerinde dişi böceklerin her iki çam türünün kütüklerine bıraktıkları ortalama yumurta sayıları ve M. galloprovincialis larvalarında *S. carpocapsae*'nin üreme kapasitesi çalışılmıştır. Nematodların etkinlik deneyleri ise tamemen arazi koşullarında yapılmıştır.

Temel sonuçlar: M. galloprovincialis dişileri yumurtlamak için sarı çam kütüklerini kara çam kütüklerine göre daha fazla tercih etmektedir. Orman zeminine bırakılan kütüklere nematod uygulandıktan sonra hem karaçamda hem de sarıçamda böcek çıkış oranı kontrol grubuna göre istatistiksel olarak daha az olmuştur.

Araştırma vurguları: Bu çalışma sonucunda *S. carpocapsae*'nin odun delici böceklere karşı etkili bir biyolojik mücadele etmeni olarak kullanılabileceği düşünülmektedir.

Anahtar Kelimeler: Entomopatojenik Nematod, *Steinernema*, *Monochamus*, Çam Kuruma Hastalığı

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Introduction

The pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934), Nickle (1970) is a serious pest that causes Pine Wilt Disease (PWD) in susceptible pine forests. This nematode is vectored by wood-boring *Monochamus* spp. beetles (Coleoptera: Cerambycidae) among susceptible *Pinus* spp. trees (Linit, 1988) during host feeding. The infective stage of the nematodes is transferred from the insect's respiratory system to young shoots of pine trees where they feed on parenchymal and epithelial cells in resin canals; here the nematodes cause tracheid cavitation and disrupt water transportation. In return, this damage caused by nematodes creates new oviposition and proliferation sites for the beetle on weakened or stressed plants (Baker, 1972; Futai, 2013; Kobayashi et al., 1984; Linit, 1988). The trees usually wilt or die within two to three months (Futai, 2013; Linsley, 1959).

Currently, there are five *Monochamus* species in Europe (Akbulut & Stamps, 2012; Schenk et al., 2020) and *M. galloprovincialis* (Olivier) is one of the most important species (Sousa et al., 2001; Inácio et al., 2014). It is widely distributed in *Pinus nigra* J. F. Arnold subsp. *pallasiana* (Anatolian black pine) and *Pinus sylvestris* L. (Scots pine) forested areas in Turkiye (Akbulut et al., 2008, Akbulut & Stamps, 2012). So far PWN has not been detected in Turkiye (Dayı & Akbulut, 2018). However, it was reported from Portugal, Madeira island and Spain in Europe (Vicente et al., 2012). Several studies have indicated that *P. sylvestris* and *P. nigra* subsp. *pallasiana* should be monitored for the possible interaction between *M. galloprovincialis* and PWN in Turkiye (Akbulut et al., 2007; Akbulut et al., 2008).

The management of *Monochamus* beetle population density is critical to control PWD, however, it is difficult to control these wood-boring insects. Chemical pesticides cannot reach the cryptic life stages within the wood; hence their use is economically unfeasible (Gumus et al., 2015). The adults of *M. galloprovincialis* have a kairomonal attraction to host tree volatiles and bark beetle pheromones, therefore these are important for monitoring or mass trapping of adult populations (Ibeas et al., 2007; Pajeras et al., 2004). Biological control with efficient natural enemies is a good option to control *Monochamus* spp. (Naves et al., 2005) as the efficacy of several biological agents such as the entomopathogenic fungi, *Metarhizium anisopliae* (Xu et al., 2015) and *Beauveria bassiana*, parasitic wasp, *Cynopterus flavator* (Naves et al., 2008) and *Scleroderma guani* (Li et al., 2015; Xu et al., 2015); entomopathogenic nematodes (EPNs) (Bin Yu et al., 2016; Yamanaka, 1993) on various larval stages of *Monochamus* spp. have been reported.

EPNs in the families Steinernematidae and Heterorhabditidae are obligate insect-pathogenic organisms that are in a mutualistic relationship with *Xenorhabdus* spp. (for steinernematidae) and *Photorhabdus* spp. (for heterorhabditidae) bacteria (Boemare, 2002; Hazir et al., 2022). These nematodes occur naturally in soil environments where they can kill a broad range of insects. Several species are mass produced using *in vitro* and *in vivo* methods and without registration are used in pest management programs as biopesticides (Lacey & Georgis 2012; Hazir et al., 2022). EPNs can either actively seek out or ambush insect hosts in soil and in cryptic habitats. The ability to seek out hosts makes certain EPN species (*S. carpocapsae* and *S. feltiae*) promising bioagents against various pests in cryptic habitats such as wood-boring Cerambycids (Solter et al., 2001; Fallon et al., 2004, Fallon et al., 2006; Bin Yu et al., 2016). Bin Yu et al., (2016) and Yamanaka (1993) reported that *S. carpocapsae* can successfully infect *M. alternatus* larvae in pine logs. Fallon et al. (2004) and Solter et al. (2001) observed that *S. carpocapsae*, *S. feltiae* and *H. marelatus* were quite effective against a different wood-boring Cerambycid, such as *Anoplophora glabripennis*. Gumus et al. (2015) reported that *Coccus coccus* larvae were highly susceptible to *S. carpocapsae* emerging from infected cadavers in the galleries of chestnut wood.

The objective of this study was to evaluate the infective potential of *Steinernema carpocapsae* on larval pop ulations of *M. galloprovincialis* found in the

Anatolian black pine and Scots pine logs in the field conditions.

Materials and Methods

Insect Cultures

A laboratory colony of *Monochamus galloprovincialis* was established from beetle-infested Anatolian black pine and Scots pine. These trees were acquired from Düzce Forest District. Newly emerged adults were kept in small cages individually for maturation feeding for ~7-10 days and then were transferred to new cages (68×60×60 cm) for oviposition. Each oviposition cage had ~20 beetles of each sex and fresh pine twigs were provided for feeding. After every 2–3 weeks healthy Anatolian black pine and Scots pine (25–30 years old) trees were felled and the boles below the live crown were cut into logs (45–55 cm long and 10–16 cm diam). The ends of these logs were waxed with hot liquid paraffin to retard desiccation (Akbulut et al., 2008). The logs were stored in the laboratory for 5–15 days (70–80% RH, 24–26°C) before oviposition. One experimental log was placed in each oviposition cage and removed when it had sufficent number of oviposition scars, usually occurring within 24–48 h. The number of oviposition scars were between the range of 21-37 for black pine and 34-64 for Scots pine respectively. The number of oviposition scars on each log was recorded before the logs were transferred to their individual PVC containers (65 cm long, 20–30 cm diam). The logs were held at 24–26°C, 70–80% R.H., and a photoperiod of 14:10 h (L:D) for beetle development (Akbulut et al., 2007, Akbulut et al., 2008). After one month from the oviposition date, logs with frass produced by the larvae during feeding were considered as evidence of the colonization success. These logs with frass (0.2-0.3 mm length) were used in the experiments.

Entomopathogenic Nematodes

Native entomopathogenic nematodes isolated from a hazelnut orchard in Gölyaka, Düzce and identified as *S. carpocapsae* (Duz-14) (Gulcu, 2018) were used in this experiment. The nematodes were reared on *Galleria mellonella* (Lepidoptera: Pyralidae) larvae in the laboratory (Kaya & Stock, 1997; Hazir et al., 2022). The infective juveniles (IJs) harvested from white traps (White, 1927) were stored in 1000 mL tetra pak containers (Gulcu & Hazir, 2012) at 13°C for <2 weeks before use. Nematode viability was >95% in all experiments.

Laboratory Experiments

In the laboratory, the productivity of *S. carpocapsae* in *M. gallopovincialis* was evaluated with larvae and adults at room temperature. For larvae, petri dishes (90 mm diam.) were filled with 5 g of pine frass. Fifty *S. carpocapsae* IJ/cm^2 were applied in distilled water to each plate to obtain a final frass moisture level of 20% (w/v). One *M. galloprovincialis* larva was added to each petri dish after IJ application. The total number of larvae used in petri assays was 10 for black pine and 11 for Scots pine. The Petri dishes were placed into plastic bags to minimize moisture loss and kept at room temperature. For adults, plastic containers (20×14×8 cm) were filled with a 200 g mixture of sterilized soil, pine frass, and pine twigs. The nematode suspension at a rate of 100 IJ/cm^2 was poured on the mixture and final moisture was 20% (w/v). Eleven plastic containers were used in the assay. The plates and the petri dishes were checked daily for 10 days and all cadavers were transferred individually to white traps (White, 1927) to confirm EPN infection and number of IJs emerging over 2 weeks. The number of IJ produced per g in the cadavers is described as productivity. The IJs were counted under a microscope and productivity was calculated as the number of IJ per insect.

Field Experiments

The infectivity of *S. carpocapsae* was tested both black pine and scots pine in field conditions. The control was black pine and scots pine without nematodes. Field experiments were conducted in black pines and Scots pines plantation areas in the Düzce University campus. So experiments were planned according to the life cycle of *M. galloprovincialis* populations (Time of adult emergence, oviposition behaviour etc.) in the natural conditions. The nematode applications were made between late April to early June in 2020. The forest floor of a

natural pine forest was simulated in the experimental area by making a bed (mixture of pine needles, twigs, and branches) that has 5 cm thickness over the ground before placing the logs. A steel net (2 mm mesh size) was used to cover the logs and prevent the escape of emerged adults. The logs infested with *M. galloprovincialis* were placed parallel to each other on the beds (Figure 1). Subsequently, one liter of distilled water was poured onto the surface of each log before nematode application. Then nematodes in another one liter water were applied at the rate of 450 IJs/cm^2 (approx. 1 million IJ per log) to the surface of pine logs using a 2 liter watering pot. Three logs were used per treatment and per control from each pine species. The experiments repeated three times. Three nematode applications were made every 15 days in the field. Weather conditions were rainy and the temperature was between 12-20℃ on the following days (approximately 10 days) after applications. Each log was monitored for adult emergence for four months since the oviposition date. At the end of the 4-month period, the bark of the logs was removed carefully using a knife and the logs were chopped with an axe to determine the number of dead or alive insects.

Statistical Analysis

The independent sample t-test was performed for the number of eggs per pine logs, the nematode productivity per pine species and the number of survived *M. galloprovincialis* for each pine species in the experiments (SPSS 22.0).

Results and Discussion

We clarified whether eggs or scars due to frass on the logs after a while. Female beetles made significantly more oviposit eggs on the logs of Scots pine than black pine (t= 14.977; df= 16; $P<0.05$). The mean number of eggs on black pine and Scots pine were 5 and 14, respectively (Figure 2).

We evaluated nematode productivity per gram using larvae and adults that obtained from control logs. The mean weight of larva from black pine and scots pine were 0,551g and 0,293g respectively. A significant difference was detected between weight of larva from black pine and scots pine (t= 3.226; df= 19; $P<0.05$). As of nematode productivity, the mean number of emerging IJs per *M. galloprovincialis* larvae were 124.300 IJs/g for black pine and 107.500 IJs/g for Scots pines (Table 1). However no significant difference was found between black pine and scots pine for nematode productivity (t=0.675; df= 11,991; $P=0.513$). The mean number of IJs per adults were 37.438 IJs/g.

The survival rates of *M. galloprovincialis* larvae in the controls were significantly higher than nematode treatment in both pine species. In Scots pine, survival rates of *Monochamus* beetles were 83.73% and 23.82% for control and nematode applied pine logs, respectively (t= 13.144; df= 16; $P<0.05$) (Figure 3). In black pine, the survival rate of *Monochamus* beetle was 81.89% in control. Whereas, there was not any alive beetle in nematode applied pine logs (t= 16.438; df= 8; $P<0.05$) (Figure 4).

Steinernema carpocapsae has shown promise as an effective EPN species for the biocontrol of several cryptic pests. As a first, this study evaluated the control potency of *S. carpocapsae* against *M. galloprovincialis* under field conditions. The data showed that this species seems efficient in parasitizing beetle larvae in the field. The IJ of this species, which usually utilizes an ambushing strategy (Griffin, 2015), can act as a cruiser to find larvae inside tunnels. This nematode was effective even at temperatures ≤13 °C on *M. alternatus* (Yamanaka, 1993). The weather conditions in our study were rainy and the temperature was between 12-20 °C; moreover the moisture level especially under the bark might have supported IJ activity and thus enhanced IJ efficacy (Kung et al., 1991; Koppenhöfer et al., 1995). Shapiro-Ilan et al. (2015) stated that ambushers are more tolerant to UV and desiccation than cruisers in aboveground applications.

Our laboratory culture was established using *M. galloprovincialis* infested logs brought from pine forest between August to September 2019. Number of larvae that obtained from each pine species were quite limited. Akbulut et al. (2008) mentioned that the female population of *M. galloprovincialis* that emerge in summer and autumn are less

fecundity compared to spring population. Moreover several factors such as seasonal changes in host plant quality (Leather, 1995), nutritional quality and moisture content of logs (Akbulut & Linit, 1999; Akbulut et al., 2008) also may impact female fecundity and immature development. This may explain the low fecundity of insect culture and obtaining the limited number of larva in the current study. Beetle females oviposited more in Scots pine logs than black pine in our study.

Figure 1. Preparing the beds (on the left), experimental design in the field (on the right)

Table 1. Nematode productivity in *Monochamus galloprovincialis*

	Larva (BP)	Larva (SP)	Adult
Number of insect	10	11	11
Weight (Gram)	0.551*	0.293	0.375
Nematode productivity (IJ/g)	124.300	107.500	37.438

BP: Black pine; SP: Scots pine; asteriks (*) indicates a significant difference between groups.

The similar oviposition preferences were observed for *M. alternatus* (Nakamura et al., 1995), *M. carolinensis* (Akbulut et al., 2004) and *M. galloprovincialis* (Akbulut et al., 2007; Akbulut et al., 2008). Fernandes et al. (2008) summarized that black pine has a thicker bark than Scots pine. The bark thickness or bark extracts (Özgenç et al. 2017) probably avoid beetle females. Because it needs more energy to lay eggs into bark. Our data might be related with this situation.

There is an obvious need for alternative control methods against *Monochamus* spp. and the PWN. These organisms are a potential threat to Turkish pine forests (Sousa et al., 2011). Türkiye has suitable conditions (geographic location, pine species, vector insect, climatic conditions, etc.,) for the introduction and distribution of the PWN (Öztürk et al., 2019). In future studies, an integrated pest management (IPM) strategy that includes various biological options (EPN, Entomopathogen fungi, pheromones, etc.) must be developed to control *M. galloprovincialis* populations in Turkish pine forests. Most likely, these biological control options against *M. galloprovincialis* larvae can be one of the key approaches for protecting pine forests from the invasion of the PWN.

Figure 2. Mean number of egg in pine logs. Asteriks (*) indicates a significant difference between groups

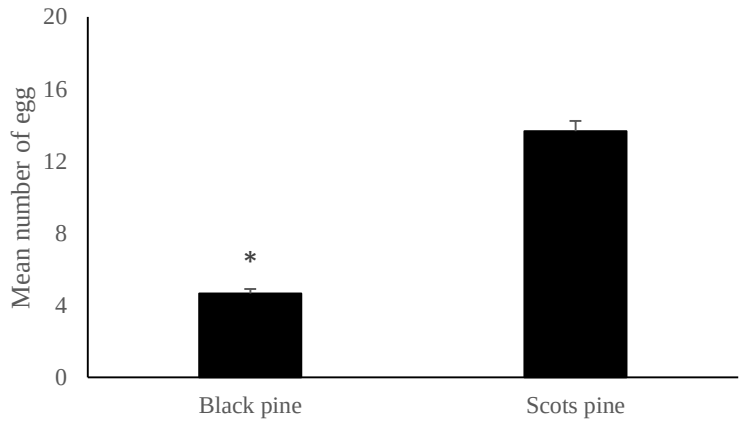


Figure 3. The survival rate of *Monochamus galloprovincialis* after nematode application in Scots pine. Asteriks (*) indicates a significant difference between groups

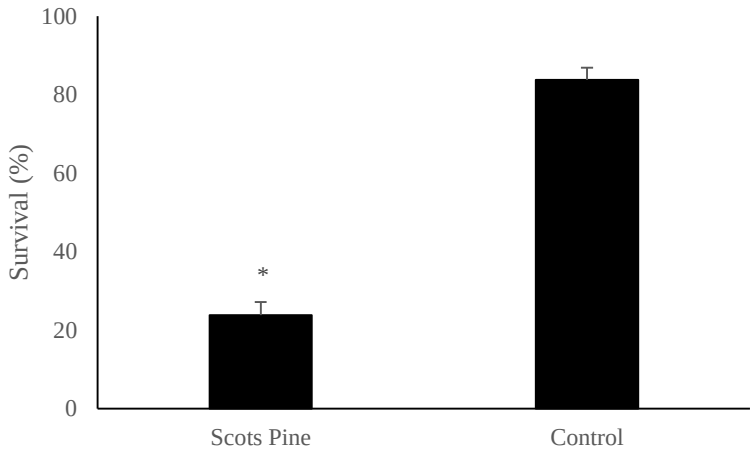
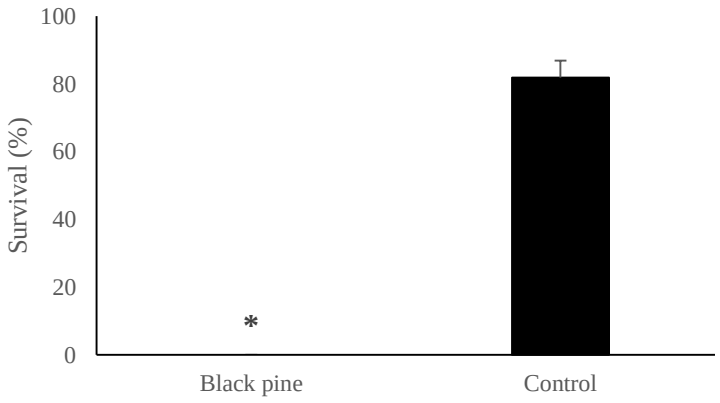


Figure 4. The survival rate of *Monochamus galloprovincialis* after nematode application in black pine. Asteriks (*) indicates a significant difference between groups



Conclusions

The results of our study confirm previous studies, suggesting that EPN can be an efficient biological control agent of wood-boring insects.

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N/A

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Author Contributions

Investigation: B. G., İ. B.; Material and Methodology: B. G.; İ. B.; Writing, review & Editing: B. G., İ. B., S. A. Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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