Türk. entomol. derg., 2020, 44 (4): 529-537
DOI: http://dx.doi.org/10.16970/entoted.768722

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Control potentials of some entomopathogenic nematodes against Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae)[1]

Asya kestanesi gal arısı, *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae)'ya karşı bazı entomopatojen nematodların mücadele potansiyelleri

Yavuz Selim ŞAHİN[2] **Nimet Sema GENÇER[2]** **İsmail Alper SUSURLUK[2*]**

Abstract

The Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae), has spread rapidly worldwide and can cause 80% product loss in chestnut. In the chemical control insecticides are ineffective because the larvae of the insect are well protected inside the chestnut galls. Various parasitoids of *D. kuriphilus* have been reared in Europe. However, native European parasitoids cannot keep the *D. kuriphilus* population below the economic threshold. The purpose of this research was to determine the potential of some entomopathogenic nematodes (EPNs) as an alternative biological control agent. Although EPNs have not been studied on *D. kuriphilus* until now, it is known that EPNs can infect some above-ground pests. In this study, two strains of *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) and one strain of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) with the four dosages were applied against *D. kuriphilus* adults in both chestnut shoots and petri dishes. The study was conducted in 2019 under laboratory conditions in Bursa (Turkey). The results of the study indicated that the EPNs can infect *D. kuriphilus* adults. In addition, the numbers of egg laying of *D. kuriphilus* adults exposed to the EPNs decreased.

Keywords: Above-ground application, biological control, *Dryocosmus kuriphilus,* entomopathogenic nematode

Öz

Asya kestane gal arısı, *Dryocosmus kuriphilus* Yasumatsu*,* 1951 (Hymenoptera: Cynipidae) dünya çapında hızla yayılmaktadır ve kestane içinde %80 ürün kaybına neden olabilmektedir. *Dryocosmus kuriphilus* larvaları kestane galleri içinde iyi korunduğu için kimyasal mücadelede, insektisitler etkisiz kalmaktadır. *Dryocosmus kuriphilus*'un çeşitli parazitoidleri Avrupa'da yetiştirilmektedir. Ancak, yerel Avrupa parazitoitleri *D. kuriphilus* popülasyonunu ekonomik zarar eşiğinin altında tutamamıştır. Bu araştırmanın amacı, *D. kuriphilus*'a karşı alternatif bir biyolojik mücadele ajanı olarak bazı entomopatojen nematodların (EPN) potansiyelini belirlemektir. Şimdiye kadar *D. kuriphilus* üzerinde EPN'ler ile ilgili çalışılmamış olmakla birlikte EPN'lerin toprak üstü zararlılarını enfekte edebileceği bilinmektedir. Bu çalışmada 4 dozda *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae)'nin iki ırkı ile *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)'nın bir ırkı hem kestane filizlerinde hem de petri kabı deneylerinde *D. kuriphilus* erginlerine uygulanmıştır. Bu çalışma, 2019 yılında Bursa (Türkiye)'da laboratuvar koşullarında gerçekleştirilmiştir. Çalışmanın sonuçları, EPN'lerin *D. kuriphilus* erginlerini enfekte edebildiklerini kanıtlamıştır. Ayrıca, EPN'lere maruz kalan *D. kuriphilus* erginlerinin yumurtlama sayısı azalmıştır.

Anahtar sözcükler: Toprak üstü uygulama, biyolojik mücadele, *Dryocosmus kuriphilus*, entomopatojen nematod

[1] This study was supported by Bursa Uludağ University, Scientific Research Unit, Bursa, Turkey, Grant Project No: KUAP(Z)- 2018/8.
[2] Department of Plant Protection, Faculty of Agriculture, Bursa Uludağğ University, Nilüfer, 16059 Bursa, Turkey
* Correspondence author (Sorumlu yazar) e-mail: susurluk@uludag.edu.tr
Received (Alınış): 13.07.2020 Accepted (Kabul ediliş): 30.10.2020 Published Online (Çevrimiçi Yayın Tarihi): 02.11.2020

Introduction

Chemical pesticides used in agricultural fields are a major cause of environmental pollution. Consequently, the importance of the biological control agents as an alternative to chemical pesticides has increased (Olson, 2015). Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are effectively used in place of some insecticides providing environmentally friendly control (Lacey & Shapiro-Ilan, 2008). EPNs, which have a symbiotic relationship with *Photorhabdus* and *Xenorhabdus* bacteria, enter insect hemolymph through natural openings. After penetration, the EPNs, release their symbiotic bacteria and the infested insects die from septicemia within 24 to 48 h (Stock & Blair, 2008). EPNs are naturally present in soil so are especially used to control soil-dwelling insect pests (Wright et al., 2005). Also, they have the potential to suppress above-ground insect pests such as grasshoppers and cockroaches (Morton & García-del-Pino, 2013; Şahin et al., 2018). The spectrum of insects susceptible to the EPNs is quite broad. Insects from 17 orders and 135 families have been found to be vulnerable to the EPNs (El-Kady et al., 2014).

The Asian chestnut gall wasp *Dryocosmus* kuriphilus Yasumatsu, 1951 (Hymenoptera: Cynipidae), native to China, is the only member of the Cynipidae family that attacks the *Castanea* genus (Stone et al., 2002; Abe et al., 2007; Gehring et al., 2018). Originally from China, *D. kuriphilus* was the first identified in Japan in 1941 and then in Korea. It was transmitted to Nepal in 1999 and to the USA with plant material imported from China in 1974. It was first identified in Europe in northern Italy in 2002, and then in Slovenia, France, Switzerland, and Hungary (Panzavolta et al., 2012). The first record of the *D. kuriphilus* in Turkey was made by Çetin et al. (2014). Over the past 20 years, the pest has spread to 25 countries in Asia, Europe and North America. Its rapid spread across different regions has resulted in considerable ecological and economic damage making *D. kuriphilus* one of the most important chestnut pests worldwide (Avtzis et al., 2019).

Dryocosmus kuriphilus females lay eggs in axillary buds of *Castanea* spp. during the summer. The eggs generally hatch in 30-40 days and the first instar of larvae overwinter inside the buds until spring comes (Avtzis et al., 2019). Larvae, which feed inside the bud during the spring, cause flower abortion and the inhibition of female flower formation (Gehring et al., 2018). Thus, fruit yield of the chestnut, *Castanea* spp., can decrease by 80% (Battisti et al., 2014; Avtzis et al., 2019).

The first attempts to limit the damage of *D. kuriphilus* were to develop resistant chestnut cultivars that were initially effective (Avtzis et al., 2019). In chemical control, contact-effect insecticides are ineffective, because the larvae of *D. kuriphilus* are well protected inside the chestnut gall (Bosio et al., 2009). Another problem that makes insecticide use unsuccessful is the difficultly of determining the best control time because *D. kuriphilus* adult emergence date can change and the adults die within 10 days (Germinara et al., 2011; Bernardo et al., 2013). For biological control, 44 species of parasitoids of *D. kuriphilus* in six families have been reared in Europe (Matošević & Melika, 2013; Quacchia et al., 2013; Kos et al., 2015). Although the parasitoid *Torymus sinensis* Kamijo, 1982 (Hymenoptera: Torymidae) is known to be effective against the *D. kuriphilus* in some countries (Moriya et al., 2003; Yara, 2006; Quacchia et al., 2014), the parasitoid cannot keep the *D. kuriphilus* population below the economic threshold, especially in Europe and Middle East (Santi & Maini, 2011; Matošević & Melika, 2013; Quacchia et al., 2013; Askew et al., 2013; Kos et al., 2015; Francati et al., 2015).

Nevertheless, EPNs can be a successful alternative for biological control of *D. kuriphilus* because, EPNs have potential to be effective against above-ground insect pests (El-Kady et al., 2014; Şahin et al., 2018). The purpose of this study is to determine the potential of some EPN strains, two strains (TURS3 and STE5) of *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) and one strain (HBH) of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae), against *D. kuriphilus* as the biological control agents.

Materials and Methods

EPNs production and application dosages

Three different strains of the EPNs, *Steinernema feltiae* TURS3, *S. feltiae* STE5, *Heterorhabditis bacteriophora* HBH, were used in this study. The 2 or 3-day-old infective juveniles (IJs) of these strains obtained by *in vivo* production were used against *D. kuriphilus*. The final instar larvae of great wax moth, *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) were used for *in vivo* production of the EPNs used (McMullen & Stock, 2014).

The EPNs were applied to adult (female) *D. kuriphilus* both in Petri dishes and chestnut shoots (Figure 1). The dosages of the EPNs applied in Petri dishes were 20, 50, 100 and 200 IJs per cm^2. The numbers of the IJs per *D. kuriphilus* adult in Petri dishes were about 57, 141, 283 and 564, respectively. Plain water was applied for the control.

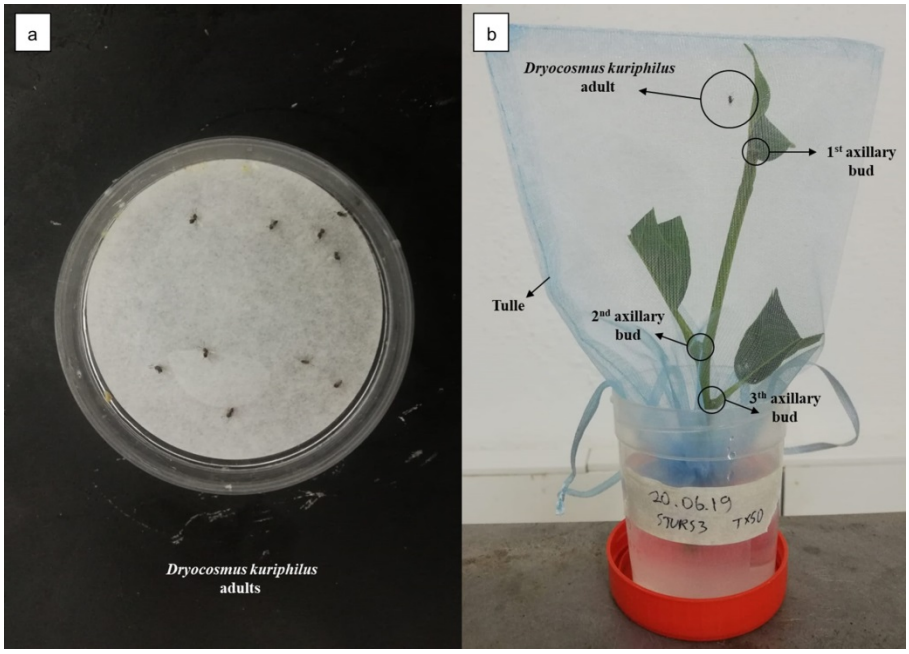

Figure 1. a) *Dryocosmus kuriphilus* adults in a plastic Petri dish; b) gall-free shoots with three axillary buds.

The solution of EPNs diluted with distilled water and were applied on the chestnut shoots by spraying (Shapiro-Ilan et al., 2006). For this, gall-free shoots containing three axillary buds were used (Figure 1). The application dosages on the shoots were 20, 50, 100 and 200 IJs/10 µl. (Susurluk, 2008). About 10 µl of the used dosages accumulated on each bud. Distilled water was applied as a control.

Dryocosmus kuriphilus adults

In spring 2019, 2-year old-shoots with galls (30-40 cm long) (a total of 50 shoots) and current shoots with buds (30-40 shoots) were collected from chestnut orchard in Bursa Province (Cumalıkızık Village, 40°10'21" N, 29°10'16" E) in northwestern Turkey, between May and June at weekly intervals. In order to obtain *D. kuriphilus* adults, we separated the galls from shoots and placed them in cardboard culture boxes. The emergence of the adults that reproduce by thelytokous parthenogenesis (Zhu et al., 2007) was checked daily and were used as soon as after hatching.

Mortality of *Dryocosmus kuriphilus* in Petri dishes

Plastic Petri dishes with a diameter of 6 cm were used for *D. kuriphilus* inoculation. Sterile moist filter paper (80% RH) in the Petri dishes were inoculated with the EPNs at the doses give above. Then 10 of *D. kuriphilus* adults were placed on the filter paper (Figure 1a). The lid of the Petri dishes was closed and it was incubated at 24°C in the dark. Water was applied for the control. The *D. kuriphilus* adults that died after 5 days were dissected to determine, whether they were infected by the EPNs. This experiment was repeated three times for each EPN strain and dosage (Figure 2).

Effects of EPN on *Dryocosmus kuriphilus* egg number in buds

As soon as *D. kuriphilus* adults emerge from the chestnut galls they begin to lay eggs in the buds and die within 7 days (Germinara et al., 2011; Bernardo et al., 2013). Even if these adults are infected by EPNs when they reach the buds, it can take up to 1 or 2 days for EPNs to kill the insects (Stock & Blair, 2008). For this reason, an egg counting method was used to determine the effectiveness of EPNs in controlling females of *D. kuriphilus.* The gall-free shoots were put in plastic bottles (100 ml) filled with water and then the EPNs were applied by spraying the shoots (Shapiro-Ilan et al., 2006). As control, distilled water was applied. Only one *D. kuriphilus* female was released on each shoot, then immediately the shoots in the bottle were covered with tulle (Figure 1b). These shoots were kept at 25°C under laboratory conditions with a 10:14 h L:D photoperiod. After 10 days, the tulle was removed and the numbers of eggs laid in buds counted. Five shoots were used in each batch. The batches were run for each EPN strain and dosage (Figure 3).

Statistical Analysis

The mortality and spawning rate of *D. kuriphilus* were examined using analysis of variance, as of the data was normally distributed. LSD test ($P<0.05$) was used to determine the difference between means in JMP® 7.0 software.

Results and Discussion

Mortality of *Dryocosmus kuriphilus* adults in Petri dishes

At 25 IJs/cm^2, TURS3 caused 20% mortality of *D. kuriphilus* adults and was statistically more effective than the control. Mortality of *D. kuriphilus* adults caused by STE5 and HBH was not significant at 13% and 6.7%, respectively. The effect of HBH was not statistically different from the control. At 50 IJs/cm^2, TURS3, STE5 and HBH caused 60, 47 and 33% mortality, respectively, differences between the mortality rates being statistically significant. At 100 IJs/cm^2, HBH and TURS3 caused 67 and 60% mortality, respectively, but these values were not statistically different. The highest mortality was 87% with STE5. At 200 IJs/cm^2, STE5 and TURS3 gave 100 and 93.3% mortality, respectively, but these values were not statistically different. However, with HBH at this dosage was 73% being significantly lower than STE5 and TURS3, and higher than control (Figure 2).

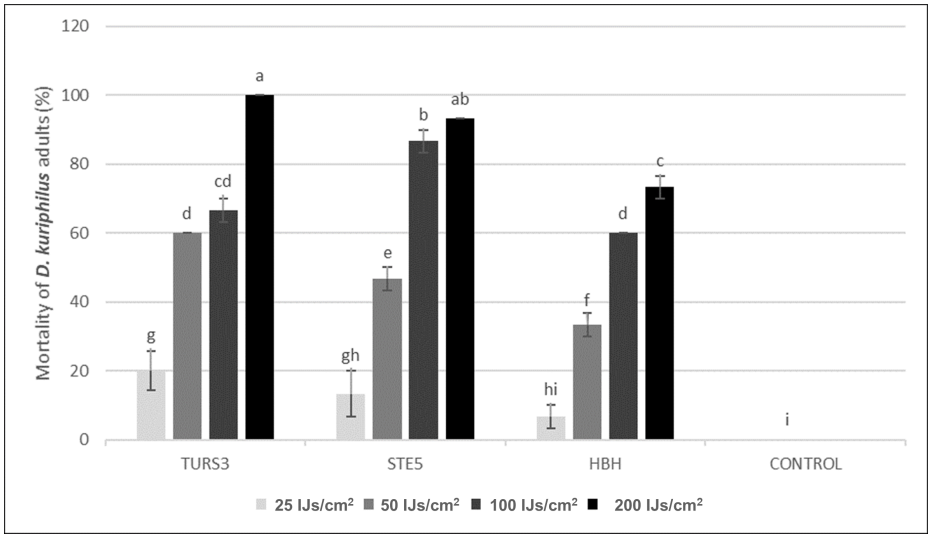

Figure 2. The mortality of *Dryocosmus kuriphilus* adults in Petri dishes. TURS3 and STE5 are strains of *Steinernema feltiae*, and HBH of *Heterorhabditis bacteriophora*. Mean ± SE followed by the same letter are not significantly different (F=95.0, df=12,26, P<0.0001).

Mean number of *Dryocosmus kuriphilus* eggs in each bud

At of 25 IJs/cm^2, the mean numbers of eggs in the buds with TURS3, STE5 and HBH applied were not significantly different from the control (34, 36 and 36, respectively). At 50 IJs/cm^2, the mean numbers of eggs in the buds with STE5 and HBH applied were not significantly different from the control (34 and 35, respectively). At 100 IJs/cm^2, the highest number of eggs determined was 37. The lowest egg number was 22 with TURS3 were applied. At 200 IJs/cm^2, the mean numbers of eggs in the buds treated with TURS3, STE5 and HBH were 15, 30 and 25, respectively, with the differences between these values being statistically significant (Figure 3). *Steinernema feltiae* TURS3 and STE5 were generally found to be more effective than *H. bacteriophora* HBH in terms of killing *D. kuriphilus.*

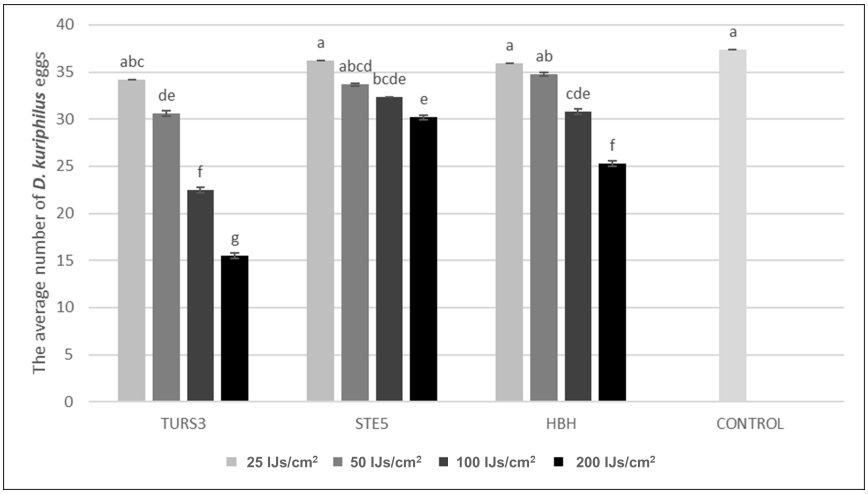

Figure 3. The average numbers of *Dryocosmus kuriphilus* eggs on each shoot [TURS3 and STE5: *Steinernema feltiae*, HBH: *Heterorhabditis bacteriophora*. Means (Means ± SE) followed by the same letter are not statistically significant (F=25.3, df=12,176, P<0.0001)].

Dryocosmus kuriphilus, an important pest of *Castanea* spp., continues to spread worldwide (Avtzis et al., 2019). The first attempts to suppress parasitism of *D. kuriphilus* initially focused on using resistant cultivars of *Castanea crenata* Siebold & Zucc. in the world. Although the use of resistant cultivars is not a sufficiently effective solution, some resistant chestnut cultivars are commercially used by some growers (Nugnes et al., 2018; Avtzis et al., 2019). However, for the control of *D. kuriphilus* recent studies have focused on biological control agents that may provide an alternative to insecticides (Avtzis et al., 2019), because of insecticides are not effective against *D. kuriphilus* larvae, since larvae of the insect are well protected inside the chestnut galls (Bosio et al., 2009).

In the application made on the chesnut shoots in this study, even if *D. kuriphilus* adults are infected by used EPNs as soon as they reach the buds, *D. kuriphilus* death occurs within 2 days. As the EPN dosages increased, the average number of eggs laid in the galls by *D. kuriphilus* adults decreased. These results show that the infected *D. kuriphilus* adults can continue to lay eggs until their death. However, the decrease in the mean numbers of eggs laid compared to the control is promising in terms of biological control. As the viability of the *D. kuriphilus* adults decreased with infection by EPNs, the amount of egg laying decreased. Thus, in the shoot experiment, numbers of laid eggs were recorded rather than the lifespan of the adults.

The use of parasitoids as biological control against *D. kuriphilus* as an alternative control method has been studied by many researchers (Speranza et al., 2008; Quacchia et al., 2013; Matošević et al., 2014; Avtzis et al., 2019). According to their results, the use of parasitoids against *D. kuriphilus* has not been effective to the needed level (Santi & Maini, 2011; Askew et al., 2013; Matošević & Melika, 2013; Quacchia et al., 2013; Kos et al., 2015; Francati et al., 2015). However, no study on the use of EPNs in the controlling of *D. kuriphilus* has been undertaken to date. Although EPNs have the potential to infect above-ground pests, they are mostly used to control pests of soil borne insect pests (Wright et al., 2005; Şahin et al., 2018) because there are important factors such as temperature, ultraviolet radiation and humidity that mostly make the above-ground application unsuccessful (Georgis et al., 2006; Lacey & Georgis, 2012). Despite all these factors, many studies are underway to enable the use of EPNs for the control of above-ground insect pests and remarkable results have been achieved (Maketon et al., 2010; Beck et al., 2013; Şahin et al., 2018; Platt et al., 2019). Similar to our study, Cutler et al. (2017) have achieved control of adults of some cockroach species [*Blaptica dubia* (Serville, 1838), *Gromphadorhina portentosa* Schaum, 1853) and *Nauphoeta cinerea* (Olivier, 1789)] using *Heterorhabditis* and *Steinernema* spp. In addition, *H. bacteriophora* were successfully used against adults of *Locusta migratoria* by Sahin et al. (2018). Schroer & Ehlers (2005), used EPNs *Steinernema carpocapsae* (Weiser, 1955) with a formulation containing 0.3% of the surfactant Rimulgan and 0.3% of the polymer, xanthan gum, on cabbage foliage to control the diamondback moth, *Plutella xylostella* (Linnaeus, 1758), larvae. With these formulations, 80% of the larvae of the insect died within 58 h. Similarly, in the present study, at a dosage of 200 IJs/cm^2, *S. feltiae* STE5 and TURS3 caused more than 80% mortality of *D. kuriphilus* adults. Also, Van Damme et al. (2016) reported that foliar application of EPNs *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* to tomato leaf for control of *Tuta absoluta* (Meyrick, 1917) larvae were effective with mortality of fourth instars being between 77 and 97%. Also, in the same study, *S. feltiae* and *S. carpocapsae* were more effective than *H. bacteriophora* against *T. absoluta*. Also, Hussein and El-Mahdi (2019) formulated three EPNs strains (*H. bacteriophora* BA1, *S. carpocapsae* BA2, *S. feltiae* OBIII) using mixed polymer based on calcium alginate to control *Thrips tabaci* Lindeman, 1889, on onion plants. Significant differences were observed in the mortality of the *T. tabaci* population. The highest mortality was caused by *S. carpocapsae* BA2 and *S. feltiae* OBIII, and the lowest mortality was with *H. bacteriophora* BA1. Consistent with these two studies, in the present study, *S. feltiae* STE5 and TURS3 were more effective against *D. kuriphilus* adults than *H. bacteriophora* HBH especially at 200 IJs/cm^2 in both Petri dish and shoot experiments. Considering studies that had been reported, in general *Steinernema* spp. appears to be more effective than *Heterorhabditis*

spp. in above-ground applications. According to the results of the present study, as similar result was found for *D. kuriphilus* and perhaps if protective formulations were used the effectiveness of the EPNs tested would be improved.

In the future, the use of EPNs against above-ground targets will become more effective. Efforts to develop the above-ground application techniques, which are currently underway, will continue to increase the effectiveness of EPNs. Control potential of used EPNs in the study against *D. kuriphilus* supports improvement of these above-ground applications.

Conclusions

This study is the first attempt to control of *D. kuriphilus* by using of EPNs. In this study, EPNs sprayed on the gall-free shoots were effective in reducing egg numbers of *D. kuriphilus* in the buds. With the EPN strains (TURS3, STE5 and HBH) used, the mean numbers of *D. kuriphilus* eggs in the buds decreased statistically as dosage increased. This study shows that the EPNs can cause a reduction in the numbers of *D. kuriphilus* eggs, which is important for reducing *D. kuriphilus* damage in chestnuts. One of the important implications of this study is that EPNs might be successfully be used for controlling of *D. kuriphilus* in the future. It is suggested that the present study will contribute to the development of the above-ground EPN application techniques.

Acknowledgments

This study was supported by the Scientific Research Projects Unit of Bursa Uludağ University (Project number: KUAP (Z)-2018/8). The master students in our laboratory are thanked for their technical support.

References

Abe, Y., G. Melika & G. N. Stone, 2007. The diversity and phylogeography of cynipid gallwasps (Hymenoptera: Cynipidae) of the Oriental and eastern Palearctic regions, and their associated communities. Oriental Insects, 41 (1): 169-212.

Askew, R. R., G. Melika, J. Pujade-Villar, K. Schonrogge, G. N. Stone & J. L. Nieves-Aldrey, 2013. Catalogue of parasitoids and inquilines in cynipid oak galls in the West Palaearctic. Zootaxa, 3643 (1): 1-133.

Avtzis, D. N., G. Melika, D. Matošević & D. R. Coyle, 2019. The Asian chestnut gall wasp *Dryocosmus kuriphilus*: A global invader and a successful case of classical biological control. Journal of Pest Science, 92 (1): 107-115.

Battisti, A., I. Benvegnu, F. Colombari & R. A. Haack, 2014. Invasion by the chestnut gall wasp in Italy causes significant yield loss in *Castanea sativa* nut production. Agricultural and Forest Entomology, 16 (1): 75-79.

Beck, B., E. Brusselman, D. Nuyttens, M. Moens, S. Pollet, F. Temmerman & P. Spanoghe, 2013. Improving foliar applications of entomopathogenic nematodes by selecting adjuvants and spray nozzles. Biocontrol Science and Technology, 23 (5): 507-520.

Bernardo, U., L. Iodice, R. Sasso, V. A. Tutore, P. Cascone & E. Guerrieri, 2013. Biology and monitoring of *Dryocosmus kuriphilus* on *Castanea sativa* in Southern Italy. Agricultural and Forest Entomology, 15 (1): 65-76.

Bosio, G., C. Gerbaudo & E. Piazza, 2009. "*Dryocosmus kuriphilus* Yasumatsu: an outline seven years after the first report in Piedmont (Italy), 65". In: I European Congress on Chestnut-Castanea (13-16 October 2009, Cuneo, Italy), 726 pp.

Çetin, G., E. Orman & Z. Polat, 2014. First record of the Oriental chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) in Turkey. Bitki Koruma Bülteni, 54 (4): 303-309.

Cutler, J., K. Hughes & R. Rae, 2017. Susceptibility of cockroaches (*Gromphadorhina portentosa*, *Nauphoeta cinerea* and *Blaptica dubia*) exposed to entomopathogenic nematodes. Biocontrol Science and Technology, 27 (4): 556-564.

El-Kady, G. A., A. F. El-Bahrawy, H. M. El-Sharabasy, Y. S. El-Badry, R. M. A. El-Ashry & M. F. Mahmoud, 2014. Pathogenicity and reproduction of the entomopathogenic nematode, *Steinernema carpocapsae* (Wieser) in the German cockroach, *Blattella germanica* L. (Dictyoptera: Blattellidae). Egyptian Journal of Biological Pest Control, 24 (1): 133-138.

Francati, S., A. Alma, C. Ferracini, A. Pollini & M. L. Dindo, 2015. Indigenous parasitoids associated with *Dryocosmus kuriphilus* in a chestnut production area of Emilia Romagna (Italy). Bulletin of Insectology, 68 (1): 127-134.

Gehring, E., B. Bellosi, A. Quacchia & M. Conedera, 2018. Assessing the impact of *Dryocosmus kuriphilus* on the chestnut tree: branch architecture matters. Journal of Pest Science, 91 (1) :189-202.

Georgis, R., A. M. Koppenhöfer, L. A. Lacey, G. Bélair, L. W. Duncan, P. S. Grewal, M. Samish, L. Tan, P. Torr & R. W. H. M. van Tol, 2006. Successes and failures in the use of parasitic nematodes for pest control. Biological Control, 38 (1): 103-123.

Germinara, G. S., A. De Cristofaro & G. Rotundo, 2011. Chemical cues for host location by the chestnut gall wasp, *Dryocosmus kuriphilus*. Journal of Chemical Ecology, 37 (1): 49-56.

Hussein, M. A. & I. F. El-Mahdi, 2019. Efficiency of three formulated entomopathogenic nematodes against onion thrips, *Thrips tabaci* under aquaculture system. Journal of Biopesticides, 12 (1): 134-138.

Kos, K., E. Kriston & G. Melika, 2015. Invasive chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), its native parasitoid community and association with oak gall wasps in Slovenia. European Journal of Entomology, 112 (4): 698-704.

Lacey, L. A. & R. Georgis, 2012. Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. Journal of Nematology, 44 (2): 218-225.

Lacey, L. A. & D. I. Shapiro-Ilan, 2008. Microbial control of insect pests in temperate orchard systems: potential for incorporation into IPM. Annual Review of Entomology, 53 (1): 121-144.

Maketon, M., A. Hominchan & D. Hotaka, 2010. Control of American cockroach (*Periplaneta americana*) and German cockroach (*Blattella germanica*) by entomopathogenic nematodes. Revista Colombiana de Entomología, 36 (2): 249-253.

Matošević, D. & G. Melika, 2013. Recruitment of native parasitoids to a new invasive host: first results of *Dryocosmus kuriphilus* parasitoid assemblage in Croatia. Bulletin of Insectology, 66 (2): 231-238.

Matošević, D., A. Quacchia, É. Kriston & G. Melika, 2014. Biological control of the invasive *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae)-an overview and the first trials in Croatia. South-East European Forestry, 5 (1): 3-12.

McMullen II, J. G. & S. P. Stock, 2014. In vivo and in vitro rearing of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae). JoVE (Journal of Visualized Experiments), 91: e52096.

Moriya, S., M. Shiga & I. Adachi, 2003. “Classical biological control of the chestnut gall wasp in Japan, 407-415”. In: 1st International Symposium on Biology Control of Arthropods (Honolulu, Hawaii, 14-18 January 2002) United States Department of Agriculture, Forest Service, Washington (US), 520 pp.

Morton, A. & F. García-del-Pino, 2013. Sex-related differences in the susceptibility of *Periplaneta americana* and *Capnodis tenebrionis* to the entomopathogenic nematode *Steinernema carpocapsae*. Journal of Invertebrate Pathology, 112 (3): 203-207.

Nugnes, F., L. Gualtieri, C. P. Bonsignore, R. Parillo, R. Annarumma, R. Griffo & U. Bernardo, 2018. Resistance of a local ecotype of *Castanea sativa* to *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) in Southern Italy. Forests, 9 (2): 94-106.

Olson, S., 2015. An analysis of the biopesticide market now and where it is going. Outlooks on Pest Management, 26 (5): 203-206.

Panzavolta, T., M. Bracalini, F. Croci, C. Campani, T. Bartoletti, G. Miniati & R. Tiberi, 2012. Asian chestnut gall wasp in Tuscany: gall characteristics, egg distribution and chestnut cultivar susceptibility. Agricultural and Forest Entomology, 14 (2): 139-145.

Platt, T., N. F. Stokwe & A. P. Malan, 2019. Grapevine leaf application of *Steinernema yirgalemense* to control *Planococcus ficus* in semi-field conditions. South African Journal of Enology and Viticulture, 40 (1): 1-9.

Quacchia, A., C. Ferracini, J. A. Nicholls, E. Piazza, M. A. Saladini, F. Tota, G. Melika & A. Alma, 2013. Chalcid parasitoid community associated with the invading pest *Dryocosmus kuriphilus* in north-western Italy. Insect Conservation and Diversity, 6 (2): 114-123.

Quacchia, A., S. Moriya & G. Bosio, 2014. “Effectiveness of *Torymus sinensis* in the biological control of *Dryocosmus kuriphilus* in Italy, 199-204”. In: II European congress on chestnut, Acta Horticulturae (ISHS) (9-12 October 2013, Hungary), 224 pp.

Şahin, Y. S., A. Bouchari, T. C. Ulu, B. Sadiç & A. Susurluk, 2018. New application method for entomopathogenic nematode *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) HBH strain against *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae). Türkiye Entomoloji Dergisi, 42 (4): 305-312.

Santi, F. & S. Maini, 2011. New association between *Dryocosmus kuriphilus* and *Torymus flavipes* in chestnut trees in the Bologna area (Italy): first results. Bulletin of Insectology, 64 (2): 275-278.

Schroer, S. & R. U. Ehlers, 2005. Foliar application of the entomopathogenic nematode *Steinernema carpocapsae* for biological control of diamondback moth larvae (*Plutella xylostella*). BioControl, 33: 81-86.

Shapiro-Ilan, D. I., D. H. Gouge, S. J. Piggott & J. P. Fife, 2006. Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. Biological Control, 38 (1): 124-133.

Speranza, S., M. Stacchiotti & B. Paparatti, 2008. "Endemic parasitoids of *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cinipidae) in Central Italy, 421-424". In: IV International Chestnut Symposium (September 2008, Beijing-China), 468 pp.

Stock, S. P. & H. G. Blair, 2008. Entomopathogenic nematodes and their bacterial symbionts: the inside out of a mutualistic association. Symbiosis-Rehovot, 46 (2): 65-75.

Stone, G. N., K. Schönrogge, R. J. Atkinson, D. Bellido & J. Pujade-Villar, 2002. The population biology of oak gall wasps (Hymenoptera: Cynipidae). Annual Review of Entomology, 47 (1): 633-668.

Susurluk, I. A., 2008. Potential of the entomopathogenic nematodes *Steinernema feltiae*, *S. weiseri* and *Heterorhabditis bacteriophora* for the biological control of the sugar beet weevil *Bothynoderes punctiventris* (Coleoptera: Curculionidae). Journal of Pest Science, 81: 221-225.

van Damme, V. M., B. K. Beck, E. Berckmoes, R. Moerkens, L. Wittemans, R. De Vis & P. De Clercq, 2016. Efficacy of entomopathogenic nematodes against larvae of *Tuta absoluta* in the laboratory. Pest Management Science, 72 (9): 1702-1709.

Wright, D., A. Peters, S. Schroer & J. Fife, 2005. "Application Technology, 91-106". In: Nematodes as Biocontrol Agents (Eds. P. S. Grewal, R. U. Ehlers & D. I. Shapiro-Ilan). CABI Publishing, Wallingford, UK, 528 pp.

Yara, K., 2006. Identification of *Torymus sinensis* and *T. beneficus* (Hymenoptera: Torymidae), introduced and indigenous parasitoids of the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), using the ribosomal ITS-2 region. Biological Control, 36 (1): 15-21.

Zhu, D. H., Y. Y. He, Y. S. Fan, M. Y. Ma & D. L. Peng, 2007. Negative evidence of parthenogenesis induction by Wolbachia in a gallwasp species, *Dryocosmus kuriphilus*. Entomologia Experimentalis et Applicata, 124 (3): 279-284.