Türk. entomol. derg., 2018, 42 (4): 305-312
DOI: http://dx.doi.org/10.16970/entoted.471095

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

New application method for entomopathogenic nematode *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) HBH strain against *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae)

Entomopatojen nematod *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) HBH hibrit ırkının *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae)'ya karşı yeni bir uygulama yöntemi

Yavuz Selim ŞAHİN[1] Ahcen BOUHARI[1] Tufan Can ULU[1]
Büşra SADIÇ[1] İsmail Alper SUSURLUK[1*]

Abstract

Entomopathogenic nematodes (EPNs) of the families Heterorhabditidae and Steinernematidae are being used as biocontrol agents against many soil borne insect pests in agriculture. Above-ground applications against the insects are usually unsuccessful due to the lack of humidity. Therefore, EPNs rapidly lose their effectiveness. In this study, conducted in 2018 under laboratory conditions in Bursa-Turkey, a new application method was developed for the use of *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) HBH hybrid strain against the migratory locust, *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae). A new trap system is coated with hydrophilic cotton fabric to provide the necessary humidity to allow the use of EPNs above-ground. Three different application rates of *H. bacteriophora* (5000, 25000 and 50000 IJs) were applied to the trap system. The fabric was inoculated with the nematodes and combined with a reservoir containing 200 ml of ringer solution. The dead and live nematodes were recorded periodically to determine their persistence on the fabric. The mortality of *L. migratoria* were also recorded to determine the infectivity of *H. bacteriophora*. The infectivity and persistence of the nematodes was sustained for more than 4 weeks by this method.

Keywords: Above-ground application, entomopathogenic nematodes, hydrophilic fabric, *Locusta migratoria*

Öz

Entomopatojen nematodlar (EPN) Heterorhabditidae ve Steinernematidae familyalarına bağlı olan, toprak altında yaşayan zararlı böceklere karşı kullanılan biyolojik mücadele etmenleridir. Toprak üzerine yapılan uygulamalar nemli ortam sağlanamaması nedeniyle başarısız olmaktadır. Bu nedenle toprak üzerinde EPN etkinliği çok kısa sürede yok olmaktadır. Bursa'da laboratuvar koşullarında 2018 yılında yapılan bu çalışmada, *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) HBH hibrit ırkının uygulaması üzerine yeni bir teknik geliştirilmiş ve *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae) üzerindeki etkinliği incelenmiştir. Yeni geliştirilen tuzak sistemi hidrofil bir kumaş ile kaplanmış ve bu kumaş sayesinde tuzak yüzeyinin sürekli olarak nemli kalması sağlanmıştır. Tuzak sisteminde *H. bacteriophora*'nın üç farklı uygulama dozu (5000, 25000 ve 50000 IJs) kullanılmıştır. Tuzak yüzeyindeki hidrofil kumaş üzerinde belirli dozlardaki nematod solüsyonları uygulanmış ve tuzak sistemi 200 ml Ringer solüsyonu içeren rezervuar ile birleştirilerek kullanıma hazır hale getirilmiştir. Belirli aralıklarla ölü ve canlı EPN bireyleri sayılarak kumaş üzerindeki kalıcılık hesaplanmıştır. Buna ek olarak çekirgelerin ölüm oranları da periyodik olarak hesaplanmış ve tuzağın etkinliği belirlenmiştir. Çalışma sonucunda bu tuzak sistemi sayesinde bu nematodun kalıcılığının artırıldığı ve toprak üzerine yapılan uygulama ile etkinlikte başarı sağlandığı tespit edilmiştir.

Anahtar sözcükler: Toprak üstü uygulama, entomopatojen nematodlar, hidrofil kumaş, *Locusta migratoria*

[1] Bursa Uludağ University, Faculty of Agriculture, Department of Plant Protection, 16059, Nilüfer, Bursa, Turkey
* Corresponding author (Sorumlu yazar) e-mail: susurluk@uludag.edu.tr
Received (Alınış): 16.10.2018 Accepted (Kabul ediliş): 29.11.2018 Published Online (Çevrimiçi Yayın Tarihi): 17.12.2018

Introduction

Locusta migratoria (Linnaeus, 1758) (Orthoptera: Acrididae) is one of the most destructive agricultural insect pests worldwide (Zhang et al., 2009; Wang et al., 2014). It is a polyphagous pest (Showler, 1995) that can migrate over thousands of kilometers (Zhang et al., 2009). *Locusta migratoria* is distributed over a larger area than any another locust or grasshopper and can spread over the entire eastern hemisphere (Australia, Africa, Europe and Asia) (Zhang et al., 2009). Locust invasions destroyed nearly 8 Mha of agricultural plants in 12 of the 30 provinces of China between 1995 and 2001 (Ma et al., 2005). Given the difficulty of predicting locust outbreaks, the affected countries use pesticides excessively, which are toxic to the environment and non-target organisms (Vos et al., 2000; Rai & Chavhan, 2015). Development of environmentally-safe alternatives to locust control using biological agents including nematodes, protozoa, bacteria, fungi and viruses have been reported (Rai et al., 2013; Rai & Chavhan, 2015).

Entomopathogenic nematodes (EPNs) from the families Steinernematidae and Heterorhabditidae are used for biological control of insects instead of using insecticides (Cross et al., 1999; Lacey & Shapiro-Ilan, 2008) to reduce the negative impact on the environment (Lewis & Clarke, 2012; Shapiro-Ilan et al., 2014). They are mostly used against soil borne insect pests (Ehlers, 1996; Gaugler, 2002; Wright et al., 2005). Various formulations have been developed to use EPNs against above-ground pests, (Schroer & Ehlers, 2005; Georgis et al., 2006; Beck et al., 2013). Some important factors that make the above-ground application unsuccessful are temperature, ultraviolet radiation and humidity. Of these, humidity is the most restrictive factor for EPNs because current formulations do not provide a moist environment for sufficient time (Georgis et al., 2006; Lacey & Georgis, 2012). It has been shown that some additives can improve EPN persistence and effectiveness on foliage, but this enhancement have not been adequate (Baur et al., 1997; Schroer & Ehlers, 2005). Many laboratory studies have been conducted to test the persistence of EPNs on the foliage. The persistence of EPNs were usually hours rather than days (Schroer & Ehlers, 2005). Although remarkable improvements have been made for the above-ground application up to date, they are still not sufficiently efficacious to be recommended for commercial use (Grewal, 2002).

This study aimed to develop a method for above-ground application with the trap system using hydrophilic cotton fabric against the *L. migratoria*. For this purpose, the HBH hybrid strain of *Heterorhabditis bacteriophora* (Poinar, 1976) (Heterorhabditidae: Rhabditida) patented by Susurluk (TPMK Patent No: TR 2013 06141 B) was used against *L. migratoria* in the new trap system.

Material and Methods

Insect, entomopathogenic nematode and trap

The HBH hybrid strain of *H. bacteriophora* was selected for this study. The hybrid strain was obtained after hybridization of Turkish native *H. bacteriophora* isolates from different climatic regions of Turkey (including hot, cold, rainy and semi-arid). The HBH strain was patented due to its superior biological characters (high effectiveness, long persistence and high reproduction capacity). Under laboratory conditions, EPN populations were reproduced as infective juveniles (IJs) using *in vivo* methods according to Kaya & Stock (1997). The final instar of great wax moth, *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae), larva was used for EPN reproduction at 25ºC. The juveniles were extracted using the White trap method (White, 1927). Freshly-harvested 2-3 d-old IJs were used in the experiment. Commercially produced adult locusts, *Locusta migratoria* L. (Orthoptera: Acrididae), was purchased from a local store (Mira Limited Şti., Aksu, Antalya, Turkey) and divided into experimental groups before trap preparation. The trap consisted a hemispherical pot with a diameter of 10 cm and a surface area of 150 cm². The surface was covered with cotton fabric and no attractants were used for the locusts.

Experimental design

Ringer solution (200 ml) was placed into the hemispherical pots to provide a liquid reservoir for the nematodes. The fabric used in this study was a 100% cotton gauze bandage with about 40 threads per cm and was applied with folds to cover the entire water reservoir. After nematode inoculation of the fabric, it was combined with the reservoir and the pot was closed with a plastic lid (Figure 1). As evaporation occurred from the trap surface, the hydrophilic fabric absorbed liquid continuously from the reservoir.

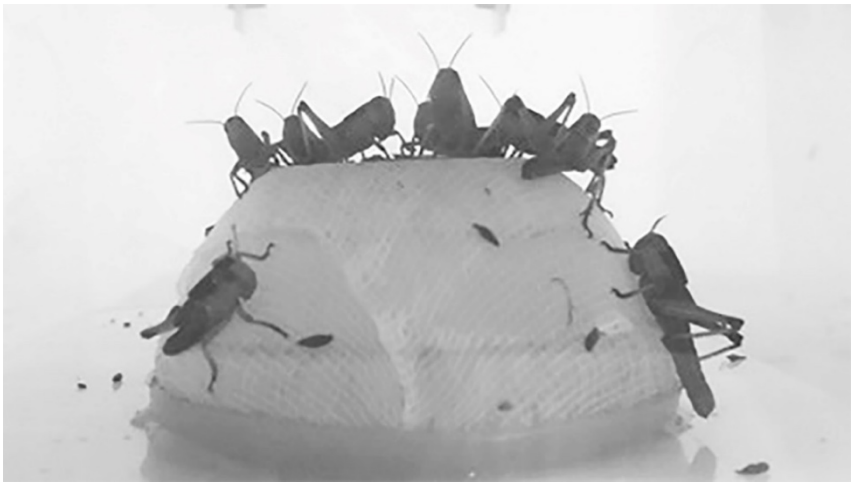

Figure 1. The trap system that contains the reservoir and fabric inoculated with the EPNs.

Three different doses of nematodes (5000, 25000 and 50000 IJs) were inoculated to three different traps and 10 adult *L. migratoria* transferred to each trap. The dead and alive locust adults were recorded 2 d after each locust placement. All locusts were removed from the trap after mortality was recorded. On days 15 and 30, a further 10 locusts were placed on the same traps. Thus, each trap had three cohorts of locusts that were transferred 1, 15 and 30 d after nematode inoculation. The timeline is shown in Figure 2. As a control, 10 locusts were transferred to the trap systems with no nematodes applied.

In addition to the effectiveness of *H. bacteriophora* HBH against the migratory locust, locust-free traps were used to detect persistence of the nematodes on the fabric. The mortality of the nematodes (persistence) in the traps was recorded 1, 15 and 30 d after inoculation. All experiments were repeated three times under laboratory conditions at 25ºC in the Nematology Laboratory in Department of Plant Protection, Faculty of Agriculture, Bursa Uludağ University, Turkey in 2018.

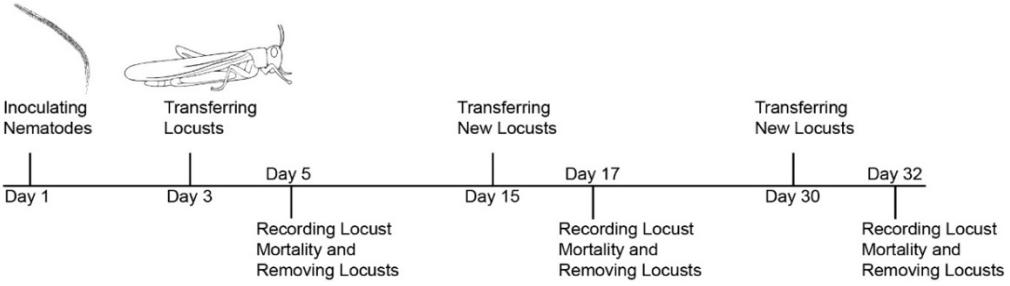

Figure 2. Trap experiment timeline.

Statistical analysis

Statistical differences of the mortality of EPNs were analyzed using one-way ANOVA with JMP®7.0 software. LSD test ($P < 0.05$) was used to determine the difference between means.

Results and Discussion

Infectivity of *Heterorhabditis bacteriophora* HBH against *Locusta migratoria*

The highest mortality of *L. migratoria* was recorded on day 3 of the experiment with 50000 IJs per trap. The effect of 25000 and 50000 IJs on day 17 was not statistically significant. On day 32, the highest mortality of *L. migratoria* was detected with 50000 IJs. On day 1, the effect of 5000 IJs was not statistically significant compared to 25000 IJs, but it was significantly lower than other doses on days 17 and 32 ($F = 35.3$, $df = 11; 24$, $P > 0.0001$).

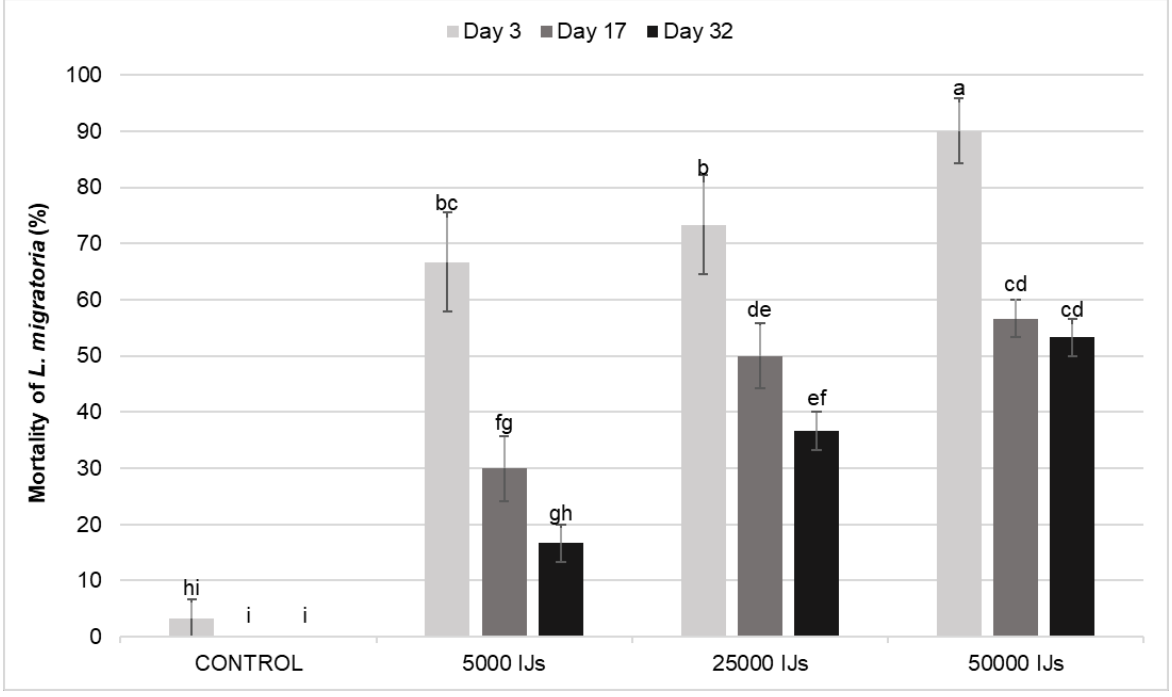

Figure 3. Mortality of *Locusta migratoria* in the doses of 5000, 25000 and 50000 IJs of *Heterorhabditis bacteriophora* HBH strain on days 3, 17 and 32 ($F = 35.3$; $df = 11, 24$; $P > 0.0001$).

Persistence of *Heterorhabditis bacteriophora* HBH on the traps

On day 1, the mortality (persistence) of IJs of *H. bacteriophora* HBH was not significantly different between inoculation rates. On day 15, the mortality of *H. bacteriophora* HBH with 25000 IJs dose was statistically higher than with 50000 IJs. On day 30, it was the opposite of this situation. The mortality of *H. bacteriophora* HBH with 5000 IJs was not significantly different from the other doses at any assessment time. The results of the persistence experiment are given in Figure 4 ($F = 66.7$; $df = 8, 18$; $P > 0.0001$). As expected, dead individuals increased over time. However, peak of the mortality was mostly below 40% of the population after 30 days, which meant that persistence of the nematode at the end of the experiment was nearly 60%. This result indicates that the new trap was effective persistence of the nematode over an extended period.

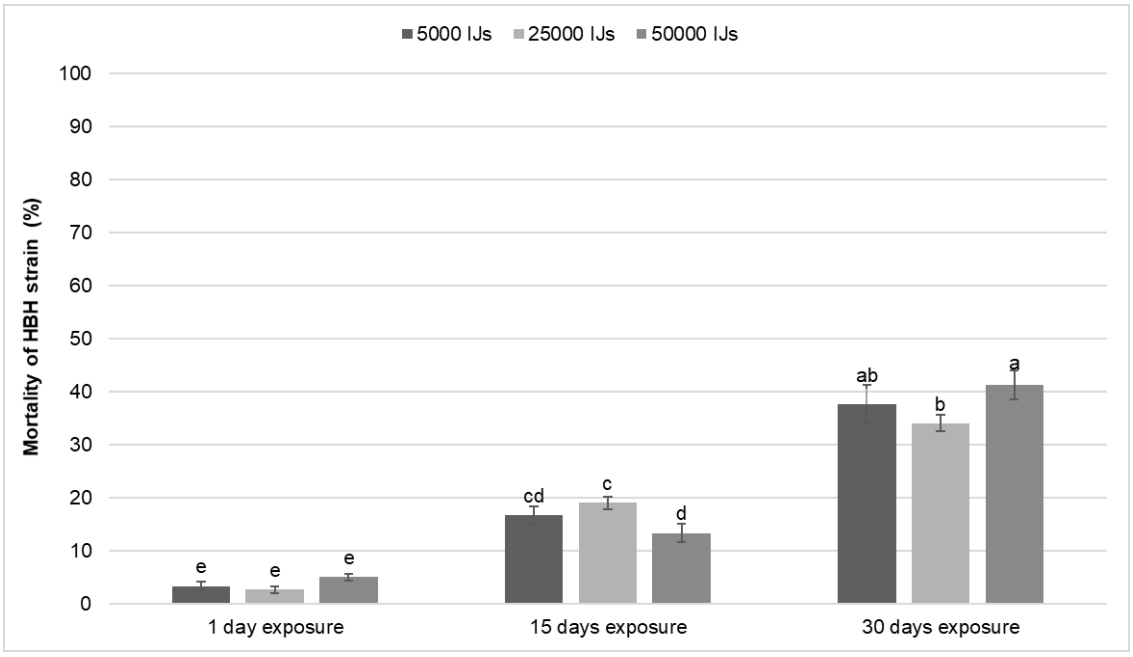

Figure 4. Mortality of *Heterorhabditis bacteriophora* HBH strain in the doses of 5000, 25000 and 50000 IJs per trap (F = 66.7; df = 8, 18; P > 0.0001).

Although there is a demand for foliar applications, EPNs are rarely used against above-ground insects because of the inability to achieve the desired success above-ground (Arthurs et al., 2004). For above-ground application, various formulations have been developed to protect EPNs from ultraviolet light and to prevent desiccation (Georgis et al., 2006; Beck et al., 2013). However, the effect of these formulations lasts only a few hours or days and the EPNs rapidly lose their effectiveness (Glazer, 2002; Arthurs et al., 2004; Schroer & Ehlers, 2005). The most critical feature of the trap system used in this experiment is the maintenance of humidity which allows it to provide a sustained effect of EPNs in above-ground application over an extended period.

In this study, the moist environment, which is necessary for EPNs, was maintained using hydrophilic cotton fabric. In several other studies, it was found that some additives usually enhanced the persistence and effectiveness of EPNs on foliage, but this improvement was insufficient (Baur et al., 1997; Schroer & Ehlers, 2005). Many laboratory studies have been conducted to test the persistence (mortality) of EPNs above ground. These demonstrated that persistence of EPNs lasted only for hours rather than days (Schroer & Ehlers, 2005). However, in the present study, the persistence of *H. bacteriophora* HBH strain extended for over 4 weeks. By day 30, the mortality of *H. bacteriophora* had reached about 40%, however, might be possible to improve persistence using better fabrics or having a larger fluid reservoir.

The method of storing and transporting EPNs in a polyether-polyurethane sponge-based formulation inspired for this work. EPNs require a thin layer of water for movement and cannot move effectively under waterlogged conditions (Grewal, 2002). The cotton fabric selected for this work provided a thin layer of water to facilitate nematode movement and also maintained a moist environment. In a study to improve the efficacy of entomopathogenic nematodes for above-ground application, Dito et al. (2016) used a protective gel covers that lasted for 8 h. In some other studies, *Steinernema feltiae* (Filipjev, 1934) was used weekly against damaging above-ground pests, *Thrips tabaci* Lindeman, 1889 and *Trialeurodes vaporariorum* Westwood 1856 (Trdan et al., 2007; Laznik et al., 2011; Beck et al., 2015).

Steinernema carpocapsae (Weiser, 1955) is considered to be more suitable for foliar application than *H. bacteriophora* because the former is more resistant to desiccation, inclined to stay in the upper soil layers

and has an ambusher host-finding strategy (Salame & Glazer, 2015). Although *H. bacteriophora* has a less active host-finding strategy and is prone to go deeper into the soil (Grewal et al., 1994), it is evident from the current study that *H. bacteriophora* can also be used for above-ground application using hydrophilic cotton fabric. Also, the chemical-physical nature of the soil may not be suitable for EPNs when they are applied inundatively (Kaya, 1990; Barbercheck, 1992; Koppenhöfer & Fuzy, 2006), so deployment of EPNs using this trap system would avoid this problem.

With the method developed in this study, *H. bacteriophora* HBH strain remained active for over 4 weeks on an above-ground surface and infected *L. migratoria*. For more realistic results, the trap system needs to be tested under field conditions, which is a more challenging environment then laboratory. Our trap system serves as a basic prototype for initial assessment, but could be modified using different fabrics, trap shapes, and different substances to protect EPNs or attract hosts. Through evaluating such modifications, application of EPNs with a trap system has a reasonable likelihood of becoming an effective option for field applications.

Conclusions

The trap system developed in this study enabled *H. bacteriophora* HBH strain to be successfully used against the *L. migratoria* above ground under laboratory conditions, with the infectivity and persistence were maintained for more than 4 weeks. In order to be successful in the field, a fabric must be selected that protects the EPNs from ultraviolet radiation and slows evaporation. In addition, enhancements such as pheromone or color could be combined with the fabric to attract target pests to the traps. Through this approach, more economical control might be achieved without the need to apply the EPNs over a large area. The findings presented here indicate that this method could be a solution to rapid desiccation of EPNs applied above ground, if confirmed by field studies.

Acknowledgments

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

References

Arthurs, S., K. M. Heinz & J. R. Prasifka, 2004. An analysis of using entomopathogenic nematodes against above-ground pests. Bulletin of Entomological Research, 94 (4): 297-306.

Barbercheck, M. E., 1992. Effect of soil physical factors on biological control agents of soil insect pests. Florida Entomological, 75: 539-548.

Baur, M. E., H. K. Kaya, R. Gaugler & B. Tabashnik, 1997. Effects of adjuvants on entomopathogenic nematode persistence and efficacy against *Plutella xylostella*. Biocontrol Science and Technology, 7: 513-525.

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: 507-520.

Beck, B., P. Spanoghe, M. Moens, S. Pollet, F. Temmerman & D. Nuyttens, 2015. Foliar-applied entomopathogenic nematodes *Steinernema feltiae* are not suitable for controlling *Thrips tabaci* in leek. Bulletin of Insectology, 68 (2): 287-298.

Cross, J. V., M. G. Solomon, D. Chandler, P. Jarrett, P. N. Richardson, D. Winstanley, H. Bathon, J. Huber, B. Keller, G. A. Langenbruch & G. Zimmerman, 1999. Biocontrol of pests of apples and pears in Northern and Central Europe: 1. Microbial agents and nematodes. Biocontrol Science and Technology, 9: 125-149.

Dito, D. F., D. I. Shapiro-Ilan, C. A. Dunlap, R. W. Behle & E. E. Lewis, 2016. Enhanced biological control potential of the entomopathogenic nematode, *Steinernema carpocapsae*, applied with a protective gel formulation. Biocontrol Science and Technology, 26 (6): 835-848.

Ehlers, R. U., 1996. Current and future use of nematodes in biocontrol: practice and commercial aspects with regard to regulatory policy issues. Biocontrol Science and Technology, 6 (3): 303-316.

Gaugler, R., 2002. Entomopathogenic Nematology. CABI Publishing, Wallingford, UK, 394 pp.

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.

Glazer, I., 2002. "Survival Biology, 169-187". In: Entomopathogenic Nematology (Ed. R. Gaugler). CABI Publishing, Oxon, UK, 394 pp.

Grewal, P. S., 2002. "Formulation and Application Technology, 265-287". In: Entomopathogenic Nematology (Ed. R. Gaugler). CABI Publishing, Oxon, UK, 394 pp.

Grewal, P. S., E. E. Lewis, R. Gaugler & J. F. Campbell, 1994. Host finding behavior as a predictor of foraging strategy in entomopathogenic nematodes. Parasitology, 108 (2): 207-215.

Kaya, H. K., 1990. "Soil Ecology, 93-111". In: Entomopathogenic Nematodes in Biological Control (Eds. R. Gaugler & H. K. Kaya). CRC Press, Boca Raton, FL, USA, 381 pp.

Kaya, H. K. & S. P. Stock, 1997. "Techniques in Insect Nematology, 281-324". In: Manual of Techniques in Insect Pathology (Ed. L. A. Lacey). Academic Press, San Diego, CA, USA, 409 pp.

Koppenhöfer, A. M. & E. M. Fuzy, 2006. Effect of soil type on infectivity and persistence of the entomopathogenic nematodes *Steinernema scarabaei*, *Steinernema glaseri*, *Heterorhabditis zealandica*, and *Heterorhabditis bacteriophora*. Journal of Invertebrate Pathology, 92 (1): 11-22.

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: 121-144.

Laznik, Ž., D. Znidarcic & S. Trdan, 2011. Control of *Trialeurodes vaporariorum* (Westwood) adults on glasshouse-grown cucumbers in four different growth substrates: an efficacy comparison of foliar application of *Steinernema feltiae* (Filipjev) and spraying with thiamethoxam. Turkish Journal of Agriculture and Forestry, 35 (6): 631-640.

Lewis, E.E. & D.J. Clarke, 2012. "Nematode parasites and entomopathogens, 395-424". In: Insect Pathology (Ed. F. E. Vega & H. K. Kaya). Academic Press, Amsterdam, The Netherlands, 508 pp.

Ma, J., X. Han, Hasibagan, C. Wang, Y. Zhang, J. Tang, Z. Xie & T. Deveson, 2005. Monitoring East Asian migratory locust plagues using remote sensing data and field investigations. International Journal of Remote Sensing, 26 (3): 629-634.

Rai, M. M. & P. H. Chavhan, 2015. Effect of weed plant extracts on migratory locust, *Locusta migratoria* (R & F). International Journal of Pharmacology and Biological Sciences, 9 (1): 27-32.

Rai, M. M., D. G. Gore, M. K. Rathod & A. M. Khurad, 2013. Evidence of transovarial transmission of *Bacillus subtilis* in the silkworm, *Bombyx mori* L. Journal of Pharmacy Research, 7 (4): 318-323.

Salame, L. & I. Glazer, 2015. Stress avoidance: vertical movement of entomopathogenic nematodes in response to soil moisture gradient. Phytoparasitica, 43: 647-655.

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., R. Han & X. Qiu, 2014. "Production of Entomopathogenic Nematodes, 321-356". In: Mass Production of Beneficial Organisms: Invertebrates and Entomopathogens (Eds. J. A. Morales-Ramos, M. G. Rojas, D. I. Shapiro-Ilan). Academic Press/Elsevier, Waltham, MA, USA, 742 pp.

Showler, A. T., 1995. "Desert Locust Control, Public Health, and Environmental Sustainability in North Africa, 217-239". In: The North African Environment at Risk (Eds. W. D. Swearingen & A. Bencherifa), West view Press, Boulder, CO., USA., 415 pp.

Trdan, S., D. Žnidaračič & M. Vidrih, 2007. Control of *Frankliniella occidentalis* on greenhouse-grown cucumbers: an efficacy comparison of foliar application of *Steinernema feltiae* and spraying with abamectin. Russian Journal of Nematology, 15 (1): 25-34.

Vos, J. G., E. Dybing, H. A. Greim, O. Ladefoged, C. Lambre, J. V. Tarazona, I. Brandt & A. D. Vethaak, 2000. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Critical Reviews in Toxicology, 30 (1): 71-133.

Wang, Y., Q. Huang, X. Zhang & B. Ren, 2014. Application of insect songs in monitoring population density level of *Locusta migratoria* migratoria (Orthoptera: Acrididae). Zoological Studies, 53 (1):55 (1-7).

White, G. F., 1927. A Method for obtaining infective nematode larvae from culture. Science, 66: 302-303.

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.

Zhang, D. X., L. N. Yan, Y. J. Ji, G. M. Hewitt & Z. S. Huang, 2009. Unexpected relationships of substructured populations in Chinese *Locusta migratoria*. BMC Evolutionary Biology, 9 (1): 144-155.