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Research Article

The Control of Turkestan Cockroach *Blatta lateralis* (Dictyoptera: Blattidae) by The Entomopathogenic nematode *Heterorhabditis bacteriophora* HBH (Rhabditida: Heterorhabditidae) Using Hydrophilic Fabric Trap

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Abstract

Chemical insecticides used against cockroaches, which are an important urban pest and considered public health, are harmful to human health and cause insects to gain resistance. The entomopathogenic nematode (EPN), *Heterorhabditis bacteriophora* HBH, were used in place of chemical insecticides within the scope of biological control against the Turkestan cockroaches *Blatta lateralis* in this study. The hydrophilic fabric traps were set to provide the moist environment needed by the EPNs on aboveground. The fabrics inoculated with the nematodes at 50, 100 and 150 IJs/cm^2 were used throughout the 37-day experiment. The first treatment was performed by adding 10 adult cockroaches immediately after the establishment of the traps. In the same way, the second treatment was applied after 15 days and the third treatment after 30 days. The mortality rates of cockroaches after 4 and 7 days of exposure to EPNs were determined for all treatments. Although Turkestan cockroaches were exposed to HBH 30 days after the setting of the traps, infection occurred. As a result of this study, the above-ground persistence and infectivity of HBH could be achieved more than 4 weeks by using hydrophilic fabric. In addition, the mortality rates of the Turkestan cockroaches were found to increase depending on exposure time and the nematode dosage.

Key Words: *Blatta lateralis*, entomopathogenic nematode, hydrophilic fabric.

Türkistan Hamamböceği *Blatta lateralis* (Dictyoptera: Blattidae)'in Hidrofil Kumaş Kullanılarak Entomopatojen Nematod *Heterorhabditis bacteriophora* HBH (Rhabditida: Heterorhabditidae) ile Mücadelesi

Özet

Halk sağlığı açısından önemli bir zararlı olan hamam böceklerine karşı kullanılan kimyasal ilaçlar insan sağlığına zararlı olup böceklerin direnç kazanmasına neden olmaktadır. Bu çalışmada biyolojik mücadele kapsamında kimyasal insektisitlerin yerine kullanılan entomopatojen nematod (EPN) (*Heterorhabditis bacteriophora* HBH) Türkistan hamam böceğine (*Blatta lateralis*) karşı uygulanmıştır. Toprak üstünde, EPN'lerin ihtiyaç duyduğu nemli ortamı sağlayabilmek amacıyla hidrofil kumaş tuzağı kurulmuştur. 50, 100 ve 150 IJ/cm^2 dozajında EPN uygulanan kumaşlar, 37 gün süren deneme boyunca değiştirilmeden kullanılmıştır. İlk uygulama, tuzakların kurulmasından hemen sonra 10 tane *B. lateralis* ergini eklenerek yapılmıştır. Benzer şekilde, ikinci uygulama 15 gün, üçüncü uygulama ise 30 gün sonra başlatılmıştır. Tüm uygulamalarda, *B. lateralis* erginleri EPN'lere 4 ve 7 gün maruz kaldıktan sonra hamamböceklerinin ölüm oranları belirlenmiştir. Türkistan hamamböceği tuzakların kurulmasından 30 gün sonra HBH'ye maruz kaldığında bile enfeksiyon meydana gelmiştir. Bu çalışma sonucunda, hidrofil kumaş kullanılarak hibrit HBH ırkının topraküstü kalıcılığı ve infektivitesi 4 haftadan daha uzun süre sağlanabilmiştir. Ek olarak, hamamböceği ölüm oranlarının maruz kalma süresine ve HBH dozuna bağlı olarak arttığı tespit edilmiştir.

Anahtar Kelimeler: *Blatta lateralis*, entomopatojen nematod, hidrofil kumaş.

Introduction

Cockroaches are a member of the phylum Arthropoda and belong to class Insecta, order Dictyoptera. More than 4500 species of cockroaches have been identified throughout the World (Hashemi-Aghdam and Oshaghi, 2015). Due to the favour of humidity and temperature, they are abundant in urban places, restaurants, food stores, bathrooms and kitchens (Nedelchev et al., 2013; Mahmoud, 2013). They can contaminate human foods with pathogenic organisms and can cause asthma, especially in children (Sohn and Kim, 2012; Hashemi-Aghdam and Oshaghi, 2015). Moreover, cockroach wastes such as faeces, salivas and cast skins can cause severe allergic symptoms (Sookrung and Chaicumpa, 2010). Chemical compounds such as fipronil, sulfonamide and imidacloprid, which are toxic to human health, are used to control cockroaches (Cutler et al., 2017). However, the use of the chemicals has been avoided in recent years because of increasing resistance to the common insecticides (Ko et al., 2016; Cutler et al., 2017). The importance of alternative methods such as biological control, which replaces the chemicals that are harmful to the environment, continues to increase (El-Kady et al., 2014; Cutler et al. 2017). Entomopathogenic nematodes (EPNs) used in place of chemical pesticides within the scope of biological control have been reported to be effective in the control of cockroaches (Morton and García-del-Pino, 2013; Cutler et al., 2017). EPNs belonging to the families Steinernematidae and Heterorhabitidae are effective biological control agents (Lacey and Shapiro-Ilan, 2008). They enter the insects' hemolymph through natural openings. The EPNs then cause the insects to die of septicaemia within 24 to 48 hours due to their symbiotic relationship with the bacteria, *Xenorhabdus* spp. and *Photorhabdus* spp. (Stock and Blair, 2008). The

EPNs inhabit naturally in soil and mainly used against soil borne insects (Wright et al., 2005). But they have the potential to control foliage-feeding and urban insect pests that inhabit above ground, such as cockroaches (Morton and García-del-Pino, 2013; Şahin et al., 2018). The spectrum of insects that are vulnerable to the EPNs is very broad (Ahmad et al., 2010; Şahin et al., 2018). Seventeen orders and 135 families of insects are known to be susceptible to the EPNs both in the field and under laboratory conditions (El-Kady et al., 2014).

Various bait techniques (Maketon et al., 2010) and gel formulations have been developed to control aboveground insect pests by EPNs (Schroer and Ehlers, 2005; Georgis et al., 2006; Beck et al., 2013). However, temperature, ultraviolet radiation and humidity are the restricting factors for EPNs in controlling pests above ground. The current EPN formulations lose their moisture within a few days and cannot support the humid condition that needs for EPN survival (Georgis et al., 2006; Lacey and Georgis, 2012). Some anti-desiccants such as rimulgan and xanthan added in these formulations could improve EPN effectiveness and persistence on foliage, but enhancements are found to be insufficient to recommend for use (Schroer and Ehlers, 2005). Recently, moisture requirement for EPNs has been provided by using hydrophilic fabric trap and aboveground persistence of the EPNs was sustained over four weeks against migratory locust, *Locusta migratoria* (Orthoptera: Acrididae) (Şahin et al., 2018).

In this study, it is aimed to control the invasive Turkestan cockroach, *Blatta lateralis* (Walker) (Dictyoptera: Blattidae) (Kim and Rust, 2013) by using fabric trap containing EPN. The hybrid HBH strain of EPN *Heterorhabditis bacteriophora* was used in the study. The fabric trap wasused to provide a moist environment which is both preferred by cockroach and EPNs.

Material and Methods

The cockroach and entomopathogenic nematode

The HBH is the hybrid strain of *H. bacteriophora* and it was obtained by hybridization of Turkish local *H. bacteriophora*

isolates from distinctive climatic zones of Turkey. It was patented by us, because of their high efficiency, long persistence and high breeding capacity (TPMK Patent No: TR 2013 06141 B). The 2 or 3-day old infective juveniles (IJs) of the strain that was obtained by in vivo production were used. The last instar larvae of great wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) were used as a host for infection at 25ºC (McMullen and Stock, 2014).
The adults of Turkestan cockroach, *Blatta lateralis* (Walker) (Dictyoptera: Blattidae), were acquired from the local company (Mira Canlı Hayvan Böcek Tur. İnş. Tarım Tic. San. Ltd. Şti.) in Antalya-Turkey. The life span of the cockroach was reported to be more than 100 days (Kim and Rust, 2013).

Experimental design

The fabric trap system designed by Şahin et al. (2018) was also used inthis study. The fabric was inoculated with the IJs in three different dosages; 50, 100 and 150 IJs/cm^2 and kept 37 days for treatments at 25 °C in dark and 70% relative humudity. This experiment was carried out in box with dimension of 40x30x30 cm (lenght x widht x hight). No further IJs additions were made to the traps after first inoculation throughout the 37 days of treatments. Only distilled water without IJs was used in the control trap.

In the 1st treatment, 10 adult cockroaches were added in the chamber trap for the infection. The mortality of the cockroaches was then determined after 4 and 7 days of exposure to the IJs. After 7 days of exposure, all the cockroaches were removed from the trap and the dead cockroaches were dissected to observe IJs development (Figure 1).

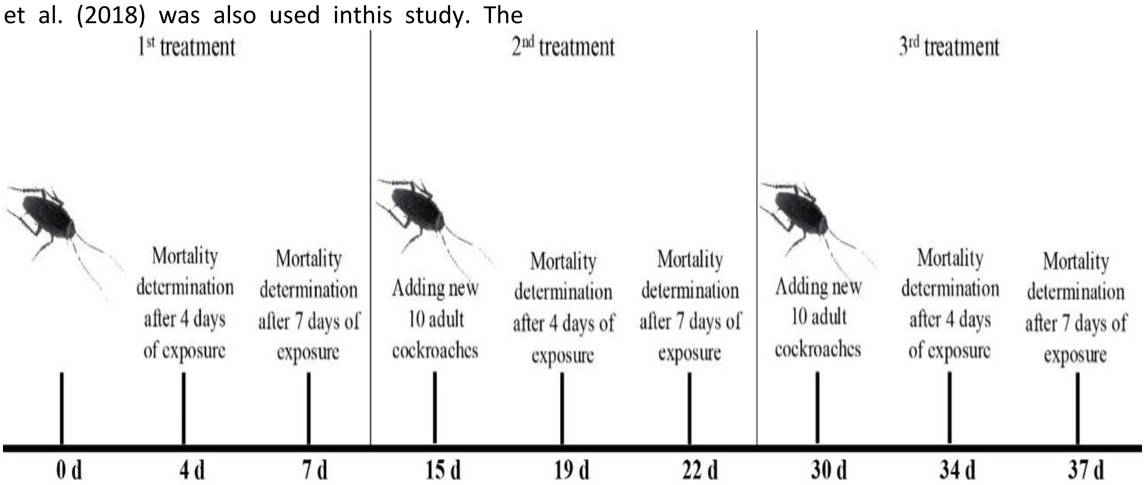

Figure 1. The timeline of the experiment procedure between 0 and 37 days (d).

In the 2nd treatment, 10 new cockroaches were replaced on 15th day after inoculation. The mortality of the cockroaches was then determined after 4 and 7 days of exposure to the IJs. After the 7 days of exposure, all insects were removed from the trap and the dead cockroaches were dissected to observe IJ development (Figure 1). In the 3rd treatment, 10 new cockroaches were replaced on 30th day after inoculation. The mortality of the cockroaches was then determined after 4 and 7

days of exposure to the IJs. After the 7 days of exposure, all insects were removed from the trap and the dead cockroaches were dissected to observe IJs development (Figure 1).

Statistical analysis

All treatments were repeated 5 times and statistical differences in the mortality of the adult cockroaches were detected by using one-way analysis of variance (ANOVA) in JMP®7.0 software. The LSD (Least Significant Differences) test ($P<0.05$) was used to determine the difference between means.

Results

On 4-day exposure of the 1st treatment, the mortality rate of the cockroaches at 150 IJs/cm^{2}was 32%. The mortality at 50 IJs/cm^2 was 16%. Differences between 50 and 150

IJs/cm^2 doses were statistically significant. However, mortality of 100 IJs/cm^2 was not significant with the mortalities at 50 and 150 IJs/cm^2. Moreover, on 7-day exposure of the 1st treatment, the highest mortality rate was detected at 150 IJs/cm^2 as 92%, and the mortality rates at 50 and 100 IJs/cm^2 were 60% and 76%, respectively. Differences of the mortalities among the dosages were statistically significant. The lowest mortality was observed in control at both periods of exposure (4 and 7 days of exposure) in the 1st treatment (Figure 2). The mortality at all dosages (50, 100 and 150 IJs/cm^2) in the 1st treatment was found statically higher than the dosages of the 3rd treatments for both 4 and 7 days of exposure. On 4-day exposure of the 2nd treatment, the statistically highest mortality was detected at 150 IJs/cm^2 as 24%. Whereas, the mortality rates of 50 and 100 IJs/cm^2 were statistically similiar and their rates were 6% and 12%, respectively. The mortality rate of 50 IJs/cm^2 dosage was statistically similiar with the control. In the 7-day exposure, the highest mortality rate was detected as 84% at 150 IJs/cm^2. However, the lowest mortality was observed at 50 IJs/cm^2 as 52%. The differences of the mortality rates were significantly important (Figure 2).

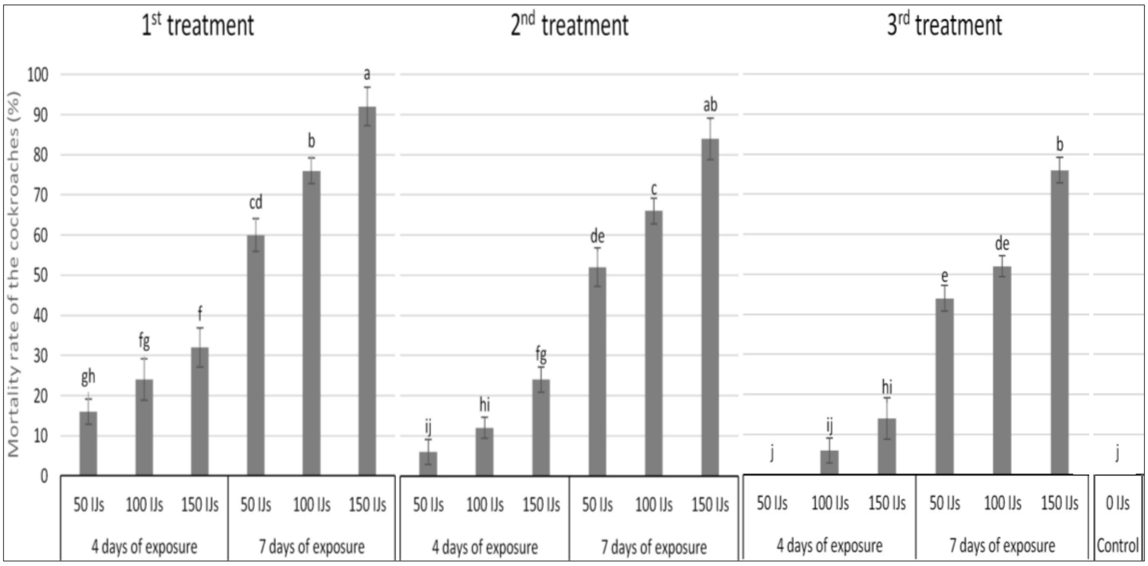

Figure 2. Mortality rates of *B. lateralis* in all treatments after 4 and 7 days of exposure to the HBH with three different dosages (50, 100 and 150 IJs/cm^2). The control covers both periods of exposure (4 and 7 days of exposure) in all treatments ($F=113.0370$, $df=18,76$, $P < 0.0001$).

On 4-day exposure of the 3rd treatment, the statistically highest mortality was detected at 150 IJs/cm^2 as 14%. The mortality rates of the 50 and 100 IJs/cm^2 dosages were 0% and 6% respectively, and were statistically similiar with the control. In the 7-day exposure, the highest mortality was obtained at 150 IJs/cm^{2}as 76%. The mortality rates at 50 and 150 IJs/cm^2 were 44% and 52%, respectively, and the differences of the mortalities among the dosages were not statistically significant (Figure 2). The mortality at all dosages (50, 100 and 150 IJs/cm^2) in the 1st treatment was found statically higher than the 3rd treatments for both 4 and 7 days of exposure.

Discussion and Conclusion

The EPNs were used more successfully against soil-born insect pests (Wright et al., 2005), because of some deterrent environmental factors such as moistureless and UV light that inhibit EPN activity on above ground (Georgis et al., 2006; Lacey and Georgis, 2012). Formulations that have been developed to increase EPN persistence and effectiveness aboveground are promising, however, they have not achieved the desired success (Schroer and Ehlers, 2005; Georgis et al., 2006). Many laboratory studies to test the persistence of EPNs aboveground have shown that their persistence lasts for hours rather than days due to moistureless (Şahin et al., 2018). In this study, the hybrid HBH strain of *Heterorhabditis bacteriophora*, were used against Turkestan cockroach (*Blatta lateralis*) adults to determine the mortality rates. Because of these negative effects in aboveground treatments, the hydrophilic fabric embodiment was set for controlling of *B. lateralis*. In controlling of invasive cricket *Locusta migratoria*, Şahin et al. (2018) has recently applied EPNs on a hydrophilic fabric to provide a moist environment. Thus, EPN persistence sustained over four weeks. Similarly, the above ground persistence of the EPNs was maintained over four weeks in this study, by using the trap designed by Şahin et al. (2018). In the 3rd treatment, when 10 adult cockroaches were added even 30 days after the setting the traps, the HBH strain of *H. bacteriophora* were able to infect the Turkestan cockroaches depending on the dosage and duration of exposure. But the mortality at all dosages (50, 100 and 150 IJs/cm^2) in the 1st treatment was found statically higher than the dosages of the 3rd treatments for both 4 and 7 days of exposure.

In all treatments in this research, the cockroach mortality was increased with the exposure time increased from 4 days to 7 days at all EPN dosages. Similarly, in some studies, cockroach mortalities have risen as the duration of exposure to EPNs was extended (Maketon et al., 2010; Baker et al., 2012; Morton and García-del-Pino, 2013; El-Kady et al., 2014). For example, Cutler et al. (2017) inoculated the *Blaptica dubia* with the EPN combination (*Heterorhabditis* spp. and *Steinernema* spp.) and then, the cockroach deaths began to occur after 6 days of exposure to EPNs. On 4-day exposure of the 1st treatment, the mortality rate of the Turkestan cockroaches at 150 IJs/cm^2 dosage was 32%. The mortality of 50 IJs/cm^2 was 16%. Likewise, Baker et al. (2012) used the *Heterorhabditis bacteriophora* to control German cockroaches *Blatilla germanica* over the exposure times of 1, 2 and 3 days. The mortality rate of the insects infected by the *H. bacteriophora* reached 20% within 3 days. Moreover, in the study of El-Kady et al. (2014), German cockroach (*Blattella germanica*) adults were exposed to the EPNs (*Steinernema carpocapsae*) for 4 days, as the dosage and exposure time increased, the mortality rate of the cockroaches increased with a similar rate to researches that were conducted by Baker et al. (2012) and Maketon et al. (2010). The increased exposure duration affects mortality, making the persistence of EPNs aboveground more important in cockroach control. Since the use of hydrophilic fabric provides a moist environment, the persistence of HBH has been sustained for more than 4 weeks aboveground in this study. Although Turkestan cockroaches were exposed to HBH on 15th and 30th days (2nd and 3rd treatments) after the first inoculation, the EPNs were still able to infect and the mortality rates were detected as 84% and 76% respectively for 7 days exposure, at 150 IJs/cm^2. It may be more effective than insecticides to use hydrophilic fabrics inoculated with EPNs against cockroaches, especially indoor environments such as warehouses, because cockroaches prefer warm, humid and dim environments. Further studies should be conducted to increase the effectiveness of EPN.

References

Ahmad, R., Hussain, M.A., Shaheen, A., Ali, S.S. 2010. Susceptibility of 23 agriculturally important insect pests to entomopathogenic nematode, *Steinernema masoodi* (Rhabditida: Steinernematidae). *International Journal of Nematology*, 20(2): 157-161.

Baker, N.R., Ali, H.B., Gowen, S. 2012. Reproduction of Entomopathogenic Nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* on the German Cockroach *Blatilla germanica* at Different Temperatures. *Iraqi Journal of Science*, 53(3): 505-512.

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

Cutler, J., Hughes, K., Rae, R. 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., El-Bahrawy, A.F., El-Sharabasy, H.M., El-Badry, Y.S., El-Ashry, R.M. A., Mahmoud, M.F. 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.

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

Hashemi-Aghdam, S.S., Oshaghi, M.A. 2015. A checklist of Iranian cockroaches (Blattodea) with description of *Polyphaga* sp. as a new species in Iran. *Journal of Arthropod-borne Diseases*, 9(2): 161-175.

Kim, T., Rust, M.K. 2013. Life history and biology of the invasive Turkestan cockroach (Dictyoptera: Blattidae). *Journal of Economic Entomology*, 106(6): 2428-2432.

Ko, A.E., Bieman, D.N., Schal, C., Silverman, J. 2016. Insecticide resistance and diminished secondary kill performance of bait formulations against German cockroaches (Dictyoptera: Blattellidae). *Pest Management Science*, 72(9): 1778-1784.

Lacey, L.A., Georgis, R. 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., Shapiro-Ilan, D.I. 2008. Microbial control of insect pests in temperate orchard systems: potential for incorporation into IPM. *Annu. Rev. Entomol*., 53: 121-144.

Mahmoud, M.F. 2013. Ecological investigation, density, infestation rate and control strategy of German cockroach, *Blattella germanica* (L.) in two hospitals in Ismailia, Egypt. *Arthropods*, 2(4): 216-224.

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

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

Morton, A., García-del-Pino, F. 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.

Nedelchev, S., Pilarska, D., Takov, D., Golemansky, V. 2013. Protozoan and Nematode Parasites of the American Coakroach *Periplaneta americana* (L.) from Bulgaria. *Acta Zoologica Bulgarica*, 65: 403-408.

Schroer, S., Ehlers, R.U. 2005. Foliar application of the entomopathogenic nematode *Steinernema carpocapsae* for biological control of diamondback moth larvae (*Plutella xylostella*). *Biological Control*, 33(1): 81-86.

Sohn, M.H., Kim, K.E. 2012. The cockroach and allergic diseases. *Allergy, asthma & immunology research*, 4(5): 264-269.

Sookrung, N., Chaicumpa, W. 2010. A revisit to cockroach allergens. *Asian Pacific journal of allergy and immunology*, 28(2-3): 95-106.

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

Şahin, Y.S., Bouchari, A., Ulu, T.C., Sadiç, B., Susurluk, A. 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.

Wright, D., Peters A., Schroer, S., Fife, J. 2005. *Nematodes as Biocontrol Agents*. In P. S. Grewal, R. U. Ehlers, & D. I. Shapiro-Ilan (Eds.), Application Technology. CABI Publishing, Wallingford, UK. pp. 91-106.9.