Orijinal araştırma (Original article)

Assessment of the susceptibility of the Turkestan cockroach, *Blatta lateralis* to Turkish isolates of entomopathogenic nematodes

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Türkistan hamamböceği *Blatta lateralis*'in Türk entomopatojen nematod izolatlarına olan hassasiyetinin belirlenmesi

Oz: Bu çalışmada iki yerel entomopatojen nematod izolatının (Steinernema feltiae-KV06 (Filipjev) (Rhabditida: Steinernematidae) ve Heterorhabditis bacteriophora-EO7 (Rhabditida: Heterorhabtidae) Blatta lateralis Walker (Blattodea: Blattidae)'in biyolojik mücadelesindeki kullanım olanakları araştırılmıştır. Çalışmada kullanılan izolatlar Ankara ili Kelebekler Vadisi ve Eğriova Tabiat Parklarından elde edilmiştir. İzolatlar, 9cm çapındaki petri kutularına bırakılan B. lateralis nimflerine 100, 250, 500 ve 1000 IJ/cm² dozlarında uygulanmıştır. B. lateralis nimflerindeki ölümler, uygulamadan 2, 4, 6, 8, 10, 12 ve 14gün sonra kaydedilmistir. 1000 IJ/cm²'de, S. feltiae-KV06 ve H. bacteriophora-EO7'nın, LT₅₀ değerleri 2.3 ve 2.6 gün ve LT₉₀ değerleri ile sırasıyla 6.6 ve 7.9 gün ile en yüksek ölüm oranına (% 100.0 \pm 0.0) neden olmuştur. Her iki izolattaki en düşük ölüm yüzdesi, 2 gün maruziyetten sonra 100IJs/cm²¹de belirlenmiştir (S. feltiae-KV06: 0.6±1.4 ve H. bacteriophora-EO7: 0.0±0.0). Farklı zaman aralıklarındaki LC_{50.90} değerleri her iki izolat arasında gözardı edilebilecek düzeyde farklılık göstermekle birlikte, S. feltiae-KV06'nın B. lateralis nimflerine karşı H. bacteriophora-EO7'dan daha etkili olduğu görülmektedir. Bununla birlikte, her iki izolatın da farklı sıcaklık, nem ve beton, parke ve fayans gibi farklı yüzeylerde de zararlı üzerindeki etkinliklerinin test edilmesi gerekmektedir.

Anahtar sözcükler: Blatta lateralis, entomopatojen nematod, biyolojik mücadele.

Abstract: Two Turkish entomopathogenic nematodes isolates, *Steinernema feltiae*-KV06 (Filipjev) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora*-EO7 (Rhabditida: Heterorhabtidae), were tested against the cockroach, *Blatta lateralis* Walker (Blattodea: Blattidae) to determine their potential for its control. The isolates were obtained

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Assessment of the susceptibility of *Blatta lateralis* to entomopathogenic nematodes from Kelebekler Vadisi and Eğriova Nature Parks in Ankara Province, Turkey. These isolates were applied at concentrations of 100, 250, 500 and 1000 IJ/cm² to *B. lateralis* nymphs in 9cm diameter Petri dishes. The mortalities were recorded at 2, 4, 6, 8, 10, 12 and 14 days after application. At 1000 IJs/cm², *S. feltiae*-KV06 and *H. bacteriophora*-EO7 caused the highest mortality (100.0 \pm 0.0%) with an LT₅₀ of 2.3 and 2.6 days, respectively, and an LT₉₀ of 6.6 and 7.9 days, respectively. The lowest mortality rate caused by both species was at 100IJs/cm² after 2 days of exposure (*S. feltiae*-KV06: 0.6 \pm 1.4; *H. bacteriophora*-EO7: 0.0 \pm 0.0). There was no significant difference in the efficacy of the isolates at all concentrations and for all time periods. The effectiveness of both isolates against this pest should be tested at different temperatures and humidities and on different surfaces such as ceramic, concrete and parquet.

Key words: Blatta lateralis, entomopathogenic nematode, biological control.

Introduction

The Turkestan cockroach, *Blatta lateralis* Walker (Blattodea: Blattidae), also known as the rusty red cockroach or red runner cockroach, has existed for about 360 million years (Shahraki et al, 2013). Although *B. lateralis* is native to the Middle East and Central Asia (Alesho, 1997), it has become a major invasive species across the world (Kim & Rust, 2013).

The majority of urban areas are infested with various populations of cockroaches. Their presence and abundance in public places such as schools, hospitals and restaurants make them a major threat to human health. Cockroaches in general, and in particular B. lateralis, not only contaminate food but also transmit a number of diseases and cause allergic reactions and psychological distress (Brenner, 1995; Shahraki et al, 2013). Also, there is a risk that *B. lateralis* will cause damage to crops and household plants (Kim & Rust, 2013). Current control methods for cockroaches are based exclusively on the use of synthetic insecticides (Appel & Smit, 2002). The application of these insecticides indoors and outdoors has become a public concern mainly due to their toxicity to non-target organisms (Kass et al, 2009). Furthermore, the continuous use of these insecticides can lead to the development of resistance (Wei et al, 2001; Chowanski et al., 2014), with some cockroach strains having developed resistance to several insecticides (Cochran, 2000; Kamyabi et al, 2006). Blattis lateralis and other cockroach species that are found most commonly in outdoor areas are best controlled by IPM (Integrated Pest management) programs that focus on biological control options. A alternative biological control method for the management of peridomestic cockroaches such as B. lateralis is the use of entomopathogenic nematodes.

Entomopathogenic nematodes (EPNs) are commonly used as biological control agents of insects in cryptic habitats (Kaya, 1990; Ramos-Rodríguez et al, 2006, Gozel & Gozel, 2016, Canhilal et al, 2017). EPNs in the genera, *Steinernema* and *Heterorhabditis*, are associated with symbiotic bacteria from the genera, *Xenorhabdus* and *Photorhabdus*, respectively (Gaugler, 1981). Infective juveniles (IJs) of EPNs enter their hosts via body openings such as the mouth, spiracles and

Türk. Biyo. Mücadele Derg.Özdemir & Evlice 2020, 11 (1):129-137anus. After the IJs enter the hemocoel, the symbiotic bacteria are released and killthe host within 48 hours (Griffin et al, 2005; Kaya et al, 2006). Due to thesecharacteristics, EPNs and their symbiotic bacteria have great potential to controlmany pests that threaten public health, including cockroaches.

The efficacy of EPNs for the control of insect pests varies, depending on factors such as the species and strain, and the application type, dose and timing. Therefore, it is essential to determine the efficacy of native entomopathogenic nematode isolates under various conditions in order to develop IPM programs based on biological control.

This study was initiated to determine the efficacy of the Turkish entomopathogenic nematode isolates, *Steinernema feltiae*-KV06 (Filipjev) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora*-EO7 (Rhabditida: Heterorhabtidae), against nymphs of the Turkestan cockroach, *B. lateralis*, under laboratory conditions.

Materials and Methods

Blatta lateralis culture

A *Blatta lateralis* culture was established in plastic containers. Dry dog food and apple were provided as food and cotton wool soaked in water provided their moisture needs. The culture was maintained in a climate chamber at $25\pm2^{\circ}$ C, $65\pm5\%$ relative humidity and 12:12 (L:D) photoperiod. Randomly selected, 60 day old late instar nymphs were used for the experiment.

Entomopathogenic nematodes

Steinernema feltiae-KV06 and Heterorhabditis bacteriophora-EO7 nematode isolates obtained in September 2018 from Kelebekler Vadisi Nature Park (40° 19'9262"N 31°91'0591"W) and Eğriova Nature Park (40° 44'1670"N 32°06'4795"W) in Ankara were used in this study. The species were identified by using morphological and morphometric characters and with DNA sequencing by amplifying the ITS1-5.8S-ITS2 region. Sequences were submitted to the NCBI database and were allocated the accession numbers MN853593 and MN853594. The nematodes were propagated in last instar larvae of the greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae) at 25°C, according to the method described by Woodring & Kaya (1998). The emerged infective juveniles (IJs) were collected in a modified version of a White's trap and were stored in Ringer solution in culture flasks at 10 °C - 12°C for a maximum of one week (Aydın & Susurluk, 2005). Nematode viability was 100% unless otherwise stated. Each new batch of IJs, which were collected daily and maintained at 4°C, were used within a week in all the experiments.

Infectivity of EPNs on Blatta lateralis

Petri dishes of 9 cm diameter were lined with two layers of filter paper (Whatman No: 2). The EPNs were then added in 2 mL of distilled water that contained 100, 250, 500 and 1000 IJs/cm². Ten, 60-day old nymphs were released into each Petri dish where dry cat food (1g / dish) had been placed as food (Appel et al, 1993). The Petri dishes were kept at 25°C for fourteen days. The number of dead nymphs was checked after 2, 4, 6, 8, 10, 12 and 14 days of exposure. All the dead cockroaches were removed from the dishes and were dissected to determine whether mortality was caused by EPN. There were three replicates for each combination of EPN species and IJ concentration. The control group was exposed to only sterile water.

Statistical analysis

One-way (ANOVA) was performed with IBM SPSS (Version 22.0) to compare the mortality of cockroaches and the efficacy of the EPNs. The differences among treatments were analysed by means of Tukey's multiple comparison test (p<0.05). Values for LC₅₀ and LC₉₀ (numbers of IJs/cm² causing 50% and 90% mortality, respectively), and LT₅₀ and LT₉₀ (time post-infection at which 50% and 90% mortality occurred, respectively) were determined with POLOPlus (Version 1.0).

Results and Discussion

The pooled results of this study showed that the mortality rate of *B. lateralis* nymphs was significantly affected by nematode concentration (F = 687.47; df = 4; P < 0.001), time (F=136.07; df = 6; P < 0.001), and nematode species (F = 11.53; df =1; P < 0.001), and the interaction between nematode concentration and time was also significant (F = 5.22; df = 24; P < 0.001). However, the interaction between nematode species (F = 1.07; df = 4; P = 0.371), and between nematode concentration, time and species (F = 0.16; df = 24; P = 1.000), were not significant.

In all treatments, total mortality was significantly different from the control treatment. The mortality of *B. lateralis* differed significantly from the control after the application of both EPN species at 250, 500 and 1000 IJs/cm² (P < 0.001). The lowest mortality percentage caused by both EPN species was at 100 IJs/cm² after 2 and 4 days of exposure to *S. feltiae* KV06 (0.6±1.4, 5.3±2.6) and *H. bacteriophora* EO7 (0.0±0.0, 5.3±2.6), respectively (Table 1). At 1000 IJs/cm², both *S. feltiae*-KV06 and *H. bacteriophora*-EO7 caused the highest mortality (100.0±0.0%) with LT₅₀ values of 2.3 and 2.6 days, respectively, and LT₉₀ values of 6.6 and 7.9 days, respectively (Tables 2, 3 and 4). Across the experiment, there were no significant differences in the LC₅₀ and LC₉₀ values between the isolates of the EPN species for all time intervals.

Türk. Biyo. Mücadele Derg.

Özdemir & Evlice 2020, 11 (1):129-137

Table 1. Mean percentage mortality (±SE) of Blatta lateralis treated with Steinernema feltiae-KV06 and Heterorhabditis bacteriophora-EO7 at
different concentrations of infective juveniles (IJs)(data are corrected for control mortality).

Isolates	Concentration	L		Days				
Isolates	(IJs/cm ²)	2	4	6	8	10	12	14
S.	100	$0.6\pm1.4 \ bE^{1}$	5.3±2.6 bDE	14.2±1.4 cCD	23.0±0.3 cBCD	36.4±0.3 cABC	46.7±0.4 bAB	60.0±0.0bA
	250	26.2±0.5 aE	43.3±0.3 aDE	56.7±0.3 bCD	70.5±0.6 bBCD	83.1±2.0 bABC	90.7±2.3 aAB	97.6±2.3aA
5. feltiae KV06	500	32.6±1.2 aE	49.7±1.2 aDE	63.9±1.0 bCDE	70.5±0.6 bBCD	85.8 ± 1.4 abABC	94.7±2.6 aAB	97.6±2.3aA
	1000	50.0±2.0 aC	67.1±0.5 aBC	90.7±2.3 aAB	97.6±2.3 aA	99.4±1.4 aA	100.0±0.0 aA	100.0±0.0aA
	Control	0.0±0.0 bA	0.0±0.0 bA	0.0±0.0 dA	0.0±0.0 dA	0.6±1.4 dA	2.4±2.3 cA	2.4±2.3cA
H. bacteriophora EO7	100	$0.0{\pm}0.0~\mathrm{cD}$	5.3±2.6 cCD	9.3±2.3 cBC	20.0±0.0 cABC	26.2±0.5 cAB	36.1±1.0 bA	43.0±1.0bA
	250	16.9±2.0 bE	36.4±0.3 bDE	53.3±0.4 bCD	63.6±0.3 bBCD	77.0±0.3 bABC	85.8±1.4 aAB	94.7±2.6aA
	500	19.7±2.5 abE	43.0±1.0 abDE	56.7±0.3 bCD	67.4±1.2 bBCD	80.0±0.0 bABC	90.7±2.3 aAB	97.6±2.3aA
	1000	43.3±0.3 aD	63.6±0.3 aCD	83.1±2.0 aBC	94.7±2.6 aAB	97.6±2.3 aAB	99.4±1.4 aA	100.0±0.0aA
	Control	0.0±0.0 cA	0.0±0.0 cA	0.0±0.0 dA	0.0±0.0 dA	0.6±1.4 dA	2.4±2.3 cA	5.3±2.6cA

¹Different lowercase letters in columns and different capital letters in rows indicate a significant difference between the means (P < 0.05, Tukey test)

Inclator	LC ₅₀ (95% CL ²)							
Isolates	21	4	6	8	10	12	14	
S. feltiae KV06	910.3 (601.8-2257.7)	470.3 (328.4-757.8)	272.1 (191.3-366.8)	203.0 (124.5- 282.7)	131.9 (76.0-182.8)	103.9 (56.9-144.1)	92.5 (23.9-147.1)	
H. bacteriophora EO7	1170.8 (774.1-3158.9)	573.194 (418.4-911.1)	328.5 (394.8-1046.1)	232.2 (158.3- 312.0)	165.2 (105.1-222.9)	126.2 (73.8-173.6)	121.2 (61.3-171.6)	

¹days of exposure. ²Confidence limits(CL) are given in parentheses.

Assessment of the susceptibility of *Blatta lateralis* to entomopathogenic nematodes

Table 3. The estimated LC₉₀ for the IJs of two entomopathogenic nematode species for the cockroach, *Blatta lateralis* at 7 time intervals

	LC90 (95% CL ²)								
Isolates	21	4	6	8	10	12	14		
S. feltiae KV06	6128.8 (2678.9- 88888.)	3153.2 (1528.4 -18814.)	1263.4 (805.5-3067.8)	1134.0 (704.0-3084.4)	560.1 (391.6-1084.7)	367.7 (267.6-646.6)	318.7 (207.5-859.6)		
H. bacteriophora EO7	5827.2 (2436.7- 72668.)	4166.3 (1829.1-36345.)	1741.1 (1021.5- 5406.2)	1064.442 (695.4-2432.9)	690.136 (479.6-1342.3)	496.6 (354.1-908.6)	334.173 (233.9-709.0)		

¹days of exposure. ²Confidence limits (CL) are given in parentheses.

Table 4. The estimated effects of dosage of the IJs of the isolates of two entomopathogenic nematode species on the LT ₅₀ and LT ₉₀ of <i>Blatta lateralis</i>
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Isolates	LT ₅₀ (95% CI ²)				LT90 (95% CI)			
	100 ¹	250	500	1000	100	250	500	1000
Steinernema feltiae KV06	12.9 (10.7-17.9)	4.3 (3.3-5.2)	3.7 (2.6-4.6)	2.3 (1.5-2.9)	40.4 (25.9-101.9)	15.2 (11.6-25.8)	14.3 (10.8-23.0)	6.6 (5.425-8.8)
Heterorhabditis bacteriophora EO7	17.0 (13.1-30.0)	5.3 (4.3-6.2)	4.5 (3.5-5.4)	2.6 (1.8-3.2)	56.6 (31.5-243.6)	16.8 (13.0-25.6)	15.1 (11.7-23.0)	7.9 (6.4-10.7)

 1Application doses

 ²Confidence limits (CL) are given in parentheses.

The host range of EPNs among the 4,600 cockroach species is poorly known (Memona et al., 2017). Only a few cockroach species have been tested to determine their susceptibility to EPNs, including Blattella germanica, Blatta orientalis, Periplaneta americana and Periplaneta brunnea (Zervos & Webster, 1989; Appel et al., 1993; Baker et al., 2012; Koehler et al., 1992; Kotlarska-Mordzinska, 2000; Susurluk & Okten, 2000; Maketon et al., 2010; Puza & Mracek, 2010; Morton & Garcia del Pino, 2013). Zervos & Webster (1989) reported that *H. heliothidis* was able to kill all instars of *P. americana*. However, Maketon et al. (2010) stated that the control of this cockroach by two Heterorhabditis species is difficult. Conversely, Appel et al. (1993) pointed out that the mortality of cockroaches treated with Steinernema species, including S. carpocapsae, was quite high. Koehler et al. (1992) determined that S. carpocapsae can effectively control B. germanica at 500,000 IJs/ml. Kotlarska-Mordzinska et al. (2000) investigated the efficacy of S. feltiae and H. bacteriophora against B. orientalis and found that at all concentrations tested (100, 200, 500 and 1000 IJs/insect), males of the cockroach were more susceptible to S. feltiae and H. bacteriophora than females.

In the majority of studies conducted, various species of cockroaches have been found to be more susceptible to entomopathogenic nematodes of the genus *Steinernema*, in particular *S. carpocapsae*. At this stage, insufficient evidence has been accumulated to make the same inference for *B. lateralis*. In this study, we evaluated the susceptibility of the nymphs of *B. lateralis* and demonstrated that both EPN species provide substantial control, even at relatively low IJ concentrations. The application of *S. feltiae* and *H. bacteriophora* even at 100 IJs/cm² resulted in mortality of over 40% and 60%, respectively, in nymphs. Moreover, to our best of knowledge, in global scale, we reported for the first time that *B. lateralis* nymphs are susceptible to EPNs

The mortality rates caused by the EPN isolates used in the study on *B. lateralis* are noteworthy. More studies are needed to investigate the efficacy of EPNs on the different life stages and genders, and also the immune system response, of the invasive cockroach species, *B. lateralis*. In this sense, it is important to continue to explore the use of entomopathogenic nematodes against this invasive pest, which is an important public health threat.

Assessment of the susceptibility of Blatta lateralis to entomopathogenic nematodes

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 Türk. Biyo. Mücadele Derg.
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