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Efficacy of entomopathogenic nematode isolates from Turkey and Kyrgyzstan against the larvae of the mosquito species *Culex pipiens* L. (Diptera: Culicidae) under laboratory conditions

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Abstract

Mosquitoes (Diptera: Culicidae) are among the most important of the pests in urban entomology, and they not only disturb people but they are also an important health hazard as they are diseases' vectors. The biological control methods against this pest, which is difficult to fight off, have recently gained importance. A total of five different entomopathogenic nematodes (EPNs), *Steinernema feltiae* (Aydın isolate), *S. carpocapsae* (Karadeniz isolate) , and *Heterorhabditis bacteriophora* (Aydın isolate) isolated from Turkey and *S. feltiae* (KG3) and *H. bacteriophora* (KG81) isolated from Kyrgyzstan, were tested against the mosquito species *Culex pipiens* L. (Diptera: Culicidae) larvae under laboratory conditions. The experimental nematode suspensions were determined as 500, 1000, and 1500 IJs ml^{-1}. The mortality rates in the trial were recorded after 24, 48, 72, 96, and 120 h. Dead mosquitoes were dissected under a microscope and confirmed to have died by the EPNs. Larval mortality was observed in all EPN species compared to the control group. *H. bacteriophora* (KG81) and *S. carpocapsae* isolates were found to be the most effective isolates with 100% larval mortality. The other isolates were *H. bacteriophora* (Aydın isolate) (70%), *S. feltiae* (KG3) (66.67%), and the most ineffective isolate *S. feltiae* (Aydın isolate) (13.3%).

Keywords: Biological control, Entomopathogenic nematodes, *Culex pipiens*, Larvae, Efficacy, Turkey, Kyrgyzstan

Background

Pests in urban entomology can be described as insects that affect human health. Insects in urban entomology include cockroaches, ants, termites, houseflies, ticks, insects and mites, bedbugs, lice, and mosquitoes (Robinson 2005).

Mosquitoes are one of the most important urban pests that belong to the Culicidae family of the Nematocera suborder. These pests are found in temperate and tropical regions of the world, except the polar regions (Lancaster and Briers 2008). These pests not only disturb people but they are also an important health hazard as diseases' vectors that spread malaria, dengue, yellow fever, and an important virus of the recent years Zika (Epstein et al. 1998). There are 112 genera and 3539 species belonging to the Anophelinae and Culicinae subfamilies of the Culicidae family (Harbach 2014). About 50 species of mosquitoes have been identified in Turkey until today (Muslu et al. 2011). Six mosquito species, including *Culex pipiens* Linnaeus, 1758; *C. martini* Medschid, 1930; *C. deserticola* Kirkpatrick, 1924; *Aedes caspius* Pallas, 1771; *Anopheles superpictus* Grassi, 1899; and *Culiseta longiareolata* Macquart, 1838, were found in Antalya; the dominant type was identified as *C. pipiens* (Çetin and Yanıkoğlu 2004).

Control methods against mosquitos' larvae include mechanical, biological, chemical, and an integrated approach where all of the above are used (Alten and Çağlar 1998). The chemical control targeted both larvae and adults, but larvae being the important ones. These pests live in water at all stages, except the adult stage. Today,

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insecticides used as larvicides are of biological origin, but they are very limited in number. Because of the environmental problems caused by insecticides and the effects of non-target organisms, alternative methods of combating these pests are sought. For a sustainable environment, the importance of biological control is increasing among these applications. But unfortunately, the high price of these biological agents at the moment makes it difficult for their broader implementation in the systems of production (Laznik and Trdan 2011).

One of the most successful groups of biological agents for controlling soil insect pests is the entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae. Nematodes in both families are obligate insect-parasitic organisms that are mutualistically associated with bacteria from the genera *Photorhabdus* (heterorhabditids) and *Xenorhabdus* (steinernematids), which are carried within the nematode digestive tracts (Kaya and Gaugler 1993). Nematodes in the infective juvenile (IJ) stages search for an adequate host in the soil and enter the insect host through natural openings (mouth, anus, and spiracles) or through the cuticle. The symbiotic bacteria are then released into the insect hemocoel (Dowds and Peters 2002) at which point the bacteria multiply and produce toxins. The nematodes also contribute to this process, and insect host is killed within 48 h by septicemia and toxemia (Kaya and Stock 1997; Duchaud et al. 2003). Once nutrients within the insect cadaver are exhausted, progeny nematodes develop into the IJ stages and emerge from the cadaver into the soil to search for another host (Griffin et al. 2005). A total of 86 species of EPN have been identified worldwide (64 belonging to *Steinernema*, 1 to *Neosteinernema*, and 21 to *Heterorhabditis*) (Kepenekci 2014).

The first EPN belonging to the genus *Steinernema*, detected in Turkey, in soil samples collected from Rize (Turkey), was identified by Özer et al. (1995) as *S. feltiae*. Kepenekci et al. (1999) identified the first nematode in Turkey from the genus *Heterorhabditis* as *H. bacteriophora*, which was found in an *Aelia* population (*Aelia rostrata* Boh.) collected from Ekecik (Aksaray, Turkey) winter quarters. To utilize EPNs in the biological control of pathogens in Turkey, the principal species and hosts, present, should be determined. Although several surveys have been conducted on this subject, there is not yet sufficient information (Kepenekci and Atay 2014).

Narksuwan et al. (2004) tested the EPNs *S. carpocapsae* (Weiser), *S. siamkayai*, *S. feltiae*, *H. indica*, and *H. bacteriophora* against *Aedes aegypti* (L.), *Culex quinquefasciatus*, *C. gelidus*, *Anopheles dirus*, and *A. minimus* mosquito species, and all of them were successfully suppressed *A. aegypti* (L.), *C. quinquefasciatus*, *C. gelidus* species (Edmunds et al. 2017). Kepenekci et al. (2014) evaluated the efficacy of *S. feltiae*, *S. carpocapsae*, *S. kraussei*, and *H. bacteriophora* against Chironomidae family members in their study. They determined that these EPN species could survive for up to 96 h in water and even after that they could parasitize the larvae of *Chironomus plumosus*. They caused more than 20% mortality after 24 h.

In this study, efficacy of five EPN species, isolated from Kyrgyzstan and Turkey, was evaluated against *C. pipiens* larvae collected from the stagnant waters in paddy fields at Samsun (Turkey)'s Bafra province under laboratory conditions.

Materials and methods

The experiment was carried out at the Entomology Laboratory of Ondokuz Mayıs University (Samsun, Turkey). Five different EPN species were applied to the larvae of *C. pipiens*. The experiment was carried out in six replications and two repetitions.

Nematode sources

Five Turkish and Kyrgyz EPN isolates, *Steinernema feltiae* (Aydin isolate) from a vegetable garden in Aydın, *S. carpocapsae* (Karadeniz isolate) from a grassland in Rize, and *Heterorhabditis bacteriophora* (Aydın isolate) from a peach orchard in Aydın (Turkey), were obtained from the Entomopathogenic Nematode Laboratory of Adnan Menderes University (Aydın, Turkey). *S. feltiae* (KG3) from an apricot orchard in Talas and *H. bacteriophora* (KG81) from a potato field in Tokmok (Kyrgyzstan) were also obtained from the Nematology and Taxonomy Laboratory of Gaziosmanpaşa University (Tokat, Turkey).

Production of nematodes

Last instar larvae of the greater wax moth (*Galleria mellonella* L., Lepidoptera: Pyralidae) was used to culture the nematodes at room temperature (23–24 °C) as described by Kaya and Stock (1997). The new-generation IJs emerged from cadavers were harvested from nematode-infected larvae which were placed on white traps (White 1927). IJs were collected and rinsed three times in sterile distilled water. Using a tetrapack juice box, each species was kept separately before refrigerating at 10 °C (Gülcü and Hazir 2012). The harvested IJs were used within 2 weeks after emergence for the experiments.

Production of *Culex pipiens*

Third and fourth larval instars of *C. pipiens* were collected from the stagnant water deposits in paddy fields located in the province of Bafra in Samsun (Turkey). The larvae were placed into 10 × 10 × 10 cm containers and 10 larvae were placed in each one. The larvae were fed fishmeal to survive. Identification of mosquitoes brought to the laboratory was carried out by

Prof. Dr. İzzet AKÇA (Ondokuz Mayıs University, Samsun, Turkey).

Bioassay

Each EPN species was applied at three concentrations (500, 1000, and 1500 IJs ml^{-1}) (approximately 7.5, 15, and 30 IJs cm^2) at 25 °C temperatures. One milliliter of distilled water without nematode was used as control. Each plastic container contained moistened filter paper on the bottom. The studies were conducted at the Entomological Laboratory of Ondokuz Mayıs Univ. in Samsun. Plastic containers were placed in incubators adjusted at 25 ± 1 °C temperatures and 90 ± 5% R.H. The data for mortality was recorded after 24-, 48-, 72-, 96-, and 120-h intervals. Dead insects were dissected under a stereomicroscope to verify that they were killed by the nematodes.

Statistical analysis

One-way ANOVA was used to compare mortality rates of *C. pipiens* treatments. Means were compared at the $P < 0.05$ level, and Tukey's test was used to separate means. Serial time-mortality data from bioassays were analyzed by probit analysis program (SPSS, Version 21) to calculate 50% lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}).

Results and discussion

The efficacy of five different EPN isolates and three EPN species [*Steinernema feltiae* (Aydın isolate), *S. carpocapsa*e (Karadeniz isolate), and *Heterorhabditis bacteriophora* (Aydın isolate) isolated from Turkey and *S. feltiae* (KG3) and *H. bacteriophora* (KG81) from Kyrgyzstan] on *C. pipiens* was determined. When counts were taken 24 h after inoculation of the EPNs, *H. bacteriophora* (KG81) 1500 ml^{-1} was found to have the highest effect

Table 1 Effect of entomopathogenic nematode isolates on mortality of *Culex pipiens*

Entomopathogenic nematodes/ concentration	Mortality % (hours)				
	24	48	72	96	120
H.b Aydın					
500*	0.00 ± 0.00f	10.00 ± 4.49ef	30.00 ± 4.49cd	36.67 ± 3.35e	53.33 ± 4.23ef
1000	0.00 ± 0.00f	20.00 ± 0.00de	26.67 ± 4.23d	40.00 ± 0.00e	60.00 ± 0.00de
1500	6.67 ± 4.23ef	26.67 ± 4.23cd	43.33 ± 6.17b	60.00 ± 0.00bc	70.00 ± 4.49ac
H.b KG81					
500	13.33 ± 6.69de	40.00 ± 7.33b	90.00 ± 4.49a	93.33 ± 4.23a	100.00 ± 0.00a
1000	23.33 ± 3.35bcd	30.00 ± 4.49bcd	50.00 ± 8.60b	93.33 ± 4.23a	100.00 ± 0.00a
1500	43.33 ± 6.17a	56.67 ± 3.35a	86.67 ± 6.69a	96.67 ± 3.35a	100.00 ± 0.00a
S.c K					
500	13.33 ± 4.23de	26.67 ± 4.23cd	43.33 ± 3.35b	50.00 ± 4.49 d	53.33 ± 4.23ef
1000	33.33 ± 6.69ab	33.33 ± 6.69bc	50.00 ± 4.49b	56.67 ± 6.17cd	90.00 ± 6.86b
1500	30.00 ± 4.49bc	30.00 ± 4.49bcd	50.00 ± 4.49b	100.00 ± 0.00a	100.00 ± 0.00a
S.f Aydın					
500	0.00 ± 0.00f	0.00 ± 0.00f	0.00 ± 0.00e	13.33 ± 4.23f	20.00 ± 0.00g
1000	0.00 ± 0.00f	3.33 ± 3.35f	10.00 ± 4.49e	16.67 ± 3.35f	20.00 ± 0.00g
1500	0.00 ± 0.00f	6.67 ± 4.23f	6.67 ± 4.23e	10.00 ± 4.49f	13.33 ± 4.23g
S.f KG3					
500	13.33 ± 4.23de	33.33 ± 4.23bc	40.00 ± 0.00bc	40.00 ± 0.00e	50.00 ± 4.49f
1000	20.00 ± 0.00cd	30.00 ± 4.49bcd	36.67 ± 3.35bcd	60.00 ± 0.00bc	60.00 ± 0.00de
1500	26.67 ± 4.23bc	26.67 ± 4.23cd	40.00 ± 0.00bc	66.67 ± 4.23b	66.67 ± 4.23cd
Control					
500	0.00 ± 0.00f	0.00 ± 0.00f	0.00 ± 0.00e	0.00 ± 0.00g	0.00 ± 0.00h
1000	0.00 ± 0.00f	0.00 ± 0.00f	0.00 ± 0.00e	0.00 ± 0.00g	0.00 ± 0.00h
1500	0.00 ± 0.00f	0.00 ± 0.00f	0.00 ± 0.00e	0.00 ± 0.00g	0.00 ± 0.00h
	F (1.25) = 15.50	F (1.25) = 17.15	F (1.25) = 43.74	F (1.25) = 119.94	F (1.25) = 154.12

Values followed by different letters in the same column differ significantly at $P < 0.05$ according to Tukey's test

**Steinernema feltiae* (Aydın isolate) (*S.f* Aydın), *S. feltiae* (*S.f* KG3), and *S. carpocapsa*e (Karadeniz isolate) (*S.c* K) and *Heterorhabditis bacteriophora* (*H.b* Aydın) and *H. bacteriophora* (*H.b* KG81) from Kyrgyzstan

(43.33%). The difference was statistically significant when compared to the control and other concentrations, while no deaths were observed in any concentration of the *S. feltiae* (Aydın isolate) and in the control group. Deaths in other isolates and concentrations were observed at different rates. After 48 h from inoculation, it was determined that the most effective concentrations and preparations were *H. bacteriophora* (KG81) 1500 ml^{-1} (56.67%), followed by the same isolates 500 ml^{-1} (40.00%), *S. carpocapsa*e (Karadeniz isolate) 1000 ml^{-1}, and *S. feltiae* (KG3) 500 ml^{-1} (33.33%). After 72 h, a mortality rate of up to 90% was determined. *H. bacteriophora* (KG81) (500 ml^{-1}) was found to be the most effective concentration and preparation. Very little efficacy was determined in all the concentrations of *S. feltiae* (Aydın isolate) isolate. The first 100% mortality rate was recorded 96 h post treatment at the concentration of 1500 ml^{-1} for *S. carpocapsa*e (Karadeniz isolate) isolate which was determined to be the most effective one, followed by 500, 1000, and 1500 ml^{-1} concentrations of the *H. bacteriophora* (KG81), respectively. According to the results obtained by the end of the experiment (120 h), 100% mortality rate at the concentration of 1500 ml^{-1} for *S. carpocapsa*e (Karadeniz isolate) and for *H. bacteriophora* (KG81) at concentrations 500, 1000, and 1500 ml^{-1} was found. This was followed by isolates and a concentration of 1500 ml^{-1} for *H. bacteriophora* and *S. feltiae*, respectively. The lowest mortality rate was determined at 500 ml^{-1}, 1000 ml^{-1} and 1500 ml^{-1} concentrations of *S. feltiae* (Aydın isolate) isolate. At least 50% mortality rate was detected in other concentrations and preparations (Table 1). In addition, the mortality rates at the LC_{50} and LC_{90} values are shown in Table 2.

As a result, the effectiveness of *H. bacteriophora* (KG81), *S. feltiae* (KG3), *S. feltiae* (Aydın isolate), *S. carpocapsae* (Karadeniz isolate), and *H. bacteriophora* (Aydın isolate) species against *C. pipiens* larvae was determined, even if at different rates. Especially after 120 h, 100% mortality rates were recorded.

Chaudhary et al. (2017) found that *S. kraussie* and *H. bacteriophora* were the most effective EPNs between 20 and 30 °C in a study on the mortality of *A. aegypti* in two different concentrations and three different temperature environments. At 100 IJs, concentration of *S. kraussie* and *A. aegypti* was found to have 100% mortality at 48 and 96 h. *H. bacteriophora* was found to have 100% mortality rate at 30 °C with 100 IJs concentration after 96 h. In the present study, the KG81 isolate caused 93.33–96.67% death after 96 h. Oğuzoğlu and Özer (2007) recorded low mortality rate of *S. feltiae* (Karadeniz isolate) and one isolate belonging to *H. bacteriophora*, collected from Turkey, on *A. aegypti* which agrees with the obtained results on *C. pipiens*. In another study,

Table 2 LC_{50} and LC_{90} for *Culex pipiens* treated with entomopathogenic nematode isolates

Entomopathogenic nematodes	Concentration	LC_{50}	LC_{90}
Heterorhabditis bacteriophora (KG81)	500	3.22	4.35
	1000	3.47	5.61
	1500	2.20	4.52
H. bacteriophora (Aydın isolate)	500	5.15	7.13
	1000	5.15	7.46
	1500	4.33	6.46
Steinernema feltiae (KG3)	500	3.47	5.61
	1000	4.44	7.20
	1500	4.23	6.90
S. feltiae (Aydın isolate)	500	5.78	6.68
	1000	6.77	9.26
	1500	8.47	12.28
S. carpocapsae (Karadeniz isolate)	500	4.60	7.30
	1000	4.27	7.97
	1500	3.30	5.35

Peschiutta et al. (2014) analyzed the effectiveness of *H. bacteriophora* on *A. aegypti* and found a mortality rate of up to 84%. Obtained data of Kyrgyzstan and Aydın isolates of *H. bacteriophora* was effective on *C. pipiens*.

Cagnolo and Almirón (2010) tested six different concentrations of *S. rarum* (OLI strain) against the larvae of *C. apicinus* and recorded that the mortality rate increased as the dose increases. They recorded a 75% mortality rate at a 400:1 dose at 25 °C. Pandii et al. (2008) tested *S. carpocapsae* and *H. indica* (local Thai strain) against *C. gelidus*. *S. carpocapsae* showed more effectiveness than *H. indica*. In the present study, 100% mortality rate for *S. carpocapsae* (Karadeniz isolate) was recorded, especially after 96 and 120 h.

In a study evaluating the efficacy of *H. bacteriophora*, *H. indica*, *S. carpocapsae*, and *S. feltiae* on *C. quinquefasciatus*, *H. bacteriophora* and *H. indica* were successfully inhibited to *C. quinquefasciatus*, while *S. carpocapsae* and *S. feltiae* showed low mortality rate (Zohdy et al. 2013). In the present study, *S. feltiae* showed low activity against *C. pipiens*, whereas *H. bacteriophora* had the highest effect.

Conclusion

Culex pipiens is a well-known species among the mosquitoes found in Turkey. Entomopathogenic nematodes (EPNs) are among the alternative methods to chemical pesticides that give hopeful results in mosquito control. In recent years, studies regarding these have gained speed around the world as well as in Turkey. The EPNs have been shown to be a fairly good alternative method for controlling *C. pipiens*. Future field trials are needed to demonstrate their effectiveness under natural conditions.

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Authors' contributions
ST and IS designed the study, supervised the work, and wrote the manuscript with input from all authors. ST and IS carried out the experiments. IS analyzed the data. Both authors read and approved the final manuscript.

Ethics approval and consent to participate
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