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Efficacyof of entomopathogenic nematode isolates from Turkey and Kyrgyzstan against black timber bark beetle, *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae: Scolytinae) adults

I. Kepenekci[1†], S. Toksöz[2], T. Atay[1] and I. Saruhan[2]

[1]*Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection, Tokat, Turkey*
[2]*Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, Samsun, Turkey*

[†] Corresponding author: kepenekci@gmail.com

Abstract

Six species of entomopathogenic nematodes (EPNs) (*Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora*) isolated from Turkey [*S. feltiae* (Aydin isolate), *S. carpocapsae* (Black sea isolate), *H. bacteriophora* (Aydin isolate) and *H. bacteriophora* (Çanakkale isolate)] and from Kyrgyzstan [*S. feltiae*(KG3) and *H. bacteriophora* (KG81)] were tested for the control of black timber bark beetle, *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae: Scolytinae) under laboratory conditions. The studies were conducted in June, 2017 at the Ondokuz Mayıs University (Samsun, Turkey). The suspensions of nematodes were applied at one concentration (1000 IJs ml^{-1}) (200 IJs $insect^{-1}$ or approximately 15 IJs cm^{-2}) at 25°C temperatures. The data for mortality was recorded after 2, 4, 6 and 8 days intervals. Insect mortality of the adult was higher in all nematode treatments than the control. Turkish EPN isolate *S. carpocapsae* (Black sea) and Kyrgyz EPN isolate *S. feltiae* (KG3) species showed the highest mortality (98.66%).

Keywords: Entomopathogenic nematodes, black timber bark beetle, *Xylosandrus germanus*, efficacy, biological control, Turkey, Kyrgyzstan.

***X**ylosandrus germanus* (Blandford) (Coleoptera: Scolytidae) (black timber bark beetle or Alnus ambrosia beetle) is a strongly invasive species. *X. germanus* should be considered a high-risk quarantine pest which attacks a very wide range of host plants, including both deciduous and coniferous trees.

In Turkey, *X. germanus* has been an important pest of nuts, *Corylus avellana* (F). It is reported that *X. germanus* is most prevalent in the Eastern Black Sea region, Samsun-Terme (Söğütlü town), Ordu (Center and Emen villages), Amasya (Ayvasıl) in hazelnut, chestnut, alder, walnut, apricot, cherry, pear and kiwi (Ak *et al*., 2005; 2006).

Entomopathogenic nematodes (EPNs) are obligate organisms that infect a wide range of insect hosts (Kaya & Gaugler, 1993). The infective juveniles (IJ) stage enters the insect via natural openings (mouth, spiracles or anus) and penetrates the hemocoel where they release their symbiotic bacteria in the presence of a susceptible insect host. Eighty six EPN species (64 from *Steinernema* genus, 1 from *Neosteinernema* genus, 21 from *Heterorhabditis* genus) have been detected worldwide (Kepenekci, 2014) and the number of species is increasing as the new studies are undertaken.

In Turkey, the first EPN species belonging to *Steinernema* genus was detected as *S. feltiae* in

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soil samples taken from Rize province by Özer *et al.* (1995). The first EPN species belonging to *Heterorhabditis* genus was detected as *H. bacteriophora* in wheat bug (*Aelia rostrata* Boh.) population obtained from Ekecik winter quarters (Aksaray province) by Kepenekci *et al.* (1999). In Turkey, eight (08) species belonging to *Steinernema* and *Heterorhabditis* have been found (Kepenekci, 2014). However, there is a limited research reported about the efficacy of native EPNs isolated from Turkey on the economically important pests found in the country (Kepenekci, 2012; Kepenekci *et al*., 2014). *S. feltiae* (KG3) and *H. bacteriophora* (KG81) which were a first report showing the occurrence of EPNs in Kyrgyzstan and first efficacy study. This is the first report regarding the effect of Turkish and Kyrghyz EPN species against black timber bark beetle (*X. germanus*). No studies have been encountered on *X. germanus* from Turkey (Kepenekci *et al*., 2014). And no work was found for the world. So this study is the first attempt for this pest.

Materials and Methods

Nematode sources: Six Turkish and Kyrgyz EPN isolates; *Steinernema feltiae* (Aydin isolate) from a vegetable garden in Aydın, *S. carpocapsae* (Black sea isolate) from grassland in Rize and *Heterorhabditis bacteriophora* (Aydin isolate) from peach orchard in Aydın (Turkey) were obtained from entomopathogenic nematode laboratory of Adnan Menderes University (Aydın, Turkey). *H. bacteriophora* (Çanakkale isolate) were obtained from nematology laboratory of Çanakkale Onsekiz Mart University (Çanakkale, Turkey). *S. feltiae* (KG3) from apricot orchard in Talas, *H. bacteriophora*(KG81) from a potato field in Tokmok (Kyrgyzstan) were obtained from nematology and taxonomy laboratory of Gaziosmanpaşa University (Tokat, Turkey). Kyrghyz EPN species were identified based on morphometric and molecular data.

Production of nematodes: 5^{th} or 6^{th}instar stage larvae of wax moth (*Galleria mellonella*, Lepidoptera: Pyralidae) were used to culture nematodes at room temperature (23-24^{o}C) as described by Kaya & Stock (1997).

Production of *Xylosandrus germanus*: *Xylosandrus germanus* adults were collected from the hazelnuts orchard from Taflan district, Kaya güney village, Samsun (Turkey).

Bioassay: Experiments were carried out in Petri-dishes (9 cm DIA.) lined with a filter paper (Shahina & Salma, 2010) and 1 ml of prepared EPN concentration was pipette onto the filter paper. Each EPN species was applied at one concentration (1000 IJs ml^{-1}) (200 IJs insect^{-1} or approximately 15 IJs cm^{-2}) (single dose screening) at 25°C temperature. 1 ml distilled water without nematode was used for control. Each Petri plate contained moistened filter paper on the bottom and added to the bait, consisted of 2 pieces of branches of hazelnut tree approximately 4 cm length (favored bait) placed on the center of filter paper. Five adult insects were placed into each Petri-dish. The bioassays had four replications. The studies were conducted in June 2017 at the Entomology laboratory of Ondokuz Mayıs University in Samsun (Turkey). Petri-dishes were placed in incubators adjusted to 25±1^{o}C temperature and 90±5% R.H. The data for mortality was recorded after 2, 4, 6 and 8 days intervals. Dead insect were dissected under a stereomicroscope to make sure that they were killed with IJs. If necessary, dead insects were removed and placed on to White-traps for nematode emergence (White, 1927).

Statistical analysis: One-way ANOVA was used to compare mortality rates for *X. germanus* treatments. Means were compared at the P=0.05 level, and Tukey's test was used to separate means (SPSS, 1999).

Results

When the results of the study were evaluated, *H. bacteriophora* (Çanakkale) (5.27%±1.33) and *S.*

feltiae (KG3) had shown positive results on the 2nd day with non-significant results (F=0.85; df=6.27; P<0.558) (Fig. 1). The highest effect on the 4thday occurred in *S. carpocapsae* (Black sea) (50%±1.34), which killed half of the insect populations that were applied, followed by the Aydin and Çanakkale isolates of *H. bacteriophora*, which had the same effect at 39%. The lowest effect was recorded at 19% in *S. feltiae* (KG3). (F=50.16; df=6.27; P<0.000) (Fig. 1).

At 6th day, the highest insect mortality rate 70.94% ±4.39 was also observed in *S. carpocapsae* (Black sea) and this was followed by *H. bacteriophora* (Çanakkale) with 65.33%±1.21 (F=17.27; df=6.27; P<0.000) (Fig. 1). The highest effect on the final day of the trial (8th day) was observed in *S. carpocapsae* (Black sea) and *S. feltiae* (KG3) as 98.66%±5.27. In addition, *H. bacteriophora* (KG81) showed 94.62%±6.99 mortality rate (F=24.35; df=6.27; P<0.000) (Fig. 1).

Heterorhabditis bacteriophora (KG81) showed 24% on 4thday and 34% on 6th day, while having a fairly good effect on 8th day as 94%. The highest efficacy (98.66%) was observed in case of the Turkish EPN isolate *S. carpocapsae* (Black sea isolate) and Kyrgyz EPN isolate *S. feltiae* (KG3) had the same effect; while the other Kyrgyz EPN isolate *H. bacteriophora* (KG81) showed a high effect (94.62%) and was found to be an important hope in control against *Xylosandrus germanus*, which is a substantial pest in Turkey, especially in hazelnut production areas.

Discussion

The studies on the effects of entomopathogenic nematodes against bark beetles were examined, though the different beetle species (*Ips sexdentatus* and *I. typographus*) have been targeted before for controlling by examining the activity of entomopathogenic fungi species (*Paecilomyces farinosus, Beauveria bassiana* and *Metarhizium anisopliae*). (Draganova *et al.*, 2006; Mudrončeková *et al*., 2013; Castrillo *et al*., 2011). The control of the same species (*Xylosandrus germanus*) by EPNs has not been hitherto reported.

Among the EPN isolates of Turkey used in the study, *S. feltiae* (Aydin isolate), *S. carpocapsae* (Black sea isolate) and *H. bacteriophora* (Aydin isolate) are shown to be highly effective. In previous studies, the efficacy of EPNs has been examined against great spruce bark beetle (*Dendroctonus micans*) at 25^{o}C and 1000 IJs concentration, the larval mortality was 98.04% and 94.04% for Aydın isolates of *S. feltiae* and *H. bacteriophora*, respectively.

Aydin isolates of *H. bacteriophora* and *S. feltiae*, which had previously been shown to have a considerably high effect in studies conducted on different pest groups in Turkey (Kepenekci *et al*., 2016; Atay *et al*., 2015; Kepenekci & Atay 2014; Kepenekci *et al*., 2013), were found to have a relatively low effect in this study (80% and 75%, respectively).

However, *S. carpocapsae* (Black sea) did not exhibit more than 40% mortality (Kepenekci & Atay, 2014). But in this study, *S. carpocapsae* (Black sea) has shown to have a high effect. In a study conducted by Atay *et al*.,(2015), *S. feltiae* (Aydin isolate), *S. carpocapsae* (Black sea) and *H. bacteriophora* (Aydin) were tested against the bean weevil, *Acanthoscelides obtectus* and *S. carpocapsae* blacksea strain was found to be effective (89.36%) and *S. feltiae* did not exhibit more than 60% mortality. Kepenekci *et al*., (2016) reported that effectiveness of Aydin isolates of *S. feltiae* and *H. bacteriophora* against larvae of *Leptinotarsa decemlineata* was 94% and 83% mortality, respectively. However, in present study, this isolate, *S. feltiae* (Aydın) did not demonstrate the desired level of effect and was not seen promising at 75%. Another study was conducted against *Phthorimaea operculella*, another important pest of the potato, the larval mortality was 96 and 80% for *S. carpocapsae* (Black sea) and *H. bacteriophora* (Aydin), respectively at 25°C and 1000 IJs concentration.

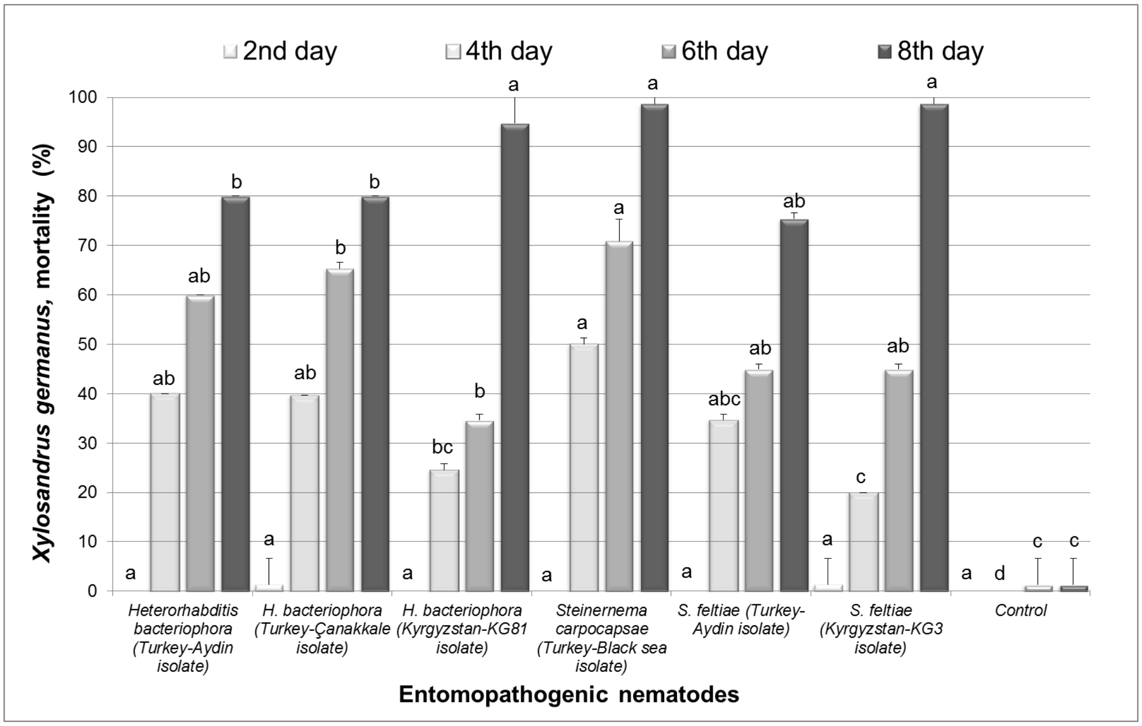

Fig. 1. Mortality (%) of *Xylosandrus germanus* adults following application of six entomopathogenic nematodes (EPNs) isolated from Turkey [*Steinernema feltiae* (Aydin isolate), *S. carpocapsae* (Black sea isolate), *Heterorhabditis bacteriophora* (Aydin isolate) and *H. bacteriophora* (Çanakkale isolate)] and Kyrgyzstan [*S. feltiae* (KG3) and *H. bacteriophora* (KG81)]. Data are expressed as mean±SEM. Different lowercase letters above the bars indicates significant difference at $P<0.05$.

S. feltiae (Aydin) did not exhibit more than 40% mortality at any temperature or concentration (Kepenekci *et al*., 2013). Similar results were observed in the present study and the highest effect was seen in *S. carpocapsae* (Black sea) and lowest in *S. feltiae* (Aydin). *H. bacteriophora* (Aydın) also showed the same effect (80%) in current study. In other study, same EPN isolates, *S. carpocapsae* (Black sea) was the most effective (80.43, 83.35 and 82.15% mortality for 500, 1000 and 2000 IJs ml^{-1} concentrations respectively). *S. feltiae* (Aydin) had higher effects (30.44, 41.93 and 35.21% mortality, respectively) than *H. bacteriophora* (Aydin) (24.15, 27.16 and 30.55 % mortality, respectively) (Atay & Kepenekci, 2016). In present study, *S. carpocapsae* (Black sea) was found effective.

Mannion & Janssson (1993) compared efficacy of five EPNs against the sweet potato weevil, *Cylas formicarius* (F.), (Coleoptera: Apionidae) in different experimental arenas. Among these *S. carpocapsae* strains were favored in the petri plate arenas while *Heterorhabditis* strains were favored in the sand arena. Similarly in our study, *S. carpocapsae* strain had the highest effect in petri dishes.

In conclusion, this study indicates that Turkish EPN isolate *Steinernema carpocapsae* (Black sea) and Kyrgyz EPN isolate *S. feltiae* (KG3) species have great potential to control the black timber bark beetle, *Xylosandrus germanus* (98.66% mortality) adults. Further work is necessary to determine the efficacy and feasibility of these EPNs against *X. germanus* under the field conditions.

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