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RESEARCH

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Efficacy of entomopathogenic nematode isolates from Turkey against wheat stink bug, *Aelia rostrata* Boheman (Hemiptera: Pentatomidae) adults under laboratory conditions

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Abstract

Background: The wheat stink bug, *Aelia rostrata* Boheman (Hemiptera: Pentatomidae), is one of the harmful insects that caused significant product losses in cereals due to outbreaks. Entomopathogenic nematodes (EPNs) are natural enemies of soil insect pests whose effects as a biocontrol agent against many harmful pests have been demonstrated by many laboratories and field/garden studies in the world. The present laboratory studies, using native EPNs [*Steinernema carpocapsae* (Black sea isolate), *S. feltiae* (isolate 09-31) (Aydin isolate) and *Heterorhabditis bacteriophora* (isolate 09-43) (Aydin isolate)] against the wheat stink bug adults, were carried out.

Results: In the application of EPNs to the soil in plastic cups, the highest mortality rates (75 and 70%) on wheat stink bug occurred at 200 IJs cm^{-2} concentration of *S. carpocapsae* isolate at 15 and 12 °C, respectively. In other tested nematode species, the highest insect mortality was observed at 200 IJs cm^{-2} concentration of *S. feltiae* and *H. bacteriophora* isolates, with mortality rates of 65–35% and 55–25%, at 15 and 12 °C, respectively. *S. carpocapsae* (Black sea isolate) was the most pathogenic among the three nematodes tested.

Conclusions: It was concluded that *S. carpocapsae* had the potential as a biocontrol agent against the wheat stink bug, *A. rostrata*. The applications of *S. carpocapsae* were the great potential for the management of the pest. Effective use of EPNs should be evaluated in integrated pest management strategies between other biological control agents. According to EPNs laboratory experiments results, field trials should be conducted in future studies.

Keywords: *Aelia rostrata*, Entomopathogenic nematodes, *Steinernema*, *Heterorhabditis*, Biological control

Background

Entomopathogenic nematodes (EPNs) are obligate parasites in the genera *Steinernema* and *Heterorhabditis*. EPNs kill insects with the aid of a mutualistic bacterium, which they carried in their intestine. The nematodes complete 2–3 generations within the host, after which free-living infective juveniles (IJs) emerge to seek new hosts (Poinar 1990). EPNs have been applied successfully against soil and foliar insect pests (Klein 1990).

The wheat stink bug, *Aelia rostrata* Boheman (Hemiptera: Pentatomidae), is one of the most harmful pests that caused significant losses in cereals. A total of nine species are found in Turkey. The harmful species in the Central Anatolia Region (Turkey) is *A. rostrata* (Iyriboz 1970). Adults overwinter at an altitude of 1500–2000 m and

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spend the winter in the form of diapauses about 4–8 cm deep under the leaves of plants such as oak (*Quercus* spp.), pine (*Pinus* spp.), milkvetch (*Astragalus* spp.) and prickly thrift (*Acantholimon* spp.). In the spring, when the air temperature is about 19–22 °C, they begin to fly toward the plain from the places where they collectively wintered. When the air temperature continues for a few days above at least 20 °C, they leave their wintering places in 3–5 days. Wheat stink bugs, descending from the winter to the plain, feed with developed cereals and spikes for 10–15 days and mate. Females lay their eggs in packages (12–18 packs) on spikes, stems, leaf surfaces, soil and weeds. One female lays about 150–180 eggs. The nymphs hatching from eggs mature after changing five shirts. The nymphal period lasts an average of 20–30 days. With some enzymes secreted from their bodies, they soften the grains and destroy their gluten. (Anonymous 2017).

Many research articles are published for controlling the brown marmorated stink bug and green stink bug. Guide et al. (2019) evaluated trials with the potential of EPNs (15 isolates of genera *Steinernema* and *Heterorhabditis*) for the control of *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae). Concentrations of (10, 20, 40, 50, and 100 IJs cm^{-2}) and greenhouse tests were carried out only with the *S. feltiae* isolate (IBCB-n 47). As results of the study, all isolates showed pathogenicity and virulence to adults of *D. melacanthus*. The isolates GL (*H. amazonensis*), IBCB-n27 (*Steinernema* sp.), and RSC05 (*H. amazonensis*) were the most virulent (80.0, 82.0, and 88.0% mortality, respectively). The highest concentrations of *S. feltiae* (50 and 100 IJs cm^{-2}) were responsible for the highest mortality rates of the green belly stink bug (88.0 and 86.0%, respectively). In the greenhouse test, *S. feltiae* caused higher mortality (38%) than the control (26%). Burjanadze et al. (2020) conducted trials in Georgia with the potential of native EPNs for the control of the brown marmorated stink bug, *Halyomorpha halys* (Stal) (Hemiptera: Pentatomidae). Two Georgian strains of *H. bacteriophora* (HRB, GEO) and *Steinernema borjomiense* and two Italian strains of *H. bacteriophora* (HRB, IT) and *S. apuliae* were used in laboratory assays at (22 °C and 80% RH) and the concentrations of 1000, 500 and 200 IJs per adult *H. halys*. At the highest concentration (1000 IJs adult^{-1}), mortality (%) of *H. halys* induced by Georgian strains HRB and *S. borjomiense* was 53.3 and 40%, respectively, while at the concentration of 500 IJs adult^{-1} caused 40 and 33.3% mortality, and 200 IJs adult^{-1} recorded 33.3 and 13.3% mortality, respectively. Italian strains HRB (IT) and *S. apuliae* proved more suppressive against the tested insects, with mortality rates of 95.3–60, 93.3–40 and 73.3–33.2%, at the concentrations of 1000, 500 and 200 IJs adult^{-1} of *H. halys*, respectively. Nanzer et al. (2021) conducted tests with *S. diaprepesi* AM163, *S. carpocapsae* All, and *S. carpocapsae* IP1 on *Euschistus heros* (Hemiptera: Pentatomidae) and observed 100% mortality of *E. heros* when the infective juveniles (IJs) were applied to sand at a concentration of 140 IJs cm^{-2} (1000 IJs insect^{-1}) under laboratory conditions. However, greenhouse tests showed lower mortality (72.5%) of *E. heros*. Cecconello et al. (2022) evaluated the potential of eight isolates of the species *Heterorhabditis amazonensis* to control *Euschistus heros* (Fabricius) under laboratory and field conditions. As results of the study, the isolates IBCB-n46, NEPET11, and IBCB-n40 caused the highest mortality in adult *E. heros* from laboratory rearing (100, 94, and 80%, respectively), and IBCB-n46 and NEPET11 were the best against the field population (71 and 47% mortality, respectively).

In this study, the efficacy of three local Turkey isolates of *Heterorhabditis bacteriophora*, *Steinernema feltiae*, and *S. carpocapsae* species was evaluated under laboratory conditions against *A. rostrata*.

Methods

Nematode cultures

Entomopathogenic nematodes (EPNs) species were obtained from Aydın Adnan Menderes University, Faculty of Sciences and Literature, Department of Biology, Department of Zoology, Aydın (Turkey). The EPNs of *S. feltiae* (Aydin isolate), *S. carpocapsae* (Black sea isolate), *H. bacteriophora* (Aydin isolate) were obtained from a vegetable garden in Aydın, grassland in Rize and peach orchard in Aydın (Turkey), respectively. Stock cultures of EPNs maintained at the Plant Protection Research Institute, Diyarbakır (Turkey).

Production of nematodes

Stock cultures of EPNs were produced at the Plant Protection Research Institute, Diyarbakır (Turkey). The wax moth larvae, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) were obtained from Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection, Tokat (Turkey). EPNs were produced on last instar larvae of the greater wax moth, at 25 °C as described by Woodring and Kaya (1988). After harvesting, the nematodes were stored at 5 ± 1 °C for two weeks.

Collection of wheat stink bug

Aelia rostrata adults were collected from Beynam overwintering areas (1480 m) in Ankara province (Turkey). Soil samples used in the study were also taken from Karacadag overwintering areas (1550 m) in Diyarbakır province (Turkey). Soil taken from the overwintering areas before the experiment was sterilized twice in the autoclave at 121 °C for 15 min and allowed to stand for 24 h between the two procedures (Smith and Onions 1994).

Laboratory trials

Experiments were carried out in plastic cups (30 g); soil taken from the overwintering areas and sterilized was placed in 60 ml plastic cups. Soil humidity was set as 15%. After adding one adult wheat stink bug into each cup, some soil was added on it. The nematode suspensions were then applied with a small hand sprayer to release 25, 125, and 200 IJs cm^{-2} to determine the most effective concentration (Glazer and Lewis 2000). In the control groups, the same experimental setup was used, but only the water was given to the cups. The prepared cups were kept in 15 °C incubators for one week (Shapiro et al. 1999). Trials were carried out for each EPN species at the same time. For each nematode concentration, four groups of five *A. rostrata* adults in soil filled cup were used. Two replicate bioassays at separate dates were tested as described above. Parallel studies at 12 °C were carried out using the same method, and mortality rates were recorded. It was preferred to establish trials at temperatures close to the overwintering temperature of wheat stink bug for future nature studies.

Statistical analysis

Analysis of variance was applied to test the significance of nematode mortality, and percent death values obtained in the studies were calculated according to Abbott formula (Abbott 1925). ANOVA was applied to the data, and Duncan multiple comparison method was compared. Data were analyzed using SPSS statistical software.

Results

Obtained results showed that all (EPNs) tested were able to kill wheat stink bug adults, expect compared to all controls. All EPNs caused significant mortality of adult *A. rostrata* relative to the controls. The mortality induced by nematodes increased, typically with increasing numbers of nematodes per adult. Results of laboratory trials showed that in the application of EPNs to the soil in plastic cups, the highest mortality rates (75 and 70%) on wheat stink bug occurred in 200 IJs cm^{-2} concentration of *S. carpocapsae* (Black sea isolate) isolate at 15 and 12 °C, respectively (Fig. 1).

According to the first repetition results of trials, the highest insect mortality was observed by *S. carpocapsae* (Black sea isolate) (75%) at the concentration of 200 IJs cm^{-2}, followed by *S. feltiae* (isolate 09-31) (Aydin isolate) (65%) at a concentration of 200 IJs cm^{-2} and then *H. bacteriophora* (isolate 09-43) (Aydin isolate) (35%) with a concentration of 200 IJs cm^{-2} in the trials carried out at 15 °C ($F = 1.83$; $df = 9.915$; $P < 0.05$). However, the highest insect mortality was observed by *S. carpocapsae* (Black sea isolate) (65%) at the concentration of 200 IJs cm^{-2}, followed by *S. feltiae* (isolate 09-31) (Aydin isolate) (55%) at a concentration of 200 IJs cm^{-2} and then

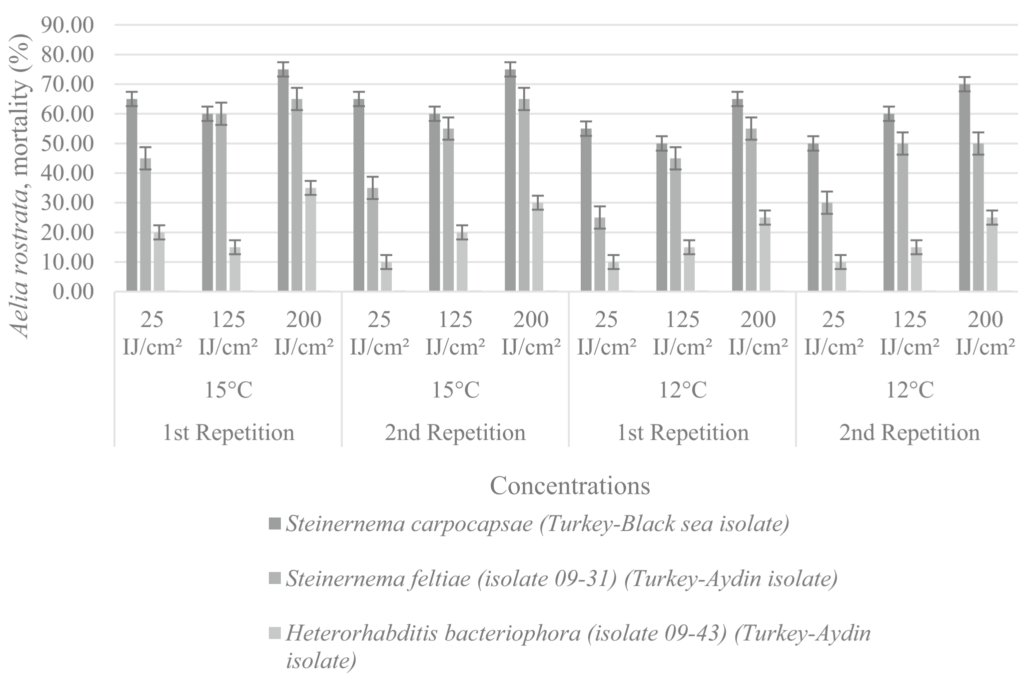

Fig. 1 Mortality (%) of three different concentrations of entomopathogenic nematodes (EPNs) from isolated Turkey [*Steinernema carpocapsae* (Black sea isolate), *S. feltiae* (isolate 09-31) (Aydin isolate), and *Heterorhabditis bacteriophora* (isolate 09-43) (Aydin isolate)] on wheat stink bug, *Aelia rostrata* adults at 15 °C and 12 °C (first and second repetitions) ($P < 0.05$)

H. bacteriophora (isolate 09-43) (Aydin isolate) (25%) at the concentration of 200 IJs cm^{-2} in the trials carried out at 12 °C ($F = 1.83$; $df = 7.383$; $P < 0.05$) (Table 1).

According to the results of the second trial, the highest insect mortality was observed *S. carpocapsae* (Black sea isolate) (75%) at the concentration of 200 IJs cm^{-2}, followed by *S. feltiae* (isolate 09-31) (Aydin isolate) (65%) with a concentration of 200 IJs cm^{-2} and then *H. bacteriophora* (isolate 09–43) (Aydin isolate) (30%) with a concentration of 200 IJs cm^{-2} in the trials carried out at 15 °C ($F = 1.83$; $df = 10.233$; $P < 0.05$). However, the highest insect mortality was observed *S. carpocapsae* (Black sea isolate) (70%) at the concentration of 200 IJs cm^{-2}, followed by *S. feltiae* (isolate 09-31) (Aydin isolate) (50%) at the concentration of 125–200 IJs cm^{-2} and then *H. bacteriophora* (isolate 09-43) (Aydin isolate) (25%) with a concentration of 200 IJs cm^{-2} in the trials carried out at 12 °C ($F = 1.83$; $df = 8.051$; $P < 0.05$) (Table 1). *S. carpocapsae* (Black Sea isolate) was found to be the most pathogenic isolate among the three nematodes tested.

Discussion

EPNs isolates [*S. carpocapsae* (Black sea isolate), *S. feltiae* (isolate 09-31) (Aydin isolate), and *H. bacteriophora* (isolate 09-43) (Aydin isolate)], which were isolated from Turkey, used in the study, were highly effective isolates.

When the efficacy studies against different pest groups by using these isolates were examined, the efficacy of EPNs has been performed against great spruce bark beetle (*Dendroctonus micans*) at 25 °C and 1000 IJs concentration, the larval mortality was 98.04 and 94.04% for Aydin isolates of *S. feltiae* and *H. bacteriophora*, respectively. However, *S. carpocapsae* (Black sea isolate) did not perform more than 40% mortality (Kepenekci and Atay 2014). But in this study, *S. carpocapsae* (Black sea isolate) showed a high effect to control the wheat stink bug. In other study, same EPN isolates at three concentrations (500, 1000, and 5000 IJs ml^{-1}) were used to control an important alfalfa pest *Holotrichapion pullum* (Gyllenhal) (Coleoptera, Apionidae). At the end of the study, *S. carpocapsae* (Black sea isolate) exhibited the highest mortality rates (80, 83, 82%, respectively) at all the tested concentrations at 20 °C, followed by *S. feltiae* (Aydin isolate) (30, 41, 35%, respectively) and then *H. bacteriophora* (Aydin isolate) (24, 27, 30%, respectively) (Atay and Kepenekci 2015). Although the same isolates (*S. carpocapsae, S. feltiae* and *H. bacteriophora*) have been used against different pests in previous studies, the efficacy values were very similar to the efficacy values obtained in this study.

In Turkey, there are very few studies on the use of EPNs against sunn pest [*Eurygaster integriceps* Puton (Hemiptera: Scutelleridae), *Eurygaster maura* L. (Hemiptera: Pentatomidae)]. In these studies, the activities of EPNs against sunn pest adults were demonstrated in laboratory trials. But no studies have been found about the use of EPNs on the control of the wheat stink bug in laboratory conditions (in vitro). Only there is one study of *H. bacteriophora* obtained from infected wheat stink bug (*A. rostrata*) adults collected from Ekecik overwintering areas in Aksaray province (Turkey).

Table 1 Percent mortality of wheat stink bug adults to three different concentrations of entomopathogenic nematodes (EPNs) from isolated Turkey [*Steinernema carpocapsae* (Black sea isolate) (*S.c*), *S. feltiae* (isolate 09-31) (Aydin isolate) (*S.f*), and *Heterorhabditis bacteriophora* (isolate 09-43) (Aydin isolate) (*H.b*)] on wheat stink bug, *Aelia rostrata* adults at 15 °C and 12 °C (first and second repetitions) ($P < 0.05$)

EPN species and concentrations	15 °C		12 °C	
	Mortality (%) (first repetition)	Mortality (%) (second repetition)	Mortality (%) (first repetition)	Mortality (%) (second repetition)
S.c (25 IJ cm^{-2})	65.00 ab*	65.00 a*	55.00 a*	50.00 ab*
S.c (125 IJ cm^{-2})	60.00 abc	60.00 ab	50.00 ab	60.00 a
S.c (200 IJ cm^{-2})	75.00 a	75.00 a	65.00 a	70.00 a
S.f (25 IJ cm^{-2})	45.00 bcd	35.00 bcd	25.00 bc	30.00 bc
S.f (125 IJ cm^{-2})	60.00 abc	55.00 abc	45.00 ab	50.00 ab
S.f (200 IJ cm^{-2})	65.00 ab	65.00 a	55.00 a	50.00 ab
H.b (25 IJ cm^{-2})	20.00 def	10.00 de	10.00 c	10.00 cd
H.b (125 IJ cm^{-2})	15.00 ef	20.00 de	15.00 c	15.00 cd
H.b (200 IJ cm^{-2})	35.00 cde	30.00 cd	25.00 bc	25.00 bcd
Control (25 IJ cm^{-2})	0.00 f	0.00 e	0.00 c	0.00 d
Control (125 IJ cm^{-2})	0.00 f	0.00 e	0.00 c	0.00 d
Control (200 IJ cm^{-2})	0.00 f	0.00 e	0.00 c	0.00 d

* Values with different letters in the same column are statistically different from each other

This is the first study in which EPNs were detected in wheat stink bug adults (Kepenekci et al. 1999).

When the efficacy studies against sunn pest by using EPN isolates were examined, different mortality rates were observed in these studies. Canhilal et al. (2007) investigated the efficacy of EPN species against sunn pest (*E. integriceps*) under laboratory conditions (25 °C). The highest mortality rate was observed at *S. riobravae* Texas isolate (90%), with 400 IJs application, and the lowest mortality rate was found in *H. bacteriophora* Musherphe isolate (30%) with 50 IJs application. In another study, Kepenekci (2004) investigated the efficacy of three races of two EPN species on adults of sunn pest (*E. maura*). As a result of the study, it was determined that *S. carpocapsae* (Anamur race), *H. bacteriophora* (Tur-H1 race), and *H. bacteriophora* (Tur-H2 race) caused 55, 69, and 95%, respectively.

In another study conducted by Koçak et al. (2007), the efficacy of different *S. feltiae* races (All and S3) against the sunn pest (*E. maura*) adults was investigated. In this study, counts were made 72 and 96 h after application at water concentrations of 25, 50, and 100 IJs 0.2 ml^{-1} and at three different temperatures (10, 15, and 25 °C). *S. feltiae* (All) showed 63.8% effect at 100 IJs 0.2 ml^{-1} water concentration and 25 °C, *S. feltiae* (S3) was found to be 74% effective at the same concentration and temperature.

In another study, Gözel et al. (2020) investigated three EPN species from Turkey, namely *S. feltiae* (Yalova isolate), *S. carpocapsae* (Sakarya isolate), and *H. bacteriophora* (Sakarya isolate) on sunn pest (*E. integriceps*) adults. Trials were conducted at two application doses (500–1000 IJs $adult^{-1}$) and 25 °C under laboratory conditions. As a result of this research, the highest recorded mortalities were 100, 100, and 92% for *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora*, respectively, at 1000 IJs dosage and 7 days after application. According to the results obtained in their study, it was observed similarly that the isolates belonging to the genus *Steinernema* used in this study showed a high efficiency in the laboratory than the isolates belonging to the genus *Heterorhabditis*.

Kepenekci et al. (1999) evaluated the isolated EPNs from wheat stink bug adults collected from overwintering areas. Their study included *H. bacteriophora*, the first species of *Heterorhabditis* genus, obtained from infected wheat stink bug (*A. rostrata*) adults, collected from Ekecik overwintering areas in Aksaray province in Turkey. This was the first study in which EPNs were detected in wheat stink bug adults.

Conclusions

This study indicated that *S. carpocapsae* (Black sea isolate) was the most pathogenic EPNs among the other tested nematode species, *S. feltiae* and *H. bacteriophora*. Therefore, EPNs isolated from Turkey may be useful for control of *A. rostrata*. One of the isolate *S. carpocapsae* (Black sea isolate) which was the most effective on wheat stink bug (*A. rostrata*) had the potential as a biocontrol agent against this pest. It seems that no studies have been carried out on the use of EPNs to the control of the wheat stink bug. *S. carpocapsae* had a potential as a biocontrol agent against the wheat stink bug, *A. rostrata*. According to EPN laboratory experiments results, field trials should be conducted in future studies.

Abbreviations

EPN: Entomopathogenic nematode; IJ: Infective juvenile; *S.c*: *Steinernema carpocapsae* (Black sea isolate); *S.f*: *S. feltiae* (isolate 09-31) (Aydin isolate); *H.b*: *Heterorhabditis bacteriophora* (isolate 09-43) (Aydin isolate).

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Author contributions

Stock cultures of EPNs (*S. carpocapsae*, *S. feltiae*, and *H. bacteriophora*) were produced, and wheat stink bug adults were collected from overwintering areas by AP. Also, AP conducted the experimental studies. AP and İK conceived and designed the research and analyzed the data. AP conducted the experiments. İK interpreted the data and corrected and revised the manuscript, corrected language mistakes and translation, and corrected references. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset(s) supporting the conclusions of this article is (are) included within the article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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