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Efficacy of the entomopathogenic nematode, *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) on *Mecorhis ungarica* (Herbst, 1784) (Coleoptera: Rhynchitidae)

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Abstract

Background: In this study, the virulances of 4 different concentrations of the entomopathogenic nematode, *Steinernema feltiae* (Filipjev) on adults of the rose weevil, *Mecorhis ungarica* (Herbst, 1784) (Coleoptera: Rhynchitidae) were tested under laboratory conditions.

Results: Ten replications for each concentration where 5 adults for each replicate were used. Suspensions prepared from each concentration, containing 3rd instar juveniles (infective juveniles) of *S. feltiae,* were sprayed on the pest adults for 20 s at 1 atm pressure. Deltamethrin was applied, at the recommended concentration (30 ml/ha), as a positive control, while pure water was used as negative control. Observations started 72 h after the applications and continued until the 15th day. The White Trap Method used for the re-isolation of nematodes from dead individuals obtained in observation days. As a result of the study, obtained mortality rates in concentration of *S. feltiae* (100, 150, 200, 250 million IJs/100 l water) were not different from each other but were found significant than control ($P < 0.05$). Additionally, among the number of re-isolated 3rd juveniles, the highest mean number was found on 200 million IJs concentration and the lowest mean was determined on 150 million IJs concentration. Tested concentrations of *S. feltiae* caused 80, 86, 82, and 92% death on adult individuals on the 15th day. Mortality rates caused by each concentration depending on the time were not significant between each other ($P > 0.05$).

Conclusion: In this study, it was determined that different concentrations of *S. feltiae* were effective on adults of rose weevil. It is thought that entomopathogenic nematodes may be an alternative and promising biological control strategy to reduce the risk of pesticide residues in oil-bearing rose production areas.

Keywords: Rose weevil, *Mecorhis ungarica*, Entomopathogenic nematodes, *Steinernema feltiae*, Virulance

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Background

The rose weevil, *Mecorhis ungarica* (Herbst, 1784) (Coleoptera: Rhynchitidae) is one of the important pests that caused damage to Rosaceae. Adult females break the rosebuds and hatched larvae feed inside the buds, so the buds do not open, produce abnormal flowers, and yield decreases. As in all conventional farming systems, the first tendency of farmers for the management of a pest such as rose weevil, unfortunately, is the use of chemical pesticides. This control strategy has led to pesticide residues in all oil-rose products that created problems in the export of the products (Kumar et al. 2004). Thus, biological control methods can be hopeful, safer, and sustainable for pest control in oil-bearing rose cultivation. The entomopathogenic nematodes (EPN); *Heterorhabditis* and *Steinernema* species are among the most known and important biological control agent that their uses have been increased in recent years (Susurluk and Ökten 2000). EPN species are symbiotically associated with *Xenorhabdus* spp. and *Photorhabdus* spp., which are harbored in the intestine of the third-stage infective dauer juvenile (DJ) (Ciche et al. 2006). They enter the insects' hemolymph through natural openings and release their bacteria inside the hosts to die because of septicemia within 24 to 48 h (Stock and Goodrich-Blair 2008). In this way, these symbiont bacteria are responsible for causing death of the host (Susurluk 2008).

The present study aimed to evaluate the efficacy of the EPN, *Steinernema feltiae* on adults of *M. ungarica* under laboratory conditions.

Main text

Four different concentrations of the entomopathogenic nematode *S. feltiae* (Bioglobal Co.; 100 (recommended concentration), 150, 200, and 250 million infective juveniles (IJs)/100 l water), and field concentration of deltamethrin (pyrethroid 3A) (Delmetrin, Tarkim Plant Protection Industry and Trade Inc., Turkey) were tested under laboratory conditions. Adult individuals of *M. ungarica* were obtained from an organic rose production area in district of Isparta in Turkey.

Method

The effects of 4 different concentrations of *S. feltiae* on adult individuals of *M. ungarica* were tested under laboratory conditions (25–30 °C, 30–40% humidity). In the trial, 10 replicates per concentration (5 adults per replicate) were used. Suspensions prepared for each concentration, containing 3rd instar juveniles (IJs) were sprayed at 1 atm pressure for 20 s (0.3 ml suspension for each Petri dish including 5 adult individuals) after then adults were placed into boxes (8 × 12.5 × 9 cm). Fresh rose flowers were brought from the organic rose production area for feeding of adult individuals and were renewed periodically in the boxes. Deltamethrin was applied at the recommended dose (30 ml/ha), as positive control and pure water was used as negative control. Observations of *M. ungarica* started 72 h after the applications of suspensions and continued until the 15th day. The White Trap Method (Kaya and Stock 1997) was used for the re-isolation of nematodes from dead individuals obtained throughout the observation days. On each observation day, dead individuals were taken from the boxes, transferred to White traps and incubated in distilled water at the dark at 20 °C for 15 days. After incubation, nematode suspensions were placed in measuring cylinders and 8 h was waited for precipitation, the supernatant discarded, and the concentrated nematodes were transferred into 15-ml tubes. Nematodes were counted under the light microscope at × 100 magnification (Olympos BX51).

Statistical analysis

One-way Anova test was applied to the parametric mortality values of *M. ungarica* and the number of the re-isolated nematodes that 3rd juvenile stage, followed by Tukey's HSD (honestly significant difference) test was performed ($P < 0.05$). The statistical software package SPSS 20.0 was used for all statistical analyses.

Results

The present study is the first experiment of EPNs on *M. ungarica*. According to the concentration and the mortality interaction, the mortality rates (%) were not different among the concentrations *S. feltiae* (100, 150, 200, 250 million IJs/100 l water), whereas these rates were significant than control. The mortality rate in case of the deltamethrin, the chemical originated insecticide studied as a positive control, was non-significant compared to all other applications.

Even though non-significant difference was found among mortality rates caused by the concentrations applied on rose weevil. There were little differences in mortality rates caused by 100, 150, and 200 million IJs/100 l water concentrations of *S. feltiae* depending on time. However, the 100 million IJs/100 l water concentrations of *S. feltiae* showed that mortality rates in the other observation days (6th, 9th, 12th, and 15th) were more significant than the 3th day ($P < 0.05$). While the 150 million IJs/100 l water concentrations of *S. feltiae* reached the highest mortality rate on the 12th day, at the 200 million concentrations occurred the highest mortality rate on the 9th day. It was determined that for only 250 million IJs/100 l water concentrations of *S. feltiae* was no difference among time-dependent mortality rates (3th, 6th, 9th, 12th, and 15th). In all observation times, no differences were found between mortality rates that occurred at the 100, 150, 200, and 250 million IJs/

Table 1 Time-dependent mortality rates (%) of different concentrations of *Steinernema feltiae* on *Mecorhis ungarica*

Time (day)	Treatments and mortality rates (%)					
	100 M IJs/100 l W	150 M IJs/100 l W	200 M IJs/100 l W	250 M IJs/100 l W	Control	Deltamethrin
3th	50 **aB**	46 **aB**	46 **aB**	74 **aA**	0 **bA**	100 **cA**
6th	72 **aA**	64 **aAB**	70 **aAB**	84 **aA**	0 **bA**	100 **cA**
9th	76 **aA**	70 **aAB**	78 **aA**	88 **aA**	0 **bA**	100 **cA**
12th	76 **aA**	80 **aA**	80 **aA**	88 **aA**	0 **bA**	100 **cA**
15th	80 **aA**	86 **aA**	82 **aA**	92 **aA**	0 **bA**	100 **cA**

"A" and "B" mean followed by different letter in the same column are different ($P < 0.05$); "a", "b", and "c" mean followed by different letter in the same row is not different ($P < 0.05$)

100 l water concentrations of *S. feltiae* ($P > 0.05$) (Table 1).

The mean number of re-isolated 3rd juvenile stage of nematodes per individual from *M. ungarica* adults treated with 4 different concentrations of *S. feltiae* is given in Fig. 1. The highest mean number of 3rd juvenile was found 200 million IJs/100 l water concentration of *S. feltiae* and the lowest mean number was also 150 million IJs/100 l water concentration. No difference found between other concentrations for mean number of re-isolated 3rd juvenile stage from adult of *M. ungarica*. Re-isolation data of the 3rd juvenile stage of entomopathogenic nematodes after reproducing in the host are of great importance in terms of maintaining the population of EPNs in the environment and thus the sustainability of the pest control.

Discussion

Entomopathogenic nematodes (EPNs) attacks usually cause death of their hosts. Studies associated with the use of EPNs against the coleopteran species under laboratory and field conditions were conducted. Laznik and Trdan (2015) determined that the most effective combination was EPFs (*B. brongniartii*, *B. bassiana*) and *H. bacteriophora* to control first and second instar larvae of the white grubs (Coleoptera: Scarabaeidae). EPNs have many superior features such as easy commercial productions in vivo or in vitro, long-term effect, easy application, application with many chemicals safety for the environment and human health, pathogenicity, host search behavior, and survivability (Canhilal 2011). Pathogenicity, invasion and reproductive capacity of EPNs are important in terms of persistence and settlement in areas where they are applied (Susurluk and Ehlers 2008; Susurluk et al. 2009). It is known that the effectiveness of EPNs species can vary according to abiotic factors such as air and soil temperature under natural conditions. However, the EPN formulations lose their moisture within a few days and cannot support enough humid condition that needs for EPN survival (Lacey and

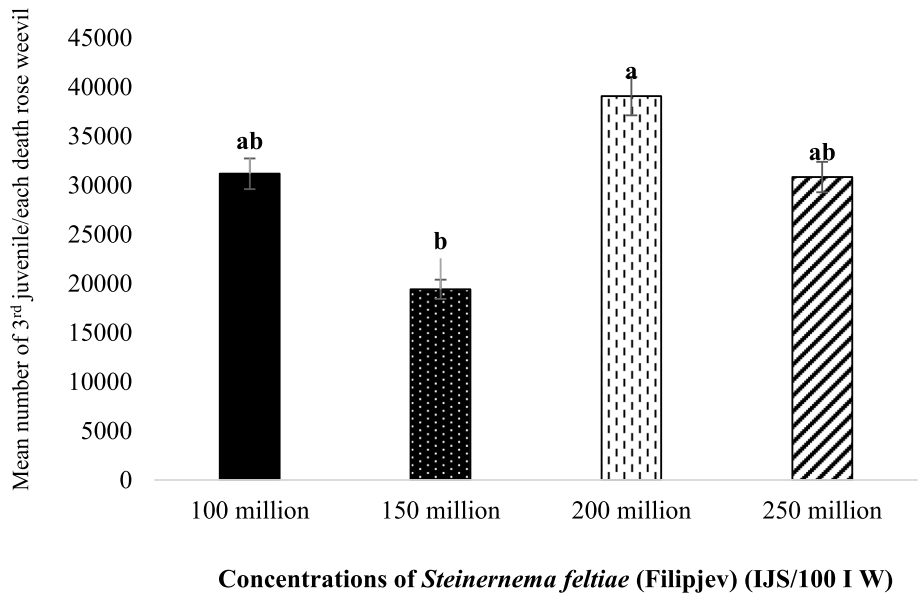

Fig. 1 Mean number of re-isolated 3rd juveniles from *Mecorhis ungarica* at different concentrations of *Steinernema feltiae*

Georgis 2012). Susurluk (2008) reported that *S. feltiae* and *S. weiseri* might be more effective against *Bothynoderes punctiventris* Germar another species of Curculionidae, early in the growing season when the soil temperature is low. However, *H. bacteriophora* might be more effective later in the season when the temperature increases, in the same study. Manzoor et al. (2017) reported that adult stage of mortality rate of *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) due to inoculation of *S. carpocapsae*, *H. bacteriophora*, and *S. feltiae* were 3.07, 0.66, and 0%, respectively. In the present study, 4 different concentrations of *S. feltiae* caused 80–92% mortality on adults *M. ungarica* 15th day after application. According to the obtained results, the time elapsed after application of the EPN, *S. feltiae* to adults of rose weevil had an effect on mean mortality rates under laboratory conditions. In addition that EPNs sensitivity to temperature and moisture in fields limit, its persistence at high densities and dramatic seasonal fluctuations and a lack of spatial association with hosts decrease the predictability of the nematodes' impact on *M. ungarica*. Therefore, more research is needed under field conditions to increase the chances of success of using EPNs in biological control programs.

Conclusion

It was concluded that *S. feltiae* can obviously be effective in control of the rose weevil. However, it was believed that this information based on the results obtained under laboratory conditions should be tested primarily in open field conditions.

Abbreviations

EPN: Entomopathogenic nematodes; *N*: Number of individuals; *S. feltiae*: *Steinernema feltiae*; *M. ungarica*: *Mecorhis ungarica*; IJs: Infective juveniles; DJ: Dauer juvenile; M: Million; W: Water; Anova: Analysis of variance; HSD: Honestly significant difference; Inc.: Incorporation

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Authors' contributions

Study conception and experimental design were performed by OD, AU, and FGGÖ. Data collection was performed by AU and FGGÖ. Data analysis was performed by OD. The manuscript was written by OD, AU, and FGGÖ. The authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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