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Efficiency of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) on the codling moth (*Cydia pomonella* L.) (Lepidoptera: Tortricidae) under controlled conditions

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Abstract

Background: The codling moth (CM), *Cydia pomonella* (L). (Lepidoptera: Tortricidae), is an important pest of apple in Turkey and other apple producing countries in the world. Several control methods are available for reducing the pest populations. Entomopathogenic nematode (EPNs), for example, can be used as a potential alternative to chemical insecticides to control codling moth larvae in the soil as eco-friendly management their hosts that can actively find them in cryptic locations.

Results: Efficacy of 4 EPN isolates, *Steinernema carpocapsae* (Bakışlı), *S. feltiae* (ES-3), *Heterorhabditis bacteriophora* (TOK-20) and *H. bacteriophora* (11-KG) for controlling the 1st instar larvae of the codling moth (*Cydia pomonella* L.) was investigated under controlled laboratory conditions. Codling moth was susceptible to different rates to all the 4 EPN isolates. All nematode trials were more effective than the control (water). The overall mortality caused by *S. carpocapsae* (Bakışlı 05) was significantly higher than the other EPN species. *S. carpocapsae* was the most effective with the highest tested concentration (100 IJs/larva), killing 82.63% of codling moth larvae, followed by *S. feltilae* (ES-3), with a mortality rate of 71.5%. *H. bacteriophora* (TOK-20) exhibited the least mortality at 25 IJs/larva concentration in all experiments.

Conclusion: The study proved that these nematodes were very efficient and could be used to control codling moth in biological control programs.

Keywords: Entomopathogenic nematodes, *Cydia pomonella*, Biological control, Efficiency

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Background

Apple is an economically important fruit crop produced mostly in countries with a temperate climate worldwide. Turkey is the third largest apple producing country in the world (FAOSTAT 2018). Codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), is one of the most serious pests of pome fruit worldwide and the most damaging one of commercial apple, pear, quince, and walnut orchards in Turkey and in some other countries in the world. Larvae bore holes, tunnels, and galleries in fruits as they feed on fruit pulps and cores (Pedigo and Rice 2009). The infestation leads to fruit dropping and decrease the market value of infested fruits. Use of organophosphate insecticides has been most of the common methods to control codling moth in apple orchards. In recent years, excessive and unnecessary insecticidal use against codling moth has increased and led to negative effects on human health and environment. Therefore, researchers are seeking alternative methods to the chemical control (Anonymous 2008; Gill and Garg 2014).

Today, in many countries, entomopathogenic nematodes (EPNs) are used in biological control of many economic insect pests (Shields et al. 1999; Laznik and Trdan 2011, Belien 2018). EPNs are a group of soil dwelling nematodes that parasitize insects pests. Use of these nematodes is economical and eco-friendly, since they are harmless to non-target organisms, human health and the environment (Gulcu et al. 2017). Other major advantages of using EPNs to control insects such as the codling moth are that they can be easily mass produced and applied to treetrunk using common irrigation and pesticide equipments. They are capable to actively locate insect pests in cryptic habitats, and aboveground pests have no opportunity to develop coevolutionary barriers toward EPNs. EPNs can serve as an effective supplementary control as part of an integrated pest management (IPM) strategy (Odendaal et al. 2016; Odendaal et al. 2015; Belien 2018; Gulcu et al. 2017).

The present study aimed to assess the efficiency of 4 indegenous EPN species against the 1st instar larvae of the codling moth under controlled laboratory conditions.

Methods

Nematode culture

Infective juveniles of *Steinernema carpocapsae* (Bakışlı 05), *S. feltiae* (ES-3), *Heterorhabditis bacteriophora* (TOK-20), *H. bacteriophora* (11-KG), obtained from the Plant Protection Department of Tokat Gaziosmanpaşa University, Turkey were used in this study. Infective juveniles of the 4 nematode species were reared, using the last instar larvae of *Galleria mellonella* (L.) according to the procedures described by Kaya and Stock (1997).

Rearing *Galleria mellonella* larvae

Galleria mellonella larvae were reared on a special diet containing 890 g of flour, 222 g of dry baker's yeast, 500 g of glycerin, 500 g of honey, 445 g of milk powder and 125 g of beeswax. Honey and glycerin were heated and then added to flour, bran, milk powder, and yeast mixture (Mohamed and Coppel 1983). *G. mellonella* eggs were placed on the food medium in 1 L glass jars and kept in an incubator with 16/8 h lighting set at 23–24°.

Rearing of entomopathogenic nematodes

Last instar larvae of *G. mellonella* was used to rear the EPNs for experiments. Ten larvae were placed into a 6-cm diameter small Petri dishes with lined Whatman paper soaked with distilled water. A suspension of infective juveniles of either nematodes were then applied on the *G. mellonella* larvae. The lid of the Petri dishes were wrapped by a parafilm and placed in the incubator at 20–23 °C. Larval mortality was controlled frequently. Infective EPN juveniles were obtained from infected *G. mellonella* larvae using the "White trap" method (White 1927). Obtained larvae were placed in culture flasks and kept in a refrigerator at + 10 °C. In order to prevent the nematodes from losing their activity, the same process was repeated by infecting new *G. mellonella* larvae every 1–2 months and thus the cultures were renewed in Nematology Laboratory.

Rearing of codling moth 1st instar larvae

Codling moth 1st instar larvae used in experiments were reared at 25 ± 1 °C, 65% R.H. with a 16L: 8D photoperiod conditions in a climate cabinet. Fifty adults were released in 2 L plastic container containing an adult diet of cotton soak with honey and water supply from a piece of sponge inserted into a container. A polyethylene sheet was placed on the bottom for oviposition and wet pieces of clothes for keeping moisture both side of the container. Adults laid eggs on polyethylene sheets. The eggs were hatched after approximately 5–6 days. Same day hatched larvae on polyethylene sheets were transferred by the help of a brush to artificial larval diets in Petri dishes. Artificial diet was bought from Southland Products Incorporated, USA. Codling moth is mass rearing in Entomology Laboratory.

Laboratory bioassays at different concentrations of EPNs

All laboratory studies were carried out under laboratory conditions (25 °C and 65% RH) using a 6-cm-diameter small Petri dish with artifical diet. Ten laboratory-reared first instar larvae of codling moth taken from the stock culture were placed in a new Petri dish in climate chamber. Four nematode concentrations (0, 25, 50, and 100 IJs/larva) were applied directly to the larvae by pipette (Fig. 1). Deltamethrin was used as a positive control.

Fig. 1 Inoculation of EPNs to 1st instar of codling moth larvae

Larval mortality was calculated after 96 h. Laboratory studies were conducted 5 times for each concentrations per EPN species. There were ten of 1st instar larvae in each petri dish. The experiment was repeated 4 times under the same conditions on different dates.

Experiments were conducted at 24 ± 5 °C and 65% ± 5% RH under a 16 h light/8 dark cycle. Dead larvae were placed on a White trap and after a week, EPN larvae were obtained from infected codling moth larvae. Insect cadavers were examined in distilled water under a stereomicroscope.

Statistical analysis

All data were analyzed, using statistical program JMP 7. Statistical analyses were done using one-way ANOVA, followed by LSMeans Student's *t* test to record the differences in the effects of different nematode species and concentrations on codling moth.

Results

Results were evaluated after 96 h EPN inoculation on the codling moth larvae. The main factors (Nematode species and concentrations) were considered, examined. Their interactions were statistically significant. The mortality rates of all EPN species increased with increasing nematode concentration ($p < 0.001$).

Overall, all EPNs and positive control were highly effective than the negative control (water). As presented in Table 1, mortality rates caused by the nematode species had a general trend, i.e., their effects increased with increasing the nematode concentration. The mortality rate caused by *Steinernema carpocapsae* (Bakışlı) was significantly higher than the others; *H. bacteriophora* (11KG), *H. bacteriophora* (TOK-20), *S. feltiae* (ES-3) ($p < 0.001$).

Data presented in Table 1 show the susceptibility of *C. pomonella* larvae to nematode infection. *S. carpocapsae* applied at 100 IJs/larva concentration was the most effective, with a mortality rate (82.63%) compared to the other species. At the lowest concentration (25 IJs/larva), mortality was (54.74%) for this EPN isolate. All EPN species mortality rates' range was significantly different among concentrations. There were significant differences on mortality rates caused by *S. carpocapsae* (Bakışlı 05) with increasing dose. *S. feltiae* (ES-3) ranked second, as its high mortality range namely 71.50%, 66%, and 40% at 100, 50, and 25 IJs/larva, respectively. *S. carpocapsae* and *S. feltiae* had similar effects at 50 IJs/larva concentration. Concentrations of 25, 50, and 100 IJs/larva of *H. bacteriophora* (11KG) caused 44.44, 61, and 68% mortality rates, respectively. *H. bacteriophora* (TOK-20) caused the least mortality rate on codling moth larvae at the 25, 50, and

Table 1 Mortality rate of 1st instar larvae of *Cydia pomonella* caused by different infective juvenile concentrations of entomopathogenic nematode species after 96 h

Concentrations IJs/larva	Nematode species			
	***Heterorhabditis bacteriophora* (TOK-20)**	***H. bacteriophora* (11KG)**	***Steinernema carpocapsae* (Bakışlı 05)**	***S. feltiae* (ES-3)**
100	53.50*	68.00	**82.63**	71.50
50	43.50	61.00	65.00	66.00
25	28.50	44.44	54.74	40.00
Control	3.00	2.00	5.00	2.50

*Mean separation within rows and columns using LSMeans Student's *t* test ($p < 0.05$)
CV: 0.27, LSD: 7.27

100 IJs/larva concentrations, where recording 28.50, 43.50, and 53.50% mortalities, respectively. A few mortality was recorded in negative control treatments in all replicates.

Deltamethrin (2,5 EC 25 g/l) treatment exhibited on average a (98%) mortality rate on codling moth larvae in all replicates. The results were shown that it was efficacious against this pest (Fig. 2). Following deltamethrin, *S. carpocapsae* had the highest effect on mortality at the highest concentration (100 IJs/larva), causing a mortality rate of 82.63%. *S. feltiae* killed 71.5% of codling moth larvae. The lowest mortality rate of (53.50%) was recorded in the treatments with *H. bacteriophora* (TOK-20). In all treatments, low mortality rates were observed at low concentrations. All nematode species were suitable for controlling the codling moth. *H. bacteriophora* (TOK-20) had the least mortality ratio at 25 IJs/larva concentration in all experiments. *S. carpocapsae* (Bakışlı 05) and *S. feltiae* (ES-3) at 100 IJs/larva concentration exhibited similar results with the Deltamethrin. These nematodes species had the potential to be used as an effective biological control agent.

Discussion

Few studies have investigated the effects of EPNs on codling moth. Curto et al. (2008) showed that *Steinernema carpocapsae* and *S. feltiae* were effective against codling moth overwintering in tree trunks after spray applications of EPNs to the trunk. The codling moth last instar larvae were effectively parasitized after autumnal EPNs application. On the other hand, Lacey and Unruh (1998) tested the effects of 3 EPNs species, at 50 IJs/cm^{2,} against cocooned larvae *C. pomonella.* The present study showed that *S. carpocapsae*, *S. riobrave* and *H. bacteriophora* infected (99, 80, and 83%) of larvae, respectively. Also, results indicated a good potential of EPNs, especially *S. carpocapsae,* for controlling *C. pomonella* under different environmental conditions. In another study, De Waal et al. (2017) reported that *Heterorhabditis zealandica* was effective against the late-instar *C. pomonella* larvae, causing 100% larval infection. Odendaal et al. (2015) showed that promising results to control diapausing *C. pomonella* with indigenous EPN strains. Their study emphasized the biocontrol potential of *S. jeffreyense*, as well as confirming that *S. feltiae* was an active nematode in cold weather, whereas other three tested EPN isolates (*H. bacteriophora*, *S. jeffreyense*, and *S. yirgalemense*) preferred warmer temperatures. Ahmad et al. (2020) found that indigenous EPN isolates *Heterorhabditis pakistanensis* NBAIR H-05 strain was effective against diapausing larvae of the codling moth and post wetting application of trunk band after 24 h resulted in significantly higher larval mortality than non-post wetting. Navaneethan et al. (2010) defined that the favor environmental conditions to perform the nematodes against the cocooned larvae as *C. pomonella* were more susceptible than non-cocooned larvae while the susceptibility of pupae were low.

Conclusions

All species used in this study were highly effective against the 1st instar larvae of the codling moth. *S. carpocapsae* (Bakışlı 05) proved to be the best candidate among the other EPN species to control *C. pomonella* larvae in controlled conditions. However, the four isolates could all be used for the control of *C. pomonella* in biological control program in apple and pear orchards. However, more field studies are needed to be conducted.

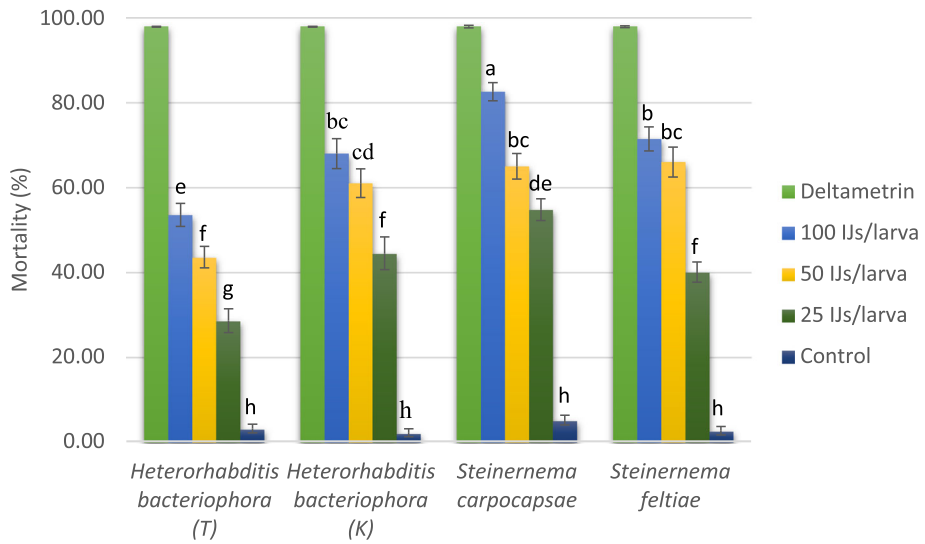

Fig. 2 Comparison the effect of deltamethrin and entomopathogenic nematodes on larval mortality rate

Abbreviations
EPNs: Entomopathogenic nematodes; *C. pomonella*: *Cydia pomonella*; *H. bacteriophora*: *Heterorhabditis bacteriophora*; *S. carpocapsae*: *Steinernema carpocapsae*; *S. fertilae*: *Steinernema feltiae*; *G. melonella*: *Galleria melonella*; CM: Codling moth

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Authors' contributions
M.Y participated in setting the work planning and executing the experimental work. A.Ö and E.A rearing codling moth larvae and participated in experimental studies. F.D.E mass rearing entomopathogenic nematodes and participated in experimental studies. All authors read and approved the final manuscript (M.Y, A.Ö, F.D.E, and E.A).

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Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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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