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Original article

Investigating the efficacies of the entomopathogenic nematodes on the last instar larvae of the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) under laboratory conditions

Entomopatojen nematodların (Rhabditida: Heterorhabditidae ve Steinernematidae) Elma içkurdu *Cydia pomonella* (L.) (Lepidoptera: Tortricidae)'nin son dönem larvaları üzerindeki etkinliklerinin laboratuvar koşullarında araştırılması

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ABSTRACT

Codling moth Cydia pomonella (L.) (Lepidoptera: Tortricidae) is the main pest of apple all over the world. Besides the usage of the broad spectrum insecticides being the main control for the C. pomonella, also there is a potential of the usage of the entomapathogen nematodes(EPN) for the same control. In this study, the efficacies of four entomopathogenic nematode species [Steinernema carpocapsae (Tokat Bakisli05), S. feltiae (Tokat-Emir), Heterorhabditis bacteriophora (TOK-20), and H. bacteriophora (11KG)] have been investigated against the last instar of C. pomonella under laboratory conditions (in vitro). Three different EPNs concentrations [250, 500 and 1000 infective juvenile (IJ)/ml-1] of the nematodes and distilled water as a control were used. Dead larvae were counted 48, 72 and 120 h after treatment, and mortality rates were calculated. H. bacteriophora (11KG) isolate was the most effective isolate causing 77.8% larval mortality at the highest concentration (1000 IJs/ml⁻¹) after 120 h. This was followed by S. feltiae (76.4%), S. carpocapsae (69.5%) and H. bacteriophora (TOK-20) (65.3%). The lowest mortality rates (respectively %5.3, 13.5, 2.7 and 10.8) were seen in all nematode species at a concentration of 250 IJs/ml-1. The isolates are found effective on the last instar larvae of C. pomonella but should be supported with detailed field studies.

INTRODUCTION

Apple (*Malus domestica* Borkh.) is a highly nutritious fruit from the Rosaceae family that is grown in a wide territory all over the world. It is accustomed to a broad of ecologies (Öztemiz et al. 2017). Turkey is among the most important apple producing countries in the world (FAOSTAT 2018). Produced 3.878,550 tons of apples in 2020 (TUIK 2020). One of the most important pests of apple, pear and walnut is the Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Barnes 1991, Howell et al. 1992, Kuyulu and Genç 2019). It's larvae feed directly on fruit, causing significant quality and quantity losses. Broad spectrum insecticides are used to control codling moth. Due to the Apple (*Malus domestica* Borkh.) is a highly nutritious fruit from the Rosaceae family that is grown in a wide territory all over the world. It is accustomed to a broad of ecologies (Öztemiz et al. 2017). Turkey is among the most important apple producing countries in the world (FAOSTAT 2018). Produced 3.878,550 tons of apples in 2020 (TUIK 2020).

One of the most important pests of apple, pear and walnut is the Codling moth, Cydia pomonella (L.) (Lepidoptera: Tortricidae) (Barnes 1991, Howell et al. 1992, Kuyulu and Genç 2019). It's larvae feed directly on fruit, causing significant quality and quantity losses. Broad spectrum insecticides are used to control codling moth. Due to the environmental and health risk associated with pesticides within the scope of Integrated Pest Management (IPM), less harmful methods of control such as the use of entomopathogens are in great demand (Lacey and Unruh 2005). Entomopathogenic nematodes (EPNs) live in the soil and are used effectively in biological control. EPNs have wide host distribution, and the ability to actively seek and infect the host. They don't have negative effects on the environment and human health and easy to use and apply (Gulcu et al. 2017). After entering the insect through the natural openings or the thin parts of the cuticle, they release the bacteria that they carry in their intestines. For these reasons, the usage of EPNs in biological control applications is increasing (Kepenekci 2012).

Codling moth larvae are known to be sensitive to EPNs (Nachtigall and Dickler 1992, Sledzevskava 1987, Unruh and Lacey 2001, Weiser 1955). There is not a single report studied that shows resistance to the entomopathogenic nematodes used to control codling moth. Entomopathogenic nematodes could be used as a supplemental agent for reducing overwintering populations (Kaya et al. 1984, Unruh and Lacey 2001). Larvae that did not die from pesticide application during the previous season could die from the application of EPNs. For this reason, involving the EPNs in the integrated pest management programs could cause reduction in the population of C. pomonella (Odendaal et al. 2018). The objective of this study was to determine the efficacy of 4 EPN isolates on the last instar larvae of codling moth.

MATERIALS AND METHODS

Entomopathogenic nematodes cultures

EPN species [(*Steinernema feltiae* (Tokat-Emir), *S. carpocapsae* (Tokat-Bakışlı05), *Heterorhabditis bacteriophora* (TOK-20), *H. bacteriophora* (11- KG)] were obtained from the Plant Protection Department of Tokat Gaziosmanpaşa University, Turkey. The infective juveniles were reared on *Galleria mellonella* (L.) last instar larvae according to Kaya and Stock (1997). Whatman paper was placed in small petri dishes 6 cm in diameter, soaked with distilled water, and ten larvae were lined up on the paper. The infective juveniles were removed from the water with a pipette and applied to the *G. mellonella* larvae. Then they were placed in the incubator at 28 ± 2 °C and checked regularly. The infective juveniles were obtained by the "White trap" method (White 1927). The recovered larvae were kept in an incubator at +10 °C.

Galleria mellonella culture

A special diet was prepared according to the relevant literature (Haydak 1936, Mohammed and Coppel 1983). First, the prepared diet was transferred to glass jars, and then *G. mellonella* larvae were placed on it. Then the jars with the eggs were placed in the incubator $(28\pm2 \text{ °C}, 16/8 \text{ photoperiod})$.

Codling moth culture

Codling moth last instar larvae using in experiment were reared in climate cabinet (25±1 °C, 65% RH with a 16L:8D photoperiod). The larvae were fed on artificial diet until the last instar larvae were obtained. Artificial diet was bought from Southland Products Incorporated, USA. 50 adults were released in 2 l plastic container containing adult diets. Eggs were laid on polyethylene sheets by adults. Hatched larvae on polyethylene sheets were placed by a brush in artificial larval diets in petri dishes.

Laboratory bioassays of EPNs

The experiments were performed using plastic containers. The last instar larvae of codling moth were transferred to each container one by one with soft forceps. A piece of corrugated cardboard (1×3 cm) was also placed in plastic containers. Then, the EPN isolates prepared in distilled water [250, 500 and 1000 infective juvenile (IJ)/ ml-1] and these isolates were applied into the plastic container with a pipette. After the application, the mouth of the container was closed with tulle and rubber. Only distilled water was used in the control. Deltamethrin 2.5 EC (at a concentration of 25 g/l) was used as a positive control. The containers were incubated under the same conditions (24±5 °C and 65%±5% RH under a 16h light/8h dark cycle). The dead larvae in plastic containers were counted at the end of 48, 72 and 120 h. The studies were performed with 3 repetitions and 10 replications. Dead larvae were taken into the "White Trap" (White 1927). After one week, EPNs were obtained from infected C. pomonella larvae.

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EPN Species	EPN Concentration	48 Hours	72 Hours	120 Hours
S. carpocapsae (Tokat Bakışlı05)	250	5.3±3.5d1B ²	6.7±3.5efB	34.7±5.0ghA
	500	9.5±3.4cdB	22.0±5.6c-eB	52.7±5.5 d-hA
	1000	16.3±2.1cdB	37.0±6.5bcB	69.4±5.0b-dA
S. <i>feltiae</i> (Tokat Emir)	250	13.5±5.2cdB	14.9±4.6d-fB	51.3±5.0 d-hA
	500	23.2±2.3bcB	30.1±0.9b-dB	61.1±7.7b-fA
	1000	34.2±2.5bB	45.2±2.4bB	76.3±5.0bcA
H.bacteriophora (Tok20)	250	2.7±1.3dB	8.1±2.2 efB	33.3±2.4hA
	500	5.4±2.7dB	13.7±1.4d-fB	40.2±2.7f-hA
	1000	12.3±4.2cdB	24.6±2.0c-eB	65.2±2.7b-eA
H.bacteriophora (KG11)	250	10.8±3.5cdB	15.0±1,2d-fB	47.2±2.7 e-hA
	500	23.2±3.3bcB	27.2±4,5b-dB	55.5±5.0c-gA
	1000	34.1±4.6bB	38.3±4.8bcB	77.7±1.3bA
	Control (Water) (-)	0.0±0.0dA	0.0±0. 0fA	0.00±0.0iA
	Control (Insecticide) (+)	97.2±1.3aA	100.0±0.0aA	100.0±0.0aA
		F=58.8; df=13,2;	F=48.8; df=13,2;	F=32.9; df=13,2;
		P<0.05	P<0.05	P<0.05

Table 1. Mortality (%) of Codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) last instar larvae caused by entomopathogenic nematode (EPN) isolates [*Steinernema carpocapsae* (Tokat-Bakışlı05), *S. feltiae* (Tokat-Emir), *Heterorhabditis bacteriophora* (TOK-20), *H. bacteriophora* (11KG)] at the end of 48, 72 and 120 h

¹Different lowercase letters following means in the same column indicate statistical significance from each other (Anova P<0.05, Tukey test) ²Different uppercase letters in the same line indicate statistically different from each other (Anova P<0.05, Tukey test

Statistical analysis:

The mortality data recorded in assays were converted to percent mortality and then transformed by arcsine transformation. One-way analysis of variance was used to test the significance, and treatment means were separated by Tukey's multiple comparison test. The statistical analyses were carried out on MINITAB (Release 18) computer program.

RESULTS AND DISCUSSION

According to statistical analyzes mortality rates increased in all entomopathogenic nematode species as the concentration increased (Table 1).

Deltamethrin which was used as a positive control, was in group A. In the assessments after 48 hours, 250 IJs/ml⁻¹ doses of S. carpocapsae and H. bacteriophora (TOK-20) were included in the same group as the negative control. In all experiments, the highest mortality rate was observed at a concentration of 1000 IJs/ml⁻¹, while the lowest mortality rate was obtained at the lowest concentrations of 250 IJs/ ml⁻¹. According to the results, the isolate H. bacteriophora (KG11) (77.8%) was the most effective isolate at the highest concentration compared to the other EPN isolates. After 120 hours, this isolate was included in group B. In addition, the mortality rates were found for other isolates such as (76.3%) for S. feltiae (Tokat-Emir), (69.5%) for S. carpocapsae (Tokat Bakışlı05) and (65.3%) for H. bacteriophora (TOK-20). The mortality rates at 250 IJs /ml-1 concentration were in S. feltiae (Tokat-Emir) isolate with (51.3%), followed by H. bacteriophora (11KG) with (47.2%), *S. feltiae* (Tokat Bakışlı05) with (34.7%) and *H. bacteriophora* (TOK-20) with (33.3%). No mortality were observed in controls treated with distilled water alone (Table 1).

There are numerous studies demonstrating the success of EPNs as a biological control agent. Orchard pests are also considered in these studies (Gaugler 2002, Kaya et al. 1984, Shapiro-Ilan et al. 2005, Unruh and Lacey 2001). *S. feltiae* has been shown to be effective against codling moth larvae in March or mid-October (Lacey et al. 2006a, 2006b, Reggiani et al. 2008). *S. carpocapsae* (Nemasys* C) provides a high level of efficacy in controlling *C. pomonella* overwintering in apple orchards (Curto et. al. 2008, Unruh and Lacey 2001). Lacey and Unruh (1998) conducted a study with *S. carpocapsae*, *S. riobrave*, and *H. bacteriophora* and reported that the most effective species was *S. carpocapsae* with 99%, followed by *S. riobrave* (83%) and *H. bacteriophora* (80%).

In another study on *C. pomonella* conducted by Odendaal et al. (2016), *S. feltiae* and *H. bacteriophora* (Hb1 and Hb2) (commercial preparations) and two indigenous species (*S. jeffreyense* and *S. yirgalemense*) were used. As a result of the study, it was reported that *S. jeffreyense* was 67% effective, followed by *H. bacteriophora* (Hb1) with 42% and *S. yirgalemense* with 41%. In laboratory bioassays involving spray application under simulated field conditions, it was found that the most effective EPN was *S. feltiae* (67%) followed by *S. yirgalemense* (58%) (Odendaal et al. 2016).

Heterorhabditis pakistanensis NBAIR H-05 strain was found to be effective against diapause larvae of codling moth (Ahmad et al. 2020). In another study investigating the efficacy of EPNs on *C. pomonella*, it was reported that pupal susceptibility was low and cocoon larvae were more susceptible than non-cocoon larvae (Navaneethan et al. 2010). *S. feltiae* was applied to *C. pomonella* larvae in four different apple fields. At the end of the study, damage was 33% less when a lower dose was applied and, correspondingly, the higher the dose, the less damage (Peters et al. 2008). It is thought that entomopathogenic nematodes can be used in biological control programs against *C. pomonella*, but more detailed field studies are needed.

The mortality data recorded in assays were converted to percent mortality and then transformed by arcsine transformation. One-way analysis of variance was used to test the significance, and treatment means were separated by Tukey's multiple comparison test. The statistical analyses were carried out on MINITAB (Release 18) computer program.

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ÖZET

Elma içkurdu Cydia pomonella (L.) (Lepidoptera: Tortricidae) dünya genelinde elmanın başlıca zararlısıdır. Mücadelesinde ağırlıklı olarak geniş spektrumlu insektisitler kullanılmakla birlikte, zararlıya karsı uygulanan biyolojik mücadele etmenleri arasında entomopatojen nematodların (EPN) kullanılabilme potansiyelleri bulunmaktadır. Bu çalışmada, dört adet EPN izolatı [Steinernema carpocapsae (Tokat Bakisli05), S. feltiae (Tokat-Emir), Heterorhabditis bacteriophora (TOK20) ve H. bacteriophora (11KG)]'nın C. pomonella'nın son dönem larvaları üzerindeki etkinliği laboratuvar koşullarında (in vitro) araştırılmıştır. Denemelerde nematodlar üç farklı konsantrasyonda [250, 500 ve 1000 enfektif larva (EL)/ ml-1] hazırlanmış ve uygulanmıştır. Kontrol olarak saf su kullanılmıştır. Nematod uygulamasından 48, 72 ve 120 saat sonra ölü larvalar sayılmış ve ölüm oranları hesaplanmıştır. H. bacteriophora (11KG) izolatı 120 saat sonra en yüksek konsantrasyonda (1000 EL/ml-1) %77.8 ölüme neden olan en etkili izolat olmuştur. Bu izolatı sırası ile S. feltiae (Tokat-Emir) (%76.4), S. carpocapsae (Tokat Bakisli05) (%69.5) ve H. bacteriophora (TOK20) (%65.3) izlemiştir. En düşük ölüm oranları (sırasıyla %5.3, 13.5, 2.7 ve 10.8) tüm nematod türlerinde 250 EL/ml-1 konsantrasyonunda görülmüştür. İzolatlar C. pomonella'nın son dönem larvaları üzerinde etkili bulunmuş ancak, detaylı arazi çalışmaları ile bu sonuçların desteklenmesi gerekmektedir.

Anahtar kelimeler: biyolojik mücadele, *Cydia pomonella*, entomopatojen nematodlar, *Heterorhabditis*, *Steinernema*

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