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***Orijinal araştırma* (*Original article*)**

The effects of some commonly used biopesticides on the survival and virulence of native Turkish entomopathogenic nematode isolates

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Yaygın olarak kullanılan bazı biyopestisitlerin yerel entomopatojen nematod izolatlarının hayatta kalma ve virülensliği üzerindeki etkileri

Öz: Bazı yerel entomopatojen nematod (EPN) izolatlarının [*Heterorhabditis bacteriophora* FLH-4H (Poinar, 1975) (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* KCS-4S (Weiser, 1955) (Rhabditida: Steinernematidae) ve *Steinernema feltiae* KMP-9S (Filipjev, 1934) (Rhabditida: Steinernematidae)] bazı biyopestisitlerle (40 g/ L Azadirachtin, 480 g/ L Spinosad) olan uyumluluğu laboratuvar şartları altında incelenmiştir. Bu biyopestisitlerin, EPN izolatlarına ait infektiv juvenillerin (IJ) canlılığı ve virülensliği üzerindeki etkisi, IJ'leri biyopestisitlerin önerilen en yüksek arazi konsantrasyonlarına 24 ve 48 saat boyunca maruz bırakılarak incelenmiştir. Biyopestisitlere 24 ve 48 saatlik sürelerde maruz kalan EPN izolatlarının hayatta kalma oranları % 5-21 arasında değişmiştir. *Heterorhabditis bacteriophora* FLH-4H izolatına ait IJ'lerin ölüm oranı her iki uygulama süresinde de %12'yi geçmemiştir ve test edilen biyopestisitlere en tolerant izolat olduğu belirlenmiştir. Test edilen biyopestisitler, EPN'ların virülensliğini önemli düzeyde etkilememiştir ve *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvalarının ölüm oranları % 70-95 arasında değişmiştir.

Anahtar sözcükler: Entomopatojen nematodlar, *Steinernema feltiae*, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, Azadirachtin, Spinosad

Abstract: The compatibility of the use of native Turkish entomopathogenic nematode (EPN) isolates, *Heterorhabditis bacteriophora* FLH-4H (Poinar, 1975) (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* KCS-4S (Weiser, 1955) (Rhabditida: Steinernematidae) and *Steinernema feltiae* KMP-9S (Filipjev, 1934), with two biopesticides, 40 g/ L Azadirachtin and 480 g/ L Spinosad, was investigated under laboratory conditions. The effects of these biopesticides on the viability and virulence of IJs of EPN isolates was studied by direct exposure to the biopesticides for 24 h and 48 h at the highest concentrations recommended for field application. Survival rates of the EPN isolates ranged between 5% and 21%. *Heterorhabditis bacteriophora* FLH-4H was the most tolerant isolate, with mortality of IJs not exceeding 12% for both exposure times. The biopesticides did not affect the virulence of the EPNs, with the mortality of *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae ranging between 70% and 95%.

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Key words: Entomopathogenic nematodes, *Steinernema sp.*, *Heterorhabditis sp.*, Azadirachtin, Spinosad

Introduction

Food safety is one of the key issues attracting the attention of people globally. The first step to ensuring food safety is the production of chemical-free products at the farm level. Therefore, environmentally-friendly control methods for pests and diseases to reduce the contamination of food with pesticides are being investigated (Tezcan et al, 2003; Kepenekci et al, 2016; Yuksel et al, 2017; Eroglu et al, 2019; Yuksel & Canhilal, 2019).

Biopesticides are produced from living things and generally pose fewer risks than chemical pesticides to human health and the environment. Recently, many biopesticides have been developed from microorganisms (bacteria, fungi, virus or protozoan) and plants, and derived from animals (pheromones, hormones, insect-specific toxins, etc.), and used across the world in the control of agricultural pests (Nawaz et al, 2016). Entomopathogenic nematodes (EPNs) are often classed as microbial pesticides and are considered one of the best biological control agents of insect pests (Grewal et al, 2005).

Entomopathogenic nematodes are often applied in conjunction with other biological and chemical pesticides and some pesticides can affect the survival and virulence of EPNs (Zimmerman & Cranshaw, 1990; Patel & Wright, 1996; Grewal et al, 1998). There are differences in the susceptibility of EPN species and isolates to agrochemicals; therefore, the compatibility of different commercial biopesticides and EPN isolates should be assessed to achieve good pest management. In this study, the effects of two commonly used commercial biopesticides on the survival and virulence of EPN isolates were evaluated.

Materials and Methods

Three EPN isolates recovered from the Cappadocia region of Turkey, namely *Heterorhabditis bacteriophora* FLH-4H (Poinar, 1975) (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* KCS-4S (Weiser, 1955) (Rhabditida: Steinernematidae), and *Steinernema felitae* KMP-9S (Filipjev, 1934) (Canhilal et al., 2016, 2017), were used in the experiments. The three isolates were cultured in the last instar of *Galleria mellonella* L. (Lepidoptera: Pyralidae) under controlled conditions at 25±2^{o}C and 60±5% RH. Newly emerged infective juveniles (IJs) of the isolates were kept refrigerated in tap water at 5^{o}C to 9^{o}C for one week before the experiments were carried out (Kaya & Stock, 1997; Ehlers, 2001). The commercial products containing the selected biopesticides that were used in this study, and their highest recommended field application rates, are given in Table 1.

The lethality of the biopesticides on the IJs of the isolates was evaluated in 24-well plates. One mL of the biopesticide prepared at the highest recommended field concentration was transferred to each well (Table 1). The suspension of IJs was prepared in tap water at 100 IJs/10µl and then added to the well. In control

treatments, the same procedure was repeated by using tap water instead of biopesticides. The well-plates were incubated on a shaker at 25 ±2 ºC and 60±5% RH in darkness in order to prevent the settling of the mixture of biopesticide and IJs (Patel & Wright, 1996; Ulu et al, 2016). Mortality was assessed after 24 and 48 hours by taking five, 50 µL subsamples from each well and observation under a stereomicroscope (Leica M125, Leica microsystems, USA).

Table 1. Biopesticides tested for their effects on the survival and virulence of three native Turkish entomopathogenic nematode isolates

Commercial name	Active substance	Classification	Manufacturer	Concentration
NİMİKS 4.5	40 g/L Azadirachtin	Insecticide	Certis USA	200 ml/ 100 L water
LASER™	480 g/L Spinosad	Insecticide	DowAgrosci. LTD.	30 ml/ 100 L water

In the virulence tests, newly emerged IJs of each isolate at 2000 IJs/mL were put into 100 mL conical flask and then 10 mL of the biopesticide at the highest recommended field concentration was added. The conical flasks were kept on a shaker at 25 ±2ºC and 60±5% RH in darkness for 24 and 48 hours. The IJs were separated from the biopesticide suspensions with a microsieve and rinsed in distilled water for 2 hours (Hara & Kaya, 1983). Using 12-well-plates, fifty live IJs of the isolate were added to individual wells, each of which contained 5 g of dry sand and one last instar of *G. mellonella* to test the infectivity of the IJs that had survived 24 or 48 hours of exposure to the biopesticide. The dead *G. mellonella* larvae were dissected under a stereomicroscope to confirm the presence of IJs. The IJs unexposed to the biopesticides were used in control treatments in the virulence testing. The experiments were replicated four times for each treatment. Statistical analyses were performed with SPSS software (22.0). Tukey's test was used to determine the differences between the effects of the applications; the level for significant difference was set at $P \leq 0.05$.

Result and Discussion

The results of this study showed that for the IJs of the EPN isolates used, there was a significant effect of isolate and biopesticide on survival, and also a significant interaction between these two factors, for both exposure times. In the virulence tests, there was a significant effect of isolate on the mortality of *G. mellonella* for both exposure times, while the interactions of EPN isolates and biopesticides were not significant for both exposure times. In addition, the efficacies of the biopesticides, as seen in the mortality rates of *G. mellonella*, were significant only after 48 hours of exposure (Table 2).

Table 2. Informations about statistical analysis ($P \leq 0.05$)

Toxic effects of two biopesticides on three Turkish entomopathogenic nematode isolates						
Resources	24 h			48 h		
	df	F	*P*	df	F	*P*
Entomopathogenic nematode isolates (N)	2	34.125	<0.001	2	64.558	<0.001
Biopesticides (B)	2	132.211	<0.001	2	121.860	<0.001
N X B	4	21.712	<0.001	4	10.816	<0.001
Virulence of three Turkish entomopathogenic nematodes against the last instar larvae of *Galleria mellonella* after 24 and 48 hours of exposure to two biopesticides						
Entomopathogenic nematode isolates (N)	2	15.831	<0.001	2	31.727	<0.001
Biopesticides (B)	2	0.877	0.428	2	5.818	0.008
N X B	4	0.808	0.531	4	2.000	0.123

Generally, the mortality rates of IJs and *G. mellonella* increased as the exposure time increased. *Heterorhabditis bacteriophora* FLH-4H was the most tolerant isolate to both of the biopesticides used for both exposure times. *Steinernema carpocapsae* KCS-4S was the most susceptible isolate to the biopesticides used and the mean mortality rates of its IJs varied between 11.8% and 21.2% for 24 hand 48 hexposure. Spinosad had the highest toxic effect on the IJs of the *S. carpocapsae* KCS-4S isolate, with mean mortality rates of 15.9% and 21.2% for 24 hours and 48 hours exposure, respectively (Table 3). The survival rate of the IJs of EPN isolates was generally higher when exposed to spinosad, except for the *S. carpocapsae* KCS-4S isolate, which had the lowest survival percentage for the spinosad treatment.

Table 3. Survival rates of three Turkish entomopathogenic nematode isolates after 24 and 48 hours of exposure to two biopesticides (%±SE)

EPN isolates	Exposure time	Mortality rates (%±SE)*		
		Spinosad	Azadirachtin	Control
Steinernema carpocapse KCS-4S	24 hours	15.9±3.0 Ba	11.8±3.7 Aa	2.7±1.6 Ab
Heterorhabditis bacteriophora FLH-4H		5.8±1.6 Aa	9.0±1.7 Ab	2.4±0.9 Aa
Steinernema feltiae KMP-9S		7.3±1.1 Aab	11.3±1.6 Aa	3.3±1.3 Ab
Steinernema carpocapse KCS-4S	48 hours	21.2±3.9 Ca	20.5±5.1 Ca	6.8±1.2 Ab
Heterorhabditis bacteriophora FLH-4H		9.8±1.6 ABa	11.4±1.7 Aa	4.9±1.6 Ab
Steinernema feltiae KMP-9S		12.0±1.4 Ba	14.9±1.9 Ba	6.1±1.3 Ab

*Mean values followed by different uppercase letters in the same column, and mean values followed by different lowercase letters in the same line, are significantly different for each exposure time; Tukey's test ($P \leq 0.05$).

All three EPN isolates showed high pathogenicity to *G. mellonella* larvae, causing mortalities ranging from 70% to 95% at 48 h. The mortality rates of *G. mellonella* in the control treatments were quite similar to those caused by the EPN isolates. Generally, there were no significant differences statistically on the virulence of EPN isolates exposed to the biopesticides when compared to the control ($P > 0.05$). Although the *S. carpocapsae* KCS-4S isolate showed the lowest survival when exposed to spinosad, there seemed to be a synergistic effect on its virulence after 24 hours of exposure to spinosad treatment when compared to the control. Maximum mortality rates (95% and 90%) were achieved with the *H. bacteriophora* FLH-4H isolate after 48 hours of exposure to Spinosad and Azadirachtin, respectively, and the *S. feltiae* KMP-9S isolate caused the lowest mortality (17% and 17%) after 24 hours of exposure (Table 4).

Table 4. The mortality of last instar larvae of *Galleria mellonella* caused by three entomopathogenic nematode isolates after exposure to two biopesticides.

EPN isolates	Exposure time	Mortality of *Galleria mellonella* (%±SE)*		
		Spinosad	Azadirachtin	Control
Steinernema carpocapse KCS-4S	24 hours	40±8 Ba	30±9 Bb	30±8.Ab
Heterorhabditis bacteriophora FLH-4H		35±12 Ba	32±9 Ba	32±5 Aa
Steinernema feltiae KMP-9S		17±5 Aa	17±5 Aa	20±2 Aa
Steinernema carpocapse KCS-4S	48 hours	70±8 Ca	75±5 Ca	85±5 Ba
Heterorhabditis bacteriophora FLH-4H		95±5 Da	90±0 Da	95±5 Ba
Steinernema feltiae KMP-9S		77±5 Ca	77±5 Ca	82±5 Ba

*Mean values followed by different uppercase letters in the same column and mean values followed by different lowercase letters in the same line are statistically different for each exposure time; Tukey's test ($P \leq 0.05$).

The survivability and virulence of the IJs of EPNs are closely associated with the species, isolate, pesticide type and dosage, and exposure time, to the pesticide (Radová, 2011; Kulkarni et al, 2013; Nitjarunkul et al, 2015; Ulu et al, 2016). Nitjarunkul et al (2015) reported that the survival rate of *S. carpocapsae* after 24 and 96 hours of exposure to neem was 99% and 96%, respectively. In another study, the survival rates of *S. carpocapsae* after combining with 0.05% Conserve (Spinosad) and 2.00% Neem Gold (Neem) were 87% and 92%, respectively (Kulkarni et al, 2013). In addition, Mahmoud et al (2016) stated that the mortalities of *S. carpocapsae* and *H. bacteriophora* did not exceed 4% after 72 hours exposure to NeemAzal T/S (1%) and Neemix (4.5%) at the lowest dose (0.1%). Krishnayyaand & Grewal (2002) reported 13% to 16% mortality of *S. feltiae* after 120 hours of exposure to Nimbecidine at 10 mL/L, which is quite similar to our findings for *S. feltiae*. The lower survival rates of *S. carpocapsae* and *H. bacteriophora* found in our study compared to earlier studies may have been due to differences in the susceptibility of EPN isolates to biopesticides and differences in the concentration rates used because the highest recommended field application rates were used in our study.

The results of the present study revealed that the IJs of EPN isolates exposed to biopesticides caused a substantial level of larval mortality. Similar mortality rates were obtained in earlier studies; Krishnayyaand & Grewal (2002) reported 60% mortality of *G. mellonella* after treatment of the IJs of *S. feltiae* with Nimbecidine, and Kulkarni et al. (2013) reported higher mortalities of *G. mellonella* caused by the IJs of *S. carpocapsae* exposed to Spinosad (83%) and Neem gold (92%). Satish et al (2018) reported 80% and 100% mortalities of *G. mellonella* larvae at 24 and 48 hours, respectively, after their treatment with IJs of *Heterorhabditis indica* Poinar, Karunakar & David that had been exposed to Neemazal. In these studies, differences in the mortality rates of *G. mellonella* larvae probably arose from different concentrations of IJs and exposure times of *G. mellonella* larvae to the IJs. However, there are also differences in the pathogenicity of EPN isolates, even in the same species, and this may have played a role in the mortality rates as well.

Conclusions

The EPN isolates tested in the present study were highly tolerant to the biopesticides used, with a lowest survival rate of 78% in the most susceptible isolate (*S. carpocapsae* KCS-4S), even though there was direct exposure to the biopesticides for 48 hours. The virulence of the EPN isolates against *Galleria mellonella* did not differ significantly from the control treatments. The overall results of this study suggest that the combinations of the EPN isolates and biopesticides tested in this study are compatible under laboratory conditions.

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