KSÜ Tarım ve Doğa Derg 26(1), 55-61, 2023
KSU J. Agric Nat 26(1), 55-61, 2023
https://doi.org/10.18016/ksutarimdoga.1029502

Biocontrol Potential of Turkish Entomopathogenic Nematodes Against the Citrus Mealybug, *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) Under Laboratory Conditions

Essa Saab NAJM[1], Ebubekir YÜKSEL[2]✉, Ramazan CANHILAL[3]
[1,2,3]Department of Plant Protection, Faculty of Seyrani Agriculture, Erciyes University, Kayseri, Türkiye
[1]https://orcid.org/0000-0003-1999-3578, [2]https://orcid.org/0000-0002-6982-5874, [3]https://orcid.org/0000-0002-0753-0077
✉: ebubekiryuksel@erciyes.edu.tr

ABSTRACT
The citrus mealybug, *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) is one of the major pests of Citrus orchards in Turkey. Management of *P. citri* is quite challenging due to its cryptic and polyphagous feeding behavior. In the current study, the control potential of native entomopathogenic nematodes (EPNs) species (*Heterorhabditis indica* 216-H, *H. bacteriophora* FLH-4H, *Steinernema carpocapsae* E-76, *S. feltiae* KCS-4S, and *S. bicornotum* MGZ-4S) against *P. citri* was evaluated under laboratory conditions at different concentrations [80, 100, 150, 200 (Infective juveniles) IJs Adult^{-1}] and temperatures (20, 25, 30 ºC). The mortality rates ranged between 16 and 58% at the highest concentration 48 hours after treatment. The highest efficacy (68%) was obtained by *Heterorhabditis indica* 216-H at the highest concentration at 25 ºC. The mortality rates were generally higher at 25 ºC than other temperatures tested and *H. indica* 216-H performed better than other EPN species tested at this temperature at all concentrations. The results indicate that *H. indica* 216-H have a great potential in the control of *P. citri*.

Plant Protection

Research Article

Article History
Received :28.11.2021
Accepted :23.03.2022

Keywords
Entomopathogenic nematodes
The citrus mealybug
Planococcus citri
Biological control

Yerel Entomopatojen Nematodların Laboratuvar Koşullarında Turunçgil Unlubiti, *Planococcus citri* (Risso, 1813) (Hemiptera:Pseudococcidae)'ye Karşı Biyokontrol Potansiyeli

ÖZET
Turunçgil unlubiti *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae), Türkiye'deki turunçgil bahçelerinin en önemli zararlılarından biridir. *Planococcus citri*'nin mücadelesi, bu türün kriptik ve polifag beslenme davranışı nedeniyle oldukça zordur. Bu çalışmada, yerel entomopatojen nematod (EPN) türlerinin (*Heterorhabditis indica* 216-H, *H. bacteriophora* FLH-4H, *Steinernema carpocapsae* E-76, *S. feltiae* KCS-4S ve *S. bicornotum* MGZ-4S) *P. citri* mücadelesindeki kontrol potansiyeli laboratuvar koşullarında farklı konsantrasyonlarda [80, 100, 150, 200 Enfektif larva (EL) Ergin^{-1}] ve sıcaklıklarda (20, 25, 30 ^{o}C) araştırılmıştır. Ölüm oranları, uygulamadan 48 saat sonra en yüksek konsantrasyonda %16 ile 58 arasında değişmiştir. En yüksek etkinlik (%68), 25^{o}C'de en yüksek konsantrasyonda *Heterorhabditis indica* 216-H ile elde edilmiştir. En yüksek ölüm oranları genellikle 25 ^{o}C'de meydana gelmiştir. *Heterorhabditis indica* 216-H 25 ^{o}C'de tüm konsantrasyonlarda test edilen diğer EPN türlerine kıyasla en yüksek etkinliği göstermiştir. Elde edilen sonuçlar, *H. indica* 216-H'nin *P. citri*'nin kontrolünde önemli bir potansiyele sahip olduğunu göstermektedir.

Bitki Koruma

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 28.11.2021
Kabul Tarihi : 23.03.2022

Anahtar Kelimeler
Entomopatojen nematodlar
Unlu bit
Planococcus citri
Biyolojik mücadele

To Cite : Najm, ES., Yüksel, E. & Canhilal, R. (2023). Biocontrol potential of Turkish entomopathogenic nematodes against the Citrus Mealybug, *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) under laboratory conditions. *KSÜ Tarım ve Doğa Derg 26*(1), 55-61. https://doi.org/10.18016/ksutarimdoga.1029502.

Atıf İçin: Najm, ES., Yüksel, E. & Canhilal, R. (2023). Yerel Entomopatojen Nematodların Laboratuvar Koşullarında Turunçgil Unlubiti, Planococcus citri (Risso, 1813) (Hemiptera:Pseudococcidae)'ye Karşı Biyokontrol Potansiyeli. KSU J. Agric Nat 26(1), 55-61. https://doi.org/10.18016/ksutarimdoga.1029502.

INTRODUCTION
Citrus cultivation occupies an important place in the Mediterranean Region of Turkey with an annual production of 4.3 million tons (TÜİK, 2020). Of the

various kinds of pests attacking citrus orchards, *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) is regarded as a major pest in Citrus orchards in the Mediterranean region of the world including Turkey (Blumberg & Van Driesche, 2001; Franco et al., 2004; Uygun & Satar, 2008; Morandi Filho et al., 2021). The citrus mealybug, *P. citri* was first discovered by Risso in 1813 from *Citrus* spp. samples in south France (Cox, 1981). The citrus mealybug is a wide spread, polyphagous and one of the the most devastating insect speices (Muştu et al., 2008). The population density of *P. citri* reaches its peak during early summer in the Mediterranean region. Newly emerged nymphs of *P. citri* settle in various parts of the plants such as the underside of leaves, twigs, and immature fruits, and start feeding by sucking plant and fruit juices. Immature fruits drop early due to feeding damages of *P. citri* on the growing fruits and fruit stalks. The sugary honeydew secreted by *P. citri* during the feeding also triggers the development of sooty mold on nearby leaves and fruits that limits the photosynthesis capacity of the leaves due to being coated with dark-colored mold. As a result of mold development and feeding damage, the yield quantity and quantity of the damaged trees drop severely. If not controlled properly, the mealybugs continue to breed and cause stunted growth, and the death of infected plants (Gill et al., 2012; Karacaoğlu & Satar, 2017). The management of *P. citri* is quite challenging. To date, attempts to control *P. citri* with chemical pesticides have met with limited success due to their cryptic feeding behavior and hidden feeding sites, coating with protective waxes, and inaccurate delivery of insecticides. Moreover, the intensive and inappropriate use of chemical insecticides lead to the development of resistance in *P. citri* populations and cause long-term severe negative effects on non-target organisms and environment (Venkatesan et al., 2016). Insecticide residues on the citrus fruits after harvest are also a cause for concern to exporters as violations of maximum residue limits (MRLs) may lead to rejected loads of product. Therefore, eco-friendly and sustainable control methods are needed in the control of *P. citri*.

Entomopathogenic nematodes (EPNs) are naturally occurring soil-borne insect pathogens and have been used successfully against many insect pest species (Karabörklü et al., 2015; Öğretmen et al., 2020; Mokrini et al., 2020; Čačija et al., 2021; Taşkesen et al., 2021; Gürkan & Çetintaş, 2022). A total of 82 species of EPN have been identified worldwide (65 belonging to *Steinernema*, 1 to *Neosteinernema*, and 16 to *Heterorhabditis*) (Kepenckci, 2014). First EPN belonging to *Steinernema* in Turkey was detected by Özer et al., (1995) as *S. feltiae* from soil samples collected from Rize. First nematode species belonging to *Heterorhabditis* in Turkey was detected by Kepenekci et al., (1999) as *H. bacteriophora* in *Aelia* population (*Aelia rostrata* Boh.) collected from Ekecik (Aksaray) winter quarters. Infective juveniles (IJs), which are the third larval stage of EPNs, are the only free-living stages outside of the host cadaver. Once a potential host is located, IJs move towards the potential host and actively penetrate into the insect body using their natural body openings/thin cuticle. Then, IJs proceed to the host's hemocoel to release the symbiotic bacteria within their intestine (*Xenorhabdus* sp. and *Photorhabdus* sp.) to assist them kill the insect in a short period time (Smart, 1995; Akhurst & Boemare, 2018; Stock, 2019). Bacterial toxins and other metabolites produced by symbiotic bacteria promote an infection process that results in the death of the host by septicemia (Griffin et al., 2005).

Although EPNs are well known to perform better in soil environment, recent studies have proven that they have also great potential in controlling the aboveground foliar pests of agricultural importance under favorable conditions (Arthurs et al., 2004; Schroer & Ehlers, 2005; Trdan et al., 2007; Laznik et al., 2010). But before initiating the field studies, EPNs are generally tested under laboratory conditions to determine the most pathogenic EPN species and isolates and achieve a successful control. In the current study, a pathogenicity screening study was conducted under laboratory conditions to reveal the control potential of different EPN species against the adults of *P. citri* at different temperatures and concentrations.

MATERIAL and METHOD

Source and Rearing of *Planococcus citri*

The initial population of *P. citri* was obtained from Assoc. Prof. Dr. Murat Muştu in the Department of Plant Protection, Faculty of Agriculture, Kayseri Erciyes University (Kayseri, Türkiye), and they were reared on sprouting potatoes (*Solanum tuberosum* L.) and pumpkin (*Cucurbita pepo* L.). Laboratory cultures were maintained in cages (650×350×590 mm) under controlled conditions (25±2°C, 60-70% RH). Only the adult female mealybugs were utilized in the experiments.

Source of nematodes

Experiments were carried out with five EPN species (*Steinernema feltiae* KCS-4S and *Heterorhabditis bacteriophora* FLH-4H, *Steinernema carpocapsae* E-76, *S. feltiae* KCS-4S, and *S. bicornotum* MGZ-4S) recovered from the same geographical region in the earlier studies (Canhilal *et al.*, 2016; 2017) (Table 1). The IJs of EPNs were reproduced last larval instar of *Galleria mellonella* L. (Lepidoptera: Pyralidae). The IJs were inoculated to Petri dishes containing 20 g autoclaved soil at the concentration of 200 IJs larva^{-1} and ten larvae were added to each Petri dish. The larvae of *G. mellonella* were cultured in 1 L glass jars under laboratory conditions (30±2°C, 60-70% RH)

using an artificial medium consisting of soybean flour, corn flour, dry bread yeast, honey, glycerin, milk powder and wheat flour (Metwally et al., 2012). Newly emerged IJs were harvested one week after inoculation and stored horizontally at the concentration of 1500 IJs ml^{-1} distilled water at 7-9ºC in culture flasks. During this period, flasks were agitated once a week to prevent the IJs from collapsing and agglomeration. Only three weeks old IJs were used in the experiments.

Table 1. Species, strain, habitat, locality, and GenBank accession numbers of entomopathogenic nematodes used in this study.

Çizelge 1. Çalışmada kullanılan entomopatojen nematodların türleri, izolatları, habitatları, lokaliteleri ve GenBank giriş numaraları.

Entomopathogenic nematodes species	Strain	Habitat	Locality	GenBank accession number
Steinernema feltiae	KCS-4S	Pine-poplar	Kocasinan-Kayseri	KX462908
S. bicornutum	MGZ-4S	Strawberry	Melikgazi-Kayseri	KX462912
S. carpocapsae	E-76	Grassland	Melikgazi-Kayseri	KX462907
Heterorhabditis indica	216-H	Olive	Dulkadiroğlu-Kahramanmaraş	KP970842
H. bacteriaphora	FLH-4H	Orchard	Felahiye-Kayseri	KX462939

Pathogenicity Bioassays

The bioassays were conducted in 24-well plates (Flat bottom, Nunc™, Cat.) (13 mm diameter) containing a circular piece of filter paper at the bottom of each well. Four multiwell plates, with each containing twelve adult female *P. citri*, were arranged (4 replicates; 48 insects) for each of the different nematode concentrations (80, 100, 150, and 200 IJs $insect^{-1}$ mealybug in 50 µl per well). Each mealybug in the control treatment was inoculated with only 50 µl of water by using a micropipette. Multiwell plates were covered with perforated parafilm to let air flow. Mealybugs were kept at 20°C after inoculation. Mortality was assessed at 24, 48, and 72 h. Previous processes were repeated for remaining temperatures 25 and 30°C. The impact of increasing concentrations of *H. indica*, *S. feltiae*, *H. bacteriaphora*, *S. carpocapsae*, and *S. bicornutum* was determined at humidity level of 95±5 RH. The bioassay was repeated on a separate date. The data of both bioassays were pooled for analysis.

Data Analysis

All data were tested for normality and the needed transformation (Arcsine) was carried out to obtain normal distribution and meet the assumptions of ANOVA. All statistical analyses were performed using Genstat software v12.1.0 (Genstat-VSNI international, 2009). Data were analyzed using RMANOVA, with a posthoc comparison of means using the Duncan method ($P \leq 0.05$).

RESULTS and DISCUSSION

All EPN species tested were able to infect and kill the adult female of *P. citri*. The analysis of data showed that the percentage mortality of *P. citri* was influenced by all main factors and their associated two-way interactions (Table 2). The virulence of tested EPN species was generally greater at 25ºC for all exposure times. The highest mortality (68.8%) was obtained after 72 hours of exposure to *H. indica* 216-H at the highest concentration (200 IJs) at 25 ºC. Only two EPN species, *S. carpocapsae* E-76 and *H. indica* 216-H were able to cause mortality over 60% at all concentrations and temperatures. *Heterorhabditis indica* 216H performed better than other EPN species at 25ºC at all exposure times and concentrations. *Steinernema carpocapsae* E-76 was the most pathogenic EPN species 24 hours post-inoculation at 20 ºC. The poorest efficacy was generally observed at *S. feltiae* KCS-4S isolate at all concentrations and exposure times.

Table 2. RMANOVA parameters for the main effects and associated interactions for mortality rates of *Planococcus citri*.

Çizelge 2. Planococcus citri'nin ölüm oranlarına ait ana faktörler ve interaksiyonlarının (Repeated Measure) ANOVA parametereleri.

Source*	df	F	P
N	4	294.12	<0.001
D	3	210.05	<0.001
T	2	79.3	<0.001
T	2	1009.33	<0.001
N x D	12	13.48	<0.001
N x T	8	17.15	<0.001
D x T	6	3.83	<0.001
N x t	8	16.77	<0.001
D x t	6	9.61	<0.001
T x t	4	4.34	0.002
N x D x T	24	0.73	0.857
N x D x t	24	0.61	0.950
N x T x t	16	1.37	0.129
D x T x t	12	0.17	0.999
N x D x T x t	48	0.09	1.000
Error	648		

*t: Time, N: Nematode species, D: Dose, T: Temperature, df: the degree of freedom, F: F-statistic, and P: Significance level (Duncan, $P \leq 0.05$).

Table 3. Mean percentage mortality (%) of *Planococcus citri* females after 24, 48, 72 hours of exposure to entomopathogenic nematode species at different concentrations and temperatures (20, 25, 30 °C).

Çizelge 3. Dişi Planococcus citri bireylerinin entomopatojen nematod türlerine farklı konsantrasyonlarda ve sıcaklıklarda (20, 25, 30 °C) 24, 48, 72 saat maruz kaldıktan sonraki ortalama ölüm yüzdeleri (%).

EPN species*	24 h				48 h				72 h			
	80 IJs	100 IJs	150 IJs	200 IJs	80 IJs	100 IJs	150 IJs	200 IJs	80 IJs	100 IJs	150 IJs	200 IJs
20°C												
216 H	4.0±2.5Aa*	4.2±2.1Aa	8.3±4.2 Aa	12.5±5.4 Aa	20.8±8.1Ba	22.9±4.5 Ba	22.9±5.1 Ba	41.7±7.3 Aa	25.0±6.3 Ba	27.1±4.3 Bab	33.3±6.5 Bab	47.9±7.4 Aa
KCS-4S	2.1±1.2Ba	2.1±1.2Ba	4.2±2.1 ABab	6.3±3.1 Aab	10.4±5.1 Cb	12.5±3.7BCb	16.7±7.2ABb	20.8±8.1Ac	16.7±7.2 Cb	20.8±8.1BCbc	25.0±5.5 ABb	31.3±6.1 Ab
FLH-4H	2.1±1.2Aa	6.3±2.5Aa	8.3±4.2 Aa	10.4±2.5 Aa	16.7±7.2 Bab	22.9±5.1ABa	25.0±5.5 Aa	31.3±6.5Aab	20.8 ±8.1Ca	25.0±5.5BCab	31.3±6.1ABab	39.6±8.1Aab
E-76	4.2±2.1Ba	8.3±4.2 ABa	10.4±2.5 ABa	14.6±5.4 Aa	18.8±8.1 Cab	25.0±6.3BCa	31.3±6.5ABa	39.6±8.1 Aa	27.1±4.3 Ca	31.3±6.1 BCa	39.6±8.1 Ba	47.9±7.4 Aa
MGZ-4	4.2±2.1Aa	6.3±3.1Aa	8.3±4.2 Aa	8.3±4.2 Aab	14.6±6.2 Bab	16.7±4.7 Bab	22.9±5.1ABab	29.2±3.2 Ab	20.8 ±8.1 Ca	25.0±5.5BCab	29.2±3.2 ABb	37.5±6.5 Ab
Control	0.0±0.0Aa	0.0±0.0Aa	0.0±0.0Ab	0.0±0.0Ab	8.3±4.2 Ab	8.3±4.2 Ab	8.3±4.2 Ac	8.3±4.2 Ad	14.6±6.2 Ab	14.6±6.2 Ac	14.6±6.2Ac	14.6±6.2 Ac
25°C												
216 H	8.3±4.2 Ca	12.5±2.5 BCa	18.8±6.3 ABa	25.0±6.3 Aa	25.0±6.3 Ca	31.3±6.1 Ca	47.9±7.4 Ba	58.3±4.2 Aa	35.4±5.7 Da	43.8±3.3 Ca	58.3±4.2 Ba	68.8±6.8 Aa
KCS-4S	2.1±1.2 Aa	4.2±2.1Abc	6.3±2.5Abc	8.3±4.2 Abc	10.4±2.5 Cb	14.6±6.2BCbC	20.8 ABc	25.0±5.4 Ac	18.8±5.4 Cb	22.9±3.9 BCc	27.1±7.3 ABc	33.3±6.1 Ac
FLH-4H	4.2±2.1Ca	8.3±4.2 BCab	14.6±5.5 ABab	20.8±8.1Aab	22.9±5.1 Ca	29.2±3.2 Ca	39.6±5.1 Ba	47.9±7.4 Aa	29.2±3.2 Ca	37.5±4.6 Cab	47.9±7.4 Bb	58.3±4.2 Ab
E-76	6.3±2.5Ca	10.4±5.4 BCab	16.7±6.1 ABab	22.9±5.1Aab	20.8±8.1Ca	29.2±3.2 Ba	37.5±6.5 Bab	52.1±8.2 Aa	31.3±6.1 Ca	39.6±4.7 Cca	52.1±6.3 Bab	62.5±8.4 ab
MGZ-4	6.3±2.5 Aa	8.3±4.2 Aab	10.4±2.5 Ab	14.6±6.2 Ab	18.8±4.2 Ba	20.8±8.1 Bb	27.1±7.3 Bbc	39.6±5.3 Ab	27.1±6.6 Ca	29.2±3.2 BCb	37.5±5.4 ABb	45.8±4.2 Abc
Control	0.0±0.0 Aa	0.0±0.0Ac	0.0±0.0Ac	0.0±0.0Ad	4.2±2.1Ac	4.2±2.1 Ac	4.2±2.1 Ad	4.2±2.1 Ad	10.4±3.2 b A	10.4±3.2 dA	10.4±3.2 Ad	10.4±3.2 Ad
30°C												
216 H	4.2±2.1 Ca	8.3±4.2 BCa	14.6±5.1 ABa	18.8±3.6 Aa	22.9±4.1 Ca	27.1±5.2 Ca	39.6±5.1 Ba	54.2±5.6 Aa	31.3±6.1 Ca	35.4±4.1 Ca	47.9±7.1 Ba	60.4±6.9 Aa
KCS-4S	0.0±0.0Aa	0.0±0.0 Aa	2.1±1.2 Abc	2.1±1.2 Abc	6.3±2.5 Bb	8.3±4.2ABCbc	12.5±5.4 Bcd	16.7 Ad	14.6±6.2Acd	16.7±6.3 Ad	18.8±4.9 Ad	22.9±5.1 Acd
FLH-4H	8.3±4.2 Ba	10.4±2.2 Ba	14.6±6.2 Aa	20.8±4.1 Aa	22.9±4.1 Ca	25.0±5.4BCab	31.3±3.5 Bab	43.7±7.4 Ab	27.1 Cab	31.3±6.1BCab	39.6±5.1 Bab	52.1±6.3 Aa
E-76	2.1±1.2 Bca	6.3±2.2 Bca	12.5±3.6 ABa	16.7±4.3 Aa	12.5±3.6 Cb	16.7±4.6 BCb	22.9±4.1ABbc	31.3±6.1 Ac	29.2±3.2Cbc	25.0±5.4BCbc	33.3±5.5ABbc	41.7±7.2 Ab
MGZ-4	0.0±0.0Aa	4.2±1.2 Aa	4.2±1.2 Abc	6.3±2.5Ab	12.5±3.6 Bb	14.6±5.2 ABb	18.8±3.6ABbc	22.9±4.3 Ad	18.8±5.2Bbc	20.8±4.7ABcd	25.0±5.4ABcd	29.2±3.2 Ac
Control	0.0±0.0Aa	0.0±0.0Aa	0.0±0.0 Ac	0.0±0.0Ac	6.3±2.1 Ab	6.3±2.1 Ac	6.3±2.1 Ad	6.3±2.1 Ae	12.5±3.5 Ad	12.5±3.5 Ad	12.5±3.5 Ae	12.5±3.5 Ad

*216-H: *Heterorhabditis indica* 216-H isolate; KCS: *Steinernema feltiae* KCS isolate; FLH: *H. bacteriaphora* FLH isolate; E-76: *S. carpocapsae* E-76 isolate; MGZ: *S. bicornutum* MGZ isolate. Different uppercase letters in the same line and different lowercase letters in the same column indicate significant differences according to Duncan test ($P \leq 0.05$).

In most cases, *Steinernema carpocapsae* E-76 showed superior performance at the lowest temperature (20 °C) compared to the other four nematode species tested (Table 3). At the highest temperature tested (30 °C), *H. bacteriophora* FLH-4H caused greater mortalities for only 24 hours of exposure and *H. indica* 216-H was the most efficient species for other exposure times. Pathogenicity screening study showed that susceptibility of the female of *P. citri* varies with EPN species under controlled conditions. The highest efficacy (68%) was obtained by *H. indica* (216 H) at the highest concentration (200 IJs) after 72 h of exposure. In most cases, *H. indica* (216 H) was also the most pathogenic nematode species, especially at 25 °C. Similar results obtained in this study, a moderate activity of EPN species against *P. citri* was reported in the earlier studies conducted by Negrisoli et al. (2013) and Stokwe and Malan (2016). Both studies reported the higher effectiveness of *Heterorhabditis* species than *Steinernema* species tested. The high virulence of *H. indica* could be explained by the small body size of the IJs of *H. indica* compared to other nematode species (Bhat et al., 2021) which is an important factor in the penetration process of EPNs into host insects. The penetration of IJs into the host body could be affected by the size of both host and IJs since most IJs use the natural body openings as an entrance (Bastidas et al., 2014). The results of this study clearly revealed that *P. citri* has a time and concentration-dependent susceptibility to both *Steinernema* species and *Heterorhabditis* species tested. The highest mortality was reached after 72 h of exposure when *P. citri* was inoculated with the highest concentration (200 IJs). These findings are in agreement with the studies conducted by van Niekerk and Malan (2012), Le Vieux and Malan (2013), Negrisoli et al. (2013), and Stokwe and Malan (2016). Increasing concentrations and exposure time to IJs had a positive effect on the mortality rates in these studies. In the current study, mortality rates were higher at 25ºC among other temperatures tested although mortality rates at 25ºC were quite similar to the ones at 30 °C. Earlier studies showed that temperature had a significant influence on the effectiveness of EPNs and the virulence of EPN species and isolates varies considerably at different temperatures (Hang et al., 2007; Radová and Trnková, 2010; Andaló et al., 2011; Yuksel et al., 2019). Low temperatures may give rise to a decrease in the movement of IJs and pathogenicity as reported in earlier studies (Dos Santos Ferreira et al., 2015). High temperatures may also cause to increase the movement of IJs leading to consumption of more energy to reach the target host (Sharmila and Subramanian, 2016).

Entomopathogenic nematode species have different foraging strategies and this is another factor causing variations in the mortality rates. Entomopathogenic nematode species with cruiser strategy search for their target host more actively following host's chemical cues compared to an ambusher nematode species (Dos Santos Ferreira et al., 2015; Lortkipanidze et al., 2016). Heterorhabditis indica is considered as a cruiser and this may have assisted them in locating their host (Lewis et al., 2006).

The overall results obtained during this study demonstrated that both *Steinernema* and *Heterorhabditis* have great potential in the control of *P. citri*. They differed in pathogencity to *P. citri*, with *Heterorhabditis* species showed more active than *Steinernema* species controlling *P. citri*. *Heterorhabditis indica* 216-H showed the more efficacy than other entomopathogenic nematodes used in this study.

CONCLUSION

All EPN species tested were pathogenic to adult females of *P. citri*. Among the EPN species tested *H. indica* 216-H and *S. carpocapsae* E-76 were the most virulent species in most cases. The results obtained in this study showed that entomopathogenic nematodes are eficcient biological control agents against *P. citri* under favorable conditions. However, further studies in field conditions are needed to evaluate the field potential of native EPN species and isolates.

ACKNOWLEDGMENT

This study was funded by the Project No. FYL-2018-8122 by the Office of Scientific Research Projects of Erciyes University.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

REFERENCES

Akhurst, R.J. & Boemare, N.E. (2018). *Biology and taxonomy of Xenorhabdus. (Entomopathogenic nematodes in biological control*, *CRC press*, Newyork, 75-90.

Anonim, (2020). Bitkisel üretim istatistikleri, 2020. https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr. (Alınma Tarihi: 20.04.2020).

Andaló, V., Moino, A., Maximiniano, C., Campos, V.P. & Mendonca, L.A. (2011). Influence of Temperature and Duration of Storage on the Lipid Reserves of Entomopathogenic Nematodes. *Revista Colombiana de entomología 37*(2), 203-209.

Arthurs, S., Heinz, K.M. & Prasifka, J.R. (2004). An Analysis of Using Entomopathogenic Nematodes Against Above-Ground Pests. *Bulletin of Entomological Research 94*(4), 297-306.

Bastidas, B., Portillo, E. & San-Blas, E. (2014). Size Does Matter: The Life Cycle of *Steinernema* spp. in Micro-İnsect Hosts. *Journal of invertebrate pathology 121*,46-55.

Bhat, A.H., Chaubey, A.K., Shokoohi, E. & Machado, R.A. (2021). Molecular and phenotypic characterization of *Heterorhabditis indica* (Nematoda: Rhabditida) nematodes isolated during a survey of agricultural soils in Western Uttar Pradesh, India. *Acta Parasitologica 66*(1), 236-252.

Blumberg, D. & Van Driesche, R.G. (2001). Encapsulation rates of three encyrtid parasitoids by three mealybug species (Homoptera: Pseudococcidae) found commonly as pests ın commercial greenhouses. *Biological Control 22*(2), 191-199.

Canhilal, R., Waeyenberge, L., Toktay, H., Bozbuga, R., Çerintas, R. & Imren, M. (2016). Distribution of Steinernematids and Heterorhabditids (Rhabditida: Steinernematidae and Heterorhabditidae) in the Southern Anatolia Region of Turkey. *Egyptian Journal of Biological Pest Control 26*(4).

Canhilal, R., Waeyenberge, L., Yüksel, E., Koca, A. S., Deniz, Y. & Imren, M. (2017). Assessment of the natural presence of entomopathogenic nematodes in Kayseri soils, Turkey. *Egyptian Journal of Biological Pest Control 27*(2).

Čačija, M., Bažok, R., Kolenc, M., Bujas, T., Drmić, Z. & Kadoić Balaško, M. (2021). Field Efficacy of *Steinernema* sp. (Rhabditida: Steinernematidae) on the Colorado Potato Beetle Overwintering Generation. *Plants 10*(7), 1464.

Cox, J.M. (1981). Identification of *Planococcus citri* (Homoptera: Pseudococcidae) and the description of a new species. *Systematic Entomology 6*(1), 47-53.

Dos Santos Ferreira, K. D., Dolinski, C., De Freitas Ferreira, T. & Moreira de Souza, R. (2015). Potential of entomopathogenic nematodes (rhabditida) for control of pink pineapple mealybug adult females, *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae), under laboratory conditions. *Nematoda 2*, e092015.

Franco, J. C., Suma, P., Da Silva, E. B., Blumberg, D. & Mendel, Z. (2004). Management strategies of mealybug pests of citrus in mediterranean countries. *Phytoparasitica 32*(5), 507-522.

Gill, H. K., Goyal, G. & Gillett-Kaufman, J. (2012). Citrus Mealybug *Planococcus citri* (Risso) (Insecta: Hemiptera: Pseudococcidae). *EDIS 2012*(9).

Gürkan B, Çetintaş R 2022. Texture, pH and Electrical Conductivity of Soils with Some Meloidogyne Species. KSU J. Agric Nat 25 (6): 1341-1349. https://doi.org/10.18016/ksutarimdoga.vi.999602.

Hang, D. T., Choo, H. Y., Lee, D. W., Lee, S. M., Kaya, H. K. & Park, C. G. (2007). Temperature effects on korean entomopathogenic nematodes, *Steinernema glaseri* and *S. longicaudum*, and their symbiotic bacteria. *Journal of microbiology and biotechnology 17*(3), 420-427.

Karacaoğlu, M. & Satar, S. (2017). Bioecological characteristics of *Planococcus citri* Risso, 1813 (Hemiptera: Pseudococcidae) under constant and alternating temperatures. *Turkish Journal of Entomology 41*(2), 147-157.

Karabörklü, S., Ayvaz, A., Yilmaz, S., Azizoglu, U. & Akbulut, M. (2015). Native entomopathogenic nematodes isolated from Turkey and their effectiveness on pine processionary moth, *Thaumetopoea wilkinsoni* Tams. *International journal of pest management 61*(1), 3-8.

Kepenekci, İ., Babaroglu, N.E., Öztürk, G. & Halıcı, S. (1999). Türkiye için yeni bir entomopatojen nematod; *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae). Türkiye 4. Biyolojik Mücadele Kongresi, Adana, Turkiye, 26-29 Ocak 1999, pp. 587

Kepenekci, İ. (2014). Entomopathogenic nematodes (Steinernematidae, Heterorhabditidae: Rhabditida) of Turkey. *Pakistan Journal of Nematology 32*, 59-65.

Laznik, Ž., Tóth, T., Lakatos, T., Vidrih, M. & Trdan, S. (2010). Control of the colorado potato beetle (*Leptinotarsa decemlineata* [Say]) on potato under field conditions: a comparison of the efficacy of foliar application of two strains of *Steinernema feltiae* (Filipjev) and spraying with thiametoxam. *Journal of Plant Diseases and Protection 117*(3), 129-135.

Lewis, E. E., Campbell, J., Griffin, C., Kaya, H. & Peters, A. (2006). Behavioral Ecology Of Entomopathogenic Nematodes. *Biological control 38*(1), 66-79.

Le Vieux, P.D. & Malan, A.P. (2013). The potential use of entomopathogenic nematodes to control *Planococcus ficus* (Signoret)(Hemiptera: Pseudococcidae). *South African Journal of Enology and Viticulture 34*(2), 296-306.

Lortkipanidze, M. A., Gorgadze, O. A., Kajaia, G. S., Gratiashvili, N. G. & Kuchava, M. A. (2016). Foraging behavior and virulence of some entomopathogenic nematodes. *Annals of Agrarian Science 14*(2), 99-103.

Metwally, H. M., Hafez, G. A., Hussein, M. A., Hussein, M. A., Salem, H. A. & Saleh, M. M. E. (2012). Low cost artificial diet for rearing the Greater wax moth, *Galleria mellonella* L.(Lepidoptera: Pyralidae) as a host for entomopathogenic nematodes. *Egyptian Journal of Biological Pest Control 22*(1), 15.

Mokrini, F., Laasli, S. E., Benseddik, Y., Joutei, A. B., Blenzar, A., Lakhal, H., Sbaghi, M., Imren, M., Özer, G., Paulitz, T., Lahlali, R. & Dababat, A. A. (2020). Potential of moroccan entomopathogenic nematodes for the control of the mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). *Scientific Reports 10*(1), 1-11.

Morandi Filho, W. J., Grützmacher, A. D., Botton, M.

& Bertin, A. (2021). Controle químico da cochonilha-farinhenta *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) em diferentes idades da videira. *Arquivos do Instituto Biológico 76*, 427-435.

Muştu, M., Kilinçer, N., Ülgentürk, S. & Kaydan, M. B. (2008). Feeding behavior of *Cryptolaemus montrouzieri* on mealybugs parasitized by *Anagyrus pseudococci*. *Phytoparasitica 36*(4), 360-367.

Negrisoli, C., Júnior, A., Botton, M., Garcia, M.S. & Bernardi, D. (2013). Evaluation of efficacy of 18 strains of entomopathogenic nematodes (rhabditida) against *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) under laboratory conditions. *Experimental parasitology 134*(3), 295-298.

Öğretmen, A., Yüksel, E. & Canhilal, R. (2020). Susceptibility of larvae of wireworms (*agriotes* spp.)(coleoptera: elateridae) to some turkish isolates of entomopathogenic nematodes under laboratory and field conditions. *Biological Control 149*, 104320.

Özer, N., Keskin, N. & Kırbas, Z. (1995). Occurence of entomopathogenic nematodes (Steinernemati dae: Heterorhabditidae) in Turkey. *Nematologica 41*, 639-640.

Uygun, N. & Satar, S. (2008). The Current situation of citrus pests and their control methods in Turkey. IOBC-*WPRS Bulletin 38*, 2-9.

Radová, S. & Trnková, Z. (2010). Effect of soil temperature and moisture on the pathogenicity of two species of entomopathogenic nematodes (Rhabditida: Steinernematidae). *Journal of Agrobiology 27*(1), 1.

Sharmila, R. & Subramanian, S. (2016). Effect of low temperature on the activity of entomopathogenic nematodes. *International Journal of Forestry and Crop Improvement 7*(1), 19-23.

Smart Jr, G. C. (1995). Entomopathogenic nematodes for the biological control of insects. Journal of nematology 27(4S), 529.

Stock, S.P. (2019). Partners in crime: symbiont-assisted resource acquisition in *Steinernema* entomopathogenic nematodes. *Current opinion in insect science 32*, 22-27.

Stokwe, N.F. & Malan, A.P. 2016. Susceptibility of the obscure mealybug, *Pseudococcus viburni* (Signoret) (Pseudococcidae), to South African ısolates of entomopathogenic nematodes. *International Journal of Pest Management 62*(2), 119-128.

Schroer, S. & Ehlers, R.U. (2005). Foliar application of the entomopathogenic nematode *Steinernema carpocapsae* for biological control of diamondback moth larvae (*Plutella xylostella*). *Biological Control 33*(1), 81-86.

Taşkesen, Y.E., Yüksel, E. & Canhilal, R. (2021). Field Performance of entomopathogenic nematodes against the larvae of *Zabrus* spp. Clairville, 1806 (Coleoptera: Carabidae). *Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi 7*(3), 429-437.

Trdan, S., Znidarcic, D. & Vidrih, M. 2007. Control of *Frankliniella occidentalis* on glasshouse-grown cucumbers: an efficacy comparison of foliar application of *Steinernema feltiae* and spraying with abamectin. *Russian Journal of Nematology 15*(1), 25.

Venkatesan, T., Jalali, S. K., Ramya, S. L. & Prathibha, M. (2016). *Insecticide Resistance and Its Management in Mealybugs*. Springer. Mani, M. & Shivaraju, C. (Eds). (2016). *Mealybugs and their Management in Agricultural and Horticultural crops*, India.

Van Niekerk, S. & Malan, A.P. (2012). Potential of south african entomopathogenic nematodes (heterorhabditidae and steinernematidae) for control of the citrus mealybug, *Planococcus citri* (Pseudococcidae). *Journal of Invertebrate Pathology 111*(2), 166-174.

Yuksel, E., Canhilal, R. & Imren, M. (2019). Potential of four turkish ısolates of entomopathogenic nematodes against three major stored products insect pests. *Journal of Stored Products Research 83*, 317-321.