Türk. entomol. derg., 2023, 47 (3): 247-257
DOI: http://dx.doi.org/10.16970/entoted.1279074

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Effects of storage temperature on viability and virulence of entomopathogenic nematodes *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* Weiser, 1955 and *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae)[1]

Depolama sıcaklığının entomopatojen nematodlar *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* Weiser, 1955 ve *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae)'nin canlılık ve virülenslikleri üzerine etkileri

Alperen Kaan BÜTÜNER[2] Merve İLKTAN[2] İsmail Alper SUSURLUK[2*]

Abstract

Entomopathogenic nematodes (EPN) are a widely used biological control agent. The aim of the study was to detect efficacy and mortalities of some EPN stored at different temperatures and periods. Three EPN species were used in the study. They were *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH hybrid strain, *Steinernema carpocapsae* Weiser, 1955 TUR-S4 isolate and *Steinernema feltiae* Weiser, 1955 (Rhabditida: Steinernematidae) TUR-S3 isolate. The species were kept at 4, 15, 25 and 35°C for 7, 14 and 21 days. Subsequently, these species were applied at a dose of 15 Infective juveniles on *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvae. The study was carried out in laboratory conditions in 2023. As a result, HBH and TUR-S4 kept at 25°C for 14 days and TUR-S3 for 21 days showed the highest virulence as 93.33%. Mortality rates of the EPN species kept at the specified temperatures were also determined. The results have showed that the highest mortality rates for the HBH, TUR-S4 and TUR-S3 isolates were 11.96% on the 14th day at 35°C, 19.81% on the 21st day at 25°C and 7.39% on the 21st day at 35°C, respectively. This study is an important step in determining suitable temperature conditions for storing and transporting EPN.

Keywords: *Heterorhabditis bacteriophora, Steinernema carpocapsae, Steinernema feltiae,* storage, temperatures

Öz

Entomopatojen nematodlar (EPN) yaygın olarak kullanılan bir biyolojik mücadele ajanıdır. Bu çalışmanın amacı, farklı sıcaklık ve sürelerde depolanan bazı EPN'lerin etkinlik ve ölüm oranlarını belirlemektir. Çalışmada üç EPN türü kullanılmıştır. Bunlar; *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH hibrit ırkı, *Steinernema carpocapsae* Weiser, 1955 TUR-S4 izolatı ve *Steinernema feltiae* Weiser, 1955 (Rhabditida: Steinernematidae) TUR-S3 izolatıdır. Bu izolatlar 7, 14 ve 21 gün boyunca 4, 15, 25 ve 35°C'de depolandı. Daha sonra bu türler *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvaları üzerine 15 Infektif Juvenil dozunda uygulanmıştır. Çalışma, 2023 yılında laboratuvar koşullarında yapılmıştır. Sonuç olarak; HBH ve TUR-S4 25°C'de 14 gün, TUR-S3 ise 21 gün süreyle %93,33 ile en yüksek virülans göstermiştir. Ayrıca, aynı sıcaklıklarda tutulan EPN türlerinde ölüm oranları da belirlenmiştir. Sonuçlar, HBH, TUR-S4 ve TUR-S3 izolatları için en yüksek ölüm oranlarının sırasıyla 35°C'de 14. günde %11,96, 25°C'de 21. günde %19,81 ve 35°C'de 21. günde %7,39 olduğunu göstermiştir. Bu çalışma, EPN'nin depolanması ve nakliyesi sırasında uygun sıcaklık koşullarının belirlenmesinde önemli bir adımdır.

Anahtar sözcükler: *Heterorhabditis bacteriophora, Steinernema carpocapsae, Steinernema feltiae,* depolama, sıcaklık

[1] This study was supported by TÜBİTAK (The Scientific and Technological Research Council of Türkiye), Grant Project No: 219O370.
[2] Bursa Uludağ University, Faculty of Agriculture, Department of Plant Protection, 16059 Nilüfer-Bursa, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: susurluk@uludag.edu.tr
Received (Alınış): 07.04.2023 Accepted (Kabul ediliş): 12.09.2023 Published Online (Çevrimiçi Yayın Tarihi): 24.09.2023

Introduction

Chemical control method in plant protection has been used for many years to reduce the impact of pests on agricultural productivity and to manage pests. However, in recent years, it has been revealed that pesticides used for chemical control have toxic effects on non-target organisms (Iyaniwura, 1991; Sánchez-Bayo, 2012; Rani et al., 2021). With the regulations being made as a result of these developments, the use of pesticides in agriculture has been restricted (Ewence et al., 2015; Gunnell et al., 2017; Robin & Marchand, 2019).

With the restriction on pesticides, which are commonly utilized for chemical control, new control methods are being promoted and brought to the forefront (Lu et al., 2012; Barzman et al., 2015). Biological control in agriculture is one of the alternatives to chemical control (Stern et al., 1959; Waage & Greathead, 1988; Baker et al., 2020). It is based on the principle of using living organisms to neutralize agricultural pests. Entomopathogenic nematodes (EPN) are among the most important organisms successfully used in biological control (Ehlers, 1996).

Entomopathogenic nematodes are endoparasitic organisms belonging to Secernentea class, Rhabditida order, Heterorhabditidae and Steinernematidae families (Ehlers, 1996, 2001; Shapiro-Ilan et al., 2006). These organisms living in the soil need to find a host and interact with it to survive (Smits, 1996; Susurluk & Ehlers, 2008; Ulu & Susurluk, 2014).

Infective Juveniles (IJs) are the third stage of the nematode juveniles, which can seek for hosts in the soil for months without any feeding (Boemare et al., 1996; Ehlers, 1996). For example, *Heterorhabditis bacteriophora* Poinar, 1976 has been observed to remain active in the soil for up to 22 months (Susurluk & Ehlers, 2008). In addition, EPN belonging to the families of Heterorhabditidae and Steinernematidae, *Photorhabdus* spp. and *Xenorhabdus* spp. establish a symbiotic relationship with the bacteria, respectively. However, although IJs can live in the soil for a long time (Ehlers, 1996), they can be affected by many abiotic factors, such as temperature, soil moisture and pesticides in the soil (Ehlers, 1996; Özdemir et al., 2021).

These factors directly affect the activity of IJs and may cause a decrease in their lethal activity on the host. Severe climatic conditions can drastically reduce the life span of EPN, which is one of the most serious threats (Glazer, 1996; Kurtz et al., 2007; Susurluk & Ehlers, 2008). Temperature, in particular, is one of the major abiotic factors with negative effects on IJs. Although IJs may live at specific temperatures, the period of exposure to these temperatures is crucial (Strauch et al., 2000; Susurluk & Ulu, 2015).

The main objective of the present study was to detect the virulence of *Heterorhabditis bacteriophora*, *Steinernema carpocapsae* and *Steinernema felitae* isolates on the larvae of *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) after storage at temperatures of 4, 15, 25, and 35°C for 7, 14, and 21 days. Moreover, it also aimed to assess the mortality rates (viability) in the used EPN isolates stored at the specified temperatures and durations.

Materials and Methods

Entomopathogenic nematode species

In the present study, the EPN utilized in this study included three species. One of them was a hybrid strain HBH of *Heterorhabditis bacteriophora* developed and patented (TPMK Patent No: TR 2013 06141 B) in Bursa Uludağ University, Faculty of Agriculture, Department of Plant Protection, Nematology Laboratory. Another EPN species was an isolate TUR-S4 of *Steinernema carpocapsae* isolated in Bursa-Turkey. The last EPN species was an isolate TUR-S3 of *Steinernema feltiae* isolated from Ankara-Turkey. These isolates used in this study were cultured on the last instar of *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) larvae, then kept at 4°C (Kaya & Stock, 1997; Ulu & Susurluk, 2014) until use. Three-day-old isolates were used for the studies.

Experimental design

The used EPN species were stored in approximately 1000±20 IJs in 60 ml of Ringer's solution in a 250 ml capacity filter capped culture flask in the experiments. The 24-well tissue culture plates (each well measuring 1.5 cm in diameter x 3 cm deep) were filled with 10% moist alluvial soil, and the larvae of *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) were placed into them. Afterwards, the EPN taken from the flask were applied on the soil. A dose of 15 IJs was used in this study because of the more objective results of performing efficacy studies at a low dose. (Ulu & Susurluk, 2014; Dede et al., 2022).

The larvae in the EPN-treated 24-well tissue culture plates were incubated at 25°C for 3 days. Then, the numbers of both dead and living insect larvae in the wells were recorded and evaluated. The detected dead larvae were carefully dissected and examined for IJs of the species in order to observe whether their deaths were a result of dead by the EPN or not. This study was carried out with the EPN species used in the experiment at 4, 15, 25 and 35°C and for 7, 14 and 21-day periods. Storage time at each temperature was made on each indicated day. Normally, EPNs are stored at 4°C, as they can maintain their viability for a long time. For this reason, 4°C was considered as a control value in this study.

The larvae in the EPN-treated 24-well tissue culture plates were incubated at 25°C for 3 days (Kurtz et al., 2007; Susurluk & Ulu, 2015; El Khoury et al., 2018). This study was carried out in 3 replications with 10 insect larvae in each replication.

Determination of the mortality rates of *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Steinernema feltiae* at indicated periods

After the species were kept in the incubator at the trial temperatures for the indicated days, at the end of the days, the mortality rates of the IJs were evaluated by means of the solutions taken from tissue culture flasks. Dead IJs were counted from the samples taken from the flask, and then results were evaluated statistically. This stage of the study was conducted in 5 replications.

Statistical analyses

JMP®16 software was used to perform analysis of variance (ANOVA) on mortality data. Furthermore, the least significant difference test (LSD) ($p < 0.05$) was used to determine the difference between means.

Results

Determination of the efficiency of *Heterorhabditis bacteriophora* HBH on different temperature and days

According to the results, the mortality rate of the *T. molitor* larvae ranged between 76.67 to 90% at 15°C. There was no statistically significant difference on mortality rates at 15°C for 7, 14, and 21 days stored. Incubation at 25°C for 14 days, revealed, it was detected that the highest mortality rate of 93.33%. There was a statistically significant difference between 7 and 21 days, as well as 14 and 21 days ($F = 19.06$; $df = 11, 24$; $p < 0.0001$) when the mortality rates of the larvae were examined after the application of *H. bacteriophora* HBH stored at the temperature for 21 days. The mortality rate of *T. molitor* larvae varied between 23.33 and 33.33% at 35°C. No statistically significant difference was found among different days at that temperature. The mortality rate of the larvae was 83.33% at 7, 14, and 21 days at 4°C, which was the control (Table 1).

Determination of the efficiency of *Steinernema carpocapsae* TUR-S4 on different temperatures and days

The results showed that the highest mortality rate of *T. molitor* larvae was 90% at 15°C. A statistically significant difference was obtained at 15°C for 7, 14 and 21 days ($F = 11.66$; $df = 11,24$; $p < 0.0001$). The highest mortality rate in this experiment was obtained on the 14th day at 25°C. This value was found to be

93.33%. At 35°C, the lowest mortality rate was 56.67% on the 21st day. At this temperature, the highest mortality rate was 83.33% on 7th. There was a statistically difference between the mortality rates at this temperature for 7, 14 and 21 days. At 4°C, which was used as a control, the mortality rate was 83.33% on all days (Table 1).

Table 1. Percentage of mortality rates of *T. molitor* larvae caused by the used EPN isolates. The statistical analysis was performed for each species separately (Mean ± SE). Means in each isolate followed by the same letters are not significant different (<0.05)

EPN Species	Time (day)	Temperatures (°C)	Mortality rates (%)
Heterorhabditis bacteriophora HBH	7	4 (Control)	83.33 ± 3.33 a
		15	76.67 ± 3.33 a
		25	80.00 ± 0.00 a
		35	33.33 ± 12.02 b
	14	4 (Control)	83.33 ± 3.33 a
		15	83.33 ± 3.33 a
		25	93.33 ± 3.33 a
		35	20.00 ± 15.27 b
	21	4 (Control)	83.33 ± 3.33 a
		15	90.00 ± 5.77 a
		25	30.00 ± 5.77 b
		35	23.33 ± 3.33 b
Steinernema carpocapsae TUR-S4	7	4 (Control)	83.33 ± 3.33 bc
		15	83.33 ± 3.33 bc
		25	86.67 ± 3.33 ab
		35	83.33 ± 3.33 bc
	14	4 (Control)	83.33 ± 3.33 bc
		15	76.67 ± 3.33 cd
		25	93.33 ± 3.33 a
		35	70.00 ± 0.00 d
	21	4 (Control)	83.33 ± 3.33 bc
		15	90.00 ± 0.00 ab
		25	60.00 ± 5.77 e
		35	56.67 ± 3.33 e
Steinernema feltiae TUR-S3	7	4 (Control)	83.33 ± 3.33 bc
		15	76.67 ± 3.33 cd
		25	73.33 ± 3.33 d
		35	70.00 ± 0.00 d
	14	4 (Control)	83.33 ± 3.33 bc
		15	76.67 ± 3.33 cd
		25	90.00 ± 5.77 ab
		35	76.67 ± 3.33 cd
	21	4 (Control)	83.33 ± 3.33 bc
		15	73.33 ± 3.33 d
		25	93.33 ± 3.33 a
		35	50.00 ± 0.00 e

Determination of the efficiency of *Steinernema feltiae* TUR-S3 on different temperatures and days

The mortality rate of *T. molitor* larvae at 15°C varied between 73.33 to 76.67% at 25°C, the mortality rate was 90% on the 14th day. The highest mortality rate was 93.33% on the 21st day at 25°C. The mortality rates obtained on the 7th, 14th, and 21st days at 25°C ranged between 50 to 76.67%. Statistically significant differences were found between these mortality rates (F = 11.11; df = 11,24; p < 0.0001) (Table 1).

In addition to these mortality rates, all the species used in the study were subjected to a single statistic to assess the level of significance between them, and statistically significant differences were found (F = 17.40; df = 35,72; p < 0.0001) (Table 2).

Table 2. Percentage of mortality rates of *T. molitor* larvae caused by the used EPN isolates. The statistical analysis was performed for all species each other (Mean ± SE). Means followed by the same letters are not significant different (<0.05)

EPN Species	Time (day)	Temperatures(°C)	Mortality rates (%)	
H. bacteriophora HBH	14	25	93.33 ± 3.33	a
S. feltiae TUR-S3	21	25	93.33 ± 3.33	a
S. carpocapsae TUR-S4	14	25	93.33 ± 3.33	a
S. carpocapsae TUR-S4	21	15	90.00 ± 0.00	ab
H. bacteriophora HBH	21	15	90.00 ± 5.77	ab
S. feltiae TUR-S3	14	25	90.00 ± 5.77	ab
S. carpocapsae TUR-S4	7	25	86.67 ± 3.33	abc
S. carpocapsae TUR-S4 (Control)	7	4	83.33 ± 3.33	abcd
S. feltiae TUR-S3 (Control)	7	4	83.33 ± 3.33	abcd
H. bacteriophora HBH (Control)	21	4	83.33 ± 3.33	abcd
S. carpocapsae TUR-S4	7	15	83.33 ± 3.33	abcd
S. carpocapsae TUR-S4 (Control)	21	4	83.33 ± 3.33	abcd
S. feltiae TUR-S3 (Control)	21	4	83.33 ± 3.33	abcd
S.carpocapsae TUR-S4	7	35	83.33 ± 3.33	abcd
H. bacteriophora HBH (Control)	14	4	83.33 ± 3.33	abcd
S.carpocapsae TUR-S4 (Control)	14	4	83.33 ± 3.33	abcd
S. feltiae TUR-S3 (Control)	14	4	83.33 ± 3.33	abcd
H. bacteriophora HBH	14	15	83.33 ± 3.33	abcd
H. bacteriophora HBH (Control)	7	4	83.33 ± 3.33	abcd
H. bacteriophora HBH	7	25	80.00 ± 0.00	bcde
H. bacteriophora HBH	7	15	76.67 ± 3.33	cde
S. feltiae TUR-S3	7	15	76.67 ± 3.33	cde
S. feltiae TUR-S3	14	35	76.67 ± 3.33	cde
S. feltiae TUR-S3	14	15	76.67 ± 3.33	cde
S. carpocapsae TUR-S4	14	15	76.67 ± 3.33	cde
S. feltiae TUR-S3	21	15	73.33 ± 3.33	de
S. feltiae TUR-S3	7	25	73.33 ± 3.33	de
S. carpocapsae TUR-S4	14	35	70.00 ± 0.00	ef
S. feltiae TUR-S3	7	35	70.00 ± 0.00	ef
S. carpocapsae TUR-S4	21	25	60.00 ± 5.77	fg
S. carpocapsae TUR-S4	21	35	56.67 ± 3.33	g
S. feltiae TUR-S3	21	35	50.00 ± 0.00	g
H. bacteriophora HBH	7	35	33.33 ± 12.02	h
H. bacteriophora HBH	21	25	30.00 ± 5.77	hi
H. bacteriophora HBH	21	35	23.33 ± 3.33	hi
H. bacteriophora HBH	14	35	20.00 ± 15.27	i

Determination of the mortality rates of *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Steinernema feltiae*

According to the results obtained, no mortality was detected on *H. bacteriophora* HBH after 7, 14 and 21 days at 15°C. A statistically significant difference was not found at this temperature value. At 25°C, the mortality rate was found to be 6.66% only on the 21st day. Mortality was not detected on the 7th and 14th days. The highest mortality rates were observed at 35°C. These mortality rates were between 4 to 11.96%. However, a statistically significant difference was not found when all HBH mortality rates were evaluated (F = 1.26; df = 11, 48; p = 0.2719). As a control, no mortality was observed on all days at 4°C. Although no mortality was detected on the 7th at 15°C for *S. carpocapsae* TUR-S4, mortality rates on the 14th and 21st days were 10.78 and 18.88%, respectively. The highest mortality rate for the isolate was determined on 21st days at 25°C. On the 14th day, this rate was 0.64%, while no mortality was detected on the 7th day. Also, a statistically significant difference was found between the days at 25°C (F = 12.02; df = 11, 48; p < 0.0001). At 35°C, the mortality was observed on all days. This value varied between 5.45 to 14.80%. There was not any mortality detected at 4°C. For *S. feltiae* TUR-S3, the highest mortality rate was found as 6.99% at 15°C. The mortality rate obtained at 25°C varies between 0 to 2.14%. The highest mortality rate for the isolate TUR-S3 was obtained as 7.39% on the 21st day at 35°C. A statistically significant difference was determined at that temperature value (F = 3.33; df = 11, 48; p = 0.0018) (Table 3).

Table 3. Percentage of mortality rates of the used EPN isolates during the experimental periods. The statistical analysis was performed for each species separately (Mean ± SE). Means in each isolate followed by the same letters are not significantly different (p<0.05)

EPN Species	Time (day)	Temperatures (°C)	Mortality rates (%)	
Heterorhabditis bacteriophora HBH	7	4 (Control)	0.00 ± 0.00	b
		15	0.00 ± 0.00	b
		25	0.00 ± 0.00	b
		35	4.00 ± 4.00	ab
	14	4 (Control)	0.00 ± 0.00	b
		15	0.00 ± 0.00	b
		25	0.00 ± 0.00	b
		35	11.96 ± 8.83	a
	21	4 (Control)	0.00 ± 0.00	b
		15	0.00 ± 0.00	b
		25	6.66 ± 6.66	ab
		35	8.33 ± 5.27	ab
Steinernema carpocapsae TUR-S4	7	4 (Control)	0.00 ± 0.00	d
		15	0.00 ± 0.00	d
		25	0.00 ± 0.00	d
		35	5.45 ± 3.63	cd
	14	4 (Control)	0.00 ± 0.00	d
		15	10.78 ± 2.18	bc
		25	0.64 ± 0.64	d
		35	7.66 ± 3.71	c
	21	4 (Control)	0.00 ± 0.00	d
		15	18.88 ± 3.48	a
		25	19.81 ± 3.55	a
		35	14.80 ± 1.85	ab

Table 3. Continued

EPN Species	Time (day)	Temperatures (°C)	Mortality rates (%)	
Steinernema feltiae TUR-S3	7	4 (Control)	0.00 ± 0.00	b
		15	0.00 ± 0.00	b
		25	0.00 ± 0.00	b
		35	0.00 ± 0.00	b
	14	4 (Control)	0.00 ± 0.00	b
		15	3.35 ± 2.07	ab
		25	0.62 ± 0.62	b
		35	2.22 ± 2.22	b
	21	4 (Control)	0.00 ± 0.00	b
		15	6.99 ± 1.77	a
		25	2.14 ± 1.43	b
		35	7.39 ± 3.46	a

In addition, mortality rates in the specified species were subjected to a single statistic to evaluate the level of significance between the species used in the study, and statistically significant differences were found (F = 4.42; df = 35, 144; p< 0.0001) (Table 4).

Table 4. Percentage of mortality rates of the used EPN isolates during the experimental periods. The statistical analysis was performed for all species each other (Mean ± SE). Means followed by the same letters are not significant different (<0.05)

EPN Species	Time (day)	Temperatures(°C)	Mortality rates (%)	
S. carpocapsae TUR-S4	21	25	19.81 ± 3.55	a
S. carpocapsae TUR-S4	21	15	18.88 ± 3.48	ab
S. carpocapsae TUR-S4	21	35	14.80 ± 1.85	abc
H. bacteriophora HBH	14	35	11.96 ± 8.83	bcd
S. carpocapsae TUR-S4	14	15	10.78 ± 2.18	cde
H. bacteriophora HBH	21	35	8.33 ± 5.27	cdef
S. carpocapsae TUR-S4	14	35	7.66 ± 3.71	cdefg
S. feltiae TUR-S3	21	35	7.39 ± 3.46	cdefgh
S. feltiae TUR-S3	21	15	6.99 ± 1.77	defgh
H. bacteriophora HBH	21	25	6.66 ± 6.66	defgh
S. carpocapsae TUR-S4	7	35	5.45 ± 3.63	defgh
H. bacteriophora HBH	7	35	4.00 ± 4.00	efgh
S. feltiae TUR-S3	14	15	3.35 ± 2.07	fgh
S. feltiae TUR-S3	14	35	2.22 ± 2.22	fgh
S. feltiae TUR-S3	21	25	2.14 ± 1.43	fgh
S. carpocapsae TUR-S4	14	25	0.64 ± 0.64	gh
S. feltiae TUR-S3	14	25	0.62 ± 0.62	gh
H. bacteriophora HBH	7	15	0.00 ± 0.00	h
H. bacteriophora HBH	7	25	0.00 ± 0.00	h
H. bacteriophora HBH	14	15	0.00 ± 0.00	h
H. bacteriophora HBH	14	25	0.00 ± 0.00	h
H. bacteriophora HBH	21	15	0.00 ± 0.00	h

Table 4. Continued

EPN Species	Time (day)	Temperatures(°C)	Mortality rates (%)
S. carpocapsae TUR-S4	7	15	0.00 ± 0.00 h
S. carpocapsae TUR-S4	7	25	0.00 ± 0.00 h
S. feltiae TUR-S3	7	15	0.00 ± 0.00 h
S. feltiae TUR-S3	7	25	0.00 ± 0.00 h
S. feltiae TUR-S3	7	35	0.00 ± 0.00 h
H. bacteriophora HBH (Control)	7	4	0.00 ± 0.00 h
H. bacteriophora HBH (Control)	14	4	0.00 ± 0.00 h
H. bacteriophora HBH (Control)	21	4	0.00 ± 0.00 h
S. carpocapsae TUR-S4 (Control)	7	4	0.00 ± 0.00 h
S. carpocapsae TUR-S4 (Control)	14	4	0.00 ± 0.00 h
S. carpocapsae TUR-S4 (Control)	21	4	0.00 ± 0.00 h
S. feltiae TUR-S3 (Control)	7	4	0.00 ± 0.00 h
S. feltiae TUR-S3 (Control)	14	4	0.00 ± 0.00 h
S. feltiae TUR-S3 (Control)	21	4	0.00 ± 0.00 h

Discussion

EPN are biological control agents used to control many insect pests. However, the survival of the EPN is directly related to some environmental factors such as temperature and humidity. One of the major limiting influences on the application of EPN under field conditions is temperature.

Nowadays, the effects of temperature on EPN have been investigated numerous times. These studies mostly focused on the virulence effects of EPN on different hosts at different temperatures (Nimkingrat et al., 2013; Ulu & Susurluk, 2014; Baimey et al., 2015; Yan et al., 2020). However, studies investigating the differences in the efficacy of EPN after storage at different temperatures for certain periods, as done in this study, are very few (Aryal et al., 2022). Similar to the present study is the study by Aryal et al. 2022, who reported using the EPN species isolated from Australia; *H. bacteriophora*, *H. zealandica, H. indica* and *S. felitae*. According to their results, *H. zealandica* had the highest virulence at 25 and 30°C. It is very important that EPN survive as long as possible under storage conditions and it is desirable that there is no decrease in the efficiency of the EPN that remain alive at the end of this period. In general, it is recommended that EPN be stored at low temperatures such as between 4 and 8°C (Burman & Pye, 1980; Georgis, 1992; Kurtz et al., 2007; Susurluk & Ehlers, 2008).

However, EPN can be exposed to higher temperatures, especially during transportations. This study was conducted to determine in more detail what lethal effects these undesirable conditions have on EPN and the change in the efficacy of survivors. The EPN species used in the study are commercially produced by firms. Knowing the reactions of these species in such conditions is very important both scientifically and commercially. In the study, it was detected that the mortality rate increased as the storage time of *H. bacteriophora* HBH increased. It was observed that the increase in temperature also increased this mortality rate. Similarly, Strauch et al. (2000) found that the survival rate of *H. bacteriophora* decreased at high temperatures. This result seems to be compatible with the present study.

In addition, in the present study, it was detected that there were some decreases in the virulence of the IJs that remained alive as the storage time extended. El Khoury et al. (2018) also found that increasing temperature decreased the pathogenicity of *H. bacteriophora.* Results from this study are in accordance with the present study. The results of the present study also indicate that as the storage time increased,

the mortality rate of *S. carpocapsae* TUR-S4 also increased. Temperature and length of storage time have significant effects on survival and efficacy of EPNs. Feng et al. (2006) observed an increase in mortality rate of *S. carpocapsae* as the storage period increased in different solutions. This result appears to be consistent with the findings of the present study.

Furthermore, it was found that an increase in temperature had a negative impact on the virulence of surviving IJs, more so than the storage period. Kung et al. (1991) found that an increase in temperature and longer storage period led to a decrease in the pathogenicity of *S. carpocapsae*. The findings of the present study are consistent with Kung et al. (1991). Similar to the other species used in the present study; the findings of this study indicated an increase in the mortality rate of *S. feltiae* TUR-S3 with an increase in storage time and temperature. Dunphy & Webster (1986) found that the mortality rate of *S. feltiae* increased at higher temperatures and longer storage periods. This result seems to be consistent with the present study. In *S. feltiae*, similar to the findings in other species, it was observed that an increase in temperature led to a decrease in the pathogenicity of the surviving IJs. This result seems to be compatible with Husin & Port (2021). In this study, a decrease in the virulence of *S. feltiae* was observed with increasing temperature, similar to the results of the present study.

In addition, the effect of a wider temperature range on the productivity and other biological activities of EPNs are still unclear. Therefore, further research on the relationship of EPN with temperature should be carried out. Consequently, the relationship of EPN with temperature is important to optimize their use in biological control, especially during transportation. Studies to date show that temperature has a significant impact on the efficiencies and survival of EPN (Ulu & Susurluk, 2014). How EPN species respond to possible high temperatures, especially during transportation, and the exposure time to these temperatures is very important in scientific and especially commercial terms. According to the present results, it is thought that providing new optimal conditions according to the response of EPN to temperatures on a species basis will increase the success of EPN in biological control.

Acknowledgements

This study was financially supported by the TÜBİTAK (The Scientific and Technological Research Council of Türkiye), Project number: 219O370. Moreover, we would like to express our heartfelt thanks to Assoc. Prof. Dr. Hilal SUSURLUK for providing valuable technical support.

References

Aryal, S., U. N. Nielsen, N. H. Sumaya, C. Wilson & M. Riegler, 2022. Effect of temperature on survival of Australian entomopathogenic nematodes and their virulence against the Queensland fruit fly, *Bactrocera tryoni*. BioControl, 67 (6): 617-628.

Baimey, H., L. Zadji, L. Afouda, M. Moens & W. Decraemer, 2015. Influence of pesticides, soil temperature and moisture on entomopathogenic nematodes from southern Benin and control of underground termite nest populations. Nematology, 17 (9): 1057-1069.

Baker, B. P., T. A. Green & A. J. Loker, 2020. Biological control and integrated pest management in organic and conventional systems. Biological Control, 140: 104095.

Barzman, M., P. Bàrberi, E. N. A. Birch, P. Boonekamp, S. Dachbrodt-Saaydeh, B. Graf, B. Hommel, J. E. Jensen, J. Kiss, P. Kudsk, J. R. Lamichhane, A. Messêan, A. C. Moonen, A. Ratnadass, P. Ricci, J. L. Sarah & M. Sattin, 2015. Eight principles of integrated pest management. Agronomy for Sustainable Development, 35 (4): 1199-1215.

Boemare, N., C. Laumond & H. Mauleon, 1996. The entomopathogenic nematode-bacterium complex: biology, life cycle and vertebrate safety. Biocontrol Science and Technology, 6 (3): 333-346.

Burman, M. & A. E. Pye, 1980. *Neoaplectana carpocapsae*: Movements of nematode populations on a thermal gradient. Experimental Parasitology, 49 (2): 258-265.

Dede, E., A. K. Bütüner, A. Susurluk, 2022. Biocontrol potential of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH hybrid strain against the beet webworm, *Loxostege sticticalis* L., 1761 (Lepidoptera: Pyralidae). Turkish Journal of Entomology, 46 (4): 399-405.

Dunphy, G. B. & J. M. Webster, 1986. Temperature effects on the growth and virulence of *Steinernema feltiae* strains and *Heterorhabditis heliothidis*. Journal of Nematology, 18 (2): 270-272.

Ehlers, R. U., 1996. Current and future use of nematodes in biocontrol: practice and commercial aspects with regard to regulatory policy issues. Biocontrol Science and Technology, 6 (3): 303-316.

Ehlers, R. U., 2001. Mass production of entomopathogenic nematodes for plant protection. Applied Microbiology and Biotechnology, 56 (5-6): 623-633.

El Khoury, Y., M. Oreste, E. Noujeim, N. Nemer & E. Tarasco, 2018. Effect of temperature on the pathogenicity of Mediterranean native entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from natural ecosystems. Redia, 101 (CI): 123-127.

Ewence, A., S. Brescia, I. Johnson & P. C. Rumsby, 2015. An approach to the identification and regulation of endocrine disrupting pesticides. Food and Chemical Toxicology, 78: 214-220.

Feng, S. P., R. C. Han, X. H. Qıu, L. I. Cao, J. H. Chen & G. H. Wang, 2006. Storage of osmotically treated entomopathogenic nematode *Steinernema carpocapsae*. Insect Science, 13 (4): 263-269.

Georgis, R., 1992. Present and future prospects for entomopathogenic nematode products. Biocontrol Science and Technology, 2 (2): 83-99.

Glazer, I., 1996. Survival mechanisms of entomopathogenic nematodes. Biocontrol Science and Technology, 6 (3): 373-378.

Gunnell, D., D. Knipe, S. S. Chang, M. Pearson, F. Konradsen, W. J. Lee & M. Eddleston, 2017. Prevention of suicide with regulations aimed at restricting access to highly hazardous pesticides: a systematic review of the international evidence. The Lancet Global Health, 5 (10): e1026-e1037.

Husin, T. O. B. & G. R. Port, 2021. Efficacy of entomopathogenic nematodes against *Tuta absoluta*. Biological Control, 160: 104699.

Iyaniwura, T. T., 1991. Non-target and environmental hazards of pesticides. Reviews on Environmental Health, 9 (3): 161-176.

Kaya, H. K. & P. Stock, 1997. “Techniques in Insect Nematology, 281-324”. In: Manual of Techniques in Insect Pathology (Ed. A. Lawrence), Academic Press, 409 pp.

Kung, S. P., R. Gaugler & H. K. Kaya, 1991. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. Journal of Invertebrate Pathology, 57 (2): 242-249.

Kurtz, B., S. Toepfer, R. U. Ehlers & U. Kuhlmann, 2007. Assessment of establishment and persistence of entomopathogenic nematodes for biological control of western corn rootworm. Journal of Applied Entomology, 131 (6): 420-425.

Lu, Y., K. Wu, Y. Jiang, Y. Guo & N. Desneux, 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nature, 487 (7407): 362-365.

Nimkingrat, P., S. Khanam, O. Strauch & R. U. Ehlers, 2013. Hybridisation and selective breeding for improvement of low temperature activity of the entomopathogenic nematode *Steinernema feltiae*. BioControl, 58 (3): 417-426.

Özdemir, E., E. Inak, E. Evlice, E. Yüksel, R. A. Delialioğlu & I. A. Susurluk, 2021. Effects of insecticides and synergistic chemicals on the efficacy of the entomopathogenic nematode *Steinernema feltiae* (Rhabditida: Steinernematidae) against *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Crop Protection, 144: 105605.

Rani, L., K. Thapa, N. Kanojia, N. Sharma, S. Singh, A. S. Grewal, A. L. Srivastav & J. Kaushal, 2021. An extensive review on the consequences of chemical pesticides on human health and environment. Journal of Cleaner Production, 283: 124657.

Robin, D. C. & P. A. Marchand, 2019. Evolution of the biocontrol active substances in the framework of the European Pesticide Regulation (EC) No. 1107/2009. Pest Management Science, 75 (4): 950-958.

Sánchez-Bayo, F., 2012. Insecticides mode of action in relation to their toxicity to non-target organisms. Journal of Environmental and Analytical Toxicology, S4: S4-002.

Shapiro-Ilan, D. I., D. H. Gouge, S. J. Piggott & J. P. Fife, 2006. Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. Biological Control, 38 (1): 124-133.

Smits, P. H., 1996. Post-application persistence of entomopathogenic nematodes. Biocontrol Science and Technology, 6 (3): 379-388.

Stern, V. M. R. F., R. Smith, R. van den Bosch & K. Hagen, 1959. The integration of chemical and biological control of the spotted alfalfa aphid: the integrated control concept. Hilgardia, 29 (2): 81-101.

Strauch, O., I. Niemann, A. Neumann, A. J. Schmidt, A. Peters & R. U. Ehlers, 2000. Storage and formulation of the entomopathogenic nematodes *Heterorhabditis* indica and *H. bacteriophora*. BioControl, 45 (4): 483-500.

Susurluk, A., R. U. Ehlers, 2008. Field persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora* in different crops. BioControl, 53 (4): 627-641.

Susurluk, I. A. & T.C. Ulu, 2015. Virulence comparisons of high-temperature-adapted *Heterorhabditis bacteriophora*, *Steinernema feltiae* and *S. carpocapsae*. Helminthologia, 52 (2): 118-122.

Ulu, T. C. & I. A. Susurluk, 2014. Heat and desiccation tolerances of *Heterorhabditis bacteriophora* strains and relationships between their tolerances and some bioecological characteristics. Invertebrate Survival Journal, 11 (1): 4-10.

Waage, J. K. & D. J. Greathead, 1988. Biological control: challenges and opportunities. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 318 (1189): 111-128.

Yan, X., M. Shahid Arain, Y. Lin, X. Gu, L. Zhang, J. Li & R. Han, 2020. Efficacy of entomopathogenic nematodes against the tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). Journal of Economic Entomology, 113 (1): 64-72.