Türk. entomol. derg., 2023, 47 (4): 469-476
DOI: http://dx.doi.org/10.16970/entoted.1331987

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Efficiency of temperature and storage duration on some morphological measurements and reproductive capacity of the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)'s Turkish HBH hybrid strain[1]

Entomopatojen nematod *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)'nın Türk HBH hibrit ırkının bazı morfolojik özellikleri ve üreme kapasitesi üzerinde sıcaklığın ve depolama süresinin etkisi

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

Abstract

Entomopathogenic nematodes (EPNs) are successfully used in the biological control of agricultural insect pests. This study aims to determine the body length of hermaphrodite individuals, egg diameter and reproductive capacity obtained from Infective Juveniles (IJs) stored at different temperatures and durations. *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)'s Hybrid Strain HBH was used in the study. IJs stored at 15, 25 and 35°C for 7, 14 and 21 days were inoculated onto *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) last instar larvae at a dose of 100 IJs. On the 2nd day of infection, hermaphrodite individuals and eggs were obtained by dissecting the larvae. The reproductive capacity was determined 10-12 days after infection. The study was conducted in Bursa Uludağ University, Faculty of Agriculture, Plant Protection Department, Nematology Laboratory in 2023. In conclusion, the longest hermaphrodite individuals and egg diameter were obtained as 6207.22 µm and 55.65 µm, respectively from the IJs stored for 7 days at 15°C. The highest reproductive capacity was also observed as 167.500 IJs per *G. mellonella* larva in IJs stored under the same conditions with respect to temperature and time. This study is important for assessing the morphological effects of different temperature values and storage durations on EPNs.

Keywords: Body length, egg diameter, hermaphrodite, *Heterorhabditis bacteriophora,* reproductive capacity

Öz

Entomopatojen nematodlar (EPN), tarımsal zararlıların biyolojik mücadelesinde başarıyla kullanılmaktadır. Bu çalışmanın amacı farklı gün ve sıcaklıklarda depolanmış olan Infektif Juvenillerden (IJ) elde edilen hermafrodit bireylerin vücut uzunluğunun, yumurta çapının ve üreme gücünün belirlenmesidir. Bu çalışmada *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)'nın Hibrit Irkı HBH kullanılmıştır. 15, 25 ve 35°C'de 7,14 ve 21 gün depolanmış olan IJ'ler 100 IJ dozunda *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae)'nın son dönem larvası üzerine inoküle edilmiştir. Enfeksiyonun gerçekleştiği 2. gün sonunda larvalar disekte edilmiş, hermafrodit bireyler ve yumurtalar elde edilmiştir. Üreme gücü ise enfeksiyon gerçekleştikten 10-12 gün sonra belirlenmiştir. Bu çalışma 2023 yılında Bursa Uludağ Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Nematoloji Laboratuvarı'nda yürütülmüştür. Sonuç olarak, en uzun hermafrodit bireyler ve yumurta çapı 15°C'de 7 gün muhafaza edilmiş olan IJ'lerden elde edilmiştir. Bu değerler sırasıyla 6207.22 µm ve 55.65 µm olarak belirlenmiştir. En yüksek üreme gücüde aynı sıcaklık ve günde tutulmuş olan IJ'lerde görülmüştür. Bu değer 167.500 IJs/*G. mellonella* larva olarak belirlenmiştir. Bu çalışma farklı sıcaklık değerlerinin ve depolama süresinin EPN'lerin üzerindeki morfolojik etkilerin belirlenmesini içeren önemli bir çalışmadır.

Anahtar sözcükler: Vücut uzunluğu, yumurta çapı, hermafrodit, *Heterorhabditis bacteriophora*, üreme gücü

[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: alperenkaanbutuner@gmail.com
Received (Alınış): 24.07.2023 Accepted (Kabul ediliş): 11.11.2023 Published Online (Çevrimiçi Yayın Tarihi): 22.11.2023

Introduction

Entomopathogenic nematodes (EPNs) are highly effective biological control agents against insect pests. These organisms are natural enemies of many economically important insect species, and in recent years, restrictions on pesticide usage have increased the importance of these organisms in the control of pests (Ehlers, 1996; Shapiro-Ilan et al., 2006; Susurluk & Ehlers, 2008; Dede et al., 2022).

Entomopathogenic nematodes which belong to the families Heterorhabditidae and Steinernematidae, are organisms that predominantly spend their life cycles searching for hosts in the soil (Boemare et al., 1996). The life cycle of individuals belonging to the Heterorhabditidae family is expressed as egg, juvenile 1, juvenile 2, juvenile 3 (Infective Juvenile), juvenile 4, and adult stages. In the first generations of this family, the adults are composed of hermaphroditic individuals (Johnigk & Ehlers, 1999). EPNs, in their Infective Juveniles (IJs) form, possess the ability to search for hosts for months without feeding (Susurluk & Ehlers, 2008). The IJs penetrate the host tissue through natural openings such as the mouth, spiracles, anus, or through wounds formed on their bodies. After entering the host tissue, the IJs release the symbiotic bacteria with which they live in a symbiotic relationship into the host tissue, leading to host septicaemia and eventual death within approximately 36-48 hours (Kaya & Gaugler, 1993; Ehlers, 2001; Ehlers & Shapiro-Ilan, 2005; Ulu & Susurluk, 2014). The EPNs kill their hosts with the help of gram-negative bacteria belonging to the Enterobacteriaceae family, with whom they have a symbiotic relationship within their bodies, and they are able to multiply within the host (Ehlers, 2001; Lewis et al., 2006). The IJs of the Heterorhabditidae family carry scattered gram-negative bacteria belonging to the species *Photorhabdus* spp. in their hemolymph, whereas the IJs of the Steinernematidae family carry *Xenorhabdus* spp. within a specialized vesicle inside their bodies (Boemare et al., 1996; Forst & Nealson, 1996; Susurluk, 2008).

Environmental factors significantly affect the lives and activities of EPNs. Additionally, they determine the distribution, mobility, infection potential and population dynamics of these organisms. Among these environmental factors, temperature is one of the most crucial factors for EPNs (Kahel-Raifer & Glazer, 2000; Shapiro-Ilan et al., 2006; Ulu & Susurluk, 2014). The increase in temperature can enhance the metabolic rate and infective abilities of EPNs while reducing their developmental periods. However, excessively high temperatures can have a detrimental effect on the activity and survival capabilities of these organisms. Similarly, low temperatures can also reduce the activity and slow down the development of EPNs (Bilgrami & Gaugler, 2007; Shaurub et al., 2015; Lillis et al., 2023). Temperature tolerance can vary among each EPN species, often being associated with their geographic distribution. While some species are more easily adapted to hot climate regions, others are better suited to cooler areas. This adaptability allows EPNs to achieve more successful results on hosts in their natural habitats and in agricultural applications (Kahel-Raifer & Glazer, 2000; Stuart el al., 2006; Vashisth et al., 2013; Lillis et al., 2023).

The main objective of this study is to determine the length of hermaphrodite individuals of *H. bacteriophora* HBH hybrid strain after its IJs are stored at different temperatures (15, 25, and 35°C) and specific time intervals (7, 14, and 21 days). Additionally, the aim is to determine the length of egg diameter and reproductive capacity.

Materials and Methods

Entomopathogenic nematode species

In this study, a single species of EPN was employed. The species under investigation was *Heterorhabditis bacteriophora*, specifically the HBH hybrid strain, which was developed and patented (TPMK Patent No: TR 2013 06141 B) at the Nematology Laboratory, Department of Plant Protection, Faculty of Agriculture, Bursa Uludağ University. HBH hybrid strain was harvested on *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) last instar larvae and subsequently stored at a temperature of 4°C (Kaya & Stock, 1997; Ulu & Susurluk, 2014) until further utilization. For this study, three-day-old isolates were utilized. The HBH hybrid strain is described as a specially adapted strain to the climatic conditions of Türkiye (Ulu & Susurluk, 2014, 2021; Şahin et al., 2018). IJs of the HBH hybrid strain were incubated at temperatures of 15, 25, and 35°C for durations of 7, 14, and 21 days. Subsequently, their reproductive capacities were determined. Additionally, the body lengths and egg diameters of the hermaphroditic individuals derived from the incubated IJs were measured. A temperature of 4°C was designated as the control group.

Experimental design

In the experiments, the hybrid strain HBH was preserved in 60 ml of Ringer's solution (Ringer, 1882) within a 250 ml culture flask with a filter cap, capable of accommodating approximately 1000±20 IJs. The study encompassed different temperature conditions, namely 4, 15, 25 and 35°C, with storage durations of 7, 14 and 21 days for each temperature setting. Specifically, designated days were assigned for the storage periods at each temperature. Typically, EPNs are stored at 4°C due to their ability to maintain viability over an extended period (Ehlers, 2001). Hence, 4°C was utilized as the control temperature in this study to establish a baseline reference. The reproductive capacity, hermaphrodite length, and determination of egg diameter were assessed using last instar (6^{th} stage) larvae of *G. mellonella*, which were employed to be hosts during the inoculation stage. The larvae were placed in 24-well tissue culture plates, with each well measuring 1.5 cm in diameter and 3 cm in depth. The plates were covered with 10% moist alluvial soil, and the inoculation process was conducted.

Measurement of hermaphrodite length and egg diameter

Heterorhabditis bacteriophora HBH hybrid strain was kept in incubators with the specified temperatures and selected days then applied to 24-well tissue culture plates containing *G. mellonella* larvae. The plates were covered with 10% moist alluvial soil, and the inoculation process was conducted. Three days after this treatment, insect larvae were transferred from 24-well tissue culture plates to white traps and two days later, the infected larvae were dissected to obtain the hermaphrodites contained within. The lengths of hermaphrodites were measured. The sizes of the eggs were also measured. The identification of obtained hermaphrodite individuals and eggs was conducted using the Leica DM500® Binocular microscope. The images captured from the microscope were instantly transferred to a computer using the integrated Leica DFC295® Digital Color Camera. Subsequently, the analyses performed on the real-time images were conducted using the Leica Application Suite Version 3.6® (LAS V3.6®) software.

Determining the reproductive capacity of HBH hybrid strain

Heterorhabditis bacteriophora HBH hybrid strain was kept at the specified temperatures (15, 25, and 35°C) for the indicated days (7, 14, and 21 days) and the reproductive capacity of these strains on *G. mellonella* was determined. This phase of the study was generally conducted as follows. Firstly, infection was performed on the last larval stage of *G. mellonella* and IJs obtained as a result of infection were stored at 4°C for 3 days. Subsequently, these IJs were removed from the storage condition and kept in an incubator at 15°C for 7 days, after which infection was performed by applying 100 IJs on *G. mellonella*. After 10-12 days, the reproductive capacity was determined on white trap. At other temperature values, these strains were kept in incubators for the specified days and experiments were implemented in the same way. The reproductive capacity was assessed using last instar *G. mellonella* larvae, which had an average weight of approximately 300±10 mg and a length of approximately 2 cm. The quantity of emerging IJs was determined as the total number obtained from the larvae with these characteristics.

Statistical analyses

JMP® Pro 16 software was used to perform analysis of variance on hermaphrodite body length, eggs diameters and reproductive capacity. Furthermore, the least significant difference test ($p < 0.05$) was used to determine the difference between means. All assessments were performed four times, with five measurements taken at each repetition.

Results

The length of hermaphrodite and eggs diameters of the HBH hybrid strain

According to the results of the study, the longest body length value observed in hermaphrodite individuals of *H. bacteriophora* HBH hybrid strain was found in hermaphrodite individuals derived from IJs incubated for 7 days at 15°C. This length value was determined to be 6207.22 µm. On the 14^{th} and 21^{st} days at 15°C, these values were obtained to be 6199.29 µm and 5637.46 µm, respectively. When the body lengths of hermaphrodite individuals derived from IJs incubated for 7, 14, and 21 days at 25°C in the incubator were examined, these values were found to be 5336.15 µm, 5335.98 µm, and 5433.51 µm, respectively. The body lengths of hermaphrodite

individuals derived from IJs incubated at 35°C for the specified days were examined, and the longest length value was observed in hermaphrodite individuals derived from IJs kept at 35°C for 7 days, and this value was determined to be 5268.51 µm (Figure 1). The body lengths of hermaphrodite individuals derived from IJs incubated for 14 and 21 days at 35°C were found to be 5213.63 µm and 4898.82 µm, respectively. Finally, the body lengths of hermaphrodite individuals derived from IJs stored at 4°C as a control were determined as a reference. This value was obtained to be 5253.41 µm. Based on all the obtained data, a statistically significant difference was observed among the values (F= 12.79; df= 9,190; p <0.0001) (Table 1).

Table 1. The lengths of hermaphrodite individuals were obtained from the incubation of IJs at the specified days and temperatures (Mean±S.E.). There is no statistically significant difference between the values represented by the same letters

EPN	Temperatures (°C)	Time (day)	Hermaphrodite Length (µm)±SE		F (df); p
Heterorhabditis bacteriophora HBH Hybrid Strain	4	7	5253.41±44.32	c	F (9,190)= 12,79; p <0.0001
	15		6207.22±164.20	a	
	25		5336.15±71.11	bc	
	35		5268.51±97.51	c	
	15	14	6199.29±225.04	a	
	25		5335.98±71.10	bc	
	35		5213.63±111.51	cd	
	15	21	5637.46±103.33	b	
	25		5433.51±94.43	bc	
	35		4898.82±97.04	d	

The diameters of the eggs within the hermaphrodite individuals derived from the stored IJs at the specified days and temperatures were also determined. According to the obtained results, the longest egg diameter was found within the hermaphrodite individuals derived from IJs kept for 7 days at 15°C. This diameter value was determined as 55.65 µm. When all the specified days and temperatures were examined, a statistically significant difference was only observed between the 7th day at 15°C and the 21st days of all temperature values used in the experiment. No statistically significant difference was found among the other temperature values and days (F= 1.71; df= 9,190; p=0.089).

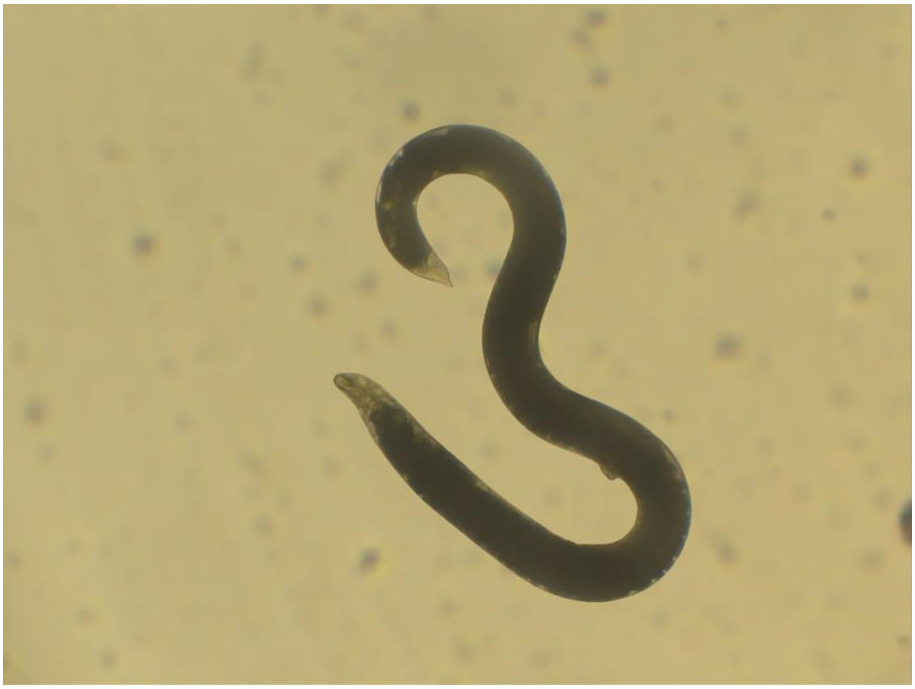

Figure 1. The microscopic image of a hermaphroditic individual derived from IJs (infective juveniles) stored for 21 days at 35°C.

The reproductive capacity of the HBH hybrid strain

The IJs of HBH hybrid strain were kept at the specified temperatures for the indicated days and subsequently, the reproductive capacity of these individuals was determined on the last instar larvae of *G. mellonella*. According to the results, the reproductive capacity was found to be higher in IJs stored for 7 days at 15°C. This value was determined to be 167.500 IJs per *G. mellonella* larva. At 15°C, on the 14th and 21st days, these values were obtained to be 157.750 and 149.500 IJs, respectively. The highest reproductive capacity at 25°C was observed in individuals kept in the incubator for 7 days, with a value of 144.500 IJs. The amounts of IJs obtained on the 14th and 21st days, at 25 ºC were found to be 139.000 and 129.000 IJs, respectively. In this study, the lowest reproductive capacity was observed in individuals kept for 21 days at 35°C. This value was determined to be 103.750 IJs. For individuals incubated at this temperature for 7 and 14 days, these values were obtained to be 133.500 and 118.750 IJs, respectively. Finally, when examining the reproductive capacity of IJs stored at 4°C as a control, this value was found to be 119.250 IJs. Statistically significant difference was found between the obtained values (F= 57.78; df= 9,190; p < 0.0001) (Table 2).

Table 2. The reproductive capacity of *H. bacteriophora* HBH Hybrid Strain IJs were obtained from the incubation of IJs at the specified days and temperatures (Mean±S.E.). There is no statistically significant difference between the values represented by the same letters

EPN	Temperatures (°C)	Time (day)	Reproductive Capacity IJ±S.E.		F (df); p
Heterorhabditis bacteriophora HBH Hybrid Strain	4		119.250±2839.50	g	F (9,190)= 12,79; p <0.0001
	15	7	167.500±3213.86	a	
	25		144.500±2111.99	cd	
	35		133.500±2812.09	ef	
	15	14	157.750±2129.83	b	
	25		139.000±2164.30	de	
	35		118.750±2711.45	g	
	15	21	149.500±1810.06	c	
	25		129.000±2039.09	f	
	35		103.750±3262.12	h	

Discussion

Entomopathogenic nematodes are commonly used in agricultural fields for the purpose of pest control through biological control (Gaugler, 1988; Gaugler et al., 1997; Shapiro-Ilan et al., 2006; Campos-Herrera et al., 2012). However, due to their physiological and morphological characteristics, EPNs are highly susceptible to extreme temperature and humidity conditions (Kung et al., 1991; Grant & Villani, 2003; Lillis et al., 2022; Lillis et al., 2023). Such environmental factors can significantly impact their various attributes, including their efficacy on the host, thereby weakening their overall effectiveness (Shapiro-Ilan et al., 2011; Ulu & Susurluk, 2014; Zhang et al., 2019). Temperature, being one of the most prominent environmental factors, plays a crucial role during the storage and transportation of EPNs. These conditions can greatly influence the survival and quality of the nematodes; therefore, temperature is recognized as one of the most influential environmental factors affecting EPNs (Susurluk & Ehlers, 2008; Ulu et al., 2016; Dede et al., 2022; Dzięgielewska et al., 2023).

Recent studies have mainly focused on the effects of temperature on the efficacy of EPNs on the host, their survival abilities under different temperature conditions, optimal temperature ranges, and longevity at different temperatures (Půža & Mráček, 2007; El-Lakwah & Yousef, 2013; Ulu & Susurluk, 2014; Lephoto & Gray, 2020; Ulu et al., 2021; Nouh, 2022). However, there is limited research on the effects of long-term exposure to different temperatures on their reproductive capacity, the characteristics of hermaphroditic individuals, and eggs (Griffin, 1996; Boff et al., 2000; Mejia-Torres & Saenz, 2013).

Similarly, in a study conducted by Boff et al. (2000) changes in the characteristics (including activity, reproductive capacity, and body length) of IJs belonging to a specific strain of *Heterorhabditis megidis* (Rhabditida: Heterorhabditidae), stored at different temperature values for nearly 70 days, were determined at biweekly intervals. The results revealed that individuals kept at 10 and 15°C exhibited the highest activity, reproductive capacity, and body length. Mejia-Torres & Saenz (2013) conducted a study in which IJs derived from a specific

isolate of the Heterorhabditidae family were incubated at different temperatures for up to 16 weeks. Subsequently, the reproductive capacity, viability, and efficacy of these IJs were determined. It was determined that the optimal temperature range for this isolate was between 20 and 25°C. This finding appears to be in line with the results of the present study.

In the study conducted by Fitters et al. (2001), a specific isolate belonging to the species *H. megidis* was incubated at different temperatures for up to three weeks. This study focused on the efficacy and reproductive capacity of IJs on *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). The findings of this study revealed that species stored below 20°C exhibited higher efficacy and reproductive capacity compared to those stored at or above 20°C. Similarly, in a study conducted by Wang & Grewal (2002), a specific isolate of *H. bacteriophora* was exposed to stress factors such as temperature and drought. Subsequently, the reproductive capacity and viability of the IJs in stock were examined. Based on the obtained data, it was determined that *H. bacteriophora* is sensitive to environmental conditions such as high temperature and drought. A decrease in the reproductive capacity of this species was revealed when exposed to prolonged periods of high temperatures. The results obtained in the present study are in accordance with these findings. The study conducted by Bütüner et al. (2023) the effect of high temperature and storage duration on *H. bacteriophora*, *Steinernema carpocapsae*, and *S. feltiae* (Rhabditida: Steinernematidae) isolates was examined. The results of the study revealed that prolonged storage at high temperatures led to a decrease in the efficacy of these species on their hosts. Additionally, it was observed that the mortality rates in the IJs (infective juveniles) increased proportionally with the duration of exposure to high temperatures. Particularly, the IJs of *H. bacteriophora* were significantly negatively affected by high temperatures and extended storage durations. The results obtained in the present study align with these findings.

According to these results, it has been observed that high temperatures and long-term storage have a negative effect on the body length of hermaphrodite individuals and egg diameters obtained from IJs preserved at different temperatures and days. However, it has been determined that the body lengths of hermaphrodite individuals and egg diameter values obtained at certain temperature values and days are longer than those values in the control group. While there have been studies examining the impact of high temperatures and long-term storage on the reproductive capacity of IJs, no study has been encountered to date regarding the effects of high temperatures and long-term storage on hermaphrodite individuals and eggs. In this respect, the present study has provided valuable data for future studies.

Acknowledgements

This study was financially supported by the TÜBİTAK (The Scientific and Technological Research Council of Türkiye), with the project number: 219O370. We would like to thank Asst. Prof. Tufan Can Ulu for the statistical support, and Merve İlktan for the technical support.

References

Bilgrami, A. & R. Gaugler, 2007. Effects of various stress factors on heat tolerance by *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. Nematology, 9 (2): 161-167.

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.

Boff, M. I., G. L. Wiegers & P. H. Smits, 2000. Effect of storage time and temperature on infectivity, reproduction and development of *Heterorhabditis megidis* in *Galleria mellonella*. Nematology, 2 (6): 635-644.

Bütüner, A. K., M. İlktan & A. Susurluk, 2023. 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). Turkish Journal of Entomology, 47 (3): 247-257.

Campos-Herrera, R., M. Barbercheck, C. W. Hoy & S. P. Stock, 2012. Entomopathogenic nematodes as a model system for advancing the frontiers of ecology. Journal of Nematology, 44 (2): 162-176.

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.

Dzięgielewska, M., K. Kaczmarek & K. Kruk, 2023. The influence of temperature on the biological activity of selected nematode species (Steinernematidae and Heterorhabditidae) under the conditions of their coexistence. Plant Protection Science, 59 (2): 193-201.

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.

Ehlers, R. U. & D. I. Shapiro-Ilan, 2005. "Mass Production, 65-78". In: Nematodes as Biocontrol Agents (Eds. P. S. Grewal, R. U. Ehlers & D. I. Shapiro-Ilan). CABI Publishing, Wallingford, UK, 523 pp.

El-Lakwah, S. F. & H. Yousef, 2013. Relation between Energy Reserves Content and Infectivity of the Heterorhabditid and Steinernematid Entomopathogenic Nematodes under Low Soil Moisture. Egyptian Journal of Biological Pest Control, 23 (1): 103-107.

Fitters, P. F., R. Dunne & C. T. Griffin, 2001. Improved control of *Otiorhynchus sulcatus* at 9ºC by cold-stored *Heterorhabditis megidis* UK211. Biocontrol Science and Technology, 11 (4): 483-492.

Forst, S. & K. Nealson, 1996. Molecular biology of the symbiotic-pathogenic bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. Microbiological reviews, 60 (1): 21-43.

Gaugler, R., 1988. Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. Agriculture, Ecosystems & Environment, 24 (1-3): 351-360.

Gaugler, R., E. Lewis & R. J. Stuart, 1997. Ecology in the service of biological control: the case of entomopathogenic nematodes. Oecologia, 109 (4): 483-489.

Grant, J. A. & M. G. Villani, 2003. Soil moisture effects on entomopathogenic nematodes. Environmental Entomology, 32 (1): 80-87.

Griffin, C. T., 1996. Effects of prior storage conditions on the infectivity of *Heterorhabditis* sp. (Nematoda: Heterorhabditidae). Fundamental and Applied Nematology, 19 (1): 95-102.

Johnigk, S. A. & R. U. Ehlers, 1999. Juvenile development and life cycle of *Heterorhabditis bacteriophora* and *H. indica* (Nematoda: Heterorhabditidae). Nematology, 1 (3): 251-260.

Kahel-Raifer, H. & I. Glazer, 2000. Environmental factors affecting sexual differentiation in the entomopathogenic nematode *Heterorhabditis bacteriophora*. Journal of Experimental Zoology, 287 (2): 158-166.

Kaya, H. K. & R. Gaugler, 1993. Entomopathogenic nematodes. Annual Review of Entomology, 38 (1): 181-206.

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.

Lephoto, T. E. & V. M. Gray, 2020. Desiccation stress tolerance of *Steinernema australe* and *Heterorhabditis bacteriophora*. Archives of Phytopathology and Plant Protection, 54 (11-12): 611-624.

Lewis, E. E., J. Campbell, C. Griffin, H. Kaya & A. Peters, 2006. Behavioral ecology of entomopathogenic nematodes. Biological control, 38 (1): 66-79.

Lillis, P. E., C. T. Griffin & J. C. Carolan, 2022. The effect of temperature conditioning (9ºC and 20ºC) on the proteome of entomopathogenic nematode infective juveniles. PloS one, 17 (4): e0266164.

Lillis, P. E., I. P. Kennedy, J. C. Carolan & C. T. Griffin, 2023. Low-temperature exposure has immediate and lasting effects on the stress tolerance, chemotaxis and proteome of entomopathogenic nematodes. Parasitology, 150 (1): 15-28.

Mejia-Torres, M. C. & A. Saenz, 2013. Ecological characterisation of the Colombian entomopathogenic nematode *Heterorhabditis* sp. SL0708. Brazilian Journal of Biology, 73 (2): 239-243.

Nouh, G. M., 2022. Effect of temperature and soil moisture on the efficacy of indigenous and imported strains of the entomopathogenic nematode, *Heterorhabditis* sp. against the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control, 32 (1): 1-7.

Půža, V. & Z. Mráček, 2007. Natural population dynamics of entomopathogenic nematode *Steinernema affine* (Steinernematidae) under dry conditions: Possible nematode persistence within host cadavers? Journal of Invertebrate Pathology, 96 (1): 89-92.

Ringer, S., 1882. Concerning the influence exerted by each of the constituents of the blood on the contraction of the ventricle. The Journal of Physiology, 3 (5-6): 380-393.

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.

Shapiro-Ilan, D. I., T. C. Leskey & S. E. Wright, 2011. Virulence of entomopathogenic nematodes to plum curculio, *Conotrachelus nenuphar*: Effects of strain, temperature, and soil type. Journal of Nematology, 43 (3-4): 187-195.

Shaurub, E. H., N. A. Soliman, A. G. Hashem & A. M. Abdel-Rahman, 2015. Infectivity of four entomopathogenic nematodes in relation to environmental factors and their effects on the biochemistry of the Medfly *Ceratitis capitata* (Wied.) (Diptera: Tephritidae). Neotropical Entomology, 44 (6): 610-618.

Stuart, R. J., M. E. Barbercheck, P. S. Grewal, R. A. Taylor & C. W. Hoy, 2006. Population biology of entomopathogenic nematodes: concepts, issues, and models. Biological Control, 38 (1): 80-102.

Susurluk, I. A., 2008. Influence of temperature on the vertical movement of the entomopathogenic nematodes *Steinernema feltiae* (TUR-S3) and *Heterorhabditis bacteriophora* (TUR-H2), and infectivity of the moving nematodes. Nematology, 10 (1): 137-141.

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

Şahin, Y. S., A. Boucharı, T. C. Ulu, B. Sadıç & A. Susurluk, 2018. New application method for entomopathogenic nematode *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) HBH strain against *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae). Turkish Journal of Entomology, 42 (4): 305-312.

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.

Ulu, T. C., B. Sadic & I. A. Susurluk, 2016. Effects of different pesticides on virulence and mortality of some entomopathogenic nematodes. Invertebrate Survival Journal, 13 (1): 111-115.

Ulu, T. C. & I. A. Susurluk, 2021. Optimization of in vitro solid culture of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH hybrid strain. Turkish Journal of Entomology, 45 (4): 441-449.

Ulu, T. C., G. Özbudak, E. Ö. Düzenli, Ş. H. Çakır & A. Susurluk, 2021. Comparison of hermaphrodites of hybrid *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH strain and its parents on reproduction capacity. Turkish Journal of Entomology, 45 (2): 185-191.

Vashisth, S., Y. S. Chandel & P. K. Sharma, 2013. Entomopathogenic nematodes-A review. Agricultural Reviews, 34 (3): 163-175.

Wang, X. & P. S. Grewal, 2002. Rapid genetic deterioration of environmental tolerance and reproductive potential of an entomopathogenic nematode during laboratory maintenance. Biological Control, 23 (1): 71-78.

Zhang, F., W. E. Makirita, L. Wu, Y. Gou, Y. Liu, L. Peng, M. Chacha, E. R. Mbega, X. Li, N. He & T. Liu, 2019. Effects of growth conditions on the forms of *Xenorhabdus nematophila*: a symbiotic bacterium of the entomopathogenic *Steinernema carpocapsae*. Journal of Biobased Materials and Bioenergy, 13 (3): 346-352.