Research Article

International Journal of Agriculture and Wildlife Science

Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi

2023, 9(2): 176 – 185, doi: 10.24180/ijaws.1297111

The Role of Storage Duration and Conditions on the Survival and Pathogenicity of Entomopathogenic Nematodes

Depolama Süresi ve Koşullarının Entomopatojen Nematodların Canlılığı ve Patojenitesi Üzerindeki Rolü

Onur Akı[1], Ebubekir Yüksel[2], Mustafa İmren[3], Refik Bozbuğa[4], Ramazan Canhilal[5]

Received: 15.05.2023 **Accepted:** 14.06.2023 **Published:** 21.08.2023

Abstract: Entomopathogenic nematodes (EPNs) (Rhabditida: Heterorhabditidae and Steinernematidae) are effective biocontrol agents for many insect pests and are generally stored for a period of time prior to their use in the laboratory or field. However, unfavorable storage conditions have a great impact on the survival and infectivity of EPNs. This study was conducted to determine the optimum storage conditions of infective juveniles (IJs) of four native EPN species (*Heterorhabditis bacteriophora* FLH-4H, *H. indica* 216-H, *Steinernema feltiae* KCS-S, and *S. bicornotum* MGZ-4S) under laboratory conditions. The survival capability of the IJs was tested at different concentrations (500, 1000, 1500, and 2000 IJs), temperatures (9 and 25 °C) and storage media [double-distilled water (ddH_2O), tap water, and sterile Ringer solution]. In general, the survival of IJs of tested EPN species was the highest at the 1st month after treatment (MAT) at the concentrations of 1000 and 1500 IJs and gradually decreased with the increasing storage periods. The survival rates of the IJs of Steirnematids were generally higher than Heterorhabditid species. The highest survival of IJs was generally obtained after 1-month storage in Ringer solution at 9°C while tap water led to poor survival for the IJs at both temperatures tested. The IJs that were stored at 9°C induced higher mortalities on the larvae of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). The results showed that the IJs of tested EPN species can remain viable for a longer period of time in Ringer solution at 1000 and 1500 IJs concentrations at 9°C without losing much of their infectivity.

Keywords: Storage, Beneficial Nematodes, Biological Control, Sustainable Agriculture

&

Öz: Entomopatojen nematodlar (EPN) (Rhabditida: Heterorhabditidae ve Steinernematidae) birçok zararlı böceklerin etkili biyokontrol ajanlarıdır ve genellikle laboratuvarda veya arazide kullanılmadan önce belirli bir süre depolanırlar. Bununla birlikte, elverişsiz saklama koşullarının EPN'lerin hayatta kalması ve infektivitesi üzerinde büyük bir etkisi vardır. Bu çalışma, dört yerel EPN türünün (*Heterorhabditis bacteriophora* FLH-4H, *H. indica* 216-H, *Steinernema feltiae* KCS-4S ve *S. bicornotum* MGZ-4S) infektif juvenillerinin (IJ) laboratuvar koşullarında optimum saklama koşullarını belirlemek için gerçekleştirilmiştir. IJ'lerin canlılıkları, farklı konsantrasyonlarda (500, 1000, 1500 ve 2000 IJ), sıcaklıklarda (9 ve 25 °C) ve depolama ortamında (ddH_2O, musluk suyu ve steril Ringer solüsyonu) test edilmiştir. Genel olarak, test edilen EPN türlere ait IJ'lerin canlılıkları, 1000 ve 1500 IJ konsantrasyonlarında depolamadan sonraki 1. ayda en yüksek iken artan depolama süreleri ile kademeli olarak azalmıştır. Genel olarak Steirnematidlerin IJ'lerinin hayatta kalma oranları Heterorhabditid türlerinden daha yüksek olduğu belirlenmiştir. IJ'lerin en yüksek hayatta kalma oranı, genellikle Ringer solüsyonunda 9°C'de 1 aylık depolamadan sonra elde edilirken, musluk suyu, test edilen her iki sıcaklıkta da IJ'ler canlılıklarında önemli bir düşüşe neden olmuştur. Patojenite testlerinde, 9°C'de depolanan IJ'ler, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) larvalarında daha yüksek ölüm oranlarına neden olmuştur. Sonuçlar, test edilen EPN türlerine ait IJ'lerin, infektivitelerinde ciddi bir düşüş olmadan 9°C'de, 1000 ve 1500 IJs konsantrasyonlarında, Ringer solüsyonunda daha uzun süre canlı kalabildiğini göstermiştir.

Anahtar Kelimeler: Depolama, Faydalı Nematodlar, Biyolojik Mücadele, Sürdürülebilir Tarım

Cite as: Akı, O., Yüksel, E., İmren, M., Bozbuğa, R. & Canhilal, R. (2022). The Role of Storage Duration and Conditions on the Survival and Pathogenicity of Entomopathogenic Nematodes. International Journal of Agriculture and Wildlife Science, 9(2), 176-185. DOI: 10.24180/ijaws.1297111

Plagiarism/Ethic: This article has been reviewed by at least two referees and it has been confirmed that it is plagiarism-free and complies with research and publication ethics. https://dergipark.org.tr/tr/pub/ijaws

[1] Onur Akı, Erciyes University, Plant Protection Department, onur_aki38@hotmail.com

[2] Assist. Prof. Dr. Ebubekir Yüksel, Erciyes University, Department of Plant Protection, ebubekiryuksel@erciyes.edu.tr (Corresponding author)

[3] Prof. Dr. Mustafa İmren, Bolu Abant Izzet Baysal University, Department of Plant Protection, mustafaimren@ibu.edu.tr

[4] Assoc. Prof. Dr. Refik Bozbuğa, Eskişehir Osmangazi University, Department of Plant Protection, refik.bozbuga@ogu.edu.tr

[5] Prof. Dr. Ramazan Canhilal, Erciyes University, Department of Plant Protection, r_canhilal@hotmail.com

* This study is a part of OA.'s Master's thesis at the Graduate School of Natural and Applied Sciences of Erciyes University

INTRODUCTION

Entomopathogenic nematodes (EPNs) in the families Heterorhabditidae and Steinernematidae are soil-borne microscopic worms that are used as a natural and eco-friendly alternative to insecticides (Hazir et al., 2003). Many studies proved the effectiveness of EPNs against a variety of agricultural pests in both laboratory and field conditions (Ansari et al., 2006; Susurluk et al., 2009; Kepenekci et al., 2016; Odendaal et al., 2016; Mokrini et al., 2020). However, the effectiveness of EPNs is closely linked to their ability to infect and kill pests, which is influenced by a variety of factors, including pre-application storage conditions (Grewal, 2000; Andaló et al., 2010, 2011). Differences in storage conditions and durations lead to a reduction in the quality of the infective juveniles (IJs) of different EPN species depending on the different storage factors such as oxygen and optimal temperature requirements of species (Qiu and Bedding, 2000). For example, high storage temperature can affect the metabolic rate of IJs, reducing stored energy reserves, which greatly affects the survival and pathogenicity of IJs (Hass et al., 2002; Andaló et al., 2011).

For research purposes, IJs are usually stored at varying concentrations from 300 to 5000 IJ/mL in tissue culture flasks as aqueous suspensions of water, distilled water, and Ringer's solutions and can remain alive for 3-12 months depending on the susceptibility of the EPN species/isolates to the above stressors (Kaya and Stock, 1997; Gülcü and Hazir, 2012). Therefore, understanding these parameters which affect the survival and pathogenicity of EPNs in storage is an important aspect to consider and crucial to achieving successful biological control (Brown and Gaugler, 1997). Hence, the main objective of this study was to determine the effect of different storage conditions and media on the viability, pathogenicity, and reproduction of local EPN species.

MATERIAL AND METHOD

Nematode culture

Four native EPN species (*Heterorhabditis bacteriophora* FLH-4H, H. indica 216-H, *Steinernema feltiae* KCS-S, and *S. bicornotum* MGZ-4S) isolated from Kayseri and Nevşehir provinces (Canhilal et al., 2017) were cultured on the last instar of the greater wax moth *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) which reared on artificial diet described by Metwally et al., 2012. To obtain new batches of IJs, the stock culture of IJs was inoculated to Petri dishes containing ten larvae at the concentration of 200 IJs/ml. The freshly emerged IJs were harvested daily after the dead larvae were transferred to modified White Traps (Prabhuraj et al., 2000). The harvested IJs were stored in double distilled water (ddH_2O). Only 1-week-old IJs were included in experiments as the age of IJs may affect the tested parameters.

Experimental set up

The survival capability of IJs of EPN species were evaluated in three different storage media [ddH_2O (Control), tap water, and sterile Ringer solution] at the concentrations of 500, 1000, 1500, and 2000 IJs/mL (Kaya and Stock, 1997). The experiments were carried out in culture flasks (Medium Volume 15-22.5 ml) filled up to 20 ml and the flasks were kept horizontally at two different temperatures (9 and 25 °C). Prior to the experiment, the mobility of IJs was checked under a stereomicroscope to determine the viability of IJs. The IJs populations having 99% population viability were included in the experiments. The number of alive IJs was counted using a Zeiss Lumar stereomicroscope (Carl Zeiss, Inc.) by withdrawing 1 mL samples from each flask and recorded after different storage periods (1, 2, 3, and 6 months) for each test temperature. During the experiment, IJs were shaken twice daily to avoid sinking to the bottom. In the pathogenicity bioassays of IJs, 1 mL of samples from flasks were taken and 200 IJs were inoculated to Petri dishes lined with two filter papers after counting the alive IJs under a stereomicroscope for each storage period. Then, ten *G. mellonella* larvae were placed into Petri dishes. The Petri dishes were incubated in the dark at 25 °C and the mortality of larvae was recorded 2 days after application (DAT). Dead larvae were transferred to modified White traps to confirm the pathogenicity of IJs. Four replicates were performed for each tested parameters (Survival and pathogenicity).

Statistical Analysis

Prior to statistical analyzes, data were subjected to normality tests and analyzed using IBM SPSS software program [Version 20.0 for Windows (SPSS Inc., Chicago, IL, USA)]. Significant differences among treatments were determined by Repeated measures ANOVA (RMANOVA) using Tukey's test at $P \leq 0:05$.

RESULTS AND DISCUSSION

Survival of infective juveniles

The survival of IJs of tested EPN species was strongly influenced by all examined parameters and their associated interactions. In general, the survival of IJs of tested EPN species was higher at the 1st MAT (Month after treatment) and gradually decreased with the increasing storage periods. The IJs stored at 1000 and 1500 IJs concentrations generally yielded the highest survival rates among other concentrations tested. Survival of the IJs of tested EPN species in the Ringer solution was higher than of those stored in tap water and distilled water. The mortality of IJs was higher at 25 °C than at 9°C in all treatments and concentrations. The survival of IJs of tested EPN species was generally lower in tap water media than distilled water and Ringer solution with a few exceptions (Figure 1, 2, 3, 4). No significant mortalities occurred in the IJs of *H. indica* at c after 1-month storage among different storage media and concentrations. After 1-month storage, the survival rates of IJs significantly decreased and the lowest survival (15%) of IJs of *H. indica* occurred in tap water at 2000 IJs concentrations at 25 °C after 6-month storage (Figure 1).

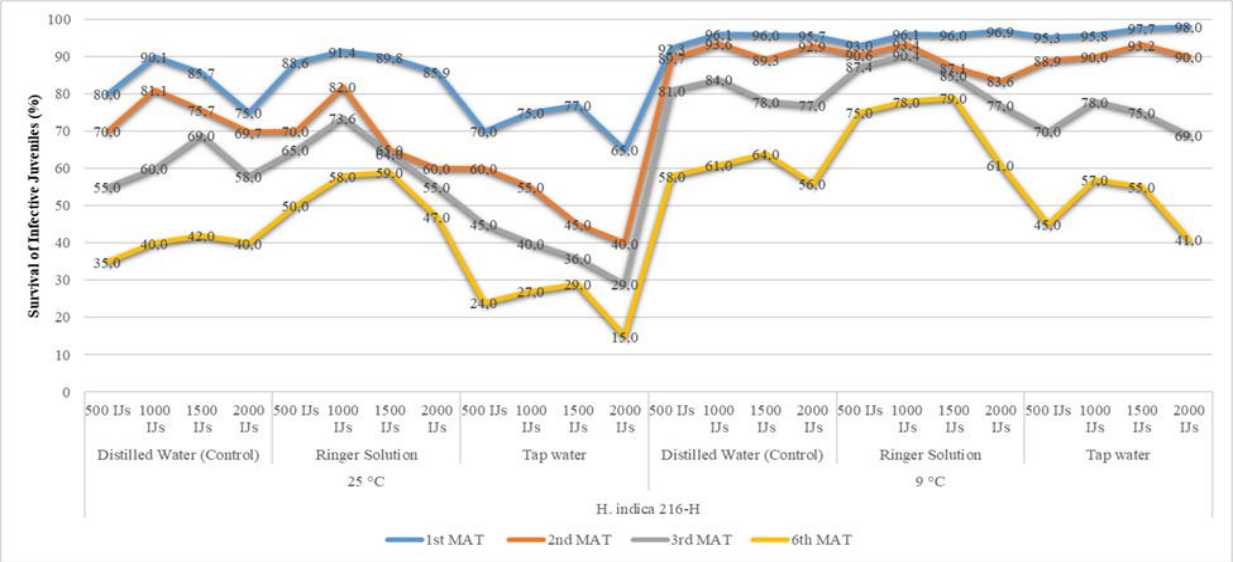

Figure 1. Survival rates (%) of infective juveniles of *Heterorhabditis indica* 216-H after different storage conditions and periods.

Şekil 1. Farklı saklama koşulları ve sürelerinden sonra Heterorhabditis indica 216-H'nin infektif juvenillerinin canlılık oranları.

Among all tested concentrations, *S. feltiae* had the greatest survival (81%) in Ringer solution at 1000 IJs at 9°C after 6-month storage (Figure 2). There were no significant chances in the survival rates of IJs of *S. feltiae* in distilled water at 500 and 1000 IJs concentrations at 9°C for the 1st, 2nd, and 3rd months after treatment.

However, survival rates decreased sharply at 9°C after 6-month storage in distilled and tap water (Figure 2). The survival of IJs of *H. bacteriophora* was higher in the Ringer solution at all storage periods than in distilled and tap water at all tested concentrations and temperatures except for 1-month storage at 9°C. After 6-month storage in tap water, the survival of IJs of *H. bacteriophora* was the lowest (8%) at 25°C at 2000 IJs concentration compared to other storage conditions. The highest survival rate (43%) of IJs of *H. bacteriophora* at 25°C after the 6-month storage period was at 1500 IJs concentration (Figure 3).

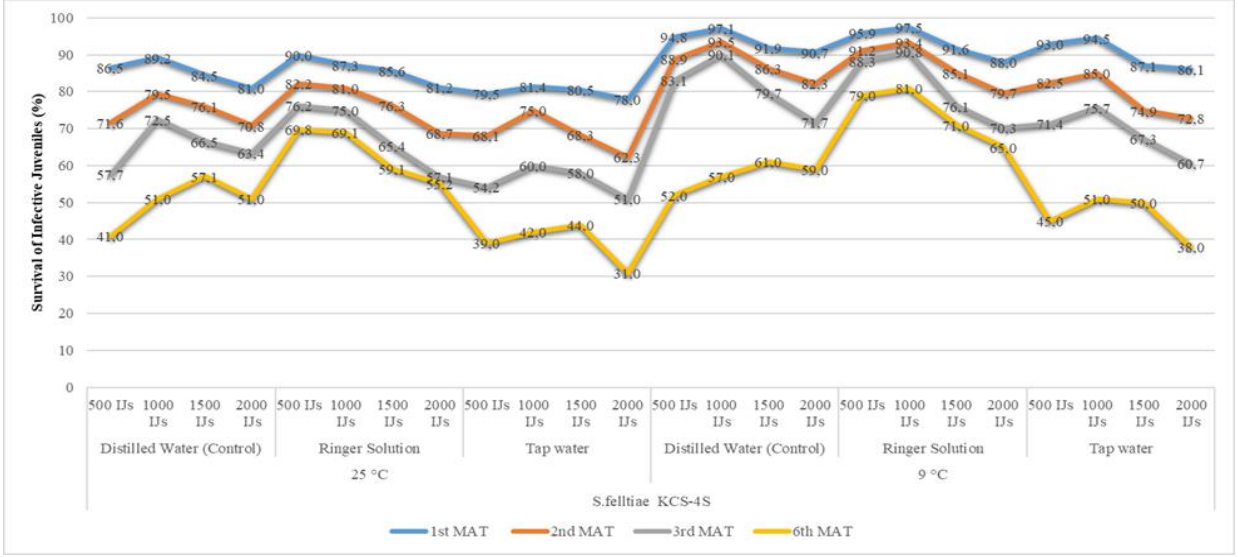

Figure 2. Survival rates (%) of infective juveniles of *Steinernema feltiae KCS-4S* after different storage conditions and periods.

Şekil 2. Farklı saklama koşulları ve sürelerinden sonra Steinernema feltiae KCS-4S'nin infektif juvenillerinin canlılık oranları.

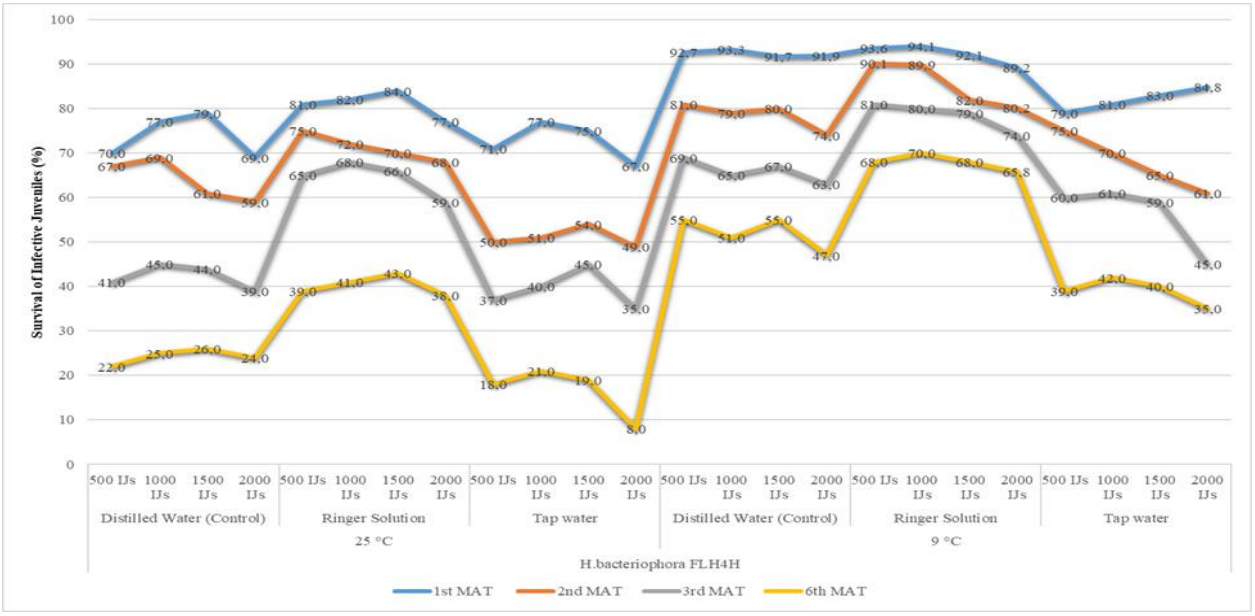

Figure 3. Survival rates (%) of infective juveniles of *Heterorhabditis bacteriophora FLH-4H* after different storage conditions and periods.

Şekil 3. Farklı saklama koşulları ve sürelerinden sonra Heterorhabditis bacteriophora FLH-4H'nin infektif juvenillerinin canlılık oranları.

The survival of IJs of *S. bicornotum* did not differ significantly at 9°C and 25°C for the 1st and 2nd-month storage at all IJs concentrations and storage media. However, after 3rd and 4th-month storage at 9°C and 25°C remarkable decrease occurred in the survival of IJs in tap water at both temperatures tested. The highest survival rate of IJs of *S. bicornotum* was recorded in Ringer solution at 9°C at 500 IJs concentration after 2, 3, and 6-month storage periods (Figure 4).

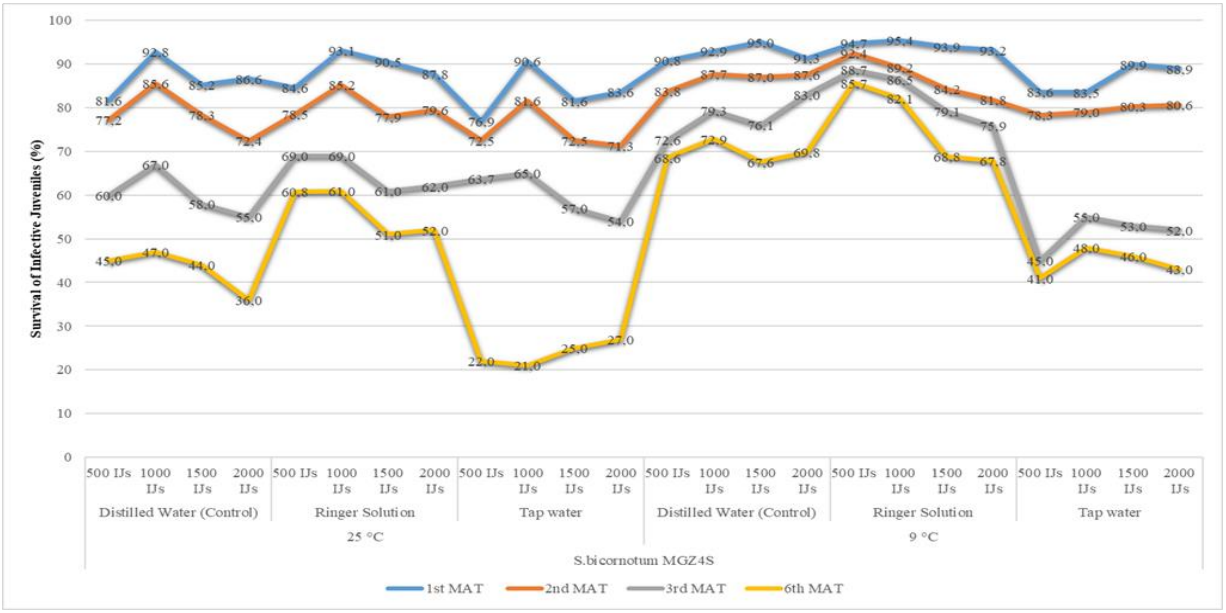

Figure 4. Survival rates (%) of infective juveniles of *Steinernema bicornotum MGZ-4S* after different storage conditions and periods.

Şekil 4. Farklı saklama koşulları ve sürelerinden sonra Steinernema bicornotum MGZ-4S'in infektif juvenillerinin canlılık oranları.

Pathogenicity of infective juveniles

In general, the IJs that were stored at 25 °C caused lower mortality rates on the *G. mellonella* larvae except for the IJs of *H. bacteriophora* which induced similar mortality at temperatures tested. The pathogenicity of IJs tended to decrease with the increasing length of storage of IJs (Figure 5, 6, 7, 8). However, there were several exceptions to this trend. For example, the IJs of *H. indica* stored for 6 months in the Ringer solution at 1500 IJs at 9 °C caused slightly higher mortality than those held for 3 months (Figure 5).

Although the pathogenicity of IJs did not vary much according to the storage environment, the highest efficiencies were generally obtained from the Ringer solution and followed by distilled water and tap water (Figure 5, 6, 7, 8). For instance, the highest effectiveness of the IJs of *H. indica* at 25 °C was achieved by the IJs stored at 1000 IJs concentration in the Ringer solution. The IJs of *H. indica* after a 6-month storage duration in Ringer solution were able to induce mortality over 70% (Figure 5).

The lowest pathogenicity (5%) on the larvae of *G. mellonella* was observed in the IJs of *S. feltiae* which was stored in tap water for 6 months at 25 °C at 2000 IJs concentration. The highest mortality (100%) was achieved by the IJs that were stored in distilled water and Ringer solution at the concentrations of 1000 IJs (Figure 6). *H. bacteriophora* was the least affected EPN species from the storage temperature in terms of pathogenicity. However, the maximum mortalities were achieved only by the IJs that were stored in the Ringer solution at 500 IJs at 9 °C (Figure 7). The pathogenicity of *S. bicornotum* on *G. mellonella* larvae did not change much for different storage concentrations at 25 °C. In addition, 3 months of stored IJs in tap water at 25 °C induced higher larval mortality compared to those stored for 1 and 2 months (Figure 8).

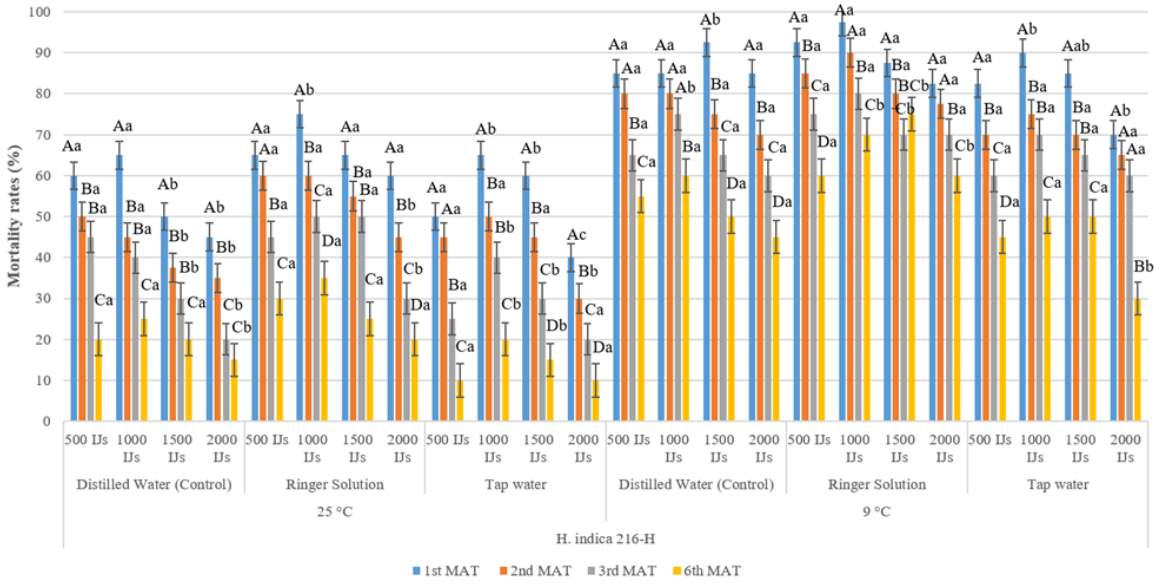

Figure 5. Pathogenicity of infective juveniles of *Heterorhabditis indica* 216-H on the *Galleria mellonella* larvae after different storage conditions and periods (Capital letters show statistically significant differences among IJs concentrations in the same storage media. Lower letters indicate statistically significant differences for the same IJs concentrations among different storage media).

Şekil 5. Heterorhabditis indica 216-H infektif juvenillerinin farklı saklama koşulları ve sürelerinden sonra Galleria mellonella larvaları üzerindeki patojenitesi (Büyük harfler, aynı depolama ortamındaki IJ konsantrasyonları arasında istatistiksel olarak önemli farklılıkları ifade etmektedir. Küçük harfler, aynı IJ konsantrasyonlarının farklı depolama ortamlarındaki istatistiksel olarak önemli farklılıklarını ifade etmektedir).

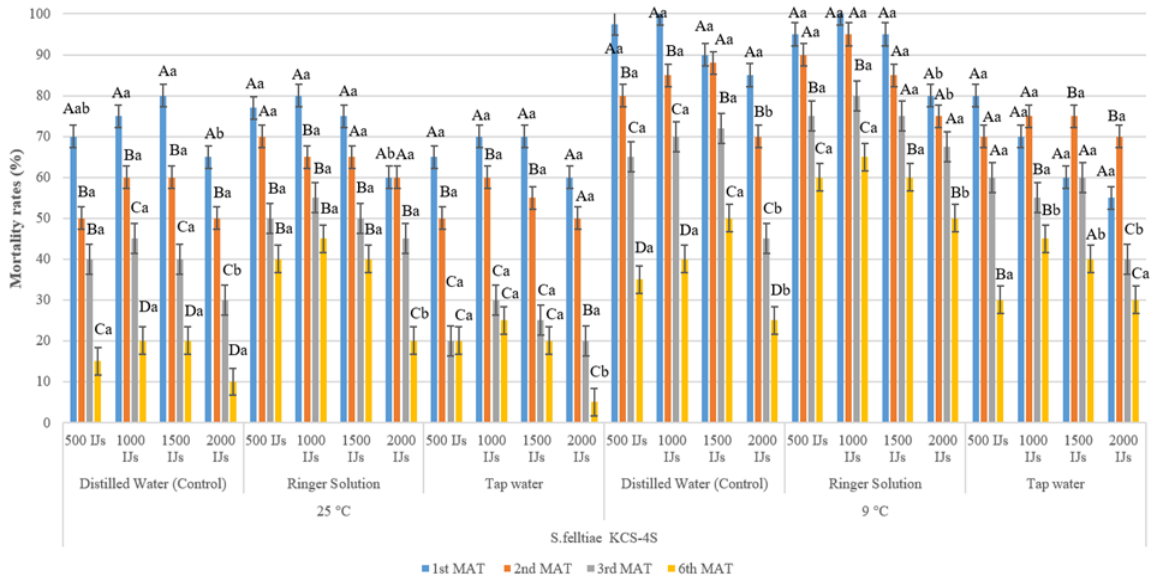

Figure 6. Pathogenicity of infective juveniles of *Steinernema feltiae KCS-4S* on the *Galleria mellonella* larvae after different storage conditions and periods (Capital letters show statistically significant differences among IJs concentrations in the same storage media. Lower letters indicate statistically significant differences for the same IJs concentrations among different storage media).

Şekil 6. Steinernema feltiae KCS-4S infektif juvenillerinin farklı saklama koşulları ve sürelerinden sonra Galleria mellonella larvaları üzerindeki patojenitesi (Büyük harfler, aynı depolama ortamındaki IJ konsantrasyonları arasında istatistiksel olarak önemli farklılıkları ifade etmektedir. Küçük harfler, aynı IJ konsantrasyonlarının farklı depolama ortamlarındaki istatistiksel olarak önemli farklılıklarını ifade etmektedir).

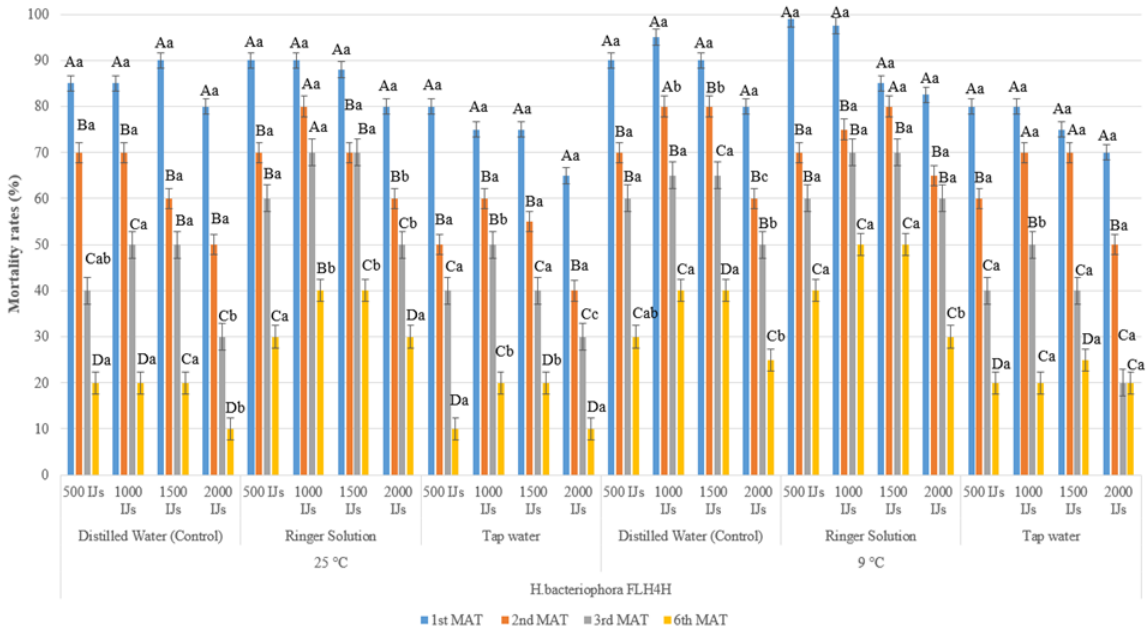

Figure 7. Pathogenicity of infective juveniles of *Heterorhabditis bacteriophora FLH-4H* on the *Galleria mellonella* larvae after different storage conditions and periods (Capital letters show statistically significant differences among IJs concentrations in the same storage media. Lower letters indicate statistically significant differences for the same IJs concentrations among different storage media).

Şekil 7. Heterorhabditis bacteriophora FLH-4H infektif juvenillerinin farklı saklama koşulları ve sürelerinden sonra Galleria mellonella larvaları üzerindeki patojenitesi (Büyük harfler, aynı depolama ortamındaki IJ konsantrasyonları arasında istatistiksel olarak önemli farklılıkları ifade etmektedir. Küçük harfler, aynı IJ konsantrasyonlarının farklı depolama ortamlarındaki istatistiksel olarak önemli farklılıklarını ifade etmektedir).

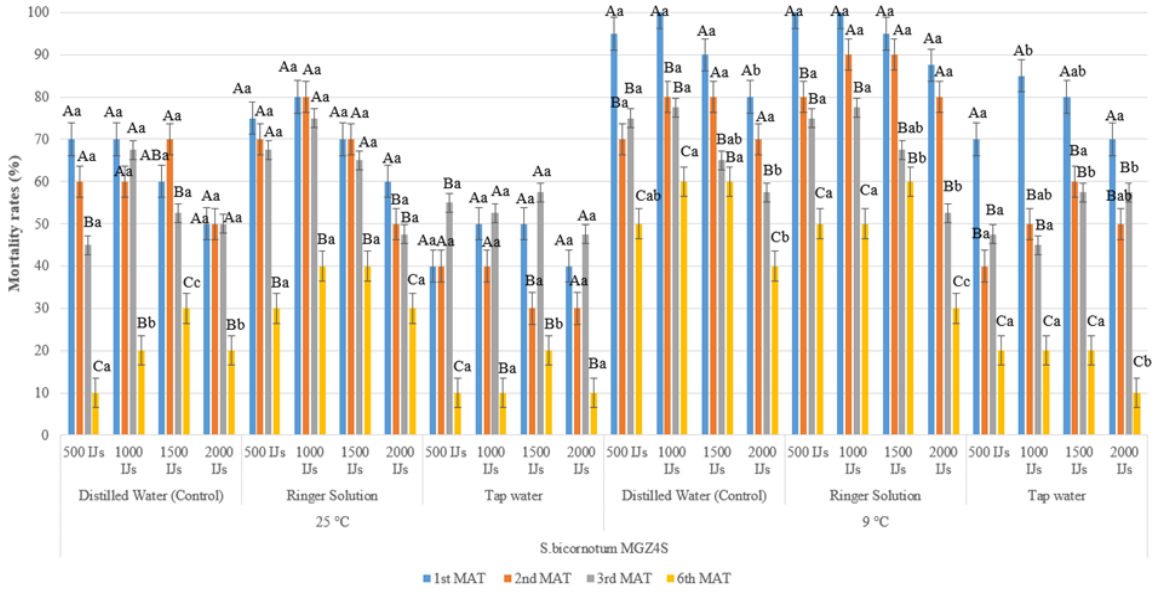

Figure 8. Pathogenicity of infective juveniles of *Steinernema bicornotum MGZ-4S* on the *Galleria mellonella* larvae after different storage conditions and periods (Capital letters show statistically significant differences among IJs concentrations in the same storage media. Lower letters indicate statistically significant differences for the same IJs concentrations among different storage media).

Şekil 8. Steinernema bicornotum MGZ-4S infektif juvenillerinin farklı saklama koşulları ve sürelerinden sonra Galleria mellonella larvaları üzerindeki patojenitesi (Büyük harfler, aynı depolama ortamındaki IJ konsantrasyonları arasında istatistiksel olarak önemli farklılıkları ifade etmektedir. Küçük harfler, aynı IJ konsantrasyonlarının farklı depolama ortamlarındaki istatistiksel olarak önemli farklılıklarını ifade etmektedir).

The correct storage of IJs, in a specific population, and at the ideal conditions, is one of the key requirements for success of EPNs. The results of this study showed the survival rate of IJs of the EPN species decreased drastically depending on the temperature and duration of storage. The highest survival of IJs was obtained from the 1-month storage duration and showed a gradual decrease with increasing storage durations. Similar findings have been reported in earlier studies. In a study conducted by Yadav (2016), the survival rates of IJs of *H. indica* and *S. thermophilum* showed a sharp decline after 4 months' storage at 25 °C and fell below 30%. However, in the same study, in contrast to these EPN species, the survival rate of IJs of *S. glaseri* was 70% at the same period which shows the difference in the adaptation capability of different EPN species in response to the same storage temperatures. Yadav (2016) also reported that the storage of the IJs of the same EPN species at a lower temperature (5 °C) led to a high decrease in the survival of IJs and *S. glaseri* was the only EPN species that survived after 4 months of storage at 5 °C. Similar to our findings, Sharmila and Subramanian (2016) reported 50 and 48% survival rates in the IJs of *H. indica* that were stored in deionized water for three months at 10 and 25 °C, respectively. However, in the current study, all tested EPN species survived for 6 months albeit with a notable decrease in survival rates of IJs. Temperature is one of the crucial factors affecting the mobility, survival and infectivity of EPN species (Glazer, 1996). The tolerance of IJs to different temperatures (heat or cold) varies greatly among EPN species and isolates (Grewal et al., 2006). In the present study, Steinernematid species showed higher survival than heterorhabditid species at 9°C which is in line with the results of Grewal (2000) and Singh et al. (2023) that reported the poor storage capability of Heterorhabditids compared to Steinernematid species. *Steinernema feltiae* is one of the species that is most commonly isolated from cold and continental climate conditions (Hazir et al., 2001; Canhilal et al., 2016; Yuksel and Canhilal, 2019). In contrast, *H. indica* and *H. bacteriophora* were generally found in tropical and subtropical climatic conditions (Bhat et al., 2020; Kour et al., 2022). EPN species that were isolated from different geographical locations may be more tolerant to survive at cool or high temperatures. In addition, EPN species have various kinds of behavioral and physiological adaptations to withstand unfavorable environmental conditions (Glazer, 1996). For instance, some EPN species such as *S. carpocapsae* was reported to be able to conserve energy during storage in water at 5°C by entering a quiescent phase in a "J" form which increases the survival chance of IJs (Grewal, 2000). The IJs of most EPN species become inactive at low temperatures to reduce metabolic costs as a survival mechanism (Griffin, 1993; Fitters and Griffin, 2004). However, there is a great variation in the metabolic responses of EPN species to different temperatures. Grewal (2000) showed that oxygen and lipid consumption of the IJs of different EPN species varied remarkably during storage in water at 25°C which also explains the differences in the survival and infectivity of IJs of different EPN species after long-term storage periods.

The IJs of EPNs naturally tend to search for a potential host in the soil environment and consume their stored energy reserves during host-seeking activity (Selvan et al., 1993; Hatab and Gaugler, 199). After different storage periods, the lipid content of the IJs may also have affected the successful penetration of the host as suggested by Hass et al. (2002) and Andalo et al. (2011). The microbial or chemical composition of the storage medium is also an important factor that influences the survival of EPN species. In the present study, tap water generally led to the lowest survival of the IJs which is similar to the findings of Grewal (2000) who tested the longevity of IJs of *S. carpocapsae* in a water-dispersible granular formulation (WG) and tap water and stated that the survival of the IJs in WG was longer than the IJs in tap water. The chlorine content of tap water may be partly responsible for the lowest survival rates which could be lethal to IJs. In addition, tap water may contain contaminants and many infectious microorganisms such as bacteria and protozoans which decreases oxygen diffusion and releases toxic metabolites (Caylak and Tokar, 2012). This may also have affected the survival of IJs in longer storage periods. On the other hand, the results indicated that the Ringer solution generally provided better survival for the IJs which is in agreement with Bai et al. (2004) who reported an increase in the survival of the IJs of *S. carpocapsae* and *H. bacteriophora* in Ringer solution. Bai et al. (2004) also noted that, up to a point, increasing concentration of IJs resulted in higher nematode survival that is similar to our findings. In the present study, IJs survival generally was higher at 1000 and 1500 IJs concentrations except for some cases. This might be due to the cryoprotectants such as trehalose and glycerol produced by the IJs as suggested by Bai et al. (2004) (Qiu and Bedding, 2002).

However, at high IJs concentrations, survival of IJs decreased remarkably which might be due to the low oxygen level.

CONCLUSION

The storage conditions of EPNs play a crucial role in the survival and pathogenicity of EPNs. The results of this study revealed that the survival IJs of tested EPN species were adversely affected by storage duration, temperature, and media. The survival and infectivity of IJs were greater after 1 month of storage. The Ringer solution and low storage temperature (9 °C), in most cases, provided a better environment for the survival of IJs of tested EPN species. However, more studies are needed to uncover the real potential of EPNs in field conditions after different storage conditions and durations.

CONFLICT OF INTEREST

The authors no conflicts of interest.

DECLARATION OF AUTHOR CONTRIBUTION

OA, **EY, Mİ**, **RB**, and **RC** designed the study, **OA** and **EY** participated in experiment, and all authors drafted the manuscript.

REFERENCES

Andaló, V., Cavalcanti, R. S., Molina, J. P., & Moino Jr, A. (2010). Substrates for storing entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). *Scientia Agricola*, *67*(3), 342-347. https://doi.org/10.1590/S0103-90162010000300013.

Andalo, 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.

Ansari, M. A., Shah, F. A., Tirry, L., & Moens, M. (2006). Field trials against *Hoplia philanthus* (Coleoptera: Scarabaeidae) with a combination of an entomopathogenic nematode and the fungus *Metarhizium anisopliae* CLO 53. *Biological Control*, *39*(3), 453-459. https://doi.org/10.1016/j.biocontrol.2006.07.004.

Bai, C., Shapiro-Ilan, D. I., Gaugler, R., & Yi, S. (2004). Effect of entomopathogenic nematode concentration on survival during cryopreservation in liquid nitrogen. *Journal of nematology*, *36*(3), 281.

Bhat, A. H., Chaubey, A. K., & Askary, T. H. (2020). Global distribution of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*. *Egyptian Journal of Biological Pest Control*, *30*(1), 1-15. https://doi.org/10.1186/s41938-020-0212-y

Brown, I. M., & Gaugler, R. (1997). Temperature and humidity influence emergence and survival of entomopathogenic nematodes. *Nematologica*, 43(5), 363-375.

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

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).

Caylak, E., & Tokar, M. (2012). Investigating chemical and microbiological contaminants in drinking water of Cankiri Province, Turkey. *Environmental Earth Sciences*, *67*(7), 2015-2025. http://dx.doi.org/10.1007/s12665-012-1641-z

Glazer, I. (1996). Survival mechanisms of entomopathogenic nematodes. *Biocontrol Science and Technology*, *6*(3), 373-378. https://doi.org/10.1080/09583159631343 .

Gülcü, B., & Hazir, S. (2012). An alternative storage method for entomopathogenic nematodes. *Turkish Journal of Zoology*, *36*(4), 562-565. https://doi.org/10.3906/zoo-1103-10 .

Grewal, P. S. (2000). Anhydrobiotic potential and long-term storage of entomopathogenic nematodes (Rhabditida: Steinernematidae). *International Journal for Parasitology*, *30*(9), 995-1000. https://doi.org/10.1016/S0020-7519(00)00080-1.

Grewal, P. S., Bornstein-Forst, S., Burnell, A. M., Glazer, I., & Jagdale, G. B. (2006). Physiological, genetic, and molecular mechanisms of chemoreception, thermobiosis, and anhydrobiosis in entomopathogenic nematodes. *Biological Control*, *38*(1), 54-65. https://doi.org/10.1016/j.biocontrol.2005.09.004.

Griffin, C. T. (1993). Temperature responses of entomopathogenic nematodes: Implications for the success of biological control programmes. In R.A. Bedding, R.J. Akhurst & H.K. Kaya (Eds.), *Nematodes and the biological control of insect pests* (pp. 115-126). CSIRO Publications.

Hass, B., Downes, M. J., & Griffin, C. T. (2002). Persistence of four *Heterorhabditis* spp. isolates in soil: role of lipid reserves. *Journal of Nematology*, *34*(2), 151.

Hatab, M. A. A., & Gaugler, R. (1999). Lipids of in vivo and in vitro cultured *Heterorhabditis bacteriophora*. *Biological Control*, *15*(2), 113-118. https://doi.org/10.1006/bcon.1999.0701.

Hazir, S., Stock, S. P., Kaya, H. K., Koppenhöfer, A. M., & Keskin, N. (2001). Developmental temperature effects on five geographic isolates of the entomopathogenic nematode *Steinernema feltiae* (Nematoda: Steinernematidae). *Journal of Invertebrate Pathology, 77*(4):243–250. https://doi.org/10.1006/jipa.2001.5029.

Hazir, S., Kaya, H. K., Stock, S. P., & Keskin, N. (2003). Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. *Turkish journal of Biology, 27*(4), 181-202.

Fitters, P. F., & Griffin, C. T. (2004). Spontaneous and induced activity of *Heterorhabditis megidis* infective juveniles during storage. *Nematology, 6*(6), 911-917. https://doi.org/10.1163/1568541044038597.

Kaya, H. K., & Stock, S. P. (1997). Techniques in insect nematology. In L. Lacey (Ed.), *Manual of Techniques in Insect Pathology* (pp. 281-324). Academic Press.

Kepenekci, I., Hazir, S., & Lewis, E. E. (2016). Evaluation of entomopathogenic nematodes and the supernatants of the in vitro culture medium of their mutualistic bacteria for the control of the root-knot nematodes *Meloidogyne incognita* and *M. arenaria*. *Pest management science, 72*(2), 327-334. https://doi.org/10.1002/ps.3998.

Kour, S., Khurma, U., Brodie, G., & Singh, S. (2022). Modeling the potential global distribution of suitable habitat for the biological control agent *Heterorhabditis indica*. *Ecology and Evolution, 12*(6), e8997. https://doi.org/10.1002/ece3.8997.

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., İmren, M., Özer, G., Paulitz, T., Lahlai, 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. https://doi.org/10.1038/s41598-020-76170-7.

Odendaal, D., Addison, M. F., & Malan, A. P. (2016). Entomopathogenic nematodes for the control of the codling moth (*Cydia pomonella* L.) in field and laboratory trials. *Journal of Helminthology, 90*(5), 615-623. https://doi.org/10.1017/s0022149x15000887.

Prabhuraj, A., Viraktamath, C. A., & Kumar, A. R. V. (2000). Modified trapping technique for the isolation of insect parasitic nematodes. *Journal of Biological Control*, *14*(2) 83-85. https://doi.org/10.18311/jbc/2000/4169.

Selvan, S., Gaugler, R., & Lewis, E. E. (1993). Biochemical energy reserves of entomopathogenic nematodes. *The Journal of parasitology, 79*(2)167-172. https://doi.org/10.2307/3283503.

Singh, M., Rani, P., Prashad, H., & Nalini, C. (2023). Effect of storage media and temperature on viability and pathogenicity of North Indian populations of entomopathogenic nematodes. *Journal of Entomological Research, 47*(1), 209-214. https://doi.org/10.1007%2Fs12639-014-0639-8.

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. https://doi.org/10.15740/HAS/IJFCI/7.1/00-00.

Susurluk, I. A., Kumral, N. A., Peters, A., Bilgili, U., & Açıkgöz, E. (2009). Pathogenicity, reproduction and foraging behaviours of some entomopathogenic nematodes on a new turf pest, *Dorcadion pseudopreissi* (Coleoptera: Cerambycidae). *Biocontrol Science and Technology, 19*(6), 585-594. http://dx.doi.org/10.1080/09583150902957348.

Qiu, L. & Bedding, R. (2000). Energy metabolism and its relation to survival and infectivity of infective juveniles of *Steinernema carpocapsae* under aerobic conditions. Nematology *2*, 551-559. http://dx.doi.org/10.1163/156854100509330.

Qiu, L., & Bedding, R. A. (2002). Characteristics of protectant synthesis of infective juveniles of *Steinernema carpocapsae* and importance of glycerol as a protectant for survival of the nematodes during osmotic dehydration. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *131*(4), 757-765. https://doi.org/10.1016/s1096-4959(02)00019-2.

Yadav, A. K. (2016). Effects of storage temperature on survival and infectivity of three indigenous entomopathogenic nematodes strains (Steinernematidae and Heterorhabditidae) from Meghalaya, India. *Journal of Parasitic Diseases, (40)*, 1150-1154. https://doi.org/10.1007%2Fs12639-014-0639-8.

Yuksel, E., & Canhilal, R. (2019). Isolation, identification, and pathogenicity of entomopathogenic nematodes occurring in Cappadocia Region, Central Turkey. *Egyptian Journal of Biological Pest Control, 29*(1), 1-7. http://dx.doi.org/10.1186/s41938-019-0141-9.