

Original article (Orijinal araştırma)

The role of drought in the efficacy of some entomopathogenic nematodes¹

Bazı entomopatojen nematodların etkinliğinde kuraklığın rolü

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Abstract

Entomopathogenic nematodes (EPNs) are endoparasitic organisms commonly used in the control of agricultural pests. There are several factors that determine the efficacy of EPNs on hosts, with one of the most significant being soil moisture. The aim of this study is to determine the effectiveness of some EPNs on hosts at different doses and under different soil moisture conditions. The study utilized 1 Hybrid Strain and 3 EPN isolates, *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 and S-Bilecik isolates. These species were applied to *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvae at 5, 10, and 15 Infective Juveniles (IJs) doses, under 1, 4, 7, 10, and 13% soil moisture conditions. The study was conducted in 2024 at Bursa Uludağ University, Faculty of Agriculture, Department of Plant Protection, Nematology Laboratory. As a result, the highest mortality rates on *T. molitor* larvae were obtained at 13% soil moisture with 15 IJs, 100% for HBH, 93.33% for TUR-S4, 86.67% for TUR-S3, and 83.33% for S-Bilecik. This study carries important implications for understanding the relationship between EPN efficacy on hosts and soil moisture.

Keywords: *Heterorhabditis bacteriophora*, soil moisture, *Steinernema carpocapsae*, *Steinernema feltiae*

Öz

Entomopatojen nematodlar (EPN) tarım zararlıları ile mücadelede yaygın kullanılan endoparazitik organizmalardır. EPN'lerin konukçular üzerinde etkinliğini belirleyen birçok faktör vardır. En önemlilerinden biri ise toprak nemi olarak bilinmektedir. Bu çalışmanın amacı, farklı dozlarda ve farklı toprak nemlerinde EPN'lerin konukçular üzerinde oluşturduğu etkinliği belirlemektir. Çalışmada 1 Hibrit İrk ve 3 EPN izolatu kullanılmıştır. Bunlar, *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH hibrit ırkı, *Steinernema carpocapsae* Weiser, 1955 TUR-S4 izolatu ve *Steinernema feltiae* Weiser, 1955 (Rhabditida: Steinernematidae) TUR-S3 ve S-Bilecik izolatlarıdır. Bu türler *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvaları üzerine 5, 10 ve 15 İnfektif Juvenil (IJ) dozunda, 1, 4, 7, 10 ve 13% toprak neminde uygulanmıştır. Çalışma, 2024 yılında Bursa Uludağ Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Nematoloji Laboratuvarında gerçekleştirilmiştir. Sonuç olarak *T. molitor* larvaları üzerinde görülen en yüksek ölüm oranları %13 toprak neminde 15 IJ' de HBH için %100, TUR-S4 için 93.33% TUR-S3 için 86.67% ve S-Bilecik için 83.33% şeklinde elde edilmiştir. Bu çalışma, EPN'nin konukçu üzerindeki etkinliğinin, toprak nemi ile ilişkisinin belirlenmesi açısından önemli sonuçlar taşımaktadır.

Anahtar sözcükler: *Heterorhabditis bacteriophora*, toprak nemi, *Steinernema carpocapsae*, *Steinernema feltiae*

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Introduction

In recent years, regulations implemented by the EU in the field of pest control in agriculture have led to restrictions on pesticide usage (Ewence et al., 2015; Robin & Marchand, 2019; Marchand, 2023). This limitation has emphasized the need for alternative methods in pest control, apart from the use of pesticides (Gerhardson, 2002; Baker et al., 2020; Dede et al., 2022; Erdoğan et al., 2023). One of the most significant and commonly employed alternatives is biological control. Entomopathogenic nematodes (EPNs) are widely utilized as biological agents in the agricultural sector for the control of pests (Shapiro-Ilan et al., 2006a; Susurluk & Ehlers, 2008; Dede et al., 2022; Bütüner & Susurluk, 2023; Ulu & Erdoğan, 2023).

Entomopathogenic nematodes are endoparasitic organisms belonging to the Rhabditida order, Steinernematidae and Heterorhabditidae families, requiring a host to complete their life cycle. Their life cycle consists of egg, juvenile 1, juvenile 2, juvenile 3 also called as infective juvenile, juvenile 4, and adult stages (Boemare et al., 1996; Glazer, 1996; Ehlers, 2001; Stuart et al., 2006). The first-generation adults of the Heterorhabditidae family are hermaphrodites, whereas subsequent generations exhibit a distinction between male and female forms. In contrast, in species belonging to the Steinernematidae family, this sexual dimorphism is observed from the first generation (Dix et al., 1992; Stock, 1998; Stock et al., 2002; Lewis et al., 2006).

Entomopathogenic nematodes can only infect their hosts when in the infective juvenile (IJ) stage. IJs enter hosts through natural openings such as the anus and mouth, releasing gram-negative bacteria belonging to the Enterobacteriaceae family, with which they symbiotically live inside their bodies (Stuart et al., 2006; Koppenhöfer et al., 2007; Ruan et al., 2018). This bacterial release causes the host to succumb to septicaemia. Species of the Heterorhabditidae family carry *Photorhabdus* spp. Boemare et al. (Enterobacteriales: Enterobacteriaceae) mixed within their hemocoels. On the other hand, species of the Steinernematidae family carry *Xenorhabdus* spp. Thomas & Poinar (Enterobacteriales: Enterobacteriaceae) within a specialized pouch in their bodies (Boemare et al., 1996; Susurluk, 2008; Bütüner & Susurluk, 2023; Ulu & Susurluk, 2024).

The underground life and effectiveness of EPNs are influenced by various factors, with soil moisture, temperature, and the physical structure of the soil being among the most crucial (Toth et al., 2022; Ulu & Susurluk, 2024). Among these, soil moisture, in particular, is known to impact the distribution of EPNs in the soil. Furthermore, it plays a significant role in the vitality, reproductive capabilities, and efficacy of these endoparasitic organisms on their hosts (Grant & Villani, 2003; Koppenhöfer & Fuzy, 2007; Salame & Glazer, 2015; Rakubu et al., 2024). Optimal soil moisture levels facilitate the movement of EPNs within the soil, enhancing their effectiveness (Bütüner & Susurluk, 2023; Garba et al., 2024). However, excessively high soil moisture levels, as in saturated soils, can have detrimental effects on the viability and development of EPNs. Therefore, it is recognized that a balanced relationship between EPNs and soil moisture is essential for their successful function and development (Koppenhöfer et al., 2020; Nouh, 2022).

In recent years, the increasing prevalence of drought globally and in our country, as well as the decrease in water usage, is of significant importance in assessing its impact on EPN effectiveness. The aim of this study is to determine the efficacy of different dose (5, 10, and 15 IJs) of EPN species, including *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH Hybrid Strain, *Steinernema carpocapsae* (Weiser, 1955) (Rhabditida: Steinernematidae) TUR-S4 isolate, *Steinernema feltiae* (Filipjev, 1934) (Rhabditida: Steinernematidae) TUR-S3, and S-Bilecik isolate, on *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) under varying soil moisture conditions (1, 4, 7, 10, and 13%).

Materials and Methods

Entomopathogenic nematode species

In this study, a hybrid strain and three distinct EPN isolates were performed. The HBH hybrid strain, selected for its high reproductive capacity, resistance to stress conditions, and prolonged durability, belongs to the *H.bacteriophora* species and is associated with the patent number TR 2013 06141 (Susurluk et al., 2013). The isolates used in the study consist of the *S. carpocapsae* TUR-S4 isolate, and *S. feltiae* TUR-S3 and S-Bilecik isolates. In this study, 3rd instar larvae (Morales-Ramos et al., 2015) of *T. molitor* were utilized in the experiment.

The *T. molitor* larvae were produced in climate chambers at Bursa Uludağ University, Department of Plant Protection, Nematology Laboratory, under controlled conditions of $26\pm 2^{\circ}\text{C}$ and a photoperiod of 16-8 (light-dark).

Experimental design

In the studies, the selected species of EPNs were kept in 60 ml of Ringer's solution (NaCl , KCl , $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, NaHCO_3 , distilled water) within a 250 ml culture flask with a filter cap, capable of storing around 1000 ± 20 IJs (Bütüner & Susurluk, 2023). The trial was conducted in 24 wells tissue culture plates (3 cm deep x 1.5 cm diameter). Each well was populated with a larva and subsequently filled with soil having different moisture levels of 1, 4, 7, 10, and 13%. Three doses (5, 10, and 15 IJs) of HBH, TUR-S4, TUR-S3, and S-Bilecik were then applied to the soil.

After 3 days, all plates were opened, and the mortality rate of larvae was determined. The identified deceased larvae underwent meticulous dissection and examination for the presence of IJs specific to the species, with the aim of determining whether their demise resulted from the influence the EPNs or not. Ten larvae were used for each soil moisture level and dose, and all assessments were repeated three times. Typically, no separate control group was established in the study, as previous investigations into the efficacy of the EPNs on the host had utilized a soil moisture level of 10% (Langford et al., 2014; Salame & Glazer, 2015; Dede et al., 2022; Nouh, 2022; Bütüner et al., 2023). Therefore, the 10% soil moisture employed in this study represents the control group for the experiment.

Statistical analyses

Mortality rates at different soil moisture (1, 4, 7, 10, and 13%) and different doses (5, 10, and 15 IJs) were analysed using one-way analysis of variance (ANOVA) and means were compared using LSD (Least Significant Differences) test ($p < 0.05$). ANOVA analysis was performed using JMP[®]Pro 16.0 software.

Results

Efficiency of HBH on *Tenebrio molitor* larvae

As a result of the conducted study, the highest mortality rate in the HBH hybrid strain was observed in larvae treated with 15 IJs at 13% soil moisture, reaching a rate of 100% (Table 1).

Table 1. Mortality rate of *Tenebrio molitor* larvae that were treated with HBH Hybrid Strain was analysed separately and statistically at each soil moisture value

EPN Species	Soil moisture (%)	Applied dose (IJs)	Mortality rates (%) \pm SE*	F (df); p
<i>Heterorhabditis bacteriophora</i> HBH Hybrid Strain	1	5	10.00 \pm 5.77 h	F (14,30) = 24,85; p < 0.0001
		10	23.33 \pm 3.33 h	
		15	46.67 \pm 3.33 fg	
	4	5	56.67 \pm 6.77 ef	
		10	56.67 \pm 6.77 ef	
		15	60.00 \pm 5.77 ef	
	7	5	56.67 \pm 3.33 ef	
		10	60.00 \pm 10.00 ef	
		15	70.00 \pm 5.77 cde	
	10	5	63.33 \pm 3.33 e	
		10	80.00 \pm 0.00 bcd	
		15	93.33 \pm 3.33 ab	
	13	5	66.67 \pm 3.33 de	
		10	83.33 \pm 3.33 bc	
		15	100 \pm 3.33 a	

* Means in columns followed by the same letters are not significantly different.

In larvae treated with 5 IJs and 10 IJs at 13% soil moisture, the mortality rates were 66.67 and 83.33%, respectively. At 10% soil moisture, the highest rate was determined in larvae treated with 15 IJs, reaching 93.33%. This rate was 63.33 and 80% for larvae treated with 5 IJs and 10 IJs, respectively. In wells covered with soil with 7% soil moisture, the highest mortality rate was observed in larvae treated with 15 IJs, reaching 70%. For larvae treated with 5 IJs and 10 IJs, the mortality rates were 56.67% and 60%, respectively. At 4% soil moisture, the highest rate was determined as 60% in larvae treated with 15 IJs, the lowest rate was obtained as 56.67% in larvae treated with 5 IJs and 10 IJs. In wells covered with soil with 1% soil moisture, the highest mortality rate was determined as 46.67% in larvae treated with 15 IJs. The lowest rate was observed as 10% in larvae treated with 5 IJs. Statistically significant differences were obtained between nearly all the values ($F= 24.85$; $df= 14, 30$; $p < 0.0001$) (Table 1).

Efficiency of TUR-S4 on *Tenebrio molitor* larvae

Results of the conducted study revealed that in the TUR-S4 isolate, the highest mortality rate was observed in larvae treated with 15 IJs at 10% and 13% soil moisture, reaching 93.33% (Table 2).

Table 2. Mortality rate of *Tenebrio molitor* larvae that were treated with TUR-S4 isolate was analysed separately and statistically at each soil moisture value

EPN Species	Soil moisture (%)	Applied dose (IJs)	Mortality rates (%) \pm SE*	F (df); p
<i>Steinernema carpocapsae</i> TUR-S4	1	5	10.00 \pm 0.00 f	$F(14,30) = 20.66$; $p < 0.0001$
		10	20.00 \pm 5.77 f	
		15	40.00 \pm 5.77 e	
	4	5	40.00 \pm 11.54 e	
		10	53.33 \pm 3.33 de	
		15	56.67 \pm 3.33 d	
	7	5	56.67 \pm 3.33 d	
		10	56.67 \pm 6.67 d	
		15	66.67 \pm 3.33 bcd	
	10	5	63.33 \pm 3.33 cd	
		10	76.67 \pm 3.33 bc	
		15	93.33 \pm 3.33 a	
	13	5	66.67 \pm 8.81 bcd	
		10	80.00 \pm 0.00 ab	
		15	93.33 \pm 3.33 a	

* Means in columns followed by the same letters are not significantly different.

At 13% soil moisture, the mortality rates for larvae treated with 5 IJs and 10 IJs were 66.67% and 80%, respectively. At 10% soil moisture, for larvae treated with 5 IJs and 10 IJs, the mortality rates were 63.33% and 76.67%, respectively. In wells covered with soil having 7% soil moisture, the highest mortality rate was observed as high as 66.67% in larvae treated with 15 IJs. For larvae treated with 5 IJs and 10 IJs, the mortality rates were 56.67%. At 4% soil moisture, the highest rate was determined as 56.67% in larvae treated with 15 IJs. The lowest rate was obtained in larvae treated with 5 IJs, as 40%. In wells covered with soil having 1% soil moisture, the highest mortality rate was determined as 40% in larvae treated with 15 IJs. The lowest rate was observed as 10% in larvae treated with 5 IJs. Statistically significant differences were obtained between nearly all the values ($F= 20.66$; $df= 14, 30$; $p < 0.0001$)

Efficiency of TUR-S3 on *Tenebrio molitor* larvae

The TUR-S3 isolate exhibited the highest mortality rate in larvae treated with 15 IJs at 13% soil moisture, reaching 86.67%. Under the same moisture conditions, the mortality rates for larvae treated with 5 IJs and 10 IJs were 66.67% and 70%, respectively. At 10% soil moisture, the highest rate was determined as 83.33% in larvae treated with 15 IJs. For larvae treated with 5 IJs and 10 IJs, the mortality rates were 66.67%. In wells covered with soil having 7% soil moisture, the highest mortality rate was observed as high as 63.33% in larvae treated with 15 IJs. For larvae treated with 5 IJs and 10 IJs, the mortality rates were 53.33% and 56.67%, respectively. At 4% soil moisture,

the highest rate was determined as 56.67% in larvae treated with 15 IJs. The lowest rate was obtained as 30% in larvae treated with 5 IJs. In wells covered with soil having 1% soil moisture, the highest mortality rate was determined as 26.67% in larvae treated with 15 IJs. The lowest rate was observed as 3.33% in larvae treated with 5 IJs. Statistically significant differences were obtained between nearly all the values ($F= 14.05$; $df= 14, 30$; $p < 0.0001$) (Table 3).

Table 3. Mortality rate of *Tenebrio molitor* larvae that were treated with TUR-S3 isolate was analysed separately and statistically at each soil moisture value

EPN Species	Soil moisture (%)	Applied dose (IJs)	Mortality rates (%) \pm SE*	F (df); p
<i>Steinernema feltiae</i> TUR-S3	1	5	3.33 \pm 3.33 f	F (14,30) = 14,05; p < 0.0001
		10	13.33 \pm 3.33 ef	
		15	26.67 \pm 3.33 e	
	4	5	30.00 \pm 5.77 e	
		10	50.00 \pm 5.77 d	
		15	56.67 \pm 3.33 cd	
	7	5	53.33 \pm 14.52 cd	
		10	56.67 \pm 6.67 cd	
		15	63.33 \pm 8.82 cd	
	10	5	66.67 \pm 6.67 bcd	
		10	66.67 \pm 8.82 bcd	
		15	83.33 \pm 3.33 ab	
	13	5	66.67 \pm 3.33 bcd	
		10	70.00 \pm 5.77 abc	
		15	86.67 \pm 3.33 a	

* Means in columns followed by the same letters are not significantly different.

Efficiency of S-Bilecik on *Tenebrio molitor* larvae

As a result of the conducted study, the S-Bilecik isolate exhibited the highest mortality rate in larvae treated with 15 IJs under 13% soil moisture, reaching 83.33% (Table 4).

Table 4. Mortality rate of *Tenebrio molitor* larvae that were treated with S-Bilecik isolate was analysed separately and statistically at each soil moisture value

EPN Species	Soil moisture (%)	Applied dose (IJs)	Mortality rates (%) \pm SE*	F (df); p
<i>Steinernema feltiae</i> S-Bilecik	1	5	6.67 \pm 3.33 g	F (14,30) = 17,57; p < 0.0001
		10	16.67 \pm 6.67 fg	
		15	26.67 \pm 3.33 f	
	4	5	30.00 \pm 0.00 f	
		10	53.33 \pm 3.33 de	
		15	53.33 \pm 3.33 de	
	7	5	50.00 \pm 5.77 e	
		10	53.33 \pm 8.82 de	
		15	60.00 \pm 5.77 cde	
	10	5	66.67 \pm 6.67 bcd	
		10	63.33 \pm 8.82 bcde	
		15	76.67 \pm 3.33 ab	
	13	5	66.67 \pm 6.67 bcd	
		10	73.33 \pm 3.33 abc	
		15	83.33 \pm 3.33 a	

* Means in columns followed by the same letters are not significantly different.

Under the same moisture conditions, the mortality rates for larvae treated with 5 IJs and 10 IJs were 66.67% and 73.33%, respectively. At 10% soil moisture, the highest rate was determined as 76.67% in larvae treated with 15 IJs. For larvae treated with 5 IJs and 10 IJs, the mortality rates were 66.67% and 63.33%, respectively. In wells covered with soil having 7% soil moisture, the highest mortality rate was observed as high as 60% in larvae treated with 15 IJs. For larvae treated with 5 IJs and 10 IJs, the mortality rates were 50% and 53.33%, respectively. At 4% soil moisture, the highest rate was determined as 53.33% in larvae treated with 15 IJs. The lowest rate was obtained as 30% in larvae treated with 5 IJs. In wells covered with soil having 1% soil moisture, the highest mortality rate was determined as 26.67% in larvae treated with 15 IJs. The lowest rate was observed as 6.67% in larvae treated with 5 IJs. Statistically significant differences were obtained between the values ($F= 17.57$; $df= 14, 30$; $p < 0.0001$) (Table 4).

Discussion

In this study, the effectiveness of *H. bacteriophora* HBH Hybrid Strain and *S. carpocapsae* TUR-S4, *S. feltiae* TUR-S3, and *S. feltiae* S-Bilecik isolates at different soil moisture levels and doses on larvae of *T. molitor* has been investigated. The results indicate that the EPN isolates lead to higher mortality rates on *T. molitor* with an increase in soil moisture and applied dose. *H. bacteriophora* HBH Hybrid Strain demonstrated a significant increase in the mortality rate as soil moisture and IJs increased, reaching 100% at 13% soil moisture per Petri dish with 15 IJs. Similarly, *S. carpocapsae* TUR-S4, *S. feltiae*, and S-Bilecik isolates exhibited mortality rates of 93.33%, 86.67%, and 83.33%, respectively, at 13% soil moisture. These findings highlight the potential of these EPN isolates in controlling pests at different soil moisture levels and doses.

The effectiveness of the EPN isolates on larvae of *T. molitor* was investigated in relation to soil moisture and IJ dose, utilizing different nematode species, further supporting the findings of this study. For instance, Nouh (2022) investigated the effectiveness of different *Heterorhabditis* spp. at various doses and soil moisture levels (10, 15, and 25%) on *Agrotis ipsilon* (Hufnagel, 1766) (Lepidoptera: Noctuidae) larvae. The study revealed that an increase in soil moisture and applied IJ dose led to a higher mortality rate on larvae. Similarly, in another study conducted by Radová & Trnková (2010), the efficacy of *S. feltiae* and *S. carpocapsae* species applied at different doses was investigated on *T. molitor* larvae at soil moisture levels of 6 and 12.5%. As a result, it was determined that an increase in both dose and moisture led to an elevated mortality rate in larvae. These results seem to be compatible with the present study.

In a study conducted by Grant & Villani (2003), the efficacy of four different EPNs (*H. bacteriophora*, *Steinernema glaseri* (Steiner, 1929), *S. feltiae*, and *S. carpocapsae*) were determined on *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) larvae at various soil moisture levels. As a result, a reduce in soil moisture was associated with a decreased mortality rate in larvae. The other study conducted by Shapiro-Ilan et al. (2006b), the suppressive effects of different isolates of *H. bacteriophora* and *S. carpocapsae* on *Curculio caryae* G.H.Horn, 1873 (Coleoptera: Curculionidae) larvae were evaluated. It was determined that an increase in soil moisture caused an intensified suppression exerted by the EPN species on the larvae. This finding appears to be consistent with the result of the present study.

Furthermore, the structure of agricultural lands varies regionally. Among the factors that undergo changes at this point, soil moisture stands out. The soil moisture, being either too low or too high, significantly influences the efficacy of EPNs on their host insects (Molyneux & Bedding, 1984; Koppenhöfer & Fuzy, 2007; Yadav & Lamramliana, 2012; Ulu & Susurluk, 2014; Bütüner & Susurluk, 2023; Stevens et al., 2023). In their study, Koppenhöfer & Fuzy (2007) investigated the activities of *Steinernema scarabaei* Stock & Koppenhöfer, 2003, *S. glaseri*, *Heterorhabditis zealandica* Poinar, 1990 and *H. bacteriophora* isolates on *Popillia japonica* Newman, 1838 (Coleoptera: Scarabaeidae) larvae at different soil moisture. As a result, an increase in soil moisture was determined to correspond to an increase in larval mortality rates. However, as the soil moisture content continued to increase, the rate of larval mortality rates eventually decreased. Similarly, in a study conducted by Rohde et al. (2010), the efficacy of EPNs on *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) larvae was determined using different isolates from the Steinernematidae and Heterorhabditidae families under various soil moisture conditions. Consequently, maximum efficacy was observed in EPNs at 75% field capacity. Mortality rates obtained at capacities exceeding or falling below this threshold were notably low. Thus, the moisture requirements of EPN isolates, which have demonstrated adaptation to the climate conditions of Türkiye based on the results obtained from the current study, have been identified.

Additionally, there is a perceived need for further research to determine the soil moisture conditions that contribute to the efficacy of EPNs on hosts, particularly for species adapted to the soil conditions of different countries or regions. Consequently, understanding the correlation between soil moisture and EPN species adapted to our country and regional conditions is crucial, especially during field studies where determining soil moisture levels can aid in identifying the potential efficacy of these species on target hosts and optimizing their impact. Furthermore, this study, conducted on the appropriateness of using EPNs in certain species at drought conditions, will shed light on which types are more suitable for the climatic conditions in our country.

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