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Özgün makale (Original article)

Virulence of two strains of entomopathogenic nematodes (Rhabditida: Steinernematidae) against adults of *Sitophilus granarius* L. (Coleoptera: Curculionidae)

Taylan ÇAKMAK [1*]

İki yerel entomopatojen nematod türünün (Rhabditida: Steinernematidae) farklı sıcaklıklarda buğday unlu biti *Sitophilus granarius* L. (Coleoptera: Curculionidae) erginleri üzerindeki etkinliği

Öz: Türkiye, buğday bitkisinin anavatanıdır. Türkiye'de buğday üretimi özellikle İç ve Güneydoğu Anadolu bölgelerinde yoğunlaşmıştır. *Sitophilus granarius* L. (Coleoptera: Curculionidae), buğdayda başlıca depo zararlılarından biri olup, Türkiye'de buğday üretiminde önemli bir sorun teşkil etmektedir. Türkiye'de Bilecik ilinden zeytin ve erik bahçelerinden elde edilen iki yerel entomopatojenik nematod türü: *Steinernema feltiae* (İzolat 8), *S. carpocapsae* (İzolat 27), dört farklı konsantrasyon (250, 500, 1000 ve 2000 IJs/ml) ve dört farklı sıcaklıkta (15, 20, 25 ve 30 °C) *S. granarius* erginleri üzerinde test edilmiştir. İki entomopatojen nematod izolatının *S. granarius* üzerindeki enfeksiyon oranları ve etkinlikleri 8 gün boyunca izlenmiş ve ölüm oranları tespit edilmiştir. Elde edilen sonuçlara göre, uygulama sonrası 8. günde *S. granarius*'un ergin canlı sayısı, *S. feltiae* ve *S. carpocapsae*'nin farklı IJs dozlarına göre karşılaştırıldığında en düşük canlı ergin sayısı 3. dozda (1000 IJs/ml) *S. carpocapsae*'de 25 °C'de 0.63±0.47 olarak gözlenirken, bunu *S. carpocapsae*'nin 30 °C'de verdiği sonuçlar (0.83±0.69) ve 20 °C sıcaklıktaki (0.83±1.06) takip etmiştir. *S. feltiae'*de ise uygulama sonrası 8. günde 2000 IJ/ml dozunda 20 °C'de 1.63±0.94, 20 °C'de ise 2.00±0.70 canlı ergin sayısı gözlemlenmiştir. Genel olarak, *S. carpocapsae*/27 izolatının *S. granarius* erginlerini *S. feltiae*/8 izolatına göre daha başarılı bir şekilde enfekte ettiği ve her iki izolatın da *S. granarius* erginlerine karşı biyolojik mücadelede kullanılma potansiyeline sahip olduğu belirlenmiştir.

Keywords: *Sitophilus granarius*, *Steinernema feltiae*, *Steinernema carpocapsae*, biyolojik mücadele.

Abstract: Türkiye is the country of origin for the wheat plant, and a significant portion of wheat production is concentrated in the southeastern and central provinces. One of the most serious pests affecting stored wheat grains in Türkiye is *Sitophilus granarius* L. (Coleoptera: Curculionidae), posing a significant threat to wheat production. This study evaluated the efficacy of two locally sourced entomopathogenic nematode (EPN) species, *Steinernema feltiae* (Strain 8) and *Steinernema carpocapsae* (Strain 27), isolated from olive and plum orchards in Bilecik, Türkiye. The experiment was conducted using four different concentrations of infective juveniles (IJs) (250, 500, 1000, and 2000 IJs/ml) at four

[1] Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology, Düzce, Türkiye
[*] Sorumlu yazar (Corresponding author): taylancakmak@duzce.edu.tr
ORCID ID (Yazar sırasıyla): 0000-0003-4151-5724
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temperatures (15, 20, 25, and 30 °C). Mortality rates of *S. granarius* adults were assessed over an 8-day period following the application of EPNs. Results showed that on the 8th day post-application, the lowest number of surviving S. granarius adults was recorded at the 1000 IJs/ml concentration of *S. carpocapsae*, with 0.63±0.47 at 25 °C. This was followed by 0.83±0.69 at 30 °C and 0.83±1.06 at 20 °C for the same nematode species. In contrast, *S. feltiae* exhibited higher survival rates, with 1.63±0.94 living adults at 20 °C and 2.00±0.70 at 2000 IJs/ml on the 8th day post-application. Overall, the *S. carpocapsae* (Strain 27) isolate demonstrated greater efficacy in infecting *S. granarius* adults compared to the *S. feltiae* (Strain 8) isolate. Both isolates exhibit significant potential for use in the biological control of *S. granarius* adults.

Keywords: *Sitophilus granarius*, *Steinernema feltiae*, *Steinernema carpocapsae*, biological control

Introduction

Wheat (*Triticum aestivum* L. emend. Fiori et Paol.) is one of the most important crops providing a staple food source for humans. As the global population rapidly increases, the demand for food, and consequently wheat, continues to grow. The world population, which was 6.1 billion in 2000, is projected to reach 9.3 billion by 2050 (Anonymous, 2009). In Türkiye, wheat holds significant importance in terms of nutritional potential. In 2021, approximately 68 million decares were planted with wheat, yielding around 18 million tonnes (Kahraman & Kahraman 2023).

Sitophilus granarius Linnaeus (Coleoptera: Curculionidae), commonly known as the granary weevil, is a major pest in grain storage facilities, particularly in continental regions (Niewiada et al. 2005). This insect feeds on wheat grains, leading to substantial reductions in both grain quality and yield (Ebadollahi 2011). The use of chemical insecticides remains a widely accepted method for pest control (Huang & Subramanyam 2005), but concerns about their environmental and health impacts have grown. As a result, there has been an increasing focus on developing environmentally safe, effective, and biodegradable alternatives, leading to a surge in biological control research (Sarkar et al. 2021).

Among the biological control agents, nematodes are known to interact with insects, with 23 families having parasitic relationships with them. Seven of these families are exclusively composed of insect-parasitic nematodes (Poinar 1975). The families Steinernematidae and Heterorhabditidae (Rhabditida) are particularly important for biological control, as these nematodes are widely used as microbial insecticides. Commercially produced by various companies, these nematodes have proven effective against a broad range of pest species (Grewal et al. 2005; Koppenhöfer 2007). Entomopathogenic nematodes (EPNs), obligate parasites of insects, play a significant role in biological control by targeting insect pests, particularly in the soil. One of the key advantages of EPNs over chemical insecticides is their host specificity (Smart 1995).

EPNs have demonstrated effective control of pests like *S. granarius*. When applied in storage environments, they actively seek out their hosts, causing no harm to vertebrates, and effectively eliminate insect pests even at low doses. Typically, EPNs kill their hosts within 48 hours by inducing septicemia through the release of symbiotic bacteria (Ünlü & Özer 2003). The infective juvenile (IJ) stage, the most

active life stage, is the third larval stage. It resides in the soil and searches for hosts, with the ability to survive for over a year without one. Infective juveniles enter the host's hemocoel through natural openings (mouth, anus, spiracles) or penetrate thinner areas of the cuticle. This is especially true for Heterorhabditidae, which have dorsal labial teeth to aid penetration (Bedding & Molyneux 1982; Wang & Gaugler 1998).

Inside the host, the nematodes and their symbiotic bacteria feed on the host's tissues, allowing the nematodes to grow and reproduce within the insect cadaver (Poinar & Grewal 2012). The nematodes feed for about 2-3 generations, until the host's resources are depleted. Eventually, infective juveniles in the J3 stage emerge from the cadaver, migrate into the soil, and begin searching for new hosts (Poinar 1979; Akhurst & Boemare 2018).

While there are concerns about the efficacy of EPNs at low temperatures, they can actively be used against *S. granarius* at temperatures above 15°C. Below this threshold, EPNs may be less effective in infecting *S. granarius* adults. The aim of this study is to investigate the virulence of two local Steinernema isolates (*S. feltiae* and *S. carpocapsae*) at four different temperatures (15, 20, 25 and 30°C) with virulence observations conducted at two-day intervals over an eight-day period, targeting the adult stage of *S. granarius* under laboratory conditions.

Materials and Methods

Mass Production of *Sitophilus granarius* Adults

The wheat weevil, *S. granarius*, used in this study was reared in the laboratory using 1-liter glass jars filled with bread wheat grains. The tops of the jars were covered with finely porous cloth to allow ventilation while preventing the adults from escaping. The cultured insects were kept in an incubator set at 27±1 °C and 60±5% relative humidity (Davis & Bry, 1985). Adult insects aged between 1 and 7 days were selected for use in laboratory experiments. The insect culture was maintained in the culture room of the Department of Agricultural Biotechnology, Faculty of Agriculture, Düzce University.

Mass Production of *Galleria mellonella*

G. mellonella larvae were reared on an artificial diet composed of 500 g of coarse bran, 50 g of honey, 65 ml of glycerin, and 25 ml of sterile water. The larvae were grown in glass jars and kept in incubators at 27±1°C (Kaya & Stock 1997). Some of the developed larvae were reintroduced into the jars to maintain the culture, while others were used for the mass production of entomopathogenic nematodes (EPNs).

Mass Production of Entomopathogenic Nematodes (EPNs)

EPNs used in this study were isolated from soil samples collected from olive and plum orchards in Bilecik, Türkiye (Table 1). The nematodes were identified through morphological and morphometric measurements. Infective juveniles (IJs) were obtained by exposing last-instar *G. mellonella* larvae to 100 g of soil. Fresh isolates were cultured and produced on late-instar *G. mellonella* larvae. The nematode cultures were renewed every two months in the laboratory, ensuring their viability.

Emergence of IJs from each *G. mellonella* larva was observed within 2-4 days under optimal conditions, depending on the EPN species.

Determination of the effectiveness of entomopathogenic nematodes on *S. granarius*

The experimental procedure followed the methodology described by Trdan et al. (2006). Freshly emerged infective juveniles (IJs) from *G. mellonella* larvae were counted and assessed for viability under an Olympus binocular microscope before use. After determining the concentration of IJs, they were placed in sterile Eppendorf tubes containing sterile water for subsequent applications.

Petri dishes (9 cm diameter) were prepared by placing filter paper and 10 wheat grains in each dish. Then, 10 adult *S. granarius* (aged 1-7 days) were introduced to the dishes. Nematodes were applied to the *S. granarius* adults using a micropipette. The dishes were incubated in the dark at four different temperatures (15, 20, 25, and 30°C) and 65% relative humidity (Model N: MCO-170AC-PE) following Kaya & Stock (1997). Each treatment (control with only sterile water, 250, 500, 1000, and 2000 IJs/ml) was replicated three times.

The cadavers of dead *S. granarius* adults were collected at each observation day (2, 4, 6, and 8 days post-application) using a needle under a stereo microscope (Olympus). The number of dead adults was recorded, and 10 grains of wheat were provided as sustenance for the control group.

Table 1. Entomopathogenic nematode species and isolate numbers used in this study (Çakmak, 2024)

Isolate	Species	Location	Host Plant
Strain 8	*Steinernema feltiae*	Bilecik	Olive orchard
Strain 27	*Steinernema carpocapsae*	Bilecik	Plum orchard

Statistical analysis

Data were analyzed using a three-way repeated measures ANOVA with STATISTICA 14.0.0.15 (TIBCO Software Inc., 2020) to assess differences in the number of living *S. granarius* adults at different nematode concentrations for the two EPN strains. All data are presented as mean ± standard error. The least significant difference (LSD) test was used to compare means, with a significance level set at $p \leq 0.05$.

Results and Discussion

The infection of *Sitophilus granarius* were assessed by counting live and dead adults at various observation points following the application of entomopathogenic nematodes (EPNs). The infection potantial varied depending on temperature and the concentration of infective juveniles (IJs) of the applied EPN species. The virulence of each EPN strain was influenced by different temperatures and EPN isolates (Figure 1 and 2).

The highest mean numbers were observed with the *Steinernema carpocapsae* (27 isolate), yielding the lowest number of live *S. granarius* adults at 0.63±0.47 on the 8th day post-application at 25 °C (Figure 2).

On the 4th day post-application, *S. carpocapsae* was more effective in reducing *S. granarius* populations compared to *S. feltiae* (1.97±0.72 at 20 °C; 1.87±1.26 at 25 °C), while *S. feltiae* resulted in higher numbers of surviving adults (6.53±1.59 at 20°C and 7.13±1.63 at 25 °C) at dose 4 (2000 IJs/ml) (Figure 1 and 2).

By the 6th day post-application, under dose 4 (2000 IJs/ml), the number of live adults was again lower in the *S. carpocapsae* group, with 0.73±1.33 at 25 °C and 0.80±1.48 at 20°C. At dose 3 (1000 IJs/ml), *S. feltiae* exhibited its highest efficacy at 20 °C with 2.87±1.50, followed by 3.33±1.46 at 25 °C (Figure 1 and 2).

On the 8th day, a comparison *of S. granarius* survival rates across different doses of *S. feltiae* and *S. carpocapsae* revealed that *S. carpocapsae* (1000 IJs/ml) was most effective at 25 °C, resulting in 0.63±0.47 live adults. This was followed by *S. carpocapsae* at 20 °C with 0.83±1.06 live adults and 0.83±1.69 at 30 °C with dose 4 (2000 IJs/ml). In contrast, *S. feltiae* resulted in 1.63±0.94 live adults at 20 °C and 2.00±0.70 at 20 °C with 2000 IJs/ml (Figure 1 and 2).

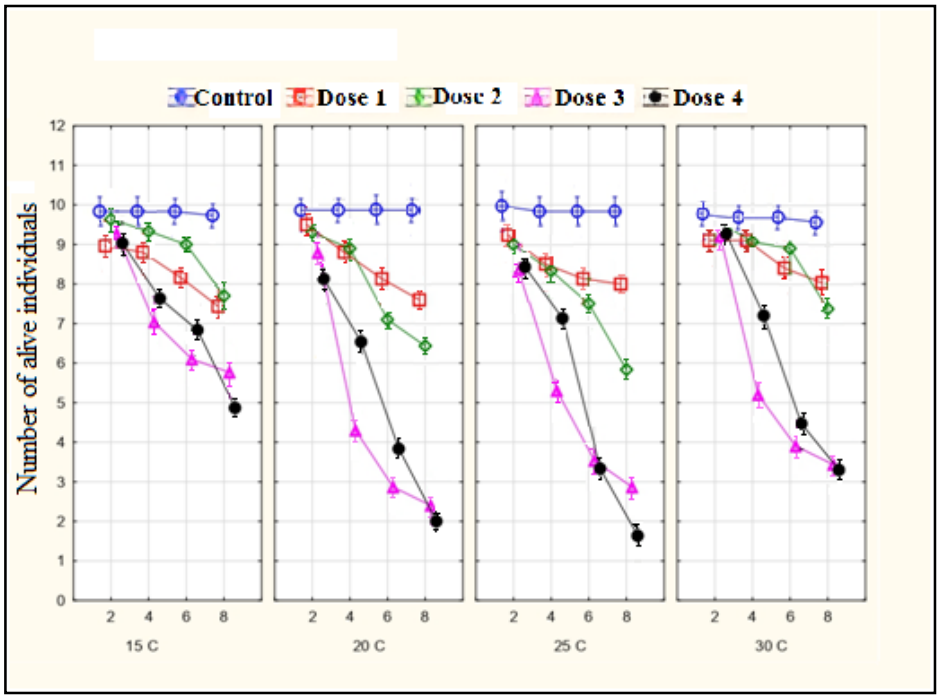

Figure 1. Virulence of *Steinernema feltiae* on *Sitophilus granarius* adults (number of alive individuals out of 10 adults) under laboratory conditions with four different temperatures (Mean±SE). Differences between means tagged with colors compared to control days were significant (P < 0.05). Control group (0 IJs); Dose 1: 250 IJs/ml; Dose 2: 500 IJs/ml; Dose 3: 1000 IJs/ml and Dose 4: 2000 IJs/ml of *Steinernema feltiae* per 10 adults of *S. granarius*. (F (36, 160)=0,116; P= 0.016) LSD test; Homogenous Groups, alpha = 0.05000 (Non-Exhaustive Search) Error: Between MS = 1,1323, df = 160.00 (Mean±SE).

Overall, both isolates performed best at 25 °C, and increasing the concentration of IJs resulted in higher mortality rates, as expected.

Recent studies on the use of EPNs against *S. granarius* adults have shown promising results. For instance, Trdan et al. (2006) tested four EPN species (*Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema feltiae*, and *S. carpocapsae*) and reported high efficacy against *S. granarius* adults. Similarly, Negrisoli et al. (2013) found that increasing temperatures led to higher mortality rates in *Sitophilus* species.

Laznik et al. (2010) evaluated the virulence of three *S. feltiae* strains against *Sitophilus oryzae* adults, with mortality rates ranging from 42% to 72% at 25 °C, and significantly lower mortality (6% to 11%) at 30 °C. In Türkiye, Yüksel et al. (2019) reported that *S. feltiae* isolates demonstrated the highest virulence (86%) against *S. granarius* adults at 25 °C.

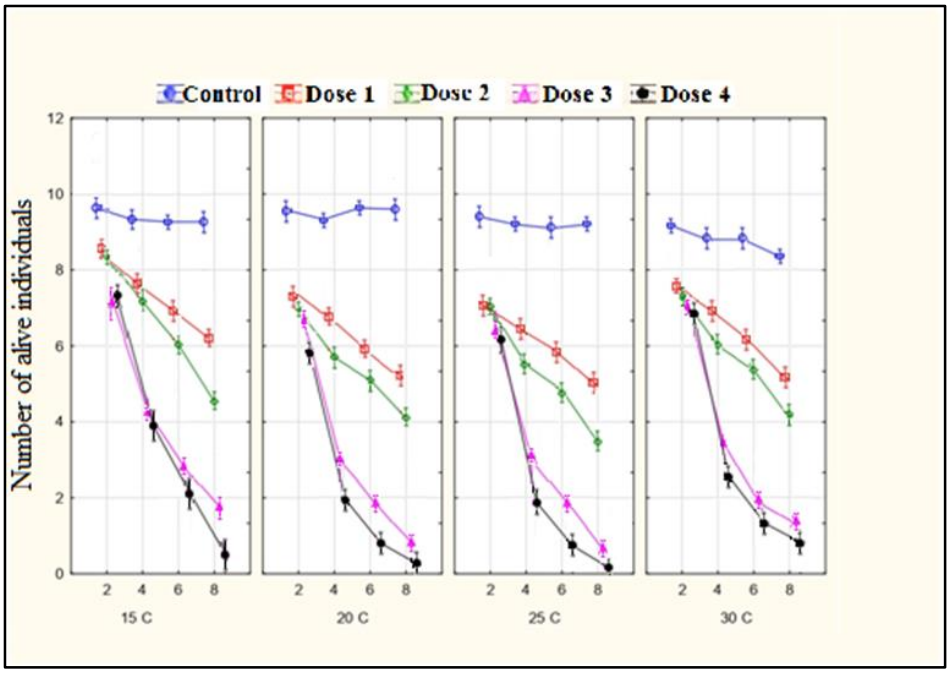

Figure 2. Virulence of *Steinernema carpocapsae* on *Sitophilus granarius* adults (number of alive individuals out of 10 adults) under laboratory conditions with four different temperatures (Mean±SE). Differences between means tagged with colors compared to control days are significant ($P < 0.05$). Dose 1: Control group (0 IJs/ml); Dose 1: 250 IJs/ml; Dose 2: 500 IJs/ml; Dose 3: 1000 IJs/ml and Dose 4: 2000 IJs/ml of *Steinernema carpocapsae* per 10 adults of *S. granarius*. ($F (36, 160)=1,555$, $P= 0.034$) LSD test; Homogenous Groups, alpha =0.05000 (Non-Exhaustive Search) Error: Between MS = 0.31458, df = 160.00.

Temperature significantly affected infection rates, with the highest mortality observed at 25 °C and reduced efficacy at 30 °C. This suggests that temperature is a critical factor in optimizing nematode applications for biological control, and strategies must be tailored to the specific environmental conditions of the application site. The findings align with those of Trdan et al. (2006) and Negrisoli et al. (2013), supporting the notion that higher temperatures increase EPN efficacy. Additionally,

the studies conducted in Türkiye by Yüksel et al. (2019) reaffirm the potential for biological control in local contexts.

Protecting stored products using chemical pesticides is challenging, and the focus should shift towards employing EPNs for pest control. In this study, infection rates of *S. granarius* adults varied based on the nematode species and the temperature at which they were applied. Higher temperatures consistently resulted in higher infection rates. While *S. feltiae* (Strain 8) showed lower activity against *S. granarius*, both isolates, *S. carpocapsae* (Strain 27) and *S. feltiae* (Strain 8), demonstrated similar levels of virulence across various temperatures (15, 20 and 25 °C). However, *S. carpocapsae* (Strain 27) exhibited the highest overall efficacy.

The increasing use of EPNs in biological control against storage pests is due to their broad host range, high mortality rates, ease of application, and minimal environmental and health impacts. This study demonstrates that EPN isolates are effective against *S. granarius*, and special application methods may enhance their efficacy in storage conditions. Notably, *S. carpocapsae* (Strain 27) showed superior performance against *S. granarius* adults, making it a promising candidate for future biological control efforts.

The present study confirms the potential of using *S. feltiae* and *S. carpocapsae* in biological control, particularly against *S. granarius*. The high infection rates of *S. carpocapsae* highlight its potential for future biological control programs. These results underscore the importance of environmentally friendly alternatives to chemical insecticides, benefiting both environmental and human health. Future research should explore the effectiveness of EPNs against different pest populations and environmental conditions. Additionally, alternative application methods may improve their efficacy in storage settings, representing an important step in advancing biological control strategies in Türkiye.

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