

Pathogenicity of Turkish Entomopathogenic nematodes, *Steinernema feltiae* and *Steinernema carpocapsae* (Rhabditida: Steinernematidae) on the rice weevil: *Sitophilus oryzae* (Coleoptera: Curculionidae)

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ABSTRACT

Stored product pests are one of the most important biotic factors that cause serious postharvest losses of food crops in the course of storage. To control these pests, pesticides have been extensively used for many years. However, it is known that pesticides and their residues have toxic effects on non-target organisms. Therefore, it is believed that Entomopathogenic Nematodes (EPNs), which have been widely employed for biological control in agricultural fields, can be used for the potential control of *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae). In this study, the biocontrol potential of *Steinernema feltiae* TUR-S3 and *Steinernema carpocapsae* TUR-S4 isolates (Rhabditida: Steinernematidae) were evaluated against the adults of *S. oryzae* at eight different concentrations [250 IJs/cm² (600 IJs/adult), 200 IJs/cm² (480 IJs/adult), 100 IJs/cm² (240 IJs/adult), 50 IJs/cm² (120 IJs/adult), 25 IJs/cm² (60 IJs/adult), 12 IJs/cm² (30 IJs/adult), 6 IJs/cm² (15 IJs/adult), and 3 IJs/cm² (7.5 IJs/adult)]. According to the results, both *S. feltiae* TUR-S3 and *S. carpocapsae* TUR-S4 exhibited the highest mortality rate (96.67%) on *S. oryzae* when applied at the concentration of 250 IJs/cm². The most effective LC₅₀ and LC₉₀ values for *S. feltiae* TUR-S3 were determined as 47.55 and 167.16, respectively. These values were obtained as 35.66 and 121.79 for *S. carpocapsae* TUR-S4 isolate.

Key words: *Steinernema carpocapsae*, *Steinernema feltiae*, *Sitophilus oryzae*, rice weevil, biological control

Türk Entomopatojen Nematodların, *Steinernema feltiae* ve *Steinernema carpocapsae* (Rhabditida: Steinernematidae) Pirinç Biti *Sitophilus oryzae* (Coleoptera: Curculionidae) Üzerinde Patojenisitesi

ÖZ

Depolanmış ürün zararlıları, tarım ürünlerin depolama sürecinde hasat sonrası ciddi kayıplara neden olan en önemli biyotik faktörlerden biridir. Bu zararlılar ile mücadele amacıyla yıllardır yaygınla pestisitler kullanılmaktadır. Ancak pestisitlerin ve ne olduğu kalıntıların hedef dışı organizmalar üzerinde oluşturduğu toksik etkiler bilinmektedir. Bu nedenle, tarım alanlarında biyolojik mücadelede yaygın olarak kullanılan Entomopatojen Nematodların (EPN), *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae)'nin potansiyel kontrolünde kullanılabileceği düşünülmektedir. Bu çalışmada *Steinernema feltiae* TUR-S3 ve *Steinernema carpocapsae* TUR-S4 izolatlarının (Rhabditida: Steinernematidae) *S. oryzae*'nin erginleri üzerindeki biyokontrol potansiyel 8 farklı konsantrasyonda [250 IJs/cm² (600 IJs/ergin), 200 IJs/cm² (480 IJs/ergin), 100 IJs/cm² (240 IJs/ergin), 50 IJs/cm² (120 IJs/ergin), 25 IJs/cm² (60 IJs/ergin), 12 IJs/cm² (30 IJs/ergin), 6 IJs/cm² (15 IJs/ergin), ve 3 IJs/cm² (7.5 IJs/ergin)] değerlendirilmiştir. Sonuçlara göre hem *S. feltiae* TUR-S3 izolatı hemde *S. carpocapsae* TUR-S4 izolatı, *S. oryzae* erginlerinde en yüksek ölüm oranını (96.67%) 250 IJs/cm² uygulama konsantrasyonunda gerçekleştirmiştir. *S. feltiae* TUR-S3 için en etkili LC₅₀ ve LC₉₀ değerleri sırasıyla 47.55 ve 167.16 dir. Bu değerler *S. carpocapsae* TUR-S4 izolatı için 35.66 ve 121.79 olarak elde edilmiştir.

Anahtar kelimeler: *Steinernema carpocapsae*, *Steinernema feltiae*, *Sitophilus oryzae*, pirinç biti, biyolojik mücadele

INTRODUCTION

One of the primary causes of product loss in agricultural areas, both during production and storage, is agricultural pests (Oerke and Dehne, 2004; Neethirajan et al., 2007; Manosathiyadevan et al., 2017). For years, pesticides have been widely used against both field and storage pests (Muda, 1986; Dubey et al., 2008; Hamel et al., 2020). However, following the emergence of toxic effects of pesticides on non-target organisms, restrictions have been imposed on pesticide use. As a result, alternative pest control methods have gained importance (Flexner et al., 1986; Pereira et al., 2009; Zaller and Brühl, 2019).

One of the widely used alternative pest control methods is biological control (Gaugler, 2002). Entomopathogenic nematodes (EPNs) are endoparasitic organisms that are used in the biological control of a wide variety of agricultural pests including stored product pests (Gaugler et al., 1997; Shapiro-Ilan et al., 2006; Şahin et al., 2018; Yüksel et al., 2019). These organisms, belonging to Heterorhabditidae and Steinernematidae families, require a host to continue their life cycle (Burnell and Stock, 2000; Gaugler, 2002; Grewal et al., 2005). Only infective juvenile (IJ) stage of EPNs can infect hosts (Strauch and Ehlers, 1998; Kunkel et al., 2006; Susurluk et al., 2018). Species belonging to the Steinernematidae family can infect hosts with *Xenorhabdus* spp., which is a gram-negative bacterium carried in a special pouch in their bodies, through a symbiotic relationship (Ehlers et al., 1990; Wouts, 1990; Lunau et al., 1993; Sunanda et al., 2012; Ulu et al., 2015). Additionally, IJs can actively search for hosts and live for several months without feeding (Susurluk and Ehlers, 2008).

Product losses in agricultural are mainly attributed to pests, and post-harvest losses of about 10% also occur due to stored product pests (Negrisoli et al., 2013; Rumbos and Athanassiou, 2017). Rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), is a significant storage pest that can cause damage to grains (Karan et al., 1974; Govindan and Nelson, 2009; Swamy et al., 2014). This pest is commonly found in warehouses, and it prefers humid storage conditions ranging from 15% to 65%, where it feeds on the endosperm of wheat grains, leading to severe damage (Kavallieratos et al., 2007). Although pesticides have been traditionally used to control this pest, their use in warehouse conditions is not recommended due to the potential pesticide residues on products. Furthermore, continuous and excessive use of pesticides triggered the development of resistant populations, rendering their use less effective (Schöller et al., 2006; Hamel et al., 2020).

The primary objective of this study was to determine the virulence of different concentrations of TUR-S3, a isolate of *S. feltiae* Filipjev (Rhabditida: Steinernematidae), and TUR-S4, a isolate of *S. cariocapsae* Weiser, (Rhabditida: Steinernematidae) on *S. oryzae*.

MATERIALS AND METHODS

Sitophilus oryzae and Entomopathogenic Nematode Species:

In the present study, two EPN species, *S. feltiae* and *S. cariocapsae* were used. The both species were isolated in Bursa, Türkiye. The infective juveniles (IJs) were obtained through infecting *Galleria mellonella* L. (Lepidoptera: Pyralidae) under laboratory conditions and stored in Riger solution at 4–6°C. *Sitophilus oryzae* adults used in the experiments were provided by the Department of Plant Protection, Faculty of Agriculture, Tekirdağ Namık Kemal University. The adults used in the experiment were kept in a plant growth chamber in dark at 23±2°C.

Experimental Design:

Firstly, filter papers were placed inside 6 cm diameter plastic Petri dishes. Then, 10 wheat grains were placed in the center of each Petri dish. Afterwards, the adults were released into the plastic Petri dishes containing wheat grains. Ten rice weevil adults were placed in each Petri dish. The IJs were applied at following concentrations: 250 IJs/cm² (600 IJs/adult), 200 IJs/cm² (480 IJs/adult), 100 IJs/cm² (240 IJs/adult), 50 IJs/cm² (120 IJs/adult), 25 IJs/cm² (60 IJs/adult), 12 IJs/cm² (30 IJs/adult), 6 IJs/cm² (15 IJs/adult), and 3 IJs/cm² (7.5 IJs/adult). EPN concentrations were applied to the filter papers in the plastic Petri dishes with a pipette in 400 µl Ringer solution. This experiment was conducted in triplicate for each EPN species. After inoculation, *S. oryzae* adults were maintained at 25°C. Four days after treatment, the mortality rates of the adults were calculated.

Statistical analysis:

The data was analyzed with ANOVA following Fisher's LSD post-hoc test ($p<0.05$). JMP®16.0 software was used for ANOVA test. The values for LC₅₀ and LC₉₀ were calculated using the Minitab® statistical software.

RESULTS

The highest mortality (96.67%) was achieved by *S. feltiae* at the concentration of 250 IJs/cm² (600 IJs/adult). The mortality rates obtained on adults with different doses of 200 IJs/cm² (480 IJs/adult), 100 IJs/cm² (240 IJs/adult), 50 IJs/cm² (120 IJs/adult), 25 IJs/cm² (60 IJs/adult), 12 IJs/cm² (30 IJs/adult), 6 IJs/cm² (15 IJs/adult) were determined as 90%, 76.67%, 70%, 60%, 46.67%, 23.33%, respectively. The lowest mortality rate on adults was obtained in the adults treated with a dose of 3 IJs/cm² (7.5 IJs/adult), and this rate was determined as 3.33%. Statistically significant differences were identified among the mortality rates of *S. oryzae* adults treated with *S. feltiae* ($F = 52.44$; $df = 8,18$; $P < 0.0001$) (Table 1).

Table 1. A percentage was calculated to determine the mortality rates of *S. oryzae* larvae caused by the utilized EPN isolates. Statistical analysis was carried out separately for each species (Mean \pm SE). Means within each isolate that share the same letters are not significantly different.

EPN Species	Dose (IJs/cm ²)	IJs/adult	Mortality rates (%)
<i>Steinernema feltiae</i> (TUR-S3)	250	600	96.67 \pm 3.33 a
	200	480	90 \pm 5.77 ab
	100	240	76.67 \pm 6.67 bc
	50	120	70 \pm 5.77 cd
	25	60	60 \pm 5.77 de
	12	30	46.67 \pm 3.33 e
	6	15	23.33 \pm 6.67 f
	3	7.5	3.33 \pm 3.33 g
	Control	0	0 \pm 0 g
<i>Steinernema carpocapsae</i> (TUR-S4)	250	600	96.67 \pm 3.33 a
	200	480	93.33 \pm 6.67 ab
	100	240	86.67 \pm 6.67 ab
	50	120	76.67 \pm 6.67 bc
	25	60	66.67 \pm 8.82 cd
	12	30	53.33 \pm 3.33 d
	6	15	33.33 \pm 6.67 e
	3	7.5	10 \pm 5.77 f
	Control	0	0 \pm 0 f

The highest mortality rate caused by *S. carpocapsae* on *S. oryzae* adults was determined as 96.67% in adult individuals treated with a dose of 250 IJs/cm² (600 IJs/adult). The mortality rates obtained on adults with different doses of 200 IJs/cm² (480 IJs/adult), 100 IJs/cm² (240 IJs/adult), 50 IJs/cm² (120 IJs/adult), 25 IJs/cm² (60 IJs/adult), 12 IJs/cm² (30 IJs/adult), 6 IJs/cm² (15 IJs/adult) were determined as 93.33%, 86.67%, 76.67%, 66.67%, 53.33%, 33.33%, respectively. The lowest mortality rate on adults was determined as 10% in adult individuals treated with the concentration of 3 IJs/cm² (7.5 IJs/adult). Statistically significant differences were identified among the mortality rates of *S. oryzae* adults treated with *S. carpocapsae* ($F = 37.19$; $df = 8,18$; $P < 0.0001$) (Table 1). In addition, in this study, LC₅₀ and LC₉₀, values were determined for *S. feltiae* and *S. carpocapsae* isolates used at different concentrations. The LC₅₀ value for *S. feltiae* was found to be 47.55, and the LC₉₀ value was 167.16. For *S. carpocapsae*, the LC₅₀ value was determined as 35.66, and the LC₉₀ value was 121.796 (Table 2).

Table 2. The LC₅₀ and LC₉₀ values were determined for *S. oryzae* infected with *S. feltiae* TUR-S3 and *S. carpocapsae* TUR-S4 isolates.

EPN Species	n	LC ₅₀ (95% CI)	LC ₉₀ (95% CI)	Slope ± SE	X ² (df)	P
<i>S. feltiae</i> (TUR-S3)	270	47.55 (31.76-63.35)	167.16 (131.38-202.93)	0.01 ± 0.001	26.02 (7)	<0.001
<i>S. carpocapsae</i> (TUR-S4)	270	35.66 (23.69-47.64)	121.79 (93.56-150.02)	0.01 ± 0.002	48.44 (7)	<0.001

DISCUSSION

Pesticides are commonly used to control pests in agricultural fields and storage areas after harvest, which can cause yield losses. However, using pesticides to control pests before harvest and in storage conditions can result in a significant residue problem on products (Dede et al., 2022). Previous studies have shown that pesticides have toxic effects on humans and non-target organisms (Ali and Jain, 1998). Entomopathogenic nematodes are commonly used to control pests that cause yield losses in agricultural fields, but studies on their use in storage conditions are limited. The present study determined the potential use of the *S. feltiae* TUR-S3 and *S. carpocapsae* TUR-S4 isolates in controlling *S. oryzae*. Eight different concentrations were evaluated for the control of *S. oryzae*. Based on the conducted study, an increase in the application of IJs/adult concentration on *S. oryzae* has resulted in a proportional increase in the mortality rate of the pest. Therefore, this study, conducted with the aim of potential control of *S. oryzae*, is promising.

In recent years, EPNs have been widely used in the control of agricultural pests and other storage pests. However, studies on the use of EPNs for the control of *S. oryzae* are quite limited. In a study similar to the present study, Laznik et al. (2010) used three different isolates of *S. feltiae* to infect *S. oryzae* adults. Results showed that mortality rates increased as the number of applied IJs/adults increased, taking into account the temperature values used in the study. The outcomes of the present study are consistent with the findings of previous studies. Similarly, in a study conducted by Canhilal and Yüksel (2020), it was determined that the mortality rate observed in *S. oryzae* increased in direct proportion to the increase in the applied IJ dose on adults. These findings align with the results derived from the present study.

In a study conducted by Trdan et al. (2006), *S. feltiae*, *S. carpocapsae*, and two other EPN isolates were used to control *Sitophilus granarius* (Coleoptera: Curculionidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) adults. According to the results, it was determined that as the IJs/adult dose applied to the insects increased proportionally, the mortality rates observed in the insects also increased, taking into account the temperature values used in the study. In this regard, these findings are consistent with the results obtained from the present study. According to a study by Javed et al. (2020), *Steinernema pakistanense*, *Steinernema bifurcatum*, *Steinernema affine*, and *Steinernema cholashanense* (Rhabditida: Steinernematidae) isolates were used to infect adults of *Tribolium confusum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae). The findings indicated that increasing the IJs/adult dose applied to the insects led to a proportional increase in mortality rates observed in the adults. These results align with the outcomes of Canhilal and Yüksel (2020) who reported a positive and accelerating trend in the mortality rates of *S. oryzae* with increasing concentrations.

CONCLUSION

In recent years, there has been a growing trend towards reducing the use of pesticides in agricultural fields and storage facilities to control pests, due to the toxic effects of pesticides on non-target organisms. This is particularly important in storage conditions, where pesticide use can lead to residues on the products. Therefore, the use of EPNs has emerged as a promising alternative for pest control in storage. This study has obtained highly effective results in controlling of *S. oryzae* using EPNs under laboratory conditions. However, some limitations arise in the application of EPNs under storage conditions. With further research and advancements, the use of EPNs is believed to be highly effective in controlling pests in storage conditions.

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