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Pathogenicity of the *Steinernema feltiae* TUR-S3 (Rhabditida: Steinernematidae) Isolate on *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae)

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ABSTRACT

For many years, pesticides have been employed to manage pests that cause losses in agricultural yield and quality during storage. However, as the harmful effects of pesticides on non-target organisms became apparent, alternative approaches gained traction. Entomopathogenic Nematodes (EPNs), commonly used in biological control, are now being considered for controlling *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae). This study evaluated the efficacy of *Steinernema feltiae* TUR-S3 (Rhabditida: Steinernema) isolate at five different concentrations (3 Infective Juveniles (IJs)/cm^2 (8 IJs/adult), 6 IJs/cm^2 (17 IJs/adult), 9 IJs/cm^2 (25 IJs/adult), 18 IJs/cm^2 (50 IJs/adult), 27 IJs/cm^2 (75 IJs/adult)) against adult *O. surinamensis* and *T. confusum*. The results indicated the highest mortality rate, reaching 83.33%, was achieved in both pests at a concentration of 27 IJs/cm^2 (75 IJs/adult). This suggests promising potential for controlling *O. surinamensis* and *T. confusum* using this approach.

Key words: *Oryzaphilus surinamensis Steinernema feltiae, Tribolium confusum*, biological control

Steinernema feltiae TUR-S3 (Rhabditida: Steinernematidae) izolatının *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) ve *Tribolium confusum* (Coleoptera: Tenebrionidae) Üzerinde Patojenisitesi

ÖZ

Depolama koşullarında tarımsal ürünlerde verim ve kalite kaybına neden olan zararlıların kontrolünde uzun yıllardır pestisitler kullanılmaktadır. Ancak pestisitlerin hedef dışı organizmalar üzerindeki toksik etkilerinin belirlenmesi ile birlikte pestisit kullanımına alternatif diğer yöntemler ön plana çıkmıştır. Bu nedenle biyolojik mücadele kapsamında yaygınlıkla kullanılan Entomopatojen Nematodların (EPNs), *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) ve *Tribolium confusum* du Val (Coleoptera: Tenebrionidae)'un potansiyel mücadelesinde kullanılabileceği düşünülmektedir. Bu çalışmada *Steinernema feltiae* TUR-S3 (Rhabditida: Steinernema) izolatının *O. surinamensis* ve *T.confusum* erginleri üzerindeki potansiyel mücadelesinde 5 farklı konsantrasyon (3 IJs/cm^2 (8 IJs/ergin), 6 IJs/cm^2 (17 IJs/ergin), 9 IJs/cm^2 (25 IJs/ergin), 18 IJs/cm^2 (50 IJs/ergin), 27 IJs/cm^2 (75 IJs/ergin) kullanılarak değerlendirilmiştir. Sonuçlara göre en yüksek ölüm oranı *O. surinamensis* ve *T.confusum*' da 27 IJs/cm^2 (75 IJs/ergin) konsantrasyonda 83.33% olarak belirlenmiştir. Bu yönüyle çalışma *O. surinamensis* ve *T.confusum* mücadelesinde umut vaad etmektedir.

Anahtar kelimeler: *Oryzaphilus surinamensis*, *Steinernema feltiae, Tribolium confusum*, biyolojik mücadele

INTRODUCTION

Agricultural production plays a significant role in sustaining the human population. With the increasing world population, the demand for food continues to rise (Grafton et al., 2015; Singh et al., 2021; Oh and Lu, 2023; Bjørndal et al., 2024). Preserving and safeguarding agricultural products is of great importance in this context in today's world (Kumar and Kalita, 2017; Kumar et al., 2021; Kusuma et al., 2024). Various factors in post-harvest storage can lead to a loss in product quality (Galstyan et al., 2019; Olorunfemi and Kayode, 2021), with storage pests being a major contributing factor to yield loss. Therefore, controlling storage pests holds significance for food supply (Hassan et al., 2023; Mantzoukas et al., 2023).

Pesticides have been widely used for many years in plant protection. However, recent studies have led to an increased understanding of the toxic effects of pesticides on human health, the environment, and non-target organisms. Consequently, there is a growing need for alternative methods of pest control (Van der Werf, 1996; Hernández et al., 2013; Kumar et al., 2023; Tan et al., 2023; Bütüner et al., 2024). One of the most important alternatives is biological control. Entomopathogenic Nematodes (EPNs) are commonly used in biological control to manage various types of insect pests (Jansson et al., 1993; Gözel and Güneş, 2013; Şahin and Gözel, 2019; Dede et al., 2022; Bütüner et al., 2023; Ulu and Susurluk, 2024).

Entomopathogenic nematodes are endoparasitic organisms that require a host tissue to complete their life cycles. These organisms belong to the Rhabditida order, Steinernematidae, and Heterorhabditidae families (Ehlers, 2001; Grewal et al., 2006; Susurluk and Ehlers, 2008; Ulu and Susurluk, 2014; Bütüner and Susurluk, 2023a). The EPNs can infect hosts only during the Juvenile 3 (Infective Juvenile) stage of their life cycles. This infective function is carried out in symbiosis with gram-negative bacteria belonging to the Enterobacteriaceae family, residing inside their bodies. In Heterorhabditidae family members, this bacterium is *Photorhabdus* spp., while in Steinernematidae family members, it is *Xenorhabdus* spp. (Peters, 1996; Shapiro-Ilan et al., 2005; Ciche et al., 2006; Stevens et al., 2023; Bütüner and Susurluk, 2023a).

Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae) is a polyphagous pest that holds significant importance among stored product pests worldwide, causing quality and yield losses in more than thirty food items (Eldeghidy et al., 2022; Gourgouta et al., 2023). The organism is known to thrive optimally at 28±2°C and 30-70% relative humidity (Arbogast, 1976; Beckett and Evans, 1994; Nika et al., 2020). Typically, this species, both in its larval and adult stages, feeds on the kernels of grains, leading to yield losses. Under favorable conditions, an adult female can lay 6-10 eggs per day and 200-375 eggs per month (Howe, 1956; Arbogast, 1976; Tremetra et al., 2000).

Tribolium confusum du Val (Coleoptera: Tenebrionidae) is a pest that, like many other stored product pests, can feed on various agricultural products in storage. Among the items it feeds on are barley, wheat, rice, sorghum, and corn flour (Estay et al., 2011; Naseri, 2017; Kavallieratos et al., 2020). Optimal conditions for its development are known to be 27±1°C and 65±5% relative humidity (Athanassiou, 2004; Stamopoulos et al., 2007; Kavallieratos et al., 2020). Similar to other storage pests, this species causes yield losses by feeding on the kernels of grains in both its larval and adult stages (Howe, 1956; Arbogast, 1976; Tremetra et al., 2000; Stamopoulos et al., 2007; Atta et al., 2020).

This study aims to determine the effectiveness of the *S. feltiae* TUR-S3 isolate on *O. surinamensis* and *T. confusum* under laboratory conditions. In this research, the TUR-S3 isolate was applied to these storage pests in five different doses to determine its efficacy.

MATERIALS AND METHODS

Entomopathogenic Nematode Species:

In this study, the entomopathogenic nematode species *S. feltiae* was utilized, specifically the TUR-S3 isolate. This isolate has been preferred due to its adaptation to the climatic conditions of our country, high virulence, ease of production, and a broad range of host compatibility (Unlu et al., 2007; Susurluk, 2008; Ulu et al., 2016; Bütüner et al., 2023).

***Oryzaephilus surinamensis* and *Tribolium confusum*:**

Adults of *T. confusum* used in this study were cultured in the Nematology laboratory of the Plant Protection Department at Bursa Uludağ University. The insects were reared at 27±1°C temperature with a 16-8 (light/dark) photoperiod (Athanassiou, 2004; Stamopoulos et al., 2007; Kavallieratos et al., 2020). Another pest species used in the study, adults of *O. surinamensis*, were cultured in the Nematology laboratory of the Plant Protection Department at Bursa Uludağ University. The culture was maintained at 28±2°C temperature with a 16-8 (light/dark) photoperiod (Beckett and Evans, 1994; Nika et al., 2020). Both insect cultures were maintained in separate environmental chambers, ensuring constant temperature and light conditions throughout the production.

Experimental Design:

Firstly, filter papers cut to fit the dimensions of 6 cm diameter Petri dishes were placed at the bottom of the Petri dishes. Ten individuals from the adult *T. confusum* culture were placed in each Petri dish, and similarly, 10 individuals of adult *O. surinamensis* were placed. Five wheat grains were positioned in the middle of each Petri dish. Infective Juveniles (IJs), concentrations for *T. confusum* adults were applied as follows: 3 IJs/cm^2 (8 IJs/adult), 6 IJs/cm^2 (17 IJs/adult), 9 IJs/cm^2 (25 IJs/adult), 18 IJs/cm^2 (50 IJs/adult), 27 IJs/cm^2 (75 IJs/adult). For *O. surinamensis* adults, the concentrations were applied as 3 IJs/cm^2 (8 IJs/adult), 6 IJs/cm^2 (17 IJs/adult), 9 IJs/cm^2 (25 IJs/adult), 18 IJs/cm^2 (50 IJs/adult), 27 IJs/cm^2 (75 IJs/adult). These prepared concentrations were individually applied to each Petri dish along with 200 microliters of Ringer's solution. In this study, different doses were prepared for each insect species and applied with three replicates. After the inoculation process, each Petri dish was incubated at 25°C for 4 days. Infected hosts were dissected to confirm that their deaths were IJ-induced. Additionally, only Ringer's solution was applied to Petri dishes with the control group.

Statistical analysis:

JMP®16 software was used of analyzing variance (ANOVA) on the mortality rate results observed in the experiment with the insects. Additionally, the Least Significant Difference (LSD) test ($p < 0.05$) was employed to determine the differences between the means.

RESULTS

The highest mortality rate of 83.33% was achieved with a concentration of 27 IJs/cm^2 of the TUR-S3 isolate applied to adult *T. confusum*. Other concentrations used in the study, namely 3 IJs/cm^2, 6 IJs/cm^2, 9 IJs/cm^2, and 18 IJs/cm^2, resulted in mortality rates of 16.67, 26.67, 46.67, and 63.33%, respectively. No dead adult individuals were found in the control group. Statistically significant differences were observed in the obtained results (Table 1) ($F = 36.92$; df= 5, 12; $p < 0.0001$).

Table 1. Percentage of mortality rates of *T. confusum*. Means in each isolate followed by the same letters are not significantly different ($p<0.05$).

EPN Species	Host	Applied Dose (IJs/cm^2)	Mortality Rates (%) ± SE	F (df); P
S. feltiae TUR-S3	*T. confusum*	Control	0 ± 0 e	F (5, 12)= 36.92; p < 0.0001
		3	16.67 ± 3.33 d	
		6	26.67 ± 8.82 c	
		9	46.67 ± 6.67 c	
		18	63.33 ± 3.33 b	
		27	83.33 ± 3.33 a	

The highest mortality rate of 83.33% in *O. surinamensis* adults infected with the TUR-S3 isolate was obtained at a concentration of 27 IJs/cm^2. Other concentrations used in the study, namely 3 IJs/cm^2, 6 IJs/cm^2, 9 IJs/cm^2, and 18 IJs/cm^2, resulted in mortality rates of 20, 26.67, 36.67, and 73.33%, respectively. No dead adult individuals were found in the control group. Statistically significant differences were observed in the mortality rates of *O. surinamensis* adults infected with the TUR-S3 isolate (Table 2) ($F = 55.92$; df= 5, 12; $p < 0.0001$)

Table 2. Percentage of mortality rates of *O. surinamensis*. Means in each isolate followed by the same letters are not significantly different (p<0.05).

EPN Species	Host	Applied Dose (IJs/cm^2)	Mortality Rates (%) ± SE	F (df); P
S. feltiae TUR-S3	*O. surinamensis*	Control	0 ± 0 d	F (5, 12)= 55.92; p < 0.0001
		3	20.00 ± 5.77 c	
		6	26.67 ± 6.67 bc	
		9	36.67 ± 3.33 b	
		18	73.33 ± 3.33 a	
		27	83.33 ± 3.33 a	

DISCUSSION

The pathogenicity of EPNs, particularly the TUR-S3 isolate of *S. feltiae*, has been investigated for its efficacy in controlling adult *T. confusum* and *O. surinamensis*. The study revealed that the TUR-S3 isolate exhibited varying mortality rates at different concentrations. For adult *T. confusum*, the highest mortality rate of 83.33% was achieved at a concentration of 27 IJs/cm2, with lower concentrations resulting in lower mortality rates. Similarly, for *O. surinamensis* adults infected with the same isolate, the highest mortality rate of 83.33% was obtained at a concentration of 27 IJs/cm2, with other concentrations yielding different mortality rates. Notably, statistically significant differences were observed in the obtained results for both *T. confusum* and *O. surinamensis* adults infected with the isolate.

Steinernema feltiae has been evaluated against various stored product pests, including *T. confusum* and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), demonstrating its insecticidal effect (Athanassiou et al., 2008; Athanassiou et al., 2010; Guru et al., 2022). Javed et al. (2020) investigated the efficacy of isolates from different species of the Steinernematidae family at three different doses on adult *T. confusum* and *Rhyzopertha dominica* (Coleoptera: Bostrychidae). The results indicated an increase in the mortality rate of adults with the application of higher concentrations of IJs. Similarly, Canhilal and Yüksel (2020) conducted a study examining the effectiveness of different isolates of *S. feltiae* species on *Sitophilus oryzae* (Coleoptera: Curculionidae) using various concentrations. The results from this study also revealed an increase in the mortality rate of adults with the application of higher concentrations of IJs. These findings are consistent with the results of the current study.

Similarly, Bütüner and Susurluk (2023b) conducted a study in which isolates belonging to different species of *S. feltiae* and *Steinernema carpocapsae* (Rhabditida: Steinernematidae) were applied at various doses on *S. oryzae*. The study revealed a proportional increase in the mortality rate of adults with an increase in the applied concentration of IJs. Laznik et al. (2010) utilized different isolates of *S. feltiae* to determine their effectiveness on adult *S. oryzae* at different temperature values. The study concluded that an increase in IJ concentration resulted in a higher mortality rate among adults. Similarly, Trdan et al. (2006) conducted a study to determine the effectiveness of different isolates of *S. feltiae*, *S. carpocapsae*, *Heterorhabditis bacteriophora*, and *H. megidis* (Rhabditida: Heterorhabditidae) on *Sitophilus granarius* (Coleoptera: Curculionidae) and *O. surinamensis*. The results indicated that an increase in the concentration of IJs in the applied dosage led to a proportional increase in the mortality rate of pests. Overall, the findings suggest a direct relationship between the increase in the applied dosage of IJs per adult and the observed increase in pest mortality rates.

CONCLUSION

In recent years, alternative methods to pesticide usage have gained popularity due to a better understanding of the toxic effects of pesticides on non-target organisms, particularly the residue problems they create on food products. Pesticides used in storage conditions, which represent the final stage of the food chain accessible to humans, have the potential to induce toxic effects on humans through residues left on products. In this regard, the utilization of EPNs as an alternative to pesticide usage holds promising results for pest control under storage conditions. This study demonstrates highly effective results in the potential control of *T. confusum* and *O. surinamensis* using low IJ concentrations of an isolate (TUR-S3) of *S. feltiae* species, under laboratory conditions. The concentration of EPNs can significantly influence their effectiveness in controlling pest populations, highlighting the need for precise application strategies in integrated pest management programs. However, there are some limitations to the application of EPNs in storage conditions. With regulations and adjustments in this field, the use of EPNs in storage conditions is believed to be highly effective in pest control.

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