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***Orijinal araştırma* (*Original article*)**

Laboratory evaluation of some native isolates of *Steinernema feltiae* (Filipjev) against the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)

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Pirinç Biti, *Sitophilus oryzae* (L.), (Curculionidae: Coleoptera)'ye karşı bazı yerel *Steinernema feltiae* (Filipjev) izolatlarının laboratuar koşullarında değerlendirmesi

Öz: Üç yerel entomopatojen nematod (EPN) izolatının (*Steinernema feltiae* UKK-1, *S. feltiae* DTK-6 ve *S. feltiae* UIP-19) *Sitophilus oryzae* (L.), (Curculionidae: Coleoptera) erginleri üzerindeki etkinliği farklı konsantrasyonlarda (50, 100, 250, 500, 1000 ve 2000 IJs /ergin) laboratuvar koşullarında (25ºC ve %65 RH) incelenmiştir. *Sitophilus oryzae* erginlerinin ölüm oranları uygulama sonrası 4. ve 8. günlerde kaydedilmiştir. Ölüm oranları artan uygulama konsantrasyonlarıyla birlikte artmıştır ve ölüm oranları %7-92 arasında değişmiştir. Uygulama sonrası 4. ve 8. gündeki en yüksek ölüm oranları sırasıyla %80 ve %92 olarak gerçekleşmiştir. Laboratuvar çalışmaları test edilen EPN izolatlarının *S. oryzae*'nin mücadelesinde iyi bir potansiyele sahip olduğunu göstermektedir.

Anahtar kelimeler: Depolanmış ürün zararlısı, entomopatojen nematod, *Sitophilus oryzae, Steinernema feltiae.*

Abstract: The efficacy of three native Turkish entomopathogenic nematode (EPN) isolates, *Steinernema feltiae* (Filipjev) UKK-1, *S. feltiae* DTK-6 and *S. feltiae* UIP-19), was investigated against the adults of *Sitophilus oryzae* (L.), (Curculionidae: Coleoptera) at different concentrations (50, 100, 250, 500, 1000 and 2000 IJs/adult) in a laboratory bioassay at 25ºC and 65% RH. The mortality rates of *S. oryzae* were evaluated after 4 and 8 days of exposure. Mortality rates increased with increasing IJ concentrations and ranged between 7% and 92%. The highest mortality rates at 4th days and 8th days after treatment were 80% and 92%, respectively. These results indicate that the tested EPN isolates have good potential for the control of *S. oryzae*.

Key words: Stored product pest, entomopathogenic nematode, *Sitophilus oryzae*, *Steinernema feltiae*

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Introduction

The rice weevil, *Sitophilus oryzae* (L.), (Coleoptera: Curculionidae), which is one of the most damaging species of stored grain products under favorable conditions (Shaaya et al., 1997). Chemical control has mainly been used in the control of this pest but control has been accompanied by the development of resistance, the presence of toxic residues in foods, and adverse effects on the environment (Fragoso et al, 2003; Pimentel et al, 2007). Recently, food safety issues have gained worldwide prominence and the need has arisen for the use of more environmentally sound methods for both the control of stored product pests and to protect food consumers from the adverse effects of pesticides (Yüksel et al, 2017)

Entomopathogenic nematodes (EPNs) are a group of successful employed biological control agents that have the ability to actively search for hosts in cryptic habitats and rapidly kill them with the help of symbiotic bacteria that they carry in their intestine (Kaya & Gaugler, 1993). In previous studies, the pathogenicity of EPNs has been reported to vary according to the species and isolate used (Trdan et al, 2009; Shahina & Salma, 2010; Yuksel et al, 2019). In this study, the potential of three Turkish EPN isolates obtained from the Nevsehir Province to control the adults of *S. oryzae* was investigated under laboratory conditions.

Materials and Methods

Adult *S. oryzae* were obtained from the Plant Protection Department of the Agriculture Faculty, Ankara University, Turkey. They were reared on rice grains and in a 1000 mL glass jar under controlled conditions (27±2 ^{o}C, 60 ± 5% RH) in the Entomology Laboratory at Erciyes University, Kayseri. Three native EPN isolates of *Steinernema feltiae* (Filipjev) obtained from the Cappadocia region of Turkey (*S. feltiae* UKK-1, *S. feltiae* DTK-6, and *S. feltiae* UIP-19) (Yuksel & Canhilal, 2019), were used in the experiments. All three EPN isolates were reared on the last instar of *Galleria mellonella* L. (Lepidoptera: Pyralidae) at 27 ± 2 ^{o}C and R.H. 65% under laboratory conditions. The harvested infective juveniles (IJs) were stored in tap water at 9 ^{o}C in a refrigerator before their use in the experiments. Newly emerged adults of *S. oryzae* and IJs less than two-weeksold were used in the experiments (Kaya & Stock, 1997; Ehlers, 2001; Trdan et al, 2006; Trdan et al, 2009).

The experiments were carried out in Petri dishes, each lined with two filter papers of 9 cm diameter, at 25 ^{o}C and 65% RH. Each Petri dish contained ten adults and twenty grains of rice inoculated with 1 mL of tap water containing one of six concentrations of IJs (0, 500, 1000, 2500, 5000, 10000 and 20000 IJs). After inoculation, the Petri dishes were sealed with parafilm to prevent the escape of the adult insects. Each treatment was replicated four times and only distilled water was used for the control treatments. Mortality rates were recorded 4th and 8th days after treatment (DAT).

A factorial experimental design, consisting of three EPN isolates, six concentration rates and two times (3 x 6 x 2), was used for the purpose of statistical analyses. Mean mortality values were compared by using the Tukey Multiple Range Test ($P \leq 0.05$).

Result and Discussion

All three tested EPN isolates killed the adults of *S. oryzae*. However, there were statistically significant differences for the mortality rates between the isolates and concentrations. The interaction between the isolates and concentrations was only significant at the 8th DAT (Table 1). In most cases, the mortality rate increased as the concentration increased. The lowest and highest mortality rates (7% and 92%) were obtained with the same isolate (UIP-19 at the lowest and highest concentrations of 50 IJs and 2000 IJs, respectively. The DTK-6 isolate had the highest efficacy at all concentrations at 4th DAT and at the lowest concentration (50 IJs) for both exposure times (Table 2). The UIP-19 isolate caused the lowest mortality (70%) at 4th DAT with 2000 IJs but it had the highest efficacy at the same concentration at 8 days, causing 92% mortality.

Table 1. A comparison of the mortality rates caused by three Turkish entomopathogenic nematode isolates tested against the adults of *Sitophilus oryzae*

R*	4 DAT			8 DAT		
	df	F	P	df	F	P
E	2	27.011	<0.0001	2	6.523	0.003
C	5	34.433	<0.0001	5	31.776	<0.0001
E x C	10	1.671	0.112	10	3.266	0.002

* R: Resources, DAT: Days after treatment, E: Entomopathogenic nematode isolates, C: Concentrations.

The results of this study showed mortality rates of the adults of *S. oryzae* that varied according to the EPN isolate and its concentration. Similar results have been obtained in earlier studies (Athanassiou et al, 2008; Athanassiou et al, 2010; Laznik et al, 2010). The efficacy of *S. feltiae* has been evaluated against *S. oryzae* under laboratory conditions and mortality rates below 30% were reported at low concentrations (Shahina & Salma 2010; Laznik et al. 2010). Negrisoli et al. (2013) studied the activity of different EPN species against the adults of *S. oryzae* at a concentration of 10 IJs per microtube and reported that mortality rates ranged from 2% to 26% at 5 days post-treatment. In the present study, the highest mortality rate (92%) was obtained at the highest concentration (2000 IJs) at 8 days post-treatment. Laznik et al. (2010) reported 72% mortality for the same concentrations and exposure time with the same EPNs. It therefore appears that the EPN isolates tested in the current study are more pathogenic to the adults of *S. oryzae* than those used in these two studies. Although a slight decrease in the mortality rate of *S. oryzae* adults with increasing concentrations of IJs has been observed in some studies increasing concentrations and exposure time generally leads to more mortality rates (Laznik et al., 2010; Shahina & Salma, 2010).

Table 2. The mortality rates (%) of the adults of *Sitophilus oryzae* at four and eight days after treatment (DAT) with 50, 100 and 250 IJs of three Turkish entomopathogenic nematode isolates

EPN isolates*	DAT*	Concentrations					
		50 IJs	100 IJs	250 IJs	500 IJs	1000 IJs	2000 IJs
	4						
UKK-1		22±5Aa	30±8Aa	35±12Aa	40±8Aa	72±15Bb	77±5Ba
DTK-6		45±12Ab	55±5ABb	57±12ABb	67±9BCb	77±5Cb	80±8Ca
UIP-19		7±5Aa	22±9ABa	37±9ABa	50±8Ba	55±12Ba	70±24Ca
	8						
UKK-1		30±8Aa	42±5ABa	57±22ABa	70±16Ba	82±9Cb	85±5Ca
DTK-6		52±9Ab	57±9Aa	62±22ABa	72±9Ba	77±5BCb	82±5Ca
UIP-19		20±8Aa	32±9aAB	55±12Ba	55±5Ba	62±17Ba	92±9Ca

* EPN isolates: Entomopathogenic nematode isolates, DAT: Days after treatment, S.f.: *Steinernema feltiae.* Mean values followed by different uppercase letters in the same line and mean values followed by different lowercase letters in the same column are significantly different for each exposure time (Tukey's test; $P < 0.05$).

The data generated in the current study demonstrated that the EPN isolates tested had high efficacy against *S. oryzae* adults under laboratory conditions but they need to be tested in storage environments. Entomopathogenic nematode isolates may be more effective in combination with compatible chemicals in the empty storage environment due to their ability to penetrate into the crevices and cracks where the storage product pests are sheltering.

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