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Mortality of the Red Flour Beetle (*Tribolium castaneum*) when Exposed to Croatian Strain of Entomopathogenic Nematodes *Steinernema feltiae* †

Ivana Majić[1*], Ankica Sarajlić[1], Damjan Veselovac[2], Doris Dorić[2], Alenka Tambolaš[2], Marina Mađar[2], Helena Ereš[2], Gabriella Kanižai Šarić[1], Anita Liška[1]

[1] University of Osijek, Faculty of Agrobiotechnical Osijek, Vladimira Preloga 1, Osijek HR31000, Croatia imajic@fazos.hr

[2] Graduate students at University of Osijek, Faculty of Agrobiotechnical Osijek, Vladimira Preloga 1, Osijek HR31000, Croatia

* Correspondence: imajic@fazos.hr

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Abstract: Complex strategies are employed to control pests of stored agricultural products. *Tribolium castaneum* (the red flour beetle) is a common pest found worldwide in stored grains. The pest has developed resistance to chemical pesticides, and biological alternatives are the only tool used as a direct control measure. Entomopathogenic nematodes are used in different agricultural systems as biological control agents whose efficiency in plant protection is often comparable to chemical pesticides. An increasing need for the development of new and enhancement of existing biological control agents led us to study the effect of the insecticidal efficacy of two species of entomopathogenic nematodes (Croatian strain: *Steinernema feltiae*) against *T. castaneum* larvae and adults. The experiment was set up under laboratory condition. The pathogenicity of IJs of entomopathogenic nematodes was tested in different nematode concentrations (0, 300 and 700 IJs insect^{-1}) under two temperature regimes (15 and 25 °C). The red flour beetle mortality was achieved up to 82% depending on the insect developmental stage, temperature and nematode concentration. *S. feltiae* were pathogenic to the red flour beetle at temperatures of 15 °C which is optimal for storing grains. The red flour beetle was susceptible to tested entomopathogenic nematodes and offspring are recovered from the insect cadavers.

Keywords: entomopathogenic nematode; the red flour beetle; stored insect pest; biological control; nematode concentration; temperature

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1. Introduction

Entomopathogenic nematodes (EPNs) are biological control agents against many insect pest species [1]. They are characterized by active search for their host and as an environmentally acceptable solution in insect pest control. Their efficacy if often comparable to the efficacy achieved with chemical pesticides [2]. The pathogenicity of EPNs is caused after nematodes penetrate inside the insect host by infective or third stage nematode juvenile (IJs). IJs transmit their symbiotic bacteria into the insect hemocoel where they produce highly virulent insecticidal toxins. The toxins kill the insect host within 24 to 72 h. Bacteria of the genus *Xenorhabdus* Boemare, Akhurts and Mourant are associated with steinernematids [3].

Insect pests of stored agricultural products, among which is *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), are economically important worldwide [4, 5]. To control stored-product insect pests, several aspects should be taken into consideration.

These insects are often found in unreachable spots, because of their ability of survive on food remains in cracks and crevices, under perforated floors, and inside machinery [6]. Most of the active ingredients of chemical insecticides have been banned or restricted globally for use because of the persistence of toxic residues in food grains and the developments of insect resistance. Therefore, an increasing trend in commercialization and application of EPNs as an efficient control agent has been observed globally [7]. EPNs from different regions are locally adapted and potentially different in terms of reproduction, infectivity, host range, and conditions for survival [8].

The aim of this study was to test the virulence of Croatian strain of entomopathogenic nematode *Steinernema feltiae* against the red flour beetle (*T. castaneum*).

2. Material and Methods

The bioassay was conducted on 4 to 6 weeks old the red flour beetle (*T. castaneum)* adults of mixed sex and larvae. The red flour beetle were reared on the medium of wheat flour and dried yeast at the ratio of 10:1, in a rearing chamber in dark at 30 °C and 70-80 % RH [9].

The sterile Petri dishes with a double layer of wetted filter papers were randomly inoculated with 0, 300, and 700 IJs per insect in sterile distilled water. To test the virulence of *S. feltiae* (Croatian strain ISO16, GenBank accession numbers MG952287), ten last instars of the red flour beetle larvae or ten adults were placed in the dishes one hour later. In control Petri dishes, insect larvae received sterile distilled water without nematodes. All treatments were replicated five times, and whole experiment was repeated once. The dishes were incubated at 15 and 25 °C (65 % RH) in the dark in a rearing chamber (BTES-e – frigomat, Termo Medicinski Aparati Bodalec). Insect mortality from each Petri dish was recorded on two days interval during seven days post treatment period. Insect cadavers were placed individually on modified White traps, kept in dark at room temperature, and monitored in 30 days period to record the IJs progeny. Insect mortality was corrected according to the Abbott's formula. A GLM analysis was performed to test effects of EPNs concentration and temperature on insect mortality. Comparisons between the means of the treatments were done using a one-way ANOVA (Tukey's studentized range test (HSD) ($P < 0,05$). Statistical analyses were done using the SAS version 9.3 software (SAS Institute, Cary, North Carolina, USA).

3. Results

The pathogenicity of *S. feltiae* was tested against the red flour beetle under laboratory conditions. All tested treatments (nematode concentration, temperature regime and insect stage) significantly influenced mortality of the insects (Table 1). Interaction of treatments did not significantly affect the mortality of the insects.

Table 1. GLM analysis of the effect of concentration of *Steinernema feltiae* and temperature on the mortality of *Tribolium castaneum*

	DF	Type I SS	Mean Square	F Value
Nematode concentration	2	260,88	3,21	0,0234
Insect stage	1	91685,00	1011,90	<,0001
Temperature	1	833,33	10,32	0,0018
Nematode concentration*Insect stage	2	96,02	1,05	0,3224
Nematode concentration*Temperature	2	168,71	1,99	0,0902
Insect stage*Temperature	1	215,33	3,16	0,1171

Mortality of the red flour beetle larvae is presented in Figure 1. Statistically significant differences between two temperature regimes were found only at lower nematode

concentrations (300 IJs insect $^{-1}$). At lower temperature, significantly higher mortality of insect larvae occurred (67 % compared to 59 % mortality). At higher nematode concentrations (700 IJs insect $^{-1}$) no significant differences were observed for the insect mortality between the temperature regimes. However, *S. feltiae* was highly virulent at both temperatures, causing 82 % insect larval mortality at 15 °C and 79 % insect larval mortality at 25 °C.

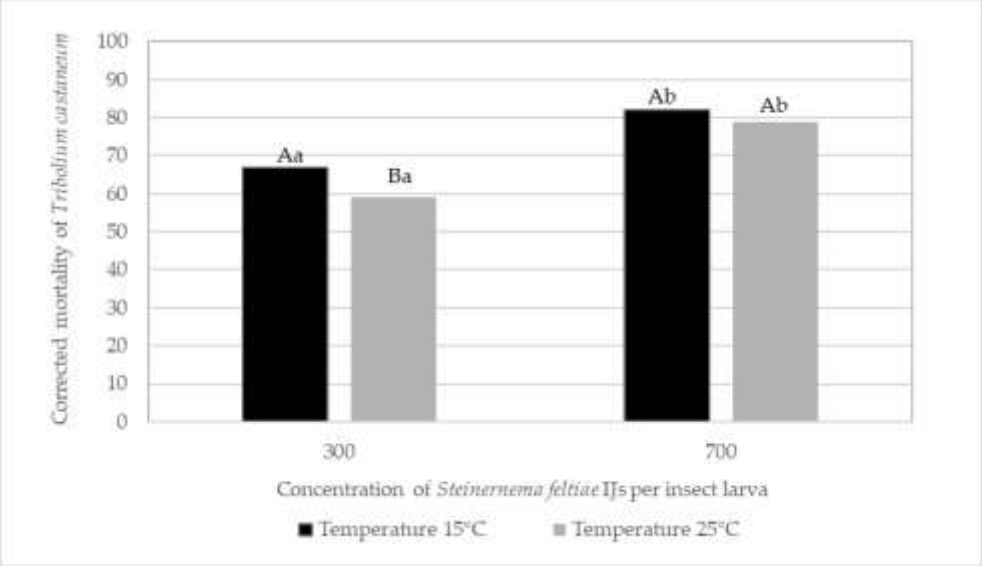

Figure 1. Corrected mortality of *Tribolium castaneum* larvae at different concentrations of *Steinernema feltiae* (Croatian strain, ISO16) and two temperature regimes. Different uppercase letters above bars indicate statistical significance between temperature regimes, and lowercase letters indicate significance between nematode concentrations (Tukey, $P < 0.05$)

Similar effects of temperature and nematode concentration were observed in a bioassay with the red flour beetle adults. Mortality of the red flour beetle adults is presented in Figure 2.

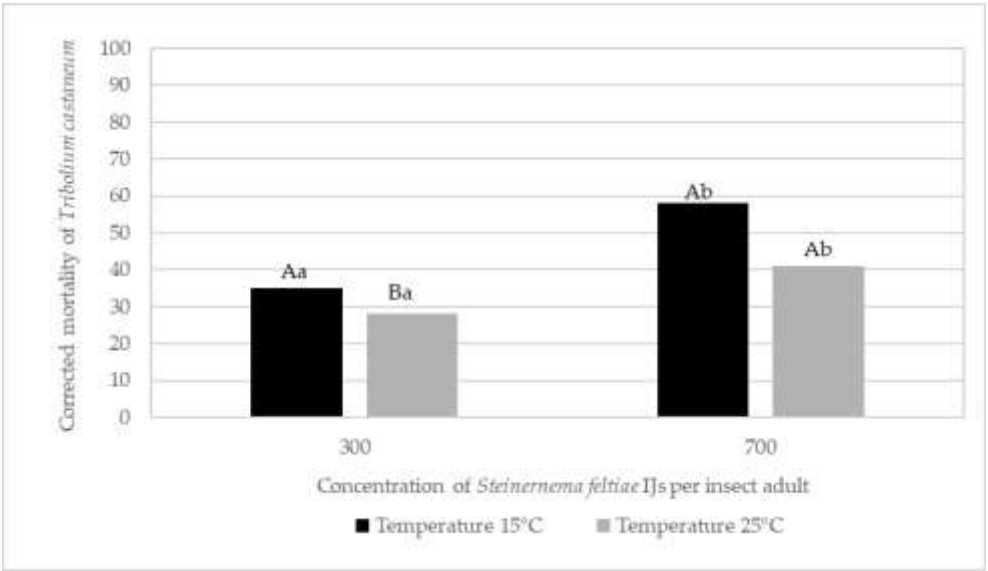

Figure 2. Corrected mortality of *Tribolium castaneum* adults at different concentrations of *Steinernema feltiae* (Croatian strain, ISO16) and two temperature regimes. Different uppercase letters above bars indicate statistical significance between temperature regimes, and lowercase letters indicate significance between nematode concentrations (Tukey, $P < 0.05$)

Statistically significant differences between the mortality of insect adults at two temperature regimes were found only at lower nematode concentrations (300 IJs insect $^{-1}$). At lower temperature, significantly higher mortality of insect adults occurred (35 % compared to 28 % mortality). At higher nematode concentrations (700 IJs insect $^{-1}$) no significant differences were observed for the insect mortality between the temperature regimes. The virulence of *S. feltiae* against the red flour beetle adults was moderate since the nematode in the highest concentration caused 58 % insect mortality at 15 °C. In the same treatment, IJs caused only 41 % insect mortality at 25 °C. The IJs progeny was moderately abundant and found from 96 % of cadavers, and this indicate that both the red flour beetle stages are good hosts for *S. feltiae*.

4. Discussion

Our study demonstrates that virulence of *S. feltiae* depends on the environmental temperature, nematode concentration and insect stage. All insect stages were susceptible to IJs of Croatian strain *S. feltiae*. Satisfactory control was achieved on insect larvae at lower temperatures, where nematodes were virulent for 82 % of insects. The insect adults were more tolerant. The highest virulence of nematodes was found in all treatments at 15 °C, indicating that Croatian strain of *S. feltiae* is more efficient in cooler conditions, as it was previously reported [10]. However, 100 % mortality was not achieved in any of the treatments. Previous studies proved that EPNs are effective against other coleopteran pest of stored products [11-13]. Among three species of EPNs, *Steinernema riobrave* Cabanillas, Poinar, and Raulston was found as the most efficient biocontrol agent against a wide range of stored pests [6]. In the same study, authors used only 10 IJs of *S. feltiae* per insect and achieved up to 90 % *T. castaneum* larvae mortality at 25 °C. The highest mortality (near 40 %) of *T. castaneum* adults in the cited study was found in treatments with 100 *S. feltiae* per insect. Similarly, we found 42 % insect adults' mortality, but we used seven times higher nematode concentrations.

Entomopathogenic nematodes are a great alternative to chemical pesticides and have already been proposed for pest control in cryptic environments outside the soil. Croatian strain S. *feltiae* proved its potential to control *T. castaneum* larvae, and moderately suppress population of the insect adults in a cooler condition that is also optimal for stored commodities. Further studies are needed to enhance control strategy using EPNs, since nematodes are prone to dehydration and dependent on the film of water.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Corrected mortality of *Tribolium castaneum* larvae at different concentrations of *Steinernema feltiae* (Croatian strain, ISO16). Different uppercase letters above bars show statistical significance between temperature regimes, and lowercase letters show significance between nematode concentrations (Tukey, $P<0.05$); Figure S2: Corrected mortality of *Tribolium castaneum* adults at different concentrations of *Steinernema feltiae* (Croatian strain, ISO16). Different uppercase letters above bars show statistical significance between temperature regimes, and lowercase letters show significance between nematode concentrations (Tukey, $P<0.05$); Table S1: GLM analysis of the effect of concentration of *Steinernema feltiae* and temperature on the mortality of *Tribolium castaneum.*

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Conflicts of Interest: The authors declare no conflict of interest.

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