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Concentration of suspension and temperature as factors of pathogenicity of entomopathogenic nematodes for the control of granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae)

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ABSTRACT

Efficacy of four species of entomopathogenic nematodes (*Steinernema feltiae*, *S. carpocapsae*, *Heterorhabditis bacteriophora*, *H. megidis*) for the control of granary weevil adults (*Sitophilus granarius*) was tested under laboratory condition. Suspensions of nematodes were applied in three concentrations (5.000, 10.000, and 20.000 IJs per ml) at three temperatures (15°C, 20°C and 25°C). After one week mortality of the beetles was assessed. No significant differences in the percentage of mortality were determined between treatments with *S. feltiae*, *S. carpocapsae* and *H. bacteriophora*, while *H. megidis* was the least efficient. Mortality of the beetles was statistically significantly higher at 20 and 25 °C. Influence of the concentration of the nematode suspension was proved to be less important in biological activity of biological agents in question.

Key words: entomopathogenic nematodes, granary weevil, *Sitophilus granarius*, efficacy, biological control.

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IZVLEČEK

KONCENTRACIJA SUSPENZIJE IN TEMPERATURA KOT DEJAVNIKA PATOGENOSTI ENTOMOPATOGENIH OGORČIC PRI ZATIRANJU ČRNEGA ŽITNEGA ŽUŽKA, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae)

V laboratorijskih razmerah smo preučevali učinkovitost štirih vrst entomopatogenih ogorčic (*Steinernema feltiae*, *S. carpocapsae*, *Heterorhabditis bacteriophora*, *H. megidis*) za zatiranje odraslih osebkov črnega žitnega žužka (*Sitophilus granarius*). Tri različne koncentracije suspenzije (5.000, 10.000 in 20.000 infektivnih ličink/ml) smo aplicirali pri treh različnih temperaturah (15°C, 20°C in 25°C). Po enem tednu smo ugotavljali smrtnost hroščkov. Med vrstami *S. feltiae*, *S. carpocapsae* in *H. bacteriophora* nismo ugotovili značilnih razlik v vplivu na smrtnost hroščkov, vrsta *H. megidis* pa je v tej zvezi pokazala najmanjšo učinkovitost. Smrtnost hroščkov je bila statistično značilno največja pri temperaturah 20 in 25 °C. Ugotavljamo, da je koncentracija suspenzije, v primerjavi s temperaturo okolja, manj pomemben dejavnik biotičnega delovanja entomopatogenih ogorčic na hroščke črnega žitnega žužka.

Ključne besede: entomopatogene ogorčice, črni žitni žužek, *Sitophilus granarius*, učinkovitost, biotično varstvo.

1 INTRODUCTION

Continuous increasing of the human population requires higher storage facilities. Some of the "health problems" may occur in these storages too. The granary weevil, *Sitophilus granarius* (L.), is a primary pest and belongs among important pests of stored grain (Ungsunantwiwat and Mills, 1985; Kucerova *et al.*, 2003). It is spread mostly in moderate climate and is fed with different kinds of cereals or their products. Wheat and barley are among the most frequent sources of food reported (Schwartz and Burkholder, 1991).

Recently more attention has been paid to preventive measures against the storage pests (Fleurat-Lessard, 2003) and in accordance with the general goal, part of which is also a reduced use of chemicals in the food production and processing, curative measures for control of storage pests also include more environmentally friendly substances (Aslan *et al.*, 2004) and organisms (Pratissoli *et al.*, 2004). Thus, entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hluchy and Samsinakova, 1989) and some parasitoids (Oliveira *et al.*, 2003) have been tested for control of *S. granarius* and some other storage pests. At the present, only a little data are available in the literature on efficacy of entomopathogenic nematodes for control of storage pests.

In the beginning, entomopathogenic nematodes were mostly known as natural enemies of the soil pests (Ishibashi and Choi, 1991; Glazer *et al.*, 1992), but now, they are more often included in studies on control of foliar pests (Head *et al.*, 2004).

In the research the efficacy of entomopathogenic nematodes for control of the adults of granary weevil was tested. Entomopathogenic nematodes are more efficient against the larvae and other preimaginal stages of insects, because they can enter their body easier (LeBeck *et al.*, 1993). In some recent studies it has also been proven that nematodes can kill adult insects too (Renn, 1998; Svendsen and Steenberg, 2000).

2 MATERIALS AND METHODS

A laboratory investigation was carried out in the Entomological Laboratory of the Chair of Entomology and Phytopathology (University of Ljubljana, Biotechnical Faculty, Dept. of Agronomy) in Ljubljana, Slovenia. Four species of entomopathogenic nematodes were tested: *Steinernema feltiae* (Filipjev), *S. carpocapsae* (Weiser) (both Rhabditida: Steinernematidae), *Heterorhabditis bacteriophora* Poinar and *H. megidis* Poinar (both Rhabditida: Heterorhabditidae) (Koppert B. V., Berkel en Rodenrijs, The Netherlands). The stock culture of granary weevil, *S. granaries*, was reared at the Chair of Entomology and Phytopathology. Population has been maintained in the wheat grain, at room temperature ($20\pm 2^{\circ}\text{C}$), in the dark, since 1998.

In the experiment adults of the granary weevil were used. They were the offspring of parent couples, which have been reared separately from the stock culture, under the same conditions. In each from 20 rearing vessels a female and a male were present and their food was the same as that of the stock culture.

After the first adults had appeared (35–45 days after males and females were put together), application of the suspensions of entomopathogenic nematodes was performed, following a preliminary tested proceeding (Ben-Yakir *et al.*, 1998). Adults of the same age (± 1 day) were used. For this purpose, one day prior to application of nematodes, all adult beetles were removed from the rearing vessels. Only beetles which emerged in the last 24 hours were used. In 5 cm-diam Petri dishes with blotting paper 10 adults and 20 grains of wheat were placed.

Efficacy of entomopathogenic nematodes was tested in three concentrations: 5.000, 10.000 and 20.000 IJs/ml. The suspensions of nematodes were prepared in glass jars and in each Petri dish 1 ml of suspension was added. Petri dishes were then closed and sealed with parafilm, to prevent beetles from escaping. Suspensions were added with a pipette and tipses were changed after every treatment. The fifth treatment was control and instead of nematode suspension distilled water was added in the Petri dishes.

Petri dishes were put into the rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia), with working capacity $0,868\text{ m}^3$ (width : height : depth = 1000x1400x620 mm), every treatment in 10 repetitions. Efficacy was tested in dark at three temperatures (15°C , 20°C and 25°C) and relative humidity 95%. After 7 days the number of the dead beetles was established. The dead beetles were examined on the presence of the nematodes under the microscope.

The multifactor analysis of variance (ANOVA) was conducted to establish the differences in percentage of adult mortality of *Sitophilus granarius*, reared in five treatments (four species of entomopathogenic nematodes – each with three concentrations - and control) at three temperatures. Duncan's multiple range test ($P \leq 0.05$) was used to separate mean differences among parameters in all treatments. All statistical analyses were performed with Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc., Maryland, USA) and figures were created with MS Office Excel 2003 (MS Corporation). Data are presented as untransformed means \pm SE.

3 RESULTS

By the group analysis was established that percentage of mortality of the granary weevil adults was statistically significantly influenced by the concentration of nematode suspension, temperature, treatment and interaction between temperature and treatment.

A suspension of 20.000 IJs/ml was the most effective in control of *S. granarius* (62.83 ± 4.21). No statistically significant differences were found between

concentration 5.000 IJs/ml and 10.000 IJs/ml (49.83 ± 4.36 in 52.00 ± 4.43), though both were more efficient compared to the control treatment (2.67 ± 1.53). No statistically significant differences in mortality of the beetles were found between temperatures 20°C and 25°C as well (71.5 ± 3.59 and 72.17 ± 4.06). Significantly the lowest values were established at 15°C (21.00 ± 2.49). The highest percentage of mortality was determined with entomopathogenic nematodes *S. feltiae* (64.22 ± 4.23), *H. bacteriophora* (62.67 ± 4.50) and *S. carpocapsae* (57.56 ± 5.70). Statistically significantly lower mortality in this pest was determined with *H. megidis* (35.11 ± 4.56). Mortality percentage was the lowest in the control treatment (2.67 ± 1.53).

By the individual statistical analysis of all treatments with entomopathogenic nematodes, a statistically significant influence of temperature on mortality of *S. granarius* adults was found. In the control treatment no statistically significant influence of temperature in this regard was stated (Fig. 1).

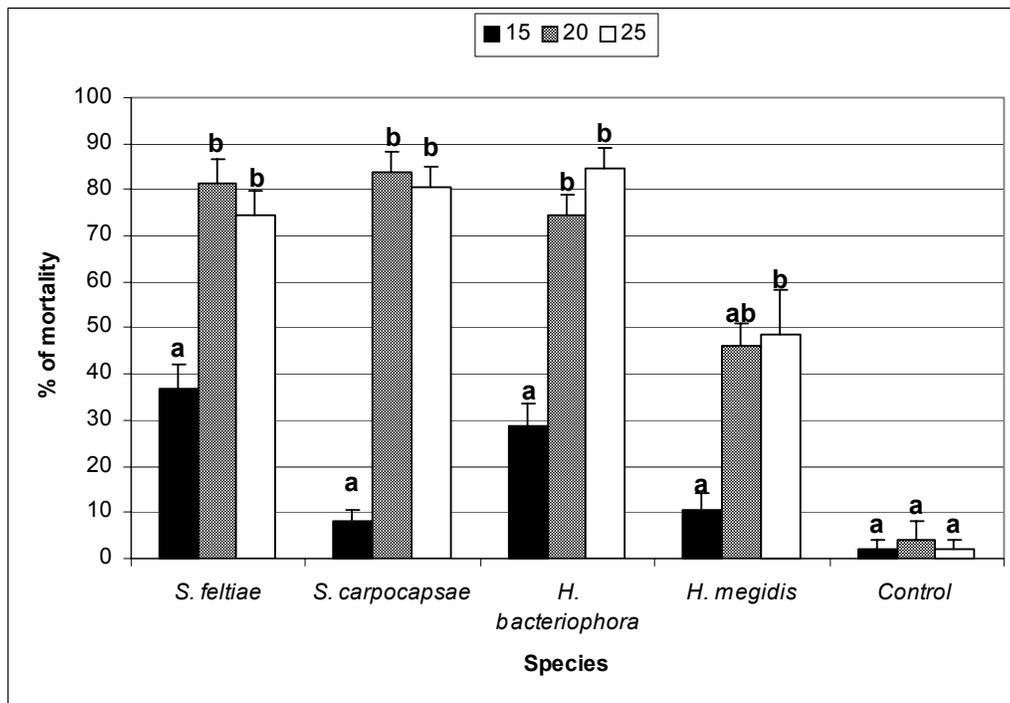


Fig. 1. Mean adult mortality of *Sitophilus granarius* treated with four different species of entomopathogenic nematodes depending on rearing temperature. In control treatment, no entomopathogenic nematodes were used. Data were analyzed by multifactor ANOVA followed by Duncan's multiple range test ($P \leq 0.05$) for separation of means. The same letters are not significantly different, and vertical lines represent standard errors of the means.

A statistically significant influence of concentration of suspension on mortality of the beetles was found with *S. feltiae* and *H. megidis*. With the other two species, *S. carpocapsae* and *H. bacteriophora*, no such influence was determined (Fig. 2). Only with *S. feltiae*, a statistically significant influence of the interaction between the temperature and concentration of suspension on mortality of the beetles was stated.

With *S. carpocapsae*, *H. bacteriophora* and *H. megidis*, the interaction between temperature and concentration was not confirmed.

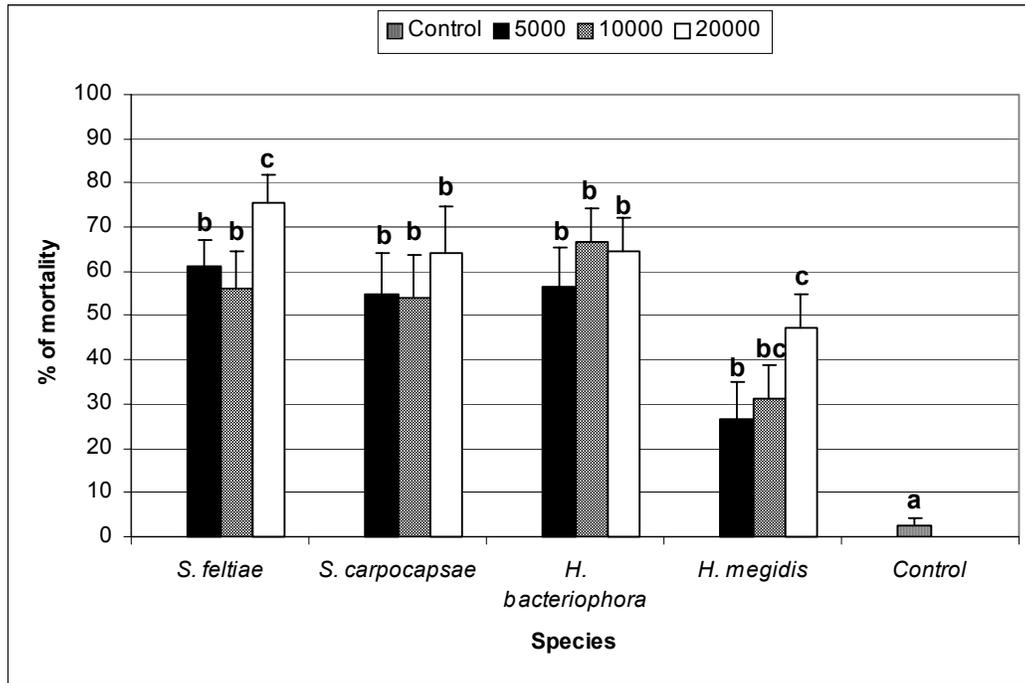


Fig. 2. Mean adult mortality of *Sitophilus granarius* treated with different concentrations (IJs per ml) of four species of entomopathogenic nematodes. All data of entomopathogenic nematodes are compared with control treatment. Data were analyzed by multifactor ANOVA followed by Duncan's multiple range test ($P \leq 0.05$) for separation of means. The same letters are not significantly different, and vertical lines represent standard errors of the means.

4 DISCUSSION

The results on efficacy of entomopathogenic nematodes for control of stored grain pest, *Sitophilus granarius*, presented in this paper, are a novelty in the field of biological control. In the scientific literature no data on efficacy of these biological agents on the tested species or any other pests of stored grain were found. A reason for this is probably a limited applicability of the results of such researches, though they present a basis for the development of strategy of storage pest control with entomopathogenic nematodes.

Application of three species of these biological agents (*S. feltiae*, *S. carpocapsae*, and *H. bacteriophora*) at two temperatures (20°C and 25°C) resulted in over 74 % mortality of the beetles. These results partly coincide with results of some related researches. An optimal biological activity of *S. carpocapsae* was determined in the temperature range from 22 to 24°C (Choo HoYul *et al.*, 2002), *H. bacteriophora* from 22 to 26°C (Doucet *et al.*, 1996), and *S. feltiae* at 25°C (Belair *et al.*, 2003).

However, these researches were tied on other hosts of entomopathogenic nematodes, so the results can not be applied on the species, used in this study. Some other results are interesting in this regard. Namely, in a study efficacy of different insecticides for control of *S. granarius* was tested and the highest mortality was determined at 25°C, while only an insignificant effect on *S. granarius* was established at 15°C (Tyler and Binns, 1982). This result is corresponding with low efficacy of entomopathogenic nematodes at 15°C. This confirms the fact that pest susceptibility to insecticides or biological agents is a complicated process and depends not only on the current physical condition of the pest organism but also on aggressiveness of the “invader”. Both, physical condition of the pest and aggressiveness of the invader, depend mostly on abiotic factors (Jian Fu Ji *et al.*, 2002).

Concentrations of the nematode suspensions used in the research were higher than is usually recommended or than is used in practice for soil pest control (Ishibashi and Choi, 1991) or control of foliar pests (Ebssa *et al.*, 2004). This is due to a very low efficacy of entomopathogenic nematodes in concentrations under 2.500 IJs/ml in our preliminary studies. The nematode species, used in the present study, have shown a certain level of efficacy against adults. The least efficient was *H. megidis*, but this was not the case in other researches (Svendsen and Steenberg, 2000). Influence of the temperature is stronger compared to the influence of the concentration of the nematode suspension. No differences were stated in adult mortality of *S. granarius* between used concentrations of *S. carpocapsae* and *H. bacteriophora*.

Considering the results of this study we concluded that entomopathogenic nematodes in higher concentrations (5.000 IJs/ml and more) could be efficient biological agents for control of adults of the granary weevil. Temperature was proven to be an important factor for their activity, but it has to be considered that it affects the pest too, especially its physical condition (Thurston in Kaya, 1994). The concentration of the nematode suspension was a less important factor of their efficacy (Arthurs *et al.*, 2004), though it depends on the nematode species. The general impression is, that the use of the highest concentration (20.000 IJs/ml) of the nematode suspension is not justified, because higher costs of the purchase of biological agents do not ensure a substantially higher efficacy compared to other two concentrations. *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* were the most effective in control of the pest, therefore when nematodes are considered for their control, all three species can be recommended.

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