COBISS Code 1.01

DOI: 10.2478/v10014-009-0018-9

Agrovoc descriptors: *Steinernema feltiae*; *Melolontha melolontha*; biological control; temperature; pest control; mortality; plant protection

Agris category code: H10

Efficacy of two strains of *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) against third-stage larvae of common cockchafer (*Melolontha melolontha* [L.], Coleoptera, Scarabaeidae) under laboratory conditions

Žiga LAZNIK[1], Tímea TÓTH[2], Tamás LAKATOS[3], Matej VIDRIH[4], Stanislav TRDAN[5]

Received June 18, 2009; accepted August 21, 2009.
Delo je prispelo 18. junija 2009, sprejeto 21. avgusta 2009.

ABSTRACT

In a laboratory experiment an efficacy of entomopathogenic nematode *Steinernema feltiae* in controlling third-stage larvae of common cockchafer (*Melolontha melolontha*) was studied. The experiment comprised of commercial product Entonem and indigenous strain C76. The efficacy of both biological agents was tested at 20 and 25 ºC and at four different concentrations of nematode suspension: 0, 250.000 infective juveniles [IJs]/m^2, 500.000 IJs/m^2, and 1.000.000 IJs/m^2. Higher mortality rate (27 %) of white grubs was obtained for strain C76 rather than for commercial product (20 %). In our experiment temperature proved to be the most limiting factor in efficacy of tested biological agents. Meanwhile, mortality rate at 20 ºC was 34 % and only 12 % mortality was achieved at 25 ºC. At highest concentration of nematode suspension and 20 ºC also the highest mortality rate (53 %) with strain C76 was obtained.

Key words: *Steinernema feltiae*, *Melolontha melolontha,* biological control, temperature, concentration of nematode suspension

IZVLEČEK

LABORATORIJSKO PREUČEVANJE UČINKOVITOSTI DVEH RAS ENTOMOPATOGENE OGORČICE *Steinernema feltiae* (Filipjev) **(Rhabditida: Steinernematidae) ZA ZATIRANJE LIČINK TRETJE LARVALNE STOPNJE POLJSKEGA MAJSKEGA HROŠČA** (*Melolontha melolontha* [L.], **Coleoptera, Scarabaeidae)**

V laboratorijskem poskusu smo preučevali učinkovitost entomopatogene ogorčice *Steinernema feltiae* za zatiranje ličink tretje larvalne stopnje poljskega majskega hrošča (*Melolontha melolontha*). V poskus smo vključili komercialni pripravek Entonem in domorodno raso C76. Delovanje omenjenih biotičnih agensov smo ugotavljali pri 20 in 25 ºC ter štirih različnih koncentracijah suspenzije ogorčic: 0, 250.000 infektivnih ličink [IL]/m^2, 500.000 IL/m^2 in 1.000.000 IL/m^2. Rasa C76 je vplivala na višjo stopnjo smrtnosti (27 %) ogrcev, v primerjavi s komercialnim pripravkom (20 %). Temperatura se je v našem poskusu izkazala kot najbolj omejujoč dejavnik učinkovitosti preizkušanih biotičnih agensov, saj smo pri 20 ºC dosegli 34 % smrtnost ogrcev, medtem ko je bila ta pri 25 ºC le 12 %. Pri najvišji koncentraciji suspenzije ogorčic in 20 ºC je bila pri rasi C76 dosežena najvišja stopnja smrtnosti ogrcev, in sicer 53 %.

Ključne besede: *Steinernema feltiae*, *Melolontha melolontha*, biotično varstvo rastlin, temperatura, koncentracija suspenzije ogorčic

[1] Young researcher, B. Sc., Jamnikarjeva 101, SI-1111 Ljubljana, email: ziga.laznik@bf.uni-lj.si

[2] Ph.D student, M. Sc., Vadastag 2, H-4244 Újfehértó, Hungary

[3] Ph. D, Vadastag 2, H-4244 Újfehértó, Hungary

[4] Teach. Assist., Ph. D, Jamnikarjeva 101, SI-1111 Ljubljana

[5] Assoc. Prof., Ph. D, Jamnikarjeva 101, SI-1111 Ljubljana

1 INTRODUCTION

Several members of beetles from the family Scarabaeidae, *Phyllophaga* spp. Harris, *Rhizotrogus majalis* (Razoumowsky), *Popillia japonica* Newman and *Melolontha melolontha* (L.), are important pests of grass sward and ornamental plants in Europe and North America (Simard *et al*., 2001). Adult beetles feed with leaves and flowers of fruit and forest trees and of ornamental plants, meanwhile larvae – white grubs - are soil pests and they feed on belowground parts of the plants (Keller and Zimmermann, 2005). In Slovenia, common cockchafer (*Melolontha melolontha* [L.]) is one of the most economically important pests of grasslands. It has a three year developmental cycle with different succession in its time and place of appearance (Vrabl, 1992). In a period from 2001 to 2007 we witnessed the massive occurrence of previously mentioned pest in the area of Črni vrh plateau (Northwest part of Slovenia) and which caused in time frames from 2002 to 2003 and 2005 to 2006 a grass sward devastation on 760 ha or on the area of 62 % of all agricultural land from that region (Poženel, 2007). The population of white grubs in abovementioned years extended to more than 200 larvae/m^2 (Poženel, 2007).

Control of common cockchafer is feasible with the application of insecticides. However, due to the appearance of insect resistance, efficacy decrease owing to soil microorganisms activity and doubts on environmentally acceptability of such kind of products, alternative solution are sought for its control (Koppenhöfer and Kaya, 1998). In controlling white grubs of common cockchafer with biological control measures most frequently the application of entomopathogenic fungus *Beauveria brongniartii* (Sacc.) Petch (Ascomycota: Hypocreales) (Keller and Brenner, 2005) is used. Main characteristic of the members of this phylum is a formation of mycelium, which carries asexual spores (conidia) on special conidiogenous cells. Conidia of majority of entomopathogenic fungus from order Hypocreales firmly fasten to insect cuticle. Host death takes place due to the suspension of nutrients transport, physical barriers and toxic extracts, as beauvericin is (Boucias *et al*., 1994).

Entomopathogenic nematodes (EPNs) from families Steinernematidae and Heterorhabditidae are important natural enemies of insects (Kaya, 1990). They are soil organisms, which live in mutualistic relationship with bacteria from the genera *Xenorhabdus* and *Photorhabdus* (Burnell and Stock, 2000). Once inside the infected insect, symbiotic bacteria are released from the bodies of infective juveniles (third larval stage of EPNs) to the host hemocoel system. And with the excretion of several toxins they cause its death in 24 to 72 hours (Forst and Clarke, 2002).

In Slovenia EPNs were till recently known as exotic species, which usage was possible only in laboratory experiments (Trdan *et al*., 2006, 2008). Since 2006 we actively examine EPNs fauna in Slovenian soils and up till now we confirmed the presence of 5 species: *Steinernema feltiae* (Filipjev) (strains B30, B49, C76), *Steinernema carpocapsae* (Weiser) (strains C67, C101, C110, C119), *Steinernema kraussei* (Steiner) (strains C46, C49), *Steinernema affine* (Bovien) (strain A12) and *Heterorhabditis bacteriophora* (Poinar) (strain D54) (Laznik *et al*., 2008ab). Strain which was used in our experiment, *S. feltiae* C76, was isolated in central part of Slovenia (Logatec area). In addition to this strain we included in a laboratory experiment for controlling common cockchafer also commercial product Entonem (Koppert B. V. Berkel en Rodenrijs, The Netherlands), which active ingredient is also *S. feltiae*.

The aims of our research was to study the efficacy of indigenous strain, *S. feltiae* C76, in a comparison to commercial product Entonem when controlling third stage-larvae of common cockchafer, and on the other hand to determine the influence of temperature and concentration of suspension on the activity of studied biological agents.

2 MATERIALS AND METHODS

2.1 Common cockchafer and entomopathogenic nematodes

In an experiment, which was conducted in an Entomological Laboratory of Chair of Phytomedicine, Agricultural Engineering, Crop Production, Pasture and Grassland Management (Agronomy Department at Biotechnical Faculty in Ljubljana, Slovenia), we studied the efficacy of EPNs in controlling third stage-larvae of common cockchafer. We collected white grubs (500) in the area of Črni Vrh above Idrija (45°55'27'' N, 14°2'37'' E, altitude 710 m) with the use of soil excavations.

We included indigenous strain of *Steinernema feltiae*, C76, which was isolated from the soil in the area of Logatec (45°54'52'' N, 14°13'33'' E, altitude 470 m) (Laznik *et al*., 2009). Strain *S. feltiae* C76 was reared using late instar larvae of *Galleria mellonella* (L.) (Bedding and Akhurst, 1975). We used only infective juveniles which were less than 2 weeks old. During the experiment, which was repeated three times, we stored the infective juveniles at 4 ºC. Product Entonem

(Koppert B.V., Berkel en Rodenrijs, The Netherlands) was supplied by the importer Zeleni hit d.o.o. (Ljubljana, Slovenia).

2.2 Laboratory bioassay

We tested the efficacy of the EPNs in controlling third larval stage of the common cockchafer by exposing individuals to either 0, 250.000 IJs/m^2, 500.000 IJs/m^2 or 1.000.000 IJs/m^2. We determined the number of infective juveniles in a previously prepared unknown concentration of nematode suspension by counting the number of such in droplets (5 µl x 5) and by diluting (adding M9 solution) or by concentrating (reduction to an adequate volume with the assistance of centrifugation) (Laznik *et al*., unpubl.). In this manner we obtained the selected concentrations of nematode suspensions (0, 750, 1.500 and 3.000 IJs/ml).

Precedently (2 weeks before inserting larvae into a place) we put in an experimental vessel (10 x 15 x 10 cm = l x w x d) 300 g of soil and 50 grains of wheat. With this we wanted to ensure enough roots which would serve as additional food for white grubs during the experiment. To each plastic vessel we then add 5 third-stage larvae of common cockchafer. Chosen concentration we applied in a 5 ml dose. Afterwards we moistured soil additionally with ordinary water (sprayer employment). Each treatment was repeated for five times. Experimental vessels were put in a rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia) with a volume of 0.868 m^3 (width x height x depth = 1000 x 1400 x 620 mm). We tested the efficacy at two different temperatures (20 and 25 ºC) and at a relative humidity of 80 %. The number of dead larvae of *M. melolontha* was determined 3, 7, and 10 days after treatment. We moistured soil daily and added supplementary feed for white grubs (carrot). The dead individuals were dissected to determine if the nematodes were present. In such a manner we wanted to prove that the insects died due to the EPNs' activity.

2.3 Statistical analysis

A multifactor analysis of variance (ANOVA) was conducted to determine the differences in mortality rates (%) between the larvae of *M. melolontha* reared in 16 different treatments (two strains of *S. feltiae* – each with four different concentrations at two different temperatures). Before the analysis, the mean mortality was tested for the homogeneity of treatment variances. The mortality data were corrected according to Abbott's formula (Abbott, 1925) and normalized using the arcsine square-root transformation. Duncan's multiple range test ($P \leq 0.05$) was used to separate mean differences among the parameters in all the treatments. All statistical analyses were performed with Statgraphics Plus for Windows 4.0 (Manugistics, Rockville, MD, USA) and the figure was created with MS Office Excel 2003. The data are presented as untransformed means ± SE.

3 RESULTS

3.1 Analysis of pooled results

Analysis of pooled results showed that larval mortality of common cockchafer was significantly influenced by the concentration of nematode suspension (F=6.88; df=2, 179; P<0.0070), temperature (F=499.91; df=1, 179; P<0.0001), nematode strain (F=59.11; df=1, 179; P<0.0001) and day after treatment (DAT) (F=5.41; df=2, 179; P<0.0161), interaction between DAT and concentration of nematode suspension (F=3.63; df=4, 179; P<0.0275), interaction between concentration of nematode suspension and nematode strain (F=64.01; df=2, 179; P<0.0001), interaction between concentration of nematode suspension and temperature F=29.39; df=2, 179; P<0.0001), interaction between nematode strain and temperature (F=251.58; df=1, 179; P<0.0001) and interaction between concentration of nematode suspension, nematode strain and temperature (F=49.94; df=2, 179; P<0.0001). Interaction between DAT and nematode strain (F=0.75; df=2, 179; P<0.4903), interaction between DAT and temperature (F=1.22; df=2, 179; P<0.3225), interaction between DAT, concentration of nematode suspension and nematode strain (F=1.46; df=4, 179; P<0.2596), interaction between DAT, concentration of nematode suspension and temperature (F=0.37; df=4, 179; P<0.8265) and interaction between DAT, nematode strain and temperature (F=0.62; df=2, 179; P<0.5511) did not have significant influence on the larval mortality rate of common cockchafer. In all treatments total mortality was significantly different from the control treatment. Corrected mortality was therefore calculated.

We found significant differences between both strains of EPNs and between both temperature values. Mortality of white grubs which were exposed to strain C76 was 26.73±2.60 %, meanwhile mortality of white grubs exposed to product Entonem was 19.64±2.15 %. Average white grubs mortality at 20 ºC was 33.49±2.49 % and at 25 ºC was 12.88±1.75 %. Concentration of nematode suspension had no influence on mortality of common cockchafer white grubs, while no statistically significant differences between individual levels of this factor (750, 1.500, and 3.000 IJ/ml) have been found (20.96±2.74, 23.48±2.96 and 25.12±3.17 %). Significant differences have not been determined between days after treatment (DAT) as an average white grubs mortality for the 3rd, 7th and 10th day was 21.11±2.87, 23.77±2.99 and 24.68±3.02 %.

Table 1: *Mean mortality (± SE) of third-stage larvae of Melolontha melolontha treated with three different concentrations of two strains of Steinernema feltiae at 20, and 25 ºC 10 DAT. The data shown are corrected for control mortality.*

Temperature (ºC)	*S. feltiae* strain	Nematode concentration (IJs/ml) 750	1500	3000
20	C76	34.57 ± 3.98	45.80 ± 4.35	52.67 ± 6.28
	Entonem	27.13 ± 6.07	30.82 ± 5.71	9.96 ± 3.74
25	C76	0.00 ± 0.00	12.90 ± 4.01	14.46 ± 3.79
	Entonem	22.15 ± 5.13	4.42 ± 2.55	23.38 ± 4.79

3.2 Individual analysis

At 20 ºC and 10 DAT, when strain C76 was applied at middle and high concentration of nematode suspension, it performed significanlty better as product Entonem (Table 1). At lowest concentration of nematode suspension (750 IJs/ml) we did not determine any significant differences between both strains when controlling white grubs. Their mortality when treated with strain C76 was 34.57 ± 3.98 % and mortality of those treated with commercial product Entonem was 27.13 ± 6.07 %.

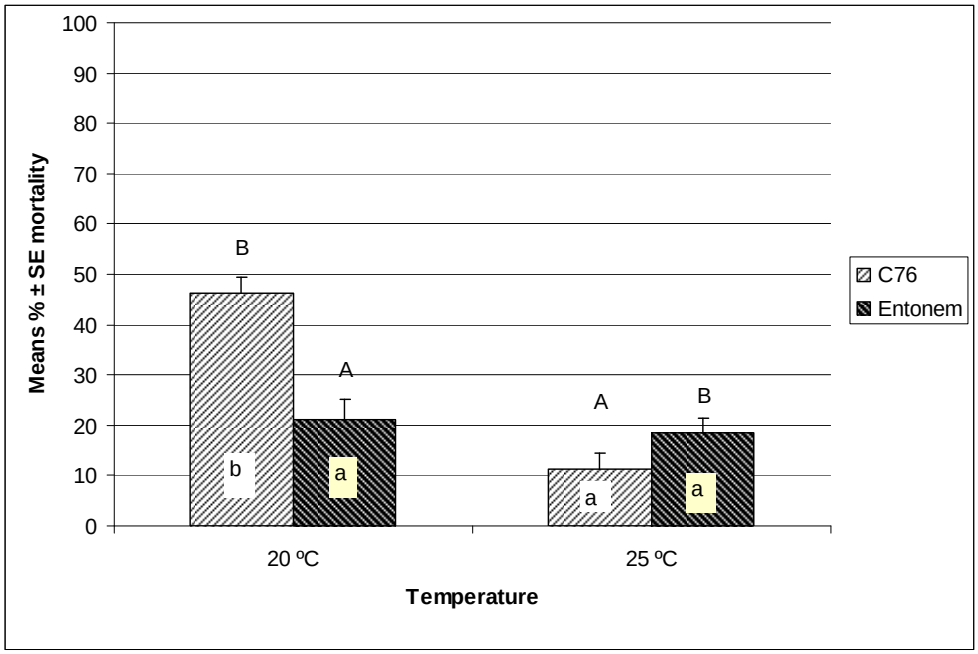

Fig. 1: *Mean mortality (± SE) of third-stage larvae of Melolontha melolontha treated with two different strains of Steinernema feltiae depending on rearing temperature at all three different concentrations 10 DAT. The data shown are corrected for control mortality and analyzed by multifactor ANOVA. Capital and lower-case letters correspond to the grouping of means by Duncan's multiple range test (P≤0.05) for EPN strains and temperature, respectively. The same letters do not differ significantly.*

At 1.500 IJs/ml concentration of nematode suspension white grubs mortality which were exposed to strain C76 was 80 ± 4.35 % and significantly smaller (30.82 ± 5.71 %) was observed when white grubs were treated with commercial product. Commercial product Entonem was the least effective (9.96 ± 3.74 %) at highest concentration of nematode suspension (3000 IJ/ml), meanwhile the strain C76 was the most effective (52.67 ± 6.28 %).

Activity of both studied strains was significantly poorer at 25 ºC than at 20 ºC (Figure 1). Commercial product was the most efficient at highest and lowest concentration of nematode suspension (23.38 ± 4.79;

22.15 ± 5.13 %), meanwhile the activity of indigenous strain C76 was at that temperature considerable poorer (from 0.0 ± 0.0 % at 750 IJs/ml to 14.46 ± 3.79 % at 3000 IJs/ml).

4 DISCUSSION

Results of our research demonstrated that indigenous strain *S. feltiae* C76 attained higher mortality rate (27 %) of third-stage larvae of common cockchafer than commercial product Entonem (20 %). In a similar research, Berner and Schnetter (2001) reported on 3 % larval mortality when *S. feltiae* strain Ehlers was applied and that as the best nematode in their experiment proved to be *S. glaseri* strain RS92 (60 %). Reason for poorer activity of *S. feltiae* we can attribute to the fact, that it goes for the species which has not been found in naturaly infected white grubs as this is documented for *S. anomali* (Kozodoi) *S. glaseri* (Steiner), *S. kushidai* (Mamiya), *S. scarabaei* (Stock), and *Heterorhabditis megidis* (Poinar) (Poinar, 1975).

Georgis and Gaugler (1991) argumented the ineffectiveness of entomopathogenic nematodes in controlling beetles from the family Scarabaeidae in most situations to unsuitable selection of strains, temperature and life cycle of insect. Several researches demonstrated that most effective strains which controlled white grubs were *H. bacteriophora* GPS11 (83-96 %), *H. zealandica* X1 (96-98 %) and *S. scarabaei* (100 %) (Cappaert and Koppenhöfer, 2003; Koppenhöfer and Fuzy, 2003; Grewal *et al*., 2004).

Constrast between strains studied in our experiment can be found due to the fact that strain C76 is much bettter adapted to the larvae of common cockchafer as we confirmed its finding in the area (Laznik *et al*., 2009), where in the past common cockchafer caused quite an extensive damage on grasslands (Urek in Milevoj, 1993). Grewal *et al*. (2004) came to similar conclusions, namely that different strains of the same EPN species might act differently on various insect pests. It was established multiple times that indigenous strains are more virulent from the exotic strains, in spite of the fact that Grewal *et al*. (2004) did not manage to confirm this in their research when studying *Popillia japonica* Newman and *Cyclocephala borealis* Arrow.

Developmental stage of insect pest influences the activity of EPNs (Georgis and Gaugler, 1991). When controlling the youngest larvae (L1) of common cockchafer with the nematode *H. downesi* strain 267 Lakatos and Tóth (2006) established 90 % efficacy at 20 ºC. When comparing L3 and L2 stages they gained higher mortality at latter one when controlling *Anomala orientalis* (Waterh.) (Lee *et al*., 2002). In a similar study, when controlling common cockchafer with the nematodes *S. glaseri* and *Heterorhabditis* sp., the most susceptible were the larval stages L1 and L2 (Deseö *et al*., 1990).

In our experiment concentration of nematode suspension had no influence on mortality of third-stage larvae of common cockchafer. In related experiments concentration of nematode suspension varied between 0.5 and 12.5 x 10^9 IJs/ha. To somehow similar results, that concentration of nematode suspension has no significant effect on mortality of exposed insects (*P. japonica*, *A. orientalis* and *Rhizotrogus majalis* [Razoumowsky]) came also Grewal *et al*. (2004), who attained similar mortality rate at 2.5 and 5.0 x 10^9 IJs/ha. Application of nematode suspension in concentration above 2.5 x 10^9 IJs/ha is not economicaly justified (Grewal and Georgis, 1998). From this view, the choice of suitable species, that is nematode species which showed superior efficacy in controlling larvae of common cockchafer in the previous experiments (e.g. *S. scarabaei*), would be the best solution.

Temperature demonstrated in our experiment as the most limiting factor which influences the activity of EPNs. At 20 ºC we attained 34 % mortality of L3 larval stage, meanwhile only 12 % mortality was found at 25 ºC. We came to likewise findings also at some other researches (Trdan *et al.*, 2006, 2008; Laznik *et al*., 2009), where we also concluded that mortality of studied insects is influenced at most by the temperature. Grewal *et al*. (2004) reports that different species of EPNs have different optimal temperatures to control pest insects. Simões *et al*. (1993) reported about nematodes *S. glaseri* and *H. bacteriophora*, which caused at 23 ºC 100 % larvae mortality of beetle *P. japonica*; meanwhile *S. carpocapsae* gained at the same conditions only 56 % mortality and at lower than 15 ºC only *S. glaseri* preserved satisfying efficacy rate.

In our experiment, the most promising activity demonstrated the strain C67 at 20 ºC and at highest concentration of nematode suspension (53 %), meanwhile the highest effect of bioproduct Entonem was attained at 20 ºC and at middle concentration of nematode suspension (31 %). At corresponding application *S. feltiae* can very satisfying control the younger larval stages of common cockchafer, but when compared to entomopathogenic fungus *Beuveria brongniartii* (Poženel, 2007), the efficacy of the nematodes is lower. Results of some researches indicate positive interaction in simultaneous application of entomopathogenic nematodes and entomopathogenic fungus (Shapiro-Ilan *et al*., 2004), but more detailed

mechanisms of their common functioning are for now poorly studied. It is well known that some species of EPNs (*S. carpocapsae* in *H. indica*) in relation to entomopathogenic fungus act antagonistically, meanwhile *H. bacteriophora* act additively (Shapiro-Ilan *et al*., 2004). Interaction between entomopathogenic fungi and entomopathogenic nematodes depends at a larger scale also from the target pest (Barbercheck and Kaya, 1991). In future researches we want to study additivity on usage of *S. feltiae* strain C76 and indigenous strain of entomopathogenic fungus *B. brongniartii* in controlling larvae of common cockchafer.

5 ACKNOWLEDGEMENTS

This work was done within Horticulture No P4-0013-0481, a program funded by the Slovenian Research Agency. Part of the research was funded within Professional Tasks in the Field of Plant Protection, a program funded by the Ministry of Agriculture, Forestry, and Food of the Republic of Slovenia Phytosanitary Administration.

6 REFERENCES

Abbott W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Barbercheck, M.E., Kaya, H.K. 1991. Competitive interactions between entomopathogenic nematodes and *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) in soilborne larvae of *Spodoptera exigua* (Lepidoptera: Noctuidae). Environ. Entomol. 20: 707-712.

Berner, M., Schnetter, W. 2001. Wirksamkeit entomopathogener Nematodes gegen Engerlinge der Maikäfer *Melolontha melolontha* und *M. hippocastani*. Mitt. Dtsch. Ges. Allg. angew. Entomol. 13: 165-167.

Boucias, D.G., S.Y. Hung, I. Mazet, and J. Azbell. 1994. Effect of the fungal pathogen *Beauveria bassiana*, on the lysozyme activity in *Spodoptera exigua* larvae. Experientia. 50: 142-147.

Burnell A.M., Stock S.P. 2000. *Heterorhabditis*, *Steinernema* and their bacterial symbionts – lethal pathogens of insects. Nematology 2: 31-42.

Cappaert, D.C., Koppenhöfer, A.M. 2003. *Steinernema scarabaei*, an entomopathogenic nematode for control of the European chafer. Biol. Control 28: 379-386.

Deseö, K.V., Bartocci, R., Tartaglia, A., Rovesti, L. 1990. Entomopathogeneous nematodes for control of scarab larvae. IOBC/wprs Bull. 14: 57-58.

Forst S., Clarke D. 2002. Bacteria-nematode symbiosis. In: Gaugler R (ed) Entomopathogenic Nematology. CABI Publ., Wallingford: 57-77.

Georgis, R., Gaugler, R. 1991. Predictability in biological control using entomopathogenic nematodes. J. Econ. Entomol. 84: 713-720.

Grewal, P.S., Georgis, R. 1998. Entomopathogenic nematodes. In: Hall, F.R. and Menn, J.J. (eds) Biopesticides: Use and Delivery. Humana Press, Totowa, New Jersey: 271-299.

Grewal, P.S., Power, K.T., Grewal, S.K., Suggars, A., Haupricht, S. 2004. Enhanced consistency in biological control of white grubs (Coleoptera: Scarabaeidae) with new strains of entomopathogenic nematodes. Biol. Control 30: 73-82.

Kaya H.K. 1990. Soil ecology. In: Gaugler R and Kaya HK (eds.) Entomopathogenic Nematodes in Biological Control. CRC Press, Florida: 93-115.

Keller, S., Brenner, H. 2005. Development of the *Melolontha* populations in the canton Thurgau, eastern Switzerland, over the last 30 years. IOBC/wprs Bull. 28: 31.

Keller, S., Zimmermann, G. 2005. Scrabs and other soil pests in Europe: situation, perspectives and control strategies. IOBC/wprs Bull. 28: 9-12.

Koppenhöfer, A.M., Kaya, H.K. 1998. Synergism of imidacloprid and an entomopathogenic nematode: A novel approach to white grub (Coleoptera: Scarabaeidae) control in turfgrass. J. Econ. Entomol. 91: 618-623.

Koppenhöfer, A.M., Fuzy, E.M. 2003. *Steinernema scarabaei* for the control of the European chafer. Biol. Control 28: 379-386.

Lakatos, T., Tóth, T. 2006. Biological control of European cockchafer larvae (*Melolontha melolontha* L.) – preliminary results. J. Fruit Ornam. Plant Res. 14: 73-78.

Laznik, Ž., Tóth, T., Lakatos, T., Trdan, S. 2008a. Entomopathogenic nematode *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) recorded for the first time in Slovenia. Acta Agric. Slov. 91: 37-45.

Laznik, Ž., Tóth, T., Lakatos, T., Trdan, S. 2008b. Entomopathogenic nematode *Steinernema carpocapsae* (Weiser) (Rhabditida:Steinernematidae), a new member of Slovenian fauna. Acta Agric. Slov. 91: 351-359.

Laznik, Ž., Tóth, T., Lakatos, T., Trdan, S (2009): First field experiment with entomopathogenic nematodes in Slovenia. Lect. pap. present. 9th Slov. Conf. Plant Prot., Nova Gorica, March 4-5 2009. Ljubl., Plant Prot. Soc. Slov. (in press). [Slovenian]

Lee, W., Choo, H.Y., Kaya, H.K., Lee, S.M., Smitley, D.R., Shin, H.K., Park, C.G. 2002. Laboratory and field evaluation of Korean entomopathogenic nematode isolates against the oriental beetle Exomala orientalis

(Coleoptera: Scarabaeidae). J. Econ. Entomol. 95: 918-926.

Poinar, G.O. Jr. 1975. Entomogenous Nematodes. E.J. Brill, Leiden, The Netherlands: 317 pp.

Poženel, A. 2007. Experiences in controlling common cockchafer (*Melolontha melolontha* L.) in Idrija region. Lect. pap. present. 8th Slov. Conf. Plant Prot., Radenci, March 6-7 2007. Ljubl., Plant Prot. Soc. Slov.: 72-77 [Slovenian]

Shapiro-Ilan, D.I., Jackson, M., Reilly, C.C., Hotchkiss, M.W. 2004. Effects of combining an entomopathogenic nematodes on mortality of *Curculio caryae* (Coleoptera: Curculionidae). Biol. Control 30: 119-126.

Simard, L., Bélair, G., Brodeur, J. 2001. Susceptibility of the European chafer (Coleoptera: Scarabaeidae) to entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). J. Nematol. Suppl. 33: 297-301.

Simões, N., Laumond, C., Bonifassi, E. 1993. Effectiveness of *Steinernema* spp. and *Heterorhabditis bacteriophora* against *Popillia japonica* in the Azores. J. Nematol. 25: 480-485.

Trdan S., Vidrih M., Valič N. 2006. Activity of four entomopathogenic nematode species against young adults of *Sitophilus granarius* (Coleoptera: Curculionidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) under laboratory conditions. J. Plant. Dis. Prot. 113: 168-173.

Trdan S., Vidrih M., Valič N., Laznik Ž. 2008. Impact of entomopathogenic nematodes on adults of *Phyllotreta* spp. (Coleoptera: Chrysomelidae) under laboratory conditions. Acta Agric. Scand., B Soil Plant. Sci. 58: 169-175.

Urek, G., Milevoj, L. 1993. Ogrčja nadloga: poljedelstvo: varstvo. Kmetovalec, 61: 7-8.

Vrabl, S. 1992. Škodljivci poljščin. Ljubljana, ČZP Kmečki glas: 19-22.