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Potential of Steinernema feltiae (Rhabditida: Steinernematidae) as a biological control agent against the cabbage maggot Delia radicum (Diptera: Anthomyiidae) in oilseed rape

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Potential of *Steinernema feltiae* (Rhabditida: Steinernematidae) as a biological control agent against the cabbage maggot *Delia radicum* (Diptera: Anthomyiidae) in oilseed rape

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Abstract: Entomopathogenic nematodes (EPNs) in families Heterorhabditidae and Steinernematidae have considerable potential as biological control agents against soil-inhabiting insect pests. In the present study, the potential of *Steinernema feltiae* as a biological control agent against cabbage maggot (CM) *Delia radicum* (Diptera: Anthomyiidae), persistence of the nematode in areas infested by CM larvae, and foraging behavior of the nematode toward CM and/or oilseed rape roots were examined in the laboratory. The results of the laboratory experiment showed that more than 80% control was achieved against the last instars of *D. radicum*. *Steinernema feltiae* persisted in the soil in high numbers for 12 weeks in laboratory assays. The foraging behavior of *S. feltiae* in the presence of *D. radicum* larvae and oilseed rape roots, offered individually and in combination, was studied using a Y-tube olfactometer filled with silver sand (Humax® U.K., particle size: 300-400 µm) at 8 and 15 °C. When insect larvae were present, more nematodes migrated toward the larvae at both temperatures.

Key words: Cabbage maggot, *Delia radicum*, efficacy, persistence, *Steinernema feltiae*, Y-tube olfactometer

Kanola bitkisinde lahana sineği, *Delia radicum* (Diptera: Anthomyiidae)'a karşı entomopatojen nematod *Steinernema feltiae* (Rhabditida: Steinernematidae)'nin biyolojik mücadele etmeni olarak potansiyeli

Özet: Heterorhabditidae ve Steinernematidae familyalarında bulunan entomopatojen nematodlar (EPN), toprak kökenli zararlı böceklere karşı biyolojik mücadele etmeni olarak önemli potansiyele sahiptir. Bu çalışmada, laboratuvardaki kanola bitkileri üzerinde lahana sineği, *Delia radicum* (Diptera: Anthomyiidae)'a karşı *Steinernema felitae*'nin mücadele potansiyeli, bu nematodun lahana sineği larvası ile bulaşık olan alanlardaki kalıcılığı, nematodun lahana sineği larvası ve/veya kanola köklerinin bulunduğu ortamda besin arama davranışları incelenmiştir. *Steinernema feltiae*, laboratuar denemelerinde 12 hafta kalıcılık göstermiştir. Laboratuar testleri, *D. radicum*'un son larva dönemine karşı % 80 oranında etkinliğe ulaşıldığını göstermiştir. Kum (firma Humax® U.K., partikül boyutu: 300-400 µm) ile doldurulmuş Y-olfaktometre düzeneği kullanılarak *S. feltiae*'nin *D. radicum* ve kanola köklerine doğru besin arama davranışları tek tek ve kombine edilerek 8 ve 15 °C' de denenmiştir. Bu düzenekteki en fazla nematod yöneliminin her iki sıcaklık değerinde de larvaya karşı olduğu tespit edilmiştir.

Anahtar sözcükler: *Delia radicum*, etkinlik, kalıcılık, lahana sineği, *Steinernema feltiae*, Y-olfaktometre

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Introduction

In the past few years, cabbage maggot (CM) *Delia radicum* (Linnaeus) (Diptera: Anthomyiidae) has become a major pest in oilseed rape, particularly in northern Europe. Control of CMs by use of chemicals is difficult and uneconomical because CMs feed around or inside oilseed rape roots, where chemicals cannot reach well. CM damage appears on older leaves of infested oilseed rape plants. The leaves turn reddish to dark purple in color and the roots of the plants are often cut approximately in the middle of the plants. In general, the infected plants become weak and smaller than healthy oilseed rape plants due to the damage in the root system (Susurluk 2005).

Entomopathogenic nematodes (EPNs) can be an alternative to chemical control of the pest. EPNs belonging to families Steinernematidae and Heterorhabditidae contain mutualistic bacteria that are lethal to many soil-dwelling insect pests (Ehlers 1996). Infective juvenile (IJ) nematodes, also called dauer larvae (DL), enter insects through the mouth, anus, spiracles, or areas of thin cuticle. After penetrating to the hemocoel, the nematodes release the mutualistic bacteria, which quickly multiply and overwhelm the hosts (Burnell and Stock 2000). As biological control agents, EPNs possess impressive attributes against many soil-inhabiting insects, particularly ease of culture and application. In addition, they are highly lethal despite their high level of safety (Gaugler 2002).

Second and third instar CMs feed on oilseed rape roots mostly in autumn. Therefore, a potential agent is required to be active at low temperatures. Since *Steinernema* species are more effective than *Heterorhabditis* species in soil below 12 °C (Griffin 1993) and CMs are most susceptible to *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) at the third instar, the most destructive stage (Sulistyanto et al. 1994), *S. feltiae* was chosen for the present study.

The objective of this study was to detect the effectiveness of *S. feltiae* against the last instar CM larvae, persistence of the nematode in soil, and the foraging behavior of *S. feltiae* for CMs and oilseed rape root systems at different temperatures.

Materials and methods

Nematode, insect, and plant

Steinernema feltiae (Filipjev) strain en02 (Nemaplus®), supplied by the company e-nema GmbH (Schwentinental, Germany), was used in the experiments and cultured according to the methods of Ehlers (1998), Ehlers et al. (2000), and Ehlers (2001). CM and oilseed rape, *Brassica napus* var. *napus* L., were obtained from oilseed rape fields in Rastorfer Passau (Kreis Plön, Germany), and the larvae were reared on the plants according to the method described by Harris and Svec (1966).

Persistence and efficacy assays

In order to detect the nematode efficacy and persistence under controlled conditions, the following experiments were conducted in the laboratory. In November, 125 young oilseed rape plants were collected from the field and individually transplanted into pots of 12 × 12 × 14 cm (l × w × h) containing a soil mixture: 337.5 g (45%) of compost [farm-sourced, Rastorfer Passau (Kreis Plön, Germany); pH: 6.0; electrical conductance (EC): 3.2 mS cm^{-1}; organic matter (OM): 6.5 g kg^{-1}], 337.5 g (45%) of sandy-loam soil [oilseed rape field-sourced, Rastorfer Passau (Kreis Plön, Germany); pH: 7.0; EC: 1.8 mS cm^{-1}; OM: 5.0 g kg^{-1}], 67.5 g (9%) of silver sand (Humax® U.K., particle size: 300-400 µm), and 7.5 g (1%) of fertilizer (10 N, 5 P_2O_5, 3 K_2O). In addition, 3 field-collected third instar *D. radicum* larvae were placed in 100 pots. All 125 pots were incubated at 8 °C for 1 day, and afterwards the 100 pots with CM larvae were treated with *S. feltiae* at a dose of 50 IJs cm^{-2}, using a hand sprayer with a nozzle of 0.5 mm in diameter and a capacity of 500 mL. The 25 pots without larvae were used as untreated controls. During the study, soil moisture was kept between 10% and 15% [weight/volume (w/v)] in the pots (Boff et al. 2001) to mimic field conditions. Soil moisture was checked using a soil moisture measuring apparatus every 2 days. Soil samples were taken from 3 different spots close to the edges for measurement. When necessary, water was added to the pots.

Once every 2 weeks after nematode application, the soil of each pot was checked for living larvae and pupae. Due to the fact that infected larvae were destroyed very soon after *S. feltiae* penetrated, the calculation of mortality was based on the number of surviving larvae and pupae in treated pots (Susurluk and Ehlers 2008). This procedure was continued until no larvae were detected or they had all pupated. The experiment was carried out at 8 °C because the larvae are active at this temperature during autumn.

In order to measure the persistence of *S. feltiae* in the oilseed rape potted soils, samples were taken using a soil core (1.5 cm in diameter × 10 cm in height) for 12 weeks and subjected to baiting with 2 last instar larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae). The water content of the collected soil was nearly 80%-90% (w/v, relative moisture). After baiting for 3 days at 25 °C, infected larvae were dissected and nematodes were monitored under a stereomicroscope. Detected IJs in each larva were recorded. This procedure was continued for a period of 12 weeks. Described assays were repeated a second time the following year, since larvae should be collected from the field only in autumn.

Detecting foraging behavior of the nematode with a Y-tube olfactometer

In order to investigate whether *S. feltiae* could migrate toward *D. radicum* larvae and/or oilseed rape roots over a longer distance than in the control, a Y-tube olfactometer (Figure 1) was used. The arena consisted of a dark grey hard PVC Y-connection manufactured for sewage water disposal. The olfactometer was filled with fine sterilized silver sand (Humax® U.K., particle size: 300-400 µm) (10% moisture w/v).

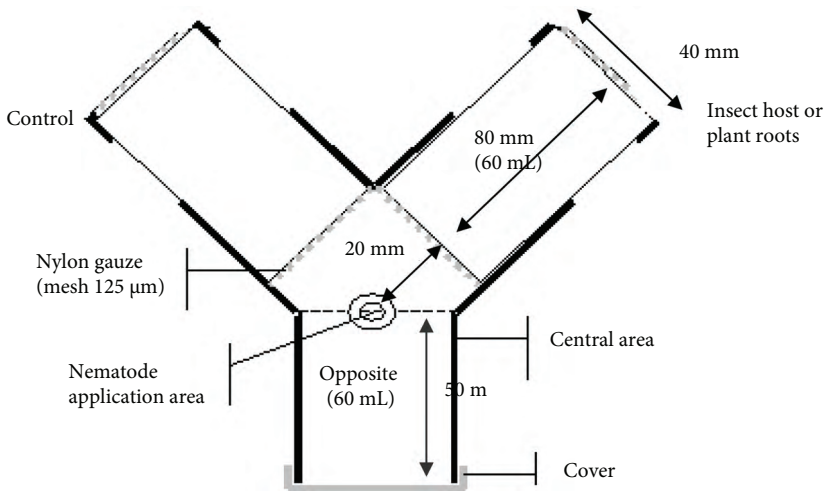

Figure 1. Schematic drawing of the Y-tube olfactometer choice arena for recording preferential responses of EPNs to insect and root stimuli in sand (modified from Boff et al. 2001).

To detect the behavioral response of the IJs of *S. feltiae*, the following 3 combinations were performed.

	Left compartment	Middle compartment	Right compartment
a.	5 CM larvae	Center	Untreated control
b.	5 CM larvae	Center	1 oilseed rape plant
c.	Untreated control	Center	1 oilseed rape plant

Control compartments contained only sand, without any larvae or oilseed rape plants. Each combination was performed in parallel with 3 olfactometers as a replication. The experiments were conducted at 8 and 15 °C. The larvae were kept in a sieve of 1-mm mesh to prevent them from migrating through the tube. Before adding nematodes, the assay units were incubated in a horizontal position in a dark room at 8 or 15 °C for 24 h to allow for the formation of a chemical gradient. Then 1 mL of Ringer's solution containing 1000 IJs (±15%) was added to the center hole, located 50 mm below the nematode application point (Figure 1), and the olfactometers were incubated for 10 days at the above temperatures. After this period, each olfactometer was taken apart and the nematodes were recovered from the sand using a modified version of Cobb's (1918) decanting and sieving method (Klein-Beekman et al. 1994). The sand in each compartment was transferred into a 1-L glass cylinder and 600 mL of tap water was poured over it. After 10 s, the supernatant was poured into a 2-L beaker. The remaining sand was mixed with 600 mL of tap water 2 more times. The supernatant was collected after 15 s and added to the beaker suspension. The total beaker suspension with the nematodes was poured through a sieve (mesh size of 0.01 mm). The nematodes remaining on the sieve were collected in a small beaker. The nematodes recovered from the sand of each compartment of the Y-tube olfactometer were counted in a counting plate under a microscope. The insect larvae were dissected and the number of IJs was determined.

Statistical analyses

The data of persistence and efficacy experiments obtained from the 2 repeated experiments in the laboratory were combined, as the data from each experiment were very similar. Because of the similarity, the combined data were analyzed using 2 data sets. Mortality comparison treatments were evaluated with the Mann-Whitney test at $P < 0.05$. Distribution of *S. feltiae* in the Y-tube olfactometer was subjected to analysis of variance (ANOVA breakdown, one-way ANOVA) and a least significant difference (LSD) test as post hoc comparisons of the mortality means. The minimum level of significance was taken as $P < 0.05$.

Results

Persistence and efficacy assays

CM pupae were not detected at the beginning of the experiments, but as the experiment continued, pupation increased from 36 ± 30% in the second week to 84 ± 41% in the fourth week and 100% in the sixth week after starting the tests. There was no mortality in the control. The results revealed significant differences according to the Mann-Whitney test (U: 152.8, $P < 0.05$). The mortality of *D. radicum* increased sharply after week 4. As pupae are not susceptible to EPNs, a major increase in the mortality cannot be expected to be due to advancing pupation. Mortality of *D. radicum* in the second, fourth, sixth, eighth, and tenth weeks was 60.87 ± 11.3%, 79.54 ± 10.9%, 84.61 ± 14.1%, 85 ± 17.0%, and 85 ± 16.8%, respectively.

The mortality of the oilseed rape plants in the control pots was higher than that of those treated with *S. feltiae* 2 weeks after treatment. While 70 ± 5.2% of 25 plants died in the untreated control 12 weeks after the start of the experiment, only 30 ± 4.5% of 100 plants died in the insect-infested pots when *S. feltiae* was added to the plant roots.

The results of the persistence study are summarized in Figure 2. Significant correlation was not observed ($r = 0.24$, $P = 0.59$) between the number of positive soil samples and the number of IJs recovered from infested *G. mellonella*. A minimum of 78 ± 9.7% and a maximum of 93 ± 11.2% of soil samples were positive for *S. feltiae*, indicating that the nematodes persisted for quite a long period at 8 °C.

Detecting foraging behavior of the nematode with Y-tube olfactometer

The average distribution of IJs in the 3 Y-tube compartments is presented in Figure 3. In combination a, 677.3 ± 35 at 8 °C and 697.3 ± 68 at 15 °C of 1000 ± 180 IJs were recovered by Cobb's sieving method. In combination b, 780 ± 58.8 IJs at 8 °C and 701.5 ± 64 IJs at 15 °C were recovered, and in combination c, 705 ± 67.8 IJs at 8 °C and 665.3 ± 71.5 IJs at 15 °C of 1000 ± 120 IJs were recovered. The distribution of IJs was calculated according to the number of recovered IJs and significant differences were recorded. For combination a: $F = 15.0$; $df = 5, 12$; $P < 0.05$. For combination b: $F = 17.8$; $df = 5, 12$;

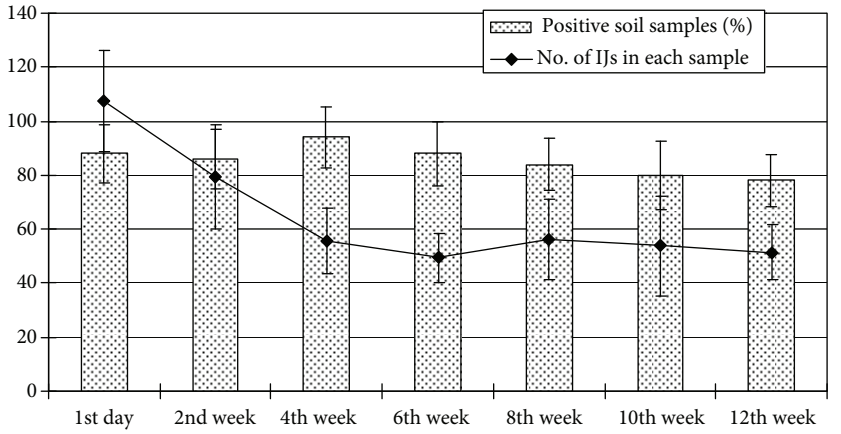

Figure 2. Percentage of soil samples positive for *S. feltiae* recorded with the *G. mellonella* baiting method, and mean number of recovered IJs from the infected insects (n = 25) over a period of 12 weeks. Error bars represent ±SE.

$P < 0.05$. For combination c: $F = 43.2$; $df = 5, 12$; $P < 0.05$. In general, a high number of EPNs remained at the point of application. Whenever insects were present, more EPNs migrated toward the insects (Figures 3a and 3b) compared to the second treatment (combination b). When oilseed rape plants were offered alone at 15 °C (Figure 3c), 28% was the highest rate IJ recovery. Thus, the positive chemotaxis toward oilseed rape roots is much lower than that toward the insect larvae at both temperatures. The response of IJs toward the stimuli increased with increasing temperature. While more IJs remained in the control at 8 °C, more IJs were found in the compartment with *D. radicum* at 15 °C. In the foraging experiments using Y-olfactometers, *S. feltiae* infected 100% of the larvae at both temperatures.

Discussion

Results of this study indicate that the larvae of *D. radicum* are excellent targets for *S. feltiae*, as was shown by Sulistyanto et al. (1994). According to the presented results, the nematodes were attracted by the insects more than by the oilseed plants in the foraging behavior experiment. Most of the nematodes in the assay remained at the point of application (Figure 3c). The opposite result was observed by Boff et al. (2002), who worked on *H. megidis* and strawberry plants. When *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) larvae and strawberry plant roots were each placed in a compartment, more nematodes (41%) moved toward the arm with the plant roots than toward the host larvae. A likely candidate for attraction is of course CO_2, a general kairomone produced as an end product of the metabolism of plants, microorganisms, and other soil animals. CO_2 has been shown to be involved in the long distance attraction of plant parasitic nematodes (Klinger 1965; Robinson 1995), and it also attracts EPNs (Gaugler et al. 1980; Lewis et al. 1993; Robinson 1995).

Chen and Moens (2003) reported that 45% mortality of *D. radicum* larva was caused by *S. feltiae* (10 IJs larva^{-1}) 4 days after application. Nielsen (2003) documented 77% mortality of third instar larvae of *D. radicum* caused by *S. feltiae*. In the present study, 60% larval mortality was obtained in the second week after *S. feltiae* application. Field trials against *D. radicum* produced variable results in persistence and larval mortalities. In all of these results, EPN dosage and environmental conditions played major roles (Hommes 1988; Bracken 1990; Simser 1992; Vänninen et al. 1992; Schroeder et al. 1996; Susurluk et al. 2001; Susurluk 2006; Yılmaz et al. 2009). Nielsen and Philipsen (2004) stated that, in a field trial, *D. radicum* populations could be suppressed by *S. feltiae*,, and *D. radicum* pupation was significantly reduced in cabbage plants when *S. feltiae* was applied early in the season.

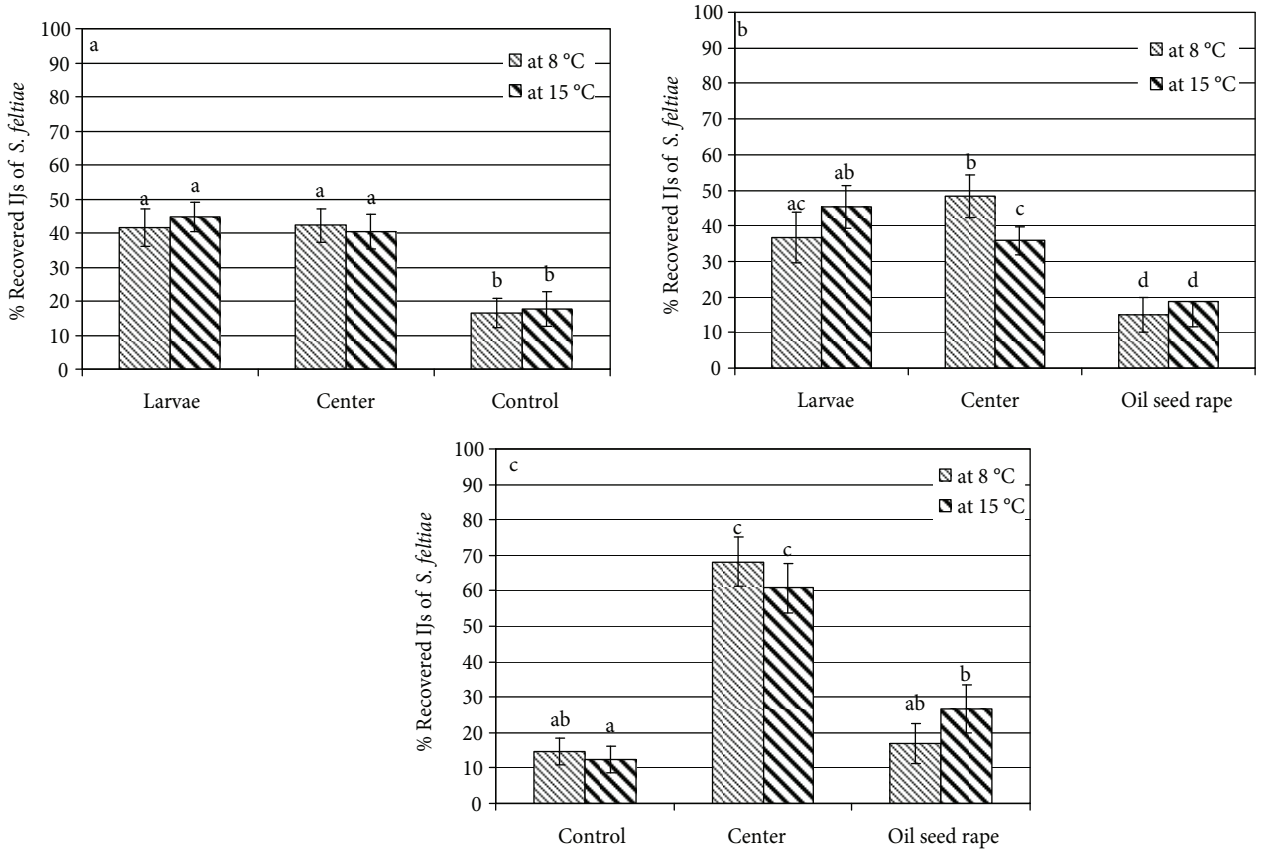

Figure 3. Distributions of IJs of *S. feltiae* washed out of the sand recovered from the different compartments of a Y-tube olfactometer that had been incubated at 8 and 15 °C for 10 days. Nematodes (1000 per olfactometer) were inoculated into the center compartment. a) In 1 compartment, 5 *D. radicum* larvae, and no stimulus in the control compartment; b) in 1 compartment, 5 *D. radicum* larvae, and 1 oilseed rape plant in the other; c) in 1 compartment, 1 oilseed rape plant, and no stimulus in the control. Columns with the same letter are not significantly different ($P < 0.05$). Error bars represent ±SE.

All of these results indicate that control of CM in oilseed rape by EPNs might be a possible strategy. At current costs, however, it is not economical to use EPNs against this pest. On the other hand, if the nematodes can be established and persist in wheat or barley crops, the inoculative application of EPNs could be economical. The introduction of EPNs into a field is less successful when the nematodes are applied in spring or early summer, when the host density might be too low due to insecticide use (Susurluk and Ehlers 2008). In contrast, application to the crop in autumn, when CM larvae are present, is a complete success. Thus, the antagonistic potential of *S. feltiae* populations can be preserved if host insects are provided by managing a suitable crop rotation regime.

In conclusion, *S. feltiae* seems to be a reasonable alternative for controlling *D. radicum*, even at lower temperatures, while most insecticides are not effective or persistent under these conditions. Further studies are needed to investigate this possibility for field treatment.

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References

Boff MIC, van Tol RHWM, Smits PH (2002) Behavioural response of *Heterorhabditis megidis* towards plant roots and insect larvae. BioControl 47: 67-83.

Boff MIC, Zoon C, Smits PH (2001) Orientation of *Heterorhabditis megidis* to insect hosts and plant roots in a Y-tube sand olfactometer. Entomol Exp Appl 98: 329-337.

Bracken GK (1990) Susceptibility of first-instar cabbage maggot *Delia radicum* (L.) (Anthomyiidae: Diptera), to strains of the entomogenous nematodes *Steinernema feltiae* Filipjev, *S. bibionis* (Bovien), *Heterorhabditis bacteriophora* Poinar, and *H. heliothidis* (Khan, Brooks, and Hirschmann). Can Entomol 122: 633-639.

Burnell AM, Stock SP (2000) *Heterorhabditis*, *Steinernema* and their bacterial symbionts — lethal pathogens of insects. Nematology 2: 31-42.

Chen S, Moens M (2003) Susceptibility of cabbage root maggot, *Delia radicum*, to entomopathogenic nematodes (Steinernematidae and Heterorhabditidae). Nematol Mediterranean 31: 157-162.

Cobb NA (1918) Estimating the nematode population of the soil. US Dept Agric Technol Circular 1: 48.

Ehlers RU (1996) Current and future use of nematodes in biocontrol: Practice and commercial aspects in regard to regulatory policies. Biocontrol Sci Tech 6: 303-316.

Ehlers RU (1998) Entomopathogenic nematodes - Save biocontrol agents for sustainable systems. Phytoprotection 79: 94-102.

Ehlers RU (2001) Mass production of entomopathogenic nematodes for plant protection. Appl Microbol Biot 56: 623-633.

Ehlers RU, Niemann I, Hollmer S, Strauch O, Jende D, Shanmugasundaram M, Mehta UK, Easwaramoorthy SK, Burnell A (2000) Mass production potential of the bacto-helminthic biocontrol complex *Heterorhabditis indica* - *Photorhabdus luminescens.* Biocontrol Sci Techn 10: 607-616.

Gaugler R (2002) Entomopathogenic Nematology. CABI Publ., Wallingford, UK.

Gaugler R, Lebeck L, Nakagaki B, Boush M (1980) Orientation of the entomogenous nematodes *Neoplactana carpocapsae* to carbon dioxide. Environ Entomol 9: 649-652.

Griffin CT (1993) Temperature responses of entomopathogenic nematodes: Implications for the success of biological control programmes. In: Nematodes and the biological control of insect pests (Eds. RAR Bedding and H Kaya). CSIRO, East Melbourne, pp. 115-126.

Harris CR, Svec HJ (1966) Mass rearing of the cabbage maggot under controlled environmental condition, with observation on the biology of cyclodiene-susceptible and resistant strains. J Econ Entomol 59: 569-573.

Hommes M (1988) Alternative possibilities of cabbage root fly control after efficiency of chlorfenvinphos and carbofuran begun to fail in some growing areas of the Federal Republic of Germany. Acta Hort 219: 47-51.

Klein Beekman MZ, Wiegers GL, Smits PH (1994) Biological control of cockchafer larvae (*Melolontha melolontha*) with the entomopathogenic nematode *Steinernema glaseri*. Medicine Faculty Landbouww, University of Gent 59: 411-419.

Klingler J (1965) On the orientation of plant nematodes and of some other soil animals. Nematologica 11: 4-18.

Lewis EE, Gaugler R, Harrison R (1993) Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. Can J Zool 71: 765-769.

Nielsen O (2003) Susceptibility of *Delia radicum* to steinernematid nematodes. BioControl 48: 431-446.

Nielsen O, Philipsen H (2004) Occurrence of *Steinernema* species in cabbage fields and the effect of inoculated *S. feltiae* on *Delia radicum* and its parasitoids. Agric Forest Entomol 6: 25-30.

Robinson AF (1995) Optimal release rates for attracting *Meloidogyne incognita*, *Rotylenchus reniformis* and other nematodes to carbon dioxide in sand. J Nematol 27: 42-50.

Schroeder PC, Ferguson CS, Shelton AM, Wilsey WT, Hoffmann MP, Petzoldt C (1996) Greenhouse and field evaluations of entomopathogenic nematodes (Nematoda: Heterorhabditidae and Steinernematidae) for control of cabbage maggot (Diptera: Anthomyiidae) on cabbage. J Econ Entomol 89: 1109-1115.

Simser D (1992) Field application of entomopathogenic nematodes for control of *Delia radicum* in collards. J Nematol 24: 374-378.

Sulistyanto D, Peters A, Hokkanen H, Ehlers RU (1994) Evaluation of entomopathogenic nematode strains for control of *Delia radicum*, *Tipula paludosa* and *T. oleracea*. IOBC/WPRS Bulletin 17: 140-143.

Susurluk A (2006) Effectiveness of the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema feltiae* against *Tenebrio molitor* (yellow mealworm) larvae in different soil types at different temperatures. Turk J Biol 30: 199-205.

Susurluk A, Dix I, Stackebrant E, Strauch O, Wyss U, Ehlers RU (2001) Identification and ecological characterisation of three entomopathogenic nematode-bacterium complexes from Turkey. Nematology 3: 833-841.

Susurluk A, Ehlers RU (2008) Field persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora* in different crops. BioControl 53: 627-641.

Susurluk IA (2005) Establishment and persistence of the entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora*. PhD Dissertation. Christian-Albrechts-Universität zu Kiel, Agrar- und Ernährungswissenschaftlichen Fakultät, Kiel, Germany.

Vänninen I, Vainio A, Hokkanen H (1992) Attempts to control *Delia* spp. with entomopathogenic nematodes. Bulletin OILB SROP 15: 143-153.

Yılmaz H, Waeyenberge L, Demir İ, Moens M, Demirbağ Z (2009) A new entomopathogenic nematode species for Turkey, *Heterorhabditis megidis* Poinar, Jackson & Klein 1987 (Rhabditida: Heterorhabditidae). Turk J Agric For 33: 385-391.