

The impact of chemical nematicides on entomopathogenic nematode survival and infectivity

Mustapha Touray^{1,*}, Harun Cimen¹,
Sebnem H. Gulsen¹, Derya Ulug¹,
Dolunay Erdogus², David
Shapiro-Ilan³ and Selcuk Hazir¹

¹Biology Department, Faculty of
Arts and Science, Aydin Adnan
Menderes University, Aydin, Turkey.

²Directorate of Plant Protection
Central Research Institute,
Yenimahalle, Ankara, Turkey.

³USDA-ARS, Southeastern Fruit
and Tree Nut Research Station,
Byron, GA 31008.

*E-mail: mtpha.touray@gmail.com

This paper was edited by
Raquel Campos-Herrera.

Received for publication
February 05, 2021.

Abstract

Entomopathogenic nematodes (EPNs) belonging to the genera, *Steinernema* and *Heterorhabditis*, occur naturally in the soil along with plant-parasitic nematodes which are important root pests of many different crops. Here, we report the effects of four registered nematicidal compounds (fluopyram, fosthiazate, metam potassium, and fenamiphos) that are used for the control of PPNs on the survival, virulence, penetration efficiency, and reproduction of *S. carpocapsae* and *H. bacteriophora*. Despite previous studies warning of the impact of nematicidal compounds on IJ survival and infectivity, none have assessed their impact on EPN chemotaxis, penetration into and reproduction in insect host or conducted longer term soil assays. Survival of EPNs, based on observing IJ movement under a stereomicroscope after incubation in different concentrations of nematicides, showed that $\geq 80\%$ of both nematode species were killed by fosthiazate, fenamiphos and metam potassium within 24 h. The recommended concentration of fluopyram killed 33% of *H. bacteriophora*, and 28% of *S. carpocapsae* after 48 h exposure. IJs exposed to the nematicides were less virulent against *Galleria mellonella* larvae in sand bioassay to non-treated IJs as significantly more control IJs of both EPN species penetrated their insect host ($\sim 47\%$ of IJs added) than IJs exposed to fluopyram and fosthiazate; and the number of IJ progeny emerging from these controls was significantly higher than the number of IJ progeny emerging from IJs treated with the nematicidal compounds. In a highly novel discovery, the chemotaxis experiments using Pluronic F-127 gel indicated that *H. bacteriophora* were repelled from nematicide-treated environments. Moreover, IJs of both species added to treated natural loamy-clay soil at 5-day-intervals for 30 days post application of nematicides were generally unable to induce *G. mellonella* mortality, except for *S. carpocapsae* IJs added to fosthiazate-treated soil. Overall, our study indicates that there were detrimental effects of the nematicides on EPN IJs in treated soil. Therefore, overlap in timing for control of PPNs and the use of EPNs for biological insect control must be avoided. Future studies should assess the optimization of timing for nematicides use and survival and infectivity of IJs in the soil.

Keywords

Chemotaxis, Entomopathogenic Nematode, Fluopyram, Fosthiazate, *Heterorhabditis*, Metam Potassium, Plant-parasitic nematodes, *Steinernema*.

Plant parasitic nematodes (PPNs) are problematic soil dwelling pests. They attack and cause severe mechanical and physiological damage to plant roots as ecto- and endo-parasites as they feed on the sap and tissues with their stylet mouthparts. Subsequent effects include negative impact on plant growth and yield and may lead to plant death due to the PPNs (Atkinson et al., 2012; Forghani and Hajihassani, 2020; McCarter, 2009). PPNs feeding activity may also lead to secondary infections by other phytopathogens like bacteria, fungi and viruses (Lambert and Bekal, 2002; Viglierchio, 1991). It is estimated that PPNs, directly or indirectly, cause substantial agricultural and economic losses that amount to \$100 billion worldwide (Atkinson et al., 2012; Kepenekci et al., 2018; McCarter, 2009). For precisely these reasons, different methods such as the use of resistant cultivars, soil solarization, chemical control, biological control approaches or combinations thereof are used to manage PPNs from damaging high valued crop plants. Among these, however, chemical control is the most frequently and readily used approach. It involves the application of non-volatile, highly toxic and broad spectrum chemical nematicides such as fosthiazate, fenamiphos, ethoprophos, metam potassium, etc., to soil through drip irrigation or soil drenching. These chemicals are less toxic to handlers and operators than fumigant nematicides (Chitwood, 2003). Use of these nematicides to manage PPNs ensures that plant root and stem systems remain healthy, thereby maintaining plant vigor and preventing associated yield loss (Chitwood, 2003; Food and Agriculture Organization of the United Nations, 2002). Yet, in order to be efficacious against PPNs, these chemicals need to persist in the soil for a prolonged period, and during this extended persistence, nematicides can pollute groundwater and have toxic effects on non-target organisms that live in soil. Accordingly, the use of more harmful nematicides is banned or substantially restricted in several developed countries (Bird and Kaloshian, 2003; Chitwood, 2003; Lambert and Bekal, 2002) but are still available for use in many developing or underdeveloped countries (Global Situation of Pesticide Management in Agriculture and Public Health, 2019; Manyilizu, 2019).

Entomopathogenic nematodes (EPNs) in the genera *Steinernema* (Rhabditida: Steinernematidae) and *Heterorhabditis* (Rhabditida: Heterorhabditidae) are lethal natural enemies of soil dwelling insects. They are ubiquitous and occur naturally nearly worldwide in many soils (Glazer, 2002; Hominick et al., 1996). Although they do occur in many soils, the natural population often does not occur in sufficient numbers to effectively control insect pests. Fortunately, EPNs

are easily mass-produced, do not require registration in many countries and are commercially available, are easily applied with standard spray equipment, are safe to humans and other vertebrates, and serve as important alternatives to chemical insecticides (Gulcu et al., 2017; Shapiro-Ilan et al., 2012, 2017). Thus, these beneficial and highly virulent organisms can be reared in laboratories and applied to suppress insect pests to augment the natural EPN populations or in soils where EPNs do not occur (e.g., nursery soils), or against insects in cryptic habitats (e.g., tree trunks, rhizomes) (Casteliani et al., 2020; Gulcu et al., 2017; Shapiro-Ilan et al., 2017). However, formulated forms of the nematicidal compounds are applied around the rhizosphere to kill PPNs actively searching for vulnerable roots of valuable plant crops; and since *Steinernema* spp. and *Heterorhabditis* spp. share many similar basic biological and physiological characteristics with other species in the Nematoda phylum (Viglierchio, 1991; Weischer and Brown, 2000) and occupy similar soil niches as PPNs (Kenney and Eleftherianos, 2016), some studies have investigated the impact of nematicidal compounds like oxamyl, avermectin, metham sodium, butyric acid and fenamiphos on EPN species like *H. bacteriophora* and *S. carpocapsae* (Browning et al., 2004; Glazer et al., 1997; Hara and Kaya, 1982; Rovesti et al., 1988, Rovesti and Deseo, 1990). These studies mostly aimed to assess the compatibility of these compounds with EPNs based on the survival and infectivity of IJs. There, however, is a need to expand on the diversity of nematicides that are tested for impact on EPN, and their effects on the penetration efficacy and reproductive capacity; chemotaxis and longer-term soil studies have not been addressed. Our objectives, herein, were to investigate the effects of four nematicidal compounds viz. fluopyram, metam potassium, fosthiazate, and fenamiphos on *S. carpocapsae* and *H. bacteriophora* survival, virulence, and penetration efficacy after exposure to nematicides in aqueous suspensions as well as in nematicide-treated soil. A novel assay measuring chemotaxis was also conducted. In addition, we included the plant-parasitic nematode (PPN) *Meloidogyne incognita*, for comparison.

Materials and methods

Nematicides

Nematicide products and their active ingredients used in this study are listed in Table 1. A stock solution of each nematicidal product was prepared in distilled water at double strength of the required concentration

Table 1. Nematicide products used in this study.

Active ingredient	Concentration (g/L)	Commercial name	Manufacturer	Recommended field rate (ml/l)	Pesticide class	Formulation
Fluopyram	400	Velum Prime® SC400	Bayer CropScience, Germany	0.6	Succinate dehydrogenase inhibitor	SC
Fosthiazate	150	Versus® 150EC	Hektaş®, Turkey	10	Organophosphate	EC
Metam potassium	510	Rotabent®	Hektaş®, Turkey	1000	Dithiocarbamate Salt	SL
Fenamiphos	400	Nemacur® 400EC	Hektaş®, Turkey	75	Organophosphate	EC

Notes: Formulation; EC: emulsifiable concentrate; SC: suspension concentrate; SL: Soluble liquid.

to represent the field recommended rate (as suggested by manufacturer) when the same volume of nematode suspension was added. Fosthiazate and fenamiphos are anticholinergic nematicides that are members of the organophosphate class of pesticides, metam potassium (Potassium n-methyldithiocarbamate) is a dithiocarbamate that are analogs of carbamate pesticides that block the activity of enzymes, such as acetylcholinesterase and Fluopyram, is an succinate dehydrogenase inhibitor (NCBI, 2021).

Maintenance of *Galleria mellonella*, EPNs, and PPNs

Galleria mellonella (Lepidoptera: Pyralidae), the greater wax moth, was used in experiments to determine the infectivity and reproduction of the two EPN species. Laboratory cultures of the wax moth were reared in 1-liter glass containers and fed an artificial diet (Touray et al., 2020). These containers were maintained in an insect rearing room (temperature 28°C). Prior to any use, the last instar larvae were heat-treated by immersing in water at 60°C twice for 10 secs each; this treatment prevents the larvae from spinning of their silken cocoon and proceeding to the pupal stage. The heat-treated larvae were kept at 10°C until further use (Touray et al., 2020). The larvae used in the experiments weighed 200 (\pm 10) mg.

Turkish strains of the EPN species, *Steinernema carpocapsae* (Rize isolate) and *Heterorhabditis bacteriophora* (isolate 09–20), were used in the experiments. These nematodes were reared in the last instar of *G. mellonella* in 9cm Petri dishes according to Kaya and Stock (1997). Infected insects were placed on White traps (White, 1927); and the freshly emerged IJs were obtained for the experiment. These nematodes were stored at 10°C in Tetra Pak containers (Gulcu and Hazır, 2012) and used within 2 weeks.

The PPN species, *Meloidogyne incognita*, was obtained from cultures maintained at the Directorate of Plant Protection Central Research Institute, Ankara, Turkey. Second juvenile (J2) stage of this nematode were obtained from egg masses extracted from the roots of tomato seedlings (Tuezza F.) (Evlice et al., 2020), stored 10°C at and used within 2 weeks.

Assessment of the direct effects of nematicides on nematodes in distilled water

Experiments were conducted using 24-well tissue culture plates as described by De Nardo and Grewal

(2003). Briefly, 1,500 IJs in 0.5 ml of water and 0.5 ml of the double strengthened stock solution of one of the nematicides were added to each well of the 24-well plates. This method was used for each nematicide and represented the recommended field rate of the nematicide. Water treatment was included as a negative control. The tissue culture plates were incubated at $25 \pm 1^\circ\text{C}$ for 24 h.

Survival of EPNs and PPNs

In the initial experiment, we tested the field rate of the nematicides on the survival of *H. bacteriophora*, *S. carpocapsae*, and *M. incognita*. After 24-hour exposure of the EPNs and PPN to the nematicide treatments at the recommended field concentration, the viability of IJs or J2 stage, respectively, was assessed by removing 0.5 ml sample from each well of the replicate and examining the first 100 IJs or J2 stage and recording the ratio of dead and alive nematodes. Before taking the sample, the contents in each well were thoroughly mixed by pipetting before removal of the sample. Nematodes were considered dead if they were immobile after prodding with blunt needle (De Nardo and Grewal, 2003; Touray et al., 2020). The ratio of dead/alive IJs was recorded. There were 12 replicates, and the experiments were conducted twice on different dates. No further assessment was made with the PPN, but the EPNs were subjected to additional evaluation.

Survival of EPNs at different nematicidal dilution rates

Serial dilutions (i.e., 50, 25, 12.5, 6.25, and 3.13% of the recommended field concentration) of the nematicides were tested to determine the threshold of where each nematicide allowed for EPN survival. After 24- and 48-hour exposure of the EPNs to the nematicide treatments, the viability of IJs was assessed by removing 0.5 ml sample from each well of the replicate and examining the first 100 IJs as described above. There were 12 replicates, and the experiment was conducted twice on different dates.

Virulence, penetration efficiency, and reproduction of EPNs

IJ infectivity was assessed against *G. mellonella* using a sand-well bioassay in 24-well plates (Touray et al., 2020). During the assessment of IJ survival, 50 live IJs exposed to a given nematicide for 24 and 48 h were collected from each treatment and rinsed with water and then were transferred with 60 μl distilled

water into wells containing 0.5 g of sterile sandy soil. One *G. mellonella* larva was added to each well. The plates were incubated at room temperature ($24\text{--}25^\circ\text{C}$). After 48-hour incubation, the mortality of the larvae was recorded, and the dead larvae were separated into two equal groups. In the first group, each cadaver was dissected in pepsin solution and the number of IJs penetrating after exposure to the nematicidal products was counted with the aid of a stereomicroscope (Mauleon et al., 1993). With the second group, each insect cadaver was placed individually on a White trap (White, 1927). The total number of emerging IJs was collected at the end of emergence (after 2 weeks for *S. carpocapsae* and 3 weeks for *H. bacteriophora*) and counted to assess EPN progeny production (Kaya and Stock, 1997). Nematodes were collected from each well in section 2.3.1 and the experiment was conducted twice on different dates.

Virulence and penetration efficacy of IJs in nematicide-treated soil

The effects of nematicides were evaluated in a soil environment as their effects may differ in treated soil and to ascertain when it is safe to apply EPNs to previously nematicide-treated soil. To this end, we applied 30 ml of nematicides at recommended concentration to 335 g of natural loamy-clay soil in 500 ml, clear hinged lid, plastic containers ($10 \times 5 \times 11$ cm). The soil was collected from a garden in Sercekoy village, Aydin, Turkey. Before the experiments, the soil was baited with *G. mellonella* larvae to check if the native EPNs were present. In the laboratory, soil was sieved to remove large particles but was not autoclaved so as to maintain its natural condition. The containers were incubated at room temperature ($24\text{--}25^\circ\text{C}$). Soil in the control group only had tap water added. After 1-, 5-, 10-, 15-, 20-, 25- and 30-days post application (dpa) of nematicides at room temperature ($24\text{--}25^\circ\text{C}$), 25 IJs/ cm^2 were pipetted on to the soil surface (110 cm^2) and the nematodes were left to acclimatize for a further 24 h in treated soil and control. The final moisture of the soil was 18% (v/v) as this is typical level of moisture expected to be encountered under field conditions. Afterwards, four *G. mellonella* larvae were added to each container to investigate the virulence and penetration rate of treated IJs after each of these periods. Larval mortality was assessed 72 h after they were added and the cadavers were dissected in pepsin solution as described above to determine the number of penetrating IJs. Each nematicide had three replicates for each time interval and the study was conducted twice.

Chemotaxis

Chemotactic response of *H. bacteriophora* towards or away from non-treated and nematicide treated areas was evaluated using a modified chemotaxis assay (Hummadi et al., 2020; Wang et al., 2009). *H. bacteriophora* was used in the chemotaxis experiments because this nematode species is a forager/cruiser so it responds strongly to cues compared to *S. carpocapsae*, which is an ambusher that can remain motionless for several hours (Lewis, 2002; Lewis et al., 2006). Pluronic F-127 (Sigma-Aldrich, Germany) was used as the substrate. First, 28.75% (w/v) aqueous Pluronic F-127 gel medium was prepared by dissolving powdered gel in 100ml distilled water and incubating mixture at 5°C overnight. Then, 2.5ml of prepared gel was poured on microscope slides (2.5×7.5cm), which were divided into three equal zones (Z1, Z2, and Z3), and allowed to solidify (Hummadi et al., 2021; Wang et al., 2009). The substrate was approximately two mm thick. In all, 30µl of an aqueous suspension of ca. 100 IJs was placed in the center of the slide (Z2), 5µl of a nematicide on the right side (Z3), and 5µl distilled water on the left (Z1) (Fig. 1). Recommended field concentration (100%), and also 1 and 0.1% dilutions of the field concentration of each nematicide were tested. Each concentration had four replicates and the study was conducted thrice on different dates. A control treatment was also added, i.e., water versus water. Each microscope slide was placed in a Petri dish, sealed with parafilm to minimize moisture loss and incubated in the dark at room temperature (23–24°C) and after 3, 6 and 24 h, the number of IJs in Z1 and Z3 zones were counted and recorded.

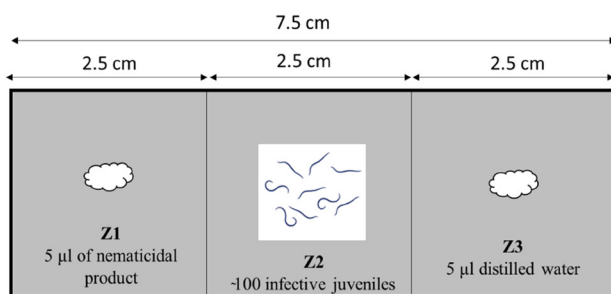


Figure 1: Experimental design using 7.5 cm glass slides to investigate the response of *Heterorhabditis bacteriophora* infective juveniles to different concentrations of nematicidal compounds and non-odor control after 3, 6 and 24 h.

Values were used for calculating the using the formula in Baiocchi et al. (2020),

$$\text{Chemotaxis index (CI)} = \frac{(\# \text{ of IJs in Z1} - \# \text{ of IJs in Z3})}{(\# \text{ of IJs in Z1} + \# \text{ of IJs in Z3})}$$

Data analysis

Data were analyzed in IBM® SPSS Statistics 23. Means were compared using analysis of variance (one-way ANOVA) followed by Tukey's Honestly Significant Difference test at $P = 0.05$ level to compare the effects of the different nematicidal products at different concentrations on the survival, virulence, insect penetration rate and reproductive capacity of *S. carpocapsae* and *H. bacteriophora*.

Results

Survival of EPNs and the PPNs

Comparison of the effects of the recommended field concentration of nematicides on the nematode species showed that *S. carpocapsae* IJs were more tolerant than *H. bacteriophora* and *M. incognita* to fluopyram ($F = 1,436.27$; $df = 2,69$; $P < 0.0001$), but all three species were adversely affected by fenamiphos ($F = 15.826$; $df = 2,69$; $P < 0.001$), fosthiazate ($F = 1,359.04$; $df = 2,69$; $P < 0.001$) and metam potassium ($F = 7.56$; $df = 2,69$; $P < 0.001$) (Figure 2). Mortality of nematodes in the control (without nematicides) was $> 93\%$.

Survival of EPNs at different nematicidal dilution rates

The survival of both *H. bacteriophora* (Fig. 3) and *S. carpocapsae* (Fig. 4) were greatly affected after exposure to different concentrations of the nematicides, especially fosthiazate, fenamiphos, and metam potassium. Treatments between the recommended concentration (100%) to 12.5% of these nematicides (i.e., fosthiazate, fenamiphos, and metam potassium) were highly detrimental to *H. bacteriophora* IJs, with no more than 20% of IJs surviving in these treatments (Fig. 3). At 6% tested concentration, the survival of *H. bacteriophora* IJs were 22, 60, and 59% for fenamiphos ($F = 1,168.29$; $df = 6,161$; $P < 0.0001$), fosthiazate ($F = 454.67$; $df = 6,161$; $P < 0.001$) and metam potassium ($F = 162.08$; $df = 6,161$; $P < 0.0001$), respectively, at 24-hour assessment, whereas the effects of 3.13% concentration ranged between 63 and 85% for these nematicides. Mortality increased significantly after 48-h exposure ($P < 0.05$) (Fig. 3).

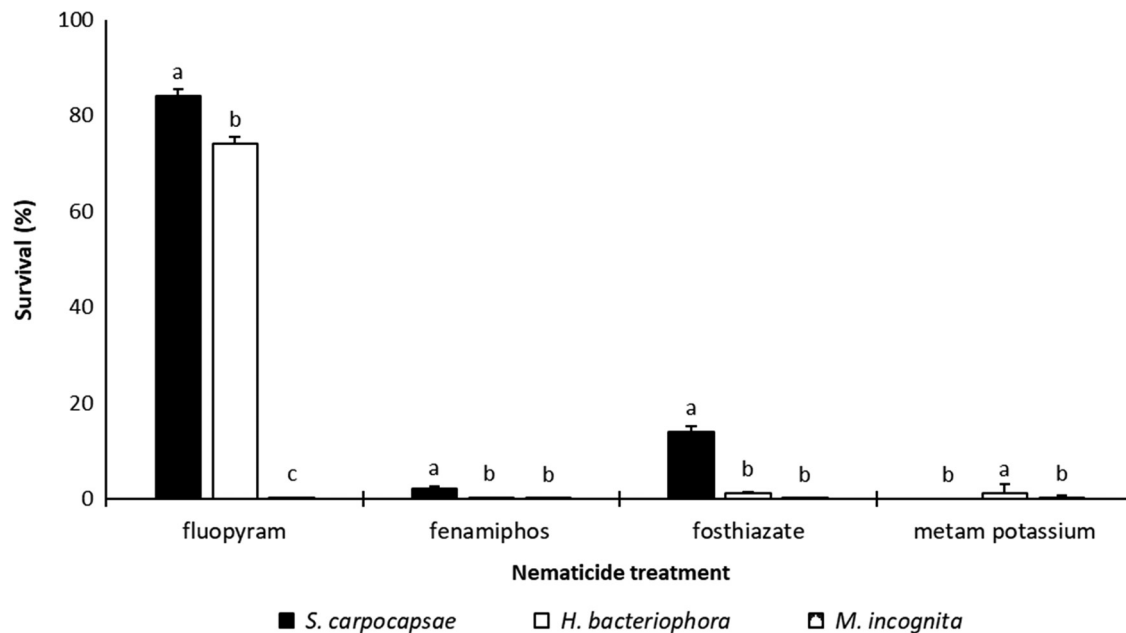


Figure 2: Comparison of the survival rates (mean \pm SE) of different nematode species (*Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, and *Meloidogyne incognita*) after 24-hour exposure to the recommended field rate of the nematicides in 24-well plates. Different letters above bars indicate statistical significance (Tukey's test $P \leq 0.05$).

Similarly, there was a downward trend in the survival rates of *S. carpocapsae* after exposure to increasing concentrations of fosthiazate, fenamiphos and metam potassium (Fig. 4). Overall, survival rate in control group was more than 90%. Following the control group, survival of IJs was highest at the 3.13% concentration for these nematicides. There were highly significant differences between the treatments for fosthiazate ($F=462.07$; $df=6,161$; $P<0.001$), fenamiphos ($F=434.73$; $df=6,160$; $P<0.0001$), and metam potassium ($F=533.15$; $df=6,161$; $P<0.0001$) (Fig. 4). As for fluopyram, the recommended field concentration killed 26% of *H. bacteriophora* and 16% of *S. carpocapsae* after 24-hour exposure. The effects of 6.25 and 3.13% of recommended field concentrations of fluopyram on *H. bacteriophora* and the effects of 12.5% 6.25 and 3.13% on *S. carpocapsae* were similar to control (water) but were statistically higher than the other tested concentrations (for *H. bacteriophora* $F=21.121$; $df=6,161$; $P<0.001$, for *S. carpocapsae* $F=38.92$; $df=6,161$; $P<0.001$) (Fig. 4).

Virulence, penetration efficiency and reproduction of EPNs

Generally, *H. bacteriophora* exposed to nematicides were less infective against *G. mellonella* larvae

compared to non-treated IJs. IJs exposed to metam potassium and fenamiphos nematicides lost their virulence even after exposure to the lowest tested concentration (3.13%) (Fig. 5). *H. bacteriophora* IJs exposed to 3.13% of recommended field concentration of fluopyram killed 63% of insect host whereas 6.25% dilution killed 29% after 24-hour exposure. Statistically more *G. mellonella* were killed by IJs in the control group (no nematicide) compared to treated nematodes ($F=32.89$; $df=6,161$; $P<0.001$). After 48-hour exposure, mortality of insect hosts declined to 13 and 50% for the 6.25 and 3.13% tested concentrations of fluopyram, respectively; IJs in the control killed 92% of insect host ($F=55.50$; $df=6,161$; $P<0.001$) (Fig. 5). As for fosthiazate, only *H. bacteriophora* IJs exposed to 3.13% nematicide concentration caused 4% *G. mellonella* death after 24-hour exposure; IJs in control killed significantly more insect host (92%) ($F=163.98$; $df=6,161$; $P<0.001$) (Fig. 5).

With *S. carpocapsae*, the percentage of *G. mellonella* larvae killed was significantly higher for IJs in control group (100% mortality) and 3.13% of fluopyram (92% mortality) compared to the higher concentrations of the nematicides (i.e., from recommended concentration to 6.25%) after 24-hour exposure ($F=32.89$; $df=6,161$; $P<0.001$) (Fig. 5). After 48-hour treatment with fluopyram, virulence of

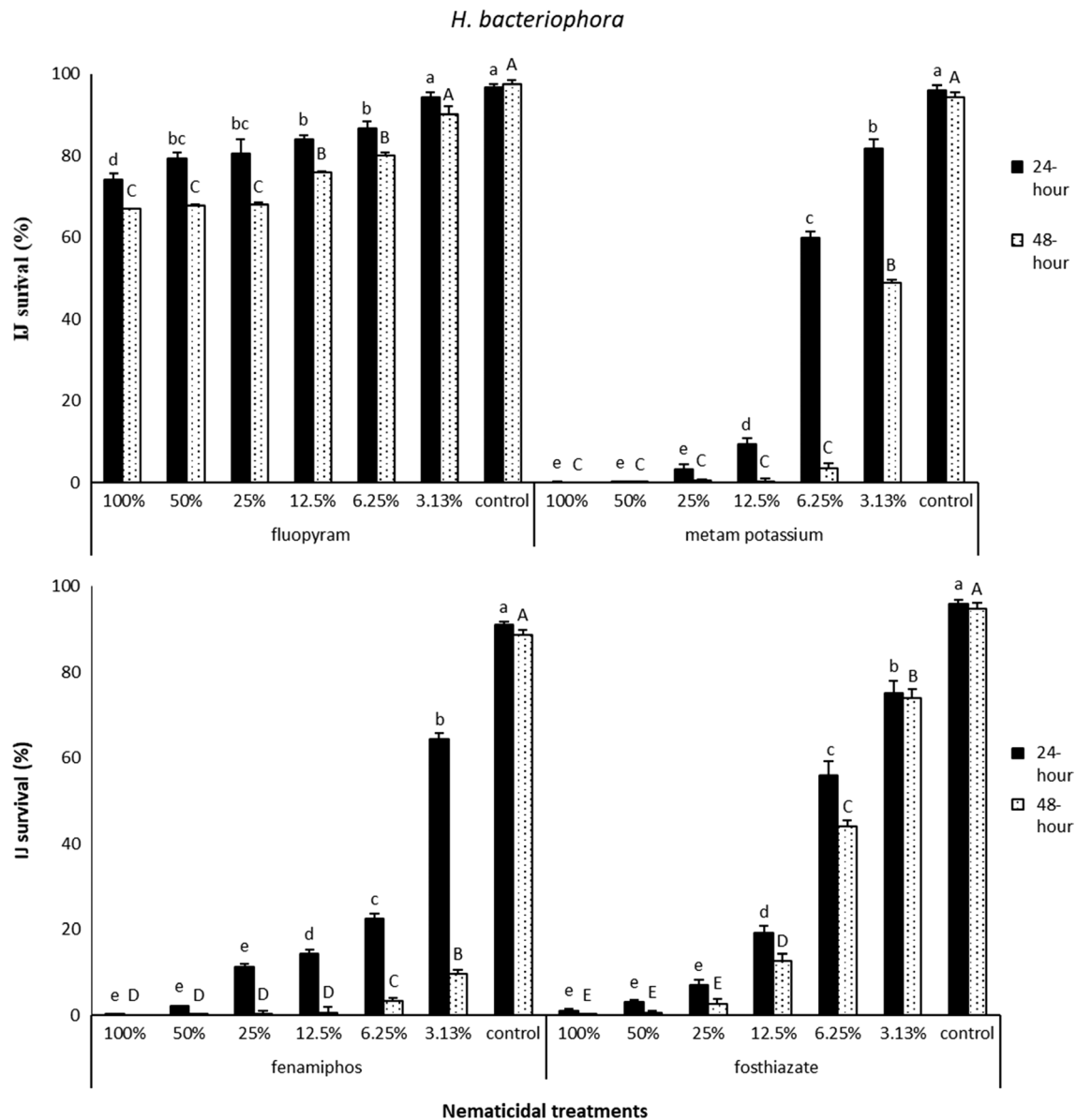


Figure 3: Survival rates (mean \pm SE) of *Heterorhabditis bacteriophora* infective juveniles (IJ) after 24- and 48-h exposure to each nematicide in wells of 24-well plates. Different letters above bars indicate statistical significance (Tukey's test $P \leq 0.05$). The percentages refer to the rate of the nematicide solutions; that is, 100% is the recommended field rate (see Table 1) and the descending percentages refer to the dilution of the field rate so that 3.13% is the lowest dilution of the nematicide tested.

S. carpocapsae IJs decreased from 92 to 72% for 3.13% tested concentration and from 42 to 25% for 6.25% concentration; this was statistically lower than the mortality caused by IJs in the control group ($P < 0.05$). For fosthiazate, IJs of *S. carpocapsae* in control group were significantly more infective than those treated with fosthiazate nematicide for 24h ($F = 31.25$; $df = 6, 161$; $P < 0.001$) and 48h ($F = 49.98$;

$df = 6, 161$; $P < 0.001$) (Fig. 5). Like *H. bacteriophora*, *S. carpocapsae* IJs exposed to metam potassium and fenamiphos nematicides lost their virulence.

For the penetration efficiency, the number of *S. carpocapsae* IJs that invaded *G. mellonella* larvae was significantly higher in the control (no nematicide) than the fluopyram ($F = 48.49$; $df = 2, 27$; $P < 0.0001$) and fosthiazate ($F = 32.65$; $df = 2, 15$; $P < 0.0001$)

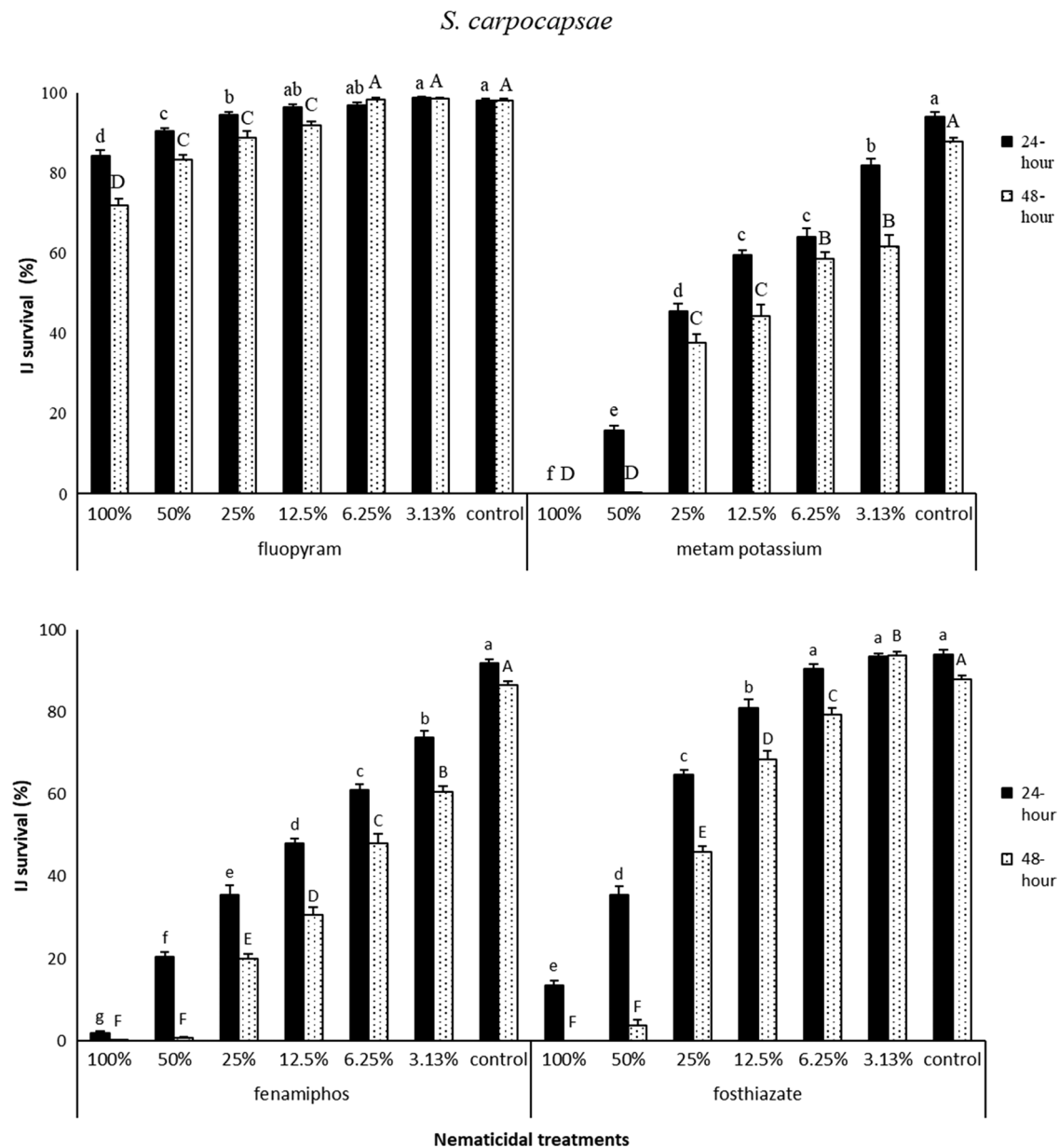


Figure 4: Survival rates (mean \pm SE) of *Steinernema carpocapsae* infective juveniles (IJ) after 24- and 48-hour exposure to each nematicide in wells of 24-well plates. Different letters above bars indicate statistical significance (Tukey's test $P \leq 0.05$). The percentages refer to the rate of the nematicide solutions; that is, 100% is the recommended field rate (see Table 1) and the descending percentages refer to the dilution of the field rate so that 3.13% is the lowest dilution of the nematicide tested.

treatments after 24-hour exposure (Fig. 6). Similarly, the number of *H. bacteriophora* IJs that penetrated host was higher in control compared to the fluopyram ($F=44.32$; $df=2,24$; $P<0.0001$) (Fig. 6).

In the case of the effects of the nematicides on the reproduction of the nematodes, a higher number of *S. carpocapsae* IJs emerged from hosts in the

control than from the fluopyram treatment ($F=6.055$; $df=3,25$; $P<0.001$). As for hosts killed by IJs exposed to fosthiazate, there was a statistical difference in the number of *S. carpocapsae* IJs that emerged from larvae in control compared to those in the nematicidal treatment ($F=0.833$; $df=2,14$; $P=0.455$) (Fig. 7). Similarly, the number of *H. bacteriophora*

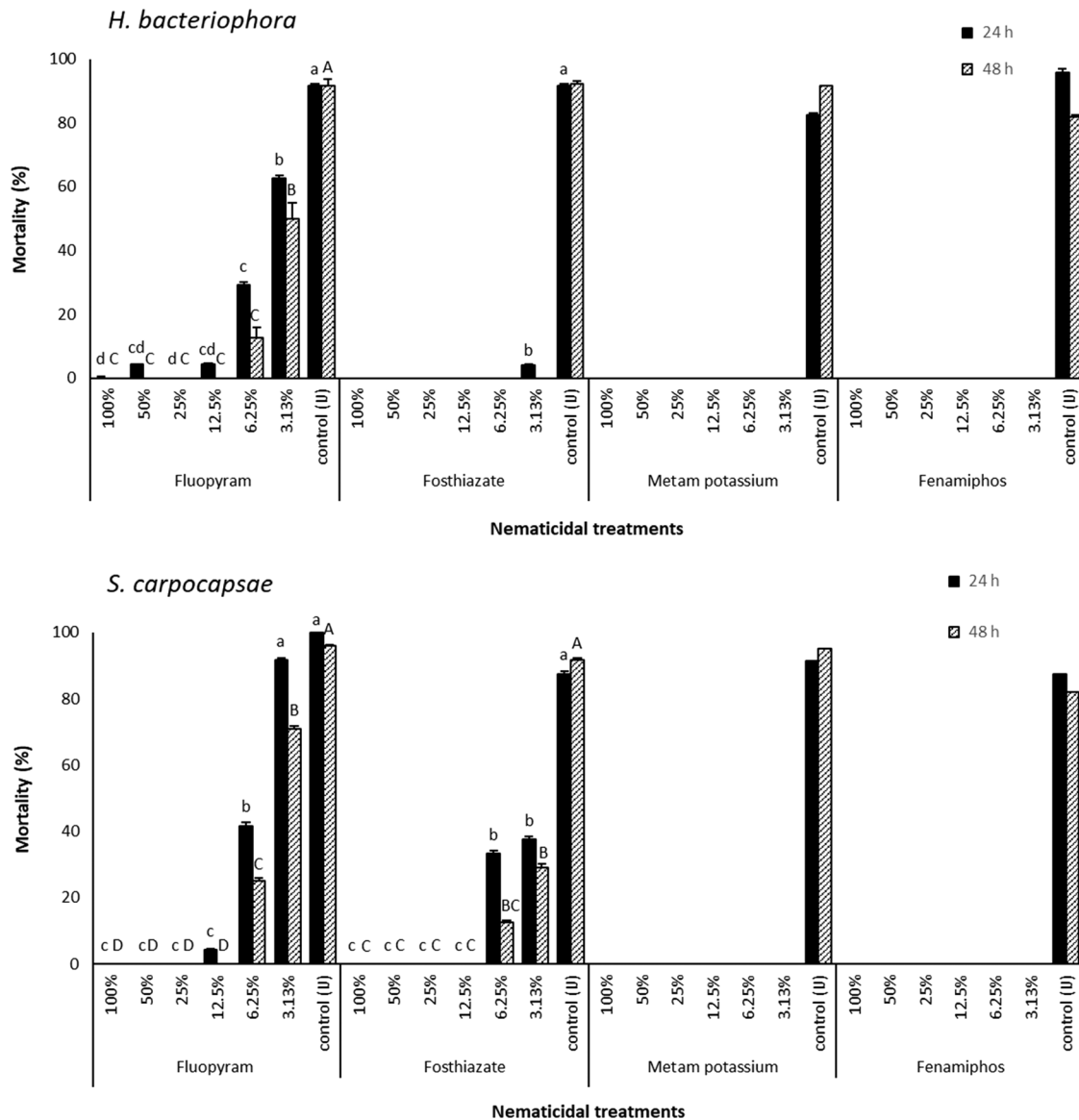


Figure 5: Mortality of *Galleria mellonella* (mean \pm SE) after exposure to nematocidal-treated *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* infective juveniles.

IJs that emerged from the hosts in the control was significantly higher than those in the nematocidal treatments as 44,560 IJs were collected from control, whereas less than 7,000 IJs was collected from the host killed by IJs exposed to different concentrations of fluopyram ($F=33.23$; $df=6,3$; $P<0.001$) (Fig. 7).

Virulence and penetration efficacy of IJs in nematocidal-treated soil

For *S. carpocapsae*, the mortality of *G. mellonella* larvae ranged between 88 and 100% at one dpa. All the insects added to containers with fosthiazate

and the control died throughout the 30-day period. Except on the 30 dpa, all the larvae in metam potassium treated soil were killed (Fig. 8A). For fluopyram and fenamiphos larval mortality was 88% at 1 dpa but gradually decreased to 33 and 16%, respectively, by 30 dpa (Fig. 8A). Correspondingly the cadavers were further dissected in pepsin solution as some in the treated group did not have the signs that distinguished EPN-infection. In total, only 13, 22, 0, and 80.9% of the dissected cadavers had *S. carpocapsae* IJs in metam potassium, fluopyram, fenamiphos and fosthiazate treated groups, respectively. At 1 dpa, on average 0.9, 26.5, and 52

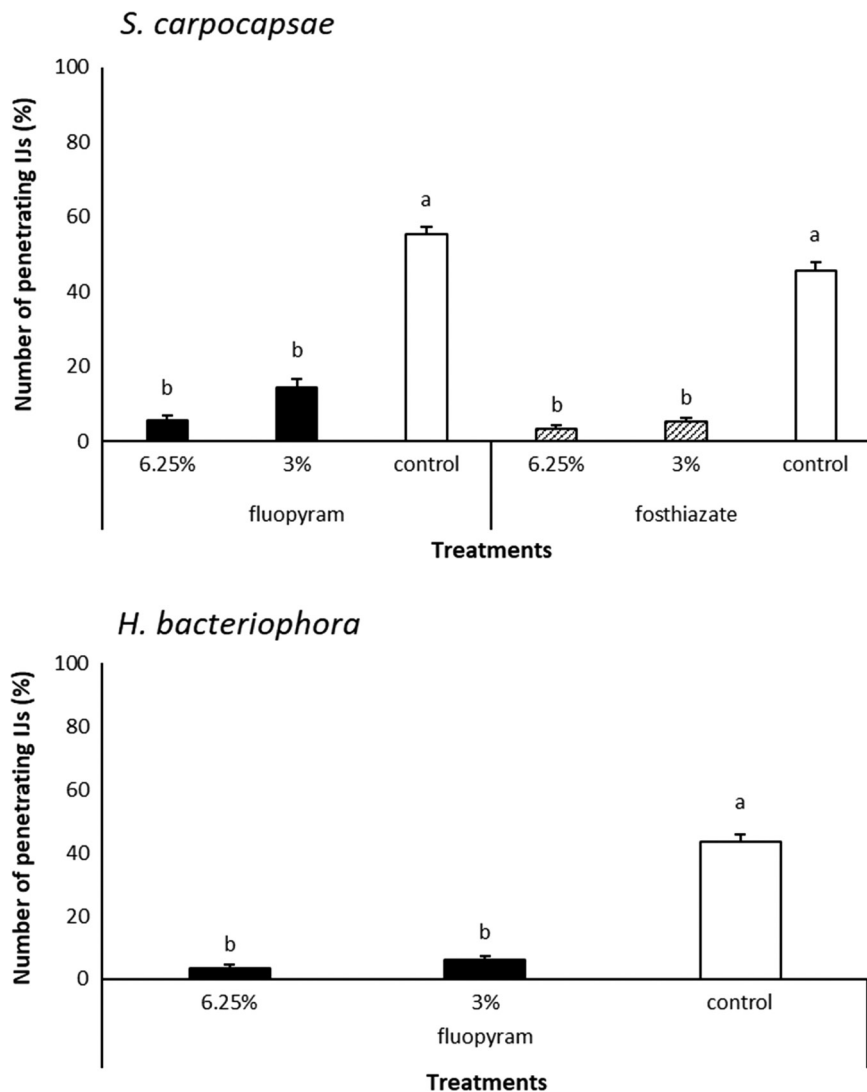


Figure 6: Percentage (\pm SE) number of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* infective juveniles (IJ) invading the insect host (*Galleria mellonella*) after 24-hour exposure to nematicides. Different letters above bars indicate statistical significance (Tukey's test $P \leq 0.05$).

S. carpocapsae IJs penetrated *G. mellonella* cadavers in fluopyram-, fosthiazate-treated soil, and control group, respectively. No IJs penetrated the insects for metam potassium and fenamiphos at 1 dpa. The number of IJs penetrating *G. mellonella* at all points of assessment for *S. carpocapsae* ranged between 52–69 in control and 26–56 for fosthiazate, whereas less than 5 IJs were found in larvae for fluopyram and metam potassium (Fig. 8B).

Similarly, 100% of the insects added to metam potassium and control were killed at all points of assessment for 30 days in the experiments for *H. bacteriophora* (Fig. 9A). For fosthiazate, 40% of *G. mellonella* were affected at 1 dpa; this decreased to

16% by the 30th day. Insect mortality fluctuated between 8.3 and 25% for fluopyram. No larva died in fenamiphos group at all points of assessment. Only 4.7, 7.1, 0, and 11.9% of the dissected cadavers had *H. bacteriophora* IJs in metam potassium, fluopyram, fenamiphos, and fosthiazate treated groups, respectively. Less than three *H. bacteriophora* IJs penetrated *G. mellonella* in all the nematicide-treated soil compared to at least 30 IJs penetrating insects in control (Fig. 9B).

Chemotaxis

Fig. 10 shows the responses of *H. bacteriophora* IJs to no odor source or sides with different

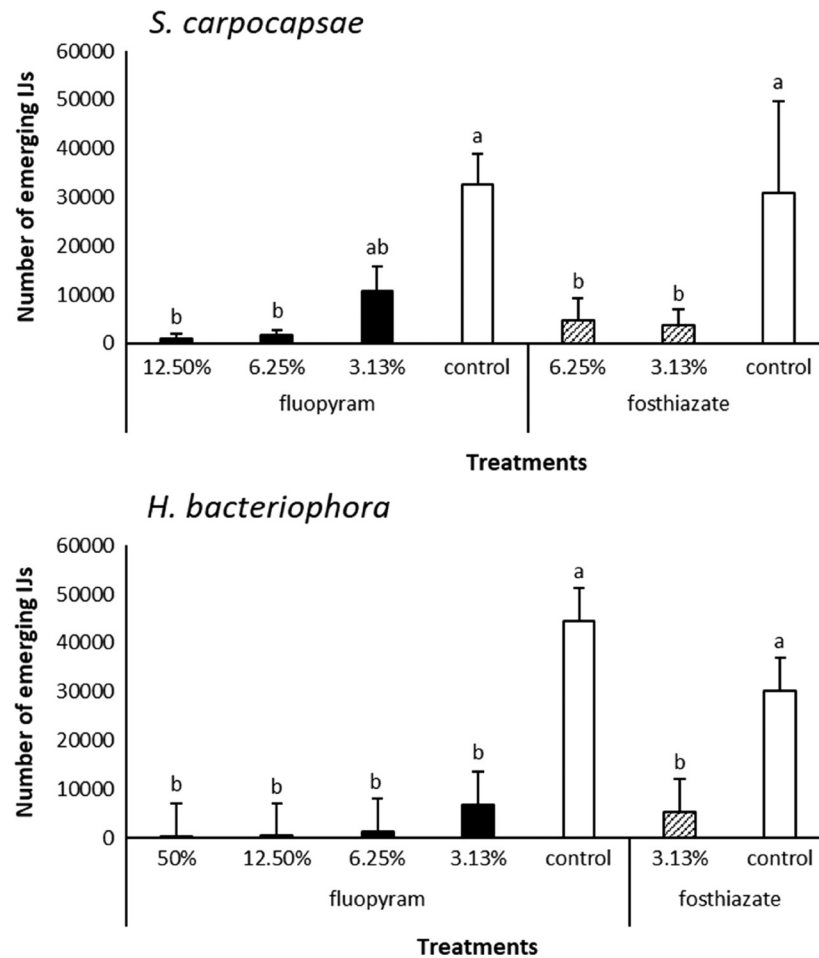


Figure 7: Mean (\pm SE) number of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* infective juveniles (IJs) emerging from the insect host (*Galleria mellonella*) after 24-hour exposure to nematicides. Different letters above bars indicate statistical significance (Tukey's test $P \leq 0.05$).

concentrations of nematicides. In the control treatment with water on both sides, chemotaxis indices at all times of assessment were zero. Overall, IJs were slightly repelled by the recommended concentrations of all the nematicides at all points of assessments. Except for 3-hour assessment of 0.1% concentration, the sides with lower concentrations (i.e., 1% and 0.1%) of metam potassium were attractive to the IJs at all other points of assessment. Preference for nematicide-treated area was also observed for 1% concentration of fluopyram at 24-hour assessment, 1% concentration of fenamiphos at 6-hour assessment and for 0.1% concentration of fosthiazate at 24-hour assessment.

Discussion

Our experimental results showed that the nematicides, fluopyram, fosthiazate, metam potassium, and

fenamiphos, had direct effects on the survival, virulence, penetration and reproduction of EPNs. In general, *S. carpocapsae* was less affected by the nematicides tested compared to *H. bacteriophora*. Grewal (2002) asserted that heterorhabditids are inclined to be more susceptible to physical challenges like pesticides than steinernematids. This has been corroborated by the experiments in our study as well as in other studies, e.g., Rovesti, et al. (1988) and Rovesti and Deseo (1990, 1991) indicated that *Steinernema* species were more tolerant to the 75 different pesticides assessed in their study. They reported that pesticides such as parathion, aldicarb, methomyl, flubenzimine, metham sodium, and fenamiphos were the most toxic pesticides against *S. carpocapsae*, *S. feltiae* and *H. bacteriophora*. Moreover, IJs of both EPNs in our study were more tolerant to the tested nematicides compared to the J2 stage of *M. incognita*. Similar observations have also

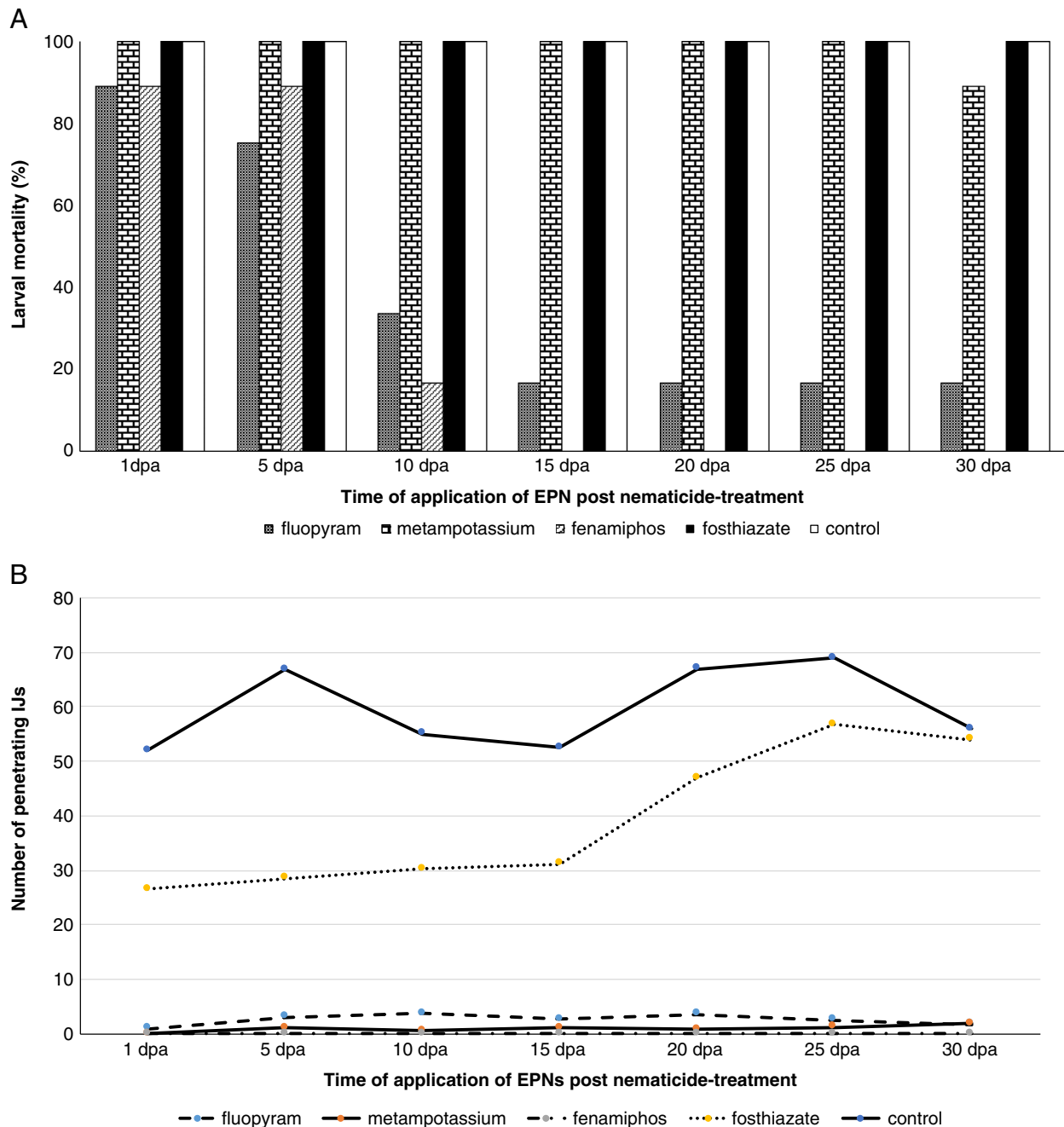


Figure 8: Mortality of *Galleria mellonella* (A) and the number of *Steinernema carpocapsae* infective juveniles (IJs) penetrating the insect host (B) after exposure to nematicides at different time periods.

been reported of *S. carpocapsae* being more tolerant to chemical pesticides such as oxamyl, permethrin, acephate, methomyl, and dichlorvos insecticides compared to *M. incognita* (Ishibashi and Ishibashi, 1985; Ishibashi and Takii, 1993).

Previous studies have pointed out that carbamate and organophosphates are the most dangerous group

among pesticides to EPNs. They can significantly kill IJs, reduce nematode reproduction capacity or negatively influence EPN activity (Gordon et al., 1996; Hara and Kaya, 1982; Ishibashi and Takii, 1993; Zhang et al., 1994). Similar effects were observed in our study. Fosthiazate and fenamiphos are anticholinergic nematicides that are members of the

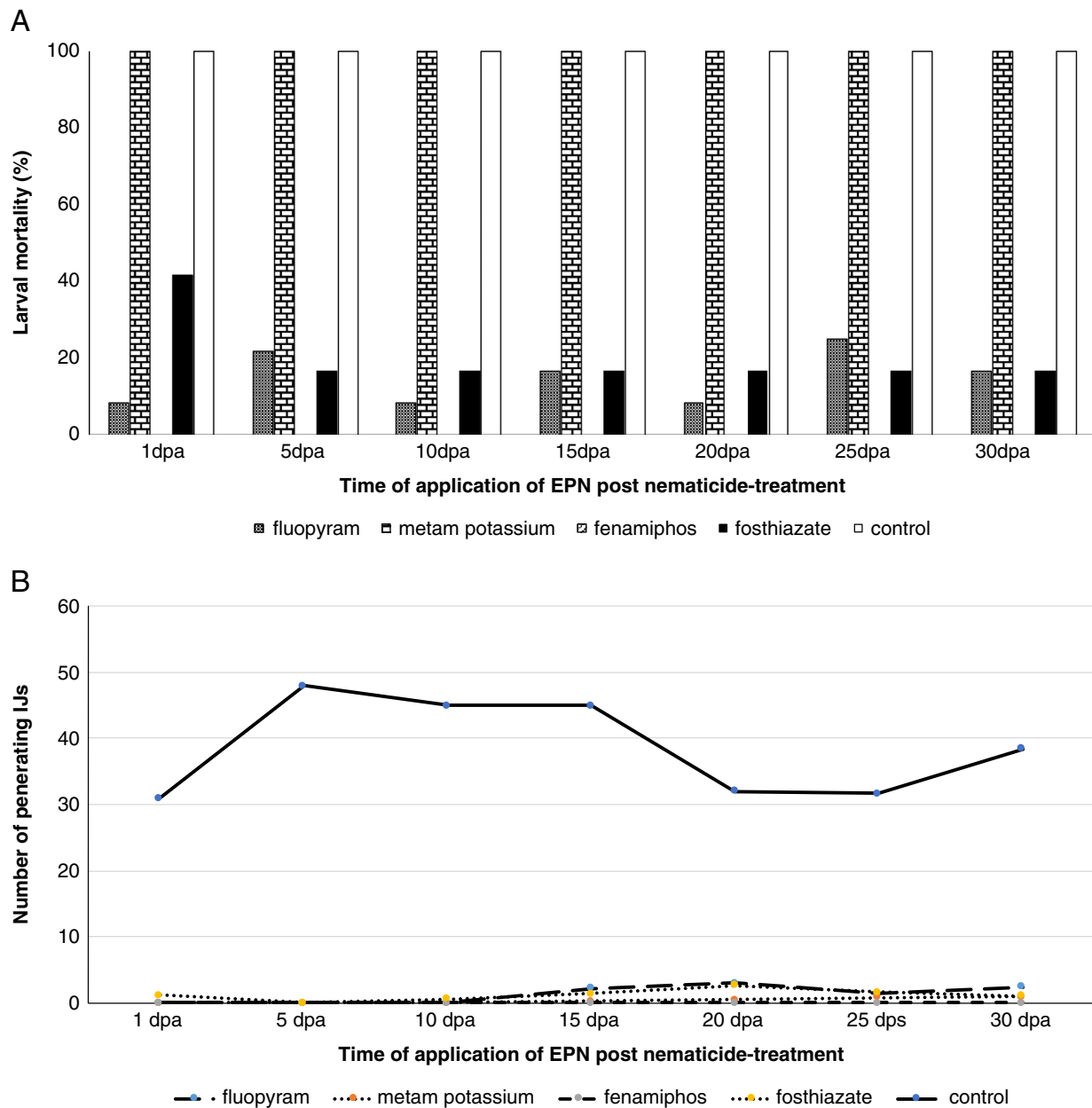


Figure 9: Mortality of *Galleria mellonella* (A) and the number of *Heterorhabditis bacteriophora* infective juveniles (IJs) penetrating the insect host (B) after exposure to nematicides at different time periods.

organophosphate class of pesticides, whereas metam potassium (Potassium n-methyldithiocarbamate) is a dithiocarbamate that block the activity of enzymes such as acetylcholinesterase (NCBI, 2021). Gordon et al. (1996) tested the toxicity of two carbamate insecticides—carbofuran and fenoxycarb—against *S. carpocapsae* and *S. feltiae* and reported that these insecticides caused the death of IJs of both nematode species.

Our results also showed that IJs exposed to all the nematicides were less virulent compared to non-treated IJs. None of the nematodes in the treated group were able to kill the *G. mellonella* larvae after exposure to recommended doses of the nematicides. Comparatively, several studies have observed that insecticides such as azadirachtin, chlorpyrifos, fipronil, imidacloprid, and thiamethoxam had no negative effects on the infectivity of *S. carpocapsae*

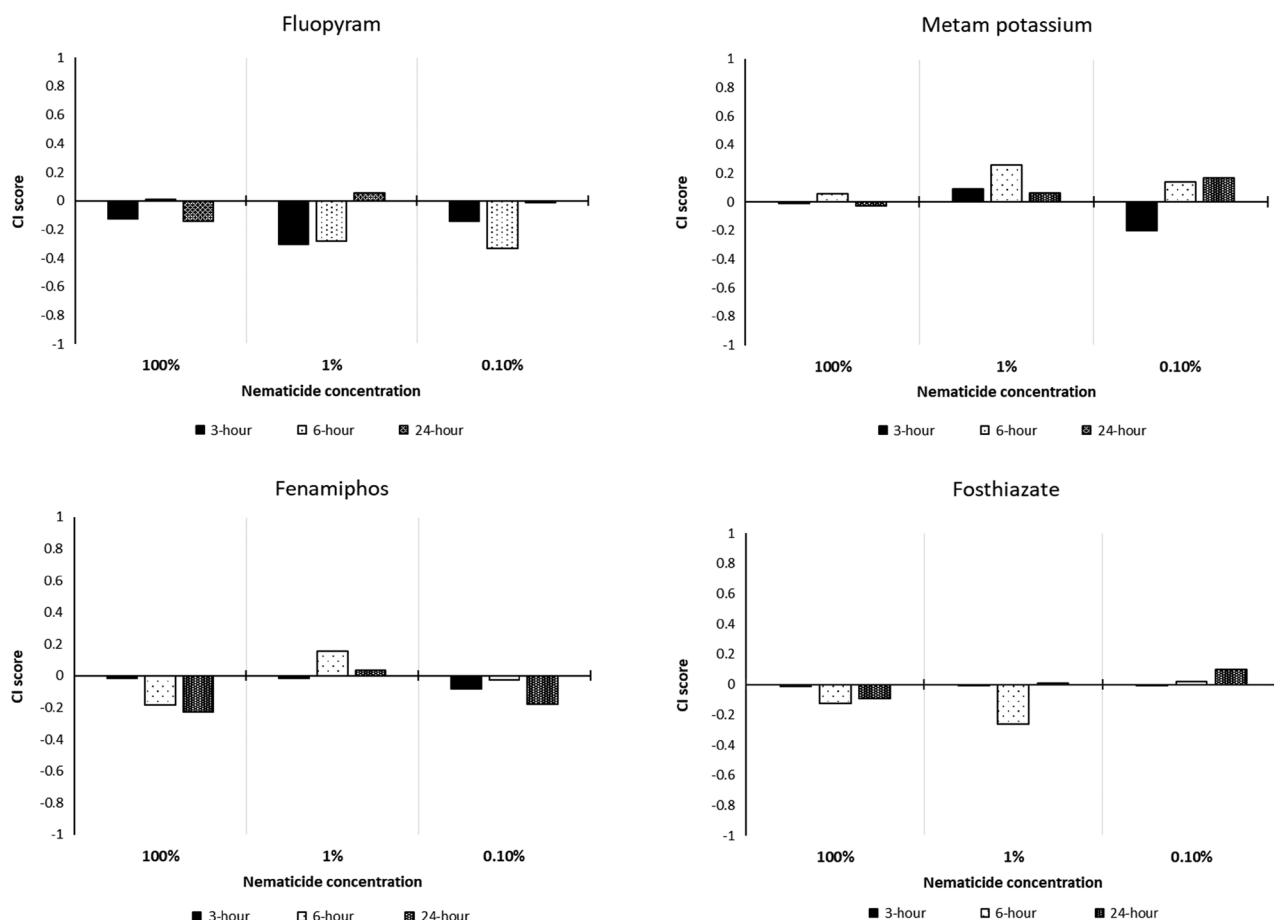


Figure 10: Response of *Heterorhabditis bacteriophora* to different concentrations of nematicidal compounds and non-treated control (distilled water) after 3, 6 and 24 h. A CI score close to -1 shows a high aversion, 0 shows neutrality, and $+1$ shows a strong attraction.

(Koppenhöfer et al., 2003; Negrisoli et al., 2010; Rovesti et al., 1988; Yan et al., 2012). We noticed that IJs exposed to low doses fluopyram, which is an organochlorine compound (benzamide) used to manage or mitigate both soil-borne fungi and plant-parasitic nematodes, were effective against insect larvae. It is a chemical that inhibits the action of succinate dehydrogenase and like other succinate dehydrogenase inhibitors (SDHI), and uniquely affects nematode motility (Faske and Hurd, 2015; Sang et al., 2018). Faske and Hurd (2015) reported that low concentrations of fluopyram had negative effects on the motility of *M. incognita* and *Rotylenchulus reniformis* and when these PPNs were removed from the fluopyram after a 1-hour treatment and rinsed, 50–60% of the nematodes recovered in motility after 24 h.

We also observed that the nematicidal compounds negatively impacted various aspects of EPN

development and behavior. IJs after exposure to the nematicide treatments had reduced penetration efficacy (~47% of IJs added in control penetrated insects in control compared to less than 15% in nematicide treatments). To the best of the authors' knowledge, no study has assessed the effects of pesticides on the penetration efficacy of EPNs.

In the case of progeny production, the numbers of emerging IJs from nematicide treatments were significantly lower compared to the untreated controls. Six-fold more *H. bacteriophora* IJs and 3-fold more of *S. carpocapsae* were collected from the controls compared to from those collected from nematicide-treated groups (6.25 and 3.13%). The number of penetrated IJs is the reason of this fewer number of emerging IJs. Likewise, Atwa et al. (2013) determined that the recommended concentrations of 11 different insecticides and fungicides had negative effects on the reproduction rates of *Steinernema*

sp. EBN-1e and *H. bacteriophora* EBN-10. Also, Ozdemir et al. (2020) showed that twice as much IJs were produced by untreated *H. bacteriophora* nematodes compared to nematodes exposed to fluxapyroxad + difenoconazole, spiromesifen, and spinosad insecticides.

This is the first study assessing the effects of pesticides, nematicides in particular, on the chemotaxis of EPNs. The chemotaxis experiments indicated that *H. bacteriophora* were repelled from nematicide-treated environments. Based on these findings, conceivably, the effects *H. bacteriophora* exposure to nematicides under field conditions may be minimized via avoidance. Indeed, chemotaxis response to nematicides should be explored further for *H. bacteriophora* as well as other EPN species. Nevertheless, a recent study (Waldo et al., 2019) evaluating the impacts of nematicides on nematode density, observed that fluopyram, abamectin, fluen-sulfone, and furfural nematicides, applied monthly to turfgrass, decreased the populations of non-target nematode. These nematodes were either killed or repelled from nematicide-treated areas.

Because EPNs are biocontrol agents whose persistence in soil is greatly affected by various abiotic and biotic factors including anthropogenic activities like application of pesticides against harmful pests (Glazer, 2002; Kaya 1990; Smiths, 1996), it is important that a case by case-based assessment of the effects of such chemical pesticides be tested (Shapiro-Ilan et al., 2012). Though the amount of documentation on the in vitro effects of various pesticides on EPN survival and infectivity has grown substantially (e.g. Hara and Kaya, 1982; Ishibashi and Takii, 1993; Nishimatsu and Jackson, 1998), these studies have mostly focused on the compatibility of insecticides, herbicides, or fungicides with different EPN species, especially for tank mixing. Importantly, our study shows that the impact of pesticides on the viability of EPNs cannot or does not directly predict their virulence/efficacy (Shapiro-Ilan et al., 2015), particularly under soil conditions. Experiments with the effects of the nematicides assayed in natural soil showed that IJs of both species, added at 5-day interval after nematicide application, were generally unable to kill *G. mellonella* except for *S. carpocapsae* in fosthiazate-treated soil. Kaya and Burlando (1989) demonstrated that fenamiphos greatly inhibited *S. feltiae* activity such that IJs in fenamiphos treated-sand were significantly unable to infect *G. mellonella*. However, in our experiments, fosthiazate did not cause substantial reductions in EPN virulence in the soil assay, which is in contrast to the viability studies showing direct toxicity on IJs. The soil appears to temper the nematicidal effects

for these EPNs. Evidently the insects added to the soil died from the effects of the nematicides, most of which are known to have broad spectrum effects and besides nematodes, can negatively impact or kill other invertebrate organisms like insects at the point of application to soil (Waldo, 2018). Interestingly, although metam potassium is a soil fumigant that turns into methyl isothiocyanate gas that moves through the soil to control the pests and may escape from soil following application and does not leave residue, we found that it was still effective on nematodes and the insects in distilled water and soil environment.

Overall, our study emphatically highlights the potential detrimental effects of nematicides on EPNs present in pesticide-treated soil during the control of PPNs. Also, it shows that IJs which are to be added in biological control programs might be negatively affected even after 30 days since most nematicides need to persist for long periods of time for effective control of PPNs—this might not be favorable for biological control programs using EPNs. But not all nematicide compounds are equally detrimental. The choice of nematicide should be considered if EPNs are to be added to nematicide-treated soil. Future studies on the effects of pesticides should also endeavor to assess all traits that impact EPN efficacy including chemotaxis, penetration into and reproduction in the host.

Acknowledgments

The authors thank Dr Harry K. Kaya for revising and editing the manuscript and Hektas Company for providing nematicides. No funds were received for this study.

Conflict of interest: The authors declare that there is no conflict of interest.

References

- Atkinson, H. J., Lilley, C. J. and Urwin, P. E. 2012. Strategies for transgenic nematode control in developed and developing world crops. *Current Opinion in Biotechnology* 23:251–6.
- Atwa, A., Shamseldean, M. and Yonis, F. 2013. Farklı pestisitlerin entomopatogenik nematodların üremesi üzerine etkisi. *Turkish Journal of Entomology* 37:493–502.
- Baiocchi, T., Li, C. and Dillman, A. R. 2020. EPNs exhibit repulsion to prenil in pluronic gel assays. *Insects* 11:457.
- Bird, D. M. and Kaloshian, I. 2003. Are roots special? Nematodes have their say. *Physiological and Molecular Plant Pathology* 62:115–23.

- Browning, M., Dawson, C., Alm, S. R., Gorrës, J. H. and Amador, J. A. 2004. Differential effects of butyric acid on nematodes from four trophic groups. *Applied Soil Ecology* 27:47–54.
- Casteliani, A., de Fatima Martins, L., Maringoli Cardoso, J. F., Oliveira Silva, M. S., Abe da Silva, R. S., Chacon-Orozco, J. G., Casteliani, A. G. B., Puza, V., Harakava, R. and Leite, L. G. 2020. Behavioral aspects of *Sphenophorus levis* (Coleoptera: Curculionidae), damage to sugarcane and its natural infection by *Steinernema carpocapsae* (Nematoda: Rhabditidae). *Crop Protection* 137:105262.
- Chitwood, D. J. 2003. "Nematicides", In Plimmer, J. R., Gammon, D. W. and Ragsdale, N. A. (Eds), *Encyclopedia of Agrochemicals*, John Wiley & Sons, Inc. NJ, doi: 10.1002/047126363X.agr1712003.
- De Nardo, E. A. B. and Grewal, P. S. 2003. Compatibility of *Steinernema feltiae* (Nematoda: Steinernematidae) with pesticides and plant growth regulators used in glasshouse plant production. *Biocontrol Science Technology* 13:441–8.
- Evlice, E., Erdoğan, F. and Yatkın, G. 2020. Bazı havuç çeşitlerinin kök-ur nematodu türlerine (*Meloidogyne* spp. Goeldi, 1982) karşı reaksiyonu. *Bitki Koruma Bülteni* 60:25–30.
- Faske, T. R. and Hurd, K. 2015. Sensitivity of *Meloidogyne incognita* and *Rotylenchulus reniformis* to fluopyram. *Journal of Nematology* 47:316–21.
- Food and Agriculture Organization of the United Nations 2002. International code of conduct on the distribution and use of pesticides. Food and Agriculture Organization of the United Nations, Rome.
- Forghani, F. and Hajihassani, A. 2020. Recent advances in the development of environmentally benign treatments to control root-knot nematodes. *Frontiers in Plant Science* 11:1125.
- Glazer, I. 2002. "Survival biology", In: Gaugler, R. (Ed), *Entomopathogenic Nematology* CABI Publishing, NY, pp. 169–82.
- Glazer, I., Salame, L. and Segal, D. 1997. Genetic enhancement of nematicide resistance in entomopathogenic nematodes. *Biocontrol Science and Technology* 7:499–512.
- Global situation of pesticide management in agriculture and public health. 2019. WHO-FAO survey. World Health Organization, Geneva.
- Gordon, R., Chippett, J. and Tilley, J. 1996. Effects of two carbamates on infective juveniles of *Steinernema carpocapsae* all strain and *Steinernema feltiae* Umea strain. *Journal of Nematology* 28:310–7.
- Grewal, P. S. 2002. "Formulation and application technology", In Gaugler, R. (Ed.), *Entomopathogenic Nematology* CABI Publishing, NY, pp. 265–84.
- Gulcu, B. and Hazir, S. 2012. An alternative storage method for entomopathogenic nematodes. *Turkish Journal Zoology* 36:562–5.
- Gulcu, B., Cimen, H., Raja, R. K. and Hazir, S. 2017. Entomopathogenic nematodes and their mutualistic bacteria: their ecology and application as microbial control agents. *Biopesticides* 13:79–112.
- Hara, A. H. and Kaya, H. K. 1982. Effects of selected insecticides and nematicides on the *in vitro* development of the entomogenous nematode *Neoplectana carpocapsae*. *Journal of Nematology* 14:486–91.
- Hominick, W. M., Reid, A. P., Bohan, D. A. and Briscoe, B. R. 1996. Entomopathogenic nematodes: biodiversity, geographical distribution and the convention on biological diversity. *Biocontrol Science and Technology* 6:317–32.
- Hummadia, E. H., Dearden, A., Generalovic, T., Clunie, B., Harrott, A., Cetin, Y., Demirbek, M., Khoja, S., Eastwood, D., Dudley, E., Hazir, S., Touray, M., Ulug, D., Gulsen, S. H., Cimen, H. and Butt, T. 2021. Volatile organic compounds of *Metarhizium brunneum* influence the efficacy of entomopathogenic nematodes in insect control. *Biological Control* 155:104527.
- Ishibashi, N. and Takii, S. 1993. Effects of insecticides on movement, nictation and infectivity of *Steinernema carpocapsae*. *Journal of Nematology* 25:204–13.
- Ishibashi, Y. and Ishibashi, N. 1985. Bioassays of the nematicides MK-166 and oxamyl in the rootknot nematode, *Meloidogyne incognita*. *Proceedings of the Association for Plant Protection of Kyushu (Japan)* 31:183–5.
- Kaya, H. K. 1990. "Soil ecology", In Gaugler, R. and Kaya, H. K. (Eds), *Entomopathogenic Nematodes in Biological Control* CRC Press, Boca Raton, FL, pp. 93–115.
- Kaya, H. K. and Burlando, T. M. 1989. Infectivity of *Steinernema feltiae* in fenamiphos-treated sand. *Journal of Nematology* 21:434–6.
- Kaya, H. K. and Stock, S. P. 1997. "Techniques in insect nematology", In Lacey, L. (Ed.), *Manual of Techniques in Insect Pathology* Academic Press, San Diego, CA, pp. 281–324.
- Kenney, E. and Eleftherianos, I. 2016. Entomopathogenic and plant pathogenic nematodes as opposing forces in agriculture. *International Journal for Parasitology* 46:13–9.
- Kepenekci, I., Hazir, S., Oksal, E. and Lewis, E. E. 2018. Application methods of *Steinernema feltiae*, *Xenorhabdus bovienii* and *Purpureocillium lilacinum* to control root-knot nematodes in greenhouse tomato systems. *Crop Protection* 108:31–8.
- Koppenhöfer, A. M., Cowles, R. S., Cowles, E. A., Fuzy, E. M. and Baumgartner, L. 2003. Effect of neonicotinoid insecticide synergists on entomopathogenic nematode fitness. *Entomologia Experimentalis et Applicata* 106:7–18.
- Lambert, K. and Bekal, S. 2002. Introduction to plant-parasitic nematodes. *The Plant Health Instructor*, doi: 10.1094/PHI-I-2002-1218-01.
- Lewis, E., Campbell, J., Griffin, C., Kaya, H. K. and Peters, A. 2006. Behavioral ecology of entomopathogenic nematodes. *Biological Control* 38:66–79.

- Lewis, E. E. 2002. "Behavioural Ecology", In Gaugler, R. (Ed.), *Entomopathogenic Nematology* CABI Publishing, NY, pp. 205–24.
- Manyilizu, W. B. 2019. Pesticides, anthropogenic activities, history and the health of our environment: lessons from Africa. pesticides – use and misuse and their impact in the environment, doi: 10.5772/intechopen.82600.
- Mauleon, H., Briand, S., Laumond, C. and Bonifassi, E. 1993. Utilisation d'enzyme digestives pour l'étude du parasitisme des *Steinernematidae* et *Heterorhabditidae* envers les larves d'insectes. *Fundamental and Applied Nematology* 16:185–6.
- McCarter, J. P. 2009. "Molecular approaches toward resistance to plant-parasitic nematodes", In Berg, R. H. and Taylor, C. G. (Eds), *Cell Biology of Plant Nematode Parasitism* Plant Cell Monographs, Springer, Berlin Heidelberg, pp. 83–113.
- NCBI 2021. PubChem Compound Summary for CID 23690432, Potassium N-methyldithiocarbamate, CID 91758, Fosthiazate and CID 31070 Fenamiphos.
- Negrisol, A. S., Garcia, M. S., Barbosa Negrisol, C. R. C., Bernardi, D. and da Silva, A. 2010. Efficacy of entomopathogenic nematodes (Nematoda: Rhabditida) and insecticide mixtures to control *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) in corn crops. *Crop Protection* 29:677–83.
- Nishimatsu, T. and Jackson, J. J. 1998. Interaction of insecticides, entomopathogenic nematodes, and larvae of the western corn rootworm (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 91:410–8.
- Ozdemir, E., İnak, E., Evlice, E. and Laznik, Z. 2020. Compatibility of entomopathogenic nematodes with pesticides registered in vegetable crops under laboratory conditions. *Journal of Plant Diseases and Protection* 127:529–35.
- Rovesti, L. and Deseo, K. V. 1990. Compatibility of chemical pesticides with the entomopathogenic nematodes, *Steinernema carpocapsae* Weiser and *S. feltiae* Filipjev (Nematoda: Steinernematidae). *Nematologica* 36:237–45.
- Rovesti, L. and Deseo, K. V. 1991. Compatibility of chemical pesticides with the entomopathogenic nematodes *Heterorhabditis heliothidis*. *Nematologica* 37:113–22.
- Rovesti, L., Heinzpeter, E. W., Tagliente, F. and Deseo, V. 1988. Compatibility of pesticides with the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae). *Nematologica* 34:462–76.
- Sang, H., Witte, A., Jacobs, J. L., Chang, H. X., Wang, J., Roth, M. G. and Chilvers, M. I. 2018. Fluopyram sensitivity and functional characterization of SDHB in the *Fusarium solani* species complex causing soybean sudden death syndrome. *Frontiers in Microbiology* 9:2335.
- Shapiro-Ilan, D. I., Han, R. and Dolinski, C. 2012. Entomopathogenic nematode production and application technology. *Journal of Nematology* 44:206–17.
- Shapiro-Ilan, D., Hazir, S. and Glazer, I. 2017. "Basic and applied research: entomopathogenic nematodes", In Lacey, L. (Ed.), *Microbial Control of Insect and Mite Pests* Academic Press, Massachusetts, pp. 91–105.
- Shapiro-Ilan, D. I., Hazir, S. and Lete, L. 2015. Viability and virulence of entomopathogenic nematodes exposed to ultraviolet radiation. *Journal of Nematology* 47:184–9.
- Smiths, P. H. 1996. Post-application persistence of entomopathogenic nematodes. *Biocontrol Science and Technology* 6:379–87.
- Touray, M., Gulcu, B., Ulug, D., Gulsen, S. H., Cimen, H., Kaya, H. K., Cakmak, I. and Hazir, S. 2020. Evaluation of different sponge types on the survival and infectivity of stored entomopathogenic nematodes. *Journal of Invertebrate Pathology* 171:107332.
- Viglierchio, D. R. 1991. The world of nematodes. agAccess, Davis, California.
- Waldo, B. D. 2018. Nematicide effects on non-target soil invertebrate populations in turfgrass systems MSc thesis. University of Florida.
- Waldo, B. D., Grabau, Z. J., Mengistu, T. M. and Crow, W. T. 2019. Nematicide effects on non-target nematodes in bermudagrass. *Journal of Nematology* 51:1–12.
- Wang, C., Lower, S. and Williamson, V. M. 2009. Application of Pluronic gel to the study of root-knot nematode behaviour. *Nematology* 11:453–64.
- Weischer, B. and Brown, D. 2000. General nematology Pensoft Publishers, Sofia.
- White, G. F. 1927. A method for obtaining infective nematode larvae from culture. *Science* 66:302–3.
- Yan, X., Moens, M., Han, R., Chen, S. and De Clercq, P. 2012. Effects of selected insecticides on osmotically treated entomopathogenic nematodes. *Journal of Plant Diseases and Protection* 119:152–8.
- Zhang, L., Shono, T., Yamanaka, S. and Tanabe, H. 1994. Effects of insecticides on the entomopathogenic nematode *Steinernema carpocapsae* WEISER. *Applied Entomology and Zoology* 29:539–47.