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Original Research

Effects of Selected Pesticides on Survival and Virulence of Two Nematode Species

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Abstract

The compatibility of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* with 22 different pesticides was determined under laboratory conditions. The mortality of nematode invasive larvae and their virulence against the insect *T. molitor* were investigated. *H. bacteriophora* showed different sensitivity from *S. feltiae* to tested pesticides, especially at pyrethrum+piperonyl-butoxide. All tested pesticides caused decreasing of virulence of *H. bacteriophora* except fenpyroximate. This ingredient decreased the virulence of *S. feltiae* significantly. Also, tebufenpyrad decreased pathogenicity of *S. feltiae*. Based on the present study, we indicate that *S. feltiae* as well as *H. bacteriophora* are tolerant to most of the tested pesticides with some exceptions.

Keywords: nematodes, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, compatibility, pesticides

Introduction

Entomopathogenic nematodes (EPNs) are considered one of the best non-chemical alternatives to insect pest control and are commercially available [1, 2]. EPNs are obligate parasites of a diverse array of insects and are present in the soils of many ecosystems worldwide [3, 4]. These nematodes possess a unique life cycle, a portion of which is spent outside of the host as non-feeding infective juveniles (IJs). IJs penetrate hosts through natural openings (mouth, spiracles, and anus) or, in some instances, directly through the cuticle and ultimately kill the host. Advantages of nematodes as biopreparations include the wide range of their host insects, the ability to kill insects fast, fast breeding, no immunization of pests, no hazard for the environment or higher animals, and the possibility to store material used for infection and its easy application.

Entomopathogenic nematode virulence is conditioned by such factors as temperature, soil moisture, soil structure, oxygen content [5, 6], and the effect of agrochemicals that are often applied together within integrated pest management (IPM) [7]. When EPNs are applied under conducive conditions, these nematodes can be as effective as chemical insecticides [8]. However, before an ecologically integrated approach to pest management involving nematode/pesticide combinations in tank-mixes, the compatibility of nematodes *Steinernema* sp. and *Heterorhabditis* sp. with new and routinely used pesticides needs to be established. This investigation is aimed at determining the effect of 21 selected agrochemicals on the survival and virulence of the IJs entomopathogenic nematodes *Steienernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* (Poinar) (Rhabditida: Heterorhabditidiae).

Material and Methods

Infective juveniles of *S. feltiae* or *H. bacteriophora* and pesticides were obtained from the company Biobest N.V. (Belgium). Stock solutions of the pesticides listed in Table 1 were prepared in distilled water at field-recommended doses.

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Table 1. Selected pesticides with active ingredient, mode of action and recommended field dose.

Trade names, Formulation*	Type	Active ingredient (a.i.)	Chemical group	Mode of action	Field dose (l/Kg/ha)
Teldor, WG	Fungicide	Fenhexamid	Amides	Sterol biosynthesis inhibitor	0.15
Tridal, SG	Fungicide	Nuarimol	Pyrimidines	Sterol biosynthesis inhibitor	0.05
Confidor, SL	Insecticide	Imidacloprid	Imidazoles	Acetylcholine mimic	0.075
Tracer, SC	Insecticide	Spinosad	Macrolides	Acetylcholine mimic	0.02
Lannate, SL	Insecticide	Methomyl	Carbamates	Acetylcholin esterase inhibitor	0.15
Spruzit, EC	Insecticide	Pyrethrum+ Piperonyl-butoxide	Pyrethroids	Sodium channel modulator	0.1
Naja, SC	Acaricide	Fenpyroximate	Pyrazoles	Mitochondrial Complex I electron transport inhibitor	0.1
Masai, WG	Acaricide	Tebufenpyrad	Pyrazoles	Mitochondrial Complex I electron transport nhibitor	0.05
Torque-L, SC	Acaricide	Fenbutatinoxide	Organotins	Mitochondrial ATP synthase inhibitor	0.05
Polo, SC	Insecticide	Diafenthiuron	Thiourea	Mitochondrial ATP synthase inhibitor	0.08
Mimic	Insecticide	Tebufenozide	Hydrazines	Ecdysone mimic	0.1
Runner, SC	Insecticide	Methoxyfenozide	Hydrazines	Ecdysone mimic	0.04
Enstar, EC	Insecticide	Kinoprene	Fatty acids	Juvenile hormone mimic	0.075
Admiral, EC	Insecticide	Pyriproxyfen	Pyridines	Juvenile hormone mimic	0.025
Insegar, WG	Insecticide	Fenoxycarb	Carbamates	Juvenile hormone imimic	0.04
Borneo, SC	Acaricide	Etoxazole	Oxazoles	Chitin synthesis inhibitor	0.05
Applaud, SC	Insecticide	Buprofezin	Thiadiazines	Chitin synthesis inhibitor	0.03
Trigard, SL	Insecticide	Cyromazine	Triazines	Chitin synthesis inhibitor	0.1
Dimilin, SC	Insecticide	Diflubenzuron	Benzoylurea	Chitin synthesis inhibitor.	0.04
Match, EC	Insecticide	Lufenuron	Benzamides	Chitin synthesis inhibitor	0.1
Apollo, SC	Acaricide	Clofentezine	Chlorobenzenes	Unknown mode of action Embryo development inhibitor	0.03

*SC – suspension concentrate, SL – soluble liquid, WG – water dispersible granules, EC – emulsifiable concentrate.

The lethal effect on nematodes was evaluated using a Petri dish (Ø 9 cm) filled with 10 ml of chosen pesticide solution and 100 µl of nematode concentrate suspension (cca 20,000 IJs/ml), using water for the control treatments. Petri dishes were kept at room temperature (22-26ºC) in darkness. Each treatment had five replicates. Nematode mortality was determined after 72 hours, by removing five 50 µl sub-samples from each Petri dish and observing under the stereomicroscope. Nematodes that did not move, even after prodding, were considered dead. To evaluate the sublethal effect of pesticides on virulence, the nematode-pesticide suspension was rinsed with sterile water 3 times to remove the rest of the pesticide. Nematodes were left 24 hours in the distilled water. Five hundred live IJs for *S. feltiae* or 1,000 for *H. bacteriophora* in 1 ml of water were applied to the Petri dish (Ø 9 cm) covered with filter paper, and 10 larvae of *Tenebrio molitor* (L) were added. Petri dishes were kept at room temperature (22-26ºC) in darkness. Each treatment had four replicates, using untreated nematode suspensions as control. Larvae mortality was determined after 5 days, and dead larvae were put onto white traps [9] to observe the nematode ability to reproduce.

Statistical analyses were performed using the Statistica 8.0 program. The percentages of IJs and *T. molitor* larval mortalities were corrected using Abbott's formula [10], and arcsine transformed before statistical analysis. One-way ANOVAs were performed and Tukey´s test at $P \leq 0.05$ assessed significant differences among groups.

Results

Nematode survival was influenced by agrochemicals. The results show that both nematodes were quite tolerant to all tested pesticides. Mortality at *S. feltiae* varied from 0.05-20.18% within 72 hours. In some cases mortality was even lower compared with control (a.i. methomyl and nuarimol). *H. bacteriophora* showed different sensitivity from *S. felti-*

ae to tested pesticides, mortality reached 1.11-17.92%. Among the pesticides, only cyromazine, diafenthurion, kinoprene, metoxyfenozide, and spinosad did not detect statistical differences ($p>0.05$) for the two nematode species. The highest IJ mortality was produced by the fenpyroximate at *S. feltiae* (20.18%) and pyriproxyfen at *H. bacteriophora* (17.92%). Nematodes also reacted differentially on some active ingredients. *H. bacteriophora* shows higher mortality at nuarimol, diafenthurion, kinoprene, methomyl, pyriproxyfen, and tebufenozide compared to *S. feltiae*. Similar sensitivity of both nematode species was detected with kinopren, as well as with other active ingredients (Fig. 1). Only diafenthurion, diflubenzuron, fenpyroximate, kinoprene pyrethrum+piperonil-butoxide, and pyriproxyfen produced a mortality higher than 10%.

The virulence test detected significant differences ($p<0.05$) in efficacy of *H. bacteriophora* and *S. feltiae* IJs previously incubated in tested pesticides. *S. feltiae* showed higher virulence against *T. molitor* larvae compared to *H. bacteriophora.* The most detected differences were recorded at nuarimol, imidacloprid, methomyl, fenpyroximate, and tebufenpyrad. The mortality of *T. molito*r larvae varied from 12.5-70.0% (Fig. 2). All tested pesticides caused decreasing of virulence of *H. bacteriophora* with methomyl (acetylcholin esterase inhibitor) showing the highest sublethal effects recording 12.5% mortality of *T. molitor* compared to the control (65.0%) after 5 days. The fenpyroximate treatment (mitochondrial Complex I electron transport inhibitor) does not affect *H. bacteriophora* IJs virulence, although this ingredient decreased virulence of *S. feltiae* significantly ($p<0.05$), with 12.5% mortality compared to the control (97.5%) (Fig. 3). A similar effect was observed with tebufenpyrad (another mitochondrial Complex I electron transport inhibitor), which significantly influenced the

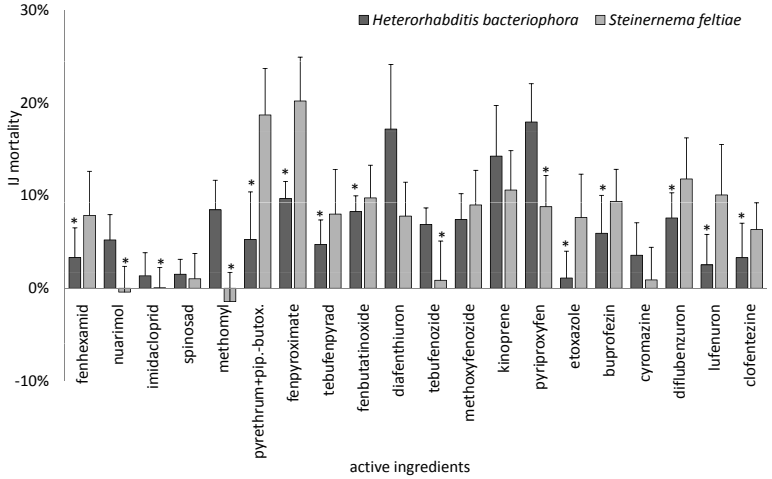

Fig. 1. Percent mortality of *S. feltiae* or *H. bacteriophora* IJs affected by tested pesticides after 72 hours of incubation in the pesticide solution.
*Mortality is corrected with Abbott's formula; negative values indicate lower mortality than in the control. Differences among pesticides between both tested nematode species are marked.

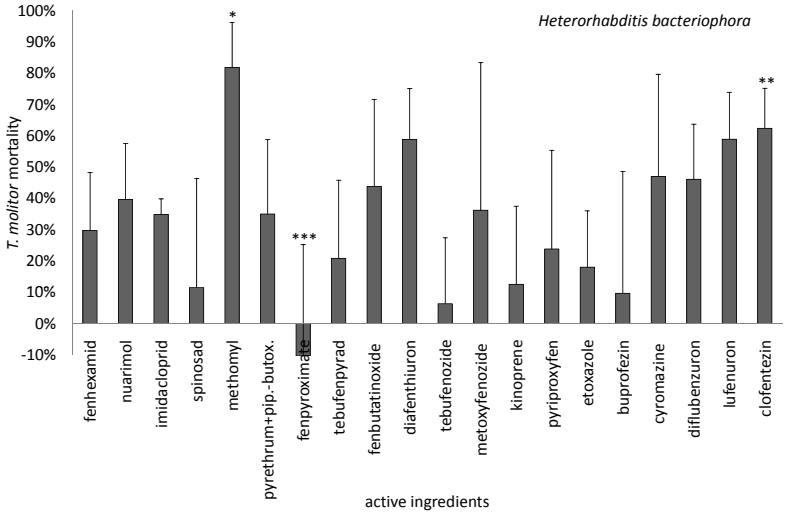

Fig. 2. Percent larvae *T. molitor* mortality affected by 1,000 IJs of *H. bacteriophora* after 5 days; nematodes were previously incubated for 72 hours in tested pesticides.
**T. molitor* mortality is corrected with Abbott's formula; negative values indicate lower mortality than in the control. Significant differences among pesticides are marked.

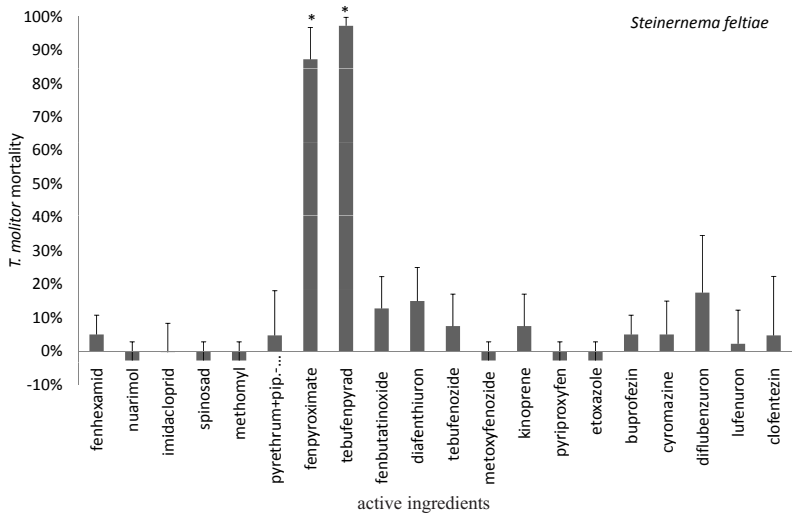

Fig. 3. Percent larvae *T. molitor* mortality (mean±SD) affected by 500 infective juveniles of *S. feltiae*; nematodes were previously incubated for 72 hours in tested pesticides.
**T. molitor* mortality is corrected with Abbott's formula; negative values indicate lower mortality than in the control. Significant differences among pesticides are marked.

virulence of *S. feltiae*. This ingredient caused a 95% decrease in efficacy. Except for these pesticides, no serious sublethal effects were observed. The efficacy of all treated *S. feltiae* invasive larvae ranged from 80-100% after 5 days. The highest mortality was recorded by etoxazole, methomyl, metoxyfenozide, nuarimol, pyriproxyfen, and spinosad.

Discussion of Results

Results of this study increase our knowledge of EPN-Pesticide interactions, and indicate that most of these presented pesticides are not toxic to both tested nematode species. These studies on compatibility are interesting when integrated pest management involves nematode/pesticide combinations in tank-mixes. Thus, is necessary to predict the application rate of the nematodes based on knowledge about the potential efficacy losses due to certain pesticides. The most interesting findings resulting from this study show that both nematodes had differential sensitivity to certain pesticides. Especially in the case of the pesticide Spruzit (pyrethrum+piperonyl-butoxide) was the difference most detectable. In the present study, piperonyl-butoxide caused 18.68% mortality of *S. feltiae,* but 5.26% at *H. bacteriophora*. Experiments examining the toxicity of piperonyl-butoxide to larvae nematodes *Haemonchus contortus* and *Trichostrongylus colubriformis* (Nematoda: Trichostrongylidae) showed that the compound was not toxic at concentrations of 20 mg/ml [11]. Helminth parasites possess a number of mechanisms for detoxification of harmful xenobiotics. More recent reports have shown that helminths also use activity of the cytochrome P450 system [12-14]. Piperonyl-butoxide acts as a synergist for insecticides by inhibiting the cytochrome P450-mediated metabolism of the insecticide [15]. This study confirmed that *S. feltiae* probably possess only restricted possibilities for metabolizing this chemical compound using the cytochrome P450 system. Also, fenpyroximate showed negative effects on *S. feltiae* vitality compared with *H. bacteriophora*. Acaricides observed significant effects both on *S. feltiae* vitality and virulence in general. Tebufenpyrad and fenpyroximate are pyrazole substances with the same mode of action (inhibitor of the mitochondrial electron transport). This fact could cause physiological failures that reduce *S. feltiae* vitality and movement, and consequently the ability to find and infect the host. This effect was not observed at *H. bacteriophora*, where fenpyroximate recorded even better results compared to control.

These results confirmed the differences in metabolism between both tested nematode species. Also, methomyl (acetylcholin esterase inhibitor) in this study showed a differential effect on both tested nematode species. A surprisingly positive effect was detected on *S. feltiae,* although many other studies confirm detrimental effects on EPNs. Rovesti [16] presented a very strong effect of this active ingredient on both tested nematode species. Methomyl as well as oxamyl belong to the same group of chemicals (carbamates). Gaugler and Campbell [17] showed that the carbamate oxamyl stimulated locomotory movement of the IJ of *S. carpocapsae* at a concentration less than 50 µg/ml, but induced partial paralysis at higher concentrations. Also, other carbamate compounds with nematicide activity such as aldicarb and carbofuran have been found to be toxic to nematodes, affecting IJ movement, although the survivor's virulence was not affected [18]. In this case, *H. bacteriophora* reacted on methomyl negatively and virulence significantly decreased. The difference in reaction of both species was detected also at other tested active ingredients (diafenthurion and pyriproxyfen), where *H. bacteriophora* showed higher mortality than *S. feltiae*. These results can also indicate that probably another system of degradation exists in those species. Imidacloprid, cyromazine, and buprofezin affected *S. feltiae* and *H. bacteriophora* vitality

only slightly. Similar results are presented by Barbara and Buss [19] or Vainio and Hokkanen [20].

It is hard to explain the differential reaction of EPNs on different pesticides, but these findings show that different nematode species can react to the same chemicals differently. Based on the present study, we indicate that *S. feltiae* as well as *H. bacteriophora* are tolerant to most of the tested pesticides and tank-mix application is possible with some exceptions. The most detrimental effect on *H. bacteriphora was* caused by methomyl. *S. feltiae* was affected by the pyrazol chemical compounds tebufenpyrad and fenpyroximate. Nowadays, pyrazole derivates are studied for the nematicidal effect in agricultural and therefore it should be pointed out the incompatibility of some chosen pyrazole chemicals with entomopathogenic nematodes. The study showed the nacessity of more side-effect studies for explanation of the nematode species susceptibility to certain pesticides.

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