Effect of Selected Pesticides on the Vitality and Virulence of the Entomopathogenic Nematode *Steinernema feltiae* (Nematoda: Steinernematidae)

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Abstract

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The survival and infectivity of infective juveniles of the entomopathogenic nematode *Steinernema feltiae* (Rhabditida: Steinernematidae) were determined after being exposed to 8 insecticides (a.i. kinoprene, lufenuron, methomyl, metoxyfenozide, oxamyl, piperonyl-butoxide, pyriproxyfen, tebufenozide), 7 acaricides (a.i. azocyclotin, clofentezin, diafenthiuron, etoxazole, fenbutatinoxide, fenpyroximate, tebufenpyrad) and 4 fungicides (a.i. captan, fenhexamid, kresoxim-methyl, nuarimol) under laboratory conditions. *S. feltiae* was tolerant to all tested insecticides and fungicides, mortality during 72 hours varied from 2.26% to 18.68 % and from 7.04% to 8.86%, respectively. Acaricides with a.i. fenpyroximate and tebufenpyrad considerably influenced the *S. feltiae* ability to infect larvae of *Tenebrio molitor*. Tebufenpyrad caused 95% and fenpyroximate 85% reduction in *S. feltiae* virulence. These results suggest that *S. feltiae* can be applied in combination with all tested pesticides except the acaricides with a.i. tebufenpyrad and fenpyroximate.

Keywords: *Steinernema feltiae*; compatibility; integrated pest management; acaricides; insecticides; fungicides

Entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) are effective insect parasites with a potential for biological control. These parasites have a broad host range, can be mass-produced using conventional fermentation technology and are exempt from registration requirements in several countries (Kaya & Gaugler 1993).

Entomopathogenic nematodes (EPNs) are often applied in conjunction with chemical pesticides, soil amendments and fertilisers. Infective juveniles (IJs) of entomopathogenic nematodes have been found to be tolerant to short exposures (2–6 h) of most acaricides, fungicides, herbicides, and insecticides (Rovesti *et al*. 1988; Rovesti & Deseö 1990) and can therefore be applied simultaneously with many pesticides. However, earlier studies have shown that some pesticides can reduce EPN viability and infectivity (Hara & Kaya 1983; Zimmerman & Cranshaw 1990; Krishnayya & Grewal 2002). The entomopathogenic nematode *Steinernema feltiae* is available commercially and is used for the control of dipteran pests in mushrooms, greenhouses and nurseries (Grewal & Georgis 1998). Nematode-pesticide combinations in tank-mixes could offer a cost-effective alternative to integrated pest management (IPM) systems. However, before an ecologically integrated approach to pest management involving nematode-pesticide combinations in tank-mixes can be developed the compatibility of these nematodes with pesticides should be discovered.

In this study, the compatibility of the entomopathogenic nematode *S. feltiae* with new and routinely used pesticides was evaluated. Therefore, the effects of a direct exposure of nematodes to pesticides at a field recommended concentration in suspension on nematode viability and pathogenicity were studied.

MATERIAL AND METHODS

Infective juveniles of *S. feltiae* and pesticides were obtained from the company Biobest N.V. (Belgium). The pesticides: 8 insecticides (a.i. kinoprene, lufenuron, methomyl, metoxyfenozide, oxamyl, piperonyl-butoxide, pyriproxyfen, tebufenozide), 7 acaricides (a.i. azocyclotin, clofentezin, diafenthurion, etoxazole, fenbutatinoxide, fenpyroximate, tebufenpyrad) and 4 fungicides (a.i. captan, fenhexamid, kresoxim-methyl, nuarimol) were tested.

Stock solutions of the pesticides were prepared in distilled water. 10 ml of chosen pesticide solution for the test was used. 100 µl nematode concentrate (ca 2000 IJ) was added to Petri dish (ø 9 cm) with pesticide solution. Water was used as a control. Petri dishes were kept at room temperature (22–26°C) in darkness. Each treatment had three replications. The vitality was checked after 24 h, 48 h and 72 h, by taking five 50 µl sub-samples from each Petri dish and observing under the stereomicroscope. The nematodes that did not move even when prodded, were considered dead. For the virulence test the nematode-pesticide suspension was rinsed with sterile water 3 times to remove the rest of the pesticide. Nematodes were left for 24 h in distilled water. 500 alive infective juveniles of *S. feltiae* were applied into Petri dish

List of the tested pesticides with active ingredient, formulation and recommended field dose

Group of pesticides	Trade name	Active ingredient (a.i.)	Formulation*	Recommended field dose (%)	Content of a.i. (g/l)
Acaricides	Apollo	clofentezin	SC	0.03	500
	Borneo	etoxazole	SC	0.05	110
	Naja	fenpyroximate	SC	0.1	50
	Perporal	azocyclotin	WP	0.1	25
	Torque-L	fenbutatinoxide	SC	0.05	25
	Masai	tebufenpyrad	WG	0.05	25
	Polo	diafenthurion	SC	0.08	250
Fungicides	Candit	kresoxim-methyl	WG	0.02	50
	Captan	captan	WG	0.15	80
	Teldor	fenhexamid	WG	0.15	50
	Tridal	nuarimol	SC	0.05	120
Insecticides	Admiral	pyriproxyfen	EC	0.025	100
	Enstar	kinoprene	EC	0.075	65
	Lannate	methomyl	SL	0.15	20
	Match	lufenuron	EC	0.1	50
	Mimic	tebufenozide	SC	0.1	240
	Runner	metoxyfenozide	SC	0.04	240
	Spruzit	piperonyl-butoxide	EC	0.1	144
	Vydate	oxamyl	EC	0.1	250

*SC – suspension concentrate; SL – soluble liquid; WP – wettable powder; WG – water dispersible granules; EC – emulsifiable concentrate

(ø 9 cm) filled with filter paper and 10 larvae of *Tenebrio molitor*. Petri dishes were kept at room temperature (22–26°C) in darkness. Each treatment had four replications and clear nematode suspension served as a control. The larval mortality was checked on the 3rd and 5th day, dead larvae were put onto the White traps (White 1927) to observe the ability to reproduce.

Statistical analyses were performed by the Statistica version 8.0 program. The percentage of IJs was corrected by Abbott's formula (Abbott 1925) and arcsine transformation was used before statistical analysis. Larval mortality data were not corrected. One-way ANOVA was used for analysis and Tukey's test at $P \leq 0.05$ was applied to assess significant differences among groups.

RESULTS

Vitality test

Nematode survival was significantly affected by agrochemicals. The results show that *S. feltiae* was quite tolerant to all tested insecticides (Table 1). Mortality varied from 2.26% to 18.68% within 72 hours. Significant differences were observed among all insecticides after 24 h ($F = 9.53$, df = 7, 115, $P < 0.05$), 48 h ($F = 11.76$, df = 7, 115, $P < 0.05$) and 72 h ($F = 27.67$, df = 7, 115, $P < 0.05$). A.i. tebufenozide showed the lowest mortality (2.94%) while the highest mortality of infective juveniles was observed in a.i. piperonyl-butoxide (18.68%) after 72 hours. Acaricides affected *S. feltiae* also

Table 1. Percent mortality (mean ± SD) of infective juveniles of *S. feltiae* affected by tested pesticides after 24 h, 48 h and 72 h of incubation

Active ingredient	24 hours	48 hours	72 hours
Fungicide			
Captan	8.68 ± 3.58^{b}	8.73 ± 2.74^{b}	8.73 ± 3.03^{b}
Fenhexamid	8.13 ± 5.06^{b}	8.15 ± 3.46^{b}	8.86 ± 4.40^{b}
Kresoxim-methyl	7.04 ± 3.58^{b}	7.33 ± 4.43^{b}	8.43 ± 4.07^{b}
Nuarimol	–1.83 ± 3.32^{a}	–1.81 ± 3.19^{a}	–0.41 ± 3.04^{a}
Insecticide			
Kinoprene	10.36 ± 4.67^{ab}	11.28 ± 4.63	11.59 ± 4.40^{ab}
Lufenuron	6.55 ± 3.39^{b}	9.86 ± 4.82^{ab}	10.06 ± 5.32^{ab}
Methomyl	3.88 ± 3.12^{d}	5.20 ± 4.31^{ab}	6.29 ± 3.62^{b}
Metoxyfenozide	8.08 ± 4.41^{ab}	8.40 ± 3.04^{ab}	8.72 ± 3.65^{b}
Oxamyl	6.33 ± 3.93^{b}	13.08 ± 3.80^{a}	17.14 ± 9.60^{b}
Piperonyl-butoxide	11.03 ± 5.26^{a}	11.07 ± 5.82^{a}	18.68 ± 4.97^{ab}
Pyriproxyfen	8.66 ± 3.17^{ab}	8.73 ± 2.91^{ab}	8.90 ± 3.19^{b}
Tebufenozide	2.26 ± 3.32^{c}	2.59 ± 3.05^{b}	2.94 ± 3.13^{c}
Acaricide			
Azocyclotin	11.08 ± 2.79^{a}	11.57 ± 4.01^{a}	12.11 ± 3.26^{ab}
Clofentezin	5.03 ± 3.41^{a}	5.25 ± 3.16^{a}	6.33 ± 2.99^{b}
Diafenthiuron	5.64 ± 2.85^{a}	6.97 ± 3.41^{a}	7.78 ± 3.73^{b}
Etoxazole	6.08 ± 3.87^{a}	6.49 ± 4.85^{a}	7.63 ± 4.09^{b}
Fenbutatinoxide	8.47 ± 3.27^{a}	9.17 ± 2.97^{a}	9.74 ± 3.62^{ab}
Fenpyroximate	5.22 ± 2.87^{a}	7.75 ± 3.77^{a}	20.18 ± 4.71^{a}
Tebufenpyrad	7.29 ± 5.53^{a}	7.59 ± 4.53^{a}	7.63 ± 4.49^{b}

Significant differences among pesticides within the group are marked with different small letters

significantly, the highest mortality reached 20.18% (a.i. fenpyroximate) after 72 hours. Fenpyroximate caused an almost 15% increase in the nematode mortality over the observed time. No significant differences were found between the times 24 h (F = 1.13, df = 6, 98, $P > 0.05$) and 48 h (F = 0.84, df = 6, 98, $P > 0.05$). After 72 h (F = 3.50, df = 6, 98. $P < 0.05$) the results showed differences in the efficacy. The results of other acaricides showed the mortality below the 15% limit. All tested fungicides had a slight influence on *S. feltiae* mortality. In the case of a.i. nuarimol *S. feltiae* mortality was even lower than the mortality in the control. Mortality ranged from 7.04% to 8.86% during 72 hours. There were statistical differences among the tested fungicides in all observed times: 24 h (F = 23.21, df = 3, 56, $P < 0.05$), 48 h (F = 29.04, df = 3, 56, $P < 0.05$), and 72 h (F = 18.70, df = 3, 56, $P < 0.05$).

Table 2. Percent mortality of *T. molitor* larvae (mean ± SD) affected by 500 infective juveniles of *S. feltiae*; nematodes were previously incubated in the tested pesticides for 72 hours

Active ingredient	3 day	5 day
Fungicide		
S. feltiae	80.00 ± 8.20^{b}	97.50 ± 5.00^{a}
Captan	82.50 ± 15.0^{ab}	82.50 ± 15.0^{a}
Fenhexamid	90.00 ± 8.20^{ab}	92.50 ± 5.00^{a}
Kresoxim-methyl	95.00 ± 5.80^{ab}	95.00 ± 5.80^{a}
Nuarimol	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Insecticide		
S. feltiae	80.00 ± 8.20^{a}	97.50 ± 5.00^{a}
Kinoprene	82.50 ± 17.1^{a}	90.00 ± 8.20^{a}
Lufenuron	92.50 ± 5.50^{a}	95.00 ± 5.80^{a}
Methomyl	97.50 ± 5.00^{a}	100.00 ± 0.00^{a}
Metoxyfenozide	87.50 ± 9.60^{a}	100.00 ± 0.00^{a}
Oxamyl	80.00 ± 8.20^{a}	85.00 ± 5.80^{a}
Piperonyl-butoxide	87.50 ± 9.60^{a}	92.50 ± 9.60^{a}
Pyriproxyfen	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Tebufenozide	87.50 ± 9.60^{a}	90.00 ± 8.20^{a}
Acaricide		
S. feltiae	80.00 ± 8.20^{b}	97.50 ± 5.00^{b}
Azocyclotin	85.00 ± 10.0^{b}	95.00 ± 5.80^{b}
Clofentezin	77.50 ± 26.3^{b}	92.50 ± 15.0^{b}
Diafenthiuron	77.50 ± 5.00^{b}	82.50 ± 5.00^{b}
Etoxazole	97.50 ± 5.00^{b}	100.00 ± 0.00^{b}
Fenbutatinoxide	85.00 ± 10.0^{b}	85.00 ± 10.0^{b}
Fenpyroximate	7.50 ± 15.0^{a}	12.50 ± 9.60^{a}
Tebufenpyrad	0.00 ± 0.00^{a}	2.50 ± 5.00^{a}

Significant differences among pesticides within the group are marked with different small letters)

Virulence test

Insecticides showed only a slight effect on the virulence of *S. feltiae* (Table 2). 100% larval mortality was reached in pyriproxyfen-treated infective juveniles compared with the control (80.0%) after 3 days (F = 1.92, df = 8, 28, $P > 0.05$). The efficacy of all treated infective juveniles ranged from 80 to 100% after 5 days (F = 2.64, df = 8, 28, $P < 0.05$) while the mortality in the control was 97.50%. Nematode virulence was seriously affected by acaricides at both observed times: 3 days (F = 36.74, df = 7, 24, $P < 0.05$) and 5 days (F = 95.99, df = 7, 24, $P < 0.05$). Fenpyroximate and tebufenpyrad influenced the *S. feltiae* ability to infect the host considerably. Tebufenpyrad caused 95% and fenpyroximate 85% reduction in *S. feltiae* virulence after 5 days compared with the control (97.5%). The efficacy of other treated infective juveniles ranged from 82.5% to 100% after 5 days. Fungicides had a slight effect on *S. feltiae* virulence after 3 days (F = 3.57, df = 4, 15, $P < 0.05$). The 5th day (F = 2.96, df = 4, 15, $P > 0.05$) had no significant effect on nematode virulence.

The nematode reproduction was not influenced by any of the chosen active ingredients.

DISCUSSION

Results of this study indicate that the majority of here presented pesticides can be successfully used for integrated plant protection systems. However, the long-term tank mix of pesticides is not a routine practice and after the application pesticides remain in contact with the nematodes for an extended period. This event is typical of greenhouse conditions due to frequent watering which may dilute or wash off the pesticides. On that ground the application rate of nematodes based on the

knowledge of potential losses in vitality due to some pesticides should be predicted.

Helminth parasites possess a number of mechanisms for detoxification of harmful xenobiotics. More recent reports have shown that helminths also use the activity of cytochrome P450 system (Kerboeuf *et al.* 1995; Kotze 1997; Alvinerie *et al*. 2001). Piperonyl-butoxide acts as a synergist for insecticides by inhibiting the cytochrome P450-mediated metabolism of the insecticide (Jones 1998). In the present study piperonyl-butoxide affects *S. feltiae* mortality (18.68%). Experiments examining the toxicity of piperonyl-butoxide to larvae of the nematodes *Haemonchus contortus* and *Trichostrongylus colubriformis* (Nematoda: Trichostrongylidae) showed that the compound was not toxic at concentrations 20 mg/ml (Kotze *et al*. 2006).

Oxamyl was moderately toxic to *S. feltiae* in this study. Gaugler and Campbell (1991) showed that carbamate oxamyl stimulated the locomotor movement of the IJs of *S. carpocapsae* at the concentration less than 50 µg/ml but induced partial paralysis at a higher concentration. Other carbamate compounds with nematicide activity such as aldicarb, carbofuran and also oxamyl have been found to be toxic to nematodes, affecting IJ movement although the survivor's virulence was not affected (Gordon *et al.* 1996). In this study methomyl showed only a slight effect on nematode vitality. Rovesti (1989) reported a very strong effect of this active ingredient on *S. feltiae* vitality and virulence. In other studies the harmful effect of methomyl on entomopathogenic nematodes was also confirmed (http://www.norganics.com/label/grubguard.pdf).

A low effect on the vitality and virulence of *S. feltiae* was found in the case of fungicides. In this study nuarimol showed a beneficial effect on nematode vitality as well as on movement (data not shown), which consequently could influence the virulence of *S. feltiae* because the *T. molitor* mortality reached a higher rate compared to the control after 5 days. Nuarimol belongs to the same chemical class (Pyrimidine) as fenarimol. Rovesti *et al*. (1988) confirmed no detrimental effect of a.i. fenarimol on the nematode *H. bacteriophora*.

A significant effect on nematode virulence was observed in acaricides. However, tebufenpyrad and fenpyroximate were not directly toxic to nematodes but the virulence was reduced consequently. Tebufenpyrad and fenpyroximate are pyrazole substances with the same mode of action (inhibitor of the mitochondrial electron transport). This fact could cause physiological failures which reduce the *S. feltiae* movement and consequently the ability to find and infect the host. Nowadays pyrazole derivatives are studied for the nematicidal effect in agriculture and therefore this problem of incompatibility of some pyrazole chemicals with entomopathogenic nematodes should be pointed out.

Based on the present study we conclude that EPN are tolerant to the tested pesticides and the tank-mix application is possible in most compounds except the acaricides (a.i. tebufenpyrad and fenpyroximate) which reduced the virulence of *S. feltiae* significantly.

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