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Effect of chlorpyrifos on infectivity and survival of *Steinernema feltiae*

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Summary. The effect of chlorpyrifos on the mortality and the infectivity of *Steinenema feltiae* was tested in suspension or sand assays. The nematode response was significantly influenced by exposure times and insecticide doses as well as by their interaction. Although in the suspension assay the nematode mortality was relatively unaffected, the nematode infectivity was highly suppressed. In the sand assay, however, both the mortality and infectivity of the nematodes were significantly suppressed. Overall results indicate chlorpyrifos and *S. feltiae* cannot be used together in insect control strategies.
Key words: compatibility, entomopathogenic nematodes, *Galleria mellonella*, insecticide.

The combined use of chemical pesticides and biological control agents in the integrated pest management (IPM) has received much interest. IPM attempts to maximise the effectiveness of chemical pesticides against the target organism while minimising their adverse effect on biological control agents and other non-target organisms (Hara & Kaya, 1982). Entomopathogenic nematodes from the Steinernematidae and Heterorhabditidae are promising biological agents that can be used in IPM (Ishibashi, 1993). They are symbiotically associated with bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively. The nematodes are attracted by insect cues (Lewis *et al.*, 1992, 1993) and penetrate the insect host directly or through a natural opening (Bedding & Molyneux, 1982), move into the haemocoel, and release the bacteria. The nematode-bacterium complex kills the insects (Akhurst & Boemare, 1990).

Chlorpyrifos is a broad-spectrum insecticide that can be used for the control of Coleoptera, Diptera, Homoptera and Lepidoptera in soil and on foliage of a wide range of crops, including fruits and vegetables as well as field crops (Tomlin, 1994). If entomopathogenic nematodes and insecticides are to be included in IPM, it is important to ascertain the degree to which the chemical may affect these nematodes.

The entomopathogenic nematode *Steinernema feltiae* is compatible with many chemical pesticides including insecticides, miticides, fungicides and herbicides (Kaya & Burlando, 1989). Certain organophosphate and carbamate pesticides, however, induce adverse effects ranging from impaired movement and infectivity to death of the infective juveniles (IJs) (Rovesti & Deseo, 1990). The infectivity of nematodes surviving an insecticide treatment was unimpaired after nematodes were freed from insecticides (Kaya & Burlando, 1989; Gordon *et al.*, 1996). Chlorpyrifos-ethyl reduced the movement of *S. carpocapsae* and *S. feltiae* but its effect on the nematode infectivity to *G. mellonella* was negligible (Rovesti & Deseo, 1990). Heungens & Buysse (1987) suggested that chlorpyrifos might be used in combination with *Heterorhabditis* sp. without serious harmful effects on the nematode. Most of these studies were conducted by exposing IJs to a pesticide solution or suspension. The present study reports on the infectivity and persistence of *S. feltiae* after exposure to both a chlorpyrifos suspension and sand treated with the same pesticide.

MATERIALS AND METHODS

Nematodes. *S. feltiae* strain Lxm31 was isolated from Belgium (Miduturi, 1997) and cultured *in vivo* on *Galleria mellonella* larvae at 22°C (Woodring & Kaya, 1988). IJs collected from the host cadaver were stored in deionised water and

kept at 5°C for a maximum of two weeks. Before being used in experiments, nematodes were passed through a Baermann funnel lined with two layers of filter paper to guarantee that only living IJs were collected. IJs were incubated at room temperature (18-22°C) for 24 hours before use.

Insects. *G. mellonella* larvae used for culturing the nematodes and for bioassays originated from a culture continuously maintained in the laboratory for several years. The insect was reared at 28°C on a diet of honey (500g), glycerol (500g), brewer's yeast (250g), and wheat flour (850g). In the bioassays only last instar larvae (weight: 2.0-2.2g) were used.

Insecticide. 25% wettable powder of chlorpyrifos (0,0-diethyl0-3,5,6-trichloro-2-pyridyl phosphoro-thioate, BASF Chimie) was used in the bioassay.

Exposure of *S. feltiae* to chlorpyrifos in water suspensions. Equal volumes (10 ml) of both a nematode suspension and a chlorpyrifos suspension were mixed in 30ml-glass bottles. Final concentrations of chlorpyrifos were 0, 0.00001, 0.0001, 0.001, 0.01, 0.1, or 1 mg active ingredient (a.i.)/ml; the final nematode concentration was 400 IJs/ml. Each insecticide concentration was replicated five times. All insecticide-nematode combinations were kept at 22°C in the dark. Mortality of nematodes was determined after 1, 3, 5, 7, 9 and 16 days by counting the surviving nematodes in 1ml of the different insecticide concentrations. Probing each non-moving nematode with a needle under the dissecting microscope identified surviving IJs. Nematode infectivity to *G. mellonella* was monitored after IJs were washed free of the insecticide by three centrifugations in water.

Exposure of *S. feltiae* to chlorpyrifos in sand. Ten ml of a chlorpyrifos suspension containing 0, 0.002, 0.01, or 0.05 g a.i. were added to 100 g sterilised sand (particle size 250-425 µm) in a plastic pot (9 cm dia. x 8 cm). Two ml of a nematode suspension containing 2000 IJs were added to each pot and mixed thoroughly with the sand. Each pot was covered with a lid but not sealed and incubated at 22°C in the dark. One day, and one, two and four weeks later, nematodes were extracted from the total pot volume using automatic nematode extraction apparatus (Hendrickx, 1995). Extracted nematodes were immediately assayed for their survival (see above). They were then washed on a 5 µm sieve to remove the magnesium sulphate residue from their body, before they were transferred to a Baermann funnel with two layers of filter paper. Nematodes that were collected after 24 h were used in the infectivity assay. Each combination of exposure time and concentration was replicated five times.

Infectivity assay. 200 IJs were transferred in one ml water to a Petri dish (90 x 15 mm) containing a filter paper. Ten *G. mellonella* larvae were added to the dishes, which were sealed with Parafilm and incubated at 22°C for 72 h in the dark. Dead insects were dissected in Ringer solution (Woodring & Kaya, 1988). The number of nematodes in the insect cadaver was counted. There were five replicates for each treatment.

Statistical analysis. The NCSS software package was used for all statistical analysis. Nematode mortality in insecticide suspension was corrected with the mortality in the untreated control using Abbott's (1925) formula; nematode mortality in the sand assay was not corrected. All the proportional data were normalised by arcsine transformation prior to a two-way ANOVA. Duncan's multiple comparison test was used to separate the means.

RESULTS

Most IJs became immobile after they were transferred to insecticide free water. When exposed to insecticide suspensions, most IJs undulated. Partial paralysis and aberrant patterns of locomotion such as tremble were observed for nematodes exposed to the higher insecticide concentrations (10^{-1}-10^{-3} ppm in insecticide suspensions and 0.01-0.05 g a.i./pot in sand treatments)

Nematode mortality in insecticide suspensions varied between 0.2% at day 1 in the 10^{-3} ppm insecticide treatments to 30.9% in the 10^{-3} ppm treatments after 16 days (Fig 1). It was significantly affected by the dose ($F=120.76$, d.f.=5, $P\leq0.01$), exposure time ($F=31.63$, d.f.=5, $P\leq0.01$) and their interaction ($F=3.17$, d.f.=25, $P\leq0.01$). In sand treatments (Fig. 2), nematode mortality increased with increasing exposure time and insecticide dose and evolved from 10.2% in the 0.002 g a.i./100 g sand at day 1 to 99.4% in the 0.05 g a.i./100 g sand after four weeks. It was significantly affected by the exposure time ($F=421.73$, d.f.=3, $P\leq0.01$), the dose ($F=62.86$, d.f.=3, $P\leq0.01$) and their interaction ($F=14.48$, d.f.=9, $P\leq0.01$).

The infectivity of surviving nematodes, as expressed by the percentage of IJs penetrating *G. mellonella*, was significantly inhibited by the insecticide treatment and negatively influenced by

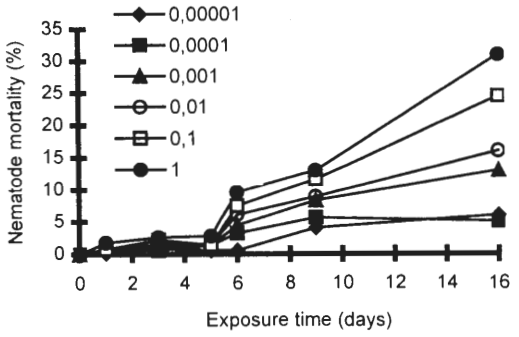

Fig. 1. Mortality of *Steinernema feltiae* after exposure to chlorpyrifos in suspension assay at different doses (mg a.i./ml) and exposure times.

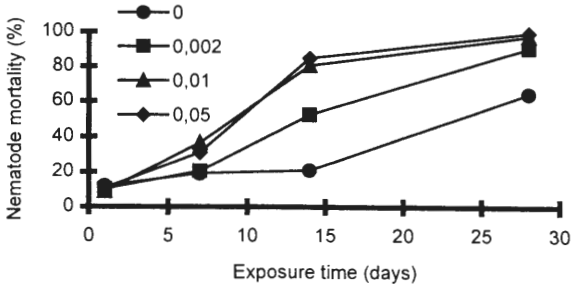

Fig. 2. Mortality of *Steinernema feltiae* after exposure to chlorpyrifos in a sand assay at different doses (g a.i/100 g sand) and exposure times.

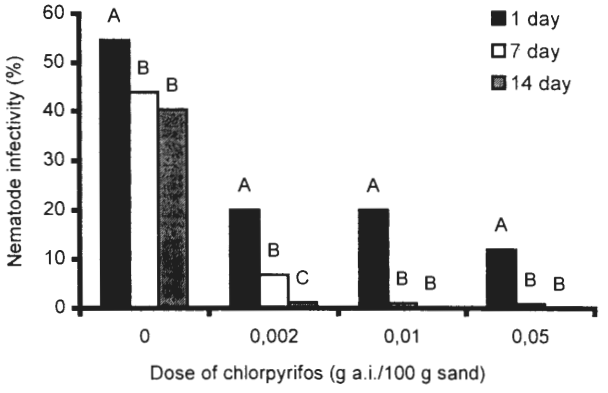

Fig. 3. Mean percentage infectivity to *Galleria mellonella* of *Steinernema feltiae* treated with chlorpyrifos at different doses and exposure times in a sand assays. Bars of the same concentration headed by the same letter are not significantly different at P≤0.01.

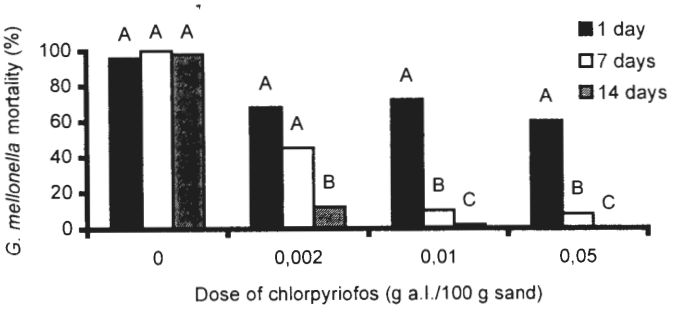

Fig. 4. Mean mortality of *Galleria mellonella* last instar larvae infected by *Steinernema feltiae* treated with chlorpyrifos at different doses and exposure time in the sand assay. For the same concentration, bars headed by the same letter are not significantly different at P≤0.01.

Table 1. Effect of chlorpyrifos doses and exposure time on the infectivity of *Steinernema feltiae* to *Galleria mellonella* (suspension assay).

Exposure time (days)	Doses of chlorpyrifos (ppm)						
	0	0.01	0.1	1	10	100	1000
1	43.4 a A	40.8 a A	41.7 a AB	33.5 ab A	17.9 b A	27.4 ab A	18.5 b A
3	59.3 a A	51.6 a AB	49.6 a B	14.0 b B	9.1 b A	10.2 b B	11.1 b A
5	45.6 ab A	61.3 a B	31.4 b AB	6.8 c BC	1.8 c B	3.5 c C	4.2 c B
7	38.7 a A	43.1 a C	24.0 b A	2.5 c C	1.3 c B	0.7 c C	1.3 c B
9	40.0 a A	45.0 a C	24.0 b A	5.1 c BC	1.0 c B	0.5 c C	0.9 c B
16	44.7 a A	44.6 a C	44.8 a AB	4.9 b BC	1.2 c B	1.0 c C	0.7 c B

Table 2. Mortality (%) of *Galleria mellonella* last instar larvae infected by *Steinernema feltiae* treated with chlorpyrifos at different doses and times.

Exposure time (days)	Doses of chlorpyrifos (ppm)						
	0	0.01	0.1	1	10	100	1000
1	100 a A	98 a A	96 a A	88 ab A	74 b A	96 a A	70 b A
3	100 a A	100 a A	100 a A	64 b AB	44 b B	54 b B	70 b A
5	100 a A	100 a A	72 b A	36 c BC	14 c C	26 c C	22 c B
7	94 a A	98 a A	84 a A	18 b C	10 b C	6 b C	14 b B
9	94 a A	96 a A	78 a A	36 b BC	10 c C	6 c C	8 c B
16	96 a A	96 a A	94 a A	28 b BC	8 c C	8 c C	6 c B

Rows followed by the same lowercase letter are not significantly different ($P \leq 0.01$). Columns followed by the same capital case letter are not significantly different ($P \leq 0.01$).

the insecticide dose and exposure time in both the insecticide suspensions ($F=36.00$, d.f.=5, $P<0.01$ for exposure time; $F=180.22$, d.f.=6, $P<0.01$ for dose; $F=3.8$, d.f.=30, $P<0.01$ for the interaction) (Table 1) and sand treatments ($F=79.60$, d.f.=2, $P<0.01$ for exposure time; $F=168.57$, d.f.=3, $P<0.01$ for dose; $F=3.68$, d.f.=6, $P<0.005$ for the interaction) (Fig. 3). Infectivity of nematodes extracted from insecticide suspensions ranged from 43.4% in the control at day one to 0.7% at the dose of 1 mg a.i./ml at day 16. The infectivity of nematodes collected from the sand ranged from 54.6% in the control at day one to 0% in the dose of 0.05 g a.i./100 g sand. Due to the small amount of nematodes extracted from the sand at week four, nematode infectivity was not evaluated.

The insecticide treatments also significantly affected the amount of *G. mellonella* killed by nematodes extracted from insecticide suspensions ($F=37.26$, d.f.=5, $P<0.01$ for exposure times; $F=119.44$, d.f.=6, $P<0.01$ for doses; $F=3.63$, d.f.=30, $P<0.01$ for the interaction) or from sand ($F=45.80$, d.f.=2, $P<0.01$ for exposure times; $F=89.65$, d.f.=3, $P<0.01$ for doses; $F=7.88$, d.f.=6, $P<0.01$ for the interaction). The smaller the insecticide dose or exposure time applied to the nematodes, both in the insecticide suspension (Table 2) and sand (Fig. 4), the greater the number of insects killed. *G. mellonella* mortality caused by nematodes originating from insecticide suspensions ranged from 100% in the untreated control to 6% in the dose of 10^{-3} ppm at day 16. Similarly, the mortality of *G. mellonella* caused by nematodes extracted from the sand ranged from 100% in the untreated control to 0% in the dose of 0.05g a.i./100g sand.

DISCUSSION

Obviously, chlorpyrifos influenced the survival and infectivity of *S. feltiae*. The greater the dose of the insecticide and the longer the time the nematodes were exposed, the greater the nematode mortality and the lower their infectivity. Chlorpyrifos stimulated *S. feltiae* to undulate. Some of the movements were even abnormal. This stimulation, however, did not enhance the nematode infectivity and insect mortality. In this study there is no evidence to support the idea that a mixture of nematodes and chlorpyrifos would increase nematode infectivity as suggested by Ishibashi (1993) who found that field applications of *S. carpocapsae*

with different insecticides yielded a better control of the insects than either the nematode or pesticide alone.

Heungens & Buysse (1987) concluded that chlorpyrifos could be used together with *Heterorhabditis* sp. but not at 22-23°C. These results were only based on the mortality of nematodes when exposed to a suspension of the insecticide. Our observations on nematode survival concur with this suggestion. Data on the infectivity and insect mortality, however, are in contrast: *S. feltiae* and chlorpyrifos are not compatible and cannot be used together in IPM. It may be possible to use both components if they are separated in space and time.

Previous research (Kaya & Burlando, 1989; Gordon *et al.*, 1996) showed that the infectivity of *S. feltiae* individuals that survived insecticide treatments was unimpaired after they were freed of insecticide. Our results demonstrate the contrary. This difference may partially be due to the difference in insecticides tested, doses and nematode isolates used. It may also be due to the difference in bioassay used. In the cited research, *G. mellonella* were exposed to the nematodes during 5 or 7 days, whereas in our study the host was challenged only during 3 days. The majority of nematodes may need a longer time to regain their infectivity after being freed from the insecticide.

Most of the experiments evaluating the compatibility of nematode and insecticide combinations are conducted by placing the nematodes in insecticide solution or suspension. That method, however, does not reflect the situation in the field. In our study *S. feltiae* was more sensitive in sand than in suspensions. Chlorpyrifos is a non-systemic insecticide with contact, stomach and vapour action (Tomlin, 1994). When exposed to insecticide suspensions, nematodes may only be affected by the contact action, whereas in the sand they may be affected by both contact and vapour action. In addition, the oxygen concentration is much higher in sand than in water. As a consequence, nematodes may have different physiological conditions that influence the nematode's resistance to the insecticide.

Gordon *et al.* (1996) reported that both *S. feltiae* and *S. carpocapsae* were equally sensitive to fenoxycarb whereas IJs of *S. feltiae* were several orders of magnitude more sensitive to carbofuran than to fenoxycarb. *Steinernema carpocapsae* IJs displayed approximately the same degree of sensitivity to carbofuran as they did toward fenoxycarb. No time-related response was observed in the combinations of *S. feltiae* with carbofuran or fenoxycarb. In our study, nematode mortality and infectivity were strongly affected by the duration of nematode exposure to chlorpyrifos. Although the insecticide inhibited the survival of nematodes, some individuals that survived the insecticide treatment still had the ability to penetrate the insect and complete their life cycle in the insect. This suggests that a genetic variation in insecticide tolerance exist. Consequently, one may think of selecting for insecticide-resistant strains, which can be used in IPM.

The present study explored the effect of chlorpyrifos on entomopathogenic nematodes. The compatibility of entomopathogenic nematodes and insecticides is affected by abiotic factors (such as nature of insecticide, temperature, soil moisture and texture) and biotic factors (e.g. species, strains, hosts). Therefore, the interaction between nematode, insecticide and host should further be studied. More information on the effect of the soil ecosystem on this interaction will be of great benefit to IPM that relies on the combination of insecticide and entomopathogenic nematodes.

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Chen S., Han X., Moens M. Воздействие хлорпирифоса на выживаемость и инвазионную способность *Steinernema feltiae.*

Резюме. Исследовали воздействие хлорпирифоса на жизнедеятельность и инвазионную способность *S. feltiae* в экспериментах с суспензиями и в колонках песка. Воздействие на нематод в значительной степени зависело от времени обработки и доз инсектицида, а также взаимодействия этих двух факторов. Хотя при экспериментах с суспензиями смертность нематод возрастала незначительно, их инвазионная способность резко снижалась. В экспериментах, проводимых в колонках песка, жизнеспособность и инвазионность *S. feltiae* были существенно ниже нормы. Полученные данные показывают, что хлорпирифос и *S. feltiae* не могут использоваться совместно для подавления насекомых-вредителей.