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RESEARCH ARTICLE

Mode of action and efficacy of iprodione against the root-knot nematode *Meloidogyne incognita*

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Abstract

The ban and restriction on the use of several synthetic chemicals for controlling plant parasitic nematodes, and concern about their side effects necessitate the availability of effective methods of control with low toxicity to humans and non-target organisms. Therefore, efficacy and mode of action of iprodione, a dicarboximide fungicide, was evaluated against the root-knot nematode *Meloidogyne incognita*, *in vitro* and *in vivo* conditions, in comparison with the nematicides fenamiphos, fosthiazate and oxamyl at 7.00, 1.66 and 1.66 mL/5 L water, respectively. *In vitro*, iprodione showed nematostatic rather than nematicidal activity against second-stage juveniles of *M. incognita* in contrast to fenamiphos, fosthiazate and oxamyl which were nematicidal. In the *in vivo* experiment with tomato, iprodione controlled *M. incognita* less than fenamiphos, fosthiazate and oxamyl. No visual symptoms of phytotoxicity were observed. Therefore, iprodione can be a useful chemical for controlling nematode populations if included in an Integrated Pest Management program.

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are among the most difficult crop pests to control (Chitwood, 2002). They cause severe yield losses worldwide, especially where crops are intensively grown. Formulations of synthetic chemicals are one of the most popular means for their management. However, the control of nematodes has become more difficult because of the ban or restricted use of nematicides, both fumigants and non-fumigants, due to toxicological and environmental concerns (Nordmeyer, 1992). Therefore, there is an increasing interest in developing nematicide or nematostatic formulations having low environmental impact.

Iprodione is a known contact dicarboximide fungicide characterised by a broad spectrum activity, and is used to control a wide range of fungal diseases (*Botrytis*, *Alternaria*, *Sclerotinia*, etc.) on vegetables, pome and stone fruits, ornamentals, root crops, sunflowers and cotton (Mendes *et al.*, 2013). In January 2013, a new formulation of iprodione was registered for use in Italy against root-knot nematodes (RKN) on solanaceous and cucurbitaceous crop plants. The efficacy of iprodione to control RKN had been reported on a wide range of crops in several Italian sites but results were unclear (Finlay *et al.*, 2012*a*,*b*). The purpose of the present study was to understand the mode of action of iprodione *in vitro* and its efficacy in *in vivo* conditions, against *Meloidogyne incognita* (Kofoid and White) Chitwood.

Materials and methods

Nematode

Eggs of *M. incognita* were collected from infected roots of tomato *Solanum lycopersicum* (L.) cv. Ikram, by shaking the chopped clean roots in a 0.5% solution of sodium hypochlorite for 3 min (Hussey & Barker, 1973). To obtain second-stage juveniles (J2s), eggs were spread on a nylon sieve (30 μm size net) in a Petri dish containing water and incubated at 28°C. J2s emerging within 24 h were used in the *in vitro* experiment.

Chemicals

Iprodione 500 g L^{-1} (Devguard® 500 SC; Cheminova), fenamiphos 240 g L^{-1} (Nemacur 240 CS; Adama),

fosthiazate 150 g L^{-1} (Nemathorin 150 EC; Syngenta) and oxamyl 100 g L^{-1} (Vydate 10L; DuPont) were used for comparison. The nematicidal solutions, made at recommended doses for field application of 30 000 L water ha^{-1}, were calculated for 5 L of water and were (mL/5 L water) 0.33 iprodione, 7.00 fenamiphos, 1.66 fosthiazate and 1.66 oxamyl.

In vitro experiment

The activity of iprodione *in vitro* was compared to that of fenamiphos, fosthiazate and oxamyl. Thirty-five *M. incognita* J2s were introduced in each well containing 2 mL of the respective chemical solution (Table 1). Then the wells were sealed with parafilm and maintained at 25 ± 2°C in the dark (Giacometti *et al.,* 2010) for 22 days. J2s exposed to distilled water served as controls. Non-motile J2s, which did not move after poking with a needle during an observation period of 10 s, were recorded every 24h and surviving nematodes are presented as relative survival per treatment where one represented 100% survival (Fig. 1). Daily, non-motile J2s were rinsed in water several times and transferred to a new well (the same well for each replicate throughout the observation period) containing distilled water and incubated at the same conditions to ascertain whether or not they resumed motility. Therefore, the percentage of non-motile nematodes that, after transferring to water, became motile was also calculated every 24 h for 19 consecutive days when all J2s were determined as dead. After the incubation period in water, non-motile nematodes were poked by a needle to check whether they were dead or alive. J2s that did not recover motility in water were considered dead. The data are presented as cumulative per cent motility (Fig. 2). Each treatment was replicated four times according to a completely randomised experimental design, and the experiment was performed twice.

In vivo experiment

The experiment was performed to compare the effect of iprodione to that of fenamiphos, fosthiazate and oxamyl on nematodes and the plant, in the greenhouse. Styrofoam boxes (40 × 60 × 20 cm), placed on soil surface covered with a polyethylene film, were filled with soil naturally and uniformly infested with *M. incognita*. The initial nematode population density of the soil was assessed processing the soil by Cobb's sieving and decanting method, followed by a modified Baermann's funnel technique for 10 days at 22 ± 2°C and then counting J2s (204 J2s per 100 mL soil). Six tomato seedlings cv. Ikram, at the four true-leaves stage (about 1-month-old), grown in alveolate polystyrene trays, were transplanted in each box. The soil was sandy (clay : silt : sand = 15:5:80; pH 8.8; organic matter <0.1%). Each box was drenched with 1.250 L solution of iprodione, fenamiphos, fosthiazate or oxamyl; controls received the same amount of water. The first application of each treatment was done at transplant (time = 0). Thereafter, applications were: iprodione once a week for 3 weeks; oxamyl only once, two weeks after transplant, according to manufacturer's recommendation (Table 1). Only applications transplant were made with the other two chemicals. Each treatment had four replicates according to a completely randomised experimental design and, the experiment was performed twice. Fertilization with water-soluble 20N-20P_2O_5-20K_2O, at a concentration of 200 ppm, was applied every 8 days at a rate of 10 L per box. Irrigation (10 L per box) was given according to crop needs, every 2 days. The appearance of phytotoxicity, if any, was monitored. Plant height, root galling index (RGI) and final population density of the nematodes at were recorded 8 weeks after transplanting. RGI was assessed according to a 0–5 scale, where 0 = no galled roots; 1 = trace of infections with a few small galls; 2 = less than 25% of galled roots; 3 = 25–50% galled roots; 4 = 50–75% galled roots; 5 = 75–100% galled roots (Ravichandra, 2010). Then, the average RGI was calculated by the following formula: Σ scores of sample plants/number of plants in the sample. The final nematode population in the soil was estimated as already mentioned.

Data analysis

Statistical analysis of the data was performed using SPSS 15.0 software (SPSS for Windows). The survival curves were plotted according to Kaplan–Meier method and differences were checked by independent Wilcoxon (Gehan) test. Means for plant height, RGI and nematode population density data were compared using one-way ANOVA. The significance of the differences between iprodione and each treatment was tested using the Student *t*-test.

Results

In vitro experiment

After 24 h of exposure, the motility of J2s was not affected by iprodione, whereas more than 60% of J2s exposed to fenamiphos and fosthiazate, and slightly less than 50% of those exposed to oxamyl, were immobilised (Fig. 1). A low percentage of non-motile J2s was recorded after 3 days of exposure to iprodione, but thereafter non-motile J2s gradually increased with time and a clear nematicidal effect was observed after an exposure of 6–7 days. During the entire period of the experiment, the percentages of

Table 1 Nematicides used, rates per ha and timing of application by soil drench

Commercial Product	Active Ingredient	Chemical Class	Rates (L ha $^{-1}$)	Application Timing (days)			
				T0	T1	T2	T3
Devguard 500 SC	Iprodione	Dicarboximide	2	AT	7 DAT	14 DAT	21 DAT
Nemacur 240 CS	Fenamiphos	Organophosphorus	42	AT	–	–	–
Nemathorin 10 G	Fosthiazate	Organophosphorus	10	AT	–	–	–
Vydate 10 L	Oxamyl	Oxime carbamate	10	AT	–	14 DAT	–

AT, at transplanting; DAT, days after transplanting.

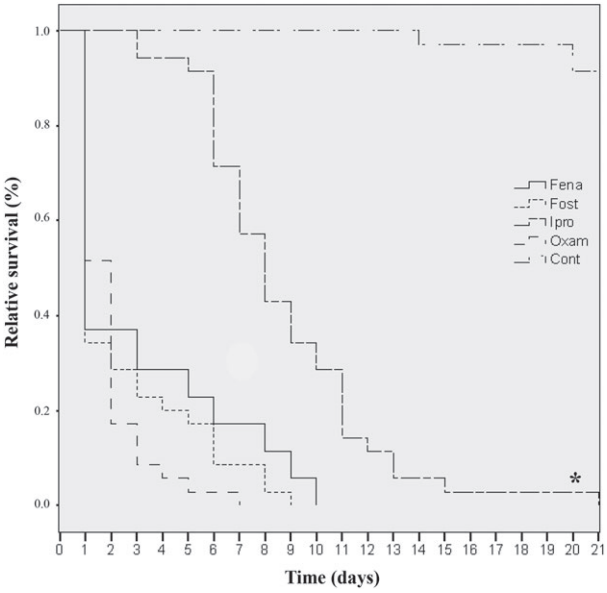

Figure 1 Relative survival (1 = 100%) of *Meloidogyne incognita* second-stage juveniles after different treatments with iprodione (Ipro), fenamiphos (Fena), fosthiazate (Fost) and oxamyl (Oxam) at doses of 0.33, 7.00, 1.66 and 1.66 mL/5 L water, respectively, and water (Cont) after 24 h. Relative survival was calculated with the Kaplan-Meier method and differences were compared by the independent Wilcoxon (Gehan) test. The *asterisk* indicates a significant difference between Ipro and the other treatments or water control (*$P < 0.05$). Data shown are from a single experiment that is representative of results obtained from two independent experiments, whereby the values were similar and not significantly different.

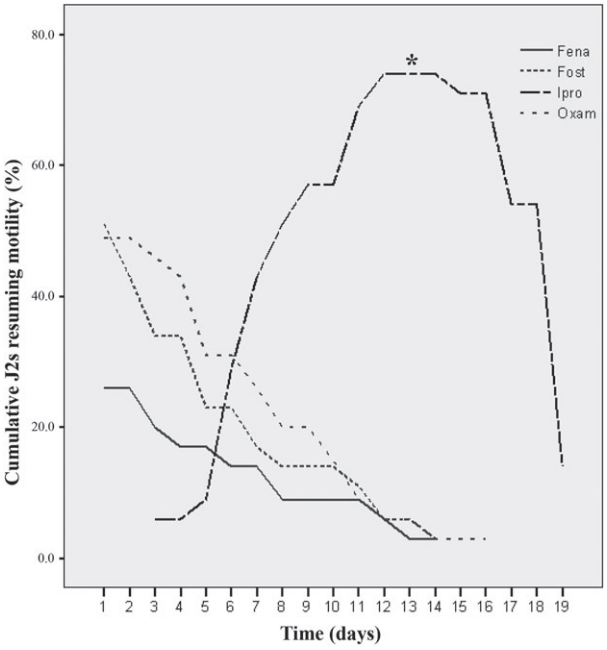

Figure 2 Cumulative second-stage juveniles of *Meloidogyne incognita* that resumed motility in distilled water after previous exposure to iprodione (Ipro), fenamiphos (Fena), fosthiazate (Fost) and oxamyl (Oxam) treatments. Observations of iprodione treated nematodes started after 4 days since non-motile J2s were not noticed during the first 3 days in the solution containing this chemical (see Fig. 1). Cumulative percent of motile nematodes was calculated with the Kaplan-Meier method and significant differences were determined by the independent Wilcoxon (Gehan) test. The *asterisk* indicates a significant difference between Ipro and the other treatments or water control (*$P < 0.05$). Data shown are from a single experiment that is representative of results obtained from two independent experiments, whereby the values were similar and not significantly different.

non-motile J2s exposed to iprodione remained always less than those exposed to fenamiphos, fosthiazate and oxamyl solutions (Fig. 1). In the control, the viability of J2s was not affected by the incubation time and the low mortality registered after two weeks was considered physiological. The relative survival time for *M. incognita* exposed to iprodione was 7.50 days, longer when compared to those of fenamiphos (0.80), fosthiazate (0.76) and oxamyl (1.04), but shorter than the 20.00 days for control.

After 24h in water, the non-motile J2s pre-exposed to iprodione recovered their motility in a shorter time than those J2s exposed to the other nematicides (Fig. 2). The greatest survival rate of the nematode was observed in iprodione and the least in fenamiphos compared to fosthiazate and oxamyl (similar values). However, the resumption of motility of J2s pre-exposed to iprodione gradually decreased over times. In all treatments, the non-motile J2s that resumed motility showed a shorter lifespan than those J2s remaining motile in the nematicide solution; and this effect was greater for fenamiphos, fosthiazate and oxamyl (Fig. 2). No significant differences

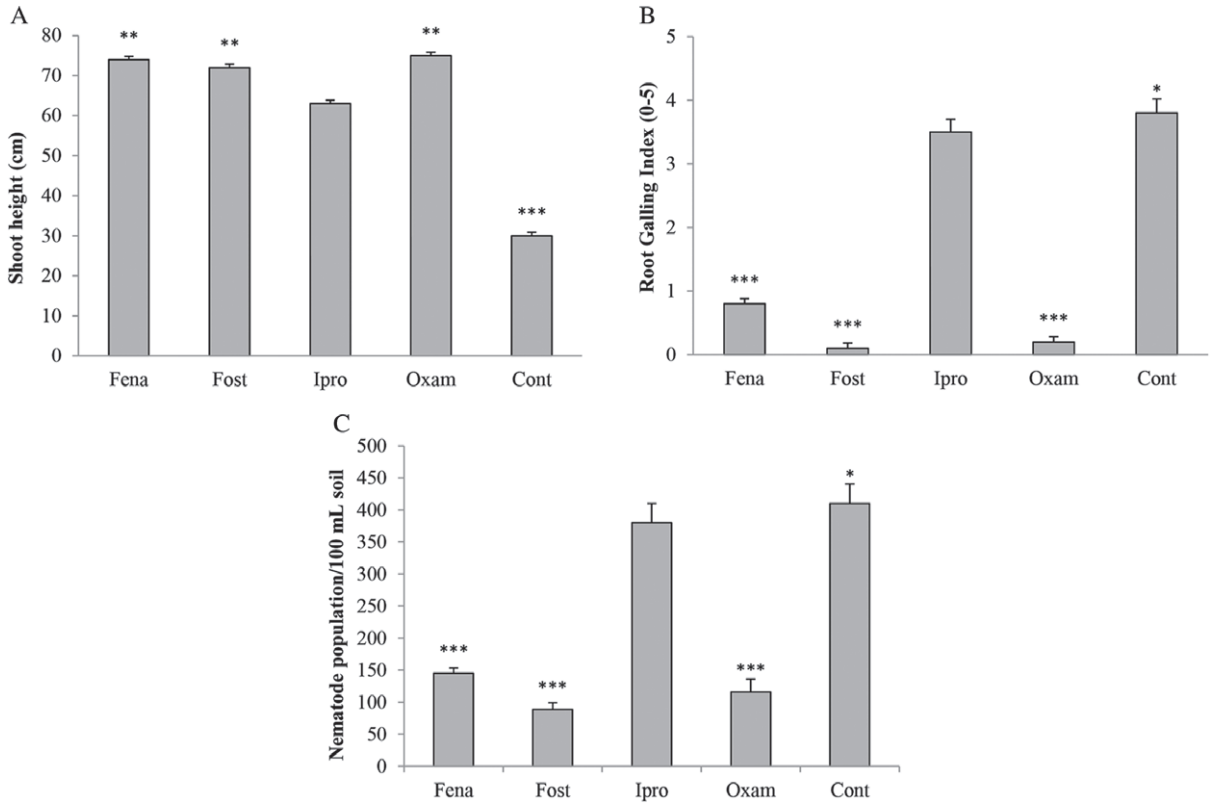

Figure 3 Effect of soil treatments with iprodione (Ipro), oxamyl (Oxam), fosthiazate (Fost) and fenamiphos (Fena), at the doses of 0.33, 1.66, 1.66 and 7.00 mL/5 L water, respectively, on tomatoes grown in boxes containing soil naturally infested with *Meloidogyne incognita* second-stage juveniles, compared to untreated soil (Cont), 60 days after transplanting: (A) plant height (cm); (B) Root galling index, according to a 0–5 scale where 0 = no galls and 5 = 75–100% of galled roots; (C) final nematode population density in the soil (J2s in 100 mL soil). Values are means ± standard deviation (SD) of four replicates, and means were separated using *t*-test. Bars indicate SD. The *asterisks* indicate significant differences between Ipro and the other treatments or water control (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$). Data shown are from a single experiment that is representative of results obtained from two independent experiments, whereby the values were similar and not significantly different.

in the recovery of motility were observed among J2s exposed to fenamiphos, fosthiazate and oxamyl solutions, but recovery of J2s pre-exposed to these chemicals was significantly smaller than those J2s pre-exposed to iprodione and control ($P < 0.05$). The experiment was repeated, but as the results were similar and not statistically different, only the data from a single experiment are shown in Fig. 1.

In vivo experiment

All treatments improved plant growth and reduced nematode severity and soil population density compared to control. Plant height was greater than the control but not significantly different among soils treated with fenamiphos, fosthiazate and oxamyl, whereas plants grown in iprodione-treated soil were significantly shorter than those of the other chemical treatments, but taller than those in the control (Fig. 3A). RGI was greater and nematode population density was greater in soil treated with iprodione when compared to fenamiphos, fosthiazate and oxamyl ($P < 0.001$). The differences of the mentioned variables between plants treated with fenamiphos, fosthiazate and oxamyl and iprodione were greater than those between plants treated with iprodione and control ($P < 0.05$) (Fig. 3B and Fig. 3C). Only fosthiazate caused visual symptoms of phytotoxicity. No significant difference was observed between the results of two independent experiments.

Discussion

Worldwide yield losses in tomato due to RKN have been estimated at 20.6% (Ravichandra, 2014). Because of the wide host range (d'Errico *et al.*, 2014) and extensive geographic distribution, host plant resistance and crop rotation cannot assure an adequate management of RKN (Johnson, 1982, 1989; Fortnum &

Currin, 1993; d'Errico *et al.*, 2016). Currently available soil fumigants are more effective than non-fumigants for controlling nematodes. However, carbamate- and organophosphate-based nematicides are highly toxic to humans and non-target organisms, so their use is restricted. Many fumigant compounds, acting mainly as fungicide, such as metham-potassium, metham-sodium and dazomet also have nematicidal activity.

Our results confirm that the effect of a nematicide on the nematodes is affected by the concentration × time application of the product, and that at a relatively low dose, the effect is of the nematistatic but becomes to nematicide at rather high dose. In our experiment, iprodione affects *M. incognita* J2s by nematostasis, paralysis and disorientation of its J2s, which consequently reduces their ability to locate and feed on host plants. For short cycle crops and in the presence of low nematode population densities, iprodione could play an important role in enhancing plant growth and could be used at transplant, followed by additional applications. Also, iprodione may be used in combination with other active compound such that the crops can benefit from its fungicide activity and by prolonging the period during which the plants remain protected from the nematode.

In conclusion, as iprodione is characterised by a safer profile (low acute toxicity, low environmental persistence, low solubility in water and low toxicity to honeybees and earthworms), it could be employed in Integrated Pest Management (IPM) program, following a knock-down treatment, to control RKN.

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