ENVIRONMENTALLY SAFE COMPOUNDS FOR CONTROLLING THE JAVANESE ROOT-KNOT NEMATODE IN POTS

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Summary. The efficacy of Vermicompost, Drangonfire-CPP, Actigard, and DiTera against the root-knot nematode *Meloidogyne javanica* was determined in a greenhouse test. These compounds with low environmental impact were applied as pre- and post-plant treatments to cowpea plants growing in a mixture of Hawaii soil and sand in pots. Nematode populations/plant and root galling, after 8 weeks, were suppressed in all treatments, although none was as effective as fenamiphos. Plant growth was also enhanced as compared to the untreated control. Pre-plant applications of Vermicompost, Dragonfire, and DiTera were more effective than their post-plant applications. Under the controlled conditions of the experiment, these compounds showed promise as safer alternatives to traditional nematicides like fenamiphos.

Key words: Acibenzolar-S-methyl, Actigard, DiTera, Dragonfire, *Meloidogyne javanica*, Vermicompost, *Vigna unguiculata.*

Root-knot nematodes, *Meloidogyne* spp., are the most widely distributed and economically important plant-parasitic nematodes in the world (Sasser, 1977). Root-knot nematodes have wide host ranges, limiting the utility of cultural control tactics like crop rotations, green manures, and cover crops. Consequently, nematicides are often used to control root-knot nematodes. The current organophosphate and carbamate nematicides, however, can contribute to environmental pollution, create human health hazards, and be toxic to nontarget animals. Several potentially safer compounds, such as the bio-rationals, botanical nematicides and soil amendments, are available as alternatives to organophosphate and carbamate nematicides. Actigard® (Syngenta Crop Protection, Greensboro, NC), acibenzolar-S-methyl, induces a systemic acquired resistance response in many plants that can affect adversely nematode infection (Owen *et al.*, 2002; Chinnasri *et al.*, 2003). DiTera® (Valent BioSciences, Walnut Creek, CA) is a natural product derived from the fungus *Myrothecium verrucaria* (Albertini and Schwein) Ditmar that has effectively controlled root-knot nematodes in field tests (Fernandez *et al.*, 2001; Giraud *et al.*, 2004). Dragonfire-CPP® (NaEx Corp-Poulenger USA, Stafford, TX) is an edible food-grade cold-pressed oil from *Sesamum indicum* that has provided promising results in nematode management in some crops (Sipes, 2006). In preliminary experiments, Vermicompost, or earthworm castings, has suppressed the populations of unidentified plant-parasitic nematodes under field conditions (Arancon *et al.*, 2002; Sipes, unpublished). There is a lack of information on the effects of sesame oil and earthworm castings on the Javanese root-knot-nematode, *Meloidogyne javanica* (Treub) Chitw. Furthermore, the adoption and use of these and other substances with low-toxicity require results and documentation from experiments conducted in different environmental conditions. The objective of the present study was to determine the efficacy of Actigard, DiTera, Dragonfire-CPP, and Vermicompost in managing *M. javanica* infections on cowpea growing in a soil mix of Hawaii soil and sand in pots.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse (temperature range of 22-28 °C and average day length of $11^1/_2$ hours) on the University of Hawaii at Manoa campus. The three products (Actigard, Dragonfire, and Vermicompost) were evaluated as pre-plant and post-plant treatments. Controls consisted of a pre- and post-plant applications of fenamiphos and water. Pre- and post-plant applications of DiTera served as a bio-rational comparative in the tests. Ten-cm-d clay pots were filled with 500 cm^3 of a mixture (1:1) of steam sterilized silty clay soil (pH 6.9) and quartz sand, which was infested with *M. javanica* using the procedure described below. In pre-plant treatments, the compounds were applied to the infested soil and the cowpea seedlings transplanted into the pots 1 week later. In the post-plant treatments, the cowpea seedlings were transplanted into the nematode-infested soil and the compounds applied 1 week after transplanting. Vermicompost, generated from earthworms fed food waste, was evaluated at a rate equivalent to 5 and 10 t/ha. For the pre-plant application, the Vermicompost was mixed with the soil:sand potting medium. The Vermicompost was applied as a top dressing in the post-plant treatment. Dragonfire-CPP was applied to the soil at 6.67 or 9.58 l/ha. The Dragonfire and its associated Emulsifier

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7509 (coconut alkanolamide) were mixed with water at a 1:5:90 (v:v:v) ratio of emulsifier to sesame oil to water. DiTera ES was applied at the rate of 12.35, 24.7, and 74.1 l/ha. The 74.1 rate was achieved with a weekly application of 12.35 l/ha for 6 weeks. Actigard 50 WG (100 mg/l water) was applied as a pre-plant spray over the soil or as a post-plant spray on the foliage. Fenamiphos (Nemacur 3®, Bayer CropScience, Research Triangle Park, NC) was applied at the rate of 9 kg/ha and drenched into the pot with 100 ml water. A 100 ml water pre- and post-plant drench served as the untreated control. Each treatment was replicated four times. Treatments were arranged in a randomized complete block design. The pots were watered regularly with tap water and the experiment was terminated 8 weeks after transplanting.

Seeds of *Vigna unguiculata* (L.) Walp. cv. California Black Eye were sown in 20-cm-d clay pots filled with vermiculite. One week later, uniformly sized seedlings were transplanted singly into the 10-cm-d clay pots.

Eggs of *M. javanica* were extracted from the infected roots and corms of *Colocasia esculenta* (L.) Schott maintained in the greenhouse as stock cultures. The corm with roots attached was cut into 2-cm^3 pieces, shaken in a 0.5% NaOCl solution (McClure *et al.*, 1973), and the nematode eggs collected on a 20 µm mesh sieve. The eggs were counted and the volume of water adjusted to give 100 eggs/ml. Each pot was inoculated with 1500 nematode eggs.

At the termination of the experiment, the cowpeas were removed from the pots, the soil was carefully shaken from the roots, and the roots then gently washed under running water. The number of galls on each root system was counted and rated according to a galling index of 0-5 (Barker, 1985). Shoot height and root length were measured. Shoots were then separated from roots. Roots were shaken in NaOCl, eggs collected, and counted. Shoot and root tissue was oven dried and the weight recorded. The nematode population density in the soil was determined by elutriation and centrifugation (Byrd *et al.*, 1976; Jenkins, 1964). The data were analyzed for variance and means separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

All of the compounds suppressed nematode populations as compared to the water control (Fig. 1). Fenamiphos was the most effective, but the high rates of pre-plant Dragonfire and both pre- and post-plant DiTera applications reduced nematodes populations to similarly low levels (Fig. 1). Vermicompost, Dragonfire and DiTera were more effective as pre-plant rather than post-plant applications. The pre-plant applications would be expected to be more effective since the compounds are not absorbed or translocated by the cowpea plant. These compounds may act by affecting the nematode ability to penetrate the host. Consequently, once the nematode has penetrated the host, the compounds are not likely to have any effect on the root-knot nema-

Table I. Effect of different compounds on the root gall index of cowpea grown for 8 weeks in pots containing soil infested with 1,500 *Meloidogyne javanica* eggs/plant.

Compound	Rate	Application time	Gall index[a]	
Vermicompost	5 t/ha	Preplant	2	abc
		Postplant	2	abc
	10 t/ha	Preplant	1	cd
		Postplant	1	cd
Dragonfire-CPP	6.7 l/ha	Preplant	1	cd
		Postplant	2	abc
	9.6 l/ha	Preplant	1	cd
		Postplant	1	cd
DiTera ES	12.4 l/ha	Preplant	1	cd
		Postplant	2	abc
	24.7 l/ha	Preplant	1	cd
		Postplant	1.25	bcd
	74.1 l/ha	Preplant	1	cd
		Postplant	1	cd
Actigard 50WG	100 µg/l	Preplant	1	cd
		Postplant	1.75	abc
Nemacur 3	9 kg/ha	Preplant	0	d
		Postplant	0.75	cd
Water		Preplant	2.5	ab
		Postplant	3	a

[a]Gall Index is based upon a 0-5 scale, where 0 = no galling, 1 = up to 10% of root system galled, 2 = between 11% and 20% of root system galled, 3 = 21% to 70% of root system galled, 4 = 71% to 85% of root system galled, and 5 = more than 85% of root system galled. Values are the average of four roots systems. Mean values in a column followed by the same letter are not significantly different (P>0.05).

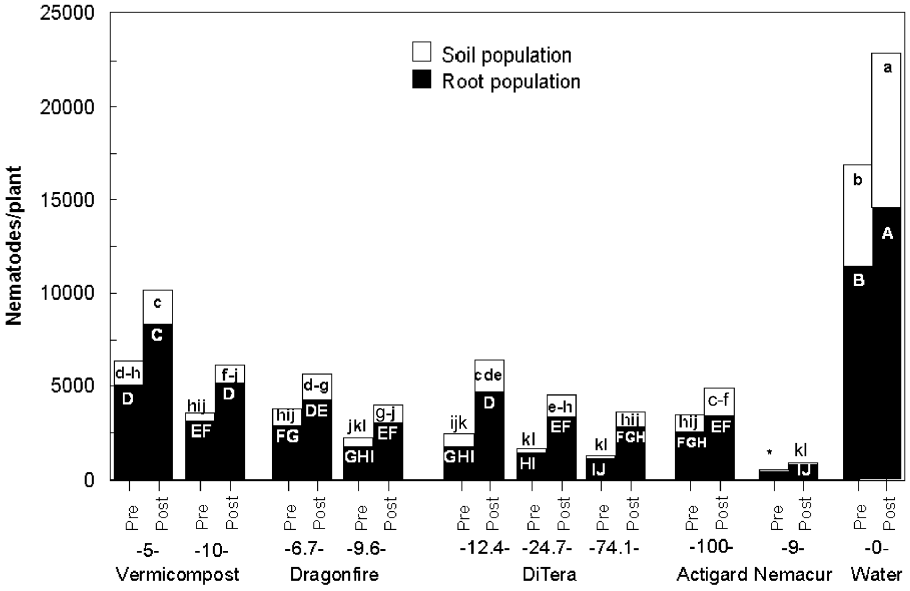

Fig. 1. Effect of different compounds on the final soil and root population levels of second-stage juveniles and eggs of *Meloidogyne javanica* on cowpea grown for 8 weeks in pots containing soil infested with 1,500 nematode eggs/plant. Bar sections with the same letters are not significantly different (P>0.05). *Letters for the pre-plant treatment with nemacur are: soil population = l, root population = j.

tode population. The magnitude of their adverse effect on nematode reproduction was greater with the higher application rates. Surprisingly, both the pre- and post-plant applications of Actigard had similar effects on the nematode. We expected the post-plant application to be much more effective since this product has systemic activity and should be applied to the plant canopy (Chinnasri *et al.*, 2003). It is possible that the Actigard remained in soil after the pre-plant application and was taken up by the cowpea plants upon transplanting. DiTera was the next most effective compound after fenamiphos, followed closely by Dragonfire.

Galling was reduced by the pre-plant application of the compounds at the higher rates as compared to the water control (Table I). The gall index tended to be lower with the high rate applications of Vermicompost, DiTera, and Dragonfire as compared to the lower rates of these compounds. Fenamiphos application resulted in the lowest number of galls and lowest galling index of all the treatments (Table I). Galling indices overall were low in the test, reaching only 3 in the untreated control.

Effects of the compounds on plant growth were less apparent. Dry root weights did not differ among treatments (Fig. 2) (P>0.05). Shoot weights, however, did

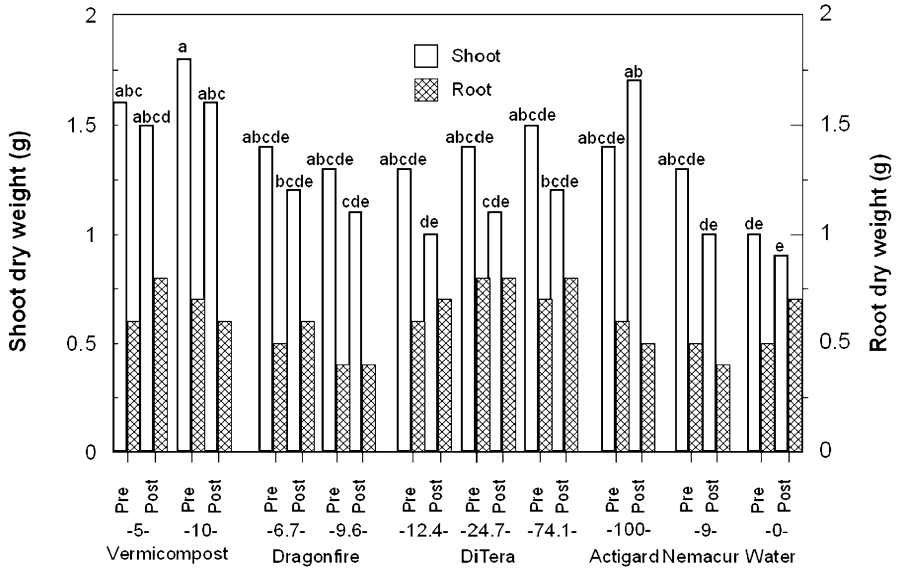

Fig. 2. Effect of different products on the shoot and root dry weight of cowpea grown for 8 weeks in pots containing soil infested with 1,500 eggs of *Meloidogyne javanica*/plant. Bars with the same letters are not significantly different (P>0.05).

vary among treatments (Fig. 2) and were greater in the pre-plant 10 t/ha Vermicompost treatment than in all of the other treatments. Only three treatments, the post-plant Actigard and both pre- and post-plant Vermicompost applications, induced more plant growth than the untreated control ($P<0.05$). The post-plant fenamiphos treatment appears to have been phytotoxic in two replicates, which reduced the average plant growth of the treatment. The relatively short growing period (8 weeks) may have precluded the manifestation of greater differences in plant growth.

The action of the tested compounds against *M. javanica* may be attributed to multiple and different effects. The efficacy of fenamiphos is well documented (Evans, 1973; Ami and Al-Sabie, 2003) and related to effects on nematode behaviour and development (McLeod and Khair, 1975; Ami and Al-Sadie, 2003). DiTera affects the nematode through contact toxicity, inhibition of egg hatching and by modifying nematode behaviour to plant roots (Twomey *et al.*, 2000; Giraud *et al.*, 2004). Actigard induces systemic acquired resistance in plants and thus retards nematode colonization of the root system without any direct effects on the root-knot nematode (Owens *et al.*, 2002; Chinnasri *et al.*, 2003). The inhibition of the nematode root infection observed with the Vermicompost and Dragonfire may be attributed to direct effects on the nematode (Alam *et al.*, 1980; Arancon *et al.*, 2004; Sipes, unpublished). The reduction in nematode damage with Vermicompost is probably not solely a function of reduced nematode infection but also an enhancement of plant growth (Arancon *et al.*, 2004).

These low-toxicity products show promise as alternatives to more traditional nematicides like fenamiphos. These products are safer for applicators, have few effects on non-target organisms, and pose little risk of environmental pollution. However, the promising results obtained by the compounds tested in this pot experiment may be not reproducible under different environmental conditions and in the open field.

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