Sting Nematode, Belonolaimus longicaudatus, Immotility Induced by Extracts of Composted Municipal Refuse[1]

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Abstract: Water extracts from saturated composted municipal refuse rendered sting nematodes, *Belonolaimus longicaudatus*, immotile after immersion for 12 hr. Extract concentrated to 33% of its original volume rendered all of the 50 sting nematodes tested immotile in 3 hr. The effect of compost extract was slightly reduced by cation exchange and greatly reduced by peroxide digestion of the organic fraction. Immotile nematodes were transferred from compost extract to distilled water after 24 hr and 60% regained motility, but after 144 hr none regained motility. *Key word:* death.

Composting municipal refuse has been considered and/or utilized by a number of municipalities to process solid wastes. Composted municipal refuse used as a soil amendment provides for solid waste disposal as well as for possible agronomic benefits. In a typical composting process, solid wastes such as household garbage, lawn trimmings and cardboard boxes are separated from the large items (auto axles, logs, etc.) and metal cans, and the former ground into small fragments. The ground solid waste is transported into a composting bin where it is moistened, continually aerated and allowed to stay approx. 6 days. During this period, considerable decomposition takes place and the temperature of the compost rises to more than 70 C. After the composting period, the refuse is transported to a compost heap where it awaits some use such as application to agricultural land. Bell (1) reported an average municipal refuse as follows: cellulose, sugar and starch, 46.6%; fats, oils and waxes, 4.5%; protein (6.25 N), 2.0%; other organics (plastics), 1.2%; ash, metals and glass, etc., 24.9%.

Suppression of plant parasitic nematodes by a variety of organic soil amendments has been reported by a number of authors. Lear (4) found that populations of root-knot nematodes, *Meloidogyne incognita*, declined when 11-22 t/ha (metric tons/hectare) of castor bean pomace were applied to the soil. Oat straw or lespedeza hay at 1% by weight was reported by Johnson (3) to control root-knot nematodes on tomato. Singh et al. (9) applied sawdust at 2.22 t/ha with supplemental N-P-K to okra and tomato plots infested with *Meloidogyne javanica*. Root galling was one-third to one-fourth that of controls, and yield increases of 70 and 125% occurred in treated okra and tomato plots, respectively. Walker et al. (12) reduced *Pratylenchus penetrans* on soybean by adding 1% by weight of soybean meal to the soil. Raspberry canes incorporated into soil by Taylor and Murant (10) reduced populations of *Longidorus elongatus*. Mankau (5) postulated that nematode suppression following the addition of organic amendments to soil partially was due to increases in nematode predators. However, Tomerlin (11) attributed the reduction of *Belonolaimus longicaudatus* in soils amended with alfalfa meal, cottonseed meal and rice straw to organic toxins rather than nematode predators, or inorganic constituents. Earlier research by Sayre et al. (8) supports Tomerlin's view. They reported that extracts of soil containing decomposing timothy and rye residues were toxic to *Meloidogyne incognita* and *Pratylenchus penetrans*. Butyric acid behaved identically, both chemically and biologically, to one of the isolated compounds. Although the point was not clear, Sayre et al. (8) apparently used motility of nematodes as a measure of toxicity. Hence, motility was used in our study as a measure of the suppressing effect of both the organic and inorganic fractions of composted municipal refuse on the sting nematode.

MATERIALS AND METHODS

During a 2-day period, three samples of composted municipal refuse (compost) were

Received for publication 12 April 1972.

[1] Florida Agricultural Experiment Stations Journal Series No. 4370. A portion of a dissertation submitted by the senior author to the graduate council of the University of Florida in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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obtained during 15- 20-min sampling periods from the conveyer belt at the final grinder of the Gainesville Municipal Waste Conversion Authority Plant. A composite of these samples was used in this experiment. Moisture levels, determined on water-saturated ceramic plates by a method similar to that of Richards (7), were approx. 400% at saturation and 100% at 0.33 bar moisture tension on a dry weight basis. Water extracts were obtained by adding distilled water to compost in 15-cm aluminum pans until the compost glistened when touched with a spatula, equilibrating for 12 hr at 5 C and filtering under vacuum through Whatman No. 41 filter paper in a Büchner funnel. The extract was reduced to one-third its volume by vacuum distillation at 35-39 C, and divided into six subsamples. The concentrated extracts contained the following percentages of major elements: K, 0.11; Na, 0.14; Ca, 0.02 and Mg, 0.02; and the following ppm of minor elements; Al, 18; Fe, 15; Mn, 6; Cu, 3 and Zn, 1.

Five treatment combinations of hydrogen peroxide digestion, cation exchange and anion exchange were used. The cation exchange resin was Amberlite IRC-50(H)AR, 16-50 hydrated mesh size; the anion exchange resin was Amberlite CA-4B(OH)AR, type 1, 100-200 hydrated mesh. The cations and anions were exchanged for hydronium and hydroxide ions, respectively. Three of the six subsamples were digested in hydrogen peroxide to destroy the organic fraction and three were not digested. In both the digested and the undigested subsamples, one subsample received anion exchange, one cation exchange and one no ion exchange treatments. The anion and cation exchanged extracts were adjusted to their original pH by use of a 1:4 mixture of distilled water to concentrated hydrochloric acid or a saturated calcium hydroxide solution. Portions of concentrated extract were diluted by 1:3, 1:9 and 1:27 with distilled water to form solutions that would approximate 33, 11 and 4% the potency of the concentrated extract.

Sting nematodes, *Belonolaimus longicaudatus* Rau, were obtained from greenhouse colonies maintained on tomato (*Lycopersicon esculentum* 'Rutgers'). The nematodes were removed from the soil by the sugar flotation technique (6), and rinsed immediately to remove the sugar solution and avoid damage from plasmolysis. One milliliter of each of the subsample treatment solutions and 10 active nematodes were placed into separate BPI dishes (Bureau of Plant Industry watch glasses), with each treatment replicated five times. Nematodes were observed under 15 X magnification at intervals over a 48-hr period in the dilution study (1:3, 1:9, 1:27) and over a 96-hr period in the fractionation study (digestion-ion exchange). If a nematode was not visibly motile, attempts were made to stimulate

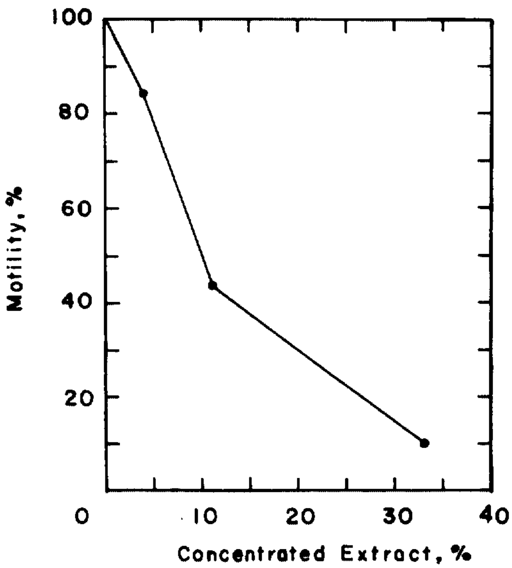

FIG. 1. Motility of sting nematodes after exposure to various potencies of concentrated compost extract.

TABLE 1. Motility of sting nematodes exposed to concentrated compost extract and various fractions.

Treatment	Nematode motility (%) Exposure time (hr)[a] 3	20	48	96
Extract	0 d	0 b	0 c	0 c
Extract, anions exchanged	0 d	0 b	0 c	0 c
Extract, cations exchanged	58 c	0 b	0 c	0 c
Extract, organic matter digested	84 b	78 a	68 a	18 b
Extract, organic matter digested and anions exchanged	88 ab	82 a	14 b	10 b
Extract, organic matter digested and cations exchanged	94 a	84 a	78 a	32 a
Tap water control	100	100	94	84

[a]Means in the same column followed by the same letter are not significantly different at the .01 level by Duncan's new multiple range test.

movement by prodding with a wire pick. If no motility was observed after considerable stimulation, including touching the nematode in the area of the nerve ring, the nematode was recorded as immotile. After 6, 24 and 144 hr in the treatment solutions, two immotile nematodes were removed from each replicate of the non-hydrogen peroxide-digested treatment solutions, placed in distilled water and observed for motility over a 48-hr period.

RESULTS

The effects on nematode motility of extract diluted 1:3, 1:9 and 1:27 with distilled water to approximate 33, 11 and 4% potency of concentrated extract are reported in Fig. 1. After 48 hr, the 33 and 11% potency solutions had reduced the motility of sting nematodes significantly but the 4% potency solution and the control had not.

When sting nematodes were placed in concentrated extract that was either untreated, digested with hydrogen peroxide and/or ionically exchanged on resins, none of the nematodes remained motile in the untreated or undigested anion-exchanged extracts after 3 hr (Table 1). None was motile in the undigested cation-exchanged extract after 20 hr. Nematode motility remained high for 20 hr in the other treatments where the organic fraction was digested by hydrogen peroxide, but not as high as in the control. There was a considerably more rapid decline in motility in the digested extracts than in the control after 20 hr, and the decline was most rapid in the anion-exchanged extract.

Over 80% of the nematodes exposed to the organic fraction for 6 hr and over 60% of those exposed for 24 hr regained motility within 1 hour after being placed in distilled water. However, after 144 hr in the organic fraction of compost extract, no nematodes regained motility when transferred to distilled water.

DISCUSSION

If the assumptions are made that all the toxic compounds were equilibrated in the aqueous phase of the saturated compost before extraction and that there was no loss of toxicity during concentration, the threefold concentrated extract would closely approximate the toxicity of the aqueous phase of compost at 0.33 bar moisture tension. If the additional assumption is made that aqueous dilutions of compost are similar to soil dilutions of compost, the 1:3, 1:9 and 1:27 dilutions would approximate the toxic effects of 33, 11 and 4% by weight of compost added to the soil. With these assumptions, the information in Fig. 1 indicated that compost applications of 5-10% (55 to 100 t/ha) may suppress sting nematodes. This prediction is in general agreement with the response of spiral nematode, *Helicotylenchus* spp., population in oat and sorghum field plot amended with compost where Hunt (2) found lower rates of composted municipal refuse - 8, 16 and 32 t/ha - to suppress spiral nematode populations. The differences may have resulted from inefficiency in extracting toxic compounds, or *Helicotylenchus* spp. may have been more sensitive than *Belonolaimus longicaudatus*. However, in both field and laboratory tests, higher rates of compost than other organic amendments have been required to suppress parasitic nematodes (3, 4, 9, 12).

The rapid loss of motility in the concentrated extract that was not digested and the continued motility of nematodes in the digested extract strongly suggest that the immotility was induced by the organic fraction of composted municipal refuse (Table 1). In addition, the higher motility of sting nematodes in extracts with cations exchanged suggests that the primary suppressing agent was a positively charged organic compound. However, there could have been a synergistic effect between the primary suppressive organic compounds and inorganic cations or toxicity from amines in the anionic resin. The more rapid decline of nematode motility in the digested fraction with the anions exchanged than in the digested fraction with cations exchanged indicates that the latter possibilities may be most likely.

Since immotility was reversible after 24 hr exposure to compost extract, immotility should not be used as a measure of death. In addition, the authors hypothesize that immotility without immediate death may be the explanation of observations such as Mankau's (5) and V. A. Perry's (personal communications, Department of Entomology and Nematology, University of Florida), where applications of organic amendments and nematicides, respectively, resulted in improved plant growth with no reduction in nematode numbers. Perry's case is particularly interesting since he was applying 16 to 21 kg/hectare of active ingredient from Dasanit® to golf greens that were so heavily fertilized and watered that increased availability of nutrients would have had little or no effect on plant growth.

Whether the mode of suppression of nematodes by various organic amendments has been through immotility, immediate death or both is not clear, but both field and laboratory experiments indicate that higher rates of composted municipal refuse are required than non-composted organic amendments for suppression of parasitic nematodes. However, even though higher rates appear to be required, soil amendment with composted municipal refuse has one large advantage – it accomplishes the last step in the recycling of solid wastes.

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