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Poultry litter compost for suppression of root-knot nematode on cacao plants

Susan L. F. Meyer*, Samuel B. Orisajo, David J. Chitwood*, Bryan T. Vinyard*** and Patricia D. Millner******

*USDA-ARS, Nematology Laboratory, 10300 Baltimore Avenue, Beltsville, MD 20705, USA
**Plant Pathology Section, Crop Protection Division, Cocoa Research Institute of Nigeria, Ibadan, Oyo State, Nigeria
***USDA-ARS, Biometrical Consulting Service, Beltsville, MD 20705-2350, USA
****USDA-ARS, Sustainable Agricultural Systems Laboratory, Beltsville, MD 20705, USA

E-mail: Susan.L.Meyer@ars.usda.gov

Abstract. Poultry litter (poultry manure mixed with the wood shavings that are used for bedding or for covering the soil floor in poultry housing units) compost was investigated as an amendment for suppressing populations of *Meloidogyne incognita* and increasing plant vigor. The greenhouse and laboratory studies were conducted with mature compost produced in a static aerated pile. Compost extract was prepared by steeping 100 g compost in 100 ml tap water and removing biomass with cheesecloth filtration followed by centrifugation. The supernatant was diluted 1:4 in water and sterile filtered and used as a 100% compost extract treatment. In microwell assays, *M. incognita* egg hatch and J2 activity were inhibited by all tested compost extract concentrations (25%, 50%, 75% and 100% extract), with > 90% inhibition in 100% compost extract after 3 and 7 day incubation periods. In greenhouse tests, cacao 'Pound 7' seedlings were transplanted into soil amended with compost. The five treatments were: 1) No poultry litter compost, no RKN; 2) No poultry litter compost, +RKN (5,000 eggs per pot); 3) 2.5% poultry litter compost (by volume), +RKN; 4) 5.0% poultry litter compost, +RKN; 5) 10.0% poultry litter compost, +RKN. Treatments (including nematodes) were placed into pots two weeks prior to seedling transplant. At harvest, stem height, stem fresh weight, and leaf fresh and dry weights were significantly greater with 5% and/or 10% compost amendment than with no compost. Stem girth in all compost treatments was greater than in controls without *M. incognita*. Compost treatments significantly decreased numbers of nematode eggs collected from pots at harvest; egg populations were suppressed by 35.1%, 14.6% and 72.5% in the 2.5%, 5.0% and 10.0% compost treatments, respectively. The results indicate that poultry litter compost amendments have potential for suppressing *M. incognita* populations on cacao seedlings and improving plant vigor.

Keywords. Cacao, cocoa, *Meloidogyne incognita*, poultry litter compost, root-knot nematode, soil amendment, *Theobroma cacao*.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) have been reported from cacao (*Theobroma cacao* L.) plants worldwide, and are considered the most important nematodes attacking this crop in terms of economic losses (Campos and Villain, 2005). These nematodes contribute to retardation of seedling growth, plant damage, sudden death, and concomitant yield losses (Campos and Villain, 2005; Orisajo, 2009; Orisajo *et al.*, 2008). Identified species on cacao include *M. arenaria*, *M. exigua*, *M. incognita*, *M. javanica*, and *M. thamesi* (Campos and Villain, 2005). *Meloidogyne incognita* in particular is a primary nematode pest of cacao (Campos and Villain, 2005; Orisajo, 2009; Orisajo *et al.*, 2008).

The practice of planting cacao seedlings under banana or plantain, commonly followed in some areas of the world, increases the risk of *M. incognita* infection on cacao seedlings, as the nematode is frequently associated with the shade crop. Recommended practices for suppressing nematodes on cacao include planting of clean seedlings, avoiding areas where nematodes infest the soil (including

under banana), use of nematicides, and development of resistant cultivars (Orisajo, 2009). However, resistant cultivars are not widely available in all cacao-growing areas, and chemical nematicides are expensive and often have toxic effects on nontarget organisms, so alternative management strategies are needed (Orisajo, 2009; Orisajo *et al.*, 2008).

One promising management strategy that cacao growers can use to suppress nematode populations and increase soil health and plant vigor is application of compost produced from poultry litter (i.e., poultry manure mixed with the wood shavings that are used for bedding or for covering the soil floor in poultry housing units). Poultry litter is readily available to many growers, and composts produced from various organic residues have been reported to suppress populations of nematodes and other plant disease-causing organisms. The mechanisms by which this suppression occurs involve improvements in soil physical, chemical and biological characteristics, including increased macronutrient content (nitrogen, phosphorus and potassium), organic matter, beneficial microbes, cation exchange capacity, pH, as well as improved soil structure and water-holding capacity (Hoitink and Ramos, 2004; Oka, 2010). Additional specific mechanisms of suppression also include reports of production and release of nematicidal substances such as thiopenes and other organic acids during soil organic amendment decomposition (Sitaramaiah, 1990) and amendment-induced resistance to nematode attack (Bridge, 1996). Plant disease suppression of compost is also attributable to the wide range and abundance of predatory and bacterivorous nematodes present in mature compost (Steel *et al.*, 2010). Studies with poultry litter or poultry litter compost demonstrated that these amendments were beneficial for suppressing populations of *M. incognita*, *Hoplolaimus columbus*, and/or *Pratylenchus penetrans* on vegetable crops and cotton, and of *Meloidogyne mayaguensis* on guava (Everts *et al.*, 2006; Gomes *et al.*, 2010; Koenning and Barker, 2004; Riegel *et al.*, 1996; Riegel and Noe, 2000). Conversely, compost composed of poultry litter and horticultural waste was not effective for suppressing *M. incognita* on pepper or tomato (McSorley *et al.*, 1999). On cacao, recent research indicated that amendment with poultry litter increased cacao seedling vigor and suppressed *M. incognita* populations on roots (Orisajo *et al.*, 2008; Orisajo and Afolami, 2009).

Clearly, much remains to be studied in this area. Composting of poultry litter has the potential to provide a product that is more stable and preferable for soil amendment than uncomposted litter, so it would be useful to determine whether the composted material is beneficial for cacao plant vigor and for nematode suppression. The goals of this study were to determine whether poultry litter compost: 1) produces substances inhibitory to *M. incognita* egg hatch and J2 activity; 2) suppresses *M. incognita* populations on cacao; and 3) enhances plant vigor.

MATERIALS AND METHODS

Nematode cultures

For greenhouse and Baermann funnel experiments, inoculum of *M. incognita* Race 1, originally isolated in MD, was prepared as in Meyer *et al.* (2008a). The nematode was grown on pepper (*Capsicum annuum*) 'PA-136' in greenhouse pots containing loamy sand, and eggs for greenhouse and microwell assay experiments were obtained from the roots of 3-month-old plants. Roots were rinsed, immersed in 0.6% sodium hypochlorite for 1 min to release eggs from egg masses, and the eggs were collected by sugar centrifugation and rinsed in water. Eggs were stored overnight at 4°C for use the following day. For the Baermann funnel test, eggs were collected from a 4-month-old plant, placed into a hatching chamber overnight, and the J2 collected and used as inoculum.

For microwell assays, eggs were collected from 3-month-old greenhouse-grown pepper plants (as above) or from 4-month-old pepper 'PA136' grown in the lab in 250 ml plastic beakers filled with sand. The egg masses were shaken by hand for 3 min in 0.6% sodium hypochlorite. Eggs were then collected and placed into a hatching chamber to collect surface-sterilized J2. The hatching chamber was kept in the incubator for 1-3 days at 4°C.

Poultry litter compost

Mature compost was produced in a 3.8-cu m static aerated pile from 3-wk old poultry litter collected from a chicken house after the chickens were removed. The compost pile self-heated to temperatures > 55°C in the central core of the pile for 4 weeks, and thereafter the temperature declined gradually; at 9 weeks the pile was turned so that the exterior material was placed in the core and the core material was placed on the exterior. The pile reheated to > 50-55°C for one week, and then gradually cooled to ambient temperature by 13 weeks. The pile was allowed to cure for an additional 3 weeks before the compost was air-dried and stored in covered containers. Composite samples were obtained and analyzed according to standard methods (Thompson *et al.*, 2001). The compost used in the experiments had the following composition: pH 8.0, soluble salts 30.7 mmhos/cm, solids 69.8%, moisture 30.2%, organic matter 37.6% (53.9% of dry weight), total nitrogen 3.18% (4.6%), organic nitrogen 3.07% (4.4%), ammonium nitrogen 0.1068% (0.1530%), carbon 18.6% (26.7%), C:N ratio 5.9, phosphorus (as P_2O_5) 4.75% (6.8%) and potassium (as K_2O) 4.35% (6.23%).

In preparation for the experiments, the compost was soaked in water (1:1 compost:water, by volume) for 2-3 minutes and clumps were broken up. The compost was placed onto nested U.S. Standard sieves (0.85 mm pore diameter over 0.106 mm pore diameter), broken further with

the bottom of a beaker, collected from the sieves, and then washed and collected twice more and air-dried.

Microwell assays

To prepare compost extract, 100 g compost was steeped for 10 min in 100 ml tap water with continual mixing to ensure that the compost pieces were broken up. The mixture was placed onto two layers of cheesecloth, squeezed through to collect the liquid, and the liquid was then centrifuged for 3 min (447×*g*). The supernatant was collected and centrifuged twice for 5 min (1,188×*g*). The resulting supernatant was diluted (1:4 in water) and then filtered twice through 0.2 µm sterile filters (Whatman, Clifton, NJ). This was considered the 100% compost extract treatment.

Microwell assays were set up in 96-well polystyrene plates, similar to methods described by Meyer *et al.* (2008b). Eggs and previously hatched J2 were assayed in separate wells. Each well received 315 µl of compost extract or of sterile distilled water control, and 35 µl of an *M. incognita* egg suspension (containing ca. 50 eggs) or 35 µl of a hatched J2 suspension (containing ca. 30 J2). Treatments were 0%, 25%, 50%, 75% and 100% compost extract in sterile distilled water (volume/volume). A plastic adhesive sheet (SealPlate®; EXCEL Scientific, Inc., CA) was placed over each plate to prevent evaporation and the plates were incubated at 26°C. Five wells were used per treatment in each trial, and each experiment was conducted twice. The total was therefore n = 10 for microwell assays. For egg assays, total numbers of hatched J2 and of active vs. inactive J2 were counted 3 and 7 days after immersion of the eggs in the treatments. For assays of previously hatched J2, counts of active vs. inactive were made 48 hours after immersion of the J2 in the treatments.

Baermann funnel tests

Enriched loamy sand soil (16:9 sand:compost that had been steamed and air-dried; composition 82.9% sand, 5.3% silt, 11.8% clay; pH 7.3; 0.8% organic matter) and poultry litter compost (described above) were placed into Baermann funnels, with a total of 50 g per funnel. Six treatments were tested: 0%, 25%, 50%, 75% and 100% compost to soil (by weight), and an additional treatment of 25 g compost layered over 25 g soil. There were five funnels per treatment and the experiment was not repeated. Each funnel received ca. 1,500 J2, and J2 were collected from the funnels three days later.

Greenhouse studies

Seedlings of cacao 'Pound 7' from Costa Rica were started on water agar, sand, vermiculite or 1 sand:1 vermiculite. When the seedlings were ca. 3 ½ weeks old, they were transplanted into 10.2 cm-diameter pots (1 seedling per pot) containing starter mix (Premier Pro-mix®, Premier Horticulture Inc., Quakertown, PA) and vermiculite and placed in a greenhouse (24-29°C; natural and supplemental lighting combined for a 15-16 hr day length). Six weeks later the cacao seedlings were transplanted into 15.2 cm-diameter pots containing the treatments and one part (volume to volume) Premier Pro-mix® to one part enriched soil (the latter is described above under "Baermann funnel Tests"). Premier Pro-mix® was added to the enriched greenhouse soil to enhance cacao seedling vigor. The five treatments were: 1) No poultry litter compost, no RKN; 2) No poultry litter compost, +RKN; 3) 2.5% poultry litter compost (by volume), +RKN; 4) 5.0% poultry litter compost, +RKN; 5) 10.0% poultry litter compost, +RKN. These treatments were prepared 2 weeks prior to the last transplant by hand mixing the components in plastic containers and placing the treatments into the pots in the greenhouse. Each nematode-inoculated pot received 5,000 *M. incognita* eggs. Six plants were transplanted per treatment (one seedling per pot), arranged in a randomized design, and the experiment was conducted twice (n = 12). The plants were watered 5 days a week with Jack's Classic liquid fertilizer (J. R. Peters, Inc., Allentown, PA) and harvested 3 months after transplant. At harvest, the numbers of viable plants, stem heights (from soil to growing tip), stem girths, leaf fresh and dry weights, stem fresh and dry weights, and root fresh weights were recorded. Dry weights were recorded after 8 days in the oven at 21°C.

Nematodes were collected and counted as described in Meyer *et al.* (2011). Following harvest, roots were removed from pots, soil was rinsed from the roots, and the root fresh weights were recorded. Roots were stored at 4°C until eggs were extracted and counted. To extract eggs, roots were cut into pieces about ½ cm long with a pair of shears, blended on low speed in 0.6% sodium hypochlorite for 1 min, and then poured onto U.S. Standard nested sieves (250 µm pore diameter over 25 µm pore diameter). The eggs were collected by sugar centrifugation, rinsed in water, and stored as aqueous egg suspensions at 4°C.

Statistical analysis

For the egg assays in microwell plates, data analyzed were total eggs hatched (based on J2 counts) and percent active J2 (of total hatched) on Day 3 and Day 7 after egg immersion. For the assays of previously hatched J2, analysis was conducted on percent active J2 at 48 h. The replicate observed number of eggs or percent active J2 were regressed onto percent poultry litter compost extract using SAS PROC GLIMMIX to fit generalized linear regression models or PROC NLMIXED to fit generalized non-linear regression models. Total eggs hatched, for both 3 and 7 days after immersion, was modelled using a negative binomial distribution and regressed onto a sigmoidal, non-linear function of percent poultry litter compost extract. Percent active J2 from hatched eggs, for both 3 and 7 days after immersion, and percent active J2 of observed J2, were modeled using a binomial distribution with Laplace optimization and regressed onto a logit (link) function of percent poultry litter compost extract.

Table 1. Effects of poultry litter compost on heights, weights, and stem girths of greenhouse-grown *Theobroma cacao* plants inoculated with *Meloidogyne incognita* (root-knot nematode).

Poultry Litter Compost*	Stem height (cm)	Leaf fresh weight (g)	Leaf dry weight (g)	Stem fresh weight (g)	Stem dry weight (g)	Root fresh weight (g)	Stem girth (mm)
0% (-)**	23.5 b	9.7 c	3.0 c	8.1 b	2.1 bc	18.0 b	7.7 b
0% (+)	23.0 b	11.0 bc	3.4 c	8.1 b	2.0 c	18.4 b	7.9 ab
2.5% (+)	25.3 ab	13.9 ab	4.2 bc	10.3 ab	2.4 abc	25.8 ab	8.6 a
5.0% (+)	26.8 ab	14.5 ab	4.9 ab	11.8 a	3.2 a	28.9 a	9.0 a
10.0% (+)	29.0 a	17.4 a	5.8 a	12.6 a	3.2 ab	24.8 ab	9.1 a

Note: Values are comparable within each column at $P \leq 0.05$.
*Percent of compost to enriched soil mix, volume/volume.
**(-) no *M. incognita*, (+) inoculated with *M. incognita*.

In all of the figures, a significant decrease in the dependent variable with increased percent poultry litter compost extract was statistically confirmed by the slope parameter (i.e., coefficient of the percent poultry litter compost extract) being statistically different from zero ($P \leq 0.05$). For the dependent variables total eggs hatched and percent active J2 from hatched eggs, results for 3 and 7 days after immersion were compared ($\alpha = 0.05$) at 5% increments of percent compost extract by specifying the above-described regression models for 3 and 7 days in a single analysis of covariance model and writing an ESTIMATE statement to test for a statistical difference between the model-predicted value for 3 and 7 days after immersion.

Percent decreases in egg hatch and J2 activity in microwell plates, and in total eggs per pot in greenhouse studies, were calculated as follows: percent decrease = [(control value minus treatment value)/control value] × 100. For greenhouse experiments, total eggs per pot were regressed onto Log_{10} (percent compost + 1). Significant decrease in the total eggs per pot with increased percent poultry litter compost extract was statistically confirmed by the slope parameter (i.e., coefficient of the percent compost extract) being statistically different from zero ($P \leq 0.05$). Differences among treatments for observed characteristics of cacao plants were identified via ANOVA using SAS PROC MIXED with the GROUP = option in the REPEATED statement to model heterogeneous within-treatment variance across treatments and the Sidak adjustment for multiple comparisons ($P \leq 0.05$).

RESULTS

Microwell assays and Baermann funnel tests

The pH values of the compost extracts and water control ranged from 7.18 to 7.66. In the microwell assays at Days 3 and 7, egg hatch and J2 activity were suppressed by poultry litter compost extract, as indicated by the statistically significant slopes of the regression models (i.e., coefficients of percent compost extract) (Figs. 1, 2).

At Day 3, egg hatch was greatest in the 0% extract (water control), with a recorded mean egg hatch of 41.0. Egg hatch at Day 3 decreased significantly with increasing extract concentration (Fig. 1), as indicated by the regression model's statistically significant slope. After three days of immersion in the treatments, egg hatch was suppressed by 19.2% in the 25% compost extract and 52.7% in the 50% compost extract, compared to water controls. Hatch was lowest in the 75% and 100% compost extracts at Day 3; mean recorded egg hatch was 5.0 and 3.7, respectively. In the 100% compost extract, this was a decrease in egg hatch of more than 90% compared to water controls.

By Day 7, more eggs had hatched in 0% extract (water controls) than in water controls on Day 3 (Fig. 1). The mean recorded egg hatch on Day 7 was 74.3 in the water controls. It should be noted that although equal amounts of egg suspension were pipetted into each well, 50 eggs per well was an estimate based on number of eggs per ml rather than an exact count. The total number of eggs per well was subsequently greater than 50. Although the regression model of egg hatch at Day 7 decreased significantly with increasing compost extract concentration, analysis of covariance comparison between egg hatch at Day 3 and egg hatch at Day 7 at 5% increments of percent compost extract indicated that total egg hatch numbers at Days 3 and 7 were statistically different from each other when eggs were immersed in 0% to 40% compost extract (Fig. 1). However, egg hatch numbers were similar between Days 3 and 7 when eggs were immersed in compost extract concentrations of 45% to 100%. At Day 7, mean recorded egg hatch in the 75% and 100% compost extracts was 6.9 and 5.0, respectively. Therefore, at Day 7, egg hatch was decreased by > 90% in

the 75% and 100% compost extracts compared to water controls.

The percent of J2 that hatched from the treated eggs and remained active also decreased with increasing compost extract concentrations for Days 3 and 7 (Fig. 2). At Day 3, mean recorded values for active J2 in the 0% and 50% compost extracts were 96.1% and 91.2%, respectively. Also at Day 3, J2 activity was lower in 75% compost (29.6% of hatched J2 were active), and in 100% compost extract only 2.2% of hatched J2 were active. J2 activity was therefore decreased by 69.2% in the 75% compost extract and by 97.7% in 100% compost extract compared with the water controls at Day 3.

At Day 7, the percent active J2 was generally lower than at Day 3. The regression models indicated that percent active J2 was statistically different between Days 3 and 7 in 0% to 85% compost extract, but statistically similar between Days 3 and 7 at 90% to 100% compost extract. For example, at Day 7, only 57.0% and 32.2% of J2 were recorded as active in 0% and 50% compost extract, respectively. However, at Day 7, 2.0% of the J2 were active in the 75% compost extract, and 4.4% of J2 were active in the 100% compost extracts. These were decreases in J2 activity of 96.5% and 92.3%, respectively, compared with water controls.

When previously hatched J2 were placed directly into poultry litter compost extracts and incubated for 2 days, the

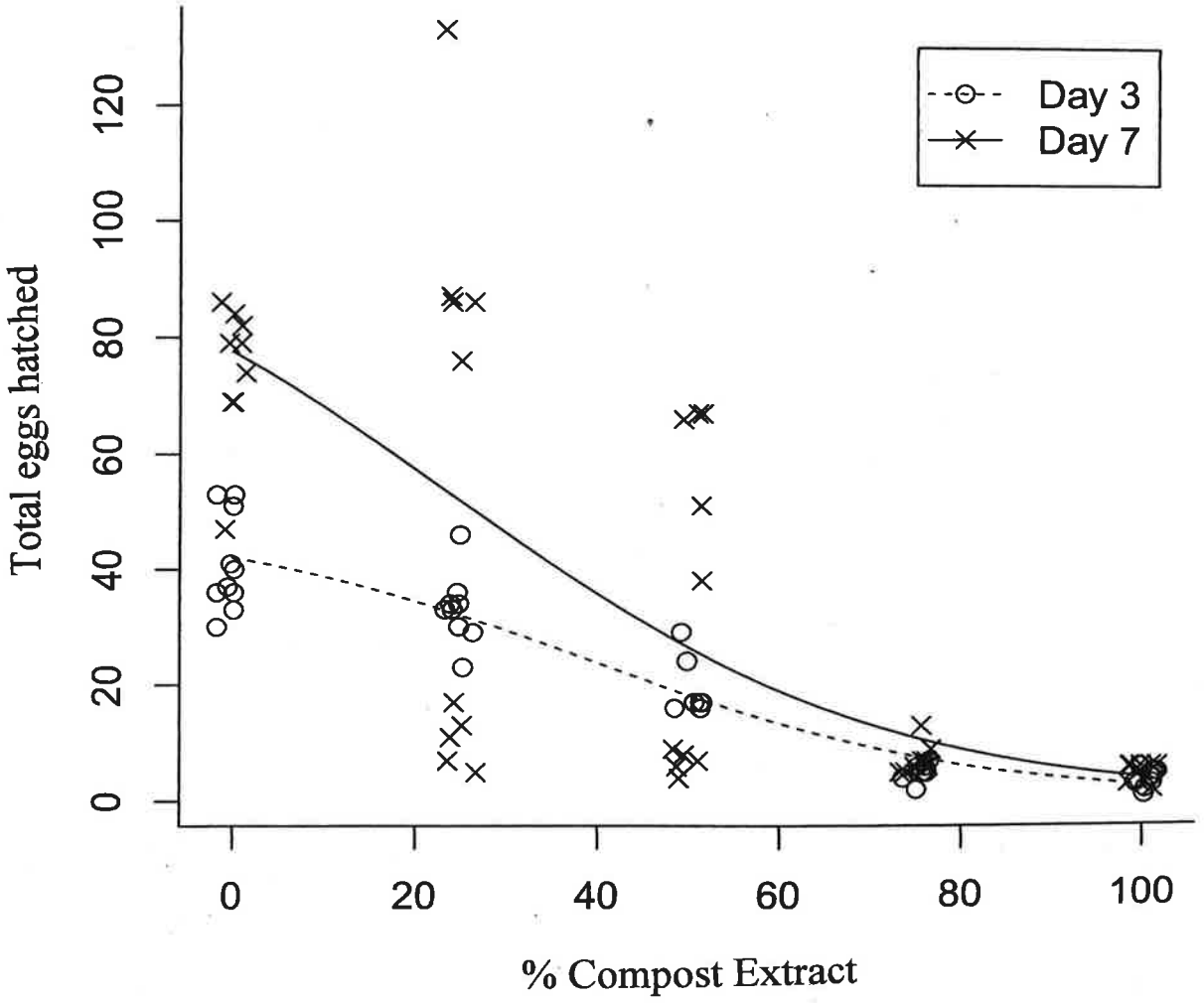

Fig. 1. Total numbers of *Meloidogyne incognita* eggs that hatched in microwell assays after 3 and 7 days of immersion in poultry litter compost extract. Shown are the values from two trials with five replicates of each treatment per trial (n = 10). The dose-response relationship is described by the sigmoidal model eggs hatched = $49.4 \cdot [1 + e^{-(1.75-0.0458) \cdot \%\ \text{Compost Extract}}]^{-1}$ for 3 days of immersion and eggs hatched = $105.6 \cdot [1 + e^{-(1.02-0.0424 \cdot \%\ \text{Compost Extract})}]^{-1}$ for 7 days of immersion; a negative binomial distribution was fitted to the total numbers of eggs hatched. The percent compost extract slope parameters were significant ($P < 0.0001$).

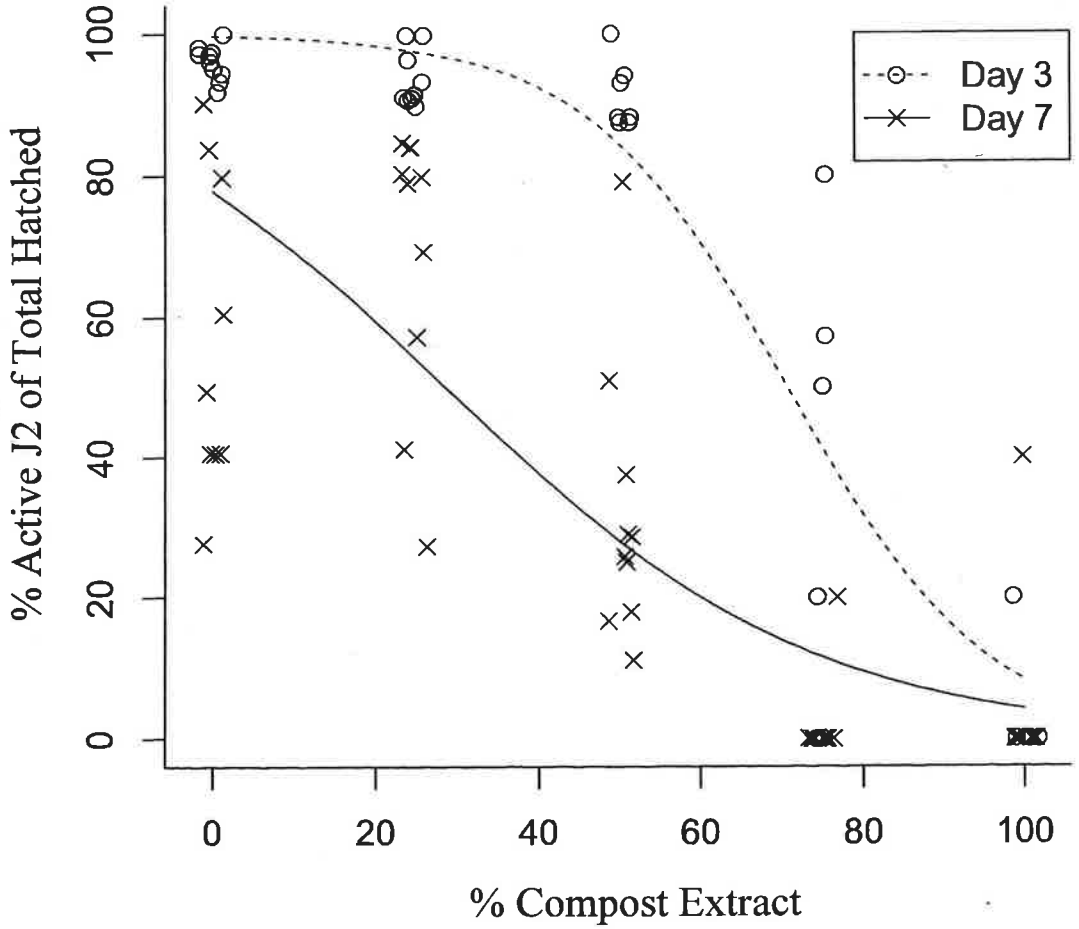

Fig. 2. The percent *Meloidogyne incognita* second-stage juveniles (J2) (of total J2 that hatched in microwell assays) that were active within 3 and 7 days of egg immersion in poultry litter compost extract. Shown are the values from two trials with five replicates of each treatment per trial (n = 10). The dose-response relationship is described by the sigmoidal model active J2 as percent of total eggs hatched = $[1 + e^{-(5.79-0.082 \cdot \%\ \text{Compost Extract})}]^{-1} \cdot 100\%$ for 3 days of immersion and active J2 as percent of total eggs hatched = $[1 + e^{-(1.26-0.0441 \cdot \%\ \text{Compost Extract})}]^{-1} \cdot 100\%$ for 7 days of immersion; a binomial distribution was fitted to the number of active J2 among the total number of eggs hatched. The percent compost extract slope parameters were significant ($P < 0.0001$).

compost extract decreased J2 activity compared with the 0% (water control) (Fig. 3). In the 0%, 25%, 50%, 75% and 100% compost extracts, recorded active J2 were 72.1%, 70.3%, 47.1%, 36.7% and 15.1%, respectively. J2 activity was therefore decreased by 79.1% in the 100% compost extract compared with the 0% compost extract.

In the Baermann funnel tests, a mean of 556 J2 was recovered from each of the control funnels that contained only soil. No J2 were recovered from any other treatment.

Greenhouse studies

In the greenhouse tests, there was an enhancement of plant vigor with poultry litter compost treatments; the measured parameters tended to be larger in the seedlings transplanted into amended soil (Table 1). Presence of *M. incognita* did not affect plant vigor in this greenhouse study. Stem height was significantly greater with 10% compost amendment than with no compost. Stem fresh weight was significantly greater in the 5.0% and 10.0% compost-amended soils than in the unamended controls, and stem dry weight in the treatments with the largest percent of compost amendment was greater than in controls with *M. incognita*. Leaf fresh and dry weights were in general significantly greater in 5.0% and 10.0% compost-amended soils than in the controls. Stem girth in all compost treatments was significantly greater than in controls without *M. incognita*.

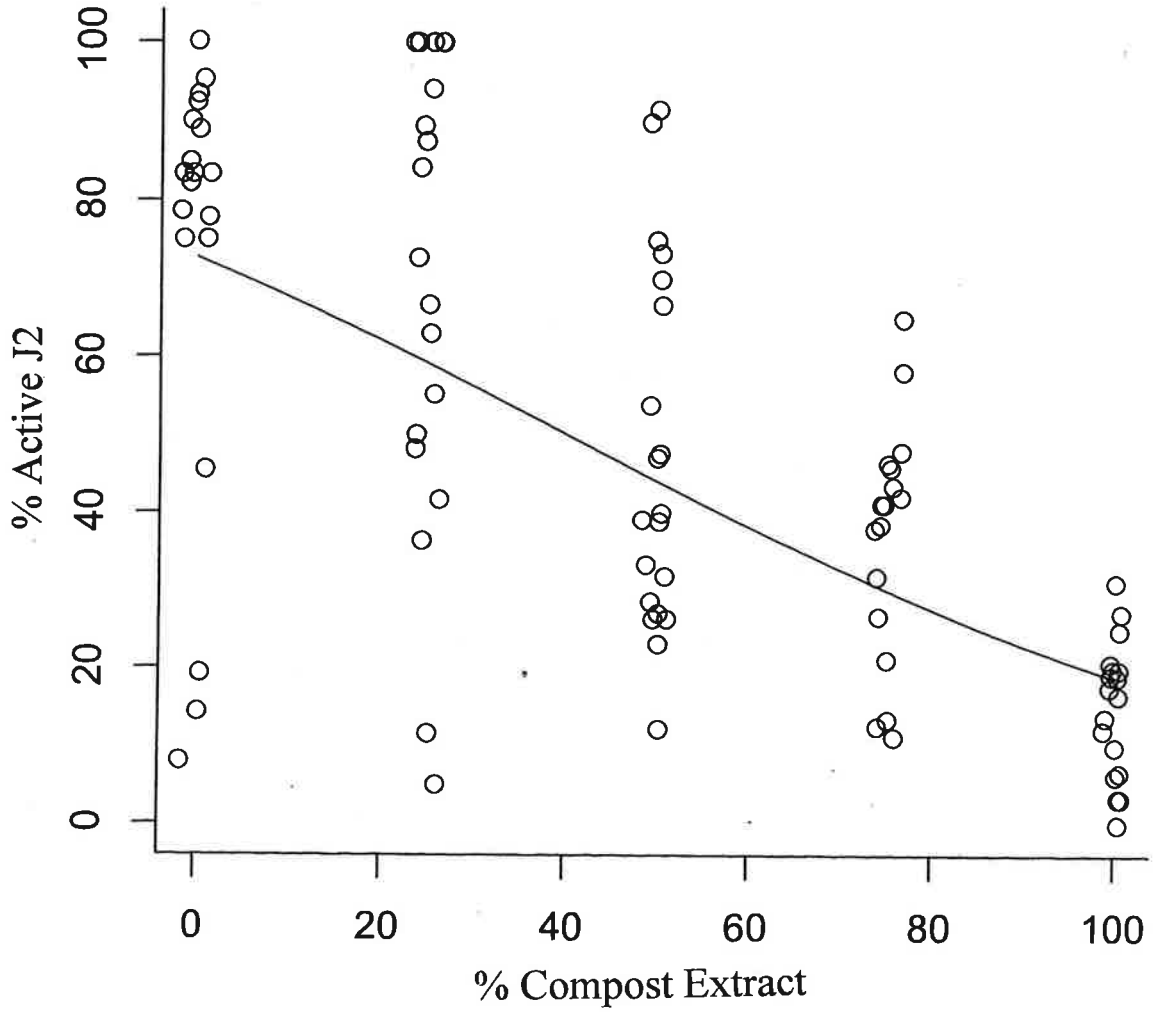

Fig. 3. The percent active *Meloidogyne incognita* second-stage juveniles (J2) in microwell assays 2 days after placement of previously hatched J2 in poultry litter compost extract. Shown are the values from two trials with five replicates of each treatment per trial (n = 10). The dose-response relationship is described by the sigmoidal model active J2 as percent of total J2 = $[1 + e^{-(0.98-0.0243 \cdot \% \text{ Compost extract})}]^{-1} \cdot 100\%$; a binomial distribution was fitted to the number of active J2 among the total number of J2. The percent compost extract slope parameter was significant ($P < 0.0001$).

Root fresh weights were highest in the 5.0% compost; this was the only measured plant parameter for which the values were not greatest in 10.0% compost. The overall indication was that treatment with compost, particularly at 5.0% and 10.0%, improved plant vigor compared to no compost treatment, whether or not nematodes were present.

Total numbers of *M. incognita* eggs per pot were also affected by poultry litter compost treatments (Fig. 4), with egg numbers significantly decreased by compost amendment. Recorded mean total eggs per pot were 5,770 in the unamended controls, 3,745 in 2.5% compost, 4,925 in 5.0% compost, and 1,585 in 10.0% compost. It should be noted that the mean for total eggs in the 5.0 % compost was substantially increased by a very large number of eggs in one pot (Fig. 4). The nematode egg populations were suppressed by 35.1%, 14.6%, and 72.5% in the three compost treatments, respectively, compared with the unamended controls.

DISCUSSION

Poultry litter compost extracts inhibited hatch of *M. incognita* eggs and immobilized J2, indicating that chemical compounds might account for some of the mode of action of the compost against the nematode. Even the lowest concentration tested inhibited egg hatch to some extent at three days of incubation, with almost no eggs hatched in the 75% and 100% concentrations of the extract. J2 activity was also strongly affected by the extracts at the 75% and 100% concentrations, and also inhibited somewhat at lower concentrations. The effects were not attributable to pH, as

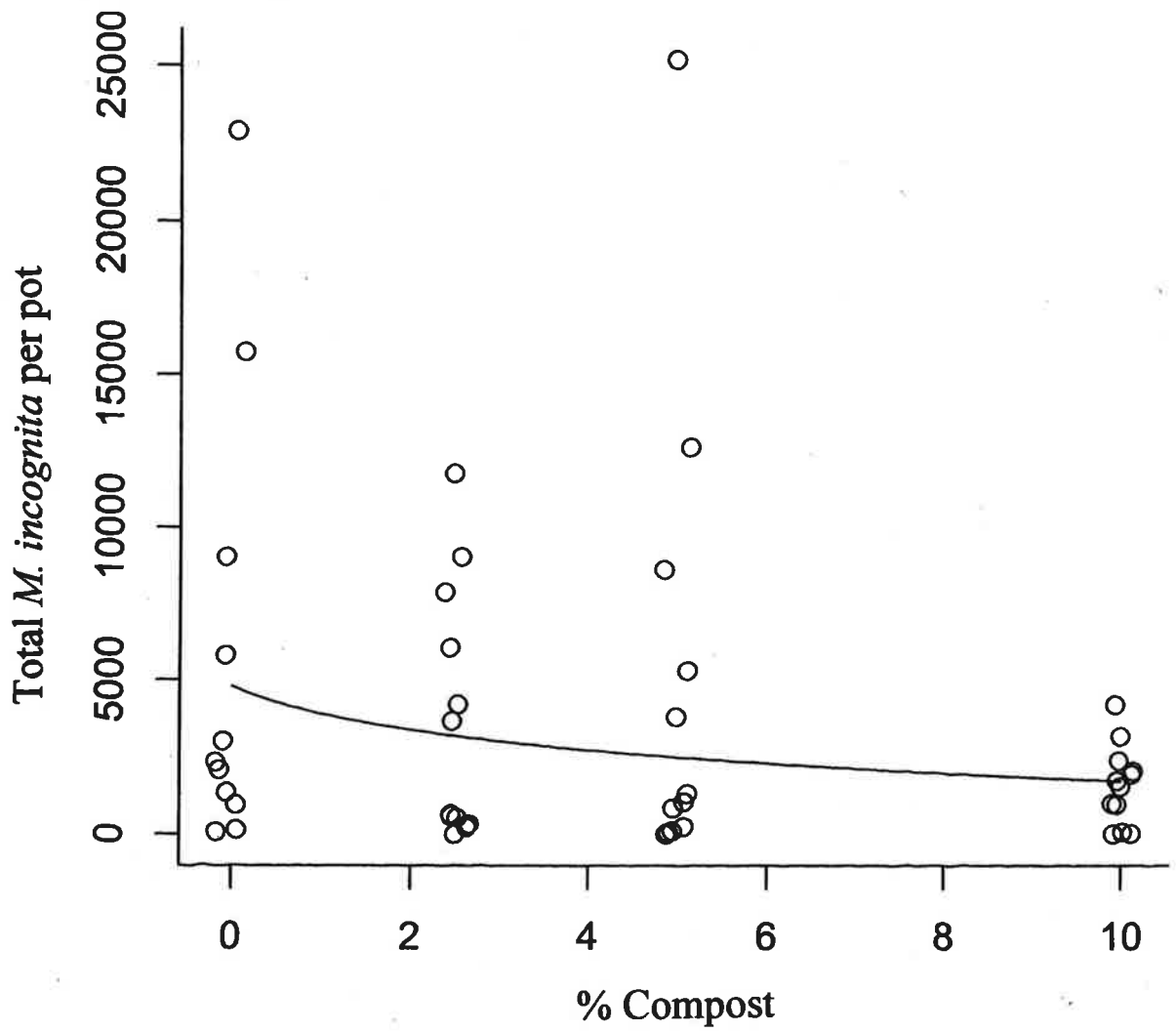

Fig. 4. Numbers of *Meloidogyne incognita* eggs on roots of greenhouse-grown cacao plants in soil amended or unamended with poultry litter compost. Percent of compost to enriched soil mix is volume/volume. Shown are the values from two trials with six replicates of each treatment per trial (n = 12). The relationship between percent compost and egg numbers is described by the model total number of eggs per pot = 4795 – 2953 x $\log_{10}$(percent Compost + 1). The log percent compost slope parameter was significant ($P = 0.0499$).

the pH of the extracts was normal. The deleterious results are similar to those from a previous study with extracts from poultry manure compost, which demonstrated that an exposure time of just 3 minutes increased the sensitivity of *M. incognita* and *H. schachtii* eggs to infection by *Verticillium chlamydosporium* (Pandey and Sikora, 2000). The authors concluded that secondary metabolites in the extract might act by affecting the egg shell or egg membrane.

The poultry litter compost was also beneficial in suppressing nematode egg populations on cacao seedling roots. In a previous study, poultry litter or poultry litter compost, combined with specific crop rotations, suppressed *M. incognita* and *Pratylenchus penetrans* populations in microplots (Everts *et al.*, 2006). Poultry litter compost was applied to the microplots at 4.7, 11.7, and 35.0 Megagrams per hectare (Mg/ha), which is approximately 0.23%, 0.57%, and 1.7% of soil weight, respectively. These are lower rates than used in our experiment with cacao, and combined with cover crops, were still efficacious for nematode suppression when applied into rotational cover crop sequences (Everts *et al.*, 2006). Poultry litter compost of 20 or 40 kg/tree also suppressed *M. mayaguensis* in guava orchards (Gomes *et al.*,

2010). Additionally, *M. incognita* population densities on cotton in greenhouse pots decreased with amendment of uncomposted chicken litter to the soil, with tested rates of 0.125% to 1.0% (weight litter to weight soil) (Riegel and Noe, 2000). Similar rates (0.25%, 0.5% and 1.0%) resulted in decreasing *M. incognita* numbers with increasing litter rate on cotton in microplots (Riegel *et al.*, 1996). Chicken litter rates of 0.5%, 1.0% and 1.5% suppressed *M. arenaria* penetration on tomato, with efficacy resulting from nonsterile litter rather than sterile litter, most likely because of microbial activity (Kaplan *et al.*, 1992).

On cacao, poultry litter was applied at rates of 0%, 0.25%, 0.35%, and 0.5% (weight litter/weight soil), with and without carbofuran (Carbofits 3G, Hockley International Limited, UK, for FITSCO Nigeria Limited, Ibadan, Nigeria) (Orisajo *et al.*, 2008). In that study, all rates of the poultry litter, with and without the pesticide, suppressed *M. incognita* population numbers. Plant height, leaf area, shoot dry weight, stem girth, and root dry weight also increased with poultry litter amendment. The rates of uncomposted poultry litter were lower than the rates we tested of poultry litter compost, and were still effective. As in the Orisajo *et al.* (2008) study with uncomposted poultry litter, the poultry litter compost used in our study was also beneficial to plant vigor. Overall, the trend in our study was for the longest stems, greatest leaf and stem weights (fresh or dry), largest stem girths, and highest root fresh weights to be recorded from the plants treated with the higher rates of poultry litter compost. While not always significantly different from data recorded from cacao seedlings without compost, the trend indicated the positive effects of the poultry litter compost treatments.

The results with poultry litter compost indicate that there is potential for use of this amendment for suppressing root-knot nematode populations on cacao seedlings. Local soils can be tested for optimal amendment rates. In addition, systems have been developed for large trees to be grown in buried containers (Hoitink and Ramos, 2004); this could show potential for use of composts to be added to soil that is not infested with nematodes, thereby decreasing nematode damage while allowing for the beneficial effects of the compost on the trees. Preparation of compost itself can be done to eradicate any plant-parasitic nematodes that might already be present in composting materials: for example, species of *Meloidogyne* and most other tested nematodes were generally killed by compost with a temperature (constant or average) of 60°C for ca. 2 days (Noble and Roberts, 2004). For compost to be routinely beneficial, composting procedures with locally available organic residues need to be established and followed so that the finished compost is a reliable product.

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