Amendment of Soil with Weathered Poultry Manure, Cow Dung and Sawdust for the Management of *Meloidogyne spp.* and Other Nematodes in Okra

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Abstract
Two separate, but identical greenhouse experiments and a field experiment were conducted to evaluate weathered poultry manure, cow dung and sawdust applied as soil amendment singly at 1.5 t /ha and 3.0 t /ha in the management of *Meloidogyne* spp. and other nematodes on okra cv. 47-4. Under greenhouse conditions, weathered poultry manure applied at 3.0 t /ha to nematode-infected okra resulted in taller plants with higher leaf number and lower root galling in comparison with other plants. This treatment also significantly reduced the soil population of *Meloidogyne* spp. second stage juveniles than other treatments. In the field infested with *Meloidogyne* spp. and other genera of plant parasitic nematodes, poultry manure-amended plants were the least galled, followed by plants amended with cow dung and sawdust. The highest fruit yield was recorded from plants amended with poultry manure. Lowest percentage *Meloidogyne* spp. population increase (12.6 %) was recorded in soil amended with weathered poultry manure at 3.0 t / ha, followed by soil amended with cow dung (17.8%) and sawdust (33.9%). The percentage change in soil populations of *Pratylenchus* spp., *Paratylenchus* spp. and *Hoplolaimus* spp. followed a similar trend. Thus application of weathered poultry manure at the rate of 3.0 t /ha to nematode-infested okra garden may have practical benefits for management of the pest.

Key words: Sawdust, Poultry manure, Cow dung, *Meloigogyne*, Management.

INTRODUCTION

Okra, (*Abelmoschus esculentus)* is a green fruit vegetable grown in temperate and tropical countries of the world. It is consumed as a vegetable in West and East Africa, Southern Asia, Central America and Australia (Singh and Sitaramaiah, 1966) and as a spice in India, China, Nigeria and Chad. Okra is known to be rich in vitamins B_2, B_{12} and E as well as minerals including magnesium, iron and calcium and consequently when added to diets, it boosts the vitamin and mineral intake of the consumer (Singh and Sitaramaiah, 1967; Babatola, 1989).

Okra thrives best mainly in loamy-clayey soil. It requires a high level of moisture, moderate relative humidity (about 56%), high temperature (about 25°-27°C) a slightly basic soil pH and plenty sunlight. These requirements which are obtainable in the tropics make it possible to increase okra production in tropical Africa (Singh *et al.*, 1967).

Plant-parasitic nematodes are cosmopolitan and important pests of agricultural crops. In particular are the root-knot nematodes, which are common in sub-tropical and tropical vegetable production. Four species have been more specifically described in these areas: *Meloidogyne incognita, M javanica, M. arenaria* and *M. hapla* (Johnson and Fassuliotis, 1984). *Meloidogyne* spp. are sedentary endoparastic plant pathogens with broad host range. Damage caused by these organisms often goes unreported or is attributed to other causes (Dun and Crow, 2001) with loss of crop revenue annually amounting to millions of dollars (Choi, 1999). Nematode management using agricultural wastes applied as soil amendments has been reported by earlier workers (Khan *et al.*, 1974; Alam et *al.*, 1978; Alam *et al.*, 1979; Mian and Rodriguez-Kabana, 1982), however the quantity of agricultural wastes recommended for nematode management are usually high (upwards of 10 t /ha), making such application impractical. The present study therefore evaluated weathered poultry manure, cow dung and sawdust applied singly at the rates of 1.5 t /ha and 3.0 t / ha for the management of phyto-nematodes infecting okra.

MATERIALS AND METHODS

Sources of agricultural wastes, okra seeds and nematodes cultures:

Agricultural wastes used in this study namely poultry manure, cow dung and sawdust, were collected fresh from the Animal husbandry unit of Obafemi Awolowo University Teaching and Research Farm, Ile-Ife, Nigeria. These were placed on concrete slabs, sun dried at 28° to 34°C for 21 days and afterwards crushed into granular form. Samples were taken to the laboratory and the total nitrogen (N), available phosphorus (P) and exchangeable potassium (K) were analyzed by standard methods (Knudsen *et al.*, 1982; Singh, 1997).

Cultures of *Meloidogyne* spp. were obtained from nematode culture plot of the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria and increased on *Celosia argentea* cv. TLV8 in the screenhouse. Nematode eggs were extracted from infected C. *argentea* root pieces using the method of Hussey and Barker (1978). Seeds of okra cv. 47-4 *(Meloidogyne* spp.-susceptible cultivar) cultivar) were obtained from National

Horticultural Research Institute (NIHORT), Ibadan, Nigeria.

Greenhouse experiment

Two separate but identical greenhouse experiments were conducted at a temperature range of 30° to 32°C. In the first, forty 5-litre plastic pots were filled with steam sterilized sandy loam topsoil. Seeds of okra cv. 47-4 were sown at two seeds per pot and thinned to one at one week after planting. There were eight treatments in five replicates arranged in a Randomised Complete Block Design. The treatments were: cow dung applied at 15 g / pot and 30 g / pot equivalent to 1.5 t / ha and 3.0 t / ha; sawdust and weathered poultry manure each applied at rates equivalent to 1.5 t / ha and 3.0 t / ha; inoculated and uninoculated control. Each of 35 out of 40 seedlings was inoculated with 2,000 fresh *Meloidogyne* spp. eggs in 5 ml water suspension nine days after planting. The inoculum was placed around the roots of okra seedlings and covered with soil. Weathered poultry manure, cow dung (in granular form) and sawdust were applied to potted plants at the rates stated above in a ring form at the base of the plant and covered with soil (depth 1-7 cm), one day after inoculation. The agricultural wastes were not applied to inoculated and uninoculated control plants; no inorganic fertilizer was applied to potted plants. Potted plants were watered regularly. The study was terminated sixty-five days after planting.

Plant height and leaf number for individual plants were recorded at two weeks after planting and thereafter every two weeks until termination of study.

Okra plants were carefully uprooted and the roots rinsed in water upon termination of the experiment. Roots of individual plants were assessed for galling according to the rating scale of Taylor and Sasser (1978), where 0 = No galls or eggs masses; 1 = 1-2 galls or egg masses; 2 = 3 -10 galls or egg masses; 3 =11- 30 galls or eggs masses; 4 = 31-100 galls or egg masses; 5 =More than 100 galls or egg masses.

Nematode Analysis

Nematode analysis was done by taking 200 ml soil sub-sample from each pot following thorough mixing of soil from the pot and nematodes were extracted using the method of Whitehead and Hemming (1965). Nematodes were killed by heat and fixed in 4% formaldehyde. The nematodes were counted under a stereomicroscope (250 x magnification) and identified. The experiment was repeated once using a new set of plastic pots.

Field Study

A plot of land, sandy loam, naturally infested with

Meloidogyne spp. was selected at the Teaching and Research Farm of Obafemi Awolowo University Ile-Ife, located on latitude 07° 28'N and longitude 04°33'E at 244 m a.s.l., in the tropical rainforest zone of Nigeria. To increase the population of *Meloidogyne* spp. on the site, Celosia cv. TLV8 was planted at the onset of early rainy season (April) and the plot was ploughed and harrowed at the onset of late rainy season (August). The plot was divided into five blocks, 30 m x 3 m; each block was divided into seven plots of 3 m x 3 m with a 1 m alley separating blocks and 0.5 m between plots.

Soil samples were collected into separate sample bags before planting from each of 35 plots. A bulk sample consisting of 20 cores (diameter 1.9 cm; depth 0-20 cm) was taken from each plot. Part of the soil sample collected was analyzed in the laboratory for pH, total nitrogen (N), available phosphorus (P) and exchangeable potassium (K) using standard methods (Knudsen *et al.*, 1982; Singh, 1997). Soil samples were again collected at harvest for analysis as described.

Nematodes were extracted from a 200-ml sub-sample of the bulk sample collected for each plot (Whitehead and Hemming, 1965) killed by heat and fixed in 4% formaldehyde. Nematodes were counted in a Doncaster counting dish (Doncaster, 1962) under a stereomicroscope (250 x magnification) and individuals from each sample were further identified using a light microscope (100-400 x magnification) up to genus (Mai and Lyon, 1960). Nematode analysis was also done at harvest as described.

The experiment was laid out in a randomized complete block design (RCBD) with seven treatments and five replicates. The treatments were: cow dung applied at 1.5 t/ha and 3.0 t/ha; sawdust applied at 1.5 t/ha and 3.0 t/ha; weathered poultry manure applied at 1.5 t/ha and 3.0 t/ha and control with no amendment applied. In August, the field was sown to okra cv. 47-4 at one seed per stand at the spacing of 50 cm within and 60 cm between rows. There were 30 plants per plot or 210 plants per block, giving a total of 1,050 plants. Weathered poultry manure, cow dung and sawdust were applied to okra plots singly at the rates of 1.5 t /ha and 3.0 t /ha, in a ring form at the base of the plant and covered with soil (depth 0-IO cm), one week after planting. Analysis of amendments shows the following results

The experiment was rainfed and maintained weed free by manual weeding. Fruits were harvested beginning from a few days after flowering and every five days thereafter until fruiting ceased. The fruits were weighed and the cumulative weights for each treatment expressed in kg / ha. Plants were rated for galls by randomly selecting ten plants per plot. Plants were carefully uprooted, washed and assessed for galling as described by Taylor and Sasser, 1978.

All statistical analysis was performed using the SAS (1985) statistical package, while treatment means were partitioned using Fisher's Least Significant Difference Test at P = 0.05

RESULTS

Greenhouse Experiment

The results show that at eight weeks after planting, the tallest plants were those amended with weathered poultry manure at 3.0 t /ha (Table 1). They were significantly taller than plants amended with other agricultural wastes. Similarly, plants amended with weathered poultry manure at 3.0 t /ha produced significantly more leaves than plants amended with other agricultural wastes and control plants (Table 2). Soil amended with weathered poultry manure at 3.0 t /ha recorded the lowest density of *Meloidogyne* spp. J2 at harvest (Table 3) and this was significantly lower than the density of the nematodes in soil with other treatments. Reduction in root gall index in weathered poultry manure amended plants was higher than in other treatments. Decline varied from 57 % in weathered poultry manure-amended plants to 40 % in sawdust amended plants and 33 % in cow dung-amended plant (Table 4).

Table 1: Effects of application of weathered poultry manure, sawdust and cow dung on height (cm) of nematode–infected okra under greenhouse conditions.

Treatment		Plant Height (cm)			
	Rate (t/ha)	2 weeks	4 weeks	6weeks	8 weeks
Cow dung	1.5	11.5	13.8	14.5	15.3
	3.0	11.2	13.8	14.5	15.3
Sawdust	1.5	9.3	11.2	12.9	13.9
	3.0	11.5	13.1	16.3	15.4
Weathered poultry manure	1.5	10.9	12.3	14.4	16.3
	3.0	11.6	14.2	16.1	17.7
Inoculated control		9.9	13.8	14.1	15.0
Uninoculated control		10.2	14.0	14.4	15.2
LSD (P=0.05)		0.60	0.62	0.60	0.50

Each value is a mean of five replicates and two separate experiments.

Table 2: Effects of application of weathered poultry manure, sawdust and cow dung on leaf number of nematode–infected okra under greenhouse conditions.

Treatment	Leaf Number				
	Rate (t/ha)	2 weeks	4 weeks	6weeks	8 weeks
Cow dung	1.5	1.6	3.2	4.4	5.2
	3.0	1.8	3.6	4.6	5.6
Sawdust	1.5	1.8	3.0	3.4	4.2
	3.0	2.0	3.2	4.6	5.4
Weathered poultry manure	1.5	1.8	3.6	5.4	6.8
	3.0	2.0	4.2	5.6	8.2
Inoculated control		2.0	3.4	4.6	5.2
Uninoculated control		2.0	3.6	4.8	5.5
LSD (P=0.05)		1.2	0.44	0.49	0.21

Each value is a mean of five replicates and two separate experiments

Table 3: Effects of application of weathered poultry manure, sawdust and cow dung on population of second-stage juveniles (J2) of *Meloidogyne* spp. infecting okra under greenhouse conditions.

	Meloidogyne spp. J2 (Number/200ml soil)	
Treatment	Rate (t/ha)	(X10)
Cow dung	1.5	165.4
	3.0	101.8
Sawdust	1.5	191.2
	3.0	222.2
Weathered poultry manure	1.5	93.8
	3.0	90.2
Inoculated control		493.0
Uninoculated control		0.05
L.S.D (P=0.05)		1.49

Each value is a mean of five replicates and two separate experiments

Table 4: Effects of application of weathered poultry manure, sawdust and cow dung on root galling of *Meloidogyne* spp.-infected okra under greenhouse conditions.

Treatment	Rate (t/ha)	Gall index **
Cow dung	1.5	2.8
	3.0	2.8
Sawdust	1.5	2.2
	3.0	2.0
Weather poultry manure	1.5	1.8
	3.0	1.6
Inoculated control		4.2
Uninoculated control		0.1
L.S.D (P=0.05)		0.50

Each value is a mean of five replicates and two separate experiments.
**Rating scale: 0 = No galls or egg masses, 1 = 1 – 2 galls or egg masses; 2 = 3 – 10 galls or egg masses; 3 = 11 – 30 galls or egg .
masses; 4 = 31 – 100 galls or eggs masses; 5 = more than 100 galls or egg masses.

Symptoms recorded on okra plants were leaf curling, leaf chlorosis and stunting. There was no evidence of phytotoxixity on the plants throughout the period of study.

Field Experiment

Analysis of soil sample and organic amendments collected before planting showed the following results: pH in calcium chloride = 4.2. N= 0.9%, P = 23.76 mg/kg, K = 19.11 mg/kg. Three species of nematodes including *Pratylenchus* spp., *Paratylenchus* spp. and *Hoplolaimus* spp. apart from *Meloidogyne* spp. that was multiplied were identified in soil collected from the experimental site. Average population densities of plant-parasitic nematodes on the experimental site at planting were as follows: *Meloidogyne* spp. J2, 1,100/200 ml soil; *Pratylenchus* spp.,220/200 m1 soil; *Paratylenchus* spp., 240/ 200 ml soil; *Hoplolaimus* spp., 156 /200 ml soil.

The levels of nematode population densities recorded were those that would normally initiate infection in a susceptible plant. Under field conditions, okra plants to which weathered poultry manure at 3.0 t /ha was applied had the least root galling, which was significantly lower than the galling on roots of other plants (Table 5).

Table 5: Effects of application of weathered poultry manure, sawdust and cow dung on root galling of *Meloidogyne* spp.-infected okra under field conditions.

Treatment	Rate (t/ha)	Gall index **
Cow dung	1.5	3.5
	3.0	3.3
Sawdust	1.5	3.8
	3.0	3.8
Weathered poultry manure	1.5	1.4
	3.0	1.1
Non-amended control		4.1
L.S.D (P=0.05)		0.38

Each value is a mean of five replicates.

The fruit yield followed a similar trend with weathered poultry manure (3.0 t /ha) amended plants producing the highest fruit yield, followed by cow dung and sawdust (Table 6). The least percentage increase in soil population of *Meloidogyne* spp. was recorded in plot amended with weathered poultry manure at 3.0 t /ha, followed by plots amended with cow dung and sawdust. The effects of the agricultural wastes on percentage change in soil populations of *Pratylenchus* spp., *Paratylenchus* spp. and

Hoplolaimus spp. followed a similar trend. Phytotoxic effects were not observed on okra plants.

Table 6: Effects of application of weathered poultry manure, sawdust and cow dung on fruit yield (t/ha) of nematode-infected okra under field conditions.

Treatment	Rate (t/ha)	Fruit weight
Cow dung	1.5	577.8
	3.0	605.5
Sawdust	1.5	522.2
	3.0	533.3
Weathered poultry manure	1.5	690.0
	3.0	724.4
Non-amended control		500.0
L.S.D (P=0.05)		2.80

Each value is a mean of five replicates

Table 7: Effects of application of weathered poultry manure, sawdust and cow dung on percentage change in soil population of plant-parasitic nematodes in nematode–infested okra field.

Treatment	Rate (t/ha)	*Pratylenchus* spp.	*Paratylenchus* spp.	*Hoplolaimus* spp.	*Meloidogyne* spp.
Cow dung	1.5	+8.2	+ 8.7	+7.6	+20.6
	3.0	+8.1	+8.1	+7.2	+17.8
Sawdust	1.5	+9.5	+9.3	+8.7	+38.3
	3.0	+9.0	+9.1	+8.2	+33.9
Weathered poultry	1.5	+6.2	+5.7	+5.9	+14.9
manure	3.0	+5.2	+5.2	+5.4	+12.6
Non-amended control		+1.24	+10.6	+12.2	+86.7
L.S.D (P=0.05)		0.64	0.44	0.27	0.64

Each value is a mean of five replicates.
+ indicates population increase.

DISCUSSION

Application of weathered poultry manure, cow dung and sawdust to nematode-infested soil resulted in increased vegetative growth, reduced root galling, increased yield of okra fruit but also an increase in soil populations of four genera of plant parasitic nematodes. The increase in nematode populations was higher however in soil to which no agricultural wastes were applied. This may be probably because the agricultural wastes applied improved the soil fertility and resulted in a lower increase in population build up of plant parasitic nematodes as also evidenced by reduced root damage of okra. The benefits of organic amendments and mulches in improving crop performance are well known (Peirce, 1987; Kostewiez, 1993). Organic amendments can be particularly useful in sites where soil organic matter is low, since increases in soil organic matter are associated with improvement in soil cation exchange capacity, water holding capacity and crop yield (Gallaher and McSorley, 1994). The findings of the current study agree with those of Kimpinski *et al.* (2003) who examined the influence of compost (cull wastes potatoes, sawdust and sheep manure mixed in ratio 3.3.1) and manure on crop yield and nematode populations. They reported that total yields of potato tubers averaged over seven growing seasons increased by 27% in plots treated with either compost or manure. Grain yields of barley also were increased by 12%, when compost was applied. Their findings indicated that organic amendments increased crop yields, but the impact on different nematode species (*Meloidogyne hapla, Heterodera trifolii*, bacteria feeding nematodes, *Diplogaster theritieri, Pratylenchus penetrans*) varied and usually increased soil population levels.

Current findings are however at variance with those of McSorley and Gallaher (1995) who investigated the effect of yard waste compost on plant-parasitic nematode densities in vegetable crops. They reported that final densities of *Paratrichodorus minor, Pratylenchus* spp., and *Xiphinema* spp., were unaffected by compost treatment in all tests. Vegetable yields were either unaffected by treatment or, in some tests, were low following the mulch treatment. They concluded that the yard-waste compost had little effect on densities of plant-parasitic nematodes associated with short-term (4 months) vegetable crops. Akhtar and Malik (2000) had reported that organic soil amendments stimulate the activities of microorganisms that are antagonistic to plant-parasitic nematodes.

The decomposition of organic matter results in accumulation in the soils of specific compounds that may be nematicidal. Management of plant - parasitic nematodes can be by improvements of soil structure and or fertility. The mode of action of organic amendments leading to plant disease control and stimulation of microorganisms is complex and dependent on the nature of the amendments (Akhtar and Malik, 2000). Studies similar to the current one have been conducted on the efficacy of poultry manure amendment applied to soil for the control of plant-parasitic nematodes on vegetable crops. No products of compost decomposition have been shown to have immediate effects on nematodes (Derrico and Maio, 1980; Kaplan and Noe, 1993; Akhtar and Mahmood, 1997). Thus a slow decomposition rate of the material used and a slow rate of release of products that affect nematodes may result in their concentrations being too low to be effective. However, if the presence of critical products is maintained for a long time, the compost or organic amendment may stimulate increased activity of biological antagonists of nematodes (McSorley and Gallaher; 1995).

It should be noted that of the three amendments used in this study, weathered poultry manure had the highest percent Nitrogen and it was the most effective of the amendments in terms of reduction in gall index, yield of okra and percentage increase in population density of four genera of plant-parasitic nematodes. It had been reported that the most important effect of organic amendments on plant-parasitic nematodes might be the reduction of populations from toxic byproducts of decomposition. Ammonia and urea were shown to suppress several nematode species in tests, and the decomposition of some forms of organic nitrogen can release byproducts, which are toxic to nematodes (Rodriguez-Kabana, 1986). The most effective of these amendments are those with low carbon-to-nitrogen ratios that can release ammonia into the soil (Rodriguez-Kabana, 1986). The results of the present study show that application of weathered poultry manure, cow dung or sawdust at the rate of 3.0 t /ha has benefits for the management of nematodes in vegetable beds. Further work is required to investigate the efficacy of similar rates of these and other organic amendments for the management of plant-parasitic nematodes on okra and other crops.

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