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MANAGEMENT OF LESION NEMATODES ON MAIZE USING GREEN AND CATTLE MANURES IN THE CENTRAL HIGHLANDS OF KENYA

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ABSTRACT

Effects of Canavalia ensiformis and Mucuna pruriens green manures and cattle manure in mitigating against Pratylenchus zeae Filipjev damage on two maize varieties, Pan 5195 and Emap11, were assessed in greenhouse and on-farm experiments. In the greenhouse test, the green manures and the cattle manure were mixed with sterilized soil at the rate of 135g kg-1 of soil. Fourteen days after incorporating the manure, pre-germinated maize seeds were planted and inoculated with 1 P. zeae g-1of soil, seven days after planting. Each treatment was replicated six times in a Randomized Complete Block Design (RCBD). In the field test, 90 day-old green manures were broadcast, ploughed in at the rate of 5 t ha-1 and irrigated before sowing the maize seeds, 14 days thereafter. Treatments were replicated three times and arranged in a RCBD. Both tests were terminated 90 days after sowing the maize. In the greenhouse test, cattle manure, C. ensiformis and M. pruriens significantly (P<0.05) increased maize dry biomass by 106, 90 and 81%, respectively, and reduced nematode associated root necrosis by up to 46%. In the on-farm test, amending soils with cattle manure and M. pruriens significantly (P<0.05) improved maize grain yield by up to 88 and 53%, respectively, and suppressed nematode population build-up by up to 68% and disease severity (root necrosis) by up to 70%.

Keywords: Canavalia ensiformis, animal manure, Mucuna pruriens, Pratylenchus zeae, root necrosis, small holder farming systems

Introduction

Maize (*Zea mays* L.) is a staple food and a source of income for smallholder farmers in Kenya. The current average production of between 1.5 - 2t ha^{-1} is far below the germplasm potential of 3-7t ha^{-1} (Muhammad and Underwood, 2004). This is partly due to low soil fertility, nematode and insect pests (Muhammad and Underwood, 2004). *Pratylenchus zeae*, a lesion nematode, is the most economically important nematode pest on maize causing up to 50% yield losses in heavily infested fields (Arim *et al*., 2002; Kimenju *et al*., 1998; Waceke *et al*., 2002). Use of conventional nematode management practices such as crop rotation and fallowing are not

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economically feasible in Kenya due to small size and scarcity of arable land. Lack of nematode resistant maize varieties is a major drawback to the use of resistance in mitigating against nematode associated maize yield losses in Kenya (Kimenju *et al*., 1998; Arim *et al*., 2002). High costs and the negative impact on the environment hinder use of nematicides on low value crops such as maize. Cattle manure, which is commonly used for soil fertility improvement by between 65-80% of smallholder farmers in Kenya would provide a viable nematode management strategy. Similarly, leguminous green manures which were introduced in maize based cropping systems in Kenya during the last 15 years would provide viable alternatives for nematode management, as they are cheap, environmentally friendly and have multiple uses (Mureithi *et al*., 2003). The suppressive effect of cattle manure on nematode pests, though well documented, is highly variable due to variability in the quality and quantities applied (Nyambati *et al*., 2003) and the target nematode and hence the need to evaluate the efficacy of the manure in the selected study areas. Although green manures have been reported to suppress phytonematodes (Al-Rehiayani & Hafez, 1998; Waceke *et al*., 2003), literature on their impact on *P. zeae* in maize is lacking. Plant Parasitic Nematode-suppressive green manures include sorghum sudan grass, rye, and mustards (Abawi and Widmer, 2000;Wang et al., 2006; Machado et al., 2007; Collins et al., 2006). This is in spite of their increased use in soil fertility improvement in maize based cropping systems in Kenya for the last 15 years (Mureithi *et al*., 2003; Wortmann *et al*., 2000). It is therefore, imperative that the impact of the green manures on nematodes be assessed.

This study, was therefore conducted to evaluate the efficacy of cattle manure and, *Canavalia ensiformis* and *Mucuna pruriens* green manures in suppressing *P. zeae* on maize.

Material and methods

The study was conducted in the greenhouse at Kenyatta University, Nairobi (1^{o} 10' 47.9" S, E 36^{o} 56' 5.8" E) and in a farmer's field, in Kibing'oti location (0^{o} 34' S, 37^{0} 11' E, 1354 masl) Kirinyaga County in the Central Highlands of Kenya.

Greenhouse Trials

Ninety day – old *C. ensiformis* and *M. pruriens* green manures were obtained from a farmers field where an experiment to test efficacy of intercropping maize with these legumes against lesion nematode was previously estabished. The fresh materials were manually chopped into small pieces, incorporated into sterilized soil contained in 15cm-diameter plastic pots at the rate of 135g kg^{-1} of soil and watered regularly. The soil a sandy- loam soil (60 % sand, 24 % silt, 16 % clay, 0.6 % organic matter, pH 5.4) obtained from Kenyatta University School of Agriculture Research Farm was sieved using a 2 mm sieve and sterilized at 121^{0}C and 11 kg cm^{-2} pressure for one hour before use. Sun-dried cattle manure obtained from the same farmer was also

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incorporated at the same rate. The mineral content of the materials was analysed at the National Agricultural Research Laboratories (NARL) and the results are depicted in Table 1.

Fourteen days after incorporating the materials, pre-germinated Pan5195 and Emap11 maize seeds were planted and inoculated with one nematode per gram of soil, seven days after planting. The nematode inoculum was isolated from maize roots obtained from the field study area and multiplied and maintained on a susceptible maize cultivar (H625) in the greenhouse (Arim *et al.*, 2002). The inoculum was extracted from 90 day-old maize roots using a maceration-filtration technique (Fallis, 1943) and standardized to a 200 nematodes ml^{-1} suspension. Inoculation involved making a 6 cm deep depression around the rhizosphere of the seedling and dispensing ten milliliters of the nematode suspension into the depression and covering with soil. Pots in which no organic materials or nematodes were added served as controls. Each treatment was replicated six times in a Randomized Complete Block Design (RCBD). The plants were watered regularly to maintain soil moisture at field capacity.

Table 1: Mineral content of cattle manure and green manures of *Mucuna pruriens* and *Canavalia ensiformis* used in the studies

	% N	% P	% K	C:N ratio	% Ca	% Mg	% Ash	% Polyphenols	% lignin
Canavalia ensiformis	3.3	0.2	1.3	14	0.9	0.2	5.8	9.9	14.4
Mucuna pruriens	5.5	0.2	1.5	21	1.0	0.3	8.7	3.4	10.7
Cattle manure	1.4	0.2	1.8	26	1.0	0.4	46.1	0.13	8.7

Data collection

The experiment was terminated 90 days after inoculation and plant growth (plant height, fresh and dry shoot, and fresh root weights) and disease assessment parameters (root necrosis, nematode population in roots and soil and reproductive factor) determined. Plant heights were measured from the first leaf node to the shoot apex. Plants were gently uprooted, shoots were cut at the first node and their fresh weights obtained. The shoots were oven dried at 60^0C for 3 days and their dry weights determined. Soil was gently shaken off from the roots to minimize damage of fine roots. The roots were gently washed, blotted dry and weighed. Thereafter the roots were cut into 5 cm- long segments and thoroughly mixed before taking a 20g root sub-sample for root necrosis index (disease severity) assessment. The Bridge & Gowen (1993) scale of 0 – 4 necrosis index was used to assess root necrosis where 0=no root damage, 1=light root damage, 2=moderate root damage, 3=severe root damage, 4=very severe root damage. After root necrosis was determined, the 20g root sample was divided into two equal sub-samples of 10g each. One sub-sample was oven dried at 60^0C for 3 days and nematodes were extracted from the other sub-sample using the Maceration - filtration technique (Fallis, 1943). Nematodes recovered were then expressed per gram dry root. Nematodes in soil were recovered from 200g soil sub-sample using the Extraction - Tray method (Thomas, 1959). A nematode reproductive rating (Rf), was determined by expressing final nematode population (P_f) as a ratio of initial population (P_i). The P_f was the total number of nematodes recovered from both the roots and the soil. The Rf was assessed according to Ferris *et al*., (1993) where: Rf <1 - most suppressive, 1 ≤ Rf <1.5 - suppressive and Rf ≥ 1.5 - least suppressive.

Field Trial

The greenhouse experiment was repeated in a farmer's field on 3m × 4m micro-plots. The fresh leguminous materials, obtained as stated earlier, were chopped into small pieces, broadcasted onto the specific treatment plots and ploughed in at a 10-15cm depth. The green manures and the sun-dried cattle manure were incorporated at the rate of 5 t ha^{-1} and plots irrigated twice per week for 21 days before planting the Pan5195 and Emap11 maize seeds. The maize seeds (one seed per hill) were planted at a spacing of 25cm × 75cm and P fertilizer applied at the recommended rate of 60 kg P_20_5 ha^{-1}. Plots in which no organic materials were added served as controls. Each treatment was replicated thrice in RCBD. The plots were irrigated once per week using overhead irrigation and kept weeds free throughout the experimental period. The trial was terminated 150 days (5 months) after planting.

Data collection

Nematode population per 200g soil sample from each plot was determined before incorporating the organic manures at the beginning of the experiment. Thereafter the soil nematode population from maize rhizosphere was determined at 45 and 90 days after planting. Root necrosis and root nematode population were determined 90 days after planting as previously described. Maize from each treatment was dried, shelled and yield determined 150 days after planting.

Data analysis

Data were subjected to Analysis of Variance (ANOVA) using Genstat 5 Release 3.2 and Least Significant Difference (LSD) was used to compare treatment means.

Results

Greenhouse test

Cattle manure, *M. pruriens* and *C. ensiformis* significantly (P<0.05) increased growth of Pan5195 relative to the controls by up to 63, 49 and 38%, respectively. While cattle manure was significantly (P<0.05) more effective in increasing dry biomass of Pan5195 by 18% compared with *C. ensiformis*, the later was as effective as *M. pruriens* (Table 2).

Table 2. Mean† plant heights, fresh root weights, fresh and dry shoot weights of Pan5195 and Emap11 grown in soils amended with *M. pruriens* (MP), *C. ensiformis* (CE) and cattle manure (CM), 90 days after inoculation, greenhouse trial.

Treatment	Plant height (cm)	Fresh root weight (g)	Fresh shoot weight (g)	Dry shoot weight (g)
Emap11+MP	113.0^{d‡}	34.9^{c}	95.8^{c}	23.5^{cd}
Emap11+CE	110.0^{c}	33.4^{c}	92.0^{c}	22.4^{c}
Emap11+CM	118.8^{e}	35.2^{c}	99.1^{d}	25.2^{d}
Emap11	107.2^{c}	29.2^{b}	75.3^{a}	13.8^{a}
Pan5195+MP	94.3^{b}	29.4^{b}	96.1^{c}	27.9^{ef}
Pan5195+CE	90.3^{ab}	29.1^{b}	95.3^{c}	26.0^{de}
Pan5195+CM	95.0^{b}	30.8^{b}	101.0^{d}	30.2^{f}
Pan5195	88.0^{a}	23.7^{a}	81.3^{b}	19.7^{b}
$SD_{(0.05)}$	4.7	1.9	5.6	2.6
CV (%)	4.0	6.0	5.3	10.6

†Mean for six replications.
‡Means followed by the same letter (s) are not significantly different (P<0.05)

Similarly, cattle, *M. pruriens* and *C. ensiformis* manures significantly ($P<0.05$) increased growth of Emap11 relative to the control by up to 106, 90 and 81%, respectively. Cattle manure was more effective in increasing growth of Emap11 compared with *C. ensiformis* by up to 14%. While cattle manure was as effective as *M. pruriens* in improving growth of Emap11, the later was as effective as *C. ensiformis* (Table 2).

Disease severity (root necrosis) of maize grown in amended soils was significantly ($P<0.05$) lower than in non-amended soil. Cattle manure, *M. pruriens* and *C. ensiformis* - amended soils significantly ($P<0.05$) reduced disease severity in Pan5195 and Emap11 by up to 22 and 46%, respectively, (Table 3). Likewise, the soil amendments significantly ($P<0.05$) reduced nematode reproduction and population in roots of Pan5195 by up to 50 and 89%, respectively. Cattle manure and *M. pruriens* were significantly ($P<0.05$) more effective in suppressing nematode reproduction and root nematode populations on Pan5195 than *C. ensiformis* (Table 3).

Table 3. Mean† root necrosis (RNI), root nematode population (Nem. g^{-1} dry root), nematode reproductive factor (Rf), and nematode suppressive ability (NSA) of *M. pruriens* (MP), *C. ensiformis* (CE) and Cattle manure (CM) amended soils, 90 days after inoculation, greenhouse trial.

Treatment	RNI*	Nem. g^{-1} dry root	Rf**	NSA***
Emap11+MP	0.7a‡	69d	0.79b	MS
Emap11+CE	0.7a	80e	0.89c	MS
Emap11+CM	0.7a	63cd	0.73a	MS
Emap11	1.3c	650g	2.91e	LS
Pan5195+MP	0.7a	51ab	0.70a	MS
Pan5195+CE	0.7a	59bc	0.82b	MS
Pan5195+CM	0.7a	46a	0.70a	MS
Pan5195	0.9b	433f	1.41d	S

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$LSD_{(0.05)}$	0.2	8.0	0.04
CV(%)	23.0	3.6	2.60

† Mean for six replications.

‡ Means followed by the same letter are not significantly different (P<0.05)

* Root necrosis index: 0 = no root damage, 1 = slight root damage, 2 = moderate root damage, 3 = severe root damage, 4 = very severe root damage (Bridge and Gowen, 1993).

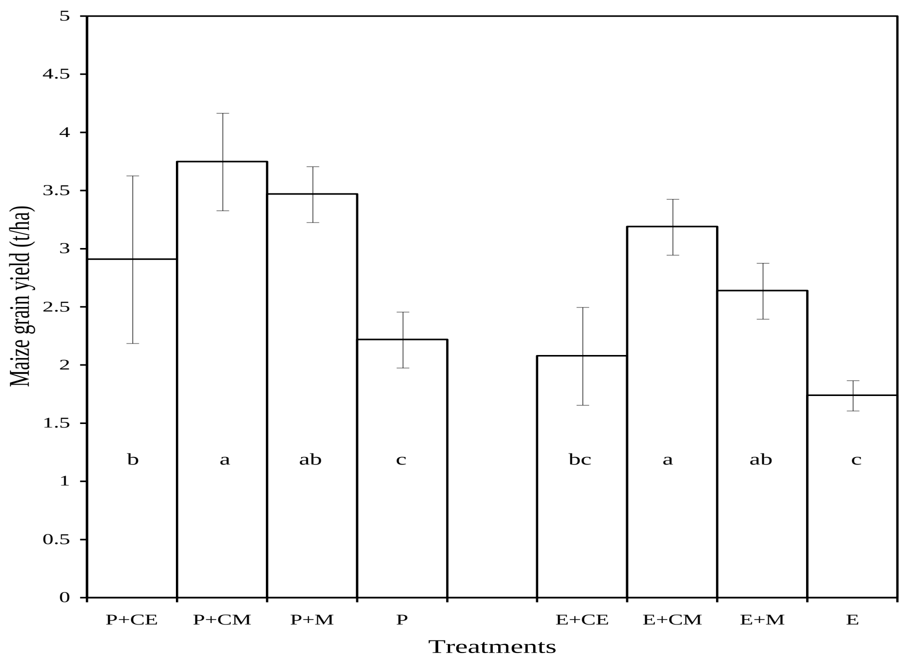
** Rf = ratio of final nematode population (P_f) to initial nematode population (P_i) (Ferris *et al.*, 1993).

***MS=Most suppressive (Rf <1), S=Suppressive (1≤ Rf<1.5), LS=Least suppressive (10 ≥ Rf ≥1.5) system (Ferris *et al.*, 1993).

Cattle manure, *M. pruriens* and *C. ensiformis* significantly (*P*<0.05) reduced nematode populations in roots of Emap11 by up to 90% (Table 3). Cattle manure and *M. pruriens* were significantly (*P*<0.05) more effective than *C. ensiformis* in reducing the nematodes (Table 3). Cattle manure-amended soils compared to non-amended, *C. ensiformis* and *M. pruriens* -amended soils, significantly (*P*<0.05) reduced nematode reproduction on Emap11 by 75, 18 and 8%, respectively (Table 3). Amending soils with *M. pruriens* significantly (*P*<0.05) suppressed nematode reproduction on Emap11 by 73 and 11% compared to non-amended and *C. ensiformis* - amended soils, respectively. Likewise, *C. ensiformis* - amended soils significantly (*P*<0.05) suppressed nematode reproduction on Emap11 by 69% (Table 3).

Field experiment

Figure 1. Mean grain yield of Pan5195 (P) and Emap11 (E) maize varieties grown in *C. ensiformis* (CE), cattle-manure (CM), and *M. pruriens* (M) - amended soils in the field experiment, 5 months after planting.



Bar means followed by the same letter (s) are not significantly ($p<0.05$) different.

Cattle manure, *M. pruriens* and *C. ensiformis* increased grain yield of Pan5195 by 73, 59 and 32%, respectively (Figure 1). Cattle manure was significantly ($P<0.05$) more effective than *C. ensiformis* in increasing the grain yield by 31%. *Canavalia ensiformis* - amended soils were as effective as *M. pruriens* – amended soils in increasing the grain yield.

Amending soils with cattle manure and *M. pruriens* significantly ($P<0.05$) increased grain yield of Emap11 by 88 and 53%, respectively (Figure 1). While cattle manure significantly ($P<0.05$) increased grain yield of Emap11 by 52% compared to *C. ensiformis*, the later was as effective as *M. pruriens* (Figure 1).

Table 4. Mean† root necrosis (RNI) and root nematode population, (Nem. g^{-1}dry root) of Pan5195 and Emap11 maize varieties grown in *M. pruriens* (MP), *C. ensiformis* (CE) and Cattle manure (CM) amended soils, 90 days after planting, on-farm test in Kibing'oti location

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Treatment	RNI[b]	Nem. g^{-1} dry root
Emap11+MP	1.0bc[‡]	98b
Emap11+CE	1.0bc	112c
Emap11+CM	0.9ab	84a
Emap11	3.0d	664e
Pan5195+MP	1.0bc	87ab
Pan5195+CE	1.0bc	89ab
Pan5195+CM	0.8a	77a
Pan5195	2.0cd	460d
$LSD_{(0.05)}$	0.14	13
CV(%)	6.0	3.6

[†]Mean for six replications.

[‡] Means followed by the same letter are not significantly different (P<0.05)

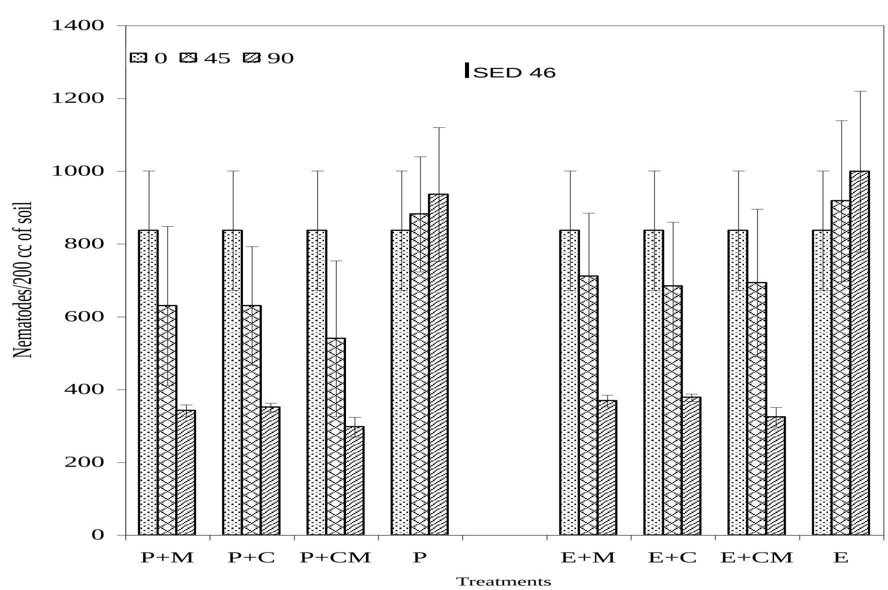
[*]Root necrosis index: 0 = no root damage, 1 = slight root damage, 2 = moderate root damage, 3 = severe root damage, 4 = very severe root damage (Bridge and Gowen, 1993).

Significant (*P*<0.05) differences in disease severity were observed among treatments (Table 4). Cattle manure - amended soils compared to non-amended and *M. pruriens* - amended soils reduced nematode disease severity on Pan5195 by 60 and 20%, respectively. *Mucuna pruriens* and *C. ensiformis* - amended soils significantly (*P*<0.05) reduced nematode disease severity on Pan5195 by up to 50% (Table 4). Similarly, the cattle and green manures significantly (*P*<0.05) reduced nematode population in roots of Pan5195 by 83 and 81%, respectively, relative to the control.

Soils amended with cattle manure and green manures significantly (P<0.05) reduced disease severity in Emap11 by 70 and 67%, respectively (Table 4). There was a significant (P<0.05) reduction of root nematode population in Emap11 by cattle manure (87%), *M. pruriens* (85%) and *C. ensiformis* (83%). Cattle manure was more effective in reducing the nematode population than *C. ensiformis* and *M. pruriens* by 25 and 14%, respectively. *Mucuna pruriens* significantly (P<0.05) reduced nematode population in roots of Emap11 by 13% compared to *C. ensiformis* (Table 4).

There was a significant (P<0.05) reduction of up to 68% in soil nematode populations of Pan5195 by the manures (Table 4). In non-amended soils, however, there was a significant (P<0.05) increase (12%) in soil nematode populations on Pan5195, ninety days after planting (Figure 2). While amending soils with cattle manure, *M. pruriens* and *C. ensiformis* significantly (P<0.05) reduced the number of nematodes in the rhizosphere of Emap11 by 68, 63 and 62%, respectively, ninety days after planting, in non-amended soils the number of nematodes in the rhizosphere of Emap11 significantly (P<0.05) increased by 19% (Figure 2).

Figure 2. Mean population of nematode obtained from the rhizosphere of Pan5195 (P) and Emap11 (E) maize at 0 (planting), 45 (mid - season) and 90 days (end - season) after planting maize in *M. pruriens* (M), *C. ensiformis* (C) and cattle-manure (CM) amended soils; Field experiments.



Discussion

The increase in maize growth and yield by the organic amendments both in the greenhouse and field experiments is consistent with findings by Chitwood (2002), Al-Rehiayani & Hafez, (1998); McSorley (2011), Waceke *et al*., (1993) and Muller & Gooch (1982). An increase in soil nutrient status, improved water holding capacity, enhanced root growth and reduced nematode damage might explain this significant increase in maize growth and yield (Chitwood, 2002). The enhanced supply of N and P macronutrients (main limiting nutrients in maize growing regions in Kenya) by the organic materials (Table 1) might have played a significant role in improving maize growth and yield (Nyambati *et al*., 2003). Nitrogen (N) inputs into the soil significantly influences not only the aboveground ecosystem productivity and but also the below-ground pools and fluxes of N such as microbial biomass N and mineralization. Anhydrous ammonia has also been shown to reduce the soil populations of many plant–parasitic nematode species, including stunt, spiral, lesion and cyst nematodes (Alam, 1992).

The differences in nutrient contents of the organic materials especially potassium and nitrogen might have explained the differences in the efficacy of the materials to improve maize growth and yield. Cattle and *M. pruriens* manures had relatively higher contents of N and K as compared to *C. ensiformis* (Table 1). The insignificant difference in grain yield of maize grown in cattle manure and *M. pruriens* - amended soils is consistent with earlier report by Nyambati *et al*. (2003) which revealed that mucuna and cattle manure were equally effective in improving maize yield. The relatively high polyphenols and lignin contents in *C. ensiformis* could partly explain their relatively low efficacy in improving maize growth and yield due to slow mineralization.

The efficacy of the organic materials to improve growth and yield of maize was influenced by the maize genotype, being more effective on Emap11 than on Pan5195. This could probably due to the difference in the root morphology, with those Pan 5195 tending to go deeper than those of Emap11 (Arim *et al*., 2006)

The negative correlation between dry shoot weight and nematodes in roots (r^2=0.6, P=0.05) and between grain yield and nematodes in roots (r^2=0.5, P=0.05) underscores the importance of nematodes in maize production.

The decline in disease severity, root and soil nematode populations in the amended soils was an indication that the amendments had suppressive effects on *P. zeae*. This corroborates findings by Baldwin & Creamer (2004), Chitwood (2002) and McSorley (2011) which revealed the high potential of organic matter to suppress plant parasitic nematodes in maize cropping systems. The nematode suppression could be attributed to toxic effects of volatile chemicals (biofumigants) produced during decomposition of organic materials (Baldwin & Creamer, 2004; Chitwood, 2002; Akhtar & Malik, 2000). Ammonia, produced in large quantities by leguminous green and animal manures that have low C: N ratio (8-20), for example, has an inhibitory effect on hatching of nematode eggs (Bello *et al*., 2000). The cattle manure, *M. pruriens* and *C. ensiformis* used in this study had 26, 21 and 14 C:N ratio, respectively (Table 1). Cattle manure in some cases can have as high as 32 C:N ratio (Bello *et al*., 2000). Nematicidal bioactive natural products such as triacontanol and tricontyl tetracosanate that are produced by *Mucuna* spp. might have reduced the mobility of juveniles and/or caused their paralysis and death (Marisa *et al*., 1996). An increase in nematode antagonists in organic - amended soils might have also contributed to the reduction of *P. zeae* populations (Al-Rehiayani & Hafez, 1998; Sikora, 1992). In addition, the high temperatures produced during the initial stages of decomposition have the potential of lowering nematode density and the inoculum potential leading to a decline in soil and root nematode populations and disease severity (Chitwood, 2002).

The differences in efficacy of the organic materials to minimize *P. zeae* damage on maize could be due to the differences in mineral contents (Table 1) and the nature of nematicidal compounds that they release on decomposition.

Although Pan5195 had been reported to be moderately resistant [Reproductive factor (Rf) = 1.3] and Emap11 susceptible (Rf = 2.9) to *P. zeae* (Arim *et al*., 2006), their ability to withstand the damaging effects of the nematode were increased by the addition of organic materials. This underscores the importance of applying an integrated approach to nematode management as opposed to a single approach.

Since *M. pruriens* green manure was as effective as cattle manure in suppressing *P. zeae* and increasing maize grain yield, it can be used as a substitute in farms where cattle manure is unavailable.

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