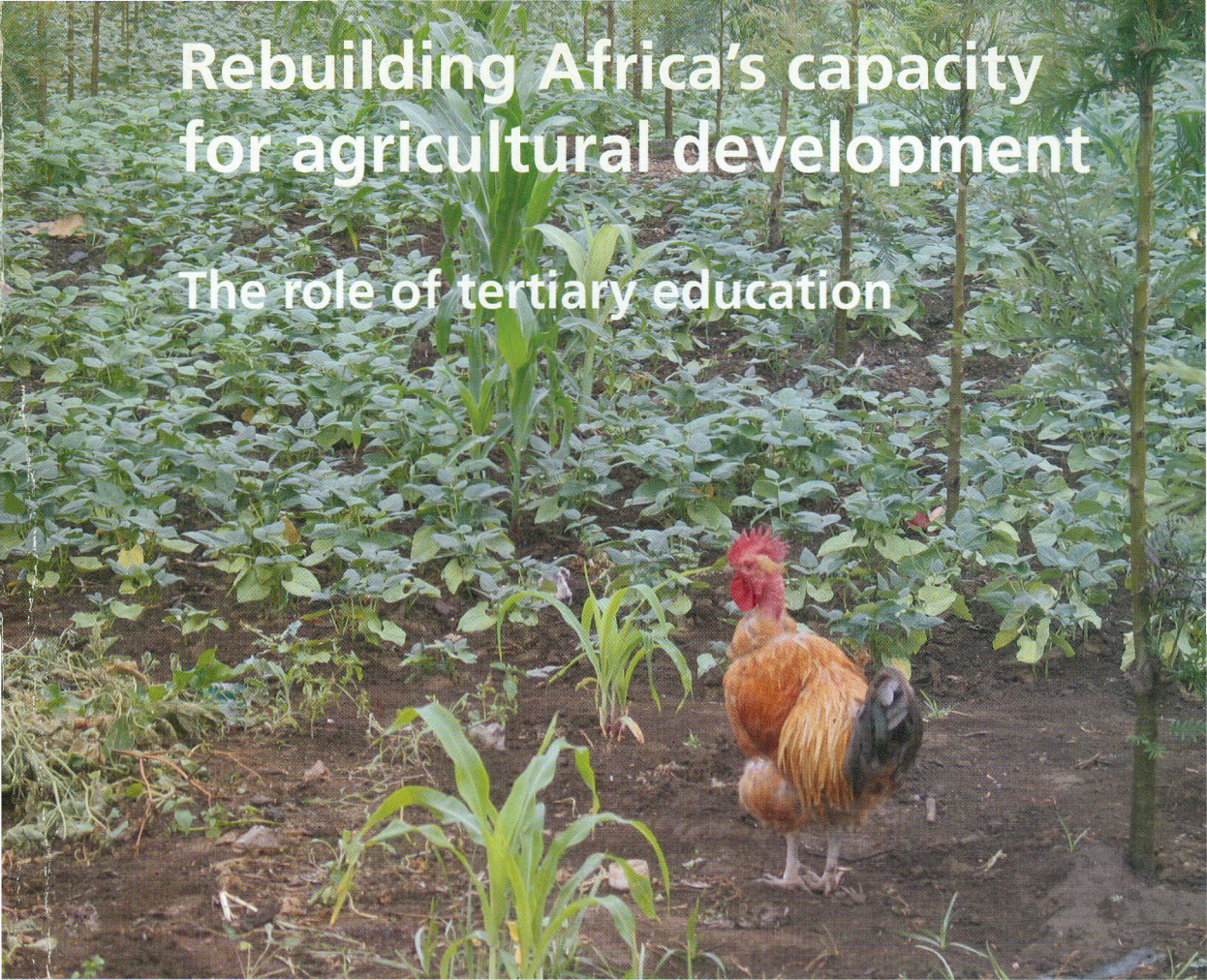


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Impact of soil nutrient management practices on plant parasitic nematodes of maize in central Kenya

J.W. Waceke,¹ D.N. Mugendi² and N.J. Mbaru¹

Abstract

Maize (*Zea mays* L.) is the staple food in Kenya. The current average production of 1.5–2 t per ha is far below the germplasm potential of 3–7 t per ha. Low soil fertility, nematode and insect pests, diseases and poor quality of seed and advisory services are some of the maize production constraints in Kenya. However, there have been major efforts to overcome these production constraints in order to increase maize production. For example, use of animal manure and green manure alone or in combination with sub-optimal levels of inorganic fertilisers and interplanting maize with leguminous trees and shrubs are being recommended to farmers for replenishing soil nutrients. Some of these soil nutrient management strategies have negative or positive effect on plant parasitic nematode populations. The objective of the study was to assess the impact of some of the soil nutrient management strategies on plant parasitic nematodes of maize, especially lesion nematodes (*Pratylenchus* spp.), which cause yield losses of up to 50% in maize. The soil nutrient management strategies whose impact on nematodes was assessed were mucuna and crotalaria alone, as intercrops or in combination with half the recommended rate of N fertiliser, and incorporation of cattle manure and green manure (*Tithonia diversifolia*, *Calliandra calothyrsus* and *Leucaena trichandra*) singly or in combination with half the recommended rate of N fertiliser. Mucuna and crotalaria intercrops reduced nematode disease severity and population by 20%. Addition of inorganic N fertiliser reduced efficacy of mucuna by 99% but not that of crotalaria against the nematodes. Incorporation of green manure had no effect or increased lesion nematode population and, consequently, disease severity. In most cases addition of N fertiliser did not affect the effects of the green manure on nematodes. Cattle manure in combination with inorganic N reduced lesion nematodes and the associated disease severity by up to 75%.

Key words: cattle manure, foliar biomass, green manure, inorganic fertiliser, legumes, maize nematodes, soil fertility

Introduction

Maize (*Zea mays* L.) is the staple food in Kenya. It contributes 3% and 12% of the national and the agricultural gross domestic product (GDP), respectively. In Kenya consumption of maize per person per annum is 120–125 kg (Agri-Forum 2001). The current average production of between 1.5 and 2 t per ha is far below the germplasm potential of 3–7 t per ha (Agri-Forum 2001) and cannot support the continued increase in demand of between 2–3% per year emanating from the high population growth rate of 3.6% per annum.

Low soil fertility ranks among the highest maize production constraints in Kenya, arising from continuous cropping, crop removals and soil erosion (Smaling 1993). Other important production constraints include nematode and insect pests, diseases, poor quality of seed, high production costs (US\$240 per t) and poor advisory services. Nematode

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pests, which increase considerably under continuous maize cropping systems, cause significant yield losses in maize. Lesion nematodes are the most important in maize, causing yield losses of up to 50%. They damage the fibrous roots, causing sloughing-off (root pruning) and severe necrosis as evidenced by small, blackish lesions on the root surface. The reduced root system impairs water and mineral uptake and translocation, resulting in stunting, leaf chlorosis, wilting and low yields. Nematode infection can also predispose maize to secondary infections by bacteria and fungi (Kimenju et al. 1998).

Soil fertility management practices have been found to affect plant parasitic nematodes and other soil-borne pathogens (Rao and Albrecht 1999). Legumes such as *Mucuna pruriens* L. (velvet beans), *Tephrosia purpurea* L., *Desmodium uncinatum* L., *Vicia villosa* L. (hairy vetch) and *Crotalaria* spp., which are known to suppress root-knot (*Meloidogyne* spp.) and burrowing (*Radopholus* spp.) nematodes on various crops would, therefore, provide an added advantage (Haroon 1993; Waceke 2000). *Calliandra calothyrsus* Meissner and *Leucaena leucocephala* Lam de Wit, which are poor hosts of the root-knot nematodes, provide viable nematode management strategies (ICRAF 1996; Desaeger and Rao 1999, 2000, 2001). *Sesbania sesban* L. and *Tephrosia vogelii* L., which suppress *Pratylenchus* spp., are other viable nematode management alternatives (Desaeger and Rao 1999, 2000, 2001).

The varying responses of nematodes to different plant genotypes, green manures and inorganic fertilisers made it necessary for this study to evaluate efficacy of soil nutrient management practices in suppressing plant parasitic nematodes of maize.

Incorporation of organic and inorganic materials also affect both plant and non-plant parasitic nematodes. Animal manure and other crop residues have been reported to either decrease or increase plant parasitic nematodes, depending on the C:N ratio of the materials (Waceke et al. 1993). Some inorganic fertilisers such as superphosphates increase *Pratylenchus* spp. populations in the soil (Yeates 1976). The varying effects of various soil fertility management practices on plant parasitic nematodes make it necessary to assess their impact on nematode species that are of economic importance to staple crops like maize. This study was conducted to investigate the impact of soil nutrient management practices on parasitic nematodes on maize, with special emphasis on lesion nematodes (*Pratylenchus* spp.), which cause maize yield losses of up to 50% in Kenya (Waceke et al. 2002; Kimenju et al. 1998).

Materials and methods

Description of sampling site

Soil and root samples for nematode assays were obtained from a field site in which trials on efficacy of a combination of soil fertility management practices were being conducted. The field site is located in Chuka, Meru South District, in the upper midland (UM2/UM3) agroecological zone, which lies 1,500 m above sea level. The area receives annual rainfall of between 1,000 and 1,400 mm. Rainfall is bimodal with long and short rain seasons occurring in March–June and October–December, respectively. The soils are poor and impoverished (Jaetzold and Schmidt 1983).

Treatments and experimental layout

There were 14 treatments, comprising the following combinations:

- Maize intercropped with *M. pruriens* (velvet/horse beans) or *Crotalaria ochroleuca* (Tanzanian sun hemp)

- Maize intercropped with *M. pruriens* or *C. ochroleuca* and supplied with half the recommended rate of N ($_{1/2}$ N). The recommended rate of fertiliser in this region is 60 kg N and P per ha (Mugendi et al. 1999).
- Maize supplied with cattle manure alone or in combination with half N.
- Maize supplied with *Tithonia diversifolia*, *C. calothyrsus* and *Leucaena trichandra* foliar biomass alone or in combination with half N.
- Maize supplied with N fertiliser at the recommended rate.
- Absolute control with no inputs.

The amount of biomass and cattle manure applied per plot was based on the N content of the organic materials. The application rate was equivalent to the amount of the respective biomass (dry weight basis) that give 60 kg N per ha. All treatments received a basal application of P fertiliser at 60 kg P per ha, to remove any P deficiencies.

The treatments were replicated three times in a randomised complete block design (RCBD). Maize was planted in plots measuring 6 x 4.5 m at inter and intra-row spacing of 0.75 m and 0.5 m, respectively. There were two maize plants per hill, according to farmers' practice, to make approximately 54,000 plants per ha.

Sampling procedures

Soil and root samples were collected mid-season during the short rains and at the silking and tasseling stage. Soil and root samples were obtained from six randomly selected maize plants per plot leaving out the two border rows. Before obtaining the samples, the topsoil (about 5 cm deep) was removed using a hand trowel and did not constitute the soil sample. Approximately 500 ml of soil was then collected from the maize rhizosphere at a depth of 15 cm and placed in labelled polythene bags to minimize desiccation. Roots collected together with the soil samples were separated and placed in labelled polythene, and formed the root sample. The root and soil samples per plot were thoroughly mixed, transported to the laboratory in cool boxes and stored at 4 °C before nematode assay.

Nematode assays

Disease severity

The root samples were thoroughly but gently washed and assessed for root necrosis (a measure of lesion nematode disease severity) using a 0–4 rating scale, where 0 = no necrosis, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100% of root system necrotic (Bridge and Gowen 1996). Six root systems per plot were assessed for disease severity, and the averages determined.

Extraction of nematodes from roots

Clean root systems were cut into 3-cm long segments and thoroughly mixed before taking 20 g fresh weight samples for nematode extraction. The samples were macerated in 100 ml of water using a 7 speed Hamilton blender for 15 seconds. Nematodes were then extracted using the Extraction Tray Technique (Fallis 1943). Nematodes were collected after every 24 hours for 3 days. Nematode suspension was adjusted to 10 ml and the number of nematodes per ml counted using a Hawksley's counter under a compound microscope.

Extraction of nematodes from soils

Soils from each experimental unit were thoroughly mixed before taking 200-ml samples for nematode extraction. Nematodes were extracted using Jenkins' Sieving-Centrifuge-Flotation method (Jenkins 1964).

Data analysis

Data were subjected to analysis of variance, and treatment means were separated using Fisher's Least Significant Difference (LSD).

Results and discussion

There were significant differences ($P < 0.05$) in necrosis indices among treatments (Table 1). Maize intercropped with *M. pruriens* plus $\frac{1}{2}$ N ($M + \frac{1}{2}$ N) or treated with *T. diversifolia* biomass plus $\frac{1}{2}$ N ($TBT + \frac{1}{2}$ N) had the highest necrosis indices, followed by that treated with *L. trichandra* (LBT) biomass alone. Plants treated with *T. diversifolia* (TBT) biomass alone, *Calliandra* plus $\frac{1}{2}$ N ($CBT + \frac{1}{2}$ N) and N fertiliser had necrosis indices that were not significantly different from each other and from those treated with $M + \frac{1}{2}$ N (Table 1). Maize plants intercropped with *M. pruriens* alone had significantly lower necrosis indices than those intercropped with $M + \frac{1}{2}$ N (Table 1).

Table 1: Mean¹ root necrosis index², total plant parasitic and non-parasitic nematodes in soil and root samples of maize

Treatment	Necrosis index	Total parasitic		Non-parasitic	
		Roots	Soil	Roots	Soil
Mucuna (M)	1.67	18.0	46.67	10.67	10.67
Crotalaria (C)	1.67	15.33	36.0	6.0	6.0
Nitrogen (N)	2.33	11.33	36.67	2.33	2.33
$M + \frac{1}{2}$ N	3.33	21.33	57	11.33	11.33
$C + \frac{1}{2}$ N	1.0	16.33	52.33	2.0	2.0
Cattle manure (CM)	0.33	19.33	82.33	9.67	9.67
$CM + \frac{1}{2}$ N	0.33	14.0	50.0	5.0	5.0
Tithonia BT (TBT)	2.33	20.33	93.33	15.0	15.0
Calliandra BT (CBT)	1.33	13.67	66.0	11.0	11.0
Leucaena BT (LBT)	3.0	20.33	49.0	11.33	11.33
$TBT + \frac{1}{2}$ N	3.33	22.67	88.67	15.67	15.67
$CBT + \frac{1}{2}$ N	2.33	16.33	74.67	18.67	18.67
$LBT + \frac{1}{2}$ N	2.0	18.67	107.0	26.0	26.0
Control	1.33	17.0	62.0	9.0	9.0
LSD _(0.05)	1.06	NS	37.1	8.4	8.4

Notes:

¹ Data are means of three replications

Significant differences ($P < 0.05$) between treatments means are calculated using Fisher's Least Significant Difference (LSD)

² Necrosis index is based on a 0-4 scale, where 0 = 0%, 1 = <25%, 2 = 26-50%, 3 = 51-75% and 4 = >76% root necrosis

BT = biomass transfer

NS- not significant

The low necrosis index associated with the *M. pruriens* intercrop alone could be an indication that the legume is a poor host of lesion nematodes. *Mucuna pruriens* has been reported to be moderately resistant to *Pratylenchus* spp. inoculation (Arim et al. 2002; Waceke et al. 2002). In addition, the velvet beans may have produced nematotoxic substances, as reported by Caamal-Maldonado et al. (2001) for *Mucuna deeringiana*. It is difficult to explain without further experimentation the high necrosis index associated with maize intercropped with *M. pruriens* in combination with the inorganic fertiliser. The relatively high P content in *T. diversifolia* and *C. calothyrsus* foliar biomass might have enhanced the build-up of lesion nematode populations and, hence, the high necrosis indices associated with the materials. Superphosphates increase *Pratylenchus* spp. populations and lesion nematode necrosis on maize (Yeates 1976).

Necrosis indices were lowest in maize intercropped with *C. ochroleuca* +_{1/2}N (C+_{1/2}N), or treated with cattle manure (CM) alone and cattle manure plus _{1/2}N (CM+_{1/2}N), and *C. calothyrsus* alone, and were not significantly different from the control (Table 1). Application of N fertiliser to maize intercropped with *C. ochroleuca* did not significantly affect lesion nematode populations. The low necrosis index associated with maize intercropped with *C. ochroleuca* could be an indication that the legume is a poor host of lesion nematode species. Although *Crotalaria agatiflora* and *C. grahamiana* have been reported to be good hosts of *Pratylenchus zaei*, *P. thornei* and *P. pseudopratensis*, other *Crotalaria* species have been reported to be poor hosts of *Pratylenchus* spp. (Desaeger and Rao 1999, 2000, 2001). *Crotalaria juncea*, *C. spectabilis* and *C. ochroleuca*, for example, have been reported to be poor hosts of *P. zaei*, *Meloidogyne arenaria* and *M. incognita*, respectively (Sundararaj and Mehta 1990; Anwar et al. 1994; McSorley et al. 1994; Arim et al. 2002). *Crotalaria juncea* also reduced the population of *P. coffeae*, *Rotylenchulus reniformis*, *Helicotylenchus multicinctus*, *M. incognita*, *Hoplolaimus indicus* and *R. similis* (Naganathan et al. 1988; Araya and Caswell-Chen 1994; Charles 1996).

The low necrosis indices associated with cattle manure with or without the inorganic fertilisers might be due to the toxic effects of ammonia associated with the decomposition of low C:N organic materials. Ammonium ions have an inhibitory effect on hatching of nematode eggs (Waceke et al. 1993).

Although there were significant differences ($P < 0.05$) in plant parasitic nematodes obtained from the soils, those obtained from roots did not differ significantly (Table 1). Maize treated with LBT +_{1/2}N had the highest nematode populations in the soil but did not differ significantly ($P > 0.05$) from those treated with *C. calothyrsus* or *T. diversifolia* alone or in combination with _{1/2}N. The number of nematodes associated with maize treated with leucaena biomass alone was significantly lower than of that associated with maize treated with both leucaena and N fertiliser. The number of nematodes in the soil associated with maize treated with the other biomass plus N fertiliser did not differ significantly from that of those in which only biomass was added. Although leaf extracts of *L. leucocephala* and *Lantana camara* L. and amending of soils with *L. leucocephala* leaves significantly reduced the number of galls and egg masses on *M. javanica* and *M. incognita* populations in tomatoes and increased plant growth (Walia et al. 1994), it appears that biomass from the species used in this study increased plant parasitic nematode populations in the soils. In addition *L. leucocephala* has been reported to be a host of *Pratylenchus goodeyi* (Walia et al. 1994). This justifies the need to screen other *Leucaena* spp. and their biomass for effects on plant parasitic nematode populations. Biomass in-

corporation in addition to inorganic fertilisers might have enhanced the build-up of plant parasitic nematode populations in the soil due to increased available soil nutrients. Maize intercropped with *C. ochroleuca* had the lowest nematode populations in the soil (Table 1). The low nematode populations could be due to the suppressive effects of *C. ochroleuca*.

There were significant differences in non-parasitic nematode populations in the soil ($P < 0.05$). Maize treated with *L. trichandra*, *C. calothyrsus* or *T. diversifolia* alone or in combination with $\frac{1}{2}$ N supported some of the highest numbers of non-parasitic nematode populations in the soil (Table 1). This supports findings by Rao and Albrecht (1999) that revealed that bacterial and fungal feeding nematodes increased with incorporation in maize-based cropping systems of biomass of *T. diversifolia*, *L. camara*, *C. calothyrsus*, *S. sesban*, *Croton megalocarpus* L. and *Senna spectabilis*. Maize treated with *C. ochroleuca* or cattle manure with or without $\frac{1}{2}$ N supported some of the lowest non-parasitic nematode populations in the soil (Table 1). The high ammonia content produced by manure during the decomposition process might have had detrimental effects on activities of both parasitic and non-parasitic nematodes (Waceke et al. 1993, 2001, 2002).

There were significant differences ($P < 0.05$) among treatments in the number of *Pratylenchus* spp. and *Paratylenchus* spp. extracted from maize rhizosphere (Table 2). There were, however, no significant differences in the unidentified parasitic nematodes and other plant parasitic nematode genera obtained from maize rhizosphere, namely *Tylenchus* spp., *Helicotylenchus* spp. and *Trichodorus* spp. (Table 2).

Maize treated with *T. diversifolia* or CM alone or with LBT or TBT + $\frac{1}{2}$ N supported some of the highest *Pratylenchus* spp. and *Paratylenchus* spp. populations in their rhizosphere.

Table 2: Mean¹ number of nematode genera extracted from the soil

Treatment	Nematode genera					
	Pr	Pa	Ty	He	Tr	U
Mucuna (M)	4.67	11.33	10.67	8.0	10.67	2.0
Crotalaria (C)	2.67	11.67	7.67	4.67	6.67	1.0
Nitrogen (N)	5.67	7.33	7.33	8.67	7.0	-
M+ $\frac{1}{2}$ N	4.0	14.33	14.67	8.0	12.33	4.33
C+ $\frac{1}{2}$ N	4.33	10.0	20.0	10.0	8.0	-
Cattle manure (CM)	13.67	20.33	30.67	5.33	11.0	1.33
CM+ $\frac{1}{2}$ N	4.67	6.0	18.0	10.0	10.0	4.0
Tithonia BT (TBT)	12.0	29.0	21.0	15.33	16.67	7.0
Calliandra BT (CBT)	4.67	18.3	21.33	10.33	11.0	1.0
Leucaena BT (LBT)	2.67	12	15.0	8.0	10.67	4.5
TBT + $\frac{1}{2}$ N	13.0	28.0	17.67	12.0	19.67	8.0
CBT + $\frac{1}{2}$ N	10.0	19.67	14.67	14.67	19.33	9.0
LBT+ $\frac{1}{2}$ N	16.3	27.33	29.0	13.0	22.0	9.0
Control	3.0	7.67	20.0	19.0	11.33	-
LSD	5.27	12.65	NS	NS	NS	NS

¹Data are means of three replications

Differences between treatment means were determined using Fisher's Least Significant Difference (LSD) at ($P < 0.05$) Pr = *Pratylenchus* spp., Pa = *Paratylenchus* spp., Ty = *Tylenchus* spp., He = *Helicotylenchus*, Tr = *Trichodorus* spp., U = uncharacterised, BT = biomass transfer, NS = not significant

It appears that *T. diversifolia*, *L. trichandra* and cattle manure have selective effects on plant parasitic nematode populations. Maize intercropped with *C. ochroleuca* and *M. pruriens* alone or in combination with $\frac{1}{2}$ N, or treated with CM+ $\frac{1}{2}$ N, *C. calothyrsus*, *L. trichandra* or N, on the other hand, supported some of the lowest *Pratylenchus* spp. and *Paratylenchus* spp. populations in their rhizosphere (Table 2). Although *Crotalaria agatiflora* and *C. grahamiana* have been reported to be hosts to *Pratylenchus* spp., it appears that *M. pruriens* and *C. ochroleuca* are poor hosts of the nematodes. This confirms reports by Arim et al. (2002) that *M. pruriens* and *C. ochroleuca* are poor hosts of *Pratylenchus* spp. Cattle manure, *Calliandra* and *Leucaena* biomass might have supported low *Pratylenchus* spp. and *Paratylenchus* spp. due to the detrimental effects of decomposition products, increase in microbial populations and mineralisation, which enhanced plant growth and improved plant resistance to nematodes (Waceke et al. 1993). Nitrogen and phosphorus might have supported low *Pratylenchus* spp. and *Paratylenchus* spp. through improved host resistance due to improved nutrient supplies (Waceke et al. 1993).

There were no significant differences ($P < 0.05$) among treatments in the number of nematode genera obtained from the roots (Table 3).

Five plant parasitic nematode genera were obtained from maize root systems: *Pratylenchus* spp., *Paratylenchus* spp., *Tylenchus* spp., *Helicotylenchus* spp. and *Trichodorus* spp. A few of the plant parasitic nematodes could not be identified (Table 3).

Conclusion

Intercropping maize with *C. ochroleuca* and *M. pruriens* reduced plant parasitic nematodes and lesion nematode-associated necrosis indices on maize. The mucuna intercrop was more effective in suppressing nematode disease severity and populations than when

Table 3: Mean¹ number of nematode genera extracted from maize roots

Treatment	Nematode genera					
	Pr	Pa	Ty	He	Tr	U
Mucuna (M)	2.67	2.67	1.67	6.0	3.0	3.0
Crotalaria (C)	4.0	3.33	2.0	2.0	2.67	5.0
Control	3.67	1.67	1.0	3.0	2.67	-
M+ $\frac{1}{2}$ N	6.33	2.0	2.67	3.67	5.0	2.5
C+ $\frac{1}{2}$ N	5.0	2.67	2.67	3.0	3.00	-
Cattle manure (CM)	5.0	3.67	1.67	3.67	4.33	1.5
CM+ $\frac{1}{2}$ N	1.3	3.0	1.33	4.33	3.0	3.0
Titihonia BT (TBT)	5.67	2.33	1.67	5.0	3.0	8.0
Calliandra BT (CBT)	2.67	2.67	1.0	3.33	3.33	2.0
Leucaena BT (LBT)	5.0	4.0	3.33	2.67	5.0	1.0
TBT+ $\frac{1}{2}$ N	4.33	3.33	2.67	3.33	5.67	5.0
CBT+ $\frac{1}{2}$ N	2.33	2.67	2.67	2.67	4.33	2.5
LBT+ $\frac{1}{2}$ N	2.33	3.0	3.33	5.67	4.33	3.0
Control	3.67	1.67	1.0	3.0	2.67	-
	NS	NS	NS	NS	NS	NS

¹Data are means of three replications

Pr = *Pratylenchus* spp., Pa = *Paratylenchus* spp., Ty = *Tylenchus* spp., He = *Helicotylenchus*, Tr = *Trichodorus* spp., U = uncharacterised, BT = biomass transfer, NS = not significant

combined with inorganic fertiliser. This was unlike *Crotalaria* intercrop, whose efficacy was not affected by addition of inorganic fertiliser.

Efficacy of organic manure against plant parasitic nematodes varies with the nature and type of the materials used. Incorporation of leucaena and tithonia revealed lower efficacy relative to calliandra and cattle manure. In most cases addition of inorganic N fertilisers did not affect efficacy of the organic materials.

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