Full Length Research Paper

Infectivity and reproduction of *Meloidogyne incognita* (Kofoid and White) Chitwood on African yam bean, *Sphenostylis stenocarpa* (Hochst *Ex*. A. Rich) Harms accessions as influenced by botanical soil amendments

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A screen house experiment was conducted to study the effects of amendment of soil with leaf powders of some plants at different levels (5, 10, 15, 20 and 25 t/ha) on the infectivity and reproduction of *Meloidogyne incognita* (Kofoid and White) Chitwood on African yam bean, *Sphenostylis stenocarpa* (Hochst *Ex.* A. Rich) Harms. The plants were neem, drumstick, lemon grass, African peach and rattle weed. Results showed that botanical soil amendments significantly ($P \le 0.05$) improved growth of plants and reduced reproduction of *M. incognita* on African yam bean in varying degrees. The effects of botanicals on growth of plants and nematode reproduction increased with increase in concentrations/ amendment levels, and were highest at 25 t/ha. *Cymbopogon citratus* produced the highest manurial properties as it supported greater vegetative growth of plants while *Moringa oleifera* was most effective in the reduction of reproduction and development of *M. incognita* in the roots. *M. oleifera* produced the least gall index and egg mass index of 1.0, though *Crotolaria retusa* also recorded egg mass index of 1.0. Results from this study are indicative of the fact that local farmers could apply these botanicals as fertilizers during cultivation to counteract the effect of root-knot nematode, and also to improve growth of crop plants but field trials are necessary before final recommendation.

Key words: Infectivity, reproduction, African yam bean, Meloidogyne incognita, amendment.

INTRODUCTION

African yam bean, *Sphenostylis stenocarpa* (Hochst *Ex* A. Rich) harms is an exceptionally nutritious pulse (Rachie, 1973) which is a member of the family Fabaceae, sub-family Papilionoidae, tribe Phaseoleae and sub-tribe Phaseolinae (Okigbo, 1973; Allen and Allen, 1981). The seeds and tubers provide good quality food for both humans and livestock. The reported protein and amino acid values for both seeds and tubers are generally higher than the contents of most leguminous and tuberous crops (Okigbo, 1973; Nwokolo, 1996;

Amoatey et al., 2000; Uguru and Madukaife, 2001). In fact, nutritionally, *S. stenocarpa* has the potential of substituting animal proteins in the diets of many people who cannot afford animal proteins. Unfortunately, one of the major setbacks to increased African yam bean production is the damage caused by root-knot nematodes, *Meloidogyne* species. Onyeke and Akueshi (2012) reported percentage yield loss of more than 70% in *S. stenocarpa* due to *Meloidogyne incognita* infection. The application of synthetic nematicides are usually recommended and even considered to be the most effective method for limiting the damage of plant parasitic nematodes in crops (Adesiyan, 1992). However, these chemicals are costly, highly toxic and present some environmental problems (Zureen and Khan, 1984;

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Adesiyan et al., 1990).

Indiscriminate and consistent use of synthetic nematicides can be toxic to beneficial soil flora and fauna, cause emergence of resistant plant parasitic nematodes and ultimately environmental degradation (Akhtar, 1991). In fact, synthetic nematicides have been reported to contaminate underground water thereby posing serious hazards to man and animals (Alam and Jairajpuri, 1990). Alternatively, research has focused on natural plant products with nematicidal or nematostatic properties because they are cheaper, readily available, biodegradable and environmentally safer (Siddiqui and Alam, 1989; Chitwood, 2002; Javed et al., 2008). Amendments of soil with organic plant material have been found to effectively suppress reproduction and the damaging impact of plant parasitic nematodes on crops (Alam et al., 1980; Muller and Gooch, 1982; Neog and Bora, 1999). Therefore, the present research was designed to study the effect of amendment of soil with some botanicals on the infectivity and reproduction of *M. incognita* on African yam bean.

MATERIALS AND METHODS

Source of botanicals for soil amendment

Leaves of Neem (*Azadirachta indica* A. Juss), drumstick (*Moringa oleifera* Lam.), lemon grass (*Cymbopogon citratus* Stapt), African peach (*Nauclea latifolia* J.E. Smith) and rattle weed (*Crotolaria retusa* Linn.) used for the experiment were collected from the Department of Plant Science and Biotechnology Garden, University of Nigeria, Nsukka and environs. The leaves (botanicals) were airdried indoors until completely dried and then ground separately into fine powders with Thomas Wiley Laboratory Mill Model 4, Arthur Thomas Company.

Maintenance and extraction of nematode eggs used for inoculation

Nsukka population of *M. incognita* maintained for up to eight weeks on susceptible African yam bean accession (TSS 112) served as source of inoculum. The galled roots of the African yam bean were carefully uprooted, washed free of soil and cut into 1 to 2 cm segments. The eggs were extracted from the galls using the NaOCL-extraction method (Barker, 1985). The extracted eggs were washed into a graduated beaker, and the volume made up to 1,000 ml with sterile distilled water. The egg suspension was calibrated in such a way that 1 ml contained 200 eggs; 20 ml of the egg suspension contained 4,000 eggs of *M. incognita*.

Screen house experiment

The experiment was conducted at the Department of Plant Science and Biotechnology Screen House, University of Nigeria, Nsukka (latitude 06° 86 39.6 N, longitude 007° 41 20.4 E and altitude 433 m above sea level). Top garden soil collected from fallow land was sterilized by heating in a large drum to a temperature of 100°C for 3 h and later air-dried for four weeks before starting the study, to avoid any possible side effects of heating (Anderson and Ingram, 1989). The physical and chemical properties of the soil were determined according to standard methods (IITA, 1989). The textural class of the soil was sandy loam (55% coarse sand, 32% fine sand, 9% silt and 4% clay) pH (in H_2O), 5.7; pH (in KCl), 4.6; OM, 1.57%; BS, 44.66%; N, 0.042%; cation exchange capacity (CEC), 8.8%; Na, 0.21; K, 0.12; Ca, 1.4; Mg, 2.2; exchangeable acidity, 0.4 and P (in ppm), 13.06.

The sterilized and air-dried soil was mixed thoroughly with each of the different botanicals at different concentration (5, 10, 15, 20 and 25 t/ha) corresponding to 6.25, 12.50, 18.75, 25.0 and 31.25 g per 2.5 kg of soil, respectively. The amended soils were poured into polyethylene planting bags of 23 cm diameter and 20 cm depth. Soils without amendments (0 t/ha) served as controls. The African yam bean seeds were planted two per bag but later reduced to one, before nematode inoculation. Two weeks after germination, the polyethylene bags were inoculated with 20 ml (4000 eggs) of the egg suspension by pouring into a shallow trench created around the root tips of each of the test plants (Hussey and Boerma, 1981), and covered immediately with top soil (Goswami and Chenulu, 1974). Plants that were not inoculated served as controls. The planting bags were arranged in a completely randomized design (CRD) with four replications. Each bag was watered (250 ml) on a day interval basis. Eight weeks after inoculation with nematode eggs, the following data were collected: number of leaflets per plant, shoot fresh weight, number of galls per root system, gall index per root system, number of egg mass per root system and egg mass index per root system. Root gall/egg mass index was determined using a rating scale according to Taylor and Sasser (1978); 0 = 0, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100 and 5 = more than 100 galls/egg masses per root system. The number of egg mass was determined using Phloxine B (MP Biomedicals, LLC, USA) to stain the roots (0.15 g/L) for 15 min and examined under a stereoscopic microscope (Daykin and Hussey, 1985).

Statistical analysis

Data collected were subjected to statistical analysis using Genstat for windows, version 3.2. Treatment means were separated using least significant difference (LSD) at 5% level of probability. Regression analysis was performed to determine the relationship between some of the parameters sampled and their responses to increasing amendment levels using Microsoft excel chart wizard 2007.

RESULTS

Result on the effects of botanical soil amendments on the number of leaflets of African yam bean plants inoculated with *M. incognita* is shown in Table 1. It was observed that the botanical amendments significantly ($P \le 0.05$) supported increases in the number of leaflets of amended plants. However, plants amended with leaf powders but without *M. incognita* infection produced the highest number of leaflets when compared with plants amended with leaf powders but with M. incognita infection. This result showed that significant differences ($P \le 0.05$) existed among different botanical amendments and concentrations. It was also observed that the number of leaflets in both infected and uninfected plants increased with increase in concentration of botanical leaf amendments. The highest number of leaflets was recorded in plants amended with C. citratus leaf powder, while the least number of leaflets was produced in plants amended with M. oleifera leaf powder in both infected and uninfected experiments.

Botanical amendment	Health	Botanical amendment level (t/ha)								
	condition	0	5	10	15	20	25	Mean		
A in alia a	Infected	25.2	40.5	48.5	54.5	68.2	79.2	52.7		
A. indica	Control	35.5	43.7	52.2	58.2	69.7	80.7	56.7		
Caltratus	Infected	26.7	54.7	67.2	77.0	98.5	121.0	74.2		
C. citratus	Control	40.7	55.7	69.5	79.5	99.5	122.2	77.8		
C return	Infected	25.2	34.0	45.7	63.7	75.2	83.7	54.6		
C. retusa	Control	33.7	36.0	48.0	67.0	78.5	85.5	58.1		
M alaifara	Infected	24.5	32.2	42.0	50.5	60.5	69.5	46.5		
M. oleifera	Control	29.5	35.2	45.5	52.5	61.7	73.2	49.6		
	Infected	22.5	30.5	47.5	63.7	88.5	101.2	59.0		
N. latifolia	Control	30.0	32.5	51.5	64.6	92.5	103.5	62.4		
Mean		29.3	39.5	51.7	63.1	79.3	92.0			

Table 1. Effects of botanical soil amendments on the number of leaflets per stand of *S. stenocarpa* 8 weeks after inoculation with 4,000 eggs of *M. incognita*.

LSD (0.05) for comparing different botanical amendment means = 1.135, LSD (0.05) for comparing different botanical amendment level means = 0.879, LSD (0.05) for comparing different botanical amendment mean × different botanical amendment level means interaction = 2.779.

Result on the effects of botanical soil amendments on the shoot fresh weight of African yam bean plants inoculated with M. incognita is shown in Table 2. It showed that botanical amendments significantly ($P \le 0.05$) increased the shoot fresh weight of amended plants. The highest shoot fresh weight was recorded in plants amended with botanical leaf powders but uninfected as against plants amended with botanical leaf powders but infected. Results also showed significant differences ($P \le 0.05$) among the different botanical amendments and concentrations. Also, shoot fresh weight per amended plant in both infected and uninfected plants increased with increase in concentration of leaf powders and was highest at 25 t/ha. The increase in the shoot fresh weight per plant was significantly ($P \le 0.05$) and positively associated with increase in concentrations in all the botanicals (Figure 1): *A. indica*, $R^2 = 0.954$; *C. citratus*, $R^2 = 0.875$; *C. retusa*, $R^2 = 0.935$; *M. oleifera*, $R^2 = 0.962$; *N. latifolia*, $R^2 = 0.880$. It was also observed that the highest shoot fresh weight was recorded in plants amended with C. citratus leaf powder while the least shoot fresh weight was produced in plants amended with M. oleifera leaf powder in both infected and uninfected experiments.

Results on the effects of botanical soil amendments on the number of galls per root system of plant showed that the number of galls per root system reduced significantly ($P \le 0.05$) in plants amended with botanicals as against plants without botanical amendment (Table 3). Infected plants amended with *M. oleifera* leaf powder produced the least number of galls per root system, while plants amended with *A. indica* leaf powder recorded the highest number of galls per root system. Also, there were significant differences ($P \le 0.05$) among botanical amendment types and concentrations. The number of galls induced by *M. incognita* decreased in linear response to increasing botanical amendment levels. The reduction in number of galls per root system was significantly ($P \le 0.05$) and positively associated with increase in concentrations of the botanicals (Figure 2): *A. indica*, $R^2 = 0.879$; *C. citratus*, $R^2 = 0.918$; *C. retusa*, $R^2 = 0.897$; *M. oleifera*, $R^2 = 0.830$; *N. latifolia*, $R^2 = 0.905$.

Results on the effects of botanical soil amendments on the gall index per root system of plant showed that gall index per root system significantly ($P \le 0.05$) reduced in infected plants amended with botanicals as against infected plant controls without amendment (Table 4). Gall index reduction as produced by botanical soil amendment increased as their concentration increased. Infected plants amended with *M. oleifera* leaf powder recorded the least gall index per root system while plants amended with A. indica and C. citratus produced the highest gall index per root system. Also, significant (P \leq 0.05) differences were recorded among botanical amendment types and concentrations. Botanical soil amendments significantly ($P \le 0.05$) reduced the number of egg masses per root system in plants as against plants without amendments (Table 5). The number of egg masses per root system of plants continued to reduce as the concentration of amendments increased and was lowest at 25 t/ha. The reduction in number of egg masses per root system was significantly ($P \le 0.05$) and positively associated with increase in concentrations of the

Botanical amendment	Health	Botanical amendment level (t/ha)							
	condition	0	5	10	15	20	25	Mean	
	Infected	5.8	8.5	9.5	11.4	13.6	14.3	10.7	
A. indica	Control	8.6	10.7	10.6	13.9	15.2	17.0	12.5	
Caitrotuo	Infected	5.7	11.3	12.3	13.7	14.8	16.5	12.4	
C. citratus	Control	10.8	16.2	18.4	19.8	21.6	23.1	18.3	
C. retusa	Infected	5.9	9.1	10.4	12.0	14.1	14.9	11.1	
	Control	8.9	11.5	12.6	14.5	15.7	18.5	13.6	
M. oleifera	Infected	5.7	8.3	10.1	11.8	12.9	14.0	10.5	
	Control	7.5	9.2	11.0	12.3	14.5	16.4	11.6	
N. latifolia	Infected	5.8	10.7	11.3	12.4	13.8	15.4	11.5	
	Control	10.2	13.7	14.8	16.1	17.7	20.2	15.4	
Mean		7.5	10.9	12.0	13.8	15.4	17.0		

Table 2. Effects of botanical soil amendments on the shoot fresh weight per stand of *S. stenocarpa* 8 weeks after inoculation with 4,000 eggs of *M. incognita*.

LSD (0.05) for comparing different botanical amendment means = 0.4672, LSD (0.05) for comparing different botanical amendment level means = 0.3619, LSD (0.05) for comparing different botanical amendment means × different botanical amendment level mean interaction = 1.1444.

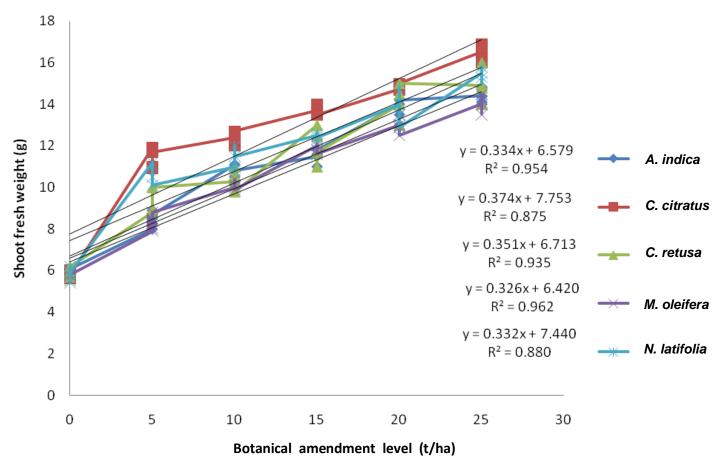


Figure 1. Effect of increasing botanical amendment levels on the shoot fresh weight per stand of *S. stenocarpa* 8 weeks after inoculation with 4,000 eggs of *M. incognita*.

Botanical	Botanical amendment level (t/ha)									
amendment	0	5	10	15	20	25	Mean			
A. indica	38.7	26.5	19.5	13.7	7.7	7.0	18.8			
C. citratus	36.0	24.7	18.5	13.2	7.5	5.7	17.6			
C. retusa	36.2	21.7	16.0	10.2	4.0	2.7	15.1			
M. oleifera	38.7	18.5	13.0	7.0	1.5	1.0	13.3			
N. latifolia	36.2	23.5	17.5	11.5	5.7	5.0	16.5			
Mean	37.2	23.0	16.9	11.1	5.3	4.3				

Table 3. Effects of botanical soil amendments on the number of galls per root system of *S. stenocarpa* 8 weeks after inoculation with 4,000 eggs of *M. incognita*.

LSD (0.05) for comparing different botanical amendment means = 1.469, LSD (0.05) for comparing different botanical amendment level means = 1.610, LSD (0.05) for comparing different botanical amendment mean × different botanical amendment level mean interaction = 3.599.

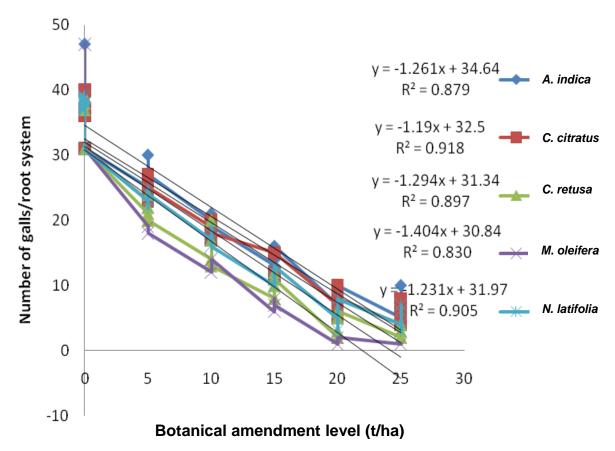


Figure 2. Effect of increasing botanical amendment levels on the number of galls per root system of *S. stenocarpa* 8 weeks after inoculation with 4.000 eggs of *M. incognita*.

botanicals (Figure 3): *A. indica*, $R^2 = 0.911$; *C. citratus*, $R^2 = 0.937$; *C. retusa*, $R^2 = 0.886$; *M. oleifera*, $R^2 = 0.891$; *N. latifolia*, $R^2 = 0.930$. Also, plants amended with *M. oleifera* leaf powder produced the least number of egg masses per root system while plants with *A. indica* leaf powder recorded the highest number of egg masses per root system. Significant ($P \le 0.05$) differences existed among botanical amendment types and concentrations.

Results on the effects of botanical soil amendments on

the egg mass index per root system showed that egg mass index per root system significantly ($P \le 0.05$) reduced in plants amended with botanicals as against plants without amendment (Table 6). The egg mass index was highest at 0 t/ha while the least was recorded at 25 t/ha. Plants amended with *M. oleifera* leaf powder recorded the least number of egg mass index per root system while the highest was produced by plants amended with *A. I ndica* leaf powder. There were also

Botanical			Botanica	l amendment le	evel (t/ha)								
amendment	0	5	10	15	20	25	Mean						
A. indica	4.0	3.0	3.0	3.0	2.0	2.0	2.8						
C. citratus	4.0	3.0	3.0	3.0	2.0	2.0	2.8						
C. retusa	4.0	3.0	3.0	2.5	1.7	1.5	2.6						
M. oleifera	4.0	3.0	3.0	2.0	1.0	1.0	2.3						
N. latifolia	4.0	3.0	3.0	2.7	2.0	2.0	2.7						
Mean	4.0	3.0	3.0	2.6	1.7	1.7							

Table 4. Effects of botanical soil amendments on the galls indices root system of S. stenocarpa 8 weeks after inoculation with 4,000 eggs of M. incognita.

LSD (0.05) for comparing different botanical amendment means = 0.1135, LSD (0.05) for comparing different botanical amendment level means = 0.1244, LSD (0.05) for comparing different botanical amendment mean × different botanical amendment level mean interaction = 0.2781

Table 5. Effects of botanical soil amendments on the number of egg masses per root system of *S. stenocarpa* 8 weeks after inoculation with 4,000 eggs of *M. incognita*.

Botanical			Botanica	l amendment le	t level (t/ha)							
amendment	0	5	10	15	20	25	Mean					
A indica	34.2	23.5	17.2	11.5	5.7	4.5	16.1					
C. citratus	35.2	23.5	16.7	10.5	5.2	2.7	15.6					
C. retusa	33.7	18.3	13.0	7.0	1.2	1.1	12.4					
M. oleifera	33.5	18.0	12.6	6.9	1.0	0.7	12.1					
N. latifolia	34.7	22.1	15.6	10.0	3.0	2.0	14.6					
Mean	34.3	21.0	15.0	9.1	3.2	2.2						

LSD (0.05) for comparing different botanical amendment means = 1.022, LSD (0.05) for comparing different botanical amendment level means = 1.120, LSD (0.05) for comparing different botanical amendment mean x different botanical amendment level means interaction = 2.504.

significant ($P \le 0.05$) differences among botanical amendment types and concentrations.

DISCUSSION

Results have shown that all the botanicals used for soil amendment posses varying degrees of manurial and antinematode/nematicidal properties. This is evident in the levels of improvement in shoot growth and reduction in number of root galls and nematode egg masses in infected and amended soils. Pakeerathan et al. (2009) reported similar reduction in *M. incognita* infection and improved growth of tomato using different green leaf manures: Gliricidia maculata, Thespesia populnea, Calotropis gigantia, Azadirachta indica and Glycosmis pentaphylla, at 25 t/ha under field conditions. Also, Muazu and Umar (2012) recorded effective control of M. incognita on cowpea by applying powder forms of the leaves of Manihot esculentum, Vitellaria paradoxa, Parkia biglobosa and Eucalyptus camaldulensis at 13.2 kg/ha in a screen house experiment. This could be attributed to past reports of nematicidal/nematostatic properties both in vitro and in vivo of some plant materials (Neog and Bora, 1999; Oka et al., 2001; Olabiyi et al., 2006; Olabiyi, 2008). Olabiyi and Oyedunmade (2003) reported that marigold (*Tagetes erecta*), basil (*Ocimum gratissimum*), nitta (*Hyptis suaveolens*) and rattle weed (*C. retusa*) had nematicidal properties, prevented egg-hatching and killed juveniles of root knot nematodes. Neem (*A. indica*) has been reported to have antinematode activities on egghatching, juvenile mortality, development and infectivity of *Meloidogyne javanica* (Javed et al., 2008). Hussain and Massood (1975) reported that some alkaloids such as nimbine, nimbinine, nimbidine and thionemone which have adverse effect on nematode biology were present in neem leaves. Also, healthy plant tissues have been reported to contain compounds with nematicidal or nemostatic principles, which include, alkaloids, phenols, thienyls sesquiterpenes, diterpenes and polyacetylenes (Gommers, 1981; Gommers and Barker, 1988).

Apart from the alkaloids and other phytochemicals contained in the botanicals used for amendments, the decomposition processes have the ability to release aldehydes and different gasses including ammonia, which are deleterious to many plant parasitic nematodes (Alam et al., 1979; Badra et al., 1979). Mian and Rodriguez-Kabana (1982) attributed the reduction in root-knot development in soils amended with organic materials due to the action of toxic compounds released during decomposition. *M. olefera* extracts have been reported to have antimicrobial activity against bacteria

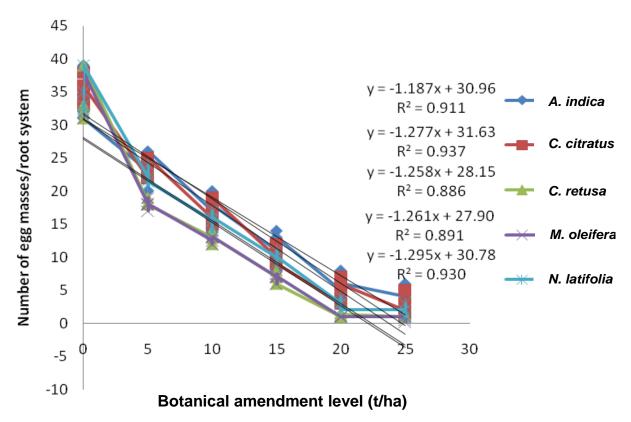


Figure 3. Effect of increasing botanical amendment levels on the number of egg masses per root system of *S. stenocarpa* 8 weeks after inoculation with 4,000 eggs of *M. incognita*.

Table 6. Effects of botanical soil amendments on the egg mass index per stand of S. stenocarpa 8 weeks after inoculation with 4,000 eggs of
M. incognita.

Botanical	Botanical amendment level (t/ha)								
amendment	0	5	10	15	20	25	Mean		
A indica	4.0	3.2	3.0	3.0	2.0	2.0	2.8		
C. citratus	4.0	3.0	3.0	2.5	2.0	1.5	2.6		
C. retusa	4.0	3.0	2.7	2.0	1.0	1.0	2.2		
M. oleifera	4.0	2.7	2.5	1.7	1.0	1.0	2.1		
N. latifolia	4.0	3.0	3.0	2.2	1.7	1.2	2.5		
Mean	4.0	3.0	2.8	2.3	1.5	1.3			

LSD (0.05) for comparing different botanical amendment means = 0.1734, LSD (0.05) for comparing different botanical amendment level means = 0.1900, LSD (0.05) for comparing different botanical amendment mean × different botanical amendment level means interaction = 0.4248.

(Rahman et al., 2009) and fungi (Jabeen et al., 2008) and in this experiment, antinematode, which is suggestive of the presence of broad spectrum antibiotic compounds in the plant. In addition to the nematicidal properties, organic soil amendment contributes to the maintenance of soil structure, is less destructive to the soil ecology, favours growth of beneficial soil microbes, facilitates rooting by crops, enhances the soil water holding capacity and ultimately leads to even distribution of nutrients in the soil profile (Arden-Clarke and Hodges, 1988). Also, the botanicals may have suppressed nematode reproduction by influencing the secretion of certain root exudates that attracted antagonistic microbes in the likes of *Pseudomonas* sp. and *Trichoderma* sp. (Kimenju et al., 2004) or changed the soil pH to be unsuitable for nematode development (Wang and Chao, 1995).

The lower mean number of leaflets and shoot fresh weight recorded in infected plants without amendments is attributed to the effect of *M. incognita* infection on plants. In fact, root-knot nematode infections on plants cause galls to form on the roots and galls are known to serve as

nutrient sinks in infected plants since nutrients are redirected from shoots to roots (Melakeberhan et al., 1990). Again, the higher number of galls, egg masses and their respective indices in the control experiments could be attributed to the absence of botanical soil amendments which allowed free penetration and reproduction of *M. incognita* in both the rhizosphere and the African yam bean roots. Results show that C. citratus had the highest manurial properties and perhaps the highest nitrogen content, as it supported greater vegetative growth of plants while M. oleifera was most efficacious in the control of nematode development in the roots. This suggests that for improved vegetative growth of plants and reduced root knot nematode activity, a mixture of both botanicals should be applied as amendment in a well worked out ratio. In some other experiments, combined use of organic matters with commercial nematicides demonstrated effective reduction in plant parasitic nematodes and improved plant growth than their separate application (Mishra and Gaur, 1989; Darekar and Mhase, 1990).

Although, *M. oleifera* leaf powder has proved to be the most effective in arresting the infectivity and reproduction of *M. incognita*, the rest of the botanicals are still good plant parasitic nematode suppressants. The effectiveness of the amendments were such that, the higher the rate of application, the lower the number of galls and egg masses, and hence the better the control without being phytotoxic. This is an indication of their compatibility and safety for use in the African yam bean farms. It could be suggested that local farmers should use these botanicals freely as manure during cultivation, to reduce the attack from root knot nematodes, improve growth, and ultimately check yield losses due to nematodes but field trials are necessary before final recommendation.

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