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Evaluation of Organic Soil Amendments on the Growth and Yield of M*eloidogyne incognita* on Cowpea Plants.

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Abstract

Pot experiment was conducted in screen house to evaluate the efficacy of the leaf powder of neem (*Azadirachta indica*), swallow-wort (*Calotropis procera*), kassod tree (*Cassia siamea*), eucalyptus (*Eucalyptus gigantea*) and locust bean tree (*Parkia biglobosa*) in the control of *Meloidogyne incognita* on cowpea and their potency in growth and grain yield improvement. Four different doses viz. 25, 50, 75 and 100g of each of the leaf powder was separately mixed with 4kg of soil in a 25cm diameter plastic pot. Non-amended pots served as control. Three cowpea seeds were sown in each pot but the seedlings were thinned to one plant per pot six days after emergence. Each seedling was inoculated with about 3000 freshly hatched juveniles of *Meloidogyne incognita* two weeks after sowing. The experiment was laid out in completely randomised design with five replications and data obtained was analysed using ANOVA and means were significant were separated using the Duncan's multiple test. The result of the study showed that all the treatments significantly ($p<0.001$) reduced root galling, nematode multiplication and improved plant growth and grain yield. Although all treatments were effective in reducing root galling and nematode population, application of Azadirachta indica leaf powder gave the highest reduction in nematode population, followed by *Calotropis procera, Cassia siamea, Eucalyptus gigantea* and *Parkia biglobosa*.

Key words: Cowpea*, Meloidogyne incognita*, *Azadirachta indica, Calotropis procera, Cassia siamea, Eucalyptus gigantea* and *Parkia biglobosa*

1 Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a dicotyledonous plant belonging to the family Fabaceae (Cronquist, It is of major importance to the livelihood of millions of people in the tropics (Quin, 1997). In Nigeria and other African countries, the most grown and eaten legume is cowpea and it is mainly cultivated in the northern states of Nigeria. It is an essential component of sustainable agriculture in marginal lands and drier regions of the tropics, rainfall is scanty and soils are sandy with little organic matter (Watt *et al*., 1985). Emechebe (1985) reported that major problem of cowpea is pests and diseases which do not only cause low yield but also discourage most farmers from cultivating the crop. One of the major limiting factors to the profitable cowpea production is the damage caused by root knot nematodes (*Meloidogyne* spp.). Root-knot nematodes infect roots of cowpea plants resulting in considerable losses. The yield loss is associated with conspicuous galls that disrupt water and nutrient uptake

1981). Rose *et al*. (1989) reported yield losses of more than 90% in high population. Similarly, Adegbite (2011a) reporte\wpea grain yield loss of 39% due to infestation by *Meloidgyne incognita.* The use of synthetic nematicides is considered the most effective practical means of combating the menace of plant-parasitic nematodes in cowpea (Adesiyan, 1992). However, chemical control of root-knot nematodes leads to environmental hazards because of the high toxicity and persistence of the nematicides (Adesiyan et al., 1990). As an alternative, organic soil amendment been found to be cheaper, less harmful to man and effective in the control of plant-parasitic nematodes (Olabiyi *et* 2007). In view of this, the Current investigation was undertaken to evaluate the nematicidal activity of five plant species: *Azadirachta indica, Calotropis procera, Cassia siamea, Eucalyptus gigantean* and *Parkia biglobosa* in the control of *Meloidogyne* spp. in cowpea.

2. Materials and Methods

2.1 Soil preparation and sterilization

Top soil (sandy loam) was used for the experiment. The soil was collected from the Biological garden of Modibbo Adama University of Technology, Yola, located at latitude 9º 14´N and longitude 12º 27´E. The soil was steam-sterilized by heating with lighted firewood in a large aluminium pot to a temperature of 100°C and maintained for one hour.

2.2 Collection and Preparation of Leaves

Leaves of *Azadirachta indica, Cassia siamea, Eucalyptus gigantea, Parkia biglobosa* and *Calotropis procera* were used for the experiment. The leaves were separately collected from different plants at different locations within the premises of Modibbo Adama University of Technology Yola and spread on polythene sheets in an open protected area for one week to dry. The dried leaves were ground separately to fine particles in a mortar and stored in a sealed container for use.

2.3 Amendment Applications Rate

Ground leaves were separately mixed with 4kg of steam-sterilized soil at the rate of 25, 50, 75 and 100 grams and the mixtures were transferred into 25 cm diameter perforated plastic pots.

2.4 Sowing of Seeds

Seeds of cowpea, cv. “Kanannado” were obtained from Monday market Maidugri. The seeds were sown into the pots filled with the mixture of steam-sterilized soil and organic matter, and the control pots which contain only steam-sterilized soil without organic matter. Three seeds were sown per pot at a depth of 2cm, but the seedlings were thinned to one per pot six days after emergence to ensure uniform plant vigour. The pots were watered once a day regularly and the potted soil around the base of the plants was loosened from time to time without disturbing plant roots using hand fork to avoid compacting.

2.5 Collection of Root Knot Nematode Samples

Samples of root knot nematodes (*Meloidogyne* spp.) were collected from tomato plants under irrigation in various farms with the permission of the farmers at Lake Alau in Borno state. Roots of diseased plants showing characteristic symptoms of 15 root knot nematode were carefully uplifted with trowel up to 15 – 20cm depth from the rhizosphere the diseased plants together with approximately 1kg of adhering soil. Samples were placed in polythene bags and brought to laboratory for analysis.

2.6 Identification of Root Knot Nematode Species

Root knot nematode species was identified on the basis of perineal pattern characteristics of mature female as described by Eisenback *et al*.1981.

2.7 Inoculum Culture

Pure culture of root knot nematode, *Meloidogyne incognita* was raised from a single egg mass obtained from a root knot nematode-infected tomato plant. The single egg mass was propagated on tomato plant by inoculating tomato seedlings grown in steam-sterilized soil. Further subcultures were made from the initial culture to increase the nematode population.

2.8 Preparation of Inoculum

Second-stage juvenile nematodes were used as inoculum. Eggs of *Meloidogyne incognita* were collected from a pure culture maintained on tomato roots using sodium hypochlorite technique (Hussey and Barker, 1973). The eggs were placed in a tap water in petri dish and incubated for 24 hours at room temperature for hatching. After hatching, the second-stage juveniles were collected and larval suspension was prepared in tap water.

2.9 Inoculation Procedure

The cowpea plants were inoculated two weeks after planting into the pots. The population of about 3000 juvenile nematodes per plant was used. Four holes about 2cm deep and 1cm wide each were made in the soil around each seedling to expose the roots. The second-stage juvenile nematode suspension was applied into each hole with a syringe and the holes were filled with moist soil. Each treatment was replicated five times and the pots were laid out in a completely randomized experimental design in the screen house. The experiment was terminated sixty days after sowing.

2.10 Data Collection

At harvest, data were collected on shoot height using measuring tape, number of leaves per plant were counted, fresh weight of shoots and grain yield per plant were determined using electronic balance, population of nematode in the soil, population of nematode in the root, number of galls and gall index.

2.11 Estimation of nematode population in the soil

The population of nematodes in the soil was determined using the modified Baermann funnel extraction technique (Barker, 1985).

2.12 Assessment of galling index

The roots were rated for the amount of galls using a rating scheme described by
Ogbuji (1981) as follows:
0 = 0 gall (no infection)
1 = 1 – 3 galls (rare infection)
2 = 4 – 10 galls (light infection)

3 = 11 - 30 galls (moderate infection)
4 = 31 - 100 galls (severe infection)
5 = > 100 galls (very severe infection)

2.13 Estimation of nematode population in the roots

The population of nematode in the roots was determined using maceration method followed by Baermann's funnel technique (Southey, 1970).

3 Results

The results show that all the treatments significantly (P<0.001) suppressed the development of *Meloidogyne incognita* population in the soil as compared to the control. The highest population of 733.34 was recorded in the control treatment, while the population dropped to as low as 320.00 - 106.67 in the soil treated with different doses of *Azadirachta indica* leaf powder, followed by the leaf powder of *Calotropis procera* (360.00 - 133.34), *Cassia siamea* (373.34 - 146. 67), *Eucalyptus gigantean* (400.00 - 173.34) and *Parkia biglobosa* (506.67 - 213.34). The highest population of *M. incognita* in the root (189.34) was recorded in the control plants, while the lowest population (28.00 - 17.34) was recorded in the plants grown in the soil amended with leaf powder of *Azadirachta indca* followed by *Calotropis procera* (37.34 - 22.67), *Cassia siamea* (41.34 - 29.34), *Eucalyptus gigantea* (54.67 - 33.34) and *Parkia biglobosa* (57.34 - 34.67). The reproductive capacity of the nematode was also significantly (P<0.001) affected by the treatments imposed on the plants. The highest rate of reproduction of *Meloidogyne incognita* (1.041) was observed in the control plants, which was significantly different from other treatments. The highest reduction in reproduction rate (0.436 - 0.149) was recorded in plants administered with the leaf powder of *Azadirachta indica* followed by *Calotropis procera* (0.493 - 0.186), *Cassia siamea* (0.506 - 0.206), *Eucalyptus gigantea* (0.552 - 0.243) and *Parkia biglobosa* (0.695 - 0.297). The number of root galls incited by *Meloidogyne incognita* on the roots of cowpea peaked at 107.75 in the control, but ranged from as low as 10.25 in *Azadirachta indica* leaf powder-treated plants to 37.00 in *Parkia biglobosa* leaf powder-treated plants. Plants treated with leaf powder of *Azadirachta indica* had the highest reduction efficacy, followed by *Calotropis procera* leaf powder, *Cassia siamea* leaf powder, *Eucalyptus gigantean,* and *Parkia biglobosa* leaf powder.

Fresh shoot weight, shoot height and number of leaves per plant were significantly (P<0.001) higher in pots treated with *Calotropis procera, Azadirachta indica, Parkia biglobosa, Eucalyptus gigantea* and *Cassia siamea* than in the control. The lowest shoot weight (10.18g) was recorded in the control plants, whereas the highest fresh shoot weight (37.45 - 53.28g) was recorded in plants treated with different doses of the leaf powder of *Azadirachta indica*. Plants treated with leaf powder of *Calotropis procera* recorded (29.88 – 44.51g), while plants treated with leaf powder of *Cassia siamea, Eucalyptus gigantea* and *Parkia biglobosa* recorded (24.22 – 37.93g), (18.26 – 35.21g) and (17.06 – 30.90g). The lowest shoot height (10.50cm) was noted in the control plants, while the maximum shoot height (45.90-93.20cm) was recorded in plants treated with leaf powder of *Azadirachta indica* followed by *Calotropis procera* (39.40 - 64.20cm), *Cassia siamea* (37.54 - 61.34cm), *Eucalyptus gigantea* (33.90 - 58.17cm) and *Parkia biglobosa* (22.54 - 48.37cm) The highest number of leaves per plant (20.67 - 30.67) was recorded in the plants treated with leaf powder of *C. procera* followed by *Azadirachta indica* (17.00 - 30.00), *Cassi siamea* (16.67 – 26.34) *Eucalyptus gigantea* (14.34 - 20.67) and *Parkia biglobosa* (12.34 – 20.34). The lowest number of leaves per plant was recorded the control plants.

Number of pods per plant, number of seeds per plant and grain yield per plant were significantly ($P<0.001$) higher in the plants treated with organic materials than in the control plants. The lowest grain yield (2.26g) was noted in the control plants while the highest grain yield (8.93 - 18.24g) was recorded in plants treated with leaf powder of *Calotropis procera*. Plants treated with leaf powder of *Azadirachta indica* recorded (8.17 - 16.90g), while plants treated with the leaf powder of *Cassia siamea, Eucalyptus gigantea* and *Parkia biglobosa* recorded (7.29 - 12.75g), (4.27 - 11.78g) and (3.51 - 12.13g) respectively. The maximum number of pods per plant (6.50 – 10.75) was observed in plants treated with the leaf powder of *C. procera* followed by *Azadirachta indica* 6.00 – 10.25, *Cassia siamea* 4.75 – 10.00, *Eucalyptus gigantea* 4.00 – 7.75, and *Parkia biglobosa* 3.50 – 7.25 whereas the lowest number of pod per plant (3.25) was recorded in the control plants. The highest number of seed per plant (39.75 – 83.00) was obtained from the plants treated with leaf powder of *Calotropis procera* followed by *Azadirachta indica* 39.50 – 77.50, *Cassia siamea* 34.50 – 54.75, *Eucalyptus gigantea* 21.00 – 54.25 and *Parkia biglobosa* 14.50 – 54.00. The lowest number of seeds per plant (10.00) was recorded in the control plants.

4 Discussion

The results of this study showed that amending soil with leaf powder of *C. procera, A. indica, P. biglobosa, E. gigantea* and *C. siamea* suppressed the population of *M. incognita* both in the soil and the roots of cowpea plants with a concomitant increase in growth and yield of cowpea. These results are in agreement with the previous findings of Ahmad *et al.* (2007) and Adegbite (2011a) who reported that application of botanicals as soil amendment cause significant reduction in root knot nematode infestation which consequently lead to increase the growth of different plants. Previous studies on phytochemical analysis revealed that the leaves of *C. procera* contained various cardiac glycosides *viz*., calotropin, calactin, calotoxin, usharin, usharidin and voruscharin are found in the latex of the plant (Rastogi and Mehrotra, 1993). Yadav *et al.* (2010) reported the presence of alkaloids, flavonoids and tannins in the methanolic extract of *Calotropis procera* leaves. *A. indica* contained a number of alkaloids and lipids associates such as nimbidol, nimbidin, nimbin, nimbinin, pyronimbin etc. In various tissues in various concentrations (Gosh, 1994c). *C. Siamea* contained anthraquinones, alkaloids, phytobatannins saponin, tannins, oxalate and phylate (Smith, 2009). *P. biglobosa* leaves contained saponin, alkaloids, tannins and cardiac glucosides (Ajaiyeoba, 2002). *E. gigantea* contained eucalyptol, cineole, pinene, phellandrene, careen and limonene (Fatemeh *et al.,* 2007). The significant reduction in nematode population and root gall formation observed in the soil amended with different leaf powder may be due to the presence of these phytochemicals in the leaves which might have been released into the soil during decomposition process. Presumably the nematicidal constituents are absorbed by the root with adverse effect on the feeding habit of the nematodes.

5. Conclusion

In conclusion, the findings of this study showed that the leaf powder of *Calotropis procera, Azadirachta indica, biglobosa, Eucalyptus gigantea* and *Cassia siamea* have strong nematicidal properties and their addition to the soil controls the population build up of *Meloidogyne incognita* and results in better growth of cowpea. This finding is important from the point of view of controlling root knot nematodes affecting cowpea since the use of synthetic nematicides by subsistence farmers is plagued with several limitations, such as prohibitive cost, lack of technical expertise in their applications and the environmental pollution they likely cause.

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Table 1. Effect of different organic amendments on the population *Meloidogyne incognita* in the rhizosphere and root system of cowpea.

Treatments	**Amendment rate /4kg of soil (g)**	***M. incognita/* 1kg of soil**	***M.incognita/* root system**	**Galls per root System**	**Rep. Factor**
	25	320.00[ef]	28.00[hi]	29.00[cd]	0.436[ef]
A. indica	50	213.34[jl]	24.00[jl]	19.50[hi]	0.293[jl]
	75	146.67[mn]	21.34[lm]	14.00[i]	0.203[mn]
	100	106.67[n]	17.34[m]	10.25[i]	0.149[n]
	25	360.00[de]	37.34[ef]	29.25[cd]	0.493[de]
C. procera	50	293.34[gh]	33.34[ef]	25.25[ef]	0.403[gh]
	75	240.00[ij]	25.34[ij]	23.00[fg]	0.329[ij]
	100	133.34[mn]	22.67[lm]	18.25[i]	0.186[mn]
	25	373.34[de]	41.34[de]	35.00[b]	0.506[de]
C. siamea	50	306.67[fg]	37.34[ef]	28.25[cd]	0.422[fg]
	75	253.34[hi]	32.00[fg]	25.50[ef]	0.349[hi]
	100	146.67[mn]	29.34[gh]	21.50[gh]	0.206[mn]
	25	400.00[cd]	54.67[bc]	35.25[b]	0.552[cd]
E .gigantea	50	320.00[ef]	48.00[cd]	29.00[cd]	0.443[ef]
	75	266.67[gh]	37.34[ef]	26.25[de]	0.368[gh]
	100	173.34[lm]	33.34[ef]	23.75[fg]	0.243[lm]
	25	506.67[b]	57.34[b]	37.00[b]	0.695[b]
P. biglobosa	50	440.00[c]	53.34[bc]	30.00[c]	0.605[c]
	75	293.34[gh]	38.67[ef]	27.50[cd]	0.405[gh]
	100	213.34[jl]	34.67[ef]	25.25[ef]	0.297[jl]
Control	0	733.34[a]	189.34[a]	107.75[a]	1.041[a]

Means in the same column followed by the same letter do not differ statistically between themselves at 5% probability level as indicated by Duncan's test.

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Table2. Effect of different organic amendments on the growth and yield of *Meloidogyne incognita*-infected cowpea plant.

Treatments	**Amendment rate (g)**	**Fresh Shoot wt.(g)**	**Shoot Height (cm)**	**leaves/ plant**	**pods/ Plant**	**Seeds/plant**	**Grain yield/ plant (g)**
A. indica		37.45^{c}	45.90^{ef}	17.00^{cd}	6.00^{cd}	39.50^{de}	8.17^{fg}
	25	46.12^{b}	49.87^{de}	17.67^{cd}	6.25^{bc}	49.25^{cd}	10.60^{cd}
	50	49.25^{ab}	55.47^{bc}	22.34^{bc}	7.75^{b}	55.00^{c}	11.94^{cd}
	75	53.28^{a}	93.20^{a}	30.00^{a}	10.25^{a}	77.50^{ab}	16.90^{ab}
C. procera		29.88^{fg}	39.40^{hi}	20.67^{bc}	6.50^{bc}	39.75^{de}	8.93^{ef}
	25	35.19^{cd}	46.97^{ef}	25.67^{ab}	6.75^{bc}	49.50^{cd}	10.44^{de}
	50	37.47^{cd}	52.70^{cd}	26.00^{ab}	7.75^{b}	66.75^{b}	15.24^{b}
	75	44.51^{b}	64.20^{b}	30.67^{a}	10.75^{a}	83.00^{a}	18.24^{a}
	100						
		24.22^{hi}	37.54^{ij}	16.67^{cd}	4.75^{ef}	34.50^{fg}	7.29^{gh}
C. siamea	25	31.39^{fg}	45.34^{fg}	17.67^{cd}	6.25^{bc}	44.50^{cd}	9.58^{ef}
	50	34.11^{cd}	56.60^{bc}	20.67^{bc}	7.00^{bc}	50.75^{cd}	10.69^{cd}
	75	37.93^{c}	61.34^{bc}	26.34^{ab}	10.00^{a}	54.75^{c}	12.75^{c}
	100						
		18.26^{jl}	33.90^{l}	14.34^{de}	4.00^{fg}	21.00^{hi}	4.27^{j}
E .gigantea	25	19.07^{jl}	42.20^{gh}	16.34^{cd}	5.75^{cd}	36.50^{ef}	7.35^{gh}
	50	22.96^{ij}	51.60^{cd}	19.34^{cd}	6.50^{bc}	44.75^{cd}	9.39^{ef}
	75	35.21^{cd}	58.17^{bc}	20.67^{bc}	7.75^{b}	54.25^{c}	12.13^{cd}
	100						
		17.06^{l}	22.54^{m}	12.34^{ef}	3.50^{fg}	14.50^{i}	3.51^{j}
P. biglobosa	25	26.18^{gh}	36.00^{jl}	16.00^{cd}	5.00^{de}	28.75^{gh}	6.61^{i}
	50	30.90^{fg}	48.37^{de}	20.34^{bc}	7.25^{bc}	54.00^{c}	11.78^{cd}
	75	23.79^{ij}	35.50^{jl}	15.67^{de}	6.00^{cd}	36.50^{ef}	6.88^{hi}
	100						
Control		10.18^{m}	10.50^{m}	9.34^{f}	3.25^{g}	10.00^{i}	2.26^{j}

Means in the same column followed by the same letter do not differ statistically between themselves at 5% probability level as indicated by Duncan's test.