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Management of Root Knot Nematodes (*Meloidogyne incognita*) on Cowpea with Plant Extracts

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ABSTRACT

Screen house experiment was conducted to test the efficacies of leaf powder of neem (*Azadirachta indica*), kassod tree (*Cassia siamea*), eucalyptus (*Eucalyptus gigantea*) and locust bean tree (*Parkia biglobosa*) in the management of *Meloidogyne incognita* on cowpea yield. In this study, 50g of each of the leaf powder was separately mixed with 4 kg of soil in a 25-cm diameter plastic pot. Non-amended pots served as the control. Cowpea seeds were sown in each pot and each seedling was inoculated with about 3000 freshly hatched juveniles of *Meloidogyne incognita* two weeks after sowing. The experiment was laid out in a completely randomised design with five replications. The obtained data were analysed using ANOVA and means, while the significance values were separated using the Duncan multiple test. The result of the study showed that all the treatments significantly ($p<0.001$) reduced root galling and nematode population, as well as improved plant growth and yield of cowpeas. Although all treatments were effective in reducing root galls and nematode population, application of *Azadirachta indica* leaf powder gave the highest reduction in root galls (0.293) and nematode population (24), followed by *Cassia siamea*, *Eucalyptus gigantea* and *Parkia biglobosa*. The findings recorded root galls and nematode population of 28.25 and 37.34, 29 and 48, and 30 and 53.34 respectively as compared to the control treatment, which recorded root galls of 107.75 and nematode population of 189.34.

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INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a dicotyledonous plant belonging to the *Fabaceae* family. It is the most important grain legume in West Africa, which provides a source of profitable revenue between 23 and 29% of the selling price (Langyintuo *et al.,* 2000). It is also valued as the cheapest dietary and high quality vegetable protein of about 25-43% (Nielson *et al.,* 1997), accounting for up to 80% of total protein intake in Nigeria (Rachie & Rawal, 1975). *Meloidogyne* spp is a major problem of cowpeas in most crop growing regions of the world (Caveness, 1992). Losses on cowpeas are attributable to *Meloidogyne* species in some West African countries, which have been put between 10% and 89% including total crop losses in some cases (Adesiyan *et al*., 1990). Iheukwumere *et al*. (1995) recognized *Meloidogyne* spp as one of the plant-parasitic nematodes of economic importance in legume production in Nigeria. The root knot nematode is estimated to cause losses ranging from 10% to 69% in Nigeria (Olowe, 2009). The symptoms of nematode infection include formation of root galls which results in growth reduction, nutrient and water uptake reduction, increased wilting, mineral deficiency, as well as weak and poor yielding plants. The use of synthetic nematicides is considered the most effective practical means of combating the menace of plant-parasitic nematodes in cowpeas (Adesiyan, 1992). However, chemical control of root knot nematodes leads to environmental hazards because of the high toxicity and persistence in the soil (Anastasiadis *et al*., 2008). As an alternative, organic soil amendment has been found to be cheaper, less harmful to man and effective in the management of plant-parasitic nematodes (Olabiyi *et al.,* 2007). In view of this, the present investigation was undertaken to assess the nematicidal activity of four plant species, namely; *Azadirachta indica, Cassia siamea, Eucalyptus gigantean* and *Parkia biglobosa*, in the management of *Meloidogyne* spp in cowpeas.

MATERIALS AND METHODS

Soil Collection and Sterilization

Top soil of 0–30 cm depth was used for the experiment in this study. The soil was collected from the Biological Garden of Modibbo Adama University of Technology, Yola, in Adamawa state, Nigeria, located at latitude 9□ 14′N and longitude 12□ 27′E. The soil, composed of 55.6% sand, 19.4% silt, 25% clay, pH 6.8 and 0.98% organic matter, was steam-sterilized by heating with lighted firewood in a large aluminium pot to a temperature of 100^{o}C and maintained for one hour. The soil was then allowed to cool and later stored in jute sacs to rest for six weeks to regain its stability.

Collection and Preparation of Leaves

Mature 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 using a mortar and stored in a sealed container for use.

Amendment Applications Rate

The ground leaves were separately mixed with 4 kg of steam-sterilized soil at the rate of 50 g. The mixtures were transferred into 25-cm diameter perforated plastic pots.

Sowing of Seeds

Seeds of cowpeas, cv. "Kanannado", were obtained from Monday market in Maiduguri, Borno, Nigeria. The seeds were sown into plastic pots filled with the mixture of steam-sterilized soil and ground leaves. Meanwhile, the control pots contained only steam-sterilized soil. Three seeds were sown per pot at a depth of 2 cm, but the seedlings were thinned to one per pot six days after emergence to ensure uniform plant vigour. The pots were watered regularly once a day, and the potted soil around the base of the plants was loosened from time to time with a hand fork to avoid compacting without disturbing the plant roots.

Collection of Root Knot Nematode Samples

The 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. The roots of the diseased plants showing characteristic symptoms of 15 root knot nematodes were carefully uplifted with a trowel up to 15–20 cm depth from the rhizosphere of the diseased plants together with approximately 1 kg of soil . The samples were then placed in polythene bags and brought to the laboratory for analysis.

Identification of Root Knot Nematode Species

The root knot nematode species was identified on the basis of perineal pattern characteristics of mature female as described by Eisenback *et al*. (1981). A 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 the tomato plant by inoculating the tomato seedlings grown in steam-sterilized soil. Further sub-cultures were made from the initial culture to increase the nematode population .

Preparation of Inoculum

Second-stage juvenile nematodes were used as the inoculum. Eggs of *Meloidogyne incognita* were collected from a pure culture and maintained on tomato roots using sodium hypochlorite technique (Hussey & Barker, 1973). The eggs were placed in the tap water in a 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.

Inoculation Procedure

The cowpea plants were inoculated two weeks after planted in the pots. The population of about 3000 juvenile nematodes per plant was used. Four holes about 2 cm deep and 1 cm 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.

Data Collection

Collection of data was carried out during harvest, whereby the shoot height was ascertained using a measuring tape, the numbers of seeds per plant were counted, and the fresh weight of shoots and grain yield per plant were determined using an electronic balance. Furthermore, the populations of nematodes in the soil and roots, as well as the number of galls and gall index were also identified.

Estimation of Nematode Population in Soil

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

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)

Estimation of Nematode Population in Roots

The population of nematodes in the roots was determined using the maceration method followed by Baermann's funnel technique (Southey, 1970). The reproduction rate of nematodes was calculated by using the formula, R= Pf/Pi, where Pf represents the final nematode population and Pi represents the initial nematode population (Oostenbrink, 1966).

Measurement Plant Height

The plant height was measured from the base of the plant to the tip of the top leaf on the main shoot. Measurements were taken from three plants in each treatment and the average height was calculated and expressed in cm.

Measurement of Shoot Weight

The weight of three randomly selected fresh shoots was measured on electronic balance and the average weight was taken as the shoot weight per plant.

Estimation of Number of Seeds per Plant

The total number of seeds produced in the three plant samples were counted, and the

average number was taken as the number of seeds per plant.

Estimation of Cowpea Grain Yield

After threshing, the grain produce obtained from each plant was cleaned and weighed in grams on electronic balance, and the average weight for each treatment was taken as the grain yield per plant.

RESULTS AND DISCUSSION

The results showed 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 213.34 in the soil treated with *Azadirachta indica* leaf powder, representing 70.91% of reduction over the control. This was followed by the leaf powder of *Cassia siamea* (306.67) with 58.18% reduction, *Eucalyptus gigantean* (320.00) with 56.36% reduction, and *Parkia biglobosa* (440.00) with 40.00% reduction as compared to the control (Fig.1). The highest population of *M. incognita* in the roots (189.34) was recorded in the control plants, while the lowest population (24.00) representing 87.32% reduction over the control was recorded in the plants grown in the soil amended with the leaf powder of *Azadirachta indca*. Likewise, this was followed by *Cassia siamea* (37.34) with 80.28% reduction, *Eucalyptus gigantea* (48.00) with 74.65% reduction, and *Parkia biglobosa* (53.34) with 71.83% reduction as compared to the control treatment (Fig. 2).

The reproductive capacity of the nematodes was also significantly (P<0.001) affected by the treatments imposed on the plants (Fig.3). 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 the reproduction rate (0.293) representing 71.85% reduction as compared to the control was recorded in plants administered with the leaf powder of *Azadirachtaindica*. This was followed by *Cassia siamea* (0.422) with 59.46% reduction, *Eucalyptus gigantea* (0.443) with 57.47 % reduction, and *Parkia biglobosa* (0.605) with 41.88% reduction as compared to the control treatment. The number of root galls incited by *Meloidogyne incognita* on the roots of cowpeas peaked at 107.75 in the control (Fig.4), but ranged from as low as 19.50 in the *Azadirachtaindica* leaf powder treated plants to 30.00 in the *Parkia biglobosa* leaf powder treated plants. The plants treated with *Azadirachta indica* leaf powder had the highest reduction efficacy (81.9%), followed by *Cassia siamea* leaf powder (73.78%), *Eucalyptusgigantea* leaf powder (73.09%) and *Parkia biglobosa* leaf powder (72.16%). Fresh shoot weight, shoot height and number of leaves per plant were significantly (P<0.001) higher in the pots treated with *Azadirachta indica, Parkiabiglobosa, Eucalyptus gigantea* and *Cassia siamea* than in the control treatment. The lowest shoot weight (10.18g) was recorded in the control plants, whereas the highest fresh shoot weight (46.12g) representing 353.05% increase over the

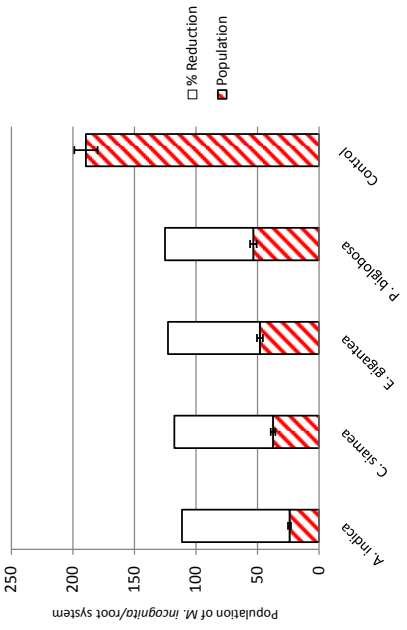

Fig.1: The effects of organic soil amendments on the population of *M. incognita* in the rhizosphere of cowpea

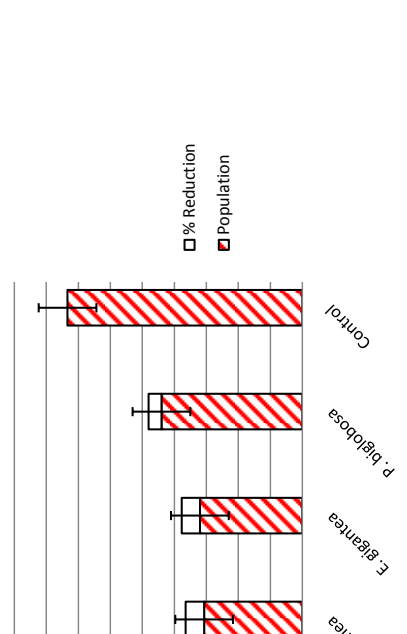

Fig.2: The effects of organic soil amendments on the population of *M. incognita* in the root system of cowpea

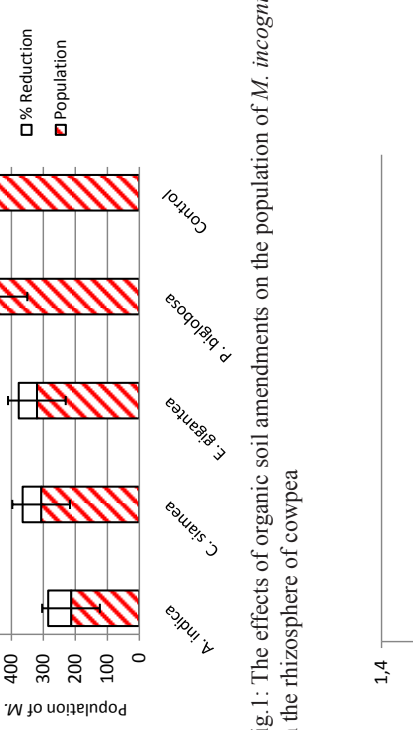

Fig.3: The effects of organic soil amendments on *M. incognita* reproduction factor in the root system of cowpea

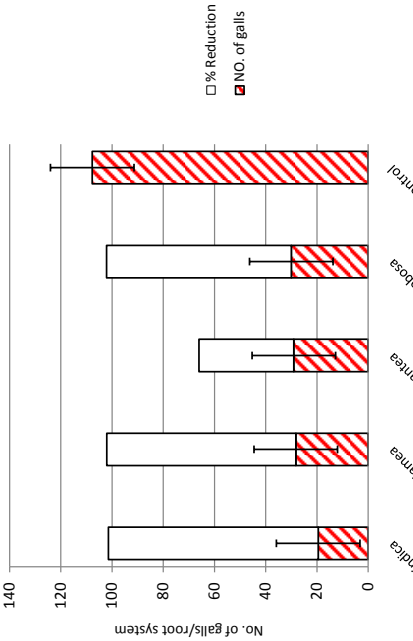

Fig.4: The effects of organic soil amendments on gall formation in *M. incognita*-infected root system of cowpea

control was recorded in the plants treated with the leaf powder of *Azadirachta indica*.

Meanwhile, the plants treated with the leaf powder of *Cassia siamea, Eucalyptus gigantea* and *Parkia biglobosa* recorded 208.35% (31.39g), 87.33% (19.07g), and 157.17% (26.18g) of shoot weight increase respectively over the control treatment (Table 1). The lowest shoot height (10.50cm) was noted in the control plants, while the maximum shoot height (49.87cm) representing 374.95% increase over the control was recorded in the plants treated with the leaf powder of *Azadirachta indica*. This was followed by *Cassia siamea* (45.34cm) with 331.81% increase, *Eucalyptus gigantea* (42.20cm) with 301.9% increase and *Parkia biglobosa* (36.00cm) with 242.86% increase over the control treatment (Table 1). The 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 highest number of seed per plant (49.25) was obtained from the plants treated with the leaf powder of *Azadirachta indica*. This was followed by *Cassia siamea* (44.50), *Eucalyptus gigantea* (36.50) and *Parkia biglobosa* (28.75). The lowest number of seeds per plant (10.00) was recorded in the control plants (Table 1). The lowest grain yield (2.26g) was recorded in the control plants, while the highest grain yield per plant (10.60g) representing 369.03% increase as compared to the control was recorded in the plants treated with the leaf powder of *Azadirachta indica*. The plants treated with the leaf powder of *Cassia siamea* recorded 9.58g representing 323.89% increase, while the plants treated with the leaf powder of *Eucalyptus gigantea* and *Parkia biglobosa* recorded 225.22% (7.35g) and 192.48% (6.61g) yield increase, respectively (see Table 1).

Means in the same column followed by the same letter do not differ statistically between themselves at 5% probability level, as indicated by Duncan multiple range tests.

Figures in parentheses indicate percentage increase as compared to the control treatment.

The results of this study showed that amending the soil with the leaf powder of *A. indica, P. biglobosa, E. gigantea* and *C. siamea* suppressed the population of *M. incognita* both in the soil and roots of cowpea plants with a concomitant increase in growth and yield of cowpeas. These results were in agreement with the previous findings of Ahmad *et al.* (2007) and Adegbite

TABLE 1
Effect of organic soil amendments on the growth and yield of *Meloidogyne incognita*-infected cowpea plant.

Treatments	Fresh Shoot wt.(g)	Shoot Height (cm)	No. of seeds/ plant	Grain yield/ plant (g)
A. indica	46.12^{b} (353.05)	49.87^{de} (374.95)	49.25^{cd} (392.5)	10.60^{cd} (369.03)
C. siamea	31.39^{fg} (208.35)	45.34^{fg} (331.81)	44.50^{cd} (345.0)	9.58^{ef} (323.89)
E .gigantea	19.07^{jl} (87.33)	42.20^{gh} (301.9)	36.50^{ef} (265.0)	7.35^{gh} (225.22)
P. biglobos	26.18 (157.17)	36.00 (242.86)	28.75 (187.5)	6.61 (192.48)
Control	10.18^{m}	10.50^{m}	10.00^{i}	2.26^{j}

(2011a), who reported that application of botanicals as soil amendment causes significant reduction in root knot nematode infestation which consequently leads to the increase in growth of different plants. The nematicidal potential of *Azadirachta indica* found in this study was supported by Yasmin *et al*.'s (2003) findings, which reported that fresh extracts of seeds, leaves and barks of neem inhibited the hatching of *Meloidogyne incognita*. Boiled water extract of fresh neem leaves was reported to be toxic to eggs and juveniles *of M. incognita* (Claudius *et al.,* 2010). The neem constituents, namely, nimbin, salanin, thionemone, azadirachtin and various flavonoids, have been reported to have a nematicidal action (Akhtar and Malik, 2000).

The results of the present study also showed that the incorporation of *Eucalyptus gigantea* leaf powder significantly suppressed the development of *Meloidogyne incognita* population both in the soil and root systems of cowpea, reduced root gall formation, and improved the growth of cowpea plant. Similar findings were also reported by Shahnazdawar *et al.* (2007) that aqueous and ethanol extracts of different parts of *Eucalyptus gigantean*, namely, leaf, stem, bark and fruit showed a nematicidal effect against *Meloidogyne javanica* on mung bean and chick pea plants when used as soil amendment.

The findings of this study also showed that amending the soil with *Cassia siamea* leaf powder significantly reduced the development of *Meloidogyne incognita* population in the soil and root of cowpea, reduced root knot disease and improved growth of cowpea plant. This result was in agreement with the findings of Bello *et al.* (2006), who reported that water extract of seed, leaf and bark of *Cassia siamea* significantly inhibited larval hatch of *Meloidogyne incognita*.

The results of this study also showed that amending the soil with *P. biglobosa* leaf powder significantly reduced nematode population, as well as improved plant growth and grain yield. This result was in agreement with the findings of Fatoki and Oyedunmade (1996), who reported the effective control of *Meloidogyne incognita* with chopped leaves of *Parkia biglobosa*. Olabiyi *et al.* (2007) also reported that the leaf extract of *Parkia biglobosa* effectively inhibited the hatching of *M. incognita* eggs.

CONCLUSION

In conclusion, the findings of this study showed that the leaf powder of *Azadirachta indica, Parkia biglobosa, Eucalyptus gigantea* and *Cassia siamea* has strong nematicidal properties. Their addition to the soil controls the population build up of *Meloidogyne incognita*, which results in better growth of cowpeas. This finding is very important from the point of view of controlling the root knot nematodes affecting cowpeas 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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