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Control of *Meloidogyne incognita* (Kofoid and White) Population on Root Knot of V*igna unguiculata* (L.Walp) (Cowpea) with Organic Soil Amendments

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Abstract

A screen house experiment was conducted to determine the efficacies of leaf powder of *Azadirachta indica* (A.Juss.), *Cassia siamea* (Lamk.) and *Eucalyptus gigantean* (Dehnh) in the control of *Meloidogyne incognita* on cowpea. Three different doses viz. 25, 50 and 75g 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. Each seedling was inoculated with about 3000 freshly hatched juveniles of *Meloidogyne incognita*. 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 population and improved growth and yield of cowpea. 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 *Cassia siamea* and *Eucalyptus gigantea*.

Keywords: *Vigna unguiculata, Meloidogyne incognita*, *Azadirachta indica*, *Cassia siamea, Eucalyptus gigantea*.

1 Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a dicotyledonous plant belonging to the family Fabaceae (Cronquist 1988). It is a food grain legume that plays a critical role in the lives of millions of people in Africa and other parts of the developing world. Both the grain and the haulm are valuable dietary proteins for the African human population and their livestock. The grain contains between 20-25 percent of protein, about twice the protein content of most cereals (Kay 1979). The crop is also a valuable and dependable commodity that produces income for many small holder farmers and traders in Sub-Saharan Africa (Langyintuo et al. 2003). Root-knot nematode, *Meloidogyne incognita* a serious pest of cowpea in most growing areas of the world (Sikora et al. 2005). Infested plants show the symptoms stunting, yellowing, aberrant development of root system characterized by the formation of typical galls, a general unthrifty appearance and poor yield, estimated yield losses ranging from 33% to 39% (Adegbite 2011a). Although, chemical nematicides hold major promise in nematode control (Adesiyan 1992), the high cost, their non-availability the time of need and the hazards they pose as environmental pollutants discourage most potential users in Nigeria. necessitates the search for pollution free and cheaper alternative control measures which are appropriate for resource-poor farmers in Nigeria. The investigation was carried out to determine the efficacies of leaf powder of *Azadirachta indica, Cassia siamea* and *Eucalyptus gigantean* in the control of *Meloidogyne incognita* 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 9° 14´N and longitude 12° 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 Amendment applications rate:

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

2.3 Sowing of seeds:

Seeds of cowpea, cv. "Kanannado" were obtained from Monday market Maiduguri. The seeds were sown in plastic pots filled with the mixture of steam-sterilized soil and organic matter, and the control experiment which contain only steam-sterilized soil. Three seeds were sown per pot at a depth of 2cm, but the seedlings were thinned to one per pot six days after emergence. 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.

2.4 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 of the diseased plants together with approximately 1kg of adhering soil. Samples were placed in polythene bags and brought to laboratory for analysis.

2.5 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.

2.6 Pure 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.

2.7 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 & 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.

2.8 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.

2.9 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.

2.10 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).

2.11 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)

2.12 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* 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 - 146.67 in the soil treated with different doses of *Azadirachta indica* leaf powder representing 56.36-80.00% reduction over the control, followed by the leaf powder *Cassia siamea* (373.34-253. 34) representing 49.09–65.45% reduction and *Eucalyptus gigantean* (400.00-266.67) representing 45.64-63.64% reduction as compared to the control. The highest population of *M. incognita* in the root (189.34) was recorded in the control plants, while the lowest population (28.00-21.34) representing 85.21–88.73% reduction over the control was recorded in the plants grown in the soil amended with leaf powder of *Azadirachta* followed by *Cassia siamea* (41.34–32.00) 78.17–83.1% reduction and *Eucalyptus gigantea* (54.67-37.34) 71.13–80.28% reduction as compared to the control. The reproductive capacity of the nematode was also ($P<0.001$) affected by the treatments imposed on the plants. The highest rate of reproduction of *Meloidogyne* (1.041) was observed in the control plants, which was significantly different from other treatments. The highest reduction in reproduction rate (0.436-0.203) representing 58.12-80.50% reduction as compared to the control was recorded in plants administered with the leaf powder of *Azadirachta indica* followed *Cassia siamea* (0.506-0.349) representing 51.39-66.47% reduction and *Eucalyptus gigantea* (0.552-0.368)46.97-64.65% reduction as compared to the control. The number of root galls incited by *Meloidogyne incognita* on the roots of cowpea peaked at 107.75 in control, but ranged from as low as 10.25 in *Azadirachta indica* leaf powder-treated plants to 35.25 in *Eucalyptus gigantea* leaf powder-treated plants.

Plants treated with leaf powder of *Azadirachta indica* had the highest reduction (efficacy of 73.09-87.01%), followed by *Cassia siamea* leaf powder (67.52 - 76.33%) and *Eucalyptus gigantean* leaf powder (67.29-75.64%). Fresh shoot weight, shoot height and number of leaves per plant were significantly ($P<0.001$) higher in pots treated with *Azadirachta indica, 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) representing 267.88–423.38% increase over the control was recorded in plants treated with different doses of the leaf powder of *Azadirachta indica*. Plants treated with leaf powder of *Cassia siamea* and *Eucalyptus gigantea* recorded (24.22–37.93g) 137.92-272.59% and (18.26–35.21g) 79.37-245.87% shoot weight increase over the control respectively. 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 (8.17-11.94g) representing 261.5-428.32% increase as compared to the control was recorded in plants treated with leaf powder of *Azadirachta indica*. Plants treated with the leaf powder of *Cassia siamea* and *Eucalyptus gigantea* recorded (7.29-10.69g) 222.57-373.01% and (4.27-9.39g) 88.94-315.49% yield increase respectively. The maximum number of pods per plant (6.00–7.75) was observed in plants treated with the leaf powder of *Azadirachta indica* followed by *Cassia siamea* 4.75–7.00 and *Eucalyptus gigantea* 4.00–6.50, whereas the lowest number of pod per plant (3.25) was recorded in the control plants. The highest number of seed per plant (39.50–55.00) was obtained from the plants treated with leaf powder of *Azadirachta indica* followed by *Cassia siamea* 34.50–50.75 and *Eucalyptus gigantea* 21.00–44.75. 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 *A. indica, 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 in the growth of different plants. Previous studies on phytochemical analysis revealed that *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, 2006). *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

The findings of this study showed that the leaf powder of *Azadirachta indica, 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 very 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 *M. incognita* in the rhizosphere and root system of cowpea.

Treatments	Amendment rate (g)	*M. incognita*/ 1kg of soil	*M.incognita*/root system	Galls per root System	Rep. Factor
A. indica	25 50 75	320.00^{ef} (56.36) 213.34^{jl} (70.91) 146.67^{mn} (80.00)	28.00^{hi} (85.21) 24.00^{jl} (87.32) 21.34^{lm} (88.73)	29.00^{cd} (73.09) 19.50^{hi} (81.9) 14.00^{i} (87.01)	0.436^{ef} (58.12) 0.293^{jl} (71.85) 0.203^{mn} (80.50)
C. siamea	25 50 75	373.34^{de} (49.09) 306.67^{fg} (58.18) 253.34^{hi} (65.45)	41.34^{de} (78.17) 37.34^{ef} (80.28) 32.00^{fg} (83.1)	35.00^{b} (67.52) 28.25^{cd} (73.78) 25.50^{ef} (76.33)	0.506^{de} (51.39) 0.422^{fg} (59.46) 0.349^{hi} (66.47)
E .gigantea	25 50 75	400.00^{cd} (45.46) 320.00^{ef} (56.36) 266.67^{gh} (63.64)	54.67^{bc} (71.13) 48.00^{cd} (74.65) 37.34^{ef} (80.28)	35.25^{b} (67.29) 29.00^{cd} (73.09) 26.25^{de} (75.64)	0.552^{cd} (46.97) 0.443^{ef} (57.47) 0.368^{gh} (64.65)
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. Figures in parentheses indicate percentage increase as compared to the control.

Table 2 Effect of organic soil amendments on the growth and yield of *M. incognita*-infected cowpea plant.

Treatments	Amendment rate (g)	Fresh Shoot wt.(g)	Shoot Height (cm)	No. of leaves/ Plant	No. of pods/ Plant	No. of seeds/ Plant	Grain yield/ plant (g)
A. indica	25	37.45^{c} (267.88)	45.90^{ef} (337.14)	17.00^{cd} (82.01)	6.00^{cd} (84.62)	39.50^{de} (295.0)	8.17^{fg} (261.5)
	50	46.12^{b} (353.05)	49.87^{de} (374.95)	17.67^{cd} (89.19)	6.25^{bc} (92.31)	49.25^{cd} (392.5)	10.60^{cd} (369.03)
	75	49.25^{ab} (383.79)	55.47^{bc} (428.29)	22.34^{bc} (139.19)	7.75^{b} (138.46)	55.00^{c} (450.0)	11.94^{cd} (428.32)
C. siamea	25	24.22^{hi} (137.92)	37.54^{ij} (257.52)	16.67^{cd} (78.48)	4.75^{ef} (46.15)	34.50^{fg} (245.0)	7.29^{gh} (222.57)
	50	31.39^{fg} (208.35)	45.34^{fg} (331.81)	17.67^{cd} (89.19)	6.25^{bc} (92.31)	44.50^{cd} (345.0)	9.58^{ef} (323.89)
	75	34.11^{cd} (235.07)	56.60^{bc} (439.05)	20.67^{bc} (121.31)	7.00^{bc} (115.38)	50.75^{cd} (407.5)	10.69^{cd} (373.01)
E .gigantea	25	18.26^{jl} (79.37)	33.90^{l} (222.86)	14.34^{de} (53.53)	4.00^{fg} (23.08)	21.00^{hi} (110.0)	4.27^{j} (88.94)
	50	19.07^{jl} (87.33)	42.20^{gh} (301.9)	16.34^{cd} (74.95)	5.75^{cd} (76.92)	36.50^{ef} (265.0)	7.35^{gh} (225.22)
	75	22.96^{ij} (125.54)	51.60^{cd} (391.43)	19.34^{cd} (107.07)	6.50^{bc} (100)	44.75^{cd} (347.5)	9.39^{ef} (315.49)
Control	0	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. Figures in parentheses indicate percentage increase as compared to the control.

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