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NEMATICIDAL EFFECTS OF *LEUCAENA LEUCOCEPHALA* AND *GLIRICIDIA SEPIUM* EXTRACTS ON *MELOIDOGYNE INCOGNITA* INFECTING OKRA

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Abstract: Two separate but identical greenhouse trials were conducted to investigate the effects of extracts of leaves and roots of *Leucaena leucocephala* and *Gliricidia sepium* each at 80,000 mg / kg and 40,000 mg / kg on *Meloidogyne incognita* on okra.

Each potted nine-day old okra seedling was inoculated with 3,000 fresh eggs of *M. incognita* and 5ml of each of the extracts was added simultaneously around the roots of the seedling. Treatment of okra plant with *L. leucocephala* and *G. sepium* extracts resulted in reduced nematode population, reduced galling, reduced nematode reproduction rate and enhanced fruit weight. Chemical analysis revealed that *G. sepium* leaves contained phenolic compound and carboxylic acid, while the roots showed the presence of aromatic amide, phenolic compound and carboxylic acid. *L. leucocephala* leaves showed the presence of phenolic compound, aromatic amide and carboxylic acid, while the roots showed the presence of phenolic compound and carboxylic acid. The results of this study suggest that leaf and root extracts of *L. leucocephala* and *G. sepium* at the rate of 40,000 mg / kg could be useful in root knot nematode management in vegetable beds.

Key words: extracts, *Leucaena leucocephala*, *Gliricidia sepium*, *Meloidogyne incognita*, okra, chemical analysis.

I n t r o d u c t i o n

Okra, *Abelmoschus esculentus* (L) Moench is one of the most important vegetables in Nigeria. It is a tropical plant, which grows best in warm climate. It is available all year round, with a peak during summer months. The pods grow

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rapidly being ready for harvest in about 30- 60 days (depending on the variety) when grown from seed. They must be picked about four to five days after flowering, when 4-5 inches or so in length before they mature and toughen. The tender unripe seedpods are used as vegetable, and have a unique texture and sweet flavour. The pods, when cut, exude mucilaginous juice that is used to thicken stews. As a result of the mucilage content, the soups made from okra fruits and young leaves blend well with many solid meals as in many homes. The mucilage is also utilized medicinally (Sigmund and Gustav, 1991). Okra is a good source of Vitamin C, A and B complex as well as iron and calcium.

Plant parasitic nematodes are important pathogens on most food, vegetable, horticultural and fiber crops and without adequate control will cause loss of yield and quality. Approximate yield losses due to plant parasitic nematodes have been estimated to be $ 100 billion worldwide each year (Sasser and Freckman, 1987). Root-knot nematodes (*Meloidogyne* species) infect almost all types of plants and may cause considerable damage. During surveys, most vegetable crops including okra were found to be infected with *M. incognita* (Khan and Khan, 1994). Nematode management is complicated and difficult and at present chemical control is employed in many crops to maintain their populations below economic threshold levels (Eapen et al. 2005). Recently, the control of plant parasitic nematodes by using conventional nematicides has declined internationally because of the inherent toxicity of many existing synthetic pesticides to non-target organisms and their persistence in the environment. There is increasing need to find more acceptable alternatives. The potential for nematicidal activity of indigenous plants and their products has been reported by earlier workers (Haseeb et al. 1981; 1984: Prot and Kornprobst, 1983; Pracer et al., 1987; Adekunle and Fawole, 2003). The nematicidal principles of plant origin in the form of substances such as isothiocyanates, thiophenics, glucosides, alkaloids, phenolics, thianins and fatty acids have been identified (Gommers 1973; Fatoki and Fawole, 2000). There may be many more plants however, not yet tested, which could prove to be effective for the management of plant parasitic nematodes. The present study was therefore designed to investigate nematicidal potential of leaf and root extracts of *Leucaena leucocephala* and *Gliricidia sepium* against *Meloidogyne incognita* on okra.

Materials and Method

Greenhouse trials were conducted to evaluate the effects of extracts of leaves and roots of *L. leucocephala* and *G. sepium* on *M.incognita* infecting okra.

Extraction of *Meloidogyne incognita* eggs

Pure cultures of *M. incognita* race 2 were maintained on okra cv. LD-88 in a greenhouse and eggs were extracted from infected okra roots as described in an earlier study (Akinsanmi and Adekunle, 2003).

Preparation of plant extracts

Ten- grams pieces each of fresh leaves and roots of gliricidia and leuceana were weighed in duplicates and placed in eight reagent bottles; 100ml of distilled water was added into each reagent bottle. The bottles were then placed in a water bath and heated at 60^0c for 90 minutes. The extracts were allowed to cool and filtered through Whatman No.1 filter paper. The filterate obtained was 100,000mg/kg (stock extract). Serial dilutions were prepared to produce 80,000mg/kg and 40,000 mg/kg extracts.

Assessment of effects of plant extracts on *Meloidogyne incognita*

Greenhouse studies were conducted in two separate but identical trials. In the first trial, seeds of okra cv-47-4 obtained from National Horticultural Research Institute, Ibadan, Nigeria were sown in 40 five litre plastic pots filled with steam-sterilized sandy loam top soil at the rate of one seed per pot. Nine days after planting each of 36 potted seedling was inoculated with 3,000 fresh *M. incognita* eggs, immediately after egg inoculation extracts of leaves and roots of gliricidia and leucaena at 80,000 mg/kg and 40,000 mg/kg were added to 32 potted seedlings. There were four inoculated control plants to which no extract was added and also four uninoculated control plants. The Experiment was laid out in a Randomized Complete Block Design with ten treatments in four replicates. After eight weeks, the study was terminated. The plants were carefully uprooted, weighed and roots of plants were assessed for galling, nematode egg population on roots of each plant was estimated (Akinsanmi and Adekunle, 2003), okra fruits were harvested and weighed and soil nematode population was estimated.

A second trial was conducted with a new set of plastic pots. All procedures and collection of data were as in the first trial without any modifications.

Assessment of root galling

Okra plant in each pot was carefully uprooted eight weeks after planting without damaging their roots. The roots of the plants were assessed for galling using the method described by Taylor and Sasser (1978) where 0= No galls or egg masses, 1= 1-2 galls or egg masses, 3= 11-30 galls or egg masses, 4= 31-100 galls or egg masses and 5= More than 100 galls or egg masses.

Nematode assay

Soil sample was collected from each of the pots and nematodes were extracted from properly mixed 200 ml soil drawn from the bulk sample collected from each pot. Extraction of nematode was by the method of Whitehead and

Hemming (1965), nematodes were killed by heat and fixed in 4% formaldehyde. Nematodes were counted in Doncaster counting dish under a stereomicroscope (250 x magnifications) and individuals from each sample were further identified under a light microscope (400 x magnification).

Infrared (IR) analysis of leaves and roots of *Leucaena leucocephala* and *Gliricidia sepium*.

For Infrared analysis, 0.1g of powders of each of leaves and roots of *L. leucocephala* and *G. sepium* were separately used for analysis. Potassium bromide (KBr) disc was prepared by grinding 0.1g of each of the samples with KBr and compressing the whole into transparent disc. The disc was then scanned in a buck scientific 500M IR machine. The IR spectrum of each sample was printed out with the aid of the machine printer (William, 1987). The IR spectrum of each plant material was compared with those of some named compounds in the process of identification of active ingredients.

Statistical analysis

All statistical analysis was performed using the SAS (1985) statistical package while treatment means were partitioned using the Duncan's Multiple Range Test (DMRT) at p= 0.05.

Results and Discussion

In Table 1 is presented the effects of *L. leucocephala* and *G. sepium* extracts on number of eggs per okra plant infected with *M. incognita.* Inoculated control plants had the highest number of eggs per plant and this was significantly higher than number of eggs on roots of other plants. Okra plants treated with *L. leucocephala* root extract at 80,000 mg/kg produced fruits with higher weights than fruits of other plants except those of plants treated with *G. sepium* leaf and root extracts. (Table 2).

The results presented in Table 3 indicate that there was no significant difference in fresh weights of okra plants infected with *M. incognita* irrespective of whether or not they were treated with plant extracts. Inoculated control soil supported a significantly higher nematode density than other soils that were treated with plant extracts (Table 4). Reproduction rate of *M. incognita* was highest in inoculated control plants, while the least reproduction rate was recorded in okra plants treated with gliricidia leaves at 80,000 mg/kg (Table 5). Roots of inoculated control plants were the most galled. There was no significant difference in root galling of plants treated with different plant extracts (Table 6).

T a b. 1 - Effect of *Leucaena leucocephala* and *Griricidia sepium* extracts on number of eggs/ okra plant infected with *Meloidogyne incognita*

Treatment	Rate (mg/Kg)	Number of eggs / plants
G. s leaves	80,000	826.0c
	40,000	2842.0b
G. s roots	80,000	2583.0b
	40,000	1819.0bc
L.l leaves	80,000	2664.3b
	40,000	2210.0bc
L.l roots	80,000	1490.5bc
	40,000	1846.0bc
Inoculated Control		26132.0a
Uninoculated Control		-

Each value is a mean of four replicates and two trials.
Treatments followed by the same alphabets are not significantly different (P=0.05) by Duncan`s Multiple Range Test (DMRT).

T a b. 2 - Effect of *leucaena leucocephala* and *Griricidia sepium* extracts on weight of fruit per okra plant infected with *Meloidogyne incognita*

Treatment	Rate (mg/Kg)	Weight of fruit / plant (g)
G. s leaves	80,000	7.2 ab
	40,000	6.8 ab
G. s roots	80,000	6.1 ab
	40,000	6.3 ab
L.l leaves	80,000	4.0 b
	40,000	3.9 b
L.l roots	80,000	10.0 a
	40,000	4.9 b
Inoculated Control		5.6 b
Uninoculated Control		6.0 ab

Each value is a mean of four replicates and two trials.
Treatments followed by the same alphabets are not significantly different (P=0.05) by Duncan`s Multiple Range Test (DMRT).

T a b. 3 - Effect of *Leucaena leucocephala* and *Griricidia sepium* extracts on fresh weight of okra plants infected with *Meloidogyne incognita*

Treatment	Rate (mg/Kg)	Fresh weight / plant (g)
G. s leaves	80,000	22.0 a
	40,000	16.9 ab
G. s roots	80,000	18.1 ab
	40,000	17.4 ab
L.l leaves	80,000	17.5 ab
	40,000	14.7 b
L.l roots	80,000	18.6 ab
	40,000	17.5 ab
Inoculated Control		18.0 ab
Uninoculated Control		17.3 ab

Each value is a mean of four replicates and two trials.
Treatments followed by the same alphabets are not significantly different (P=0.05) by Duncan`s Multiple Range Test (DMRT).

T a b. 4 - Effect of *Leucaena leucocephala* and *Griricidia sepium* extracts on soil nematode population per okra plant infected with *Meloidogyne incognita. (*Number /200m/ soil)

Treatment	Rate (mg/Kg)	Soil nematode population
G. s leaves	80,000	1577.0 b
	40,000	2167.3 b
G. s roots	80,000	1851.5 b
	40,000	2138.3 b
L.l leaves	80,000	2133.5 b
	40,000	1871.0 b
L.l roots	80,000	2712.0 b
	40,000	2192.5 b
Inoculated Control		34,472.5 a
Uninoculated Control		-

Each value is a mean of four replicates and two trials. Treatments followed by the same alphabets are not significantly different.(P=0.05) by Duncan`s Multiple Range Test(DMRT).

T a b. 5 - Effect of *Leucaena leucocephala* and *Griricidia sepium* extracts on nematode reproduction rate on okra plant infected with *Meloidogyne incognita*

Treatment	Rate (mg/Kg)	Reproduction factor (Pf / Pi)
G. s leaves	80,000	2.8 c
	40,000	9.5 b
G. s roots	80,000	6.1 bc
	40,000	8.6 b
L.l leaves	80,000	7.4 bc
	40,000	8.9 b
L.l roots	80,000	5.0 bc
	40,000	6.2 bc
Inoculated Control		87.1a
Uninoculated Control		-

Each value is a mean of four replicates and two trials. Treatments followed by the same alphabets are not significantly different. (P=0.05) by Duncan`s Multiple Range Test (DMRT)

T a b. 6 - Effect of *Leucaena leucocephala* and *Griricidia sepium* extracts on gall index of okra infected with *Meloidogyne incognita*

Treatment	Rate (mg/Kg)	Gall index
G. s leaves	80,000	1.54 b
	40,000	1.50 b
G. s roots	80,000	1.61 b
	40,000	1.32 b
L.l leaves	80,000	1.57 b
	40,000	1.61 b
L.l roots	80,000	1.61 b
	40,000	1.72 b
Inoculated Control		2.28 a
Uninoculated Control		-

Each value is a mean of four replicates and two trials. Treatments followed by same alphabets are not significantly different (P=0.05) by Duncan`s Multiple Range Test..

Analysis of variance is based on $\sqrt{n+1}$ -transformed data

Infrared spectra of the samples were recorded using KBr pellet technique. The diagnostic peaks of the spectra were interpreted using William (1987) method. *G. sepium* leaves contained phenolic compounds and carboxylic acid, G. sepium roots showed the presence of aromatic amide, phenolic compounds and carboxylic acid. *L. leucocephala* leaves showed the presence of phenolic compounds, aromatic amide and carboxylic acid while *L. leucocephala* roots showed the presence of phenolic compounds and carboxylic acid (Figures 1, 2,3 and 4)

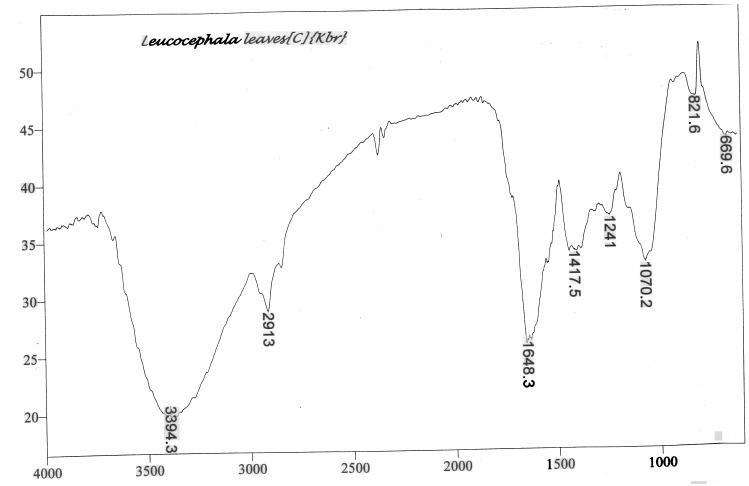

Fig. 1. – Infrared spectrum of leaves of *Leuceana leucocephala*

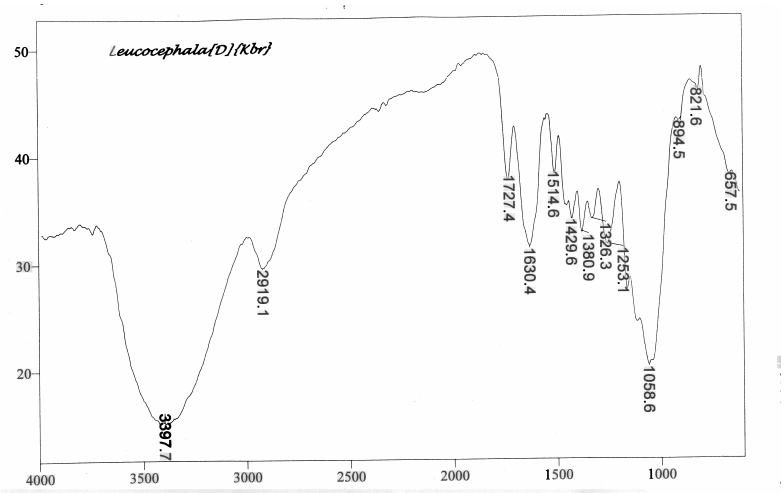

Fig. 2. – Infrared spectrum of roots of *Leuceana leucocephala*

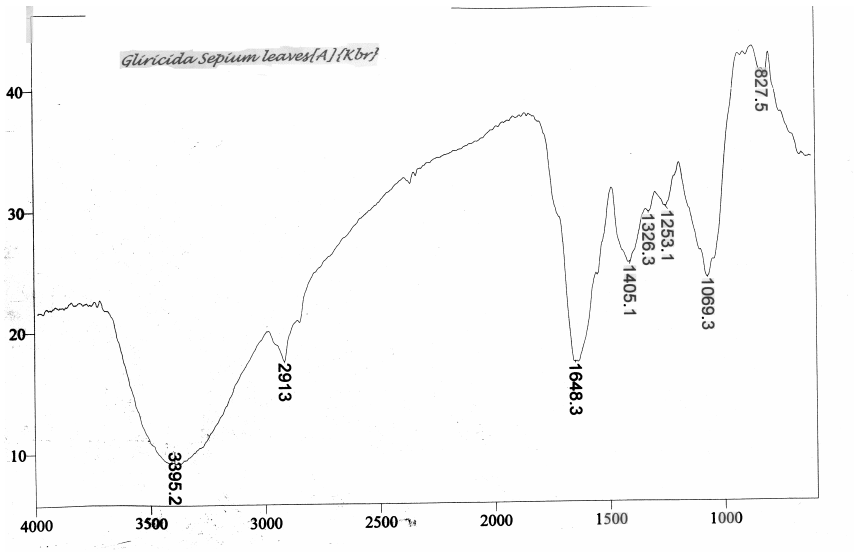

Fig. 3. – Infrared spectrum of leaves of *Gliricidia sepium*

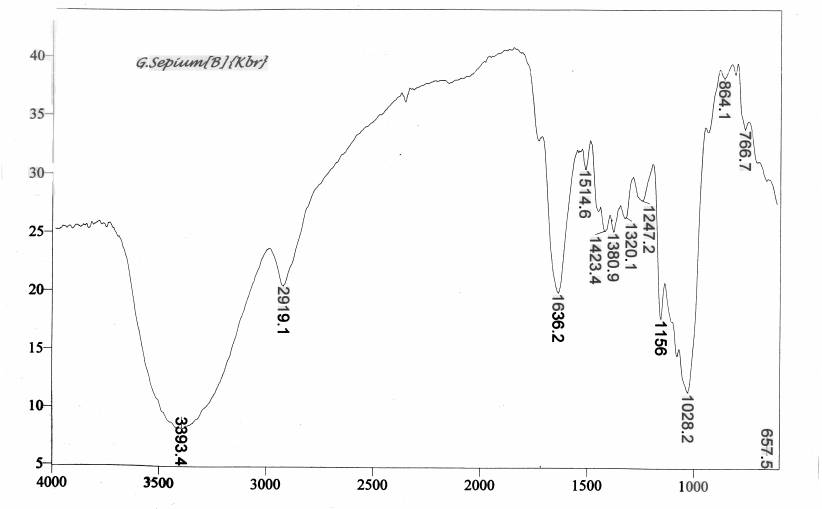

Fig. 4. – Infrared spectrum of roots of *Gliricidia sepium*

Treatment of nematode –infected okra plants with root and leaf extracts of *G. sepium* and *L. leucocephala* resulted in a reduction in *M. incognita* population, reduced root galling which implied reduced pathogenicity of the nematode, reduced nematode reproduction as well as an increase in fruit weight. The extracts of the two plants used in the current study were equally effective at 40,000 mg/kg

and 80,000 mg/kg in the management of *M. incognita* on okra. Similar findings were reported by Akhtar (1993) on the utilization of plant-origin waste materials for the control of plant-parasitic nematodes. He studied nematicidal properties of several waste materials of plant origin in soil infested with plant-parasitic nematodes. Among the materials, press mud, vegetable- and fruit-processing waste and tobacco wastes were the most effective in reducing the incidence of root knot and development of plant parasitic nematodes on tomato. However amendments to soil with spent tea, wheat straw, paddy husk, paddy straw, sugarcane trash, domestic garbage, dead vegetation and pigeon pea stubble were also found to be beneficial in nematode control. He further asserted that as a consequence of nematode control, plant growth improved, with a few exceptions where higher levels (5% (w/w)) of waste materials were phytotoxic. In the same vein, Adekunle and Fawole (2003) reported that application of water extracts of neem leaves, siam weed leaves and roots at 20,000 mg/kg and 40,000 mg/kg or carbofuran at 1.5 kg a.i/ha and 2.5 kg a.i/ha to potted tomato plants, delayed development and consequently reduced population of *M. incognita* evidenced by a prolonged generation time of the nematode. Same treatments were said to reduce number of nematode egg masses and eggs as well root galling of tomato plants. In another study with plant leaf extracts, Lilane et al. (1998) reported that direct exposure of *Aphelenchoides saprophilus* to leaf extracts of *Cassiope tetragona*, *Empetrum hermaphroditum* and *Betula pubescens* spp. *turtuosa* in agar cultures resulted in a lower reproduction rate of the nematode. Also, leaf extract treatment in potted soil altered nematode species composition and dominance structure and generally reduced species number and maturity index. Nematode population density was reported to be adversely affected after application of *Betula* extracts. Our findings are also corroborated by those of Akhtar and Farzana (1996) who reported that standard extracts of roots and shoots of *Datura arborea*, *D. stramonium, Hyoscyamus albus, H. muticus, Solanum incanum, S. indicum* and *S. nigrum* exhibited 100 % mortality of *M. incognita*, with less effects of root extracts. They also found that larval hatching and nematode mortality were strongly influenced by the concentration of the extract, plant species and the duration of exposure.

In the current study, addition of plant extracts to the soil enhanced the fruit production of okra. The addition of organic matter to soil for improvement of soil fertility and crop yield is a practice almost as old as agriculture. However beneficial effects of organic materials with respect to the suppression of plant pathogens including nematodes have been recognized only in the recent past (Akhtar and Alam, 1993). There is a need for utilizing plant extracts and waste materials for supplementing chemical fertilizers as an additional benefit to their pesticidal properties. The active ingredients identified in the plant extracts may have been contributory to their nematicidal activities as recorded in this study.

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DELOVANJE EKSTRAKTATA LISTOVA I KORENA *Leucaena leucocephala* I *Gliricidia sepium* NA FITOPARAZITNE KORENOVE NEMATODE *Meloidogyne incognita* U PROIZVODNJI BAMIJE

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R e z i m e

Delovanje ekstraktata listova i korena *Leucaeana leucocephala* i *Glicidia sepium* na fitoparazitne korenove nematode *Meloidogyne incognita* u proizvodnji bamije, ispitivano je u Nigeriji u saksijskim ogledima u kontrolisanim stakleničkim uslovima. Sejanci su inokulisani sa po 3000 jaja nematode, i potom je dodavano po 5 ml biljnog ekstrakta. Tretman ispitivanim ekstraktima rezultirao je, u odnosu na kontrolu, smanjenom populacijom nematode, manjim i većim prinosom plodova bamije. Hemijska analiza utvrdila je fenole i karboksilnu kiselinu u ekstraktima listova i korena *G. sepium*, a u listovima *L. leucocephala* još i aromatične amide. Rezultati ukazuju da ispitivani ekstrakti imaju potencijal za suzbijanje *M. incognita*.

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