

PATHOGENICITY OF *Meloidogyne Incognita* IN SELECTED GROUNDNUT VARIETIES AND ITS MANAGEMENT WITH AQUEOUS LEAF EXTRACTS OF WILD SAGE (*Lantana Camara* L.) AND LOCUST BEAN (*Parkia Biglobosa* JACQ.)

*Baba, H. S.¹, Izuogu N. B.¹, Olajide, M. C.¹, Dosunmu, O. O.², Ahmed, O.¹, and Saliu, A. R.¹

¹Department of Crop Protection, University of Ilorin, Ilorin, Nigeria.

² Department of Organic Chemistry, University of Ilorin, Ilorin, Nigeria.

* Corresponding Author: (+2348068821543, bhalimatshola@gmail.com)

ABSTRACT

Investigations were conducted to evaluate the pathogenicity of the root-knot nematode, Meloidogyne incognita, on four groundnut (Arachis hypogaea L.) varieties. The efficacy aqueous leaf extracts of Lantana camara and Parkia biglobosa in the management of M. incognita infecting the groundnut varieties was also investigated. A preliminary study was first carried out from November 2014 – January 2015 in a screen house to access the pathogenicity of M. incognita on the four varieties of groundnut. Results obtained from soil and root nematode population led to field trials which involved the management of M. incognita using botanicals. The field experiment which was a 4x3 factorial fitted into Randomized Complete Block Design (RCBD) commenced in August, 2015. The effects of treatment on plants' height, number of leaves, weight of fruits and on the population of root and soil nematodes were determined. Phytochemical screening of the active components in the test plant extracts was also conducted. Data collected were subjected to analysis of variance (ANOVA) and significant means were separated using Fisher's protected LSD. Results from the study revealed that the growth parameters, yields and nematode population were significantly higher ($p < 0.05$) for the treatment combinations of L. camara and P. biglobosa than the control L. camara leaves extract at 100% performed better than P. biglobosa with respect to all the data measured. Phytochemical analysis revealed the presence of alkaloids, saponin, tannin, flavonoids, phenol and glycosides as the active chemical components in the test plants. This bio-active components were responsible for the nematotoxic effect of the leaf extracts. However results indicate that both aqueous extracts of L. camara and P. biglobosa can be used to manage the root-knot nematodes and increase the yield of groundnut without any toxic effects on the plants.

Key words: *Lantana camara*, *Parkia biglobosa*, *Meloidogyne incognita*, Aqueous leaf extract and Pathogenicity

INTRODUCTION

Groundnut (*Arachis hypogaea L.*) is a leguminous seed crop cultivated in the tropics and other part of the world (Ajeigbe *et al.*, 2015). Nigeria was the third largest producer of groundnut in 2011 with 2,962,760 tons following China (16,114,231 tons) and India (2,962,760 tons) (Zekeri and Tijjani, 2013). The crop is currently grown throughout Nigeria with the exception of the riverine and swampy areas. Cultivation of groundnut in Nigeria has been reported to occupy between 1.5 and 2 million ha of land (Zekeri and Tijjani, 2013).

Groundnut is a rich source of calcium, potassium, phosphorus, magnesium and vitamin E (Ibrahim *et al.*, 2014) and the oil is used for cooking, soap making, cosmetics and furniture.

From an agronomic point of view, groundnut contributes significantly to nitrogen fixation (Adam, 2000).

Groundnut production is faced with different constraints such as lack of improved seed varieties, inadequate soil nutrient, poor crop management practices, pests and diseases (Ahmed, *et al.*, 2010). In Nigeria, groundnut yield loss to plant diseases could be as high as 51% (Etejere *et al.*, 2013). Important biotic factors that limit groundnut production include – fungal diseases like early leaf spot, late leaf spot, rust and *Sclerotium rolfsi*; viral diseases like bud necrosis virus, tomato spotted virus, peanut stripe virus and rosette and insect pests like jassids, thrips, termites, leaf minor, spodoptera and white grub. Many nematode species have also been reported to negatively affect the production of groundnut. Amongst the important ones is *M. arenaria* whose infestation results in the yellowing of foliage and stunting (Osei *et al.*, 2013).

Nematodes are soil dwelling round worms that limit the production of groundnut all over the world. The pest is less than 1mm long and bores into roots and pods. Their presence in the roots is known to decrease the number of nodules and activity of nitrogen fixing bacteria. Damaged pods are characterized by the appearance of small brown spots which become larger and darker as nematodes grow. Other symptoms include premature wilting and slow recovery to improved soil moisture condition. Nematodes attack plant roots, by limiting their development and restricting the uptake of water and nutrients making them silent enemies of most food, vegetables, horticultural and field crops (Izuogu and Abiri, 2015).

Losses due to phyto-nematodes can however be minimized by some recommended practices which include application of pesticides. Chemical control has proven to be the most effective method of controlling root-knot nematodes but the high cost, health hazards and lack of synthetic pesticides when needed discourage their use by resource poor farmers. This has led plant nematologists in recent times to give more attention to the use of organic amendments. Therefore, efficient approaches that utilize a wide range of botanicals are required. Begum *et al.*, (2000) reported that *L. camara* leaf extracts have antimicrobial effect

and has been used to protect Mungbean against galling of roots and was also used against juveniles of *Meloidogyne* spp by many authors (Qamar *et al.*, 2005; Shaukat and Siddiqui, 2001). Ajaiyeoba (2002) also confirmed antimicrobial activities of *P.biglobosa* leaf extracts.

The objectives of this study were to assess the efficacy of *Lantana camara* (Lantana or Wild Sage) and *Parkia biglobosa* (monkey cutlass tree or African locust bean) against root – knot nematode and to determine the effect of the treatments on the growth and yield of the four groundnut varieties and to assess the resistance or susceptibility of the groundnut varieties to the root–knot nematode.

MATERIALS AND METHODS

Screen house experiment

A preliminary screen house experiment was carried out from November 2014 to January 2015 to assess the pathogenicity of root-knot nematodes on four groundnut varieties namely Samnut 10, Samnut 11, Nut (IT288) and Nut (KSV-6). The experiment was laid out in a Completely Randomized Design. Galled roots of *Meloidogyne* infected-roots were incorporated at 10g/pot of sterilized soil. Each groundnut variety was replicated five times giving a total of twenty pots.

Field experiment

The result on soil and root nematode population obtained from the screen house experiment led to field trials which involved the management of *M. incognita* using botanicals on four groundnut varieties at the University of Ilorin Teaching and Research farm.

The experimental design was a 4×3 factorial fitted into a Randomized Complete Block Design (RCBD). The field size was 35m × 30m, ploughed, harrowed, ridged and then divided into three blocks each with 12 plots including the control in each block.

Soil samples were collected in a zigzag pattern at 0-15cm depth from plots with the aid of soil auger for initial soil nematode population using the method of extraction described by Whitehead and Hemming (1965). The nematodes extracted were taken to International Institute for Tropical Agriculture for identification and population enumeration. Soil samples were also collected for physico–chemical analyses using the Kjeldahl method. Leaves of *L. camara* and *P. biglobosa* were collected from Tanke area in Ilorin before the sun comes out and were identified at the herbarium of the Department of Plant Biology, University of Ilorin. The leaves were separately air-dried for seven days at room temperature of 25⁰-27⁰C. The dried leaves were pulverized using mortar and pestle and separately soaked in hot water at 100⁰C at the rate of 1kg per four liters of water following the technique described by Oyedunmade and Izuogu (2011). The suspension was sieved in a muslin cloth in both cases and the aqueous extracts were collected in plastic containers. Samples from the leaf extracts

were taken to Chemistry Department of University of Ilorin for preliminary phytochemical screening to identify bioactive compounds of nematocidal importance such as alkaloids, saponin, tannin, phenol, terpenoid and flavonoids adopting the techniques described by Cuilei, (1984), Harborne (1998) and Trease and Evans (1989). Many researchers have followed the trend (Joshi *et al.*, 2013; Ayoola *et al.*, 2008; Chang *et al.*, 2002; Banso and Adeyemo, 2006).

Galled roots of *Celosia argentea* were collected at Oyun area (Lat: 8.5° N and Log: 4.55° E) in Ilorin Kwara State. The root-knot nematode had been previously identified at the Pathology Laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan as *Meloidogyne incognita* Race 2. Galled roots weighing about 10kg that has been carefully washed to remove soil particles were cut and chopped into pieces with knife before incorporating into the soil evenly for both the control and treated plots. This was carried out a week before planting and the objective was to increase the initial root-knot nematode population in the soil. Groundnut seeds were planted at the rate of two seeds per hole at 5cm depth. One week after planting 100ml of the leaf aqueous extract were applied per plant. First weeding at 4th week was carried out manually, followed by fertilizer Single Super Phosphate (SSP) application. Second soil sampling was conducted after one month of planting for nematodes identification and enumeration followed by second treatment application. At harvest, data on weight of fresh shoot, root and yield were taken. Soil and root samples were collected in zigzag pattern for final nematode assay. Soil chemical analyses were carried out to determine the final nutrient composition of the soil.

All data collected were subjected to analysis of variance (ANOVA) using GENSTAT statistical package 12th edition and significant means were separated using Fisher's protected LSD at alpha = 0.05.

RESULTS

Screen house results revealed high number of soil nematode population in all the varieties of groundnut. There was ingress of some juveniles into the roots of all the tested varieties as revealed from the extraction (Table 1). The field results showed that all the treated plants performed significantly higher ($p < 0.05$) than the control in terms of vegetative growth parameters and yield as well as reduction of nematode population. Both treatments were effective from the third week of planting to the tenth week. *L. camara* treated plants recorded significantly higher mean plant height than and *P. biglobosa* in untreated plots (Table 2a). However, treated plots were significantly taller ($p < 0.05$) than control plots. Table 2b shows no significant difference in mean plant height of groundnut variety. There were no significant differences ($p > 0.05$) among varieties as shown in Table 3a. Table 4a shows that the mean fresh shoot, root and fruit weights were significantly higher in *L.camara* treated plots than

those treated with *P. biglobosa*. There were no significant differences ($p > 0.05$) between *P. biglobosa* treated and untreated plots. Table 4b shows that there were no significant differences ($p > 0.05$) between varieties with respect to mean fresh shoot, root and fruit weights of the plant. Table 5 indicates that the treated plots performed significantly better than the untreated plots in terms of soil and root nematode population. The physicochemical analyses of soil in Table 6 revealed a significant increase in soil nutrient composition after harvest than the initial nutrient composition before planting. Phytochemical screening of the aqueous leaf extracts showed that the phytochemical constituents of *L. camara* aqueous leaf extracts were significantly higher than phytochemical constituents of *P. biglobosa* aqueous leaf extracts (7).

DISCUSSION

Screen house trial showed that some *Meloidogyne incognita* juveniles ingressed the roots of all the tested groundnut varieties but they did not produce galls. A significant reduction in Nematode population densities in the soil and roots was observed in this study, following the treatment with *L. camara* and *P. biglobosa* which translated to increased growth and yield of the crop. This is in line with Chitwood (2002) who reported that plants are important sources of naturally occurring pesticides containing nematicidal compounds such as alkaloids, fatty acid, diterpens, glucosinolates, isothiocyanates, phenols, sesquiterpenes and thienyls that have given satisfactory nematode control. The reduction in nematode population could be attributed to the effect of these phyto-compounds in the aqueous leaf extracts used. Additional nematostatic or nematicidal effect might be due to the release of allelo-chemicals from groundnut roots thus reducing the ingress of these nematodes and associated damage or yield loss. Other factors responsible for the reduced nematode population may be attributed to the inability of female root-knot nematode to produce eggs or large number of eggs as a result of nematostatic effect of botanicals. Neeraj, *et. al.*, (2017) had demonstrated that botanicals have the ability to inhibit egg hatchability in root-knot nematodes. Groundnuts are generally known to be resistant to *Meloidogyne incognita*, however, there was evidence from this study that the nematode ingressed the roots but could not cause infection as there were no symptoms of galled roots. The leaf, stem, root and seeds extracts have been reported to contain some phytochemicals such as alkaloids, glycosides, tannins flavonoids, sterols, phenols, quinines and saponins and these exhibit some nematicidal properties (Rajinikanth *et al.*, 2013). The higher level of tolerance exhibited by the treated varieties might be due to chemicals produced by the treatments, that were translocated systematically throughout the plants and possibly activated the plant's defense mechanisms.

It is also possible that the concentration of the bioactive ingredients in the roots were not high enough to totally ward off these nematodes from ingressing the roots. Hence, their presence inside the roots possibly caused some physiological disruption in the system which resulted in

malfunctioning of the organs translating to poor growth and development. Varietal differences among these groundnuts could be another factor responsible for its tolerance. Addition of aqueous leaf extract could serve as soil amendment which contributes to the plant development.

CONCLUSION

Meloidogyne incognita ingressed the roots of the test groundnut varieties and caused yield reduction. *L. camara* and *P. biglobosa* are effective in the management of root-knot nematodes and improved yield. The botanicals investigated in this work could have practical application in the protection of groundnut from attacks of *M incognita* due to their inherent advantage such as environmental safety, easy handling, low toxicity to plants and mammals and low cost.

Table 1: Screen house test for the pathogenicity of groundnut variety with *Meloidogyne incognita*

Variety	Soil Nematode Population at end of pot trial	Root Nematode Population at harvest
V1	183 ^{ab}	13.67 ^a
V2	170 ^a	14.33 ^{ab}
V3	185 ^{ab}	12.77 ^a
V4	186 ^{ab}	12.67 ^a

V1= Samnut 10, V2 = Samnut 11 , V3= NUT KSV – 8, V4 = NUT IT – 288

Table 2a: Effect of plant extracts on mean plant height (cm) of groundnut varieties infected with *Meloidogyne incognita*

Plant Extract	weeks after planting									
	1	2	3	4	5	6	7	8	9	10
<i>Parkia biglobosa</i>	3.24	6.41	9.14 ^a	11.84 ^a	14.40 ^a	15.13 ^a	17.21 ^a	24.40 ^b	25.80 ^b	28.62 ^b
<i>Lantana camara</i>	3.03	6.11	8.70 ^a	11.61 ^a	13.61 ^a	15.30 ^a	16.84 ^a	27.80 ^a	31.90 ^a	32.80 ^a
Control	2.80	5.73	6.50 ^b	7.44 ^b	8.70 ^b	9.80 ^b	11.12 ^b	17.72 ^c	19.40 ^c	20.12 ^c
S.E.M	0.14	0.27	0.23	0.54	0.52	0.56	0.62	0.68	1.26	0.94
	NS	NS								

Note: Values with the same superscript(s) down the column are not significantly different at P=0.05 (means were separated with Fisher's Protected LSD)

NS = Not significant

Table 2b: Effect of groundnut variety on mean plant height (cm) of groundnut varieties infected with *Meloidogyne incognita*.

Groundnut Variety	weeks after planting									
	1	2	3	4	5	6	7	8	9	10
Samnut 10	3.31	6.61	8.76	11.01	13.20	14.32	15.93	24.04	25.01	27.50
Samnut 11	3.22	6.40	8.41	11.00	12.40	13.60	15.14	24.30	27.61	28.30
NUT KSV -8	2.70	5.53	7.70	9.63	11.13	12.30	14.14	22.90	26.70	28.00
NUT IT -288	3.10	6.11	7.91	9.70	13.41	14.22	16.01	23.40	26.23	27.44
S.E.M	0.18	0.38	0.74	1.68	1.75	1.60	1.66	2.84	3.19	3.23
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values with similar superscript (s) down the column are not significantly different at P=0.05 Fisher's Protected LSD. NS = No significance

Table 3a: Effect of plant decoctions on mean number of leaves of groundnut varieties infected with *Meloidogyne incognita*.

Plant Extract	Weeks After Planting									
	1	2	3	4	5	6	7	8	9	10
<i>Parkia biglobosa</i>	14.11	25.11	36.12	51.82	60.11	75.90	85.11	98.33	118.44 ^a	130.78 ^a
<i>Lantana camara</i>	16.44	23.22	30.22	42.44	57.60	65.70	78.22	86.22	95.114 ^b	104.90 ^b
Control	13.22	21.90	32.33	42.22	55.11	60.70	68.90	79.11	93.44 ^b	101.44 ^b
S.E.M	1.30	0.79	1.37	4.09	5.01	6.04	5.53	6.25	3.96	3.49
	NS	NS	NS	NS	NS	NS	NS	NS		

Note: Values with the same superscript (s) down the column are significantly not different at P=0.05 Fisher's Protected LSD

NS = Not significant

Table 3b: Effect of groundnut variety on mean number of leaves of groundnut varieties infected with *Meloidogyne incognita*.

Groundnut Variety	Weeks After Planting									
	1	2	3	4	5	6	7	8	9	10
Samnut 10	12.11 ^b	22.22	30.44	38.90	49.80 ^b	58.11	68.92	78.60	96.44	106.80
Samnut 11	16.33 ^a	24.22	34.60	48.11	65.11 ^a	72.80	82.74	92.80	105.80	114.22
NUT KSV -8	16.44 ^a	25.21	34.90	49.70	63.70 ^a	68.80	75.70	83.90	95.70	105.90
NUT IT -288	13.80 ^{ab}	23.80	33.60	48.70	57.90 ^{ab}	71.33	81.33	92.33	104.80	116.11
S.E.M	0.87	1.10	1.60	3.71	3.19	5.57	5.64	6.90	8.54	9.35
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values with similar same superscript(s) down the column are not significantly different at P=0.05 according Fisher's Protected LSD

NS = Not significant.

Table 4a: Effect of plant extracts on mean shoot, root and fruit weight (gm) of groundnut varieties infected with *Meloidogyne incognita*.

Plant extracts	Shoot	Root	Fruit
<i>P. biglobosa</i>	389.44 ^{ab}	183.33 ^b	95.50 ^b
<i>L. camara</i>	578.33 ^a	268.33 ^a	228.90 ^a
CONTROL	240.60 ^b	115.90 ^b	41.70 ^b
S.E.M	58.1	22.9	38.40

Values with similar superscript (s) down the column are not significantly different at P=0.05 according to Fisher's Protected LSD

NS = Not significant

Table 4b: Effect of groundnut variety on Shoot, Root and Fruit weight (gm) of groundnut varieties infected with *Meloidogyne incognita*.

Groundnut Variety	Shoot	Root	Fruit
Samnut 10	444.44	203.33	141.11
Samnut 11	432.	200.10	144.44
NUT KSV -8	321.11	153.33	78.90
NUT IT -288	342.22	173.33	81.11
S.E.M	96.0	42.2	79.3
	NS	NS	NS

Values with the same superscript(s) down the column are not significantly different at P=0.05 using Fisher's Protected LSD

NS: Not significant

Table 5: Soil Nematode Population

	Initial Soil Nematode Population Before Treatment	Soil Nematode Population at one month after Treatment	Soil Nematode Population at harvest	Root Nematode Population at harvest
V1T1	142.10ab	123.0 cd	51.33 c	4.667 bc
V2 T2	145.23 b	115.0 bc	54.33c	1.333ab
V3 T1	152.11 c	113.7 bc	55.00c	4.000 b
V4 T1	149.23 c	106.3ab	56.67c	4.000 b
V1 T2	148.14 b	94.0ab	30.33bc	1.33ab
V2 T2	146.15 b	80.7 a	23.33b	0.333a
V3 T2	152.25 c	84.0a	23.67b	0.333a
V4 T2	149.26 c	81.7a	19.67 a	0.667ab
V1C	152.36 c	134.3d	77.33d	12.333d
V2C	143.60ab	131.3d	76.33 d	13.333d
V3C	150.40 ac	125.0cd	73.67d	11.667d
V4C	135.50 a	125.7cd	76.33 d	12.667d

V1T1= Samnut 10 +*P.biglobosa*, V1T2= Samnut 10 + *L. camra*,V2T1 = Samnut 11+ *P. biglobosa*, V2T2= Samnut 11+*L. camara*,V3T1= NUT (KSV – 8),+*P. biglobosa*, V3T2= NUT (KSV – 8)+*L. camara*,V4T1 = NUT (IT – 288) + *P. biglobosa*, V4T2= NUT (IT – 288) + *L.camara*

Table 6: Soil nutrient composition

Nutrients	Treated Plots		Control Plots	
	BP	AH	BP	AH
Na ⁺ (cmol/kg)	0.37	0.12	0.45	0.33
K ⁺ (cmol/kg)	0.16	0.003	0.16	0.008
Ca ⁺⁺ (cmol/kg)	0.0026	0.0002	0.003	0.002
Mg ⁺⁺ (cmol/kg)	0.0015	0.0004	0.0018	0.0006
Phosphorus (ppm)	0.0016	0.0018	42.7	0.0004
%N	11.24	13.25	11.20	8.09

Keys: N⁺ = Nitrogen ions, Ca⁺⁺ = Calcium ions, Mg⁺⁺ = Magnesium ions, P = Phosphorus,
 Na⁺ = Sodium ions, K⁺ = Potassium, BP = before planting and AH = At harvest

Table 7: Phytochemical constituents of *Parkia biglobosa* and *Lantana camara*

Constituents	<i>Parkia biglobosa</i>		<i>Lantana camara</i>	
	Ethanol Extract	N-Hexane Extract	Ethanol Extract	N-Hexane Extract
Alkaloid	++	++	+++	++
Tannin	+++	++	-	++
Saponin	++	-	+++	++
Flavonoid	+	+	+++	++
Steroid	-	+	+	+
Terpenoid	-	++	+	+
Phenol	+++	++	+	++

Absent (-), present at low levels (+), present at moderate levels (++), present at high levels (+++).

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