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Original Research

Efficacy of Aqueous Leaf Extracts of Negro Coffee (*Cassia occidentalis*) and Lemon Grass (*Cymbopogon citratus*) in the Management of Nematode Pests of Okra (*Abelmoschus esculentus* L. Moench)

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Abstract

A two-year rain-fed field trials to evaluate the efficacy of aqueous leaf extracts of *Cassia occidentalis* and *Cymbopogon citratus* at different levels in the management of nematode pests of okra was conducted. The levels of treatments used were 25, 50, 75 and 100 %, where 0% served as control. The experimental design was a randomized complete block design (RCBD). Effects of treatments on growth, yield, soil nematode population, root weight and root gall indices were determined. Phytochemical screening and infra-red spectrum to determine the secondary metabolites in the leaf extracts were also carried out. It was observed that the treated plants especially those that received 50% level and above performed significantly better (p=0.05) than the control with respect to the measured parameters. The phytochemical result revealed the presence of tannin (7.4%), crude alkaloids (2.5%), saponin (0%) and crude oxalates (42.28 mg/g) in cassia while lemon grass contained tannin (4.5%), crude alkaloids (0.52%,), saponin (1.76%) and oxalates (0%). The infra-red spectrum revealed that the two plant extracts contained very strong and broad absorption bands ~3400^{-1}cm region, assignable to ~NH stretching mode probably of alkaloid family. The medium absorption bands were due to ~CH bands which are common in natural products. The medium absorption bands are also strong indicators of other compounds in the leaf extracts. The use of botanically derived crude leaf extracts of cassia at 50% and lemon grass at 75% in the management of nematode pests of okra will therefore be of economic benefit, ensuring food security.

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INTRODUCTION

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. Okra ranks high amongst the economical important vegetables of the world. The immature fruits of Okra, which are good sources of vitamin C, are used for the preparation of certain soups and sauces (Diouf, 1997). Successful production of okra in Nigeria has been hampered to some extent by nematode pests, especially the root- knot nematodes *Meloidogyne* spp. (Enopka *et al.,* 1996; Agu and Ogbuji, 2001). According to Sikora and Fernandez (2005), root-knot nematodes are particularly damaging vegetables in tropical and subtropical countries of the world and cause losses up to 80% in heavily infested fields. Three species of root-knot nematodes, M. *javanica*, M. *incognita* and M. *arenaria*, are found in Nigeria and they attack over 140 species of cultivated plants amongst which are important food crops and vegetables (Enokpa *et al.,* 1996).

In the Tropics, *M. incognita* very frequently attack okra (Singh *et al.,* 1993; Khan *et al.,* 1998). Khan and khan (1994) reported that *M. incognita* elicited leaf browning, suppression in plant growth, fruit yield and photosynthetic pigments in okra.

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, 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 (Adekunle and Fawole, 2003; Izuogu *et al*., 2009, 2010, 2011, 2012; Yakubu and Izuogu, 2013; Abolusoro *et al.,* 2013; Olabiyi *et al.,* 2013). 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 (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 aqueous leaf extracts of *Cassia occidentalis* and *Cymbopogon citratus* against nematode pest of okra.

MATERIALS AND METHODS

A rectangular piece of land measuring 25m by 40m on the Teaching and Research Farm of the University of Ilorin was ploughed and harrowed in April, 2009. Seeds of root-knot nematode susceptible *Celosia argentea,* L.c.v.TLV-13 were broadcast on the harrowed plot to increase initial build-up of inoculum between April and July 2009. Twelve weeks after planting, the heavily infected root systems of the crop residues were ploughed 0.20 cm into the plot to increase the primary source of inoculum for the two year trials in 2009 and 2010. The land was ridged and divided into 4 blocks and 40 plots with alleys measuring 1m in between blocks and 0.5m in between plots. Soil samples were collected for identification of nematode genera present and their initial population. The treatments which comprise aqueous extracts of *Cassia occidentalis* and *Cymbopogon citratus* at 0, 25, 50, 75 and 100 % were used for the trials. While 0% which received no plant extract served as the control treatments, the other levels of treatment were incorporated into the soil at the time of seed planting. Seeds of okra Clemson spineless were sown at a spacing of 60cm on the ridges at the rate of 3seeds/hole. Initial and final soil nematode population were extracted using the method described by Southey (1986). Data collection commenced at two weeks after planting on plant height and number of leaves for eight weeks. At harvest, fruit weight, shoot weight, root weight and final soil nematode population were taken. Root gall index using a scale of 0-5 described by Makete (2000) was rated. The leaf extracts were tested for the presence of bioactive ingredients/ secondary metabolites adopting the techniques described by Guilei, (1969), Harborne, (1976), Trease and Evans (1989). Infra-red spectroscopy was carried out at the Ladoke Akintola University of Technology, Ogbomosho, Oyo state, Nigeria.

RESULTS

Effect of Cassia and Lemon grass on mean plant height (Table 1) shows that there were no significant differences in plant height among the treatments and levels of application at two weeks after planting(WAP) but there was significant differences among the treatments from 4-8WAP. Plants treated with higher levels (50, 75 and 100 %) of both extracts had highest plant height than the control (Nematode only) plants in 2009 and 2010. It was however observed that plants treated with Cassia extract recorded significantly higher plant height than the Lemon grass treated plants in both years of experimental trial.

The number of leaves (Table 2) was higher in Cassia than Lemon grass treated plants from 4-8WAP in 2009 than 2010. Though there were no significant differences at 2WAP in leaves number in both treatments. Meanwhile, plant treated with higher levels (50, 75 and 100 %) of both extracts recorded significantly higher leaves number than the lower level (25%). The least number of leaves was recorded in the control (nematode only) plants of both extracts in both years.

Table 1: Effect of Cassia and Lemon grass leaf extracts on Mean Plant Height (cm) of Okra

TREATMENT: Cassia	2009				2010			
	2 WAP	4 WAP	6 WAP	8 WAP	2 WAP	4 WAP	6 WAP	8 WAP
Control (Nematode + 0% Cassia extract)	11.2	22.0^{a}	30.8^{b}	34.8^{bc}	17.5	25.0^{a}	33.5^{c}	39.7^{b}
Nematode + 100% cassia extract	11.1	25.0^{a}	44.0^{a}	50.5^{a}	19.3	30.0^{a}	50.5^{a}	55.2^{a}
Nematode + 75% cassia extract	11.0	23.4^{a}	36.3^{ab}	42.0^{b}	19.9	27.8^{a}	42.0^{b}	51.3^{a}
Nematode + 50% cassia extract	11.1	22.1^{a}	35.5^{b}	41.2^{b}	18.0	27.6^{a}	41.5^{b}	50.3^{a}
Nematode + 25% cassia extract	10.7	25.6^{a}	31.0^{b}	37.0^{b}	20.4	28.0^{a}	37.0^{bc}	46.8^{b}
TREATMENT : Lemon Grass (LG)								
Control (Nematode + 0% LG extract)	10.0	20.0^{b}	24.7^{c}	31.8^{c}	16.1	23.2^{b}	30.1^{c}	37.4
Nematode + 100% LG extract	11.4	22.5^{a}	34.5^{b}	44.0^{ab}	16.5	28.9^{a}	44.0	49.8^{a}
Nematode + 75% LG extract	11.2	21.6^{a}	33.6^{b}	39.0^{b}	15.7	28.2^{a}	39.0^{b}	43.2^{b}
Nematode + 50% LG extract	10.1	20.4^{a}	32.5^{b}	36.6^{b}	18.2	23.6	32.8^{c}	40.5^{b}
Nematode + 25% LG extract	10.2	21.0^{a}	31.3^{b}	35.3^{bc}	17.0	22.8	31.9^{c}	40.2^{b}
S.E	**1.23 N.S**	**1.74**	**1.82**	**1.65**	**1.64 N.S**	**1.78**	**1.75**	**1.56**

N.S = Not significant; WAP = Weeks after planting; Means in the same column followed by different letters are statistically different at P = 0.05 according to Duncan's multiple range test

Table 2: Effect of Cassia and Lemon grass leaf extracts on Mean Number of Leaves of Okra

TREATMENT: Cassia	2009				2010			
	2 WAP	4 WAP	6 WAP	8 WAP	2 WAP	4 WAP	6 WAP	8 WAP
Control (Nematode + 0% Cassia extract)	5.3	10.3^{ab}	14.0^{c}	21.3^{c}	6.0	11.3^{a}	19.3	23.0^{c}
Nematode + 100% cassia extract	5.5	11.0^{a}	23.0^{a}	30.6^{a}	6.0	10.3^{ab}	27.1	35.6^{a}
Nematode + 75% cassia extract	5.3	9.6^{b}	20.7^{ab}	28.0^{ab}	5.7	9.3^{b}	26.3	30.7^{b}
Nematode + 50% cassia extract	6.0	10.3^{ab}	19.0^{b}	27.0^{ab}	5.6	9.8^{b}	24.0	28.3^{b}
Nematode + 25% cassia extract	5.5	9.4^{b}	17.0^{bc}	23.6^{bc}	5.5	9.2^{b}	23.8	24.6^{c}
TREATMENT : Lemon Grass (LG)								
Control (Nematode + 0% LG extract)	5.5	9.3^{b}	12.5^{d}	19.0^{c}	5.2	10.6^{ab}	16.0	20.0^{d}
Nematode + 100% LG extract	5.4	11.0^{a}	20.1^{b}	27.7^{ab}	5.3	10.4^{ab}	25.4	31.2^{ab}
Nematode + 75% LG extract	6.1	10.4^{ab}	18.5^{b}	25.5^{b}	5.9	9.2^{b}	22.3	29.6^{b}
Nematode + 50% LG extract	5.2	9.3^{b}	17.0^{bc}	24.0^{b}	6.0	9.0^{b}	22.0	25.6^{bc}
Nematode + 25% LG extract	5.3	9.0^{b}	14.6^{c}	23.3^{bc}	5.3	8.5^{b}	21.5	24.8^{bc}
S.E	**0.37 N.S**	**0.39**	**1.25**	**1.62**	**0.36 N.S**	**0.82**	**2.37**	**1.45**

N.S = Not significant; WAP = Weeks after planting; Means in the same column followed by different letters are statistically different at P = 0.05 according to Duncan's multiple range test

At harvest, data recorded included fruit weight, shoot weight and root weight (Table 3). Significant differences were recorded among the treatments in 2009 and 2010. Cassias treated plants recorded higher fruit and shoot weight with least root weight record as compared to Lemon grass treated plants with lower fruit and shoot weight but higher root weight record in both years. However, there was no statistical difference in levels of application of both extracts in both years of the experimental trial. The root gall index record (Table 3) shows higher gall rating in the control plants of both treatments in both 2009 and 2010 while least gall rating index was recorded in plants that received higher levels of the treatments.

Table 3: Effect of Cassia and Lemon grass leaf extracts on Mean Fruit Weight, Shoot Weight, Root Weight & Root Galling Index of Okra

TREATMENT: Cassia	Mean Fruit Weight (g)		Mean Shoot Weight (g)		Mean Root Weight (g)		Root Galling Index	
	2009	2010	2009	2010	2009	2010	2009	2010
Control (Nematode + 0% Cassia extract)	35^{b}	33^{c}	23.6	23.8	6.5^{b}	7.2^{b}	3.6^{b}	3.9^{b}
Nematode + 100% cassia extract	50.6^{a}	56^{a}	45.4^{a}	45.7^{a}	4.2^{a}	4.6^{a}	0.9	1.0^{a}
Nematode + 75% cassia extract	45^{a}	52^{a}	40.7	42.9^{a}	4.6^{a}	4.2^{a}	1.4	1.1^{a}
Nematode + 50% cassia extract	44^{a}	50^{a}	36.0	39.0	4.6^{a}	4.5^{a}	1.6	1.3^{a}
Nematode + 25% cassia extract	40^{a}	45^{a}	30.4	32.8	5.0^{a}	4.8^{a}	1.8	1.3^{a}
TREATMENT : Lemon Grass (LG)								
Control (Nematode + 0% LG extract)	30^{b}	30^{c}	20.2	20.5	6.3^{b}	7.5^{b}	3.5^{b}	3.8^{b}
Nematode + 100% LG extract	44^{a}	46^{a}	40.5	39.0	4.8^{a}	4.5^{a}	1.2	1.4^{a}
Nematode + 75% LG extract	41^{ab}	41^{b}	37.4	35.6	5.1^{a}	4.8^{a}	1.7	1.8^{a}
Nematode + 50% LG extract	41^{ab}	40^{b}	32.0	32.9	5.1^{a}	4.9^{a}	1.9	2.1^{a}
Nematode + 25% LG extract	39^{ab}	40^{b}	30.4	31.7	5.3^{ab}	5.2^{ab}	2.0	2.2^{a}
S.E	**1.78**	**1.56**	**1.66**	**1.72**	**0.84**	**1.12**	**0.35**	**0.62**

Means in the same column followed by different letters are statistically different at P = 0.05 according to Duncan's multiple range test.

Data on nematode population at the initial and final stages of the experiment were also recorded (Table 4). The main nematodes identified were *Meloidogyne spp*, *Pratylenchus*, *Helicotylenchus*, *Radopholus*, *Rotylenchus* and *Xiphinema*. The parasitic nematode population recorded in descending order was *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, *Radopholus*, *Rotylenchus* and *Xiphinema* . Plants treated with lower levels of extracts had significantly higher nematode populations than those that received higher levels of the treatments. Whereas, the final nematode population recorded in the control plants was significantly higher than the treated plants in both years.

Table 4: Effects of *C. occidentalis* and *A. citratus*on soil nematode population at Harvest in 200g soil samples

TREATMENT: Cassia	*Meloidogyne*		*Pratylenchus*		*Helicotylenchus*		*Radopholus*		*Rotylenchus*		*Xiphinema*	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Control (0% Cassia extract)	440	720	15	38	37	42	18	39	10	28	7	7
100% cassia extract	426	89	19	3	34	12	19	6	12	2	6	3
75% cassia extract	375	126	14	5	30	12	15	8	11	3	8	3
50% cassia extract	400	140	20	6	28	10	16	8	8	3	9	4
25% cassia extract	385	148	17	8	26	11	18	12	10	6	5	3
TREATMENT : Lemon Grass (LG)												
Control (0% LG extract)	365	798	17	42	36	49	20	36	9	30	6	5
100% LG extract	411	110	18	10	36	15	17	8	7	4	6	3
75% LG extract	370	136	21	11	31	16	18	10	10	5	7	3
50% LG extract	329	144	13	7	28	14	18	12	8	4	9	4
25% LG extract	420	180	16	12	29	27	16	13	10	7	8	5
S.E	**15.35**	**13.7**	**1.71**	**1.56**	**1.42**	**1.14**	**1.20**	**0.93**	**0.86**	**1.02**	**1.10**	**0.90**

DISCUSSION

From the results obtained, the plant height and number of leaves of the treated plants were significantly higher than the control plants. The reason for this better result recorded might be attributed to the high quantity of active ingredients or the plant secondary metabolites present in the plant materials. Cassia extract treated plants gave better height and leaves number than the lemon grass extract treated plants at both levels of concentration and year of experiment. This might also be due to higher composition of plant metabolites on cassia than lemon grass which could be associated with optimum nutrient uptake by the plant for growth and development. Similar observations were also recorded by Izuogu *et al.,* (2011) on the control of nematode pests of okra with two plant materials.

The fruit weight, shoot weight, root weight and root galling index recorded in both years of the experimental trial were comparatively different in Cassia extract treated plants and lemon grass extract treated plants. The variation in the results can be attributed to the effect of the toxic chemicals in the plant materials on the soil nematode reduction which invariably will increase the activities of soil microorganisms responsible for plant growth and provision of the necessary nutrients required by the plant for optimum yield. Such results have also been reported by Izuogu and Oyedunmade, 2009; Izuogu *et al*., 2012; Abolusoro *et al*., 2013, on the efficacy of selected plant materials on nematode pests of some plants.

Meloidogyne, Pratylenchus, Helicotylenchus, Radopholus, Rotylenchus and *Xiphinema* nematode populations were recorded at the initial and final stages of the experiment at both years. The significant reduction in the population of these nematodes is likely due to the toxicity of the active ingredients on the plant extracts. Plant secondary metabolites such as flavonoids, tannins, saponins, and steroids have been found to be toxic to nematodes at certain levels of concentrations in the plant materials. The results of this experiment corroborate with those of Yakubu and Izuogu, (2013) on the evaluation of botanicals on root-knot nematodes where such active ingredients were found to suppress nematode populations in the field. Olabiyi *et al.,* (2013) also reported similar findings of the efficacy of neem compost on nematodes in spinach. Several other plants and organic materials have also been reported to contain different metabolites necessary for plant growth, better yield and at same time toxic to pathogenic microorganisms in the soil.

CONCLUSION

However, from the results obtained in this experiment, Cassia and Lemon grass plant extracts can be used to control nematode pest of okra in areas where they are hazardous to the growth of the crop. The use of botanically derived crude leaf extracts of cassia at 50% and lemon grass at 75% in the management of nematode pests of okra will therefore be of economic benefit, ensuring food security.

Conflict of Interest

All the authors declared no conflict of interest

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