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Evaluation of plant extracts in the management of root-knot nematode *Meloidogyne incognita* on cowpea [*Vigna unguiculata* (L) Walp]

A.O. Claudius-Cole*, A.E. Aminu and B. Fawole

Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria
** Corresponding author's e-mail: bi_cole@yahoo.com*

Abstract

Water extracts of *Ocimum gratissimum, Azadirachta indica, Vernonia amygdalina* and *Moringa oleifera* were evaluated for their effect on pathogenicity of *Meloidogyne incognita* race 2 and on the growth and yield of cowpea. Eggs and juveniles of *M. incognita* were exposed to water extracts from leaves of these indigenous plants for ten days in a completely randomized design with four replicates. Data on egg hatch inhibition and juvenile mortality were taken daily. Three cowpea cultivars were inoculated with *M. incognita* and later drenched with the botanical extracts at 10,000 mg/kg and 20,000 mg/kg in pots. The experiment was laid out in a randomized complete block design with five replications. Data collected were number of leaves, plant height, grain yield at harvest; nematode population in soil and roots, and the reproductive factor of the nematodes. Egg hatch inhibition ranged from 40% - 63.7% in the extracts compared to the control with 0%. Juvenile mortality in extracts was from 82% - 93.8% compared to the control with 25%. Grain yield of plants treated with *V. amygdalina* at 10,000 mg/kg and 20,000 mg/kg; and 20,000 mg/kg of *A. indica, O. gratissimum* and *M. oleifera* were significantly higher than in the untreated plants. These plants also had nematode reproductive factors comparable to the uninoculated control. This study therefore shows that low to moderate concentrations of these indigenous botanicals are effective in reducing the pathogenicity of the root-knot nematode and is accompanied by a yield increase in cowpea.

Keywords: African basil, cowpea, leaf extracts, Moringa, neem, root-knot nematode management.
Running title: *Ocimum* sp. and *Vernonia* sp. for nematode control in cowpea.

Introduction

Currently in Africa, cowpea (*Vigna unguiculata* (L.) Walp) is one of the priority crops selected for active production, research and utilization. It is highly nutritious, thus enhancing good human and livestock nutrition throughout the world. Being a drought- tolerant and warm weather crop, cowpea is well-adapted to the drier regions of the tropics where other food legumes may not perform well (Hall *et al*., 2002). It also has the ability to fix atmospheric nitrogen through its nodules and it grows well even in the soils with more than 85% sand and with less than 0.2% organic matter and low levels of phosphorus (Singh *et al*., 1997). Also, it is shade-tolerant and, therefore, compatible as an intercrop with maize, millet, sorghum, sugarcane and cotton, as well as with several plantation crops thereby forming a valuable component of the traditional cropping systems. Coupled with these attributes, its quick growth and rapid ground cover checks soil erosion and *in situ* decay of its roots and nitrogen rich residues improve soil fertility and structure which together have made cowpea an important component of subsistence agriculture, particularly in the dry savannas of sub-Saharan Africa (Philips, 1990; Langer and Hill, 1993; Singh *et al*., 1997). Cowpea is widely traded outside the production area; providing cash for the farmer and cheap nutrition for rural and urban dwellers. Other uses are for ornamental purposes and extraction of dyes and soil reclamation (Mangala and Mauria, 2006).

Meloidogyne spp, is a major problem wherever cowpea is grown in most parts of the world. Iheukwumere *et al*. (1995) recognized *Meloidogyne* spp as one of the plant-parasitic nematodes of economic importance in legume production in Nigeria. Sasser (1980), and Afolami and Fawole (1991) documented the nature of plant-parasitic nematode interaction and subsequent crop damage and yield losses. The most destructive ones on local cowpea are the three species of root-knot nematodes; *Meloidogyne incognita, M. arenaria* and *M. javanica*. The root-knot nematode is estimated to cause losses ranging

from 10% to 69% (Ogunfowora, 1976; Olowe, 2009). The nematode also adversely affects nodulation in cowpea at low populations while nodulation may be completely prevented in cowpea plants under high nematode population (Babatola and Adelamo, 1988). Nematode control is necessary in order to reduce crop losses and ensure self-sufficiency in the requirements for food and industrial raw materials. Among the several ecologically-based approaches in nematode management is the use of pesticides of plant origin (Olowe, 1992; Adesiyan *et al*., 1990; Mangala and Mauria, 2006). Extracts from parts of certain plants such as neem (*Azadirachta indica L*.), African basil (*Ocimum gratissimum.*), bitter leaf (*Vernonia amygdalina L*.) and moringa (*Moringa oleifera*, Lam) have been reported to contain pesticidal properties that inhibit egg-hatch and development of *Meloidogyne* spp. (Salawu, 1992a; Aralepo, 1989; Jahn 1989 and Ajayi 1990). It was the objective of this study, therefore, to evaluate the effects of water soluble extracts of leaves of moringa (*Moringa oleifera*), African basil (*Ocimum gratissium*), neem (*Azadirachta indica*) and bitter leaf (*Vernonia amygdalina*) on egg hatch and mortality of second stage juveniles of root-knot nematode in *in vitro* studies; and to determine the effects of the extracts in the management of *Meloidogyne incognita* on cowpea (*Vigna unguiculata*).

Materials and Methods

Preparation of plant extracts and nematode inoculum

Leaves of moringa (*Moringa oleifera*), African basil (*Ocimum gratissium*), neem (*Azadirachta indica*) and bitter leaf (*Vernonia amygdalina*) were collected from the Department of Forest Resources Management, University of Ibadan. The leaves were thoroughly washed to remove soil and other debris. They were left to air dry for five days at room temperature in the laboratory and then ground to powder using a Warring® laboratory dry mill. A stock solution was made by mixing 100 g each of the powdered plant leaves with 1000 ml of distilled water in heating bottles. Heating was done for one hour over a water bath. The extract was allowed to cool and sieved through Wattman No. 1 filter paper placed in a funnel. This initial concentration was 100,000 mg/kg from which dilutions of 10,000 mg/kg and 20,000 mg/kg were made and used as treatments in the experiment. Eggs of *M. incognita* were extracted from galled roots of *Celosia argentea* using the method described by Hussey and Barker (1973). The nematode eggs in the suspension obtained were counted using a counting slide under a stereomicroscope. The number of eggs per ml was adjusted to about 2000 by concentrating the suspension.

***In-vitro* Laboratory Study**

This study was conducted in the Nematology Research Laboratory of the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria. Twenty (20) eggs of *M. incognita* were placed in labeled glass blocks containing two mls of the 10,000 and 20,000 mg/kg extract concentration as well as in a distilled water control. The eggs were picked from the concentrated nematode suspension with a flat tipped picking instrument. There were nine treatments in all (two concentrations of each of the four plant extracts and the distilled water control). Each treatment was replicated four times and laid out in a completely randomized design (CRD). The number of eggs that hatched into juveniles in the test solutions was observed every 24 hours for 10 days. The same extracts were tested on the mortality of 20 newly hatched juveniles of the nematode. The experiment was laid out in a completely randomized design with four replicates and nine treatments as above. The number of dead and living juveniles in the test solutions was observed every 24 hours. Inactive nematodes were noted as 'dead' when they assumed characteristic death position and failed to react to touch with a handling needle. Juveniles that appeared dead were removed from the glass blocks and placed in distilled water for a few minutes for confirmation. The cumulative number of dead juveniles was recorded at the end of 10 days. Both experiments with eggs and juveniles were repeated a second time for verification.

Pot Studies

The pot experiment was laid out on a platform at the roof top garden of the Department of Crop Protection and Environmental Biology (CPEB) University of Ibadan, Nigeria while sandy-loam top soil was collected from the Crop Garden of the department and steam-sterilized for 1 hr 30 min in a soil sterilizer. The soil was allowed to cool and then filled into 20 cm diameter (5 litre capacity) pots. The three cowpea cultivars; IT-93K-1140, IT-85D-2865 and IT-97K-497-2 were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan. The cowpea seeds were planted in pots containing sterilized soil at the rate of two seeds per pot and later

thinned to one seed per pot. The pots were arranged in randomized complete block design (RCBD) with each treatment replicated five times. The plants were watered daily and kept weed-free by hand weeding.

Two week old seedlings of the cowpea cultivars were inoculated with 5,000 eggs of *M. incognita* race 2. Inoculation was done by slowly dispensing 2.5 ml of the previously prepared nematode suspension into holes made in the soil around the plant and as close to the roots as possible. A week after inoculation (3 weeks after planting), the soil in each pot was drenched with 250 ml of the respective plant extract and that of control with distilled water. There were two nematode treatments, inoculated and uninoculated; nine botanical treatments, the four (*O. gratissimum, A. indica, V.amygdalina and M. oleifera)* extracts in two concentrations (10,000mg/kg and 20,000mg/kg) plus the distilled water control. These were applied to each of the three cowpea cultivars.

Data on number of leaves and plant height were taken weekly while grain yield was taken at termination of the experiment. At harvest, the pots were upturned over a polythene sheet and the root system was gently separated from the soil. Roots were thereafter washed and weighed and gall rating was done using the method described by Coyne *et al*. (2007). Extraction of nematodes from the roots was carried out using the sodium hypochlorite (NaOCl) technique (Hussey and Barker, 1980) for eggs and maceration and sieving for nematodes within the roots (Coyne *et al*., 2007). The soil from each pot was properly mixed, from which a 200 ml sample was taken for extraction using the pie pan method of Whitehead and Hemming (1965). Data on counts were transformed using square root transformation. Data collected were analyzed using Analysis of Variance (ANOVA) and means were separated using standard errors of means.

Results

All the extracts used inhibited egg hatch and increased juvenile mortality significantly ($p \leq 0.05$) more than the distilled water control (Figures 1 and 2). The most effective were *M. oleifera* (moringa) and *V. amygdalina* (bitter leaf). Bitter leaf at 20, 000 mg/kg inhibited egg hatch the most at 50% followed by moringa at 20,000 mg/kg (40%), the least effective extract was moringa at 10, 000 mg/kg. All the extracts caused high juvenile mortality of the root-knot nematode when compared with the distilled water control, they were however not significantly different from each other.

The cowpea cultivars showed varying responses to nematode infestation and consequently the effect of the botanical treatments also varied, although all three cultivars were susceptible. The uninoculated plants of all three cultivars had better growth and yield compared to the inoculated plants (Table 1). IT-97K-497-2 had the highest ($P \leq 0.05$) yield of the three cultivars with IT-85D-2865 having the lowest yield, being the most susceptible. Plants treated with bitter leaf and moringa at 20, 000 mg/kg consistently had significantly high ($p \leq 0.05$) number of leaves in the three cultivars of cowpea. Neem treated plants at 20,000 mg/kg concentration produced leaves comparable to the uninoculated control in IT-85D-2865 and IT-93K-1140. All the plants treated with botanical extracts were significantly taller ($p \leq 0.05$) than the untreated inoculated plants, although they were not as tall as the uninoculated control. The tallest plants were those treated with bitter leaf and moringa at 20,000 mg/kg in all the three cultivars. Grain yield of plants treated with moringa at 20,000 mg/kg and both concentrations of bitter leaf were comparable to the uninoculated control. These had significantly higher yields than other treatments.

All the extracts significantly ($p \leq 0.05$) reduced total nematode population except for neem at 10,000 mg/kg in IT-93K-1140 (Table 2). The most effective extracts for reducing the nematode populations were bitter leaf (20,000 mg/kg) and both concentrations of moringa. The characteristic galling symptom of the nematode on cowpea roots was less in plant extract-treated roots ($p \leq 0.05$) than in untreated plants, with bitter leaf and moringa-treated plants having significantly fewer galls than other treatments. Neem at 20,000 mg/kg also significantly reduced nematode damage. The nematodes reproductive ability was reduced most effectively by bitter leaf and moringa at 20,000 mg/kg with the least effective being neem at 10,000 mg/kg.

Discussion

The use of various parts of indigenous plants as botanical extracts has become important in pest management in recent years following the environmental hazards caused by chemical control measures (Olowe 1992; Mangala and Mauria. 2006). It is apparent from the result of this investigation that water soluble extracts from leaves of bitter leaf (*Vernonia amygdalina)*, neem (*Azadirachta indica*), African basil (*Ocimum*

gratissimum) and moringa *(Moringa oleifera)* were toxic to *Meloidogyne incognita* both *in-vitro* and under greenhouse conditions, although, with different degrees of nematotoxicity, even at the relatively low concentrations. Direct contact of the extracts with the eggs and juveniles ensured that the active ingredients in the leaf extracts were effectively delivered to the nematode. The inoculated control which recorded the highest percentage egg hatch was due to the fact that the normal life cycle and activities of the nematode were not interfered with the way the plants extracts did. Percentage mortality of *M. incognita* exposed to extracts revealed that both concentrations of *M. oleifera*, *A. indica* and *V. amygdalina* inhibited juvenile survival and these can be described as the most effective of the plant extracts.

The extracts were not phytotoxic to cowpea; rather they caused increased plant growth, vigour and yield by suppressing nematode population. Yield from IT-97K-492-2 was more than in the other two cultivars in addition to showing some amount of tolerance while IT-93K-1140 and IT-85D-2865 had lower yields and showed more sensitivity to the nematode. Observation on the lack of effect on the number of leaves in this study is similar to that of Egunjobi and Afolami (1976), who observed that there were no significant differences in total number of leaves of plants treated with neem leaf extract of different concentrations. The ability of *M. oleifera, V. amygdalina* and *A. indica* to inhibit nematode activity was expressed in increased grain yield of the cowpea cultivars. Since the objective of every farmer is to make profit via yield, there may be a preference for these botanicals in nematode management, especially when combined with a less susceptible cultivar of cowpea.

Vernonia amygdalina, bitter leaf, inhibited egg hatch and juvenile mortality in *in- vitro* and reduced nematode population in pot trials which was accompanied by increased growth and improved yields. *Vernonia amygdalina* was also found to effectively reduce nematode populations and increase yield of eggplant (Afouda *et al*., 2008). The antifungal properties of *V. amygdalina* was reported by Ekpo (1991) against many fungi attacking maize. Owolade *et al*. (2000) also found *Vernonia amygdalina* to be a good seed treatment for managing seed borne-diseases in maize. This property may also be what is responsible for the extracts efficacy in nematode management. Furthermore, Ajayi (1990) reported that extracts of bitter leaf at 25% and/or 50% concentration compared favourably with carbofuran in inhibiting *M. incognita* egg hatch and juvenile survival to the extent of 100% after 48 hours of exposure.

Moringa oleifera, moringa is widely used in water treatment and in this study was found to be a good inhibitor of nematode egg hatch and juvenile survival. It was also effective in reducing nematode population in plants with a subsequent increase in plant growth and yield. Guzman (1984) found water extracts of moringa to be as toxic to *Meloidogyne incognita* as standard pesticides.

Ocimum gratissimum, African basil, effectively reduced egg hatch and juvenile mortality compared to the control in this study. The concentration used for treating plants in the screenhouse trials, however were only moderately effective in controlling the nematode. Hasabo and Noweer (2005) found that water extract of basil was effective in reducing populations of root-knot nematodes in eggplant to a moderate degree at 5% concentration. Their finding also indicated that these *Ocimum*-treated plants had lower yields, meaning that there was still some effect of the nematode population on the plant. The oils of a composite of *Ocimum* spp whereas proved to be as effective as carbofuran at 25% concentration (Onifade, 2007). This suggests that a water soluble component of the extracted oil may deliver better control of the nematode.

Seed extracts of neem have also been found to be excellent suppressor of *Meloidogyne incognita* (Salawu, 1992a) and *Heterodera sacchari* (Salawu, 1992b). Salako *et al*. (2008) found that many farmers in Nigeria use neem for various crop protection practices with a lot of success. Seed dressing of cowpea with neem reduced seedling infestation of disease by as much as common seed dressing pesticides (Salako *et al*. 2008). Fatoki (2001) also found that neem leaf extracts compared with carbofuran in reducing nematode population in infected cowpea plant with an accompanied yield increase.

The nematicidal effect of the tested extracts may possibly be attributed to their high contents of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knoblock *et al.,* 1989). The mechanisms of plant extracts action may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation (Konstantopoulou *et al*., 1994).

In conclusion, the use of these extracts may well provide one of the efficient and cheap

methods of nematode control that are necessary and environmentally safe to farmers and produce end users. Other researchers (Puri, 1999; Oka *et al.*, 2000; Afouda, 2008) have reported successes in using various plant extracts in nematode management. If low concentrations can be effective in nematode management, as demonstrated by this study, then a given quantity of plant material can be better utilized over a larger area. Therefore, the use of indigenous plant extracts should be considered in integrated disease management strategies. It is suggested that further trials be conducted in the field on the basis of the promising results from these studies.

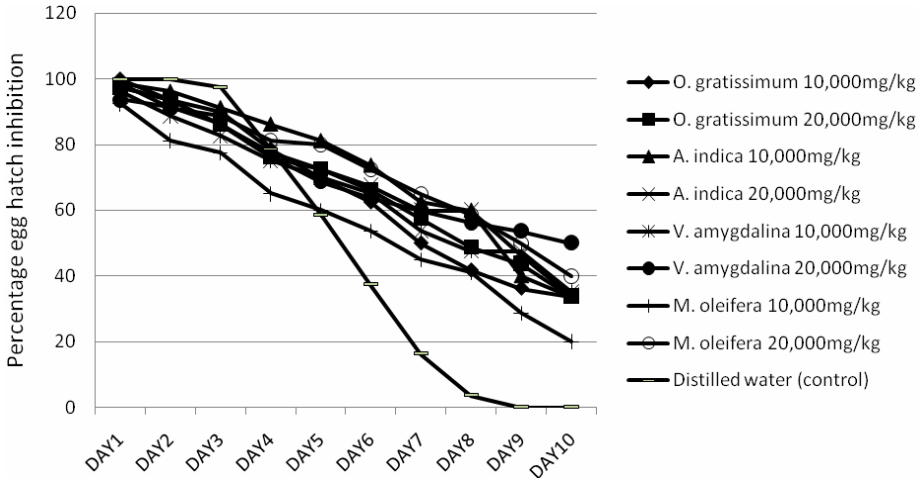

Fig. 1: Cumulative percentage eggs hatch inhibition of *Meloidogyne incognita* exposed to concentrations of botanical leaf extracts.

Values are means of four replicates; bar = standard error; not visible on chart

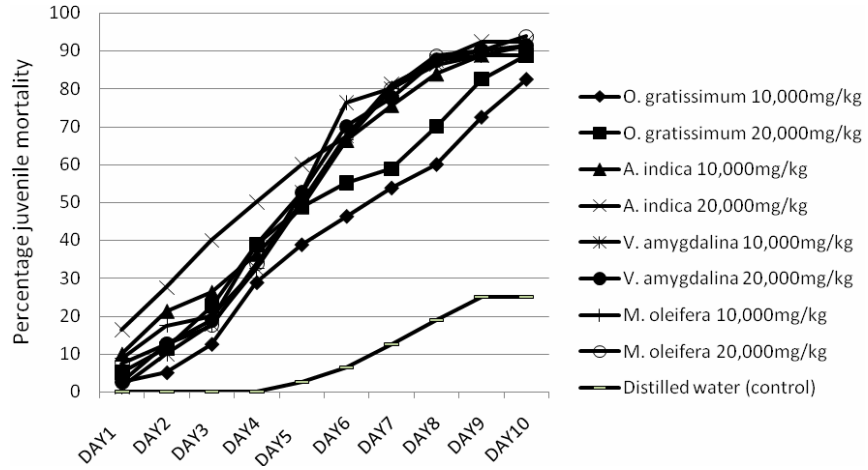

Fig. 2: Cumulative percentage juvenile inhibition of *Meloidogyne incognita* exposed to concentrations of botanical leaf extracts.

Values are means of four replicates; bar = standard error; not visibleon graph

Table 1: Mean number of leaves, plant height and dry grain weight of three cowpea cultivars inoculated with *M incognita* (5000 eggs) and treated with two concentration of four botanical extracts

Botanical extracts	Number of leaves			Plant height (cm)			Dry seed weight (g)		
	IT–93k – 1140	IT–85D –2865	IT– 97k – 497-2	IT–93k – 1140	IT– 85D – 2865	IT–97k – 497-2	IT-93K-1140	IT-5D-2865	IT97K-497-2
O. gratissimum (10,000 mg/kg)	50.4	48.2	40.6	21.7	18.0	17.8	0.5	8.6	12.4
O. gratissimum (20,000 mg/kg)	51.4	49.4	41.9	22.5	19.7	19.8	9.9	8.2	13.1
A. indica (10,000 mg/kg)	50.7	51.5	42.4	21.2	17.9	18.2	9.9	8.2	12.4
*A. indica (*20,000 mg/kg)	55.8	54.1	42.1	21.0	19.2	19.3	11.8	10.2	13.3
V. amygdalina (10,000 mg/kg)	53.1	54.7	56.7	22.1	19.2	20	10.8	8.8	14.1
*V. amygdalina (*20,000 mg/kg)	58.1	53.1	65.9	25.4	23	24.6	11.2	10.1	15.3
M. oleifera (10,000 mg/kg)	51.7	42.2	49.6	24.7	19.3	20.4	11.8	10.7	12.4
*M. oleifera (*20,000 mg/kg)	54.9	42.7	54.6	26.6	23.2	23.8	12.9	11.1	14.1
Distilled water with nematodes	50.1	41.3	40.2	17.2	14.9	15	8.8	8.1	12.3
Distilled water with no nematodes	55.2	54.6	69.4	30.1	26.6	28.1	12.1	10.9	15.2
Mean	53.1	49.2	50.3	23.3	20.1	20.7	10.0	9.5	13.5
Standard Error	0.9	1.7	3.4	1.1	1.0	1.2	1.1	0.4	0.4

Values are means of five replicates.

Table 2: Mean total nematode number per plant and reproductive factor of *M incognita* (5000 eggs) in the cowpea cultivars treated with two concentration of four plant leaf extracts.

Botanical extracts	Total no. of nematodes /plant[1]			Galling index			Reproductive factor[2]		
	IT-93K-1140	IT-85D-2865	IT-97K-497-2	IT-93K-1140	IT-85D-2865	IT-97K-497-2	IT-93K-1140	IT-85D-2865	IT-97K-497-2
O. gratissimum (10,000 mg/kg)	4.5	4.6	4.4	6.1	8.0	5.3	2.7	3.1	3.0
O. gratissimum (20,000 mg/kg)	4.5	4.7	4.3	6.9	9.3	4.2	2.1	2.5	2.3
A. indica (10,000 mg/kg)	4.6	4.7	4.4	7.1	9.6	5.0	2.0	2.3	2.4
*A. indica (*20,000 mg/kg)	4.6	4.7	4.2	7.5	9.8	2.9	1.8	2.1	2.0
V. amygdalina (10,000 mg/kg)	4.0	4.3	4.3	1.9	3.7	4.1	2.2	2.5	2.4
*V. amygdalina (*20,000 mg/kg)	4.0	4.3	3.9	2.1	4.0	1.4	1.7	2.0	1.9
M. oleifera (10,000 mg/kg)	3.9	4.3	3.9	1.7	3.7	1.5	2.0	2.3	2.8
*M. oleifera (*20,000 mg/kg)	4.0	4.3	4.1	2.0	3.9	2.6	1.6	1.8	1.8
Distilled water with nematodes	5.0	5.0	5.0	19.9	22.2	19.7	3.8	4.3	4.6
Distilled water without nematodes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	3.9	4.1	3.8	5.5	7.4	4.7	**1.99**	**2.29**	**2.31**
Standard Error	0.4	0.5	0.4	1.8	1.9	1.8	**0.30**	**0.34**	**0.36**

Values are means of five replicates;
[1] Square root transformed means presented
[2] Reproductive factor = P_f/P_i

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