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Nematicidal Activities of Aqueous Extracts of *Moringa Oleifera* Leaf and Seed on Root-Knot Nematode, *Meloidogyne incognita* Infecting Cucumber

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ABSTRACT
Screenhouse and field experiments were conducted to assess the efficacy of the aqueous extracts of *Moringa oleifera* leaves and seed on the root-knot nematode, *Meloidogyne incognita* infecting cucumber. The experiments were designed as factorial in the screenhouse and on the field. In each case, two concentrations each of the tested plant materials (100% and 50% concentration) were evaluated. The effects of treatments on vegetative growth, weight of fruits as well as nematode population were determined after analysis using ANOVA and the Duncan's New Multiple Range Test. Plant growth was best at 100% of the aqueous extract of *Moringa* leaves. All the treated plants were less galled and had reduced nematode population compared with the control. In the screenhouse and field, 100% and 50% aqueous extracts of *M. oleifera* leaves were more effective in controlling the nematode than the seed extracts. Chemical analysis revealed the presence of flavonoids, alkaloids, saponin, tannins as the active chemical components in the test plant. These components are toxic to micro-organisms including nematodes. The results suggest that both the aqueous extracts of *M. oleifera* leaves and seeds can be used to control the menace of *Meloidogyne incognita* in cucumber in nematode–endemic fields.

Keywords: Aqueous extracts, cucumber, growth, *Meloidogyne incognita*, *Moringa oleifera*.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important economic crops, which belongs to family Cucurbitaceae. It is a native of Asia and Africa where it has been consumed for 3000 years (Alan, 2014). It is grown all over the world as a good source of vitamins, minerals, fiber and roughages. The fruit is used as a vegetable or salad. The immature fruit is cooked and given to children for dysentery. The seeds have a cooling effect when consumed and used as a diuretic. The edible portion, which is about 80% of the fruit, contains 95% water, 0.7% protein, 0.1% fat, 3.4% carbohydrates, 0.4% fiber and 0.4% ash (Chartzoulakis, 2014).

Cucumber yields are frequently reduced by myriads of insects, pathogenic disease, and weed pests (Archana *et al*., 2014). This vegetable crop is attacked by several fungi, bacteria, viruses and nematodes. The root-knot nematode (*Meloidogyne incognita)* is identified as one of the major pests of cucumber, which feeds on the

roots of the plants. Foliage symptoms from the affected root system include stunting, wilting, and leaf yellowing. Infested roots develop galls prevent the normal water and nutrient uptake by roots. (Bernhardt *et al.,* 2013).

Nematode management can be defined as a practice whereby plant parasitic nematode populations are maintained at levels that do not cause economic losses. There are two broad categories of management practices: Chemical and Non chemical. The chemicals used earlier to control plant - parasitic nematodes were usually fumigant and non-fumigant nematicides. These are not only expensive but also cause environmental pollution, phytotoxicity, contamination of ground water and adversely affect the land and its biotic environment. The disadvantages of hazardous chemicals have created interest in searching for alternative methods for plant-parasitic nematode management (Devi, 2002).

Botanical pesticides possess a spectrum of properties including insecticidal activity, repellence to pests, antifeed, insect growth regulation, toxicity to nematodes, mites, snail and slugs, and other pests of agricultural importance (Deka *et al*., 2002). Botanical pesticides are available in many plants for which deep search and testing is required as many of them are still unexplored (Korunic, 2004). There is a need to exploit the toxic components of promising botanicals especially those, which have shown nematicidal properties.

Moringa oleifera, known popularly as drumstick tree is a fast growing, drought resistant deciduous tree, native to the southern foothills of the Himalayas in north western India, and widely cultivated in tropical and subtropical areas where its young seed pods and leaves are used as vegetables. The fruits (pods) are initially light green, slim and tender, eventually becoming dark green, firm and up to 120 cm long, depending on the variety (Martin, 2000). Among its numerous benefits, Moringa leaves and seeds have been found to possess pesticidal properties (Fahey *et al*., 2005).

The acceptance of alternative control measures in the use of botanicals, such as *Moringa oleifera*, which is economical, is therefore inevitable. Nematicidal properties of *M. oleifera* have been reported by several scientists . In tests carried out in the greenhouse at the University of Ilorin, growth parameters of maize plants treated with *M. oleifera* were improved, while the untreated control plants recorded poor growth. There were no statistical differences between the soil nematode population for the treatments, although soil nematode population was lower in treated soils (Izuogu *et al*., 2013). Murslain *et al*. (2013) reported the efficacy of *Moringa oleifera* at various concentrations on the infectivity of root-knot nematode on eggplant. Though *Moringa oleifera* has been shown to possess a high level of efficacy against myriads of commercially important pathogens, more information is needed on their potential as botanicals in the management of root-knot nematodes. The objectives of this study were to: determine the active phytochemical components present in Moringa leaf and seeds; assess the effect of the aqueous extracts of the test plant on the growth and yield of root-knot nematode -infected cucumber in both screenhouse and field; and evaluate the effects of aqueous extracts of *Moringa oleifera* leaf and seed on the soil population of root-knot nematode in the screenhouse and field

MATERIALS AND METHODS

Site description

The study was conducted at the University of Ilorin Teaching and Research Farm (8 29N, 435E), the Southern Guinea Savannah Zone, Nigeria between August and November, 2015.

The source of cucumber seeds and *Moringa oleifera*

The variety of cucumber (Market more) seeds

used was sourced from an agro-chemical outlet in Ilorin metropolis, Ilorin, Kwara State. The leaves and seeds of *Moringa oleifera w*ere collected from Lao area, Ilorin, Kwara State. The seeds were detached from their dry pods and collected in a polythene bag; they were then spread thinly and air-dried (room temperature of 27±2°C) for seven days. The fresh leaves were also collected and air-dried.

Preparation of *Moringa oleifera extracts*

Both dried seeds and leaves of *Moringa oleifera* were ground to powder form (Agbenin *et al.,* 2005). Aqueous extracts of Moringa leaf and seed powders were prepared respectively by thoroughly mixing 100 g powder of each plant material in 1000 ml of boiled water. The resultant mixture from each plant material was left for 48 hrs at laboratory temperature. Thereafter, the mixture was sieved out through cheese cloth, then 15 mm. diam. Whatman No.1 filter paper. Obtained filtrates were used as 50% (initial filtrates diluted to half its strength) and 100% (initial filtrates undiluted) concentration at the rate of 100 ml per plant.

Phytochemical Analysis

The powdered leaf and seed samples (100 g) were respectively extracted with ethanol, n-Hexane, ethyl acetate and water, and the defatted extracts were tested for flavonoid using the method of Bohn and Kopcipal-Abyazan (1994), saponins according to Sofowora (1982), alkaloids using Harborne (1973) method, glycosides, tannin according to Van and Robinson (1981) method, and phenols using diethyl ether reagent according to Adamu *et al*. (2007).

Field layout and planting of cucumber seed

The experiment was designed as a 2 x 3 factorial fitted into a Randomized Complete Block Design (RCBD) and Completely Randomized Design (CRD) for the field and screenhouse studies, respectively and both replicated five times. The factors were: two extracts (leaf and seed) and three concentration levels (0%, 50%, and 100%). The experimental field, which was well-drained coarse sandy-loam, was ploughed, harrowed, ridged and divided into four blocks, each block measuring 100 m × 4 m (400 m^2). There was a 1 m alley between the blocks to avoid bio-pesticide interference. Each block was further divided into 10 plots to accommodate the five treatments outlined with 2 m alley between plots. The blocks were replicated four times.

The soil samples were collected before inoculating with galled roots, randomly from all the plots to assess the initial population of nematodes using modified Baerman's method as described by Whitehead and Hemming (1965). Chopped galled roots were added to the soil before planting to increase the nematode population of the soil. The seeds were planted at the rate of three seeds per hole at the depth of 4-5 cm and separated at the distance of 50 cm. One week after the planting, the seedlings were thinned to a two -plants per stand before application of the treatments.

The treatments applied to cucumber plants in both field and screenhouse were 0% (control), 50% and 100% concentration each of moringa leaf and seed extract. The treatments were applied at the rate of 100 mls in split doses (50 mls at each time) at one week after planting and at four weeks after planting. The plants were weeded every three weeks to enhance their growth as well as remove weeds that might serve to harbor other pests and pathogens.

Screenhouse soil sterilization, layout and planting

Topsoil used for the study was collected from the field in the Faculty of Agriculture, University of Ilorin, Nigeria. The topsoil was sieved and steam sterilized 2 hours at a temperature of 90°C and allowed to cool down for 72 hours using the method described by Gautam and Goswami (2002). The cooled soil

was then transferred into 50, 15-litre perforated buckets each containing 12 kg of sterilized soil and were arranged on slabs to avoid contamination from the ground. Three seeds of cucumber were planted in each bucket. The buckets were arranged in a completely randomized design with five replications then labeled with the specific treatments. Chopped root galls of 100 g were inoculated in the pot to serve as the nematode inoculum before planting. Three seeds of cucumber market more cultivar were planted per pot then later thinned, leaving only the most vigorous plant in each bucket.

Data collection and analysis

The data collected were plant height, the number of branches, the number of leaves, from 2 to 8 weeks after planting, the number of flowers, number of fruits, yield (fruit weight) per plot at harvest, mean root gall and soil nematode population at harvest.

The number of galls induced by *M. incognita* on the entire root system were counted at the termination of the experiment. For nematode soil population counts, composite soil samples from each replicate were sent to International Institute of Tropical Agriculture (IITA), Ibadan for counting at planting, one month after planting and at harvest.

Data collected were subjected to a 2-way Analysis of variance (ANOVA). Separation of means was done using the New Duncan Multiple Range Test at 5% level of significance.

RESULTS

The results from the two trials (field and pot) generally followed a similar trend. Significant differences between the treated plants and the untreated ones were observed (Tables 1-3). All the levels of the test plant extracts performed significantly better in terms of vine length, number of branches, and number of leaves than the control plants. The highest growth of cucumber was recorded with the leaf extracts at 100% and the lowest, with 50% seed extracts. Seed extracts at 100% compared favourably with leaf extracts at 50%.

Table 1: Effect of extracts on the vine length of cucumber inoculated with Root-Knot nematode in field and pot trials at four times intervals

Moringa extracts	Field				Pot			
	2WAP	4WAP	6WAP	8WAP	2WAP	4WAP	6WAP	8WAP
Seed Extract at 100%	14.35b	39.30b	85.65b	130.20b	12.70b	23.40b	37.65b	54.55b
Seed Extract at 50%	10.95d	26.75d	66.85d	117.20d	9.80d	17.75c	32.95d	45.30c
Leave Extract at 100%	17.30a	46.85a	102.70a	156.15a	14.55a	30.00a	47.00a	65.10a
Leave Extract at 50%	12.90c	32.00c	83.85b	133.30c	11.40c	24.65d	40.10b	56.05b
Control	7.10e	12.30e	17.35d	25.05e	6.45e	11.40d	15.85e	22.65d
S.E.M	0.351	0.64	1.18	1.54	0.24	0.45	0.42	0.72

Each value is a mean of five replicates. The figures with the same letter in the same column are not significantly different using new Duncan's Multiple Range Test (DMRT) at P=0.05
WAP: weeks after planting

Table 2: Effect of extracts on the number of branches of cucumber inoculated with Root-Knot nematode in field and pot trials

	Field				Pot			
Moringa extracts	2WAP	4WAP	6WAP	8WAP	2WAP	4WAP	6WAP	8WAP
Seed Extract at 100%	4.60b	11.10c	20.30b	29.90c	4.10bc	9.40b	19.00b	27.10c
Seed Extract at 50%	4.80b	9.30d	16.00c	23.80d	3.70c	8.00c	14.90c	21.80d
Leaf Extract at 100%	5.30a	14.60a	28.00a	43.20a	4.80a	11.40a	23.70a	37.80a
Leaf Extract at 50%	5.40a	11.90b	21.40b	33.10b	4.50ab	9.70b	18.40b	29.70b
Control	3.40c	6.60e	10.90d	16.60e	2.50d	6.00d	9.80d	15.30e
S.E.M	0.16	0.21	0.47	0.87	0.16	0.21	0.39	0.66

Each value is a mean of five replicates. The figures with the same letter in the same column are not significantly different using new Duncan's Multiple Range Test (DMRT) at P=0.05
WAP: weeks after planting

Table 3: Effect of extracts on the number of leaves of cucumber inoculated with Root-Knot nematode in field and pot trials

	Field				Pot			
Moringa extracts	2WAP	4WAP	6WAP	8WAP	2WAP	4WAP	6WAP	8WAP
Seed extract at 100%	6.00b	13.60b	25.10b	33.90c	5.00b	12.40b	23.00b	21.70c
Seed extract at 50%	5.60b	10.90c	19.10c	27.30d	4.40c	9.80c	17.00c	25.50d
Leaf extract at 100%	6.60a	16.70a	32.30a	48.90a	5.90a	15.50a	28.30a	44.50a
Leaf extract at 50%	6.60a	13.70b	24.10b	37.90b	5.30b	12.90b	22.30b	35.30b
Control	4.40c	7.80d	13.50d	20.20e	3.50d	7.00d	11.90d	17.30e
S.E.M	0.17	0.23	0.48	0.98	0.18	0.37	0.52	0.88

Each value is a mean of five replicates. The figures with the same letter in the same column are not significantly different using new Duncan's Multiple Range Test (DMRT) at P=0.05;
WAP: weeks after planting

There were significant differences in the yield parameters among different treatments applied on cucumber planted on *M. incognita* infested soil in the screenhouse and field (Table 4). All the yield parameters (fruit weight, fruit girth, shoot weight, and number of plants with 20% flowering) in the control plants were significantly reduced as compared with the treated plants. Highest yield parameters were recorded in plants treated with leaf extracts at 100%. There was no significant difference in yield parameters in plants treated with *M. oleifera* leaf at 50% and seed at 100%, while *M. oleifera* seed at 50% recorded the lowest means among plants treated with the extracts.

Table 4: Effect of *Moringa oleifera* aqueous extracts on the yield, shoot weight, fruit girth and 20% flowering of Cucumber inoculated with root-knot nematode in field and pot trials

Moringa extracts	Yield (kg)		Shoot weight (g)		Fruit girth (cm)		20% flowering	
	Field	Pot	Field	Pot	Field	Pot	Field	Pot
Seed extracts at 100%	7.70b	6.90a	30.00b	24.00b	6.41b	6.29b	8.20b	7.80b
Seed extracts at 50%	7.20c	5.80b	24.00c	19.00c	5.89c	5.64c	6.90c	6.60c
Leaf extracts at 100%	9.00a	7.10a	37.00a	31.00a	6.73a	6.56a	10.40a	9.10a
Leaf extracts at 50%	7.90b	6.20b	29.00b	26.00b	6.42b	6.18b	8.50b	7.20bc
Control	5.70d	4.20c	17.00d	15.00d	4.73d	4.69d	3.40d	2.80d
S.E.M	1.00	2.00	1.00	1.00	0.07	0.06	0.35	0.33

Each value is a mean of five replicates. The figures with the same letter in the same column are not significantly different using new Duncan's Multiple Range Test (DMRT) at P=0.05

Table 5 shows the mean soil nematode population before planting, four weeks after planting, nematode population at harvest and the number of root galls of both field and pot trials. It was observed that the different application rates had significant effect on parameters describing plant damage by *Meloidogyne incognita*. *Moringa oleifera* leaves and seed extracts were effective in significantly reducing the nematode population and number of root galls as compared with the untreated control. The highest reduction level was recorded from application of *M. oleifera* leaves (100%) followed by *M. oleifera* leaves (50%), *M. oleifera* seeds (100%) and *M. oleifera* seeds (50%).

Table 6: The result of the phytochemical screening of *M. oleifera* leaf and seed extracts are shown in table 6. The screening showed that the plant leaves contained flavonoids, saponins, tannins, glycosides, steroids and phenols. However, the alkaloids were found to be absent in the leaf extracts. While saponins, alkaloids and tannins were found in the seed extracts.

Table 5: Effect of extracts on the nematode population and root gall of Cucumber inoculated with Root-Knot nematode in Field and Pot trials

Moringa extracts	Nematode population						No. of galls on roots	
	Before planting		4 WAP		At Harvest		At harvest	
	Field	Pot	Field	Pot	Field	Pot	Field	Pot
Seed extract at 100%	177.00a	216.00a	132.00c	159.00a	97.5c	81.00c	25.34cd	25.00c
Seed extract at 50%	178.50a	223.50a	135.00cd	160.50a	108.00d	91.50d	27.70d	27.00c
Leaf extracts at 100%	177.60a	220.50a	117.00a	156.00a	61.50a	57.00a	18.00a	17.20a
Leaf extracts at 50%	186.00a	229.50a	120.00ab	151.50a	84.00b	72.00b	22.50b	21.10b
Control	174.00a	229.50a	360.00e	437.50b	462.50e	485.00e	71.70e	27.10c
S.E.M	5.22	5.37	4.41	4.74	3.27	3.42	1.07	1.19

Each value is a mean of five replicates. The figures with the same letter in the same column are not significantly different using new Duncan's Multiple Range Test (DMRT) at P=0.05.
WAP= Weeks after planting.

Table 6: Phytochemicals present in *Moringa oleifera* leaf and seeds

Phytochemicals	Leaf extracts using solvents				Seed extracts using solvents			
	Ethanol	n-Hexane	Ethyl acetate	Water	Ethanol	n-Hexane	Ethyl acetate	Water
Flavonoids	-	+	-	+++	-	-	-	-
Saponins	+	+	-	+	+	+	-	+
Alkaloids	-	-	-	-	-	-	+	+
Tannins	+++	-	++	-	-	-	-	+
Glycosides	-	+	-	+	-	-	-	-
Steroids	-	+	-	+++	-	-	-	-
Phenols	-	-	-	++	-	-	-	-

Note: +++ appreciable amount; ++ moderate amount; + trace; - complete absence

DISCUSSION

Results of plant extracts tested against *Meloidogyne incognita* in the screenhouse and on the field showed that the nematode soil population of cucumber at harvest was suppressed and that of number of galls was reduced in plots where Moringa leaf extract (100 and 50%) and Moringa seed extract at 100 and 50% were applied. These observations show that the leaves and seeds of *Moringa oleifera* were effective in controlling *M. incognita* at various concentrations at which they were tested. Moringa leaf powder was not phytotoxic, improved plant growth and number of leaves (Guzman, 1984). The nematicidal effect of Moringa leaf powder could be attributed to its high content 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 (Knobloch *et al*., 1989).This is in accordance with Pandey (2002) who studied the effects of neem cake and *Cymbopogon flexuosus* (leaves), *Cymbopogon winterianus* (leaves), *Spilanthes acmella* [*Blainvillea acmella*] (shoots), and *Costus speciosus* (shoots and rhizomes) dried powder against root- knot nematode (*Meloidogyne incognita*) population. All treatments significantly suppressed nematode population and enhanced plant dry and fresh weights.

The results obtained in this study are consistent with the findings of Belay *et al*. (2015) who reported the efficacy of the aqueous extracts of the leaf and seed of the *Brassica napus* L. (Rape seed), *Lantana camara* L. (Lantana), *Tagetes erecta* L. (African marigold), and *Azadirachta indica* L. (Neem) at 5% and 10% concentration resulting in 84.67 - 100% mortality of the juveniles of *M. incognita* after 72 hours of exposure *in vitro*.

According to Oladokun *et al.* (1987), growth can be evaluated by measuring certain vegetative characters of the plant. These include shoot length or plant height, number of leaves, number of branches, leaf area and days to 50% plant flowering, shoot weight, number of fruit, less root galling index. This study showed that the plant vine length, number of leaves and of branches and yield were increased in the cucumber plants that were treated with the test plant extracts. It was observed that cucumber that were treated with 100 and 50% leaf extracts, and 100% seed extracts of the test plant in most cases grew taller, had higher number of leaves, more branches and yield when compared with cucumber that were treated with lower concentration (50%) of seed extracts of the test plant.

This result is in agreement with that of El-Sherif *et al*. (2014) who reported the nematicidal impacts of magnetic iron, *Bacillus thuringiensis* (B.t) and dry leaf powder of moringa singly and integrated as dual or triple treatments comparing with oxamyl on adjusting *M. incognita* infecting eggplant under greenhouse conditions. The results of this study reveal that all tested treatments significantly ameliorated eggplant

growth and reduced tested nematode parameters . The bioactive agent, flavonoid was found in the moringa leaf extracts. This is probably responsible for the efficiency of the plant material in reducing nematode population in the soil and hence enhanced growth and yield of all the treated plants. This is in agreement with the findings of Deveral (1972) who reported the pesticidal astringency and repellant nature of flavonoids on nematode pests. Olabiyi (2004) also reported the efficacy of flavonoid in reducing *M. incognita* population as well as enhancing egg-hatch inhibition of *M. incognita*. The result obtained in the qualitative analysis of the test plant extracts showed that both leaf and seed of moringa contained saponin. This also accounts for effectiveness in reduction of soil population of *M. incognita* and enhanced growth and yield of cucumber, this observation is in line with that of Barry *et al.* (1986) . One can infer that alkaloid, flavonoid, saponin and tannin are the major bioactive chemical component of the test plant materials. These basic phytochemicals could be bio-nematicidal in nature and have been reported to confer pesticidal abilities on plant (Izuogu and Oyedunmade, 2009). Some of these phytochemicals such as tannins, saponins, amongst others are reported to have nematicidal properties that caused disruption of membranes in organisms thereby facilitating penetration of toxic principles to the detriment of such organism (D'Addabbo *et al.,* 2010).

From the study conducted in the screenhouse and field, it was discovered that the leaf and seed of Moringa plant hold a promise as affordable, environmentally friendly, biodegradable, available and effective nematicides for controlling root- knot nematode, *M. incognita*. It is therefore concluded that the production in large quantities of these ecofriendly and biodegradable plant materials should be encouraged and the synthetic nematicide be replaced with the use of these plant materials in the control of root-knot nematode, *M. incognita*, in all areas where the nematode is problematic.

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