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EVALUATION OF THE NEMATICIDAL AND ANTIFUNGAL ACTIVITY OF AQUEOUS EXTRACTS OF *MORINGA OLEIFERA* LEAVES AND SEED IN CUCUMBER FIELD

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ABSTRACT. This aim of the two-year study was to evaluate the nematicidal and antifungal activity of *Moringa oleifera* extracts against *Meloidogyne incognita* and fungi infestation in cucumber field. The aqueous extracts of leaves and seeds of *M. oleifera* were used to treat the plants. The findings of the present study revealed that the plant extracts were active against the test pathogens. All treated plants were significantly higher than the control with respect to number of leaves and branches, vine length, fruit weight, and yield. Of the two varieties of cucumber used, combination of cucumber market with moringa aqueous leaf extracts gave higher results. The phyto-chemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins, and tannins. These possess nematicidal and antifungal activities. Combination of variety 2, Market More with *Moringa* leaves aqueous extract is being recommended to farmers for management of nematode and fungal diseases. Organic amendments have the advantage of controlling environmental effluence.

Keywords: *Meloidogyne incognita;* anti-fungi; phytochemicals; *Cucumis sativus*.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most valuable economic crops, which belongs to family *Cucurbitaceae*. It is native to 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 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). The

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production of this crop is severely affected due to some biological and agrochemical constraints in the recent years. Among the biological constraints, the root-rot fungus (*Fusarium solani*) and root-knot nematodes (*Meloidogyne* spp.) rank high among all pathogens attacking cucumber, as they cause tremendous yield losses (Archana and Prasad, 2014).

The root-knot nematodes (*Meloidogyne* spp.) 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). The other soil-borne pathogen, like root-rot fungus, *Rhizoctonia solani* caused root-rot symptoms on cucumber. In addition to the cavities caused during PPN invasion, nematodes play important and destructive role in disease complex, where they either act as stimulant, magnifiers or as vectors, and also produce other forms of mechanical damage to plant roots that are open to exploitation by soil-borne fungi.

The importance of disease complex has been a matter of serious concern from the time when wilt resistant cotton became susceptible in presence of root knot nematode which was first reported by Atkinson (Atkinson, 1892) in Alabama on cotton. The damage caused by nematode alone is less, as compared to damage caused by association of one or more than one pathogen with nematode, which may result in extensive crop loss (Devi *et al.,* 2014).

To overcome this problem, the management of such important pathogens could be achieved with the use of chemicals, fertilizers, broad spectrum pesticides, etc. Pesticides and chemical fertilizers are considered to be the most effective control strategies to date. Their continuous use has resulted in direct toxicity to predators, fishes, man and cattle population and caused adverse effect on soil health and environment (Diwedi and Diwedi, 2007). More attention has been paid to safe and eco-friendly management of such soil-borne pathogens in integrated manner. The excessive use of pesticides in-formed the supplementation or substi-tution of these hazardous chemicals fertilizers with low priced and easily available nutrient sources, such as organic and bio-organics components of environment. The organic matters, like farm yard manure, composts and botanical residues, are being used in various crops. These are store houses of nutrients and found not only in enhancing crop production (Jamwal, 2005), but also had the capability to increase soil fertility (Pathak *et al.,* 2005) and control of pests and diseases of crops. For these reasons, the choice of *Moringa oleifera* for nematode and fungi management was made.

Leaves of this plant are reported to possess various biological active-ties, including hypocholesterolemic, antidiabetic, hypertensive agent and regulate thyroid hormone (Mehta *et al.,*

2003). *Moringa oleifera* is also being studied for its antiinflammatory, antimicrobial, diuretic (Udupa *et al.,* 1994), antibiotic (Eilert *et al.,* 1981), and antimicrobial properties (Palaniswamy, 2004).

Keeping in view of the importance of cucumber and associated pathogens, a preliminary soil-survey was conducted to ascertain the presence of root-knot nematode (*M. incognita*) and fungi in cucumber infested field.

The aim of this study was to assess the effect of *Moringa* seed and leave crude extracts on disease complex involving *M. incognita* and soil-borne fungi in cucumber field.

MATERIALS AND METHODS

Collection of plant materials and extraction

The experiment was conducted in the year 2015 and 2016 in a naturally nematode and fungi infested area in Ilorin metropolis. Leaves and seed were collected from the *Moringa oleifera* plant from Lao area, Ilorin, Kwara State, Nigeria.

The leaves and seeds were air-dried under room temperature for 7 and 14 days, respectively, and pulverized to powder. *M. oleifera* leaf and seed powder aqueous extracts were prepared respect-tively by thoroughly mixing 1 kg powder of each plant material in 4 L of boiled water.

The resultant mixture from each plant material was left for 48 hrs at laboratory temperature. Thereafter, the residue was sieved out through whatman No.1 filter paper. Obtained filtrates were used at the rate of 100 ml per plant.

Sources of root-knot nematodes

Roots of *Celosia argentea* plant infected with root-knot nematodes (*M. incognita*) were collected from a vegetable garden in Lao area, Ilorin, Kwara state. One hundred kilograms of roots were carefully washed to remove soil particles and then cut into small pieces which were evenly incorporated in the plots to increase the initial soil nematode population.

Isolation and identification of fungi from soil sample

One gram each of the four soil samples were suspended in 10 ml of distilled water in four different labelled conical flasks to make microbial suspensions (10^{-1} to 10^{-5}). Dilution of 10^{-3}, 10^{-4} and 10^{-5} were used to isolate fungi. One ml of microbial suspension of each concentration were added to sterile Petri dishes (triplicate of each dilution) containing 15 ml of sterile Potato Dextrose Agar. Streptomycin solution at 0.2 g l^{-1} concentration was added to the medium before pouring into Petri plates to inhibit bacterial contamination. The Petri dishes were then incubated at 28 ± 20°C in dark. The plates were observed everyday up to three days. Six fungal colonies were picked from the mixed culture and sub cultured on fresh plates to obtain the pure culture.

Identification of the soil fungi:

The six fungal isolates obtained before planting and after harvest were taken to the international institute of tropical agriculture (IITA), Ibadan, for identification.

The source of cucumber seeds

Two different varieties of cucumber (Cucumber Market More and Roma-vf) were sourced from Agro-Chemical outlet

in Ilorin metropolis, Ilorin, Kwara State Nigeria.

Experimental design and field layout

The experiment was designed as a 2×3 factorial fitted into a Randomized Complete Block Design (RCBD) and replicated four times. For the two-year trials, the experimental field used was a diseased field infected with fungi and nematodes, which was divided into four blocks serving as replicates. There was a 1m alley between the block to avoid bio-pesticide interference. Each block was further divided into six plots to accommodate the six treatments (variety one treated with *Moringa* leaf and seed extracts with the third plot serving as control, same for variety two). The soil samples were collected randomly from all the plots to assess the initial population of nematodes using modified Baerman's method as described by Whitehead and Hemming (1965) and initial soil fungal population were cultured and isolated. 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 -plant stand before application of the treatment.

One hundred ml each of the treatment filtrates of both samples were applied on the soil to each cucumber plant in the field. The treatment was applied twice; 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 would compete for nutrients and also harbor pests and disease causal agents.

Phytochemical screening

The powdered leaf and seed samples (100 g) were respectively extracted with ethanol, n-Haxane, ethyl acetate and water, and the defatted extracts were tested for flavonoid using the method of Bohm and Koupai-Abyazani (1994), saponins according to Sofowora (1982), alkaloids using Harborne (1973) method, glycosides, tannin according to Van Buren and Robinson (1969) method, and phenols using diethyl ether reagent, according to Adamu *et al.* (2007).

Root-knot development and nematode soil populations

The numbers of galls induced by *M. incognita* on the entire root system were counted and rated according to Taylor and Sasser root gall rating 1978. For nematode soil population counts, composite soil samples from each replicate were sent to International Institute of Tropical Agriculture for counting at planting, one month after planting and at harvest.

Antifungal activity

Agar well diffusion assay

The vulnerability of the fungi to *Moringa* aqueous extract was estimated on Potato Dextrose Agar (PDA) by measuring the diameter of zone inhibition and values as average of three replicates, according to Albuquerque *et al.* (2006).

Data collection and analysis

The data collected include: vine length, the number of branches, the number of leaves, fruit yield, mean inhibition, mean number of root gall and soil nematode population. Data collected were subjected to a two-way Analysis of variance (ANOVA). Separation of means was done using Duncan's new multiple range test (DMRT) at 5% level of significance.

RESULTS

The result of the two years of experiment followed a similar trend and therefore were pooled together. Significant differences were recorded between the treated and the untreated plants (*Tables 1-3*). All the test plant extracts amended in the soil performed significantly higher than the control plants in terms of vine length, number of branches, and number of leaves. Amongst the treatments, the maximum growth of cucumber was obtained with plants treated with the leaf extracts and the lowest with plants treated with seed extracts. Meanwhile, there were increase in the vine length, number of branches and the number of leaves in all the plant extracts, as compared with the control. In all, variety two (Marketmore) performed significantly higher than variety one (Roma VF) from week two to week eight. It was thus apparent that variety two, treated with leaf extracts amidst the other treatments, caused maximization of growth.

Table 1 - Effects of variety and *Moringa* extracts on the vine length of cucumber

Moringa extracts		Number of weeks after planting			
		2	4	6	8
Variety one	Seed extract	8.70a	24.95b	60.90b	105.05c
	Leaf extracts	7.00c	16.70d	46.30d	84.05d
Variety two	Seed extract	7.95b	22.40c	53.20c	111.80b
	Leaf extracts	8.85a	36.85a	72.55a	127.35a
Variety one control		5.40e	8.45f	12.73f	16.21f
Variety two control		5.65d	9.10e	15.40e	18.90e
S.E.M		0.164	0.66	0.96	1.23

Each value is a mean of five replicates. The figures with the same letter in the same column are not significantly different using Duncan's new multiple range test at *P*=0.05.

Table 2 - Effects of variety and *Moringa* extracts on the number of branches of cucumber

Moringa extracts		Number of weeks after planting			
		2	4	6	8
Variety one	Seed extract	2.10c	7.60b	16.30b	24.60c
	Leaf extracts	2.10c	6.70b	12.10c	20.00d
Variety two	Seed extract	2.60b	8.00b	13.80c	27.70b
	Leaf extracts	2.80a	9.20a	20.40a	37.00a
Variety one control		2.00e	4.96d	7.45e	12.21f
Variety two control		2.20d	5.10d	8.50d	13.10e
S.E.M		0.15	0.23	0.62	0.67

Each value is a mean of five replicates. The figures with the same letter in the same column are not significantly different using Duncan's new multiple range test at *P*=0.05.

Table 3 - Effects of variety and *Moringa* extracts on the number of leaves of cucumber

Moringa extracts		Number of weeks after planting			
		2	4	6	8
Variety one	Seed extract	3.10c	9.20b	20.10b	29.90b
	Leaf extracts	3.10c	7.90c	14.70d	23.00c
Variety two	Seed extract	3.60ab	9.50b	17.30c	32.60b
	Leaf extracts	3.80a	11.40a	23.90a	43.60a
Variety one control		3.20c	4.49f	8.00f	14.92e
Variety two control		3.33c	6.30d	10.50e	16.70d
S.E.M		0.15	0.26	2.43	1.02

Each value is a mean of five replicates. The figures with the same letter in the same column are not significantly different using Duncan's new multiple range test at *P*=0.05.

The treatment effects of variety and the aqueous extracts of test plants in the field are shown in *Table 4*. Significant difference was recorded in all plants treated with the test plant extracts on the yield parameters (fruit yield, shoot weight, and fruit girth) of cucumber plant as there were higher total yield compared with the control. The highest total yield was recorded in variety two treated with *M. oleifera* leaves extract, while the lowest yield was obtained in variety one control. From the table, it was observed that all the yield parameters in the control plants were significantly reduced, as compared with the other treatments. There were also significant differences in the varietal response, as variety two performed significantly higher than variety one.

Table 4 - Effects of variety and *Moringa* extracts on the yield, shoot weight and fruit girth of cucumber

Moringa extracts		Field		
		Yield (kg)	Shoot weight (g)	Fruit girth (cm)
Variety one	Seed extract	7.50d	23.00d	6.54bc
	Leaf extract	7.60c	31.00c	6.24c
Variety two	Seed extract	8.30b	36.00b	6.78b
	Leaf extract	9.60a	44.00a	6.88a
Variety one control		5.00f	15.00f	4.32e
Variety two control		6.20e	19.00e	5.14d
S.E.M		0	0	0.11

Each value is a mean of four replicates. The figures with the same letter in the same column are not significantly different using Duncan's new multiple range test at *P*=0.05
V1 = Roma vf; V2 = Cucumber Market More

The treatment effect of variety and *Moringa* showed that all plant extracts amended in the soil suppressed the development of *M. incognita* root galls, and nematode population density (*Table 5*). The highest reduction level of soil nematode population and root gall was recorded from application of leaf extracts on variety two plants and the

lowest at variety one, plants treated with seed extracts. Meanwhile, all the treated inhibited the multiplication of the soil nematode population, compared with the control. The root-knot nematode multiplied well without plant extracts in treated cucumber plants (varieties one and two control).

Table 5 - Effects of variety and *Moringa* extracts on the nematode population

Moringa extracts		Nematode pop. before planting	Nematode pop. 3WAP	Nematode pop. at harvest	Root galls
Variety one	Seed extract	189.00a	129.00d	73.00bc	22.20bc
	Leaf extract	189.00a	123.00c	64.00b	20.00ab
Variety two	Seed extract	174.00a	111.00ab	53.00a	19.40ab
	Leaf extract	177.00a	108.00a	49.00a	16.00a
Variety one control		188.00a	350.00e	465.00e	78.00e
Variety two control		183.00a	270.00d	460.00e	68.4d
S.E.M		2.22	5.52	11.13	2.87

Each value is a mean of five replicates. The figures with the same letter in the same column are not significantly different using Duncan's new multiple range test at *P*=0.05

Table 6 shows the inhibition zone of the aqueous extracts of the test plant. The aqueous extracts of *Moringa oleifera* leaves and seeds showed activity against all tested strains of fungi isolated from the infected field. The leaves extract showed maximum activity against *Aspergillus flavus* and *Penicillium* sp., while the lowest activity was found to be against *Aspergillus niger*. The seed extract was more effective against *Phytophthora* sp. and the lowest activity against *Aspergillus niger*. The largest zone of inhibition was produced by aqueous extract of *M. oleifera* leaves against *A. flavus*.

Table 6 - The inhibition zones of aqueous extract of *M. oleifera* on fungi isolates

Tested fungi	Mean inhibition, % (diameter, mm)	
	Seed extract	Leaves extract
Phytophthora sp.	17.3±0.32	23.4±0.95
Rhizopus sp.	15.9±0.45	29.1±0.74
Penicillium sp.	13.8±0.37	32.3±0.60
Aspergillus niger	6.9±0.26	20.0±0.19
Aspergillus parasidicus	9.5±0.72	24.2±0.33
Aspergillus flavus	14.4±0.67	39.4±0.67

Mean of three replicates ± standard error

Phytochemical screening of *Moringa* leaves and seeds revealed the presence of bioactive ingredients (*Tables 7 and 8*). The aqueous extracts of *Moringa* leaves contained appre-ciable amount of flavonoids and steroids, moderate amount of phenols, and trace amount of saponin and glycosides, while alkaloids and tannins were absent. In the aqueous extracts of the seeds however, saponin was present in moderate amount, while alkaloids and tannins hitherto absent in aqueous leaf extracts were present in trace amounts.

Table 7 - Qualitative analysis result for *M. oleifera* leaf

Parameter	Ethanol	n-Hexane	Ethyl acetate	Water
Flavonoid	-	+	-	+++
Saponin	+	+	-	+
Alkaloids	-	-	-	-
Tannin	+++	-	++	-
Glycosides	-	+	-	+
Steroids	-	+	-	+++
Phenols	-	-	-	++

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

Table 8 - Qualitative Analysis result for *M. oleifera* seed

Parameter	Ethanol	n-Hexane	Ethyl acetate	Water
Saponin	+	+	-	++
Alkaloids	-	-	+	+
Tannin	-	-	-	+

++ moderate amount; + trace; - complete absence

DISCUSSION

Evaluating the nematicidal and antifungal activity of *M. oleifera* leaf and seed extract against nematodes and fungi isolates was carried out in this study.

For this, the aqueous extracts of seeds and leaves of *M. oleifera* were tested against microbial pathogens. The findings of the present study reveal that the plant extracts were active against the test pathogens. It was observed that plant treatment with *Moringa* leaves and seed extract increased number of leaves and branches, vine length, fruit weight, and subsequently higher produce at harvest. This could be as a result of the presence of bioactive ingredients in the extracts of *Moringa* leaves and seeds, which may have been instrumental in suppressing nematode and fungal activities. These phytochemicals may have further helped to improve soil fertility, hence improving growth and yield of the cucumber plants. Foidl *et al.* (2001)

reported that foliar spraying of some plant leaves with *Moringa* extract produced some notable effects as overall increase in plant yield between 20 and 35% and higher sugar and mineral levels.

Among the two *Moringa* extracts examined, the leaf extract was found superior to the seed towards the vegetative growth and yield of cucumber plants. The leaf extracts of *M. oleifera* were more active against varying microorganisms. The maximum growth (vine length, number of leaves and branches), as well as increase in yield of cucumber was obtained with plants treated with the leaf extracts and the lowest with plants treated with seed extracts. The present knowledge is in line with previous studies that ascertained the fact that *Moringa* (leaves and seeds) contain appreciable amounts of specific plant pigments with demonstrated potent antioxidant properties such as the carotenoids (lutein, alphacarotene, beta-carotene and xanthin) and chlorophyll (Owusu, 2008). Besides that, the leaves have high nutritional potentialities of several macro elements as Mg (Yameogo *et al.,* 2011). All treatments were significantly higher than the control. Sivakumar and Ponnusami (2011) indicated that organic manures are fairly good source of nutrients, which boosted plants to uptake progressively beneficial elements, to increase the leaf nutrient status and eventually attain optimum growth and productivity. Different part of *M. oleifera* plants have been reported to be a rich source of important minerals as Ca, Mg, K, Fe, Zn, P, S, Cu, Mn , Se and Na, which can be valorized for a balanced nutrition of populations (Yameogo *et al.,* 2011; Moyo *et al.,* 2011). This also corroborate with a result which showed that foliar treatment with *Moringa* extract increased flowering, drymatter, fruit weight, produced larger flowers and fruits and consequently higher yield at harvest time, greater number of shoots per plant and higher percentage of sugars and minerals and eventually caused plants to be firmer and more resistant to pests and diseases (Foidl *et al.,* 2001).

All plant extracts applied in the soil significantly suppressed the development of *M. incognita* root galls, and nematode population density. Maximum reduction level of soil nematode population and root gall were obtained from application of leaf extracts on variety two plants and the lowest on variety one plants treated with seed extracts. All treatments inhibited the multiplication of the soil nematode population, compared with the control. The root-knot nematode multiplied well without plant extracts in treated cucumber plants demonstrating the fact that the two varieties are susceptible to the organisms. The ability of some plants to exert nematicidal and nematostatic activity has been known for a long time. Many secondary metabolites of plants with nematicidal activity against plant pathogenic nematodes have been reported (Kim *et al.,* 2008).

The results of this study also demonstrated the antifungal activity of the aqueous extracts from *M. oleifera* leaves and seeds against strains of fungi isolated from the infected field. Antifungal potential of aqueous extract of *Moringa* leaves and seed (100 ml) was tested against fungi using mean growth inhibition.

The results obtained showed that *Moringa* leaves and seed aqueous extract exhibited variable antifungal activity ranging from high (39.4± 0.67 mm) moderate (32.3±0.60 mm) and low (20.0±0.19 mm) for leaves and high (17.3±0.32 mm) moderate (15.9±0.45 mm) and low (6.9±0.26 mm) for seed extracts, respectively. These results corroborate the antifungal activity of the essential oil and crude extracts of seeds, leaves, flowers and stems of Moringa against dermatophyte fungi, *Aspergillus* spp., *Penicillium sclerotigenum, Cladosporium cladosporioides* and *C. albicans* (Rocha *et al.,* 2011).

The application of The *Moringa* leaves and seed aqueous extract can be used as inhibitor of *Phytophthora* sp., *Rhizopus* sp., *Penicillium* sp., *Aspergillus niger, Aspergillus parasidicus* and *Aspergillus flavus.* The development of plant extracts and phytochemicals as an alternative to synthetic chemicals has been favoured because many of them are selective and are of little harm to non-target organisms and the environment (Hedin *et al.,* 1997). According to Dahot (1998), *M. oleifera* leaf extracts contain small peptides, which could play an important role in the plant's antimicrobial defense system. The proteins/peptides are believed to be involved in a defense mechanism against phytopathogenic fungi by inhibiting the growth of microorganisms through diverse molecular modes, such as binding to chitin or increasing the permeability of the fungal membranes or cell wall (Chuang *et al.,* 2007).

As for the varietal response, variety two (Cucumber Marketmore) was found to have higher performance than variety one (Roma-vf) and the synergistic effect of variety two and *Moringa* leaves aqueous extracts resulted in the outstanding cucumber vegetative growth and yield obtained in this study.

The present study reveals that *Moringa oleifera* plant shows the presence of phytochemical constituents, like alkaloids, flavonoids, glycosides, saponins, and tannins. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs (Rhoades, 1979). Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes (Korkina and Afanas'ev, 1997). They have been found to be effective antimicrobial substances against a wide array of microorganisms *in vitro* and are known to be synthesized in response to microbial infection by plants. They have the ability to bind with

extracellular and soluble proteins and complexes with bacterial cell walls. Steroids are known for their antibacterial activity specifically associated with membrane lipids and cause leakage from liposomes (Epand *et al.,* 2007). Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal medicines and are under investigation for antibacterial, antineoplastic and other pharmaceutical fun (Yamunadevi *et al.,* 2011). They are also responsible for dissolution of the cell wall of microorganism by weakening the membranous tissue (Hernandez *et al.,* 2000). Tannins have shown potential antiviral, antibacterial and antiparasitic effects. Saponins cause hemolysis of red blood cells (Winter *et al.,* 1993). They also cause inhibition in the cell wall synthesis by forming irreversible complexes with proline rich protein (Mamtha *et al.,* 2004). The saponins have the ability to cause leakage of proteins and certain enzymes from the cell (Zablotowicz *et al.,* 1996). The antifungal activity was screened because of their great medicinal properties towards the pathogenic organisms.

Phytochemical components are responsible for both pharmacological and toxic activities in plants. These medicinally bioactive components exert antimicrobial action through different mechanisms and thus support the antifungal activity of the plant extracts used in this study. From results obtained, the effectiveness of nematicidal and antifungal activities of *M. oleifera* leaf and seeds in cucumber was established. However, in terms of superiority, the combination of variety two, Marketmore and *Moringa* leaves aqueous extract is being recommended to farmers for management of nematode and fungal diseases.

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