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(RESEARCH ARTICLE)

Inhibitory effect of neem (*Azadirachta indica*) and moringa (*Moringa oleifera*) leaf extracts on egg hatch of root knot nematode *Meloidogyne incognita*

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Abstract

This experiment was conducted in the laboratory of the Department of Crop Production of the Federal University of Technology Minna to evaluate the inhibitory effect of *Azadirachta indica* and *Moringa oleifera* leaf extracts at different level of concentrations S (100 %), S/2 (50 %), S/10 (10 %), S/100 (1 %) on egg hatch of *Meloidogyne incognita*. The experiments were laid out in a completely randomized design (CRD) and replicated four times. All the concentrations of *Moringa oleifera* and *Azadirachta indica* and their various combinations inhibited egg hatch of *Meloidogyne incognita* throughout the period of observation with the standard solution. As the egg masses were exposed to these concentrations and the larvae hatched were recorded at time intervals of 3, 6, 12, 24, 48, 72 and 96 hours respectively. It was observed that the standard solution (S) of the leaf of both extracts significantly ($p \leq 0.05$) inhibited egg hatch of *Meloidogyne incognita* in which the S, S/2, S/10 inhibited egg hatch of this parasite compared to S/100 and the control (distilled water) in which high number of larvae of 50, 137 and 75, 133 in *A. indica* and *M. oleifera*, respectively.

Keywords: *Azadirachta indica*; Egg masses; *Moringa oleifera*; *Meloidogyne incognita*; Leaf extracts

1. Introduction

Nematodes popularly called round worms are elongated, cylindrical worms parasitic in flora and fauna or free living in the soil, water. Over 2, 500 species have been described despite being quite difficult to distinguish [1].

Meloidogyne incognita is one of the most widespread nematode pests of tropical and subtropical regions of the world [2]. It belongs to the family Heteroderidea. It is an endoparasite which affect so many species of crops Some of its host are okra, pineapple, bell pepper, pawpaw, coconut, coffee, melon, cucumber, carrot, yam, cotton, rubber, potato, lettuce mango, cassava, banana, plantain, rice, sugarcane, tomato, spinach, cocoyam, maize, tobacco. The economic importance of root knot nematode cannot be overestimated as farmers worldwide lose millions of dollars due to their attack on crops. Plants with root knot nematode show signs like stunted growth, root galls and in chronic cases death of plant [3].

The root knot nematodes have very large host ranges, cultural control methods required careful planning especially in a vegetable fields infested with *M. hapla* can strongly be planted to a non-host crop such as corn, but grower's short-term economic return could be diminished [4]. Plants extract that are less harmful to man but still as effective as the synthetic one are being developed.

The attack of plant parasitic nematodes is most severe on crops when the roots of the seedlings are attacked just after germination [5]. The loss of yield can be as high as 80 % as reported by Siddiqi (2000) [6]. Some of the symptoms exhibited by plants infected by root knot nematode include poor growth, yellowing of leaves and wilting, Characteristic galls can be found along the length of the root, causing swollen roots and reduced root system. In severe case, the roots rot leading to death of the plant. It also makes plant susceptible to fungi and bacterial attack especially bacterial wilt.

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The poor growth and yellow leaves is as a result of the inability of the root to absorb essential element. Even water uptake and transport is affected because of the damage done on the vascular tissues of plants during galling.

Azadirachta indica is native to India where it was given the name "neem". It belongs to the family Meliaceae meaning it's a relative of mahogany. Neem is an attractive broad-leaved, fast growing tree which can grow up to 30 m tall and 2.5 m in girth. Its trunk generally straight is 30-80 cm in diameter. Its spreading branches form a rounded crown of deep-green leaves and honey- flowers as much as 20 m across. It is drought tolerant and ever-green but may shed most of its leaves in severe drought. It thrives mainly in tropical and subtropical region [7].

The neem tree thrives best in sub arid and sub humid regions with annual rainfall of 400-1200 mm. with well drained sandy soil. The dry leaves of neem have long been known to prevent insects from attack store foods, and eating clothes. The leaves are also burnt to chase away mosquito [8]. Neem has long been known for its medicinal value in treating ailments like scratches and skin rashes to malaria and diabetes. Neem oils are also prescribed for healthy hair, balancing of sugar level of blood, detoxifying the blood, and proper liver function.Neem products are reported to be antifungal, antidiabetic, antibacterial, antiviral and sedative by medical practitioners [8].

The seeds can be crushed into powder, soaked overnight in water and sprayed on crops. It acts as a repellant, and egg laying deterrent to insects. In India, urea is coated with neem and used as fertilizer because it reduces pollution, improves the soil and fertilizer potency. It is known to contain some biological active substance like azadirachtine, nimbinekemferol, thionemone etc. that are nematicidal in nature [8].

Moringa oleifera is a perennial tree popularly known as drumstick tree. It is deciduous, short, and slender, possess brittle stems and branches. The tree can grow to a height of about 12 m while its trunk can be 30 cm wide. Its leaves are pale green with small leaflet. The flowers have a pleasant fragrance and, are white or creamy-white in color while the stamens are yellow. The pods are brown, triangular, hanging pendulously and split open lengthwise in three parts when dry. The pod contains seeds about twenty (20) in its piths.

M. oleifera is basically a tropical plant and grows favorably on lowlands but can thrive in highland. It can be found in a wide range of vegetation from rainforest to savannah. It is drought resistant and serves as ingredient in the preparation of many traditional medicines throughout the world. The phytochemical screening of moringa shows that phenolics, flavonoids, tannins, glycosides etc., are present in the extracts [9]. On screening the leave for elements, the predominant mineral elements in the fresh and dried moringa leaf powder were calcium, magnesium, potassium, iron and copper. They accounted for the strong bones and good growth of animals that are feed with moringa products.

The qualitative phytochemical analysis of the aqueous extraction of neem leaves indicates the presence of alkaloids, carbohydrates, reducing sugars, flavonoids saponins, tannins and phenolic compounds. The main mineral elements found in neem are sodium, potassium and calcium, others are magnesium, iron, zinc, cobalt, chromium, nickel, copper, lead, barium, cadmium and aluminum [10].

Nematodes greatly affect agricultural production around the world. Nematodes threaten agricultural produce as they reduce nutrient uptake by roots, feed on the stem, roots, leaves, buds seeds and infected plants. The types of nematodes we have also determine the level of damage caused, others are environmental pH and level of infestation. Nematodes affect so many crops such as tomatoes, okra, maize, banana etc. The final result of nematode infestation is reduction in yield of produce both in quality and quantity.

The use of plan extract is fast gaining popularity in the treatment of parasites. This is because synthetic chemical control is becoming expensive and also toxic to man and his environment [11].

Another form of botanical control is the use of amendment. This is simply the powder form of the plant or part to be used. Amendment is made by first drying the material (plant or it part) under shade and the grinding to powder. Umar and Aji (2013) [1] compared the effect of two amendments; one from biter leaf and the other from cashew seed kernel on the soybean which had been infested with root knot nematode. Both amendments had significant effect on the plant compared to the control experiment which was not treated. It was record that the soybean on the plot that was treated with cashew seed kernel amendment had more leaves, pods and seed weight than plants treated with bitter leaf.

Saravanapriya and Sivakumar (2005) [12] compared the nematicidal tendency of botanical extract viz: leaf powder of Marigold (*Tagetes erecta* Linn), Neem (*Azadirachta indica*), Aak (*Calotropis gigantes* Ait) and seed powder of Watermelon (*Citrullus lanatus* Thumb), and Betel nut (*Areca catechu* Linn). The seed of the test crop tomato was first sprinkled with the dry powder of the botanicals before being planted on nursery bed and the effect was visible as the

more seeds treated with the extracts geminated. This might be because the powder extract acted as a shield on the seed thus prevent nematode attack. During transplant the root of the seedlings were dipped in the water extract before planting. The result showed that Aak and neem are very effective against root knot nematode. This corresponds with the report of John and Hebsy (2000) [13] where neem leave extract significantly reduced galling in plants and improve plant growth.

When neem seed oil and moringa seed oil were combine in the ratio 1:3 and administered to albino rats by Ilesanmi et al., (2017) [14], the rats showed no sign of renal or hepatic injury even though they were immobilized at first. This means that the oil administered sedated the rats and could be further researched as possible cure for insomnia. Also these prove that neem seed oil and moringa seed oil is safe for human even though it's toxic to insects can be used as insecticide.

Botanical come in different parts of plants seed, root, leave, stem, in different forms powder also called amendment, water extract, alcohol extract and can be applied in various ways like sprinkling on seed, root dip, spreading at the base of the plant, or put in holes close to plant root.

The use of chemicals in the control of plant parasitic nematode involves the use of nematicides, mostly inorganic chemicals which may have adverse effect on the produce and the environment. Nematode being one of the major constraints to world agricultural production causing loss in yield, quality and quantity, In view of these, the research work was carried out to determine the efficiencies of botanicals for the control of plant parasitic nematodes.

2. Material and methods

Research work was carried out in Crop Production Department laboratory of Federal University of Technology Gidan Kwano Campus, Minna, Niger State of Nigeria. Location is between Latitude 9° 37′ N and Longitude 6° 28′ E in the Southern Guinea Savannah zone.

The leave of *Azadirachta indica* were collected from a fully grown and matured tree at Bosso campus of Federal University of Technology, Minna Niger State through the use of cutlass while the leave of *Moringa oleifera* were collected from a fully grown matured tree in Gidan Kwano campus of Federal University of Technology, Minna Niger State through the use of cutlass and both were taken to the Crop Production laboratory for further use.

2.1. Preparation of extract from the leaves

The leaves of *Azadirachta indica* and *Moringa oleifera* were detached from their stalk, washed under a tap of running water and weighed with weighing balance in the laboratory. 2 kg each of the detached leaves were then chopped into smaller sizes using a knife, pound with pestle and mortal into a paste. Each paste separately blended in a Philip electronic blender and 2 L of distilled water was added from the required 6 liter to each paste and blended for 2 minutes. The paste was then poured into a plastic container and the remaining 4 liters each added and stirred using a clean glass rod for proper mixing

The plastic containers were covered with aluminum foil paper and left on the laboratory bench for 24 hours. After 24 hours the paste was filtered using Muslim cloth and the filtrate was tagged as standard concentration "S", about ten drops of Streptomycin was added to prevent bacteria growth.

2.2. Preparation of different dilution levels from the standard extract (S).

Four different concentration levels were prepared, undiluted standard solution "S", half concentration (S/2). One-tenth of the standard solution (S/10), and one hundredth of the standard solution (S/100).

They were prepared as follows;

S (100%); this is the standard solution (undiluted)

S/2 (50%); to the standard solution (S) and equal volume of distilled water was added.

S/10 (10%); to each volume of the standard concentration measured ten times of the distilled water was added.

S/100 (1%); to every volume of standard concentration a hundred times its equivalent was added.

0% (C); this treatment is termed which is the distilled water [11].

The egg masses for the experiment were collected from the culture of heavily infested *Cochorus olitorius* (Jews mallow) cultured in the screen house. The plant was uprooted carefully and taken to the laboratory in a polythene bag. The roots were thoroughly washed under running tap water to remove adhering soil to get the egg masses, they were then cut into short pieces for convenient and easy handling and placed in a Petri dish for observation under the aid of light microscope to locate the egg mass. Two egg masses were inoculated into each Petri dish. Proper care was taken to ensure the use of the egg masses within 1-2 hours to avoid hatching before inoculation.

Plastic Petri dishes of 10 cm in diameter were used for this research work. The petri dishes were arranged in a Completely Randomized Design (CRD) on the laboratory desk. For each concentration of extract, 15 ml of solution was poured into the Petri dishes using a calibrated pipette. At the beginning of the experiment, two set of freshly removed egg masses were transferred into the solution of each of the labeled Petri dishes. Equal volume of distilled water was poured into similar sized Petri dishes and inoculated to serve as control. There were four replicates of each treatment and kept at ambient temperature in the laboratory during this research. The total number of larval hatched were recorded at 3, 6, 12, 24, 48, 72 and 96 hours intervals. The data collected were analyzed using the Analysis of Variance (ANOVA) and Least Significant Distance (LSD) at $P \geq 0.05$ was used for comparing mean differences.

3. Results

3.1. Effect of *Azadirachta indica* leaf extract at different levels of concentration and time of exposure on egg hatch of *Meloidogyne incognita*

Table 1 shows the effect of *Azadirachta indica* leaf extract on varying concentrations of the leaf extract on egg hatch of *Meloidogyne incognita* and time of exposure. The result from the experiment carried out indicated that egg hatch decreased with increase in the concentration of *Azadirachta indica* extract. Generally, the total number of larvae hatched differs from each other base on their level of concentration. At 3, 6, and 12 hours there was no significant difference ($p \leq 0.05$) between S, S/2, S/10, S/100 and control. However, at 24 hour, significant differences of egg hatch were recorded in S/100 and S, S/2, S/10 in which between 17 larvae were hatched after 24 hours. The control recorded 78.50 larvae of *M. incognita* at 24 hour. Similar trend were recorded with high level of significant difference ($p \leq 0.05$) of egg hatch at 96 hours of observation.

Table 1 Effect of *Azadirachta indica* leaf extract at different levels of concentrations and time of exposure on egg hatch of *Meloidogyne incognita*

Concentration levels	Period (hours)						
	3	6	12	24	48	72	96
S	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S/2	0.0	0.0	0.0	1.25[a]	1.50[a]	1.75[a]	2.00[a]
S/10	0.0	0.0	0.0	1.50[a]	2.00[a]	2.50[a]	3.50[a]
S/100	0.0	0.0	0.0	17.00[b]	24.00[b]	36.75[b]	50.25[b]
Control	0.0	0.0	0.0	78.50[c]	93.25[c]	115.00[c]	137.00[c]
SE±	0.0	0.0	0.0	6.94	8.19	10.12	12.37

Means of the same letters within the same column are not significantly different, at probability of 5% using Duncan Multiple Range Test (DMRT)

3.2. Effect of *Moringa oleifera* leaf extract at different levels of concentration and time of exposure on egg hatch of *Meloidogyne incognita*

The result shows the effect of *Moringa oleifera* leaf extract and varying concentrations of the leaf extract on egg masses of *Meloidogyne incognita* and the time of exposure. Similarly, egg hatched decreased with increase in the concentration of *Moringa oleifera* leaf extract (Table 2). It was also observed that as the time of exposure of egg masses, the number of egg hatched increased after 24 hours with significant difference between S, S/2, S/10 and S/100 in which 59.5 larvae were recorded. As the time of exposure increased, the number of larvae hatched in the extracts increased significantly

($p \leq 0.05$) with the control recording the highest number of larvae 133.75) at 96 hours of observation. However, the most effective of the concentrations were S, S/2 and S/10 after 96 hours of exposure of the egg masses to the extracts.

Table 2 Effect of *Moringa oleifera* leaf extract at different levels of concentration and time of exposure on egg hatch of *Meloidogyne incognita*

Concentration levels	Period (hours)						
	3	6	12	24	48	72	96
S	0.0	0.0	0.0	1.25^{c}	1.75^{c}	2.00^{c}	2.75^{d}
S/2	0.0	0.0	0.0	2.00^{c}	3.00^{c}	4.00^{c}	5.00^{d}
S/10	0.0	0.0	0.0	2.75^{c}	5.25^{c}	10.00^{c}	14.75^{c}
S/100	0.0	0.0	32.50^{b}	41.25^{b}	54.00^{b}	70.25^{b}	75.00^{b}
Control	0.0	0.0	59.50^{a}	87.75^{a}	115.75^{a}	130.00^{a}	133.75^{a}
SE±	0.0	0.0	5.73	7.92	10.23	11.62	11.79

Means of the same letters within the same column are not significantly different, at probability of 5% using Duncan Multiple Range Test (DMRT)

4. Discussion

From this study, egg hatch of *Meloidogyne incognita* decreased as the concentrations of the leaf extract increased. This could be due to some nematicidal properties such as alkaloids, amides, flavonoids and ketones associated with *Moringa oleifera* and *Azadirachta indica*. The effective performance of *Azadirachta indica* may be as a result of some biological active substances like azadirachtine, nimbine, kemferol, thionemone etc. that are nematicidal in nature [8, 15]]. It is evident that as the diluted extract toxicity decreased, it resulted in correspondent decrease in the inhibition and the least effective was recorded in the control (distilled water) while the most effective of both extracts were recorded in the standard solutions respectively, the result collaborated the findings of Bello *et al.,* (2006) [11] in which egg hatch were inhibited by some botanical extracts.

5. Conclusion

This study shows that *M. oleifera* and *A. indica* leaf extracts inhibited egg hatch respectively. They both possessed nematicidal properties. The egg hatch was dependent and also a function of the length and duration of exposure. The standard concentration of the used plant extract (*M. oleifera* and *A. indica*) in the inhibition of nematode egg hatch was found to be more effective compared to when the level of concentration decreased as in S/100 and the control (distilled water). Based on the result obtained, these plants can be used for the control of *M. incognita* to reduced parasitisation of susceptible crops as well as boosting agricultural production for the sustenance of human population.

Compliance with ethical standards

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Disclosure of conflict of interest

Within the authors of this research work, there was no conflict of interest.

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