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Nematocidal Activity Of African Pumpkin (Momordica Balsamina) Powder And Extract Against Root-Knot Nematode (Meloidogyne Javanica) On Okra (Abelmuscus Esculentus) In Yola, Nigeria

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Abstract
An experiment was conducted in 2022 to find out the nematocidal effects of aqueous extract and powder of Momordica balsamina (African pumpkin) on M.javanica on okra in Yola Nigeria. For the laboratory experiment, there were six treatments comprising the control (Tc-distilled water), crude extract (T1) and four dilutions of the crude extracts as T2 (5 ml), T3 (10 ml), T4 (15 ml) and T5 (20 ml). For the juvenile mortality test, these treatments were applied to 18 perti dishes containing 1000 M. javanica J2 juveniles. These treatments were also applied to 18 petri dishes containing 1000 J2 eggs of M. javanica all arranged in CRD. For the screen house experiment, 18 plastic pots each containing 4 kg sterilized loamy soil incorporated with six levels of M. balsamia leaf powder treatment (Tc (0 g), T1 (5 g), T2 (10 g), T3 (15 g), T4 (20 g) and T5 (25 g)) arranged in CRD. Okra seeds were planted in the pots and one week after emergence were inoculated with 1000 M. javanica juveniles. Data collected were analysed. Results for juvenile mortality showed the crude extract treatment (T1) the highest mortality with 91.67 % followed by T2 (5 ml dilution) with 81.50 % after 72 hours. For egg hatchability test, T1 recorded highest egg hatch inhibition with only 19.30 % hatch after 72 hours. For the screen house test, the 25 g powder treatment (T5) produced the least soil nematode population (228.57) and RF (0.22) showing M.javanica reproduced most poorly in this treatment. Therefore, the experiment reveals the nematocidal potency of extract and powder of M. balsamina leaf and can be used to replace toxic chemical nematicides. Also, field trial of its performance is recommended.

Keywords: *African pumpkin, Powder, Extract, Juvenile, M.javanica*

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I. Introduction

Okra (Abelmoschus esculentus L.), a member of the family Malvaceae, is commonly known as Lady's finger, as well as by several vernacular names, in the different areas of the world where it is cultivated (Jain, 2012). Apart from its use for food, okra fruits have also been recommended to cure dysentery, gonorrhea, and urinary complications (Islam, 2019) its seeds reportedly possess anticancer and fungicidal properties (Durazzo, 2019).

With all the benefits derivable from okra, pests and disease organisms damage still plague the crop and there by reducing its potential yield (Jones 1996). Production of okra at an economically viable level has been hugely curtailed by the incidence and severity of root-knot nematode disease caused by Meloidogyne spp. (Adebowale et al; 2014 and ClaudiusCole 2018). The species that cause the most damage are *M. arenaria*, *M. incognita*, *M. javanica* and *M. hapla* (Moens *et al*., 2009) plus recently added *M. enterolobii* (Philbrick *et al*., 2020).

Presently, synthetic pesticides are the main means of controlling nematode, but they are neither economical nor environmentally friendly (Aji, 2010). While the use of plant extract as alternative

nematicides is becoming widespread, the medicinal and pesticidal value of any plant has been reported to be due to their phytochemical (Ogwudire et al., 2022). Therefore, this study was undertaken to find out the nematicidal potential locked up by nature in M.balsamina leaves.

II. Materials And Methods

Experimental site

The experiment was carried out in the Laboratory of Department Crop Protection and Landscape Garden of Modibbo Adama University, Yola, Adamawa State, Nigeria.

Source of Plant Leaves and Preparation of Powder and Extract

The Momordica balsamina was sourced from the Mandza'a, village in Hong Local Government Area, Adamawa State. The fresh leaves were cut from the matured plants, shade-dried on polythene sheets, ground to powder using mortar and pestle and stored in polythene bags as described by Umar, 2013.

For the laboratory experiment, 50g of M.balsamina was turned in to a 5 litre plastic bucket and 500 ml distilled water was poured onto it. The set up was allowed to stand for 48 hours and filtered through Whatman No.1 filter paper Umar (2009). The filtrate obtained was designated as crude extract. There were six treatments made up of control (Tc-distilled water), crude extract (T1) and four dilutions of the crude extracts as T2 (5 ml), T3 (10 ml), T4 (15 ml) and T5 (20 ml).

For the juvenile mortality test, these treatments were applied to 18 perti dishes containing 1000 M. javanica J2 juveniles. These treatments were also applied to 18 petri dishes containing 1000 J2 eggs of M. javanica all arranged in CRD.

The phytochemical analysis of the Momordica balsamina was carried out in the Biochemistry Laboratory of the Faculty of Life Science, Modibbo Adama University, Yola to determine the presence of phytochemicals present in the leaf powder. These included tannins, saponins, alkaloids, flavonoids, cardiac glycosides, oxalate, phenols and steroids (Palermo et al., 2014).

III. Laboratory Experiments

Source of Inoculums and Extraction of Nematode

The inoculums for these experiments were the second stage of juvenile (J2) and eggs of M. javanica extracted from pure culture of infested tomato roots. The extraction of juveniles was done using the modified Baermam Method (Whitehead and Hemming, 1965), where shallow trays were lined had placed in them, sieves lined with tissue paper and macerated okra roots. Water was poured into tray from the side to a level just submerging the materials on the sieve. The setup was allowed to stand for 24 hours and the nematode juveniles were collected by decanting into a beaker. Precisely 10ml of the nematode suspension was taken in syringes and counted under a stereoscopic microscope using a grid counting dish and 1000 juveniles were used for each inoculation of juvenile mortality test and screen house experiment.

Egg masses from infested okra plant roots were used to prepare nematode egg suspension at a concentration of 100eggs/ml. Nematode eggs were extracted by cutting the roots into 1cm pieces and agitating in 0.05% NaOCl (sodium hypochlorite) for 2 – 3 minutes in a beaker (Hussey and Barker, 1973). The eggs were released from the egg masses and then rinsed with tap water on nested 150- and 25-um pore sieves (Dong *et al.*, 2007).

Juvenile Mortality Test

Eighteen petri dishes each containing 1000 second stage juveniles (J2) of M. javanica in 10 ml of water were used for the experiment. Crude extract was diluted with 5 ml, 10 ml, 15 ml, 20 ml and 25 ml of distilled water in treatment T1, T2, T3, T4, T5 respectively Tc, contains only distilled water which was the control and observation were made after every 24 hours for three (3) days. Identification of dead nematodes were done by touching them with a needle to see if they moved or not while dead nematodes were counted and recorded. There were six treatments replicated three times and arranged in the completely randomized design (CRD)

Egg Hatchability Test

Eighteen petri dishes each containing 1000 eggs of M. javanica in 10 ml of water were used for the experiment. Crude extract was diluted with 5 ml, 10 ml, 15 ml, 20 ml and 25 ml of distilled water in treatment T1, T2, T3, T4, T5 respectively while Tc, which contained only distilled water which was used as control. The number of hatched juveniles were counted after 24, 48 and 72 hours.

Pot Experiment

The seed of okra variety used for the experiment was the EX-Gombo okra which is susceptible to root knot nematodes and were obtained from Hong Central Market, Adamawa state.

The potted experiment was carried out at the landscape unit of Modibbo Adama University, Yola.

Top soil was collected from the Landscape Garden of the Modibbo Adama University, Yola. The soil was bulked and steam sterilized by the introduction of water vapor (100^0C) into the soil to elevate soil temperature to lethal (71 – 82 0C) that can destroy soil borne pest for about 30 – 45 minutes. The soil was allowed to rest for two weeks after which 4 kg of it was put into each of 18 perforated plastic pots and separately mixed thoroughly with amendment at different levels prior to planting of okra in each of the pots as follows: 0 g/pot, 5 g/pot, 10 g/pot, 15 g/pot, 20 g/pot and 25 g/pot designated as Tc, T1, T2 T3, T4 and T5 respectively. This was allowed to decompose for two weeks before two okra seeds were planted in each pot and thinned down to one seedling per

pot. There were six treatments replicated three times, giving a total of 18 pots and arranged in the Completely Randomized Design (CRD).

Meloidogyne javanica second stage juveniles (J2) extracted above were used to inoculate all potted plants. A 10 ml suspension containing approximately 1000 J2 of M. javanica was applied two weeks after emergence using a 10 ml syringe. The inoculation was done by removing soil to expose the roots on two sides of the plant and emptying the content of the syringe and covering up the soil All agronomic practices required were carried out as at when due.

At harvest, the soils sample from similar treatments were mixed in a plastic bucket and 250 cm^3 taken from each treatment and used for nematode extraction (Mohammed and Umar, 2012). Gall index was determined using the rating scale of Anwar *et al.* (2007) as follows:0 = no galls, 1 = 1 - 2 galls, 2 = 3 – 10 galls, 3 = 11 – 30 galls, 4 = 31 – 100 galls and 5 = >100 galls.

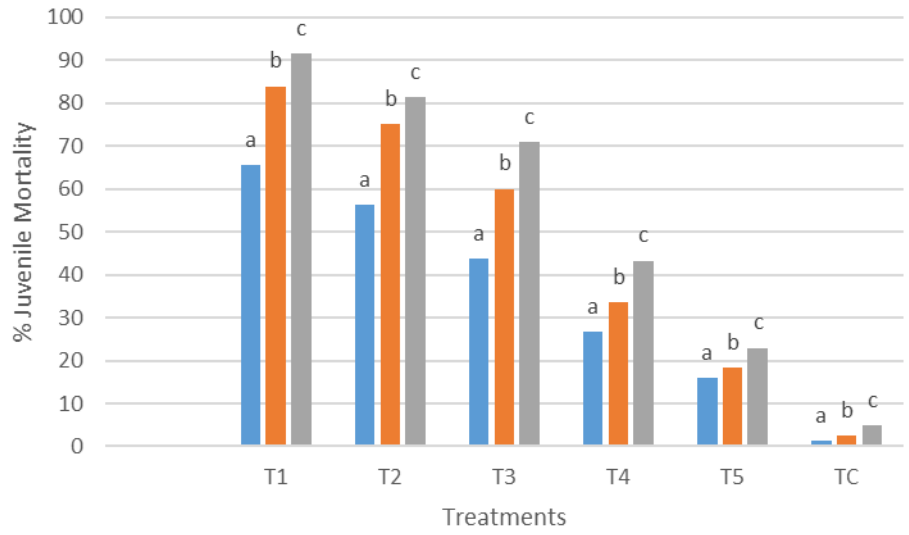
Data collected was subjected to analysis of variance (ANOVA) according and means were separated using New Duncan's Multiple Range (DMRT) at 5 % level of probability. Data collected on juvenile mortality and egg hatchability was analyzed using simple percentage.

IV. Results

Result of the phytochemical tests of M. balsamina leaf powder showed the high presence of tannins, flavonoids, cardiac glycosides and oxalate, low presence of saponins, alkaloids and phenols and absence of steroids. The presence of these phytochemicals could have accounted for the results obtained in the study. Knoblock *et al.* (1989), stated that nematicidal effect of tested extracts may have resulted from the high amount of certain oxygenated compounds they contain, which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their groups interfering with the enzyme protein structure.

The result of juvenile mortality test showed that after 24 hours of exposure of M.javanica juveniles to extracts of M.balsamina, the crude extract (T1) recorded the highest mortality of these juveniles with 65.67% followed by the 5 ml dilution (56.33%) and least was control (1.27). After 48 hours of exposure, the crude extract still recorded the highest juvenile mortality with 83.92% of the juveniles dead with least being control with 2.50%. the trend continued after 72 hours of exposure to M,balsamina extracts with the crude extracts still recording the highest mortality of 91.67%, followed by the 5 ml dilution (81.50%) and least was control (5.00%) (Fig. 1).

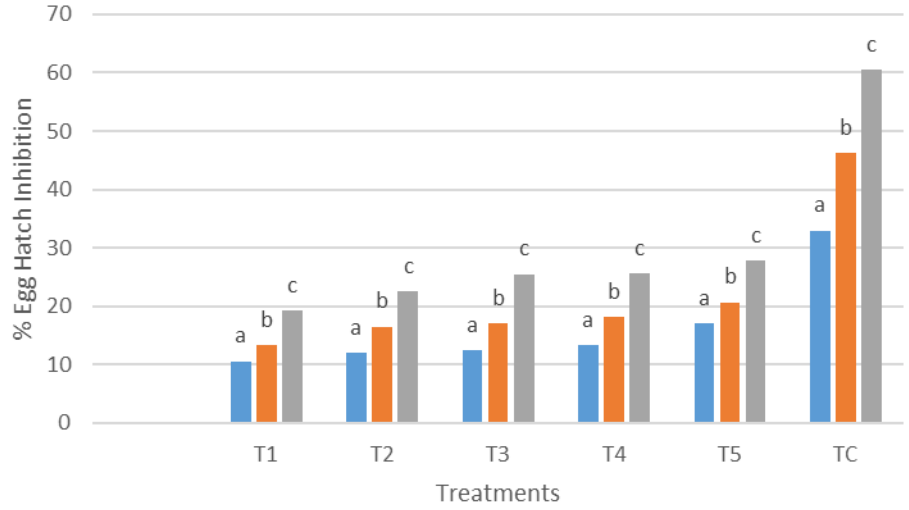
Fig,1: Effect of M.balsamina Leaf Extracts on the Mortality of M. javanica Juveniles



Key: a = 24 hours, b = 48 hours, c = 72 hours

Result of the egg hatchability test showed the crude extract (T1) recorded the highest M.javanica egg hatch with 19.30% hatched after 24 hours of exposure to extracts of M.balsamina. This was followed by the 5 ml dilution (T2) (12.00%), 10 ml dilution (12.50%) and the least hatch inhibition was control (Tc) with 33% of the eggs hatched. After 48 hours of exposure, the crude extract recorded the least percentage eggs hatched with 13.40% while control had the highest egg hatch with 46.30%. At the end of 72 hours, the least egg hatch was still recorded by the crude extract with 19.30% while the highest was control with 60.60% (Fig.2).

Fig,2: Effect of Extracts of M.balsamina Leaf Extract on the Inhibition of M.javanica Egg Hatch



Key: a = 24 hours, b = 48 hours, c = 72 hours

In the pot experiment, result of the plant height showed that the 25g M.balsamina leaf powder (T5) treatment produced the tallest okra plants at 23.00cm, followed by the 20g powder treatment (T4) at 22.33cm. Both treatments were significantly (p=0.05) taller plants than all the other treatments. They were followed by T3 (15g Treatment) at 20.33cm, T2 (19.00cm), T1 (17.67cm) and least was control (Tc) at 16.67cm tall (Table I).

The result on Table I showed that as the amount of M.balsamina leaf powder increased, the mean number of fruits produced by the okra plants increased. The 25g treatment recorded significantly (p=0.05) higher mean number of okra fruits than all other treatment with 12.03 fruits followed by 20g (10.23 fruits), 15g (8.30 fruits), 10g (7.03 fruits), 5g (6.66g fruits) and least was the control with 6.50 fruits. Here also, increase in powder quantity led to increased inhibition of M.javanica activity allowing the okra plants to produce more fruits.

The result for mean fresh fruit weight showed that the highest powder level (T5-25g) recorded significantly (p=0.05) heavier okra fruits with 13.00g per fruit, followed by T4 (11.33g) and least was control with 8.66g per fruit (Table 1).

Table I: Effect of Powder of M.balsamina Leaves on M.javanica on Okra

Treatment	Plant height (cm)	Number of Leaves	Number of Fruits	Fresh fruit weight (g)
T1 (5g)	17.67	6.33	6.66	9.33
T2 (10g)	19.00	6.67	7.03	9.66
T3 (15g)	20.33	7.36	8.30	10.67
T4 (20g)	22.33	8.00	10.23	11.33
T5 (25g)	23.00	10.5	12.03	13.00
TC (0g)	16.67	6.03	6.50	8.66
Mean	19.83	7.48	8.45	10.44
SEM (±)	1.03	0.67	0.91	0.64

SEM = Standard error of the mean

M.javanica produced more galls on the roots of control okra plants that received no powder of M.balsamina leaves. The control plants (TC) recorded significantly (p=0.05) higher galling index (3.35) than all other treatments followed by T1 (1.55), T2 (1.33) and least was T5 (25g treatment) with 0.83 (Table 2).

The nematode M.javanica, reproduced more as the quantity of powder decreased. The least nematode population was recorded in the 25g M.balsamina leaf powder treatment with 228.57 nematodes, followed by 20g treatment (822.85), 15g (834.28) and the highest by a highly significant (p=0.05) margin was the untreated control with a whopping 17,657.14 (Table 2). The reproduction factor result showed that the powder curtailed the capacity of the nematode to invade and cause damage to the roots of okra plants thereby ensuring good growth and fruiting. At highest concentration of rosemary extracts (40%) in tomato soil, the lowest number of juveniles (45) with least Rf (reproduction factor (0.33) was observed while in marigold extract highest number of juveniles (225) with highest Rf value (1.56) was observed at lowest concentration (10%) as compared to control (J2= 290; Rf= 2.02) (Bajestani *et al.* 2017).

Table II: Effect of Powder of M.balsamina Leaves on M.javanica on Okra

Treatment	Galling index	Nematode population	Reproductive factor
T1 (5g)	1.55	1142.85	1.14
T2 (10g)	1.33	1017.14	1.01
T3 (15g)	1.23	834.28	0.83
T4 (20g)	1.03	822.85	0.82
T5 (25g)	0.83	228.57	0.22
TC (0g)	3.35	17657.14	17.65
Mean	1.55	3617.14	3.61
SEM (±)	0.37	2811.48	2.81

SEM = Standard error of the mean

V. Discussions

M.balsamina extract and powder ensured a high level of mortality of M.javanica juveniles particularly the crude extract. This is similar Maximum mortality of juveniles (100, 97.1 and 95.6%) was recorded for extracts of eucalyptus, cinnamon and nerium respectively and minimum mortality % (65.2%) was recorded for garlic all in 10% concentrations with control having highly reduced mortality of juveniles of 13% (Hussein *et al,* 2016). Similarly, Umar and Mamman, 2014, Ahmad and Monjil, 2019, Mamman et al. (2022, Mamman, (2024) and others obtained good mortality of M.javanica juveniles with extracts of plants. The result further showed that as the period of exposure increased, the percentage mortality of M.javanica juveniles increased. This trend was also obtained by other workers (Oka et al, 2014; Aji et a., 2022; Mamman and Igbadu, 2023).

This result of the egg hatch inhibition test is an indication that the presence of these extracts especially the crude extract in a medium that contains eggs of M.javanica will likely see fewer than normal juveniles hatch out due to the inhibitory effect of the extracts. Jidere and Oluwatayo (2018) reported that Jatropha leaf extracts (>40%) produced higher inhibition of nematode egg-hatch, followed closely by *Ricinus communis, Moringa oleifera* leaves and 0% egg-hatch inhibition was recorded by untreated control. Furthermore, they found a significant difference ($P<0.05$) between the different concentrations used on percentage egg-hatch inhibition and juvenile mortality and the higher concentration of the various botanicals, the greater their effect on egg-hatch inhibition and juvenile mortality. This is the kind of effects recorded in this study.

The amount of powder treatment received made the difference because, as the quantity increased, the plant height increased indicating that the powder reduced the activity of M.javanica on okra allowing the plants to grow better. The plant parts of A. marina (leaves, stem and pneumatophore) used as soil amendment at 5% w/w for the suppression of root knot nematodes showed that, germination of seed, root weight, shoot weight, root length, and shoot length increase in both mash bean ($P <0.001$) and okra ($P < 0.001$) (Tariq et al., 2007).

The result on Table I showed that as the amount of M.balsamina leaf powder increased, the mean number of fruits produced by the okra plants increased. The 25g treatment recorded higher mean number of okra fruits than all other treatment with followed by 20g, and least was the control with 6.50 fruits. Here also, increase in powder quantity led to increased inhibition of M.javanica activity allowing the okra plants to produce more fruits. By limiting the activity of M.javanica, M.balsamina leaf powder apart from adding nutrients to the soil, made it easier for okra to produce larger heavier fruits. Adekunle and Akinlua (2007) observed that treatment of okra plant with Leucerna leucocephala and G. sepium extracts resulted in enhanced fruit weight, reduced nematode population, reduced galling and reduced nematode reproduction rate.

M.javanica produced more galls on the roots of control okra plants that received no powder of M.balsamina leaves. The control plants recorded higher galling index than all other treatments. Bawa *et al.* (2014) also reported that soil treated with ethanol extracts of plant leaves, red bell pepper fruits, ginger rhizomes and African locust bean seeds extracts have a significantly lower root gall as against the untreated control.

The nematode M.javanica, reproduced more as the quantity of powder decreased. The absence of any check in the control treatment allowed M.javanica to have a field day by multiplying freely while this freedom was curtailed in the T5 with the presence of 25g of M.balsamina leaf powder. This points to the fact that M.balsamina leaf powder in the quantum of 25g per 4kg per pot could drastically reduce M.javanica proliferation. Incorporating various manures in the soil at 60g per plot of tomato var UTC359IT, caused a reduction in the populations of *M.javanica* and improved tomato growth which compared favourably with carbofuran (Umar and Chubado, 2009). Incorporation of 10% fresh vetiver into the soil significantly reduced M. incognita by 46-67% (Jindapunnapat et al., 2019).

At highest concentration of rosemary extracts (40%) in tomato soil, the lowest number of juveniles (45) with least Rf (reproduction factor (0.33) was observed while in marigold extract highest number of juveniles (225) with highest Rf value (1.56) was observed at lowest concentration (10%) as compared to control (J2= 290; Rf= 2.02) (Bajestani *et al.* 2017).

VI. Conclusion

The results clearly show how M. balsamina leaf extract and powder caused the mortality of M.javanica eggs and inhibited the hatching of the nematode's eggs as the powder particularly the 25g treatment recorded vert low galling index, nematode population and reproduction factor, that it has the potential to be a good nematicide. It is therefore concluded that M.balsamina leaf crude extracts and 25g powder can be used as control material in areas infested with M.javanica. Field trial is also recommended.

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