

Use of Leaf Extract and Powder of *Ipomoea asarifolia* for Control of Root-knot Nematode (*Meloidogyne javanica*) on Okra

**Abstract:** As the unseen enemy, nematodes have caused untold damage to crops and farmers' income across the world. A study was conducted in 2019 and 2020 to determine the effect of leaf extract and powder of *Ipomoea asarifolia* on rootknot nematode, *Meloidogyne javanica* on okra in the laboratory and pot experiment. Juvenile mortality and egg hatch inhibition tests were conducted in the laboratory. Result of the juvenile mortality test showed that the crude extract (100 % extract) cause the highest mortality (95.33 %) of juveniles while the same crude extract inhibited the hatching of more *M. javanica* eggs (89.67 %) than the dilutions and control. For the pot experiment, results show that the 60 g powder treatment gave the least galling index (1.0), nematode population (146.96), reproduction factor (0.25) and the highest yield (1568.3 kg/ha) in 2019. The same trend was observed in 2020. Based on these findings, *I. asarifolia* leaf extract and powder (60 g) have the potential to put in check the menace posed by *Meloidogyne javanica* on okra.

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Keywords: *Ipomoea asarifolia*; *Meloidogyne javanica*; Extract; Okra; Larva

## INTRODUCTION

Plant-parasitic nematodes have been reported to cause huge damage to crops across the world. Nimbalkar and Rajurkar (2009) made it clear that among various pests and diseases which damage crops, plant-parasitic nematodes present a formidable pest problem for different crops and root-knot nematode (*Meloidogyne* spp.) is an important pest of vegetables. Al-Banna *et al.* (2020) reported that root-knot nematode is the most economically important plant parasitic nematode species that cause serious damage to most agricultural crops and more than 3000 plant species are hosts to this pest. Saifullah *et al.* (1990) stated that in some developing countries like Pakistan, root-knot nematode infection is so common and wide-spread that galled roots are considered to be normal. The use of nematicides is fast and effective but hazardous to man and the environment. De-registration of some hazardous nematicides, has put more pressure on farmers to use non-chemical pest control methods that do not pollute the environment (Sidhu *et al.*, 2017). Interest has now shifted to the use of botanicals which do not pollute the environment.

Okra (*Abelmoschus esculentus* L. Moench) belongs to the hibiscus family Malvaceae and is a popular vegetable in the world, which originated from Africa (National Research Council, 2006).

Okra is usually grown in Nigeria under irrigation during the rainy season especially in the Fadama vegetation (Aladele *et al.*, 2008). The young pods are particularly rich in nutrients with 86.1% moisture content, 9.7% carbohydrates, 12.2% protein, 0.1% fibre, 0.2% fats and 0.9% ash (Ariyo and Aken'ova, 1986). Sasser (1977) reported that root-knot nematodes cause annual losses in tropics up to 29% in tomato, 22% in okra, 24% in potato, 23% in egg plant, 25% in pepper and 28% in beans. *M. javanica* is also a member of the root-knot nematodes and is known to attack okra leading to losses.

## **MATERIALS AND METHODS**

The experiments were conducted in the laboratory of Department of Agronomy, Taraba State University, Jalingo in 2019 and 2020.

**Preparation of Extracts:** Leaves of *Ipomoea asarifolia* were collected, washed and shade-dried after which they were ground to powder using mortar and pestle. The powders were stored in plastic containers. Extracts were prepared using the method described by Adegbite and Adesiyan (2005). Fifty grams of the *I. asarifolia* leaf powder was soaked in 500 ml distilled water contained in 1000 ml plastic container and left for 48 hours. It was then filtered through Whatman No.1 filter paper. The filtrate obtained was designated as crude extract (100 % filtrate). Dilution was carried out with 5 ml (80 %), 10 ml dilution (66.7 %), 15 ml dilution (57 %) and 100% distilled water was used as control and designated as CT. This gave a total of five treatments each for both the egg hatchability and juvenile mortality tests.

**Extraction of Nematode Eggs and Juveniles:** Second stage juveniles of *M. javanica* were isolated from tomato roots through the modified Baermann method (Whitehead and Hemming, 1965). This involved sieves lined with tissue paper placed on shallow plastic trays, the blended tomato roots were placed on the sieve and water was poured in the tray by the side of the sieve. The set up was left to stand for 24 hours and the juveniles were collected by decanting the nematode suspension into a beaker. Nematode suspension of 10 ml aliquots were taken and counted under a stereoscopic microscope using a grid counting dish and an average of 500 nematode juveniles were used for juvenile mortality and egg hatchability tests and pot experiment.

**Juvenile Mortality Test:** A 10 ml suspension of 500 second stage juveniles (J2) of *M. javanica* was dispensed into a petri-dish into which was added 10 ml concentration of treatment. This was carried out for each of the five treatments (crude extract, 5 ml, 10 ml and 15 ml distilled water and 100% distilled water was used as control and designated as CT) and replicated three times. In all, 15 petri-dishes were used and the set up was arranged in complete randomized design (CRD). Observation was done every 24 hours and dead nematodes was counted and recorded.

**Egg Hatchability Test:** A 10 ml suspension of 500 eggs of *M. javanica* was dispensed into a petri-dish into which was added 10 ml concentration of treatment. This was carried out for each of the five treatments (crude extract, 5 ml, 10 ml and 15 ml dilutions and 100% distilled water as control and designated as CT) and replicated three times. In all, 15 petri-dishes were used and the set up was arranged in complete randomized design (CRD). This set up was allowed to stand for three days after which hatched nematodes were counted under a microscope (Ononuju and Nzenwa, 2011).

**Pot Experiment:** Loamy soil from Taraba State University Jalingo Teaching and Research Farm was collected and sterilized and left for two weeks. This soil was put into 15 perforated 20 cm diameter plastic pots at the rate of 6 kg per pot. The powder of *I. asarifolia* was incorporated into the soil in the pots at four levels which include 60 g/pot, 50 g/pot, 40 g/pot and 30 g/pot with 0 g/pot as control (CT). The set up was arranged in complete randomized design (CRD) and in three replications. Also, the set up was placed in a secluded area beside the laboratory building. Incorporation of powder was done two weeks before planting during which the soil was watered. Seeds of okra variety cv 'Yar Kodon', a local variety commonly cultivated in the area, was planted at the rate of three seeds per pot and thinned down to one seedling per pot a week after emergence. All cultural practices were carried out as required.

Two weeks after the emergence of okra seedlings, the soil in the pots was inoculated with 500 J2s of *M. javanica* contained in a 10 ml suspension through two holes drilled in the soil down to the roots on both sides of the okra seedlings. Data was collected on plant height, number of leaves, shoot weight, root length, root weight, number of fruits, fruit weight, yield, galling index, final nematode population and reproduction factor.

All data collected was subjected to analysis of variance (ANOVA) by SAS processes and MS Excel.

## RESULTS AND DISCUSSIONS

The phytochemical analysis of *Ipomoea asarifolia* leaf powder showed the presence of alkaloids, cardiac glycosides, flavonoids and saponins.

Result of the juvenile mortality test (Fig. 1) showed that after being exposed for 72 hours to extracts of *I. asarifolia*, the crude extract recorded significantly ( $P = .05$ ) higher mortality of juveniles of *M. javanica* (95.33 %) than all other treatments. This was followed by the 5 ml dilution with 75.33 %, 10 ml dilution with 53.33 % and 15 ml dilution with 22.33 % mortality. The least was the control (CT) with 15 % juvenile mortality (Figure 1). Mortality of juveniles increased with time as it was lowest the first 24 hours and highest after 72 hours. Mahlootjie *et al.* (2019) recorded similar results when they reported a significant mortality rate of *Meloidogyne javanica* J2 treated with fermented *Lantana camara* at varying extract concentration. This mortality rate may have resulted from the presence of the phytochemicals in the leaves of *I. asarifolia*.

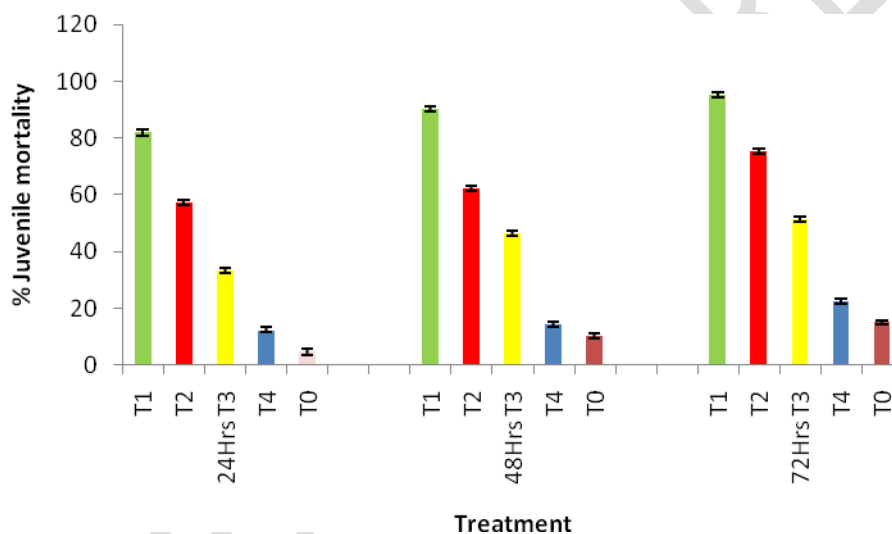


Figure 1: Effects of Extracts of *Ipomoea asarifolia* on Mortality of Juveniles of *M. javanica*

Key: T1-100% extract, T2-5 ml dilution (80 %), T3-10 ml dilution (66.7 %), T4-15 ml dilution (57 %), T0-Control (100 % distilled water)

Results of the egg hatch inhibition test (Fig 2) showed that extracts of *I. asarifolia* have the capacity to reduce the hatching of eggs of the root-knot nematode, *M. javanica*. The percentage egg hatch inhibition recorded by the extracts was significantly ( $p = .05$ ) higher than control. The

crude extract (100 % extract) recorded the highest inhibition with 89.67 %, followed by the 5 % dilution with 82.33 %, 10 ml dilution (58.33 %) and lowest was the 15 ml dilution with 41.33 % egg hatch inhibition. The least was control with 14.33 % hatch inhibition. The extract contains phytochemicals that are reported to be able to inhibit nematode egg hatch. Afzal *et al.* (2021) reported that aqueous extracts of *Amaranthus viridis*, *Chenopodium album*, *Solanum nigrum*, *Carica papaya* and *Euphorbia hirta* all inhibited egg hatching and caused larval mortality of rootknot nematodes and that *C. album* recorded the maximum reduction of 24.3% in egg hatching.

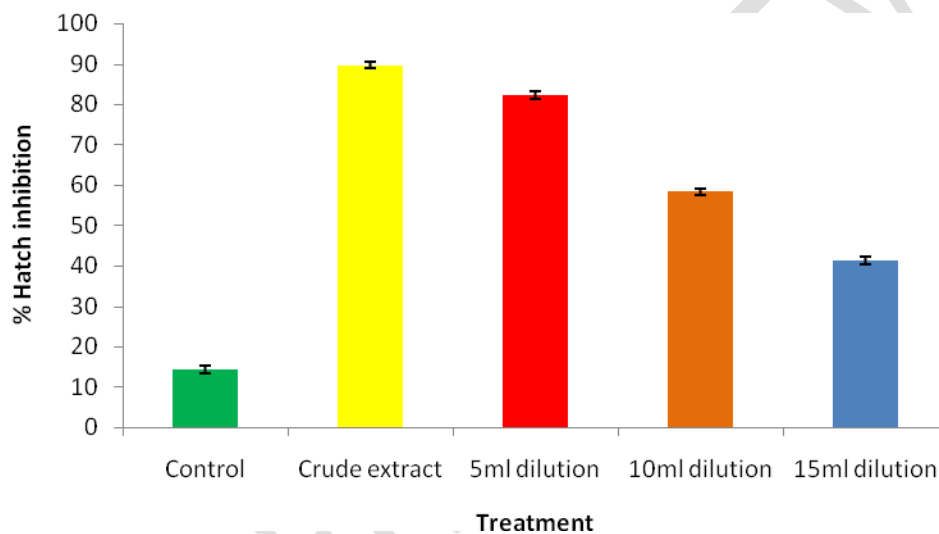


Figure 2: Effects of Extracts of *Ipomoea asarifolia* on Hatching of *M. javanica* Eggs

Key: T1-100% extract, T2-5 ml dilution (80 %), T3-10 ml dilution (66.7 %), T4-15 ml dilution (57 %), T0-Control (100 % distilled water)

In the pot experiment, results reveal a significant ( $p = .05$ ) difference between all levels of *Ipomoea asarifolia* leaf powder and control for plant height, number of leaves, root weight, root length, number of fruits, yield, galling index, nematode population and reproduction factor in 2019 and 2020. The 60 g treatment recorded taller okra plants (77.86 cm for 2019 and 76.83 cm for 2020) than other treatments though there was no significant ( $p = .05$ ) between it and the 50 g

treatment (69.66 cm for 2019 and 70.73 cm for 2020). The least was control okra plants with (26.33 cm for 2019 and 25.85 cm for 2020) (Table 1). The 60 g and 50 g treatments recorded significantly ( $p = .05$ ) higher yield than other treatments but the difference between the two was not significant. The 60g treatment recorded 1568.3 kg for 2019 and 1572.6 kg for 2020 as shoot weight. There were fewer galls on roots of okra plants treated with 60 g powder of *I. asarifolia* than other treatments as it recorded a galling index of 1.00 and 1.10 for both years respectively with the highest being recorded by control (3.66 and 3.64 for 2019 and 2020 respectively).. *M. javanica* reproduced more poorly on okra plants that received 60 g powder treatment as indicated by the nematode population at the end of the experiment (146.96 and 140.83 for 2019 and 2020 respectively) and reproduction factor (0.26 and 0.25 for 2019 and 2020 respectively) (Table 2).

Plants are a repository of compounds of natural origin that have nematocidal effect. This has been demonstrated by many workers (Abolusoro *et al.*, 2020; El-Nuby *et al.*, 2020; Das *et al.*, 2021; Mamman *et al.*, 2021; El-Ashry *et al.*, 2021). The 60 g treatment showed more promise in reducing the negative activities of *M. javanica* on okra than other treatments. It had the lowest final nematode population and lowest reproduction factor and galling index than all other treatments. Das and Bahera (2019) in a pot culture study reported that two bio-fumigants (Cabbage and Cauliflower leaves) reduced root knot nematode (40.7%), spiral nematode (49.1–79.7%), lance nematode (40.8–80.1%) and stunt nematode (40.8–81.3%). There was improvement in growth parameters of okra plants like shoot length (23.3–54.6%), fresh shoot weight (28.4–81.9%), dry shoot weight (11.6–85.7%), root length (14.1–46.5%), fresh root weight (22–38.7%) and dry root weight (24–39%) of okra plant as compared to untreated control. Leaves of both cabbage and cauliflower exhibited similar levels of reduction in nematode population and enhancement of plant growth parameters.

**Conclusion:** In conclusion, the reduced galling and nematode population and improved growth parameters above untreated plants shows *Ipomoea asarifolia* has nematicidal qualities and may be used as a substitute for harmful and environmentally unfriendly nematicides.

Table 1: Effect of Extract of *Ipomoea asarifolia* on *M. javanica* on Eggplant in 2019 and 2020

	2019					2020				
	PH (cm)	NL	SW (g)	RW(g)	RL (cm)	PH (cm)	NL	SW (g)	RW(g)	RL (cm)
<b>60g</b>	77.86	18.67	121.56	16.86	29.20	76.83	19.30	122.90	16.23	30.63
<b>50g</b>	69.66	18.67	112.40	18.03	26.33	70.73	18.70	111.06	18.63	26.26
<b>40g</b>	64.45	14.00	66.33	22.66	20.83	64.47	14.46	67.06	21.26	20.20
<b>30g</b>	47.23	13.00	53.66	26.33	15.33	46.43	12.83	53.73	25.46	15.63
<b>CT</b>	26.33	10.33	50.00	30.10	13.33	25.80	10.56	49.90	31.20	12.46
<b>Mean</b>	57.10	14.93	80.79	22.80	21.01	56.85	15.17	80.93	22.56	21.04
<b>SEM</b>	9.18	1.63	15.09	2.48	3.05	9.28	1.68	15.10	2.64	3.33

All means in the same column bearing the same letters are not significantly different at  $p=0.05$

PH-Plant height, NL-Number of leaves, SW-Shoot weight, RW-Root weight, RL-Root length, LSD-Least significant difference, SE-Standard error

Table 2: Effect of Extract of *Ipomoea asarifolia* on *M. javanica* on Eggplant in 2019 and 2020

	2019						2020					
	NF	FW (g)	Yield (kg/ha)	GI	NP	RF	NF	FW (g)	Yield (g)	GI	NP	RF
<b>60g</b>	3.66	48.03	1568.3	1.00	146.96	0.26	3.92	47.02	1572.62	1.10	140.83	0.25
<b>50g</b>	2.67	35.23	1411.7	1.66	203.40	0.36	2.69	34.45	1461.37	1.88	209.73	0.37
<b>40g</b>	2.33	32.70	1211.7	2.33	394.36	0.86	2.52	32.48	1235.10	2.66	386.10	0.81
<b>30g</b>	2.00	31.20	1043.3	3.33	602.23	1.16	2.39	30.73	1040.20	3.48	610.90	1.20
<b>CT</b>	2.00	14.20	476.3	3.66	707.73	1.40	2.22	13.69	479.77	3.64	715.10	1.45
<b>Mean</b>	2.40	32.27	1142.26	2.40	410.93	0.80	2.75	31.67	1157.81	2.55	412.53	0.81
<b>SEM</b>	0.30	5.40	188.71	0.49	109.02	0.22	0.30	5.33	192.84	0.48	111.03	0.23

All means in the same column bearing the same letters are not significantly different at  $p=0.05$

NF-Number of fruits, FW-Fruit weight, GI-Galling index, NP-Nematode population, RF-Reproduction factor, CT-Control, LSD-Least significant difference

## COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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