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EFFECT OF *JATROPHA CURCAS* TANNINS ON ROOT-KNOT NEMATODE DISEASE OF OKRA, MUCILAGINOUS PROPERTY AND NUTRIENT COMPOSITION

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ABSTRACT

Tannins have been reported to possess anthelmintic properties especially for gastrointestinal nematodes in ruminants. Tannins extract from physic nut (*J. curcas*) plant parts were evaluated for their nematicidal control on the root-gall nematode disease of Okra and its mucilaginous property and nutrient composition in 2019 and 2020 cropping seasons The experiment was a 3x6 factorial experiments arranged in Completely Randomized Design with five replications in the screen house. Tannins extracted from the seeds, leaves and roots of physic nut plants were separately applied at 0, 1, 2, 3, 4 and 5 ml / potted okra plant inoculated with 1,200 infective (J2) larvae of *Meloidogyne incognita*. Viscometer and standard official methods of analysis were used in the determination of mucilaginous property and nutrient composition on immature fresh fruits harvested 6 days after anthesis. Results showed that Tannins application significantly (P=0.05) reduced root-gall nematode infection and increased the moisture contents, fat, calcium, sodium, and mucilaginous property of okra fruits. Phosphorus and nitrogen contents were on the other hand enhanced by the infection. Neither Okra nor root-gall nematode infection affected ash, magnesium, and potassium contents of the fruits.

Keywords: Okra, Tannins, Root-gall nematode infection, mucilaginous property, nutrient composition, *Jatropha curcas*,

INTRODUCTION

Okra, *Abelmoschus esculentus* (L) moench belongs to the family *Malvaceae*. It is an important vegetable crop especially in West Africa, India, Brazil, and the United States where it is widely cultivated. (ECHO, 2003; Alimi, 2004). Schippers (2000) reported that the crop can be found in almost every market all over Africa including Nigeria. In Nigeria, it is among the most commonly grown vegetables because of the mucilaginous or slimy property of the fruit.

Okra fruit is an important source of protein, carbohydrates, minerals, vitamins, and dietary fibre. In fact, the immature fruits (pods) which are consumed as vegetables can be used in salads, soups, and stews, fresh or dried, fried, or boiled (Habtamu *et al*., 2014). FAO (2006) reported that okra seeds serve as a non-caffeinated substitute for coffee and at the same time a rich source of seed oil. This may be the reason why the seed contains approximately 21% protein, 14% lipids and 5% ash (Iyagba *et al*., 2012). Funke (2012) further confirmed the importance of this crop by stating its invaluable medicinal use in treating peptic ulcers.

The crop is an erect annual, herbaceous plant growing up to 2m in height. It is propagated by seed and requires a monthly average temperature range of 20-30$^{\circ}c$ for growth, flowering, and pod production. Okra production requires a well-drained sandy loam with high organic matter free of soil borne disease pests such as southern stem blight, wilts, and nematode (Sanders, 2001)

Despite the numerous benefits associated with the yield potential of okra, pests and disease organisms cause damage that reduces the potential output of this crop (Jones 1996). Profitable production of okra has been greatly limited due to the incidence and severity of root-knot nematode disease caused by *Meloidogyne* spp. (Oyedunmade, 2000; Adebowale *et al*; 2014 and Claudius-Cole 2018).

Although, chemical control methods frequently used for controlling root-knot nematodes may have led to increased quantity and quality of yields of our crops, the drawback caused by its high toxicity and damage to environment has fuelled the need for relatively safer alternative since pesticides residues have been reported in vegetables such as okra, onions, lettuce, cabbage, spinach and tomato (EL-Nahhal, 2004).

While the use of plant extract as alternative nematicides is becoming widespread. The medicinal value of any plant has been reported to be due to their phytochemical constituents. This study is therefore concerned with evaluating the efficacy of *J. curcas* tannins for root-knot nematode disease control and improved Okra yield.

MATERIALS AND METHOD

Site Location

The experiments were conducted at the Teaching and Research Farm of Federal University of Technology, Owerri (FUTO) located on Lat $5^{0}27$ 50.23" North and Longitude $07^{o}2$ 49.33" East at 55 msl (Handheld global positioning system). Owerri has a rain forest agro ecology

characterized with more than 2500 mm annual rainfall, 27-29^{o}C annual temperature and 89-93 % humidity. The Soil was loamy sand and naturally infested with root-gall nematode, *Meloidogyne* Specie (*M. incognita)* (Agu, 2008).

Plant Materials and Preparation of Crude Extracts

Fresh plant materials of physic nutplants (leaves, roots, and seeds) collected from Federal University of Technology, Owerri were dried under shade before grinding into coarse powder using mortar and pestle. This was later sieved into fine particles and then stored in a cool dry place until required. The purification procedure was essentially according to that of Strumeyer and Malin (1975). Extracts of the dried ground plant part was prepared (20 g) in 400 ml of 70 % aqueous acetone containing 0.1 % ascorbic acid using an ultra-sonicator. The supernatant obtained on centrifugation for 15 minutes at 2500 rpm was evaporated under vacuum at about 30^{o}C to remove acetone. The aqueous sample was lyophilized. The dried sample was dissolved in 80 % aqueous methanol containing 0.1 % ascorbic acid.

After the filtration of the lyophilized sample, Sephadex-20 slurry was prepared in a chromatography column (by addition of 300 ml of 80 % aqueous methanol to 25 g Sephadex LH-20 in the column and 0.1. % Ascorbic acid). The filtrate was poured into the column and the eluted sample without tannins was collected using conical flask. The tannins remained on the sephadex LH-20 and gave it a brown colour. The tannins were eluted by adding 100 ml of 5 % aqueous acetone containing 1 mg/ml ascorbic acid into the column.

The volume of the eluted tannins was recorded in mls. And the amount of ascorbic acid added during the isolation process was calculated as (x)mg of ascorbic acid. The acetone was removed under vacuum at 30^{o}C using rotary vacuum evaporator, and then the aqueous solution containing the pure tannins was lyophilized in a pre-weighed beaker (W_1) to get weight of beaker plus tannins solution (W_2). The difference in $W_2$and W_1gives the weight of the tannins containing ascorbic acid (Y, mg). Hence, the weight of the tannins was obtained by (Y, mg-X, mg.). This preparation was free of ascorbic acid.

Test For Tannins

Five milliliters of the extract and 3 drops of 1 % lead acetate addition indicated a yellow precipitate formation indicating the presence of tannins.

Experimental Design and Layout

The experiments were carried out as factorial in completely randomized Design (CRD) with six treatment applications in five replications. The Tannins under study had six treatment levels of applications while physic nut parts had three (root, seed & leaves). Thus, a 3x6 factorial experiment arranged in Completely Randomized Design with 5 replications was conducted in the screen house thereby giving a total of 90 pots containing 4kg of steam sterilized soil. The treatments were randomly assigned to the experimental pots and applied at various rates: 0.00ml (control), 1.00ml, 2.00ml, 3.00ml, 4.00ml and 5.00mlon okra plant

cultivar (NHA_e47 - 4). All the test plants were inoculated and treated with various rates of the Tannins under study. Inoculated plants without treatment or zero rate of treatment applications served as the control.

Planting

Before planting, one hundred seeds of the test okra cultivar (NHA_e47-4) were surface sterilized. This was done by soaking in 10% concentration of commercial sodium hypochlorite solution (NaOcl) and later rinsed several times in tap water. The seeds were then plated on moistened filter paper contained in sterilized Petri-dishes to sprout. The Petri-dishes were appropriately labelled. Two sprouted seeds were planted in each potted soil but later thinned down to one plant per pot before inoculation. The test okra cultivar used was obtained from National Horticultural Research Institute, Okigwe in Imo State (NIHORT).

Inoculum Preparation

The inoculum source was the *M. incognita* population which has been maintained and built up on Okra (*Abelmoschus esculentum*) in pot cultures. The infected roots of the Okra plants were recovered intact by inverting the pots to free the root system. Galled roots were also chopped into smaller pieces and put into warren blender containing about 1 litre of water. In other to avoid inactivating nematode larvae, the warren blending was allowed to run for three seconds intervals. The slurry was made up to 1200ml by adding more water. Thirty milliliters (30ml) of this mixture was taken and poured into a Petri-dish and the number of larvae counted using a stereomicroscope. The mean number of larvae in three counts was 430.

Inoculum and Phytochemical Application

Ten days after planting, all test okra cultivars were inoculated by measuring and pouring out 30ml of blended slurry of the infected galled roots of okra at the root base of each plant and then covered up with thin layer of the sterilized soil. Tannins where simultaneously applied at the base of the potted plants at the following rates: 0.00 [control], 1.00, 2.00, 3.00, 4.00, and 5.00 ml per plant. The inoculated plants were kept in a well-ventilated screen house. The average soil temperature ranged from 30 – 32^0C. In order to forestall any fungal problem 0.1% benlate (Benomyl) was incorporated to the potted soil.

Mucilaginous Property and Nutrient Composition Measurement

The mucilaginous property of okra fruits was measured using a Brookfield RVT viscometer (Brookfield Engineering Laboratories mass, USA) and the procedure as described by Akingbala *et al*., (1981). Nutrient composition was determined on all immature fresh fruit samples harvested 6 days after anthesis using the standard methods of Association of Official Analytical chemists (AOAC, 1990) methods. Nitrogen was determined by the micro Kjeldahl method (AOAC, 1990). The minerals were analysed from solutions obtained after ashing samples at 525^{o}C and dissolving the ash in distilled de-ionized water containing a few drops of concentrated hydrochloric acid. Sodium and potassium were determined using a flame photometer (Corning. UK Model 405),

while phosphorus was determined colorimetrically using spectronic 20 (Gallenkamp, UK) as described by Pearson (1976). Calcium and Magnesium was determined using atomic absorption spectrophotometer (P Y E Unicorn UK, Model SP 9). Moisture content was calculated by difference and Fat determined using Soxhlet extractor.

Infection Assessment

The experiment was terminated 12 weeks after inoculation. By this time all the plants have produced fruits and most growth and yield parameters measured. In assessing the extent of root infection by *M. incognita* in each treatment level, the root systems were recovered intact. This was done by inverting the pots to free the root system. Adhering soils were gently removed from the roots by rinsing in water. Root systems were individually scored according to Agu and Ogbuji (1996) in which

0 = No infection (no galls present)
1 = Rare infection (1-3 galls present)
2 = Light infection (4-10 galls present)
3 = Moderate infection (11-30 galls present)
4 = Severe infection (30 and above galls present)

Data Analysis

Data obtained were subjected to analysis of variance using GENSTAT Edition 4. Differences between means were separated using Fisher's Least Significant Difference (F - LSD) at 5% level of probability.

RESULT AND DISCUSION

Result of this study in figure 1 and 2 showed that application of Tannins extracts led to reduced galls and better yield performance on treated okra plants when compared to the severely galled untreated control okra plants in both years (2019 and 2020). This could be attributed to the nematostatic effect of Tannins. Maistrello *et al*., (2010) further asserted that Tannins are compounds that could be involved in the passive defence of plant as chemical barriers to the invasion of the parasite in the roots and they might increase host resistance to nematode infection. Okra plants treated with leaf (4.95 g) and seed (4.80 g) tannins produced okra pod weight significantly ($p<0.05$) higher than those treated with root tannins (3.46 g) in 2019 (Fig 1). The same was true for 2020 (Fig 2).

Highest okra pod weights were however produced with leaf (5.19 g) and seed (5.04 g) tannins treatment application in 2020 than with root tannins (3.70g) and the untreated control. This agrees with the works of Mbah *et al*., (2005) who found the yield of tomato plants treated with plant leaf extracts higher than those of untreated (control) plants. Ojiako *et al.*, (2015) also reported that the seed extract, irrespective of the solvent used had better repellency action than the root extracts.

The effect of Tannins on Okra Mucilaginous property and root-gall incidence is shown in Table 1. Mucilaginous property significantly ($p<0.05$) increased with increased rates of Tannins treatment applied at reduced galling. Agu *et al*., (2009) noted a steady increase in mucilaginous property in okra pods as okra galling response to *M. incognita* decreased at increased rate of Carbofuran. Mucilaginous property of plants treated with 5 mls of tannins were significantly ($p<0.05$) higher at reduced

galling than those in the severely galled untreated control pots in 2019 and 2020.Agu*et al*., (2013) also reported that root gall nematode decreases with corresponding increase in application rate of *J. curcas* .The Effects of Tannins on percentage nutrient composition of Okra showed that neither root-gall disease, *J. curcas* tannins nor parts affected Magnesium (Mg) and Potassium (K) and Ash contents of Okra fruits.(Table 2). Phosphorus (P) and Nitrogen (N) contents were on the other hand significantly ($P<0.05$) enhanced by root-gall nematode infection when compared with the treated control (Table 3).

Enhanced Phosphorus and Nitrogen content observed in the root-gall nematode infected plants could be due to uninterrupted translocation of phosphorus. Carneiro *et al*., (2002) reported that phosphorus translocation was not interrupted in okra plants infected with *M. incognita* and that the use of Phosphorus by the parasite was not greater than 10 % of the nutrient uptake by the plant. The significant increases observed in this study may be because Phosphorus enhances nitrogen uptake, efficiency, and utilization during such important growth process as seed and fruit development in plants (Huber 1980). This was more so with plants treated with 5 mls of Root tannins whose phosphorus (P) and Nitrogen (N) contents were significantly ($p<0.05$) enhanced than those treated with seed and leaf tannins, respectively.

Fat, Sodium (Na), Calcium (Ca) and Moisture were all significantly($p<0.05$) reduced by root-gall nematode infection in the untreated control plants (Table 4). This confirms the work of Kirkpatrick *et al*., (1991) who reported xylem vessels disruption in infected roots thereby causing interruption of nutrient translocation to the shoot and consequent interference with carbohydrate synthesis. Plants treated with 5 mls of leaf tannins produced okra pods with significantly ($P<0.05$) higher percentage of Fat, Sodium (Na), Calcium (Ca) and Moisture contents. This was followed by seed and root tannins.



Fig 1: Shows the relationship between root-gall index, okra pod weight and Tannins in 2019

KEY: L0-L5 = Leaf Tannins (0 ml, 1 ml, 2 mls, 3 mls, 4 mls, 5 mls); R0-R5 =Root Tannins (0 ml, 1 ml, 2 mls, 3 mls, 4 mls, 5 mls); S0-S5 = Seed Tannins (0 ml, 1 ml, 2 mls, 3 mls, 4 mls, 5 mls)



Fig 2: Shows the relationship between root-gall index, okra pod weight and Tannins in 2020

KEY: L0-L5 = Leaf Tannins (0 ml, 1 ml, 2 mls, 3 mls, 4 mls, 5 mls); R0-R5 = Root Tannins (0 ml, 1 ml, 2 mls, 3 mls, 4 mls, 5 mls); S0-S5 = Seed Tannins (0 ml, 1 ml, 2 mls, 3 mls, 4 mls, 5 mls)

Table 1: Effect of Tannins and *Jatropha* parts on Okra mucilaginous property as influenced by root-gall nematode

	2019								2020							
	Mucilaginous property (mpa*s)				Root-gall Index (0-4)				Mucilaginous property (mpa*s)				Root-gall index (0-4)			
	Jatropha Parts				*Jatropha* Parts				*Jatropha* Parts				*Jatropha* Parts			
Tannins (ml)	L	R	S	Mean	L	R	S	Mean	L	R	S	Mean	L	R	S	Mean
Untreated (control)	1025	1004	1011	1013	4	4	4	4	1025.6	1005	1012	1014	4	4	4	4
1	1103	1115	1104	1107	3.4	3.8	3.2	3.46	1104	1116	1105	1108	3	3.6	3	3.2
2	1124	1205	1194	1174	3	3.8	2.8	3.2	1124.6	1205.6	1195	1174.93	2.8	3.8	2.8	3.13
3	1235	1393	1105	1244	2	3	2	2.33	1235	1394	1106	1245	1.8	2.8	1.8	2.13
4	1310	1705	1669	1561	1	2.4	1.2	1.5	1311	1706	1669.6	1561.93	1	2	1.2	1.4
5	1605	2124	2105	1944	1	2	1	1.33	1606	2125	2106	1945	1	1.8	1	1.26
Mean	1233	1424	1364		2.4	3.16	2.36		1234.28	1424.93	1365.26		2.26	3	2.3	
LSD $_{0.05}$(*Jatropha* parts)		0.36				0.29				0.46				0.33		
LSD $_{0.05}$ (Tannins)		0.51				0.42				0.65				0.46		
LSD $_{0.05}$(J. parts X Tannins mls)		0.89				ns				1.12				ns		

ns = not significant, L = leaf, R= root and S = seed

Table 2: Effect of Tannins and *Jatropha* parts on percentage nutrient composition of Magnesium, Ash and Potassium as influenced by root-gall nematode

	% Magnesium				% Ash				% Potassium				Root-gall index (0-4)			
	Jatropha Parts				*Jatropha* Parts				*Jatropha* Parts				*Jatropha* Parts			
Tannins (ml)	L	R	S	Mean	L	R	S	Mean	L	R	S	Mean	L	R	S	Mean
Untreated (control)	0.32	0.31	0.3	0.311	4.1	4.11	4.11	4.12	0.34	0.32	0.322	0.3273	4	4	4	4
1	0.33	0.31	0.32	0.32	4.1	4.11	4.11	4.12	0.34	0.322	0.318	0.3273	3.6	3.2	3.4	3.4
2	0.33	0.32	0.32	0.325	4.2	4.11	4.12	4.13	0.344	0.318	0.32	0.3273	3.8	3	3.2	3.33
3	0.34	0.32	0.33	0.329	4.2	4.12	4.12	4.13	0.346	0.32	0.322	0.3293	3	2	2	2.33
4	0.34	0.33	0.33	0.334	4.2	4.13	4.13	4.14	0.348	0.322	0.328	0.3327	2.4	1.2	1.4	1.66
5	0.34	0.33	0.34	0.337	4.2	4.13	4.14	4.14	0.35	0.328	0.33	0.336	2	1	1.2	1.4
Mean	0.33	0.32	0.32		4.2	4.12	4.12		0.345	0.3217	0.3233		3.13	2.4	2.5	
LSD $_{0.05}$(*Jatropha* parts)		Ns				0.02				0.0087				0.31		
LSD $_{0.05}$ (Tannins)		Ns				Ns				Ns				0.44		
LSD $_{0.05}$(*Jatropha* parts X Tannins)		Ns				Ns				Ns				0.76		

ns = not significant, L = leaf, R= root, and S = seed

Table 3: Effect of Tannins and Jatropha parts on percentage nutrient composition of Nitrogen, Phosphorus and Fat as influenced by root-gall nematode

	% Nitrogen				% Phosphorus				% Fat				Root-gall index (0-4)			
	Jatropha Parts				*Jatropha* Parts				*Jatropha* Parts				*Jatropha* Parts			
Tannins (ml)	L	R	S	Mean	L	R	S	Mean	L	R	S	Mean	L	R	S	Mean
Untreated (control)	2.46	2.47	2.44	2.457	0.0059	0.0059	0.0059	0.0059	0.078	0.0777	0.0776	0.0778	4	4	4	4
1	1.98	2	2.02	2.001	0.0050	0.0054	0.0051	0.0051	0.12	0.0777	0.0891	0.0956	3	3.6	3	3.2
2	1.53	1.97	1.83	1.774	0.0039	0.0050	0.0044	0.0044	0.123	0.081	0.11	0.1047	2.8	3.8	2.8	3.13
3	1.42	1.53	1.44	1.462	0.0036	0.0040	0.0031	0.0036	0.131	0.088	0.152	0.1237	1.8	2.8	1.8	2.13
4	1.35	1.51	1.42	1.427	0.0029	0.0037	0.0030	0.0032	0.21	0.097	0.2	0.169	1	2	1.2	1.4
5	1.33	1.47	1.37	1.389	0.0028	0.0033	0.0030	0.0030	0.213	0.15	0.201	0.188	1	1.8	1	1.26
Mean	1.68	1.82	1.75		0.0041	0.0046	0.0041		0.1458	0.0952	0.1383		2.26	3	2.3	
LSD $_{0.05}$(*Jatropha* parts)		0.03					0.0020			0.0001				0.33		
LSD $_{0.05}$ (Tannins)		0.04					0.0020			0.0001				0.46		
LSD $_{0.05}$(*Jatropha* parts X Tannins mls)		0.06					0.0040			0.0002				ns		

ns = not significant, L = leaf, R= root and S = seed

Table 4.: Effect of Tannins and *Jatropha* parts on percentage nutrient composition of Sodium, Calcium and Moisture as influenced by root-gall nematode

	% Sodium				% Calcium				% Moisture				Root-gall index (0-4)			
	Jatropha Parts				Jatropha Parts				Jatropha Parts				Jatropha Parts			
Tannins (ml)	L	R	S	Mean	L	R	S	Mean	L	R	S	Mean	L	R	S	Mean
Untreated(control)	0.0384	0.0377	0.0376	0.0379	0.204	0.202	0.202	0.203	64.5	63.3	62.5	63.433	4	4	4	4
1	0.0403	0.038	0.0381	0.0388	0.307	0.297	0.302	0.302	65.8	64.82	65.5	65.373	3	3.6	3	3.2
2	0.0413	0.0381	0.0382	0.0392	0.402	0.304	0.4	0.369	65.9	65.5	66.5	65.967	2.8	3.8	2.8	3.13
3	0.047	0.0382	0.0401	0.0418	0.431	0.356	0.406	0.397	70.54	69.5	70.5	70.18	1.8	2.8	1.8	2.13
4	0.0502	0.0397	0.0442	0.0447	0.504	0.46	0.497	0.487	76.7	72.5	76.5	75.233	1	2	1.2	1.4
5	0.0591	0.0432	0.05	0.0507	0.563	0.494	0.561	0.539	89.55	76.5	85.5	83.85	1	1.8	1	1.26
Mean	0.0461	0.0391	0.0419		0.402	0.352	0.395		72.165	68.687	71.167		2.26	3	2.3	
LSD $_{0.05}$(*Jatropha* parts)		0.0001				0.0012				0.3525				0.33		
LSD $_{0.05}$ (Tannins)		0.0001				0.0017				0.4986				0.46		
LSD $_{0.05}$(*Jatropha* part X Tannins mls)		0.0002				0.0029				0.8636				ns		

*** = Significant, ns = not significant, L = leaf, R= root, S = seed**

CONCLUSION

J. curcas Tannins application reduced root-gall nematode infection and enhanced okra pod yield, mucilaginous property, and nutritional composition of okra. However, since the crude tannins used in this study was found to be nematicidal to the *M. incognita* membrane, further research should be undertaken on its utilization in the field and for the development of new environmentally friendly nematicidal agents.

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