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USE OF PHYSICNUT ALKALOIDS TO CONTROL ROOT-GALL NEMATODE AND TO IMPROVE MUCILAGINOUS PROPERTY AND NUTRIENT COMPOSITION OF OKRA

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ABSTRACT

In the quest for a sustainable nematode control strategy, alkaloids of physic nut (*Jatropha 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 season. The experiment was a 3×6 factorial experiments arranged in Completely Randomized Design with five replications in the screen house. Alkaloids extracted from the seeds, leaves and roots of physic nut plants were applied separately at 0, 1, 2, 3, 4 and 5 ml / potted okra plant inoculated with 1,200 infective (J2) larvae of *M. incognita*. Results showed that 5 ml of Root and Seed Alkaloids application significantly (P=0.05) reduced root-gall nematode infection to a gall index of 1 and 1.2 (rarely galled) when compared to the untreated control (4 -severely galled) plants thereby achieving more than 100 percent increase in mucilaginous property of okra over the control for both years of study. Moisture content, fat, calcium and sodium also increased more than the untreated control at 5 ml of root and seed alkaloids. Phosphorus and nitrogen contents were on the other hand rather enhanced by the infection. Neither Okra nor root-gall nematode infection affected ash, magnesium and potassium contents of the fruits. Nevertheless, bioactive active constituent of Physicnut plant parts such as root and seed alkaloids can thus be developed as eco-friendly non-synthetic nematicide for improved yield.

Keywords: Alkaloids; *Jatropha curcas;* mucilaginous property; nutrient composition; Okra; Physic nut; Root-gall nematode infection.

INTRODUCTION

"Okra, *Abelmoschus esculentus* (L) moench belongs to the family *Malvaceae*. It is regarded as an economically important vegetable crop because of its nutritional value that has the potential to improve food security" [1]. "It contains valuable food ingredients, which can be successfully utilized to build up and repair the body" [2,3]. "The fruits are very mucilaginous and Carbohydrates are mainly present in the form of mucilage" [4,5].

"Okra contains proteins, carbohydrates and vitamin C and plays a vital role in human diet" [6]. "Consumption of young immature okra pods is important as fresh

fruits can be boiled, fried or cooked. The composition of okra pods per 100 g edible portion (81% of the product as purchased, ends trimmed) is: water 88.6 g, energy 144.00 kJ (36 kcal), protein 2.10 g, carbohydrate 8.20 g, fat 0.20 g, fibre 1.70 g, Ca 84.00 mg, P 90.00 mg, Fe 1.20 mg, β-carotene 185.00 µg, riboflavin 0.08 mg, thiamin 0.04 mg, niacin 0.60 mg, ascorbic acid 47.00 mg" [7,8]. Therefore, promoting the consumption of okra pods could provide cheap sources of nutrients that can improve the nutritional status and general well-being among resource-constrained households.

Following interest generated by okra as an invaluable tropical crop, efforts have been made to increase its production and yield per unit area. However, the yield potential of this crop has not been achieved in this part of the tropics due to disease factor [9] reported that "in fields infested with *Meloidogyne incognita* and planted with Okra (*Abelmoschus esculentus*), Tomato (*Lycopersicon esculentus*) and Eggplant (*Solanum melongena*), the nematode population at harvest increased by 69% under okra, 40% under tomato and 32% under eggplant". This implies that okra is a good host, tomato a moderate host and eggplant a poor host. Root-galling of *M. incognita* decrease the ability of infected plants to translocate nutrients and water from soil and this cause symptoms of water and nutrient deficiencies on the above ground parts of the plants thereby affecting growth and yield of crop.

"Over the years several nematode management strategies which have been introduced. This included use of certain agronomic measures, cultivation of resistant cultivars, predators and parasites, allelochemicals of cover crops, organic amendments, sanitation, solarization, nanotechnology, resistant cultivars, plant extracts and synthetic nematicides" [10].

"However, with diminishing options for use of synthetic nematicides for control, *Meloidogyne spp* problems are steadily beginning to resurface. Disadvantages associated with the use of synthetic chemicals in agriculture cannot be over emphasized. Pesticides residues have been reported in vegetables such as onions, lettuce, cabbage, spinach, okra and tomato" [11]. This clearly indicates the need for alternative control measures which are environmentally safe and effective against the pests.

"Although, some higher plants have been examined as sources of novel compounds with activity against parasitic nematodes [12], the use of plant extract as alternative nematicides is becoming widespread and gaining prominence. The lack of scientific validation of the active compounds of such plants has hampered the optimization of its use. Little is however, known on the relative efficacy of *J. curcas* and its phytochemical constituents in the control of root-knot nematode disease for improved okra yield".

MATERIALS AND METHODS

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 (Hand held 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)"* [13].

Plant Materials and Preparation of Crude Extracts

"Fresh plant materials of twelve month old physic nut plants (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" [14].

Extraction of the Alkaloids was done using the continuous extraction methods using soxhlet apparatus. Four hundred grams (400 g) of 100 □m size powder samples were weighed and packed in a cheese cloth bag which served as extraction thimble.

The thimble was then placed into a conical flask with cover and the sample was moistened with 650 ml amount of 95 % Ethanol. This was made alkaline with sufficient quantity of ammonia and mixed thoroughly. The sample in the thimble was submerged over night in ethanol and then placed in the soxhlet extractor on the next day and the sample was extracted for about 3-4 hours. The ethanol extract was filtered and was evaporated in soxhlet distillate apparatus at 60^{o}C. The crude alkaloid extract was further treated with 40 ml of 0.1 N Hydrochloric acid. This was filtered and the filtrate was collected in conical flask. The filtrate was alkalinified with 2 ml of ammonia and placed in a separatory funnel. 300 ml of of chloroform was added into the separatory funnel, mixed and shaken for about ten minutes and allowed to separate into two layers. The lower layer of the chloroform contained the alkaloids and the upper layer the aqueous portion the remaining alkaloids was extracted from the upper layer until the last chloroform extract tested negative to Dragendoff's reagent. The combined alkaloids extract was evaporated in soxhlet distilling apparatus at 60^{o}C and also evaporated in water bath maintained at the same temperature until semi-dry. The residue was weighed and percentage yield of alkaloids was calculated using the formula reported by [15].

$$\% \text{ Yield} = \frac{\text{Weight of the alkaloids residue x .100}}{\text{Weight of sample}}$$

Test for Alkaloids

Five millilitres of the extract was added to 2 ml of HCl. To this acidic medium 1 ml of Dragendroff's reagent was added. An orange or red precipitate produced immediately indicated the presence of alkaloids.

Experimental Design and Layout

"The experiments were carried out as factorial in completely randomized Design (CRD) with six treatment applications in five replications. The Alkaloids 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 4 kg of steam sterilized soil. The treatments were randomly assigned to the experimental pots and applied at various rates: 0.00 ml (control), 1.00 ml, 2.00 ml, 3.00 ml, 4.00 ml and 5.00 ml on okra plant cultivar (NHA_e47 - 4). All the test plants were inoculated and treated with various rates of the phytochemical under study. Inoculated plants without treatment or zero rate of treatment applications served as the control" [14].

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 labeled. 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 and Application

"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 1200 ml by adding more water. Thirty millilitres (30 ml) 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.Ten days after planting, all test okra cultivars were inoculated by measuring out and pouring 30 ml 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. The inoculated plants were kept in a well ventilated screen house" [14].

Phytochemical Application

"The Pure Alkaloids substrate extracted were diluted in distilled water to obtain a final concentration of 500 mg/ml using the formula $V_1C_1=V_2C_2$" [16]. Alkaloids, were applied at the base of the potted plants at the same time plants were inoculated (i.e. ten days after planting) at the following rates: 0.00, 1.00, 2.00, 3.00, 4.00, and 5.00 ml per plant.

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 [17]. "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 [18] methods". "Nitrogen was determined by the micro Kjeldahl method" [18]. "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 [19]. 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 [20] 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

RESULTS AND DISCUSSION

Figs. 1 and 2 shows the effect of Alkaloids extract on root-gall nematode disease *M. incognita* and okra pod weights in 2019 and 2020 cropping season. Okra plants on pots treated with 5 mls of seed and root alkaloids produced okra pod weights significantly ($p<0.05$) higher than leaf alkaloids and the severely galled untreated control in 2019. "The efficacy of the seeds and root parts of *J. curcas* have been variously reported [21-23] to have insecticidal and cytonematicidal activities on stored seeds and field crops". The same was true for 2020 cropping season which produced highest Okra pod weights on application of 5 mls of seed and root alkaloids at reduced gall index. The observed increases obtained in both years may due to the nematicidal effect of the alkaloids which interfered with nematode metabolism thereby suppressing nematode activity. Ibrahim et al. [24] reported that "Alkaloids interfere with processes such as DNA replication and RNA transcription which are vital to micro-organisms".

Plants treated with alkaloids produced okra pod with significantly ($p<0.05$) higher mucilaginous property than the severely galled untreated plants in both years (2019 and 2020) (Table 1). Mucilaginous property significantly ($p<0.05$) increased with decreased root galls at increased rates of alkaloids treatment. Root alkaloids at 5 mls produced pods with higher mucilaginous property compared to other rates and the untreated control in both years. This was followed by plants treated with 5 ml of seed alkaloids. Plants treated with leaf alkaloids had the least mucilaginous property in okra pods.

Table 2 showed that neither root-gall disease *nor J. curcas* alkaloids affected magnesium (Mg) and potassium (K) and ash contents of okra fruits. Phosphorus (P) and nitrogen (N) content contents were significantly ($P<0.05$) enhanced by root-gall nematode infection when compared with the untreated control (Table 3). "The enhanced Phosphorus and Nitrogen content observed in the root-gall nematode infected plants could be due to uninterrupted translocation of phosphorus [25] 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 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" [26].

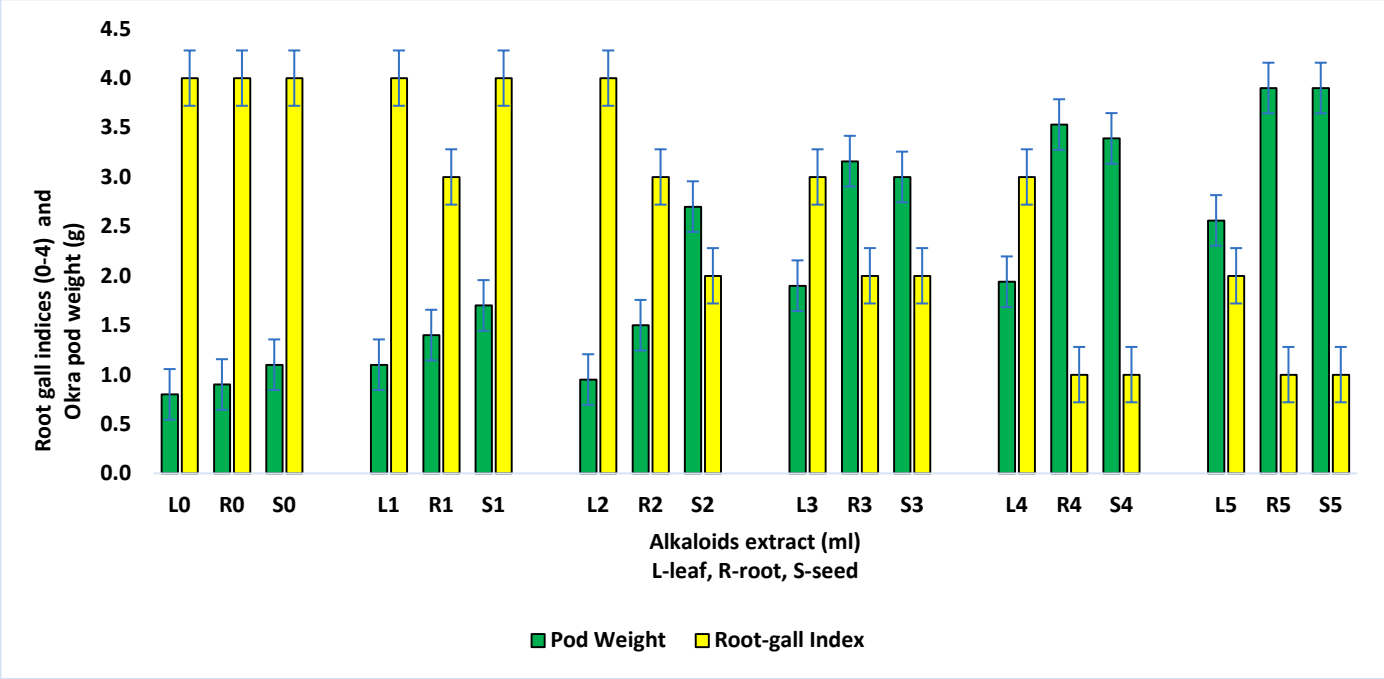

Fig. 1. Shows the relationship between root-gall index, okra pod weight and Alkaloids in 2019

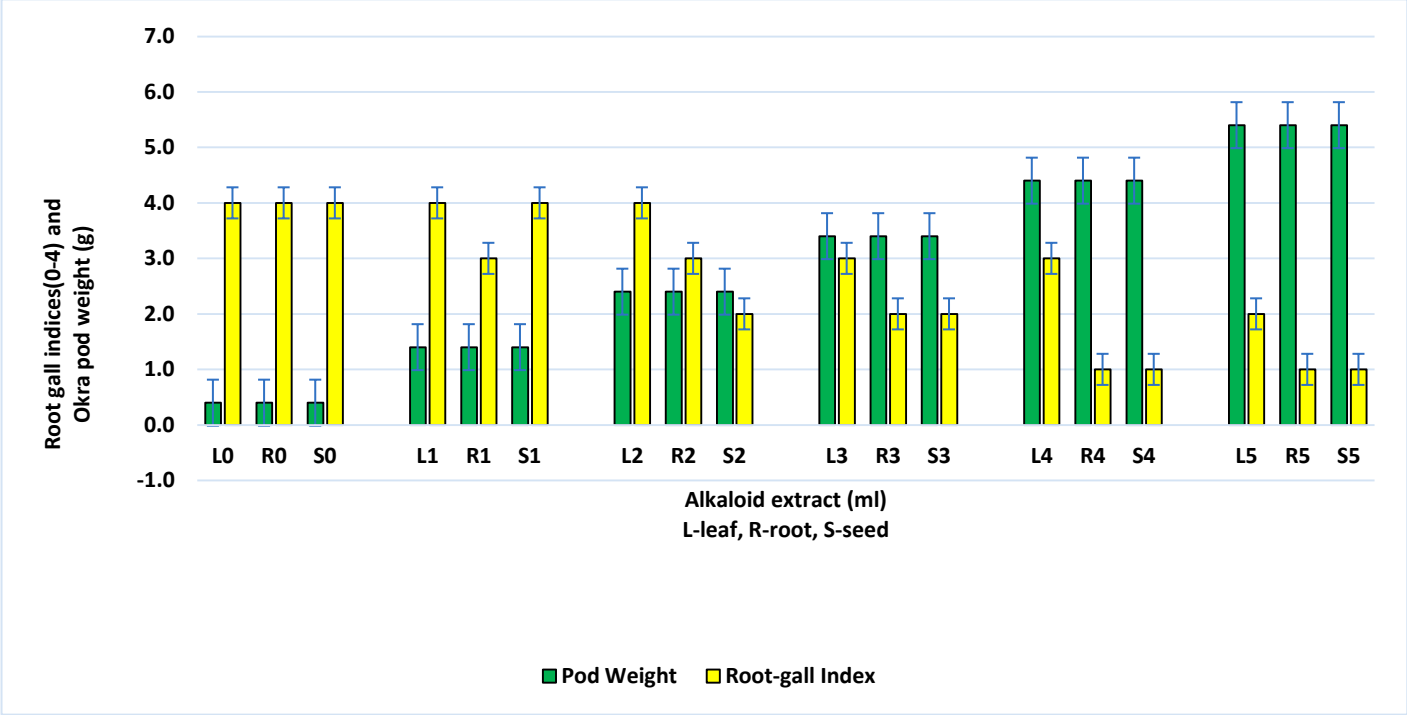

Fig. 2. Shows the relationship between root-gall index, okra pod weight and Alkaloids in 2020

Table 1. Effect of Alkaloids and *Jatropha* parts on Okra Mucilaginous as influenced by root-gall nematode [14]

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**			
Alkaloids (ml)	**L**	**R**	**S**	**Mean**	**L**	**R**	**S**	**Mean**	**L**	**R**	**S**	**Mean**	**L**	**R**	**S**	**Mean**
Untreated **(control)**	1021.00	1000.00	1010.00	1010.30	4.00	4.00	4.00	4.00	1024.00	1003.00	1013.00	1013.00	4.00	4.00	4.00	4.00
1	1099.00	1111.00	1101.00	1103.70	3.80	3.40	3.60	3.60	1102.00	1114.00	1104.00	1106.00	3.60	3.20	3.40	3.40
2	1120.00	1201.00	1190.00	1170.30	3.80	3.00	3.20	3.33	1123.00	1204.00	1193.00	1173.00	3.80	3.00	3.20	3.33
3	1231.00	1389.00	1301.00	1307.00	3.00	2.00	2.20	2.40	1234.00	1392.00	1304.00	1310.00	3.00	2.00	2.00	2.33
4	1306.00	1701.00	1664.00	1557.00	2.60	1.20	1.40	1.73	1309.00	1704.00	1667.00	1560.00	2.40	1.20	1.40	1.66
5	1601.00	2180.00	2101.00	1960.70	2.20	1.00	1.20	1.46	1604.00	2183.00	2104.00	1963.00	2.00	1.00	1.20	1.40
Mean	1229.70	1430.30	1394.50		3.23	2.43	2.60		1232.00	1433.00	1397.00		3.13	2.40	2.53	
LSD $_{0.05}$(*Jatropha* Parts)		6.64				0.28				6.64				0.31		
LSD $_{0.05}$ (Alkaloids)		9.40				0.40				9.40				0.44		
LSD $_{0.05}$(*Jatropha* Parts × Alkaloids)		16.28				ns				16.28				ns		

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

Table 2. Effect of Alkaloids 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			
Alkaloids (ml)	**L**	**R**	**S**	**Mean**	**L**	**R**	**S**	**Mean**	**L**	**R**	**S**	**Mean**	**L**	**R**	**S**	**Mean**
Untreated**(control)**	0.1050	0.1042	0.1058	0.1046	3.2650	3.2430	3.2400	3.2500	0.1142	0.1144	0.1136	0.11407	4.0000	4.0000	4.0000	4.0000
1	0.1048	0.1042	0.1046	0.1042	3.3020	3.3240	3.3690	3.3310	0.1142	0.1148	0.1138	0.11427	3.6000	3.2000	3.4000	3.4000
2	0.1042	0.1032	0.1044	0.1041	3.3740	3.4410	3.4410	3.4190	0.1142	0.1148	0.1142	0.1144	3.8000	3.0000	3.2000	3.3300
3	0.1032	0.1038	0.1044	0.1036	3.4110	3.5650	3.5700	3.5150	0.1146	0.1150	0.1138	0.11447	3.0000	2.0000	2.0000	2.3300
4	0.1042	0.1036	0.1022	0.1032	3.4880	3.6140	3.6180	3.5730	0.1148	0.1154	0.1144	0.11487	2.4000	1.2000	1.4000	1.6600
5	0.1042	0.1034	0.1032	0.1036	3.5840	3.9200	3.8590	3.7880	0.1152	0.1154	0.1146	0.11507	2.0000	1.0000	1.2000	1.4000
Mean	0.1040	0.1032	0.1048		3.4040	3.5180	3.5160		0.115	0.115	0.1141		3.1300	2.4000	2.5300	
$LSD_{0.05}$(J.Parts)		ns				ns				ns				0.3100		
$LSD_{0.05}$(Alkaloids)		ns				ns				ns				0.4400		
$LSD_{0.05}$(J. Parts × Alkaloids)		ns				ns				ns				0.7600		

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

Table 3. Effect of Alkaloids 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			
Alkaloids (ml)	**L**	**R**	**S**	**Mean**	**L**	**R**	**S**	**Mean**	**L**	**R**	**S**	**Mean**	**L**	**R**	**S**	**Mean**
Untreated **(control)**	2.4500	2.4400	2.4500	2.4467	0.0058	0.0036	0.0059	0.0051	0.0681	0.0778	0.0780	0.0746	4.0000	4.0000	4.0000	4.0000
1	1.9600	1.7100	1.8800	1.8500	0.0049	0.0037	0.0049	0.0045	0.8002	0.1000	0.0890	0.3297	3.6000	3.2000	3.4000	3.4000
2	1.8800	1.6100	1.7000	1.7300	0.0004	0.0038	0.0004	0.0039	0.0900	0.1060	0.1000	0.0986	3.8000	3.0000	3.2000	3.3300
3	1.6900	1.5000	1.6600	1.6170	0.0039	0.0039	0.0025	0.0034	0.1000	0.1300	0.1400	0.1233	3.0000	2.0000	2.0000	2.3300
4	1.5300	1.4660	1.5000	1.4987	0.0038	0.0010	0.0038	0.0030	0.1500	0.1945	0.1845	0.1763	2.4000	1.2000	1.4000	1.6600
5	1.4300	1.3720	1.4200	1.4070	0.0037	0.0016	0.0036	0.0030	0.1960	0.2056	0.2050	0.2022	2.0000	1.0000	1.2000	1.4000
Mean	1.8233	1.6830	1.7683		0.0044	0.0029	0.0039		0.2340	0.1356	0.1327		3.1300	2.4000	2.5300	
LSD $_{0.05}$(J.Parts)		0.0185				0.0001				0.0001				0.3100		
LSD $_{0.05}$ (Alkaloids)		0.0262				0.0001				0.0001				0.4400		
LSD $_{0.05}$(J. Parts × Alkaloids)		0.0454				0.0002				0.0001				0.7600		

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

Table 4. Effect of Alkaloids 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			
Alkaloids (ml)	**L**	**R**	**S**	**Mean**	**L**	**R**	**S**	**Mean**	**L**	**R**	**S**	**Mean**	**L**	**R**	**S**	**Mean**
Untreated **(control)**	0.0375	0.0386	0.0376	0.0379	0.2026	0.2050	0.2040	0.2038	62.9000	63.8000	61.1000	62.6000	4.0000	4.0000	4.0000	4.0000
1	0.0377	0.0400	0.0379	0.0385	0.3000	0.3030	0.3000	0.3010	63.9000	65.2000	64.2000	64.4330	3.6000	3.2000	3.4000	3.4000
2	0.0380	0.0409	0.0380	0.0389	0.3890	0.3990	0.3900	0.3926	64.6000	66.3000	66.1000	65.6670	3.8000	3.0000	3.2000	3.3300
3	0.0400	0.0432	0.0400	0.0410	0.4200	0.4200	0.4010	0.4126	68.2000	70.0300	69.3000	69.1770	3.0000	2.0000	2.0000	2.3300
4	0.0444	0.0460	0.0440	0.0448	0.4500	0.5010	0.4956	0.4822	70.2000	76.1000	75.1000	73.8000	2.4000	1.2000	1.4000	1.6600
5	0.0488	0.0589	0.0489	0.0522	0.4610	0.5620	0.5606	0.5278	75.3000	91.3200	85.1000	83.9070	2.0000	1.0000	1.2000	1.4000
Mean	0.0410	0.0446	0.0410		0.3704	0.3983	0.3918		67.5170	72.125	70.1500		3.1300	2.4000	2.5300	
LSD $_{0.05}$(J.Parts)		0.0016				0.0003				0.0532				0.3100		
LSD $_{0.05}$ (Alkaloids)		0.0023				0.0004				0.0752				0.4400		
LSD $_{0.05}$(J. Parts × Alkaloids)		0.0040				0.0008				0.1302				0.7600		

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

"Fat, sodium (Na), calcium (Ca) and moisture were significantly reduced by root-gall nematode infection in the untreated control plants (Tables 4). [27] reported increased degradation of fat into fatty acids in nematode infected *Arachys hypogeal*. Reduced Sodium (Na) and Calcium (Ca) had also been reported in the leaves of vetch infected with *M*. *hapla"* [28]. "Percentage moisture of okra fruits was affected by root-gall nematode infection and alkaloids from Jatropha plant parts. There was moisture reduction in fruits of infected plants. This agreed with the report of [29] who stated that xylem vessels disruption in infected roots causes interruption of nutrient translocation to the shoot and consequent interference with carbohydrate synthesis. Plants treated with root alkaloids however produced okra pods with significantly ($P<0.05$) higher percentage of Fat, Sodium (Na), Calcium (Ca) and moisture content".

CONCLUSION

The present study showed that physicnut plant parts can be exploited phytochemically for nematode control and improved yield as 5 ml of root and seed alkaloids reduced root-gall nematode infection of Okra to a gall index of 1 and 1.2 (rarely galled) when compared to the untreated control (4 - severely galled) plants thereby achieving more than 100 percent increase in mucilaginous property of okra over the control for both years of study. Improved pod weights and nutritional composition over the control were also recorded at 5 ml. Since the crude alkaloids extract was observed to be nematicidal in activity, further research efforts is suggested in field experimentation and evaluation toward the development of an eco-friendly nematicide.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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