Australian Journal of Science and Technology

ISSN Number (2208-6404)
Volume 6; Issue 2; June 2022

Original Article

Assessment of *Jatropha curcas L.* as alternative nematicide for root knot nematode (*Meloidogyne incognita*) management

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ABSTRACT

Chemical control of plant parasitic nematodes is associated with many drawbacks and potential dangers to the environment, human and animals. The application of plant extracts has been considered as an ecologically friendly alternative control measure against nematode pest. However, little is known on their bioactive efficacy in the management of root-knot nematodes. The aim of the present study was to assess the nematicidal activity of phytochemicals extracted from *Jatropha curcas* seeds on hatchability and mortality of the root-knot nematode, *Meloidogyne incognita*. Results showed that seed extracts were statistically significant in the control of *M. incognita* at ($P = 0.05$). Most egg hatchability inhibition (0.00, i.e., 100%) was achieved with Saponins after 12 h treatment exposure time at 10 ml, while at 5 ml (0.08) egg hatchability inhibition was also obtained with Saponins after 12 h treatment exposure time. For nematode mortality, alkaloids applied at 10 ml recorded highest mortality (99.73 %) after 72 h treatment exposure while alkaloids at 5 ml also recorded highest mortality (94.73 %) after 72 h treatment exposure time when compared to the control (in distilled water) and other phytochemicals. Least egg mass hatchability and juvenile mortality of nematodes occurred with tannins and flavonoids, respectively. Nematode egg mass hatchability decreased with increased rate of phytochemical dosage while juvenile mortality increased with an increase in the rates of phytochemical extracts applied. *J. curcas* seed extracts exhibited satisfactory control and can successfully be developed to a nematicidal agent in the management of *M. incognita*.

Keywords: Hatchability, *Jatropha curcas*, *Meloidogyne incognita*, mortality, phytochemicals, root-knots

Submitted: 04-05-2022, **Accepted:** 21-05-2022, **Published:** 30-06-2022

INTRODUCTION

Plant-parasitic nematodes are major pathogens on most vegetables in many countries especially in the tropics and subtropics where they are obviously regarded as the cause of serious yield losses on a wide range of crops.[1] Yield losses due to parasitic nematodes are reported to be more than 100 Billion annually.[2] Among all plant parasitic nematodes, root-knot nematodes caused by *Meloidogyne incognita* are the most destructive species which cause serious problem to a great number of economically important crops.[3] Its approximate distribution in agricultural soils of Nigeria is 75 % among *Meloidogyne* species.[4]

Management practices employed to check the incidence and severity of these plant pathogenic organisms have been focused on cultural practices, crop rotation, plant resistance, and chemical nematicides.[5] Most chemical control of plant parasitic nematodes, essentially involves the use of synthetic nematicides. However, in view of the many draw backs and potential dangers associated with its use such as high cost of chemicals, potential pest resurgence, secondary pest outbreaks as well as human and animal poisoning and environmental pollution,[6-8] it has become necessary to view pesticides use as a threat to clean environment and to sustainability in crop production.

One of the prominent, ecologically friendly alternative control measures against nematode pest is the application of plant extracts.[9] Anaele[10] investigated the efficacy of the extract of Siam weed on *M. incognita* while Javad *et al.*[11] reported the efficacy of ginger extract for egg inhibition and larval mortality

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of *Meloidogyne javanica*. Other researchers also have found that neem cake and Rakshak gold (neem-based product) were effective against eggs of *M. incognita*.[12] Phytochemical screening of different plants has revealed numerous bioactive compounds including alkaloids, tannins, flavonoids, glycosides and saponins. These plant secondary metabolites serve as defense mechanism against many pathogenic micro-organisms. However, little is known on their relative bioactive efficacy in the management of root-knot nematodes. In view of this fact, this study was undertaken to evaluate the effect of phytochemical seed extracts from *J. curcas* against root-knot nematode.

MATERIALS AND METHODS

Experimental Design and Layout

The experiment was carried out in the Centre for Agricultural Research Federal University of Technology, Owerri, Nigeria.

Plant Materials and Crude Extracts Preparation

Fresh samples of *J. curcas* fruits with dehisced seeds and seed coats were collected from Federal University of Technology, Owerri. The seeds were dried under shade and ground into coarse powder using mortar and pestle. This was later sieved into fine particles and then stored in a cool dry place until required.

Extraction and Isolation of Alkaloids

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 overnight in ethanol and then placed in the Soxhlet extractor on the next day and the sample was extracted for 4 h. The ethanol extract was filtered and was evaporated in Soxhlet distillate apparatus at 60° 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. Three hundred milliliter (300 ml) of chloroform was added into the separatory funnel, mixed, and shaken for about 10 min and allowed to separate into two layers. The lower layer of the chloroform contained the alkaloids and the upper layer contains the aqueous portion. The remaining alkaloids were 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° 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 Dimaandal.[13]

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

Flavonoids Extraction and Isolation

Each of the air-dried powdered sample was weighed and treated with 80% methanol for 24 h using Soxhlet extractor. The extract obtained from each sample was subsequently extracted in petroleum ether, diethyl ether, and ethyl acetate following the method of Subramanian and Nagarajan (1969).[14] Petroleum ether was discarded due to it being rich in fatty substance. The ether fraction was used for free substance. The ether fraction was used for free flavonoids, whereas ethyl acetate fraction was used for bond flavonoids. Ethyl acetate fraction of each sample was hydrolyzed further with 7% H_2SO_4 for 24 h and was then re-extracted with ethyl acetate. The fraction obtained was repeatedly washed with distilled water to neutrality, dried, and weighed before the yield was calculated.

Extraction and Isolation of Saponins

Five hundred grams (500 g) of seeds sample was taken and ground to obtain 2 mm mesh size. It was subjected to successive Soxhlet extraction first with petroleum ether and subsequently with methanol. The extract was vacuum dried using Rotary Vacuum Flask Evaporator to yield solid residue. Weight of the solid obtained after drying was 60 g. Solid residue was refluxed with 80% w/v ethyl acetate for half an hour. The solvent was decanted off and discarded. The residue was then dissolved in 90% w/v methanol. It was further filtered, concentrated and was precipitated in acetone to yield saponins glycosides. The solvent was decanted off. Precipitate was filtered, dried and was weighed to estimate total saponins content of the plant drug as described by Amin *et al*.[15]

Extraction and Isolation of Tannins

The purification procedure was essentially according to that of Strumeyer and Malin.[16] 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 min at 2500 rpm was evaporated under vacuum at about 30° 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 color. 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 xmg of ascorbic acid. The acetone was removed under vacuum at 30° 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_1 gives the weight of the tannins containing ascorbic acid (Y, mg). Hence, the weight of the tannins was obtained by (Y, mg-X, mg.).

The isolated phytochemicals were tested using various confirmatory tests.

Extraction of Juveniles

Roots of Indian spinach severely galled by *M. incognita* were harvested from the pot culture, washed and cut (1-2 cm) in pieces. The cut roots were shaken vigorously in a flask bottle containing 3 l of water and 0.5 % NaOCl for 5 min. The eggs were passed through a 75 and 25µm pore sieve mesh. Then the water containing eggs through the 25µm pore sieve mesh was collected. The eggs obtained were viewed under microscope to ascertain the population of the overall eggs and this was later kept under cool temperature for 1 week to hatch thirty milliliters (30 ml) of larvae suspension was taken and poured into a counting dish placed under an electronic stereomicroscope and the number of larvae counted. Mean number of three counts was 30 active larvae per 30 ml suspension. Therefore, 30 ml suspension containing 30 larvae (J_2) was introduced in each Petri dish.

Extraction of Eggs

Infected roots of Indian spinach maintained in pots were harvested, washed well and chopped into smaller pieces. The chopped roots were soaked in a solution containing 5.2% sodium hypochlorite, (NaOCl) and shaken vigorously for 2 min according to Adegbita and Adesiyan.[4] Eggs contained in the suspension were sieved by placing it on a 75µ and 25µ sieve mesh. The eggs were then viewed under compound microscope and the number of eggs counted.

Nematicidal Assay

The effects of alkaloids, saponins, flavonoids, and tannins extracted from *J. curcas* seeds, were separately applied at 0, 5, and 10 ml to 30 juveniles (J_2) of *M. incognita* in distilled water suspension in sterilized Petri dishes. Completely Randomized Design (CRD) experiment was adopted and each treatment was replicated 3 times. All the Petri dishes were kept on laboratory/incubation table at ambient temperature (±30° C). With the aid of electronic stereomicroscope at magnification 100×, dead and living juveniles (J_2) were counted after 12, 24, 48, and 72 h of exposure to each treatment. The juvenile (J_2) was considered dead when it did not move on physical stimulus with a fine needle.[17] Percentage mortality was calculated as ratio of number of dead juveniles to original number of juveniles and expressed in percentage.

$$\text{i.e., } \%\ \text{Mortality} = \frac{X}{N} \times \frac{100}{1}$$

Where
X = Number of dead juveniles (J_2)
N = Original number of juveniles (J_2)

To determine the effect of *J. curcas* parts extracts on egg masses, 20 eggs were placed in each Petri dishes containing 0, 5, and 10 ml of different extracts. Each treatment was replicated 3 times. After 12, 24, 48, and 72 h of exposure, the number of juveniles hatched was counted with the aid of inverted microscope at magnification 40×.

Data Analysis

The data were analyzed as factorial experiment in CRD by using ANOVA (Genstat Edition 4). Least significant differences were calculated at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of the Extracts on the Hatchability of *M. incognita*

Effect of phytochemical seed extracts, rates, and hours of exposure on hatchability of *M. incognita* eggs is shown in Table 1. The various phytochemical seed extracts reduced hatchability of the nematode egg masses compared to control. The previous researchers have reported similar inhibition of egg hatching and mortality of *M. incognita* juveniles after application of phytochemicals.[18] Phytochemicals either affect embryonic development or kill the eggs or even dissolve the egg masses.[4]

Table 1: Effect of phytochemical seed extracts, rates and hours of exposure on hatchability of *Meloidogyne incognita* eggs

Phytochemical seed extracts (Treatments)	Rate (ml)			
	0	5	10	Mean
Alkaloids	4.42	2.58	1.92	2.97
Flavonoids	4.42	1.00	0.33	1.92
Saponins	4.42	0.08	0.00	1.50
Tannins	4.42	3.08	1.75	3.08
Mean	4.42	1.69	1.00	
$LSD_{0.05}$ (Rate)		0.52		
$LSD_{0.05}$ (Phytochemical)		0.60		
$LSD_{0.05}$ (Phytochemical×Rate)		1.04		

Highest hatchability inhibition occurred on application of saponins which significantly ($P < 0.05$) differed from the effects of flavonoids, alkaloids and tannins [Table 1]. It has been reported that the biological effect of saponins may be due to their specific interaction with cell membrane which causes changes in cell permeability.[19] Hatchability significantly ($P < 0.05$) reduced with increased rates of phytochemicals applied. Highest hatchability inhibition (100%) was obtained with saponins at 10 ml when compared to other treatments after 12 h of incubation period [Table 1]. Nematode egg mass hatchability increased with increased hours of exposure to the phytochemical treatments. This is in conformity with similar work by the previous researchers on the effect of plant extracts on egg hatching of the nematode *Rotylenchulus reniformis*.[20]

Phytochemical rates correlated negatively with hatchability. However, hatchability inhibition was strongest at 12 h period of treatment exposure or incubation [Table 1]. The previous researchers also reported strongest hatchability inhibition at 12 h when compared 24, 48, and 72 h treatment exposure time.[21] Least egg mass hatchability inhibition occurred at 72 h of incubation on application of 5 ml of phytochemical treatments [Table 2].

Effect of the Extracts on Mortality of *M. incognita* Juveniles

The effect of phytochemical seed extracts and hours of exposure on mortality of *M. incognita* juveniles is shown in Table 3. The seed extracts applied caused mortality of *M. incognita* juveniles (J_2), compared to the control. Highest mortality occurred on application of alkaloids and saponins which differed significantly ($P = 0.05$) from those of flavonoids and tannins. The mortality level achieved with alkaloids may be attributed to mechanisms such as disruption of protein synthesis, stability of biomembranes, and metabolically important enzymes.[22] Alkaloids interfere with processes such as DNA replication and RNA transcription which are vital to micro-organisms. More so, saponins from *Medicago sativa* have been reported to inhibit cholesterol accumulation in egg and/or larva.[23] The mechanism of action of the phytochemicals might be due to their cytotoxicity.[24,25]

Juvenile mortality increased with increased rate of phytochemical treatment. Highest mortality rate of 99.73% and 98.9% occurred at 10 ml of alkaloids and saponins after 72 h incubation period, respectively [Figure 1]. This study agrees with that of previous researchers who reported that increased concentrations of 5 and 10% of aqueous plant extracts significantly increased mortality of nematode juveniles.[26]

Nematode juvenile mortality not only increased with increased hours of exposure to the phytochemicals but was highest at 72-h incubation time both at 5 ml and 10 ml application [Table 4]. This work agrees with the earlier report that mortality of

Table 2: Effect of hours of exposure intervals and rates on hatchability of *Meloidogyne incognita* eggs

Hours of exposure	Rates (ml)			
	0	5	10	Mean
12 h	2.67	0.92	0.58	1.39
24 h	4.00	2.08	1.33	2.47
48 h	4.67	2.00	1.25	2.64
72 h	6.33	1.75	0.83	2.97
Mean	4.42	1.69	1.00	
$LSD_{0.05}$ (Rate)		0.52		
$LSD_{0.05}$ (Hours of Exposure)		0.60		
$LSD_{0.05}$ (Hours of Exposure×Rate)		1.04		

Table 3: Effect of phytochemical seed extracts and rates on mortality of *Meloidogyne incognita* juveniles

Pytochemical seed extracts (Treatments)	Rates (ml)			
	0	5	10	Mean
Alkaloids	0.00	26.5	29.92	18.81
Flavonoids	0.00	16.83	21.75	12.86
Saponins	0.00	26.17	29.67	18.61
Tannins	0.00	17.92	24.58	14.17
Mean	0.00	21.85	26.48	
$LSD_{0.05}$ (Rate)		1.18		
$LSD_{0.05}$ (Phytochemical)		1.36		
$LSD_{0.05}$ (Phytochemical×Rate)		2.36		

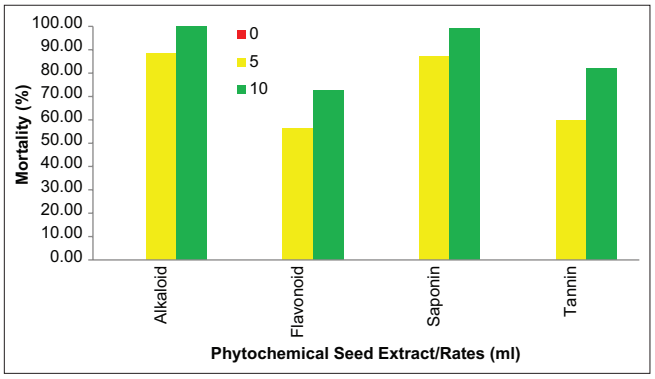

Figure 1: Relationship between phytochemical seed extracts and juvenile mortality of root-knot nematode, *Meloidogyne incognita*

M. incognita juveniles was highest at 72-h treatment exposure time when compared to 12, 24, and 48-h exposure time.[27] It was also reported that juvenile mortality increased with corresponding increase in time of exposure to *Citrus medica* leaf extracts.[28]

There was a positive correlation between phytochemical rate, period of incubation, and Juvenile mortality rate. Least

Table 4: Effect of hour of exposure intervals and rates on mortality of *Meloidogyne incognita* juveniles

Hours of exposure	Rates (ml)			
	0	5	10	Mean
12 h	0.00	14.67	22.08	12.25
24 h	0.00	20.08	26.33	15.47
48 h	0.00	24.33	28.50	17.61
72 h	0.00	28.33	29.00	19.11
Mean	0.00	21.85	26.48	
$LSD_{0.05}$ (Rate)		1.83		
$LSD_{0.05}$ (Hours of exposure)		1.36		
$LSD_{0.05}$ (Hours of exposure×Rate)		2.36		

nematode mortality (16.83) occurred at 5 ml of flavonoids treatment application while 12 h of incubation time recorded the least mortality of juvenile nematodes for all phytochemicals studied [Table 3 and 4].

CONCLUSION

This study shows that the phytochemical seed extracts of *Jatropha curcas* affected both the mortality and egg mass hatchability of the root-knot nematode, *M. incognita*. Crude alkaloids and saponins were found to be most active in this study and, therefore, could be useful in the development of new nematicidal agents for root-knot management. In view of the environmental challenges associated with the use of synthetic conventional nematicides, the crucial role played by these phytochemicals in this study is very promising toward provision of an ecologically friendly alternative control measure against nematode pests.

ACKNOWLEDGMENT

The authors are thankful to the staff of the Centre for Agricultural Research, Federal University of Technology, Owerri, Nigeria and the New Concept Analytical Laboratory, Obinze, Owerri, Nigeria for their assistance.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that no competing interest exists.

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