# EVALUATION OF *Jatrophacurcas* LEAF EXTRACTS FOR NEMATICIDAL PROPERTIES AGAINST ROOT-KNOT NEMATODE *Meloidogyn incognita.*

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**ABSTRACT**
Alkaloids, Saponins, Flavonoids and Tannins extracted from *Jatrophacurcas* leaves were tested for nematicidal action against a root-knot nematode, *Meloidogyne incognita*. These phytochemicals were applied at 0,5 and 10 mls per thirty (30) juveniles ($J_2$) of *M. incognita* suspended in 10 mls of distilled water in sterilized petri-dishes. The petri-dishes were later laid out in a 3x4x4 factorial experiment in Completely Randomized Design (CRD) with three replications and incubated for 12, 24, 48, and 72hours.Results showed increased juvenile ($J_2$) mortality rate at increased phytochemical rate and incubation period. Highest mortality rate of 89.4% (i.e. 26.83 dead juveniles) occurred at 10 mls of Alkaloid at 72hours incubation period. This differed significantly (P=0.05) from those of Flavonoids (69.80% i.e 20.92 dead juveniles) and Tannin (69.40 i.e. 20.83 dead juveniles).No juvenile mortality occuredin petri-dishes with no phytochemical application.

**Keywords**:*Meloidogyne incognita*, *Jatrophacurcas*, phytochemicals, Root-knots, Mortality

## INTRODCUTION

Plant parasitic nematodes have been reported to cause serious constraints to increased crop production (Afolami and Adigbo, 1999). Abad *etal*., (2008) reported an estimated global annual loss of $157 to Root-knot nematode (*Meloidogyne* spp.) attack.In Africa, *Meloidogynearenaria*, *M.javanica* and *M. incognita* are regarded as the most dominant species (IITA, 1981; De Waele and Elsen, 2007).

The population of these plant parasitic nematodes have been controlled over the years in the field through several approaches such as the use of natural enemies, adoption of cultural practices, cultivating resistant cultivars and pesticide application (Khan and Kim, 2007; Khan *etal.*, 2007; Okada and Harada, 2007; Williamson and Kumar, 2006; Browning *etal.*, 2006).

However, the disadvantages associated with the use of pesticides in agriculture cannot be over emphasized. Pesticide residues have been reported in vegetable (EL-Nahhal, 2004). Wahab, (2009) reported that the extensive and very indiscriminate use of these synthetic plant protectants on pests and diseases have often aided development of resistance and ensured serious damage to their natural enemies (Cui *etal*., 2013). This clearly indicated the need for alternative control measure which is environmentally safe and effective against the pest.

An increasingly promising alternative to the use of chemical nematicides is the exploitation of higher plant as a source for novel compounds with naturally occurring nematicides (Adesiyan*etal*., 2000).

Susan *etal.*, (2005) studied the leaf extract of *Ocimumbasilicum, Tageteserecta, Chrysanthemum cinerariafoluim, Meliaazadirach* and seed extract of *Azadirachtaindica* against the root knot nematode *Meloidogyne incognita* on egg plant.Javed*etal*; (2003) also reported the efficacy of ginger extract for egg inhibition and larval mortality.

Although, some higher plants have been examined as some of the novel compounds with activity against nematodes (Adesiyan*etal*., 2000) not much is known on the efficacy of the individual phytochemicals present in *Jatrophacurcas*. The objective of this study was therefore to determine the nematicidal effect of some phytochemicals, extracted from*Jatrophacurcas* leaves against a root-knot nematode, *Meloidogyne incognita*

## MATERIALS AND METHODS

### Plant Materials And Crude Extracts Preparation

Fresh samples of the leaves of *Jatrophacurcas* were collected from Federal University of Technology, Owerrilocated on Lat $5^0$27 50.23" North and Longitude $07^o$2 49.33" East at 55 msl (Hand held global positioning system) and 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 and sieved into fine particles and then stored in a cool dry place until required

### Extraction and Isolation of Alkaloid

The extraction of the alkaloids was done using the continuous extraction methods using soxhlet apparatus, four hundred grams (400g) of 2mm size powder samples was weighed and packed in a cheese cloth bag which served as extraction thimble.The thimble was then placed into a suitable jar with cover and the sample was moistened with sufficient amount of 95% Ethanol. This was made alkaline with sufficient quantity of ammonia and mixed thoroughly. The sample in the thimble was macerated overnight 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 concentrated in soxhlet distillate apparatus at 60 $^{o}$c. The crude alkaloid extract was further treated with 0.1 N Hydrochloric acid. This was filtered and the filtrate was collected. The filtrate was alkalinified with ammonia and placed in a separatory funnel. Measured quantities of chloroform was added into the separatory funnel, mixed and shaken for about five times and allowed to separate into two layers. The lower layer of the chloroform contained the alkaloid and the upper layer the aqueous portion the upper layer was extracted until the last chloroform extract was found negative to Dragendoff's reagent. The combined chloroform extract was concentrated in soxhlet distilling apparatus at 60$^{oC}$ and evaporated in water bath maintained at the temperature until semi-dry. The residue was weighed and percentage yield of alkaloids was calculated using the formula reported by Dimaandal (2003).

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

**Flavonoids Extraction and Isolation**

Plants collected were washed in running tap water to remove dust and other unwanted particles various plant parts (seeds, roots, leaves) of collected plants were separated, air dried, powdered, weighed and stored separately for extraction. Each of the air dried powdered sample was weighed and extracted with 80% methanol for 24hrs 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). 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 for bond flavonoids. Ethyl acetate fraction of each sample was hydrolyzed further with 7% $H_2SO_4$ for 24hrs 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 ofSaponins**

Five hundred grammes of seed sample was taken and ground to obtain coarse particles, it was subjected to successive soxhlet extraction first with petroleum ether and subsequently with methanol. The extract was vacuum dried using rotary vacuum flask evaporatory to yield solid residue. Weight of the solid obtained after drying was 60gm. Solid residue was refluxed with 5=80% w/v ethyl acetate for half an hour. 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 saponin glycosides. The solvent was decanted off precipitate was filtered, dried and was weighed to estimate totalsaponins content of the plant (Amin*et al*., 2016).

**Extraction and Isolation of Tannin**

The purification procedure was essentially according to that of Strumeyer and Malin (1975). Extracts of the dried ground plant parts (seed, root and leaves) was prepared (16-20g) in 100ml of 70% aqueous acetone containing 0.1% ascorbic acid using an ultra-sonicator. The supernatant obtained on centrifugation was evaporated under vacuum at about 30$^{0c}$ to remove acetone. The aqueous sample was lyophilized. The dried sample was dissolved in 80% aqueous methanol containing 0.1% ascorbic acid.

After filtration, added to swollen slurry of sephadex LH – 20 prepared in 80% aqueous methanol constraining 0.1% ascorbic acid. It was stirred for about 5 minutes and then filtered through sintered glass crucibles. The sephadex LH -20 was washed slowly on the crucible with 80% aqueous methanol containing ascorbic acid under gravity without applying vacuum until absorbance of the elute at 280nm was <0.05. The procedure removed non tannins from the sephadex. The tannins remained on the sephadex LH -20 and gave it a brown colour. Tannins were eluted using 5% aqueous acetone containing ascorbic acid (1mg /ml). The volume of theelute was recorded (xml which contained xmg of ascorbic acid). Acetone was removed under vacuum at about 30$^{0c}$ and then the aqueous solution containingtannin was lyophilized in a container of a known weight. The lyophilized sample (ymg) had x mg of ascorbic acid and the rest (Ymg-X mg) was the tannins. In our preparation 40-60% (100x/y) ascorbic acid was present. A portion of this preparation was stored in desiccators in the cold room and the rest was used for removal of ascorbic acid.

**Extraction of Juveniles**

*Meloidogyne incognita* was identified on the basis of perineal pattern (Eisenback*etal*., 1981). Infected roots from Indian spinach were washed and chopped to 1.2cm. The chopped rootswere shaken vigorously in a flask bottle containing water and 0.5% NaOcl for 5 mins. The eggs were passed through a 75□m and 25□m □pore sieve mesh. Then the water containing eggs through the 25□m □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 one week. Later about 90% of the eggs hatched into larvae (J2). 1ml of larvae suspension was found to have 30-36 juveniles. Mean number of larvae was counted using a stereomicroscope. The mean number of larvae in three counts approximated to 30 and was used for nematicidal assay.

**Nematicidal assay**

Nematicidal evaluation was carried out in sterilized Petri dishes. For each experiment, phytochemical crude extracts were Alkaloids, Flavonoids, Saponins and Tannin. For studying the juvenile mortality, 30 juveniles of *M. incognita* were suspended in 0, 5 and 10mills of different extracts. The Petri dishes with no

phytochemical extract served as control. Each treatment was replicated 3 times. All the Petri dishes were kept at ambient temperature (±30$^{o}$C). After, 12, 24, 48 and 72h incubation, all dead and living juveniles (J2) were counted with the aid of inverted microscope at magnification 100. The nematodes were considered to be dead when they did not move on physical stimuli with a fine needle (Hong *etal*., 2007). The mean percentage of mortality was then calculated. The ratio of dead nematodes/number of total nematodes expressed the percentage mortality.

**Statistical Analysis**

The data were analysed using ANOVA (Genstat Edition 4). Least significant differences were calculated at P=0.05

**RESULT AND DISCUSSION**

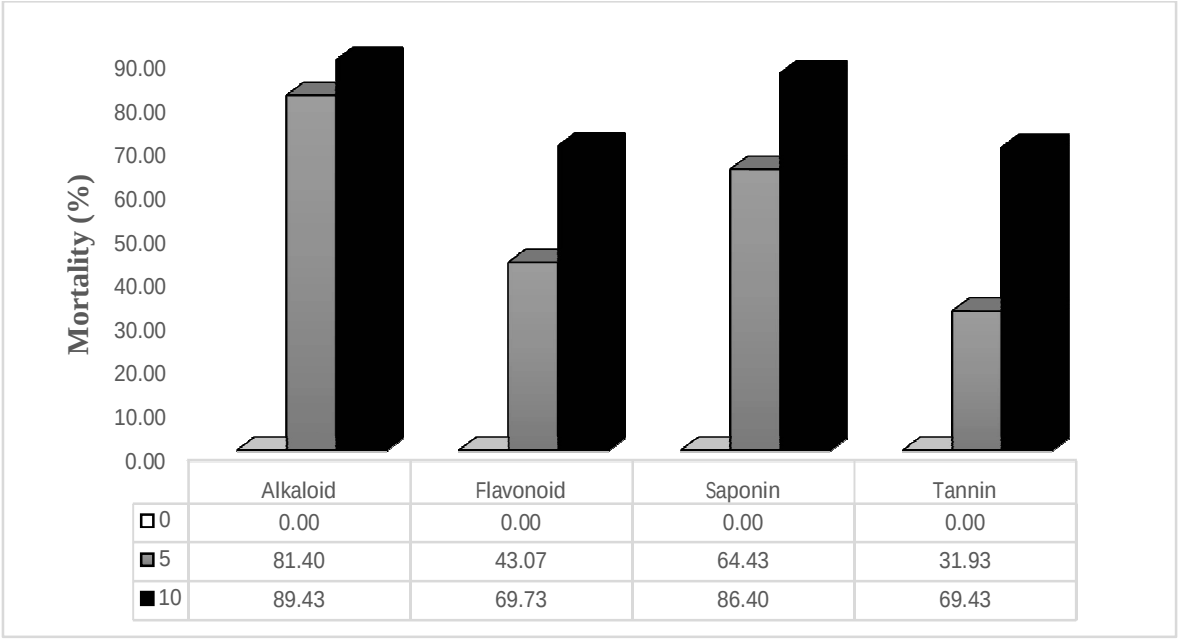
Effect of Phytochemical Leaf Extracts and Hours of Exposure on mortality of *Meloidogyne incognita* juveniles are recorded in Table 1. Phytochemical Leaf extracts applied caused mortality of*M. incognita*juveniles ($J_2$), compared to the control. Highest mortality occurred on application of Alkaloids and differed significantly (P=0.05) from those of Flavonoids and Tannin. The mortality level achieved with Alkaloids may also be attributed to other mechanisms such as disruption of protein synthesis, stability of biomembranes and metabolically important enzymes (Oyedeji*etal*., 2011). Alkaloids interfere with processes such as DNA replication and RNA transcription which are vital to micro-organisms.The mechanism of action of the phytochemicals might be due to their cytotoxicity (Matsuhashi*etal*., 2002; Kuljanabhagavad and Wink, 2009).

Juvenile mortality increased with increased rate of phytochemical treatment. Highest mortality rate of 89.4% (i.e 26.83 dead juveniles) occurred at 10 mls of Alkaloid at 72hours incubation periodThis study agrees with that of Hussein *et al*; (2016) who stated that concentrations of 5 and 10 percent of aqueous plant extracts significantly increased mortality of nematode juveniles compared to the control. Nematode juvenile mortality increased with increased hours of exposure to the phytochemicals. Okeniyi*etal*., (2010) also reported that juvenile mortality increased with corresponding increase in time of exposure to Citrus medica leaf extracts (Table 1).

A positive correlation existed between phytochemical rate, period of incubation and Juvenile mortality rate (Table 1). Least nematode mortality (9.92) however occured at 12hours of incubation at 5mls of Tannin application.

**Table 1: Effect of Phytochemical Leaf Extracts, Rates and Hours of Exposure on mortality of *Meloidogyne incognita* juveniles**

| **Pytochemical Leaf Extracts** | **Rates (ml)** | | | |
|---|---|---|---|---|
| **(Treatments)** | **0** | **5** | **10** | **Mean** |
| Alkaloids | 0.00 | 24.42 | 26.83 | 17.08 |
| Flavonoids | 0.00 | 12.92 | 20.92 | 11.28 |
| Saponins | 0.00 | 19.33 | 25.92 | 15.08 |
| Tannin | 0.00 | 9.58 | 20.83 | 10.14 |
| **Mean** | 0.00 | 16.56 | 23.62 | |
| $LSD_{0.05}$ (Rate) | | 1.31 | | |
| $LSD_{0.05}$ (Phytochemical) | | 1.51 | | |
| $LSD_{0.05}$ (Phytochemical X Rate) | | 2.62 | | |
| **Hours of Exposure** | | | | |
| 12 | 0.00 | 9.92 | 16.08 | 8.67 |
| 24 | 0.00 | 14.83 | 23.33 | 12.72 |
| 48 | 0.00 | 19.50 | 26.92 | 15.47 |
| 72 | 0.00 | 22.00 | 28.17 | 16.72 |
| **Mean** | 0.00 | 16.56 | 23.62 | |
| $LSD_{0.05}$ (Rate) | | 1.31 | | |
| $LSD_{0.05}$ (Hours of Exposure) | | 1.51 | | |
| $LSD_{0.05}$ (Hours of Exposure X Rate) | | 2.62 | | |




| | Alkaloid | Flavonoid | Saponin | Tannin |
|---|---|---|---|---|
| 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 5 | 81.40 | 43.07 | 64.43 | 31.93 |
| 10 | 89.43 | 69.73 | 86.40 | 69.43 |

Figure 1: Relationship between Phytochemical Leaf Extract and Juvenile Mortality of root-knot nematode *Meloidogyneincognita*

## CONCLUSION

The result of study clearly showed that individual phytochemicals from Jatropha*curcas*have nematicidal properties which could be developed further as alternative non synthetic and environmentally-friendly nematicides. However, studies are to be continued to evaluate nematicidal efficacy under field condition since crude phytochemical extracts found to be active in this study could be useful in the development of new nematicidal agents.

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