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Phytochemical Characterization and Insecticidal Property of *Jatropha* Plant

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Abstract- Phytochemical analysis on the pulverized leaf sample of *Jatropha curcas* as well as the insecticidal activity of the ethanolic leaf extract on *Callosobruchus maculatus* was carried out. The study investigated the mortality rate as a result of treatment of grains with the leaf extract at different dilutions of 20, 40, 60, 80 and 100mg/ml. These were tested against *C. maculatus* by treating 20g of bean sample with the extract and then infesting each in a plastic container with 20 adults of the insect and the untreated grains were used as the control. The plant extract resulted in a significant increase ($P<0.001$) in adult mortality at the end of 96 hours but there was no significance difference ($P=0.084$) after 24 hours. The result had a general dose-response characteristic. There was also significant variation in the phytochemical composition of the plant leaf. Compared to other phytochemicals, the concentration of saponin and tannin were higher in the plant leaf.

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I. Introduction

Jatropha curcas often referred to as 'Jatropha' and is also known as 'physic nut'. The seeds contain between 35 to 40% oil (50-55% oil on kernel basis) (Kaushik *et al*, 2006), which can be processed to produce a high quality biodiesel fuel useable in a standard diesel engine (Kumar *et al*, 2008; Koyejo *et al*, 2010). Besides biodiesel production, *J. curcas* has numerous other uses as it is a multipurpose plant. Among other benefits are health and environmental values. Though extensive work was done on alternative uses of *Jatropha*, there is not much information available on its use as a pesticide (Dowlathabad *et al*, 2010). Within nature, man depends on plants for his food supply. Yet, as a result of population increase, the equilibrium between human beings and their food supply is considered unsound. This results from serious damages caused by insects. Insect and pest control has for long being controlled by majorly chemical method. Other methods such as biological (e.g. natural enemies and predators), cultural and legislative means have been employed as well. However chemical method of insect pest control is the most effective and its result is almost immediate. Control of insect pests is very important especially in agricultural practice as they adversely affect crop yield, as well as in medical practice for most insects act as a vector for most of the life threatening disease like malaria, yellow fever, dengue fever, Chikungunya fever, filariasis, encephalitis, etc (Anupam *et al*, 2011). Hence the control of most pests can indirectly control spread of certain diseases, improve crop yield, longevity of stored crops especially grain, reduce discomfort and so on. With chemical method of insect control being the major means, synthetic insecticides such as organochlorines and organophosphate compounds among others have been employed. But this has not been very successful due to human, technical, operational, ecological and economic factors. Insect pest control by use of chemical pesticides is fraught with various problems like environmental pollution, development of resistance, adverse effect on non-target organisms, residual toxicity, increased cost of chemicals, potential hazards to man and increased demand for hygienic food supplies, clearly demanding the need for alternative approaches (Ghosh *et al*, 2007). Due to the deleterious effect of chemical pesticides, though most effective, a search for natural-product based agrochemicals which are biodegradable, eco-friendly and safe to the environment has intensified (Adebowale and Adedire, 2006), which this study is committed to. However, most previous reports on plants as potential insecticides (eg. mosquitocides) centred on the larvae for *Jatropha sp* (Sakthivadival and Daniel, 2008; Rahuman *et al*, 2007). This approach is usually efficacious for it prevents proliferation by cutting down already developing larvae population in their number, especially in their breeding sites. With this, much population of the insects are targeted before they reach their adult stage. However there are meager reports on insecticidal activity of *J. curcas* leaf extract on adult insects which should actually complement on instances where the breeding sites were hidden or distant or situation where the larvae development was not truncated. Therefore there is need for this study which actually aimed at investigating the phytochemical properties and insecticidal activity of the leaf extract of *J. curcas*. In this work, a null hypothesis was tested which was stated thus: Null hypothesis (H_0): After 96 hours, there was no significant difference in the mortality rate by the various treatments (concentrations). Alternative hypothesis (H_1): There was a significant

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difference in the effect (mean mortality rate) of the various treatments after 96 hours.

II. Materials and Methods

a) Collection and Identification of Plants

Experiments in this study were conducted under laboratory conditions. Fresh healthy leaves of *Jatropha curcas* L. were collected from school of Agriculture and Agricultural Technology, Federal University of Technology Owerri, Imo State in July, 2014 and botanical identification was carried out in the Crop Science Technology Department of the Federal University of Technology, Owerri. The taxonomical identification of the plant was confirmed by Dr. S.O. Ojiako, a plant taxonomist of the Department of Crop Science, Federal University of Technology Owerri, Nigeria. The leaves were washed in tap water and air dried to constant weight at room temperature after which they were ground into powder using an electric blender.

b) Phytochemical analysis of plant material

Phytochemical characterization of the leaves of *J. curcas* was carried out by screening for the presence and percentage concentrations of saponin, flavonoid, alkaloids, tannin, cyanogenic glycoside, oxalate and phytate according to the methods described by Trease and Evans (1989) and AOAC (1990).

c) Saponin

100ml of 20% v/v aqueous solution of ethanol was added to 2.0g of the pulverized sample in an Erlenmeyer flask and heated in a water bath at 55°C for 4 hours with occasional stirring. The mixture was filtered and the residue re-extracted with a fresh 100ml of 20% v/v ethanol solution. Both extracts were combined and volume reduced to about 50ml on a water bath set at 90°C. The mixture was cooled and extracted using 20ml of diethyl ether in a 250ml seperatory funnel. The ethereal layer was discarded followed by addition of n-butanol into the lower layer, then shaken and allowed to separate. The organic layer was shaken twice with 10ml of 5% w/v sodium chloride solution in a seperatory funnel and poured into a pre-weighed beaker. The mixture was evaporated to dryness on a boiling water bath, cooled and reweighed. Percentage saponin content was calculated.

d) Flavonoid

100ml of 80% v/v methanol solution was added to 2.0 g of powdered sample, stirred on a magnetic stirrer for 3 hours and later filtered using a filter paper. The residue was re-extracted and filtered again. Both extracts were combined and put into a pre-weighed beaker and then evaporated to dryness on a boiling water bath. It was cooled and reweighed, and percentage flavonoid computed.

e) Alkaloid

100ml of 20% v/v ethanolic acid solution was added to 2.0g of the powdered sample in a flask, covered and allowed to stand for 3 hours with stirring on a magnetic stirrer. The mixture was filtered while the residue re- extracted as before. Both extracts were combined and concentrated on a water bath to about one-quarter of the original volume. Drops of concentrated ammonium hydroxide were added upon cooling till complete precipitation of the alkaloids had occurred. The resulting mixture was kept in a cool place overnight and filtered using a pre-weighed filter paper which was later dried in an oven set at 70°C for at least 6 hours. Weight of the filter paper and content was reweighed after drying in a desiccator and percentage alkaloids calculated.

f) Tannin

50ml of distilled water was added to 0.5g of powdered sample and stirred for 2 hours on a magnetic stirrer. The mixture was filtered into a 50ml volumetric flask and made up to the mark. Three test tubes (blank, sample and standard) were set up containing 1ml of water, 1ml of sample filtrate and 1ml of 2mg/ml tannic acid solution respectively.

1ml of each of 0.1M hydrochloric acid, ferric chloride and potassium ferrocyanide solution were added to each of the three test tubes. Their absorbances were measured using spectrophotometer set at 720nm within ten (10) minutes and the percentage tannin content was calculated.

g) Cyanogenic glycoside

1.0g of powdered sample was placed in a quick fit round bottom flask. 200ml of deionized distilled water was added to it and allowed to stand for 2 hours. The flask was then connected to complete the distillation setup. 150ml of the mixture was distilled into a 250ml receiver flask containing 20ml of 2.5% w/v sodium hydroxide solution. 100ml of the resulting mixture was measured out into another flask and 8ml of 6.0M ammonium hydroxide solution was added followed by 2ml of 5% potassium iodide solution. The contents were mixed and titrated with 0.02N silver nitrate solution till no further turbidity upon addition of the silver nitrate solution. Percentage cyanogenic glycoside was then calculated.

h) Oxalate

To 5.0g of dry powdered sample, 20ml of 0.3N hydrochloric acid was added in a flask and stirred at 50°C for 1 hour. The mixture was filtered and the process repeated two times while combining the filtrates and making it up to 100ml with distilled water. To 20ml of filtrate, 3 drops of phenolphthalein indicator was added. 5.0N ammonium hydroxide was added in drops till the reaction mixture was alkaline. Glacial ethanoic acid was then added in drops till the pink colouration

disappeaed and then a few more drops just to make the mixture acidic. 5ml of 5% calcium chloride solution was added and mixture allowed to stand for 3 hours, after which it was centrifuged at 300rpm for 15 minutes. The residue was washed three times with hot water using centrifugation method. The residue was then dissolved in 2ml of 3.0N tetraoxosulphate (VI) acid solution with warming at 70°C followed by titrating the resultant solution with freshly prepared 0.01N potassium permanganate solution till permanent pink colouration that lasted for 30 second was obtained. Blank titration was carried out using the same volume of 3.0N tetraoxosulphate (VI) acid as that used in dissolving the oxalate residue. The test was done in duplicates and percentage oxalate content calculated.

i) Phytate

2.0g of dry sample was placed in a flask and 50ml of 0.18M trichloroacetic acid solution added to it. The mixture was stirred for 1 hour and centrifuged at 3000rpm for 10 minutes. 10ml of centrifugate was added to 4ml of 0.036M Iron (III) chloride solution in a boiling tube and placed in boiling water for 45 minutes. Resulting mixture was centrifuged and ferric phytate was collected, washed twice and with 20ml of 0.18M trichloroacetic acid solution and 30ml of water each time. 3ml of 1.5 M sodium hydroxide solution was added to the residue followed by 25ml of water and placed in boiling water till coagulation of ferric hydroxide was complete. The ferric hydroxide was collected by centrifugation and washed twice with water. Ferric hydroxide thus obtained was dissolved in 40ml of 3.2M trioxonitrate (V) acid and made up to 100ml using distilled water. Iron content was determined and content Absorbance was measured in a spectrophotometer at 540nm and percentage phytic acid was then computed.

j) Preparation of plant extract

The powdered plant material (100g) was for ethanolic bulk extraction. Ethanol was added to the sample and shaken intermittently for at least three hours and left standing overnight. The mixture was filtered into a clean dry beaker, obtaining a dark green filtrate. The ethanol was then gently evaporated by placing the beaker in an oven set at 75°C to obtain a thick dark green plant extract. Using an analytical balance, required quantities were weighed into different beakers and diluted appropriately with methanol (Sanis *et al*, 2012) giving rise to five different dilutions of 20, 40, 60, 80 and 100mg/ml.

k) Maintenance of the experimental insect

Bean weevil, *Callosobruchus maculatus* was collected in bulk from bean sellers and identified by Mrs Usenwunne Chinwe, an entomologist in the Animal Science Department of Federal University of Technology, Owerri. They were acclimatized in the laboratory in a clean uninfected jar. The jar was covered with a clean thin cloth and tightly held in place with a rubber band to allow for aeration and prevent entry or exit of insects. It was kept at room temperature 37°C with 12 hour light and dark regimes.

l) Toxicity of Jatropha curcas leaf extract on C. maculatus in grains

About 400g of beans was washed with clean water and then dried in an oven. 20g of the grain was weighed out into different clean petri - dishes. The various dilutions of the leaf extract were applied to them and left to air dry. Each dilution or concentration was applied to three sets of beans, each weighing 20g. The same was done for other treatments while the control had no treatment.

The beans were put into various transparent plastic jars and each container was then infested with 20 *C. maculatus* adults selected at random. The jars were covered with a thin cloth each and held with a rubber band to facilitate aeration and prevent contamination. Each treatment was replicated three times. The adult mortalities were counted and recorded for a period of four days at a 24 hour interval.

m) Statistical analysis

Statistical analysis was performed with GraphPad Prism version 5.0 using one-Way analysis of variance (ANOVA) followed by Dunnet multiple comparison test. Results were expressed as mean ± S.E.M. Groups of data were considered to be significantly different if $P < 0.05$. Schneider-Orelli formula was used to adjust the data where there were deaths in the control treatment while Probit analylsis was employed in the calculation of LC_{50}.

III. Result and Analysis

At the end of 24 hours, after subjection to various concentrations of the plant extract, there was no significant difference ($P=0.084$) when compared to the untreated control. However at the end of 48 hours the treatments resulted in a significant ($P<0.001$) increase in the mean mortality rate, and after 72 hours there was even more significant increase in mortality ($P<0.001$) among the various treatments relative to the control. At the end of three days (72 hours) mortality rate increased significantly ($P<0.001$), approaching a median value. Mortality rate was proportional to the concentration and duration. There was no significant difference in mortality for 20mg/ml over the period of four day which was obviously different in other treatments at some point during the period of 96 hours when compared with the control (Table 1).

Table 1 : Effect of ethanolic extract of *Jatropha curcas* leaves on the mortality rate of *Callosobruchus maculatus*

Treatments (mg/ml)	Mortality count			
	24hours	48hours	72hours	96hours
Control	0.000±0.00	0.000±0.00	0.333±0.3333	0.667±0.3333
20	0.000 ± 0.00^{ns}	1.000 ± 0.5774^{ns}	1.667 ± 0.3333^{ns}	3.000 ± 0.5774^{ns}
40	0.667 ± 0.333^{ns}	1.333 ± 0.3333^{ns}	$2.6\,67\pm0.3333^{a}$	4.667 ± 0.3333^{b}
60	0.667 ± 0.3333^{ns}	1.333 ± 0.3333^{ns}	3.333 ± 0.8819^{b}	5.333 ± 0.8819^{c}
80	1.000 ± 0.5774^{ns}	2.333 ± 0.3333^{b}	4.333 ± 0.3333^{c}	7.000 ± 0.5774^{c}
100	1.333 ± 0.3333^{ns}	3.333 ± 0.3333^{c}	5.667 ± 0.3333^{c}	9.333 ± 0.8819^{c}

Values are expressed as Means ± SEM with n = 3; a, b, c, ns represent $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}p < 0.01$ and no significance respectively compared to control. Means in the same column having the same letter (superscript) are not significantly different at $p < 0.05$.

Mean percentage mortalities were computed (Table 2) and also corrected using the Schneider-Orelli formula (Püntener, 1981) in cases where death was recorded in the control treatments (Table 3), which occurred after 72 and 96 hours as follows:

$$\text{Corrected \%} = \left(\frac{\text{Mortality \% in treatment plot} - \text{Mortality \% in control plot}}{100 - \text{Mortality \% in control plot}}\right) \times 100$$

Result for the phytochemical analysis carried out is shown below in Table 4. Results were expressed as percentages. The plant *J. curcas* showed a higher content of tannn(7.58%) than other phytochemicals.

Table 2 : Percentage Mortality of *Callosobranchus maculatus* after 96 hours of exposure in ethanolic extract of *J. curcas*

Treatments (mg/ml)	% Mortality			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	1.665	3.335
20	0	5	8.335	15
40	3.335	6.665	13.335	23.335
60	3.335	6.665	16.665	26.665
80	5	11.665	21.665	35
100	6.665	16.665	28.335	46.665

Table 3 : Percentage Mean Mortality Corrected using Scheider – Orelli Formulae

Treatments (mg/ml)	Corrected % Mortality			
	24	48	72	96
Control	0	0	0	0
20	0	5	6.783	12.067
40	3.335	6.665	11.868	20.69
60	3.335	6.665	15.254	24.135
80	5	11.67	20.339	32.757
100	6.665	16.67	27.122	44.825

Table 4 : Phytochemical composition of J. curcas

Phytochemical	Concentration (%)
Saponin	4.89
Flavonoid	3.56
Alkaloid	4.50
Tanin	7.58
Cyanogenic glycoside	4.19
Oxalate	3.62
Phytate	4.10

IV. Discussion

Insecticidal activity of J. curcas

The result from this study showed that the plant, *J. curcas* exhibited an insecticidal action on *C. maculatus* adults with varying susceptibility. After 96 hours there was a significant increase in mortality rate among treatments, hence the null hypothesis was rejected thereby accepting the alternative hypothesis.

From Table 1, there was no significant difference in insecticidal mortality rate by the plant extract compared to the control treatments for all

concentrations (P=0.084). However, by the end of 48, 72 and 96 hours, there were significant differences in mortality rates; P<0.001and P<0.005 respectively. Comparing the various doses after every 24 hours, there is a marked increase showing a positive correlation between concentration and the percentage of insect mortality which confirms the report of Shadia *et al* (2007). Highest mortality rate was 44.83% compared to 12.07% (Corrected %) in 100 and 20mg/ml respectively at the end of 96 hours (Table 3). Death in the control treatment accounted for error as shown in Tables 1 and 2, which is sometimes a part of an experiment. However, the percentage was corrected with that in mind (Table 3).

Tables 1 and 3 showed that the efficacy of the plant extract is both dose and time dependent, for a higher mortality rate is expected from the above trend. Either higher concentration of the extract at a given time and/ or time lapse for a given concentration will both produce better results. This shows that the efficacy of the plant can be maximized by employing higher concentration or allowing a particular treatment to act for a longer period time. However due to the toxic effect of the plant, lower concentration is necessary considering human health especially when used as grain protecting agent. The median lethal concentration (LC_{50}), computed using the Probit analysis (Finney, 1952), gave 149.6mg/ml. This is the concentration that will kill 50% of the insect population after 96 hours. This implies that a higher concentration will be needed to yield a 50% mortality rate within 96 hours, unless allowed for a relatively extended period of time.

Major insecticidal works on *Jatropha* centred on the seed oil (Ebtisam *et al*, 2013; Dowlathabad *et al*, 2010; Adebowale and Adedire, 2006) while meager reports on the leaf are found (Sanis *et al*, 2012). Studies on the seed oil showed higher percentage mortalities for even relatively lower concentrations of the *Jatropha* seed oil extract. Constance *et al* (2013) reported a wide gap between the mortality rates of *Sitophilus zeamais* at the end of 42 days after being treated with leaf, juice and seed oil extracts exclusively. The result from the study showed that the insect mortality increased with the concentration of the extract as expected, but at concentrations more than 10ppm, the seed oil showed significantly (P<0.001) higher insecticidal effect compared to others. In fact 100% mortality was recorded in grains pre-treated with seed oil compared to 58.9% and 55.6% mortality observed for grains pre-treated with leaf extract and juice of *J. curcas* respectively after 42 days.

The phytochemical analysis of the leaf of *J. curcas* in this study revealed the presence of saponin, tannin, flavonoid, alkaloid, oxalate, phytate and cyanogenic glycosides in varying concentrations as shown in Table 4. Some of these secondary metabolites account for the toxicity of the plant extract conferring in it its characteristic insecticidal property, especially phytate, saponin and cyanogenic glycosides. Achten *et al*, (2008) reported high concentration of phorbol esters (phorbol-12-myristate-13-acetate) present in *Jatropha* seed which had been identified as the main toxic agent responsible for *Jatropha* toxicity. Presence of these phytochemicals also confers taxonomic importance on the plant. Among the phytochemicals investigated from the leaf, tannin was found to be the most abundant followed by saponin and alkaloid. flavonoid and oxalate were the lowest in concentration. A similar sequence with tannin being the most abundant phytochemical in plant parts, followed by saponin, alkaloid and so on as seen in this study has been reported by Mallikharjuna *et al* (2007). Other recent investigation showing presence and importance of these phytochemicals similar with the results of this investigation include Ogunkunle and Ladejobi (2006), Ferreira *et al* (2009) and Kumar *et al* (2009).

The presence of phytochemicals also lays credence to the fact that this specie is a potential source of these important phytochemicals. For example, flavonoids are one of the most popular secondary metabolites possessing a variety of biological activities at nontoxic concentrations (Irshad *et al*, 2010). Flavonoids together with other secondary metabolites identified in *J. curcas* has been severally reported in other plants to show curative activity against diverse pathogens, used traditionally as analgesic, antimicrobial (Hassan *et al*, 2004; Singh *et al,* 2009) and insecticidal, as this study has shown. Cardiac glycoside was found to have acaricidal effect against larva and adult stages of the camel tick (Al-Rajhy et al, 2003).

However, *J. curcas* leaf extract have shown adulticidal activity in this study and in fact could serve as an effective replacement for chemical insecticides. The insects used were adults which also explain the relatively low mortality rates recorded. This study has demonstrated that the use of *J. curcas* ethanolic leaf extract can significantly increase mortality rate in *C. maculatus* infesting treated grains and thus reduce the extensive use of synthetic organic chemical insecticides which result in environmental hazards and resistance in major insects, especially insect vectors.

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