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TOXICITY ACTIVITIES OF *Jatropha curcas* SEED EXTRACT AGAINST THE 4TH INSTAR LARVAE AND PUPAL STAGES OF *Aedes aegypti*

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AUTHOR'S CONTRIBUTION

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Mosquitoes are vectors of several diseases of public health importance. Their control with synthetic insecticides has faced challenges of resistance by target insects and consequent environmental pollution. There is therefore need to find alternative ecofriendly plant derived insecticide. Therefore toxicity activities of *Jatropha curcas* seed extract against the 4th instar larvae and pupal stages of *Aedes aegypti* was studied *in vitro* with increasing doses of *Jatropha curcas* seed extract for 96 hours. The result showed significant difference between mortality among treated animal and control ($P < 0.05$). Mortality of both larvae and pupae of *Aedes aegypti* treated with *Jatropha curcas* seed extract was dose and time dependent. At the highest (9%) concentration (wt/vol), mean larval mortality was 100%, while at the lowest (1%) concentration, the mortality decreased to 50%. Mean pupal mortality increased to 100% at highest concentration of 9% (g/100ml) but decreased to 40% at lowest concentration. The duration of incubation also influenced mortality. At 72 and 96 hours of incubation all treated larva died in highest concentrations of the extract (9% and 7%) while the entire treated pupa died out in 96 hours of incubation at the highest concentration (9%) of the extract. Survived larva and pupa after 96 hours in lower concentration could not develop to adult stage. In conclusion *Jatropha curcas* seed water extract has toxicity effects on larvae and pupae of *Aedes aegypti* and can be deployed for the control of the insect.

Keywords: Toxicity; *Jatropha*; vector; insecticides; *Aedes aegypti*.

1. INTRODUCTION

Many of the neglected tropical diseases such as malaria, yellow fever, dengue fever etc., are vectored by insects especially mosquitoes [1]. One of the mosquitoes, *Aedes aegypti* is the intermediate host and vector of dengue and yellow fever virus and instrumental in the spread of the diseases [2,3,4,5]. *Aedes aegypti* bite human for blood that they need for the development of their eggs [6], and unfortunately during such blood meal, transmit parasites and viruses to human. Approaches to the control of diseases transmitted by insect vectors have been through measures that target the adult stage of the insects or any of the developmental stages [7]. One of such measures is the use of synthetic insecticides, which kill the insect but not the disease agent [7,8]. Unfortunately, synthetic insecticides have their own environmental problems associated with their use [9,10]. They are non-biodegradable, and contaminate the environment as well as affect non-target organisms therefore destroying the ecosystem [10].

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In a bid to resolve this problems, interest in insecticides of natural origin, specifically plant-derived product has recently received close attention [11]. Several studies have emphasized the importance of research and development of herbal substances for controlling mosquitoes. Their results may vary, but natural plant products may be possible alternatives to synthetic substances, as they are effective and compatible with human and animal life and the environment [12]. One of the plants with promising potency as an alternative phyto-insecticide is *Jatropha curcas*.

Jatropha curcas is a drought resistant shrub of the family Euphorbiaceae, which is predominant in Central America and today is found throughout the world in the tropics [13,14]. Different extracts of *J. curcas* seeds, leaves, stem and bark have been used as an antiseptic, diuretic, purgative, larvicides as well as for treating cancer, gout and skin disease [15,16]. This plant shows high agro-industrial potential in many places such as India because the seed produces non-edible oil that can be used as a biofuel [17]. Apart from the biodiesel production, *Jatropha* possess insecticidal properties and act as antifeedant against some insects [18], growth inhibitors of several Lepidoptera Species and *Sitophilus zeamais* [14]. Jatropha leaves and seeds contains a broad ranges of bioactive metabolites especially phobol esters [19,20]. These metabolites are ecofriendly due to their low toxicological activities [16]. *Jatropha curcas* extracts have been shown to cause no harmful problem on the environment. The plant is readily available and can be easily sourced by the local people. Therefore, there is need to investigate the toxicity activity of seed and leaves extract of *J.curcas* on the developmental stages of *Aedes aegypti*.

2. MATERIALS AND METHODS

2.1 Collection and Processing of *Jatropha* Seeds

Mature seeds of *Jatropha curcas* were collected from the Biological garden of Applied Biology and Biotechnology Department, Enugu State University of Science and Technology Enugu State, Nigeria. The seeds were dehaulled and sorted manually. Spoilt seeds were removed while quality seeds were stored. The seeds were air dried to constant weight and later grounded into fine powder. Powdered seeds were measured out in 1, 3, 5, 7, 9 grams and each placed in five different 250ml flasks. Each was added 100 ml of distilled water to give concentrations of 1%, 3%, 5%, 7%, and 9% solutions (wt/vol); shaken vigorously and left overnight. The suspension was later strained through double-layered sieve cloth and stored as stock until use. The various flasks constitute concentrations of 1%, 3%, 5%, 7%, and 9% respectively.

2.2 Source of Animal and Maintenance

Eggs of *Aedes aegypti* were kindly donated by the National Arbovirus Research Center Enugu. The egg ribbon was gentle immersed in an enamel white background bowel containing 400 ml of distilled water. 10 gram of yeast was added to the container as food for emerging larvae. The set up was kept in the dark inside a $60x30x30cm^3$ net-screened wooden cage. The eggs were allowed to hatch. The mosquito stages were maintained at temperature of $28\pm2\ ^0C$, 75-80% relative humidity, with 12:12 light and dark photoperiod. Larvae were fed yeast and fish fingering feed. Larval instars were monitored and separated into a new container with the aid of a plastic pipette. Emerged 4^{th} instar larvae and pupae were used for this study.

2.3 Mortality Assay

Larval or pupal mortality was tested with either 4^{th} instar larvae or 12 hour old pupae using 1%, 3%, 5%, 7%, and 9% concentrations of *Jatropha curcas* seed water extract in six 500 ml conical flasks, each containing 200 ml of distilled water. Each treatment received 10 larvae or pupae and was replicated three times. Control larvae or pupae for mortality test were kept in 200 ml of distilled water only. Larvae were fed with yeast and fish fingering feed. Mortality was recorded every 24 hours for 4 days. Mortality was measured according to Abbot's formula (1925). Abbot formula = percentage of test mortality - percentage of control mortality +100 – percentage of control mortality x 100. The differences between test mean mortality values and control mortality values was further analyzed using one-way analysis of variance (ANOVA).

3. RESULTS

The results of the treatment of larval or pupal stages of *Aedes aegypti* with *Jatropha* seed water extracts are presented in the following tables. In Table 1 the extract exhibited toxicity activity on 4^{th} instar larvae of *Aedes aegypti*. At the highest (9%) concentration (wt/vol), the extract showed a mean mortality of 100%, while at 7%, 5%, 3% and 1% concentrations, the mortality decreased in a dose dependent manner with 100%, 90%, 63% and 50% mean mortality respectively. There were also significant difference between each treatment level and control ($P< 0.05$). In Table 2 the mean mortality of pupal stage after exposure to *Jatropha* seed water extract was also dose dependent. At highest concentration of 9% (g/100 ml)

Table 1. Mean mortality of 4th instar larvae of *Aedes aegypti* after exposure to *Jatropha* seed water extract

Extract conc. (g/100 ml)	Initial No. of larvae	Mean mortality in 24 hrs	Mean mortality in 48 hrs	Mean mortality in 72 hrs	Mean mortality in 96 hrs	Total mean mortality	SEM	% Mean Mortality
Control	30	0.0	0.0	0.6	0.0	0.06	± 0.02	6%
1	30	0.0	1.7	1.3	2.0	0.50	± 0.38	50%
3	30	1.7	1.3	2.3	1.0	0.63	± 0.47	63%
5	30	4.7	1.3	1.7	1.3	0.90	± 1.26	90%
7	30	6.7	1.7	0.0	0.0	1.00	± 2.39	100%
9	30	8.3	1.7	0.0	0.0	1.00	± 3.60	100%

Table 2. Mean mortality of pupal stage of *Aedes aegypti* after exposure to *Jatropha* seed water extract

Extract Conc (g/100 ml)	Initial No. of pupae	Mean mortality in 24 hours	Mean mortality in 48 hrs	Mean mortality in 72 hrs	Mean mortality in 96 hrs	Total mean mortality	SEM	% Mortality
Control	30	0.0	0.0	0.0	0.0	0.0	± 0.00	0%
1	30	0.0	0.0	2.3	1.7	0.40	± 0.39	40%
3	30	0.0	1.7	1.3	1.0	0.40	± 0.25	40%
5	30	1.3	2.0	3.3	1.7	0.83	± 0.84	83%
7	30	3.7	1.7	2.7	1.7	0.96	± 1.11	96%
9	30	7.7	2.3	0.0	0.0	1.00	± 3.16	100%

mean mortality was 100%, 7% (g/100 ml) concentration exhibited 96% mean mortality, while 5%, 3%, and 1% (g/100ml) concentrations recorded 83%, 40%, and 40% mean mortality respectively. There was significant difference when treatments were respectively compared to control ($P< 0.05$).

The duration of incubation influenced mortality. At 72 and 96 hours of incubation all treated larva died in highest concentrations of the extracts (9% and 7%) (Table 1) while the pupae died out in 96 hours of incubation at the highest concentration (9%) of the extract (Table 2).

4. DISCUSSION

Toxicity activities of *Jatropha curcas* seed water extract was studied using the 4th instar larvae and pupal stages *of Aedes aegypti*. The result revealed that extract of Jatropha seeds exhibited toxicity on larval and pupal stages of test animal. There was no such mortality in the control indicating that mortality observed in the study was caused by *Jatropha curcas* seed water extract. Toxicity was dose and time dependent with highest mortality at highest concentration of *Jatropha curcas* seed water extract and at longer incubation period. Treated larvae at low concentration exhibited delayed pupal development. Those larvae that survived after 96 hours at lower concentration molted or otherwise to different stages but could not develop to adult stage, while pupae treated with low concentration of extract showed delayed adult emergence or did not develop into adult. The findings of this study agree with previous reports of [21,1,13], who in their respective studies observed the toxicity activities of Jatropha plant on mosquitoes and other insects.

The toxicity activity of *Jatropha* against the test animal as observed in this study is due to the phytochemical composition of *Jatropha*. *Jatropha* has been reported to contain alkaloids, tannin, saponins [22,23], steroids, flavonoids, cardiac glycosides, oleic acid and linoleic acid [24]. Tannin exerts its action by a combination of mechanisms that include iron chelation and enzyme inhibition [25]. Insect molting process is aided by enzymic actions. Delayed development into next stage may be due to inhibition of enzymes necessary for break down of old cuticle and synthesis of new one. Chaieb [22] extensively reviewed insecticidal effects of saponins, linking their insecticidal activity with their interaction with cholesterol, which results in impaired ecdysteroid synthesis, which probably resulted in either death or delayed development of mosquito larvae and pupae in treatments with low concentration of *Jatropha curcas* seed water extract. Also, higher larvicidal activity of *J. curcas* seed extract could be an attribute of toxic phorbol esters and other compounds, which have been reported to be high in Jatropha seeds [19].

5. CONCLUSION

We therefore conclude that *Jatropha curcas* seed water extract has toxicity effects on larva and pupa of *Aedes aegypti* and can be deployed for the control of

the insect. Despite the toxic effects against the test animal, however, the extract is environmental friendly and biodegradable. Therefore, the extract can be spread on water bodies, in the farm ecosystem and at any habitat where the vector is breeding. The impact therefore will be profound when combine with other good practices of mosquito control.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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