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Comparative Larvicidal Efficacy of Leaf and Stem Extract of *Jatropha Curcas* against *Culex Pipiens Pipiens*

Olayemi I K[1], Busari J[1], Adeniyi K A[1] and Ukubuiwe A C[1*]

[1]Applied Entomology and Parasitology Research Unit, Department of Biological Sciences, Federal University of Technology, Minna, Nigeria.*

**For correspondence e-mail: azubuike383@gmail.com*

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ABSTRACT

The present study aimed to investigate the larviciding efficacy of stems and leaves extract of *Jatropha curcas*, against larval stage of the *Culex pipiens pipiens* mosquito. Methanolic extracts of the plant material were prepared and bio-assayed against the larvae as per standard World Health Organization's (WHO) procedures. The larvae were exposed to a series of concentrations of the extracts ranging from 0.25 - 3.75 mg/l; and mortality recorded post-24 hours exposure. The results showed that larval mortality increased significantly ($P<0.05$) with increasing in extracts concentration. Mortality ranged from 2.00 ± 1.40% in the lowest leaf-extract concentration (i.e., 0.25 mg/l) to 96% in the highest (3.75 mg/l). Generally, mortalities were relatively higher among larvae exposed to stem extract than that of leaf. Probit regression analysis of the mortality data gave LC_{50} and LC_{90} of the leaf extract as 2.65 and 3.45 mg/l respectively; while those of the stem were 2.33 and 3.25 mg/l respectively. These results indicate that the stem extract of *J. curcas*, in particular, is a potential source of a mosquito larviciding lead-agent, and its further exploitation should fast-track the development of a cost-effective larvicide for integrated mosquito vector control.

Keywords: *Bio-assay, Extracts, Larva, Lethal Concentration, Mortality and larviciding efficacy.*

1. INTRODUCTION

Mosquitoes are insect vectors of human diseases including, Malaria, Filariasis, Dengue fever, Japanese encephalitis, etc. [1]. These diseases cause several million cases of morbidity and mortality per annum, in their various regions of distribution [2]. For example, Filariasis transmitted by the mosquito genus *Culex*, threatens the health of over a billion people world-wide with about 2.5 million deaths reported yearly [3]. In addition, the *Culex* mosquitoes constitute the most abundant Culicids in urban areas [4]. This development has made the control of *Culex* mosquitoes imperative. Yet, the conventional mosquito control strategy in sub-Saharan Africa (i.e., the use of insecticide-treated bed nets), is targeted at taking advantage of the behaviour of anopheline mosquitoes that bite mostly indoors and late at night [5]. This vector control strategy is not effective against *Culex* mosquitoes that bite principally outdoors before people go to sleep under the protective bed-nets. Therefore, larval control measures would be more effective against *Culex* mosquitoes, taking its adult behavior into consideration.

However, the effective mosquito larviciding interventions often involve the use of synthetic chemicals that are expensive, scarce and harmful to the ecosystem [6, 7]. These challenges have called for the search for mosquito larvicides from cheap and readily available sources, especially, natural products of plants origin [8]. To this end, various plant taxonomic families have been screened for insecticidal properties, with encouraging results obtained in many cases [9-11]. Studies have revealed that *Jatropha curcas* (Physic nut) is rich in bio-active phytochemicals that have found applications in medicine [12-14].

Insecticidal efficacies of extracts of *Jatropha curcas* have been demonstrated against a wide range of insect species including, the Tobacco hornworm (*Mandu casexta*), cotton bollworm *(Herlicoverpa armigera*), melon Aphid (*Aphis gossipii*), leafhopper (*Enapoascabi guttula*), maize weevil (*Silophilus zeamays*), etc. [15-17]. However, information on larviciding activities of *J. curcas* against mosquito species in general, *Culex pipiens pipiens* in particular is very scanty. Therefore, this study was carried out to determine the larvicidal efficacies of the extracts of *J. curcas* against the *Culex pipiens pipiens* mosquito. This is against the back drop that *J. curcas* is widely distributed in the Tropics, where its cultivation is highly encouraged for medicinal uses and production of bio-diesel.

2. MATERIALS AND METHODS

2.1. Source of Plant Materials and Preparation of Extracts

Fresh leaves and stems of *Jatropha curcas* were obtained from plants growing in the field, around the Temporary Campus of Federal University of Technology, Minna, Nigeria. The taxonomic identity of the plant materials was authenticated by a Botanist in the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria; after which voucher specimens of the plant materials were deposited in the Herbarium of the Department.

For preparation of extracts, the plant materials were air-dried at ambient Laboratory conditions. Then, the dried plant materials were pulverized using an electric grinder (Model: WPB80BC), and the powder stored at 28ºC until used. Thereafter, 25g of the individual plant material (i.e., leaves or stems) was extracted in 300ml methanol, using Soxhlet apparatus, at 80ºC for a period of 16hours [18]. The crude extracts were purified using a rotary vacuum evaporator, and the stock solutions, subsequently prepared, were stored in a refrigerator at 4ºC.

The *Culex pipiens pipiens* mosquitoes used for this study were originally obtained from the wild in Minna, Nigeria, and maintained in the Laboratory of the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria. Laboratory handling and maintenance of the mosquitoes, followed standard mosquito rearing protocols [19].

2.2. Preparation of Working Solution of Extracts

The stock solution was serially diluted in ten-fold with distilled water (i.e., 2 ml stock solution to 18 ml solvent), following recommended guidelines [20]. Test concentrations were then obtained by adding a range of 0.25 - 3.75 mg/l (in multiples of 0.50 mg/l), to 100ml of distilled water.

2.3. Bioassay of Plant Extracts against Cx. p. pipiens Larvae

Larvicidal bioassays were conducted for 24 hours in plastic containers of 250ml capacity. Batches of 25 healthy 4th instar larvae of the mosquito were transferred, by means of a strainer, to plastic containers holding 100ml distilled water. The appropriate volume of extract was added to 100ml of water in the test containers, to obtain desired extract concentration.

Four replicates were set up for each test concentration and an equal number of Controls were also set up simultaneously; with distilled water to which only 1ml of the methanol solvent was added. The experiment was carried out at ambient Laboratory conditions of 28.00±1ºC, 82.00±2% relative humidity and 12 hr.: 12hr light: darkness photoperiod. Larval mortality was recorded post-24 hours exposure to the extracts. The whole experiment was repeated within 24 hours of termination of the first exercise.

2.4. *Statistical Analysis*

Larval mortality data obtained were subjected to Analysis of Variance (ANOVA) using software ware package SPSS 16 version. Statistical differences between the treatments was determined by Duncan multiple range test ($P<0.05$). Probit regression analysis [21], was done using Biostat 2012 software for the determination of LC_{50} and LC_{90} of the different extracts.

3. RESULTS

Table 1 shows the concentration-dependent effects of leaf and stem extracts of *J. curcas,* against 4th instar larval stage of *Culex pipiens pipiens* mosquitoes, under Laboratory conditions. For both extracts, mortality rates of the larvae increased significantly ($P<0.05$) with rising extract concentration. Mortality ranged from 2.00±1.40% in the lowest leaf-extract concentration tested (i.e., 0.25mg/l) to 96.00±0.00%. in the highest concentration (3.75mg/l). However, the respective percentage ranges were 0.00±0.00% and 100.00±0.00% for the stem extract. Except for the first three extract concentrations tested (i.e., range = 0.25 - 1.25mg/l), larval mortalities recorded among mosquitoes exposed to the stem extract were significantly higher than those of leaf-extract.

The lethal concentrations (LC) of the extracts required to kill 50 and 90% ((i.e., LC_{50} and LC_{90}, respectively) of the larvae are highlighted in Table 2. The LC_{50} of the leaf and stem extracts were, respectively, 2.65 and 2.33mg/l while, the LC_{90} values were 3.45 and 3.25 mg/l, respectively.

4. DISCUSSION

The extracts of *J. curcas* bio-assayed in this study, demonstrated significant larvicidal activities against *Cx. p. pipiens*, and such activities were dose-dependent. The concentration-dependent effects of the extracts indicate the presence of mosquito larvicidal bio-active phytochemicals in the leaves and stems of *J. curcas.* This observation supports the results of Rahuman *et al.* [22], who reported significant larvicidal activities of *J. curcas* against different mosquito species. The larvicidal activities of the extracts may be due to the presence of Flavonoids, Saponins, Glycosides, etc [23], which, according to literature, are highly toxic to mosquito larvae [11, 24, 25]. On the whole, the stem extract elicited much higher mortalities in the larvae, than the leaf-extract. These differential larvicidal activities of the leaf and stem extracts may be due to variations in phytochemical composition and/or concentrations of such phytochemicals in the two organs of *J. curcas.* According to Oseni and Alphonse [26], while Flavonoid and Phenolic phytochemicals were present in the stem, they were absent from the leaf; also, alkaloids occurred in a higher concentration in the stem than leaf of *J. curcas*. These phytochemicals, that are either present only in the stem or occurred there in higher concentrations, have been reported as responsible for the insecticidal of many plant extracts [11, 27].

Table 1. Mean mortality of *Culex pipiens pipiens* mosquito larvae, exposed to varying concentrations of leaf and stem extracts of *J. curcas*

Extract concentration (mg/ml)	Plant material extracted	
	Leaf	Stem
0.00 (Control)	0.00±0.00[a]	0.00±0.00[a]
0.25	2.00±1.40[b]	0.00±0.00[a]
0.75	10.00±3.46[c]	6.00±1.64[b]
1.25	18.00±4.68[d]	18.00±2.60[c]
1.75	38.00±4.88[f]	47.00±3.28[d]
2.25	28.00±4.52[e]	55.00±4.32[d]
2.75	56.00±2.24[g]	75.00±5.88[e]
3.25	80.00±2.70[h]	85.00±5.88[f]
3.75	96.00±0.00[i]	100.00±0.00[g]

*Values followed by different superscript alphabets in a column are significantly different at P =0.05.

Table 2. LC_{50} and LC_{90} (mg/ml) of methanolic extract of *Jatropha curcas* against 4th instar larval stage of *Culex pipiens pipiens* mosquitoes

Extract	LC_{50} (Confidence Limits)	LC_{90} (Confidence Limits)
Leaf	2.65 (2.19 – 2.79)	3.45 (3.22 – 3.60)
Stem	2.33 (2.14 – 2.51)	3.25 (3.08 – 3.37)

5. CONCLUSION

The findings of this study have shown that extracts of the stems and leaves of *J. curcas* possess significant larvicidal potentials against the *Cx. p. pipiens* mosquito and, therefore, may be a veritable source of insecticidal lead-agents for mosquito vector control. However, the stem extract appears to be more

promising as a larvicide, due to its richer qualitative and/or quantitative phytochemical diversity. This finding, therefore, suggest that more attention should be focused on the stem extract of *J. curcas*, through fractional bio-assay, for optimum larvicidal efficacy. The results of this study have provided base-line information that should fast-track and guide the development of cost-effective eco-friendly larviciding agents for integrated mosquito vector control.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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