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Efficacy of Some Plant Extracts on *Anopheles gambiae* Mosquito Larvae

S.A. Aina, A.D. Banjo, O.A. Lawal and K. Jonathan

Department of Plant Sci. and App. Zoology,
Olabisi Onabanjo University, Ago-Iwoye, Nigeria

Abtract: The efficacy of both the ethanolic and aqueous extracts of the fruits of *Physalis angulata* L. (Solanaceae); *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) and seeds of *Piper guineense* Schum and Thonn (Piperaceae); *Jatropha curcas* Linn. (Euphorbiaceae) were tested on the second instar larvae of *Anopheles gambiae* (L) at varying concentrations. With percentage mortality, *P. guineense* (83.33%) in ethanol was the most effective followed by its aqueous form (71.67%), *P. angulata* (36.94%) in ethanol, *X. aethiopica* (34.44%) in ethanol, *J. curcas* (33.06%) in ethanol, aqueous extracts of both *P. angulata* and *X. aethiopica* at 29.44% while the least active was *J. curcas* (20.56%) in water. On the basis of 24hrs LC_{50} values, *P. guineense* (0.028 mg ml^{-1}) ethanolic extract acted most followed by its aqueous form (0.09 mg ml^{-1}), ethanolic extract of *P. angulata* (2.5 mg ml^{-1}), ethanolic form of *J. curcas* (3.25 mg ml^{-1}), X. *aethiopica* (3.57 mg ml^{-1}) ethanolic extract, aqueous forms of both *P. angulata* and *X. aethiopica* with 4.5 mg ml^{-1} while the aqueous forms of *J. curcas* (12 mg ml^{-1}) was the least active against the *Anopheline* larvae. For all the plants used, there were significant difference among the ethanolic extracts and the aqueous forms. This could also make mosquito control in rural area become easier than before.

Key words: *Physalis angulata* L. · *Xylopia aethiopica* (Dunal) A. Rich · *Piper guineense* Schum and Thonn · *Jatropha curcas* Linn · *Anopheles gambiae* (L) · Ethanolic extract

INTRODUCTION

There are approximately 3,500 species of mosquitoes grouped into 41 genera. Human malaria is transmitted only by females of the genus *Anopheles*. Of approximately 430 species of *Anopheles*, only 30-40 transmit malaria in nature [1].

The mosquito *Anopheles gambiae* is the principal vector of malaria in Africa. According to the latest WHO statistics, this parasitic disease infects from 300 to 500 million persons per year in the world and kills more than a million and a half each year, mainly African children. Together with AIDS, malaria is one of the causes of mortality in the populations of African, South Asia and Latin America; it contributes a large part of the continued impoverishment of these populations [2].

Okorie and Lawal [3] tested the larvicidal properties of ethanolic extracts of fruits of *P. guineense* (African black pepper) on larvae of *Aedes aegypti* (L) at different concentrations.

Scott *et al*. [4] also reported that the extracts from three species of the plant family Piperaceae; *P. nigrum* (L), *P. guineense* and *P. tuberculum* (Jacq.) were effective against insects from five orders. All the three species contain isobutylamides, plant secondary compounds that act as neurotoxins in insects. The materials were considered safe for mammals because *Piper* spp. were used for centuries for spice and medicinal purposes. *P. guineense* oils also prevented the emergence of F_1 brunchids of *Callosobruchus maculatus* [5]. Formulation of 1% of essential oil of *X. aethiopica* was also said to be toxic on *Sitophilus zeamais* [6]. The seeds of J. *curcas* are considered antihelmintic in Brazil and the leaves are for fumigating against bed-bugs (Cimicidae). Also, the ether extract shows antibiotic activity against *Styphylococcus aureus* and *Escherichia coli* [7].

This study is carried out to know the effects aqueous and ethanolic extracts of these plants on larvae of *Anopheles gambiae* (L) with a view to discover more plant products that can be used to control the prevalence of malaria fever in developing nations.

Corresponding Author: S.A. Aina, Department of Plant Sci. and App. Zoology, Olabisi Onabanjo University, Ago-Iwoye, Nigeria

MATERIALS AND METHODS

Experimental Site: The research was conducted at the Nigerian Institute of Medical Research (NIMR), Yaba (Long. 3°25'E, Lat. 6°35'N) in the Lagos state from the month of May to December, 2007.

Collection of Plant Materials: The fruits of *X. aethiopica* and seeds of *P. guineense* were bought while the fruits of *P. angulata* and the seeds of *J. curcas* were collected at Sagamu and Ago-Iwoye respectively, both in the Ogun State, Southwestern Nigeria. The materials were also dried in the Gallemhamp oven and identification of the plants was done at the Elikaf Herbarium, Department of Plant Science and Applied Zoology, OOU, Ago-Iwoye, with the assistance of Dr. M.O. Soladoye.

Collection/culture of Mosquito: The *Anopheles gambiae* mosquito larvae used for this study were collected from a culture maintained in the insectaries of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos.

Aqueous Extraction Preparation: The plants were blended using the Moulineax blender. 200 grams of each grounded botanical was then soaked separately in 400 to 1 litre distilled water for 1hour to dissolve the active components. The suspensions were latter filtered using the Whatman's No. 1 filter paper. The filtrates were then freeze-dried to remove the water solvent in each case using the Edwards Modulyo Freeze-drying machine. From the freeze-dried (Stock), serial dilutions were made to obtain different concentrations of 20, 15, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05 and 0.02 mg ml^{-1}.

Ethanolic Extraction Preparation: 200 grams of each blended material was mixed with 70% ethanol in separate jars and allowed to stay for 1 hour. They were later filtered into conical flasks using the Whatman's No.1 filter paper and the filtrates were put into the Gallenhamp Vacuum oven to evaporate the extraction solvent. Serial dilutions were made from the stock to obtain different concentrations of 20, 15, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05 and 0.02 mg ml^{-1}.

Bioassay of Extracts: Ten active second instar larvae of the *Anopheles gambiae* were transferred into (100 ml) containers containing 2 ml of distilled water and 50 ml from each graded concentrations of each extract was added. In the controls, the larvae were put in 50 ml of distilled water and 2% ethanol respectively. 3 replicates were set-up for each concentration including the control. Observations were made over 24 hours, after which the larvae were introduced into distilled water to notice recovery. A recovery time of 5minutes was allowed [8]. Larvae were counted as dead when they were not coming to the surface for respiration and were probe insensitive [9].

Statistical Analysis: The data recorded from the bioassay tests were analyzed by probit analysis based on the Statistical Analysis System (SAS) version 8. Comparison among seeds, fruits, between seeds and fruits and all the plants were also sorted out using the Analysis of Variance (ANOVA) which was carried out using the Statistical Package for Social Sciences (SPSS) for windows version 14.

RESULTS

Table 1 shows that of *P. guineense* (83.33%) seeds extracted with ethanol give the highest percentage mortality followed by its aqueous extract with 71.66%, ethanolic extract of the fruits of *P. angulata* (36.94%), ethanolic extract of *X. aethiopica* (34.72%), ethanolic extract of *J. curcas* (33.06%) seeds, aqueous extracts of *P. angulata* and *X. aethiopica* both at 29.44% while the aqueous form of *J. curcas* had the least mortality with 20.56%.

Table 1: Percentage Mortality of *A. gambiae* larvae tested with ethanolic extracts of the four plants

Plant species	Part used	Extraction medium	Total mortality(360) and % mortality
P. guineense	Seed	Ethanol	300 (83.33%)
	v	Water	258 (71.67%)
J. curcas	v	Ethanol	119 (33.06%)
	v	Water	74 (20.56%)
P. angulata	Fruits	Ethanol	133 (36.94%)
	v	Water	106 (29.44%)
X. aethiopica	v	Ethanol	125 (34.72%)
	v	Water	106 (29.44%)
Control	-	Ethanol	0 (0.00%)
	-	Water	0 (0.00%)

Table 2: LC_{50} of ethanolic extracts of plants on *A. gambiae* second instar larvae

Plant species	Part used	Extraction medium	LC_{50}
P. guineense	Seed	Ethanol	0.028
	v	Water	0.09
J. curcas	v	Ethanol	3.25
	v	Water	12.00
P. angulata	Fruits	Ethanol	2.50
	v	Water	4.50
X. aethiopica	v	Ethanol	3.57
	v	Water	4.50
Control	-	Ethanol	0.00
	-	Water	0.00

Table 3: Independent Samples T-Test for *P. guineense* extracts

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Mortality	Equal variances assumed	4.642	0.035	1.314	70	0.043	1.1667	0.88775
	Equal variances not assumed			1.314	68.293	0.043	1.1667	0.88775

Table 4: Independent Samples T-Test for *J. curcas* extracts

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Mortality	Equal variances assumed	6.321	0.014	1.403	70	0.016	1.2500	0.89066
	Equal variances not assumed			1.403	66.192	0.016	1.2500	0.89066

Table 5: Independent Samples T-Test for *P. angulata* extracts

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Mortality	Equal variances assumed	1.371	0.246	0.720	70	0.474	0.7500	1.04103
	Equal variances not assumed			0.720	69.551	0.474	0.7500	1.04103

Table 6: Independent Samples T-Test for *X. aethiopica* extracts

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Mortality	Equal variances assumed	0.110	0.741	0.542	70	0.589	0.5278	0.97299
	Equal variances not assumed			0.542	69.999	0.589	0.5278	0.97299

Table 7: ANOVA for ethanolic extracts of the plants

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	635.910	3	211.970	12.499	0.000
Within Groups	2374.250	140	16.959		
Total	3010.160	143			

Table 8: ANOVA for aqueous extracts of the plants

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	570.222	3	190.074	12.225	0.000
Within Groups	2176.667	140	15.548		
Total	2746.889	143			

Table 9: T-test for the two extracts of the plants

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	61.420	1	61.420	3.051	0.082
Within Groups	5757.049	286	20.130		
Total	5818.469	287			

The LC_{50} values shown in Table 2 indicated that the ethanolic extract of *P. guineense* (0.028 mg ml^{-1}) was the most active followed in descending order by the its aqueous extract with 0.09 mg ml^{-1}, ethanolic extract of *P. angulata* (2.5 mg ml^{-1}), ethanolic form of *J. curcas* (3.25 mg ml^{-1}), ethanolic extract of *X. aethiopica* (3.57 mg ml^{-1}) and aqueous extracts of *P. angulata* and *X. aethiopica* (4.5 mg ml^{-1}) while the aqueous form of *J. curcas* (12 mg ml^{-1}) was least in performance.

In Table 3 and 4, show that there was significant difference between the ethanolic and aqueous extracts of *P. guineense* and *J. curcas* respectively while Table 5 and 6 also show that there were no significant differences between the ethanolic and aqueous extracts of both *P. angulata* and *X. aethiopica*. In Tables 7-9; Table 7 shows that there was significant difference in the toxicity of the ethanolic extracts of all the plants, Table 8 also reveals that there was significant difference among the aqueous extracts of all the plants while Table 9 shows that there was no difference between the ethanolic and aqueous groups for all the plants.

DISCUSSION AND CONCLUSION

The problem of high cost and development of resistance in many vector mosquito species to several of the synthetic insecticides have revived interest in exploring the pest control potentials of plants [10]. Also, economic and environmental concerns have encouraged a tendency recently towards the use of "soft" pesticides [11].

The assessment of botanicals for the plant extracts show that the ethanolic extract of *P. guineense* was the most effective for the control of *A. gambiae* larvae while the aqueous extract of *J. curcas* was the least to pose mortality which is in line with the report of Oke *et al.* [12] in which the hexanolic extract of *P. guineense* kill both 77 and 95% of the *Aedes aegypti* larvae in 1 and 24 hours, respectively. Also the extract of *Cannabis sativa* (Moraceae) tested on *Anopheles stephensi* within 24 and 48 hours gave LC_{50} of 15.58 and 8.04 ppm, respectively [13].

Also Fafioye *et al*. [14] reported that the ethanolic extracts of *Parkia biglobosa* and *R. vinifera* were more potent against the juveniles of *Clarias gariepinus* than the aqueous forms. This is due to the polarity, volatility and its (ethanol) power to dissolve more of the active ingredients.

Although the statistical analysis revealed that the ethanolic extraction is better in performance which does not mean that we can not also use the aqueous form for such control. There is need to still investigate on the use of other volatile solvents in order to really discover the unknown properties of these plants. Invariably, botanical insecticides may serve as suitable stand alone alternatives to synthetic insecticides in future as they are relatively safe, degradable and are readily available in many areas of the world [15].

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