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The Toxicity of Extracts of *Tetrapleura tetraptera* (*Aridan*), *Delonix regia* (Flame of the Forest) and *Raphia vinifera* (Raffia Palm) on the Larvae of *Anopheles gambiae*

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Abtract: The ethanolic and aqueous extracts of the fruits of *Tetrapleura tetraptera* (Fabiaceae) and seeds of *Delonix regia* (Bojer ex Hook.) Raf (Fabiaceae) and *Raphia vinifera* L. (Arecaceae) were tested on the second instar larvae of *Anopheles gambiae* (L) at varying concentrations. With percentage mortality, *T. tetraptera* (59.17%) in ethanol was the most effective followed by *D. regia* (42.78%) in ethanol, *T. tetraptera* (31.67%) in water, *D. regia* (20.56%) aqueous extract and *R. vinifera* (19.44%) in ethanol, while the aqueous extract of *R. vinifera* (16.39%) was the least active. On the basis of 24hrs LC_{50} values, *T. tetraptera* (0.08g/ml) ethanolic extract was the most toxic followed by the ethanolic extract of *D. regia* (1.4mg/ml), aqueous extract of *T. tetraptera* (5.37%), ethanolic form of *R. vinifera* (10%), *D. regia* (11.5%) aqueous extract while the aqueous forms of *R. vinifera* (12.5%) was the least active. 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 and will also be less toxic to other non-target species.

Key words:*Tetrapleura tetraptera* (Fabaceae) · *Delonix regia* (Bojer ex Hook.) · *Raphia vinifera*(L) · *Anopheles gambiae*(L) · Aqueous extracts and 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 (www.wikipedia/Anophelesgambiae, [2]).

Udo, [3] reported that among five local spices, *T. tetraptera* powdered form was found to only possess repellent action against the maize weevil, *Sitophilus zeamais*. The fruits of the native *Aridan* tree were used as an emulsifier for neem oil. The emulsifiable concentrations were more effective than the insecticide Lindane, Fenithrothion and Carbaryl to suppress grasshoppers (Olaifa and Adenuga, [3]). Isolated compounds from *T. tetraptera* either from fruits or other parts were found to exhibit strong molluscicidal properties against the schistosomiasis-transmitting snails, *Biomphalaria glabrata* [4].

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.

MATERIALS AND METHODS

Experimental Site: The research was conducted at the Nigerian Institute of Medical Research (NIMR), Yaba (Long. 3°25^{1}E, Lat. 6°35^{1}N) in the Lagos state from the month of May to December, 2007.

Collection of Plant Materials: The fruits of *T. tetraptera* and the seeds of *R. vinifera* were bought while the seeds

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of *D. regia* were collected at Sagamu 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. 200grams of each grounded botanical was then soaked separately in 400 to 1litre 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, 0.02mg/ml.

Ethanolic Extraction Preparation: 200g of each blended material was mixed with 70% ethanol in separate jars and allowed to stay for 1hour. 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, 0.02mg/ml.

Bioassay of Extracts: Ten active second instar larvae of the *Anopheles gambiae* were transferred into (100ml) containers containing 2ml of distilled water and 50ml from each graded concentrations of each extract was added. In the controls, the larvae were put in 50ml of distilled water and 2% ethanol respectively. 3 replicates were set-up for each concentration including the control. Observations were made over 24hours, after which the larvae were introduced into distilled water to notice recovery. A recovery time of 5minutes was allowed [5]. Larvae were counted as dead when they were not coming to the surface for respiration and were probe insensitive [6].

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

T. tetraptera (59.17%) fruits extracted with ethanol gives the highest percentage mortality followed by the ethanolic extract of the seeds of *D. regia* (42.78%), aqueous extract of *T. tetraptera* (31.67%), aqueous extract of *D. regia* (20.56%) seeds, ethanolic extract of *R. vinifera* (19.44%) while its aqueous form had the least mortality with 16.39% as shown in Table 1 below.

The LC_{50} values shown in Table 2 indicated that the ethanolic extract of *T. tetraptera* (0.08mg/ml) was the most active followed in descending order by the ethanolic extract of seeds of *D. regia* (1.40mg/ml), aqueous form of *T. tetraptera* (5.37 mg/ml), ethanolic extract of *R. vinifera* (10mg/ml) and aqueous extract of *D. regia* (11.50mg/ml) while the aqueous form of *R. vinifera* (12.5mg/ml) was least in performance.

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

Plant species	Part used	Extraction medium	Total mortality(360) and % mortality
D. regia	Seed	Ethanol	154 (42.78%)
	v	Water	75 (20.56%)
R. vinifera	v	Ethanol	70 (19.44%)
	v	Water	59 (16.39%)
T. tetraptera	Fruit	Ethanol	213 (59.17%)
	v	Water	114 (31.67%)
Control	-	Ethanol	0 (0.00%)
	-	Water	0 (0.00%)

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

Plant species	Part used	Extraction medium	LC_{50}
D. regia	Seed	Ethanol	1.40
	v	Water	11.50
R. vinifera	v	Ethanol	10.00
	v	Water	12.50
T. tetraptera	Fruit	Ethanol	0.08
	v	Water	5.37
Control	-	Ethanol	-
	-	Water	-

Table 3: Independent Samples T-Test for *D. regia*

		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	22.020	.000	2.338	70	.022	2.1667	.92672
	Equal variances not assumed			2.338	61.350	.023	2.1667	.92672

Table 4: Independent Samples T-Test for *T. tetraptera*

		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	1.038	.312	2.784	70	.107	2.7500	.98792
	Equal variances not assumed			2.784	69.613	.107	2.7500	.98792

Table 5: Independent Samples T-Test for *R. vinifera*

		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	1.131	.291	.421	70	.675	.3056	.72495
	Equal variances not assumed			.421	68.629	.675	.3056	.72495

Table 6: ANOVA for ethanolic extracts of the plants

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32.889	2	16.444	.986	.377
Within Groups	1751.778	105	16.684		
Total	1784.667	107			

Table 7: ANOVA for aqueous extracts of the plants

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	265.574	2	132.787	7.969	.001
Within Groups	1749.639	105	16.663		
Total	2015.213	107			

Table 8: T-test for the two extracts of the plants

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	163.630	1	163.630	10.595	.001
Within Groups	3305.019	214	15.444		
Total	3468.648	215			

In Table 3, there was significant difference between the ethanolic and aqueous extracts of *D. regia* while Tables 4 and 5 show that there were no significant differences between the ethanolic and aqueous extracts of both *T. tetraptera* and *R. vinifera*. While in Tables 6-8; Table 6 shows that there was no difference in the toxicity of the ethanolic extracts of the plants, Table 7 also reveals that there was significant difference among the aqueous extracts of the plants while Table 8 show that there was difference between the ethanolic and aqueous groups respectively.

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 [7]. Also, economic and environmental concerns have encouraged a tendency recently towards the use of "soft" pesticides [8].

The assessment of botanicals for the three plant extracts show that the ethanolic extract of *T. tetraptera*

was the most effective for the control of *A. gambiae* larvae while the aqueous extract of *R. vinifera* was the least to pose mortality which is in line with the report of Oke *et al.*, [9] in which the hexanolic extract of *P. guineense* kill both 77% and 95% of the *Aedes aegypti* larvae in 1hour and 24 hours respectively. Also the extract of *Cannabis sativa* (Moraceae) tested on *Anopheles stephensi* within 24 and 48hours gave LC_{50} of 15.58 and 8.04ppm respectively [10].

Also Fafioye, *et al,* [11] 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 [12].

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