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TOXICITY EFFECT OF VARYING CONCENTRATIONS OF *Raphia vinifera* FRUIT EXTRACT ON AFRICAN CATFISH (*Clarias gariepinus* Burchell, 1822) FINGERLINGS

*¹Idowu, A. A., ²Ayoola, S. O., ¹Nwekoyo, V. E., ¹Adeosun, F. I. and ¹Adesiyun, G. E.

¹Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, Nigeria

²Department of Marine Sciences, University of Lagos, Nigeria

*Corresponding author, email: idowudoyin@yahoo.com

Abstract

This study is aimed at evaluating the toxicity effect of *Raphia vinifera* on the hematological and histopathological parameters of *Clarias gariepinus* fingerlings. Fish samples with an average weight of $\pm 2.00\text{g}$ and an average length of $\pm 8.00\text{cm}$ were obtained from Eweje Fish Farm, Odeda Ogun State. The fruits of *Raphia vinifera* were sourced from the fresh water swamp forest of Tata village in Ilaro Local Government Area Council of Ogun State, Nigeria. The fruit extract was used in determining the range test for 24hrs. A range of 5g/L, 3.75g/L, 2.5g/L, 1.25g/L and 0g/L (control) were obtained and used in determining the toxicity effect for 96 hrs on fingerlings catfish (*C. gariepinus*), by studying the mortality rate of fish exposed to *R. vinifera* fruit extract, and recording the number of mortalities every 12hrs. The susceptibility of *C. gariepinus* to the effects of the fruit extract was duration and concentration-dependent. Fish mortality was found to be associated with the decrease in pH and DO values. Although histopathological lesions were observed in the exposed and unexposed fish, the lesion in the unexposed fish was at a lesser extent compared to that of the exposed fish. The water in which the fish were stocked was analyzed to be at DO $5.34 \pm 0.05\text{ mg/L}$; pH, 6.75 ± 0.02 ; temperature, $20 \pm 0.02^\circ\text{C}$; conductivity, $91.4 \pm 1.01\text{ }\mu\text{S/cm}$; TDSs, $53.4 \pm 0.81\text{ mg/L}$ levels respectively. There were significant differences ($P < 0.05$) in all the concentrations as mortality increased with time of exposure. The study shows that the use of *R. vinifera* fruit extract at higher doses produced 100 % mortality rate in *C. gariepinus*. It is therefore advised that 100% dose of the extract should be prohibited as it can destroy both egg and fingerlings of fish species.

Keywords: Hematology, histopathology, *Raphia vinifera*, *Clarias gariepinus*

Introduction

Raphia vinifera (P. Beauv) known as raffia or bamboo palm, called Igi Ope in Yoruba land, and ayon udin in Edo land, is abundant along the creeks of Niger Delta, Cross River, Lagos and Ikorodu in Nigeria (Keay, 1989). The nut is called Iregbe Ako in Yoruba land. It contains bitter oil and has the property of stunning fish (Burkill, 1985). However, the palm is not efficiently utilized. Mostly, it is exploited for the production of palm wine, which is commonly fermented into ethanol and less reported for their ethno-botanical uses (Ukwubile *et al.*, 2013; Ndon, 2003). In Bayelsa state and many coastal communities in the Niger Delta, the fruits of raffia palm are milled and used as bait to stupefy and poison fish for easy catching. Ukwubile *et al.* (2013) reported that most fishes killed by raffia palm fruit extracts were pelagic species, whose opercula are not always tightly closed, suggesting that it may be due to respiration process.

The gills of the affected fishes were also reported by the authors to change colour within 20 minutes, initially from red to pale red to almost whitish, after being in contact with the fruit. Two possible mechanisms of action were also suggested by the authors; first the blockage of the blood capillaries supplying blood to the gills and other respiratory organs of the fish, which resulted in the change of the colour of the gills, caused by the extract. Secondly, phytochemicals especially tannins, saponins and alkaloids, react with oxygen to form fish poison and/or decrease oxygen tension, when in an aqueous solution complex with basic salts, and could suffocate fishes.

Many toxicity studies have been carried out using *Raphia vinifera* on fish. This study therefore focuses on the toxicity effect of *Raphia vinifera* on the haematological and hispathological parameters of African catfish, *Clarias gariepinus*. African mud catfish, *Clarias gariepinus* Family: Clariidae, is one of the most commonly cultured fish species



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in the world (FAO, 1977). Changes in the behavior of the fish are to be monitored, as behavioral changes are good indicators of damage to the fish, as a consequence of exposure to toxic agents (Almeida *et al.*, 2009). Hence, this study is aimed at investigating the effect of raffia palm, *Raphia vinifera* on the haematological and histopathological characteristics of the *Clarias gariepinus* fingerlings.

Methodology

Collection and Processing of Experimental Plant

Fresh fruits of *Raphia vinifera* were collected from the fresh water swamp forest of Tata village in Ilaro Local Government Area Council of Ogun State, Nigeria. The nuts were oven dried at 60°C for 48 hours for easy removal of the pulps/nuts from the shells. The removed pulp was then oven dried for 96 hours (4 days), at 60°C. Other materials used for the project were: Rectangular plastic tanks with dimension 1m x 1m x 1m, Distilled water, Weighting scale with capacity 1kg and Electrical oven.

Phytochemical Determination

Quantitative and qualitative analyses were carried out to determine the presence of tannins, saponin, phenol, alkaloids, steroids, phytate, oxalate, glycosides, flavonoids and anthraquinone according to the methods described by Okwu and Nnamdi (2008) and Akpabio *et al.* (2008).

Collection, Stocking and Management of Experimental Fish

A total of 150 fingerlings African catfish with an average weight of $2.0 \pm g$ and an average length of $8.0 \pm cm$ was purchased from Eweje Fish Farm, Odeda, Ogun State, Nigeria. The fish were transported to the laboratory in plastic tanks of 50L capacity, filled to half and acclimated for 10 days in two 14cmx14cm rectangular tanks containing dechlorinated tap water after the tanks were washed and disinfected with salt prior to the day of stocking. During this period, the fish were fed at 3% body weight twice daily using Skretting feed (2mm) grade. The routine change of water was carried out every two days.

Design and Setup of Experiment

A Randomized Complete Block Design (RCBD) was used, the plastic tanks were labeled into Treatment 1-5 with 3 replicates each. Each replicate contains 10 fishes stocked, at the rate of 30 fishes per treatment.

Range Test

The range test was carried out in the fish hatchery by soaking 50grams of the ground *Raphia vinifera* in a liter of distilled water for 24 hours. After which the extracts were sieved out using clean white handkerchief so as to get the stock solution, the solution was then added to 100ml into each plastic tank containing ordinary water in the following concentrations in this order 100ml, 200ml, 400ml, 600ml and 800ml.

Extracts Dosage and Design Range

A range of 5g/L, 3.75g/L, 2.5g/L, 1.25g/L and 0g/L (control) was obtained and used in determining the toxicity test for 96 hrs on *C. gariepinus* fingerlings by studying the behavioural changes of the fish exposed to the fruit extracts.

Haematological Test

The blood sample for this analysis was collected using 5ml syringe and needle into heparinised EDTA bottle from the caudal fin region of the fish. The blood sample was then taken to the Veterinary Teaching Hospital Laboratory, Federal University of Agriculture, Abeokuta. The following haematological tests were carried out on the fish samples at the experiment using the following standard procedures; Packed cell volume (PCV) and Haemoglobin concentration (Hb) as described by Ezzelle *et al.* (2008), RBC, WBC and its differential count were determined using Hoffbrand *et al.* (2006) method of blood analysis.

Histopathological Analysis

At the end of the experiment, the remaining fishes were scarified (Fafioye *et al.* 2005). The liver was harvested, fixed in 10% formalin for 24hrs prior to paraffin embedding, sectioning at 5µm and staining with haematoxylin and eosin according to the methods of Roberts, (1978; Bancroft and Cook, (1994) for histopathological examinations under light microscopy.



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Statistical Analysis

The data obtained was analyzed using SPSS according to Finney's Probit Analysis, (1971) using Microsoft Spreadsheet computer programme. The statistical difference of means between test groups was estimated using Analysis of variance (ANOVA) using Statistical Programme for Social Sciences (SPSS) software, version 20.

Results and Discussion

Phytochemical Screening

Among the phytochemical contents analyzed as shown in Table 1, the fruit was found to contain high concentration of saponin, alkaloid and oxalate, a moderate concentration of tannin, flavonoid and steroid, and a low concentration of phytate, phenol and glycoside while anthraquinone was not detected.

Table 1: The phytochemical screening of *Raphia vinifera* fruit pulp

Phytochemicals	Qualitative Analysis	Quantitative Analysis (%)
Tannin	++	8.96
Saponin	+++	12.18
Flavonoid	++	4.55
Alkaloid	+++	16.81
Phytate	+	1.16
Oxalate	+++	21.62
Steroid	++	3.44
Phenol	+	0.76
Glycoside	+	0.58
Anthraquinone	Not detected	0.08
+ = Low present ++ = Moderately present +++ = High present		

Range Finding Test

The occurrence of mortality during the range test shows that the fish in the 100ml solution extract died at the 6th hour, that of the 100ml solution extract + 100ml solution died at 12th hour, that of the 100ml solution extract + 200ml of ordinary water died at the 18th hour, while those of 100ml solution extract + 400ml of ordinary water, 100ml solution extract + 600ml of ordinary water and 100ml solution extract + 800ml of ordinary water did not die till the 24hours expiration. The range for the experiment was found to be at 5g/L, 3.75g/L, 2.5g/L, 1.25g/L and also 0g (which is the control).

Water Quality Analysis

During the toxicity tests, the monitored water parameters included; Dissolved Oxygen (DO), Hydrogen ion concentration (pH), Temperature, Conductivity and Total Dissolved Solids (TDS). The results recorded according to Table 2, showed that the values for DO of the water in the plastic tanks were

5.34±0.05mg/L; pH (6.75±0.02); Temperature (20±0.02°C); Conductivity (91.4±1.01 µS/cm); TDSs (53.4±0.81 mg/L), respectively. The physical and chemical parameters of the water used showed that there were both slight and wide fluctuations in the parameters of the culture water during the experimental period. However, the slight fluctuations were not significantly different ($p < 0.05$) and could not have produced serious effect on fish mortality, however, the wide fluctuations produced significant difference ($p > 0.05$) since they might have been altered by the experiment, this is in concurrent with the findings of (Mackereth, 1963). Aside, neurological dysfunction which brought about abnormal behavioural pattern, deficiency of dissolved oxygen causes hypoxic condition in fish which results in an increased breathing rate and to cope with the condition, the fishes gulp air by frequent surfacing. Furthermore, it is possible that the excess of extract gets accumulated in the gills, reducing gaseous and ionic exchanges.



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Table 2: Mean water quality parameters measured during the experimental period

Concentration (g/L)	DO (g/L)	pH	Temp. (°C)	Conductivity (µS/cm)	TDS (g/L)
5.00	4.56±1.32	6.49±0.17	19.5 ± 0.5	100 ± 0.5	66.2 ± 0.2
3.75	4.95 ± 1.3	6.58±0.21	20 ± 0.2	96.8 ± 0.45	64.7± 0.17
2.5	5.67 ± 1.3	6.6 ± 0.2	20 ± 0.2	96.3 ± 0.45	62.1± 0.17
1.25	5.62 ± 1.2	6.72 ± 0.3	20 ± 0.3	95 ± 0.3	63.3± 0.2
0	5.65 ± 1.4	6.75 ± 0.3	20 ± 0.2	91.4 ± 0.2	53.4± 0.15

Mortality Recorded

The toxicity test of *R. vinifera* on *C. gariepinus* fingerlings led to a progressive stress across all the treatment tanks except the control as higher concentration levels of the botanical fruit extract resulted in higher rates of mortality. The percentage mean mortalities recorded for the experimental fish within the 96 hours are shown in Table 3 and denoted on

the probity graph shown in Figure 1. The two results indicated that at the end of the 96 hours, the 5g/L concentration produced 100% mortality, 3.75g/L concentration produced 70% mortality, 2.5g/L concentration produced 50% mortality, 1.25g/L concentration produced 10% mortality while the control recorded 0% mortality.

Table 3: Mortality recorded during the experiment

Concentration (g/L)	Time (Hours)								% Mortality
	12	24	36	48	60	72	84	96	
5.00	4	2	3	1	0	0	0	0	100
3.75	0	2	2	1	1	1	0	0	70
2.50	0	0	0	2	1	1	1	0	50
1.25	0	0	0	0	0	0	1	0	10
0	0	0	0	0	0	0	0	0	0

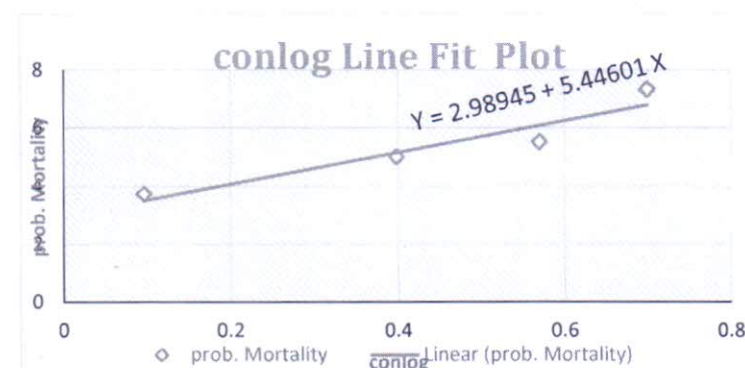
Probit Analysis**Figure 1:** Linear relationship between probit mortality and log concentration of *Clarias gariepinus* fingerlings exposed to varying concentrations of *Raphia vinifera* fruit extract.**Haematological parameters**

Table 4 shows the haematological changes in the test and control *C. gariepinus* fingerlings after exposition. The mean Haemoglobin (Hb) ranges from 6.68±0.03 to 10.68±0.03g/L while Packed Cell Volume (PCV) mean ranged from 17.98±0.02 to 32.16±0.02g/L. The values of the mean for Red blood cell, White Blood Cell (WBC) value ranges from 0.78±0.02 to

14.58±0.03g/L. Hetophilis (HET), Lymphocyte (LYM) and Eosinophil (EOS) has its mean values ranging from 36.94±0.10 – 46.91±0.16, 49.99±0.02 – 58.96±0.07 and 1.99±0.02 – 3.01±0.01g/L respectively. The mean range for Monocyte was between 0.00±0.00 to 1.00±0.00g/L. The mean at the highest concentration (5g/L) and 3.75g/L were significantly different for PCV, meanwhile for HB except for 5g/L



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concentration and control all the other remaining concentrations are not significantly different from each other. Also, concentrations 5g/L and 3.75g/L for RBC and WBC 5g/L, 3.75g/L and 2.5g/L were significantly different from each other as well. Furthermore, there were significant

differences at concentrations 5g/L, 2.5g/L and 1.25g/L for HET, and all the concentrations including the control for LYM but for eosinophil only concentration 3.75g/L is significantly different at ($P < 0.05$) from the rest of the concentrations.

Table 4: Haematological parameters of *C. gariepinus* fingerlings exposed to varying concentrations of *R. vinifera* fruit extract

Parameters	Concentration				
	5g/L	3.75g/L	2.5g/L	1.25g/L	0
PCV	25.99 ± 0.01 ^b	17.98 ± 0.02 ^c	31.97 ± 0.04 ^a	31.99 ± 0.01 ^a	32.16 ± 0.02 ^a
HB	8.79 ± 0.02 ^c	6.68 ± 0.03 ^d	10.68 ± 0.03 ^b	10.78 ± 0.03 ^a	8.88 ± 0.03 ^c
RBC	1.10 ± 0.01 ^b	0.78 ± 0.02 ^c	1.35 ± 0.02 ^a	1.38 ± 0.02 ^a	1.51 ± 0.01 ^a
WBC	14.09 ± 0.02 ^b	10.29 ± 0.02 ^c	9.39 ± 0.02 ^d	14.58 ± 0.03 ^a	14.19 ± 0.03 ^a
HET	36.94 ± 0.10 ^d	46.91 ± 0.16 ^a	37.96 ± 0.06 ^c	43.66 ± 0.59 ^b	43.76 ± 0.45 ^b
LYM	58.96 ± 0.07 ^a	49.99 ± 0.02 ^d	57.93 ± 0.12 ^b	52.92 ± 0.14 ^c	57.99 ± 0.15 ^b
EOS	2.98 ± 0.03 ^a	1.99 ± 0.02 ^b	2.99 ± 0.02 ^a	2.99 ± 0.02 ^a	3.01 ± 0.01 ^a
MON	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Means with different superscripts along the row are significantly different ($P < 0.05$)

PCV = Packed Cell Volume, WBC = White Blood Cell

HB = Haemoglobin, RBC = Red Blood Cell

HET = Eosinophil, LYM = Lymphocyte

EOS = Eosinophil, MON = Monocyte

BAS = Basophil.

Histology of the liver of *C. gariepinus* fingerlings exposed to varying concentrations of *R. vinifera* fruit extract

The Histopathological changes observed in the liver of experimental fish are shown in plates 1-5, indicating different lesions, ranging from congestion of the liver to vacuolation of the hepatocyte. The observed histopathological changes seen in the photomicrograph were as a result of various clinical factors resulting from increased concentrations of *R. vinifera*, which brought about severe damages to the fish liver. According to the photomicrograph (Plates 1-

5), Plate 1 shows the liver of the unexposed (control) *Clarias gariepinus* fingerlings central vein (black arrow) with hepatocyte vacuolations (red arrow), in Plate 2 at concentration 1.25g/l the central vein got congested (black arrow). However, Plate 3 indicated mononuclear cellular infiltration (black arrows) and vacuolation of the hepatocyte (red arrows) within the liver while Plate 4 showed vacuolation of the hepatocyte (red arrows) and Plate 5 showed the central vein was congested (black arrow) and vacuolation of the hepatocyte (red arrows) with loss of liver architecture.

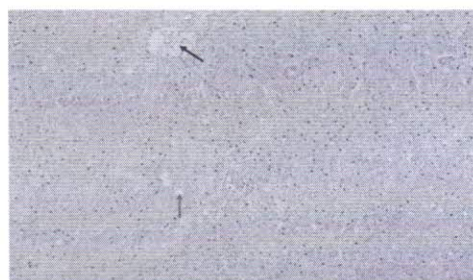


Plate 1: Control Fish Liver



Plate 2: The Fish liver at 1.25g/L

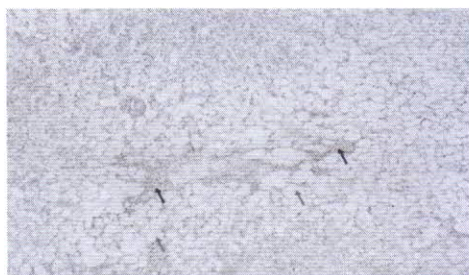


Plate 3: The liver at 2.5g/L



Plate 4: The Fish liver at 3.75g/L

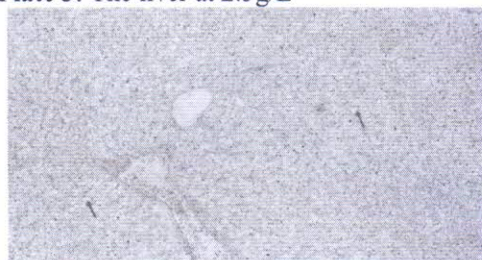


Plate 5: The Fish liver at 5.00g/L

Behavioural Patterns of Fish

The observation from the control tank showed that the experimental fishes exhibited normal behaviour and swimming patterns without any mortality. The fishes exposed to different concentrations of the solution extract, on the other hand, showed abnormal behaviour which was observed only at 24 hrs after the addition of the fresh extract. The fishes became alert, stopped swimming, and remained static in position in response to the sudden changes in the surrounding environment. Frequent surface-to-bottom movements and faster opercula activity were observed as surfacing and gulping of air increased with the increase in the concentration of the extract. Also, it was observed that the fish remained in a vertical position for a few minutes with the anterior side or terminal mouth up near the surface of water trying to gulp air, and the tail was in a downward position. Soon, the fish settled to the bottom of the tank and were found lying at the bottom of the plastic tanks before they died. This behavioural pattern observed in this study agreed with the findings of Lin and Liu (1990), who reported that clinical signs such as abnormal movement and high respiration rate in hybrid tilapia (*Oreochromis mossambicus*) induced by ammonia suggested neurological dysfunction and gill damage.

The abnormal behaviour, which was observed only at 24 hrs after the addition of

the fresh extract agrees with the findings of Yunis *et al.* (2014), who reported that after 24 hrs of exposure to aqueous *Uncaria tomentosa* plant extract, the fishes exhibited clinical and behavioral alterations, which is also noticed in fish exposed to the fruit extract of *R. vinifera*.

Some toxicity study of other plants' extracts on fish having similar results have been reported such as *Blighia sapida* and *Kigelia africana* on *C. gariepinus* (Onusiriuka and Ufodike, 1994 and 1998), *Parkia biglobosa* and *Raphia vinifera* on *C. gariepinus* and Tilapia (Fafioye *et al.*, 2004), Tobacco on *O. niloticus* and *C. gariepinus* (Omoniyi *et al.*, 2002), *Raphia hookeri* on *C. gariepinus* (Adeogun *et al.*, 2002). It can be observed from water analysis results that strong relationships exist between water parameters and behavioural pattern of the fish. These wide fluctuations with significant difference might have been altered by the experiment and hence produced deleterious effect such as stressful conditions of abnormal behaviours prior to death and mucus secretion on the gills of the moribund fish.

The fruit extract of *R. vinifera* was toxic to the exposed *C. gariepinus* with evidence of histopathological and haematological study. The results in Table 4 indicated varying values with significant difference across the table at ($p < 0.05$) with increased or decreased concentrations. Histopathology showed vacuolation of the liver hepatocyte, however



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as concentration increased from 1.25g/l-5g/l the fish liver suffered central vein congestion, cellular infiltration and loss of liver architecture, this result concurred with Fafioye *et al.* (2004) that observed histological changes were as a result of various clinical factors resulting from use of high concentrations of *R. vinifera* fruit extract. The histopathology and haematological changes in the various organs especially the liver is similar to epithelial damages caused by cadmium according to Oronsaye, (1997) and endrin (Eller, 1971). The various alterations seen is definitely as a result of several physiological stresses in the fish while there were struggling. The control liver had normal internal arrangement components with presence of brownish granular pigment within the parenchyma.

Conclusion and Recommendation

The study showed that the presence of higher concentration of saponin, alkaloid and oxalate in *R. vinifera* fruits extract were responsible for the ichthyotoxicity. However, the fruits extract of the plant represents a safe and easy way of harvesting fish from a large fresh water body at a controlled level especially during fish stock assessment in cove area. This is because *R. vinifera* fruit extract at higher doses produced 100 % mortality rate in this study. It is therefore advised that 100% dose of the extract should be prohibited as it can destroy both egg and fingerlings of fish species.

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