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Research Article

Ichthyotoxic Assessment of Methanolic Extract of *Raphia hookeri* Exposed to Different Concentrations of African Clariid Mud Catfish (*Clarias gariepinus*) Fingerlings

Andem A B*, Joseph A P, Ibor O R, John E R

Department of Zoology and Environmental Biology, University of Calabar, P.M.B. 1115 Calabar, Cross River State, Nigeria

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ABSTRACT

The importance of studying the toxicity effects of methanolic extracts of *Raphia hookeri* on the survival of *Clarias gariepinus* cannot be over-emphasized, because they are mostly used locally for catching of fishes, because of its ichthyotoxic properties. These extracts end up killing excess fishes; including fingerlings, fry, sub-adults and adults, which could end up making the fish population unsustainable on the long run. This study will help to sensitize the fishermen over the long term effects of methanolic extracts of *R. hookeri* on fish population. 25 fingerlings of *C. gariepinus* were exposed to methanolic extracts of *R. hookeri* in concentrations of 0.00 (control), 30.0, 50.0, 70.0 and 100.0ppm for 96 hrs. All experiments were set up in triplicates. The *C. gariepinus* fingerlings exposed to different concentrations of methanolic extracts of *R. hookeri* exhibited signs of stress and erratic movement. All the time-effect relationship was found to be concentration dependent. A log-concentration probit regression analysis was significant ($p<0.05$), yielding a co-efficient of determination, r^2 of 0.913. The 96 hours LC_{50} and 95% confidence limits for *C. gariepinus* exposed to different concentrations of Methanolic extract of *R. hookeri* was 1.70 ± 0.25ppm, having an interval of 10.15 – 2.0.077ppm. In conclusion, the study revealed that extracts of *R. hookeri* has cytotoxic and ichthyotoxic properties on the fingerlings of *C. gariepinus*.

Keywords: Ichthyotoxic, Assessment, *R. hookeri*, Methanolic extract, African catfish

INTRODUCTION

In Africa, the cheapest protein sources come from fish[1]. *Clarias gariepinus* is one of the most widely distributed fish in Nigerian waters, and belong to the Clariidae family[2]. They have a high tolerance and high survivability[3] and are capable of adapting to unfavourable environmental conditions[4]. The good taste of *Clarias gariepinus*, have made it to have a high market value in Nigeria[5], and this has led to the increase in the culture of the fish in commercial quantities, thereby boosting the finances of the fish farmers. Most traditional fishermen make use of various toxic plants, in order to increase the catch yields[6,7]. *Raphia hookeri* (Raffia palm) are mainly found in the tropical rainforest, and is the largest palm in Africa[8]. Economically, *Raphia hookeri* has multiple uses in Africa. Local gin and alcohol are produced from fermented sap of *Raphia hookeri*. Their trunks are used as firewood, the leaves are used for shelter and the stems are used for the production of palm sap used as beverage, while the leaf petiole produces fibrous piassava. Infected palms contain oily larvae of weevils and beetles, which are used as delicacies. Edible oil is also produced from the mesocarp of the ripe fruit[4]. Pounded and fermented fruits of *Raphia hookeri* have been reported to have ichthyotoxic properties[9-11]. The toxic effects of Raphia palm pulp extracts in *C. gariepinus* fingerlings was studied by[12], the author reported that Raphia palm have lethal haematological effects on *C. gariepinus*[13] reported that methanolic extracts of *R. hookeri* extracts have various effects like; hyperventilation, sneezing, and rapid opercula movement in *C. gariepinus*, due to the damaging effects of saponins on the respiratory epithelia, which is the active component of Raphia palm[14,11,15] have all reported that various plants extracts are used for fishing in south-eastern Nigeria. [16]reported that these toxic plants are capable of causing physiological dysfunction and reduced growth in fish. The cytotoxic properties of *R. hookeri* fruit mesocarp was reported by[17]. The toxicity effects of toxicants depend on the species, sex, size, age and general health condition[4]. The irritation and impairment of the respiratory epithelia of fish exposed to toxicant reduces the capacity of fish gill epithelia to diffuse oxygen across the membrane[18,19] reported that saponins exhibit potent anti-cell adhesive activity. [20]reported that *R. hookeri* extracts has the capacity of killing fingerlings of *Oreochromis niloticus* and *Clarias gariepinus*. The study is aimed at determining Ichthyotoxic Assessment and LC_{50} of Methanolic Extract of *Raphia hookeri* exposed to different Concentrations of

*Author for Correspondence: a.b.andem@unical.edu.ng

Table 1: Manifestation, Overturning and Survival Time of *Clarias gariepinus* Exposed to Concentrations of Methanolic extract of *Raphia hookeri*.

Concentration (ppm)	Manifestation Time (Hrs)	Over-turning Time (Hrs)	Survival Time (Hrs)
0.00	0.00	0.00	0.00
30.0	3.00	9.03	12.00
50.0	2.00	4.00	6.00
70.0	1.40	2.30	3.00
100.0	1.27	1.40	2.00

African Clariid Mud Catfish (*Clarias gariepinus*) Fingerlings.

MATERIALS AND METHODS

Test Chemical

The methanolic extract of Raphia hookeri used for this research was gotten from Ikot Abasi Effiom in Akpabuyo Local Government Area of Cross River state.

Collection and Transportation of Test Fish

Clarias gariepinus fingerlings were collected from hatchery complex of the University of Calabar fish farm, Calabar, Cross River state with the aid of a scoop net in the early hours of the morning to avoid heat, high intensity, stress and were transported to the laboratory in an open plastic basket containing aerated habitat water. The container containing the fingerlings was handled with care to avoid agitation which may cause damage to fingerling and possibly death.

Acclimation and Maintenance of Test Fish

The *Clarias gariepinus* fingerlings were kept in glass holding tanks for at least 21 days (three week) to allow them acclimatize to the prevailing laboratory conditions in one tank (30 x 30 x 50) cm 2/3 filled with water which was continuously aerated with electrical air pumps. During the period of acclimation, fingerlings were fed with coppens feed. The fingerlings holding water was changed daily to avoid accumulation of toxic waste products and metabolite and food particles. Tap water was dechlorinated by vigorously aerating and allowing the water to settle for at least 48 hours before use to avoid stress caused by chlorine in the tap water.

Preparation of Test Chemicals

Air-dried and grounded fruit of *R. hookeri* (20kg) was extracted by percolation using hexane (2.13% yield) and then with methanol (24.93% yield). Methanolic extraction yielded more extract. The percolate was evaporated to dryness using a low-high vacuum pump that creates the required vacuum for the removal of methanol and moisture. Desiccation containing calcium chloride salts were then used to further absorb methanol and moisture from the extract. Stock solutions of the extract were prepared by dissolving 100g of the extract in 10litres of water and from this, serial dilutions were made.

Range Finding

Range finding tests were inducted prior to the experiment in order to determine the appropriate test concentration

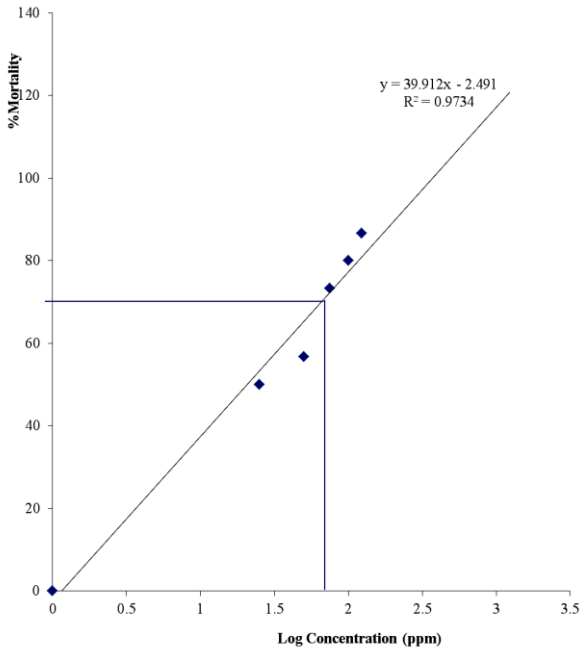

Figure 1: Probit graph of *Clarias gariepinus* fingerlings exposed to different concentrations of Methanolic extract of *Raphia hookeri*

range of the fingerlings. A wide range of concentrations were tested including one which killed all organisms within 96 hours and another concentration which did not kill the organisms within 24 hours. Following the procedure, appropriate test concentrations spaced at logarithmic intervals selected for the research experiment[21].

Test procedure

Appropriately graded test concentrations spaced at logarithm intervals to include one concentration showing 100 percent mortality, another with no mortality and intermediate concentrations with partial mortality were used for the test. Tests were conducted in rectangular glass aquaria with four litres of test solution. Twenty five (25) fingerlings of *Clarias gariepinus* were exposed to methanolic extracts of *Raphia hookeri* in concentrations of 0.00 (control), 30.0, 50.0, 70.0 and 100.0ppm for four (4) days. All experiments were set up in three replicates. The volume of water to the weight of fishes was calculated in accordance with[22]. Careful observations were made to note the number of mortalities of the test organisms during the four days of exposure. *Clarias gariepinus* was considered dead if it remains motionless when touched with a glass rod.

Statistical Analysis

The mortality-concentrations data were subjected to probit transformation, regression analysis and LC_{50} values were computed using Predictive Analytical Software (PASW) version 20. The significant of the slope were tested using Chi-square. Graphs were drawn using Microsoft excel (MSE) version 2013[21].

RESULTS

Table 2: Probit Transformation/analysis of mortality data of *Clarias gariepinus* exposed to concentration of Methanolic extract of *Raphia hookeri.*

Conc (ppm)	Log Conc (x)	N	R	p	M_R	Y	R_P	*P*
0.00	0.00	25	0	0	0	0	0.00	0.00
30.0	1.477	25	12	0.48	48	13.6	-1.56	0.54
50.0	1.699	25	16	0.64	64	14.13	-1.87	057
70.0	1.845	25	19	0.76	76	19.91	-0.91	0.79
100.0	2.000	25	25	1.00	100	24.80	0.24	0.99

n = Number of fish fingerling tested at each concentration, r = Number of fish fingerling responding, p = Response rate, r/n, M_R = Mortality rate, Y = Expected probit from visual regression line, R_P = Residual probit, *P* = Probability.

Table 3: Results of regression analysis of Log Concentration – probit relationship of *Clarias gariepinus* fingerlings exposed to concentration of Methanolic extract of *Raphia hookeri.*

Conc. (Log Unit)	Response rate, p	Equation	Co-efficient of determination, r^2	Significant level, α
0.00	0.00			
1.477	0.48			
1.698	0.64	4.431 + 44.174x	0.913	0.05 (S)
1.845	0.76			
2.00	1.00			

Table 4: Chi-square Tests of *Clarias gariepinus* fingerlings exposed to different concentration of Methanolic extract of *Raphia hookeri.*

	Chi square	df[a]	Sig.
PROBIT Pearson Goodness-of-FitTest	1.410	1	0.235[b]

Table 5: Covariance's and correlation of *Clarias gariepinus* fingerlings exposed to different concentration of Methanolic extract of *Raphia hookeri.*

PROBIT	Concentration	Natural Response
Concentration	62.217	0.863
Natural Response	1.023	0.023

Table 6: LC_{50} with 95% confidence limits of Clarias gariepinus fingerlings exposed to different concentrations of Methanolic extract of Raphia hookeri.

LC_{50} with ± 95%CL	Confidence limits	
	Lower	Upper
1.70 ± 0.25	10.15	20.08

Manifestation time

Time – concentration effect relationships such manifestation, overturning and survival times is shown in Table 1. Manifestation time (the time interval between the initial exposure of fish to toxicant and the first appearance of symptoms of toxicity in fish) was observed to decrease with increase in concentration of Methanolic extract of *Raphia hookeri*. *Clarias gariepinus* exposed to 30.0, 50.0, 70.0 and 100.0ppm of Methanolic extract of *Raphia hookeri* indicated manifestation times of 3.00, 2.00, 1.40 and 1.27 hours respectively, decreasing steeply from 3.00 to 1.27 hours as the concentration of plant extract increased (Table 1).

Overturning time

Overturning time, (the time between the introduction of the toxicant and the loss of equilibrium or righting balance) showed a similar trend with manifestation time, decreasing with increase in toxicant concentration. In all concentrations, overturning was preceded by uncoordinated, fast swimming bursts, swimming head up and tail down and swimming upside down. Overturning times of 0.00, 9.03, 4.00, 2.30 and 1.40 hours were recorded for to 0.00, 30.0, 50.0, 70.0 and 100ppm of Methanolic extract of *Raphia hookeri* respectively (Table 1).

Survival time

Survival time, the time interval between the initial exposure of *Clarias gariepinus* to toxicant and the time the first mortality occurs. Survival time of *Clarias gariepinus* exposed to concentrations of Methanolic extract of *Raphia hookeri* decreased as exposure concentration increased (Table 1). *Clarias gariepinus* exposed to 0.00, 30.0, 50.0, 70.0 and 100.0ppm of Methanolic extract of *Raphia hookeri* yield survival time of 0.00, 12.00, 6.00, 3.00 and 2.00 hours respectively. Drops in survival times were steeper from 30.0 to 70.0ppm but ended more gradually towards 100.0ppm.

Probit Analysis Results

Results of regression analysis of Log Concentration – probit relationship of *Clarias gariepinus* fingerlings exposed to different concentration of Methanolic extract of *Raphia hookeri* is shown in Table 2 and 3. The trends in mortality data of fingerlings indicate that fingerling's mortality rate increased with increasing concentration of Methanolic extract of *Raphia hookeri* (Figure 1). Probit transformation of mortality rate-log concentration relationship determined to be linear by regression analysis (Figure 1 and Table 3). A log concentration probit regression analysis was significant ($p<0.05$) yield a co-efficient of determination, r^2 of 0.913 (Figure 1 and Table 3), and the LC_{50} with 95% confidence limit was also determined to be 1.70 (Figure 1). Chi-square shows insignificant difference at $p<0.05$ (Table 4). Covariances and correlation of *Clarias gariepinus* fingerlings exposed to different concentration of Methanolic extract of *Raphia*

hookeri is presented in Table 5. The LC_{50} (medium lethal concentration) the concentration of the toxicant that kills 50% of exposed organisms) was determined by the method of [23]and the 95% confidence limit computed (Table 6). The LC_{50} and 95% confidence limits for *Clarias gariepinus* exposed to concentration of Methanolic extract (*Raphia hookeri*) was determined as 1.70 ± 0.25ppm and LC_{50} interval of 10.15 – 2.0.08ppm as shown in Table 6.

DISCUSSION

Raphia palm have lethal haematological effects on *C. gariepinus*[12]. [16]reported that toxic plants are capable of causing physiological dysfunction and reduced growth in fish. The irritation and impairment of the respiratory epithelia of fish exposed to toxicant reduces the capacity of fish gill epithelia to diffuse oxygen across the membrane[18]. The study revealed that *Clarias gariepinus* fingerlings displayed various signs of stress and restlessness, when exposed to the methanolic extracts of *Raphia hookeri*, due to the damaging effects of saponin present in the *Raphia hookeri* on the respiratory epithelia. The damaging effects of saponins on organisms are due to the fact that saponins exhibit potent anti-cell adhesive activity[19]. Similar results were observed by[13], who reported that methanolic extracts of *R. hookeri* extracts had various effects like; hyperventilation, sneezing, and rapid opercula movement on *C. gariepinus* fingerlings. Methanolic extracts of *Raphia hookeri* displayed its cytotoxic and ichthyotoxic properties, as mortality was observed when the fingerlings of *Clarias gariepinus* were exposed to the extracts of *Raphia hookeri*, and this corroborated with the report of[9-11,17]. The trends in mortality data of fingerlings indicate that fingerling's mortality rate increased with increasing concentrations of Methanolic extract of *Raphia hookeri*, and this corroborated with the report of[4], who reported that the morphological, behavioural and mortality responses of *Clarias gariepinus* exposed to extract of *Raphia hookeri* was concentration dependent. Mortality of the test fingerlings exposed to the methanolic extracts of *Raphia hookeri* increased with increase in concentration and this was similar to the findings of[20], who reported that *R. hookeri* extracts has the capacity of killing fingerlings of *Oreochromis niloticus* and *Clarias gariepinus*. Similar observations were also made by[13,24], who reported that exposure of fish continuously to saponins pollutants causes' death, due to the increase in the permeability of the membranes of the red blood cells. The LC_{50} of 1.70 ppm for *Clarias gariepinus* fingerlings exposed to the methanolic extracts of *Raphia hookeri* in the present study was higher than that reported by[4,25]. The variations could be due to the differences in species, sex, toxicants, size, age and general health condition[4]. Also, the susceptibility of different organisms to toxic substances differs[4]. The study revealed that the exposure of *C. gariepinus* to the methanolic extract of *R. hookeri* resulted in hyperventilation, froth production from the mouth of the fish, and possibly rapid opercula movement and some damaging effect on the respiratory epithelia due to the saponins. The mortality of *Clarias gariepinus* exposed to extracts of *Raphia hookeri* was concentration dependent. Also, the extracts of *Raphia hookeri* showed it's cytotoxic and ichthyotoxic properties on the fingerlings of *Clarias gariepinus*.

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