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Research Article

Sublethal Effects of Methanolic Extract of *Raphia hookeri* on the Reproductive Capacity of *Clarias gariepinus*

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Raphia hookeri fruits are used for fishing in Nigeria due to their ichthyotoxic properties. This study investigated the toxic effects of *R. hookeri* on the reproductive capacity of *Clarias gariepinus*. The results from both short-term (96-hour test) and long-term (3-month sublethal test) bioassays revealed a linear relationship between *R. hookeri* extract dose and negative effects on the catfish. The percentage survival of both sexes of the catfish decreased with increasing extract concentration at short-term exposure, with LC_{50} values of 600 mg/L and 800 mg/L for male and female, respectively. At long-term exposure, the reproductive capacity of 10–12-month-old male and female brood-stocks diminished at relatively higher concentrations of *R. hookeri* fruit extract, with the gravid females producing fewer and mostly unviable eggs. The fruit extract also affected the eggs' hatchability and fry survival when the exposed gravid females were treated with pituitary hormone and sperms from unexposed males, while the exposed males were unable to sexually stimulate female brooders. Sperms and pituitary hormone from exposed males were infertile, leading to low percentage of hatched eggs and mortality of the few hatched fries within 24 hours. These results confirmed the ethnobotanical use of this fruit extract for fishing in Nigeria.

1. Introduction

Fish is one of the cheapest protein sources in sub-Saharan Africa [1], and the importance of fish in developing countries increased greatly after the Sahelian drought of 1971 to 1974, which decimated the cattle population and hence shot up the prices of beef [2]. *Clarias gariepinus* (Burchell, 1822) belongs to the family Clariidae, and is widely distributed in Nigerian waters [3]. The fish is known for its high tolerance and high survivability [4] and resilience in harsh habitat conditions [5]. There is a huge market value for the fish in Nigerian markets due to its taste [6], which resulted in the proliferation of fish farming business in Nigeria using different fish production methods. However, wild catch still remains important. In order to increase catch yields in traditional fishing, different toxic plants that can stupefy fish have been exploited [7, 8].

Chopra et al. [9] reported at least 112 plants with poisonous effects on fish. Seigler [10] reported the piscicidal effect of some common plants of India that are frequently used in freshwater bodies against target organism while Neuwinger [11] also observed that there is a wide variety of wild and cultivated poisonous plants available to coastal fishermen for capturing fish. Most recently, Fafioye [12] reported 40 plants with piscicidal effects being used in Southwest Nigeria for fish harvesting. However, it can be expected that the use of these plants for fishing in Nigerian freshwater bodies would have negative effects on aquatic biodiversity. Several studies have shown the specific effects of plant extracts that have toxic properties: *Azadirachta indica* have been known to be toxic to *Gambusia affinis* [13]; Charkraborty et al. [14] reported that *Croton tighim* seeds, *Millettia pachyarpa* roots, *Annona squamosa*, *Dodonaea viscosa*, *Camellia sinensis*, and *Nicotiana tabacum* have some toxic properties; acute concentrations of the bark of *Balanites sapida and Kigelia africana* to *Clarias gariepinus* have been reported to cause physiological dysfunction and reduced growth [15]; Aguigwo [16] reported that cassava peel extracts caused stressed behavior in *Clarias anguillaris* fingerlings.

Obuotor and Onajobi [17] reported the cytotoxic properties of *R. hookeri* fruit mesocarp.

Raphia hookeri (Raffia palm) is the largest palm in Africa and is restricted to the tropical rainforest [18]. It is one of the most economically useful plants in Africa; the trunk serves as firewood, the leaves are used for shelter and the stem produces palm sap, which is drank as beverage. The fermented sap can be distilled into alcohol or local gin [19] while the leaf petiole yields fibrous piassava. Succulent, oily larvae of weevils and beetles are obtained from infected palms and serve as delicacy. The mesocarp of the ripe fruit yields edible oil [20, 21]; however, the fruit mesocarp is also reported to have potent toxic properties [17].

Ekelemu [22] studied the toxic effects of Raphia palm pulp extracts in *C. gariepinus* fingerlings and showed that Raphia palm have lethal haematological effects on *C. gariepinus*. Furthermore, Adeogun [23] reported multiple negative biological effects of methanolic extracts of *R. hookeri* extracts on *C. gariepinus* includinghyperventilation, sneezing, and rapid opercula movement which was reported to be due to the damaging effects of the active component of Raphia palm (saponin) on the respiratory epithelia. Negative effects of toxicants depend on the species, sex, size, and age as well as the general health condition. In this study, we wished to investigate the effect of sublethal doses of *R. hookeri* extracts on reproductive functions of male and female catfish broodstock.

2. Materials and Methods

2.1. Extraction Procedure. Plant extraction followed the procedure of Adeogun et al. [24]. Air-dried and grounded fruit of *R. hookeri* (20 kg) 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. Desiccators 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 100 g of the extract in 10 L of water and from this, serial dilutions were made.

2.2. Acquisition of Test Organisms. For the bioassay, 10–12-month-old male (342.2 ± 17.05 g) and female brood stocks (250±24.58 g) of *C. gariepinus* were obtained from a commercial fish farm in Ibadan. They were then transported to the laboratory in well-aerated plastic containers. At the laboratory, the fish were kept in rectangular glass tanks (1800 cm^2) throughout the experiment for easy visibility.

2.3. Acclimatization Procedure. Acclimatization of the fish samples was done for a period of 7 days. During the acclimatization period, the fish were fed once daily with a 40% protein fish-feed at the rate of 3% body weight for adult fish/day. Well aerated dechlorinated municipal tap water was used as the holding medium (temperature 28.5 ± 2.5°C; pH 7.1 ± 0.3). The tank water was monitored and changed twice a week to avoid contamination. The fish were well-adapted to laboratory conditions and no death was recorded for 4 consecutive days [25].

2.4. Exposure of Test Organisms. The fish were weighed and the average weight obtained for the male fish was 342.2 g and 250.0 g for females. Feeding was discontinued 24 hours prior to the commencement of the experiment and during the 96-hours range-finding test [25]. A preliminary range-finding test was carried out for 96 hours (4 days) using diluted extract concentrations ranging from 250 mg/L–1200 mg/L. This was done to determine the concentration at which the toxicant was lethal to the test organisms. Distribution of fish into tanks was done and 4 fish were kept in each experimental tank. The control fish tank was not exposed to the *R. hookeri* treatment (only water). Standard procedures for bioassay as described by APHA [26] were used with slight modifications (12 g of fish to 1 L of water for brooders). Fish behavioral changes were reported at 1, 2, 3, 4, 8, 16, 48, 72, and 96 hours. Dead fish were promptly removed from the experimental tank. Dose response for mortality response was analyzed by the Probit method [27]. The index of toxicity measurement derived from this analysis was 96-hour LC_{50} values (median lethal concentration that causes 50% response (mortality) in exposed organisms).

2.5. Toxicity Experimental Procedure. Fractions of 1/5, 1/10, and 1/15 of the LC_{50} were used in a 30-day sublethal exposure for male and 20 days for female brood stocks. Two replicates of each fish sex (4 fish per tank) were kept in each concentrations prepared. For the male brood stocks, the concentrations used were 40, 60, and 120 mg/L while for the female, 53.3, 80, and 160 mg/L were used. Control experiments with no extract exposure were also set up for each case.

2.6. Fertilization Procedure. After the exposure for 30 days, *R. hookeri* exposed male catfish were randomly selected and their sperm sacs were collected and kept in labeled clean containers with 9% saline solution. It had been observed that the life-span of fish sperm varies with the substrate within which the testes are kept and sperm also live longer periods in lower temperatures [28]. These containers were then kept in the refrigerator to extend their lifespan according to Nahagama [29] and Viveen et al. [30]. The pituitary gland of each exposed male was also removed and gently grounded in a mortar before adding 9% saline solution.

Then, 0.1 mL of this preparation, which contained pituitary hormone (PH), was injected into equivalent weighted unexposed female fish (control). These females were kept for 12 hours to aid the maturation of the eggs. After 12 hours, the eggs were stripped and the stored sperm milt from the exposed male fish was used in the fertilization of these stripped eggs by gentle mixing in a plastic bowl containing 9% saline solution to ensure longer sperm viability. Stripping of eggs for female brooders was done by applying gentle pressure to the abdominal area [30]. The fertilized eggs were then spread in the nursery bowls containing tiny shredded ropes to simulate nursery grounds, with water running in and out

Table 1: Morphological and behavioural responses of *C. gariepinus* in test solution.

Studied characteristic	Observations on fishes prior to exposure	Observations on fish after exposure
Skin colour	Black dorsally and grayish white ventrally	Colour changed to a mottled grayish colour dorsally with dark spots occurring all over body
Response to stimuli	Rapid response to external stimuli that is touching	Relatively sluggish with occasional spiral movement
Swimming	Normal swimming (dorsal side up)	Alternate swimming on the lateral side and rapid and erratic swimming behavior
Breathing mechanisms	Normal breathing	Gasping for breath
Opercula movement	Normal opercula movement	Rapid opercula movement

of the bowls to ensure constant aeration as well as to avoid contamination.

2.7. Estimation of Brood-Stock Fertility. The total number of stripped eggs was counted during distribution into nursery tanks and recorded. Continuous observation of the nursery tanks revealed that hatching occurred about 15 hours after induced fertilization. The unhatched eggs were siphoned out, counted, and recorded to determine percentage hatchability. Egg viability rates were determined based on the percentage number of hatched eggs [31], where

$$\text{Egg viability rate} = \frac{\text{Number of hatched eggs}}{\text{Total number of eggs in a batch}} \times 100. \quad (1)$$

The same process was repeated for the exposed female fish using the unexposed control male fish PH preparations and sperm. Control experiments involved the use of unexposed male fish PH and sperm on unexposed female fish.

2.8. Data Analysis. Toxicity measurement index was LC_{50}, which is the median lethal concentration that causes 50% response (mortality) in exposed organisms. The LC_{50} was determined by Probit method of analysis for the male and female brood-stocks [27]. Data on fish wet weight, egg count, and number of hatchlings across exposure concentrations were analyzed by one-way ANOVA (differences between means were considered significant when $P < 0.05$).

3. Results

3.1. General Observation. The morphological and behavioral responses of the fish prior and after introduction to the plant extracts are shown in Table 1.

All exposed fish demonstrated rapid and erratic swimming behavior on introduction of diluted concentrations of the extract. Other observations included being stunned (for 20–30 seconds), gasping for breath, rapid opercula movement, and emission of froth from the mouth. Behavioral changes were less apparent at lower *R. hookeri* concentrations (data not presented as they were not quantified). The fishes were noted to swim away from point of plant extract administration; they appeared stunned for a period of 30 seconds. After 24 hours, fish gradually became sluggish, especially in the highest concentrations with their mouth agape. None of these responses was seen in control groups.

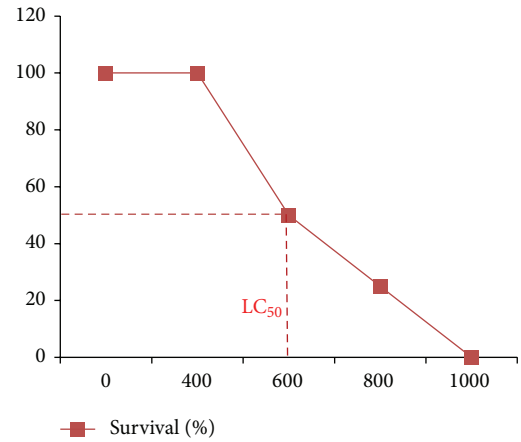


Figure 1: Determination of median lethal concentration (LC_{50}) of methanolic extract of *R. hookeri* by arithmetic graphic method for the male brood-stock of *C. gariepinus*.

3.2. Mortality Response. The results also indicated increased mortality rate with increasing concentrations of *R. hookeri* methanolic extracts. Dead specimens had increased mucus secretions. For the male brood-stock, first death was recorded at 42 hour time point in the highest concentration (1000 mg/L). By 92 hours, all the fish in this group were dead (Tables 2(a) and 2(b)). For the female brood-stock, first death was recorded at 28 hours in the highest concentration (1200 mg/L) and by 96 hours, 75% of the fish in the highest concentration were dead (Tables 2(a) and 2(c)). There was no mortality in the lowest concentrations for both the male (400 mg/L) and the female fish (600 mg/L) (Table 2(a), Figures 1 and 2).

Furthermore, it was observed that exposing *C. gariepinus* to increasing doses of *R. hookeri* methanolic extracts sharply reduced either viability of sperm or production of PH or both in exposed male brood-stock. This conclusion is based on indirect observation from the performed long-term bioassay. Number of unhatched eggs from control females treated with PH and sperm taken from exposed males was used to evaluate this effect. The eggs originating from control fish had the best viability score (96.46% and 94.42%), while eggs originating from exposed males scored poorly (0.21%, 0.41%, 20.09%, 22.51%, 32.34%, and 33.37%) (Table 3, Figure 3).

Table 4 shows the negative effect of the increasing doses of *R. hookeri* extracts on egg production and quality from exposed female *C. gariepinus* brood-stock. Prolonged periods

Table 2: (a) Effects of different *R. hookeri* methanolic extract concentrations on male and female brood stock of *C. gariepinus*. (b) Time dependence of the toxicity of different *R. hookeri* methanolic extract concentrations on male brood stock of *C. gariepinus* over 96 hours. (c) Time dependence of the toxicity of different *R. hookeri* methanolic extract concentrations on female brood stock of *C. gariepinus* over 96 hours.

(a)

Concentration (mg/L)	Number of survivors		% survival	
	Male	Female	Male	Female
Control	4	4	100	100
400	4	n/a	100	n/a
600	2	4	50	100
800	1	2	25	50
1000	0	1	0	25
1200	n/a	1	n/a	25

Male LC_{50} = 600 mg/L; Female LC_{50} = 800 mg/L.
n/a: not applicable.

(b)

Concentration (mg/L)	Mean % mortality over exposure period Time (hours)								
	1	2	4	8	16	24	48	72	96
1000	0	0	0	0	0	0	100	100	100
800	0	0	0	0	0	0	75	75	75
600	0	0	0	0	0	0	50	50	50
400	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

(c)

Concentration (mg/L)	Mean % mortality over the exposure period Time (hours)								
	1	2	4	8	16	24	48	72	96
1200	0	0	0	0	0	0	75	75	75
1000	0	0	0	0	0	0	75	75	75
800	0	0	0	0	0	0	50	50	50
600	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

Table 3: Effect of pituitary gland preparation and sperm from exposed *C. gariepinus* male brood-stock on eggs' fertilization and hatchability.

Conc. (mg/L)	Replicates	Weight of exposed male broods (g)	Total number of hatched eggs	Total number of unhatched eggs	% viability
0	C1	296.0	5073	300	96.42
0	C2	302.3	6050	222	96.46
40	R1A	124.0	1743	3647	32.34
40	R1B	374.4	2422	4835	33.37
40	R2A	220.5	728	2896	20.09
40	R2B	279.0	1405	4836	22.51
60	R1A	498.7	28	13176	0.21
60	R1B	449.5	0	10980	0
60	R2A	368.0	20	4812	0.41
60	R2B	431.5	0	5260	0
120	R1A	299.0	0	4055	0
120	R1B	299.0	0	4055	0
120	R2A	376.6	0	3405	0
120	R2B	376.6	0	3405	0

Note: Weight of the unexposed female fish used for fertilization ranged from 179 g to 320 g with a mean weight of 248.7 g.

Table 4: Effect of *R. hookeri* methanolic extracts on egg production and quality in exposed female *C. gariepinus*.

Conc (mg/L)	Replicates	Weight of exposed female broods (g)	Total number of eggs	Total number of hatched eggs	% viability
0.0	C1	230.0	2415	1950	80.75
0.0	C2	243.0	2114	1665	78.76
53.3	R1	149.0	1893	1243	65.66
53.3	R2	237.0	2748	1742	63.39
80	R1A	215.5	2850	35	1.23
80	R1B	240.0	2114	0	0
80	R2	220.5	2453	0	0
160	R1	280.5	2275	0	0
160	R2	224.0	2850	0	0

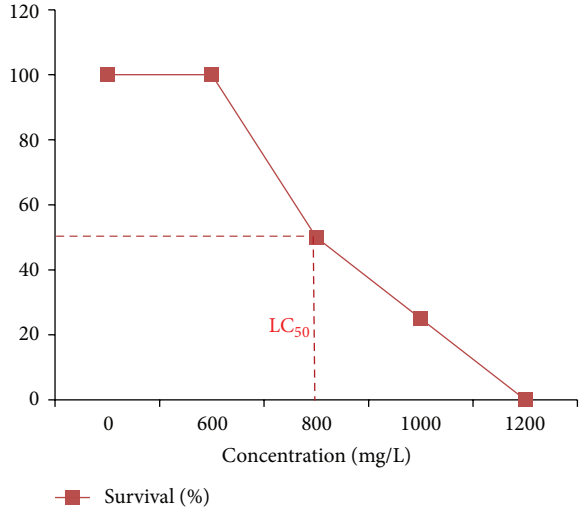


Figure 2: Determination of median lethal concentration (LC_{50}) of methanolic extract of *R. hookeri* by arithmetic graphic method for the female brood-stock of *C. gariepinus*.

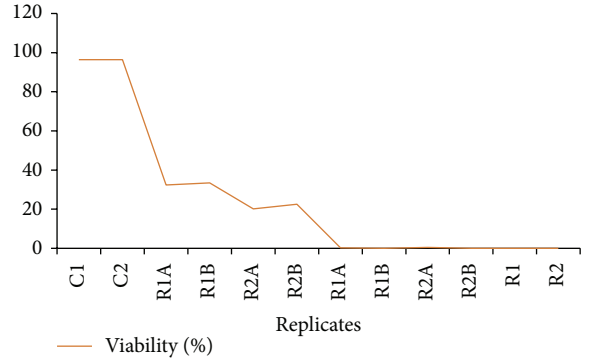


Figure 3: Percentage viability of eggs from female brood stock of *C. gariepinus* in different concentrations of *R. hookeri* methanolic extracts.

of hatching were recorded and this increased with increase in exposure concentrations. Egg maturation was observed to occur at about 15 hours. There was delay in egg maturation (15 hours rather than the normal 10–12 hours) of the unexposed female injected with the pituitary hormone of exposed males. Significantly ($P < 0.05$) lower values were recorded for the total number of eggs that hatched from control exposures to the highest exposure. The highest exposure concentrations prevented egg viability in exposed fish as there were no hatchlings (0%) in this exposure concentration (Tables 3 and 4).

Furthermore, the weight of *C. gariepinus* males exposed to *R. hookeri* methanolic extracts ranged from 124.0 g to 498.7 g (Table 3), while the average weight of the unexposed male fish was 626.5 g (Table 4).

4. Discussions

The reproductive output of a species is an integral endpoint in fish toxicology studies and an important aspect of risk assessment for aquatic ecosystems [32]. Survival of a species is highly dependent on the reproductive success of the species in question and for economically relevant species; water quality monitoring is necessary to aid evaluation of challenges posed by incidence of pollution [33]. Behavioral responses to poor water quality in exposed *C. gariepinus* brood-stock, is an indication of the toxic effect of the extract of the botanical. Consequently, it is evident from the results obtained that an increase in the concentration of *R. hookeri* methanolic extract gave rise to a series of morphological and behavioral responses from the exposed mud-catfish (*C. gariepinus*). These responses were found to be concentration-dependent. The different stressful conditions observed in this study could be due to the negative effects of the extract on the general physiology of the test organism. The gasping for breath resulted in the continuous darting up and down in the test-water and prolonged stays at the water surface. Similarly, gasping for breath and rapid opercula movement reported in this study may be due to the damaging effect of the saponins contained in *R. hookeri* extract on fish gill epithelia. This observation is coherent with the report of Adeogun [34] that saponins are present in *R. hookeri* and it causes hyperventilation, coughing/sneezing, rapid opercula movement, and mortality in mud-catfish. The observation also agrees with the report of Roy et al. [35] that saponins are highly toxic to fish because of their damaging effect on the respiratory epithelia. The froth produced from the mouth of the test organisms confirms the presence of saponin as an

active component of *R. hookeri*. This is due to the fact that Raphia-saponin 1 that is found in *R. hookeri* fruit belongs to a group of triterpenoid glycoside saponins, which have a distinctive foaming characteristic resulting from the combination of the nonpolar sapogenin and their water soluble side chain [36]. The resulting irritation and impairment of the respiratory epithelia will reduce the capacity of fish gill epithelia for diffusion of oxygen across the membrane [37]. According to Adeogun [23, 34], the continuous exposure of such fish to saponins-rich pollutants as is found in this study plant and some others used in capturing fish by local fisherfolks, asphyxiation (resulting in death) may occur. This is mainly due to increase in permeability of the membranes of the red blood cells (RBC), which leads to their destruction and consequently causes the hemoglobin to be released into the blood stream (hemolysis) [34]. As a result, the oxygen carrying capacity of RBCs within the organism circulatory system will be reduced. He et al. [38] confirmed that the saponins derived from the seed of *Camellia oleifera* may induce sneezing due to the stimulation of the nasal mucous membrane. Other observations such as color changes, sluggish and occasional spiral movement, alternate swimming on the lateral side, and rapid and erratic swimming behavior are additional effects resulting from the discomfort that the exposed test organisms were experiencing during the duration of the exposure. The different behavioral responses of the fish can also have a response to a nervous reaction of the fish to the extract (Table 1). Though this study was not designed to determine the effects on the nervous system, Thomas's [39] study comparatively linked such responses to the effect of toxicant on the cholinesterase activity in various parts of the nervous system. Also Quist [40] agrees with these observations in his study using *O. niloticus* exposed to *Piper guineense*. Similarly, Onwubuya [41] recorded similar responses with juveniles of *C. gariepinus* exposed to the aqueous extract of *R. hookeri*.

These deviations from normal behavior in fish were further confirmed when such responses were not observed in the control setup with no extract. According to Matthiessen and Logan [42] as well as Scott and Crossman [43], fish behavior is an endpoint that is not often used in toxicological assessment in spite of the fact that this parameter has the potential for use in toxicological studies as a sensitive indicator of reproductive impairment or other expressions of toxicity. Little et al. [44] observed that behavioral changes in fish occur 75% earlier than the onset of significant mortality in fish. Some other authors have reported similar observations in different fish species exposed to nonbotanical materials. For example, Adeogun and Victor [45] reported uncoordinated swimming movement, hyperventilation, coughing, and occasional darting movement in their study of *Clarias gariepinus* exposed to industrial effluent. Similarly, Srivastava et al. [46] reported hyperexcitation, convulsions and rapid opercula movement in *Labeo rohita* and *Channa punctatus* exposed to paper mill effluent. Also, Pathan et al. [47] observed hyperexcitation, erratic swimming, convulsions, and jerky movement in *Rasbora daniconius* exposed to paper mill effluent. Consistent with these reports are temporary loss of equilibrium, hyperventilation, and hyperactivity in juvenile members of the same species (*C. gariepinus*) exposed to textile industry effluent [48].

The mean 96-hour LC_{50} value of 600 mg/L and 800 mg/L obtained for adult male and female brood-stock of the mud-catfish to the methanolic extract (Tables 2(a)–2(c); Figures 1 and 2) in this study was lower than 1590 mg/L recorded by Onwubuya [41] for mud-catfish juveniles using aqueous extract. This indicates that the active ingredients, though polar, were extracted more effectively by methanol than water. The values however were less (for male brood-stock) and equal (for female brood-stock) to the values obtained by Adeogun et al. [24] for aqueous extract of *R. hookeri*. The different LC_{50} values recorded for the different sexes used showed that tolerance to the toxicant differs from one sex to the other and it is lower in the male fish than the female. This agrees with the observation of Fryer and Illis [49]. Similarly, according to Parish [50], organisms differ in their susceptibility to toxicant. Rand and Petrocelli [51] related these differences to accessibility rates, patterns of metabolisms, and excretion mechanisms as well as genetic composition. Comparing with other studies with toxicants other than botanicals, the 96 h LC_{50} value reported in this study is higher than that of Vanerker et al. [52] for physicochemically treated herbal pharmaceutical effluent (96 h LC_{50}; 35.00–41.00%) on *Lebistes reticulatus* and 20.06% for juvenile *C. gariepinus* exposed to textile industry effluent. However, higher LC_{50} value is expected in adult *C. gariepinus* because the magnitude of toxic effect is directly proportional to corporal surface/body weight of organisms and advancement in physiology may confer better adaptation/coping strategies to environmental stressors in adult fish than juvenile/fingerlings fish [53].

The mottled skin color and poor outlook of *C. gariepinus* may be results of depressed feeding or starvation in fish during the exposure period. According to McCue [54], starvation can be referred to as a biological condition wherein a postabsorptive animal, otherwise willing or able to eat, is unable to do so as a result of some external influence such as anthropogenic disturbance. Severe anthropogenic disturbance among other factors like long-term ecological change can lead to long and frequent bouts of starvation. Despite the continuous use of energy for the purpose of survival, most animals do not process food all the time, hence leaving them dependent on endogenous physiological fuels for important processes such as survival and reproduction [45, 55–58]. In other words, situations such as change in water quality due to the introduction of botanicals into water for harvesting by artisanal fisher-folks, or as the case applies in this study, can induce starvation leading to reliance on endogenous fuel, hence disrupting the balance between the body mass and energy flux within the organism. Exposures of these catfish to *R. hookeri* extract could have resulted in poor water quality as evidenced by the gasping for breath and occasional darting up and down the water column which may be due to low dissolved oxygen in exposure concentrations with the exception of the control.

According to Adeogun and Victor [45], reproductive success is a key factor in determining species survival. Reproductive impairment in fish by a variety of xenobiotics

has been well reported [32, 59–65] and may manifest in adults in form of decreasing quality and/or quantity of gametes, which in turn may affect fertilization success, embryonic development, larval viability, and subsequently species fitness and survival [45]. Lahnsteiner and Patarnello [66] and Bonnet et al. [67] are of the opinion that the representative criterion of the quality of a batch of eggs produced during spawning is the proportion of those eggs which successfully complete development into the next distinct biological stage of its species. For this study, significant decreases ($P < 0.05$) in number of hatched eggs and percentage viability of eggs with increase in concentration was observed. Decreased egg production was observed also in different studies carried out by other authors using different pollutants apart from botanicals [32, 45, 62, 63, 68–72]. Ankley et al. [32] reported a significant decrease in fecundity of the fathead minnow (*Pimephales promelas*) exposed to multiple point discharges from eleven Canadian industry effluents and concluded that egg production was negatively affected at concentrations of effluent lower than approximately half the effluent concentrations that negatively impacted in vitro steroid production indicating that variations in egg production may be a cheaper and early indicator of fish response to environmental stressors. The highest concentration in this study prevented egg viability as there were no hatchlings at this concentration. This indicates reproductive failure and a negative reproductive end point at concentrations that did not affect fish survival. Rickwood et al. [62] exposed mature adult fathead minnow to bleached Kraft pulp mill effluents and reported that egg production was completely halted by one of the effluents used in the 11-mill survey from Jackfish bay in which effluent concentration did not exceed 40%. On-site exposures were subsequently used to test the effects of various process streams within the mill effluent before secondary treatment and the combined alkaline stream caused decreased spawning events and decreased egg production [62].

The results of the breeding indicated that egg maturation of the normal unexposed female injected with the pituitary hormone of exposed males took place at about 15 hours rather than the normal 10–12 hours. The delay in egg maturation could be attributed to the negative impact of the exposure to *R. hookeri* extract. Though this is not conclusive, it could be as a result of loss of nervous coordination which could have further led to disruption in the endocrine system. According to Robinson [68], the reproductive behavior of many fish is influenced by the senses through their perception of environmental changes. He further stated that this could lead to impairment of several endocrine glands and in turn, these could act on ovaries and testes. From his observations, it is obvious that there is high interdependence between the nervous and endocrine systems. As such, these two systems often act together. This implies that anything affecting one will consequently affect the other. Furthermore, the induction of the unexposed female brood-stocks with the pituitary hormone of exposed male brood-stocks showed that the viability of the eggs produced decreased with the increase in concentrations of the extract to which the male has been exposed to (Table 4; Figure 3). Hence, it can be inferred that the extract had a significant effect on the pituitary hormone of the male brood-stocks. Also, the exposed female mudcatfish's result showed that despite the induction of these exposed brood-stocks with the pituitary hormone of unexposed male brood-stocks, the eggs they produced were immature and nonviable (Table 4). This also indicated that the concentration of the extract, to which the female brood-stocks had been exposed, significantly affected the maturation of eggs. This is corroborated by the observation of Lamai et al. [73], who states that there is a possibility that dieldrin accumulation in the central nervous system could block or disrupt the hypothalamic to pituitary link. The hypothalamic to pituitary link is the section that usually controls egg development and maturation. Also, Sorensen [74] noted that exposures to toxicants like copper have systemic effects as well as reproductive effects such as blockage of spawning activities, reduced egg production, deformity of fry, and low ability of the young to survive.

Table 5: Summary of the analysis of variance (t-test) of mean percentage viability of exposed *C. gariepinus* brood stock.

Concentration of extract (mg/L)	Df	t-test value	Sig (2 tailed) $P < 0.05$
R40	2	11.649	0.007
R60	2	89.316	0.000
R53.3	2	10.007	0.010
R80	2	76.147	0.000

The t-test values all indicated significant response at $P < 0.05$ except for R40 (Table 5) and this further corroborates the fact that there is possibility of acute or sublethal effects of the different concentrations of the *R. hookeri* extract. It further confirms that higher concentrations of the extract could negatively impact the viability of the eggs and quality of the sperm of the different exposed brood-stocks.

5. Conclusion

This study has demonstrated that exposure of *C. gariepinus* male and female brood-stock to the methanolic extract of *R. hookeri* resulted in reproductive impairment in catfish that were exposed to lower concentrations and reproductive failure in the highest exposure concentration. Furthermore, it is also possible that the disruption of nervous coordination occurs. This may depend on the concentration of the extract used and the length of time that the test organisms are exposed to the toxic botanical extract. However from this study, it was observed that higher concentration induced greater impact on the catfish than lower concentrations. Thus, it could be said that frequent exposures to lower concentrations may not cause any permanent damage to reproductive ability in fish due to biodegradability of most botanicals. But, if the fish are exposed to higher concentrations of the botanical as seen in this study, more deleterious effects could occur. These effects may linger for a longer period of time before the population is restored back to a healthy life. Thus, the negative effects that arise, such as on their reproductive

ability, as a result of such exposures could lead to a more great impact on these fish biodiversity as well as the nontarget aquatic organisms.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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