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Effect of Lethal Concentrations of Rubber Extract (*Hevea brasiliensis*) on the Survival on Fingerlings of *Clarias gariepinus* under Laboratory Condition.

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Abstract
The water soluble fraction (WSF) of *Hevea brasiliensis* was tested against *Clarias garienpinus,* at 0, 30, 40, 50, 60 and 70mg/l in glass aquaria stocked with ten animals for 96 hours under observation for changes. Moribund swimming, restlessness, respiratory difficulties, depigmentation and mortalities were observed in the WSF exposure groups, but not in the controls. LC_{50} values were estimated at 50.12mg/l. There was no significant difference in mortalities between the replicate group (P>0.05), leading to the conclusion that the WSF of *Hevea brasiliensis* had same toxic effects on both batches of the test organism.

1.0 INTRODUCTION

Rubber extract (*Hevea brasiliensis*) is a complex mixture, which contain substance like hydrocarbon, protein and phospholipids, while the serum phases is mainly water, with small amount of soluble compounds including carbohydrate, amine, inorganic ions and metallic ions (Jacob et. al*;* 1993) Rubber in the wild grows in the tropical rainforest, often in periodically flooded areas, but larger trees are found on the well – drained plateaus (Bekele, et. al*.,* 1993). A typical example is the Cross River Rubber plantain were the sample was collected for study. The Cross River Rubber plantation is surrounded by aquatic environment which makes it a concern in eco-toxicological studies. In Nigeria, the high production season for rubber tapping lies between May and September which corresponds to wet seasons, while the low production season lies between November and February which correspond with dry season. It is a general believed that rubber extract (*Hevea brasiliensis*) may finds its way into the aquatic ecosystem through surface run–off during the wet season, which is believed to be a high production season for rubber extract in Nigeria. (Pers.com).

Catfish are dominant fish species in the Cross River and its tributaries, and contributes significantly to the fisheries of the area. (Pers. com). African catfish (*Clarias gariepinus*) is an important aquaculture candidate in most part of the world, including Nigeria, it lives in fresh water as larvae and also as adult. (Pers.com).The objective of this study was to investigate the effects of water soluble fraction of rubber extract (*Hevea brasiliensis*) on the African catfish (*Clarias gariepinus*)

2.0 Material and Method

2.1 Collection of Sample

A total of 240 healthy *Clarias gariepinus* used in this study were collected from the University of Calabar fish farm, Calabar, Cross River state, located within the University of Calabar at latitude $04^0$5, 02^0N and longitude 008^0 20' 450'E respectively. The climate of the area is tropical and is characterized by distinct wet and dry season (Asuquo and Bassey, 1999 and Akpan et al., 2002).

2.2 Acclimatization of Specimens

The organism were collected along with habitat water, the organism were selected into size classes of 2.5-4.5cm, and acclimatized in filtered habitat water for 24 hours prior to the toxicity experiment.

2.3 Monitoring of Water Quality Parameters

The initial water parameter, Dissolved oxygen, Temperature, pH, Nitrite and Ammonia were determined using mercury – in- glass thermometer and Lurton Do and pH meters. The battery operated meters were calibrated according to manufacturer's instructions before being used for measurement (Boyd 1989, 1990).

2.4 Preparation of Water Soluble Fraction (WSF) of Rubber Latex

The water soluble fraction (WSF) of Rubber Extract obtained from Cross River Rubber plantation was obtained by vigorously shaking Rubber extract with filtered habitat water in a separatory funnel. The system was allowed to stand for six hours to effect complete phase separation, after which the lower aqueous layer containing the WSF was collected for the toxicity tests. The concentration of hydrocarbon was determined by spectrophotometric measurement of a n-hexane extract at 430nm wavelength using a Hatch direct reading (DR) 3000 spectrophotometer (Stuermer et a*l.,* 1981).

2.5 Stocking of Specimen

After the 24 hours acclimatization, the fish were randomly distributed into a rectangular glass aquaria measuring 25 X 10X 15cm, filled with two (2) liters of habitat water. 10 fingerlings was stocked in each of the aquarium, and the fish were exposed to varying concentrations (0, 30, 40, 50, 60 and 70mg/l). The experiment was replicated with control aquaria receiving filtered habitat water without the addition of WSF of Rubber extract. The Rubber extract (1g) was first dissolve in 5ml of DMSO and made up to 1,000ml with distilled water before being shaken in a separatory funnel to produce the water soluble fraction (WSF). The stock solution was used in the preparation of different concentrations of the working solution by dilution with distilled water.

2.6 Monitoring of Specimen for Mortality

The test was conducted for 96 hours with daily observations of abnormality and mortality of test organisms. Dead organisms were quickly removed from the test medium to avoid decay and contamination. The test tanks were aerated with air stones connected to electrically powered aquarium pumps.

2.7 Determination of Mortality Lethal Concentration (96 hours LC_{50})

The concentration in which 50% mortality (Lc_{50}) occurred was obtained graphically by probits analysis, plotting log concentration against fish mortality (Finney 1971; Stephan, 1977).

2.8 Statistical Analysis

The homogeneity of the replicate samples was checked by the Krus- Kal-Wallis test, before data of the replicate were pooled together and treated as single group. Significant difference in the no. of dead organism between control and experimental group were evaluated using ANOVA. Significance was accepted when ($P<0.05$) Statistical analysis was powered by SPSS 18.0 (SPSS Inc, Chicago, USA).

3.0 RESULTS

The test organism (*Clarias gariepinus*) showed pathological changes and mortalities in a concentration dependent manner. Sub-lethal changes observed were moribund and erratic swimming behaviour, weakness, paleness and depigmentation of the skin. Similar changes were not observed in the control. *Clarias gariepinus* was less sensitive to *Hevea brasiliensis* contamination with 50% (Lc_{50}) at 50.12mg/l after 96hrs exposure (Table 1) (Fig 1). The means (± SD) water parameters of the test medium were 27.45 ± 0.35 ^{0}c (Temperature), 6.33 ± 1.17 (pH), 0.1 ± 0.0mg/l (Nitrite), 3.9 ± 2.7mg/l (DO) and 0.0 ± 0.0mg/l (Ammiona). (Table 2). The mortality patterns of the species in the replicate were similar in the WSF of *Hevea brasiliensis* latex. (Table 3). The concentration of total hydrocarbon in the WSF was 2.196mg/l. Statistical analysis using ANOVA method showed that there was no significant difference ($p> 0.05$) in mortalities between the replicate. Organism that survive in the test medium to the end of the experiment were highly stressed as shown in their non-agile movements, compared to their counter-part in the controls which were all active and normal.

4.0 DISCUSSION

The percentage mortality of *C. gariepinus* in the water soluble fraction of the latex ranged from 0-100% in both batches A, and B at the end of 96 hours of test. No mortality was recorded in the 0 - 40 mg/l concentration of toxicant. However, 60 % mortality was recorded in the 50 mg/l concentration in each of the batches, while 100 % mortality was recorded in the 60 and 70 mg/l concentrations of toxicant. Between 0 -100 % mortality was reported in *Clarias gariepinus* juveniles exposed to varying lethal concentrations of detergent effluent with 0% mortality recorded in the control tanks A and B, 30 and 50 % mortalities in 0.01 mg/l, 40 and 80 % mortalities in 0.02 mg/l concentrations in A and B, 90 and 70 % mortalities in 0.03 mg/l concentration in A and B, 80 and 100 % mortalities in 0.04 mg/l concentration in A and B and 100 % mortalities each in 0.05 mg/l concentration in A and B in Ogundiran et al*.,* (2010) report.

In the present study, percentage mortalities were concentration-dependent. The higher the concentration, the higher the percentage mortalities. Similar report was presented by Ogundiran et al.,(2010) when investigating toxicological impact of detergent effluent in juveniles of African catfish *Clarias gariepinus*, Calta et al*.,* (2004) when studying acute toxicity of the synthetic pyrethroid deltamethrin to young minnow carp, *Cypinus carpio*, Ayotunde et al*.,* (2011) when investigating on the toxicity of *Carica papaya* on adult *Clarias gariepinus*, Ayuba and Ofojekwu, (2002) when investigating on the acute toxicity of the root of Jimson's weed, *Datura innoxia* to the African catfish *Clarias gariepinus* fingerlings, Adedeji et al*.,* (2008) when investigating acute toxicity of diazinion to African catfish *Clarias gariepinus.*

Clarias species generally are ecologically adapted to muddy environments in which temporary changes in water chemistry are more rapid and the contaminant concentration are usually higher (Koivisto, 1995; Ayotunde et al., 2011). Such environmental stress may facilitate tolerance to increase concentrations of contaminants (Ayotunde et al., 2011). This view may however not be supported by some contaminants or toxicants such as rubber latex which produced 100% mortality of the fish in 96 hours. Oh et al., (1991) gave three factors for the selective toxicity of toxicants for various fish species as: different inhibition of acetyl

cholinesterase, different detoxification and absorption. The above factors might have probably been responsible for the different toxic reaction showed by the fish in the different concentration and time during the period of experiment. The reaction are usually more pronounced at higher concentrations due to increased inhibition of acetyl cholinesterase (Oh et al., 1991) which eventually results in the death of the test organism (Adedeji et al., 2008; Ayotunde et al., 2011). The results of this work also agrees with the work of Ayuba and Ofojekwu (2002) when investigating acute toxicity of the root of Jimson's weed, *Datura innozia* to the African catfish, *Clarias gariepinus* fingerlings.

The 96 hours LC_{50} was 50.12 mg/L representing log concentration of 1.70 for both batches (batches A and B). The 96 hours LC_{50} of toxicants are known to vary with toxicant (Samabaswa & Rao, 1985; Arimoro, 2009; Ogundiran et al, 2010). For instance, Ogundiran et al .,(2010) reported 96hrs LC_{50} of 0.0166 mg/l and 0.0038 mg/l for batch A and B *Clarias gariepinus* juvenile under the toxicity effect of detergent effluent, A 96 hours LC_{50} of 0.1 mg/l and 0.03 mg/l was reported by Adewoye et al., (2010) when working on the effect of soap and detergent effluents on *Clarias gariepinus*. Again, Ayotunde et al., (2011) reported the 96 hours LC_{50} of 0.033 - 0.33mg/l on *Clarias gariepinus* adult using *Carica papaya* extract. The varied 96 hours LC_{50} values usua.lly obtained from different toxicants and test organisms is again reported by Ekanem et al., (2011), when they reported a 96 hours LC_{50} of 5.0 ± 1.76 and 4.0 ± 1.76 mg/l for *Macrobrachium macrobachion* and *M. vollenhovenii* using crude oil.

In this study the 96 hours LC_{50} of 50.12 mg/l obtained for the Batch A and B *Clarias gariepinus* might have depend on the ranges of the toxicant after series of preliminary tests which produced the concentrations finally used for the test.

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TABLE 1: Log-transformation of the toxicant on *C. gariepinus* for the determination of probit level of the toxicant at the end of experiment (96 hours)

		%M	
Toxicant conc. (mg/l)	Log values of concentration	Batch A	Batch B
0	0	0	0
30	1.48	0	0
40	1.60	0	0
50	1.70	60	60
60	1.78	100	100
70	1.85	100	100

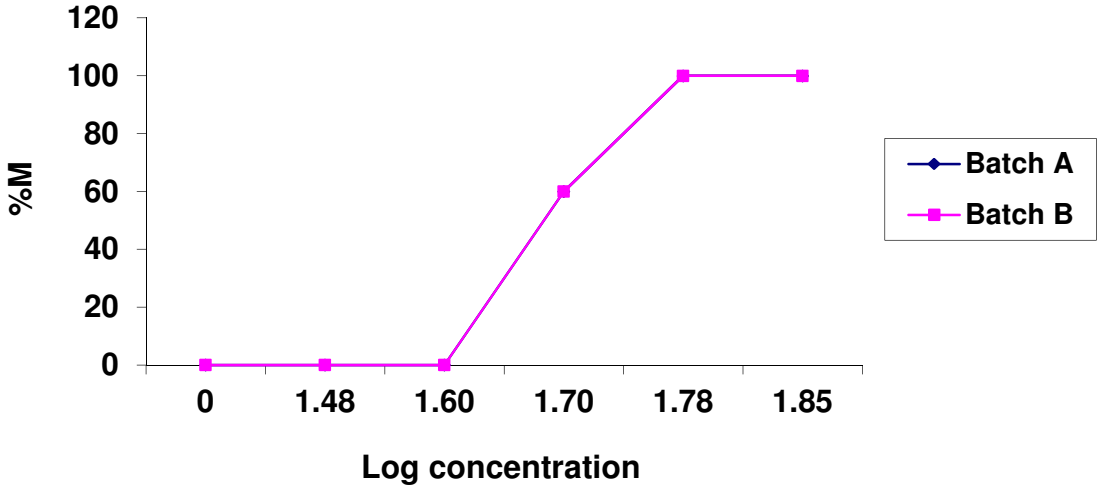

FIG. 1: Log-transformation of the toxicant on *C. gariepinus* for the determination of probit level of the toxicant at the end of experiment (96hrs)

Table 2: Means Water Parameter of Habitat Water Used for the Test.

Parameter	Min	Max	Mean	SD
Temperature (^{0}c)	27.1	27.8	27.45	0.35
pH	5.16	7.50	6.33	1.17
Nitrite (mg/l)	0.1	0.1	0.1	0.0
Dissolve oxygen (mgll)	1.2	6.6	3.9	2.7
Ammonia (mgll)	0.0	0.0	0.0	0.0

TABLE 3: Summary of the percentage mortality and survivors of *C. gariepinus* in the toxicant at the end of the experiment (96 hours)

Conc. of toxicant (mg/l)	Batch A				Batch B			
	Mortality M	% M	Survivors (S)	%S	Mortality M	% M	Survivors (S)	%S
0	0	0	10	100	0	0	10	100
30	0	0	10	100	0	0	10	100
40	0	0	10	100	0	0	10	100
50	6	60	4	40	6	60	4	40
60	10	100	0	0	0	100	0	0
70	10	100	0	0	10	100	0	0