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A Laboratory Bioassay of the Potential Effect of Rubber Extract (*Hevea brasiliensis*) on the Survival of Fingerlings of *Oreochromis niloticus*

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Abstract
The potential effects of *Hevea brasiliensis* on the survival of fingerlings of *Oreochromis niloticus* were investigated in duplicate (A and B) using the water soluble fraction of the latex under laboratory conditions for 96 hours. The WSF of *Hevea brasiliensis* was tested against *Oreochromis niloticus* at 0, 8, 16, 24, 32 and 40mg/l in glass aquaria stocked with ten animals for 96 hours under observation for changes. Behavioural pattern exhibited by the fish include, loss of balance, restlessness, attempt at jumping out and hemorrhaged gills, respiratory difficulties and mortalities were observed in the WSF exposure groups, but not in the controls. LC_{50} values were estimated at 28. 50 ± 0.2mg/l. There was significant difference in mortalities between the replicate group ($p < 0.05$), leading to conclusion that the organism in each batch responded differently to the toxic effect of WSF of *Hevea brasiliensis* latex.

1.0 INTRODUCTION

*Hevea l*atex is a biological product of complex composition. The basic component of a freshly tapped natural rubber latex, other than water which constitute about 22 to 48%, are dry rubber (20-45%), proteinous substance (1.5%), resinous substance (2%), carbohydrate 1%, inorganic matter 0.5% and other component (CHIN, 1979). The use of rubber is wide spread ranging from household to industrial products entering the production stream at the intermediate stage or as final product. Tires and tubes are the largest products of rubber, the remaining 44% are taken up by the general Rubber goods (GRG) sector, which include all products excepts tires and tubes. It is very pertinent to observe the Environmental problem associated with *Hevea brasiliensis* latex because of it wide spread usage.

In this study an attempt has been made to evaluate the potential effect of *Hevea brasiliensis* latex on *Oreochromis niloticus*. Since fish often respond to toxicants in a manner similar to higher invertebrates, they can be used to screen for chemical that have potential to cause tetratogenic and carcinogenic effects in humans. (Solbe, 1995). Test organisms to be used for acute toxicity test must be ecologically important, occupy trophic position leading to humans or other important species, and have adequate background biology, be widely distributed, be genetically stable, have it early stage (larvae, fry fingerlings and juveniles) available throughout the year and be sensitive (Ernest, 2004).

Tilapia (*Oreochromis niloticus*) is one of the most important freshwater finfish in aquaculture world. Among the numerous regions now inhabited by Tilapia, many are under threat from various pollutants, especially botanical pollutant. This fish species is commonly used in experimental work for its rusticity and good adaptation to the captivity conditions (Burkill, 1985). As a result of their great adaptability, high fecundity and rapid growth, they are used extensively for fish culture. This study intends to determine the toxic effects of *Hevea brasiliensis* latex on fingerlings of *Oreochromis niloticus*.

2.0 MATERIALS AND METHOD

2.1 Collection of Specimen

A total of 240 healthy *Oreochromis niloticus* fingerlings were used for this study. The fingerlings were 2.5-4.5cm in size. The fish were bought from the University of Calabar fish farm, Cross River State located within the University of Calabar at latitude $04^0$5, 020'N and longitude $008^0$20' 450' E respectively. (Asuquo and Bassey, 1999 and Akpan et al., 2002).

2.2 Acclimatization of Specimen

Oreochromis niloticus fingerlings were allowed to acclimatize to laboratory conditions for 24 hours in the stock tank. The test tank were aerated with air stone connected to electrically powered aquarium pumps to prevents accumulation of toxic wastes for the maintenance of the stocked and serial dilution for bioassay test, dechlorinated tap water was used.

2.3 PREPARATION OF TOXICANT SOLUTION

The water soluble fraction (WSF) of *Hevea brasiliensis* latex was obtained by vigorously shaking rubber extract with flittered habitat water in a separatory funnel. The system was allowed to stand for six hours to effects complete phase separation, after which the lower aqueous layer containing the WSF was collected for the toxicity test.

2.4 Stocking of Specimen

Oreochromis niloticus fingerlings of approximately the same size were gently caught using a hand net in order to avoid stress, into stock tanks measuring 25 X 10 X 15cm from an acclimatized tank.,. The stock tank was filled with 2 liters of dechlorinated water.

2.5 Monitoring of Specimen for Mortality

Test animals were taken as dead if failed to move their bodies. They float or sink into bottom when probed gently with a glass rod. During assessment for mortality each fish was removed from a test media with a pair of forceps, placed in a clean empty petri dish and recorded.

2.6 Preliminary Test

The concentration ranges chosen for the preliminary test of WSF of *Hevea brasiliensis* latex on *Oreochromis niloticus* were 0, 10, 20 ,30,40 and 50mg/l. Ten fish were randomly introduced into each of the reconstituted latex and each concentration was set in duplicate with control containing declorinated water without the addition of WSF of *Hevea brasiliensis* latex.

2.7 Definitive Test

The concentration ranges chosen for the WSF of *Hevea brasiliensis* latex for the toxicity test on *Oreochromis niloticus* after the preliminary tests were 0, 8, 16, 24, 32 and 40mg/l. The duration of the experiment was 96hours. After 96 hours the LC_{50} determination was caculated using a modified method (Finney 1971, Stephan, 1977). The fish were starved in order to minimize waste production. The distress behaviour and the deaths were closely monitored and recorded from the onset of the experiment 6h, 12h, 24h, 48h, 72h and 96h respectively. The initial water parameter and daily water parameter dissolved oxygen, temperature; pH, nitrite and ammonia were monitored 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).

2.8 Statistical Analysis

The number of deaths organism between control and experimental group were analyzed 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 (*Oreochromis niloticus*) showed pathological changes and mortalities. Sub-lethal changes observed were erratic swimming behaviour, restlessness, loss of balance, attempt at jumping out and haemorrhaged gills, respiratory difficulties and mortalities were observed in the WSF exposure groups but not in the control. *Oreochromis niloticus* was more sensitive to *Hevea brasliensis* contamination with 50% (Lc_{50}) at 28.50 ± 0.2mg/l after 96hrs exposure (table1, fig 1). The means (± SD) water parameters of the test medium were 30.45 ± 1.45 ^{0}c (temperature), 7.54 ± 0.78 (pH), 1.7 ± 1.6mg/l (Nitrite), 3.85 ± 2.65mg/l (DO) and 0.15 ± 0.15mg/l (Ammonia). (table 2). The mortality pattern of the species in the replicate varies in the WSF of *Hevea brasliensis* (table 3). Statistical analysis using ANOVA method showed that there was significant difference ($p<0.05$) in mortalities between 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 *O. niloticus* in the toxicant in this study ranged between 10 – 100 % in batch A and between 0 – 100 % in batch B. In batch A, 10 % mortality was recorded in the control with none in batch B. Normally, the control is not expected to have any mortality. But if such mortality occurs, it might have been attributed to stress on the organism. Udo et al., (2006) recorded 20 % mortality in the control experiment in batch B, when investigating toxicity of crude oil to early life stages of *Heterobrachus longifilis*, which they attributed to stress. They did not record any mortality in the batch A group of organisms in the control. Similar observation was reported by Stuermer et al., (1981) when working on the toxicity of Santa Barbara seep oil to starfish embryos.

The percentage mortalities shown by *Oreochromis niloticus* were generally observed to show variations in the same concentration of toxicant. For instance, in 16 mg/l concentration of toxicant, 60 % mortality was recorded in batch A with 40 % in batch B, and in 24 mg/l concentration of toxicant, similar percentage

mortalities as recorded in 16 mg/l concentration of toxicant in batches A and B were also observed showing similar percentage mortalities for different concentration. Similar observations were reported by Omotoso and Fagbenro (2005), when comparatively investigating the toxicity of three commercial detergents on the survival of the Nile tilapia, *Oreochromis niloticus*, Ural and Saglam (2005) when investigating on the acute toxicity of Pyrethroid deltamethrim on the fry of rainbow trout (*Oneorhyncus mylass*), Ayotunde et al*.,* (2011) when investigating the toxicity of *Carica papaya* on the haematological and piscicidal effect on adult catfish (*Clarias gariepinus*).

The mortality of organisms as a result of toxicants effect has been linked with the ability of the individual organism to withstand the toxicity of the toxicant, coupled with the ability of the organism to accumulate the toxicant for a longer time without the expression of death. (Ayuba and Ofojekwu, 2002; Cagauan et al., 2004; Udo et al., 2006), which of course is related to the concentration of the toxicant (Paris-Palacios et al., 2000; Ogundiran et al., 2010; Adewoye, 2010).

In the present study, it was generally observed that the percentage mortality of *O. niloticus* was not concentration-dependent as varied percentage mortalities were recorded in same concentration and time. This might have been due to the ability of each organism in each batch to respond differently to the concentration of toxicant with time as was previously reported by Omoregie et al*.,* (1990), Okwuosa and Omoregie (1995), Omoniyi et al*.,* (2002) when respectively studying the sub-lethal effects of Gammalin-20 and Actellic 25EC on *Oreochromis niloticus*, effects of lethal and sub-lethal concentrations of tobacco (*Nicotiana tobaccum*), leaf dust extract on weight and haematological changes in *Clarias gariepinus* and acute toxicity of Alkyl benzene sulphate on *Clarias gariepinus*.

The 96 hours LC_{50} obtained for *O. niloticus* was 24.55 mg/l for batch A individuals and 33.11 mg/l for batch B individuals. This may be attributed to the varying concentrations of the toxicants as previously reported by other authors (Samabaswa and Rao, 1985; Adewoye et al., 2010; Ayotunde et al., 2011). This situation is not uncommon in toxicity experiments as different organisms are known to respond differently to the different concentrations of toxicant with time.

The result of this study is however different from those of Wannee et al., (2002), Omotoso and Fagbenro (2005), Ayoola et al*.,* (2011), who independently reported 96 hours LC_{50} of 17.5, 17.1, 16.9 and 16.8 ppm respectively when investigating the toxic effects of roundup, a glyphosate herbicide on the Nile tilapia, *O. niloticus*, 12.04 ± 1.22 mg/l and 41.88 ± 10.81 mg/l when comparing the toxicity of three commercial detergents on the survival of the Nile tilapia *O. niloticus* and 96 hours LC_{50} of 2.65 mg/l and 0.19 mg/l when working on the acute toxicity of Nile tilapia (*O. niloticus*) juveniles exposed to aqueous and ethanolic extracts of ipomoea aquatica leaf.

There varying 96 hours LC_{50} values may not be unconnected with the use of different concentrations of same toxicant as was the case in the present study.

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TABLE 1: Log-transformation of the toxicant on *O. niloticus* for the determination of probit level of the toxicant at the end of experiment (96hrs)

		%M	
Toxicant conc. (mg/l)	Log values of concentration	Batch A	Batch B
0	0	10	0
8	0.90	40	50
16	1.20	60	50
24	1.38	60	70
32	1.51	70	90
40	1.60	100	100

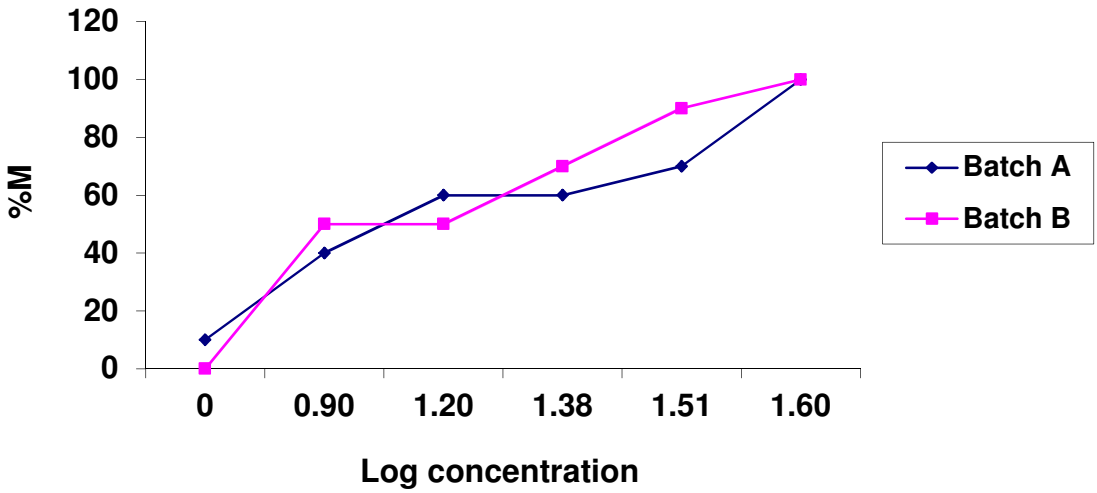

FIG 1: Log-transformation of the toxicant on *O. niloticus* for the determination of probit level of the toxicant at the end of experiment (96hrs)

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

Parameter	Min	Max	Mean	SD
Temperature (^{0}c)	29.0	31.9	30.45	1.45
pH	6.76	8.32	7.54	0.78
Nitrite (mg/l)	0.1	3.3	1.7	1.6
Dissolve oxygen (mgll)	1.2	6.5	3.85	2.65
Ammonia (mgll)	0.0	0.3	0.15	0.15

TABLE 3: Summary of the percentage mortality and survivors of *O. niloticus* 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	1	10	9	90	0	0	10	100
8	4	40	6	60	4	40	6	60
16	6	60	4	40	5	50	5	50
24	6	60	4	40	7	70	3	30
32	7	70	3	30	9	90	1	10
40	10	100	0	0	10	100	0	0