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Icthyotoxic assessment of water extract from fresh *Euphorbia heterophylla* (L) plant stem to *Barbus occidentalis* (Pisces: Cyprinidae) (Boulencer, 1920) fingerlings

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ABSTRACT: Icthyotoxic assessment of water extract from fresh *Euphorbia heterophylla* (Limnaeus) plant stem on *Barbus occidentalis* (Boulenger, 1920) fingerlings was conducted using static bioassay tests over a period of 96 hours. The range finding test was userd to determine the lethal concentration of the botanical on *B. occidentalis* and was found to induce varying behavioural response in the fish. The 96h median lethal concentration (LC_{50}) of 2.42g/l and safety level of 0.24g/l were determined for *B. occidentalis fingerlings exposed to water extract from fresh* Euphorbia heterophylla *(*Linnaeus) plant stem. A concentration dependent relationship was established for the effect of the toxicants on the test organisms. Percentage survival of the test organisms followed a regular pattern increasing with decreasing concentration. Prior to death of fish darkening of fish, erratic swimming and respiratory distress were observed.

Key words: *Euphorbia heterophylla*, *Barbus occidentalis,* Icthyotoxic assessment; Behavioural responses; Water extract.

Introduction

Fishermen, particularly sector of the fishing industry, in an attempt to maximize each indulge in the use of some poisonous chemicals and extract from plants in estuaries, creeks, lagoons and reservoirs that reduce the activeness of the fish. Icthyotoxic plants commonly used by fishermen include plants from families such as Acathcaceae, Anacardiaceae, Euphorbiaceae, Fabiaceae, Labiatecae, Solaneaceae etc.(Adewole, 2002). Many works have been carried out on the responses of fish to toxicants from some

plants and other obnoxious chemicals (Omoregie and Okpanachi, 1992; Omoregie and Ufodike, 1994; Ufodike and Omoregie, 1994; Wade et al., 2002 but the effect of *E. heterophylla* (L) toxic chemicals on fish has not been given much attention despite its economical and ornamental importance. This plant belongs to the family Euphorbiaceae which embraces about 7,000 species distributed all over the temperate and tropical world (Bradley, 1979) and produces milky irritating which contains some bioactive ingredients like diterpene together with aleuritolic acid, oleanolic acid, and betulin diacetate sesquiterpene-coumarin and a quinoid-type diterpenoid which negate physiological activities in fish (Madureira et al., 2004a and 2004b).

The test organmisms, *Barbus occidentalis* is an ornamental fish species of great economical value and belongs to the family *Cyprinidae*. It is qa common freshwater species, slivery black in colour and often tinged with yellow or pink and hardy (Reed et al., 1969). The choice of the species was based on its ecological and economic significance.

The primary objective of this research work is to establish the environmental consequence of the use of this Icthyotoxic plant (*E. heterophylla)*, in harvesting fish and to determine the 96 hour median lethal concentration (LC_{50} of the plant extract to the test organism.

Materials and Methods

Fingerlings of *B. occidentalis* with a mean weight and total length of 3.1g and 6.2cm respectively, were obtained from fish farm of the University of Ibadan. They were transported to the laboratory in plastic bucket containing cool water, in the morning before sunrise to prevent the fish from being stressed. The fishes were acclimatized in the laboratory by being held in tanks (30cm x 20cm x 15cm) containing clean dechlorinated water for seven days. The water in each tank was replaced thrice a week.

The fishes were fed with crushed pellets twice daily. The left over feed and faeces were siphoned off promptly and dying or dead fish were promptly removed to avoid contamination. The percentage of death recorded during acclimatization was less than 5% as such the fishes were accepted as being adapted to the laboratory conditions. Feeding was discontinued 24 hours prior to the commencement of the bioassay test (Ruparelia et al., 1990).

Specimen of the fresh stem of *E. heterophylla* was collected from a cassava farm near Oba reservoir in University of Ibadan. The test solutions were prepared prior to initiation of the experiment. The stems of *E. heterophylla* were pounded into a paste using mortal and pestle. 500g of fine particulate powders were dissolved in 2 litre-distilled water to warm temperature 23.3 ± 0.5°C for 24 hours. The extract was filtered using Whatman/E Filter Paper No. 1. Using a vacuum pump. The filtrate was freeze-dried and store in the refrigerator for use. A range finding test was conducted to determine the concentration to be used in the definitive tests. Test concentration in the range finding tests were selected at intervals based on logarithm ratio 0.00, 1, 0.01 and 0.1 (Parish, 1985). The fishes were distributed randomly at the rate of ten per tank using hand net. Six aquaria were used in all comprising five treatment aquaria and one control glass tank. Each tank contained five liters of water for each test concentration. Observations were made on the behaviour of the test organism at 1, 2, 4, 8, 16, 24, 48, 72, and 96 hours. Dead specimens were removed immediately death was confirmed. After the range finding test, some concentration were chosen for the definitive tests. A stock solution of the freeze extract was made and delivered into the experimental aquaria as 0.0mg/l (control), 1.2g/l, 1.8g/l, 2.4g/l, 3.0g/l and 3.6g/l concentrations. Eighteen aquaria of dimension 0.20m x 0.15m x 0.10m were used. The fishes were distributed randomly at the rate of ten per tank to each of the five concentrations in triplicates using hand net. The toxicant solutions and test water were renewed after 48 hours in each bioassay. Water characteristics were monitored after 24 hours using methods described by APHA (1985). Test aquaria were examined for fish mortality on a daily basis. Fish were confirmed dead when they showed no response to touch by glass rod. Dead specimens were removed immediately and recorded. The 96h LC_{50} for each extract was determined as summary of percentage mortality data as a probit analysis using arithmetic method. The lower and upper confidence of limits of the LC_{50} was determined as described by UNEP (1988). Observations were made on the behavioural response of the test organism at 24, 48, 72, and 96 hours. Results were subjected to statistical analysis using SPSS10 Windows 2000 to test for the significant difference (P!”!#$%&’0”*%(+’%./012% concentrations of *E. heterophylla*.

Results

The water quality parameters within the treatment tanks as shown in Table 1 did not vary significantly ($P \geq 0.05$) to what is obtained from the control. The mean water quality values for temperature were 24.12±0.27^{o}C, dissolved oxygen was 6.36±0.14mg/l, pH was 6.95±0.03, Total alkalinity 30.18±0.39mg/l and free carbon dioxide is 4.84±0.01mg/l. At concentration of 3.6g/L, 3.6g/L, 3.0g/L and 2.4g/L fishes were observed to swim actively at the bottom, came up to the surface of the water to gasp for air, there was erratic swimming behaviour, spiral uncoordinated movement.

Table 1: Water Quality Parameters During the Acute Bioassay Exposure of *B. occidentalis* to *E. heterophylla*.

Parameters	**Control 0.0(mg/l)**	**1.2g/l**	**1.8g/l**	**2.4g/l**	**3.0g/l**	**3.6g/l**	**Mean**
Mean Temp. (^{0}C)/SEM	23.0±1.5	25.0±0.5	24±1.0	24.4±1.5	24±1.5	24.2±1.1	24.12±0.27
Mean dissolved oxygen mg/l/SEM	5.95±0.8	6.02±0.04	6.27±0.06	6.45±0.05	6.67±0.06	6.82±0.06	6.36±0.14
Mean pH/SEM	6.84±0.01	6.92±0.03	6.91±0.04	7.00±0.01	7.02±0.03	7.02±0.04	6.95±0.03
Mean Free Carbon-dioxide mg/l/SEM	4.88±0.02	4.86±0.02	4.83±0.01	4.80±0.01	4.79±0.01	4.86±0.01	4.84±0.01
Mean Total Alkalinity mg/l/SEM	29.15±1.55	29.20±1.85	29.80±1.75	30.40±2.10	31.05±1.85	31.45±1.65	30.18±0.39

Table 2: Mortality rate of *B. occidentalis* fingerlings exposed to acute concentrations of *E. heterophylla* (L.) water extract.

Conc. g/l	**Log Conc. g/l**	**Mean Mortality (%)**				**96h corrected mortality**	**Survival**	**Probit**
		24h	**48h**	**72h**	**96h**			
0.0	0.0000	0.0	0.0	0.0	0.0	0.0	100.0	-
1.2	0.0792	3.3	3.3	3.3	3.3	3.3	96.7	3.12
1.8	0.2553	10.0	20.0	20.0	23.3	23.3	76.7	4.26
2.4	0.3802	23.3	33.3	40.0	53.3	53.3	46.7	5.06
3.0	0.4771	53.3	63.3	63.3	70.0	70.0	30.0	5.52
3.6	0.5563	70.0	73.3	83.3	86.7	86.7	13.3	5.13

In the definitive test, at concentration 3.6g/l, 87% mortality was recorded at the 96 hour exposure time. While at concentration of 3.0g/l and 2.4g/l, 70% and 53% mortality were recorded respectively. The toxicity of the plant varied with concentration and time of exposure of test organisms. In the control group no mortality was recorded (Table 2). The 96h LC_{50} was determined using probit values and regression analysis 2.42g/l (Figure 1) with a safety concentration of 0.24g/l for the exposed organism. At any concentration of the icthyotoxic plant, the regression equation Y = 8.2803log conc. X + 1.4359 and r = 0.7805 will produced a projected mortality.

During each bioassay, colour changes were observed in the test solution. A light green coloration was observed immediately the test solution was introduced into the experimental tanks with water. The intensity of the colouration was also observed to decrease at about 48 hour after the start of the bioassay test and precipitates of the plant were observed to settle at the bottom of the tank experimental tank. There was production of foul odour from the test solutions, which increase with increasing concentration. The intensity of colouration was observed to decrease with decreasing concentration of the test solution.

The 96h LC_{16} and LC_{84} were also determined using probit method as 1.88g/l and 2.82g/l respectively. 2.82 remains the MATC.

Discussion

The Icthyotoxic potential and phytotoxic properties of plants extracts have been reported by many authors (Reed, et al., 1969, Ufodike and Omerege, 1994 and Adewole et al., 2002). Various forms of abnormal behaviours were observed in *B. occidentalis* when exposed to different concentrations of *E. heterophylla*. These include erratic swimming, occasional darting of fish up and down the water column, aggregation of fishes below the water surface gasping for breath and change in colour from slivery black to pale colour. Similar behavioural response was reported by Chlayvareesalla et al., (1997), for *Clarias* and *Puntius gonionotus*, when exposed to different concentration of piscicidal plant (*Maesa ramenticae*). Wade et al., (2002) also reported similar behavioural response for *Oreochromis niloticus* when exposed different concentration of cassava effluent under laboratory conditions.

The behavioural response of the test organism was observed to be close dependent reducing with decreasing concentration. Chin et al., (1987) also observed similar response in *Penaeus monodon* when excposed to different concentrations of ammonia. Adeogun (1994) reported similar response *O. niloticus* and *Clarias gariepinus* due to the effect of *Raphia hookeri*. Loss of balance and direction in the test organisms, uncoordinated spiral movement and occasional jumping which are exhibited to a nervous reaction of the test organism to the toxicant (Adeogun, 1994). The stressful breathing behaviour exhibited by the fish may be as result of respiratory impairment due to effect of toxicant on the gills and the inability of the gills surface to actively carry out gaseous exchange might be responsible for the recorded mortalities which is shown to be significantly different (P>0.05) and directly proportional to the exposure concentration and period. The increase in mortality reported in this work as a result of increase in the concentrations of the plant extract was due to the impairment of normal metabolism by the inhibitory parents in the extract which could produce digitalis-like action that may result into arrhythmia, loss of normal heart beat rhythm, leading to serious disorder and severe cases may be fatal resulting to death (Oti, 2003).

The various colour changes observed in experimental tanks with different concentrations of fresh *E. heterophylla* is concentration dependent and this is not far from the observation made by Oti (2003) which was also reported as having effect on the skin coloration of the fish exposed to such icthyotoxic plants. This means that such plant will not only be harmful in causing mortality but will negatively affect the chromatophores of the surviving fish. The darkening patches on the skin is as a result of the dispersion response of the melanin pigments in the chromatophores which move towards the periphery, by pituary hormone intervention also known as melanocyte stimulating hormone (MSH) (Oti, 2003). It has been stated earlier by Novales (1959) that MSH is the most important pigment movement determinant factor within the chromatophores. So this experiment has been able to put up a postulate that chemicals present in the *E. heterophylla* like others toxicants constituents affects dispersion and as well induces melanocyte stimulating hormones, since dark patches and other changes in colourations were only observed on fish in the treatments group and not dependent on the variation of the concentration of the extract (toxicants). Emission of strong foul odour from the best solution by the end of the 96h test may be attributed to oxygen depletion and death of fish.

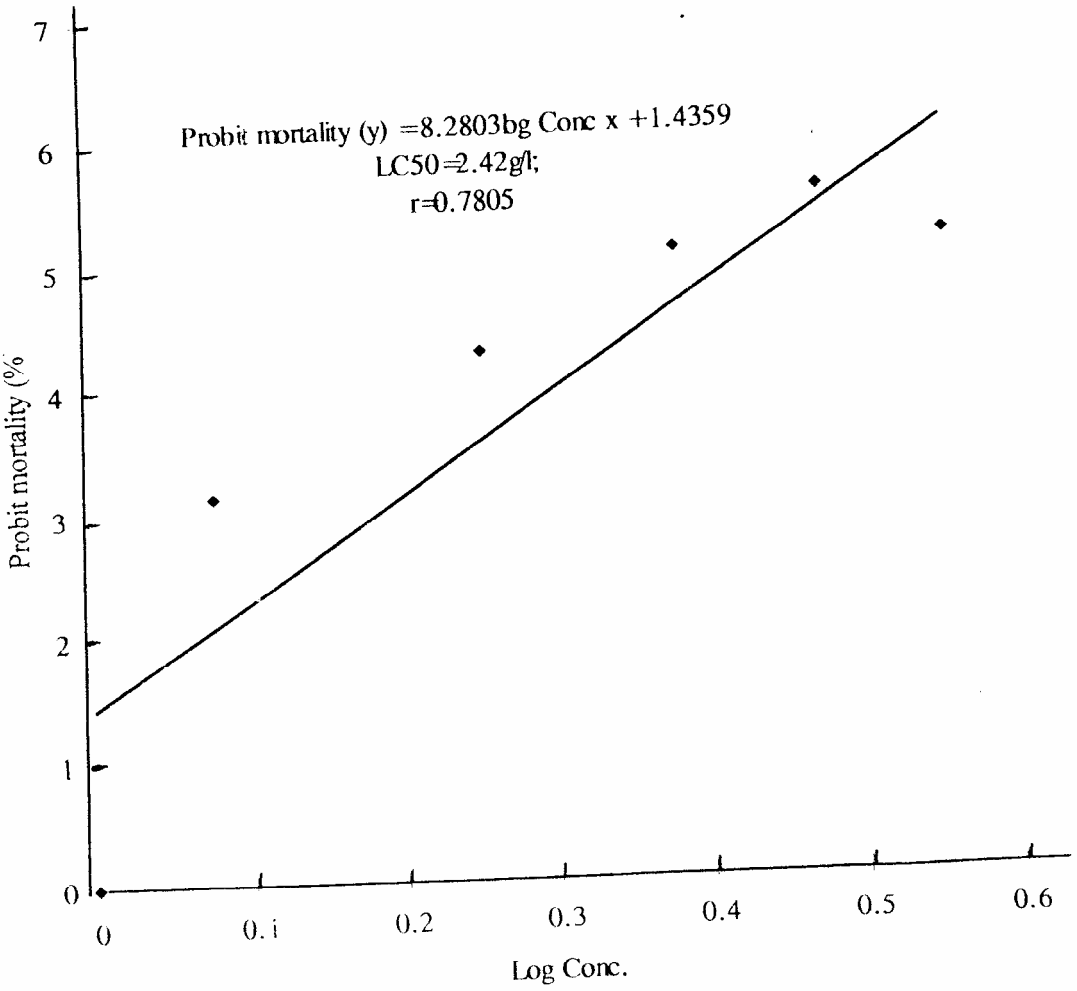

Figure 1. Probit mortality of *B. occidentalis* exposed to water exctract of fresh *E. heterophylla*

The 96h LC_{50} lower and upper limit for the toxicity of the toxicant to the test organism were found to overlap there making the 96hrs LC_{50} valid (Reish and Oshida, 1986). The confidence limits for the 96hrs LC_{50s} were also found to overlap, which implies that the 96hrs LC_{50s} are statistically insignificant (P3!"!#$). Based on the 96h-$LC_{50}$ (2.48g/l) and an empirical applicable factor of 0.1 (Sprague, 1971), the safety level was 0.25g/l water extract of ***E. heterophylla*** concentrations on ***B. occidentalis***. The threshold concentration that produces statistically significant deleterious effect as seen in Probit mortality (Table 2, and Figure 1) is commonly expressed as the maximum acceptable toxicant concentration (Wickins, 1976).

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