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Phytochemical Screening and Lethal toxicity of *Balanite aegyptiaca* bark (aqueous extract) on juvenile of *Clarias gariepinus.*(Burchell, 1822)

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Abstract

Plant with poison effect have widely been identified for a number of years, hence, there is need for the determination of a safer concentration, highest and lowest since some of this plants are used in rivers to ease catching of fish. The qualitative and quantitative analyses of the aqueous extract were conducted. This research assessed the lethal toxicity effect of aqueous extract of B. aegyptiaca on C. gariepinus juveniles. C. gariepinus were exposed to the acute concentrations of aqueous extract of the bark of B. aegyptiaca to determine the 96h-LC_{50}.The acute toxicity of water extract from dry B. aegyptiaca plant bark was conducted using the static renewal bioassay technique. The lethal concentration in which Ten (10) Juvinile fish were stocked in 50cm x 50cm x 95cm glass tank for 96hr. at concentration of 0.0g/L, 0.02g/L, 0.04g/L, 0.06g/L and 0.08g/L. The result of the experiment shows that saponin, tannins, glycoside and anthraquinones are found present in the plant extract, while steriod is absent.nd absent. The 96h-LC_{50} for C. gariepinus was estimated to be 0.069g/l and physiological response of the test fish was highest at 0.08g/l with lowest at 0.02g/l. The results of the study showed the toxicity effect of B. aegyptiaca on C.gariepinus juveniles. The safety level of 0.069g/l can be recommended to be applied in water body for the purpose of catching fish and also because it is biodegradable and leave no adverse effect on the environment.

Keywords: Balanite aegyptiaca; Clarias gariepinus; phytochemical; and lethal toxicity

Introduction

Piscicidal plants have been observed to increase the opercular ventilation rate of fish as their concentration increases (Dan-Ologe and Sogbesan, 2007). The importance of some of these piscicidal plants is unique in the sense that their chemical compositions enhanced their properties as medicinal plants, preservatives and insecticides. Strong concentration than necessary of these toxic plants are used by the fishermen which could lead to physiological disturbance of the aquatic organisms and ultimately reduction in aquatic productivity (Mondal *et al.*, 2007). Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscidal which are not likely to have residual effect on the organisms since most of the bioactive chemicals arc biodegradable (Stalin *et al., 2008).*

*Balanite aegyptiaca*also known as the Desert date in English, 'dattier du desert' in French, 'heglig' in Arabic, 'mjunju' in Swahili, 'tanni' in Fulfulde, 'adua' in Hausa and 'cungo' in Kanuri. Although found almost everywhere in the continent, very high concentrations of the tree are most prevalent in Sahel and Sudan savanna zones of West Africa and semi-arid regions of East Africa (NRC, 2008). Two accessions of the tree with fruit, nut and kernel shapes that corroborate the findings are common in North Eastern Nigeria. Every part of *Balanite aegyptiaca* tree has economic importance. Its roots and bark are pounded and dipped in rivers for fishing, the wood as yoke for draught animals and hand implements, while humans eat the leaves and flesh of the ripe fruit because they are very rich in carbohydrates and vitamins (Stalin *et al., 2008).* The research was conducted to assess the toxicity effect of *B.aegyptiaca* extract on juvenile of *Clarias gariepinus* and the composition was also determined.

Materials and Methods

Pilot Study

Pilot studies were carried out to determine the maximum concentration that did not produce death and the minimum concentration that produced 100% death. In between these concentration values were selected for acute toxicity studies. The varied

concentrations were chosen to prepare the stock solutions (Babatunde, 1997).

Sample Collection and Preparation

The bark of *Balanite aegyptiaca* was obtained from Sangere, Modibbo Adama University of Technology Yola and dried in shade for three days. The dried bark was then crush into powdered form using pestle and mortal and sieved using a 0.1mm sieved.

Extraction procedure of Balanite aegyptiaca

Two hundred grames of the fine powder was soaked in 1litre distilled water and was allowed to stand in this condition for two days with occasional concentrated by drying in an oven at 40^0C for one week to obtain a pest (Stalin *et al., 2008).*

Preparation of Stock Solution

The following stock solutions were prepared from the extract 0.00g/L, as control, 0.02g/L, 0.04g/L, 0.06g/L, and 0.08g/L which was introduce into each of the tanks in duplicate for the exposure of *C gariepinus* in accordance with the criteria set by the American Society for Testing and Materials (ASTM, 2007; Babatunde, 1997).

Experimental Design

A Complete randomized design was used. Ten tanks with dimension 30cm X 30cm X 45cm were set up. The bioasssay was conducted in duplicate.

Fish Sample Collection

200 pieces Juveniles of *Clarias gariepinus* with average length of 6.0± 21cm and weight of 9.20± 13g, body weights were obtained locally from Divine Fish Farm, Yola were taken to the Fisheries Laboratory of Modibbo Adama University of Technology, Yola in polyethine bag in the morning before sun rise to prevent rapid change in temperature. The fishes were held in the laboratory for one week prior to the commencement of the experiment. During this period, three quarters of the test water was changed daily by siphoning. The fishes were fed with 3mm multi feed Pellet thrice daily. The uneaten feed were siphoned out to avoid contaminating the water. The Tanks were checked daily for fish mortality at time intervals as recommended by Sprague (1971) and dead fish removed and recorded.

Acute Toxicity Test

Acute toxicity test was performed according to the OEC guideline No. 203 for semi-static tests (APHA, 1985). Fish was exposed to range of balanite concentration for 96 Hours. Mortality was recorded at 12, 24, 48 and 96 Hours. The lethal concentration causing 50% mortality (LC_{50}) was calculated by the Litchfield. According to this criteria, Concentrations of the test compound used in short term definitive tests were between the highest concentration at which there was 0% mortality and the lowest concentration at which there was 100% mortality. For the 96hr-acute toxicity test, the concentrations of the working solution used are: 0.00g/L, 0.02g/L, 0.04g/L, 0.06g/L, and 0.08g/L. Observation were made on the behavioural response of the test organism and the result were subjected to statistical analysis using SPSS10 Window2000 and the significant different ($p<0.05$).

Results

Mortality occurred in all the experimental tanks except in the 0.00g/L tank which had 0% mortality rate (Table 1). The first mortalities of *C. gariepinus* were observed in 12 hrs in tanks containing 0.02g/L with mortality value of 1; followed by 48 hrs with a mortality value of 2, and 96hrs recorded a value of 3 resulting into a total value of 6 (60% mortality rate). Concentration of 0.04g/L had 70% mortality rate. This was recorded from the 48hrs, 72hrs, and 96 hrs respectively with a corresponding mortality value of 1, 2, and 4 totalling 7. The total value of 8 was observed at the concentration of 0.06g/L, this resulted to the mortality value of 2 observed at 12hrs, 48hrs (2), 72hrs (4) and 96hrs (0). At 0.08g/L mortalities were recorded at the 12hrs (7), and 48hrs (3) only while no mortality was recorded in 72 and 96 hrs respectively. The highest percentage mortality of 100 was recorded in 0.08g/L while the lowest of 0% was recorded in the control set-up.

The presence of phytochemicals in the bark of *Balanite aegyptiaca* observed in this study confirmed qualitatively the presence of saponins, tannins, glycoside, steroids, and anthraquinones while flavonoids were shown to be absent as in the Table 2 below. The presence of these phytochemicals are classified as moderate (++) and highly present (+++).

Table 2 shows the quantitative and qualitative of the phytochemical screening of the bark of *Balanite aegyptiaca* isolates used for this study. Saponin is highly present 0.06mg/L; tannin 0.046mg/L, glycosides 0.044mg/L. and Anthraquinones0.05mg/L are moderately present while steroids and flavonoids were absent.

Table 1: Percentage Mortality of C. gariepinus Juvenile exposed to different concentration of *B. Aegyptiaca*

Duration concentration	Log Concentration	12Hr	48Hr	72Hr	96Hr	Total	%Mortality	Probit
0.00g/L	0.00	0	0	0	0	0	0	0
0.02g/L	-1.6989	1	2	0	3	6	60	5.25
0.04g/L	-1.3979	0	1	2	4	7	70	5.52
0.06g/L	-1.2218	2	2	4	0	8	80	5.84
0.08g/l	-1.0969	7	3	0	0	10	100	8.09

Table 2: Quantitative and Qualitative Screening of Phytochemical of the bark of *B. aegyptiaca*

Test	Qualitative	Quantity Per 0.5g Extract	Percentage (%)
Saponin:	+++	6mg	1.2
Tannins:	++	4.5mg	0.9
Glycoside:	++	5mg	1.0
Anthraquinones	++	4.4mg	0.88
Steroid	---	NO	NO
Flavonoid	---	NO	NO

Key

High present +++

Moderate ++

Absent --

Not detected NO

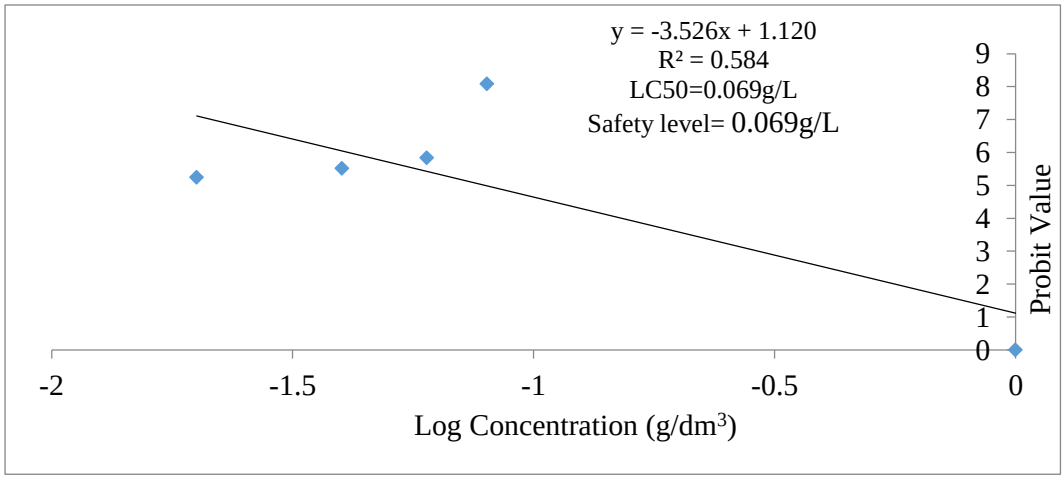

Figure 1: Mortality of *Clarias gariepinus* Exposed to Varying Concentration of *Balanite aegyptiaca*

Discussion

Fish response negatively with increase in the concentration of extract of *B. aegyptiaca* (Hall and Walker, 1991), however, the following physiological response were at increase from lower concentration of 0.02g/L to tank with highest concentration of 0.08g/L. increase in the concentration of *balanite aegyptiaca* extract also can give rise to a series of morphological 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 and spiral swimming with head up with excess mucus secretion .The percentage mortality was recorded highest in 0.08g/l (100 %) and lowest in 0.02g/l (60 %) after 96hr of exposure. The mortality rate increase with increase in the concentration of the extract. Hence, No mortality was recorded in the control experiment throughout the experimental period

The presence of phytochemicals in the bark of *Balanite aegyptiaca* observed in this study confirmed the presence of saponin which was observed to be higher , followed by Glycoside ,then tannin also and lastly Anthraquinones .while steroid and flavonoid were found to be absent. This agrees with (Fasola, 2000).

The quantitative estimation of phytochemical (bark) of *Balanite aegyptiaca* (Aqueous extract) revealed the presence of active phytochemical constituents. The phytochemical active compounds of *B. Aegyptiaca* were qualitative and quantitatively analysed and the results are mentioned in Table 1 and Table 2 respectively. The quantitative estimation of primary metabolites revealed that the various phytochemical constituents present in the plant extract (Table-2). In the bark sample are: Saponin 1.2%. Tanning 0.9%. Glycoside 1.0%. Anthraquiones 0.88%. While Steroid and Flavonoid were absent. This agrees with the result of Emad *et al.* (2012).

Conclusion

The Study has demonstrated that exposure of *C. gariepinus* juveniles to the aqueous extract of *B. aegyptiaca* resulted in organ impairment in catch fish. Hence the resultant damage been done by such extract as well as increases in mortality which increased with increase in concentration of the extract.

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