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Effects of *Balanites aegyptiaca* on tadpoles and *Oreochromis niloticus*

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ABSTRACT
Objective: Fingerlings of *Oreochromis niloticus* and tadpoles were exposed to the acute concentrations of aqueous extract of the bark of *Balanites aegyptiaca* (date palm desert) to determine the 96h-LC_{50} (lethal concentration 50, concentration that will kill half of the test animals exposed).
Methodology and results: The *O. niloticus* (Nile tilapia) was further subjected to sub-lethal concentrations of the plant extract to determine the effect on growth and haematological indices. The time for toxicity disappearance (TTD) was also estimated. The experiments were conducted using the static renewal bioassay technique at UNAAB Fish Laboratory. The 96h-LC_{50} for *O. niloticus* was estimated to be 26.22 mg l^{-1} while that of Tadpole (*Rana species*) was13.77 mg l^{-1}. The TTD of *B. aegyptiaca* was estimated to be 48 hours. During sub-lethal exposure, there was a slight decrease in the mean body weight and all the haematological parameters as the concentration of the toxicant increased except MCV, MCH, MHC that were more or less equal. However, statistical analysis on these values of haematological indices did not show any significant difference at 5%. The use of *Balanites aegyptiaca* is recommended in control of predators e.g. tadpoles in pond fish culture system because the product is non-toxic to fish at lower concentration, biodegradable and very cheap. The uncontrolled use in open water body for fishing should be prohibited, as the resultant deleterious effects will subsequently lead to death of not only target fish but also other aquatic organism. Hence, contribute to reduction in the biodiversity.
Conclusion and application of results: The results of this study show the toxic effect of *B. aegyptiaca* on *O. niloticus* and Tadpoles. The lethal doses (96h-LC50) obtained are 26.22 mg l^{-1} and 13.80 mg l^{-1} respectively. Therefore, *B. aegyptiaca* powder can be use in selective eradication of aquatic organism to control unwanted predatory species i.e. tadpoles. *B. aegyptiaca* bark powder can be recommended because it is biodegradable and leave no adverse effect on environment.
Keys words: *Balanites aegyptiaca, Oreochromis niloticus, Tadpoles, haematological parameters*

INTRODUCTION

Fish in Niger is a luxury product, especially the big Tilapia (*O. niloticus*) and Nile perch (*Lates niloticus*), because only wealthy citizens have access to this costly product in Niamey (Niger). The consumption (per caput) of fresh fish in Niger was 2.79 kg/inhabitant/m in 1990 and decrease to 1.13 kg/inhabitant/m in 1993 (Bouari, 1995). While the word capture fisheries and aquaculture were of 122 million metric tons in 1997 and decrease to 117 million of tons in 1999 (FAO, 2000). In Niger Republic however, the national fish production decrease from 1600 tons in 1986 to 975 in 1995 (Bouari, 1995). This decrease was due to low aquaculture production and over exploitation of the resource by the use of unorthodox fishing methods. The most commonly used unorthodox method is the application of *Balanites aegyptiaca* (Del., 1813) (date palm desert) powder along the River Niger, which is the unique permanent river that traverses the country with a length of over 550 km. It has toxic properties, which has been found to be useful in the control of intermediate host of some diseases that are waterborne, for example, snail, etc. (Maydell, 1991). The toxicity of the plant extract on fish and some other aquatic organisms has been reported by many authors (Beal & Anderson, 1993; Saha & Kaviraj, 1996; Anguigwo, 1998; Ofojekwu *et al.*, 1989; Agbon *et al.*, 2002). The effect of bark extract of *Azadirachta indica* (A. Juss, 1830) (Neem) on *Tilapia zillii* were investigated by Omoregie & Okpanachi (1997). Agbon *et al.* (2002) studied the effect of Tobacco leaves (*Nicotiana tabacum*, L. 1753) on *O. Niloticus*. The toxicity of *B. aegyptiaca* and *Kigelia africana* (Lam.) Benth., 1849, on *O. niloticus* was investigated by Ufodike & Omoregie (1994). The need therefore arises to investigate the effects of the bark of *B. aegyptiaca* alone, on *O. niloticus* and one of its fry predators (Tadpoles), with a view of using *B. aegyptiaca* as a biocide for the control of the predators in culture systems. *Oreochromis niloticus* is one of the most popular culture fish species in many tropical countries (Yi, 1999) such as Niger Republic (Ibrahim, 2002). The objective of this study is to evaluate the toxic effect of *Balanites aegyptiaca* on Tadpoles and *Oreochromis niloticus*.

MATERIAL AND METHODS

Experimental fish: The fish were obtained from the reservoir of the department of aquaculture and fisheries management and taken to the outdoor fibreglass tank of 2 m x 1m x 0.5 m dimension in the laboratory for acclimatization. Ten aquaria of 60 cm x 30 cm x 30 cm dimension were prepared indoor for the experiment. The fingerlings of *O. niloticus* of mean weight and standard length of 5.38 ± 4.32 g and 5.32 ± 1.23 cm respectively were seined and transported to the department early in the morning. Mortality recorded was less than 1% and the dead fish were removed immediately. The acclimatized lasted for more ten (10) days into the fibre tank during which they were fed with compounded diet of 35% crude protein at 3% of the biomes, three times a day.

Experimental *Tadpoles:* The tadpoles were collected from a pool of water in the gutter dug round the wildlife domestication unit of department of forestry and wildlife management of University of Agriculture, Abeokuta (UNAAB). The length of the tadpoles was between 16-30 mm.

Preparation of experimental material: Bark sample of *B. aegyptiaca* were collected from Niger Republic at the lift side of Niger River bank at Niamey level. The sample were collected with a machete and sun dried for two days before packaging for transport to Abeokuta, Nigeria. The sample was oven dried to constant weight for three days at a constant temperature of 60°C. The sample was pounded, in a wooden mortar, and sieved with a 1 mm mesh size sieve. The powdered samples were then wrapped, into nylon bag, and stored at room temperature in airtight container. An electric weighing balance (model: Mettler 60 KB) was used to determine the weight. The wrapped samples were packaged in units weights of 10 g, 5 g, 1 g and 0.5 g respectively.

Acute toxicity determination: A stock solution of the sample was prepared by putting 10 g of the powder in 1 litre of deionized water and allowed to stand for 24 hours at room temperature. 50 ml of this stock solution was added to 200 ml of declorinated, well-aerated tap water to determine the toxicity of the extract on the fish (FAO, 1982). Trial and error method were used to determine, the highest concentration, at which the acute toxicity test will start. The direct application of the powder into the water was found to be more reliable and potent hence, this method was adopted. A static renewable bioassay method was adopted for this study.

Acute toxicity determination in O. niloticus: Eight (8) aquaria were randomly labelled and neatly arranged on a stack in the laboratory. They were filled with 20 l of dechlorinated, well-aerated municipal tap water. Ten (10) fish of a mean total length, standard length and weight of 7.80 cm, 5.32 cm ± 1.20 and 5.38 g ± 4.32 respectively, were collected from the fibre glass holding tank and put into each aquarium. The fish were acclimatized for 10 days and fed at 3% of their biomass, three times daily prior to exposure. The following concentrations of *B. aegyptiaca* powder 25 mg l^{-1} ; 27.5 mg l^{-1}; 30 mg l^{-1}, and a control (that is 0.50 g in 20 l, 0.55 g in 20 l, 0.60 g in 20 l, and 0.00 g in 20 l) representing treatments one, two, three and four respectively were replicated. The toxicant was applied each day after changing the water. To determine the 96 h LC_{50}, the experiments were conducted for 4 days.

Acute toxicity determination in Tadpoles: Four treatments were used with the following concentrations: 12.5 mg l^{-1}, 15.00 mg l^{-1}, 17.50 mg l^{-1} and control. To determine the 96 h LC_{50}, the experiment was also conducted for 4 days. The tadpoles used were about 4 days to 9 weeks old or 16 to 30 mm long.

Determination of time for toxicity disappearance (TTD); Four treatments were prepared for both fish (*O. niloticus*) and tadpoles with a concentration of 30, 40, 50 mg l^{-1} and control. The treatments were exposed for 4 days. The samples of *B. aegyptiaca* bark powder were put at approximately the same time. 10 fish and 10 tadpoles were introduced each day. The dead animals were removed the following day, others batch of fish, and tadpoles were introduced in the same toxic water.

Sub-lethal exposure: For the sub-lethal test, after determination of 96 hr LC_{50} and time disappearance of the toxicant, three treatment of concentrations of 15 mg l^{-1} (0.30 g/20 l), 20 mg l^{-1} (40 g/20 l) and control (0.00 mg l^{-1}) were prepared in 20 l of in glass aquaria labelled A, B and C respectively. 10 fingerlings of average weight of 10.25 g (+ 1.25), 10.20 g (+6.17) and 9.28 g (+1.30) representing A, B, and C respectively, were introduced into each aquarium. The water was changed every two days and the treatment of *B. aegyptiaca* bark powder was renewed. This exercise continued for 5 weeks (35 days). During the exposure period, the temperature, the pH, dissolved oxygen and conductivity of the water were measured using standard laboratory methods. The fish were weighed weekly during this period of sub-lethal exposure.

Haematological analysis: At the end of 35 days of sub-lethal exposure, 5 fingerlings representing five replicates were picked from each aquarium for haematological test. The fish will be labelled A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, C1, C2, C3, C4, C5 representing specimen from the experimental media A, B, C respectively. These fish were sacrificed and blood collected with aid of a heparinized micro capillary tubes. The fish were killed with a blade razor by cutting the head region above the operculum. The haemoglobin (Hb); packed cell volume (PCV), red blood cell (RBC); mean cell volume (MCV), mean cell haemoglobin (MCHC); total protein (TP) and glucose were analysed using various appropriate laboratory methods as described by Blaxhall & Daisel (1973).

Analysis; The lethal concentration (LC50) at 96 hrs was computed using the probit analysis (Ikele *et al.*, 2011). The mean values of the five fish taken from media A and C and three fish from B were recorded for statistical analysis of variance (ANOVA) at 0.05 level of probability.

RESULTS AND DISCUSSION

Effect of *Balanites aegyptiaca* on *O. niloticus:* The acute lethal concentration after 96 hours showed that 15%, 80% and 100% mortality occurred at concentration of 25 mg l^{-1}, 27.5 mg l^{-1} and 30 mg l^{-1} respectively representing the treatment T_1, T_2, T_3. No mortality was recorded in the control (treatment 4) (Table 1).

Table 1: Mortality of *O. niloticus* fingerlings on 24 – 96 hours exposure to varied concentrations of *B. aegyptiaca* (hs = hours, R = replicate)

Concentration of *B. aegyptiaca*	Log concentration	Treatment	Exposure time (h)				Total mortality	% of mortality
			24 hs	*48 hs*	*72 hs*	*96 hs*		
25 mg l^{-1}	1.39	R_1	0	1	0	1	2	15
		R_2	0	1	0	0	1	
27.5 mg l^{-1}	1.43	R_1	7	1	0	0	8	80
		R_2	5	3	0	0	8	
30 mg l^{-1}	1.47	R_1	6	3	1	0	10	100
		R_2	8	2	0	0	10	
Control (00 mg l^{-1})	0	R_1	0	0	0	0	0	0
		R_2	0	0	0	0	0	

The highest mortality was recorded in the 30 mg l^{-1} concentration within the first 24 hours. However, no mortality was recorded in the control during the 96 hours test period. The simple arithmetic mean was used to determine the percentage of mortality of the *O. niloticus*. Using probit table, percentage of probit was also known and these were used to establish the LC_{50} of the treatment effect on *O. niloticus*. These were simply done by plotting percentage probit against logarithm of concentration (mg l^{-1}) and the results of 96 hr LC_{50} were determined to be 26.22 mg l^{-1}.

Effect of *Balanites aegyptiaca* on Tadpoles: The acute lethal concentration (LC_{50}) toxicity test carried out on tadpoles for 96 hours showed that 50%, 60%, 70%, 100% of mortality at concentration of 12.5 mg l^{-1}, 15.00 mg l^{-1}, 17.50 mg l^{-1} and 20 mg l^{-1} respectively representing the treatment T_1, T_2, T_3, T_4 respectively. No mortality was recorded in the control (T5) and the treatments were not replicated (Table 2). Ten (10) tadpoles were introduced in each medium.

Table 2: Mortality of tadpoles on 24 – 96 hours exposure to varied concentrations of *B. aegyptiaca* (hs = hours, R = replicate)

Concentration of *B. aegyptiaca*	Log concentration	Exposure time (h)				Total mortality	Total Number survived	% of mortality
		24 hs	*48 hs*	*72 hs*	*96 hs*			
12.5 mg l^{-1}	1.09			2	3	5	5	50
15 mg l^{-1}	1.17			3	2	5	5	50
17.5 mg l^{-1}	1.24	1	1	3	2	7	3	70
20 mg l^{-1}	1.30	10				10	0	100
Control (00 mg l^{-1})	0					00	10	00

The high mortality was observed at 20 mg l^{-1} within 24 first hours. However, no mortality was recorded in the control during the 96 hours of the test period. The simple arithmetic calculation was also used to determine the total mortality of the tadpoles. Using probit table, percentage of probit was also known and these were used to establish the LC_{50} of the treatment effect on tadpoles. This was done by plotting percentage probit against logarithm of concentration (mg l^{-1}) and the result of the 96 hr LC_{50} determined to be 13.80 mg l^{-1}.

Effect of *B. aegyptiaca* at sub-lethal concentrations: The experiment on sub-lethal effect of *B. aegyptiaca* bark's on fish (*O. niloticus)* showed no mortality both in control and other treatment media but the fish showed distress sign for some few hours after exposure and become active as the number of hours increased. The fish swam freely the following day which revealed that the extract has loss it potency.

Effect of *B. aegyptiaca* on physico-chemical parameters: Mean of physic-chemical parameters during sub-lethal exposure of *O. niloticus* on ***B. aegyptiaca*** bark's (powder) are presented below (Table 3). There were slight fluctuations in the parameters.

Table 3: Water quality parameters measured during the exposure period

Concentration (mg l^{-1})	Dissolved oxygen (mg l^{-1})	pH	Conductivity (µS cm^{-1})	Temperature (°C)
0.00	3.90	7.06	142.6	27.1
20	2.6	7.16	142.6	27.1
15	3.00	7.13	140	27.2

Effect of *B. aegyptiaca* on fish weight: There were reduction in weight in all treatment including the control but higher reductions were recorded in the higher concentration (Table 4). The reduction of weight should be also due to stressful treatment of changing the water every two days, weighing the fish each week and the feeding system, which were done just for fish subsistence.

Table 4: Weight of fish during sub-lethal exposure

Concentration (mg l^{-1})	1st wk (g)	2nd wk (g)	3rd wk (g)	4th wk (g)	5th wk (g)	Initial weight (g)	Weight Gain/loss (g)
00.00	10.08±1.26	10.87±1.22	9.85±1.09	9.84±1.28	9.24±1.28	9.28	-0.04
20.00	10.70±3.07	10.80±2.96	10.83±3.08	10.73±3.09	9.23±3.12	10.20	-0.97
15.00	10.64±1.80	10.20±1.84	10.05±1.84	9.73±1.80	9.33±1.84	10.25	-0.92

The results obtained from this investigation show that putting *B. aegyptiaca* directly in fish environment led to an increase in mortality of *O. niloticus*. The 96 hrs LC_{50} value of 26.22 mg l^{-1}, reported in this work is much higher than those reported by Omoregie & Okpanachi (1997) (6.03 mg l^{-1}) when the exposed *Tilapia zilli* (Gervais) to water extract of the bark of neen plant, *Azadiractha indica* (Lodd). This shows that the bark of *B. aegyptiaca* is not as toxic as those of *Azadiractha indica*. However, this value (26.22 mg l^{-1}) is far less than those reported (109.6 mg l^{-1}) by Agbon *et al.* (2002), when they exposed *Azadiractha indica* to water extract of Tobacco (*Nicotiana tobaccum*) leaf dust. This shows that the bark of *B. aegyptiaca* is more toxic than the leaf dust extract of Tobacco. The mortality rate increased with the increase of concentration of *B. aegyptiaca* bark in fish medium, i.e. during the bioassays all fish died within an interval of 2 to 8 hours at concentration of 2.00 g in 20 l and 1.00 g in 20 l respectively. However, in 0.50 g in 20 l, 15% of mortality was recorded after 96 hours of exposure. The darkening of the fish, tail beat, respiratory distress, the initial hyperventilation and erratic swimming observed in the bioassays are indications that mortality of the exposed fish is not only due to impaired metabolism but could in addition be due to nervous disorder as reported by Omoregie & Okpanachi (1997). These abnormal behavioral response in fish exposed to toxicants were earlier reported by Marking & Terry (1974), Kulakkottoliekal & Kramar (1987), De Silva & Ranasingle (1989), Ufodike & Omoregie (1990, 1994), Okwuasa & Omoregie (1995) and Agbon *et al.* (2002). The toxicity time disappearance either in fish or in tadpoles is about 48 hours. The sub-lethal concentration of toxicants in the aquatic environment will not necessarily result in outright mortality of aquatic organism but Omoregie *et al.* (1990) reported that the sub-lethal concentration have significant effects which can result in several physiological dysfunction in the fish. During the sub-lethal exposure no mortality were recorded but slight loss of weight in fish exposed *B. aegyptiaca* and the control fish. The reduction in weight is marked differently in the exposed fish and probably proportional to the concentration. The loss weight could be due to stress of changing the water every two days and the feeding practice as the fish were fed just for subsistence. As reported by Marking and Terry (1974), toxicity could be influenced little by temperature of 7 to 22°C, hardness of 10 to 30 mg l^{-1}, or by pH of 6.5 to 9.5. The mean values of physico-chemical parameters recorded during sub-lethal exposure of *O. niloticus* in *B. aegyptiaca* powder concentration shows slight relationship with dissolved oxygen and pH while there are more or less very little fluctuation of hardness and temperature (Table 3).

Effect of *B. aegyptiaca* on some haematological parameters of *O. niloticus:* The table below indicates the results of haemotoglobin (Hb), packed cell volume (PCV), Red blood cell (RBC), mean cell volume (MCV), mean cell haemotoglobin (MCH), mean cell haemotoglobin concentration (MCHC), the total protein and glucose level values (Table 5). These parameters

followed the same trend. They were all decreasing as the concentration increased except MCV, MCH and MHC values that were equal. There was no significant difference (P > 0.05) when subjected to ANOVA.

Table 5: mean of haematological indices of *O. niloticus* exposed to different sub-lethal concentration of *B. aegyptiaca*.

Haematological parameters	*B. aegyptiaca* concentration (mg l^{-1})			Calculated F. Ratios
	0.00	20	15	
PCV	18.4	12.25	16.2	0.71
Hb	6.2	5.25	5.44	0.81
RCB	2.08	1.80	1.84	0.59
MCV	88	86.5	88.4	0.71
MCH	80	29	30	0.91
MCHC	34	33	34	1.04
Total protein	29.5	24.5	25.6	0.90
Glucose	35	25.6	29	1.17

The haematological test carried out on the fish blood showed slight reduction in PCV, Hb, RBC, total protein and glucose level and more or less in MCV, MCH and MCHC of exposed and control fish. Similar reductions have been reported by Kabir (2009), Omoregie *et al.* (1994); Omoniyi *et al.* (2002) when they exposed fish to polluted environment render laboratory conditions. There is no significant difference (P > 0.05) in value calculated of haematological test. This is an indication that the values were all within the tolerance range. Observation also showed that the water quality parameters in the test aquaria did not vary significantly from those of the control aquaria hence all the loss in weights, erratic behaviours and change in haematological parameters were the results of introduction of Balanites bark into the medium.

CONCLUSION

The study on acute toxicity of *Balanites aegyptiaca* bark on *Oreochromis niloticus* and tadpoles shows 96 hrs LC_{50} of 26.22 mg l^{-1} and 13.80 mg l^{-1} respectively. It also revealed low percentage (15%) of *Oreochromis niloticus, Tadpoles succumb to higher concentration of 25 mg l-1 and 12.5 mg l-1 respectively* and 100% of mortality was recorded at higher concentration of 30 mg l^{-1} and 20 mg l^{-1} respectively. The time for toxicity disappearance was found to be about 48 hours. Thus, it is possible to use *B. aegyptiaca* powder in selective eradication of aquatic organism. This can also be used to control unwanted predatory species i.e. tadpoles, other Cichlid (e.g. *Hemichromis fasciatus* (Peters, 1852); *H. bimaculatus* (Gill, 1862). For clearing ponds of unwanted predatory fish direct application at 30 mg l^{-1}, ten fish can be newly stocked after 48 hours. *Balanites aegyptiaca* bark powder can be recommended because it is biodegradable and leave no adverse effect on environment. In addition, it is economical and cheap. This will eliminate predators and reduce cost of management such as expenditure on chlorinated hydrocarbon, other chemical to kill predators. However, the use of *B. aegyptiaca* by local fishermen in rivers, streams and lakes, is ill advised, as the resultant deleterious effects will subsequently lead to death of not only target fish but other aquatic organism, hence reduce the biodiversity. If the present rate at which these extracts are being used is not checked, the continuous existence of the aquatic fauna, including biologically important fish species, will be in serious jeopardy as reported by Omoregie and Opanachi (1997). The environmental authorities in Niger Republic need to ban the use of this plant extracts in the open aquatic environment.

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