Jos Journal of Applied Animal Science & Environmental Stability 2026; 1(1):160-171

Jos Jornal of Applied Animal Science and Environmental Stability
Department of Animal Science. University of Jos.

ACUTE TOXICITY EFFECTS OF CRUDE SEED EXTRACTS OF DESERT DATE (*Balanite aegyptiaca)* ON CAT FISH (*Clarias gariepinus)* JUVENILES

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ABSTRACT

This study investigates the toxicity effects of *Balanites aegyptiaca* crude seed extract on juveniles of *Clarias gariepinus*, as it affects the water quality and haematological parameters. The fresh fruits of *Balanites aegyptiaca* were collected from Jos, Nigeria, and processed to obtain a fine powder for analysis. Juvenile *Clarias gariepinus*, averaging 3g in weight and 9cm in length, were acclimatized to laboratory conditions for one week before exposure to varying concentrations (0.05, 0.10, 0.15, 0.20, and 0.25 g/L) of the extract. Water quality parameters such as temperature, pH, dissolved oxygen, and carbon dioxide were monitored daily throughout the experiment. Results indicated that while pH levels remained stable across treatments, significant variations in carbon dioxide levels were observed. Haematological assessments revealed a dose-dependent response in red blood cell counts and hemoglobin levels at lower concentrations, but a non-linear trend at higher concentrations. Overall, findings indicate that *Balanite aegyptiaca* extract can influence both water quality and fish health parameters.

Keywords: Water Quality, Haematological Indices, *Clarias gariepinus* Juveniles, Acute Concentrations, *Balanite aegyptiaca*

INTRODUCTION

All water pollution affects organism and plants that lives in these water bodies and in almost all cases (Chris Dinesen, 2022). The effect is damaging not only to the individual species and populations, but

also to the natural biological communities, and that this occurs when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful constituents (Agrawal *et al*., 2010). Many plant components have the potential effect to produce adverse effects on productivity of farm animals (Novak & Haslberger 2000). They have an array of chemicals noxious or toxic to fish and other animals including man, this chemicals diversity also includes many other compounds with medicinal values (Novak & Haslberger 2000).

Plants extracts have been used by man to collect fish in lentic and lotic waters. Indigenous tribes of South America have long used plants as piscicides (Power, Fuentes, & Harrison, 2008). According to Power *et al*. (2008) macerated material of plant origin is thrown into rivers, streams or shallow ponds, and then the fish which are stupefied float to the surface and can be collected. After analysing the phytochemicals, the plant material revealed the presence of active compounds such as rotenoids and saponins (Power *et al*., 2008).

The bark of *Balanite aegyptiaca* is used as a fish poison, but it is not poisonous to man (Heuze & Tran, 2012). The stem bark and the fruits are used in Morocco, Nigeria and Senegal as a laxative or purgative for colic and stomach aches (Nkunya, Weenen, & Bray, 2020). It is reported that the edible bitter-sweet pulp is used as food or confectionery, and laxative as well as for the treatment of constipation, the leaves are used to make soup by some tribes in Nigeria while in Chad the fresh twigs are burned to keep insect away (Nkunya *et al*., 2020). Therefore, this study seeks to evaluate the toxicity effects of aqueous crude seed extract of *Balanite aegypiaca* on *Clarias gariepinus* juveniles.

MATERIALS AND METHODS

Collection Area and Preparation of Toxicant

The fresh part (fruits) of the experimental plant was collected from Jos, Jos North Local Government Area of Plateau State North-central Nigeria. The *Balanites aegyptiaca* fruits was transported to Hydrobiology and Fisheries research Laboratory of University of Jos, Nigeria where the seed was removed and dried under shed and pound using pestle and mortar and sieved with 0.5mm sieve into fine powder before taking the sample for phytochemicals and proximate analysis in the department of biochemistry University of Jos.

Collection and Acclimation of Experimental Fish (*Clariasgariepinus*) Juveniles

Live and healthy, mixed sex Juveniles of *Clarias gariepinus* with an average weight of 3g and length of 9cm were purchased from a Private Fish Farm in Zarmaganda, Jos South, Plateau State and transported to Hydrobiology and Fisheries Research laboratory of University of Jos, Nigeria. Fish were transferred

into six (6) plastic tanks each with 10L dechlorinated tap water and allowed to acclimatize to laboratory conditions for a period of one week. Fish were fed 3% body weight with commercial feed (Blue crown) twice daily. Three quarters of the water in the tank were siphoned out on daily basis to remove left over feed and faecal matter and replaced with fresh water. Mortality observed during acclimation period were replaced and allowed to stabilize to zero. Feeding stopped 24 hours prior to exposure to the bioassay media.

Determination of Definitive Acute Concentrations of *B. aegyptiaca* Crude Seed Extract

Series of preliminary investigations was conducted to obtain the acute concentrations (Ayorinde, Audu, Ogundeko, & Ujah, 2020). Median lethal concentration that causes 50% mortality of exposed fish was determined and the five definitive tests concentrations of 0.05, 0.10, 0.15, 0.20, and 0.25g/L of *Balanite aegyptiaca* were obtained.

Experimental Design

A total of twelve (12) transparent circular plastic tanks of 16 liters capacity each with 10 liters of de-chlorinated tap water were used in a complete randomized block design. Each test tank was replicated and designated as A1-C2, E1-B2, C1-A2, D1-F2, B1-E2 and F1-D2. Each tank contained ten (10) mixed sex juveniles of *C. gariepinus*. Fish were sorted into cohort and distributed in batches of ten fish/per experimental tank with the control.

Monitoring of Water Quality Parameters for Acute Toxicity

Water quality parameters of the tanks exposed to acute concentrations of *Balanite aegyptiaca* crude seed extract such as: Temperature (^{o}C), pH, Dissolved oxygen (g/L), and Free carbon (IV) oxide (g/L) were monitored daily, following the procedures described by APHA (2005).

Haematological parameters of *Clarias gariepinus* juveniles exposed to Crude Seed extract of *Balanite aegyptiaca.*

Haematological parameterswas carried out according to the method described by Maheswaran, Devapaul, Muralidharan Velmurugan, & Ignacimuthu (2008).

RESULTS

Acute Bioasssay of Aqueous Crude Seed Extract of *Balanite aegyptiaca* on *Clarias gariepinus* juveniles

The results of the acute bioassay on *Clarias gariepinus* using different concentrations (0.05, 0.10, 0.15, 0.20, and 0.25g/L) of aqueous crude seed extract from *Balanite aegyptiaca* over 96 hours are summarized in table 1 below. Mortality rates increased with higher concentrations: 100% at 0.25g/L, 80% at 0.20g/L, 60% at 0.15g/L, 40% at 0.10g/L, and 20% at 0.05g/L. No mortality was observed in the control tanks (0.00g/L).

Figure 1 elaborate the linear relationship between the probit mortality and the logarithm of concentration for *Clarias gariepinus* juveniles exposed to the aqueous crude seed extract of *Balanite aegyptiaca.* The calculated 96-hour LC_{50} value for the extract on *Clarias gariepinus* juveniles is 0.111g/L, with upper and lower confidence limits of 0.080g/L and 0.153g/L, respectively.

Table 1: Acute Bioassay of *Clarias gariepinus* Juveniles Exposed to Aqueous Crude Seed Extract of *Balanite aegyptiaca*

Conc. (g/L)	Log. Conc.	No of Fish	Mortality Time (Hours)						Percentage Mortality (%)	Probit Mortality
			12hrs	24hrs	36hrs	48hrs	72hrs	96hs		
0.25	0.6021	10	7	6	4	-	-	-	100	8.7190
0.2	-0.6990	10	5	4	2	2	-	-	90	6.2816
0.15	-0.8239	10	3	-	1	3	2	-	60	5.2533
0.1	-1.0000	10	-	-	1	2	1	-	40	4.7467
0.05	-1.3010	10	-	-	-	1	-	1	20	4.1584
0	0.0000	10	0	-	-	-	-	-	0	0.0000

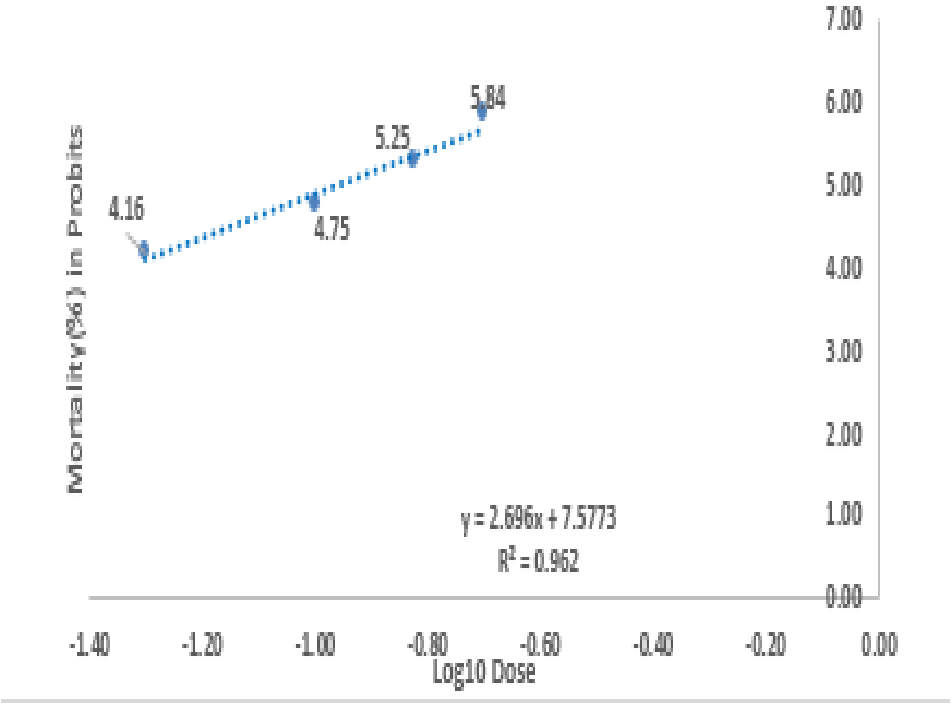


Figure 1: Graph of probit mortality against log concentrations

The water quality parameters recorded during the acute toxicity experiment are presented in Table 2. Among the measured parameters, dissolved carbon dioxide (CO_2) and dissolved oxygen (DO) differed significantly ($P < 0.05$) among the treatment groups, whereas pH and temperature were not significantly affected ($P > 0.05$). The pH of the experimental water ranged from 6.98 in the control to 7.69 in treatment B, with no significant differences ($P = 0.948$) among treatments. Similarly, water temperature remained relatively stable throughout the experimental period, varying between 26.19°C and 27.20°C, and was not significantly influenced by the concentrations of the extract ($P = 0.830$). Carbon dioxide concentration differed significantly ($P < 0.05$), with the highest value (56.00 mg/L) recorded in treatment A (0.05 g/L), followed by 43.75 mg/L in treatment B (0.025 g/L). The lowest CO_2 concentration (23.00 mg/L) was observed in treatment D (0.00625 g/L), while treatments E and the control recorded intermediate values of 33.25 mg/L and 32.75 mg/L, respectively. Dissolved oxygen also varied significantly ($P = 0.018$) among treatments. The control group recorded the highest dissolved oxygen concentration (5.40 mg/L), which differed significantly from treatments A, B, C, and D. The lowest dissolved oxygen concentration (2.10 mg/L) was recorded in treatment A, while treatments C, D, and E recorded values of 3.50 mg/L, 3.20 mg/L, and 3.90 mg/L, respectively.

Table 2: Water Quality Parameters of Experimental Tanks During Acute Exposure of Aqueous Crude Seed Extract of *Balanite aegyptiaca* on *Clarias gariepinus* juveniles

Parameters	A	B	C	D	E	Control	SEM	P-value
pH	7.66	7.69	7.31	7.37	7.22	6.98	0.21	0.948
Carbon dioxide	56.00^{a}	43.75^{b}	27.25^{d}	23.00^{e}	33.25^{c}	32.75^{c}	2.67	0.000
Temperature	27.2	27	26.78	26.75	26.7	26.19	0.21	0.830
Oxygen	2.10^{b}	2.30^{b}	3.50^{b}	3.20^{b}	3.90^{ab}	5.40^{a}	0.33	0.018

$^{a, b}$ Means in the same row not sharing superscript are significantly different at $P<0.05$.

SEM = Standard error mean

The haematological indices of *Clarias gariepinus* juveniles following 96-hour exposure to acute concentrations of aqueous crude seed extract of *Balanites aegyptiaca* are presented in Table 3. Among the haematological parameters evaluated, only white blood cell (WBC) count was significantly affected ($P < 0.05$) by the extract concentration, whereas lymphocyte (LYM), red blood cell (RBC), haemoglobin

(HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT), mean platelet volume (MPV), and platelet distribution width (PDW) did not differ significantly ($P > 0.05$). The WBC count increased significantly from 25.30 ± 1.20 in the control group to 89.00 ± 2.30, 97.00 ± 1.30, 90.34 ± 1.54, and 88.90 ± 1.10 at extract concentrations of 0.05, 0.10, 0.15, and 0.20 g/L, respectively, before declining to 66.10 ± 31.20 at 0.25 g/L. Although not statistically significant, lymphocyte percentage varied from 17.56 ± 1.86% at 0.05 g/L to 47.66 ± 24.65% at 0.10 g/L. Similarly, RBC counts ranged from 0.92 ± 0.44 to 2.26 ± 0.13, while haemoglobin concentration varied between 64.50 ± 0.05 g/L and 189.00 ± 1.00 g/L across treatments. Haematocrit values ranged from 13.14 ± 2.62% to 29.39 ± 1.66%, whereas MCV values ranged between 118.35 ± 2.95 fL and 138.85 ± 1.35 fL. Likewise, MCH, MCHC, platelet count, MPV, and PDW exhibited numerical variations among treatment groups but were not significantly influenced by exposure to the extract ($P > 0.05$).

Table 3: Mean Haematological Indices of *Clarias gariepinus* Juveniles Exposed to Acute Concentrations of Aqueous Crude Seed Extract of *Balanite aegyptiaca* for 96 Hours

Parameters	0(g/L)	0.05(g/L)	0.1(g/L)	0.15(g/L)	0.2(g/L)	0.25(g/L)	P-values
WBC (µL)	25.30±1.20	89.00±2.30	97.00±1.30	90.34±1.54	88.90±1.10	66.10±31.20	0.040
LYM (%)	23.52±1.27	17.56±1.86	47.66±24.65	19.31±0.10	20.81±0.74	23.67±5.30	0.420
RBC ((µL)	1.44±0.33	2.26±0.13	2.22±0.80	1.98±0.95	0.92±0.44	1.93±0.15	0.370
HGB(g/L)	86.00±8.00	189.00±1.00	160.50±12.50	148.00±99.00	64.50±0.05	119.00±32.00	0.410
HCT(%)	20.09±4.71	28.76±1.70	29.39±1.66	23.16±10.66	13.14±2.62	24.54±0.64	0.320
MCV (fL)	138.85±1.35	127.30±0.50	131.95±2.75	118.35±2.95	120.35±4.45	127.55±6.55	0.060
MCH(pg)	64.05±19.96	84.15±5.25	72.70±8.30	66.33±18.01	62.86±15.15	63.46±21.46	0.910
MCHC(g/L	462.50±148.50	661.00±44.00	552.00±166.00	564.00±166.00	518.00±107.00	490.00±143.00	0.880
PLT (µL)	36.50±3.50	141.50±27.50	90.00±7.00	170.50±27.50	169.50±39.50	221.50±139.50	0.430
MPV (fL)	12.33±0.09	11.93±0.30	12.13±0.36	11.38±1.67	10.37±0.05	11.00±1.14	0.610
PDW (%)	16.05±0.45	16.20±1.60	17.10v1.10	15.90±1.30	14.80±0.20	11.00±0.00	0.570

WBC=White blood cells; LYM= Lymphocytes; RBC= Redblood cells; HGB= Haemaglobin; HCT= haematocrit; MCV= Mean corpuscular volume; MCH= Mean corpuscular haemoglobin; MCHC= Mean corpuscular haemaglobin concentrations; PLT= platelets; MPV= mean platelets volume; PDW= platelets distribution width

DISCUSSION

The analysis of water quality at acute concentration shows that the pH levels across treatments indicate the water remains generally within the optimal range for most freshwater fish species, which is typically between 6.5 and 8.5. Treatments 1 and 2 displayed the highest pH values, while Treatment 6 showed the lowest pH at 6.98. Despite the variation, the high p-value suggests no statistically significant difference among the treatments. Johnson *et al*. (2020) highlights that while fish can tolerate slight fluctuations in pH, significant deviations can lead to stress and impaired metabolic functions. Some species, such as salmonids, are particularly sensitive to acidic conditions, potentially experiencing higher mortality rates when exposed to pH levels below 6.5 (McDonald & Adams, 2018).

Carbon dioxide (CO_2) levels showed significant variation across treatments, with Treatment 1 having the highest CO_2 concentration at 56.00 mg/L, which is indicative of poor water quality and potentially detrimental to fish health. Lower CO_2 levels in Treatments 4, 5, and 6 are more favorable for fish. Elevated CO_2 levels are known to impair fish respiration and metabolic processes, leading to stress and altered behaviours (Thompson *et al*., 2020). Some fish species, like tilapia, have shown adaptability to higher CO_2 levels compared to more sensitive species, indicating variability in responses to increased CO_2 concentrations (Carter & Smith, 2021).

The temperature values across treatments were relatively stable and within a suitable range for many freshwater fish species. However, it shows no significant differences in temperature among the treatments. Optimal temperatures for many fish species fall between 20°C and 30°C, and studies have shown that temperature significantly affects fish growth and reproduction (Liu *et al*., 2022).

Dissolved oxygen levels exhibited significant variation, with Treatment 1 (2.10 mg/L) indicating hypoxic conditions. Treatment 6 showed the highest level of 5.40 mg/L, which is more conducive to fish health. Studies have established that oxygen levels below 3 mg/L can cause stress and increased mortality rates in fish (Garcia *et al*., 2021). Different fish species exhibit varying tolerances to low oxygen levels; for example, catfish can survive in low oxygen conditions better than trout, which are more susceptible to hypoxia (Wang & Hu, 2020).

Haematological parameters are used in medical analysis of fish body function which is determined by the effect of the surrounding water (Ojutiku, Asuwaju, Kolo, Obande, &Agbelege,

2013), thus reveal the level of damage in fish. Fish exposed to toxicants can exhibit a variety of physiological responses, including blood disturbances (Osman, Koutb& Sayed, 2010; Thangam, Jayaprakash &Perumayee, 2014). The anisocytosis of red blood cells in this study indicates stress mediated release of blood cells by haematopoietic tissues (Al-Zaidan, 2017) and poikilocytosis implies destruction of blood cells due to the effects of the toxicant on the exposed fish (Ajima, Ogo, Audu, &Ugwoegbu, 2014) while normocytic and normochromic cells indicate that concentrations of the plant extract had no effect on the blood cells. Decrease in WBC and LYM recorded in this study opposed the findings of Adewoye, (2010), which reported an increase in WBC and LYM due to the fish's attempt to fight against the toxicant by producing more WBC and LYM to recover their health condition. The decrease in RBC as concentrations of the test material increase in this study is similar to the findings of Ajima *et al*. (2017) that RBC decreased after chronically treating C. gariepinus with urea fertilizer. It also agrees with the findings of Suely, Zabed, Ahmed, Mohamad, Nasiruddin, Sahu, & Ganesan (2015) after exposing Heteropneustesfossilis to concentrations of Terminalia arjuna. A significant decrease in RBC and HGB level after exposure to pesticides and other toxicants were reported by several investigators (Adewoye, 2010). The present study also corroborates the findings of Adedeyi & Odo (2017) who reported decline in HCT following elevation in concentration of selenium on C. gariepinus. Similar reduction had been reported by Thangam, *et al*. (2014) after exposing C. carpio to copper. In contrast with results of the present study, Riaz-ul-Haq, Javeed, Iram, Rasheed, Amjad, & Iqbal, (2018) have previously observed an increase in HCT (or PCV) of freshwater fish Channa punctatus exposed to endosulphan pesticide. The present study also agree with the findings of Nnamdi, Benjamin, Abdulbashir, Kingsley, Tam-Miete, Opeyemi, & Micheal, (2020), which reported a significant decrease in the MCH and MCHC level of fishes exposed to sub-lethal concentrations of Abamectin and Fipronil. The observed decrease in these parameters could be due to bioaccumulation of the plant extract in the body (Dahunsi &Oransu, 2013) and the haemolytic properties of the toxicant owing to the presence of saponins (Francis, Kerem, Makkar, & Becker, 2006). The recorded mortality in this study could be due to stress induced by the aqueous crude seed extract of B. aegyptiaca on the immune system of C. gariepinus which might have slowed toxic progress and resulted in acute toxic response which agreed with the report of Adedeyi & Odo (2017).

Conclusion:

The findings of this study indicated that the crude seed extract of *Balanite aegyptiaca* contains a variety of phytochemical components with distinct effects on fish physiology. These phytochemicals adversely impact water quality and fish organs, leading to changes in blood parameters and increased mortality among fish populations. Consequently, the crude seed extract of *Balanite aegyptiaca* could be considered an aquatic pollutant. Therefore, it is recommended that the indiscriminate use of this extract in aquatic environments is capable of affected non-targeted aquatic biota negatively, and as such should be discouraged. Moreover, the extract has to be cautiously applied in fish water environment.

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