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Acute Toxicity of Aqueous Crude Leaf Extract of *Azadirachta indica* (NEEM) on The Gill Biochemistry of *Clarias gariepinus* (African catfish) Juveniles

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Abstract

The indiscriminate discharge of pollutants into the water system by human activities is causing a momentous threat to the aquatic environment and leading to scarcity of fish and other aquatic fauna. This study was carried out to determine the acute toxicity test of the aqueous crude leaf of Azadirachta indica extract on the gill of Clarias gariepinus (African Catfish) juveniles by assessing the phytochemicals, investigation of changes in water quality parameters and biochemical parameters. A total of 120 mixed-sex juveniles of C. gariepinus with (mean weight of 11.4g and mean length 12.5cm) were used for the experiment. Acute toxicity test (96hrs static non reneweable bioassay) was carried out with ten (10) fish each in six (6) circular tanks, each duplicate replicated. After a range finding test was conducted, fish were exposed to various concentrations of 1.00, 1.20, 1.50, 2.00, 2.50 and 0.00g/L which served as the control tank. Fish exposed to the various concentrations showed abnormal behaviour and mortality increased as the toxicant concentrations increased throughout the exposure period. The result of the phytochemical screening of the toxicant showed the presence of saponins, tannins, steroids, cardiac glycosides and anthraquinones, flavonoids with the absence of alkaloids. The result of the water quality parameters monitored during the experiment varied significantly ($P<0.05$). Biochemical parameters varied significantly ($P<0.05$), the activities of the Aspartate aminotransferase, Alanine transaminase and Alkaline phosphatase increased with increase in toxicant concentrations. The study revealed that the substance is toxic to the exposed fish at various concentrations and the result showed that the 96hrsLC_{50} caused 50% mortality. The toxicant Azadirachta indica had adverse effects on the water quality, behavioural signs, and the biochemical parameters of the exposed fish compared to the control tank. Therefore, the use of A. Indica in water bodies can lead to contamination of the aquatic ecosystem by causing threat to fish well-being and survival.

Keywords: Toxicity, Azadiracta indica, Clarias gariepinus, Biochemistry and Gill

INTRODUCTION

According to the Food and Agricultural Organization (FAO), more than 60,000 plant species are used for various purposes all over the world (F.A.O., 1991). They are used to control parasites and to conserve or restore native species, but their uses are not encouraged because of their adverse effects on aquatic organisms (F.A.O.,1991). However, traditional fishing with plant substances has been shown to have negative consequences on aquatic organism, which roots to a significant ecological imbalance and does not encourage continuous fishing (Mohotti & Epa, 2016). Increase in anthropogenic activities and pollution has affected aquatic environment and exposure of these aquatic organisms to various toxicants have shown to have adverse effects on fish physiology thereby, causing mortality (Henry et al., 2004).

MATERIALS AND METHODS

EXPERIMENTAL PLANT (*Azadirachta indica*) Leaf.

Fresh leaf of *A. indica* (Plate 1) were collected from the Federal Ministry of Forestry, Bauchi Road Jos, Plateau State using a sharp knife. It was air-dried in the Research Laboratory Department of Zoology, University of Jos. The dried sample was pulverized using mortar and pestle, sieved with 0.5mm sieve and the powder stored in a glass bottle container until when needed.

COLLECTION AND ACCLIMATION OF EXPERIMENTAL FISH (*C. gariepinus*) JUVENILES

Apparent healthy One hundred and twenty (120) juvenile of *Clarias gariepinus* with mean weight and length of 21.4g and 14.5cm respectively were used for this investigation. The fish were purchased from Integrated Fish Farm at Zarmaganda, Jos South, Plateau State, Nigeria and transported to the Research Laboratory Department of Zoology, University of Jos, Nigeria. Twelve (12) circular plastic tanks (40 × 30 × 20cm) were used and ten (10) fish were placed in each of the tanks. They were allowed to acclimatize to laboratory conditions for a period of one weeks during which the fish were fed with Vital feed® once daily at 3% of their body weight. Three quarters of the water in the tank was siphoned out on a daily basis to remove left-over feed and faecal matter and replaced with fresh borehole water. Mortality observed during the acclimation period was removed, replaced and allowed to stabilize to zero. The photoperiod was natural (12hrs day: 12hrs night) and feeding was stopped 24 hours prior to exposure to the bioassay media.

EXPERIMENTAL DESIGN

A 24-hour preliminary test was conducted to obtain a realistic toxic concentration for the acute toxicity test in a static nonrenewable bioassay. The test was carried out to determine the 96hrs LC_{50}. A total of six (6) circular plastic test tanks (40 × 30 × 30cm) were used, each duplicate replicated in a randomized block design. The tanks were labelled Al - A2, Bl - B2, Cl - C2, Dl - D2, El - E2 and F1 –F2 which served as the control tank. Each of the tanks was filled with 20L (twenty litre) of bore hole water.

During the bioassay which lasted for 96 hours (4 days), water quality parameters such as temperature was measured every 24 hours while the dissolved oxygen, total alkalinity, pH and free

carbon dioxide were all determined using standard laboratory methods as described by APHA (2005) at the beginning and end of the bioassay.

Preparation of stock solution

Azadirachta indica leaf was air-dried and weighed using a Metler weighing machine. The dried sample was crushed using a mortar and pestle. *Azadirachta indica* powder (1g) was macerated in 1L of distilled water and allowed to stand for 24hrs (Audu et al., 2020). The mixture was filtered using 0.5mm sieve containing a funnel choked with non-absorbent cotton wool and the filtrate formed the stock solution (Audu et al., 2020).

RANGE FINDING TEST (RFT)

Range finding test (RFT) was conducted to determine the concentrations of aqueous crude extract of *A. indica* that would cause 50% mortality of the test fish after 96 hours (4 days). The RFT involved exposing five (5) fish to different concentrations of 3.00, 2.50, 2.00, 1.50 1.20 and 1.00g/L of *Azadirachta indica* of aqueous crude leaf extract of *A. indica* with 0.00g/L which served as the control without the test material. Based on the results of the RFT, a definitive concentration of 2.50, 2.00, 1.50 1.20 and 1.00g/L of *Azadirachta indica* of aqueous crude leaf extract of *A. indica* with 0.00g/L (control) were prepared in six (6) circular plastic tanks and replicated for bioassay (Audu et al., 2020).

BIOCHEMICAL ANALYSES

At the end of the 96hours exposure of *Clarias gariepinus* juveniles to acute concentrations of aqueous crude leaf extract of *Azadirachta indica,* the exposed and control fish were dissecte to remove the gills. The organs were rinsed in distilled water to remove traces of blood (Ogueji & Auta, 2007). The gill sample was homogenised in normal saline using a ceramic mortar and pestle. The sample was centrifuged for 5 minutes at 1000rpm to obtain supernatants which was used for the analyses using the methods of Ogueji & Auta, (2007). The activities of the enzymes; Alkaline Phosphatase (ALP), Aspartate Transaminase (AST) and Alanine Transaminase (ALT) were analyzed using Reitman & Frankel (1957).The data were analyzed using One- Way ANOVA. Results were considered significant at $P<0.05$.

RESULTS

The result of water quality parameters during acute toxicity tests showed that the mean values of water quality parameters varied significantly ($P<0.05$) compared to the control. Dissolved oxygen (DO) decreases as toxicant concentration increased, the highest toxicant concentration 2.5g/L recorded 0.23mg/L, while the lowest concentration 1.00g/L recorded 4.37mg/L and the control tank 0.00g/L recorded 7.25mg/L. Free carbon dioxide (CO_2) increased as toxicant concentrations increased, the highest toxicant concentration 2.50g/L recorded 4.73ppm while the lowest concentrations 1.00g/L recorded 2.88ppm and the control 0.00g/L recorded 2.88mg/L. Temperature decreased as toxicant concentrations increased, the highest toxicant concentration 2.50g/L recorded 21.80^{o}C while, the lowest

concentration 1.00g/L recorded 22.23ºC and the control 0.00g/L recorded 23.50ºC. pH increased as toxicant concentrations increased, the highest toxicant concentration 2.50g/L recorded 4.83 while the lowest toxicant concentration 1.00g/L recorded 4.16 and the control 0.00g/L recorded 4.08. Alkalinity increased as toxicant concentration increased, the highest toxicant concentration 2.50g/L recorded 29.40mg/L while the lowest concentration 1.00g/L recorded 21.75mg/L and 10.43mg/L was recorded in the control tank (table 1). **The m**ean mortality rate of *Clarias gariepinus* Juveniles exposed to various concentrations of Aqueous crude leaf extract of *Azadirachta indica* (96hours) (Table 2).

The results of the biochemical study carried out on the gills of *C. gariepinus* juvenile exposed to various concentrations of crude leaf extract of *A. indica* showed significant difference ($P<0.05$) compared with the control. ALP, AST and ALT were observed to increase as toxicant concentration increased Fig 1 -3.

TABLE 1.
Mean Values of Water Quality Parameters of Tanks with *C. gariepinus* Juveniles Exposed to Various Concentrations of Aqueous Crude Leaf of *A. indica* Extract (96hrs)

Concentration (g/L)	**Water Quality Parameter**				
	Dissolved oxygen (mg/L)	**Free carbon dioxide (ppm)**	**Temperature (°C)**	**pH**	**Alkalinity (mg/L)**
Control (0)	7.25±0.07 ^{e}	2.18±0.12 ^{a}	23.50±0.10 ^{c}	4.08±0.03 ^{a}	10.43±0.17 ^{a}
1.00	4.37±0.21 ^{d}	2.88±0.24^{ab}	22.43±0.01 ^{b}	4.16±0.05^{ab}	21.75±0.05 ^{b}
1.20	3.35±0.10 ^{c}	2.80±0.47^{ab}	22.28±0.05 ^{b}	4.33±0.04^{bc}	23.15±0.25 ^{c}
1.50	2.11±0.01 ^{b}	3.70±0.13^{bc}	22.28±0.05 ^{b}	4.44±0.02 ^{c}	22.10±0.00 ^{b}
2.00	0.34±0.06 ^{a}	3.75±0.24^{bc}	22.19±0.13^{ab}	4.74±0.05 ^{d}	26.50±0.00 ^{d}
2.50	0.23±0.51 ^{a}	4.73±0.08 ^{c}	21.80±0.18 ^{a}	4.83±0.04 ^{d}	29.40±0.06 ^{e}

Note: Values are mean ± standard error of the mean
Values in the same column followed by different lower case super script are significantly different at $P<0.05$.

Table 2.
Mean mortality rate of *Clarias gariepinus* Juveniles exposed to various concentration of Aqueous crude of *Azadirachta indica* extract (96hours)

S/N	Concentration	Log Concentration (g/L)	Number of fish	Mortality in (hrs)					Mean Mortality	% Mortality	Probit Mortality
				12	24	48	72	96			
1.	0.00	0.0000	10	-	-	-	-	-	0	0	0.0000
2.	1.00	0.0000	10	-	-	-	-	2	2	20	4.1584
3.	1.20	0.0791	10	-	-	-	1	2	3	30	4.4756
4.	1.50	0.1760	10	-	-	2	2	1	5	50	5.0000
5.	2.00	0.3010	10	-	2	3	3	-	8	80	5.8416
6.	2.50	0.3979	10	1	3	3	3	-	10	100	8.7190

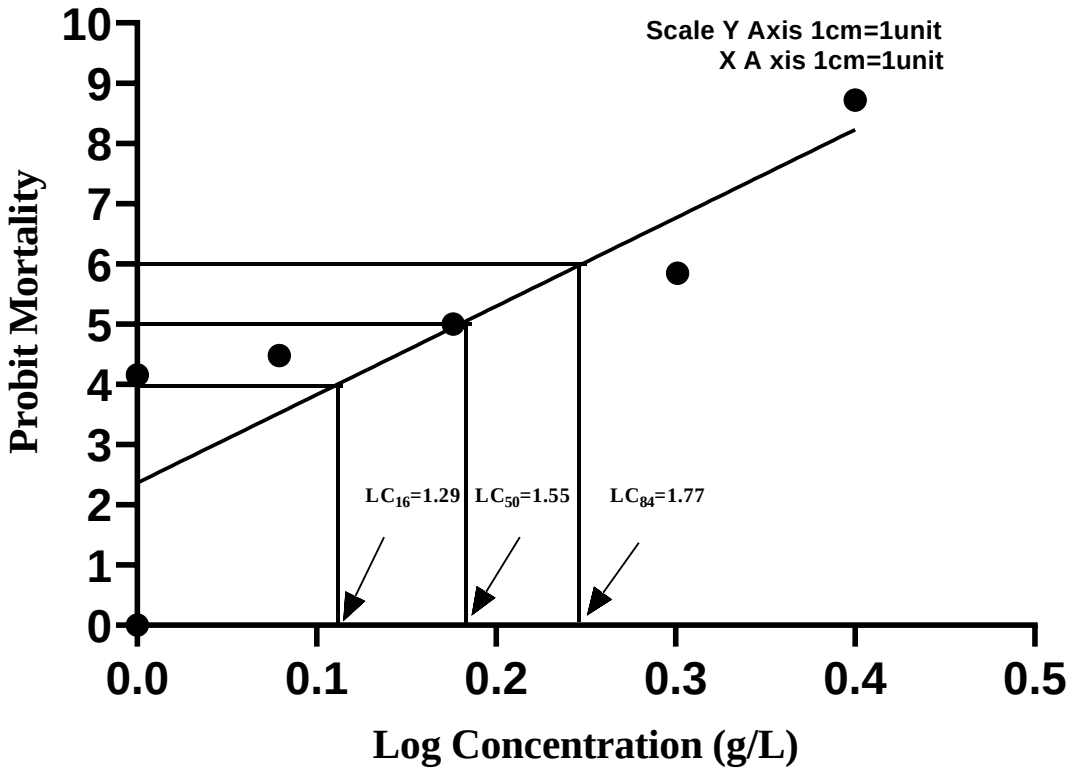

Figure 1. Linear Relationship Between Probit Mortality against Log Concentration.

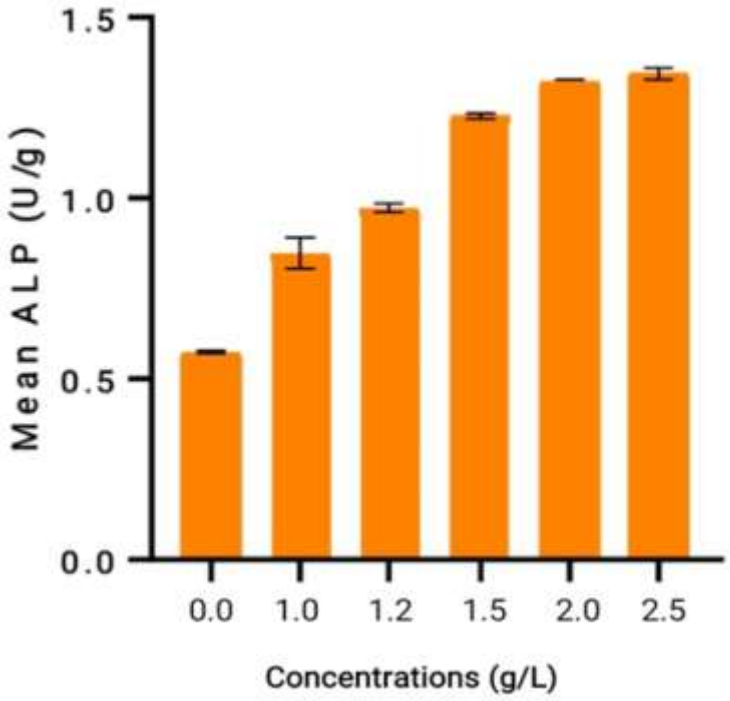

Figure 1 Alkaline Phosphatase (ALP) of Gills of *Clarias gariepinus* Juveniles exposed to various concentration of Aqueous Crude Extract of *A. indica*

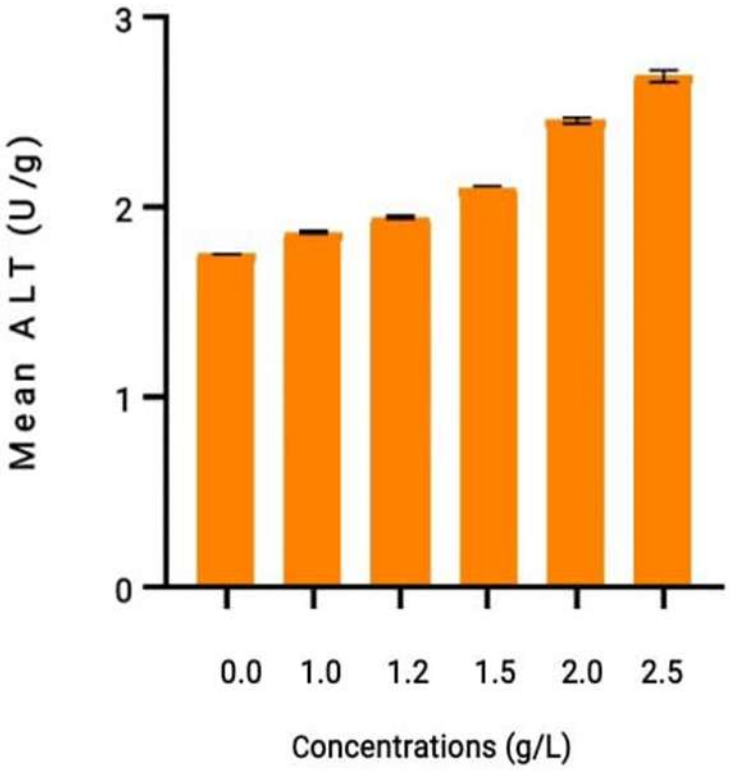

Figure 2 Alanine Transaminase (ALT) of Gills of *Clarias gariepinus* Juveniles exposed to various concentration of Aqueous Crude Extract of *A. indica*.

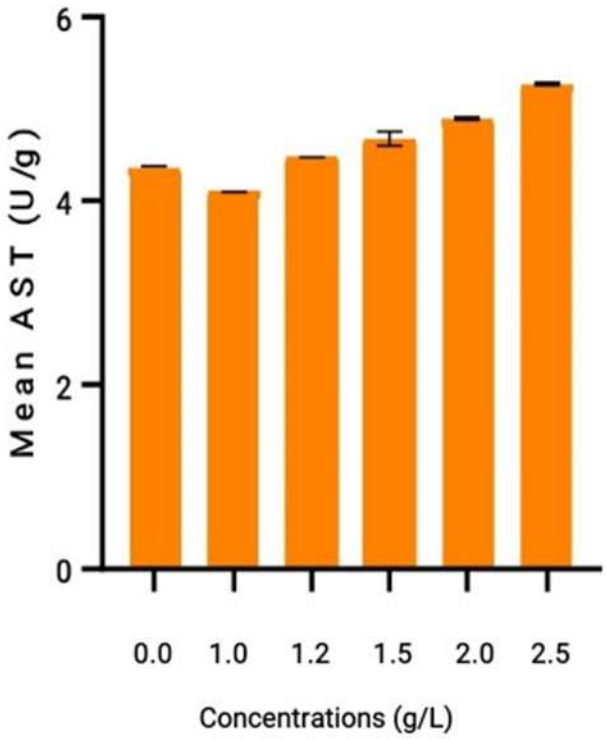

Figure 3 Aspartate Aminotransaminase (AST) of Gills of *Clarias gariepinus* Juveniles exposed to various concentration of Aqueous Crude Extract of *A. indica*.

DISCUSSION

The result of the acute toxicity experiment carried out showed that aqueous crude leaf extract of *A. indica* caused significant changes in water quality and biochemical parameters of *C. gariepinus* juveniles. Mortality increased as toxicant concentration increased, 100, 80 and 50% mortalities were recorded in the higher concentrations after 96hours which is similar to the finding of (Audu et al., 2020; Ayorinde et al., 2020) who reported increased in mortality of *C. gariepinus* after 96 hours exposure of waste dry cell battery and crude fruit endocarp extract of Calabash (*Lagenaria siceraria*).

The 96hrs LC_{50} of *A. indica* aqueous crude leaf extract to the test fish (*C. gariepinus*) was found to be 1.55g/L with the upper and lower confidence limits of 1.66 g/L and 1.45 g/L respectively. Several scientists reported higher 96hrs LC_{50} (55.76mg/L, 242 mg/L and 332 mg/L) of different plant extract on different fish species compared to that of *A. indica* aqueous crude leaf extract (Ayotunde et al., 2011; Suresh, Shailender, & Krishna, 2013). This is probably due to the constituent of the phytochemicals in the plants and the fish species.

There was significant difference ($P<0.05$) in pH values between treatment tanks compared to the control in the 96hours acute bioassay. The Hydrogen ion concentrations (pH) of the experimental media were observed to increase with increase in toxicant concentration.
There was significant difference ($P<0.05$) in the total alkalinity recorded between test tanks compared to the control. The total alkalinity content in this experiment was observed to increase with increase in the concentrations of the toxicant. Capkin, Altinok & Karahan (2006) reported that alkalinity levels above 20mg/L can significantly increase the survival of fishes. High alkalinity can cause direct physical damage to skin, gills and eyes of fish. Dissolved oxygen content was observed to decrease with increase in concentration of the toxicant. Dissolved oxygen values of all the test media were significantly different ($P<0.05$) decreased with increase in concentrations of the toxicant compared with the controls. Low dissolved oxygen value can increase the susceptibility of fish to toxic effects of toxicant. Free carbon (IV) oxide content increased with increase in the amount of the toxicant. There was significant difference ($P<0.05$) in the amount of carbon (IV) oxide in the test tanks compared with the control. Because of the anesthetic properties of carbon dioxide, it has the ability to disrupt the normal physiological function of the fish (Malcolm, 1994).

Alteration of fish enzyme is an indicative of unsuitable environmental conditions or the presence of stress factors (Yang & Chen, 2003; Kamal & Omar, 2011). Therefore, the study of serum biochemical parameters is a useful biomarker in toxicology (McDonald & Grosell, 2006). In this study, ALP, AST and ALT profiles in the gill of *C. gariepinus* juveniles exposed to aqueous crude leaf extract of *A. indica* shows a significant difference ($P<0.05$) compared with the control. This study revealed increase in enzyme activities with increased in toxicant concentration. The result agrees with the findings of several authors, who recorded an increase in enzyme activities with increased in toxicant concentration of Nile tilapia and African catfish exposed to pesticides, anaesthetics and polluted water (Akinrotimi, Edun, & Ukwe, 2018) (Osman et al., 2018).

CONCLUSION

The result of this research showed that the aqueous crude leaf extract of *A. indica* contain various phytochemicals constituents that perform different roles in a living fish. The leaf extract has negative effects on both water quality parameters and enzymes leading to mortality and alteration in the fish physiology. Therefore, aqueous crude leaf extract of *A. indica* could act as an aquatic pollutant. The physiological changes shown in the biochemical parameters of the exposed *C. gariepinus* juveniles might have affected the general health status of the fish resulting in death of fish. Thus, unusual use of aqueous crude leaf extract of *A. indica* into the aquatic environment should be discouraged, effluent from households and industries should be properly disposed to avoid being washed down directly into the aquatic environment.

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