EFFECT OF AQUEOUS SOLUTION OF AFRICAN LOCUST BEAN TREE *(Parkia biglobosa)* LEAF EXTRACTS ON THE HAEMATOLOGY OF AFRICAN CATFISH *(Clarias gariepinus)* JUVENILES AND WATER QUALITY OF CULTURE MEDIUM.

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INTRODUCTION

Haematological analysis can be used for monitoring the health status of fish from the information's it provides (Ayanda et al., 2015). Hematological indices can vary depending on so many factors ranging from environmental factors to fish species, age, sexual maturity, the toxicant, length of exposure, water quality, and health condition (Ayoola, 2018). Clarias species is a widely distributed fish which constitutes one of the major fisheries in Asia and Africa. Some records have shown that Clarias species contributes about 14% of over 6,000 tonnes of annual fish production from all fisheries sectors in Nigeria (Akin-obasola, 2019). The common species found in Nigeria are Clarias gariepinus, Clarias anguillaris, Clarias pachynema, Clarias macromystax,Clarias agboyiensis, Clarias jaensis, Clarias buthupogon and Clarias camerunensis. (Idodo-Umeh 2003). The Clarias gariepinus is a prominent cultured species because of its hardiness and fast growth rate. Several investigations have been carried

ABSTRACT

The acute toxicity of aqueous extract of African Locust Beans tree (Parkia biglobosa) leaf on African catfish (Clarias gariepinus) juveniles was investigated in a static bioassay to determine the median lethal concentrations (LC50) at 96 hours of exposure. The bioassay was conducted in the water quality laboratory of Prince Abubakar Audu University Anyigba, Kogi State. Six graded concentrations of 0, 85.5, 90.5, 95.5, 100.5 and 105.5 mg/l of the aqueous extract were applied to Clarias gariepinus juveniles in plastic containers. The 96 hours LC50 values (with 95% confidence limits) estimated by probit analysis was 90.241 mgl-1.Fishes exposed to extract of P.biglibosa leaf (0.0, 85.5, 90.5, 95.5, 100.5 and 105.5 mg/l) for 96 hours revealed that aqueous extract of Parkia biglobosa leaf causes alterations in various blood parameters. Red blood cell (RBC) and white blood cell (WBC) counts, haemoglobin concentration and haematocrit values were decreased. The toxicity of the aqueous extract of the plant against Clarias gariepinus was both time and dose dependent. It is thought that this plant extract, the leaf and other parts such as the bark and the roots, would be useful in aquaculture to eradicate predators and competing wild fish from nursery, rearing and stocking ponds prior to the stocking of commercially important fry and fingerlings of desired species

Key words:
African catfish, haematology,
leaf extract, Parkia biglobosa,
water quality

out on various toxicants with Clarias sp. (Ani *et.al.,* 2017). Parkia biglobosa (Jacq), African Locust Beans tree is a perennial deciduous tree of high commercial value; it is used as a source of food, medicinal agents, timber, fuel and fibre (Orwa, *et al.,* 2009). In West Africa, the seeds of P. biglobosa provide a rich source of vegetable protein for humans; the husks are not eaten by humans, but they serve as feed for livestocks (Ajibade and Soetan 2012). The fruits are fermented to a condiment called 'dawa-dawa' or 'iru' used as a flavour intensifier for soups and stews (Ladokun and Adejuwon 2013). The extracts from P. biglobosa have been used as natural pesticides against different pests and diseases due to the phytochemicals present in different parts of the plant. The study therefore investigate the hematological changes in C.gariepinus exposed to P. biglobosa leaf extract as a natural cure for fish disease

Experimental Fish: Two hundred healthy Clarias gariepinus juveniles (mean weight of 18 ±0.02g) were purchased from Ifeoma farms, Lokoja, Kogi State. The fishes were acclimatized for two weeks under laboratory conditions, during the period, fish were fed 3% of their body weight with Blue Crown commercial fish feed (2mm)

Blood Sampling: Haematology analysis was conducted on the fishes from each treatment in triplicate after the acute toxicity bioassay. The fish used were thoroughly washed according to the procedure of Ayoola (2018) to avoid contamination before their blood samples were collected. They were bled from caudal artery by severing the caudal fin and the blood were collected into anticoagulant bottle (EDTA bottle) labeled to represent each treatment (Ayoola 2018). All the samples collected were taken to Biochemistry Laboratory, Kogi State University, for Haematological analysis using Sysmex (KS-21N) digital. Parameters determined include direct measurement of erythrocyte otherwise known Red Blood Cells (RBCs) (10^{12}/L), White Blood Cells (WBCs) (10^{9}/L) Packed Cell Volume (PCV)(g/L) and Platelets. Haemoglobin (Hb) (g/L), Mean Corpuscular Haemoglobin Concentration (MCHC) (g/L), Mean Corpuscular Haemoglobin (MCH) (pg) and Mean Corpuscular volume (MCV)

Haematological Analysis

Experimental set-up: Extracts was introduced into 18 circular plastic containers initially filled with 20 liters of dechlorinated, bore hole water at a concentration of 105.50, designated as (T6), 100.50 (T5), 95.50 (T4), 90.50 (T3) and 85.50 (T2) mg/L and 0.00 (T1) mgL-1 served as control. Each concentration had 3 replicates. Fish was randomly be selected and stocked at 10 juveniles per container. Fish was starved for 24hr prior to the commencement of the bioassay and throughout the exposure period which lasted 96hr.

MATERIALS AND METHODS

Study Location: The study was conducted at the water quality laboratory of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Prince Abubakar Audu University, Anyigba, Kogi State, located at latitude 7024'16''N and longitude 7037'50.6''E., within guinea savannah middle belt zones of Nigeria. The distribution of trees, grasses and other things in the area is determined by factors such as; fire, demographic pressure, patterns of cultivation, clearing and relief. Trees found in Anyigba do adapt to dry conditions (deciduous) and they shed their leaves in the dry season to control evapotranspiration (Ifatimehin et al., 2012).

Sample Collections and Preparations: Fresh samples of Parkia biglobosa leaf was obtained from Anyigba, Dekina, Kogi State, washed and air dried to constant weight. The dried samples were pounded using a clean laboratory mortar to a fine powder which was then sieved through 0.25 mm sieve. 500 g of the resultant powder was dissolved in two litres of water at room temperature (23± 0.5°C) for 24 h. The extract was filtered using a sieve.

Mean Corpuscular Volume (MCV)

(fL) and Procalcitonin Test (PCT) were calculated according to the formulae described by Fagbenro et al. (2020) and Oladimeji et al. (2022).

(%) = Hb/PCV× 100.

The following were determines as;

Haemoglobin (Hb)

= PCV (%)/3.

Mean Corpuscular Haemoglobin Concentration (MCHC)

Mean Corpuscular Haemoglobin (MCH)

(pg) = Hb/RBC× 10.

(fl) = PCV/RBC× 10

Statistical Analysis: Data collected were subjected to one-way analysis of variance (ANOVA) using SPSS version 20. Significant means were set at $p<0.05$ and separated using Duncan multiple range test. Data also on LC50 (96hrs) were analyzed using the probit-logit transformation method

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Water Quality Determination: During the experiment, physico-chemical parameters of bioassay water namely temperature, hydrogen-ion concentration (pH), total dissolved solids (TDS), electrical conductivity (EC) and dissolved oxygen (DO) were monitored (Okeke et, al,.2020). These were measured during the acute toxicity test. Temperature was measured using mercury in glass thermometer and is calibrated in degree Celsius (oc). pH was determined using pH meter (pH model 2602), while electrical conductivity and total dissolved solids were determined using Hanna “Combo” portable hand instrument (Hi 98129 Hanna Instruments, Mauritius). DO (mg/l) was measured using digital oxygen meter (portable DO model JPB 607).

Haematological Analysis: Results of the haematological analysis (Table 1) shows significant differences ($P<0.05$) in the values of MCH, MCHC and WBC. In MCH, T3 was the highest with 33.37 and the lowest is 19.64 in T5. In MCHC, T5 was the highest with 33.47 while T6 was the lowest with 33.30 mean value. The analyzed result of haematology is shown in table 1.

RESULTS

Table 1 : Haematological analysis of *Clarias gariepinus* exposed to aqueous solution of *Parkia biglobosa* leaf extract

PARAMETERS	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
PCV(%)	10.00±1.41a	8.50±0.70a	11.00±4.2a	10.00±2.8a	8.50±0.71a	8.50±2.12a
RBC(10^6 cells/μL)	1.67±0.23a	1.42±0.13a	1.77±0.75a	1.66±0.49a	1.45±0.17a	1.42±0.35a
HB(g/dL)	3.34±0.47a	2.84±0.24a	3.67±1.41a	3.34±0.94a	2.85±0.25a	2.83±0.71a
MCV(fL)	60.22±0.31a	60.02±0.01a	62.31±3.13a	60.54±0.76a	58.67±1.91a	59.86±0.04a
MCH(pg)	20.03±0.04b	20.00±0.10ab	20.92±0.087a	20.19±0.27ab	19.64±0.56b	19.93±0.02ab
MCHC(%)	33.35±0.02ab	33.41±0.04ab	33.37±0.01ab	33.36±0.04ab	33.47±0.13a	33.30±0.01b
WBC(10^3cells/μL)	5465.00±1187b	3222.50±837.c	3570.00±763.c	6375.00±1212a	6715.00±636.a	2660.00±912.c
PCT(mm)	109.91±15.98a	109.77±0.53a	144.36±28.17a	118.10±12.59a	132.77±11.99a	129.65±12.07a

The value is expressed in mean and standard deviation. Values with different letter superscript in the rows are not significantly different (p>0.05).

Keys: PVC = Packed Cell Volume, RBC = Red Blood cell, Hb = Haemoglobin, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemogblobin, MCHC = Mean Corpuscular Haemogblobin Concentration, WBC =White Blood Cell, PCT =Procalcitonin Test.

Water Quality Parameters: The physiochemical parameters of the test water measured during acute toxicity bioassay; it can be seen that there was significant difference in all the parameters except in the total dissolved solids. pH was highest in control concentration (T1) (6.90) while the lowest is seen in T6 (6.10); Temperature was highest in T5 (28.43oc) and lowest in the control T1 (27.20oc); Electrical conductivity (µS/cm) was lowest in 841.33 in the control concentration while the highest is seen in 991.33 in T6; Dissolved oxygen was highest in the control concentration T1 and lowest in concentration of T6 This is shown in table 2. Below.

Table 2. Water quality parameters during the ex periment.

Water Quality Parameter Treatment (mg/L)	**Ph**	**Temperature (oc)**	**Electrical Conductivity (µS/cm)**	**Total Dissolved Solids (mg/L)**	**Dissolved Oxygen (mg/L)**
Control	6.90±0.00a	27.20±0.01f	841.33±0.00f	420.33±0.00	3.91±0.00a
85.5mg/L	6.41±0.01b	27.43±0.00e	870.00±0.01e	434.67±0.00	3.56±0.00b
90.5mg/L	6.34±0.01c	27.65±0.00d	912.33±0.00d	456.33±0.00	3.55±0.01c
95.5mg/L	6.27±0.01d	27.86±0.00c	919.67±0.00c	465.33±0.00	3.42±0.00d
100.5mg/L	6.17±0.00e	28.43±0.00a	953.33±0.00b	478.67±0.00	3.24±0.00e

105.5mg/L 6.10±0.01f 28.30±0.00b 991.33±0.00a 496.67±0.00 3.10±0.00f

Mean in the same column with different superscripts differ significantly ($p<0.05$).

DISCUSSION

The result showed haematological changes in the blood parameters. The changes in blood variables suggest that there was osmotic disturbance and change of oxygen carrying capacity during the exposure of Parkia biglobosa leaf extracts. Similar values were reported for RBC, haemoglobin and hematocrit when fish was exposed to fiazinon (an organophosphate pesticide) (Banae et al. 2011). This was related to destruction of cells and/or decrease in size of cells due to adverse effects of pesticide. Zaki et al. (2009) also reported a decrease RBC count, haemoglobin concentration and packed cell volume (PCV) values in the fish exposed to extract of medicinal plant. Okeke, et.al. (2020) reported decreased haemoglobin, RBC count and haematorit values in Clarias gariepinus exposed to lead nitrate. Toxicants might cause adverse effect on the haemotopoietic organs which reduces the supply of RBC either due to less production and/or increased rate of removal from circulation. Fall in the level of haemoglobin may be the consequence of toxic effects of the extracts on the synthesis of this molecule. The Parkia biglobosa leaf may disrupt the synthesis pathway by affecting the activity of enzyme involved in the synthesis of haemoglobin. Blood cell indices seem to cause change that are more sensitive and cause reversible changes in the homeostatic system of fish. Fluctuations in these indices correspond with value of RBC counts and haemoglobin concentration. A similar respond was noted in common carp and other freshwater fish exposed to acute toxic level of pesticides (Rao, 2010). Physico-chemical parameters of the test water measured during acute toxicity bioassay were within suitable range for the survival and normal growth of C. gariepinus. Hence changes in fish behavior and subsequently death could not have arisen from poor water quality of the test water. On the optimum pH scale for fish growth developed by Badiru (2005), the range for this study (6.10-6.90) corresponds with the desirable range (6.5-9) for the fish production. However, dissolved oxygen range for this study (3.10-3.90mg/L) spans the range for slow growth following long term exposure (1-5mg/L) of the dissolved oxygen scale for warm water fishes by Badiru (2005). Similarly, the temperature range for this study (27.20-28.43) is within the normal range of temperature in the tropics to which fish are

CONCLUSION

This plant (Parkia biglobosa) is recommended to be used as a biological control in eradicating predators and unwanted organisms in the ponds by farmer instead of using agrochemical, but using for obnoxious methods of fishing (poison) in the wild should be discouraged and caution should be observed when it is being used as insecticides.

This result shows that aqueous extract of P. biglobosa leaf caused some clinical haematological change and ultimate death when exposed to C. gariepinus juvenile. This mean that the extract of P. biglobosa leaf equally have pesticide property like the extract of the barks of the same plant on C. gariepinus and can therefore, be used to eradicate unwanted fishes from ponds prior to the stocking of desired fish species. One can also deduce from this research work that the introduction of P. biglobosa into water bodies would threaten the life and existence of fish

RECOMMENDATION

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adapted (22-350C) as reported by Okeke et.al, (2020).The fact that mortality of fish during acute bioassay was concentration dependents could be directly linked with the direct effect of the toxicant. This is similar to the finding of Babalola and Oni (2018) where a direct relationship between mortality in C. gariepinus and concentration of diethyl phthalate was recorded as dose and time-dependent in such that as the exposure time increased from 12 to 96 hours, the median concentration reduced. The lack of mortality in the control group was also reported by Ayuba and Ofojekwu (2002) who exposed Clarias gariepinus fingerlings to the acute toxicity of the root of Jimsons weed (Datura innixia) seed extract. The concentration dependent nature of fish mortality in this study also agreed with the work of Fafioye et al. (2004) who exposed C. gariepinus to P. biglobosa extracts. However there is difference in the result of the present study and that of Abalaka and Auta (2010), which may be due to the difference in age, parts of the plants used (toxicants) and environment conditions. The result of this investigation agrees with the earlier works of Oti (2002) and Oshode et al. (2008).when they exposed fish to acute concentrations of different toxicants. This is similar to the findings of Okomoda and

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