Effects of Hypoestes forskalei Schult Roem leaf extract on the behavior of *Clarias gariepinus.*

Ubaha G.A[1] , Idowu B. A [1]. and Omoniyi I.T [2]

1.Department of Biological Sciences, University of Agriculture Abeokuta.
2.Department of Aquaculture and Fisheries Management, University of Agriculture, Abeokuta.
E mail: gaubahamrs@gmail.com

ABSTRACT: The e**ffects of *Hypoestes forskalei Schult Roem* leaf extract with** piscicidal **activity on *Clarias gariepinus* used for fishing was studied. F**ingerlings, juvenile and adult of the African mud cat fish (*Clarias gariepinus)* were collected and kept at 27^0 C to acclimatize, and their standard length and weight were taken. *Hypoestes forskalei* extract was obtained by water extraction method from its leaves and stem. Various concentrations of the plant extract were prepared as bioassay media and water qualities parameters were determined before and after the experiment. Freshly prepared concentrations of the plant extract were introduced into the water containing 10 fishes each while the haematological analysis of the fish were also determined accordingly**.** The temperature of the control water was the least at 27^0C while water sample treated with the highest concentration of plant extract recorded the highest value of 28.8^0C with significant reduction of the pH of the water and the dissolved oxygen concentration in the water medium significantly decrease as concentration of the plant increase. All the fishes were rendered immotile for about 8 seconds followed by irrational behaviours. Mortality decrease with decreasing concentration of the plant extract in all the stages of the treated fishes. Haemoglobin concentration, PCV and RBC count of treated fishes irrespective of their developmental stages were markedly lower when compared to the control while the WBC count recorded in most of the treated adult fishes were lower than those recorded in juvenile and fingerlings. *Hypoestes forskalei* is a plant with high piscicidal properties and highly toxic to aquatic lives and can induce long term health hazards on the internal organs of the fish consumers.
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INTRODUCTION

Since prehistoric times, various cultures throughout the world have used piscicidal plants for fishing. Plants are regarded as inexhaustible sources of structurally diverse and biologically active substances (1). Fossil record dates back the use of plants by man for various purposes including medicinal use (2). However, their uses as poison for obtaining fish from water bodies is of great concern because of their adverse effects on the aquatic organisms especially fish. Most fish poisons also called icthyotoxins or piscicides has been identified in related plant species, a variety of chemicals found in these plants will stun fish when it passes through the gills or in some cases when ingested making the fish to float to the surface for easy capture (3).The chemical also caused destruction of the erythrocyte membrane and the primary and secondary lamellae of the gill filament (4). There are various methods to capture fish from water. These include the use of hooks, net, setting of traps with baits, use of chemicals substances and the use of plants and plant products (5, 6, 7).These methods seem cheap and affordable hence commonly practiced by fishers folk all over the world. *Hypoestes forskalei* (Roem Schult) of the family *Acantheceae* is one of such plant with piscicidal properties which is being used by the local fish farmers in Cross River State of Nigeria to kill fish. Therefore, the effect of the use of the extract of *H. forskalei* on the behaviour of the fish was studied.

MATERIALS AND METHOD

COLLECTION OF THE FISH: Three stages (fingerlings, juvenile and adult) of the African mud cat fish (*Clarias gariepinus)* were used in this study and collected in plastic bowls and kept in 20 litres glass aquaria (60 x 30 x 30cm). The fish were kept under normal temperature (27^0 C) and were allowed to acclimatize for seven days during which they were fed twice daily with compounded food while the water in the aquaria was changed every other day. Total mortality during this period was recorded and the standard length (SL) of the experimental fish was measured using a meter rule. The weight was taken using triple beam balance and recorded.

COLLECTION OF PLANT MATERIALS: Fresh leaves and stems of *Hypoestes forskalei* were in a sac and oven dried to a constant weight using Gallen Kamp Oven at 60^oC for 24hours. The dried materials were grounded with the aid of sterile mortar and pestle and then stored in an air tight dry container at room temperature.

PREPARATION OF THE STOCK SOLUTION: 500g of the grounded material was weighed and wrapped in a clean dry white Muslin bag and soaked in one litre of distilled water for a period of ten hours to obtain the stock solution. Various concentrations of 500mg/l, 250mg/l, 125mg/l, 62.5mg/l, 31.25mg/l,15.6mg/l, 7.8mg/l and 3.9mg/l were prepared from the stock solution while the control was also set up without the plant material (0.00mg/l). The bioassay media were replicated.

COLLECTION AND DETERMINATION OF WATER PARAMETERS: The following water qualities parameters were determined; temperature, pH and dissolved oxygen content, before and after the experiment. The temperature was taken both at the point of collection of the water and at the project location using thermometer. The pH of the water was determined using the digital pH meter (Jenway) while, the dissolved oxygen content of the water was determined using Azide winklers titrimetric method.

APPLICATION OF THE TOXICANT: Freshly prepared concentrations of the plant extract were introduced into the water in the aquaria containing 10 fishes. For each concentration, 500ml was added to 20 litres of water and the fish were observed for 48 hours and the behavioral reactions and time of mortality were noted and recorded accordingly.

HAEMATOLOGICAL AND HISTOPATHOLOGICAL ANALYSIS: At the end of the experiment, the blood was taken from the region of the heart using sterilized needle and syringe from both the experimental and control into heparinized tubes to prevent coagulation and labeled appropriately. Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) and Heamoglobin (Hb) were determined according to methods described by Blaxhall and Diasley (1973) (8). The mean value of the two fish were taken from each of the experimental media and recorded.

The animals were then dissected and the internal organs such as kidney, liver, heart, gills and brain were collected and stored in 1% formalin for histopathological studies.

Statistical analysis: two-way analysis of variance (ANOVA) was used to determine the significance the test.

RESULTS:

EFFECT OF TREATMENT ON WATER QUALITY PARAMETERS: Table 1 shows that the temperature of the control water was the least (27^0C) while water sample treated with the highest concentration of plant extract recorded the highest value of 28.8^0C. There was significant increase in temperature of the water in the tank as the concentration of plant extract increased. The hydrogen ion concentration (pH) of the water was observed to significantly reduce with increasing concentration of the plant extract indicating a slight acidity. The control recorded the highest pH of 6.5 while water medium treated with 500mg/l recorded the least pH of 6.1. Dissolved oxygen concentration in the water medium significantly decrease as concentration of the plant extract while least concentration was recorded in water medium treated with 500mg/l of the plant extract, just as control recorded the highest value of 9.1.

Table 1. WATER QUALITY PARAMETERS OF TREATED WATER SAMPLES

Conc. (mg/l)	*Dissolved oxygen (Mg/l)*	*Ph*	*Temp. (^{0} C)*
Control	9.1^a	6.5^a	27.0^d
31.25	8.6^a	6.4^a	27.7^c
62.5	7.8^b	6.39^b	28.0^c
125	6.4^c	6.3^c	28.4^b
250	4.5^b	6.2^d	28.5^a
500	3.4^e	6.1^d	28.8^a

Values with similar superscript denote no significant difference

Values with dissimilar superscripts denote significant difference

All the fishes irrespective of their developmental stages responded immediately to the presence of various concentrations of the plant extract and were immotile for about 8 seconds. Then, they settled at the base of the aquarium for about 5-7 minutes, followed by violent and random movement in the water coupled with several attempts to jump out of the water. Irrational behaviours were later observed such as restlessness, fast backstroke movement and rapid respiratory movement. The duration of fish reactions to the presence of extract varies with the concentration of the extract applied and the developmental stages of the fishes while the time recorded for first mortality in all the stages of the treated fishes, increased with decreasing concentration of the plant extract as shown in Table 2 below.

EFFECT OF TREATMENT ON HAEMATOLOGY OF FISHES: Haemoglobin concenterations of treated fishes irrespective of their developmental stages were markedly lower when compared to the control. The PCV and RBC count of all treated fishes irrespective of their developmental stage; were markedly lower than the control while the WBC count recorded in most of the treated adult fishes were lower than those recorded in juvenile and fingerlings fishes treated with corresponding concentration of the plant extract as shown in the graph below.

Statistical analysis revealed that, there was significant difference between the duration of reaction recorded for the fingerlings and juveniles when

compared to the duration of reaction of the adult fishes (P > 0.05). Time recorded for the first mortality in the fingerlings was significantly higher (P>0.05) than those recorded for the juvenile and adult, while duration for total mortality was higher in adult fishes than the fingerlings and juvenile (Table 3).

Table 2; Duration of reaction and mortality of various developmental stages of the *Clarias gariepinus* fish

	Adult fishes		*Juveniles*		*Fingerlins*	
Concentration (Mg/l)	**Time recorded for 1st mortality (sec)**	**Duration for total mortality (min)**	**Time recorded for 1st mortality (sec)**	**Duration for total mortality (min)**	**Time recorded for 1st Mortality(sec.)**	**Duration for total mortality (min.)**
0.0	0.0	0.0	0	0	0	0
500	18	76	15	23	12	20
250	26	60	25	35	24	29
125	40	90	32	44	25	35
62.5	90	90	31	60	24	42
31.25	0	0	40	90	40	90
15.62	0	0	70	180	110	2880
7.8	0	0	90	0	118	2880

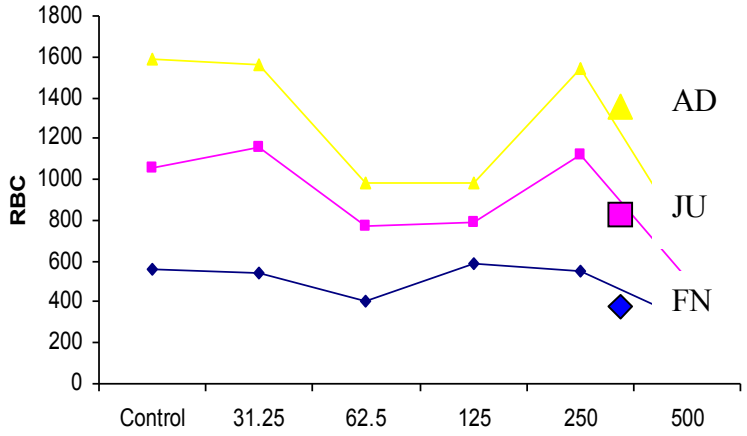

Fig. 1: Plot of Red Blood Cells of different stages of *Clarias gariepinus* treated with different concentration of *Hypoestes forskalei*

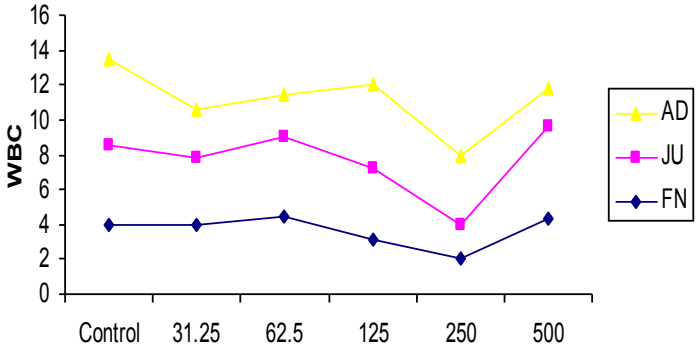

Fig 2; Plot of White Blood Cells of different stages of *Clarias gariepinus* treated with different concentration of *Hypoestes forskalei*

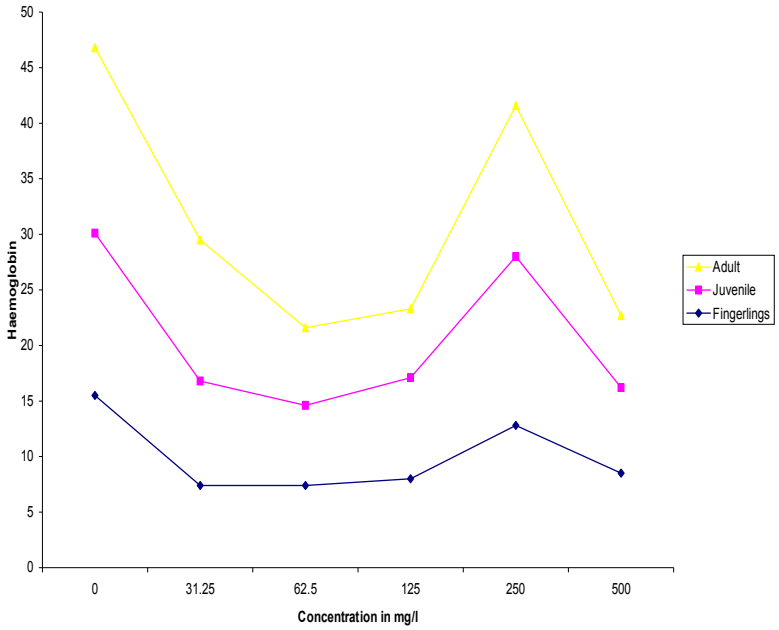

Fig. 3 Plot of Haemoglobin counts of different stages of *Clarias gariepinus* treated with different concentration of *Hypoestes forskal*

DISCUSSION

The effects of *Hypoestes forskalei* Schult Roem leaf extract on the behavior of *Clarias gariepinus* was studied**.** The fish used in the study displayed irrational behaviour on the application of the plant (*Hypoestes forskalei*) extract to different developmental stages of *Clarias gariepinus*. These include vigorous movement, fast back stroke movement, restlessness, increased opercular movement and jumping. These observations agree with the report of Agbon *et al,* (2002) (9), when Nile tilapia (*Oreochromis niloticus*) was exposed to aqueous extract of dry tobacco dust *(Nicotiana tobaccum*), the fish exhibited stressful behaviour such as erratic spiral movement and showed signs of respiratory distress suggesting that the reaction exhibited by *Oreochromis niloticus* might have been induced by the stimulant nicotine present in the tobacco extract. In addition, Ogbeibu *et al* (2002) (10), also reported that the skin of the experimental fish became darker in colour with the display of signs of respiratory distress with increased opercula movement. However, the skin of the fish treated with *H. forskalei* in this study show no observable change in skin colour. Again, the display of the irrational reaction by the fish may be due to the fact that *H. forskalei* contained a stimulant that affect the nervous system of the fish there by causing excitation, restlessness and jumping exhibiting rapid respiratory movement with increased opercula beat which could be attributed to respiratory distress (11).

Burkill in 1985 (12) reported that the active ingredients present in members of the family Acantheceae to which *Hypoestes forskalei* belongs include saponins and alkaloids. Kritzon (2003) (3) reported that alkaloids toxin belongs to a group called flavonoids which stun fish by impairing their oxygen consumption. The presence of saponins act on respiratory organs of the fish. The extract of *H. forskalei* contains alkaloids and saponins and therefore could have caused the same reaction as observed by Kritzon (2003). This may explain the various responses of the different developmental stages of *Clarias gariepinus* used in this study to the presence of various concentrations of the stimulant present in the plant extract. The signs showed by the experimental fish in this study are indications that mortality of the exposed fish may have been due to impaired respiratory activity. Alkahem *et al* (l3), reported similar obsevation on *Oreochromis niloticus* exposed to trichloroform while Omotoyin *et al* (14*), reported* similar observation with *Sarotherodon galilaeus* (Tilapia) fingerlings exposed to piscicidal plant extracts of *Tetrapleura tetraptera*. Fafioye (2001) (15) also reported similar changes in fish exposed to *Parkia bioglobosa* and *Raffia vinifera*. The behavioural change also agrees with the report from Jothivel and Paul (2008) (16) on the seeds of *Animirta cocculus* on *Clarias gariepinus.* The high level of

mortality of fishes of all developmental stages treated with different concentrations of the leaf extract of *H. forskalei* has confirmed that *Hypoestes forskale*i is a plant with piscicidal properties due to its ability to kill fish even at a very low concentration.

The application of the extract caused a proportional decrease in the pH level of the water and this decrease had been reported to be a major cause of fish death (17). The report on Cuyahoga river water quality monitoring programme (18) has also shown that low pH is harmful to immature fish and insects. This again probably explains increase mortality of fingerlings and juveniles treated with the plant extract at the lowest concentration. According to Haya (19), temperature usually affects the rate of metabolism and growth of aquatic organisms, rate of plant photosynthesis, sensitivity to diseases, parasites and toxic materials and this increase rate of death of fishes at higher temperature. It was observed that the application of the extract of *H. forskalei* caused an increase in the level of temperature and this may have caused the high mortality rate recorded by the various developmental stages of *C. gariepinus* treated with various concentrations of the extract. The marked increased in respiratory rate observed within the first two hours of this study suggests respiratory impairment probably due to hypoxic environment of the toxicant and the subsequent effects on the gill filament. Similar observations were made by Oti and Ukpabi (2000) (20) using milk extract of *Thevetia peruviana* on *Clarias gariepinus*. The PCV and RBC count of the treated fishes were markedly lowers than the control irrespective of their developmental stages. There was also reduction in haemoglobin concentration in all the treated fishes except at concentration of 250mg/l indicating a high deleterious effect to oxygen transport. These observations could suggest possible reason for the irrational behaviour, restlessness and the subsequent mortality observed in all the experimental fishes.

Conclusion:

From the study, *Hypoestes forskalei* is a plant with piscicidal properties and highly toxic to aquatic lives especially fish and the water body due to its negative effects on the water parameters (temperature, pH, dissolved oxygen level) and hence can disrupt the balance of the ecological system. Therefore, fishes killed by the application of this extract to water body, should not be consumed due to the tendency of the extract to induce long term health hazards on the internal organs of the consumers.

Corresponding e mail:
gaubahamrs@gmail.com

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