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Haematological Effect of Sub-Lethal Concentrations of Neem Leaves (*Azadirachta indica*) on *Heterobranchus bidorsalis*.

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Abstract

This study examined the impact of *Azardirachta indica* leaves extract on blood composition and flesh of *H. bidorsalis* under laboratory conditions over a 96 h exposure period. Concentrations of *A.indica* leaves extract used were 0.00 (control),5.00, 10.00, 15.00, 20.00 and 25.00mg/l. A total of one hundred and twenty juveniles of *H.bidorsalis* (wt.15.91 + 0.96g) were selected randomly at 10 fish per aquarium for the experiment with duplicate of each treatment.The extract led to increase in the opercular ventilation rates, erractic swimming, respiration distress in the test fish at the highest concentrations (20 and 25 mg/l). With the exception of PCV, Hb, RBC, the values of all the parameters in the control were lower than those in the treatments. WBC, MCHC,MCH and MCV values in the treatments increased with increase in concentrations of neem leaves extract. There is significant difference in the haematological parameters between in the control and the treatment levels ($P<0.05$). There was a significant reduction in the neutrophils of the fish exposed to 10.00, 15.00, and 25.00 mg/l of the extract. Significantly ($P<0.05$) low fat and low protein were observed while there was a significant increase in %Ash as the concentrations of *A. indica* leaves extract increased.

Key words : Neem leaves, *Azadirachta indica* , *Heterobranchus bidorsalis*,Haematology ,Leaves extract.

Introduction

Neem is a tree in the mahogany family meliaceae. It is one of two species in the genus azadirachta and is a native to India, Myanmar, Bangladesh, and Pakistan. It grows in the tropical and semi-tropical regions of the world.It is called the tree of the 40, as it is said to treat 40 different diseases (Ganguli, 2002). Neem contains various compounds that have insecticidal and medicinal properties; it contains salannin which has been proved to be an effective insect repellant. Neem is a medicinal plant containing diverse chemical active substances of several biological properties (Mamdouh et.al.,). Neem is bitter in taste; the bitterness is due to an array of complex compounds called triterpenes or more specifically limonoids. The most important bioactive principle is azadirachtin. The leaves are used as general antiseptics, treatment of urinary disorders, diarrhoea ,malaria fever and skin diseases. The anti-malaria action is attributable to gedunin, a limonoid.

Fish haematology continues to offer the potential of a valuable tool in evaluating fish. Some factors such as temperature change, stress, change in chemical composition of water, food and environmental factors (coldness or severe hotness of water) have adverse effects on fish health .

Haematological characteristics have been widely used in clinical diagnosis of diseases and measurement of heamaglobin concentration, heamatocrit, erythrocytes and leucocytes counts proved valuable in fish biology, in assessing the health of fish, and monitoring stress responses due to sub-lethal concentration and pollutant. The blood supplies each cell with the required water oxygen, nutrients and hormones, and the blood receives the waste products of matebolism for transport to the organs of excretion.

Pollutants can come from various sources: heavy metals, cyanide, chlorinated phenols, insecticides, herbicides, or sewage. In all cases of toxicant pollution, the course of the illness as well as the lesions is related to the amount of toxicant found in the water. The effect of the pollutant on the fish is variable. There may be direct damage to gills or skin leading to metabolic asphyxia. The pollutant may be concentrated within the body causing liver and kidney damage.

There are two ways that wild or cultured fish can accumulate toxic materials. One is by direct uptake from the water and the other is through their food. Any toxic materials can be taken up from water by the fish's gills. The large exposed surface area, the thin membrane, the profuse blood supply and the large volume of water that pass over the gills, all of which make

the gills very effective at removing oxygen, also make the gill a very efficient mechanism for filtering certain toxic materials from water. Toxicants that have caused the most problems in aquatic environments are those that are not easily broken down in the environment, because of their resistance to breakdown these materials tend to accumulate in the environment and can build up to very high concentrations in fish. This is especially true of toxic materials that are very soluble in fat, such as some of the insecticides and polychlorinated biphenyl (PCB) compounds. These materials are taken up very rapidly from water and food by fish and are accumulated in the fatty tissues (Evan and John 1980).

Materials and Methods

Juveniles of *H. bidorsalis* (mean wt. 15.91+ 0.96 g) of the same brood stock were collected from a fish farm in Akure, Nigeria and transported to the Research Laboratory of Fisheries and Aquaculture Department, Federal University of Technology, Akure, Nigeria in a well oxygenated container so as to reduce the stress which the fish went through. The fish were acclimated to laboratory condition for a period of 3 days in well aerated glass aquaria; thereafter the fish were randomly distributed at 10 fish per aquarium for the experiment. Two aquaria acting as replications for each treatment (concentrations) were set up.

The leaves of *A.indica* were plucked from the neem trees within the campus of Federal University of Technology, Akure, Nigeria .The leaves were air-dried for two weeks, the well-dried leaves were squeezed with hand before being ground with an electric grinding machine. The pulverised neem leaves were sieved, using a 0.1mm sieve and kept in desiccators. The fine particulate powder (100g) was dissolved in distilled water (1litre) for 24 h at room temperature (29°C) as described by Cruz et.al.,(2004) The mixture was filtered with filter paper and the extract (100 g/l) was used for the experiment. The following concentrations were prepared and introduced into each of the experimental aquaria: 0.0 (control) ,5.00, 10.00, 15.00, 20.00 and 25.00 mg/l in duplicates. The fish were examined at every 3 h for the first 24 h, then 6 h interval up to 48 h and every 12 h up to 96 h. All laboratory conditions were maintained and abnormal behaviour in the fish were recorded. Temperature, pH , dissolved O_2, salinity and conductivity in each aquarium were monitored during the exposure period (96 h) .

At the completion of the 96 h of exposure, 5–6 fish were removed from each treatment and about 5ml of blood was collected from the caudal peduncle using separate heparinized disposable syringes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant and centrifuged at 3000 rpm for 10 minutes. Packed Cell Volume (PVC), haemoglobin (Hb),White Blood Cell (WBC) , Red Blood Cell (RBC), Mean Corpuscular Haemoglobin Concentration (MCHC) , Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were determined differently using various appropriate laboratory methods as described by Blaxhall and Daisley (1973).

After 96 hours of the experiment, the fish were killed and oven dried at a temperature of 105°C for 5 hours. Dried samples of each treatment were ground and kept in nylon packages wrapped properly and throughout the period of analysis to prevent moist and dehydration from he environment. Proximate analysis was carried out according to the methods of A.O.A.C. (1990) to determine the protein, lipid, moisture and ash content of the samples.

The results obtained were subjected to one way analysis of variance (ANOVA) while mean separation was done using Duncan's multiple range test.

Results

The water quality parameters measured during the experiment period is presented in Table 1. There were slight fluctuations observed in the overall quality of the experimental water. There was a decrease in dissolved oxygen of the water from the control to the highest concentration of the leaf extract used in the experiment. The salinity and conductivity of the water did not really change.

The percentage mortality increased significantly with increasing concentration of neem leaves extract . No mortality was recorded in the control experiment. Opercular ventilation rates, erractic swimming, respiration distress were observed at higher concentrations (15.00., 20.00, and 25.00 mg/l) of the extract; such changes were not observed in control (0.0) aquaria and it was very minimal at lower extract concentrations (5.00, 10.00 mg/l).

PCV,HB .WBC, RBC, MCHC, MCH, and MCV of *H. bidorsalis* exposed to different concentrations of neem leaves extract showed significant haematological alterations and changes

after the 96 h exposure period (Table 2). It was observed that PCV (28.36 - 24.36%,), Hb (10.39-8.59gm/dl), RBC (4.39 -3.66µL) and MCHC (33.49-33.40gm/dL) of the fish exposed to neem leaves extract decreased as concentrations of extract increased (0.0 – 25.0 g/l) from the mean value. There is significant difference (P<0.05) in MCV and MCH while there is no significant difference in WBC as neem extract concentrations increases from the lowest concentration to the highest concentration.The result of the proximate analysis of the experimental fish is presented in Table 3. The analysis of variance shows that the effect of the neem leaves extract on the body of the experimental fish is highly significant (P<0.05) as indicated in all the parameters of the proximate analysis (including moisture, ash, fat, protein and carbohydrate).

Table 1: Water quality parameters obtained during exposure of the *H. bidorsalis* to 96 hours concentration of *A.indica* leaves extract.

Parameter	Control	5.00	10.00	15.00	20.00	25.00
Temperature	27.38±0.00a	27.39±0.34a	27.36±0.34a	27.37±0.33a	27.41±0.32a	27.42±0.32a
pH	6.99±0.01a	6.98±0.01a	6.97±0.00a	6.98±0.00a	6.93±0.01a	6.98±0.01a
DO2	5.83±0.01b	3.82±0.08a	3.72±0.07a	3.73±0.13a	3.72±0.08a	3.59±0.08a
Salinity	0.13±0.03a	0.65±0.51a	0.15±0.01a	0.15±0.01a	0.16±0.01a	0.16±0.01a
Conductivity	0.0029±0.00a	0.0031±0.00a	0.0031±0.00a	0.0031±0.00a	0.0031±0.00a	0.0032±0.00a

Means ± S.E on the same row with different superscript are not significantly different from each other (p<0.05)

Table 2 : Values of haematological parameters of *H. bidorsalis* juveniles exposed to different sub-lethal concentrations of *A. indica* leaves extract for 96 hours

Parameter	Control	5.00	10.00	15.00	20.00	25.00
Hb	10.39±0.04d	9.54±0.26c	9.58±0.08c	9.18±0.03ac	8.53±0.32a	8.59±0.07ab
MCHC	33.49±0.01b	33.17±0.03a	33.11±0.04a	33.30±0.10ab	33.16±0.09a	33.4±0.05b
MCH	22.83±0.30ab	23.89±0.04c	22.43±0.22bc	23.65±0.25a	22.51±0.44a	23.53±0.03bc
MCV	69.72±0.67ab	73.02±1.17c	68.69±0.29a	71.93±1.33ba	68.62±0.52a	70.61±0.11abc
PVC	28.36±0.15d	28.36±0.15d	27.01±0.01c	27.21±0.21c	25.73±0.28b	24.36±0.14a
RBC	4.59±0.07c	4.39±0.11c	4.24±0.04bc	3.88±0.20ab	3.83±0.07ab	3.66±0.13a
WBC	7825.0±575.0a	8390.0±1490.0a	8135.0±1135.0a	9105.0±205.0a	5209.0±3941.0a	5316.5±4033.5a

Means ± S.E on the same row with different superscript are not significantly different from each other (p<0.05)

Table 3 :Proximate analysis of *H. bidorsalis* exposed to *A. indica* leaves extract during the 96 h experiment.

Composition	Control	5.00	10.00	15.00	20.00	25.00
Moisture	6.19±0.02b	5.87±0.15a	10.84±0.05c	10.75±0.07c	10.74±0.07c	11.39±0.03d
Ash	14.91±0.03a	18.78±0.35d	16.31±0.08b	15.47±0.52ab	17.27±0.11c	19.59±0.17d
Fat	9.58±0.06e	5.40±0.20c	6.48±0.21d	3.39±0.01a	3.71±0.04a	4.22±0.02b
Protein	59.64±0.89ab	58.29±0.49ab	55.35±0.45a	60.88±0.78b	57.79±0.39ab	56.33±2.58a
NFE	9.68±0.84a	11.68±0.21a	11.03±0.12a	9.52±1.36a	10.49±0.54a	8.47±2.71a

Means ± S.E with different superscripts are significantly different from each other (p<0.05)

Discussion

The results obtained from this investigation revealed that sub-lethal concentrations of the leaves extract of *Azadirachta indica* did not necessarily result in outright mortality of the fish but may have significant effects which can result in several physiological dysfunctions in the fish. Hence concentrations of *Azardirachta indica* leaves extract higher than the one used in this investigation may result in total mortality of the fish as given in the range finding test carried out.

The changes in the water parameters of the various experimental media reported in this investigation indicated that the sub-lethal concentration of *Azadirachta indica* leaves extract did not adversely affect water quality. Where slight changes were observed, the values were all within tolerance range.

Exposure of *Heterobranchus bidorsalis* to sub-lethal concentrations of *A.indica* leaves also did not lead to death of the fish, but there was malfunctioning of their body system whichmay be due to the pharmacological properties that are present in *A. indica* leaves (Biswas et.al.,2002 and Das et.al., 2002). Exposure of *H. bidorsalis* to sub-lethal concentrations of *A. indica* caused a significant decrease in PVC, Hb, RBC and also MCHC with MCV. The significant reduction in these parameters is an indication of severe anaemia caused by *A.indica* leaves on the exposed fish.

In this investigation, there was a slight fluctuation in the lymphocytes of the exposed fish and this is attributed to the stimulation of the immune mechanism of the fish to eliminate the effects of the pollutants. This is also reported by Sampath et al (1993) in the increase of lymphocytes of the Nile tilapia, *Oreochromis niloticus* exposed to toxic environment. There was also a reduction in the neutrophil of the experimental fish exposed to *A.indica* leaves extract and a slight fluctuation in MCH. There was absence or a little change in monocytes and eosinophils of the fish. There was also a slight increase in the WBC.

In the proximate analysis the significant increase in % moisture, % Ash ,slight changes in the carbohydrates, decrease in % fat and % protein recorded may be as a result of chemical stress that the fish were exposed to during the 96 h of exposure to the various concentrations of A. indica leaves extract.This result agrees with Martinez et.al.,(2004) who reported that fish under stress may mobilize protein to meet every energy requirements needed to sustain increased of the physiological activities.

From the present study, it was observed that the application of *Azadirachta indica* in aquaculture could be dangerous to fish at high concentrations because of its active components that are repellent and disruptive to living organisms. Higher concentrations of neem leaves extract above the one used in the experiment will result in convulsion, somersaulting of fish, difficult in swimming and loss of balance in the fish, which will subsequently lead to death , if the present rate at which neem leaves are being used is not checked, the continuous existence of the aquatic fauna, including biologically important fish species, will be in serious jeopardy thus its safe use in aquaculture should be fully investigated.

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