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Acute Toxicity of Piscicidal Plant Extracts (*Adenia cissampeloides*) on Tilapia (*Sarotherodon galilaeus)* Juveniles

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Abstract: The piscicidal quality of water extracts of *Adenia cissampeloides* leaves on Tilapia (*Sarotherodon galilaeus*) juveniles was investigated in a static renewal bioassay to determine the median lethal concentration (LC_{50}) at 96hour of exposure. Five graded concentrations of 800, 600, 400, 200 and 100 mg/litre of aqueous solution of A. *cissampeloides* and a control 0 mg/10L were applied to *S. galilaeus* fingerlings in plastic tanks. The 96h LC_{50} of aqueous extract of *A. cissampeloides* to *S. galilaeus* under laboratory condition was 317mg/l. Behavioural changes such as erratic swimming, loss of reflex, hyper ventilation, increased surfacing frequency and jerky movements were observed prior to death. Histopathological changes in the liver of *S. galilaeus* juveniles observed are severe widespread vacuolar degeneration and necrosis, hepatocytes, hyperplasia and presence of large numbers of megalocytes. In the gills, there were denudation of gill filaments, swelling of chondrocytes and rarefication of cartilage within gill filament. These damages became severe with increasing concentration of the plant extracts.

Key words: Toxicity test · *Adenia cissampeloides* · *Sarotherodon galilaeus*

INTRODUCTION

In fish farming operation, it is a common management practice to eradicate predatory and competing wild fish from nursery, rearing and stocking ponds prior to the stocking of commercially grown fry and fingerlings of desired species. This is sometimes done by application of synthetic toxins including chlorinated hydrocarbons such as aldrin, eldrin, dialdrin and organophosphate like glyphosate and cypermethin. Indiscriminate use of these piscicides have been reported to pose a great risk to aquatic organisms, especially food fishes and consequently to humans [1].

The use of synthetic organic compounds in the control of aquatic system has been found to cause contamination of waterways thus endangering organism living in this system [1].

The use of plant piscicides is very common around the world from times immemorial for poisoning and stupefying fish [2]. These plant piscicides are biodegradable without any major side effects as observed with the use of synthetic chemicals.

Convectional Pesticides from natural products or phototoxic plants, such as tea seed cake and derris root are widely used and toxic effects of these have been documented [2-4]. All these convectional plant pesticides are however either not within the reach of the fish farmers or their uses may not be cost effective especially for farmers in developing countries such as Nigeria. There is therefore need for more information on the piscicidally useful plants which have been reported to be of great biodiversity in countries such as Nigeria where aquaculture is currently recording good growth [5]. *A.cissampeloides* is one of such plants with good piscicidal quality and the active ingredient of this plant is mainly alkaloids. Fafioye [5] reported the piscicidal quality of this plant however there is need for more information on the right dosage of A. *cissampeloides* that can be used in pond management without creating serious ecological imbalance in the pond ecosystem in relation to the fish.

Sarotherodon galilaeus is one of the most widely cultured and valued food fish among the tilapias. However due to their prolific breed ability and hardly nature, completely harvest and removal from the pond

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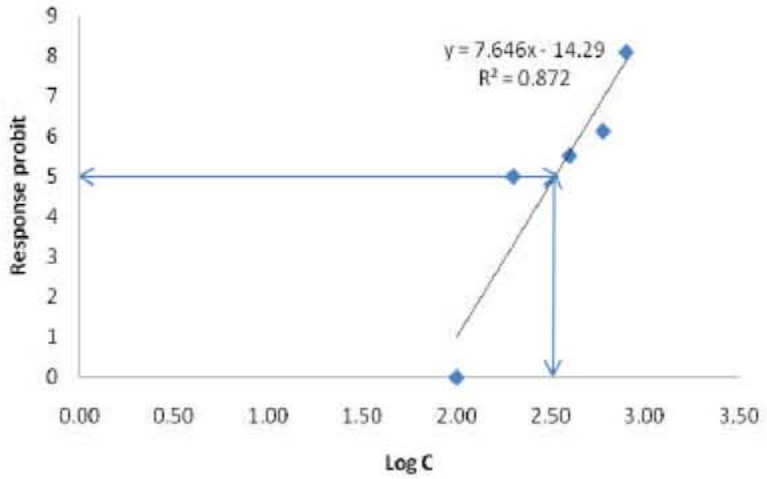

Fig. 1: Linear relationship between probit response and log concentration of Piscicidal plant extract (*Adenia cissampeloides*) on *Sarotherodon galilaeus* Juveniles

during pond preparation before stocking can create a serious management problem. Most farmers resort to the use of non degradable synthetic materials which often create more management problems that it is solving. Study into the ecological management of toxic biodegradable piscicidcal plants will go a long way in providing the needed solution in management problem.

This work was undertaken to investigate the piscicidal actions of *A. cissampeliodes* on *S. galililaeus* and also to examine toxic impacts of the plant on the liver and gills of the experimental fish.

MATERIALS AND METHODS

Collection of Species: The fresh samples of *A. cissampeloides* (Figure 1) were collected from University of Ibadan, Southwestern part of Nigeria. The area of collection was Technology road, located in the University of Ibadan, Nigeria.

The test fish *S.galilealus* fingerlings (mean weight, 2.2 g) were obtained from Departmental of Wildlife and Fisheries Management, University of Ibadan, Nigeria fish farm. These fish were acclimated to laboratory conditions for two weeks using 30 litres capacity plastic tanks (60cm x 45cm x 45cm). The tanks were filled with 10 litres of water, well aerated and kept at room temperature of 30°C. The fingerlings were unfed for 24 hours prior to and during the experiment.

Aqueous Extraction of *A. cissampeliodes* Plant: One hundred gram of *A.cissampeliodes* plants was weighed, rinsed and spread to drain (Plate 1). 1000 mls at 40°C of cold water was measured with the aid of the 100 ml measuring cylinder into a bowl. The *A.cissampeloides* plant was transferred into the bowl containing the 100ml distilled water and the plants were thoroughly squeezed with hand along with the 100mls distilled water containing in the bowl, so that the toxins in the plant could diffuse out turning the water into a green colour and the plants shrunken to its minimum size. The plants were then removed and squeezed again so that all the water in the plants could drain out into the bowl. The extract collected was afterwards filtered using net with fine mesh size to remove all sediments and little plants parts that might be in the extract. The filtered extracts were then transferred into different collecting bowls according to the desired concentrations.

Experimental Procedure: The aqueous extract of *A. cissampeloides* plant was measured. The range finding test was determined according to the method described by [6].

From the range finding test, five different concentrations of the toxicant were obtained for the definitive test. A complete randomized design was used in the experiment with 10 fish/10-litre freshwater.

Five concentrations of *A. cissampeloides* (100,200,400,600 and 800) were prepared and introduced into each of the experimental plastic tanks (1, 2, 3, 4, 5) Another experimental tank(6) without toxicant was employed as control. The experimental observations were performed at 3 hours intervals for 4 days (96 hours) for any abnormal behaviour and death. The behaviour of the fish was observed after the introduction of the plant extracts.

Plate 1: The Piscicidal plant-*A. cissampeloides*

The mortality was recorded daily up to 96hours and fish were considered dead if the gills did not respond to touching by a probe and floating belly up or sideways without movement, also sometimes, dead fish may sink to the bottom of the experimental tank. Dead fish were promptly removed and preserved in formaldehyde before cutting on slide for histopathological examination. Static-renewal method was used and the solution was changed to maintain concentration as described by [7].

Histopathological Test: Liver and gill tissues were collected from the dead fish and fish in the control treatment for histopathology tests. Fish were randomly selected and dissected to extract the tissues. Gills were preserved in 10% formalin and liver in Bouin's fixative. Tissues were processed, sectioned and stained with hematoxylin and eosin, using standard histological techniques.

Determination of Physico-chemical Parameters of Water Samples: Water quality was analyzed at the beginning and end of the experiment. Temperature was determined using mercury-in-glass thermometer calibrated in degree centigrade (°C). Horiba pH meter D-51 was used to measure hydrogen ion concentration. The electrode was directly dipped into the water samples and the readings were taken.

Dissolved oxygen was determined using Rex portable Dissolved Meter Model-JPB-607.

Statistical Analysis: The result obtained was subjected to One-way analysis of variance (ANOVA), Logic and probit analyses were also measured by using STATISTICA for windows XP on PC.

RESULTS

The linear relationship between probit response and log concentration of *A. cissampeloides* are presented in Figure 1. The 96 LC_{50} of *A. cissampeoides* was 317mg/l. When aqueous extract of *A. cissampeloides* was administered, *Sarotherodon galilaeus* juveniles were observed to be stunned for about 2-3 minutes and eventually swam away from the point at which toxicant was added to water. Fish exposed to different concentrations of aqueous extract of *Adenia cissampeloides* exhibited hyperactivity characterized by rapid and erratic swimming, occasional darting up and down water column, spiral movement and opercular movement. Other behavioural changes included rapid respiration and slow-down of reflexes when touched with a glass probe. In acute concentrations prior to death, juveniles aggregated at the air-water interface gasping for air with their mouths permanently opened. *S. galilaeus* juveniles exhibited these behavioural deviations and these were more pronounced with increasing concentrations. The colour of fish became progressively darker with increasing concentrations and fish sank to the bottom of the test container shortly before death.

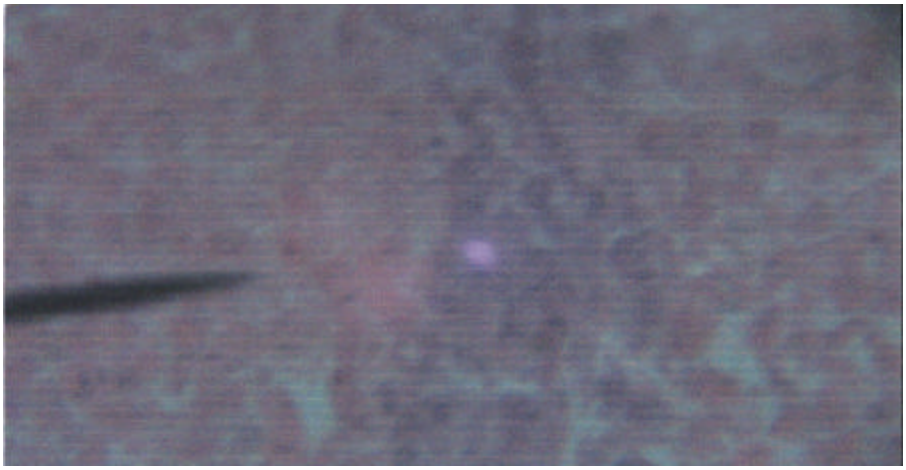

Plate 2: Liver of Tilapia exposed to 0mg/l(control)(mg:x100)

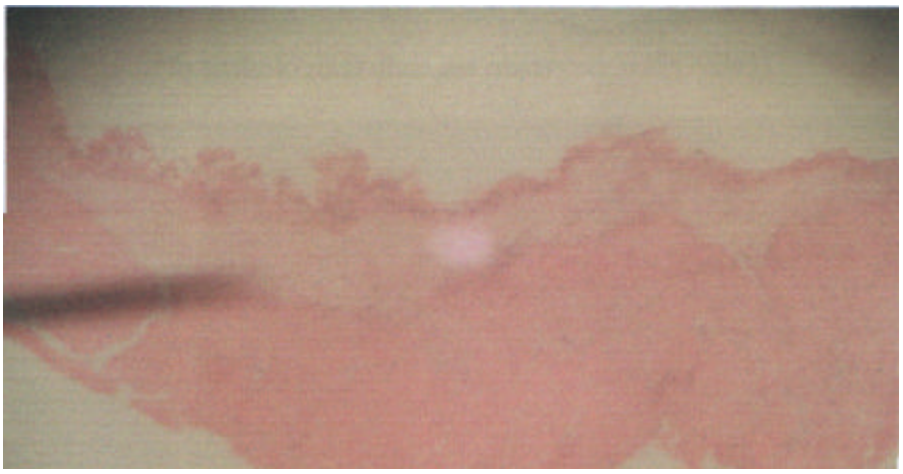

Plate 3: Liver of the test fish on exposed to 100mg/l of *Adenia cissampeloides* - No pathological changes observed (mg: x100)

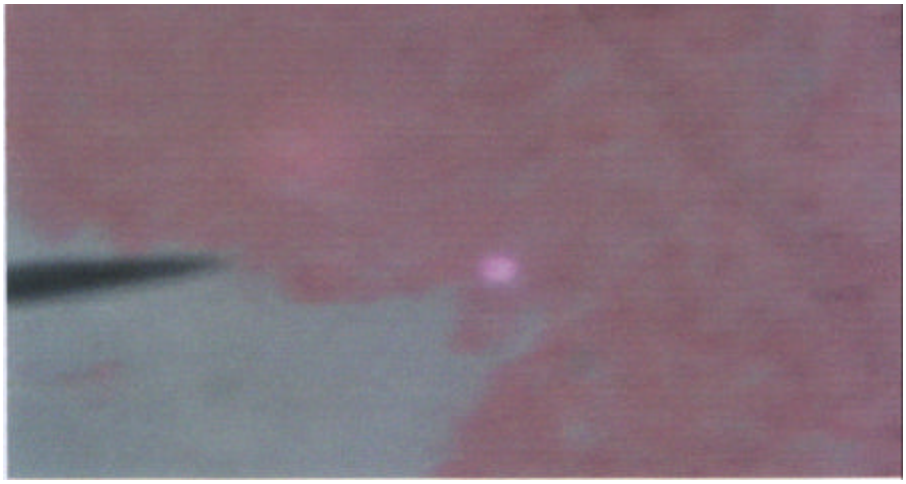

Plate 4: Liver of the test fish exposed to 200mg/l of *Adenia cissampeloides*-No pathological changes observed (mg: x100)

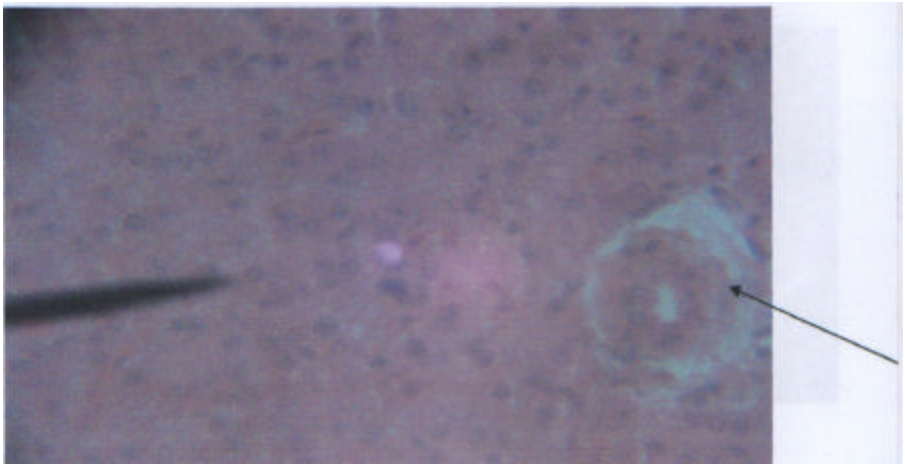

Plate 5: Liver of the test fish exposed to 400mg/l of *Adenia cissampeloides*-Severed wide spread vacuolar degeneration of Hepatocytes (Mg: x100)

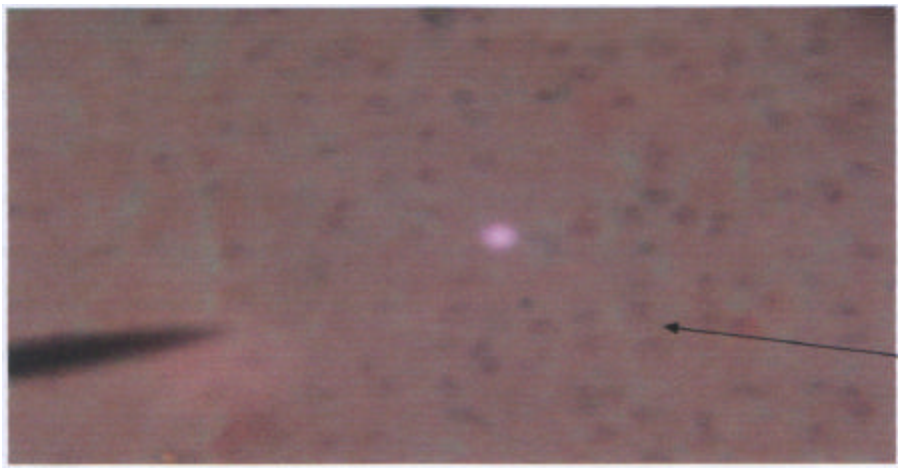

Plate 6: Liver of the test fish exposed to 600mg/l of *Adenia cissampeloides*-Severed wide spread vacuolar degeneration of Hepatocytes (Mgx100)

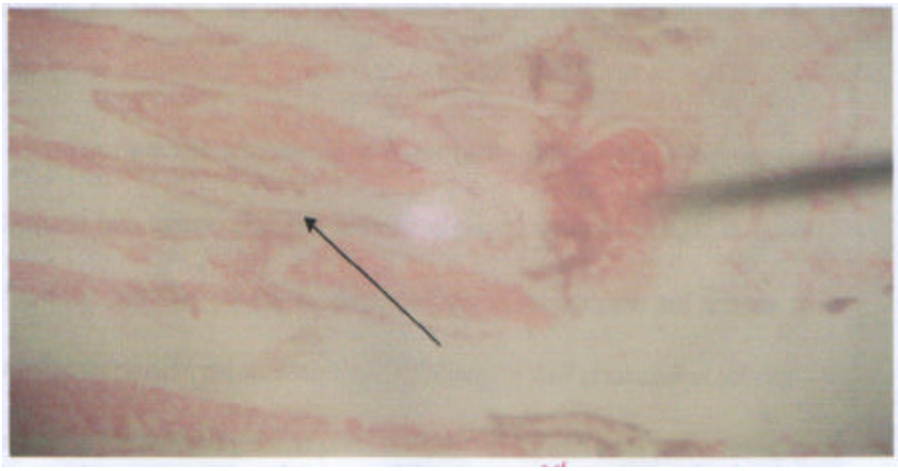

Plate 7: Liver of the test fish exposed to 800mg/l of *Adenia cissampeloides*-Severed wide spread vacuolar degeneration of Hepatocytes and necrosis (Mgx100)

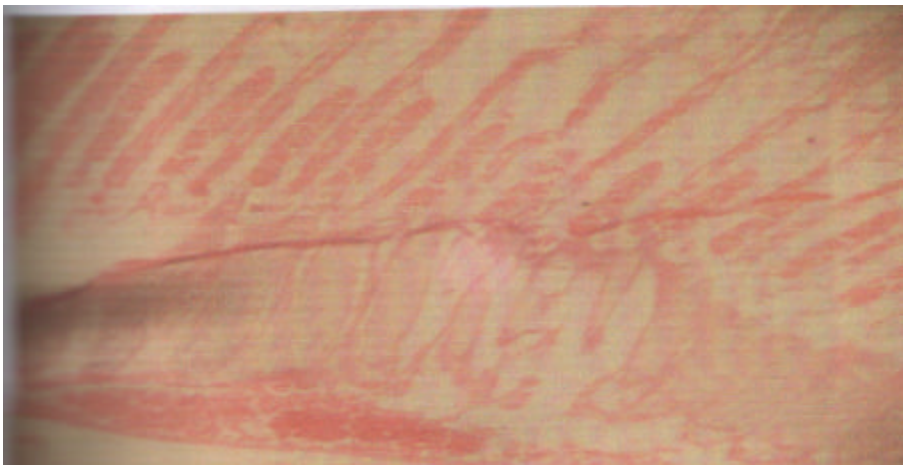

Plate 8: Gill of the test animal exposed to 0mg/l of *Adenia cissampeloides* -No pathological change observed (Mg: x100)

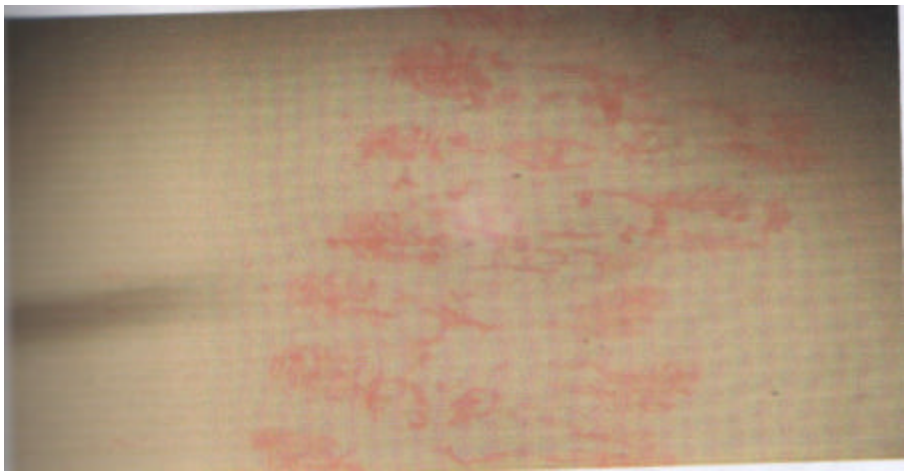

Plate 9: Gill of the test animal exposed to 100mg/l of *Adenia cissampeloides* -No pathological change observed (Mg: x100)

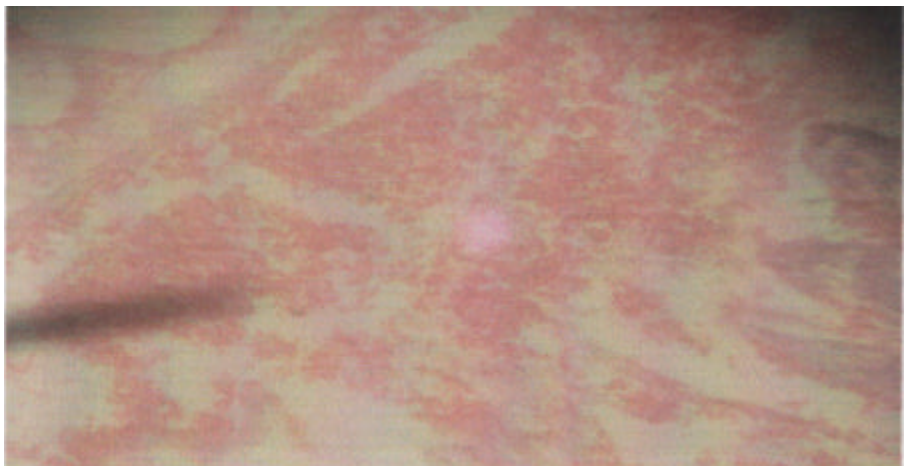

Plate 10: Gill of the test animal exposed to 200mg/l of *Adenia cissampeloides* -No pathological change observed (Mg: x100)

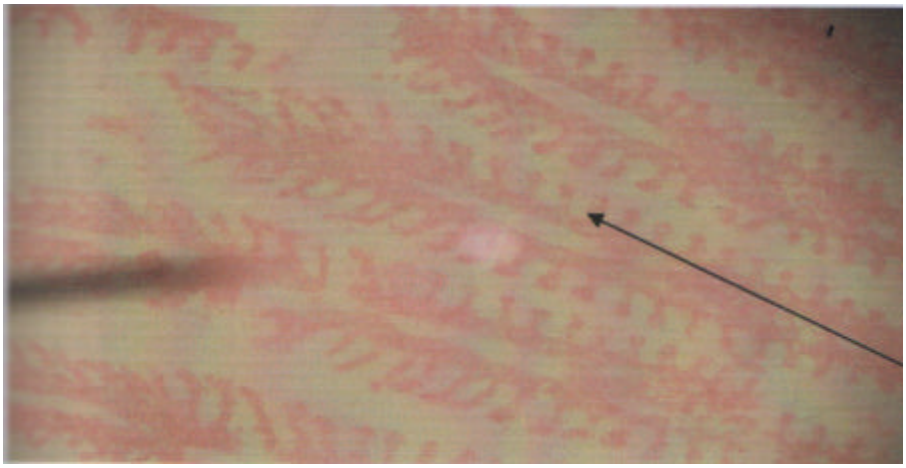

Plate 11: Gill of the test animal exposed to 400mg/l of *Adenia cissampeloides* -Severe heamorrhage, denudation and thinning of gill lamella (Mg: x100)

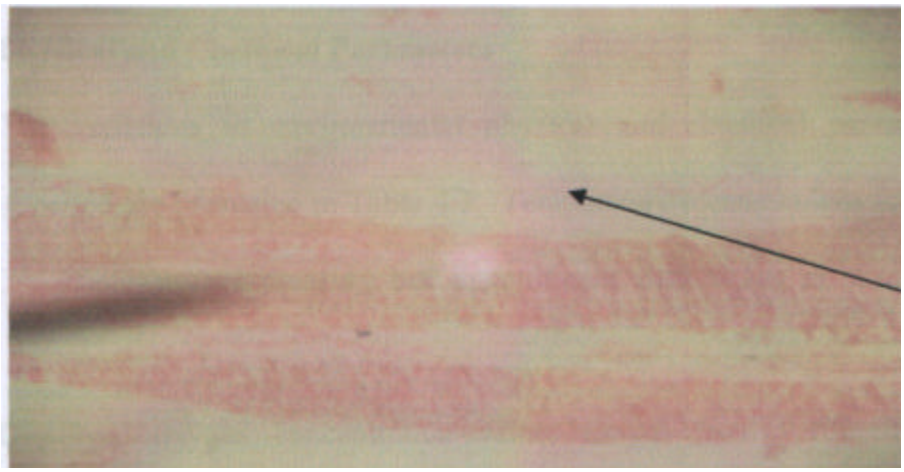

Plate 12: Gill of the test animal exposed to 600mg/l of *Adenia cissampeloides* - Denudation of gill filaments and swelling of chondroncytes (Mg: x100)

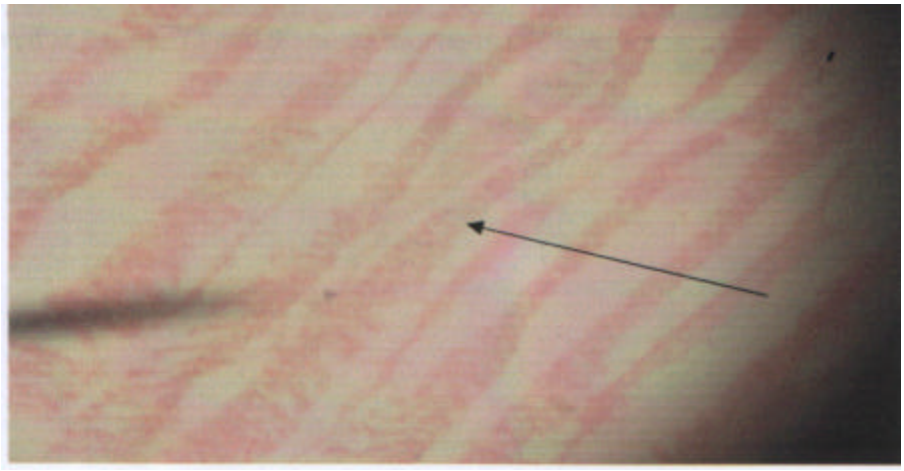

Plate 13: Gill of the test animal exposed to 800mg/l of *Adenia cissampeloides* - Denudation of gill filaments and swelling of chondroncytes (Mg: x100)

Table 1: Mean physico-chemical parameters of test concentrations of *Adenia cissampeloides* for 96hrs of acute toxicity test

Concentration (Mg/l)	Physico-chemical Dissolved O_2 Mg/ l	parameters pH	Temp°C
0.0	6.2±0.1	7.0±0.1	27.0±1.0
100	6.0 ±0.4	7.1±0.2	27.0±1.3
200	6.0 ±0.2	6.9±0.2	26.9±0.5
400	5.9 ±0.1	6.9±0.2	27.0±1.5
600	6.0±0.4	7.0±0.2	26.9 ±0.6
800	5.9±0.5	6.9+0.2	26.8± 0.3

Toxic Effects of Acute Concentration: Juveniles of *S. galilaeus* were generally very sensitive to acute concentrations of aqueous extract of *A. cissampeloides*. No mortality was recorded in the control (0 mg/l) and in 100 mg/l, 50% mean mortality in 200 mg/l, 70% mean mortality in 400 mg/l, 86.67% mean mortality in 600 mg/l and 100%. Mean mortalities of 50, 70, 86, 67% were recorded in the experimental tanks containing respectively 200, 400, 600 mg/l of toxicant. Finally, the 100% mortality was recorded at the of 96 hours in the tank with 800 mg/l of toxicant. A 96hours LC_{50} value for *S. galilaeus* juveniles obtained by probit analysis was 250 mg/l (Fig. 1).

Histopathological Changes in the Organs of Test Animals

Liver: The changes that occurred to the liver of the test animal on 96h exposure to varying concentrations of *A. cissampleoides* are presented in plates 2-7. The liver of unexposed or control fish had a reddish-brown colouration. It was however pale in specimens exposed to the concentrations of 100, 200, 400, 600 and 800 mg/l increasing with increasing concentrations. Histopathological investigations of the liver of unexposed control *S. galilaeus* juveniles revealed hepatocytes arranged in cords around central veins in all the lobules. No pathological changes were observed. Varying degrees of degeneration had however taken place in the liver of exposed fish. These ranged from widespread vacuolar degeneration of hepatocytes through necrosis to periportal fibrosis. Degenerative changes were however more pronounced in the highest concentrations while no pathological changes were recorded for the lowest concentration (100 mg/l).

Gills: The unexposed fish (control) gill filaments had a reddish colouration due to diffusion with blood capillaries. The gills of exposed fish were pale compared with unexposed (control) fish and this pale colouration increased with increasing concentrations in the gills (Plate 8-13). There was also accumulation of mucus on the gill filaments of exposed fish compared with control. The gills filaments of control fish were arranged as fronds (finger-like structures) on gill filaments, swelling of chrondrocytes and thinning of lamellae with swollen and oedematous gill filaments were some of the changes observed in the gills.

Physico-Chemical Parameters: The variations in environmental physical and chemical parameters for the sampling period are presented in Table 1.

DISCUSSION

The stressful and erratic behaviors of the *S. galilaeus* juveniles tend to show the feeling of respiratory impairment probably due to the effect of the toxicants on the gills. This agreed with [3] that who reported the clinical signs such as abnormal movement and high respiration rate induced by *Oreochromis niloticus* to *Derris elliptica* suggested neurological dysfunction and gills damage. The fishes became inactive at higher concentration for a much longer time. This is normal observation in acute and chronic toxicity test [4]. The initial reaction of the fish was to swim actively due to the effect of *A. cissampeloides* aqueous plant extract on the nervous system; an increased in the swimming activity increase with concentrations. The time of toxicity disappearance was observed from the record of relative median death time in different concentration of *A. cissampeloides* aqueous plant extract for 96hours. This is consistent with observation of earlier work with fish species with bimodal respiratory system [8] and thus is possible to use *Adenia cissampeloides* aqueous plant extract in selective eradication of aquatic organisms.

Physiological changes in fish exposed to toxic substances are observed in organs like the liver and the gills with considerable histopathological changes [1]. Fish showing severe gill epithelial hyperplasia, separation of the gill epithetial layers from supportive tissues, necrosis of liver hepatocytes gave the highest mortality while alteration of the gills leading to epithelial hyperplasia and

separation of the epithelial layer from supportive tissues are usually directly related to gill function disorders, which may affect the physiology or cause the death of fish [1].

With increasing toxicity, liver parenchymal necrosis, fatty degeneration, blood cell congestion and fibroses are specific liver lesions were prominent in the fish samples, this occurrence is usually linked to pesticides toxicity [3]. The histopathological alterations found in the gills and liver of the test fish may be linked to the toxic effects of the piscicide *(A. cissampeloide* extracts).

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