

## Piscicidal potential of mesocarp of neem plant (*Azadirachta indica* L.) fruit on hybrid, "Heteroclaris"

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(Received: May 19, 2006 ; Revised received: December 17, 2006 ; Accepted: January 30, 2007)

**Abstract:** The piscicidal potential of water-extract mesocarp of *Azadirachta indica* (L) was studied in static bioassay experiment with continuous aeration to determine its acute toxicity. The mortality rate and opercular ventilation under laboratory conditions over 96 hr exposure were monitored. The 96 hr LC<sub>50</sub> was 81.28 mg l<sup>-1</sup> while the threshold value was 21.13 mg l<sup>-1</sup>. The fish exhibited respiratory distress (such as gasping air), loss of appetite, loss of balance and erratic swimming prior to mortality.

**Key words:** *Azadirachta indica*, Piscicidal potential, Mudcatfish hybrid, Toxicity, Behavioural responses  
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### Introduction

Toxicology remains the science, which promises real opportunities to unravel some of the fascinating problems of biology by identifying chemicals and physical tools with which to probe living processes. Some of these chemicals are phytotoxic and piscicidal. The usefulness of plants for piscicidal and medicinal purposes has been reported (Akobundu, 1987; Adewole *et al.*, 2002; Sambasivam *et al.*, 2003; Rahman *et al.*, 2005; Singh *et al.*, 2005; Budak and Budak, 2006). The importance of some of these piscicidal plants is unique in the sense that their chemical compositions had enhanced their properties as medicinal plants, preservatives, insecticides, molluscicides to mention a few and in these ways have been useful to man and aquatic animals.

Due to their narcotic, pesticidal and molluscidal properties, many fishermen and fish farmers indiscriminately use various parts of these plant extracts to weaken and kill the fishes for easy catch and clean up the aquatic systems of some pests. Invariably stronger concentration than necessary are used and this could lead to physiological disturbance of the aquatic organism and ultimately reduction in aquatic productivity (Wareen, 1977; Mondal *et al.*, 2007).

Some of these plants used are non-selective in their destruction, thereby interfering with the ecological balance of the immediate environment. Oti (2003a) reported the acute toxicity of water extract of the bark of milk bush, *Thevetia peruviana* to African mud catfish hybrid (*Clarias gariepinus* X *Heterobranchius bidorsalis*) fingerlings. This plant was observed to increase the opercular ventilation of the fish as the concentration increased.

The neem plant, *Azadirachta indica* (L) of the Family Meliaceae and native of eastern Asia is a known medicinal plant that

contains margosine, eriterperenoid, azatin, rotinine and quinine among other active ingredients as reported by Ade-Serrano (1982) and Adewole *et al.* (2002). The leaves, barks, fruits and roots of the plant have been highly appraised for their medicinal purposes. As natural insecticide it contains tetranitroterpenoid compounds known as meliatoxins that are highly toxic to insects and mammals (Ascher *et al.*, 1992; Ascher, 1993). Omoregie and Okpanachi (1992) and Oti (2003 a, b) reported on the sublethal and acute effects of water extract of the bark of *A. indica* on *Tilapia zillii*, mudcatfish hybrid and African pike.

Jacobson (1989) and Schmutterer (1990), reported reduced longevity on rainbow trout exposed to Neem seed. It is generally accepted that the tetranitroterpenoid (also called limonoid) compound azadirachtin is responsible for the majority of biological effects observed in organisms exposed to Neem plant (Isman *et al.*, 1990; Mordue and Blackwell, 1993; Verkerk and Wright, 1993). Simmonds *et al.* (1990) reported that Azadirachtin contained 0.25% of margosin and 3% azatin. However, 25 different biologically active compounds have been isolated from neem seeds (Lee *et al.*, 1991). Little attention has been given to the fruit which usually drops into the river and fish ponds, since most aquaculturist admire the plant for providing shade. Our attention was driven to this due to mortality recorded in such ponds by local fish farmers who planted them as shady plants in their fish farms. In addition, animals like bats have often been seen to feed on the mesocarp of this plant.

Hence, the present study was conducted to evaluate the piscicidal potential of the mesocarp of *A. indica* fruit extract on the mud catfish hybrid (*C. gariepinus* X *H. bidorsalis*) commonly called 'Heteroclaris' which is widely cultured in Nigeria because of its remarkably fast growth rate and high market value.



## Materials and Methods

180 fingerlings of "Heteroclaris" ( $\delta$  *C. gariepinus* x  $\phi$  *H. longifilis*) (mean weight  $5.04 \pm 1.1$  g) were collected from the Hatchery Unit of Federal College of Freshwater Fisheries Technology, New Bussa, Nigeria for this study. The fish were acclimatized for one week in a water trough of dimension 2.0 m x 2.0 m x 1.0 m and were fed twice a day (08.00 and 14.00 hr) with a 40% crude protein prepared pebbled feed at 5% of their body weight. The fish were not fed for 48 hr prior to the exposure period, which lasted for 96 hr.

Methods of acute toxicity as recommended by UNEP (1985) were employed. Fruits of *A. indica* were collected from the premises of Federal College of Freshwater Fisheries Technology, New Bussa, Nigeria. The mesocarp was removed washed and sun dried to constant weight. The well-dried samples were pounded in clean mortar and sieved using 0.1mm sieve. 500 g of the fine powder was dissolved in 2 litre-distilled warm water of 32.3°C for 24 hr. The extract was filtered using whatman's filter paper no.1 using a vacuum pump. The filtrate was freeze-dried and stored in the refrigerator for use. Four concentrations of the extract viz. 50 mg l<sup>-1</sup>, 100 mg l<sup>-1</sup>, 150 mg l<sup>-1</sup> and 200 mg l<sup>-1</sup> were prepared and delivered into experimental tanks of 0.6 m x 0.3 m x 0.3 m. A fifth treatment of distilled water (0.0 mg l<sup>-1</sup>) only was used as control.

Ten fish were exposed to each of the five concentrations in triplicates. The toxicant solutions and control were renewed after 48 hr in each bioassay. Water quality parameters namely temperature, dissolved oxygen and free carbon dioxide were monitored every 24 hr using methods described by APHA (1980). Mortality was recorded every 3 hr and dead fish were immediately removed.

The lethal concentration that will cause 50% mortality *i.e.* 96 hr LC<sub>50</sub> was estimated by probit analysis as described by Wardlaw

(1985). The opercular ventilation rate per minute was recorded at the beginning of the experiment and every 24 hr thereafter and the mean of six readings taken (Oti, 2003a). Safety level was estimated using an empirical applicable factor of 0.1 as described by Sprague (1971). Results obtained were subjected to statistical analysis with Duncan's multiple range F-test (Duncan, 1955) to determine significant differences between the various treatments.

## Results and Discussion

The water quality parameters in each treatment during the exposure period are shown on Table 1. Mean temperature varied from 24.0°C to 26.0°C, dissolved oxygen 5.07mg l<sup>-1</sup> - 5.86 mg l<sup>-1</sup>, free carbon dioxide 0.79 mg l<sup>-1</sup> - 0.88 mg l<sup>-1</sup>, while pH ranged from 6.6 to 7.3 for all the concentrations. The water quality parameters in the extract concentrations did not vary significantly ( $p < 0.05$ ) from what was obtained in the control. Fish mortality increased with increase in plant extract concentrations. Mortality was highest, 100% in 200.0 mg l<sup>-1</sup> and lowest, 30% in 50 mg l<sup>-1</sup> after 96 hr of exposure. No mortality was recorded in the control experiment throughout the experimental period as shown in Table 2.

The lower and upper limits of 96 hr LC<sub>50</sub> were 34.35 mg l<sup>-1</sup> and 192.30 mg l<sup>-1</sup> while the mean 96 hr LC<sub>50</sub> was 81.28 mg l<sup>-1</sup>.

The computed regression equation illustrated in Fig. 1 is:

$$Y = -5.57 + 5.721 \text{ Log } C \quad (r=0.8)$$

where Y = mortality and C = concentration of extract.

The safety level estimated was 8.13 mg l<sup>-1</sup> while acceptable toxicant concentration was 21.13 mg l<sup>-1</sup>.

There was a significant decrease ( $p \leq 0.05$ ) in the opercula ventilation rate as the period (*i.e.* days) of exposure of

**Table - 1:** Mean and standard deviation values of physico-chemical parameters of the various concentrations of *A. indica* fruit extracts media used for the experiment

Parameters / Extract	Control 0.0 (mg l <sup>-1</sup> )	50.0 mg l <sup>-1</sup>	100 mg l <sup>-1</sup>	150 mg l <sup>-1</sup>	200 mg l <sup>-1</sup>
Mean temp. (°C)	24.0±1.5	26.0±0.5	25±1.0	25.5±1.5	25±1.5
Mean dissolved oxygen (mg l <sup>-1</sup> )	5.86±0.8	5.52±0.04	5.37±0.06	5.14±0.05	5.07±0.06
pH	6.6 - 6.9	6.7 - 7.0	6.7 - 7.3	6.8 - 7.0	6.6 - 7.1
Mean free carbondioxide (mg l <sup>-1</sup> )	0.88±0.02	0.86±0.02	0.83±0.01	0.80±0.01	0.79±0.01

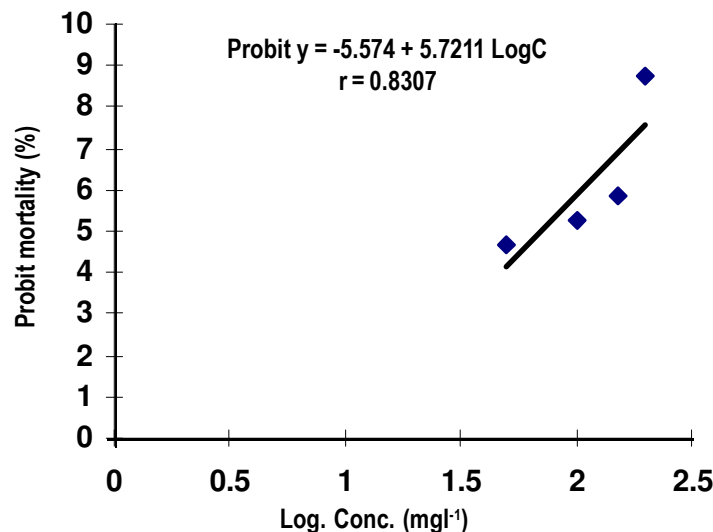
**Table - 2:** Mortality rate of "Heteroclaris" fingerlings exposed to various concentration of *A. indica* fruit extract

Conc. mg l <sup>-1</sup>	Log conc. (mg l <sup>-1</sup> )	Mortality				Mortality rate (%)	Survival rate (%)	Probit
		24 hr	48 hr	72 hr	96 hr			
0.0	0.0000	0	0	0	0	0.0	100.0	-
50.00	1.6990	0	2	2	3	30.0	70.0	4.67
100.00	2.0000	2	4	4	6	60.0	40.0	5.25
150.00	2.1751	4	5	7	8	80.0	20.0	5.84
200.00	2.3010	4	5	9	10	100.0	0.0	8.72

**Table - 3:** Mean and standard deviation of opercula ventilation per minute (mean of six readings) of "Heteroclaris" exposed to various concentrations of *A. indica* fruit extract

Concentration (mg l <sup>-1</sup> )	Time of opercula ventilation/minute				
	Start	24 hr	48 hr	72 hr	96 hr
00.00	82±0.44	81±0.32	81±0.11	80±0.21	81±0.11
50.00	90±0.14	88±0.22	86±0.41	85±0.26	95±0.31
100.00	108±0.26	102±0.31	100±0.31	90±0.26	103±0.41
150.00	119±0.21	110±0.40	109±0.16	94±0.30	101±0.31
200.00	122±0.17	114±0.22	110±0.31	96±0.32	102±0.31

All data on the same column are significantly different at  $p < 0.0001$ ,  $F = 13.99$ ,  $N = 150$

**Fig. 1:** Mortality of "Heteroclaris" fingerlings exposed to varying concentrations of *Azadirachta indica* fruit extract

the fish increased in all the toxicant concentrations. The opercula ventilation rate was highest, 122 / minute in 200 mg l<sup>-1</sup> while the least value 80/minute was recorded in the control (Table 3). No significant difference ( $p \geq 0.05$ ) occurred in the opercula ventilation rate of the control experiment throughout the period of exposure (96 hr).

The piscicidal potential and phytotoxic properties of plants extracts have been reported by many authors (Akobundu, 1987; Okorie *et al.*, 1993; Ufodike and Omoregie 1994 and Adewole *et al.*, 2002). The estimated 96 hr LC<sub>50</sub> (81.28 mg l<sup>-1</sup>) of the neem fruit extract for the hybrid, "Heteroclaris" in the present study is far higher than 8.0 mg l<sup>-1</sup> reported by Oti (2003 b) when he exposed the African Pike, *Hepsetus odoe* to water extract of neem plant bark. This implies that the bark of this plant could be more toxic than the fruit and also that there could be variations in the tolerance limit of different fish species to the same toxicant. The 0.51 mg l<sup>-1</sup> 96 hr LC<sub>50</sub> documented by Oti (2003 a) when "Heteroclaris" was exposed to the water extract of the bark of the milk bush, *Thevetia peruviana* is lower than the observation from this study, which further indicates variation in the tolerance limit of the same species of fish to different toxicants.

The high 96 hr LC<sub>50</sub> recorded in this present study may be related to the hardy nature of African mud catfishes since they could tolerate considerable amount of toxicants without serious side effects. The *A. indica* fruit would be required to elicit 50% mortality of this fish.

The stressful breathing behaviour exhibited by the fish reflected by increased opercula ventilation with increased concentration of extract may be as result of respiratory impairment due to effect of rotenone (which is a fuming biocide) on the gills (Ade-Serrano, 1982 and Adewole *et al.*, 2002). In addition, the inability of the gills surface to actively carry out gaseous exchange might be responsible for the recorded mortalities which was shown to be significantly different ( $p > 0.05$ ) and directly proportional to the extract concentration and exposure period.

The darkened patches observed on the skin could be as a result of the dispersion response of the melanin pigments in the chromatophores which move towards the periphery, by pituitary hormone intervention also known as melanocyte stimulating hormone (MSH) (Oti, 2003a). Novales (1985), earlier stated that MSH is the most important pigment movement determinant factor within the chromatophores. So from this study, it can be said that chemicals

present in the *A. indica* contains melanocyte stimulating hormones, since dark patches were observed only on fish in the treatment groups and not dependent on the variation of the concentration of the extract (toxicant).

Isman *et al.* (1990), Mordue and Blackwell (1993) and Verkerk and Wright (1993), had reported that tetranotriterpenoid (also called limonoid) compound, azadirachtin is responsible for the majority of biological effects observed in organisms exposed to neem compounds. Other behavioural abnormalities which include erratic swimming and loss of balance observed in this investigation are indications that mortality of the fish exposed may not only be due to impaired metabolism but could in addition be due to nervous disorder as earlier reported by Agbon *et al.* (2002), Aguigwo (2002), Oti (2003 a, b), Sambasivam *et al.* (2003), Oti and Ukpabi (2005) to mention a few. The threshold concentration that produces statistically significant deleterious effect as seen in probit mortality is commonly expressed as the maximum acceptable toxicant concentration (MATC) (Wickins, 1976).

The present study has revealed the ill effect of extract of the fruit of *A. indica* when it is above safety limit of 8.13 mg<sup>l</sup><sup>-1</sup> or threshold level of 21.13 mg<sup>l</sup><sup>-1</sup>. Therefore, based on the result from this study, *A. indica* fruit mesocarp has piscicidal potential and local fishermen should be discouraged in using it as a piscicide to catch fish or shade plant in fish farms especially since the plant has been reported to be non-target specific (Koul and Isman, 1991) in its action.

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