African Scientist Vol. 7, No. 1 March 31, 2006
Printed in Nigeria

1595-6881/2006 $12.00 + 0.00

http://www.klobex.org

AFS 2005028/7105

Acute toxicity of mesocarp of *Azadirachta indica* (L.) (Neem plant) on fingerlings of *Heterobranchus bidorsalis*

A. A. Akinwande[1]*, A. A. Dada[2] and I. O. Umar[3]

[1]Federal College of Freshwater Fisheries Technology, P.M.B. 1500, New Bussa, Nigeria
[2]National Institute for Freshwater Fisheries Research, P.M.B. 6006, New Bussa, Nigeria
[3]Department of Science Laboratory Technology, Federal Polytechnic, Idah, Nigeria

(Received October 12, 2005)

ABSTRACT: The piscicidal potential of water-extract mesocarp of *Azardirachta indica* was investigated in static biossay experiment with continuous aeration to determine its acute toxicity. The 96-hLC_{50} was 97.42mg/l while the threshold value was 31.12mg/l. The extract led to an initial increase in opercula ventilation rates which then decreased significantly. The computed regression equation (y) = 4.624 + 6.352 Logc. (r=0.72) and the fish exhibited respiratory distress, loss of balance and erratic swimming prior to mortality. The usefulness of *A. indica* as piscicidal plant is discussed.

Key words: *Azadirachta indica*, Acute toxicity, threshold value, *Heterobranchus bidorsalis.*

Introduction

Certain plants contain or produce substances capable of harming or killing animals both on land and in water. The usefulness of some of these plants for piscicidal and medicinal purpose has been reported (Adewole *et al*., 2002). Phytochemical analysis of plants containing poisons has shown that they contain diverse toxic substances. Such as alkaloids, sponginess, solanine etc. (Gills, 1992). The position and importance of some of these piscicidal plants is unique, whereby most fish farmers and fishermen indiscriminately use various kinds and parts of those plant extracts due to their narcotic, pesticidal and molluscidal properties in order to weaken and kill fish for easy catch or clean up the aquatic system of some pests. Some of these plants used are non-selective in their destruction, thereby interfering with the ecological balance of the immediate environments. Lethal and sublethal concentrations of plant poisons are known to have toxic effects on fish behaviour, haematology, listopathology and physiological processes of exposed organisms (Buttler, 1971).

The Neem plant, *Azadirachta indica* is a known medicinal plant that contains margosine, eriterpereoid, azatin, rotinine and quinine among other active ingredients as reported by Ade-Serranno (1982) and Adewole (2002). The leaves, barks, fruits and roots of the plant have been highly appraised for their medicinal purposes. Omoregie and Okpanachi (1992) and Oti (2003) reported on the sublethal and acute effect of water extract of the bark of *A. indica* to *Tilapia zilli*. Little attention has been given to the fruit

which usually drops into the river and ponds, since aquaculturist admire the plant for providing shade. Hence, this present study was conducted to evaluate the lethal effect of the mesocarp of *A. indica* extract on *heterobranchus bidorsalis* which is commonly cultured in Nigeria because of its remarkable fast growth rate.

Materials and Methods

One hundred and fifty fingerlings of *H. bidorsalis* (mean weight 3.0g ± 1.2g) were obtained from federal College of freshwater Fisheries Technology, hatchery Complex, New Bussa. The fishes were acclimatized for five days in water troughs (1.5m x 0.3m x 0m) containing dechlorinated municipal water and fed with 40% pelleted feed at %% of their body weight. Static 96 hour toxicity bioassay was carried out as described by Sprague (1977) and APHA (1980). The mesocarp of *A. indica* fruits collected locally was removed, washed and sundried to constant weight. The well-dried samples were pounded in clean mortar and sieved using 0.1mm sieve. 300g of the fine powder was dissolved in1.5 litre of distilled war water at 32°C for 24 hours. The extract was filtered using Whatman's filter paper No. 1 using a vacuum pump. Ten experimental fish were exposed to the test solution at the following extract concentrations: 25mg/l, 75mg/l, 100mg/l and 125mg/l. The control did not contain any extract and each treatment was in duplicates. The test water were renewed after 48 hours in each bioassay. Physico-chemical parameters of the test water were monitored using standard methods as described by APHA (1980). Experimental fish were not fed during the period of exposure which lasted for 96 hours. Mortality was recorded every 3 hours and dead fish were removed. The lethal concentration that will cause 50% mortality (i.e. the $96hLC_{50}$) was estimated by probity analysis as described by Wardlaw (1985). The opercula ventilation rate per minute was recorded at the beginning of the study and every 24 hours thereafter and the mean taken at every six readings (Oti, 2000). Safety level was estimated using an empirical applicable factor of 0.1 as described by Sprague (1971). Sections of gill tissues of exposed fish were used to histopathological examination at the end of experiment.

Results obtained were subjected to one way analysis of variance(ANOVA) and Duncan multiple test for significant differences between the various levels of treatment.

Results and Discussion

The temperature, dissolved oxygen, free carbon dioxide and pH of the experimental aquaria ranged from 24.0° - 27.0°, 5.16mg/l – 6.06mg/l, 0.68mg/l – 0.7mg/l and 6.3 – 7.0 respectively. There was no significant difference ($P \geq 0.05$) in the water quality parameter within the treatment to what was obtained from the control (Table 1).

Mortality increased with increase in the plant extract concentrations. It was highest (90%) in 12.5% and lowest (20%) in 50mg/l of the total concentrations (Table 2). No mortality was recorded in the control treatment. The $96\text{-}hLC_{50}$ was determined as 97.42 while the computed regression equation was found to be Y = -4.62476.352logC (r=0.72). The safety level estimated was 9.24mg/l. The opercula ventilation rate recorded within each treatment is shown in Table 3.0%. The highest (120) was in extract 125mg/l concentration and the least (81) in 50mg/l extract concentration. The opercula ventilation rate in the exposed fish initially increased sharply and the increase was directly proportional ($P<0.05$) to the extract concentration and then decreased steadily.

Histopathological examination of exposed fish showed distortion of epithelial cells of the gills in all concentrations of the plant extract except in the control.

Table 1: Range and mean values of physicochemical parameters of the various concentrations of *A. indica* fruit extracts used for the experiment.

Parameter	**Control 0.0mg/l**	**50.0mg/l**	**75mg/l**	**100mg/l**	**125mg/l**
Mean temp. (°C)	6.0 ± 0.6	25.0 ± 1.1	24.0 ± 1.2	27.0 ± 1.2	26.5 ± 0.2
Dissolved oxygen (mg/l)	6.06 ± 0.4	5.96 ± 2.1	5.16 ± 0.8	5.18 ± 6.7	5.62 ± 1.2
pH range	6.5-6.7	6.4-6.8	6.3±6.9	6.5-6.9	6.5-7.0
Mean free Carbon dioxide mg (mg/l)	0.79 ± 1.4	0.69 ± 1.1	0.73 ± 2.4	0.7 ± 1.2	0.68 ± 1.7

Table 2: Mortality rate of *H. bidorsalis* fingerlings exposed to varying concentrations of *A. indica* fruit extract.

Conc. mg/l	**Log conc. mg/l**	**MORTALITY**				**Mortality rate (%)**	**Probity**
		24	**48**	**72**	**96**		
0.0	0	0	0	0	0	0.0	-
50.0	16990	0	1	2	2	20	5.10
75.0	1.875	0	1	2	4	40	5.64
100.0	2.0000	2	2	4	8	80	6.10
125.00	2.097	2	4	8	8.5	90	7.24

Table 3: Opercular ventilation (mean of six readings) per minute of *H. bidorsalis* exposed to various concentrations of *Azardirachta indica* fruit extract.

Concentration (mg/l)	Opercular ventilation per minute				
	Start	Start 24 hours	48 hours	72 hours	96 hours
0.00	81 ± 0.21	80 ± 0.76	81 ± 0.94	81 ± 0.55	80 ± 1.2
50.0	89 ± 1.26	91 ± 1.12	90 ± 1.42	88 ± 0.74	90 ± 1.20
75.0	104 ± 1.30	106 ± 2.2	102 ± 1.40	100 ± 0.50	97 ± 0.80
100.0	106 ± 0.40	108 ± 1.22	101 ± 1.20	96 ± 0.40	100 ± 1.80
125.0	112 ± 1.40	114 ± .84	105 ± 0.40	102 ± 0.50	104 ± 0.68

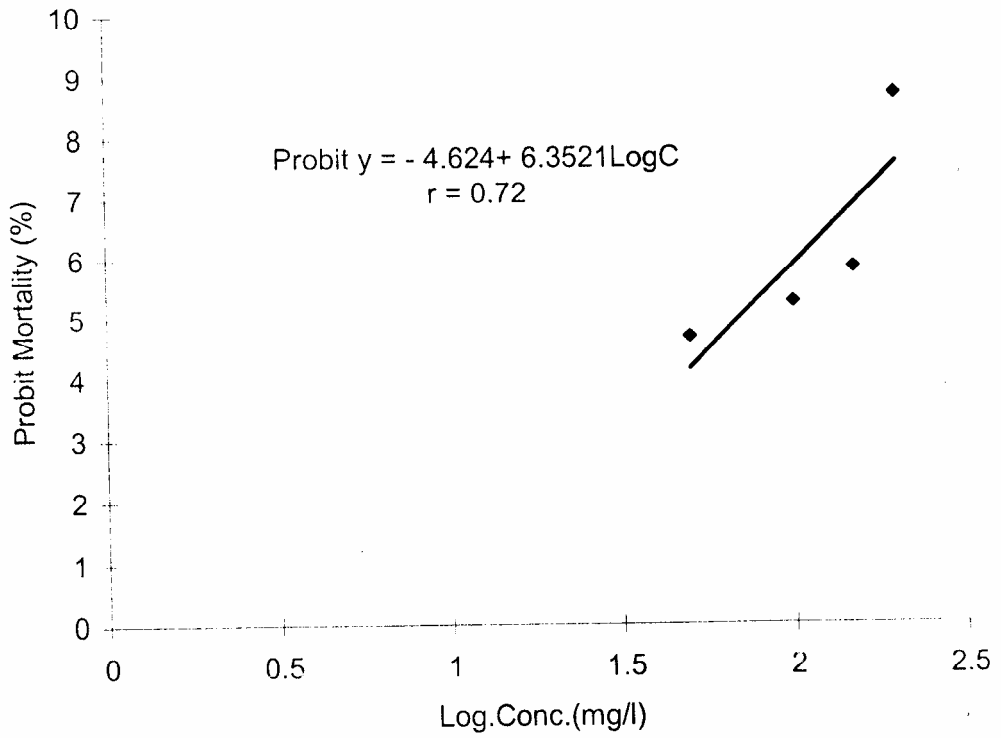

Fig. 1: Mortality of "Heteroclarias" fingerlings exposed to varying concentrations of *A. indica* fruit extract

The piscicidal potential and phytotoxic properties of plant extracts have been reported by many authors (Reed 1967; Ufodike and Omoregie 1994). The value of 96-LLC_{50} 97.42 mg/l estimated in this work was higher than 8.00 mg/l reported by Oti (2000) when *Hepsetus odoe* was exposed to water extract of Neem plant bark. This implies that there is the possibility of the bark to be of higher toxic value than the fruit and also that there could be variations in the tolerance limit of different fish species to the same toxicant.

The high 96h LC50 (97.42 mg/l) recorded in this study may be related to the handy nature of African mud catfish species that they could tolerate considerable amount of toxicants without serious side effects. It also shows that high concentrations of *A. indica* fruit would be required to elicit 50% mortality of this fish of high economic value.

The respiratory distress reflected by increased opercular ventilation with increased concentration f extract may be as a result of respiratory impairment due to the effect of rotinine (which is a fuming biocide) on the gills. In addition, the inability of the gills surface to actively carry out gaseous exchange might be responsible for the recorded mortalities which was significantly different ($P < 0.05$) and directly proportional to the extract concentration and exposure period. The distortion and necrosis of epithelial cells of the gills observed may be due to the direct exposure of the gills to the toxicant.

The threshold concentration that produces significant deleterious effect as seen in probity mortality is commonly expressed as the maximum toxicant concentration (MATC) (Wilkins, 1976).

Conclusion

The present study has revealed the ill effect of extract of the fruit of *A. indica* above the safety limit of 9.24 mg/l. Therefore, the practice by local fishermen of using *A. indica* to catch fish from our inland waters should discouraged.

References

Ade-Serrano, S. (1982) Growth inhibitory and lymphocytotic effect of *Azadirachta indica*. J. Med. Plants 5, 137 – 130.

Adewole, A. M. (2002) Evaluation of chemical components of some toxic plants in Ibadan, Southern Nigeria. M.Phil. Dissertation, University of Ibadan, Ibadan, Nigera, 229pp.

Adewole, A. M.; Faturoti, E. O.; Oladehinde, O. F. and Ayelaagbe, O. O. (2003) A survey of some indigenous fish phytotoxic plants in Ibadan, Southwestern Nigeria. Book of Abstracts, 1st Annual Conference of the Zoological Society of Nigeria. p. 17.

Duncan, D. B. (1955) Multiple Range and Multiple F-test. Biometrics 11: 1 – 42.

Omoregie, E. and Okpanach, M. A. (1992) Growth of *Tilapia zillii* exposed to sublethal concentrations of crude extracts of *Azadirachta indica*. Acta Hydrobiol. 34: 281 – 286.

Gills, L. S. (1992) Ethnomedical uses of plants in Nigeria. Macmillan Press Ltd. p. 229.

Butler, P. A. (1971) Influence of pesticides on marine ecosystems. Proc. Roy. Soc. (London) 177: 321 – 329.

Oti, E. E. (2000) Acute toxicity of water extracts of the bark of the *Thevetia peruviana* to the African freshwater catfish "Heteroclarias" hybrid fingerling. J. Fish Tech. 2: 124 – 130.

Reed, W.; Burchard, J.; Hopson, A. J.; Jennes, J. and Yaro, I. (1967) Fish and Fisheries of Nothern Nigeria. Gaskia Corporation, Zaria, Nigeria. 226pp.

Sprague, J. B. (1971) Measurement of pollutant toxicity to fish bioassay methods for acute toxicity. Water Research 5: 245 – 266.

Ufodike, E. B. C. and Omoregie, E. (1994) Acute toxicity of water extracts of barks of *Balanites aegyptica* and *Kigela africana* to *Oreochromis niloticus* (L.). Aquatic and Fish Management 25: 873 – 879.

Wardlaw, A. C. (1985) Practical Statistics for Experimental Biologists. John Wiley and Sons. New York, 290pp.

Wickins, J. F. (1976) The tolerance of warm water prawns to recirculated water. Aquaculture 9: 19 – 37.