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Acute toxicity of tobacco (*Nicotiana tobaccum*) leaf dust on the African catfish: *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

The acute toxicity of tobacco (Nicotiana tobaccum) leaf dust, on the juvenile African catfish (Clarias gariepinus) was assessed in a static renewal bioassay system for 96 hours. Five graded concentrations of tobacco leaf dust were prepared as 25.00, 20.00, 15.00, 10.00, 05.00 mg/l and a control experiment (0.00mg/l). The 96hour LC_{50} value was 10.96mg/l, r^2 value of 0.68 with 95% lower and upper confidence limit of 7.50 and 16.00 mg/L respectively. At varied concentrations of tobacco leaf dust, the fish showed erratic swimming, secretion of mucus, respiratory distress, and darkening of skin. Mortality was recorded in all concentration of tobacco leaf dust tested.

Keywords: Acute toxicity, *Nicotiana tobaccum*, tobacco leaf dust, *Clarias gariepinus*, 96hour $LC_{50,}$ Nigeria.

INTRODUCTION

The use of tobacco (*Nicotiana tobaccum*) leaf dust to clear fishponds of predators and weed, before stocking, has been documented [1]. It has also been reported that it is used to fish by local fishermen in Nigeria [2]. The common predators in ponds include predatory insects and insects' larvae like water scorpions, nymphs of odonates tadpoles and frogs, leeches, and fish species like *Clarias* species, which could predate on small fingerlings of stockfish. These predators are large enough to take significant toll on the stocked fish if not eradicated before the ponds are stocked [3]. The attraction of tobacco dust as a biocide is because it is inexpensive, locally available and easily degradable [4]. The active ingredient in Tobacco leaves is nicotine, which contribute between 2%-5% dry weight of leaves [5].

The acute toxicity of tobacco leaf dust on *Oreochromis niloticus* has been studied and reported to have a 48h LC_{50} of $109.6mgl^{-1}$ [6]. This value is far lower than that estimated in this study in which *Clarias gariepinus* was found to have $626mgl^{-1}$, thus indicating that *C. gariepinus* was more resistant to tobacco toxicity than *O. niloticus* [7].

Experiment were conducted using dry tobacco (*Nicotiana tobaccum*) leaves aqueous extracts to determine the acute toxicity and sub lethal effect on some haematological indices of *Oreochromis niloticus* using static renewable bioassay method .the extract was found to be toxic with a 48 hours $_{LC50}$ value 109.6mgl^{-1}.sublethal concentration of extracts were found to have an inverse relationship with the heamatolgical indices assessed. Statistical analysis using ANOVA revealed that there was a significant difference ($P<0.05$) in the value of red blood count (RBC) and haemoglobin (Hb). A maximum acceptable toxicant concentration (MATC) of 5mgl^{-1} was found while a safety level of 10.96mgl^{-1} was estimated [6].

Fish are intimately associated with the aqueous environment; physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish [8]. The African catfish *Clarias gariepinus* is one of the commercial important species of fish for rapid aquaculture expansion in Nigeria and elsewhere in the developing world. African catfish grow quickly, are omnivores and are desirable as food; they are valuable species worldwide. They posses accessory air breathing organs which enable them to tolerate adverse aquatic condition where other cultivable fish species cannot survive [9] it is widely cultivated used as experimental fish [10-13]. Often ponds in which *Clarias gariepinus* is cultivated are infested with aquatic mollusk. Plants extracts have been shown to be useful for the control of water mollusk responsible for the transmission of water borne disease [14].

Pesticides are compounds, which have biocide properties and are applied in other to killed or control certain organisms and many other. Few are absolutely specific to their target organisms and many other species including fish may therefore be at risk because even sub lethal concentration may significantly affect the physiological behaviours, nutrition and reproductive function. The acute toxicity of pesticides of fish has involved the determination of the LC_{50}, which is the concentration that kill 50% of group of fish under specified conditions [15].

Botanicals are natural biocide [16] and their contamination of natural waters has become inevitable in Nigeria because of recent wide use piscicidal plant like *Blighia sapida*, *Kigelia africana*, *Tetrapleura tetraptera*, *Raphia vinifera*, *Parkia biglobosa* and *Tephorosia vogelli* are frequently in use by the fisher folks because they are highly potent[17]. The presence of these botanicals in high concentration may have adverse effects on aquatic organism.

The study was under taken to determine the median lethal concentration of tobacco on *Clarias gariepinus* during the 96 hours exposure period. It is therefore hoped that the results obtained herein, will add to our knowledge of the mechanism of the action of tobacco on the fresh water fish.

MATERIALS AND METHODS

Experimental Fish

Healthy juvenile of the African catfish *Clarias gariepinus* mixed sex and the same brood stock mean weight and length of (10.87±0.23g) and (2.40±0.14cm) respectively were obtained from Akia fishpond in Igbide Isoko South L.G.A. Delta State. The fish were held in the laboratory in large plastic aquaria of 60L capacity with well-aerated borehole water. They were fed daily with Catfish feed (Dizengoff; 4.5mm; Protein 42%, Fat 13%, Fibre 1.9% and Ash 1.2%) at 3% of their body weights. Feeding was done twice daily (0800 and 1800 hours) with the diet during acclimatization period but stopped prior to exposure. The fish were not fed 24hours prior to the experimental period, as well as during the experimental period, which lasted 96 hours. Natural photoperiod was maintained during the acclimation and experimental period.

Toxicant Preparation

Tobacco (*Nicotiana tobaccum*) leaf was obtained from Uzere, Isoko North LGA of Delta State Nigeria. The leaf was sun dried up for 7 days and grinded into tobacco dust or powder 25.00, 20.00, 15.00, 10.00, 5.00 and 0.00mg/L of tobacco powder were measured and homogeneously mixed in 1 liter of water to give (25.00, 20.00, 15.00, 10.00, 5.00 and 0.00mg/L) concentration of tobacco leaf dust. The concentrations were arrived at after several preliminary investigations. These concentrations were introduced in three 3 aquaria with their replicates.

Experimental Procedure

Forty (40) litres aquaria were maintained throughout the exposure period. Ten (10) juvenile fish each were placed in the 40L plastic aquaria. Well-aerated borehole water was used during acclimatization and exposure. In order to monitor the toxicant strength, level of dissolved oxygen, the effects of evaporation, Ammonia concentration during experimentation, the entire test water and concentration of toxicant in each aquarium was renewed 24hours interval. The water quality parameters of the experimental set up bioassay, with tobacco leaf dust toxicant and control, were conducted at every sampling time according to the APHA [18] procedures. The water quality parameters measured included pH 6.58 $\pm$ 0.34, temperature 28.4 $\pm$ 1.2^{o}C, dissolved oxygen 7.89 $\pm$ 1.16mgL^{-1}, free carbon dioxide 4.95 $\pm$ 0.08 mgL^{-1} and total alkalinity 38.6 $\pm$ 1.34 mgL^{-1}.

Visual Examination

This was conducted on the behavioral pattern of the fish, which include the erratic movement, leaping and instabilities.

Data Analysis

The results obtained were analyzed with the use of version 15.0 SPSS (Statistical software). Logarithm and arithmetic graph method [19], was used to determine the median lethal concentration and median lethal time of tobacco exposed for 96hours while the confidence limit was determined two know the limit of the toxicant efficacy. The one-way analysis of variance (ANOVA) at 5% probability level was used to test for significant difference between various physiochemical parameters mentioned.

RESULTS

Acute Toxicity

Acute toxicity test of *Clarias gariepinus* exposed to acute concentrations (25.00, 20.00, 15.00, 10.00, 5.00 and 0.00mgL^{-1}) of tobacco leaf dust during the 96 hours exposure period reveal 100 percent mortalities in 25mgL^{-1} and 20mgL^{-1} concentration of tobacco leaf dust during the 96 hours exposure. However, 15.00mgL^{-1} concentration revealed 75 percent mortality after 96 hours. No mortality was recorded in the control exposure as represented in Tables 1. and 2. illustrating the logarithm concentration, total mortality, and probit mortality from for further analysis.

Table 1: Cumulative Mean mortality of *Clarias gariepinus* exposed to various acute concentrations of tobacco (*Nicotiana* tobaccum) leaf dust during the 96 hours exposure period.

Concentration of tobacco leaf dust (mgL^{-1})	Mean number of juvenile	Exposure Period (Hours)			
		24	48	72	96
0.0 (Control)	10	0.0	0.0	0.0	0.0
5.0	10	0.0	0.0	0.5	1.0
10.0	10	0.0	0.5	2.0	3.5
15.0	10	0.0	1.0	2.5	4.5
20.0	10	1.0	2.5	4.5	7.5
25.0	10	1.0	4.0	6.5	10.0

Table 2: Logarithm concentration, mean total mortality and probit mortality of the *Clarias gariepinus* exposed to various acute concentrations of tobacco leaf dust during the 96 hours exposure period.

Concentrations of tobacco leaf dust (mgL^{-1})	Logarithm concentration	Mean total mortality	Mean total mortality (%)	Probit mortality
0.00 (Control)	0.000	0.00	0.00	0.00
5.00	0.699	1.00	10.00	3.72
10.00	1.000	3.50	35.00	4.62
15.00	1.176	4.50	45.00	4.87
20.00	1.301	7.50	75.00	5.67
25.00	1.398	10.00	100.00	8.72

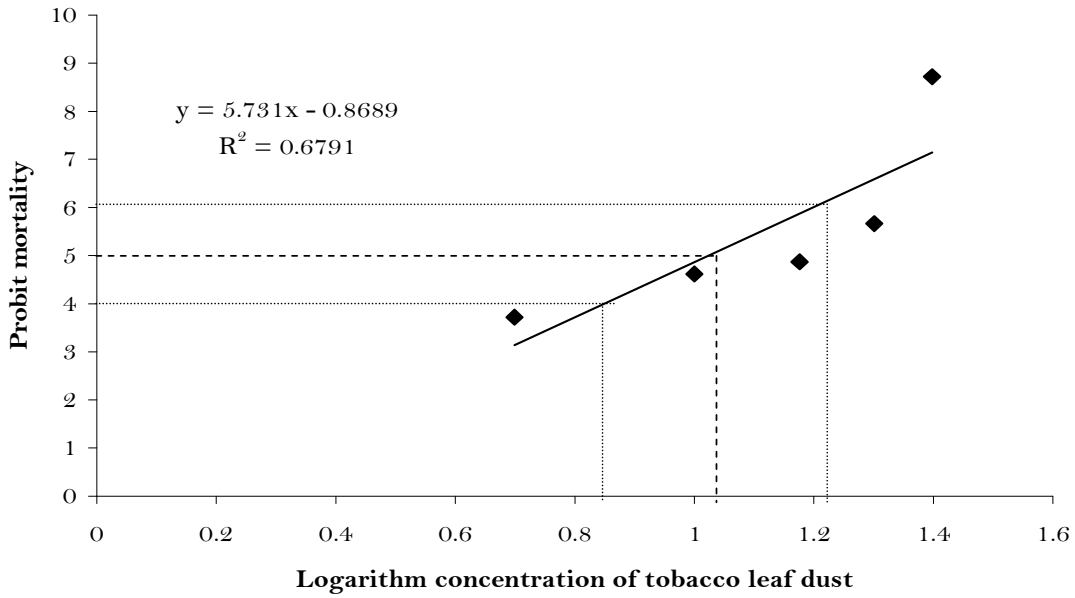

Fig 1: Linear relationship between probit mortality and logarithm concentration of tobacco leaf dust concentrations to which *Clarias gariepinus* was exposed during the 96 hours exposure period.

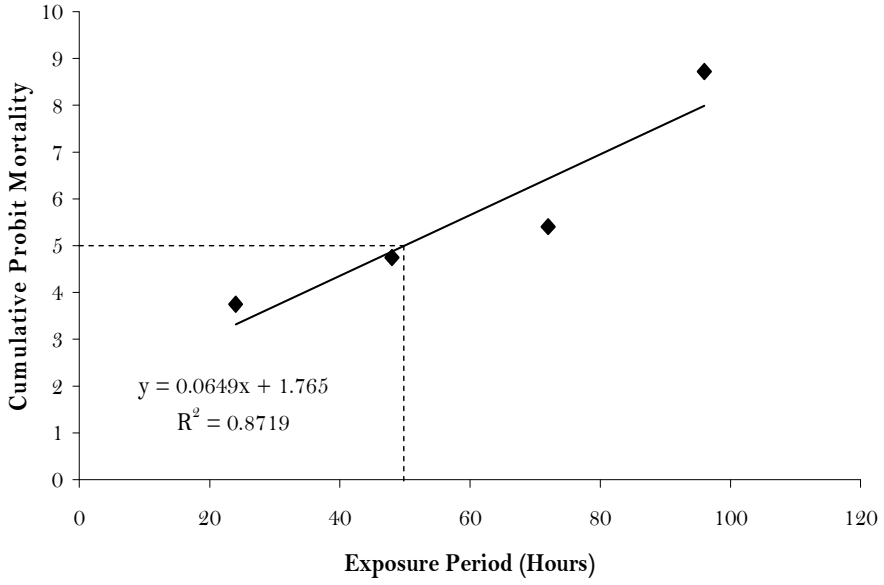

Fig 2: Linear relationship between probit mortality and exposure periods of *Clarias gariepinus* exposed to 25.0mg/L concentration of tobacco leaf dust during the 96 hours exposure period.

Mean Lethal Concentration

The median lethal concentration of various acute concentration of tobacco leaf dust exposed to African Catfish, *Clarias gariepinus* during the 96 hours period was 10.96mgL^{-1} with line of best fit equation: 5.731x – 0.8689 as represented in Fig 1. The 95% lower and upper confidence limit was 7.50 and 16.00mgL^{-1} respectively. Regression analysis implies that 68% of the mortality correlates with acute concentrations of tobacco leaf dust used.

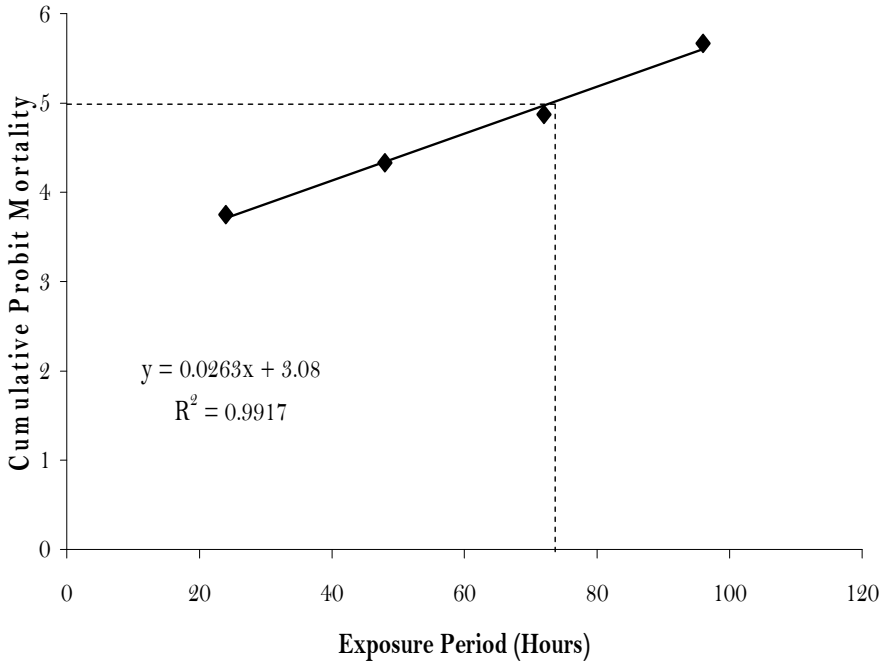

Fig 3: Linear relationship between probit mortality and exposure periods of *Clarias gariepinus* exposed to 20.0mg/L concentration of tobacco leaf dust during the 96 hours exposure period.

Mean Lethal Time

The median lethal time for 25.0 mgL^{-1} tobacco leaf dust on the test fish (Fig.2.) showed that at 49.50 hours 50% of the test fish was killed by the 25.0 mgL^{-1} concentration of tobacco leaf dust. Similarly, the median lethal time for 20.0 mgL^{-1} tobacco leaf dust on the test fish (Fig 3.) showed that at 77.80 hours 50% of the test fish was killed by the 20.0 mgL^{-1} concentration of tobacco leaf dust.

Behavioural changes such as erratic swimming and increase in operculum ventilation, secretion of mucus and uncoordinated movement, respiratory distress, the skin and gill surfaces of the fish were observed to be covered with mucus, strong spasm and darkening of skin were observed during exposure of skin to tobacco leaf dust.

DISCUSSION

The acute toxicity of plant extracts on fish has been reported by many authors [20 -22](Ufodike and Omoregie, 1994; Onusiruika and Ufodike, 1994; Aguigwo, 1998). The 48-h LC_{50} of the water extract of tobacco leaves was estimated to be 109.6 mg/L. This value is far higher than that reported by Ufodike and Omoregie [20] in their study of the acute toxicities of the extracts from the bark of *Balanites aegytiaca* and *Kigelia africana* on *Oreochromis niloticus*. The strange behaviours exhibited by the fish may be as a result of the respiratory impairments due to the effect of Nicotine on the gills reduce respiratory activity in fish [1] and the inability of

the gill surface to actively carry out gaseous exchange might be responsible for the recorded mortalities which is shown to be dependent on the concentration of the tobacco extracts in the bioassay. The observed increase in swimming activity shown by the fish in exposure to the toxicant was directly proportional to the concentration of the tobacco extracts in the bioassay. There was gradual decrease in activities as time progressed until a state of Calmness, which was subsequently followed by death.

A similar pattern of behaviour was reported in a study of *Brachydanio rerio* [23]. This might be attributed to the effect of Nicotine, which is a stimulant that affects the nervous system and thus caused excitation and the resultant increase in swimming activity.

The acute toxicity test of tobacco leaf dust on the African catfish *Clarias gariepinus* is dose-dependent. As the concentration of the tobacco leaf dust increase the rate of the mortality of *Clarias gariepinus* also increase, which is directly proportional. Median lethal concentrations of 50.12mgc, 3.4 and 3.2ppm and $0.002mgl^{-1}$ have been reported when *Heteroclarias* and *Tilapia guineansis* were exposed to various concentrations of Cassava mill effluent [22], *Parkia biglobosa* and *Raphia vinifera* [24] and *Chloropyrifo* [25] respectively.

In conclusion, this study confirms that the extracts of tobacco leaf dust have piscicidal properties and this could be welcomed information to tobacco farmers in view of their worries over the Federal Ministry of Health campaign against smoking. The study has shown that tobacco leaf dust extract can be applied in aquaculture management to reduce the undesirable fish populations, so as to improve production of desirable fish species for the country.

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