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Toxicity of Tobacco (*Nicotiana tobaccum*) Leaf Dust on Enzymatic and Protein Synthesis Activities of African Mud Catfish (*Clarias gariepinus*)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NNN and OAU designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AAAU and IKE managed the analyses of the study and performed the statistical analysis. Author IKE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The toxicity of tobacco (*Nicotiana tobaccum)* leaf dust with piscicidal and pesticidal properties was investigated on some enzymatic and protein synthesis activities of juvenile African mud catfish (*Clarias gariepinus*). Fish were exposed to both lethal and sub-lethal concentrations of tobacco leaf dust for 21 days in a renewal bioassay procedure. The median lethal concentrations (LC_{50}) were derived using Finney probit method while protein and enzymatic activities were determined using Biuret and Randox methods respectively. The LC_{50} values for 48 hours acute bioassay test was 2.11g/l for tobacco leaf dust. Tobacco exposed fish showed significant increase (P≤0.05) in serum AST, ALT and ALP levels. Significant decrease (p≤0.05) were observed for liver AST (141.00 ± 2.52 to 154.67 ± 0.67), serum and liver total protein (6.47 ± 0.03 to 7.80 ± 0.40 and 3.30 ± 0.00 to 3.57 ±0.12) and serum albumin (4.23 ± 0.09 to 4.83 ±0.20) across all concentrations. The study showed that exposure of *C. gariepinus* juvenile to sub-lethal concentrations of tobacco (*Nicotiana*

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tobaccum) leaf dust can induce various toxicological effects in the form of enzymatic alteration. Therefore, the longer exposure of tobacco leaf dust in aquatic ecosystem is dangerous to fish and subsequently human health.

Keywords: Toxicity; tobacco; enzymes; protein; Clarias gariepinus.

1. INTRODUCTION

The use of tobacco (*Nicotiana tobaccum*) leaf dust to clear fishponds of predators and weed before stocking of fish juveniles has been documented [1]. This method is attractive because tobacco leaves have high potency, are inexpensive, locally available and easily degradable [2,3]. The most active ingredient of the plant is the nicotine [4]. Nicotine is made up of pyridine and pyrroliding ring which is produced in the root and transported to the leaves for storage. This material is soluble in water, alcohol, chloroform, kerosene and some fixed oil [5].

The acute toxicity of plant extracts on fish has been reported by many authors [6,7,8] and it was suggested that at sub-lethal levels of toxicants, lower protein levels are observed, indicating that the synthesis of protein is inhibited under such conditions [9]. The behaviours exhibited by fish in response to exposure to tobacco leaf dust have been associated with respiratory impairments due to the effect of nicotine on the gills causing the inability of the gill surface to actively carry out gaseous exchange which could lead to mortality [8]. It is reported that xenobiotics compounds usually concentrate in the tissues of aquatic biota and are known to produce cumulative deleterious effects [10,11,12,13].

However, the presence of predatory fishes, Chaoborus larvae, tadpoles, frogs, and leeches in fish ponds is a serious problem in aquaculture, as they share and better utilize cultured habitats and their food [14]. In controlling these predators, fish farmer in Nigeria often use tobacco leaf dust in eradicating these unwanted organisms [5,15]. Despite the use of this plant material by fish farmer, eco-toxicologists are interested in the ecotoxic properties of this plant origin.

Clarias gariepinus is mostly widely used because; it is hardly, and is able to tolerate both well and poorly oxygenated waters. It respires bimodally with wide cultivation in Nigeria freshwater bodies. They are widely used to evaluate the health of aquatic ecosystem and physiological changes which serve as biomarkers of environmental pollution [16]. The knowledge of sub-lethal effects of xenobiotic compounds on enzymatic activities is very important to delineate the health of fish status and also to provide an understanding of it ecological impacts, hence the need to evaluate the effect of toxicity of tobacco (*N. tobaccum*) leaf dust on enzymatic and protein synthesis activities of African mud catfish (*C. gariepinus*).

2. MATERIALS AND METHODS

2.1 Specimen Collection

Juveniles of a tropical fish species, African mud catfish, *C. gariepinus* (Burchell,1822) with mean weight 17.23 ± 3.59 g and mean standard length 13.7 ± 0.9 cm were obtained from the Oyo State Fish Farms, Mokola, Ibadan and transported in unaerated container to Zoology Laboratory, University of Ibadan, Ibadan, Nigeria. Selection of this species was based on availability, adaptability to laboratory conditions, convenient handling size, in-depth knowledge of species biology and ecology, economic values. The fishes were acclimatized for at least two weeks during which they were fed with dried commercial fish food containing 40% crude protein at 2.5% of body weight twice daily [17,18].

2.2 Measurement of Physico-chemical Parameters of Water

The physico-chemical parameters of water determined were; Temperature, pH, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD) and Conductivity. These parameters were all measured daily, throughout the test period. Temperature was measured using a mercury glass thermometer. pH was measured using Jenway pH meter. DO, BOD and Conductivity were determined by standard methods as described by APHA [19].

2.3 Preparation of Tobacco Leaf Dust

The leaves of tobacco (*N. tobaccum*) were obtained from a farmyard in Eleyele, Ibadan and

identification of the leaf was carried out in the herbarium of the Department of Botany, University of Ibadan, Ibadan, Nigeria. The collected sample leaves were sun dried for 14 days (as practised by the fish farmers) and grounded into powder with the use of laboratory Qlink Blender and Grinder, and sieved before being stored in a sealed plastic container until required for the bioassay test.

2.4 Bioassay Techniques

A 100 mg of tobacco leaf dust were measured and then mixed in 1 litre of water to give 100 mg/L concentration of the tobacco leaf dust. The dilution was made with the aerated water. Ten fully acclimatized juveniles of fishes were exposed each to five, 50 litre capacity plastic containers. Each plastic container was aerated with mechanical pumps. Feeding was discontinued 24 h before the start of the experiment [20]. The catfish juveniles were observed for 96 h and any behavioural changes were recorded. A static renewal bioassay procedure [21] was adopted in which the test media was regularly renewed every 24 hours at the same set of concentrations for a period of 21 days to study the effect of sub-lethal concentration on the enzymatic and protein synthesis activities of African mud catfish as described by Rahman et al. [22]. The concentrations used for the acute toxicity test were 1.4 g/L, 1.6 g/L, 1.8 g/L, 2.0 g/L and control (0.00 g/L).

2.5 Blood Sampling

After 21 days, three fishes from each tank were randomly caught gently with hand net in order to avoid/minimize handling stress. The mucus and water from the body from the fish was dried off using a clean cloth. Fish head was wrapped in a towel to allow a better grip. Blood was taken from the vein running ventrally along the vertebral column using 1ml sterile plastic syringes equipped with a 26G hypodermic needle. The blood was transferred into EDTA Heparinized tubes and was immediately taken to clinical pathology laboratory of the Department of Veterinary Pathology, University of Ibadan for analyses. The serum was then removed by subjecting the tubes to centrifugation at 3000 rpm for 5 min and then stored at −80°C until further analyses. After blood collection, fishes were immediately sacrificed and the desired organs (Liver and Heart) were removed to prepare post-mitochondrial fractions for enzymatic and biochemical analyses.

2.6 Preparation of Post-Mitochondrial Fraction (Supernatants0)

The Liver and Heart were immediately excised and rinsed in ice-cold 1.15% KCl buffer, blotted on filter paper and weighed appropriately. The tissues were then macerated and homogenized in four volumes of homogenizing buffer (pH 7.4) using laboratory mortar and pestle. The homogenized tissues were later centrifuged at 3000 g, 4^{o}C for 10 minutes and the supernatant obtained was aliquoted and stored at -20^{o}C for biochemical analysis.

2.7 Biochemical Parameter Analyses

Albumin, Creatinine Globulin, Aspartic Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), glucose, cholesterol and total protein were all measured for serum, liver and heart. Alanine Aminotransferase (ALT) was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4 dinitrophenyldrazine. Aspartate Aminotransferase (AST) was measured by monitoring the concentration of oxaloacetate formed with 2, 4 dinitrophenyldrazine, while the Alkaline Phosphatase (ALP) was measured by using 4-nitrophenol phosphate as a substrate and 2-amino-2-methyl-1-propanol (AMP) or diethanolamine (DEA) as a buffer.

2.8 Statistical Analysis

Toxicological concentration data involving quantal response (mortality) for the acute tests were analyzed by probit analysis [23]. The indices of toxicity measurement derived from this analysis were;

LC_5 = Sub-lethal concentration that causes 5% response (Mortality) of exposed organisms.

LC_{50} = Median concentration that causes 50% response (Mortality) of exposed organisms.

LC_{95} = Lethal concentration that causes 95% response (Mortality) of exposed organism and their 95% Confidence Limits. One way Analysis of Variance (ANOVA) was used to test for statistical difference in the enzymatic and plasma protein composition. Duncan Multiple

Ranges Test (DMRT) was used in determination of significance at 0.05 level of probability.

3. RESULTS

The mean and standard error values for the physico-chemical parameters of pH, dissolved oxygen, temperature, conductivity, biological oxygen demand were 7.58 ± 0.04, 9.20 ±0.30 mg/L, 27 ±0.03°C, 181 ± 1.20 µSc/cm and 185.78 ± 0.07 mg/L respectively.

In the tobacco leaf dust treated fishes, several abnormal behavioural responses were observed and recorded such as incessant jumping and gulping for air, restlessness, frequent surface to bottom movement, sudden change of direction during movement, resting at the bottom, loss of skin colouration, loss of equilibrium and gradual onset of inactivity, while no adverse behavioural changes or mortality were recorded in the control experiment throughout the period of the bioassay. Feeding was also reduced in the organisms. Linear relationship between the probit mortality and the log concentration indicated a positive relationship. Probit transformation of mortality-log concentration analysis yielded a linear equation of y= -0.62+19.37x after 12 hr exposure and y= -0.34+16.42x after 48 hr exposure. The concentration of tobacco leaf dust that will cause 50% mortality (LC_{50}) at 12, 24, 36, and 48 hours were 1.95 g/L, 2.01 g/L, 2.32 g/L and 2.11 g/L respectively. Mean LC_{50} obtained after 48 hr exposure was 1.84 g/L. Table 1 is the probit analysis, showing the $LC_{50,}$ LC_{95} and LC_5 and their confidence limit (95% CL) at different exposure time.

There was a significant increase (p≤ 0.05) in Serum AST level ranging from 42.67 ± 0.33 to 47.00 ± 0.58 across all concentrations when compared with the control measuring 39.67 ± 0.33 only. There was however, a significant decrease (p≤0.05) in the Liver AST levels ranging from 141.00 ± 2.52 to 154.67 ± 0.67 across all concentrations as compared with control measuring 166.00 ± 5.51. There was a significant decrease in the heart AST levels at sub-lethal concentrations 0.20 g/L and 0.40 g/L measuring 144.33 ± 2.84 and 150.67 ± 2.66 respectively as compared with control measuring 156.67 ± 0.33 in the tobacco exposed fish. There was a significant increase (p≤0.050) in the Serum ALT levels measuring from 42.67 ± 0.33 to 47.00 ± 0.58 across all sub-lethal concentrations of fish exposed to tobacco leaf dust as compared with control measuring 39.67 ± 0.33. Also noticed was a significant decrease (p≤0.05) in the liver ALT level measuring 28.33 ± 1.86 of in fish exposed to 0.20 g/L of tobacco leaf dust as compared to control measuring 35.00 ± 0.58. A significant increase (p≤0.05) was observed in the serum ALP levels ranging from 110.00 ± 4.60 to 132.00 ± 1.70 in fish exposed to all sub-lethal concentrations of tobacco leaf dust as compared with control measuring 93.67 ± 3.80. (Table 2).

A significant decrease (p≤0.05) ranging from 6.47 ± 0.03 to 7.80 ± 0.40 was observed in the Serum total protein of fish and Liver total proteins of fish ranging from 3.30 ± 0.00 to 3.57 ± 0.12 across all concentrations of tobacco exposure when compared with controls measuring 8.00 ± 0.40 and 3.60 ± 0.10. There was a significant decrease (p≤0.05) in the levels of Serum albumin ranging from 4.23 ± 0.09 to 4.83 ± 0.20 across all concentrations as compared with control measuring 5.03 ± 0.18. Also noticed was a significant decrease (p≤0.05) in the heart albumin levels of fish exposed to 0.20 g/L and 0.40 g/L measuring 1.23 ± 0.03 and 1.20 ± 0.00 except for 0.80 g/L where there was a significant increase measuring 1.50 ± 0.00 as compared with control measuring 1.37 ± 0.03. A significant decrease (p≤0.05) in liver globulin level measuring 1.70 ± 0.20 was observed in fish exposed to 0.20 g/L of tobacco leaf dust when compared with control measuring 2.43 ± 0.13. There was however, no significant difference in liver globulin levels of fish exposed to 0.40 g/L and 0.80 g/L of tobacco leaf dust. There was a significant increase (p≤0.05) in the serum, liver and heart creatinine levels of fish exposed to 0.20 g/L of tobacco leaf dust measuring 0.80 ± 0.12, 1.73 ± 0.03, and 0.83 ± 0.07 respectively when compared with their respective controls measuring 0.57 ± 0.07, 0.60 ± 0.20, 0.40 ± 0.00. Also noticed was a significant increase (p≤0.05) measuring 1.23 ± 0.03 in the heart creatinine level of fish exposed to 0.80 g/L of tobacco leaf dust when compared with control measuring 0.40 ± 0.00 (Table 3).

Table 1. Probit and log dose of Tobacco Leaf dust (*Nicotiana tobaccum*) on *C. gariepinus* for 48 hours

Exposure time	LC_{50}(95%CL) g/L (range)	LC_{95}(95%CL) g/L (range)	LC_{5}(95%CL) g/L (range)	Slope ± S.E	Probit Equation
12 Hrs	1.95 (0.61 – 1.98)	2.37 (2.01 – 2.39)	1.60 (0.42 – 1.70)	19.37 ± 7.02	y = -0.62 + 19.37x
24 Hrs	2.01 (0.91 – 2.30)	2.71 (2.11 – 2.80)	1.50 (0.31 – 1.65)	12.70 ± 4.25	y = 1.15 + 12.70x
36 Hrs	2.32 (1.10 – 2.38)	3.64 (2.99 – 3.85)	1.47 (0.38 – 1.60)	8.37 ± 4.72	y = 1.94 + 4.72x
48 Hrs	2.11 (0.95 – 2.20)	2.66 (2.20 – 2.70)	1.68 (0.49 – 1.75)	16.42 ± 5.31	y = -0.34 +16.42x

***CL** = Confidence Limit*

Table 2. Enzyme Levels of *Clarias gariepinus* exposed to tobacco leaf dust for 21 days

Concentration (g/l)	ALT (µl)			AST (µl)			ALP (µl)		
	Liver	Serum	Heart	Liver	Serum	Heart	Liver	Serum	Heart
Control (0)	35.00±0.58^{b}	25.00±1.00^{a}	36.00±2.00^{a}	166.00±5.51^{c}	39.67±0.33^{a}	156.67±0.33^{b}	69.00±12.00^{a}	93.67±3.80^{a}	87.33±10.33^{a}
0.20	28.33±1.86^{a}	31.33±0.68^{b}	33.66±1.66^{a}	141.00±2.52^{a}	47.00±0.58^{d}	144.33±2.84^{a}	84.00±1.00^{a}	132.00±1.70^{d}	72.66±6.33^{a}
0.40	35.00±2.52^{b}	31.33±0.33^{b}	35.33±2.66^{a}	157.00±2.00^{bc}	45.33±0.33^{c}	150.67±2.66^{a}	97.00±1.00^{a}	121.00±0.58^{c}	83.66±3.84^{a}
0.80	32.67±0.67^{ab}	31.00±1.00^{b}	33.33±3.66^{a}	154.67±0.67^{b}	42.67±0.33^{b}	157.33±0.66^{b}	77.50±15.50^{a}	110.00±4.60^{b}	93.00±0.61^{a}

Means with the same superscript letter in a column are not significantly different in the DMRT test (P≤ 0.05)

Table 3. Plasma protein Levels of *Clarias gariepinus* exposed to tobacco leaf dust for 21 days

Concentration (g/l)	Total Protein (µl)			Albumin (µl)			Globulin (µl)			Creatinine (µl)		
	Liver	Serum	Heart	Liver	Serum	Heart	Liver	Serum	Heart	Liver	Serum	Heart
Control (0)	3.60±0.10^{b}	8.00±0.40^{b}	3.40±0.20^{a}	1.10±0.06^{a}	5.03±0.18^{b}	1.37±0.03^{b}	2.43±0.13^{b}	2.97±0.23^{a}	2.03±0.23^{a}	0.60±0.20^{a}	0.57±0.07^{ab}	0.40±0.00^{a}
0.20	3.30±0.00^{a}	7.50±0.17^{b}	3.20±0.20^{a}	1.40±0.20^{a}	4.53±0.23^{ab}	1.23±0.03^{a}	1.70±0.20^{a}	2.97±0.12^{a}	2.13±0.17^{a}	1.73±0.03^{b}	0.80±0.12^{b}	0.83±0.07^{b}
0.40	3.43±0.03^{ab}	6.47±0.03^{b}	3.20±0.00^{a}	1.13±0.13^{a}	4.23±0.09^{a}	1.20±0.00^{a}	2.23±0.09^{b}	2.20±0.12^{a}	2.00±0.00^{a}	0.67±0.07^{a}	0.47±0.03^{a}	0.47±0.07^{a}
0.80	3.57±0.12^{ab}	7.80±0.40^{b}	3.30±0.00^{a}	1.40±0.00^{a}	4.83±0.20^{ab}	1.50±0.00^{c}	2.17±0.21^{b}	2.97±0.43^{a}	1.80±0.00^{a}	1.07±0.23^{a}	0.57±0.03^{ab}	1.23±0.03^{c}

Means with the same superscript letter in a column are not significantly different in the DMRT test (P≤ 0.05)

4. DISCUSSION

The stressful and erratic behaviour of the fish in this investigation gives a signal to respiratory impairment and this may probably be as a result of the effect of the tobacco leaf dust (*Nicotiana tobaccum*) on the gills. This result is in agreement with Adewoye et al. [24] and Ayoola [25]. Respiratory hyperactivities observed in this study are attributed probably to the disturbances in the metabolic state resulting in the waste of energy. It is possible that animals which have higher metabolic activities could require higher level of oxygen and thus would embark on higher respiratory activities.

It was observed that the lethal concentrations (LC_{50} and LC_{95}) obtained in this study were increasing with increased in time of exposure except at the last 12 hr that witnessed a remarkable drop. This time dependent increment in the lethal concentration may be indicative of the fact that the toxic active principle (nicotine) is rapidly degraded in the water and as such requires much higher concentration over time to produce the same lethal effect on *Clarias gariepinus*. Oxidative biodegradation of the extract over time might be responsible for the observed decrease in the toxicity of the extract with increasing exposure period as earlier suggested by Kela et al. [26]. This finding disagreed with the work of Fafioye et al. [27] who reported an increase in toxicity with increasing exposure period. Nevertheless, *Nicotiana tobaccum* leaf dust was observed to possess a piscicidal effect. Bahavioual changes due to the effect of the toxicant were similar to those observed with formalin as toxicant [28]; Cadmium sulphate as toxicant [29]; Synthetic pyrethroid, Delta methrin as Toxicant [27,30] and *Raphia vinifera* extract as toxicant [27].

The calculated LC_{50} values of 2.11 g/L over 48 hr exposure period was far higher than the LC_{50} value of 0.0028 g/L reported by Fafioye et al. [27]. The observed differences in the toxicity pattern may be due to the nature of the toxicity bioassay, difference in the types and concentration of the phytochemical constitute of the plant used, age and parts of the plants used, difference in the genetic make-up of species of plant used as well as source of the plant nutrition [31,32,33].

The observable increase in the Serum AST, ALT and ALP levels of fish exposed to tobacco leaf dust (*Nicotiana tobaccum*) indicates that there was an increased demand for energy due to tissue impairment. Tiwari and Sing [34] reported an increase in Serum AST and ALT of fish exposed to piscicidal activity of alcoholic extract of *Nerium indicum* due to demand for energy. Moreover, elevation of AST, ALT and ALP reflect hepatic disease, some inflammatory disease or injury to the liver –hepatocellular damage. It is possible that under tobacco induced stress, the damage of tissues and organs may occur with concomitant elevation and liberation of these enzymes into circulation. Plasma proteins which include albumin and globulin serve a vital function in carrying materials from one part of the body to another via circulation. The protein makeup of an organism is of important because of proteins involvement in enzymes, hormones and antibiotics as well as osmotic pressure balance and in maintaining acid-base balance [35]. In this study, fishes exposed to tobacco leaf dust (*Nicotiana tobaccun)* showed lower total protein levels in the serum and liver indicating that at sub- lethal levels, the synthesis of protein may be inhibited. Similar observations were made by Magdy et al. [9] for DDT and Malathion in *Sarotherodon melanotheron.* According to Das and Mukherjee [36], exposure of fish for a long time to most toxicants interferes with protein metabolism. Gluth and Hanke [37] reported that decrease of total protein in fish could be attributed to either a state of hydration and change in water equilibrium in the fish or a disturbance in the liver protein synthesis or both. Albumin is the most abundant protein in the plasma. It is synthesized in the liver at a rate that is dependent on protein intake subject to feedback regulation by plasma albumin level. Albumin is a useful indicator of the integrity of glomerular and other membranes. A decrease in serum and heart albumin levels of fish exposed to tobacco leaf dust (*Nicotiana tobaccum*) when compared with the control is probably an indication of poor liver function or impaired synthesis. This observation is similar to that of Abd-El-reheem [38] in albino mice exposed to cadmium chloride. Proteins include globulins, which are produced in the liver and others organs of the immune system and represent most of the immumologically active proteins in the blood [39]. The results of this study shows a decrease in the liver globulin levels of fish exposed to tobacco leaf dust (*Nicotiana tobaccum*) and this suggests a depressed immune response due to the effect of the

toxicant. Creatinine is a biomarker of muscle purine metabolism, liver damage and kidney acid. In this study, an increase in Serum, Liver and heart creatinine of fish exposed to 0.20 g/L and heart of fish exposed to 0.80 g/L of tobacco leaf dust (*Nicotiana tobaccum*) is suspected to be due to tobacco induced effect of muscle metabolism and impairment of carbohydrate metabolism. Hadi et al. [35] reported an increase in creatinine level of *Tilapia zillii* exposed to Aluminium and this might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrate metabolism.

5. CONCLUSION

The study showed that exposure of *C. gariepinus* juvenile to sub-lethal concentrations of tobacco (*N. tobaccum*) leaf dust can induce various toxicological effects in the form of enzymatic alteration, which may make them vulnerable to disease, and eventually lead to death. Interestingly, the results of this work suggest that the leaf dust of *N. tobaccum* contained some toxicologically active bio-constituents and the longer exposure of tobacco leaf dust in aquatic ecosystem is dangerous to fish and human health too.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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