



Effect of Lethal and Sub-lethal Concentrations of Tobacco (*Nicotiana Tobaccum*) Leaf Dust Extract on Weight and Hematological Changes in *Clarias Gariepinus* (Burchell)

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ABSTRACT: Lethal and sub-lethal bioassays on *Clarias gariepinus* were conducted to evaluate the toxicity of tobacco (*Nicotiana tobaccum*) leaf dust on weight gain and haematological indices of *Clarias gariepinus* (mean weight $10.5 \pm 0.70_g$) in glass aquaria with aeration system. The concentrations used during the lethal exposure are: 2.00, 1.00, 0.50, 0.25g/l and a control (0.00g/l); while the concentrations of the toxicant used during the 21-days sub-lethal exposure were 0.30, 0.15, 0.10, 0.05g/l and a control (0.00g/l). The 48-h LC_{50} estimated by probit analysis during acute exposure was found to be 626.0 mg/l. The results obtained from the sub-lethal exposure showed that there was a progressive decrease in weight of fish as the concentration of the toxicant increased. Also, haematological indices indicated that the fish became anaemic and the severity of this condition was directly proportional to the tobacco dust concentrations. @JASEM

Fish flesh is about the best source of animal protein due to the low level of connective tissue that makes it more digestible than other animal protein source and moreover, it is well documented in the literature that fish is a better source of vitamins (A, B1, B6, D and E) and minerals than meat (Al-Jedah *et al*, 1999; Egan *et al*, 1981). Fish is an alternative cheap source of animal protein in Nigeria, which is sought after by many due to short supply of other animal products as highlighted by Omoniyi (1995). Unfortunately, the average annual supply comprising both domestic production and import of about 750,000 tonnes in 1997 is far below the demand which is estimated at 1.86 million tones annually (Otubusin, 1999). Fish production through aquaculture has been identified as a more practical and promising approach to meeting the fish demand, but there are a number of factors militating against the development of culture fisheries. Predatory animals such as chaoborus larvae, tadpoles, frogs, leeches and 'weed' fish species which compete and predate on fry and fingerling of stocked fish constitute one of the problems of the Nigerian fish culturists have to contend with. This aspect of culture management must be addressed if Nigeria is to meet her animal protein demand through fish production. It is a common preliminary practice in fish culture operations to use synthetic toxins including chlorinated hydrocarbons and organophosphates to eradicate these predatory and competing fish from nursery, rearing and production ponds prior to the stocking of preferred commercial fish species. However, the application of synthetic toxins is not advisable due to their toxicity to other non-target aquatic species, persistence in the environment (Mason, 1998) and even to the consumers of the fish.

The use of specific natural biocides with pesticide properties, derived from plants, has been reportedly used by some artisanal fishermen in Nigeria (Reed *et*

al, 1967). Tobacco plant (*Nicotiana tobaccum*) leaf is one of such with pesticidal properties. The active ingredient in tobacco leaf is nicotine which constitutes 2-5% dry weight on the leaf (Hassal, 1982). Agbon *et al* (2002) studied the toxicity effect of tobacco leaf dust extract on *O. niloticus* one of the culturable fish species in Nigeria. No study has yet been reported on the effect of tobacco on *Clarias gariepinus*, another culturable species, which is very popular because of the price it commands. Therefore, this study is necessary. The effect of toxicants on fish can be assessed by the use of haematological indices as it has been reported to be a routine procedure in toxicological research, environmental monitoring and fish health conditions (Blaxhall, 1972; Smit *et al*, 1979). Also, Sampath *et al* (1993) observed that studies on fish blood could reveal conditions within the body of fish long before an outward manifestation of disease condition. This study was therefore conducted to investigate the effect of water extract of tobacco leaf dust on *Clarias gariepinus* to determine the 48-h LC_{50} and the effect of sub-lethal concentrations on weight gain and haematological indices.

MATERIALS AND METHODS

Fingerling (average weight $10.5 \pm 0.7g$) of pure *C. gariepinus* were procured from CHI Nigeria Limited, Lagos. They were transported in oxygenated polythene bags to the Department of Aquaculture and Fisheries Management, University of Agriculture, Abeokuta where they were acclimated in out-door glass aquaria for seven days before experimentation. The dried leaves of tobacco (*Nicotiana tobaccum*) were purchased at a local market in Abeokuta, Ogun State. The leaves were oven-dried to a constant weight at a temperature of 60°C in less than 4 hours using a Gallenkamp oven. It was powdered with the

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aid of Moulinex electric blender and stored in dry airtight container.

The acclimated fingerlings were randomly allocated to glass aquaria, each with dimension of 60 x 30 x 30 cm. Dechlorinated tap water from storage tank was used and these parameters: temperature, dissolved oxygen, NH_3 -Ammonium, pH and conductivity were monitored in each aquarium, with the aid of portable water quality analysis kit. A stock solution of the tobacco leaf extract with a concentration of 25g/l was prepared and test solutions were obtained from it by serial dilutions. The volume of each test solution used in the bioassay per replicate in all treatments was 20litres. The concentrations of the water extract of tobacco dust in each treatment were: 2.0, 1.0, 0.5 and 0.25g/l. There was also a control. For each treatment, there were two replicates. 10 fish were put into each aquarium for the acute bioassay test, which lasted for 48 hours. Fresh preparations of the test solutions were introduced into the aquarium for toxicity test as described by Buikema *et al* (1982). During the exposure period, dead fish observed was removed and recorded. The lethal concentration that caused 50% mortality (48-h LC_{50}) was estimated by probit analysis as described by Wardlaw (1985).

The sub-lethal tests were conducted in the aquaria with test solutions of 0.3, 0.15, 0.10, 0.05g/l concentration and a control. Five (5) fish were put into each aquarium and fed 3% of body weight once a day with a diet of rice-bran and fish meal. The test solutions were changed every three days and the experiment lasted for 21 days. The temperature, pH and dissolved oxygen were determined with Jenway 3150 dual-purpose meter, conductivity was determined with a TOA model CM-205 conductivity meter while the total ammonia was estimated with TOA model 1M-5s ion-meter. The initial weights of the fish prior to sublethal exposure and final weights at the end of twenty-one days, were taken with the aid of Mettler balance model PB602.

At the end of 21- days, blood was collected from fish by cardiac puncture with the aid of hypodermic needle and syringe (2ml), put into EDTA vials and taken to laboratory for analysis using method described by Blaxhall and Daisley (1973). The haematological parameters analysed were Haemoglobin (Hb), packed cell volume (PCV), Red blood count (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), total protein and glucose. The values for each treatment concentration were subjected to statistical analysis using a two - way analysis of variance at 5% level of probability.

RESULTS

The acute toxicity test showed that all fish in the control medium survived during the experiment while mortalities were observed in all other treatments. The mean mortality in each treatment was converted to percentage mortality that was transformed into percent probit with the aid of probit table (Wardlaw, 1985). The probit mortality curve shown in Figure 1 was subjected to regression analysis and from the trend line the 48h LC_{50} of the tobacco leaf dust extract was estimated to be 0.626g/l. The fish were also observed to be restless and jumping in the bioassay media. The frequency of jumping and degree of restlessness were noticed to be high in the bioassays as the concentrations of toxicant increased. The toxicity disappearance time for tobacco leaf dust extract in the highest concentration was found to be five days.

The sub-lethal effect on the weight revealed that as the concentration of the extract increased there was a corresponding decrease in body weight gained by fish during the 21- days exposure period (Figure 2). The effect of the tobacco dust extract on some haematological parameters of fish shown in Table 1 reveals significant difference ($p < 0.05$) among the treatments except for the mean cell haemoglobin concentration (MCHC) which showed no differences.

Table 1. Mean values (with standard errors in parentheses) of haematological parameters of *Clarias gariepinus* exposed to sub lethal concentrations of tobacco leaves extract.

Haematological Parameters	Concentration of tobacco leaves extract (g/l)				
	Control 0.00	0.05	0.10	0.15	0.30
PCV (%)	12 (1.00)	11.50 (0.50)	11.00(2.00)	9.00(1.00)	8.50(1.00)
Hb (g/dl)	4.10(0.20)	3.95(0.50)	3.70(0.30)	3.05(0.25)	2.85(0.15)
RBC($\text{mm}^3 \times 10^6$)	1.35(0.05)	1.30(0.10)	1.30(0.20)	1.10(0.20)	0.95(0.05)
MCV (μm^3)	89.50(2.20)	89.50(1.60)	89.50(1.20)	84.50(2.40)	81.50(2.50)
MCH (μg)	30.50(0.50)	30.50(1.30)	30.00(2.10)	28.50(0.80)	28.00(1.60)
*MCHC (%)	34.50(1.00)	34.50(0.50)	34.00(1.00)	33.50(1.50)	33.50(0.50)
Total protein (mg/dl)	20.00(1.80)	19.50(2.10)	18.00(0.70)	16.50(1.55)	12.00(2.20)
Glucose (mg/dl)	16.00(1.40)	14.00(0.80)	12.50(0.50)	11.00(1.10)	9.50(0.30)

* No significant difference.

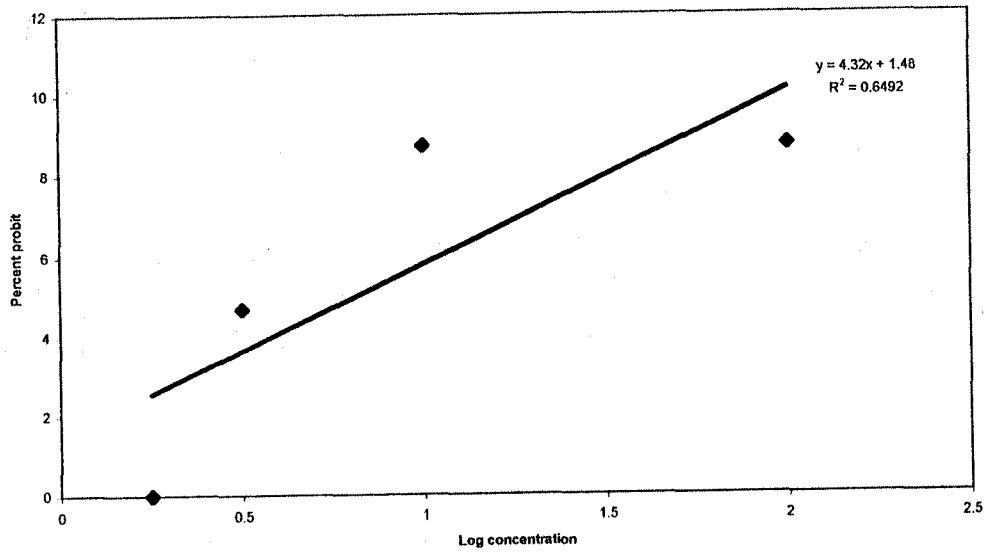


Figure 1. Probit mortality curve of *C. gariepinus*

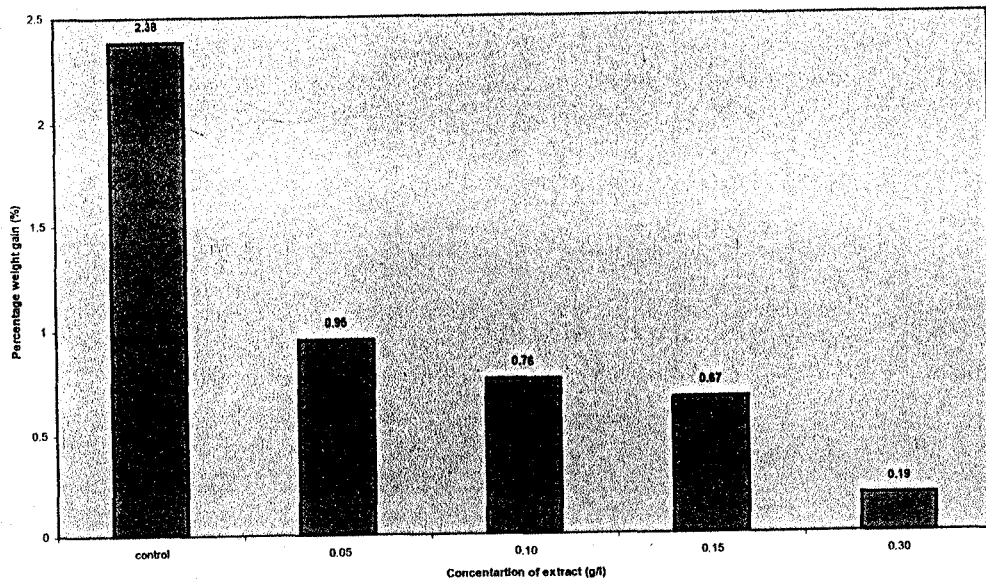


Figure 2: Percentage weight gain of *Clarias gariepinus* exposed to sub-lethal concentrations of tobacco leaves extract

The mean values of water quality parameters measured during sub-lethal exposure are presented in Table 2. The slight variations observed in these values did not reveal any statistically significant difference ($p > 0.05$).

Table 2: Mean* values of water quality parameters observed during sub-lethal exposure.

Parameter	Concentration of extract (g/l)				
	0.00(control)	0.05	0.10	0.15	0.30
Temperature(°C)	28.75(0.04)	28.50(0.07)	28.50(0.05)	28.50(0.03)	28.60(0.02)
Dissolved oxygen (mg/l)	6.50(0.30)	5.30(0.20)	4.50(0.15)	4.30(0.05)	3.50(0.10)
NH ₃ -Ammonium(mg/l)	3.60(0.40)	3.60(0.60)	1.56(0.22)	1.80(0.10)	1.68(0.80)
pH	6.31(0.03)	6.33(0.15)	6.19(0.20)	6.12(0.04)	6.25(0.85)
Conductivity(us/cm)	146.30(1.20)	157.20(2.10)	150.00(1.00)	160.80(3.40)	168.80(2.30)

DISCUSSION

The acute toxicity of tobacco leaf dust on *Oreochromis niloticus* has been studied (Agbon et al, 2002) and reported to have a 48h LC50 of 109.6mg/l. This value is far lower than that estimated in this study in which *C. gariepinus* was found to have 626mg/l thus indicating that *C. gariepinus* was more resistant to tobacco toxicity than *O. niloticus*. The toxicity of other plant extracts on fish has been reported. Onusiriuka and Ufodike (1994 and 1998) reported that when they exposed *C. gariepinus* to the bark extract of *Blighia sapida* and *Kigelia africana* they recorded 8.317 mg/l and 26.913mg/l respectively. This observation implies that tobacco leaf dust extract is less toxic to *C. gariepinus* than *B. sapida* and *K. africana*.

Mucus was observed to have accumulated on the gills of the moribund fish which might be responsible for the mortalities recorded in this study. Konar (1970) reported that accumulation of mucus on the gills reduces respiratory activity in fish. The inability of the gill surface to actively carry out gaseous exchange might be responsible for the recorded mortalities. The observed restlessness and jumping of fish in bioassay media might be due to the effect of nicotine, which is a stimulant, in tobacco leaf (Hassal, 1982). Yamamoto *et al* (1962) reported that this stimulant could bind on to acetylcholine receptors in the nervous system thus causing the excitation and the resultant frequent jumping and restlessness.

During the sub-lethal exposure, a decrease in weight (figure 2) gain by the fish with increase in extract concentration was observed. This observation was not unexpected as Omoregie *et al* (1990), had reported that sub-lethal concentrations of toxicants in aquatic environment often result in several physiological dysfunctions instead of an outright mortality of fish. The retardation of growth, as shown in figure 2, might be due to interactions of the nicotine with normal metabolism of the fish. This finding also agrees with the report of Onusiriuka and Ufodike (1998) on *C. gariepinus* and Omoregie *et al* (1998) on *Oreochromis niloticus* when working with different plant-derived piscicides. The water quality parameters in the different treatments showed no significant difference hence the effects on this study could be negligible.

The statistically significant ($p < 0.05$) decrease in values of the haematological parameters (Table 1) studied was not uncommon in fish exposed to sub-lethal concentrations of toxicants. Similar reduction of haematological indices was reported by Musa and Omoregie (1999) when *C. gariepinus* was treated with sub-lethal doses of malachite green. Omoregie *et al* (1994) had earlier reported similar observations when they subjected *O. niloticus* to sub-lethal concentrations of formalin. The reduction in

these blood parameters is an indication of anaemia caused by exposure to the extract of tobacco leaf dust. This anaemic response could be as a result of destruction of erythrocyte or inhibition of erythrocyte production (Wintrobe, 1978) or haemodilution as reported by Sampath *et al* (1993).

Conclusion: This study confirms that the extract of tobacco leaf exerts piscicidal property, an encouraging information for tobacco growers/farmers in view of their worries over anti-smoking campaign by the medical regulations. This study has shown that tobacco leaf dust extract can be applied in pond management to eradicate stunted and unwanted fish populations so as to enhance production of desirable fish species for the country.

ACKNOWLEDGEMENT: The authors are grateful to the Department of Aquaculture and Fisheries Management, University of Agriculture, Abeokuta, Nigeria for providing the facilities used in this study.

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