**HAEMATOLOGICAL RESPONSE OF NILE TILAPIA (*Oreochromis niloticus*) JUVENILES EXPOSED TO TOBACCO (*Nicotiana tobaccum*) LEAF DUST**

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**ABSTRACT**

Tobacco (*Nicotiana* tobaccum) leaf dust has piscicidal properties thus there is a need to study its haematological effects on Nile tilapia juveniles. A 96hour bioassay was conducted on *Oreochromis niloticus* juveniles (mean wt., 30g) to determine the median lethal concentrations (LC50) .The fish were exposed to various concentrations of tobacco leaf dust (0.5g-2.5g/l).Water quality parameters and physiological parameters were monitored/determined according to standard procedures. Water quality parameters were monitored after 96hours. The LC50 at the end of 96hours was 1.35g/l. The monitored water quality parameters such as temperature, pH and dissolved oxygen were significantly decreased while total alkalinity and conductivity increased significantly in the exposed media, compared to the control test. The fish showed hypeventilation, erratic swimming, loss of reflex during the period of exposure and this increased with increase concentrations of tobacco leaf dust. Haematological analysis of the blood revealed significant haematological changes, the intensity of haematology damages increased with increasing concentrations and exposure to tobacco leaf dust. The reduction in blood parameters could be as a result of destruction of erythrocyte or haemodilution. However, the monitored water quality parameters revealed that the plant dust has effects on the blood parameters of the test fish and consequently the biodiversity of the organisms. The result provided baseline information and established safe limits of using tobacco leaf dust in fish ponds, hence 1.0g/l concentration of tobacco leaf dust is recommended for the use on *O. niloticus juveniles*

**INTRODUCTION**

Aquaculture is increasingly becoming one of the fastest growing aspect of the agricultural industry worldwide (FAO, 2004). Fish farmers often use tobacco leaf in controlling these unwanted organisms/pests (Konar, 1970; Tobor, 1990). According to Aleem (1987), the use of tobacco leaf dust is due to its inexpensiveness, local availability and easier degradability. Despite, the effective use of this plant material, eco-toxicologists are interested in the ecotoxic properties of plant origin pesticides/piscicides, such that plant origin pesticides/piscicides cannot be used directly in freshwater bodies unless their toxicity and sub-lethal long term effect have been studied on non-target animals, sharing the habitat with the target animals.

The active ingredient of the plant used, is the nicotine (Hassal, 1982), It is soluble in water, alcohol, chloroform, ether, kerosene and some fixed oils (Vogue, 1984). Tobacco leaf dust has been used in Nigeria as an effective insecticides and treatment of predators/pest in water (pond) since it is completely biodegradable (Aleem, 1987; Nile tilapia is of the commercially important species of fish for rapid aquaculture expansion in Nigeria. The choice of the test fish is attributed to the report of Rand *et al.,* (1995) that in order to extrapolate meaningful, relevant and ecological significant results from aquatic toxicity tests, not only appropriate test but also appropriate organism should be used, whenever possible, species should be studied or representative of the ecosystem that may be impacted; thus the choice of the *Oreochromis niloticus* which is of economic importance in Nigeria as an abundant cultural fish species in Nigeria and is very popular with fish farmers and consumers. The knowledge of sub-lethal effects of tobacco is very important to delineate the health of fish status and to provide a future understanding of ecological impacts (Radhaiah *et al.,* 1987). The aim of this research is to ascertain the assumption whether tobacco leaf dust (*Nicotina tobaccum*) in a sub-lethal concentration and in a medium exposure time can influence changes in the blood of *O. niloticus* after the 96 hours exposure period.

**MATERIALS AND METHODS**

Juvenile *O. niloticus* of the same brood stock (30.01±0.34g) were obtained from the Federal University of Technology, Akure fish farm. They were acclimatized in a glass tank for 24 hours. The mortality and later transferred to the experimental plastic aquaria 10 fish/48L aquaria). The leaves of tobacco were sun-dried for 10 days and milled into powder, sieved and stored in a sealed plastic container until required. The concentrations of tobacco used were calculated as 50% 96h LC50 (96h LC50 of tobacco leaf dust on *O. niloticus* obtained from preliminary investigation). Thus 100 mg of tobacco leaf dust were measured and mixed in 1 litre of water to give 100 mg/L concentration of the tobacco leaf dust. These concentrations were introduced into 12 sets of aquaria with one replication.

Forty (48) liters capacity aquaria were maintained throughout the exposure period. Ten (10) juveniles each were placed in the 48L plastic aquarium. Bore-hole water was used during the acclimatization and exposure period. In order to monitor the toxicant strength, level of dissolved oxygen, the effects of evaporation; ammonia concentration and reduce stress during experimentation, the test media were replaced by 50% prepared – concentrations of the same quantity after removing its equivalent along with defaecation every 6 hours to maintain the requisite level and potency of the concentration. The exposure period lasted for 96hours during which some water quality parameters were monitored daily using APHA (1998) methods. After 96 hours, 60 fishes were sacrificed and analyzed for the haematological examination. Blood was obtained from randomly selected fish from the control and the exposed test after the 96hours, using 2.0ml plastic syringe, as described by Kori-Siakpere (1998). The blood was transferred into a lithium heparin anticoagulant tube at room temperature for 30-40 minutes (Mahoba, 1987) and stored at refrigerator until analyses.

Fish mortality data were analyzed using complete randomized design with equal replication (one-way ANOVA test) at 5% level probability. All data were presented as means ± standard error, the data from the 96hours tobacco leaf dust exposure was first analyzed using a one-way ANOVA test, after which individual means were compared, using Bonferoni multi-sample correction/test.

**RESULTS AND DISCUSSION**

Mean values of water quality parameters of the different sub-lethal concentrations of tobacco leaf dust and control media to which the test fish *O. niloticus* were exposed over the 96hours exposure period is as presented in Table1. The value of temperature, pH and dissolved oxygen were found to significantly (*p*<0.05) and (*p*<0.01) decreased as the concentrations of tobacco leaf dust increased. However, the values of total alkalinity and conductivity in the exposed media were significantly (*p*<0.01) increased as the concentrations of tobacco leaf dust increased, compared to the control test. Exposure of *O. niloticus* juveniles to tobacco leaf dust solution clearly disrupted haematological parameters. Haematocrit, haemoglobin values, erythrocyte and leucocyte counts, total protein and albumin of the fish exposed to different concentrations of tobacco leaf dust revealed significant haematological alteration and changes (Table 2). Erythrocyte reduces from mean value of 1.67 – 1.0mm3 with increase in concentration of tobacco leaf dust, the decrease in these values were also observed to be both a factor of time and concentration of tobacco.

**Table 1**. Water quality parameters of the sub-lethal concentrations of tobacco leaf dust after 96 hours

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Concentration (g/l) | Temperature (0C) | Dissolved oxygen (mg/l) | pH | Conductivity |
| 0.0 | 24.20 ±0.00 | 6.20±0.10 | 6.80±0.05 | 117.9±0.40 |
| 0.5 | 24.30 ±0.00 | 5.90±0.20 | 6.50±0.00 | 134.3±1.10 |
| 1.0 | 24.30 ±0.00 | 4.10±0.10 | 6.40±0.00 |  |
| 1.5 | 24.30 ±0.00 | 3.80±0.10 | 6.40±0.05 | 141.0±1.20 |
| 2.0 | 24.30 ±0.00 | 3.30±0.00 | 6.30±0.05 | 145.8±2.05 |
| 2.5 | 24.30 ±0.00 | 3.10±0.10 | 6.20±0.00 | 150.1±1.05 |

\*mean ± Standard Error (SE).

**Table 2**: Blood parameters of *O. niloticus* exposed to tobacco leaf dust concentrations for 96 hours.

|  |  |
| --- | --- |
| Concentrations(mg/l) |  Haematological Parameters  |
| PCV(%) | RBC(mm3) | WBC(mm3) | HB(g/dl) | Total protein (mg/dl) | Albumin(mg/dl) |
| 0.0 | 15.00(1.00) | 1.67(0.27) | 3.88(0.10) | 6.93(2.57) | 5.13(0.12) | 3.43(0.21) |
| 0.5 | 14.00(1.00) | 1.73(0.06) | 3.82(0.10) | 4.67(0.31) | 4.80(0.20) | 2.93(0.12) |
| 1.0 | 11.7(0.58) | 1.21(0.04) | 5.12(0.10) | 4.00(0.00) | 3.43(0.21) | 2.73(0.12) |
| 1.5 | 10.7(0.58) | 1.21(0.01) | 5.00(0.10) | 2.20(0.35) | 2.80(0.20) | 2.37(0.32) |
| 2.0 | 9.33(1.16) | 1.10(0.03) | 5.12(0.08) | 1.33(0.35) | 2.20(0.20) | 2.00(0.20) |
| 2.5 | 9.00(1.00) | 1.00(0.03) | 5.27(0.76) | 0.70(0.10) | 1.97(0.15) | 1.87(0.23) |

\*Mean ± Standard Error (SE).

Haemoglobin values reduced from 6.93-0.70g/dl with increase in concentration from 0.0g /l control - 2.5g/ l (Table2). The erythrocyte and leucocyte counts showed intra- concentration variations, the number of the leucocytes increased as the concentration of the toxicants increases while erythrocytes deceased with increasing concentrations of tobacco leaf dust , In different concentrations of tobacco leaf dust (0.0, 0.5, 1.0, 1.5, 2.0, and 2.5g/l), PCV values varied from 15.0 ,14.00, 11.67 ,10.67, 9.33 and 9.0%, respectively, at concentrations 0.5 and 2.5g/l, and the PCV values reduced with increase in concentration of test dye and this is traceable to different fishes having different blood parameters unlike human blood that is constant (Baker and Silverton, 1982). Total protein (g/L) varied with different concentrations of tobacco leaf dust, the mean value for total protein varies from 1.97-4.80mg/dl and 1.87-3.43mg/dl in albumin, this shows that total protein decreases with increase in concentration of tobacco leaf dust when compared with the control (0.0) with mean value of 5.13mg/dl (Table2). The 96-h LC50 was 1.35 g/L tobacco leaf extract, compared to other synthetic pesticides used in fish farming, such as carbamates and organophosphates, tobacco based products are certainly less toxic to fish (Wan et al., 1996). Results indicated that tilapia is more sensitive to tobacco leaf water extract than other fishes (Mamdouh et al., 2008). Changes in the water quality parameters showed that the concentrations affected the water quality, but the values were within tolerance range (Table 1).

Houston et al. (1971) reported changes in the blood parameters of fresh water fish exposed to various handling procedure before experiment and the effect of stress on the fish. The disrupted haematological parameters observed in this experiment also agreed with Akinbulumo (2005) who reported that fish showed toxic reaction to *Derris elliptica* root powder by surfacing jaws and becoming stupefied*.* Reduction in oxygen level in this study is in line with Lloyd (1961) who reported that toxicity of several poison on rainbow troutincreased inversely to the oxygen concentrations of water .A number of poisons become more toxic at low oxygen concentrations because of an increasing respiratory rate, increasing the amount of poison to which the animal is exposed.

Haematological examination revealed adverse effect of tobacco leaf dust on the blood of *O*. *niloticus*, and this is similar to Mason et al. (1994) who had earlier reported similar observations when subjected *O. niloticus* to sub-lethal concentration of formalin. The result from statistical analysis shows that there is reduction in some of the blood parameters, this is an indication of anemia which is a condition characterized by a deficiency of haemoglobin, packed cell volume and erythrocytes.

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