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Anesthetic And Haematological Response of Tobacco (*Nicotiana tobaccum*) Extract On African Catfish (*Clarias gariepinus* Burchell 1822) Fingerlings.

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Abstract
Anesthetic and Haematological investigation was made on the effect of tobacco extract on Clarias gariepinus fingerlings (4.03±2.20g) using bioassay tests over a period of 96hours. Water quality parameters like Dissolved Oxygen and PH decreased significantly while alkalinity increases and temperature was almost at the same level. There were no similarities in the time of anesthesia and time of recovery at different concentrations of ethanol extract of tobacco for Clarias gariepinus. Anesthesia time decreases on increase of anesthetic dose. Fish showed marked behavioural changes like erratic swimming, aggression, jumping, air gulping, hyperventilation and settling at the bottom which indicates anesthesia time. There was increase in White Blood Cell (WBC) counts with increase in tobacco concentration, Packed Cell Volume (PCV) and Red Blood Cell (RBC) counts decreases with increase in tobacco concentration. Haematologically, there was destruction of Red Blood Cell and low concentration of Haemoglobin (HB) in the exposed fish compared to fish in the control tank.

Keywords: *Anesthetic, Anesthesia time, Recovery time, Nicotiana tobaccum, Haematology, Fish*

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I. Introduction

Piscicidal plants are frequently used by fisher folks of various African countries extensively for capturing fish and reported to be of high potency (Fafioye *et al., 2004*). Piscicides are often used to control competing species in fish production especially in small water bodies/ enclosures, eradicate fish to control parasites, and conserve or restore native species, but their uses are not encouraged because of their toxicity to aquatic organisms and the degradation of the environment (Olufayo, 2009).

The Introduction of most chemicals into the aquatic environment occurs both directly and indirectly and the accumulative non- lethal effect of the toxicants of aquatic organisms is extremely hazardous to fish (Olufayo, 2009).The aquatic ecosystem like the terrestrial environment is continuously subjected to changes in quality that are due to the introduction of substances of diverse characteristics arising from man's cultural activities (Oluah, 2001). The accumulation of toxicants in an aquatic environment can result in reduced reproductive capabilities, alter growth rates and reduce ability to withstand variations in PH, temperature and dissolved oxygen (Adamu *et al;* 2008).

Various parts of plants have been extracted and used as poisons including bark, flower, seeds, fruits, leaves, roots, stems, pulp and even the entire plant (Fafioye, 2005) and many authors ascribed the piscicidal activities of plants to be due to the presence of Phytochemicals such as tannins glycosides, resins, flavonoids, anthraquinones, saponins, nicotine, pipanne, ricin, amnrine, pyrethrum and diosgenin (Sogbesan and Emmanuel, 2015).

Fish are regularly handled during various management practices such as weighing, strippling, tagging, sorting, treatment for and against disease and transportation. These processes may result in undesirable outcomes such as immune-suppression and growth retardation (Heo and Shin, 2010) and decreased fish performance, increased susceptibility to diseases and mortality in extreme cases (Mesa *et al; 2000*). In order to avert these effects, it is necessary to sedate the fish before handling.

Conventional anesthetics such as tricaine methane sulphate (MS-222), benzocaine and quinaldine are expensive and not readily available in some third world countries (Agokei and Adebisi, 2010) and this has necessitated the search for alternative use of plant materials that are cheap, available and abundant which can

perform anesthesia function effectively (Audu *et al* ;2013).However, there is need to identify other plants that will have more effect to the conventional anesthesia thus the choice of another plant (*Nicotiana tobaccum*) that may been effective anaesthesia.

Heamatological study is important for toxicological research, environmental monitoring of fish and their health conditions during culture because fish generally are so intimately associated with the aquatic environment (Olufayo, 2009). The determination of haematological values of fishes are carried out for a variety of purpose; to establish a 'normal range' of blood parameters (klontz,2005).A thin epithelial membrane seperates fish blood from the water and any unfavourable changes in the water body is reflected in the blood (Olufayo,2009). Several authors have documented the effect of tobacco dust on blood of fish (Omoniyi *et al.,* 2002, Agbon *et al*, 2002).

Tobacco (*Nicotiana tobaccum*) a natural biocide is native to tropics and subtropical America but it is now planted profitably globally (Knapp,2004).The active ingredient in tobacco leaves is nicotine, which contributes between 2%-5% dry weight of leaves (Hassal,1982). Nicotine and related alkaloids contained in tobacco are generally recorganized as being narcotic (Agokei and Adebisi, 2010). In the quest for safer, more effective, readily available, affordable, eco-friendly and easily adaptable anaesthetic which is comparable to conventional chemical anaesthetics, tobacco, a popular narcotic, is hoped to be the suitable alternative (Temitope, 2014).

Fish have been the most popular choice as test organisms because they are presumably the best understood organisms in the aquatic environment (Buikema *et al.*,1982). 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 (Kori-Siakpere and Oviroh,2011). This research is therefore aimed to investigate the viability of using *Nicotiana tobaccum* leaf extract as anesthetics on *Clarias gariepinus* fingerlings and its haematological effect on the fish blood.

II. Materials And Methods

Experimental Fish

Seventy healthy fingerlings of *Clarias gariepinus* mixed sex and the same brood stock (4.03±2.20g) were obtained from a private fish farm in Owerri, Imo State. The fish were transported in an aerated bag to Fisheries and Hydrobiology Research laboratory of Imo State University, Owerri were the research took place. The fish were transferred to a large plastic aquarium of 60L capacity with well-aerated borehole water and were allowed to acclimatize to laboratory conditions for 7 days. Feedings was stopped 48 hours to experimental day to minimize the amount of waste in the test media decomposition and oxygen depletion.

Collection and preparation of experimental plant

Leaves of tobacco were obtained from Ekiti State, Nigeria, shade dried at ambient temperature, milled into fine particles size (<250 µm) and stored in a clean, dry and air-tight transparent plastic bottle.

Anaesthetic experimental procedure

A range finding test was conducted to determine the concentration at which *Clarias gariepinus* fingerlings reached anesthesia level. After the range finding test, five different aquaria were filled with 10 litres of water, then extract of tobacco (0.0, 1.5, 2.5, 3.5 and 4.5) g/5L were added to each and stirred properly to ensure even disposal of anesthesia. Water quality parameters like Temperature, Dissolved Oxygen, pH and Alkalinity were measured at 24 hours intervals for 96 hours. Fish were sorted into cohort and added in batches of ten fish per aquarium in replication. Behavioural changes were monitored and recorded, anesthesia time were taken with stop watch from when the fish losses equilibrium, swims erratically, jumps, gulp air, settled at the bottom of the aquaria and stopped responding to stimulus. Fish are then transferred to recovery aquarium without tobacco and recovery time also taken with a stop watch when fish appears to exhibit normal behaviour, and water quality parameters were measured at the end of the experiment.

Haematology experimental procedure

After 96 hours, fish from different aquarium with different concentration of tobacco extract were randomly selected and used for further haematological experimentation. Fish were sieved out of the water after reducing the water volume, this is because stress has been identified as a factor affecting physiological conditions and haematology of fish (Ololade and Oginni,2010). The caudal peduncle of fish was cut with a sharp pair of scissors and blood collected by means of disposable sterile plastic syringe fitted with needle (Gaafer *et al*.,2010). The blood was transferred into a litium heparin anticoagulant tube at room temperature for 30-40minutes and stored in the refrigerator for analyses. Red blood cell (RBC) count and White blood cell (WBC) counts were counted under light microscope with an improved neubauerhaemocytometer (Mgbenka and Oluah,2003, Shah and Altindag,2005). The Packed cell volume (PCV) and haemaglobin (Hb) concentration

values were determined by the microhaematocrit capillary tube and cyanomethaemoglobin methods (Hesser,1960) respectively. RBC and WBC percentage were determined by the RBC count from the haemocytometer to determine the WBC absolute count (Musa *et al*.,2013). WBC differential count were determined manually as recommended for avian (Zinkl, 1986) and fish (Stoskopf,1993) blood, because nucleated RBC prevents accurate enumeration using automated analysis (Huffman *et al.,*1997).

Statistical Analysis

The data obtained were subjected to One-way Analysis of Variance (ANOVA) using SPSS version 10 and variation between anesthetic dose, anesthesia time and recovery time were compared with Fisher's pairwise comparison.

III. Results

The mean values of the water quality parameters for the different concentrations of tobacco extract for 96 hours are shown in Table 1. Mean value of the water temperature were not significantly affected ($P>0.05$) by the concentrations of tobacco extract. Total Alkalinity significantly increases ($p<0.05$) while Dissolves Oxygen and pH decreased significantly ($p<0.05$). The results of the various experiments on the different concentrations of leaf extract of tobacco are shown in Table 2. The time of anesthesia (in minutes) decreased from 54-42 minutes with increase in concentration of tobacco leaf extract. Also increase in tobacco concentration led to rise in recovery time from 24-40 minutes. Fish in aquarium with 3.5 and 4.5g/5L concentration showed marked behavioural changes like aggression, jumping, air-gasping, settling at the bottom of the aquaria and stopped responding to stimulus showing anesthesia time. No mortality in control, 1.5 and 2.5g/5l aquaria but mortality was noticed in 3.5 and 4.5g/5l concentration aquaria, that is 15 and 40 deaths respectively. That is to say that mortality increases with increase in concentration of tobacco leaf extract.

The mean value of Haematological indices of *Clarias gariepinus* fingerlings exposed to different concentration of tobacco leaf extract for 96 hours were presented in Table3. The result showed that there was increase in White Blood Cell (WBC) counts from 4.10 to 6.40 mm^3 with increase in concentrations of tobacco extract. Haemoglobin values decreased from 7.05 to 0.64g/dl^{-1} with increase in concentration of tobacco extract, also Packed Cell Volume (PCV) and Red Blood Cell (RBC) counts decreased from 18.0 to 7.20% and 1.73 to 1.18mm^3 respectively with increase in concentration of tobacco extract.

Table 1.Mean values of water quality parameters for the different concentrations of tobacco leaf extract for 96hours

Parameters	control	1.0 mg/l	1.5mg/l	2.0 mg/l	2.5 mg/l
Temperature (^{0}C)	23.34±0.0	22.3±0.2	22.3±0.1	22.3±0.2	22.3±0.1
Dissolved Oxygen (mg/l^{-1})	8.5	8.0	6.7	5.1	4.9
PH	6.9	6.6	6.2	5.4	5.0
Alkalinity (mg/l^{-1})	74.1	84.4	103.7	129.3	142.7

Table 2.Dosage, Recovery and Mortality time of *C.gariepinus* fingerlings anesthetized with tobacco leaf extract

Concentration (g/5l)	Anesthesia time (mins.)	Recovery time (mins)	Mortality (%)
Control (0)	-	-	0
1.5	54	24	0
2.5	51	27	0
3.5	48	30	15
4.5	42	40	40

Table 3.Mean values of haematological indices of *C. gariepinus* fingerlings exposed to different concentrations of tobacco leaf extract for 96 hours.

Parameters	control	1.0 mg/l	1.5mg/l	2.0 mg/l	2.5 mg/l
PCV (%)	18.00±0.00	15.20±0.10	10.00±0.04	8.20±0.08	7.20±0.05
Haemoglobin	7.05±0.00	5.82±0.10	4.20±0.10	1.40±0.34	0.64±0.10
WBC (mm^3)	4.10±0.02	5.25±0.10	6.16±0.08	6.24±0.03	6.40±0.04
RBC (mm^3)	1.73±0.04	1.50±0.03	1.34±0.66	1.24±0.08	1.18±0.02

(PCV-packed cell volume, HB -haemoglobin, WBC- white blood cell, RBC- red blood cell)

IV. Discussion

The findings of this study are similar to the observations of (Musa *et al*., 2013) who reported that changes in the water quality parameters showed that tobacco leaf dust concentrations significantly affected the water quality. pH value in the highest concentration of leaf dust was 5.0 which is lower than the recommended pH value for fresh water fish (Noga,1996) recommended pH range of 6.5 to 8.5 for fresh water fishes, indicating acidic condition as toxicant strength increases. The acidity of the water probably could be the cause of the decrease in Dissolved Oxygen content of the water and increase in Alkalinity.

Based on the present study, it was observed that tobacco leaf extract served as an anesthetic agent on the experimental fish. Therefore tobacco appears to meet five of the eight criteria used to define an ideal anesthetic according to (Marking and Meyer, 1985). Anesthesia time decreased significantly ($p<0.05$) with increase in concentration of tobacco extract. This is in line with the work of (Weber *et al*., 2011) which suggested that the total loss of reflex time decreases inversely proportional to the concentration of anesthetic agent in teleost fish.

From this study, the fish in 1.5 and 2.5g/5l concentrations of tobacco leaf extract recovered within 24 and 27 minutes respectively with 0% mortality, this showed that tobacco is an anesthesia as (Burka *et al.,* 1997) reported that an appropriate anesthesia depends mainly on its effectiveness in immobilizing fish with good recovery. So the best range for the use of tobacco leaf extract on *Clarias gariepinus* fingerlings as anesthetic was suggested to be 1.5 to 2.5g/5l because there will be no mortality.

The study revealed that there was significant decrease in Packed Cell Volume (PVC), Haemoglobin (Hb) and Red Blood Cell (RBC) as the concentration of tobacco extract increases, this is an indication of severe anaemia caused by destruction of erythrocytes (Omoniyi *et al*., 2002) or haemo-dilution as reported by (Sampath *et al*.,1993, Adeyemo,2005). Adamu and Audu (2008) reported that the significant decrease in PVC may be attributed to gill damage and/or impaired osmoregulation causing anaemia and haemodilution. Increase in tobacco leaf extract caused decreasein Packed red cell volume values and this is traceable to different fishes having different blood parameters unlike human blood that is constant (Baker and Silverton,1982). The decrease in Haemoglobin may be due to increase rate of breakdown of Red Blood Cells and reduction in the rate of formation of red blood cells (Mossa, 2004).

White Blood Cell (WBC) values increased with increase in concentration of tobacco leaf extract, this could be attributed to the increase in leucocytes synthesis as a defense mechanism against the destruction of erythrocytes (Musa *et al*., 2013). The increase in WBC value could probably indicate that the fish were less stressed by the increase in concentration of tobacco extract because leucocyte profiles are altered by stress and can be directly related to stress hormone levels (Dhabhar *et al*., 1996).

V. Conclusion

From this study, tobacco as an anesthetic is a better option because it is readily available, cheap and highly effective with few or no mortality and 1.5-2.5g/5l of the leaf extract can readily anesthetize fish with a short recovery time and no mortality. Haematological characteristics of *Clarias gariepinus* fingerlings showed that leaf extract of tobacco increases the water alkalinity and decreases the pH and dissolved oxygen content of the water which affected the blood parameters of the experimental fish.

References

[1]. Adamu, K.M., Audu, B.S. and Audu,E.L.(2008). Toxicity and Histopathological effects of Portland cement powder in solution on the structure of the gill and liver tissues of the Nile tilapia, *Oreochromis niloticus*:A microscopic study, Tropical Freshwater Biology,17(1): 25-36.

[2]. Adeyemo, O.K. (2005). Haematological and histopathological effects of cassava mill effluent in *Clarias gariepinus*. *African Journal of Biomedical Research*, 8: 179-183.

[3]. Agbon A. O., Omoniyi I. T., Teko A. A. (2002). Acute toxicity of tobacco (*Nicotiana tobaccum)* leaf dust on *Oreochromis niloticus* and haematological change resulting from sub-lethal exposure. *J. Aquatic. Sci.* 17(1): 5-8.

[4]. Agokei, O. A., & Adebisi, A. A. (2010). Tobacco as an anesthetic for fish handling procedures. *Journal of Medicinal Plants Research, 4*(14), 1396-1399.

[5]. Audu, B.S, Adamu, K.M and Ufodike, E.B.C (2013). Behavioural response and opercular ventilation rate on nile tilapia (*Oreochromis niloticus*) fingerlings after anesthesia with crude leaf extract of marijuana (*Cannabis sativa) Applied Science Research Journal.* 1(2):66-77.

[6]. Baker F. J. and Silverton, R.E (1982). *Introduction toMedical Lab Tech*, Butterworth Scientific, London, UK, 5th edition,

[7]. Buikema, A.L (Jr).Niederlehner , B. R and Cairns, J.(Jr). (1982). Biological monitoring: Part IV- Toxicity Testing Water Resource, 16: 239-262.

[8]. Dhabhar, F. S. Miller, A. H. McEwen, B. S. and Spencer R. L. (1996). "Stress induced changes in blood leukocyte distribution—role of adrenal steroid hormones," *Journal of Immunology*, vol. 157, no. 4, pp. 1638–1644.

[9]. Fafioye O. O. (2005) Plants with piscicidal activities in southwestern Nigeria. *Turkish Journal of Fisheries and Aquatic Sciences 5: 91-97.*

[10]. Fafioye O. O., Adebisi A. A. and Fagade S.O. (2004) Toxicity of *Parkia biglobosa* and *Raphia vinifera extracts* on *Clarias gariepinus* juveniles. *Afri. J. Biotechnol*., 3:627-630.

[11]. Gafaar, A.Y., El-manakhly, E.M., Soliman, M.K., Soufy, H., Monas, Z., Mohammed, S.G. and Hassan, S.M. (2010).Some pathological, biochemical and Haematological investigation on Nile tilapia (Oreochromis niloticus) following chronic exposure to edifenphos pesticide. *Journal of American Science*, 6(10) 542-551.

[12]. Hassal K.A(1982). The chemistry of pesticides. Macmillan Press, London, UK., 372p.

[13]. Heo, G.J and Shin, G (2010). Efficacy of benzocaine as an anaesthetic for crucian carp (*Carassius carassius*). *Vet. Anaesth, Analg*. 37: 132-135.

[14]. Hesser, E. F.(1960). "Methods of routine fish hematology," *Progressive Fish Culturist*, vol. 22, pp. 164–171.

[15]. Huffman, P.A. Arkoosh, M. R. and Casillas, E. (1997). "Characteristics of peripheral blood cells from rainbow trout evaluated by particle counter, image analysis, and hemocytometric techniques,"*Journal ofAquaticAnimalHealth*, vol. 9, no.4,pp. 239– 248.

[16]. Knapp E. (2004) Nomenclatural changes and a new sectional classification in *Nicotiana* (Solanaceae) Taxon. 53(1):73-82.

[17]. Kori-Siakpere O. and Oviroh E. O. (2011) Acute toxicity of tobacco (*Nicotiana tobaccum*) leaf dust on the African catfish: *Clarias gariepinus* (Burchell, 1822). *Archives of Applied Science Research*, 3 (2): 1-7.

[18]. Marking, L.L. and Mayer, F. (1985). Are better anaesthetics needed in fisheries. Fisheries. 10(6):2-5

[19]. Mese, M. G., Maule, A. G., & Schreck, C. B. (2000). Interaction of infection with *Reinbacterium salmoninarum* and physical stress in juvenile Chinook salmon: physiological responses, disease progression and mortality. *Tras. Am. Fish. Soc., 129*, 158-173. http://dx.doi.org/10.1577/1548-8659(2000)129<0158:IOIWRS> 2.0.CO;2

[20]. Mgbenka, B.O. and Oluah, N.S.(2003). Effect of Gamalin 20(lindane) on differential white blood cell counts of Afrcan catfish, *Clarias albopunctatus.* Bull. of Environmental Contamination Toxicol. 71:248-254.

[21]. Mossa, A.H. (2004). Genotoxicity of pesticides. PhD Thesis. Pesticides chemistry and toxicity, Faculty of Agriculture, Damantiour Alexandria University.

[22]. Noga, E.J. (1996). *Fish Disease*: Diagnosis and Treatment Mosby Yearbook, Incoporated. Weslin industrial Drive: St. Louis, Missouri, 367pp.

[23]. Ololade, I.A. and Oginni. O. (2010). Toxic stress and haematological effects of nickel on African catfish, *Clarias gariepinus*, fingerling. *Journal of Environmental Chemistry and Ecotoxicology*. Vol.2(2). Pp.014-019.

[24]. Olufayo,M.O.,2009. Haematological characteristics of *Clarias gariepinus* (Burchell,1822) juveniles exposed to *Derris elliptica* root powder. *African Journal of Food,Agriculture,Nutrition and Development.* AJFANDONLINE.9(3).ISSN:16845374.Availablefrom:URL:http//www.ajol.info/index.p hp/ajfand/article/view File/43115/26663. Accessed:21-08-2018.6.42pm.

[25]. Oluah, N. S. (2001). The effect of sublethal cadmium on the haematology of the freshwater catfish, *Clarias gariepinus* (Pisces: Clariidae), *Journal of Science, Agriculture, Food Technology and Environment*, 1: 9 -14.

[26]. Omoniyi, I.A., O. Agbon and S.A. Sodunke, 2002. Effects of lethal and sublethal concentrations of Tobacco (Nicotiana tobacum) leaf dust extraction on weight and haematological changes in *Clarias gariepinus* (Burchell 1822). *Journal of Applied Science and Environmental Management*. 6: 37-41.

[27]. Sampath, K., Velamnical, S. Kennedy, I. J. and James, R. (1993). "Haematological changes and their recovery in *Oreochromis mossambicus* as a function of exposure period and sub- lethal level of Ekalus," *Acta Hydrobiologica*, vol. 35, pp. 73–83.

[28]. Shah, S.L, Altindag, A. (2005).Alteration in the immunology parameters of tench (*Tincatinca* L) after acute and chronic exposure to lethal and sub-lethal treatments with mercury, cadmium and lead. Turk.J.vet.Anim.Sci.29:11163-11168.

[29]. Stoskopf, M. K.(1993). *Clinical Pathology in Fish Medicine*, W.B. Saunders Company, Hartcourt Brace Jovanourah Inc.,

[30]. Zinkl, J. G. "Avian hematology," in *Schalm'S Veterinary Hematology*, N. C. Jain, Ed., pp. 256–260, Pai hea and Febiger, Philadelphia, Pa, USA, 1986.

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