International Journal of Fisheries and Aquaculture Sciences.
ISSN 2248-9975 Volume 3, Number 2 (2013), pp. 151-160

http://www.irphouse.com

Effect of freeze-dried bark extract of *Tephrosia vogelii* on some haematological parameters of *Heterobranchus longifilis* juveniles Val. (pisces: 1840) in Nigeria.

Anju, T.D.; Solomon, S.G. and Cheikyula, J.O.

Department of Fisheries and Aquaculture
Federal University of Agriculture Makurdi, Nigeria.
Correspondence Author: e-mail: solagabriel@yahoo.co.uk

Abstract

This study was carried out to assess the effect of the freeze-dried bark extract of *Tephrosia vogelii* as a tranquilizer on the haematological indices in *Heterobranchus longifilis* juveniles. Experimental fish were obtained from the River Benue at Makurdi and transported to the Department of Fisheries and Aquaculture, University of Agriculture, Makurdi, Nigeria. *Heterobranchus longifilis* weighing 115.25+25.00g were selected randomly and injected intramuscularly (IM) with the freeze-dried bark extract concentrations (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 g/l). Control fish were injected with distilled water. Injected fish were observed for behavioural responses and transferred to 70- litre plastic tanks containing 40 litres of water for recovery. Blood samples were analysed using the Mindray Auto Heamatology Analyzer for white blood cells (WBC), red blood cells (RBC), heamoglobin (Hgb), heamatocrit (Hct), mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH) and mean corpuscular heamoglobin concentration (MCHC). WBC, RBC and Hgb increased marginally as concentration of extract increased but not statistically significant ($P>0.05$) indicating that the freeze-dried bark extract of *T. vogelii* did not impact negatively on the internal environment of the fish. This result reveals that the freeze-dried bark extract of *T. vogelii* could be used as a tranquilizer in live fish transportation.

Key words: Anaesthetics, tranquilizer, haematological, induction, recovery, time.

Introduction

Anaesthesia is a biological reversible state, induced by an agent which results in the partial or complete loss of voluntary neuromotor control through chemical or non–chemical means (Summerfelt and Smith 1990). Anaesthesia is frequently applied in aquaculture to help minimize fish stress and to prevent injuries. Anaesthesia is also required for measuring or weighing fish, sorting and tagging, administration of vaccines, life transport, sampling and collection of gametes (Colye et al. 2004, Maricchiolo and Genovese 2011). Anaesthesia increase safety for both the fish and the handler during minor procedure and allows them to be performed out–of–water with decreased stress for the fish and during major surgical procedures it minimizes movement and physiological changes in response to nociception (Harms and Bakal 1995). It has been observed that because fish are considered as sentient animals and are thus included in ethical codes, they should from legal and ethical points of view be anaesthetized whenever they are to be subjected to stressful handling (Ramanyaka and Atapatu 2006).

A wide range of chemical anaesthetics are now used in aquaculture and fisheries for various purposes. However, in spite of the growing number of chemical anaesthetics, only MS–222 has been approved for use with food fish in the USA by the Food and Drug Administration (FDA) and in the European Union (EU) (Yushimura et al. 1981, Brown 2011). This approval of MS-222 for use on edible fish not withstanding, fish treated with MS-222 must be held for 21 days as required by law in those countries before human consumption. Other chemical anaesthetic agents commonly used in fish anaesthesia, include 2–phenoxythanol, benzoncaine, quinaldine, quinaldine sulphate (Immersion anaesthetics) and katamine hydrochloride, xyline, metomidate (Parenteral anaesthetics) etc.

According to Marking and Meyer 1985, Yanar and Kumlu 2001, Solomon and Amali 2004, Agokei and Adebisi, 2010 chemical substances currently used as fish anaesthetics suffer several disadvantages such as poor solubility in water and long induction time (e.g. quinaldine), acute toxicological effects at high concentration and potential health risk on humans including development of rashes (e, g. 2-phenoxyethanol), scarcity and high cost especially in developing nations (e.g. Benzocaine and MS–222).

Natural plant extracts have been used for thousands of years to kill and demobilize fish (Ramanayaka and Atapatu 2006). For instance, the Vietnamese were known to use a plant called *Derris tonkinensis* to anaesthetize fish long ago (Brown 2011). In Nigeria several piscicidal plants like *Blighia sapida*, *Kigelia Africana, Tetrapleura tetraptera, Raphia venifera*, *Parkia biglobosa* and *Tephrosia vogelii* are frequently used by fisherforks because they are highly potent due to the presence of active ingredients such as saponins and rotenones (Obomanu et al. 2007). Owing to the many disadvantages of chemical anaesthetic agents research effort into plant extracts that could be used as fish anaesthetics with less toxicological impact on the fish, handler and environment has been intensified. As result, clove oil, a plant extract distilled from clove plant *Eugenia caryophyllata* (Soto and Burhaudinn 1995) has been defined by FDA as GRAS (Generally Recognized as Safe) product and is now widely used as fish anaesthetic in aquaculture.

Some of the advantages of clove oil include effectiveness at low dosage, low cost, easy availability and reduction of stress for both the fish and handler (Iversen et al. 2003, McGovern-Hopkins et al. 2003). Its main disadvantage is the relatively low therapeutic index: the ratio between the therapeutic and the toxic concentration (Velisek et al. 2005).

Tephrosia vogelii is a tropical African perennial shrub. Locally known as "Kuhwa Indyar" (in Tiv language), has long been used by the Tiv people of Benue State in Nigeria to kill fish in natural waters. It grows up to 3-4 metres high, branching low and ramified. The bark is grey–brown with yellowish or more or less fissured and lenticellate, stems are grey–brown with yellowish or rust coloured dense pubessence. *Tephrosia vogelii* contains rotenoids (deguelin, rotenone, tephrosin etc.) as its main active ingredients (Ingham 1983, Marson et al. 1984). According to Lungu (1987) and Michael (2002) it is highly toxic to poikilothermic animals like mulluscs, frogs, toads, and worms and an effective poison for killing fish. The purpose of the study is to determine the effect of the freeze–dried bark extract of *Tephrosia vogelii* on the haematology indices of *Heterobrunchus longifilis.*

Materials and methods

Fresh samples of *T. Vogelii* bark were collected during the rainy season between July and September 2011. The samples were air-dried for 21 days under shade and then, Oven – dried for 3-4 hours to constant weight following Ominiyi et al. (2003). The dried samples were pulverized to powder using an electric kitchen blender and stored in air-tight laboratory bottles. 200g of the stored bark samples of *T. vogelii* was weighed and transferred into a flat–bottom flask of 2.5 litre capacity and 1 litre of distilled water was added to cover the samples. The flask was covered, shaken and allowed to stand for 24 hours. The mixture was then filtered using muslin cloth and suction filtration. The filtrate was concentrated using Rotary Evaporator and then dried using Iyovat gt3 freeze-drying machine. The dried extracts were weighed into sample bottles and stored. The anaesthetic solution of the freeze-dried bark extract of *T. vogelii* was prepared by dissolving graded series of stored freeze – dried samples of *T. vogelii* (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/l) in de-ionized water under laboratory condition for 24 hours at room temperature ($27.00 + 0.04^0$C) and the mixture filtered using No. 1 whatman filter paper.

The administration of the anaesthetic solution of the freeze – dried bark extract of *T. Vogelii* was carried out using the parenteral method of anaesthesia. Before sedation the fish were starved for 24 hours to prevent regurgitation from the gastro-intestinal tract, and observation and recovery baths provided with aeration. Some water quality parameters were measured as shown in Table 1. Three healthy *Heterobranchus longifilis* juveniles were selected randomly from both the control and the treatment groups. Each was weighed and injected 0.5ml of the extract concentrations (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06g/l) using No. 23 needle and a 2ml syringe. Injection was done intramuscularly (IM) at the dorsal saddle, just above the lateral line, behind the operculum following Neiffer and Stamper (2009). Injected fish were observed for behavioural responses and transferred into 70- litre tanks containing 40 litres of water for recovery and time taken to recover was noted.

Table 1: Mean values of water quality parameters during sedation of *H. longifilis* with *T. vogelii* freeze-dried bark extract

Conc. (g/ℓ)	Water quality parameters			
	Temperature ˚C	Dissolved oxygen (mg/l)	PH	Alkalinity (mg/l)
0.00	25.40±0.03^{a}	6.00±0.03^{e}	7.11±0.01^{b}	44.18±0.02^{d}
0.01	25.28±0.01^{b}	6.10±0.01^{d}	7.11±0.01^{b}	44.29±0.02^{c}
0.02	24.64±0.01^{c}	6.14±0.03^{cd}	7.14±0.01^{a}	44.28±0.01^{c}
0.03	24.59±0.01^{c}	6.17±0.02^{c}	7.15±0.01^{a}	44.32±0.02^{c}
0.04	24.45±0.01^{d}	6.19±0.02^{c}	7.15±0.01^{a}	44.39±0.02^{ab}
0.05	23.57±0.02^{e}	6.31±0.02^{b}	7.15±0.01^{a}	44.34±0.02^{bc}
0.06	23.33±0.02^{f}	6.39±0.01^{a}	7.15±0.01^{a}	44.45±0.04^{a}

Means in the same column followed by different superscript differ significantly ($P<0.05$)

The collection of blood samples from anaesthetized fish was carried out three hours after anaesthesia. The fish were stunned via application of pressure on the head with a blunt object and the lower lip cut open with a pair of scissors and blood was collected from the heart with No 23 needles and a heparinised syringe in order to stabilize the blood. The collected blood was immediately transferred into EDTA (6% ethylene Diamine Tetra Acetic Acid) bottles. The samples of blood were immediately analyzed using Mindray Auto Heamatology Analyzer. The various haematological variables analyzed for include; white blood cells (WBC), red blood cells (RBC), haemoglobin (HB), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Statistical analysis

Statistical analysis of the results obtained was carried out using Genstat Discovery Edition 4 for one–way Analysis of variance (ANOVA) to determine differences in haematological variables of experimental fish treated with the freeze – dried bark extract of *T. Vogelii.* Graph Pad Prim 5 and SSC stat V2.18 were used to test if differences existed between the haematological variables measured. Summary statistics were obtained for the variables using Minitab 14 for windows. Significant difference was accepted if the P-value was less than 0.05.

Results

The result showed that the various concentrations of freeze–dried bark extract of *T. Vogelii* were effective enough to anaesthetize *H. Longifilis* up to stage 3 of anaesthesia. The induction time to reach stage 3 of anaesthesia was 57.33 and 32.00 seconds with the corresponding recovery time of 140.62 182.00 minutes at

concentration 0.01 and 0.06g/l respectively. Induction time decreased at higher concentration of freeze-dried sample.

Effect of freeze–dried bark extract of *T. Vogelii* on the haematological indices of *Heterobranchus longifilis* is shown in Table 2. The result shows the highest value of white blood cells (WBC) was 1.749 x 10^{11} cells/l at concentration 0.6g/l while the lowest value was 1.292 x 10^{11} at concentration 0.01g/l. The highest value of red blood cells was 1.795 x 10^{12} cells/l at concentration 0.06g/l while the lowest value was 1.235 x 10^{12} cells/l at concentration 0.01g/l. The highest value of haemoglobin was 7.65g/dl at concentration 0.05g/l while the lowest value was 5.80g/dl at concentration 0.01g/l. The highest value of heamatocrit (Hct) was 24.90% at concentration 0.06g/l while the lowest value was 17.50% at concentration 0.01g/l. The highest mean corpuscular volume (MCV) was 141.30FL at concentration 0.04g/l while the lowest was 134.45FL at concentration 0.03g/l. The highest value of mean corpuscular haemoglobin (MCH) was 47.850pg at concentration 0.03g/l while the lowest value was 46.70pg at concentration 0.05g/l. The highest value of mean corpuscular heamoglobin concentration (MCHC) was 34.700g/dl at concentration 0.03g/l while the lowest value was 33.250g/dl at concentration 0.01g/l.

Table 2: Mean Values of Haematological variables in *H. longifilis* injected various concentrations of *T. vogelii* Freeze dried Bark Extracts

Conc (g/l)	Weight of Fish (g)	Haematological Parameters						
		WBC ($x10^{11}$/L)	RBC ($x10^{12}$/L	Hbg (g/dL)	HCT (%)	MCV (FL)	MCH (Pg)	MCHC (g/dL)
0.00	75.00±0.00^{a}	1.494±1.76x10^{10a}	1.555±2.95x10^{11a}	7.450±1.15^{a}	21.400±4.40^{a}	137.80±2.10^{a}	48.200±1.80^{a}	35.150±1.85^{a}
0.01	67.00±0.00^{a}	1.297±3.10x10^{9a}	1.235±1.50x10^{10a}	5.800±0.40^{a}	17.400±0.80^{a}	46.900±2.70^{a}	33.250±0.75^{a}	33.250±0.75^{a}
0.02	70.00±0.00^{a}	1.406±1.04x10^{10a}	1.425±5.00x10^{9a}	6.300±0.30^{a}	19.350±0.05^{a}	136.20±0.40^{a}	47.650±1.25^{a}	34.650±0.05^{a}
0.03	75.00±0.00^{a}	1.440±2.23x10^{10a}	1.440±1.15x10^{11a}	6.400±0.90^{a}	20.100±0.90^{a}	134.45±2.25^{a}	47.850±0.45^{a}	34.700±0.05^{a}
0.04	66.00±6.00^{a}	1.492±9.90x10^{9a}	1.450±1.30x10^{11a}	6.400±0.50^{a}	22.400±1.50^{a}	141.30±0.50^{a}	47.150±0.55^{a}	33.750±0.15^{a}
0.05	72.50±2.50^{a}	1.577±1.11x10^{10a}	1.730±1.20x10^{11a}	7.650±0.05^{a}	24.35±1.95^{a}	140.95±1.75^{a}	46.700±0.30^{a}	33.650±1.05^{a}
0.06	67.00±3.00^{a}	1.749±1.15x10^{10a}	1.795±3.50x10^{10a}	7.600±0.30^{a}	24.900±2.30^{a}	137.15±0.85^{a}	47.550±0.85^{a}	33.450±0.45^{a}

WBC = White Blood Cell
RBC = Red Blood Cell
Hgb = Haemoglobin
HCT = Haematocrit
MCV = Mean Corpuscular Volume
MCH = Mean Corpuscular Haemoglobin
MCHC = Mean Corpuscular Haemoglobin Concentration

Discussion

Haematological studies have been widely used as a means of assessing the health of fish and the trend of the haematological characteristics of fish generally serve as a standard for physiological or toxicological studies (Olufayo 2009). Haematological and biochemical profiles of fish provide important information about the internal environment of the organism (Masopust 2000) and are often determined an index of their health status (Oshode et al. 2008). Fish blood can also serve as a valuable tool in

detecting physiological changes that take place in the animal (Tilak et al. 2007) and such indices are closely related to response of animals to their environment (Val and Kapoor 2003). The values of WBC obtained with the freeze–dried bark extract revealed a two-fold fashion: value at concentration 0.01 – 0.04g/l increased (P>0.05) with increasing concentration while values at concentration 0.05 – 0.06g/l increased above control (P>0.05) with increasing concentration in accordance with Ucar and Atamanalp (2010) as reported for Brown trout and Rainbow trout treated with 2–Phenoxyethanol and clove oil as anaesthetics respectively. The result, however, differs from Adewoye (2010) for *Clarias gariepinus* exposed to *T. Vogelii* aqueous leaf extract. The difference in the results of these two experiments is due to biological factors such as species, stage of the life cycle and age, lipid content and body condition, all of which affect the metabolic rate and therefore the pharmacokinetics of the anaesthetic compound (Iversen et al. 2003). Other factors likely to be responsible for this difference include concentration (Hseu et al. 1998), environmental factors like the pH of immersion anaesthetics (Ross and Ross 1984), size and weight of the experimental fish (Colye et al. 2004), and nitrogenous compounds particularly ammonia and nitrite (Neiffer and Stamper 2009). Adeyemo (2005), Gabriel et al. (2007), Shahi and Singh (2011) reported significant increases in the values of WBC and attributed the result to excitement of the defense mechanism of the fish to fight against the antigens which augmented the production of more WBC to improve the health status of the fish. However, in the present study, the fact that the increases in WBC values were not significant among the concentrations (P>0.05) suggests that the health of the experimental subjects was not negatively impacted to warrant an excitement of the defense mechanism to fight against the antigens.

The values of blood variables involved in oxygen transport (RBC, Hb and Hct) in *H. longifilis* treated with freeze-dried bark extracts appear to show a positive correlation similar to Tilak et al. (2007) between red blood cells (RBC) and heamatocrit (Hct) in three major Indian Carps exposed to 2-Phenoxyethanol. The RBC count and heamoglobin (Hb) values decreased (P>0.05) below their controls at concentration 0.01–0.04g/ℓ and then increased (P>0.05) above control at concentration 0.05–0.06g/ℓ. Similar reductions in RBC and Hb values have been reported by other researchers (Tilak et al. 2007, Sudagara et al. 2009, Ucar and Atamanalp 2010, Ayuba and Ofojekwu 2010). Velisek et al. (2007) also reported decrease in the values of RBC in sheatfish (*Silurus glanis*) immediately after treatment with 2-phenoxyethanol anaesthetic without significant differences. Gabriel et al. (2009) reported the absence of significant changes in the blood variables associated with oxygen transport when catfish hybrids were exposed to aqueous leaf extract of *Lepidagathis alopecuroides* and suggested that the levels of toxicant used did not interfere with erythropoiesis or cause heamolysis. Consequently, in the present investigation the absence of changes in the heamatological variables associated with oxygen transport in *H. longifilis* following treatment with the different concentrations of freeze-dried bark extracts of *T. vogelii* could be attributed to the fact the levels of the dose and concentration of the freeze-dried bark extract used as a tranquilizer on the experimental fish neither caused changes in the blood variables associated with oxygen transport in *H, longifilis* nor lead to erythropoiesis or heamolysis.

Absolute Red Cells indices (MCV, MCH and MCHC): The mean values of MCV and MCH obtained after administration of the freeze–dried bark extract appear to fluctuate. These results are similar to that of Gabriel et al. (2011) in *Clarias gariepinus* following anaesthesia with metomidate where neither a definite pattern nor significant differences were found in values of MCH; Ucar and Atamanalp (2010) in Rainbow trout (*Onchorynchus mykiss*) and Brown trout (*Salmo trutta furio*) treated with clove oil anaesthetic where significant changes were not observed in the values of MCV and MCH and Sudagara et al. (2009) in Roach (*Rutilus rutilus*) anaesthetised with clove powder where no differences were observed in the values of MCV and MCH in two experimental subjects groups. Mean values of MCHC obtained with the freeze-dried bark extracts declined with increasing concentration of anaesthetic extracts with no significant differences ($P>0.05$) similar to Gabriel et al. (2011) in *Clarias gariepinus* following anaesthesia with metomidate where no differences were found in values of MCHC; Ucar and Atamanalp (2010) in Rainbow trout (*Onchorynchus mykiss*) and Brown trout (*Salmo trutta furio*) treated with clove oil anaesthetic and Sudagara et al. (2009) in Roach (*Rutilus rutilus*) anaesthetised with clove powder where significant differences were not observed in values of MCV, MCH and MCHC respectively. Plant- derived toxins are known to cause changes in the blood variables (Hb, RBC, MCV, MCH and MCHC) associated with oxygen transport in fish (Agbon et al. 2002, Ominiyi et al. 2003). This may result in anaemic condition due to the destruction (lysis) of erythrocytes or inhibition of erythropoeisis by active ingredients in the extracts (Brown 1980). Significant increase in the values of MCV and MCH were reported in Siberian sturgeon (*Acipenser baerii*) treated with both eugenol and MS-222 immediately after anaesthesia compared with controls (Gomulka et al. 2008). In this case, the researchers suggested erythrocyte swelling due to significant increase in MCV. Ayuba and Ofojekwu (2010) reported significant decrease in MCV in *Clarias gariepinus* exposed to *Datura innoxia* extract and attributed the result to anaemic condition resulting from erythrocyte destruction. In the present study lack of significant changes in the absolute red blood indices obtained with freeze-dried bark extract of the *T. Vogelii* suggests that these variables did not impact negatively on the experimental fish. The result thus indicates that the freeze-dried bark extract of *T. vogelii* could be used as a tranquilizer on *H. Longifilis* without adverse effects on the life of the animal.

References

[1] Adewoye SO, 2010. Heamatological and biochemical changes in *Clarias gariepinus* exposed to *Tephrosia vogelii* extract. *Advances in Applied Science Research* 1: 74 - 79.

[2] Adeyemo OK. 2005. Haematological and histopathological effects of cassava mill effluent in *Clarias gariepinus*. *African Journal of Biomedical Research* 8: 179 – 183.

[3] Agbon AO, Ominiyi IT, Teko AA. 2002. Acute toxicity of tobacco (*Nicotiana tobaccum*) leaf dust on *Oreochromis niloticus* and haematological change resulting from sub-lethal exposure. *Journal of Aquatic Science* 17: 5 – 8.

[4] Agokei OE, Adebisi AA, 2010. Tabacco as an anaesthetic fish handling procedures. *Journal of Medical Plant Research* 4:1396-1399.

[5] Ayuba VO, Ofojekwu PC. 2010. Growth and Haematological changes in *Clarias gariepinus* exposed to sub-lethal concentrations of *Datura innoxia* extract for 12 weeks. *Hamdard Medicus* 53: 91 – 98.

[6] Brown LA. 1980. Haematological Principles and Procedures. Lea and Fabiger Philadelphia pp 319.

[7] Brown, L. A. (2011). Anaesthetic for fish. Seminar on application of anaesthetic agents in aquaculture held in Cantho on 1st December, 2010. Available at http://www.vietfish.org./49c70/aqua.htm. [accessed 17th June, 2011].

[8] Colye SD, Durborow RM, Tidweu JH. 2004. Anaesthetics in aquaculture. SRAC (Southern Regional Aquaculture Center) publications No. 3900.

[9] Gabriel UU, Amakiri NE, Ezeri GNO. 2007. Haematology and gill pathology of *Clarias gariepinus* exposed to refined petroleum oil, kerosene under laboratory conditions. *Journal of Animal and Veterinary Advances* 6: 461 – 465.

[10] Gabriel UU, Obomanu FG, Edori OS. 2009. Haematology, plasma enzymes and organ indices of *Clarias gariepinus* after intramuscular injection with aqueous leaf extracts of *Lepidagathis alopecuroides. African Journal of Biochemistry Research* 3: 312 – 316.

[11] Gabriel UU, Deelae SN, Akenrotimi OA, Orokotan OO. 2011. Haematological responses of *Clarias gariepinus* exposed to anaesthetic metonidate. *Continental Journal of Pharmacology and Toxicology Research*. 4: 18 – 29.

[12] Gomulka P, Wlasow T, Velisek J, Svobodova Z, Chemielinska E. 2008. Effects of Eugenol and MS-222 anaesthesia on Siberian Sturgeon *Acipense baerii* Brandt. *Acta Veterinaria Brno* 77: 447 – 453.

[13] Harms CA, Bakal RS. 1995. Techniques of fish anaesthesia. *Journal of Exotic Animal Medicine* 3: 19-25.

[14] Hseu JR, Yeh SL, Chu YT, Ting YY. 1998. Comparison of efficacy of five anesthetic in goldiined sea bream, *Sparus sarba. Acta Zoologica Taiwanica* 1: 11-18.

[15] Ingham JL. 1983. Naturally occurring Iso-flavonoids (1855-1981). *Progress in the Chemistry of Organic Natural Products* 43:1 - 266.

[16] Iversen M, Finstad B, Mckinley S, Elisanssen RA. 2003. The efficacy of metomidate, clove oil, Aqui-SK and Benjoak R as anaesthetics in Atlantic Salmon (*Salmo salar*) smolts and their potential stress-reducing capacity. *Aquaculture* 221: 549 – 566.

[17] Lungu S. 1987. An Ecological Evaluation of Zambia Trees and Shrubs in Relation to Their Potential uses for Agroforestry in Zambia. MSc Thesis, University of Zambia, Zambia.

[18] Maricchiolo G, Genovese L. 2010. Some contribution to knowledge of stress responses in innovative species with particular focus on the use of the anaesthetics. *The Open Biology Journal* S: 24-33.

[19] Marking LL, Meyer FP. 1985. Are better anaesthetics needed in fisheries? *Fisheries* 10: 2 – 5.

[20] Marson A, Msonthi JD, Hostettmann K. 1984. On the reported Molluscidal activity from *Tephrosia vogelii* leaves. *Phytochemistry* 23: 1824 – 1825.

[21] Masopust J. 2000. Heamatological and biochemical profile of blood. Clinical biochemistry. Karolinum para Czech Republic pp 832.

[22] McGovern-Hopkins K, Tamaru CS, Takeshita G, Yamamota M. 2003. Procedural guide for the artificial insemination of lyretoil swordtail (*Xiphophorus helleri)*. University of Hawaii sea Grant College Program, school of Ocean and earth science and Technology. Available at http://www.soest.hawaii.edu/SEAGRANT. [accesed on 18th July, 2011]

[23] Michael M. 2002. Trees, shrubs and lianas of West African Dy zones. CIRAD: Centre de cooperation international en recherché agronomique pour le development. Maragrapgh publishers GMBH pp 328.

[24] Neiffer DL, Stamper MA. 2009. Fish sedation, Anaesthetic, Analgesia and euthanasia: considerations, methods and types of Drugs. *Institute for Laboratory Animal Research Journal* 50: 343-360.

[25] Obomanu FG, Ogbalu OK, Gabriel UU, Fakarurhobo SGB, Agadi SU. 2007. Piscicidal effects of *Lepidagathis alopecuroides* on mudskipper, *Periophthalmus papillio* from the Niger Delta. *Research Journal of Applied Science* 2: 382-387.

[26] Olufayo MO. 2009. Heamatological characteristics of *Clarias gariepinus* (Burchell 1822). Juveniles exposed to Derris euptica Root powder. *African Journal of Food, Agriculture, Nutrition and Development* 9: 921-933.

[27] Ominiyi I, Agbon AO, Sodunke SA. 2002. Effect of lethal and sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust extract on weight and haematological changes on *Clarias gariepinus* (Burch). *Journal of Applied Science* 2: 382 – 387.

[28] Oshode OA, Bakara A.A, Adeogun AO, Eluntoye MO, Sowunmi AA. 2008. Ecotoxicological assessment using *Clarias gariepinus* and microbial characterization of leachate from municipal solid waste landhill. *International Journal of Environmental Research* 2: 391 – 400.

[29] Ramanayaka JC, Atapatu NSBM. 2006. Fish anaesthetic properties of some local plant materials. Tropical Agricultural Research Extension 9. Available at http://www.agri.ruhrac.UC/tare/pdf/v-9/A.G.9.14pdf: [accessed 24th March 2011].

[30] Ross LG, Ross B. 1984. Anaesthetic and Sedative Techniques for Fish. Glasgow: Nautical Press.

[31] Shahi J, Singh A. 2011. Effect of bioactive compounds extracted from euphorbious plants on hematological and biochemical parameters of *Channa punctatus*. *Revista do Instituto de Medicina Tropical de São Paulo* 53: 259 – 263.

[32] Solomon SG, Amali EI. 2004. Preliminary investigation into the use of a local plant, *Datura innoria* as tranquilizer for Musfish, *Clarias gariepinus* Burchell (1822). In: 18th Annual Conference of the Fisheries Society of Nigeria (FISON) 8-12th December, 2003. Pp 43-47.

[33] Soto CG, and Burhanuddin CG, 1995. Clove oil as a fish anaesthetic for measuring length and weight of Rabbitfish, *Signus lineaus*. *Aquaculture* 135:149-150.

[34] Sudagara M, Mohammdizarejabada A, Mazandarania R, Pooralimotlagha S. 2009. The efficacy of Clove powder as an anaesthetic and its effect on haematological parameters onf Roach (*Rutilis rutilis*). *Journal of Aquaculture Feed Science and Nutrition* 1: 1 – 5.

[35] Summerfelt RC, Smith JS. 1990. Anaesthetic surgery and related techniques. In: C.B. Shreck and P.M. Moyle (eds). Methods of fish biology. American Fisheries society, Bethesda MD, Pp 213-172.

[36] Tilak KS, Veeraiah K, Butchiram MS, 2007. Effect pf phenol on heamatological components of Indian major carps *Catla catla, labeo rohita and Cirrhinus mrigala. Journal of Environmental Biology*, 28:177-179.

[37] Ucar A, Atamanalp M. 2010. The effects of Natural (clove oil) and synthetical (2 – phenoxyethanol) Anaesthetic substances on haematology parameters of Rainbow Trout (*Onchorynchus mykiss*) and Brown Trout (*Salmo trutta fario*). *Journal of Animal and Veterinary Advances* 9: 1925 – 1933.

[38] Val AL, Kapoor BG. (eds.). 2003. Fish adaptation: Environmental pollution and fish morphology. Science Publishers.

[39] Velisek J, Svobodova Z, Piackova V, Groch L, Nepejchaova L. 2005. Effects of clove oil Anaesthesia on common carp (*Cyprinus carpio* L.). *Veterinary Medicine Czech* 50: 269-275.

[40] Velisek J, Wlasow T, Gomulka P, Svobodova Z, Novotnyi L. 2007. Effects of 2-phenoxyethanol anaesthesia on Sheathfish (*Silurus glanis* L.) *Veterinary Medicina* 52: 103 – 110.

[41] Yanar M, Kumlu M. 2001. The Anaesthetics Effects of Quinaldine Sulphate and/or Diazepam on Sea Bass (*Dicentrarchus labrax*) Juveniles. *Turkish Journal of Veterinary and Animal Science* 25: 185 - 189

[42] Yushimura H, Nakamura M, Koeda T. 1981. Mutagenic screen of anaesthetics for fishes. *Mutagenic Research* 90:119-124.