EFFECTS OF PROPHYLATICS ON GROWTH PERFORMANCE AND PHYSIOLOGY OF AFRICAN CATFISH *Clarias gariepinus* (BURCHELL, 1822) FINGERLINGS

BY

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MARCH, 2019.

DECLARATION

I, FATOYINBO OLUWABUNMI VICTORIA hereby declare that this project was written by me and it is a record of my own research work. All borrowed ideas were duly and properly acknowledged.

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CERTIFICATION

I certify that this project work was carried out by **VICTORIA, OLUWABUNMI FATOYINBO** with the **MATRIC NUMBER FAQ/13/0991** under the supervision of **MR. J.O JIMOH** in the Department of Fisheries and Aquaculture, Federal University Oye-Ekiti, Ekiti State, Nigeria. The thesis has been read and approved as meeting the requirements for the award of Bachelor of Science (Fisheries and Aquaculture).

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DEDICATION

This project work is dedicated to Almighty and All-sufficient GOD, who has been my source of help and has been my strength right from my first year of my course to the final year.

ACKNOWLEDGEMENTS

All the glory and honour goes to the Almighty God, my maker and stronghold, who has shown me his unlimited grace, mercy and favour all through the course of my five years degree programme.

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ABSTRACT

Three different chemicals: formalin, hydrogen peroxide and potassium permanganate were used for intermittent prophylactic treatment of *Clarias gariepinus* fingerlings (2.43±0.04) for 10weeks. The experiment was made up of four treatments: T1 (Control), T2 (Potassium permanganate at 2ppm concentration), T3 (Hydrogen peroxide at 1000ppm concentration), and T4 (Formalin at 1000ppm concentration) with each treatment having 15 fingerlings of catfish. Each treatment was in triplicates. The fish bath lasted for 30minutes. The process was repeated biweekly while some parameters such as mean weight gain, specific growth rate, daily weight gain, and percentage survival were taken weekly. Haematological parameters such as white blood cells, red blood cells, heamoglobin, Mean corpuscular heamoglobin concentration, mean corpuscular volume, and parcked cell volume were analyzed. The histology of the liver and the gills were also carried out. The data were later subjected to one way analysis of variance and the mean were separated using Duncan Multiple range test.

Mean final weight gains of 3.96±0.67, 5.31±1.66, 3.70±0.28, and 5.27±1.84 were recorded for treatments 1, 2, 3 and 4 respectively. The specific growth rate ranged between 0.57±0.06 and 0.68±0.14%/day, the Average Daily Weight Gain (g/day) between 0.05±0.01 and 0.08±0.02. The percentage survival is 72.33±11.45%, 56.00±4.81%, 61.91±2.38% and 45.22±10.02% for treatments 1, 2, 3 and 4 respectively.There were significant differences in the weight gain and the percentage survival of the experimental fish ($p<0.05$). The white blood cells count ranged between 4.13±0.32 and 6.23±0.25 from T1 to T4. The white blood cells count ranged between 9.20±9.40 and 27.60±0.88. The heamoglobin values were 12.45±2.03, 13.09±0.98, 11.34±1.76 and 10.52±3.04 for T1, T2, T3 and T4 respectively. The highest MCV (356.85±2.45) was recorded in T4 and the lowest in T1. Apart from PCV that decreased in values in T3 and T4, other red blood indices increased significantly ($p<0.05$) when compared to the control.

It was concluded that the three chemicals effected the growth, survival, haematology, and histology of C. gariepinus fingerlings. Good management practices that prevent disease infections in fish are better for fish farmers. However, if chemical is to be applied to treat disease in fish, $KMnO_4$ is recommended as its negative effects on fish haematology and histology according to this study is the lowest when compared to the other chemicals.

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CHAPTER ONE

1.0 Introduction

1.1 Aquaculture Production in Nigeria

Aquaculture is a controlled cultivation of aquatic fauna and flora (FAO, 2012). Fish is an important and the cheapest source of animal protein and account for about 37% of Nigeria total protein requires (Solomon *et al.,* 2016). Fish as a cheapest source of protein provides approximately 16% of the protein consumed by the world population (Omeru *et al.,* 2016). Fish is a reliable source of thiamine, riboflavin, vitamins A and D, phosphorus, calcium and iron (Eyo and Ekanem, 2011). Consumption and demand for fish protein is increasing due to its affordability in Nigeria (Jamabo *et al.,* 2017). For past years, there has been an increase in aquaculture production, especially in the tons of fish from 13 million tons of fish in 1990 to 37.9 million tons in 2001, 51.65 million tons in 2006 and 62.7 million tons in 2011 (FAO, 2013).As at 2015, the estimated fisheries production was 1 027 000 tonnes, to which marine catches contributed 36%, inland waters catches contributed 33% and aquaculture 31% (FAO, 2016). Fishery sector contributed to 0.5% of national GDP in 2015.

Constraints/challenges faced by the aquaculture industry are inadequate quality fish seed for stocking ponds, dearth of information on modern technologies in aquaculture due to poor extension services, lot of skill gaps between the managerial-supervisory unskilled personnel, lack of fishermen's cooperative societies, poor infrastructural facilities, poor funding by government and high cost of fish feed (Adeogun, *et al*, 2007; Ugwumba, 2005) cited by Adedeji, *et al.,*(2011). According to Ugwumba and Orji (2007), the constraints to investment in aquaculture in the Niger delta include lack of capital, lack of land for pond establishment, scarcity of quality seeds (fingerlings), high cost of feeds, high cost of labor, inadequate water supply, lack of

modern technologies, poor storage facilities, high cost of transportation, mortality of fish due to diseases and water pollution and poaching. This has posed security challenges to investment in aquaculture in the area (Ayodeji *et al.,* 2012). An observation was made by FAO, (2000) cited by Jerimoth, (2012) that the concept of fish farming is perceived as a foreign technology by the small scale, resource poor farmers, most especially in sub-Saharan Africa and it is seen as a donor driven development.

Clarias gariepinus belongs to the family Clariidae and is the most commonly cultivated fish in Nigeria (Omeru, 2016). *Clarias gariepinus* is one of the most important tropical fresh water fish species for aquaculture whose aquaculture potential have been documented (Omeru *et al.,* 2016). It is one of the most important aquaculture candidates because of its ability to tolerate a wide range of environmental conditions, high stocking densities under culture conditions, fast growth rate, high yield potential, high fecundity, air breathing characteristics and high market value (Hetch,2007; Babalola and Apata, 2006) cited by Jamabo *et al.,* (2017).

Hematology is the study of the cells and proteins found in the blood (Alan H Turner *et al.,*2008). Haematology is the study of medicine concerning the study of blood, the blood-forming organs, including the diagnosis, treatment, and prevention of diseases of the blood, bone marrow, and immunologic, hemostatic, and vascular systems (Gerald *et al.,* 2012). The study of haematology in fish plays an important role in understanding variation of blood characteristics in relation to factors like food selection, ecological habitat, phylogenetic position, pollutants, etc (Healio 2017). Fish haematology is gaining increasing importance infish culture because of its importance in monitoring the health status of fish (Dienye *et al.*, 2014)

Histopathology, the study of lesions or abnormalities on cellular and tissue levels is useful tool for assessing the degree of pollution, particularly for sublethal and chronic effects (Bernet *et al.*, 1999) cited by (Imam *et al.*, 2014).

Formalin is a generic term which describes an aqueous solution of 37-50% formaldehyde gas dissolved in water (Jimmy *et al.*, 2014). Solutions of formalin for use on fish should contain 10 to 15% mathanol, which inhibits formation of paraformaldehyde, a highly toxic compound. (Ruth, 1996) cited by (Yisa *et al.*, 2015).

Hydrogen peroxide is a chemical with the formula H_2O_2. In its pure form, it is a pale blue, clear liquid, slightly more viscous than water (Lewis, 2015). Hydrogen peroxide is the simplest peroxide (a compound with an oxygen–oxygen single bond). (Yanong, 2011). Laboratory tests conducted by fish culturists in recent years have demonstrated that common household hydrogen peroxide can be used safely to provide oxygen for small fish (Roy, 2010).

Potassium permanganate is a strong oxidizing agent and an inorganic chemical compound $KMnO_4$,a water soluble salt consisting of equal mole amounts of potassium (K^+) and permanganate(MnO_4-) (Kovi-Siakpere *et al.*, 2008) cited by (Badger, 2017). It dissolves in water to give deep purple solutions, evaporation of which gives prismatic purplish-black glistering crystals (Matthew, 2017). It has been used as a therapeutant and prophylactic for fish diseases since 1918 when the first controlled test of its efficacy against myxobacteriosis was performed by Davis (1922) cited by (Kovi-Siakpere, 2008) and (Archarya *et al.*, 2014).

1.2 Justification

In past years, aquaculture in Nigeria has played a major role in production and nation's economic development at a time when government is seeking for ways to diversify the economy, from being purely oil based (Jerimoth, 2012). Thus, serving as one of the most productive source of economic development in Nigeria (Kesena, 2012). Aquaculture is a potential means of contributing to the food security of the nation, directly by producing fish for food and indirectly by generating employment for the teaming unemployed populace, save foreign exchange and generate foreign exchange through export of fish and fish products (Ekelemu, 2012). However, there are certain constraints that is hindering the development of aquaculture production in Nigeria. Such challenges include scarcity of quality seeds (fingerlings}, high cost of feeds, high cost of labor; inadequate water supply (Kesena, 2012). Others are lack of land for pond establishment, lack of capital, lack of modern technologies, poor storage facilities, high cost of transportation, mortality of fish due to diseases andwater pollution and poaching, inadequate site selection, poor designs and construction of fish pond, lowlevel of fish farm management techniques, high cost of pelleted fish feeds, inadequate hatchery facilities and poor record keeping (Ajana, 2007). To solve the problem of disease infection,various chemicals have been used in the treatment of fish, especially *Clarias gariepinus*. Rasowo *et al.*, (2007) used formaldehyde, sodium chloride, potassium permanganate, and hydrogen peroxide to prophylatically treat *Clarias gariepinus* eggs. Kori-Siakpere, (2008) used potassium permanganate to treat fingerlings of the African Catfish, Clarias gariepinus. Jimmy, (2014) used formalin to treat catfish fingerlings. Akpoilih, (2010) used formalin to improve the hatchability of eggs and survival of catfish frys. However, none of the stated works above was able to

determine the effects of these chemicals on the heamatological and histological characteristics of *Clarias gariepinus.*

1.3 Objectives

The objective of this study is to determine the effect of the intermittent prophylatic treatment with potassium permangante, hydrogen peroxide and formalin on the growth performance, histology and haematology of *Clarias gariepinus* fingerlings.

CHAPTER TWO

2.0 Literature Review

2.1 Aquaculture production in Nigeria

Aquaculture is the fastest growing food producing industry in the world (Subasinghe, 2005 and Akeem *et al.,* 2015). It was stated by Ayinla, (2012) that global aquaculture production has quadrupledover the past twenty years and that aquaculture production is likely to double in the next fifteen years, as a result of wild fisheries approaching their biological limits an the world demand for cultured fish continuing to increase. Aquaculture is gaining attention all over the world as means of improving world fish production which iscurrently on decline due to dwindling output from capture fishery (FAO, 2010) cited by (Nene *et al.,* 2017). In Nigeria the annual fish demand as at 2012 is 2.66 million metric tonnes with supply being only 1.32 million metric tonnes. Out of this figure (1.32 million metric tonnes) local production is 0.62 million metric tonnes while 0.7 million metric tonnes is from importation (Ekelemu, 2012). Aquaculture account for only 200,000 metric tonnes of the total fish supply. The current aquaculture production, is a far cry from its potential production of 2.5- 4.0 million metric tonnes (Jerimoth, 2012). The aquaculture sub-sector is considered a very viable alternative to meeting the nation's need for self sufficiency in fish production. This is based on its high reliability in return on investment and low capital intensity, relative to capture fisheries (Kesena, 2012). The major aim of the Federal government is to achieve self-sufficiency in fish production and ultimately to have fish products available for export. Unfortunately, the fisheries sector is still under-developed despite being the major source of livelihood and income for many of the coastal populace. In developing countries such as Nigeria, there has been rapid population growth which eventually lead to increased disposable income and changing consumer preferences has drastically

increased the annual demand for aquatic food source. Proliferation of more efficient capture technologies, decades of government subsidies, increased market access even for remote fishing communities, and development programs aimed at increasing production from the fragile open-access resource has led to large scale depletion of fish resources.

2.2 African catfish (*Clarias gariepinus*)

Clarias gariepinus, The African catfish is a very popular fresh water fish in Nigeria (Eyo *et al.*, 2012). *Clarias gariepinus* at various geographical locations bears different names. It is called *Clarias lazera* in Northern and central Africa, *Clarias gariepinus* in South Africa (Viveen *et al.,* 1986) cited by Omeru *et al.,* (2016). *Clarias gariepinus* is characterized with nated skin and dougate with fairly long dorsal aid anal fins (Chen *et al.,* 2016). The dorsal fin has 61-80 soft rays and anal fin has 45-65 soft rays. They have strong pectoral fins with spines that over serrated on the outer side (Wang *et al.,* 2017). Based on research it posses nasal and maxillary barbells and somewhat smallish eyes, their coloring is dark frey or black dorsally and green coloured ventrally (Duan *et al.,* 2017).

Clarias gariepinus is very popular to fish farmers and also regarded as the most aquaculture candidates because of its ability of high fecundity rate, fast growth rate, air breathing characteristics, high yield potential, high stocking densities under culture conditions, high market values, tolerates high density and environmental extremes, resistance to diseases infection and ability to withstand adverse paid conditions especially low oxygen content and high turbidity, good food conversion ratio in which it accepts wide range of natural and artificial food and then adapts to a variety of feeding modes in expanded niches (Babalola and Apata, (2006), Sogbesan, (2006), Hetch, (2007) cited by (Adewole *et al.,* 2008) and (Jamabo *et al.,* 2017). Acccording to

Bamidele, 2007, the culture of *Clarias gariepinus* as seed for fish production is becoming increasingly essential as the fish is contributing to the food abundance and nutritional benefit to the family health, income generation and employment opportunities. *Clarias gariepinus* is generally considered as one of the most important tropical species of the aquaculture and also as an ecologically important and commercially valued in Nigeria (Ayuba *et al.,* 2014).

2.3 Disease Infections in fish

Disease issues are of great concern in aquaculture production (Idowu *et al.,* 2017). Disease is a condition in living organisms in which normal physiological functions are being impaired due to alteration in the body systems and typically manifested by distinguishing signs and symptons.(Adedeji *et al.,* 2017). Fish diseases affect the survival and growth rates of fishunder culture. Given that drug treatments are expensive, fishdiseases invariably lead to lower harvest and higher cost (Sogbesan *et al.,* 2017). Fish farmers often suffer hefty economic losses due to fish diseases. To alleviate such losses, it is crucial to take precautions to prevent fishdiseases and reduce pathogen levels in water bodies (Assefa *et al.,* 2018). It is also important to prevent water quality from deteriorating and to strengthen the natural resistance of the fishstock. Regular monitoring of fish health is an effective way to identify disease causes and appropriate treatments (Abunna *et al.,* 2018). One major cause of serious fish kill is overlooking the contagiousness of fish diseases and thus delaying treatment. As such, adequate care and treatment should be given to infected fish promptly. A range of disease could be found in farmed aquatic animals and hence farmers are using chemicals and antibiotics for the traetment of the diseased fish and other cultured aquatic animals as well as pharmaceutical companies and

chemical sellers are influencing fish and prawn farmers to buy their products (Belal *et al.,* 2012). Fish like other humans and humans suffer from diseases and parasites. Fish defences against disease are specific and non-specific. Non-specific defences include skin and scales, as well as the mucus layer secreted by the epidermis that traps microorganisms and inhibits their growth (Woo, 2011).

Bacterial infection of fish population reared in hatcheries is quite common (Oladele *et al.,* 2015). The most frequent pathogen is Flavobacterium columare (Verma *et al.,* 2011). Fish reared in overstocked condition or in tanks with insufficient cleaning and also the starving fish populations are susceptible to such type of bacterial infection (Plumb *et al.,* 2018). Color of infected fish is whitish. Bacterial colonies can develop mainly on epithelium of fins and barbels and around the mouth of fry. Sometime small pieces of epithelium between the rays of fins become loose (Jansen, 2010). Position of barbels can draw attention to weak health condition of fish. Barbels of healthy fry are pointed forward, while barbels of sick fish are curved backward. Infected fish stay sometimes in vertical position, "hanging" near surface of water (Richter, 2010). After outbreak of disease same antibiotic and formalin treatments with higher frequency are required than for prophylaxis (Jansen *et al.,* 2010).

Fungal and parasitic infections may occur among different size of fry and fingerlings. Malachite green, Formalin or Dipterex baths are the best measures to control them. Suggested concentration of malachite green treatment is maximum 0.07 mg/liter. Frequency of therephautic treatment is 4–5/day (Van *et al.,* 2010). Formalin is a good tool for controlling bacterial and protozoan infections at the rate of 5–10 ml/100 liter applied as it was detailed before. Suggested frequency of formalin baths is 4–6 times daily (Huisman *et al.,* 2010). Organophosphate baths at

the rate of 0.1 g/liter for 20–30 minutes are effective against worm-type gill or skin parasites. The allowed frequency of such treatment is not more than two-three days (Viveen *et al.*, 2010).

2.4 Causes of Fish Diseases

1. Natural factors:

Temperature of natural fresh water will change with the seasons change, but this change will cause a lot of fish sick and dying in large numbers, because some of the parasites that threaten the health of fish disappear or appear to be affected by temperature change (Bonnie, 2016). Dissolved oxygen is the content of oxygen in water, will have a very large impact on the survival of fish. pH in 8.5-7 is the most suitable for the growth of fish, that means neutral and weakly alkaline environment is more suitable for the survival of fish, if pH is less than 5 or higher than 9, it will lead to a large number of fish dead (Cheng, 2016).

2. Water pollution:

With the rapid development of township enterprises, leading to a lot of sewage into rivers and lakes, and these sewage is the cause of the fish disease, sewage contains a large number of heavy metals and a variety of micro-organisms, will pose a threat to the survival of fish (Bonnie, 2016).

3. Feeding fish with fish feed pellets of low nutrition:

In different fish growth stages, different formulation should be used to satisfy its nutrition demands (Cheng, 2016).

2.5 Common Fish Diseases & Symptoms in Freshwater Cultured Fish

1. Bacterial Disease

Bacterial diseases are caused by the infection of bacteria in fish body. Bacterial hemorrhagic disease is most likely to harm grass carp. In early stage of illness, fish will show signs of bleeding, which usually occurs in the fin base, gill cover, mouth and lower jaw, if it continues to develop the fish body will have bleeding, the abdomen will be expanding, and red spots will appear at liver and kidney (Bonnie, 2016). While Gill rot disease is most likely to harm grass carp and common carp. In early stage of illness, the fish will loss of appetite, the head will go black with increased mucus, if it continues to develop, the gill part will appear the wax yellow and mud gray spot.

2. Parasitic disease

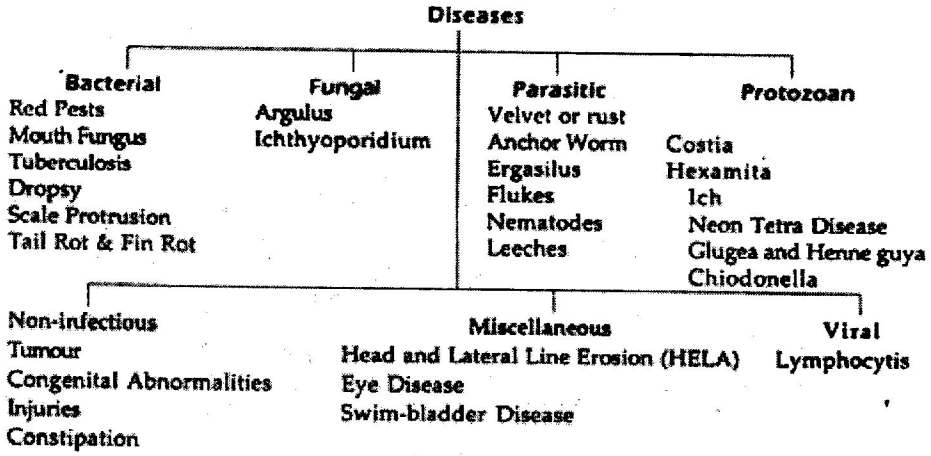
The most common parasitic diseases isMyxospore disease, Dactylogyrusis, Trichodiniasis, Lernaeosis, etc. Common carp were the most susceptible to the Myxospore disease, the carp and Bighead are most susceptible to the Dactylogyrusis, wheel worm is the main invasion of common carp, it is not easy to find in the early stage, and the biggest victims of the Lernaeosis is grass carp, especially the most popular in the summer (Cheng, 2016).

3. Viral diseases

Virus disease is a disease caused by a variety of viruses that invade the body of a fish. Hemorrhage of grass carp is the most serious disease in breeding stage, virus will invade the gills

of fish, around the eyes, head, jaw and other parts, and the whole fish is dark black. This disease is more likely to occur in small grass carp, once suffering from the disease, the mortality rate is very high, can reach 80%, and will die in 3 - 2 hours (Bonnie, 2016).

2.6 Fish disease organism



Source: Saraswati, (2016). Seven(7) Major Diseases of Fish

Table 1: List of Chemicals with their purpose of use and doses

No.	Name	Trade name	Chemical formula	Purpose	Dose
1.	Lime	lime/chun/ agricultural lime	CaO, hydrated or slake lime, $Ca(OH)_2$ and $CaCO_3$	Improved liberation of bases, biological activity, oxygen decomposition, maintain pH of pond water, remove tubidity	0.5-1kg/dec
2.	Rotenone	Rotenone /aquatin /aqurte-gold	$C_23H_{22}O_6$	Fish poison or toxicants	20-35 g /dec in nursery pond and 15-30g / dec in culture pond
3.	Phostoxin	Quickphos / phostoxin tablet	Aluminium phosphide	Fish poison	3-5 tablets/dec
4.	Formalin	Formalin	40 % HCHO	Employed as an antifungal agent, and in the control of ectoparasites.	1-3 ppm as disinfectants and 3-5ppm for disease treatment.
5.	Potassium Permang-anate	Potash	KmnO4	Active against saprolegniasis, dactylogyrosis, gyrodectylosis, Argulosis.	5-15 mg/dec
6.	Malachite green	Malachite green	$C_2H_2O_4$	Active against the oomycete, Saprolegnia, which infects fish eggs in commercial aquaculture.	1-5 mg/dec, 1-2 ppm
7.	Copper sulphate	Tut/ Copper sulphate	$CuSO_4$	Effective against external parasites.	15-25 mg/dec

2.7 Potassium permanagante

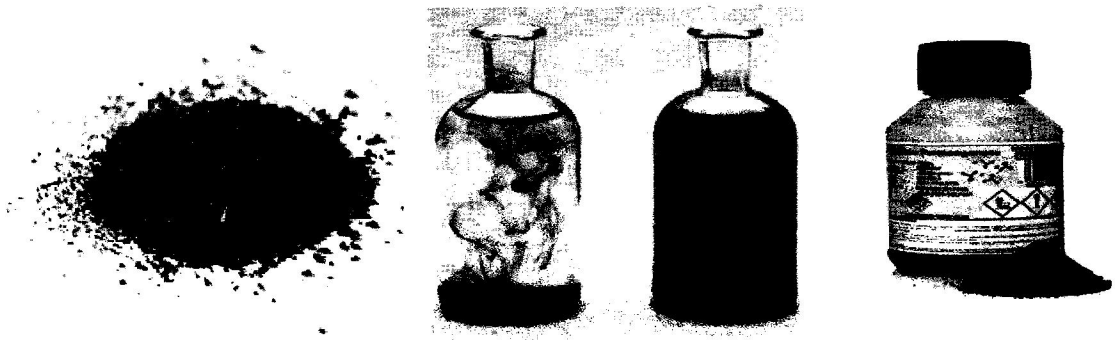


Plate 1: Potassium permanganate in crystal and liquid solution

Source: Michael, (2014). Potassium permanganate-similar structures and other chemical information

Regulatory action on the use of $KMnO_4$ has been deferred pending the outcome of ongoing research. Straus and Griffin (2002) described the four legal options for using aquatic chemotherapeutics in the United States as follows (Kovi, 2008):

1) the FDA has approved the use of compound as a therapeutant;

2) the therapeutant is the subject of an Investigational New Animal Drug (INAD) exemption;

3) the therapeutant has been determined by the FDA to be of low regulatory priority; or

4) the therapeutant is not of low regulatory priority but regulatory action has been deferred pending the outcome of research.

Currently only formalin (Formalin-F, Paracide-F, and Parasite S), oxytetracycline (Terramycin), sulfadimethoxine and ormetropin (Romet 30), and sulfamerazine (no longer manufactured) are FDA approved therapeutants; though each approval is for specific uses (Greenlees, 1997) cited by (Ovie, 2010). Users of aquaculture chemicals have been frequently advised to use chemicals sparingly and only when needed to avoid stressing the treated fish and possibly introducing

8. Salt	Lobon/ Nun/ Salt	NaCl	active against for coastiasis, trichodiniasis, chilodonelliasis, dactylogyrosis	500-1000 g/dec
9. Hydrogen peroxide	Oxyflow/ Oxymax/ Bio care/ Bio- Ox/ Oxy plus	10% H_2O_2	Oxygen supplier	5-10 g/dec
10. Oxytetra-cycline	Oxy-dof-f/ Aquamycine/ Renamycin	Oxytetracycline 20%, doxycycline 10%. Oxytetracycline-hydrochloride 25%, Oxytetracycline	Effective against a wide range of gram-negative and	2-6 mg /kg feed

Source: Jilani *et al.*, (2012). African Journal of Basic & Applied Sciences 4 (4): 110-114.

greater harm than benefit (Wellborn, 1985; Tucker and Robinson, 1990) cited by (Siakpere, 2010). For most fish, potassium permanganate can be administered at a concentration of 2 mg/L as along-term bath (four-hour minimum) in fresh water or salt water systems(Ellen *et al.,* 2010). Potassium permanganate is also reasonably safe to use in recirculating systems and has minimal impact on biofilters when used at 2 mg/L. Treated water should retain the purple coloration for at least four hours (Ruth *et al.,* 2010). Potassium permanganate can be used as a surface disinfectant at concentrations of 10 mg/L (30–60minutes contact time) to 500 mg/L (30 secondscontact time) in a fish room or hatchery, however,quaternary ammonium compounds are better suited to this purpose. Potassium permanganate will kill bacterial, fungal and many parasitic agents, but it is not viricidal (Klinger *et al.,* 2010). As mentioned above, potassium permanganate is an indiscriminate oxidizer, and as such, can burn gill tissue and mucus of treated fish if too much chemicalis applied. A good rule of thumb to prevent excessive damage to fish is to avoid treating them with potassium permanganate more than once a week (Floyd *et al.,* 2010). Potassium permanganate is potentially toxic to humans and other organisms including several fish species (Kegley *et al.,* 2010), but is not included in regulatory categories. Therefore, there is no pattern or established limit to this compound and it may be used freely and without restriction in aquaculture.

2.8 Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is a drug of low regulatory priority status that is effective in treating fish and fish eggs infected by fungi. (Rach *et al.,* 1997) cited by John (2017). Laboratory tests had been conducted by different fish culturists using different fish species to check their sensitivity to hydrogen peroxide treatments. Hydrogen peroxide is one of the potential

alternatives that are as well introduced which has been used in aquaculture as an immersion (bath) treatment against many different disease-causing organisms, including external parasites, bacteria and fungi on different species and life-stages of fish (Fath *et al.,* 2017). Hydrogen peroxide is produced naturally in surface water by a photochemical process involving dissolved light absorbing organic matter and molecular oxygen (Shehab *et al.,* 2017). Hydrogen peroxide (H_2O_2) is an obvious disinfectant candidate, for it has antimicrobial effects and easily degrades to harmless by-products. At present, the use of H_2O_2 in aquaculture is comparatively moderate compared to the use of other disnifectants such as formalin and salt (Fouad *et al.,* 2017). In order to increase the practical use of H_2O_2. Fish farmers have to become familiar with its benefits and risks. When added to water, H_2O_2 breaks down into O_2 and H_2O overtime and the formation of these by-products is one reason that hydrogen peroxide is considered to be relatively safe for the environment (El-Din *et al.,* 2017). Hydrogen peroxide is highly reactive in nature, similar in some respect to the reactivity of potassium permanganate, makes it ideal for use in aquaculture against numerous external fish-diseases-organisms but unfortunately too expensive (El-Bab *et al.,* 2017).

2.9 Formalin

Plate 2: Formalin

Source: Evans, (2010). Formaldehyde in trailers

Formalin is a generic name which describes a solution of 37% formaldehyde gas dissolved in water (Ruth, 2010). It is effective intreating fungi, external parasites, including protozoans and monogenic trematodes and widely used in therapeutic and prophylactic treatment by aquaculturists (Floyd, 1996) cited by (Adebayo *et al.,* 2010). Solutions of formalin for use on fish should contain 10 to 15% methanol, which inhibits formation of paraformaldehyde, a highly toxic compound. Formalin is used as a bath treatment to control external parasitic infections of fish (Francis, 2010). It is said to be extremely effective against most protozoans, as well as some of the larger parasites such as monogenetic trematodes (Ruth, 2010). Formalin effectively kills parasites on the gills, skin and fins. It is not the preferred treatment for external bacterial or fungal infections. High concentration of formalin are used to control fungi on fish eggs. Formalin is not effective against internal infections of any type (Floyd, 2011). When parasites become a

problem, the use of formalin is common worldwide due to its highly treatment efficiency without affecting the fish at normal dosage rates (Fouad *et al.*,2017). However, due to the recent focus on formalin-related worker safety issues as well as potential environmental discharge effects of formaldehyde, there are needs to apply proper management practices (Sheba *et al.*, 2017). Formalin is applied as a bath treatment. It can be applied as a prolonged bath, which means it is placed into the water indefinitely, or it can be applied as a short-termbath, which means fish are placed into the bath for arelatively short period of time (30 to 60 minutes) and then placed into clean (untreated) water (Adebayo *et al.*, 2010). The concentration of chemical used is determined by the period of time the fish are to be in contact with the chemical, the temperature of the water, and the condition of the fish (Akpoilih *et al.*, 2010). The use of synthetic chemicals such as formaldehyde as a means of increasing agricultural productivity has pose a serious treat and great consequences to the water bodies. The application of formalin to fish pond to kill bacteria, fungi and others harmful micro-organism pose a great problem to human after the consumption of the affected fishes (Andem *et al.*, 2015). Owing to the increasing demand for fishes, the world market farmers continue to use Formalin and other chemicals to maximized harvest efficiency of the fish, although this may be detrimental to human health (Abosi *et al.*, 2015). Ayuba *et al.*, (2013) showed that increase concentration of Formalin onaquatic water reduce the amount of oxygen circulated, causes respiratory distress among the fishes, loss of balance, gulping for air, vertical movement of fishes, excessive accumulation of mucus and death.

Table 2: List of chemicals and rules guiding their application

Chemical	Rule
Hydrogen peroxide hydrogen peroxide (i.e. 500 ml of hydrogen peroxide for 1 ton of water)for 1 hour.	Immerse fish in a solution of 1 :2,000
Formalin/Formaldehyde solution (i.e 100ml of fúrmaldehyde for 1 ton of water) for 1 hour. If necessary,increase the concentration to 1: 4000 (i.e 100ml of formaldehydefor 0.4 ton of water). Do not use any formaldehyde solution with white sediments.	Immerse fish in a 1:10,000 formaldehyde
Potassium permanganate permanganatesolution (i.e 4g of potassium permanganate for 1 ton of water) for 1-3 hrs For extended immersion, use a 1 :400,000 potassium permanganate solution (i.e. 2.5g potassium permanganate for 1 ton of water) for 24 hours.	Immerse fish in 1 :250,000 potassium

Source: Agriculture, Fisheries and Conservation Department (2010)

2.10 Fish hematology

According to G. William Klontz (2011), Hematology is the science of studying the anatomical, physiological and pathological aspects of blood. Blood is a fluid tissue contained within the cardiovascular system. Plasma is said to be the fluid element of blood, blood also form some elements which include erythrocytes, leukocytes, and thrombocytes. The primary funtions of blood include:

- Maintenance of acid-base balance
- Oxygenation of tissues
- Removal of metabolic waste products from tissues.
- Nutrition of tissues
- Therefore, any dysfunctions of blood can have serve effects on the physiological activities of the entire system of man or animal.

Therefore, when hematological methods are used as aids in fish disease diagnostics, the following variables must be taken into account:

1. Species of fish
2. Sex of fish
3. Origin (gene pool) of fish

4. Water temperature

5. Water chemical nature, especially alkalinity, pH, and hardness

6. Diet

7. Physiological age of fish

8. Sampling techniques

9. Cell counting techniques

10. Staining techniques

11. Personnel

Based on research done on the haematological changes in Catfish juveniles when exposed to toxic substance such as premium motor spirit(PMS), in which some of the parameters are Red blood cells(RBC), White blood cells(WBC), haemoglobin(Hb), Packed Cell Volume(PVC) and these blood samples are collected from the fish by cardiac puncture (Fapohunda *et al.,* 2016). Other parameters includes Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean corpuscular volume (MCV) which are calculated using the formula Seiverd (Adebayo *et al.,* 2016). Thus, The Haematological analysis revealed a significant reduction in Red Blood Cell(RBCs) count from 3.78+0.46/µl in the control fish to 2.68+0.18/µl in the highest concentration (Fapohunda *et al.,* 2016). Also a significant decrease was recorded in haemoglobin (Hb) from 12.25±0.5g/L in control to 7.83±0.99g/L in the highest PMS concentration (Adebayo *et al.,* 2016).

Also based on previous work, on the histological examination of catfish relating to its gills, liver and kidney when exposed to premium motor spirit, with fish separated in different tanks to show results. Thus, results are shown that the kidney of fish in the second tank used showed no visible changes and there was degeneration in the renal cells of fish in the third tank (Fapohunda *et al.*, 2016).

2.11 Haematological Changes in fish

Changes in haematological parameters such as, Red Blood Cell (RBC), White blood Cell (WBC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) in *Clarias gariepinus* juveniles, both in control and sub-lethal concentrations under 96 hours exposure to Premium motor spirit (PMS) were shown in the Table below. The Haematological analysis revealed a significant reduction in Red Blood Cell (RBCs) count from 3.78+0.46/µl in the control fish to 2.68+0.18/µl in the highest concentration. Also a significant decrease was recorded in haemoglobin (Hb) from 12.25±0.5g/L in control to 7.83±0.99g/L in the highest PMS concentration (Adebayo *et al.*, 2016)

Table 3: Haematological changes in *C. gariepinus* exposed to different concentrations of PMS

Parameters	Concentration (m/L)			
	0	45	55	65
H b	12.25±0.5[a]	10.85±0.21[a]	9.80±0.28[b]	7.83±0.99[b]
P C V	33.5±3.54[a]	30.0±1.41[a]	26.5±0.71	23.0±2.83[c]
W B C	6000±282.84[c]	6150±353.55[c]	7700±1414.21[b]	8400±1272.79[a]

Source: Adebayo *et al.*, 2016.

2.12 Histological examination of African Catfish

Various researches have been done on the histology of fish.

2.12.1 Histopathological changes in the liver

The liver of the control fish *Clarias gariepinus* appears as a continuous mass of hepatic cells; hepatocytes (h) which cord-like pattern interrupted by blood vessels and sinusoids (bs). The cords of hepatocytes are arranged around the central vein (cv). The hepatocytes are large in size, polygonal in shape with centrally located nuclei. The hepatocytes have homogenous eosinophilic cytoplasm. The sinusoids are seen as communicating channels occupied by blood cells with Küffer cells (kc) (Imam *et al.*, 2010).

2.12.2 Histopathological changes in the gills

Histologically, the gills of the adult catfish *Clarias gariepinus* are composed of primary lamellae (pl), secondary lamellae (sl), epithelial cell (epc), mucous cell (mc), and chloride cell (chc) y blood cells with Küffer cells (kc)

CHAPTER THREE

3.0 Materials and methods

3.1 Experimental site

The experiment was carried out in the wet laboratory of the Department of Fisheries and Aquaculture, Federal University Oye Ekiti, Ekiti state Nigeria.

3.2 Source of Experimental fish

180 fingerlings of Clarias gariepinus were purchased from ABUAD (Afe Babalola University Ado-Ekiti) fish farm, Ado-Ekiti. The fish were acclimatized for 14 days in the laboratory before the commencement of the experiment.

3.3 Source of Experimental feed

Vital feed (0.2mm and 0.5mm) was purchased from Metrovet Consultants Limited, Ado-Ekiti.

3.4 Chemical and reagents

All chemicals and reagents used were of analytical grade. Formalin was sourced from Federal University Oye Ekiti, Faculty of Agriculture Laboratory. Hydrogen peroxide was procured from Real Petbos Pharmacy, Ado-Ekiti and potassium permanganate was procured from Destiny Scientific Enterprises, Ado-Ekiti. The concentrations were applied

3.5 Experimental Design and Layout

The experiment was a complete randomized design (CRD) consisting of four treatments with each representing different chemicals: Potassium permanganate, Hydrogen peroxide and Formalin (treatments 2, 3 and 4 respectively) and a control (treatment 1). Each treatment had three replicates. The concentrations of the chemicals are presented in table 3.1 as recommended by Rasowo *et al*, (2007).

3.6 Experimental Procedure

Fingerlings were obtained from a reputable fish farm in Ado Ekiti, Ekiti State, Nigeria. The fish were acclimatized for 14 days in the wet laboratory of the Department of Fisheries and Aquaculture, Federal University, Oye Ekiti. The fish were raised in 12 (36x25x25cm) plastic aquaria with each aquarium stocked with 15 fingerlings. The first prophylactic treatments were given on the first day of the experiment. The treatments were repeated at two weeks interval for the duration of ten weeks that the experiment lasted. The weights of the fish were taken every fortnight using an electronic weighing balance Kerro BL10001 compact scale. The survival of the fish was monitored daily. To ensure good water quality, the aquaria water was changed every other day while the uneaten feeds siphoned every day.

Table 4: Chemical allocation for Treatments and their Concentration

Treatment	Chemicals	Concentration	Duration (Minutes)
1	Control Treatment	-	-
2	Potassium permanganate ($KMnO_4$)	2(ppm)	30
3	Hydrogen peroxide	1000(ppm)	30
4	Formalin	1000(ppm)	30

***ppm: parts per million**

Source: Rasowo *et al.*, (2007).

3.7 Water Quality Analysis

Water quality parameters such as dissolved oxygen (DO), hydrogen ion concentration (pH), and temperature were measured weekly. The calibrated mercury thermometer was used to measure water temperature; the pH and dissolved oxygen concentration was measured using the Jenway meters (model 3050 for DO and 9070 for the pH).

3.8 Feed and Feeding of Experimental fish

The fish were fed with 0.2mm vital feed at 5% body weight for the first five weeks. This was later substituted for 0.5mm at the same body weight for the remaining period of the experiment. Feeding was done twice daily (08.00h and 09.00h for morning session and 17.00h and 19.00h for evening session). Table 3.2 below gives the proximate compositions of the vital feed given to the fish throughout the duration of the experiment.

Table 5: The Proximate Composition of the feed used for the experimental fish

Nutrients	0.2mm	0.5mm
Crude Protein	45	38
Lipids	11	9.5
Crude fibre	2.8	3.5
Calcium	2.7	2.0
Phosphorus	1.1	0.9
Moisture	12	12

Sources: Vital feed

3.9 Growth performance and Nutrient utilization of *Clarias gariepinus*

The mean weight of the fish was taken weekly while the record of fish mortality in each treatment was recorded daily. The growth performance parameter that was evaluated were mean weight gain, specific growth rate, average daily weight gain, relative growth rate and percentage survival while the nutrient utilization parameters includes feed conversion ratio (FCR) and feed efficiency ratio (FER),

3.9.1 Mean weight gain (MWG) (g)

$$MWG = WF - W_1$$

where
WF = Final average weight of fish W_1 = Initial average weight at the beginning of the experiment

3.9.2 Specific Growth Rate (SGR) %

$$SGR = \frac{Log_e WF - Log_e W_1 \times 100}{\text{Time (days)}}$$

3.9.3 Average Daily Weight gain (ADWG)

$$ADWG = \frac{\text{Final mean body weight} - \text{Initial mean body weight}}{\text{Cultured Period ((days)}}$$

3.9.4 Percentage Survival

$$\text{Survival Rate} = \frac{\text{Total number of fish} - \text{Mortality}}{\text{Total number of fish}} \times 100$$

3.9.5 Feed conversion ratio (FCR)

$$FCR = \frac{\text{Dry feed consumed (g)}}{\text{Gain in weight (g)}}$$

3.10 Haematological analysis/Collection of blood samples

Blood samples was collected at the end of the experiment from the caudal vein into an EDTA litium tube. The blood was analysed to determine the packed cell value (PCV) with microhaematocrit using heparnized capillary tube (25mm). Red blood cell (RBC) and white blood cell (WBC) counts were determined as described by Blaxhal and Diasley (1973) and cited in Ogunji *et al.,* (2014). Hemoglobin (Hb) concentration was determined by the methods described by Wedemeyer and Yasutake (1977).

3.11 Histological examinations

At the end of the experiment, gill and liver samples were collected from four fish randomly selected from all the treatments. The organs were preserved in 10% formalin to retain the structural integrity of the cells and tissue. The dealcoholized tissues were inserted in molten paraffin wax for 3 hours. After this, the tissues were embedded using a deposable embedding mold and allowed to cool. Before sectioning the embedded tissues was placed on ice for proper sectioning and processed for histological examinations using standard histological techniques. Sections of organs were cut at 5µm and stained with heamatoxylin and eosin (H & E staining) procedure prescribed by Bancroft & Layton (2013). The slides were later viewed under a light microscope with 400x image magnification.

3.12 Statistical analysis

The analysis will be carried out in triplicates for all determinations and the results of the triplicate will be expressed as mean ± SEM. Statistical Package for Social Sciences (SPSS) 2.0

computer software was used to analyze the data. Mean differences were separated using Duncan multiple range test. Differences were considered significant at 0.05 level.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Growth performance of *Clarias gariepinus* fingerlings

The best weight gain (5.31±1.66g) was observed in the potassium permanganate treated fish (T2), while the hydrogen peroxide treated fish (T3) gained the least weight (6.13±0.29g). There was significant difference in the weight gain of all the treated fish and the control (Table 4.1).The feed conversion ratio (FCR) of the fish in this study ranged btween 1.47±0.41 and 1.91±0.28 with treatment 2 (T2) having the best FCR while the least was seen in the control experiment (T1), however, there was no significant difference in the FCR of all the fish used in this study. Thehighest survival rate(72.11%) was recorded in the control experiment (T1), followed by treatment 3 (61.91±2.38), treatment 2 (56.00±4.81) and treatment 4 (45.22±10.02).The specific growth rate (SGR) ranged between 0.57 %/day and 0.68 %/day. Although treatment 2 have the best SGR, there was no significant difference in the SGR value across all the treatments (Table 6.0).

Table 6: Growth Performance of the *C. gariepinus* fingerlings

Parameters	T1	T2	T3	T4
Mean Initial weight (g)	2.43±0.04[a]	2.45±0.03[a]	2.43±0.01[a]	2.46±0.10[a]
Mean Final weight (g)	6.39±0.68[a]	7.76±1.65[a]	6.13±0.29[a]	7.73±1.94[a]
Weight gain (g)	3.96±0.67[a]	5.31±1.66[b]	3.70±0.28[a]	5.27±1.84[b]
ADWG (g)	0.05±0.01[a]	0.08±0.02[a]	0.05±0.00[a]	0.07±0.03[a]
FCR	1.91±0.28[a]	1.47±0.41[a]	1.82±0.07[a]	1.59±0.16[a]
SGR (%/day)	0.57±0.06[a]	0.68±0.14[a]	0.62±0.03[a]	0.67±0.14[a]
Survival Rate (%)	72.11±7.45[b]	56.00±4.81[ab]	61.91±2.38[ab]	45.22±10.02[a]

Mean ± S.E with different superscripts along the same row are significantly different at $p < 0.05$

4.2 Haematological parameters of *Clarias gariepinus* fingerlings

There was significant difference among the white blood cell (WBC) count across the treatments. The highest count (6.23±0.25 x 10^9/L) was observed in the treatment 4 fish while the least (4.13±0.32 x 10^9/L) was recorded in treatment 1 (Table 4.2).. The Haematological analysis also revealed a significant reduction in Red blood Cell (RBCs) count from 27.60±0.88 in treatment 2 (KMnO4) to 9.20±9.40 in treatment 4 (Formalin). Also, a significant decrease was recorded in haemoglobin (Hb) from 13.09±0.98 in treatment 2 (KMnO4) to 10.52±3.04 in treatment 4 (Formalin). A significant decrease was also recorded in Packed Cell Volume (PCV) from 40.33 ± 0.67^b in treatment 2($KMnO_4$) to 27.83±1.88 in treatment 4 (Formalin). Treatment 4 (formalin) had the highest MCV (Mean Corpuscular volume) and MCH(Mean Corpuscular volume). Significant differences were notice between the MCV values in treatment 4 and the other three treatments. significant variation was observed between the MCH values in treatment 1 and treatment 2. The Mean Corpuscular Haemoglobin Concentration (MCHC) values of the entire treatments were statistically the same (Table 7.0).

Table 7: Heamatology of *Clarias gariepinus*

Treatments	T1	T2	T3	T4
WBC (10^9/L)	4.13±0.32^c	4.57±0.21^c	5.50±0.50^b	6.23±0.25^a
RBC (10^{12}/L)	26.70±4.04^a	27.60±0.88^a	14.00±13.54^b	9.20±9.40^c
Hb (g/L)	12.45±2.03^a	13.09±0.98^a	11.34±1.76^{ab}	10.52±3.04^b
MCV (10^{-15}L/cell)	138.58±4.53^c	146.12±3.45^{bc}	202.36±8.34^b	356.85±2.45^a
MCH (10^{-12}g/cell)	46.63±0.56^a	47.43±0.76^a	81.00±7.04^b	114.35±2.34^c
MCHC (g/L)	33.65±0.45^b	32.46±0.55^b	40.03±2.45^c	37.80±0.45^a
PCV (%)	37.00±1.15^b	40.33±0.67^b	28.33±1.76^a	27.83±1.88^a
N	65.00±10.40^c	26.67±1.20^b	27.03±1.67^a	27.33±5.75^a
L	30.00±10.40^a	69.33±1.20^b	70.33±1.67^c	70.67±2.33^c

Mean ± S.E with different superscripts along the same row are significantly different at $p < 0.05$

4.3 Water quality parameters

The results of this study indicate that the mean pH value ranged from 6.92 to 7.00 while temperature of water is within the range of 22.63^{0}C and 22.70^{0}C. Mean dissolved oxygen varied from 9.40mg/l to 9.43mg/l. There was no significant difference among all the tested water quality parameters in this study.

Table 8: Water quality parameters

Parameters	T1	T2	T3	T4
Temperature (^{0}C)	22.70±0.03^a	22.70±0.06^a	22.70±0.10^a	22.63±0.33^a
DO (mg/L)	9.42±0.02^a	9.40±0.00^a	9.42±0.02^a	9.43±0.02^a
pH	6.92±0.03^a	7.00±0.06^a	7.00±0.50^a	6.98±0.03^a

Mean ± S.E with the same superscripts along the same row are not significantly different at $p < 0.05$

4.4 Histological analysis of the Clarias gariepinus fingerlings

4.4.1 The gills of treated C. gariepinus fingerlings and control

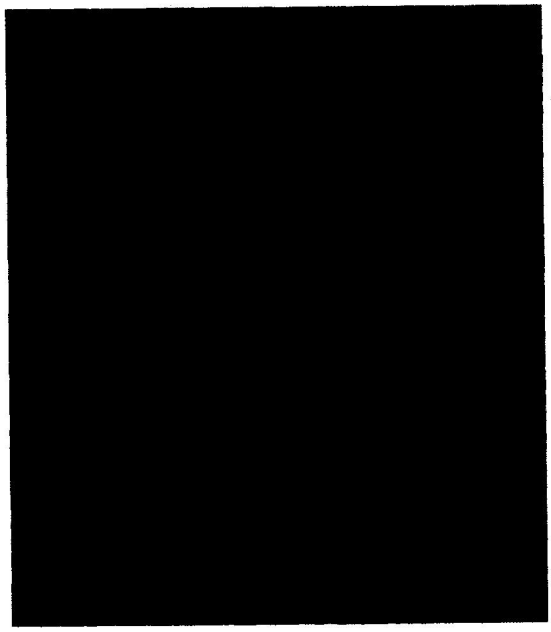


Plate 3: Gills of *Clarias gariepinus* fingerlings in the control showing no pathological lesion(x400).

Plate 4: The photomicrograph of *Clarias gariepinus* gills exposed to 2ppm of $KMnO_4$ showing severe degeneration in the gill structure. Section also showing that some of the gill rakers have been eroded(x400).

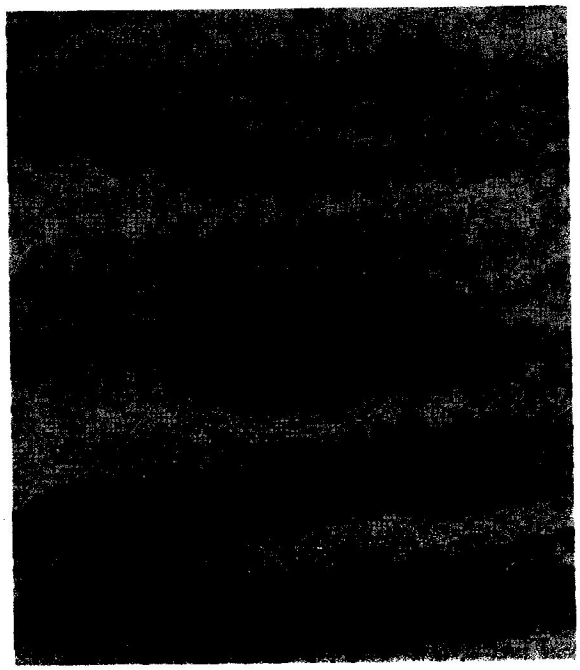


Plate 5: The photomicrograph of *C. gariepinus* gill exposed to 1000ppm of H_2O_2 showing severe degeneration in the gill structure, section also showing that all the gill rakers have been eroded(x400).

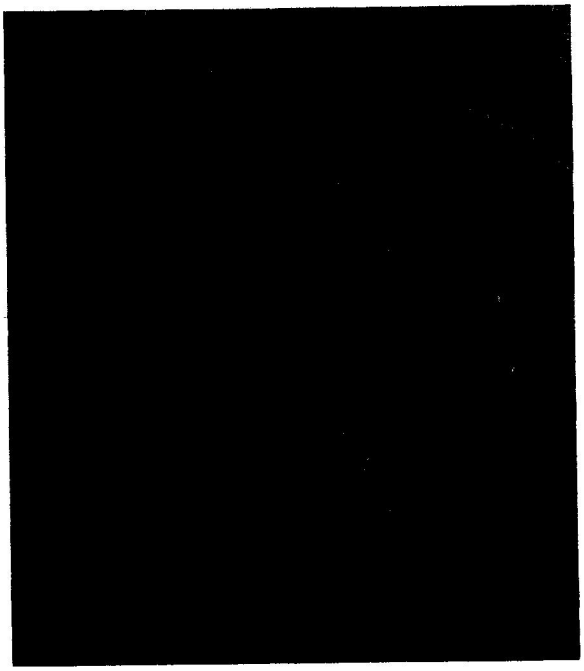


Plate 6: The photomicrograph of C. gariepinus gill exposed to 1000ppm of formalin, showing fusion of the gill rakers and there is an alteration in the gill structure(x400).

The control treatment (0ml/L) had no pathological lesion (plate 3). While gill exposed to 2ppm of $KMnO_4$, showed severe degeneration in the gill structure with a section showing some eroded gill rakers (plate 4). There was a severe degenaeration in the gill structure with section showing that all of the gill rakers have been eroded (plate 5). Plate 6 showed a fusion of the gill rakers and there was alteration in the gill structure.

4.4.2 Liver Histology of *Clarias gariepinus* exposed to different treatments

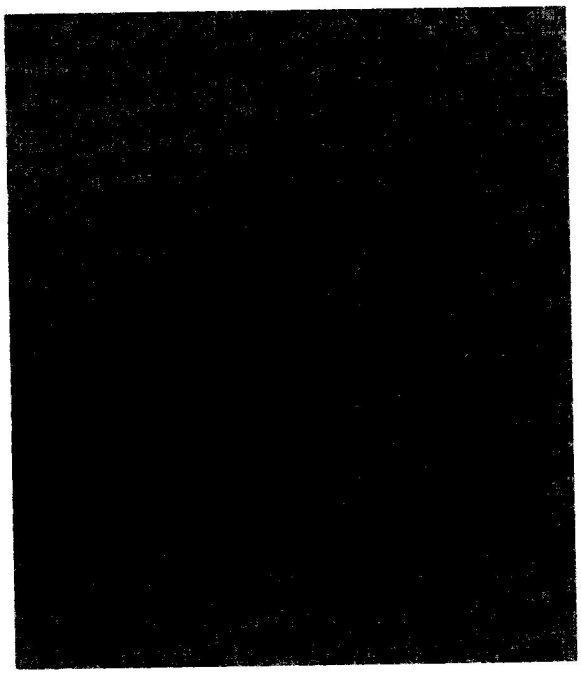


Plate 7: Liver of *C. gariepinus* fingerlings in the control treatment showing no pathological Lesion and hepatocyte arranged in grandular pattern and other cells normal and systematically arranged(x400).

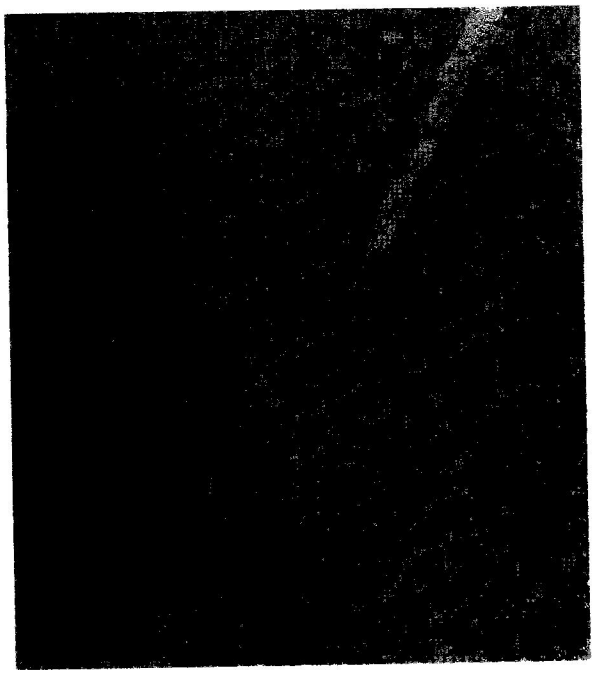


Plate 8: The photomicrograph of *C gariepinus* liver exposed to 2ppm of $KMnO_4$ showing slight changes in the hepatocytes(x400).

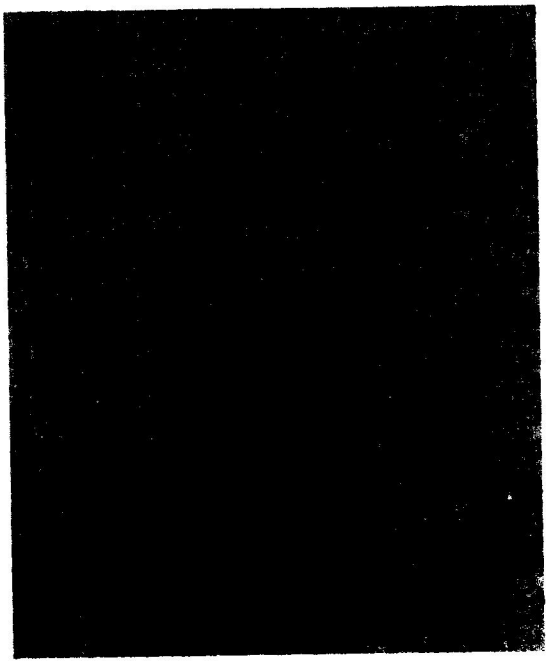


Plate 9: The photomicrograph of C*larias gariepinus* liver exposed to 1000ppm of H_2O_2 showing an increase in the interstitial cell and diffuse vacuolation of hepato-cytes(x400).

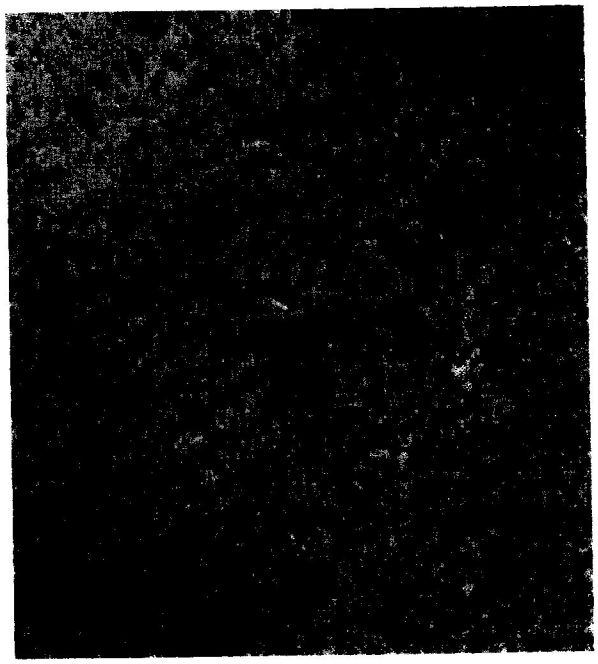


Plate 10:The photomicrograph of *C gariepinus* liver exposed to 1000ppm of formalin showing a very mild diffuse vacuolation of hepatocytes (x400).

The control treatment (0ml/L) showed no pathological lesion (plate 7). The photomicrograph of C*larias gariepinus* liver exposed to 2ppm (T_2) of potassium permangante showed slight changes in the hepatocytes (Plate 8). In Plate 9(1000ppm) of hydrogen peroxide, there was a an increase in interstitial cell and vacuolation of the liver. In Plate 10, there was a very mild diffuse vacuolation of hepatocytes.

4.5 Discussion

4.5.1 Growth performance

The specific growth rate for treatment 2 ($KMnO_4$) was the highest. This may be connected to the fact that Potassium permanganate, is a chemical oxidizing agent that will react with any organic matter in a pond including algae, bacteria, fish, particulate and dissolved organic, and organic bottom sediments according to Andrew and Roy, (2013). The fact that the fish in treatments 2 and 4 were afforded more space in the tanks due to mortality in them could not be ruled out as reason why they have better weight gain than the rest as stocking density is known to have a profound influence on growth, survival and behaviour of fish according to Boujard *et al.*, (2002) also supported by Jamabo and Keremah, (2009). The specific growth rates observed in this study are better when compared to what was observed by Walakira *et al*, (2014) in *C. gariepinus* larvae treated with salt and formalin at 3000ppm and 400µL/L concentration respectively but they are similar to the ones observed in banana leaf extract treated fish (5.82±2.16). There was no difference in the FCR of the fish in this present study. This is an indication that all the fish utilized the feed given well to gain weight.

4.5.2 Water parameters

The water quality parameters showed little variation.The range of temperature (22.63°C-22.70°C), pH (6.92-7.00) and dissolved oxygen (9.40mg/l-9.43mg/l) obtained is favorable for fish culture, they are within the range described as optimal by Boyd (1979) and this agrees with similar work by Jamabo *et al.*,(2015) and Jamabo *et al.*, (2017). Therefore the mortality recorded might not be connected to poor water quality.

4.5.3 Haematological parameter

Haematological characteristics are essential tools that are used as indicators for monitoring physiological status and changes in fish (Erhunmunse and Ainerua, 2013, Ogueji *et al.,* 2017). Haematological studies have provided reliable information on metabolic disorders, chronic stress status and health status before and after clinic examination of specimens (Bahmani *et al.*, 2001 and Nwani *et al.,* 2017). In this study, there was a sharp decrease in RBC values in *Clarias gariepinus* treated with the chemicals except for potassium permanganate. This might be as a result of an impairment of erythropoietic process. Velisek *et al.,* (2011) reported a significant reduction in RBCs when rainbow trout (*Oncorhynchus mykiss*) were exposed to verapamil. Ogueji *et al.,* (2017) also reported a significant reduction in RBCs when African catfish (*Clarias gariepinus*) were exposed to diazepam. Significant reductions in PCV and Hb values were also observed across the three chemicalswhen compared to the control except also for $KMnO_4$.These reductions might be as a result of significant decrease in hematopoietic activity. Nwani *et al.,* (2017) reported significant reductions in PCV and Hb values observed across diazepam treated fish specimens when compared to the control. Ajima *et al.,* (2016) made it known that *O. niloticus* juvenile exposed to varying concentrations of verapamil significantly reduced RBC, Hb and PCV values. The decrease in Hb values could also be as a result of the adverse effect on Hb biosynthesis.This was supported by Nwani *et al.,* (2013) as they reported that Hb biosynthesis when adversely altered, could limit the oxygen-carrying capacity of the fish blood. Low Hb value has also been associated with the low active fishes (Satheeshkumer, *et al,* 2011). *Clarias gariepinus* juveniles exposed to sub-chronic concentrations of some toxic chemicals significantly decreases RBC, Hb, and PCV values (Ibrahim *et al.,* 2017).

A significant increase in white blood cells(WBC) values of the fish exposed to the chemicals in this study, could be as a result of an immune system response to the toxic effect of the chemicals as WBCs have been involved in immune function regulation in many organisms (Nwani *et al.,* 2013). Saravanan *et al.,* (2012) reported a significant increase in WBCs when *Cirrhinus mrigala*was exposed to various concentrations of ibuprofen drug. Similar result has been reported (Ajima*et al.,* 2016) when *O. niloticus* were exposed to verapamil.Oguej*iet al.,* (2017)also reported a significant increase in WBCs observed in *Clarias gariepinus* juveniles after exposure to various concentrations of diazepam.

There were significant alterations in the values of red cell indices (MCV, MCH, and MCHC) of the treated fish in this study when compared with the control experiment. Red cell indices are important for the diagnosis of anemia in most animals including fish (Cole, 1986) cited by Mbah*et al.,* (2017). Dacie and Lewis, (2011); Iheanacho *et al.,* (2017); Nwani *et al.,*(2017) reported a significant increase or decrease in red cell indices which may indicate macrocytic or microcytic anemia. Increase in red cell indices as observed in the current study might implies possible macrocytic anemia. Cole, (1986) reported an increase in MCV, MCH, HcT and Hb values when *Oncorhynchus mykiss* was exposed to pharmaceutical drug (verapamil). Similar report was made by Saravanan *et al.,* (2012) indicating a significant increase in MCV and MCH values when Indian major carp (*Cirrhinus mrigala*) were exposed to Ibuprofen drug. Ogueji *et al.,* (2017) also reported a significant increase in MCV and MCH and MCHC values when African Catfish (*Clarias gariepinus*) were exposed to diazepam .

Alteration in WBC differential count is an insightful indicator of environmental stress (Cole *et al.,* 2001) cited by Nwani *et al.,* (2017). Significant increases ($p<0.05$) in neutrophil and lymphocytes contents were seen in Potassium permangante ($KMnO_4$), Hydrogen peroxide, and

Formalin treated fish above the control in this study. Similar finding was observed when *C. gariepinus* were exposed to chlorinfenicol drug (Nwani*et al.,* 2013). Barros-Beeker *et al.,* (2012) reported a significant increase in neutrophil values when Zebra fish (*Danio* rerio) larva were exposed to oxytetracycline. (Ogueji *et al.,* 2017) also reported a significant increase in neutrophil, lymphocytes, monocytes, eosinophils and basophils values when African catfish (*Clarias gariepinus*) juveniles were exposed to diazepam.

4.5.4 Histological analysis

The histopathological examination of the gill and liver of the exposed fish indicated that the gills and liver were affected. In fish, gills are critical organs for their respiratory, osmoregulatory, and excretory functions (Lawrence *et al.,* 2010). Gills are considered good indicator of water quality, being models for studies of environmental impact, since they are the primary route for the entry of chemicals (Emmanuel *et al.,* 2010). Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply. Damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress. Temiotan *et al.,* (2010) reported that if gills would be destroyed due to xenobiotic chemicals or the membrane functions are disturbed by a changed permeability, oxygen uptake rate would be rapidly decreased. Gill exposed to $KMnO_4$, showed slight degeneration in the gill structure (an expansion of the lamellae) with a section showing some eroded gill rakers (plate 2). There was a severe degeneration in the gill structure with section showing that all of the gill rakers have been eroded (plate 3) in the fish treated with hydrogen peroxide. Plate 4 also showed a fusion of the gill rakers and alteration in the gill structure for fish treated with formalin. This is similar to the observation of Adebayo *et al.,* (2016) when gills were exposed to sub-lethal concentrations of premium motor spirit(PMS) which led to impairment in gaseous exchange efficiency of the gills.

Vascular changes in the gills of exposed fish could be attempts by the fish to supply more blood to the gills, to increase oxygen uptake and supply to the internal organs (Guilio and Hinton, 2008). According to Ogbomida *et al.,* (2010),the liver is the main organ for detoxification. The liver is the main organ that suffers serious morphological alterations in fish exposed to pesticides and chemicals (Rodrigues and Fanta, 1998; Ezemonye *et al.,* 2010). Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors. The liver of the exposed fish had slight changes in the hepatocytes and vacuolated cells showing evidence of fatty/severe degeneration. This result conforms to reports of the study in Temiotan and Lawrence (2010). Same observation was made by Olukunle (2011); Jimoh (2012) and Adeleke *et al.,* (2015).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Disease infection can happen in fish culture. Farmers are bound to look for ways to treat their fish in cases of disease infection. Various chemicals are available for various diseases. This study has revealed that exposure of *Clarias gariepinus* to potassium permangante, hydrogen peroxide and formalin can produce significant changes in the physiology of *Clarias gariepinus* as manifested in the growth performance, haematological and histological parameters. Persistent exposure of fish to these chemicals especially formalin may lead to the mortality of *C. gariepinus* due to disruption of internal physiology.

5.2 RECOMMENDATION

It is recommended that good management practices that would not warrant disease infections in farms should be observed constantly. However if a farmer must use chemicals to treat fish, especially in cases of fungal infections, potassium permanganate would be recommended as its effect on fish heamatology and histology were not as severe as the other two chemicals. Similar research should be carried out by students and other researchers using other chemicals that farmers do use to treat their fish in cases of disease infection.

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