FEED ADDITIVE POTENTIALS AND ANTIBACTERIAL EFFECTS OF ALLIUM SATIVUM, CHROMOLAENA ODORATA AND TALINUM TRIANGULARE AGAINST PSEUDOMONAS AERUGINOSA INFECTION IN CLARIAS GARIEPINUS BURCHELL, 1822

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CERTIFICATION

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DEDICATION

I Appreciate Your Aid, O ALLAH, Through Unaffected Humbleness.
I Recognize Your Grace, O ALLAH! In The Real Soul of Islam,
I Thank You With All My Heart, O ALLAH!
This Work Would Not Have Been Possible, Without Your Protection And Guidance.
For Your Cause, And Help, This Work Become A Reality.
Oh Mighty ALLAH, Please Accept It, And Bless It.
It Is Dedicated To You, Oh Mighty ALLAH.
To my beloved wife, Mrs. Taiwo, M. Tiamiyu and my children for their encouragement and persistent prayer.

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ABSTRACT

Emergence of antibiotic-resistant bacteria in fish is of public health concern. Assessment of the suitability and safety of medicinal plants as alternatives to antibiotics in aquaculture is imperative. However, there is limited information on the use of *Allium sativum, Chromolaena odorata* and *Talinum triangulare* as feed additives due to their antimicrobial potentials. This study was designed to investigate the use of these plants as feed additives, and their antibacterial effects on *Pseudomonas aeruginosa* infection in *Clarias gariepinus* (African catfish).

African catfish fingerlings (n=360, 1.10±0.01g) and juveniles (n=210, 117.30±1.57 g) were randomised into 10 groups each and fed for 70 and 42 days, respectively. Formulated rations containing three concentrations (A=0.5%, B=1.0%, and C=3.0%) of different treatments: *A. sativum* (T_1A, T_1B, T_1C), *C. odorata* (T_2A, T_2B, T_2C), *T. triangulare* (T_3A, T_3B, T_3C) and control (no additive, CC) were fed to fish at 5% body weight. Growth parameters of the fingerlings were monitored, while haematology and histopathology of gills, liver, kidney and intestine of the juveniles were carried out. *In vitro* antibacterial effects of 25.0, 50.0 and 100.0% aqueous extracts (60g of the chopped dried leaves extracted with 300mL of distilled water) of the plants against *Pseudomonas aeruginosa* were determined. Catfish fingerlings (n=150; 53.1±0.23g) randomised into four groups were fed with pre-tested effective rations T_1A, T_2B, T_3B and CC. All fish were inoculated with *Pseudomonas aeruginosa* (0.2 mL culture containing 1.4 x10^6 cfu/mL) intraperitoneally and their survivability was evaluated by using mortality rate. Twenty four catfish juveniles (146.4±0.74g) divided into four paired sub-groups: Q1 and Q2, Q3 and Q4, Q5 and Q6, Q7 and Q8 were fed rations CC, T_1A, T_2B and T_3B, respectively. Sterile incision of 45.0 mm by 1.0 mm was created on the dorso-lateral part of each fish and sub-groups Q2, Q4, Q6 and Q8 were inoculated with *Pseudomonas aeruginosa*, while Q1, Q3, Q5 and Q7 were not inoculated. Percentage healing rates were measured on days 3, 6, 9, 12 and 15 post-incision. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Catfish on T_3B and T_1B had the least (4.70±0.11) and highest (6.32±1.01) feed conversion ratios, respectively. Values for red blood cell, packed cell volume, haemoglobin

concentration, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, lymphocytes and neutrophils were within normal limits across the groups. No lesions were observed in fish fed with T_1A and T_3B; however, other groups had mild gill lamellae hyperplasia and hepatic necrosis. The highest antibacterial effect (inhibitory zone 12.50±1.26 mm) was recorded in 100% aqueous extract of *C. odorata*. In the challenged groups, survival rates of 20% and 80% were obtained for CC and T_2B, respectively. At day 15 post-incision, T_3B had significantly highest healing rate in inoculated (86.7%) and uninoculated (100%) fish, with CC being 0.0% and 64.4%, respectively.

The plants were established as growth promoters with antibacterial effects against *Pseudomonas aeruginosa* infection in *Clarias gariepinus*. Inclusion rates at 1.0% of *Talinum triangulare* or *Chromolaena odorata* is recommended to enhance growth, survival and wound healing in *Clarias gariepinus*.

Keywords: *Clarias gariepinus*, *Talinum triangulare*, Antibacterial, Feed additives, *Pseudomonas aeruginosa*

Word count: 498

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemist
API	Analytical Profile Index
AqE	Aqueous Extract
ARB	Antibiotic Resistant Bacteria
AST	Aspartate Aminotransferase
AWG	Average weight Gain
AXO	arabinoxylooligosaccharides
BD	Basal Diet
CAM	Crassulacean Acid Metabolism
CAR	Central African Republic
CDC	Centre for Disease Control and Prevention
CFU	Colony Forming Units
CL^-	chloride ion
CO_2	carbon dioxide
CP	Crude Protein
CYP	Cyclophosphamide

DM	Dry Matter
DNA	Deoxyribonucleic Acid
DO	Dissolve Oxygen
dsRNA	double Stranded Ribonucleic Acid
EDTA	Ethylene – diamine Tetra acetic Acid
EU	European Union
EUF	Erythrocytes Volume Fraction
FAO	Food Agricultural Organization of the United States
FCA	Freund's Complete Adjurant
FCR	Feed Conversion Ratio
FDA	Food and Drug Administration
FDF	Federal Department of Fishery
FOS	fructooligosaccharides
FSN	Food Security and Nutrition
GDP	Gross Domestic Product
GE	Gross Energy
GIT	Gastro Intestinal Tract
GOS	Galactooligosaccharides
H & E	Haematoxylin and Eosin
H^+	Hydrogen ion
Hb	Haemoglobin
HIV	Human Immune Deficient Virus
IMO	Isomaltooligosaccharides

K	Condition Factor
$KMnO_4$	Potassium Permanganate
LPSs	Lipopoly saccharides
MAFF	Ministry of Agriculture and Food Manual
MCHC	Mean Corpuscular Haemoglobin Concentration
MCN	Mean Corpuscular Haemoglobin
MCV	Mean corpuscular Volume
MDP	Muramyl Dipeptide
MFBW	Mean Final Body Weight
MIBW	Mean Initial Body Weight
MS	Methane Sulphonate
MT	Metric Tonne
MOS	Mannanoligosaccharides
NA	Not Available
NFE	Nitrogen Free Extract
NFS	Nigeria Fishery Statistics
NH_3	Ammonia
NO_2	Nitrite
NRC	National Research Council
ODNs	Oligodeoxynucleotides
OH^-	Hydroxyl ion
OIE	Office International des epizooties /International Office of Epizooties
PCV	Packed Cell Volume

PER	Protein Efficiency Ratio
PPM	Part Per Million
RBC	Red Blood Cells
RPC	Replicate Plate Counts
ROS	Reactive Oxygen species
SCFA	Short Chain Fatty Acid
scFOS	short-chain Fructooligosacharides
SEM	Standard Error of Mean
SGR	Specific Growth Rate
SR	Survival Rate
T^0	Temperature
TAN	Total Ammonia Nitrogen
TDS	Total Dissolved Solids
TOS	Trans-galacto Oligosaccharide
UIHB #	University of Ibadan Herbarium Number
USA	United States of America
WBC	White Blood Cell
WHO	World Health Organization
XOS	Xylooligo-saccharide

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background information

Aquaculture being one of the activities that contribute to food production is very important and it grows very fast in the world (Ayinla, 2012). Globally, aquaculture makes important contribution in closing the breach between supply and demand for fish production (Dada and Odugbemi, 2013). With the increasing in human population and the global wild fish supply becoming stagnant, research showed that production from aquaculture would need to be increased by 133% by 2050 so as to meet consumers demand globally (FAO, 2010).

Nigeria is one of the nations where aquaculture is rising very fast and the most widely farmed species is African Catfish, *Clarias gariepinus* (Akinrotimi *et al*., 2014a). *Clarias gariepinus* is a tremendous species for aqua-farming as it is predatory, develops fast, and endures water with poor physico-chemical parameters (Rad *et al*., 2003). According to Pruszyński (2003) quality of the meat of African catfish was appreciated by consumers especially when used in pepper soup and or smoked and used in soups. A great request for aquaculture produces exist in Nigeria by ever increasing population of the citizenry (Ojutiku, 2008). The effort to meet the demand resulted in fish culturing expanding in different ways, rising and expanding. Madu *et al*. (2003) observed that the issue of suitable feedstuff become a major source of fear and phobia to many prospective fish farmers in Nigeria. The complementary and complete feed amount to forty and sixty percent of production budgets correspondingly to the intensive and semi-intensive fish cultivation schemes (Fagbenro *et al*., 2003) and can sometimes negate the economic viability of a farm if suitable feed are not used. In order to provide quality feed at reduced cost, farmers started to compound feed locally in which antibiotics were incorporated as growth promoter without adhere to tenet of using antibiotics. Antibiotics were equally introduced into aquaculture since disease occurrences are increasingly being recognised as a main set back in aquaculture invention and occupation. African Catfish are aggressive,

they inflict wounds on their bodies especially when stock at high densities. The issue of wound inflicted on the body of cultured fish become problematic as wounded fish are vulnerable to predation and more so open wound facilitate the spread of infection hence the use of antibiotics.

Rahman *et al*. (2009) reported that pathogens are fundamental part of any water environs and frequently been establish on bare fish. Soocean *et al*. (2010) also concluded that in aquaculture wherever situations are sub-standard, probabilities of infections are augmented by elevated tension situation in fish and this straight affect immunity. The capability of fish to combat disease causing agent reduces due to poor immune response (Narnaware *et al*., 1994) and this consequently results in cultured fish become additionally vulnerable not only to pathogenic but also to adaptable bacteria (Rahman *et al*., 2009). Poor immune response in fish provides parasites the chance to multiply producing grave healthiness and this could ultimately be deadly to the host fish if it is not treated. Smith *et al*. (2003) reported that disease outbreaks led to huge financial forfeiture either through death or cheap meat value hence causing reduced profits edges and this impending both financial and societal improvement in many nations. The chief cause of massive deaths in fish and financial losses amongst aqua farmers according to Toranzo *et al*. (2005) was bacterial infections. Pseudomonades which are gram negative, opportunistic pathogens, naturally occurring in aquatic environment, normal gut flora of healthy fish are among these bacteria. They cause outbreak of diseases when the normal environmental conditions changed and fish stressed (Angelini and Seigneur, 1988). *Pseudomonas species* were recognized in several kinds of fish as causal of pseudomonas septicemia (Austin and Austin, 2007). Abdominal ascites, darkness of the skin, exophthalmia, detached scales and petechial haemorrhage are some of the characteristics of the disease (Toranzo *et al*., 2005). Austin and Austin (2007) stated that fish might be protected from disease over dual ways; eiher by invulnerable control of the animal to combat the attack of pathogens and also through drug. Fighting the invasion of the pathogen can be achieved through innate immune reaction of the organism (Harikrishnan *et al*., 2010). Enormous usage of antibiotics for infection management has repressed the development of water faunas. It is also one of numerous possible causes of the antimicrobial-resistant microbes which feast from faunas to Man through the foodstuff chain.

Herbal medicine has been used as alternative to chemotherapy globally (Denev, 2008). Many plants species have been documented to possess several medicinal value and the phytochemicals which are naturally present in them, work through nutrient plus nutritive fibres to safeguard animal and man against infections (Soladoye *et al*., 2012). The medicinal plants were used as growth promoters, stress resistant boosters, preventative of infection, control of diseases and it can also act as immunostimulant (Citarasu, 2010), this pointed out clearly that medicinal plants constitute the main source of health care products and new pharmaceutical drugs (Ogbonnia *et al*., 2008). There have been little investigations on the therapeutic floras that can be used to promote growth, to resist stress and to prevent infection, control of diseases in view of abundance of these plants in our immediate environments that may have significant effects in aquaculture. Hence an urgent quest for search of medicinal plants and herbal medicine useful in aquaculture in this part of the world in view of economic loss in aquaculture as a result of death, cost incurred in treatment and more importantly food safety.

Allium sativum (Garlic) has been described to have numerous valuable effects including antimicrobial possessions, antioxidant and antihypertensive (Sivam, 2001). Metwally (2009) stated that incorporation of garlic in fish feedstuffs caused increase growth in fish. Metwally (2009) also concluded that adding garlic in fish feedstuff upsurges the wellbeing of fish and it aids in the regulation of disease causing agent particularly fungus and bacterium.

Chromolaena odorata (Siam weed) had been used globally for medicinal and nutritional purposes. Hataichanok *et al.* (2013) pointed out that extracts of the fresh leaves of *Chromolaena odorata* have been traditional used to treat soft tissue injuries, burns and membrane infections. Reduction in bleeding and clotting times has been effected by *Chromolaena odorata leaves* and according to Akomas and Ijioma (2014) this may be beneficial in handling hemorrhage complications and hastening wound healing.

Talinum triangulare (Waterleaf) has been reported to have highly elevated antioxidant values and this could be as a result of important mineral deposits such as magnesium, potassium, calcium also vitamins, for example C, E, and beta-carotene and soluble fibres which are present in it (Ezekwe *et al*., 2013).

1.2 Statement of the problem

Desire for growth promotion, challenges of the bacteria and parasite infections and inflicted wound on the cultured fish necessitate the usage of antibiotics and other chemotherapeutic agents in the aquaculture industries. The prevention and treatment of both fish as well as human diseases by the extensive use of antibiotics and other chemicals uncontrollably have no doubt added to an upsurge in the occurrence of hardy bacteria strains (Nya and Austin, 2011) causing problem for human as well as animal wellbeing along with environment. Negative impacts of antimicrobials as well as other chemotherapeutants used for checking infections and growth promotion has been condemned (Thiyagaran *et al*., 2014). However, intensity in aqua farming resulted in the elevation of circumstances which warrant the usage of extensive collection of compounds comprising antimicrobials, anesthetics, insecticides, hormones and numerous dyes. It has been found out that compounds like formalin, hydrogen peroxide, malachite green and others offer not long term solution against parasitic hitches in aquaculture (Kurva and Gadadhar, 2013). Bathing fish with medicaments is a labour intensive and costly operation, and this only cause temporary relief. The treatments cause pressure to the fish and increase probabilities of contaminations. Several of the compounds used in aquaculture have been prohibited and banned in many countries as a result of not being environmental friendly (Thiyagaran *et al*., 2014).

1.3 Research questions

i. Is there any effects on growth performance of African Catfish fed *A. sativum, C. odorata* and *T. triangulare* as feed additives?

ii. Is there any protective effects on African Catfish against *Pseudomonas aeruginosa* infection as a result of feeding *A. sativum, C. odorata* and *T. triangulare* as feed additives?

iii. Do *A. sativum, C. odorata* and *T. Triangulare* fed separately to African Catfish as feed additives heal similar wounds at different rates?

1.4 Study aim and objectives

The main objective of this study is to investigate the use of *A. sativum, C. odorata* and *T. triangulare* as feed additives, and their antibacterial effects on *Pseudomonas aeruginosa* infection in *Clarias gariepinus* (African Catfish)

The specific objectives are to:

1. Determine the phytochemical constituents of *A. sativum, C. odorata* and *T. triangulare*.
2. Investigate the effects of *A. sativum, C. odorata* and *T. triangulare* fed separately on growth, blood characteristics parameters and histopathological studies in African Catfish.
3. Evaluate the efficiency of the *A. sativum, C. odorata* and *T. triagulare* in improving the survival of African Catfish against *P. aeruginosa* infection.
4. Investigate the effects of *A. sativum, C. odorata* and *T. triangulare* on the wound healing rate in the African Catfish.

1.5 Justification

The break out of communicable infections in addition to use of antimicrobials as growth promoters led to uses of wide range of chemicals and antimicrobials (Smith *et al.,* 2003). Uses of excessive chemotherapeutants have been extensively condemned because of their undesirable effects like drug resistance being developed and immunosuppression, accumulation of drug residue in the tissue, thus result in less inclination for diet fish treated with antibiotics by consumer (Madhuri *et al*., 2012). There is increase in awareness of consumers demand for perfection in fish and shellfish food to ensure safety. More so, the cost of hormone and antibiotics used for hindrance and management of infections as well as growth elevation is reported to be high (Rhodes *et al*., 2000). Therefore, the assessment of the suitability and safety of medicinal plants as alternatives to antibiotics in aquaculture is imperative. However, there is inadequate statistics on the usage of *Allium sativum, Chromolaena odorata* and *Talinum triangulare* as feed additives despite their antimicrobial potentials. This work raises questions about antibiotics and other chemotherapeutants usage in aquaculture and the study intends to add to knowledge by

assessing the phytochemicals of these three selected herbal plants (*Allium sativum, Chromolaena odorata* and *Talinum triangulare*.), the effects of their bioactive compounds on growth, blood characteristics, survival to challenges of bacterial infection especially *Pseudomonas aeruginosa* and wound healing in African Catfish (a hardy, indigenous, widely cultivated, commercially viable tropical fish).

Phytobiotics have been testified to stimulate several actions comprising antistress, growing upgrade, tonic, hunger stimulus, immune stimulation as well as antibacterial possessions in fish cultivation (Citarasu, 2010). Phytobiotics use as herbal medication have a lengthy history, mostly in Asia country (Ji *et al*., 2009) and in Africa countries (Soladoye *et al*., 2012) and might offer a valuable basis of innovative pharmacological units as well as medicine for enhancing growth promotion, effective management of communicable infections thereby improving fish well-being and ensure foodstuff safety and superiority while preserving the aquatic environments as well (Suman and Csaba, 2011). The herbs do not have many negative impact which are connected with synthetic antimicrobials (Manal *et al*., 2014). Ji *et al*. (2009) reported that phytochemicals provide better exactitude than chemotherapeutants, causing no toxicity as well as a cheaper source for a treatment. This study will contribute knowledge to ethno-veterinary therapeutic herbal resources and their usages in handling health problem, as well as to ensure food safety. As it was recounted by Citarasu (2010), the usage of natural produces like plants extracts in aqua farming as alternative to chemotherapy is innovative and evolving endeavour which requests advance study.

1.6 Study hypotheses

Ho1. A. *sativum*, *C. odorata*, and *T. triagulare* do not have effect on growth parameters of the African Catfish.

Ho2. *A. sativum*, *C. odorata*, and *T. triagulare* do not have effect on blood characteristics and integrity of the tissues of the African Catfish.

Ho3. *A. sativum*, *C. odorata*, and *T. triangulare* do not improved survivability of the African Catfish against *P. aeruginosa* infection.

Ho4. *A. sativum*, *C. odorata*, and *T. triangulare* do not heal similar wounds at different rates in the African Catfish.

1.7 Data analysis

Analysis of variance (ANOVA, one way) was employed to analyze all data with Graph Pad Prism Software Version 5.1. Mean of the water quality parameters, weight measurements, haematological and biochemical parameters were measured. The outcomes were presented as mean ± SEM (standard error). The means were ranked by Tukey test. All differences were regarded as significant at α value of 0.05 among treatment groups.

CHAPTER TWO

LITERATURE REVIEW

2.1 Aquaculture in the world

Water covers over 70 percent of the earth surface. Fish are pervasive inhabitant of this ecological unit. With above 30,000 known species and numerous that may yet be revealed, fish are the greatest prosperous vertebrate collection and show an extremely significant ecological part (Noga, 2000). Arable farming, fisheries and aquaculture are the three key groups of events that add to food production. Among these, aquaculture is the world's latest revolution in foodstuff making. The research has shown that the worlds natural stock of fish and shellfish have fixed production bounds, though renewable, cannot be surpassed even in the finest controlling managements. The highest maintainable fishing boundary has been exceeded in most of our lakes, rivers and oceans (FAO, 2000). Aquaculture is therefore means to bridge the gap of fish supply (FAO, 2014).

Aqua farming productivity is increasing quicker than any other animal-centered food segment as production in capture fisheries is stagnating worldwide (FAO, 2014). In the preceding five years, world fish stock has grown dramatically with continuous growth in fish production and enhanced delivery networks. The regular growing level of 3.2 per hundred annually recorded in the epoch between 1961and 2009, outpaced the growth of 1.7 percentage annually recorded in the populace globally.

FAO (2016) reported that about 126 million tons of fish obtainable for human ingestion in 2009, 9.1 million tons (9.1 kg per capital) fish was consumed in Africa which was lowest in the world, while Asia consumed 85.4 Mt (20.7 kg/capital) that accounted for two thirds of over-all ingestion while 42.8 Mt were expended outside China (15.4/capital). Total fish production amounted to 3.09 million tons in the Arab World signifying 2.2 percent of global fish making in 2012. In the similar year, 7 Arab nations jointly made 88.2 percent

of overall fish making in the Arab nations. Egypt, a nation of fish producer in Arab generating 28.8 %. In the Arab World, most modern knowledge are now in practice in aquaculture, still marketable aquaculture only occurs in limited Arab nations nevertheless 91.9 percent of Arab aqua-farming is created in Egypt. By year 2012, the total aquaculture making in the Arab world total 587,195 tons demonstrating only 1.23% of worldwide aquaculture in that year. By year 2011, the global fishing segment was predicted to yield 149 Mmt, and that 89 million metric tons (60%) would originate from fish hunting and 60 million metric tons (forty percent) from cultivated fish (FAO, 2011). Annual projection of 1.3% in global production up to year 2020 was estimated and this was a dawdling rate than compared with the preceding decade. This was owing to a lesser growing level in aqua-farming making (2.8 % in that epoch likened with 5.6 percent for 2001-2010) as well as a diminishing or still in open fishery. Aqua-farming will progressively be the backbone of revolution in the fish industries segment as this development indicated a new era. FAO (2016) forecast that in 2015, aquaculture will exceed fish hunting as significant basis of fish for human ingestion. Fish considerably added to human food source and diet for more than 660 million fish-workforces and their relatives. At least 15 per hundred of per capital ingestion of animal protein of above 4.5×10^6 consumers was provided from fish (FAO, 2016). The past five decades shown a steady progress in fish production globally (Figure 2.1) through a regular yearly growing level of 3.2 % of diet fish resource overtaking world populace growth at 1.6 per hundred. Apparently fish eating increased to 19.2kg in 2012 from 9.9 kg in the 1960s (preliminary estimate) (Table 2.1), all figures offered are rounded up. This inspiring improvement was motivated by amalgamation of urbanization, populace growing, and increasing revenues and eased by more efficient distribution of catch fish and fish products from expanded fish production systems.

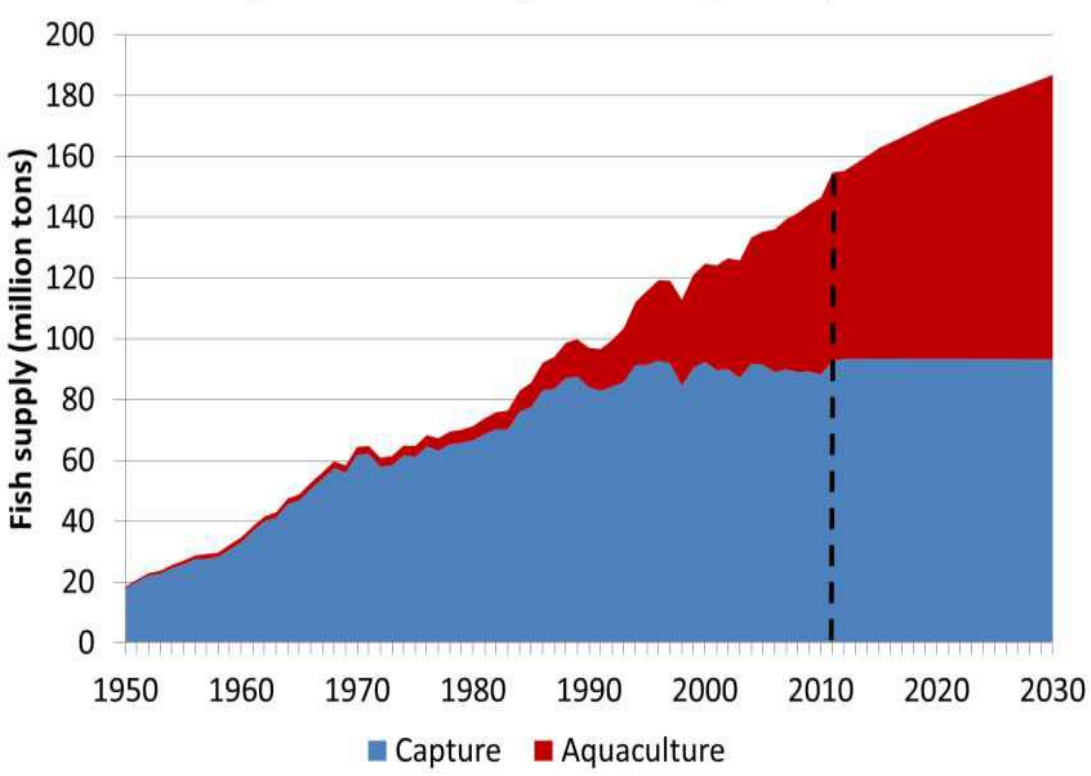


Figure 2.1. Showing Global Fish Supply: 1950-2030 (Source: FAO, 2016)

Worldwide, fish presently contributes approximately 16.6 per hundred of protein from animal as well as 6.5 % of entirely protein expended by Man (FAO, 2012). Fish is a basis of excellent proteins comprising all vital amino acids which can be easily digested. In many developing and least-developed countries, average per capital fish ingestion was little however little amounts of fish had a substantial affirmative nourishing influence than diets made from plants. More so, fish delivers health welfares in defense against cardiovascular maladies and supports in expansion of the brain in the foetus and newborns because fish is rich in unsaturated lards, predominantly long-chain omega-3 fatty acids. FAO (2016) reported that the benefits of plenty fish intake basically overshadow the possible adverse effect connected with adulteration or well-being dangers.

Table 2.1. The State of World Fishery and Aquaculture, 2009-2014

Year	Capture (Million tonnes)			Aquaculture (Million tonnes)		
	Inland	Marine	Total	Inland	Marine	Total
2009	10.5	79.7	90.2	34.3	21.4	55.7
2010	11.3	77.9	89.1	36.9	22.1	59.0
2011	11.1	82.6	93.7	38.6	23.2	61.8
2012	11.6	79.7	91.3	42.0	24.4	66.5
2013	11.7	81.0	92.7	44.8	25.5	70.3
2014	11.9	81.5	93.4	47.1	26.7	73.8

Source: Report of global fisheries and aqua-farming (FAO, 2016).

2.2 Aquaculture in Africa

Aquaculture is one of the latest frontlines to contribute to foodstuff security in the emerging and least developed world. In some countries, it now signifies the fastest developing agricultural business, with freshwater aqua-farming leading entire agricultural industries. This universal happening is replicated in Africa where aqua-farming resource as shown in Table 2.2 provide high class food at little cost to populaces, produce earnings for arable and fishing families and display a vital role in several home and abroad markets (Kitessa, 2014). Despite the huge investment in aquaculture in Africa, proper governance of aquaculture has been prevented and this is caused by a number of external problems hence Africa has little aquaculture tradition, unlike Asia. Several nations in sub-Saharan Africa are branded by little agrarian making, poor organization of funds, tenacious political unpredictability, financial stagnation, increasing environmental damage, dearth of technical expertise, and stark poverty (Ozigbo *et al.,* 2014). Nigeria with production of 15,489 tonnes annually, tracked by Egypt with yield of approximately 5,645 tonnes is the largest Africa nation in aquaculture (Ekunwe and Emokaro, 2009), and this is in contradiction to FAO (2012) conclusion that Egypt is the chief maker of aqua-farming in 2010 with 919,585 tons of overall Africa produce trailed by Nigeria by 200,535 tons. Kenya Madagascar, Togo, Sudan and Zambia were only five other countries that yield over 1,000 tonnes each. The outputs demonstrate generally that Africa is far-off in aqua-farming making.

Table 2.2. Food fish production capacity in Africa from freshwater aquaculture and mariculture* (Unit: tonnes, live weight)

Year	Freshwater aquaculture	Mariculture	Total
2004	546 229	12 659	558 888
2006	739 383	15 096	754 480
2008	928 296	14 632	942 929
2010	1 273 583	12 858	1 286 441
2012	1 467 979	17 408	1 286 441
2013	1 594 069	21 539	1 615 608

Source: FAO aquaculture newsletter 54, March, 2016.www.fao.org/publications

2.3 Aquaculture in Nigeria

Nigeria consumed above 1.5 million tonnes of fish yearly. This made Nigeria a principal fish purchaser nation in Africa and amid the leading fish buyers worldwide. According to NFS (2016) the entire fish request in Nigeria calculated on the inhabitants estimation of 180 million (2014) is 3.32 mmt; and quantity of fish produced as of aqua-farming, artisanal as well as industrialized fisheries the same year amount to 1.123m Mt. Nigeria fish importations are above 900,000 Mt though its local harvest is valued at 450,000 Mt annually (FDF, 2008). The advance in fish making is owed to improved engagements of aquaculture, as well as the requisite for aqua-farming rose as a consequence of reduction in quantity from ocean fisheries owing to over-fishing, pollutions and habitat destruction (Adedeji and Okocha, 2011). Owing to over utilization of the hunted fisheries, the anticipation of the Nigeria fish industries is in aqua-farming expansion and suffice to say that Nigerian fishery segment is categorized by opulent resource base. The sector contributes majorly to livelihood security of over 3 million individuals in Nigeria (Adedeji and Okocha, 2011). Fish farming bustle in this nation took place approximately 5 decades ago (Olagunju *et al*., 2007) and till now aqua-farming in the country is still in the emerging period, since the request of the constantly increasing populace has not been met (Ojutiku, 2008). This nation has the normal wealth (for instance lands, watercourses, rivers, lakes and lagoons as well as human capacity) and capacities to contend with other aqua-farming nations.

Fish is in high demand in Nigeria, it makes up of almost 41 per hundred of the meat in the typical Nigerian food because fish foodstuffs are fairly economical likened to pork, beef and other animal protein that can be sourced in the country (FDF, 2008). In addition, a comprehensive array of minerals, amino acids and vitamins are embedded in fish which make it high in nutritional value (Akinrotimi *et al.*, 2007a). The four major sources of fish supply in Nigeria are artisanal fisheries, industrial trawlers, imported frozen fish and aquaculture. FDF (2007) reported that the production from aquaculture is increasing between 2000 and 2007 by 5 – 22% in domestic fish production compared to artisanal sources. Aqua-husbandry stands as a sub-divisions of food creation, being recently pursued by the FGN so as to meet up with the high request for fish and fish foodstuffs in the country and finally to

have fish merchandises accessible for overseas trade and this is in line with her effort to diversify its oil-based economy.

The aqua-farming in Nigeria is principally that of catfish husbandry (Adewumi and Olaleye, 2011). However there are over hundred varieties of catfish in the world, *Clarias gariepinus* signifies the most prevailing of these fish species and have worthy marketable importance in Nigerian shops (Adewolu and Adoti, 2010). Aquaculture, most especially rearing of *Clarias gariepinus* is important to the Nigerian commercial activities. It decreases the level of joblessness, provide incomes and upsurges the Gross Domestic Product (GDP). *C. gariepinus* could be vended alive at the open market, price worth 2 to 3 times that of tilapia hence it fetches a higher price than tilapia in most countries (Olagunju *et al*., 2007). According to Emokaro *et al*. (2010) it has a higher feed conserving rate and requires less space, time and money. In all states in Nigeria, both small scale commercial fish agriculturalists were involved in the culture of Clarias catfish since 1985. Catfish production has prospective for development to meet the national fish request and this can reduce fish importation, providing employment and alleviating poverty. Akinrotimi *et al.* (2014b) reported that ninety percent of the overall fish making in Nigeria come from intensive cultivation of Catfish. The catfish species that are cultivated in Nigeria comprise *C. anguillaris, C. gariepinus, H. bidorsalis, Chrysichthysnigro digitatus* and *Synodontis sp* among others. However, *C. gariepinus* is undoubtedly the utmost farmed species in this country.

2. 4 *Clarias gariepinus*

Clarias gariepinus belong to the family Clariidae, a class of catfish which was discovered and named in 1822 by Burchell. African Catfish, a piercing tooth catfish, characteristically with accessory lung for air-breathing, scaleless, gaunt, lengthened physique, having prominent dorsal and anal fins, and a hood like skull. It is easily recognized as a result of its prominent dorsal and anal flippers that make it look like eel. It has a slim physique, uniform bony skull as well as expansive buccal cavity with 4 duos of tentacles. The projecting tentacles makes African Catfish look like cat whiskers. Skelton (2001) observed that colour of African Catfish usually varies from white to a pale cream ventrally and light brown to dark dorsally and is often mottled with shades of grey and olive (Plate 2.1). Robins *et al.*

(1991) reported that it can grow maximally to weight of 60kg and according to IGFA (2001) to lengh of 170 cm. African Catfish is naturally cultivated in earthen fishponds nevertheless some other systems such as hapas and tanks can be used. In the feral water, the fish breeds naturally nevertheless effort is being intensified on artificial reproduction by inducing spawning. The African catfish tolerates relatively poor water quality, omnivorous and grows fast hence an excellent species for aquaculture **(**Rad *et al*., 2003).

Plate 2.1. African catfish (*Clarias gariepinus*) (Source: Dr. Rutaisire, 2005)

2.4.1 Natural distribution and habitat

African Catfish is ubiquitous in several Africa nations, except Maghreb, the Cape countryside of Southern part of Africa and Guinea (Upper and Lower) (Picker and Griffiths, 2011). Skelton (2001) reported that African Catfish is extensively spread among other fish in Africa. African Catfish spread from the river umtamvuna in South Africa (eastern part) to the far south of river orange system in the western part of South Africa (Jubb, 1967). *Clarias gariepinus* is agreed to be a freshwater species however it tolerates countless diverse environments, including the uppermost part of creeks. It survives well in dawdling flowing watercourses, floodplains, ponds and dams (Skelton 2001). It is a fish that can be found in remnant pools of drying rivers as it withstand little DO_2 as well as waters high in turbidity (Van der Waal, 1998). *Clarias spp* can be found in streams, rivers, swamps to floodplains, and calm waters from lakes, some of which are subject to periodic parching. *Clarias spp* can stay alive during the dry seasons because it possesses the auxiliary air inhalation structures which made it possible (Bruton, 1979a).

2.4.2 Natural feed and feeding technique

Many research works on food composition of *Clarias gariepinus* have conclusions that the fish are omnivores or predators. Catfish from the river Ubangui in Central African Republic (CAR) had been studied (Micha, 1973) and study concluded that *Clarias lazera* (*Clarias gariepinus*) nourished mostly on benthics, water beetles, other fish and higher plants remains. They also forage on mollusks, land-dwelling insects and fruits. Bruton and Allanson (1980) pointed out that crustacean or fish were main food of the Catfish in Lake Sibaya (South Africa) and that aquatic and terrestrial creatures were significant parts of the food of adult fish and juvenile which live in low zones. Though, plant debris, diatoms, mollusk and arachnids were the petty food stuffs expended in the lake. Feeding habit of *Clarias gariepinus* in Lake Mcllwaine (Zimbabwe) was studied and it was concluded that as fish became larger, the food composition changes. The big fish depends more on Zooplankton. *Clarias gariepinus* are omnivorous fish. They depend on different food items ranging from tiny zooplankton to smaller fish which is about half of their own dimension or about 10% of their physique weight. Bruton (1979a) reported that *C. gariepinus* is equipped with different anatomical adaptations which allow them to feed under low visibility. These anatomical adaptations include wide mouth which can be displaced

vertically to engulf bulky target or enormous capacities of H_2O when filtering food. On their jaws are a broad band of recurred teeth, also possess pharyngeal teeth which prevent grabbed prey from escaping. Also possess an extensive curved caudal fin, characteristic for fish which waylay their victim and five branchial arches with long gills rakers. *C. gariepinus* has capability to shift from depending on one category of victim to another type of victim.

2.4.3 Reproduction in African catfish

2.4.3.1 Natural reproduction

Clarias gariepinus has separate sex. Large number of eggs are produced by females on an annual basis and fertilization is external. The primary sex characteristics of the fish is the gonad refers to the testes in the male and ovaries in the female.

2.4.3.2 Artificial reproduction

Attempts were made previously to acquire seeds of the maximum weight and the finest quality under controlled conditions for artificial reproduction in *Clarias gariepinus.* Inyang and Hettiarchichi (1994) used various preparations to stimulate ovulation experimentally with aim of finding stimulators that would ensure such effects. Satisfactory results for stimulated fish breeding require that appropriate paternal (and maternal) material should be used. Brzuska (2003) used carp pituitary homogenate to stimulate ovulation. Brzuska *et al*. (1999) also investigated the effects of reproduction after ovulation stimulation using different preparations (of both synthetic and natural origin).

2.4.4 Rearing

2.4.4.1 Water quality in catfish farming

The entirety of biological, chemical and physical factors that upset the welfare and growth of cultivated organisms in their environs is referred to as water quality. The universal state of cultivated organism is impacted by water superiority and this determines the health and growth of their conditions. Increased production requires more feed and since only about 50% of the feed is converted into fish flesh, the problem associated with water quality management intensified. The non-draining techniques have probably also increased disease and off-flavour problem. The emphasis should be laid on management of water quality as

relates to improved productivity and disease outbreaks. The commercial aquaculture enterprise is successful if there are provision of the optimal environs for fast development with lowest rate of funds. Superiority of H_2O is important when planning for high aqua-farming production. Among the several water quality variables found in the complex environment of culturing fish, some play significant role. Factors which are grave are; amount of liquefied O_2, temperature, NO_2, ammonia, CO_2, alkalinity as well as suspended solids. Dissolved O_2 is mandatory for aerobic metabolism in fish hence the utmost significant and critical parameter in culturing fish. Dissolved oxygen needs to be monitored continuously in aquaculture production systems. Water quality needed for culturing of *Clarias gariepinus* is indicated as shown in Table 2.3.

2.4.4.2 Temperature of the water

Next to oxygen is water temperature in the water quality variables. The action, behaviour, nourishing, development and reproduction of entire fishes are affected by water heat. It may be the distinct most significant influence that disturbs the well-being of a fish throughout its life period. The temperature regulates the quantity of dissolve oxygen (DO) the water can hold. The amount of DO the water can hold is inversely proportional to the temperature of the water. Water has a high heat engrossing capability i.e it can engross huge quantities of heat with slight rise in temperature. Sudden changes in water temperature is prevented because of this characteristics. Momentary day-to-day stratification in profounder water may lead to confined DO reductions in bottommost waters, or the advance of provisional but tense levels of compound for example carbon dioxide and hydrogen sulfide thereby leading to disease condition.

2.4.4.3 Dissolved oxygen

The number of fish kills in the fish farms increased in number as a result of Oxygen depletion. Oxygen depletion also occurs as a result of heavy feeding of fish in limited volumes of water in case of heavy stocking. An early morning dissolved oxygen (DO) of 2.0 ppm has been accepted by most managers as being the critical level below which it should not be allowed to fall. However, level below 5ppm are considered to be stressful for most warm water fishes including channel catfish. Dissolve Oxygen can be maintained at adequate levels to support the fish if good aquaculture practice is maintained even if waste products

are used to maintain fertility. In a single night if there was incidence of poor water quality, the entire fish population in a farm can be killed. Over production and adverse weather conditions may lead to oxygen depletion in the fish farms. Many diseases in fish farms are aggravated by chronically low DO. The fish producers need to understand the factors affecting production and removal of DO in the ponds. Water in fish pond is seldomly 100% saturated with DO because temperature of water is constantly changing and biological or biochemical, chemical and physical activities in the pond continuously use or produce oxygen.

2.4.4.3.1 Effect of oxygen on fish growth

The processes of breathing, anabolism and catabolism by some animal require oxygen. The metabolism of fish is greatly affected by oxygen concentration of the cultivation environs. When the concentration of liquefied oxygen decreases, the breathing and nourishing actions of the fish decreases and this result in reduction in growth rate and increase in incidence of diseases. Tom (1998) reported that low DO caused inability of the fish to assimilate consumed food. Keeping dissolved oxygen closer to saturation ensures better physiological conditions and good health for fish. Low DO results in swimming activities being decreased, tissue hypoxia, stressful condition and decreased immunity in fish. According to Tom (1998) oxygen demand are significantly different in different species of fish and also oxygen requirements for fish significantly decreases as individual weight of the fish increases.

2.4.4.4 Acidity and alkalinity (pH)

The association among H^+ as well as OH^- ions is referred to as pH. It is the numerical expression of the alkalinity or acidity of a matter. It is described scientifically as the -ve logarithm of the H^+ level. The gauge of pH ranges from 0 to 14. H^+ and OH^- ions are equal at pH 7.0 that is regarded as neutral solution. Values below pH 7.0 indicate increasing acidity (H^+ ions) while increasing alkalinity (OH^- ions) is denoted by values above pH 7.0. The monitoring of water superiority factors, for instance pH, alkalinity, CO_2, NH_3, nitrite and hardness are important in fish culturing. These variables are interrelated, when photosynthesis is high, CO_2 concentrations are low and pH values become high. The toxicity of ammonia in turn increases as a result of this combination. The pH, alkalinity and

hardness of the water are generally raised when agricultural lime in the form of calcium carbonate is added to the pond. A pH value of 9 to 10 greatly increased an un-ionized toxic ammonia. The pH can be lowered by the addition of alum or gypsum to the pond.

2.4.4.5 Ammonia (NH_3)

Ammonia in form of ionized and un-ionized forms are present in water. The total ammonia nitrogen (TAN) concentration present in pond water is the addition of ionized and un-ionized ammonia ($NH_4 + NH_3 = TAN$). A major component of protein is Nitrogen (N) and is required in all life forms. Ionized ammonia (NH+) is not toxic to fish however un-ionized ammonia (NH_3) present in the pond is toxic to fish. Feed remains the key source of ammonia in the pond however there other ways. Ammonia can be effectively removed from the pond using biological processes.

2.4.4.6 Nitrites ($N0_2$)

Nitrites is built up in the water as a result of breakdown of the nitrogen cycle, however the exact mechanism is not yet known. Nitrite is normally not present in natural waters. Nitrites combine with haemoglobin after entering through the gill membranes to form methaemoglobin, a compound that cannot transport oxygen. The signs are like that of reduction of O_2 in the fish pond. Amount of nitrite toxic to catfish is determined by the amount of chlorides, oxygen level present in the river and temperature of the river. Concentrations of Nitrite as little as 0.5 ppm can cause problems.

2.4.4.7 Chlorides (CI^-)

Presence of Chlorides (Cl^-) in the water can prevent Nitrite toxicity. Chloride and nitrite in ratio 3:1 can protect fish, however ratio of 5:1 or 6:1 will protect better even if the fish is immunocompromised. Anytime nitrites is found in pond water, the chloride concentration in the pond water can be checked to know the amount of chloride to be added.

Table 2.3. Water quality needed for culturing of *Clarias gariepinus*

Parameters	Spawns, larvae fry	Progressive fry	Progressive fry /grown-ups
Oxygen	80–100%	3–5 part per million	>3 part per million
Temperature	Optimal 30.0°C	Optimal 30.0°C	Optimal 26.0 – 8.0°C
Ammonia		0.10 ppm (1.0 ppm)	
Nitrite		0.50 part per million	
NO3-N		100.0 part per million	
pH		6.0 – 9.0	
Carbondioxide		6.0 part per million (10.0 – 15.0 part per million)	
Salinity		10 part per thousand. (15–16 part per thousand)	

Source: FAO repository paper, 2006.

2.4.5 Production systems

Catfish husbandry is categorized into 2 groups based on the inputs and activities. One is sole business; agriculturalist who carry out activities on a small scale or on a weekend basis without using sophisticated methods. Most fish farmers practice this method. Household and or farm wastes, compounded feeds are main source of food and production depend on natural fertilization. The level of intensification is low hence the yields are also low. The second groups are the big commercial farmers. They use tanks with varying degrees of aeration and recirculation. Foreign technical partners give assistance to these groups to develop intensive re-circulation systems which are highly sophisticated. These big farms which are found in and nearby city hubs make usage of imported progeny stock and feedstuff. Investigation on the likelihoods of launching resourcefully comprehensive sole catfish initiatives in Nigeria is much needed.

2.4.6 Feed and nutrition

Fish food represent not less than sixty percent of the overall budget of aqua-farming business (Gabriel *et al.,* 2007). *C. gariepinus* require relatively high protein. Feed need to be formulated for intensive monoculture of the African catfish. According to ADCP (1983) diets containing planned consumable energy level of 12 kilo Joule per gram and crude protein of 30-42 percent are required to give best growth rates and food conversions in Afican Catfish. Fagbenro and Adebayo (2005) reviewed animal and aqua food industries in Nigeria and reported that most catfish feeds were compounded from nearby obtainable feed components for instance soya bean, maize, wheat offal, rice roughage, fishmeal, blood meal and fish oil. Crickets, earthworms, maggots and termites constitute other unusual feedstuffs in African catfish culturing.

2.5 Biological hazards associated with aquaculture

FAO (2012) stated that the main impediments of aqua-farming particularly in the nations that are yet to develop are adverse environmental conditions and diseases.

2.5.1 Bacteria

The bacteria are a large group of prokaryotes, unicellular, micro-organisms. Their lengths are of a few micro-meters. Bacteria shapes range from spheres to rods and some are spirals.

Bacteria are found everywhere on earth, they live on bodies of animals and plants, growing in earth, liquid, radioactive discarded, acid, scorching mainsprings as well as in organic matter. One gram of soil contains approximately forty million bacteria and as many as 1 million bacteria surviving in 1litre of fresh water. World's biomass of bacteria cells on earth is approximately five nonillion 5 x 10^{30}. Vital processes in nutrients recycling such as the fixation of nitrogen from the atmosphere and putrefaction are useful activities that are carried out by bacteria.

2.5.1.1 Enterobacteriaceae.

These include many of the more familiar pathogens that are gram negative bacteria. The intestinal tract of the animals contain several of these bacteria. Human waste and animal (including bird) manure are different source of pathogenic bacteria in aquaculture ponds and natural water bodies. Edward and Pullin (1990) reported that viruses and enteric organisms are rapidly die-off in the fish ponds which are well managed. Fish harvested from waste-fed systems are rich in numbers of organisms and products derived from such harvest pose potential health risk (Buras, 1993).

2.5.1.1.1 *Salmonella species*

Among the bacteria that caused human gastrointestinal diseases globally is *Salmonella spp*. Products contaminated with *Salmonella sp* are not accepted by many countries importing sea food. *Salmonella spp* are naturally present in some tropical aquatic environments and studies indicated that the prevalence of it in tropical water is higher than in temperate waters, though seasonal variations usually occur (Fenion, 1983). Water birds usually feast *Salmonella spp* and additional pathogens in the environs (Beveridge, 1998). Scavenging birds and other animals also spread this organism and this has been proved by isolating the organism from the humid aquaculture schemes where faecal trashes are not used as fertilizer. Although, *Salmonella sp* is established to be the gut flora of homeothermic faunas, they have been found in the digestive tract of carp and tilapia developed in free leftover and left-over fed aqua-farming (Buras, 1993).

2.5.1.1.2 *Escherichia coli*

Escherichia coli is ubiquitous bacterium in the tropics. It is frequently used as gauge for faecal contagion, though association is questionable. Food borne diseases such as mild enteritis which could lead to serious illness and death are associated with some strains of *Escherichia coli.* Risk of disease causing strains of *Escherichia coli* is high in the pond fertilized by animal manure especially bovine manure. *E coli* 0157: H7 has been implicated in infection associated with with water. The strain has been established to occur in cattle and infective dose is low hence fertilizing pond with bovine manure poses potential risk to public health.

2.5.1.1.3 *Vibrio species*

In temperate and tropical regions, Vibrios which are largely briny accepting organisms are found certainly in the oceanic and saline-water, although *Vibrio mimicus* and *Vibrio cholerae* can be found in fresh water. Vibrios was sequestered from fin fish, mollusks, crustaceans and sediment plankton. The occurrence of Vibrios in the water correlated positively with the admixture of contaminated human waste in the water however not correlated with number of faecal coli forms present. There is also an affirmative correlation between the quantity of infections described and water temperature. Some species of *Vibrio* pathogenic in human may also be pathogenic in fish. Generally, the infective dose is great and the danger connected with ingestion of fish is expected to be little, therefore a millon organisms must be consumed to originate cholera in human, (Dalsgard, 1996).

2.5.1.1.4 *Aeromonas* and *Plesiomonas spp*

Among the normal aquatic bacteria flora are *Aeromonas* and *Plesiomonas sp* and in tropical waters *Plesiomonas* are common. *A. hydrophila* is the species most often implicated in food borne disease (Morgan and Wood, 1988) and *P shigelloids* has likewise been described to cause outbreaks of gastroenteritis as consequence of consumption of fish (Tsukamoto, 1978) however, epidemiological evidence showed that community health hazards from *Aeromonas and Plasiomonas spp* in cultivated fish are little.

2.5.1.1.5 *Clostridium botulinum*

Cl. botulinum is an anaerobic, spore forming organisms, ubiquitous in nature. It produces a neurotoxin life threatening food borne illness. Based on the antigenic nature of the neurotoxin produced, the organism can be grouped into seven types. *Cl. botulium* type E has been isolated in fish being naturally resident in aquatic environments. Risk of botulism can be prevented if the fish are properly processed so that organism will not grow and toxin will not be produced.

2.5.1.1.6 *Listeria monocytogenes*

Listeria monocytogenes is pathogen associated with food. There is no epidemic outbreaks associated with consumption of crustaceans and farmed fin fish. However, sporadic cases of Listeriosis has been associated with seafood including smoked salmon and smoked mussels in vulnerable populations. *L. monocytogenes* occasionally reported in tropical fishery products but commonly sequestered from aqua-farming harvests from temperate areas. Fish harvested in temperate area aquaculture system might be polluted with *Listeria spp* and this pose a possible health hazard once ingested uncooked or devoid of heat treatment.

2.5.1.1.7 *Campylobacter species*

Campylobacter species causes diarrhoea illness in humans. They are not normally found in unpolluted aquatic environments but normal flora in the gastrointestinal tract of warm blooded faunas especially poultry. The statistics on the incidence of *Campylobacter sp* in aquatic environments is scanty. Fertilizing ponds with poultry manure might establish a public health threat. Some reports indicated presence of *Campylobacter sp* in bivalves but information on its occurrence in crustaceans and finfish is rare. The hazard of *Campylobacter* infections connected with ingestion of cultivated fish products is little as suggested by available data.

2.5.1.1.8 *Shigella species*

Shigella species have rarely been sequestered in aquaculture schemes and produces. The hazard of contamination connected with the ingestion of farmed fish foodstuffs contaminated with *Shigella sp* is little based on epidemiological evidence.

2.5.1.1.9 *Pseudomonas*

Pseudomonas is a group of G-ve, aerobic Gamma proteobacteria in the clan Pseudomonadaceae with almost 191 species been defined **(**Abdullahi *et al*., 2013). The memberships of the genus have a great pact of metabolic variety and have been found in many niches (Oni *et al*., 2013)**.** Many *Pseudomonas* strain genome sequences are available and genus can be easily cultured in vitro hence an excellent focus for scientific research. Roberts (2001) reported *Pseudomona aeruginosa* as an unscrupulous human pathogen, *Pseudomona syringae* as plant pathogen, *Pseudomona fluorescens* as the plant growth-promoting organism and *Pseudomona putida*, the soil bacterium are the best studied species. *Pseudomonas spp* are common in natural water bodies and is implicated in septicaemia of aquatic animals (Roberts, 2001). These bacteria cause disease in the stressed host being an opportunistic pathogens. The most common bacterial infection among fish which are associated with stress under culture conditions is *Pseudomonas* infection (Kitao *et al.*, 1993).

2.5.1.1.9.1 *Pseudomonas aeruginosa*

P. aeruginosa is rod shape, G-ve bacterium fit in to the family Pseudomonadaceae. This species can survive in different environments including aquaculture environment and it is opportunistic pathogen (Abdullahi *et al*., 2013). *P. aeruginosa* was sequestered from the mouth, gill as well as membrane of African Catfish juvenile (Akinyemi, 2012). Oni *et al*. (2013) also reported that *Pseudomonas aeruginosa* was sequestered from gills, stomach material and skin of fingerlings of *Clarias gariepinus* cultured in the country. Akinyemi (2012) had established that *P. aeruginosa* was a G –ve bacterium, positive to catalyze test, affirmative to citrate consumption test, not positive to coagulase test, negative to indole test, positive to motility test, and formed acid in sugar fermentation test by means of lactose, sucrose and glucose sugar. Oni *et al*. (2013) described difference in sensitivity of *Pseudomonas aeroginosa* to different antibiotics and he reported that the bacterium is inherently hardy to numerous antimicrobials and this might be owing to low penetrability of its exterior-membrane, its ability to produce enzymes that can inactivate antibiotic (e.g cephalosporinases) and the constitutive expression of various efflux pumps.

2.5.1.1.10 Other bacteria

Other bacteria reported to be pathogenic to human including *Leptospira interogans, Erysipelothrix rhusiopathiae, Streptococus iniae, Yersinia enterocolitica* and *Mycobacterium sp* are widely distributed in different habitats including aquatic environments. Disease outbreaks in ornamental fish and food fish has been described to be triggered by *M. marinum.* Ingestion of crustaceans and cultivated fin fish had not being reported to be associated with any cases of illness. However, *Streptococus iniae* and *Mycobacterium marinum* infected fish may present professional dangers to personnel.

2.5.2 Parasites

Large number fresh water and marine fish species are potential sources of parasites which are medically important. Consumption of inadequately cooked or raw fish which are contaminated with these highly pathogenic zoonoses are the main cause of human infection. The prevalence of these infections is high in a few countries of the world where eating inadequately cooked or raw fish is a traditional practice. The intermediary host of the parasites is fish and when the parasites are consumed, human become the definitive host. The major human infections are cestodiasis, nematodiasis and trematodiasis.

2.5.2.1 Trematodiasis

Trematodiases are important fish-borne disease reported globally. Trematodiases are rarely lethal but can initiate sickness as well as impediments resulting in death. The consumption of life encysted Trematode metacercariae when existing in the dermis of fresh water fish that were minimally processed, inadequately cooked or raw result in infection. *Opisthorchis* and *Clonorchis* are two major genera that are importance in human health.

2.5.2.2 *Clonorchis metacercariae*

Clonorchis metacercariae migrate to the bile duct from the small intestine and they originate clinical infection. Clonorchiasis was studied broadly, and the contagion was implicated in recurring cholangiocarcinoma, cholangiohepatis pyogenic and cholangitis (WHO, 1995). Chen and Light (1994), reported that metacercariae can remain in muscle of fish for long, persist for days in desiccated fish, and remain in salted or prickled products for few hours however killed by adequate cooking.

2.5.2.3 Opistorchiasis

Opisthorchias is prevalent in societies that eat inadequately cooked or slightly treated or raw fresh water fish. Bithynia, a snail species usually found in the rice fields is commonest intermediate host in the endemic areas. WHO (1995) pointed out that the danger of getting Opistochiasis through ingestion of cultivated fish is adjudged rare.

2.5.2.4 Paragonimiasis

Paragonimia westermani is the most common among 40 species of Paragonimus reported. Mammals including human that forage on crabs are the final hosts. The lung is affected by the parasite and often showed symptoms of tuberculosis. The infection of Paragonimiasis is more in people than Opistorchiasis and Clonorchaiasis combined (WHO, 1995).

2.5.2.5 Intestinal trematodiasis

Echinomiases and Heterophyiases are intestinal infection initiated by parasites (intestinal trematode) of families Echinostomatidae as well as Heterophyidae. Other categories including Metagonimus and Heterophyses have been reported to cause disease. The clinical symptoms including anorexia, diarrhea, abdominal pain and lethargy. Eating of inadequately cooked or raw brackish water or fresh water fish has been attributable to human infections.

2.5.2.6 Nematodiases

In human, fish-borne nematodiases is incidental infections. The natural definitive hosts of nematodes include birds, pigs and marine mammals. Intermediate hosts can be oceanic, fresh water or brackish-water fish. Ingestion of fish containing infective larvae is the cause of the infection.

2.5.2.7 Capillariasis

Capillaria phillipnensis caused Capillariasis in the definitive hosts which led to gastroenteritis. Fish eating birds are also final hosts and they spread waste polluted with parasite eggs along their migratory route in freshwater fish ponds. Capillariasis is usually a minor infection of intestine and untreated infections can be dangerous.

2.5.2.8 Gnathostomiasis

Gnathostoma spinigerum caused human gnathostomiasis. Frogs and fish harbor larvae of the parasite. Comsumption of partially processed or raw inadequately cooked fish transmit this infection to human. The occurrence of wandering bumps in diverse parts of the body and eosinophilia are characteristics of the disease. Excision of the worms surgically, is the only effective treatment of the disease.

2.5.2.9 Anisakiasis

The larval of escarididoid nematodes caused anisakiasis in marine mammals which are normal definitive hosts. *Anisakis simplex* is the species causing disease in humans (Kim, 1990). The parasite is killed by freezing (-20^0C for 24 hours) and normal cooking. Fishery products consumed raw is potential source of infection. However, freezing and cooking may not guard against sensitive responses due to ingestion of *A. simplex* (Audicana, 1997).

2.5.2.10 Cestodiases

Cestode infections associated with the ingestion of fish are uncommon in human. The cestodes which established in the human gastro intestinal tract are mild pathogenic and the illnesses not deadly. Various species of freshwater, amadromous and marine fish transmitt major human cestodeans in human that caused Diphyllobothriasis.

2.5.2.11 Diphyllobothriasis

Diphllobothrium latum found mainly in cold water caused Diphyllobothriasis when fish infected with the tapeworm is consumed. Mammals that eat fish and human are the final hosts and intermediary hosts are fish as well as fresh water copepods.

2.5.3 Viruses

Finfish and crustacean are not connected in the feast of viral food borne infection however consumption of raw mollusca bivalves has been reported to be a main source of viral infection associated with water harvest. Virus producing diseases in fish are not infective in humans.

2.5.4 Other biological hazards (toxin)

Aquatic organisms have been pointed out to produce large amount of toxic mixtures and these pose significant human health risks (Pulin *et al*., 1993). Aquatic micro-organisms such as detritus bacteria and microscopic algae which are food for larvae of commercially important finfish and crustaceans produce toxic compounds. Paralytic shellfish poisoning in crabs and lobsters has been described recently (Price *et al*., 1997). Challenges of these bacteria and parasite infections necessitate the usage of antimicrobial and additional healing agents.

2.6 Antibiotics

Antibiotics are well-defined as matters which is capable to hinder the growing or kill microbes. They can also be defined as any matter that are of natural, man-made or semi synthetic derivation; that at *in vivo* concentrations hinders the growing or kills microbes through networking through exact goal (Cerf *et al*., 2010). Antibiotics have been played a role in the battle between man and microbes for a long time and these agents have been present in the environment. Morbidity and mortality have been greatly reduced through innovation of antibiotics aimed at the management of communicable infections. Antibiotics contributed significantly to improvements in the health of the general populace. Fleming discovered Antibiotics in 1928 and since then they have become indispensable drugs for animal health and human well-being. Antibiotics can be of synthetic origins or derived from natural sources. Antibiotics have been used in human, food animals as well as aquaculture. The usage of antibiotics could be considered as metaphylactic, prophylactic or therapeutic. The usage of antibiotics in any entities or groups to preclude the growth of infections is prophylactic, the treatment of established infections is therapeutic while metaphylaxis is a procedure of crowd-medicine that target to treat both sick and other exposed faunas.

2.6.1 Mode of operations of antibiotics.

Chemical structures of antimicrobial drugs are different types. Actions are on diverse portions of bacterial mechanism. Antimicrobial act based on one of these two merchineries.

i. Bactericidal consequence: in this group antimicrobial destroys the microbes by distrupting any of the foundation of bacterium‘s cell contents or its wall.

Fluoroquinolones, Metronidazole and Penicillin are examples of antibiotics in this group.

ii. Bacteriostatic effect: DNA replication, production of bacterial protein and other cellular metabolisms of the bacteria are stopped when the antibiotics interfere with host. Chloramphenicol, tetracyclines, sulfonamides and macrolides are the examples.

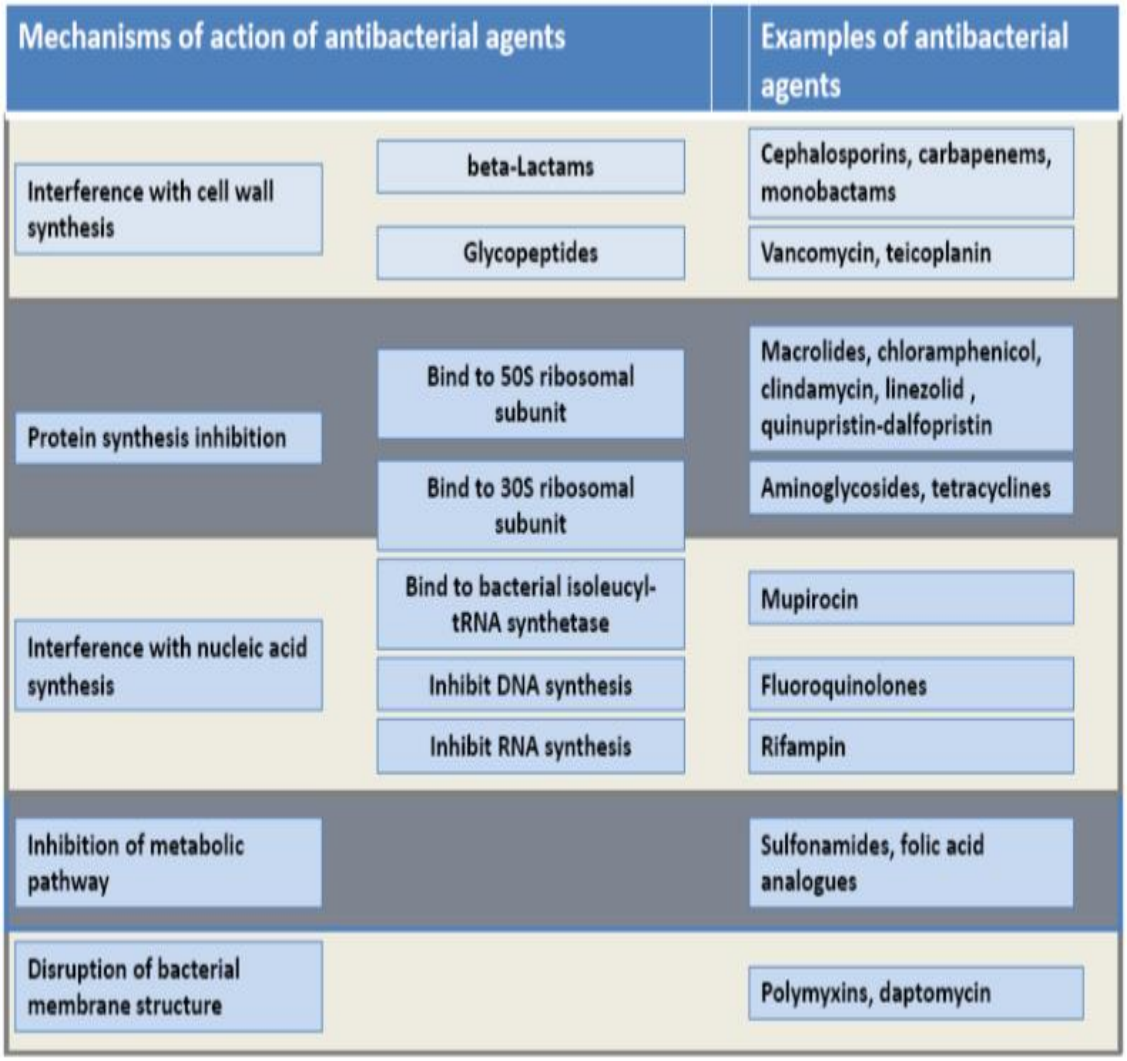


Figure 2.2. The various mechanisms of action of antibiotics

2.6.2 Antibiotics in aquaculture

Antimicrobials are used to control bacterial, fungal and viral infection outbreaks in aqua-farming system. They are used to correct water quality problems, disinfect equipment and eggs, used as growth promoters as well as controlling aquatic weeds and free-living molluscs. Successful usage of antibiotics hinge on accurate opinion, appropriate dose level and correct method of management (Millanao *et al*., 2011). Antibiotics are used in aqua-farming to preclude and treat microbial infections just as it was used in other animal production sectors (FAO, 2002b). Intensive animal husbandry systems involved animals being kept in nearby vicinity within the same zone of air or water and antibiotics are commonly used for prophylaxis. The intensity in aqua-farming led to break out of microbial infections which now requires the intensive use of antibiotics. Antibiotics are used in aqua-farming as feed additives, as a prophylactic or therapeutic measures and uses of animal and human wastes in ponds in integrated fish farming system also increase quantity of antibiotics that gain access into the pond environment. In developed world, there are guidelines on usage of antimicrobials in aqua-farming industries. The government agencies responsible for veterinary medicine must approve drugs before it can legally be used, for instance, FDA in the United State of America

2.6.2.1 Means of administration of antimicrobial in aquaculture

2.6.2.1.1 Medicated feed

Medication of feeds is a successful way of treating many bacterial diseases in ornamental or food fish. Medication of feeds has also been used recently to treat some parasites such as sea lice. Medicated feeds are either prepared as floating or sinking pellets in commercial quantity though such feedstuffs have a restricted shelf life. One of the challenges of medicated feed is reduction of palatability of such feed as a result of incorporated drug. Antibiotics are incorporated into feed via a powdered premix using binder such as fish or vegetable oil or gelatin (up to 5%). The medicated feed is fed for particular time as recommended by veterinary practitioner to cure a certain disease or prevent certain infection. Withdrawal period recommended by veterinarian must be followed before the fish is cropped for food.

2.6.2.1.2 Injection

Injection is one of the effective methods of administration of antibiotics in aquaculture industry. However, this method is more useful in valued decorative fish for instance koi carp or stock used for brooding instead of being employed in big scale fish production. The intramuscular and intraperitoneal cavity are normal routes for injection. The quantity of antibiotic to be used is usually premised on the individual weight of the fish, dosage recommended for the antibiotic as well as the concentration of the antibiotic.

It is expressed as:

$$\text{Volume required of the antibiotics} = \frac{\text{dosage recommended (mg/kg) X fish weight (kg)}}{\text{Concentration of the antibiotic (mg/ml)}}$$

2.6.2.1.3 Topical

Topical treatments can be useful in brood stock or ornamental fishes. Iodine-based solution like antiseptic microbicide can be used to treat ulcers or open sores and if necessary followed by topical antibiotic. Improvement of water quality and the elimination of parasites can allow ulcers to heal themselves.

2.6.2.1.4 Baths and dips

The antibiotics used in baths and dips for systemic infections are poorly absorped internally. Baths and dips are useful in treatments of surface infections like superficial fungal infections, bacterial gill disease, fin rot and external parasite infestations. Baths and dips management for fish require distinct tank with the flow stopped and extra ventilation must be provided. The capacity of water in the pond, tank is needed to be calculated (Millanao *et al*., 2011).

2.6.2.2 Antibiotics resistance in aquaculture

The intensification of aqua-farming (fish and shrimp farming) has resulted in broken out of infections most especially bacterial diseases which required the use of antimicrobials. Infectious diseases that are not treated may lead to disturbance of animal welfare and eventually significant stock losses. There is limitation in the usage of vaccines to preclude

bacterial infections in fish (Kumar *et al*., 2006), hence increased usage of antimicrobials for prophylactic and therapeutic measures in fish farming (Millanao *et al*., 2011). Millanao *et al*. (2011) noted the adverse consequences of using antibiotics in aqua-farming and Fernández-Alarcón (2010) has studied *in situ* the antimicrobial resistance in the aquaculture industries. Fernández-Alarcón (2010) reported that the biochemical mechanism by which bacteria display resistance include the production of enzymes that destroy or modify the antibiotic, possession of a permeability barrier that prevent access to the bacterial cell or alteration of the target site that is normally attacked by the antibiotics. Genetic mechanisms by which bacteria acquire resistance include chromosomal mutation and the acquisition of extra chromosomal elements (resistance plasmids).

2.7 Prebiotic

Prebiotics can be well described as s-chain CHO that moderate the configuration and metabolic rate of the microbes of the digestive gut in a useful way (Mahious *et al*., 2006). Mahious *et al*. (2006) also pointed it out as a diet component which are not digestible and in a beneficial way impact the host by carefully modulating the activity and growth of amount of microbes in the digestive gut of the host and therefore improves host well-being. They are fibers that are not digestible by enzymes, salts and acids produced in the intestine of animals for digestion process. They act as a substrate and stimulate growth of bacteria which are beneficial in the intestine of the host animal and thereby protecting the intestinal mucosa of the host animal by decreasing the amount of microbes (Song *et al*., 2014). This aids the host by improving nutrients digestion, growth, immunity and resistance to disease (Burr *et al.*, 2005). Prebiotics were originally selected to arouse lactobacilli and bifidobacteria in human microbiota (Gatesoupe, 2005). It is not bacterium, not like probiotic and its effect in ordinary environs is less. Nowadays, several food which are mainly carbohydrates are used as prebiotics. Gibson *et al.* (2004) pointed out that a prebiotic is a food component which fulfill the following criteria: that cannot be hydrolysed by digestive enzymes, able to resist gastric acidity, unabsorbed by gastro-intestine, able to be fermented by abdominal organisms and can carefully motivate the growth and the action of microbiota associated with well-being. Any food that touches the colon for example some peptides and proteins, carbohydrates and some lipids that are not digestible could be candidate for prebiotics (Mahious and Ollevier, 2005). Some authentics prebiotics are non-digestible

carbohydrates such as TOS, IMO, soyabean oligosaccharide, XOS, lactulose, lactosucrose and gluco-oligosaccharides. Prebiotics are selectively fermented by Bacteroides, Bifidobacteria, and Lactobacillus.

2.7.1 Prebiotic in aquaculture

The idea of using prebiotics as alternative to antibiotics in aquaculture has been gaining ground since usage of antibiotics for growth promotion, prevention and control of diseases were criticized for the growth of antibiotic-resistant bacteria, occurrence of antibiotic residue in aquaculture production, obliteration of bacterial population in the aquaculture environ as well as destruction of the water animal's immunity (Sapkota *et al*., 2008). Nicholas *et al*. (2007) pointed out that it is more applicable to influence the digestive tract organisms in water faunas by using prebiotics which modify the situations to favour certain bacterial species that might augment fish growth effectiveness as well as decreasing infection vulnerability of the host animal.

2.8 Immunostimulants most commonly used in aquaculture

2.8.1 Glucans

Among the most popular immunostimulants used in aqua-farming are glucans. They are sourced from wall of yeast cell and some complex floras. Glucans gave orally or inoculated to the fish possess exceptional immunostimulatory power. The non-specific immune responses of carp were effectively stimulated by ßeta-1, 6, branched Beta-1, 3 Glucans (Jeney and Anderson, 1993). Jeney and Anderson (1993) pointed out that the usage of Glucans augment action in innate defense mechanism as well as in defense against *Yesinia ruckeri*. Atlantic salmon (*Salmo salar*) treated with glucan protected against *Vibrio salmonicidia.* The highest antibody response was measured against *E. tarda* when healthy fish was nourished with ß-1, 3 glucan. The aflatoxin-treated fish fed with immunostimulant also showed significantly increased antibody titre. FCR and SGR were improved when 500 mg and 250mg Beta-glucan per kg diet were included in fish diet (Misra *et al*., 2006). Administration of Beta-glucan at rate of 500µg glucan per fish as an immune booster in carp against the contagion of *A. hydrophila* increased the survivability to 100 percent when likened to the uninfected fish (Selvaraj *et al*., 2005)

2.8.2 Levamisole

Levamisole is an artificial phenylimidazolthiazole and it has been used as anti-helminthic means in both veterinary and human medicine (Siwicki *et al*., 1990). Levamisole has been attested to advance the reaction of mammalian macrophages and T- lymphocytes of both strong and weakened individuals (Ogunbiyi *et al*., 1988). It is used in fish to augment the innate immunity or as booster with a vaccine (Siwicki *et al*., 1990). The potential of levamisole as an immunostimulant in fish has been proved however doses and time of administration need to be considered because outcome of levamisole depend on dose and time. Lower dosages of levamisole might not be effective and high dose has been reported to suppress the immune response (Jeney and Anderson, 1993). Recently, the prospective of levamisole as immunostimulant has been demonstrated in the common carp against the encounter of *A. hydrophila* by ways of improved lysozyme and neutrophil actions (Maqsood *et al*., 2009).

2.8.3 Chitin

Chitin is a polysaccharide in nature, not soluble, linear ß-1, 4-linked polymer of N-acetyl-D-glucosamine, shared component of crustacean and insect external skeleton and cell wall of fungi. The crab and shrimp shells are used for manufacturing of Chitin commercially. Chitin are widely used in agriculture, chemistry and medicine because of its material, chemical and mechanical properties. Aside numerous uses in agriculture and medicine, chitin plays chief part in aqua-farmine. Injection of chitin into brood trout has been observed to protect against *Aeromonas salmonicida.* Esteban *et al*. (2000) equally reported that chitin incorporated into fish diet incite the immune response in a very little time.

2.8.4 Chitosan

Chitosan is manufactured by the basic deacetylation of chitin sourced from crab casing. Esteban *et al*. (2000) reported that level of protection for brook trout (*Salvelinus fontinalis*) was high for 1-3 days after administration of chitosan by immersion and injection but by day 14, the level of protection greatly reduced. More so, Esteban *et al*. (2000) pointed out that simple immersion of brook trout into Chitosan was not as effective as injection of chitosan. The protection of carps and salmonids against bacterial diseases by Chitosan used as an immune booster in aquaculture has been established (Siwicki *et al*., 1994).

2.9 Wound

Wound is defined as a physical injury which may involve piercing, torning and cutting; blowing, burning or puncturing of the skin or any other external surface which result in the disruption of regular continuity of the structure (Shafiuddin, 2009). It is also described as a disturbance of normal functional configuration and more significantly utility (Shafiuddin, 2009). Assault on the tissue by chemical, physical, thermal, microbial and or immunological may produce wound (Raina *et al.*, 2008). Wounds are characterized as the disturbance of cell and anatomic progression of a tissue. The cell and anatomic progression of the tissue will be disturbed usually led to incapability. The continuity of epithelium is disrupted and underlying connective tissue may or may not be lost. The occurrence of wounds cannot be avoided in normal life.

2.9.1 Classification of wound

Wound is broadly classified into two groups: opened wound (e.g skin is torn, expurgated or perforated) and closed wound (e.g blunted forces trauma originated a contusion) (Ganeshan, 2012). Wound also classified as acute or chronic based on injury curative physiology and it can also be classified based on its etiology, location, or duration (Rashmi *et al*., 2016).

- **Based on open and closed wound**

  When hemorrhage is obviously noticeable as a result of blood seepages from the body, it is open wound. Accoring Ganeshan (2012) open wound can be further grouped as abrasions or superficial wounds, cuts, incision, laceration, penetration injury, avulsions and gunshot injuries.When the blood seepages the vascular system but remains in the body, it is said to be close wound. Bruises, hematomas or blood tumor, crush injury are examples of this type of wound

- **Based on wound healing physiology**

  Wound is classified as acute and chronic based on healing physiology. A flesh damage that normally advance through a logical and well-timed reparative course to restore functional and anatomical integrity of the tissue is said to be acute. Example of this is cuts or surgical incisions and within expected time frame wound healing is complete. Menke *et al*. (2007) described wounds that enter the condition of pathologic swelling as result of failure to advance through the regular phases of healing as chronic wounds. Wound in this case either re-occur frequently or require

a prolonged time to heal. Diseases such as diabetes mellitus, medications, case of malnutrition and immune-depression are implicated as causes of chronic wounds. Other factors include trauma, hypoxia, local infection and presence of foreign bodies (Menke *et al*., 2007).

2.9.2 Causes of wound in African catfish.

Conspecific biting which mostly occur in catfish especially when crowded together during feeding, sorting and transportation cause tissue damage. African Catfish behaves aggressively. They have pectoral spines that are shrill and firm anterior to the soft-rayed part of dorsal as well as pectoral appendages that can be used to inflict wounds on one another especially at high stocking densities. Cook and Zumla (2009) reported that different species of fish can inflict dangerous and painful stings by using dorsal or caudal spines which have complex venom glands. The pectoral fins in Catfish sometimes used as defense mechanism against predators (Haddad and Martins, 2006). Catfish can impose excruciating injuries by means of their pectoral and dorsal bites and this has been established for several years. Arciuli *et al*. (2012) reported poor welfare of the fish as a result of inflicted wound on the body part and also down grading of the fillets as a result of poor visual appeal to the consumer. The incidence of bacterial or parasitic infection as a result of inflicted wound which have a detrimental effect on flesh quality of the fish had been reported (Ingerslev *et al*., 2012).

2.9.3 Mechanism of wound healing

Regeneration of dermal epidermal tissue is a natural process that is involved in healing or repair of wound. Skin is composed mainly of epithelial and connective tissues, forming tough pliable covering over vertebrate body surface. The major organ in the mammalian body is the skin and it has been described as the most extensively distributed tissue. Skin serves as physical barrier for entry of microbes into the body and also serve as excretory organ. The two parts of the skin are epidermis (outer layer) and dermis (inner layer) and these parts always at state of equilibrium. The constant pressure which epidermis is always exposed to as a result of being in contact with external environment make it prone to injury. According to Nagori and Solanki (2011) a chain of intricate biochemical proceedings take occur in a carefully coordinated chute resulting in contraction and closure of the wound in

the injured skin to restore the function. Rosenberg and De la Torre (2006), reported that the healing process of the wound begins immediately once the skin is broken and this process can last for months. This normal physiological process has different phases which include the following that are overlapping phases in orderly healed wounds.

a) Haemostasis phase
b) Inflammation phase
c) Proliferation phase
d) Remodelling phase

a) Hemostasis phase: Certain bio-chemical events which are complex set in motion immediately the injury is on the skin. It occurs in a carefully coordinated cascade to restore the impairment. The two major processes that take place are coagulation and fibrin clot development. Clotting disorders can interfere with this phase. After the assault on the skin the blood vessels contract immediately until the spasm relaxes. Damaged blood vessels become sealed after the platelets sealed. The damaged blood vessels is sealed when the platelets released a vasoconstrictive substance to form a stable clot. Nagori and Solanki (2011) explained that the platelets seeping from injured tissues collected and adhered to the exposed tissue due to the effect of ADP (Adenosine-di-Phosphate). The fibrin is formed from fibrinogen when the substance secreted by platelets intermingle and arouse intrinsic coagulation cascade through the generation of thrombin. A stable hemostatic plug was then formed when fibrin mesh strengthens the platelets aggregates.

b) Inflammatory phase: This stage overlapped with the first stage and it instantly begins after the assault. The stage may last for two days or in prolonged cases last for two weeks (Schultz, 1999). Erythema, warmth and swelling associated with agony are characteristics of the phase. Rosenberg and De la Torre (2006), reported that factors platelet derived growth factor (PDGF) and tranforming growth factor beta (TGFβ) are free at this stage and this lead to the movement as well as cells division of proliferative phase. Microscopic organisms and debris are phagocytized at this stage as well. The phase may last for four days.

c) Proliferative phase- Proliferative stage is a stage characterized by angiogenesis, tissue formation, and deposition of collagen, epithelialization and wound closure. This phase usually overlap with second phase, lasting up to 2 days to 3 weeks. Proliferative phase come

into existence as the site of the wound is debrided. Nagori and Solanki (2011) reported that deposition of new extracellular matrix occur and dermal regenerative process take place as proliferation of fibroblasts (the cells that secret the collagen framework) occurs. The final remodeling phase involved novel collagen matrix develops cross linked and organized. Pericytes regenerate the outer layer of capillaries, the lining will be produced by the endothelial cells and keratinocytes distinguish to form the defensive external stratum.

d) Remodelling phase- New collagen is molded in this stage and it can last for 3 weeks to 2 years. Tissue tensile strength of the wound is increased as outcome of inter-molecular cross-linking of collagen that formed via vitamin C-dependent hydroxylation (Rosenberg and De la Torre, 2006). Mark tissues eventually turn out to be 80% as tough as the initial tissue and finally flattened.

2.9.4 Factors affecting wound healing

A typical biologic process in the vertebrate body is wound healing. Kerstein (2007) pointed out that wound restorative can be unfavorably influenced by several aetiological components. Understanding the factors that influence the wound healing as observed by Kerstein (2007) is key for faster and better restorative of wound. Factors that affect rate and quality of wound healing include the following:

i) **Improper diet**. Proper diet which serves as energy and nutritive substrates is required for wound healing. Amount equal or greater to 3.5 gram per decilitre of serum albumin is required for perfect restorative of wound. Kumar *et al.* (2006) reported that decreased rate of collagen synthesis at wound site leading to reduced tensile power of the wound occur as a consequence of inadequate amount of protein consumption. This also promoted chance of contamination at the site of the wound.

ii) **Contamination at the injury site**. Wound infection leads to impairment of wound restorative. *S. aureus, P.aeruginosa*, and *S. pyogenes* are among some essential organisms causing wound contamination (Kumar *et al.*, 2006)

iii) **Drugs.** Several preparations are recognized to distrupt wound restorative. Franz-*et al*. (2007) reported that chemotherapeutic medications used in treatment of tumor are the main collection of drugs that were acknowledged to interrupt

wound restorative. Normal healing process is distrupted as a result of reduction in proliferation of fibroblast and collagen synthesis when systemic glucocorticoids is administered.

iv) **Lack of enough oxygen supply to the tissue in the wound area**. Sufficient blood into the wound is required for proper wound restorative. Extreme pain, anxiety or cold can result in poor blood supply locally and prolong restorative time (Cuzzell and Stotts, 1990). Reducing tissue supply of oxygen at the site of the wounds can be decreased by smoking and use of tobacco (Kumar *et al.*, 2006).

v) **Selinity**. Old age is implicated in delaying of wound healing. Collagen creation and wound shrinkage is slowing down in elderly people due to diminishing growth and activity of the fibroblast (Kumar *et al.,* 2006).

vi) **Disease conditions**. Wound infection are more rampant in diabetic patients. Investigation carried out by Greenhalgh in 2003 revealed that diabetic patients have wound contamination level of 11% more than patients that are not diabetic generally. Severe and prolonged liver disorders were similarly implicated in delay injury restorative. Patients with weakened immunity are more vulnerable to wound contamination.

2.9.5 Effect of pharmacological activities in wound restorative:

i) **Anti-inflammatory action**. Inflammatory (acute) reaction produces elements that are critical for tissue growth and restorative during the early stages of injury (Thomson, 2000**)**. However, Pierce (2001) **r**eported that wound closure can be delayed due to the prolonged inflammation which could be detrimental, as it precluding wound transformation and matrix production, and this result in an increased pain. Akihisa *et al*. (1996) stated that some extracts obtained from plants and animals having anti-inflammatory outcome also retain wound healing action.

ii) **Activity of anti-oxidant.** The creation of unrestricted radicals destroy collagen, proteins, lipids, proteoglycan and hyaluronic acid on or nearby the injury surrounding and can lead to interruption in wound restorative. Viable tissue may be preserved and wound healing could be facilitated by the extract that

posseses a significant anti-oxidant activity (Yeoh, 2000). Dissemond *et al*. (2002) reported that important strategy in healing of chronic wounds was elimination of reactive oxygen species (ROS) and Halliwell (1988) pointed out that antioxidants accelerate the procedure of wound restorative by terminating the unrestricted radicals.

iii) **Activity of anti-microbial.** Wound restorative can be hindered by presence of large numbers of microorganisms (Rijswik, 2000). Reduction of the bacterial load on the place of injury could facilitate injury restorative. Rijswik (2000) concluded that proper means of preclusion and managing of wound contamination ought to be able to destroy the pathogens and likewise be able to stimulate immune activity.

iv) **Analgesic activity**. Pain must not be increased when applying the dressing to open wound, and if possible, it should lessen the pain since exposed wounds can create pain and subsequent ill health (Gupta and Jain, 2010).

2.9.6 Role of phyto-constituents in wound healing

Many plants possess secondary bioactive compounds that are useful for treatment of ailments have been detected (Oz, 2010). The management and treatment of wound can be effected by plants extracts. Omale and Isaac (2010) had reported some medicinal plants such as walnut leaf and onion bulb that have antimicrobial effects to combat infection and have prospective for wound restorative in animals. Bello *et al.* (2013) reported addition of nutritional walnut greenery and residues of the bulb of onion as supplements at different addition quantity that enhance positive special effects on skin wound restorative of *Clarias gariepinus*. The phytomedicine for wound healing are safe and hypersensitive reactions are rarely encountered and more so they are cheap and affordable. Some medicinal plants such as garlic (Deresse, 2010), water leaf (Liang *et al*., 2011) and Siam weed (Anyasor *et al*., 2011) were described to possess remarkable antioxidant activity hence could be useful to hasten wound healing processes. Some chemical entities such as Tannins, Flavonoids, Saponins, Sterols and Polyphenols and Tri-terpenoids derivative of floras suppose to be acknowledged and expressed for handling and treatment of injuries.

i) **Tannins**. Tannins stimulate wound restorative due to the mordant and antimicrobial activities they possess and also act as unrestricted radical foragers (Soni and Singhai, 2012).

ii) **Flavonoids**. Flavonoids improving vascularity and decrease fat peroxidation via precluding or reducing the commencement of death of the cell. It possesses anti-microbial and astringent activities that appears to be accountable for injury shrinkage and improve level of epithelialization thereby contribute to promotion of the wound restorative procedure (Soni and Singhai, 2012)

iii) **Saponins.** Injury contraction as well as elevated rate of epithelialization is promoted by Saponins (Soni and Singhai, 2012). Anti-oxidant and antimicrobial action of saponin could be accountable for this.

iv) **Sterols and polyphenols**. Sterols and Polyphenols possess anti-oxidant activity and ability to scavenging for free radical which result in reduction of lipid peroxidation, cell necrosis and improving vascularity responsible for their wound healing (Soni and Singhai, 2012).

v) **Tri-terpenoids**. Increased rate of epithelialization as well as wound shrinkage could be owing to anti-microbial as well as mordant property possessed by Tri--terpenoid and this responsible for promotion of wound healing (Soni and Singhai, 2012).

2.10 Medicinal plant therapy

Herbal plants have been used for management of illnesses traditionally for many years all over the world and till today modern drugs are still being developed from plants (Ates and Erzdogrul, 2003). The interest in medicinal plants has increased significantly in recent years not only to cure humans but also to cure animals. Medicinal plants are major components of the natural medicine and alternate to orthodox treatments worldwide. Herbal medicine is recognized as alternative medicine and almost 60% of the world inhabitants, both in the emerging nations and in the advanced nations used it (Ogbonnia *et al*., 2008). The WHO encouraged uses of herbs particularly in countries where access to the orthodox management is not satisfactory (WHO, 1980). Globally, plant extracts are used for their antimicrobial activities (Aibinu, 2006). Ogbonnia *et al*. (2010) stated that the origin of some

of the western manufactured drugs is medicinal plants. In the Asian countries, phytobiotics, in the form of plant biomedicine have been used for long (Ji *et al*., 2009). Great contribution has been made to the health care by Nigeria flora (Soladoye *et al.,* 2012). Indigenous medicinal plants in Nigeria equally contribute to the natural wealth.

There is increase interest in screening of plants to find new pharmaceuticals globally. New drugs with little or no side effect are now being manufactured by analyzing and evaluating plants that were once considered of no value. The screening for various herb materials to find naturally occurring antioxidants to be used in medicinal preparations or food to replace potentially harmful synthetic additives has been embraced globally in the last few decades (Ritcher *et al*., 2003). Many herbal preparations are now being scrutinized scientifically for their therapeutic actions and this is gaining prominence because of side effects associated with western orthodox medicine (Gupta *et al.,* 2010).

2.10.1 Medicinal plants used in aquaculture

The natural compounds derived from plants termed' Phytobiotics' when embedded into animal diets enhanced animal productivity. There is a growing awareness among researchers and feedstuff businesses for the integration of basils in fish foods as immune booster (Alisahi *et al.,* 2010). The plant bioactive compounds have different possessions such as: analgesic, antioxidant, and appetite enhancement, digestive enzyme activity hepatoprotective, insecticidal, stimulant of secretion of bile, laxatives and antidiarrhea (Alisahi *et al.,* 2010). Several research works have demonstrated that phyto- additives protected fishes from diseases and enhanced their growth (Johnson and Banerji, 2007). The comparative effect of basils was described in several fishes, comprising *Clarias gariepinus* (Turan and Akyurt, 2005) and Japanese flounder (Ji *et al*., 2007).

2.10.1.1 *Allium sativum*

Allium sativum L. (Garlic) is of Alliaceae family. *A. sativum* is popularly known as a therapeutic plant and a valued seasoning. It is used for various physiological disorders and different ailments. In the Celtic word, garlic means strong. *Allium sativum* has been considered as one of mankind plants cultivated for over 5000 years in the Middle East, though believed to have been sourced from Asia (Central part). Presently, it is grown around the world and used for medicinal purposes for hundreds of years (Singh *et al.,* 2008). Garlic

is being used in Chinese medication not less than 3,000 years ago and about 5000 years ago in Sanskrit.

2.10.1.1.1 Biology of *Allium sativum*

The common names of garlic worldwide are: alii sativi bulbs, ail, ajo, ayu (Yoruba), ayo-ishi (Igbo), da suan (Chin), garlic, taisan (Jap), inniku (Jap), tafanuwa (Hausa), taesan (Kor), (Sanskrit), lasan (Hindi), lobha (Nepalese) **(**http://www.mcp.edu/herbal/ default. htm**).** Garlic is a perpetual basil, bulbous and closely associated to the onion. The rhizome is separated into several plump segments called cloves. The erect flowering stem is tall with height of about 2-3 feet. A number of active compounds are present in garlic bulbs especially sulphur containing mixtures that are accountable for its pharmacological actions. *Allium sativum* rhizome distilled by steam yields vital oil comprising allyl methyl, diallyl and dimethyl mono to hexa sulfide (Singh *et al.,* 2008)**.**

2.10.1.1.2 Distribution of *Allium sativum*

Garlic has been cultivated around the world, from Siberia to Mediterranean climates. *Allium Sativum* is assumed to be originated from central part of Asia. Garlic had been planted in the Middle East for more than 5000 years. It is now being planted in most countries of the world. In the US, garlic is produced mostly in California.

2.10.1.1.3 Phytochemicals of *Allium sativum*

The chemical examination of methanolic extract of garlic shown higher quantities of flavonoids and phenolics and alkaloids, glycosides and tannin present in little quantities (Odutuga *et al*., 2014).

2.10.1.1.4 Medicinal Uses of *Allium sativum*

Garlic is a unique ancient plants employed in treatment, ranks the highest of all the herbal remedies consumed for its health benefits. *Allium sativum* has been used for ages in many nations to fight bacterial, fungal, parasitic and viral contaminations (Citarasu *et al*., 1998). Among the earliest documented plants use for management of infection and preservation of health is garlic (Rivlin, 2001). The antibacterial effect of garlic juices to both G -ve and G +ve bacteria was originally described by Louis Pasteur (Rivlin, 2001). *Allium sativum* was found to be valuable in the management of elevated blood pressure (Krishnaraju *et al*.,

2006), rheumatic disorder, asthma, cold, chronic fever, diabetes, paralysis and also documented to be stimulant (Nwokocha *et al*., 2011). It was also documented to be potent in the prevention of cancer (Wattenberg, 1990) as well as having hypocholesterolemic effect (Silagy and Neil, 1994)

2.10.1.1.5 Nutritional Uses of *Allium sativum*

Allium sativum is documented to be a great vegetable because it is used as cooking ingredient and it can also be eaten delightfully. The part of the garlic most often used for medicinal uses and cooking is the bulb. Garlic is most often used as raw after being sliced, minced, chopped, juiced and it can also be eaten raw. When it is cooked, it enhances flavor and also add to nutritional benefit. It is being used as spice or seasoning in many nations today. Garlic is used in many cultures primarily as an herb to enhance many food dishes.

2.10.1.2 *Chromolaena odorata*

C. odorata (L.) K and R 1987, acknowledged as Siam weed in English is a species in Asteraceae Family (McFadyen *et al.,* 2003). The intrusive nature of *C. odorata* made it a grave weed in western and central Africa, Southeast Asia, Pacific Islands, India and Australia (Mgobozi *et al*., 2008). It has been originally introduced as decorative herb in the middle nineteenth century in north eastern India and from there spread to Asia and Oceania (Muniappan *et al.,* 2005), and into Central and West Africa (Prasad *et al*., 1996).

2.10.1.2.1 Biology of *Chromolaena odorata*

C. odorata is a brittle stems plant which branch readily and with a low, leathery root structure (Henderson, 2001). The plants corollas of the florets vary in colour from pale blue to white or mauve and achenes are dark with a pastel pappus (McFadyen, 1989). *Chromolaena odorata* develops to 2–3m in tallness and it could reach 5–10m tall when reinforced by other vegetation in open-land field. A marked morphological variability is observed in *C. odorata* in terms of leaf shape, flower color, plant architecture, hairiness and smell of the crushed leaves when observed within its native range.

2.10.1.2.2 Distribution of *Chromolaena odorata*

Chromolaena odorata is widely dispersed in several nations in the damp subtropics and tropical part of the ancient World. Intrusive ways of *C. odorata* in these countries of the

ancient World have been described by Gautier (1992) and McFadyen (1989). *C. odorata* was reported to have the first record of naturalization in the Ganges floodplain and Dacca (present-day Bangladesh and India) in the 1870s. In the early 1840s, *C. odorata* was said to be make known to Asia as a decorative plant as it was seen in Calcutta, India. Gautier (1992) reported that presently *C. odorata* is widespread in Thailand but it was reported to be widespread in Bengal, Assam, (Bangladesh and India), Myanmar (Burma) in the early twentieth century (Rao, 1920). The Verbenaceae imported from Sri Lanka in 1937 to Nigeria probably accidentally introduced *C. odorata* (Ivens, 1974) meaning that *Chromolaena odorata* appeared in Asia earlier than Western part of Africa. The biotypes that were grown in West and South Africa and Asia though invasive, vary from one another in biology, structure and ecology and difference within each form is little (Von Senger *et al*., 2002). Consequently, they are meaningfully separate individuals, and they were branded as sub-species (Zachariades *et al*., 2004)

2.10.1.2.3 Phytochemicals of *Chromolaena odorata*

The qualitative phytochemical analysis of *Chromolaena odorata* extracted with methanol and petroleum ether showing the following biological active compounds: alkaloids, diterpenes, flavonoids, saponins, steroids, tannins as well as triterpenes. From the chloroform extract: steroids, alkaloids, flavonoids, saponins, tannins, and glycosides were identified (Anyasor *et al*., 2011)

2.10.1.2.4 Medicinal Uses of *Chromolaena odorata*

Chromolaena odorata has been reported to be a healing herb in tropical Africa for different ailments comprising of fever, toothache and dysentery (Olajide *et al.,* 2000); fever, skin diseases, diarrhoea, diabetes and wound dressing (Zachariades *et al*.,2009). The phytochemical components of plants which include flavonoid, tannins alkaloids and other phenolic compounds have been claimed to responsible for their medicinal values and these exert a certain biological action on the a human body (Akinmoladun and Akinloye, 2007). Nabavi *et al*. (2008) claimed that technical reports had revealed that greeneries of floras are main bases of antimicrobials, antioxidants and other phytobiotics with therapeutic standards. Cough has been treated with a decoction of the *C. odorata* leaf and the leaf with lemon grass and guava leaves as decoction has been used for the management of malaria

(Phan *et al*., 2001). Other traditional therapeutic uses include anti-inflammatory, anti-diarrheal, antispasmodic astringent, antipyretic e.t.c (Vital and Windell, 2009). The extract of new greeneries of *C. odorata* has been traditionally used as herb for treatment of skin infections, soft tissue wounds and burns in some developing countries (Zachariades *et al*., 2009). Nose bleeding and bleeding from fresh cuts had been reported to be stopped by fresh juice from the leaf using as haemostatic agent (Phan *et al*., 2001).

2.10.1.2.5 Nutritional Uses of *Chromolaena odorata*

Usage of Siam weed as leaf meal in garden-fresh form is hindered by unpleasant scent and its expected poisonousness and information on its utilization by livestock as leaf meal is scanty (Vital and Windell, 2009). Nwokolo (1987) compared mineral composition of Siam weed and cassava leaf meals and concluded that Siam weed leaf meal had higher nutritional values than cassava leaf meal. The exact digestive process of proteins and minerals in the foliage meals of both Siam weed as well as cassava was analysed by Nwokolo (1987) and it was discovered that the average to low digestibility of the two leaves was due to presence of the antinutritional factors in both plants. The conversion of *C. odorata* to nitrite either in the feed or within the digestive tract is said to be responsible for poisoning and death obsevered in livestock (Sajise *et al.,* 1974).

2.10.1.3 *Talinum triangulare*

2.10.1.3.1 Biology of *Talinum triangulare*

Water leaf belongs to the family, Portulaceae. It is a perpetual plant which is extensively developed in humid areas as a leafy plant (Ezekwe *et al*., 2013). The leaves which are arranged helixically opposite one another are often packed at the upper part of the stem. Once waterleaf established itself, it grows fast and easily reseeds itself. It is mainly self-pollinating and flowers early and round the year. The colour of the flowers is pink and always open in the morning. The leaf is succulent and greenish in colour. *T. triangulare* is a cosmopolitan wild plant abundant throughout the humid tropics. *T. triangulare* originated from tropical Africa, a non-conventional vegetable crop, which are extensively developed in Asia, West Africa and South America (Schippers, 2009).

2.10.1.3.2 Phytochemicals of *Talinum triangulare*

The qualitative analysis of both wet and dry samples of *Talinum triangulare* revealed occurrence of ancillary metabolites for instance alkaloids, saponins, flavonoids, and tannins (Aja *et al*., 2010). *Talinum triangulare* is rich in crude protein, cardiac-glycosides, essential oils, flavonoids, polyphenols and total lipids (Aja *et al*., 2010). In a research work carried out by Akachuku and Fawusi (1995); 29.4% and 13.4% crude protein content were respectively found in the leaves and tender stems of *Talinum triangulare.* Aja *et al*. (2010) provided an account that alpha-tocopherols, beta-tocopherols, essential oils and total lipids were present in high quantity in *Talinum triangulare*. Ezekwe *et al*. (2002) evaluated *T. triangulare* and reported the presence of substantial amount of vital natural resources for example calcium, magnesium and potassium; pectin (soluble fibres), vitamins for example alpha as well as beta- tocopherols, beta-carotene, C, as well as omega -3-fatty acids all of which are prerequisite for development as well as growth. *T. triangulare* leaf extracts have revealed to have significant antioxidant action (Liang *et al*., 2011) and high kaempferol content (Andarwulan *et al*., 2010).

2.10.1.3.3 Medicinal uses of *Talinum triangulare*

Talinum triangulare contained high amount of flavonoids and alkaloid (Ezekwe *et al*., 2013). Flavonoids was recognized to be decreasing oxidative pressure thereby useful in the controlling of cardiovascular diseases (Aja *et al*., 2010) and occurrence of alkaloid in the foliage adds to the therapeutic importance of *T. triangulare.* Stimulation of central nervous system, topical anaesthetic in opthamology, anti pyretic action, powerful pain relivers among other uses have been attributed to Alkaloids (Oloyede, 2005). Joshua *et al*. (2012) also reported that *T. triangulare* leaf have been connected therapeutically in the treatment of cardiovascular ailments like diabetes mellitus, obesity and stroke. *T. triangulare* was reported to have valuable therapeutic capabilities for purgative, gastro-intestinal diseases and treatment of diarrhoea (Mensor *et al.*, 2001). Waterleaf has been used traditionally to manage polyuria (Khare, 2007), measles (Liang *et al*., 2011), gastrointestinal disorders and hepatic ailments (Mensah *et al*., 2008).

2.10.1.3.4 Nutrition potential of *Talinum triangulare*

Talinum triangulare is a regularly consumed eatable flourishing plant kind in Nigeria and also are eaten throughout the year (Opabode and Adeboye, 2005). The flourishing foliage are usually eaten as boiled (cooked) plant and remarkable antioxidant activity have been reported to be possessed by the leaf extracts of waterleaf (Liang *et al*., 2011). They are soft and watery and not to be cooked for lengthy time. Mbang *et al*. (2008) reported that the greeneries as well as new sprouts are used to congeal pottage and it is eaten in bulky amounts in Nigeria especially southern part. Water leaf were established to be rich in protein (crude, 22.10%), crude fiber and ash at 11.12% and 33.98%) respectively (Ofusor *et al.*, 2008). 'Gbure' as well as 'Afang', soups which are native to the Yorubas and Efiks, correspondingly are preparations from *Talinum triangulare* in Nigeria.

2.11 Haematology and biochemical parameters

2.11.1 Haematology

Haematological factors have been used to evaluate the health of Man and Livestock. Health of an organism can be determined using blood indices as indicators. The functional status of animals exposed to toxicants can be assessed using haematological parameters as diagnosing factors as it reflects pathology of the whole body when evaluated. According to Oshode *et al*. (2008) the well-being of fish is often determined using indices of hematological parameters. Blood characteristics factors could be used to assess fish conditions, suitability of feeds or feed mixtures, toxicity of substances as well as diagnosis of diseases (Akinrotimi *et al., 2007b*). Physiological variations in the fish as a consequence of chemical and physical variations in the environs often quickly revealed as quantifiable because of the intimacy of the organisms with the aqueous environment (Wilson and Taylor, 1993). Increases or decreases in hematological levels can be detected in fish as an outcome of exposure to chemical compounds in the environment. Akinrotimi *et al.* (2007b) reported that timely opinion is likely when evaluating hematological data, mainly blood factors as blood tissues truly reveal chemical and physical changes occurring in organisms. Akinrotimi *et al.* (2007b) concuded that alterations in the basic components of blood sample of the animal while likened with the normal values gives the interpretation of state of health of the animal.

2.11.1.2 Haematology parameters

Important tools for diagnosis and prognosis of fish ailments are life blood parameters. Adebayo *et al*. (2007) stated that the important blood characteristics parameters observed during the course of stress are erythrocytes count, haemoglobin content, hematocrit value and leucocytes count. Changes in blood cells response is an indication of variations in the external and or internal milieu of animals. According to Adebayo *et al*. (2007), numerous cultured fish species were considered in order to establish normal value ranges for blood parameters, and any deviance from these values may show a disorder in the normal physiology of the fish. Some of these investigations were efforts to decide if noteworthy deviations from typical standards of these factors occur that might be attributable to more or less external or internal parameters (Gabriel *et al*., 2001). Inquiries in fish blood to create 'normal' values for haematological parameters have been carried out by Adebayo *et al*. (2007). In higher vertebrates haematological values were affected by various factors as it was concluded by Schalm (1967) and this was corroborated to happen in fishes as it was pointed out by Adebayo *et al*. (2007). According to Svobodova *et al*. (1993) variables such as dietary state, age, sex, disease, season, pollutants, and stress affect blood parameters values. Seasonal variations effect change in blood parameters of many teleost. Ibrahim *et al*. (2003) re-counted that a significantly different was detected in the blood factor levels of 3 species of cyprinid fish measured in warm months to those measured in cold seasons. Ibrahim *et al*. (2003) also reported that the Hb concentration measured was reported to be lowest within summer in tench and also a lower values were equally reported in mirror carp. According to Jawad *et al.* (2004) the seasonal variation did not affect the haematological parameters of both sexes of Tenualosa ilisha. In sexually matured gold fish, males steadily had higher PCV values than the females, and this equally applicable in *Salmo gairdneri* and brook trout according to Clement (2002) and these differences had been proposed to be used as a means of sexing fish. Clement (2002) pointed out that the male Rainbow trout from the wild has lower haematological response than female when exposed to handling stress or being trapped though Etim *et al.* (1999) did'nt detect a little dissimilarity amid masculine and feminine of Chrysicthysm *spp* when exposed to similar conditions. Jawad *et a1*. (2004) reported that more packed cell volume values were observed in males compared with females and these values were independent of body dimension in Tenualosa ilisha

(Indian shad).Akinrotimi *et al*. (2007b) and also Ezeri *et al.* (2004) recounted haematology values of Sarotherodon melanotheron and Clarias, respectively, with the male had lower values of Haemoglobin, Haematocrit, White Blood Cells, Mean Corpuscles Haemogloblin, Mean Corpuscles Volume, Mean Corpuscles Haemogloblin, neutrocytes, thrombocytes and monocytes than the female before and after acclimation.

Haematocrit and haemoglobin concentration, erythrocytes count are blood parameters which are related to environmental paramrters for instance salinity and water temperature; gonado somatic fluctuations affected the haematological parameters (Guijarro *et al*., 2003). The environs where fish lives could influence the blood chracteristics of the fish and this is line with observation of Femandes and Mazon (2003) who reported that the reaction of the animal to the surroundings are closely related to the characteristics of its haematological parameters. Bayir (2005) observed that factors like water temperature and reproductive period affected WBC. Denson *et al.* (2003) reported that exposure of Rachyccntron canadum to different degree of salinity reduced RBC and Hb values. The blood parameters of *Clarias gariepinus* had been studied by number of authors and it had been reported that exposure to attack by pathogens, diseases and enviromnental pollutants have caused stress in the fish hence changes in haematological parameters (Saravanan *et al*., 2003). Gabriel *et al*. (2001) detected an upsurge in erythrocyte and leucocytes counts in fish from contaminated environs however Simonato *et al*. (2007) described a reduction in hematocrit values and erythrocyte counts of the fish exposed to pollutants. Higher hematocrit percentages have been recorded in the fish harvested from the polluted site (Gabriela *et al*., 2009). Yaji and Auta (2007) reported that a reduction in PCV values, erythrocyte counts and upsurge in leucocytes counts may occur in infected fishes. Upsurge in the quantities of WBCs and neutrophils have been re-counted in fish that are parasite infested (Ghiraldelli *et al*., 2006) and a decrease in hematocrit values and RBC numbers had been described in infected fish (Martins *et al*., 2004). Erythrocyte count and percentage of haematocrit values have been observed to differ due to seasonal changes. Peripheral stressors like subjection to adaptation have pronounced effect on the blood characteristics of fishes. Decrease in haemoglobin values during acclimation have been observed in fish by Yagi and Auta (2007) and it was reported that the female are less reactive to the pressure of adaptation than males. Akinrotimi *et al.* (2007a) reported that in *Sarotherodon*

melcmotheron on acclimation the haemoglobin value decrease and also Gabriel *et al*. (2004) reported decrease in the Hb and PCV values in the fish blood. Stress due to capture have resulted to Hyperglycaemia in fish (Yagi and Auta, 2007). Rehulka and Adamec (2004) reported that variations in haematological parameters of rainbow trout was due to activity, diet and metabolic adaptations and not as result of water temperature. The age and length of the fish increase the values for haemoglobin concentration and red blood cells counts as it was reported by Rehulka and Adamec (2004). Unfavorable exogenous factors like overstocking, poor water quality induced changes in haematological parameters in the Indian shad (*Tenualosa ilisha*) and these changes were indicators of the ill health in cultivated fish.

2.11.2 Blood chemistry

Health of fish can be determined by evaluating blood biochemistry parameters (Cnaani *et al*., 2004). Exogenous influences for instance stress induced key deviations in life blood composition (De-Pedro *et al*., 2005), ailments (Chen *et al.,* 2005) and system of culturing (Svobodova *et al*., 2008). The rudimentary biological features for instance feeding system and number stocked have direct effect on some biochemical parameters (Coz-Rakovac *et al*., 2005). Jawad *et al*. (2004) also recounted that serum biochemical could be swayed by many non-living factors like age, food, water temperature, periodic pattern and gender of the fish hence the values differ among breed to breed. Aminotransferase activity could be reduced due to the structural liver alternations and this increase plasma protein concentration with simultaneous decrease in deamination capability (Hrubec *et al*., 2001). Kavadias *et al*. (2004) specified that great percentage of fat in the chemical configuration of the diet responsible for higher cholesterol concentration of L. calcarifer and great protein level in striped bass amplified as it grows. Basten (2010) reported that the level of alkaline phosphatase (ALP) tend to be higher and acute renal dysfunction led to high level of urea when there was a blockage in the bile system. Overall quantity of protein in the blood stream is the combination of the albumin and globulin values (Uyanik *et al.,* 2001). Higher or lower values than normal ranges established for organisms is an indication of serious health conditions.

The use of blood characteristics in diagnosis of the health conditions in fish is accepted globally. Adebayo *et al*. (2007) re-counted that fish culturing can be enhanced by

consistent checking of the blood factors of farmed fish. These studies have largely been used as a sensitive index and effective means to check biological and pathological deviations in fish to evaluate the health status of the experimental African Catfish nourished with varying additive levels of *Allium sativum, Chromolaena odorata* and *Talinum triangulare* as feed additives.

CHAPTER THREE

DIETARY EFFECTS OF *Allium sativum, Chromoleana odorata* AND *Talinum triangulare* FEED ADDITIVES ON GROWTH PERFORMANCE OF *Clarias gariepinus* (AFRICAN CATFISH) FINGERLINGS

3.1 Introduction

Agriculture business outputs, initiating from livestock and fisheries, together with aquaculture, must grow by over sixty percent (FAO, 2010) in order to feed the world in 2050. Accomplishing this goal is challenging for the global community bearing in mind the fact that several people, mostly in unindustrialized nations, still suffer from food shortage and paucity. The inexpensive sources of animal protein and micronutrient for many people in Africa is fish (Bene and Heck, 2005). The key challenge is that entire world fishery produce has reduced and the human consumption for aquatic product has increased over the year (FAO, 2014). While open water fisheries have been declined there is great opportunity to enlarge aqua-farming in Africa so as to advance food security (Ozigbo *et al*., 2014). Cultured fish needs high superiority balanced food for quick development so as to be harvested earlier in order for aquaculture industry to compensate for the reduction in capture fisheries. There is need to produce local fish food for the sustainability and progress of aqua-farming in Africa particularly in the countryside. The role played by locally made fish food cannot be over emphasized in reducing production cost for aqua-farming to prosper and close the gap at present prevailing in demand and supply for fish particularly in Africa. The need to improve feed productivity, growth performance and infection resistance of cultured organisms in various sectors of this industry necessitate the use of feed additives. There are different food additives obtainable to increase fish development in the market places and some of these additives are antibiotics and hormones that may impact animals and handlers negatively. Antiseptics as well as antibiotics were used for growth promoting, precluding or management of infections originated from water but with

partial achievement (Yunxia *et al*., 2001). The excess usage of antimicrobials for growth promotion and to prevent bacterial infections in aqua-farming, have led to proliferation of bacteria that resisted antibiotics. FAO/WHO/OIE (2006) also confirmed that the usage and misuse of antiseptics and antibiotics in aquaculture have resulted in occurrence of bacteria that resisted antibiotics and residues of drugs in preserved fish. Current reality on the use of different food additives mostly to guide against the side effect of chemotherapeutant in fish and to decrease price of feedstuff has made it compellingly to study the consequence of feeding of medicinal plants as feed additives. Aqua-farming needs high superiority feedstuffs that encompass essential nutrients as well as balancing feedstuff additives to keep animal's healthy, quicker growing and eco-friendly. Search for natural growth promoters and alternative health products to be used in aquatic feed is as a result of the worldwide request for safe food (Denev, 2008).

Recently, phytobiotics have been described as possible replacements, amid other feed additives, to antimicrobial in aquaculture foods (Citarasu, 2010). Previously, some works carried out by some researchers (Thiyagarajan *et al*., 2014), indicated the helpful effects of nutritional therapeutic herb in aquaculture production. There is urgent need to investigate many medicinal plants within our reach that can be useful in promoting growth, increased feed utilization and without causing adverse effect on the cultured organisms and human consumers in aquaculture industries in Nigeria. This work was designed to study some herbs that could be used as feed additives in aquaculture industries to promote growth with hope of guiding against some side effects attributed to chemically produce commercial feed additives which might be harmful to culture organisms and human consumers.

3.2 Materials and methods

3.2.1 Experimental fish

Three hundred and sixty apparently healthy African catfish, fingerlings sourced from a home-grown fish farm in Olodo, Oyo State of Nigeria. Fingerlings were acclimatized for 14 days in 100L capacity circular tank. The tank was filled with clean bore-hole water. The biochemical and organic potentials of the water were measured for appropriateness for fish rearing. Preceding stocking, the fish were treated with 1milligram per Littre KMnO4 to eliminate exo-parasites. Throughout the time of adaptation the fish were nourished

sufficiently (Anibeze and Eze, 2000) using a computed ration of 35% crude protein without herbal extract and no history of herbal feeding from the farm where fish were sourced.

3.2.2 Collection of plant and grounding of plant powder

Healthy, new foliage of *Chromolaena odorata* and *Talinum triangulare* were harvested from wild and bulb of *Allium sativum* were sourced from Bodija markets in Southwestern part of Nigeria. The plants were documented by the technical staff of Department of Botany University of Ibadan. The voucher samples were kept with herbarium of University of Ibadan for future references. The foliage were rinsed thoroughly with clean water. The foliage was air dried in the shade for three weeks at room temperature of 25 ± 2^{o}C on side bench in the laboratory and then ground to powder with a mechanical grinder. Pieces of garden-fresh garlic rhizome were skinned and cut up into lesser pieces then oven-dehydrated at 70^{o} C till a perpetual weight was gotten. The dried out garlic was grounded with electrical food processor into a powdered form. The plant powder was kept in air tight plastic containers and label appropriately (Amisah *et al*, 2009)**.** The scientific name, shared name, part of the plant used, and herbarium number assigned to these plants powder for the purpose of this experiment were shown in Table 3.1. The results of phyto-qualitative screening of the plants shown in Table 3.2 and the outcomes of chemical analysis carried out on the dried samples of the medicinal plants using normal procedure as adapted by Sofowora (1993) were presented in Table 3.3. Plant extracts were evaluated chemically in line with the official procedures of analysis styled by the association of official analytical chemist (AOAC, 18TH Edition, 2005). The entire investigations were carried out in duplicate.

Table 3.1. Details of the Medicinal Plants Used as Feed Additives in the Experiment

S/N	Family	Scientific Names	Common Name	Local Names	Parts of the plants Used	Voucher* Number Specimen HB #)
1	Liliaceae	*Allium sativum* (Lin.)	Garlic	Ayuu	Bulb	UIH - 22536
2	Compositae	*Chromolaena odorata* (Linn.) K. R.	Siam weed	Akintola	Leaf	UIH - 22521
3	Portulacaceae	*Talinum triangulare* (Jacq) Wild	Water Leaves	Gbure	Leaf	UIH - 22522

*****UIHB# =University of Ibadan Herbarium Number

Table 3.2. Phytochemical analysis of the Medicinal Plants Used as Feed Additives

Phytochemicals:	***Allium sativum***	***Chromolaena odorata***	***Talinum triangulare***
Alkaloids	PPP	ppp	ppp
Tannin	ppp	ppp	pp
Phlobatannin	p	pp	p
Saponin	pp	ppp	ppp
Flavonoids	P	P	P
Anthraquinones	P	A	P
Steroids	P	P	P
Terpenes	P	P	P
Cardenolides	A	A	A
Phenol	PPP	PPP	PPP
Chalcones	A	A	A
Cardiac glycoside	A	A	PP

Observation Remarks:

PPP = Substantial Quantity Present, PP = Moderate Quantity Present

P = Trace Quantity Present, A = Absolutely Absent

Table 3.3. Proximate analysis of *Allium sativum, Chromolaena odorata and Talinum triangulare*

	Allium sativum	***Chromolaena odorata***	***Talinum triangulare***
Moisture (%)	9.88	9.26	10.37
Fiber (%)	4.57	15.28	16.43
Fat (%)	2.89	3.56	2.23
Protein (%)	3.67	18.86	11.88
Ash (%)	3.78	11.76	13.29
Carbohydrate (%)	75.21	41.28	45.80

3.2.3 Experimental diet preparation

Fishmeal, maize, wheat bran, soya bean meal, calcium carbonate and limestone were sourced from Bodija market. All materials were crushed into powder by means of a mechanical crusher, and then mixed with vegetable oil to form basal diet for the experiment. The materials and proximate chemical composition of basal feed (Table 3.4) was valued through the systems adapted by the AOAC (2005) to define the basic protein content. Ten different foods with or without additives, representing ten dietary variants (Table 3.5) were then prepared by incorporating prepared *Allium sativum* powder at levels of 5, 10 and 30 g/kg into basal food, indicated as treatment No T_1A, T_1B and T_1C respectively. Prepared *Chromolaena odorata* powder was integrated into basic diet at levels of 5, 10 and 30 g/kg to form treatment No T_2A, T_2B and T_2C and prepared *Talinum triangulare* powder was also incorporated into basal food at levels of 5, 10 and 30 g/kg to form treatment No T_3A, T_3B and T_3C, respectively. Feed not containing any herbal additives was used as the control (0.0 g/kg) referred to as treatment, CC. Manually, the materials were painstakingly mixed together. The mixed materials was standardized to a dough-like paste using warm water. Pellet press of 0.5 mm width was used to pelletise the food. The foods were dried up for four days and kept in sealed vessels during the course of the investigational period.

3.2.4 Experimental technique

3.2.4.1 Fish feeding and culture

Experimental fish with mean body weight of 1.10 ± 0.01g and average length 4.85 ± 0.04cm were arbitrarily prearranged at the proportion of 12 fish for each of the 30 plastic tanks, measuring 27.0 cm x 27.0 cm x 40.0 cm set up in triplicates. The fish were randomly assigned to the ten different diets (CC, T_1A, T_IB, T_1C, T_2A, T_2B, T_2C, T_3A, T_3B and T_3C) at 36 fish per treatment. Every plastic aquaria tanks were three-quarter filled with water, well aerated with an aquarium pump (Farrari^R model). The fish were fed the trial diets two times everyday, within 8.0 - 9.0 am and 15.0 - 16.0 pm at 5% body weight during the course of the trial which last for a period of 70 days except on sampling days. Serving of food was deferred 24 hours prior to the feeding trial to upturn hunger and response for fresh food (Madu and Akilo, 2001). The trial containers were checked day-to-day to take out dead fish. Waste food as well as faeces in individual tank were drain off daily. Water in the containers

was similarly replaced on a daily basis. Water quality parameters were taken weekly all through the period of the trial. Temperature of the water (T^oC), Oxygen (O_2), pH, Total Dissolved Solids (TDS), Ammonia (NH_3) as well as Nitrite (NO_2) were gauged by means of a handy Hanna^R H198186 meter and Aqua chek® (USA) water quality test stripes. The probe of Hanna^R H198186 meter was immersed into the water in the containers, and then steadied readings of DO_2, pH, T^oC, as well as TDS shown on the meter screen instantly and these were documented accordingly. Aqua check® streak were physically plunged into water inside the tanks. In lieu of pH readings, the streak was removed promptly; for NH_3 and NO_2; the streak was briskly agitated up and around within the water for half a minute. Colour alteration for pH, NH_3 and NO_2 were compared with the established colours in quarter of a minute, half a minute and half a minute correspondingly. The average readings for water quality parameters recorded for each of experimental groups during feeding trials were shown in Table 3.6.

Table 3.4. Proximate chemical analysis of the material of the basic diet

Ingredients	**%**
Yellow Corn	35.0
Soyabean Meal (44%)	28.5
Fish Meal (65%)	17.0
Wheat Bran	9.5
Calcium Carbonate	0.3
Ground lime stone	0.7
Vegetable Oil	6.5
Mineral Mix	1.7
Vitamin Mix	1.0
Nutrients opus	**%**
DM	90.40
CP	30.65
EE	11.73
Ash	2.70
CF	10.11
NFE	44.81
GE (Kcal/100 g DM)*	467.77
Protein/Energy ratio (mg CP/Kcal GE)*	65.52

Key. DM=Dry matter, CP=Crude protein, EE=Ether extract, CF=Crude fibre, NFE=Nitrogen free extract, GE=Gross e nergy

NFE = 100 – (protein + lipid + ash + crude fibre). GE (Kcal/100 g DM) = CP x 5.64 + EE x 9.44 + NFE x 4.11 calculated according to NRC (1993), * not in percentage.

Table 3.5. Details of the experimental treatments (different inclusion rates)

Groups	Details
CC	Basal Diet (BD) + 0% (0g/kg) (as a control)
T_1A	Basal Diet (BD) + 0.5% (5g/Kg)
T_1B	Basal Diet(BD) + 1.0% (10g/Kg)
T_1C	Basal Diet(BD) + 3.0% (30g/Kg)
T_2A	Basal Diet(BD) + 0.5% (5g/Kg)
T_2B	Basal Diet(BD) + 1.0 % (10g/Kg)
T_2C	Basal Diet(BD) + 3.0% (30g/Kg)
T_3A	Basal Diet(BD) + 0.5% (5g/Kg)
T_3B	Basal Diet(BD) + 1.0% (10g/Kg)
T_3C	Basal Diet(BD) + 3.0% (30g/Kg)

CC = Control (0%), $T_{1=}$ (Group of fish fed with *Allium sativum* additives), $T_{2=}$ (Group of fish fed with *Chromolaena odorata* additives), $T_{3=}$ (Group of fish fed with *Talinum triangulare* additives). A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively.

Table 3.6. Average values of water quality parameters monitored during the experiment.

Plant Species	Group	Temp (^{0}C)	DO (mg/l)	pH	NH_3	NO_2	TDS (ppm)
	CC	28.03±0.28[a]	6.42±0.06[a]	7.10±0.0[a]	0.0±0.0 [a]	0.01±0.01[a]	70.67±5.46[a]
Allium sativum	T_1A	28.20±0.32[a]	6.45±0.01[a]	7.13±0.03[a]	0.0±0.0 [a]	0.03±0.0 [a]	74.67±0.33[a]
	T_1B	27.87±0.22[a]	6.44±0.02[a]	7.10±0.0[a]	0.03±0.03 [a]	0.02±0.01[a]	75.00±2.08[a]
	T_1C	28.43±0.37[a]	6.45±0.03[a]	7.17±0.07[a]	0.0±0.0 [a]	0.01±01[a]	75.67±0.33[a]
Chromolaena odorata	T_2A	27.97±0.58[a]	6.44±0.03[a]	7.07±0.03[a]	0.0±0.0 [a]	0.02±0.01[a]	76.33±0.88[a]
	T_2B	28.07±0.37[a]	6.52±0.03[a]	7.10±0.0[a]	0.0±0.0 [a]	0.02±0.0 [a]	76.67±0.33[a]
	T_2C	28.37±0.34[a]	6.42±0.02[a]	7.17±0.07[a]	0.0±0.0 [a]	0.01±0.0 [a]	70.00±2.65[a]
Talinum triangulare	T_3A	28.00±0.30[a]	6.51±0.04[a]	7.10±0.0[a]	0.0±0.0 [a]	0.01±01 [a]	75.67±1.20[a]
	T_3B	27.97±0.33[a]	6.45±0.02[a]	7.10±0.0[a]	0.0±0.0 [a]	0.01±0.01[a]	72.67±0.67[a]
	T_3C	28.00±0.30[a]	6.45±0.02[a]	7.07±0.03[a]	0.0±0.0 [a]	0.02±0.01[a]	76.33±1.45[a]

Average of 10 weeks readings. Average values with the similar superscript letter in the same row are not meaningfully changed (P>0.05). CC = Control (0%), T1= (Group of fish fed with Allium sativum additives), T2= (Group of fish fed with Chromolaena odorata additives), T3= (Group of fish fed with Talinum triangulare additives). A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively

3.2.4.2 Collection of data

Fish in individual tank were collectively assessed for weight at the beginning of the study and weekly afterward by means of digital electronic weighing scale to the nearest gram. The portion of the food was adjusted weekly as soon as fresh weights of fish for the different trial groups were measured. The average length was measured with a graduated tape. Figures on new body weight changes, relative weight gain, specific growth rate, feed conversion ratio, condition factors (Fasakin *et al*., 2001) and survival percentage (Bagenal, 1978) were determined by means of these formulae:

i. Weight gained (g) = Final Weight of fish - Initial Weight of fish (1)

ii. Relative Weight Gain (RWG, %) = $\frac{\text{Weight gain X 100}}{\text{Weight (Initial)}}$ (2)

iii. Specific Growth Rate (SGR) was measured as:

$$\text{SGR (\% per day)} = \frac{(\text{net Log } W_2 - \text{net Log} W_1) \times 100}{T_2 - T_1} \quad (3)$$

Where:

W_2 = Final Weight of fish

W_1 = Initial Weight of fish

iv. Feed Conversion Ratio (FCR).

Measured from the association of food ingested as well as gained weight (wet)

$$\text{FCR} = \frac{\text{Total Feed Ingested by fish (g)}}{\text{Weight Gain by Fish (g)}} \quad (4)$$

v. Survival Rate (SR) was calculated as:

$$\text{SR (\%)} = \frac{(\text{No} - \text{N t})}{\text{No}} \text{ X } 100 \quad (5)$$

Since:

No = Sum of fish at the beginning of the trial

Nt = Sum of fish at the end of the trial

vi. Condition factor (K) was measured as:

$$K = \frac{W}{L^3} X 100 \quad (6)$$

Since:

W = Weight of fish (g)

L = Normal length of fish (cm)

3.2.4.3 Analysis of data

All data were subjected to analysis of variance (ANOVA) by means of Graph Pad Prism Software Version 5.1 Average values of the water quality factors and mean values of weight measurements were measured. The outcomes were presented as mean ± SEM. All measurements were subjected to Analysis of variance (ANOVA) and Tukey Test was used to rank the means. All changes were considered as meaningfully different at $P<0.05$ amid treated groups.

3.2.5 Ethical approval (EA)

The EA was acquired from the University of Ibadan Animal Care and Use for Research Ethical Committee. The approval number is **UI-ACUREC/App/2015/066.** Experiment was conducted according to ACUREC approved protocol.

3.3 Result

The growth performances of *Clarias gariepinus* in reaction toward various addition levels of *Allium sativum, Chromolaena odorata* and *Talinum triangulare* as feed additives in the fish feed were offered in the subdivisions that follow**:**

3.3.1 Growth performance

Growth responses of the *Clarias gariepinus* fingerlings served locally made feed comprising varying quantities of *Allium sativum, Chromolaena odorata* and *Talinum*

triangulare as feed additives for a 70-day growth period was offered in Table 3.7. Mean of the original body weight of *C. gariepinus* nourished with the trial foods at the begining did not vary, indicative of homogenous groups. Fish were capable of consume the test diets at varying degrees but AWG, FCR, SGR, SR, as well as K values of fish were only marginally vary ($P > 0.05$). Nevertheless, the mean final body weight (MFBW), was meaningfully ($P < 0.05$) advanced than the original fish weight in all diet trial groups (Table 3.7). Fish fed with *Allium sativum* as feed additives (T_1C) at 3% inclusion level provided the best growth performance (2.53 ± 0.02g) which was slightly better than those fed on basal diet minus herbal feed additives (1.91±0.13g). The highest mean average weight gain (AWG) value (1.44 ± 0.07g) was achieved by the diet variant (T_1C), containing 3.0% *Allium sativum* inclusion and the least mean average weight gain (AWG) value (0.84±0.11g) was recorded by the diet variant (CC) containing no herbal additives (Table 3.7). The best SGR was found in fish nourished with 3% *Allium sativum* (T_1C) whereas the lowermost was observed in fish nourished with 3% *Chromolaena odorata* (T_2C). The highest and least FCR were recorded in fish nourished with 1% *Allium sativum* (T_1B) and 1% *Talinum triangulare* (T_3B) respectively. There was no significance alteration ($P > 0.05$) in SR however highest SR of 94.45 ± 2.78% was observed in fish nourished with 3% *Allium sativum* (T_1C) while lowest SR of 75.0 ± 4.81% were recorded in both control group and fish fed with 3% *Talinum triangulare* (T_3C), however, SR exceeded 80.0 % in all other treatment groups.

3.3.2 Water quality measurements

The water superiority factors monitored in plastic aquaria tanks under laboratory conditions, as indicated in Table 3.6 were fairly stable in all the treatments. Water quality factors were not meaningfully vary ($P>0.05$) among trial groups and were within the endorsed ranges for the cultivating of African Catfish (Viveen *et al*., 1986).

Table 3.7. Growth performance of African catfish fingerlings fed diets containing different levels of herbal additives

Parameters	Diets									
		Allium sativum			*Chromolaena odorata*			*Talinum triangulare*		
	CC	T_1A	T_1B	T_1C	T_2A	T_2B	T_2C	T_3A	T_3B	T_3C
MIBW[1] (g)	1.07 ±0.240[a]	1.08 ±0.003[a]	1.18 ±0.003[a]	1.09 ±0.052[a]	1.09 ±0.007[a]	1.13 ±0.041[a]	1.13 ±0.022[a]	1.05 ±0.029[a]	1.10 ±0.015[a]	1.09 ±0.049[a]
MFBW[2] (g)	1.91 ±0.127[b]	2.38 ±0.025[b]	2.36 ±0.205[b]	2.53 ±0.015[b]	2.25 ±0.213[b]	2.39 ±0.041[b]	2.05 ±0.147[b]	2.14 ±0.084[b]	2.38 ±0.096[b]	1.96 ±0.439[b]
AWG[3] (g)	0.84 ±0.107[a]	1.30 ±0.028[a]	1.18 ±0.208[a]	1.44 ±0.066[a]	1.16 ±0.212[a]	1.26 ±0.022[a]	0.91 ±0.128[a]	1.09 ±0.113[a]	1.28 ±0.085[a]	0.87 ±0.110[a]
RWG[4] (%)	78.9 ±8.87[a]	121.1 ±3.03[a]	100.4 ±17.94[a]	133.0 ±12.46[a]	106.7 ±19.48[a]	111.2 ±9.18[a]	80.3 ±10.11[a]	104.9 ±13.63[a]	116.3 ±6.82[a]	97.9 ±5.69[a]
SGR[5]	0.36 ±0.029[a]	0.49 ±0.009[a]	0.43 ±0.055[a]	0.52 ±0.032[a]	0.45 ±0.062[a]	0.46 ±0.024[a]	0.36 ±0.035[a]	0.44 ±0.043[a]	0.48 ±0.019[a]	0.42 ±0.017[a]
FCR[6] (gg^{-1})	5.77 ±0.53[a]	5.71 ±0.43[a]	6.32 ±1.01[a]	5.60 ±0.38[a]	5.64 ±0.99[a]	4.79 ±0.40[a]	5.74 ±1.11[a]	5.14 ±0.45[a]	4.65 ±0.10[a]	5.45 ±0.69[a]
SR[7] (%)	75.00 ±4.81[a]	88.89 ±7.35[a]	91.67 ±4.81[a]	94.45 ±2.78[a]	86.11 ±10.01[a]	80.55 ±2.78[a]	80.56 ±7.35[a]	83.34 ±8.33[a]	80.56 ±7.35[a]	77.09 ±4.78[a]
CF[8] (K)	0.80 ±0.03[a]	1.11 ±0.06[a]	0.97 ±0.11[a]	0.91 ±0.03[a]	0.90 ±0.04[a]	1.11 ±0.05[a]	0.91 ±0.04[a]	0.87 ±0.06[a]	0.94 ±0.02[a]	0.86 ±0.10[a]

Data are represented as mean of three samples replicates ± standard error of mean. Mean values with the same superscript letter In the same row are not significantly different. ($p>0.05$). [1] Mean Initial body weight, [2] Mean Final Body Weight, [3] Average Weight Gain, [4]Relative Weight Gain, [5]Specific Growth Rate, [6]Feed Conversion Rate, [7]Survival Rate, [8]Condition Factor. * CC = Control (0%), $T_{1=}$ (Group of fish fed with *Allium sativum* additives), $T_{2=}$ (Group of fish fed with *Chromolaena odorata* additives), $T_{3=}$ (Group of fish fed with *Talinum triangulare* additives). A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively

3.4 Discussion

The necessity to appreciate the importance of phyto-additives in aqua-farming has led to various investigation of different herbal plants in aquaculture feed. It is a well-known fact that a diversity of therapeutic herbs have been fruitfully used in cultivation of fish to increase the growth as well as immune energizer (Irkin *et al*., 2014). The problem of acceptability is often encountered when medicinal plants are used in fish diets usually in connection to the palatability of the food (Rodriguez *et al*., 1996). In this current work, *Clarias gariepinus* fingerlings accepted all the trial foods, a signal that the palatability of the diets was not affected by different concentrations of medicinal plants incorporated. This might be ascribed to the handling method which include air drying and oven dehydration (70^{o}C, in case of garlic bulb) techniques that might have reduced the anti-nutrient factors that may be present in these plants thereby not affecting the palatability of the diets. Siddhuraju and Becker (2003) concluded that different processing like soaking and drying techniques reduces anti-nutrient in feed leading to better palatability and growth in fish. Several efforts have been made to investigate medicinal plants as spices for human and additives in foods of faunas, however, investigation on feed additives in fish nutrition has received slight attention (Lawhavinit *et al*., 2011) and more so medicinal plants as feed additives in rearing of African catfish in this part of the world. The aqueous, ethanol extracts and dried powder forms of garlic (*Allium sativum*) have been studied and it has been found to contain a variety of organo sulfur compound such as ajuene, s-allylcysteine, diallyldisulfide, S-methylcysteine sulfoxide, allicin and S-allyyylcysteine with medicinal properties (Kyo *et al*., 2001). Metwally (2009) also re-counted beneficial effects of garlic as growth promoter in livestock production. Fish fed garlic supplemented diets has been equally described to have improved growth performance (Metwally, 2009). The potential effect of garlic as a medicinal plant promoting growth and physiological enhanchers in African Catfish (Nya and Austin, 2011), *Oncorhynchus mykiss* (Thanikachalam *et al*., 2010) and *Oreochromis niloticus* (Shalaby *et al*., 2006) had been reported. Shalaby *et al*. (2006) stated a noteworthy increase in weight, upsurge in feed efficiency, improved SGR and PER in Nile tilapia when nourished with food comprising 30 g per kg A. *sativum* powder ration, although this has not been compared with lower or greater inclusion rates. The level of *A. sativum, C. odorata, T. triangulare* supplementation in fish feed as additives

especially in *Clarias gariepinus* fingerlings has not yet been tested. In this experiment different inclusion rates (0.5%, 1.0% and 3.0%) of *A. sativum, C. odorata, T. triangulare* had been investigated on growth performance of African Catfish, the effect showed that *Allium sativum* (garlic) enhanced growth better than *Talinum* (Water leaf) and Chromolaena (Siam weed) (Table 3.7). The best performance of *Allium sativum* could be due to the presence of allicin which is one of the key active ingredients in *A. sativum* powder and it had been reported to enhance appetite and thus stimulate growth in fish (Amagase and Milner, 1993). The outcome of allicin as an agent promoting growth had been documented in many reports (Hu *et al.*, 1999). The result of our findings in this experiment agreed with work of Agatha (2012) who recorded non-significant difference in weight gain but a significant difference in final weight compared with initial weight of African Catfish fingerlings fed different inclusion rates (0.5%, 1%, and 3%) of garlic. Similarly, the outcome of the work agreed with account of Aly *et al*. (2008) who inspected the growing levels of *Orechromis niloticus* after nourishing with *A. sativum* (ten and twenty gram per kilogram food served), and established to be statistically not significant after one or two months but Metwally (2009) reported the best growth performance of *O. niloticus* when nourished with food comprising 32 gram per kilogram food of garlic fine particles. In this experiment, 30g/kg of *Allium sativum* only enhanced the growing of the experimental fish likened with placebo but not significantly increased. However, Aly *et al.* (2008) recorded a significant increase in Nile Tilapia after eight months feeding, an indication that high doses of *Allium sativum* for a long period might be desirable to increase the growth rate.

Chromolaena odorata (Siam weed) is a perennial herb which is about 1.5-2.0 metre in height, forms dense tangled bushes with a distinguishing aroma (Phan *et al*., 2001). Yorubas referred to the plant as "Ewe Akintola" or Epo Agatu. In Tropical Africa, *C. odorata* has attained a repute as a therapeutic plant for different diseases comprising fever, malaria, diabetes and wound dressing (Zachariades *et al*., 2009). *C. odorata* usefulness as growth promoter in aquaculture industries has not been investigated. In an experiment conducted by Igboh *et al.* (2009) on chemical analysis of *Chromolaena odorata*, the result shown that Siam weed is rich in high quality protein. The outcome of proximate analysis of *C. odorata* in this trial showed a high protein content of 18.86% compare to *T. triangulare* 11.88% and *A. sativum* 3.67% as shown in proximate analysis of the plants used (Table

3.3). The implication of this in nutritional protein supplementation cannot be over emphasized. Aro *et al*. (2009) reported that *C. odorata* function as a good basis for protein, minerals, dietary fibre and energy thus favour the deliciousness of animal feed and indicated this as reason why the plant have been used in livestock production. However, *C odorata* have not been used in aqua-farming for growth promotion, therefore this work tested the effectiveness of *C odorata* as growth promoter in African catfish fingerlings. The result showed that *C. odorata* marginally enhanced growth in the experimental fish at inclusion rate of 0.5%, 1.0% and 3% compared to control diet that did not contain *Chromolaena odorata* as feed additives. This effect mighty be owing to great protein content of *C odorata* as well as the increase in fiber content (15.28%) that mighty upsurge faecal bulk and this enhance level of intestinal transit that have prebiotic effect (Igboh *et al*., 2009). 1.0% inclusion rate of *C. odorata* showed a better result compared with 0.5% and 3.0% inclusion rates.

Talinum triangulare (Jacq) Wild is of Talinum genus, in the family Portulacaceae, develops well under humid conditions is one of vegetables common in Nigeria (Burkill, 1994), It is a cosmopolitan weed extensively nurtured and used up in Africa especially Southern Nigeria (Imoh and Julia, 2000). *T. triangulare* is greatly valued as a medicinal, therapeutic vegetable and nutritious plant across Central Africa (Fasuyi, 2006). Mensor (2001) reported its usefulness as potential medicinal plant for purgative, laxative, gastro-intestinal diseases, treatment of diarrhea as well as in the managing of cardiac maladies for instance obesity and stroke (Aja *et al.*, 2010). Many livestock farmers and aquaculturists are fond of feeding *T. triangulare* to their animals without any scientific basis for this act. Effort has not been made to examine the outcome of *T. triangulare* on growing recital of fish in aquaculture industries. In this study different inclusion rates (0.5%, 1.0% and 3%) of water leaf had been investigated as feed additives to enhance growth in African Catfish fingerlings. The three different inclusion rates marginally enhanced growth in the experimental fish compared with control (Table 3.7) though not significantly increased. The enhancement in growth of the experimental fish could be due to *Talinum triangulare* found to be rich source of some fat soluble and water soluble vitamins as reported by Ogbonnaya and Chnedum (2013). In this experiment, the inclusion rate of 1% (10g/kg) *T. triangulare* showed better result compared with 0.5% (5g/kg) and 3.0% (30g/kg) and control diet.

Specific Growth Rate (SGR) is one of the essential factors in assessing diet utilization efficiency in fish, thus the observed higher values of SGR in all diet variants except cluster of fish fed with 30g/kg of *C. odorata* which showed the same effect as control showed that food were utilized for growth better than the control diet in other diet variants. Experiment carried out by Cho and Lee (2012) revealed the affirmative effects of nutritive therapeutic plant as feedstuff additives on feed utilization and growth in fish. The values of FCR observed among the treatments were not significantly different however the finest FCR (4.79 ± 0.40) was detected in group of fish fed with 10g/kg *T. triangulare* as feed additive likened with the control group with the value of 5.77 ± 0.53 (Table 3.7). The best Feed Conversion Ratio values observed in treatment cluster of fish fed with 10g/kg *T.triangulare* suggested that addition of 10g/kg *T. triangulare* as feed additive improved feed utilization better than other treatment groups. The Condition Factors (CK) observed were marginally dissimilar (P>0.05) amongst treatment collections. Similar results were achieved by Bichi and Ahmad (2010) who fed different dietary levels of treated leaves to African Catfish fingerlings. The best condition factors were detected in the cluster of fish nourished with 5g/kg of *A. sativum* and 10g/kg of *C. odorata* compared with other the treatment groups and control group.

Generally, increase in body weight observed in this experiment were low, the finest growing outcome was achieved in the fish nourished 30g/kg *A. sativum* as feed additive and the least was documented in the fish nourished with basic food (0g/kg) without feed additive. The fish were reared solely on the locally made food fed under laboratory conditions without contact to natural food as may be established in riverine or pond surroundings, this could explain the slow weight gains observed in the experimental fish. The African catfish is predatory fish and can feed on an extensive range of natures under natural conditions. The percentage survival could be adjudged to be good during the course of the trial epoch. This might be as a consequence of good water superiority supervision occasioned by daily changing of the water in the aquaria and more so, the suitability of *A. sativum, C. odorata, T. triangulare* inclusion in *Claria gariepinus* diet. Occasional mortalities occurred throughout the trial epoch might be credited to cannibalism among the fish and could not be attributed to deleterious effects of herbal extracts as minimum survival rate recorded during the experiment was 75 % (Table 3.7). All through the trial period,

water qualities factors data were taken at 9.00 to 10.00am in the morning and 3.00 to 4.00pm in the afternoon. The average water temperatures of the aquaria were found to be 28.53 ± 0.67°C. Water T^{o} extend from 29^{o}C to 35°C is appropriate for culturing of fish according to Aminu (1996). DO content of the aquaria were established to be 6.30±0.04 mg/L. The rate of DO in the water body is one of the significant parameters in culturing fish. Low dissolved oxygen in the water body makes fishes inhabited such type of water to be physiologically weak and easily suscesptible to diseases (Chanda *et al*., 2013). The dissolved oxygen level of 5 part per million or more is desirous for fish making while levels equals or lesser than 3 part per million is regarded as hazardous to lethal for fish production (Ellis *et al*., 1952). pH measures the acidity and alkalinity condition of the water, described as the efficiency index of a water body, is one of the essential elements in fish cultivation. The pH values observed in all the aquaria in this experiment were neutral to very slightly alkaline (7.0 - 7.1) an indication of good pH conditions for fish cultivation. pH range from 6.5 to 9.0 is appropriate for fish while pH greater than 9.5 is inappropriate for fish culturing (Rahman, 2005). pH below 6.5 decreases biological actions of the fish, acceptance to lethal materials and fish growth. Not more than 0.2mg/l of ammonia was noted in all the aquaria water all through the trial period and this could be due to daily changing of aquaria water and constant aeration of the water. Ammonia (NH_3), un-ionized form, can be deadly to fish at concentration of 0.4 to 3.1 ppm in 96 hours. The principal source of ammonia in catfish production ponds is protein a major constituent in feeds. The amount of Nitrite observed in this experiment range from 0.01- 0.04mg/dl and this was within accepting range suggested for fish culture (Boyd, 1999). Nitrite above tolerant level could interfere with oxygen uptake by blood haemoglobin, the symptoms of nitrite poisoning are very similar to an oxygen depletion. Total solids measures production output of a water body and is affected by the suspended ingredients, deposit as well as microbes; also specifies the existence or non-existence of fish food particles in the water. During the experimental period total dissolved solids of 72 ppm were recorded and this means the water body in the aquaria is productive according to Choudhury *et al*. (2005) because aquaria water was not turbid due to feaces, food particles and soil particles and this could be as a result of frequent change of the aquaria water.

CHAPTER FOUR

ASSESSMENT OF BLOOD PROFILES AND HISTOPATHOLOGICAL EXAMINATION OF AFRICAN CATFISH (*Clarias gariepinus*) FED DIFFERENT HERBAL PLANTS EXTRACTS AS FEED ADDITIVES

4.1 Introduction

There is rapid ongoing development in all facets of aquaculture in Nigeria. Among culturable fin fish species in Nigeria that is in high demand is *Clarias gariepinus* (Burchell, 1822) (Akinrotimi *et al*., 2014a). Among other culturable species, *Clarias gariepinus* is capable of withstanding adverse environmental conditions than other species such as tilapia (Singh and Lakra, 2011). This, together with its fast growth rate has made it species of choice for fish farming in Nigeria hence its intensive management for the past two decades in Nigeria (Akinrotimi *et al.*, 2014b). Infectious diseases among other several problems limiting the production in intensive aqua-farming with resulting undesirable influence on production output. The disease control strategy of the culturists is the use of antibiotics and chemotherapy which unfortunately led to development of multiple antibiotic resistant bacteria (FAO/WHO/OIE, 2006). There is now grown awareness in the use of therapeutic floras or spices as food improver in fish foods as an alternative to chemotherapeutants in order to evade undesirable effects of synthetic products (Raa, 2000).

Innate defense system of fish is very vital in order to fight infectious diseases, thereby, using immune promoter to control fish diseases are better alternative to antibiotics (Ortuno *et al*., 2002). Modulation of innate immune system of fish has been gaining increasing interest of recent as both prophylactic measures and treatment against diseases in fish (Masoud and Mostafa, 2013). Amid other food additives in aqua-farming foods, probiotics and phytobiotics have been described recently to be potential alternatives to antibiotics. Natural immunostimulants like herbal plants are reported to be bio-friendly,

decomposable and nontoxic for human well-being as well as the environment (Ortuno *et al*., 2002). The phagocytic cells, lysozyme, complement and antibody reactions of fish can be stimulated by herbal immune booster which are constituents which stimulate WBC and can make fishes to be resistant to communicable infections (Secombes and Olivier, 1997). Medicinal plants have been used in inoculations to intensify the exact immune reaction or given as food additives to modify innate immunity according to Badrelin *et al*. (2008) and these have been confirmed to play part in safeguard against infections in fish. Natural organic immune booster which are herbal extracts that can be used against the growth of bacterial resistance with no health-intimidating effects on organisms and environmental friendly have been widely reported (Prit-Benny *et.al*., 2010) however the actual risks of this medicinal plants have to be evaluated regularly through skillful and constant experimental trials adding to wide field survey and epidemiological statistics gathering.

The report of some studies carried out as pointed out by Audu *et al*. (2014) have proved that investigation of life blood indices is an approximate way for monitoring the health position of water faunas as these parameters make available dependable evidence on deficiencies, metabolic ailments and chronic stress status. According to Aderemi (2004) blood indices is important in the monitoring of nutrients, physiological as well as pathological status of an animal. According to Olafedehan *et al.* (2010), analysis of life blood for their components could make available essential facts for the diagnosis and prediction of ailments in faunas. Lifeblood is accountable for essential biological functions like nutrient transport, conveyance of gases, metabolic wastes and immune responses in all vertebrates, (Álvarez-Mendoza *et al*., 2011). Consequently, any change of these indices is an indication of impending health complications in the examined animal, and by extension, difficulties with the adjoining surroundings. Davis *et al*. (2008) pointed out that the overall stress levels of vertebrate animals can be measured by counting specific leukocyte types present in circulation. The diagnosis and reaction to management in diversity of infections in animals can be confirmed by chemical and biological analysis of the blood. Hematological and serum biochemical variables had been reported to be a useful tools to estimate the physiological stress special effects of herbal additives in fish (Yılmaz and Ergün, 2012). In humans and domestic animals, biochemical data of blood serum have been routinely used in health care system.

The non-specific protective mechanism is more essential in fish than mammals and a vital role is played by immunostimulant in well-being and administrative strategies of the aquatic creatures. Some medicinal plants have been proved to improve nonspecific defense mechanism of fish. Previously, it had been proved that *Allium sativum* had effect in enhancing growth in African Catfish (Agatha, 2012). Some medicinal plants frequently described have proved to possess some bio-active compounds for example alkaloids, saponins e.t.c and they have effect on the growth and/or biological composition of the fish. The toxicity of the plants can be proved by hematological, biochemical and histopathological studies. Examination of hematological and biochemical factors is one of the utmost instructive techniques to monitor biological status since these factors are specific amid divergent species (Celik, 2004). Many investigations relating to the blood characteristics and serum enzyme actions of diverse fish species exist (Gharaei *et al*., 2010). This trial was therefore designed to examine the toxic effect of using herbal extracts (*Allium sativum, Chromolaena odorata* and *Talinum triangulare*) as feed additives by evaluating blood characteristics, serum biochemistry and histopathological reactions of *Clarias gariepinus* fingerlings after 42-day exposure.

4.2. Materials and methods

4.2.1 Experimental method

Two hundred and ten *Clarias gariepinus* juveniles, mean 117.3 ± 1.57 gram and average length 26.70 ± 0.26 cm were sourced from a local fish farm in Olodo in Oyo State. They were acclimatized in a large round plastic bowl of 150 litters, fill to half capacity with clean bore-hole water. The fish were accustomed to laboratory situations for 14 days nourished with formulated feed containing Yellow corn (35), Soya-bean meal-44% (28.5), Fish meal-65% (17.0), wheat bran (9.5), Calcium carbonate (0.3), Ground limestone (0.7), Vegetable oil (6.5), Mineral combination (1.7) and Vitamin Combination (1.0) as percentage composition of basal diet that did not contain any herbal extract at the commencement of the trial. At the commencement of the trial the fish were randomly distributed into 4 groups (21fish/group) in three replicates and reared in plastic aquaria measuring 40.0cm x 27.0cm x 27.0 cm filled with borehole water. First group (CC) tagged as control was fed with basic food without herbal extract. Second group was re-distributed into 3 sub-groups and they

were fed *Allium sativum* supplemented diet at rate of 0.5%, 1.0%, and 3.0% respectively representing G_1A, G_1B and G_1C. 3rd group was re-distributed into 3 sub-groups and they were fed *Chromolaena odorata* supplemented diet at rate of 0.5%, 1.0%, and 3.0% respectively representing G_2A, G_2B and G_2C and finally, 4th group was re-distributed into 3 groups and they were fed *Talinum triangulare* supplemented diet at rate of 0.5%, 1.0%, and 3.0% respectively representing G_3A, G_3B and G_3C. The experimental fish were nourished at a feeding amount of 5.0% body mass, divided into 2 feeding times for 42 days. Water superiority was preserved by exchanging water on a daily basis. The experimental tanks filled to three-quarter of their capacities with clean borehole water and roofed with a net prepared of good polyethylene gauze screen of 1millimetre net dimension, to avert the fish leaping out.

4.2.2 Monitoring of water quality

The water superiority factors were careful controlled all through the experiment according to Boyd (1979). The water superiority factors were measured using Hanna® H198186 meter and Aqua check® stripes.

4.2.3. Blood collection

Three fish were haphazardly taken from each replicate at termination of the experiment and then sedated according to Horvath *et al*. (1984) using tricane methane sulphonate (MS-222) at rate of 200mg/litre of clean water. Then, two millilitre of blood was drawn from the caudal vein by means of a two millilitre syringe impregnate with heparin. Part of the blood collected was used for plasma indexes by centrifuging (3000 round per minute for 5 minutes) and the other part was used for blood characteristics analysis according to MAFF (1984). The plasma indexes (glucose measured in milligram per decilitre, cholesterol measured in milligram per decilitre, triglyceride measured in milligram per decilitre, and total protein measured in milligram per decilitre) were determined using spectrophotometer (Technicon, RA-1000, USA) by means of standard equipment (RX MONZA CH200, AP 542, TP 245, AB 362, GL364, AS 101, AL 101, Randox Laboratories Limited, UK.). The PCV was measured with the regular microhematocrit technique and expressed in part per hundred. Haemoglobin measured in gram per deciliter spectrophotometerically at 540 nm absorbance by means of the cyanmethemoglobin procedure with a salable equipment (Pars

Azmoon) as described by Hayatbakhsh *et al*. (2014). The differential leukocyte computation was accomplished through blood slurs marked with Giemsa solution. Slurs were inspected through compound microscope at hundred times magnification (oil immersion).

4.2.3.1 Estimation of haematological parameters

4.2.3.1.1 Erythrocytes count

The blood was collected into a vials impregnated with EDTA to prevent clotting. Blood was filled up to 0.5 mark in Red Blood Cell pipette and instantaneously, the Hayem's solution (fluid used for dillution) was filled up to the 101 mark (1:200 dilution). Pipette was shaken carefully, two drops were cast-off, and diluted blood was then discharged into the counting chamber. The number of Red Blood Cells was calculated in five small squares of the red blood cell column under high power microscope after the solution was permitted to resolve for few seconds and the quantity of Red Blood Cells per cubic mm was considered with equation:

$$\frac{\text{Number of cells x Dilution factor x Depth factor}}{\text{Area counted}}$$

(MAFF, 1984)

4.2.3.1.2 Measurement of haematocrit

Haematocrit was measured by means of the microhaematocrit technique. Capillaries that were not heparinized was used to collect blood. It was permitted to fill 1/2 to 3/4 intervals of capillary tube and the capillaries were closed with sealant at both ends. The capillaries were then moved to a fast speed microhaematocrit centrifuge and were positioned in the furrows of capillary head. Samples were spinned at 12000 round per minute for 300 seconds. Packed Cell Volume was calculated conventional on a microhaematocrit reader.

4.2.3.1.3 Determination of haemoglobin (Hb) content

Haemoglobin was measured by the Cyanomethemoglobin technique. In this technique haemoglobin was transformed initially to methemoglobin and then and there to cyanmethemoglobin that was calculated colorimetrically. 0.02 millilitre of blood was drawn into five ml of Drabkin's reagent. It was thouroughly shook well and permitted to settle for

600 seconds. Occasionally a gelatin like material was found in the solution caused by the broken cell partition of Red Blood Cells. It was detached by centrifugation. Optimal density was calculated at 540 nm. By means of a profitable cyanomethemoglobin standard, a standard graph was arranged so that the values of Haemoglobin were read straight and recorded as gram per decilitre.

4.2.3.1.4 White blood corpuscles (WBC) count

Blood was placed in phials that impregnated with ethele diamine tetra acetic acid. The blood was filled up to 0.5 marks of White Blood Cell tube and instantly thinned fluid, Turk's 50 solution was filled up to l1 inscriptions directly above the bulb. Solution was shaken painstakingly and was permitted to settle for 120 seconds. A drop of fluid from the solution was permitted to run below the cover slip. It was permitted to settle for 120 seconds and the WBCs were calculated in the 4 conner sq mm. The amount of white blood cells/mm^3 per was measured consequently.

$$\frac{\text{Number of cells x Dilution factor x Depth factor}}{\text{Area counted}}$$

(MAFF, 1984)

4.2.3.1.5 Determination of red blood cell constants

Constructed from the outcomes of the tests which determine total RBC, Hb and PCV, a number of measurements have been derivative which give quantifiable evidence around the red blood corpuscles. The calculative values are MCV, MCH and MCHC are called RBC constants. They were calculated using standards formulae according to MAFF (1984).

4.2.3.1.5.1 Mean corpuscular volume (MCV)

MCV is the volume of the average cell or the average cell volume of all the Red Blood Cell.

$$\text{Mean Corpuscular Volume} = \frac{\text{PCV x 10}}{\text{Red Blood Cell}} \quad (\mu^3) \qquad (1)$$

4.2.3.1.5.2 Mean corpuscular haemoglobin (MCH)

The MCH is the quantity of Haemoglobin in the average Red Blood Cell or average quantity of Haemoglobin in a cell in all the red cells.

$$\text{Mean Corpuscular Haemoglobin} = \frac{\text{Hb x 10}}{\text{RBC}} \quad \text{(pg)} \qquad (2)$$

4.2.3.1.5.3 Mean corpuscular haemoglobin concentration (MCHC)

MCHC is the share of the average red blood cell comprising haemoglobin or the concentration in the average cell.

$$\text{Mean corpuscular Haemoglobin Concentration} = \frac{\text{Hb (g/dl) X 100}}{\text{PCV (\%)}} \quad \text{(\%)} \qquad (3)$$

4.2.4 Histopathological evaluation

Tissue samples from liver, gill, kidney and intestine of experimental fish were collected and preserved in bouin fluid for histopathological analysis.

4.2.4.1 Slide preparation for sections

Chromic acid was used to wash new slide and then followed by comprehensive rinsing with diluted NaOH, distilled water and then desiccated in a hot air oven.

4.2.4.2 Preparation of paraffin sections

The harvested tissues were fixed and embedded in paraffin wax for histological evaluation. The tissues were carefully not over fixed. Section of about 5 micron thick were cut from the paraffin embedded tissue and floated in a hot water bath. The flattened sections were mounted on clean glass slides and heat fixed.

4.2.4.3 Haematoxylin and eosin (H and E) staining

Sections already mounted on glass slide were stained with haematoxylin and eosin stain. Staining was carried out by initially dewaxing by means of xylene and dehydrated by descending grade of 90.0% and 70.0% rankings of ethanol and then dipped in water. This procedure was followed by standard staining process as styled by Bancroft and Gamble (2007).

4.2.5 Analysis of data

Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

4.3 Result

4.3.1 Water quality parameters

The water quality factors measured revealed a mean of pH 7.1± 0.0, temperature 28.4 ±1.2 ^{o}C, dissolved Oxygen 5.53 ± 0.02 mgL^{-1}, ammonia concentration of 0.0 ± 0.0, Nitrite of 0.01±0.0 and Total dissolved solid of 70.67±5.46.

4.3.2 Hematological parameters

The outcomes on blood characteristics factors of trial African Catfish juveniles fed *Allium sativum*, *Chromolaena odorata* and *Talinum triangulare* as feed additives were shown in Tables 4.1, 4.2, 4.3 and figures (4.1-4.5). The results of hematocrit value, hemoglobin concentration, erythrocytes count (RBCs) leukocytes (WBCs), lymphocytes, MCV, MCH, and MCHC are summarized as follows. In the group of fish nourished with *Allium sativum* and the group of fish fed *Chromolaena odorata*, the hematocrit value and hemoglobin concentrations showed similar result ($P>0.05$) as shown in Table 4.1 and Table 4.2 respectively. However, a noteworthy increase ($P<0.05$) in hematocrit value and a significant increase ($P<0.01$) in Hb concentration were detected in group of fish nourished with 10g/kg *Talinum triangulare* (G_3B) when compared with initial values in the group of fish treated with *Talinum triangulare* (Table 4.3). Comparing the values amid the groups, the uppermost hematocrit value was observed in the G_3B group (34.67 ± 0.67%) while the lowest value was recorded in G_2B (23.67 ± 1.45%) and the initial value was 23.03±2.08% as shown in figure 4.1. For haemoglobin concentration, when comparison was made between the groups, G_3B increased meaningfully ($P<0.01$) when likened with initial values nevertheless there was a decrease noteworthy difference ($P<0.01$) in G_1B, G_1C, G_2A, G_2B, G_3A treatments when compared with the G_3B (Figure 4.1). Erythrocytes count decrease meaningfully ($P<0.05$) in fish nourished on 10g/kg of *A. sativum* equated with control in the group of fish fed *A. sativum* (Table 4.1). A non- significant ($P>0.05$) result showed in erythrocytes count within the group of fish fed *C. odorata*, however highest value of 3.42 x 10^6 µl was observed in G_2C (Table 4.2). A decrease significant difference ($P<0.05$) was noticed in erythrocytes count of G_3A and G_3C in the group of fish nourished with *Talinum*

triangulare (Table 4.3). Amongst the clusters, the erythrocytes count increase significantly in G_1B compared with G_2C and G_3B and G_3C decreased significantly ($P<0.05$) compared with G_2C and G_3B (figure 4.2). The result of erythrocyte indices showed the average values of MCV and MCH of fish nourished on ration containing 10g/kg *A. sativum* improved significantly ($P<0.01$) when likened with the fish whose diet containing no herbal extracts (i.e CC, control group) as shown in Table 4.1. No substantial difference ($P>0.05$) was detected in MCV and MCH of fish fed diet containing *C. odorata* however highest value was observed in G_2B (111.0 ± 6.75 fl) and lowest value in G_2A (87.82 ± 1.20 fl) for MCV and highest value of 34.62 ± 2.14 pg in G_2B and lowest value of 28.12 ± 1.71 pg in G_2C for MCH (Table 4.2). Furthermore, the group of fish fed on diets containing 30g/kg *Talinum triangulare* additives (G_3C) showed increase noteworthy difference ($P<0.01$) in the average values of MCV and MCH when likened with control group (Table 4.3). Comparison between groups showed that the Mean values of MCV of G_2A, G_2C, G_3B decreased significantly ($P<0.01$) when compared with mean value of MCV in fish fed on diet containing 30g/kg *Talinum triangulare* additives, G_3C (figure 4.3). More so, mean value of MCH of G_1B improved meaningfully ($P<0.01$) when equated with mean values of MCH of G_2A and G_2B and mean values of MCH of G_2A and G_2C decrease significantly ($P< 0.01$) compared with mean values of G_3C as shown in figure 4.3. A non-significantly different ($P>0.05$) average values of MCHC was detected within and between all the treatment groups when compared with the control group during the experiment. However, the uppermost values of 32.83 ± 0.82% (G_1A), 33.04 ± 1.15% (G_2A), 34.71 ± 0.34% (G_3B) and lowest value of 31.46 ± 0.98% (CC), 31.22 ± 1.13% (G_2B) and 31.46 ± 0.98% (CC) for MCHC were detected in the groups of fish nourished with *A. sativum. C. odorata* and *Talinum triangulare* feed additives respectively (figure 4.3).

A significant increase ($P<0.05$) in the leukocyte counts of fish nourished with a ration incorporated with 5g/kg garlic (G_1A) when likened with the control group was noticed. Within the groups, leukocyte counts in G_1A increased significantly ($P<0.01$) when compared with fish fed a diet containing 10g/kg (G_1B) and 30g/kg, G_1C (Table 4.1). Within the groups of fish fed with *Chromolaena odorata* and *Talinum triangulare* no substantial alteration was detected in leukocytes count when likened with control but G_2B, G_2C and G_2A showed decrease significant ($P<0.05$) when likened with initial values (Table 4.2) and

leukocytes count of G_3B, G_3A and G_3C decrease meaningfully ($P< 0.01$) when compared with initial values (Table 4.3) respectively. Comparison among the groups showed that leukocyte count of fish nourished with ration mixed with 5g/kg *A. sativum* (G_1A) was meaningfully increased ($P<0.01$) when compared with G_2A, G_2B and G_2C and also G_3A, G_3B and G_3C ($P <0.001$) as shown in (figure 4.4). Lymphocyte counts were similar ($P>0.05$) in group of fish fed *A. sativum* (Table 4.1) and group of fish fed *C. odorata* however highest value of lymphocyte counts 69.67±1.80% was documented in control group and lowermost value of 57.67±4.37% in G_1A (Table 4.1) and lowest value of 59.33±2.33 % was documented in G_2C of the group nourished with *C. odorata* (Table 4.2). In the group of fish nourished with *Talinum triangulare*, a decrease significant ($P <0.001$) value in lymphocyte counts was observed in group of fish fed a ration incorporated with 5g/kg (G_3A) compared with the control group (Table 4.3). Generally, among the groups, lymphocyte count improved meaningfully ($P<0.05$) in fish nourished on ration containing G_1B, G_1C, G_3B and G_3C when likened with fish nourished on diets containing G_3A doses (figure 4.5). A non-significant difference ($P>0.05$) in the number of monocytes, neutrophils and eosinophils was detected within all the groups however the group of fish nourished with 30g/kg of *C. odorata* (G_2C) revealed an increased noteworthy difference ($P<0.05$) compared with the group of fish nourished with10g/kg of *Talinum triangulare* (G_3B) as feed additives (figure 4.5).

Table 4.1. Comparative haematological parameters of different African catfish fingerlings fed diets containing different levels of *Allium sativum* (mean ± SEM)

Parameters	Initial value	Control	*Allium sativum*		
			G_1A	**G_1B**	**G_1C**
PCV (%)	23.03 ± 2.082[a]	30.0 ±1.528 [a]	28.33 ± 5.175 [a]	24.33 ± 0.667[a]	24.33 ± 0.882 [a]
Hb (g/dl)	7.53 ± 0.742 [a]	9.37 ± 1.856 [a]	9.37 ± 0.384 [a]	7.97 ± 0.384[a]	7.97 ± 0.433 [a]
RBC(x 10^6µl)	2.12 ± 0.643 [a]	3.35 ± 0.174 [a]	2.71 ± 0.597[a]	1.76 ± 0.306[b]	2.03 ± 0.203[a]
WBC (x 10^3µl)	20.75 ±0.226[a]	16.52 ± 0.956 [a]	22.87 ± 0.232 [b]	15.1 ± 1.563[a]	12.17 ± 0.882[ac]
LYMPH	59.67 ± 3.283 [a]	69.67 ± 1.801 [a]	57.67 ± 4.372 [a]	64.00 ± 3.786[a]	69.00 ±0.577 [a]
HET	34.0 ± 2.65 [a]	25.0 ±1.86 [a]	34.0 ±4.37 [a]	30.0 ±3.79 [a]	23.3 ±3.79 [a]
MCV (fl)	120.9 ± 21.07[a]	90 .40 ±5.18[a]	106.9 ±5.76[a]	144.8 ±19.66[b]	121.2 ±7.81[a]
MCH (pg)	39.42 ± 6.54 [a]	28.32 ±1.43 [a]	35.05 ±1.44 [a]	47.16 ±5.90[b]	39.53 ±1.78 [a]
MCHC (g/dl)	32.72 ± 0.46 [a]	31.46 ± 0.98 [a]	32.83 ±0.82 [a]	32.70 ±0.71 [a]	32.70 ±0.70 [a]

Variables are PCV (packed cell volume), Hb (haemoglobin), RBC (red blood cell), WBC (white blood cell), LYMPH (lymphocyte), HET (heterophils), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), and MCHC (mean corpuscular haemoglobin concentration). Mean±standard deviation of data for triplicate groups with different scripts in the same row differ significantly (One way Anova and Tukey's multiple range test, α0.05). G1 =Group of fish fed with Allium sativum additives (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively).

Table 4.2. Comparative haematological parameters of different African catfish fingerlings fed diets containing different levels of *Chromolaena odorata* (mean ± SEM)

			Chromolaena odorata		
Parameters	**Initial value**	**Control**	**G_2A**	**G_2B**	**G_2C**
PCV (%)	23.03 ± 2.082[a]	30.0 ±1.528 [a]	26.33 ± 1.453 [a]	23.67 ± 1.453 [a]	30.33 ±0.667 [a]
Hb (g/dl)	7.53 ± 0.742 [a]	9.37 ± 1.856 [a]	8.47 ± 0.176 [a]	7.40 ± 0.611 [a]	9.60 ±0.379 [a]
RBC(x 10^6µl)	2.12 ± 0.643 [a]	3.35 ± 0.174 [a]	3.00 ± 0.199 [a]	2.16 ± 0.258 [a]	3.42 ±0.080 [a]
WBC (x 10^3µl)	20.75 ±0.226[a]	16.52 ± 0.956 [a]	14.13 ± 1.272 [b]	14.38 ± 1.653 [b]	14.37 ±1.013[b]
LYMPH	59.67 ± 3.283 [a]	69.67 ± 1.801 [a]	60.00 ± 2.517 [a]	60.00 ± 1.155 [a]	59.33 ± 2.333 [a]
HET	34.0 ± 2.65 [a]	33.0 ±1.86 [a]	33.0 ±3.22 [a]	33.7 ±0.88 [a]	34.3 ±2.19 [a]
MCV (fl)	120.9 ± 21.07 [a]	90 .40 ±5.18 [a]	87.82 ±1.20 [a]	111.0 ±6.75 [a]	88.77 ±3.74 [a]
MCH (pg)	39.42 ± 6.54 [a]	28.32 ±1.43 [a]	29.04 ±1.37 [a]	34.62 ±2.14 [a]	28.12 ±1.71 [a]
MCHC (g/dl)	32.72 ± 0.46 [a]	31.46 ± 0.98 [a]	33.04 ±1.15 [a]	31.22 ±1.13 [a]	31.67 ±1.33 [a]

Variables are PCV (packed cell volume), Hb (haemoglobin), RBC (red blood cell), WBC (white blood cell), LYMPH (lymphocyte), HET (heterophils), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), and MCHC (mean corpuscular haemoglobin concentration). Mean±standard deviation of data for triplicate groups with different scripts in the same row differ significantly (One way Anova and Tukey's multiple range test, α0.05). G_2 =Group of fish fed with *Chromolaena odorata* additives (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively).

Table 4.3. Comparative haematological parameters of different African catfish fingerlings fed diets containing different levels of *Talinum triangulare* (mean=SEM)

			Talinum triangulare		
Parameters	**Initial value**	**Control**	**G_3A**	**G_3B**	**G_3C**
PCV (%)	23.03 ± 2.082[a]	30.0 ±1.528 [a]	24.00 ± 1.155[a]	34.67 ± 0.667[b]	23.67 ±0.333 [a]
Hb (g/dl)	7.53 ± 0.742 [a]	9.37 ± 1.856 [a]	7.93 ± 0.521[a]	12.03 ± 0.260[b]	7.67 ±0.176 [a]
RBC (x 10^6µl)	2.12 ± 0.643 [a]	3.35 ± 0.174 [a]	1.99 ± 0.223[c]	3.54 ± 0.048[a]	1.58 ±0.020[c]
WBC (x 10^3µl)	20.75 ±0.226[a]	16.52 ± 0.956 [a]	12.25 ± 0.983[b]	12.93 ± 0.581[b]	11.55 ±0.293[b]
LYMPH	59.67 ± 3.283 [a]	69.67 ± 1.801 [a]	49.33 ±5.239[b]	70.33 ± 2.186[a]	66.00 ±0.577 [a]
HET	34.0 ± 2.65 [a]	33.0 ±1.86 [a]	33.7 ±0.88 [a]	26.1 ±3.10 [a]	22.1.0 ±0.88 [a]
MCV (fl)	120.9 ± 21.07 [a]	90 .40 ±5.18 [a]	122.5 ±7.95 [a]	97.90 ±2.84 [a]	149.5 ±1.21[b]
MCH (pg)	39.42 ± 6.54 [a]	28.32 ±1.43 [a]	40.37 ±2.38 [a]	33.99 ± 1.15 [a]	48.41 ±0.50[b]
MCHC (g/dl)	32.72 ± 0.46 [a]	31.46 ± 0.98 [a]	33.00 ± 0.61 [a]	34.71 ±0.34 [a]	32.39 ±0.49 [a]

Variables are PCV (packed cell volume), Hb (haemoglobin), RBC (red blood cell), WBC (white blood cell), LYMPH (lymphocyte), HET (heterophils), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), and MCHC (mean corpuscular haemoglobin concentration). Mean±standard deviation of data for triplicate groups with different scripts in the same row differ significantly (One way Anova and Tukey's multiple range test, α0.05). G_3 =Group of fish fed with *Talinum triangulare* additives (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively).

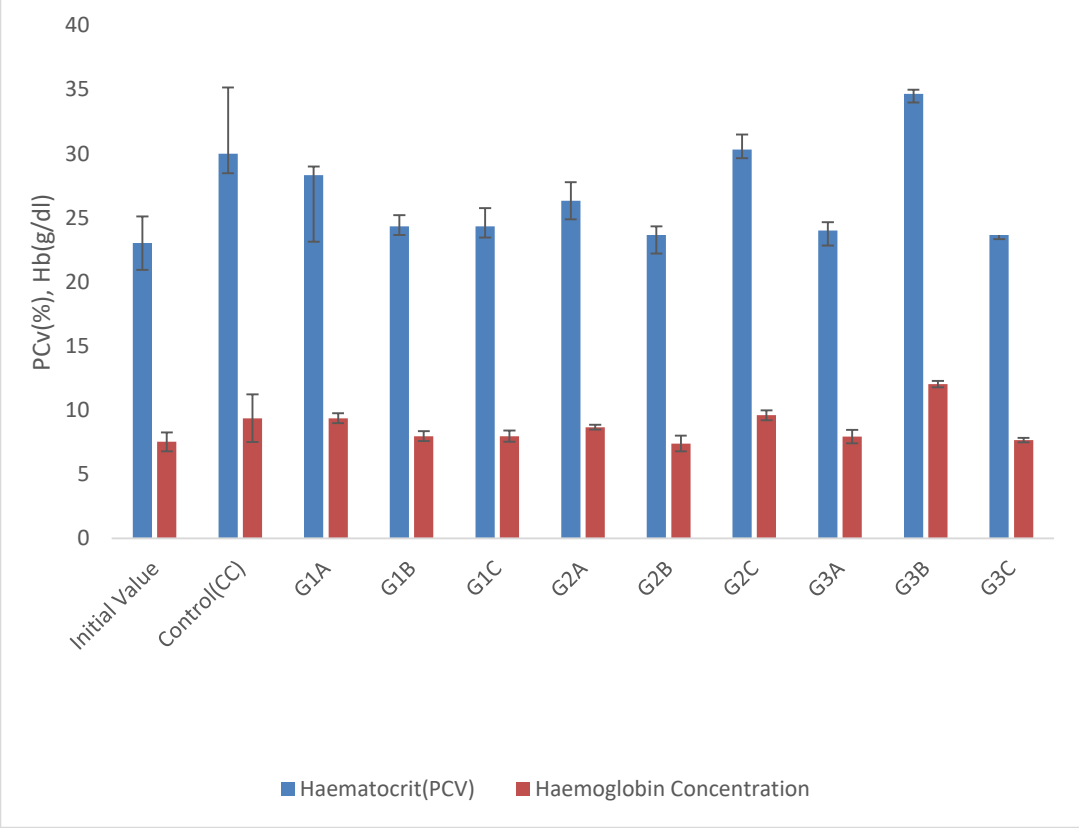


Figure 4.1. Different levels of hematocrit (PCV) and hemoglobin concentrations (Hb) in initial, control and other treated fish. G_1= Group of fish fed with *Allium sativum* additives; $G_{2=}$ Group of fish fed with *Chromolaena odorata* additives; $G_{3=}$ Group of fish fed with *Talinum triangulare* additives. (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively)

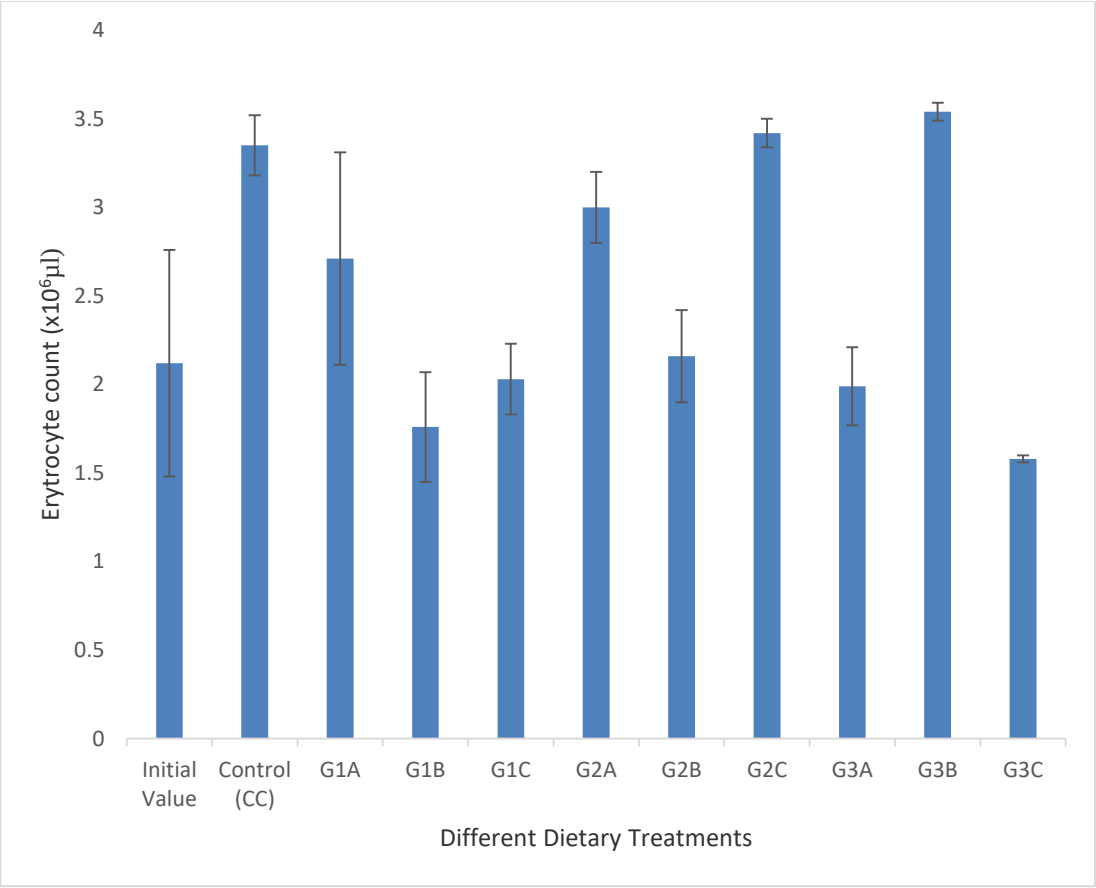


Figure 4. 2. Erytrocytes count (RBC) in initial, control and other treated fish. G_1= Group of fish fed with *Allium sativum* additives; $G_{2=}$ Group of fish fed with *Chromolaena odorata* additives; $G_{3=}$ Group of fish fed with *Talinum triangulare* additives. (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively

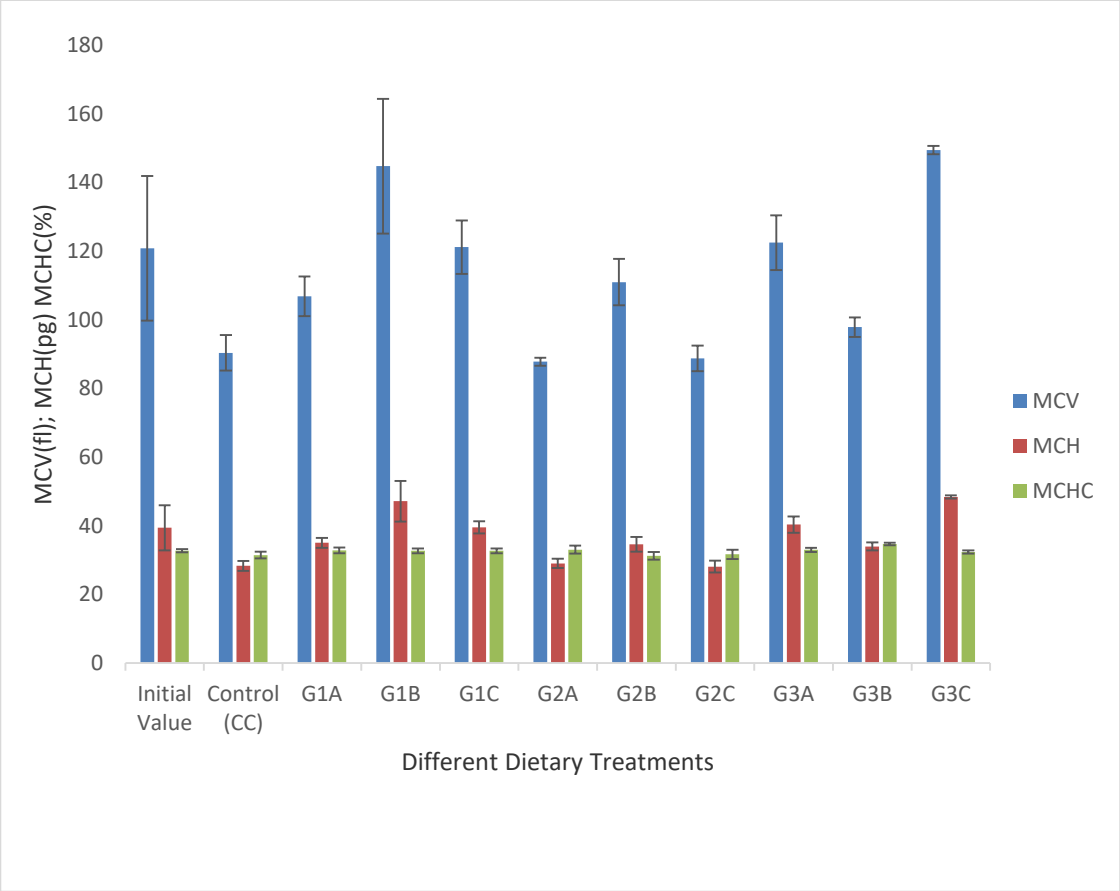


Figure 4.3. Different levels of mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentrations (MCHC) in initial, control and other treated fish.G1= Group of fish fed with *Allium sativum* additives; G2= Group of fish fed with *Chromolaena odorata* additives; G3= Group of fish fed with *Talinum triangulare* additives. (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively).

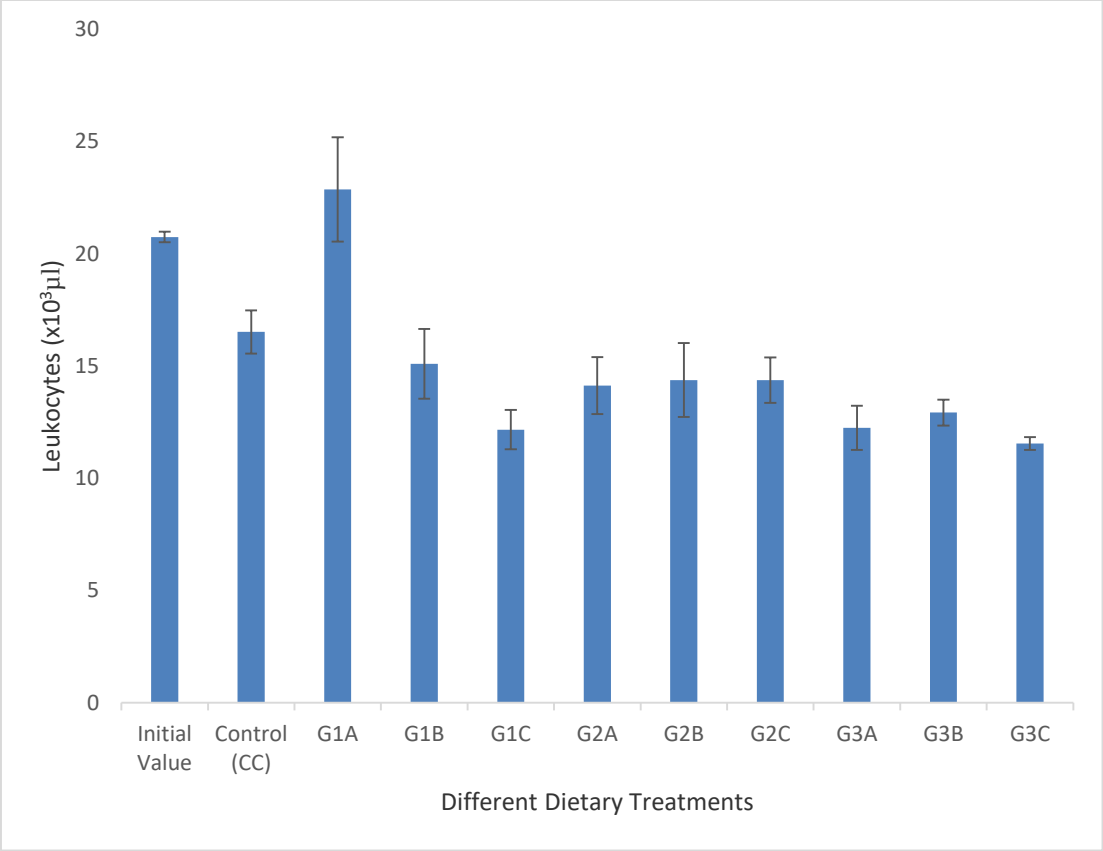


Figure 4.4. Different leukocytes count (WBC) in initial, control and other treated fish. G_1= Group of fish fed with *Allium sativum* additives; $G_{2=}$ Group of fish fed with *Chromolaena odorata* additives; $G_{3=}$ Group of fish fed with *Talinum triangulare* additives. (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively.

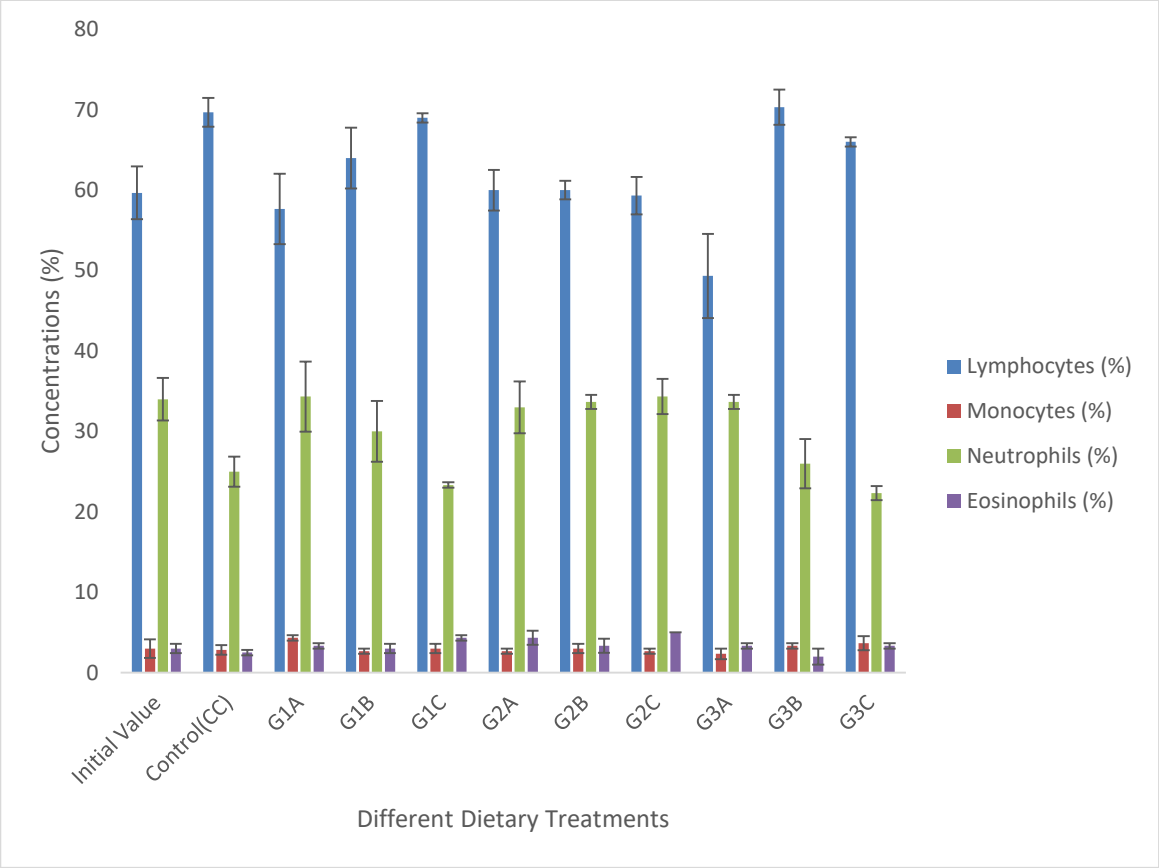


Figure 4.5. Differential leukocytes in initial, control and other treated fish. G_1= Group of fish fed with *Allium sativum* additives; $G_{2=}$ Group of fish fed with *Chromolaena odorata* additives; $G_{3=}$ Group of fish fed with *Talinum triangulare* additives. (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively.

The results of biochemical findings observed in this study were presented in Tables 4.4 - 4.6 and figures 4.6 – 4.8 as follows; the level of total protein detected in group of fish nourished with *A. sativum* feed additives showed an increased significant difference ($P<0.001$) in group fed with 10g/kg *A. sativum* (G_1B) compared with control group (no herbal supplementation) and initial value and more so within the group, G_1B increased significantly compared with G_1A (5g/kg) and G_1C (30g/kg) (Table 4.4). The values of total protein detected in group of fish nourished with *C. odorata* and *Talinum triangulare* were similar ($P>0.05$) however uppermost value of total protein was recorded in G_2B (6.43±0.07mg/dl) and lowest value recorded in G_2C (5.60±0.58 mg/dl) for group fed with *C. odorata* (Table 4.5) while highest value of 6.40±0.12 mg/dl was recorded in G_3B and lowest value of 5.87±0.27 mg/dl was documented in the control of the group fed with *T. triangulare* (Table 4.6). Comparison of total protein across the groups showed that G_1B increased significantly ($P<0.05$) with respect to G_2A, G_2B, G_2C, G_3A, G_3B and G_3C (figure 4.6). The value of Albumin increased meaningfully ($p<0.001$) in G_1B compared with Control group and initial value and more so within the group, G_1B increased significantly ($P<0.05$) when equated with G_1A and G_1C (Table 4.4). The values of Albumin attained in group of fish nourished with *C. odorata* and *T. triangulare* was insignificant ($P>0.05$) and uppermost value of 2.10±0.20 mg/dl were recorded in control of both groups and lowest values of 1.23±0.22 mg/dl in G_2C (Table 4.5) and 1.47±0.04 mg/dl in G_3C (Table 4.6) were recorded in group of fish fed *C. odorata* and *T. triangulare* respectively. Among the three experimental groups, Albumin in G_1B increased meaningfully ($P>0.001$) when equated with G_2A, G_2B, G_2C, G_3A, G_3B and G_3C (figure 4.6). The globulin values in all treatment clusters when compared with the control group revealed no noteworthy difference ($P>0.05$). However, highest value of 4.90 ± 0.0 mg/dl was recorded in G_1B (Table 4.4), highest value of 4.77±0.07mg/dl was recorded in G_2B (Table 4.5) and highest value of 4.87±0.03 mg/dl was recorded in G_3A (Table 4.6) and lowest values of 3.78±0.46 mg/dl was recorded across the three experimental groups. An examination of the A-G ratio showed no substantial difference ($P>0.05$) among the treatment groups (figure 4.6). No noteworthy difference ($P>0.05$) was observed in the values of the glucose within and between the experimental fish groups, however highest values of glucose were recorded in G_1B (309.3 ± 4.70 mg/dl), G_2C (322.7 ± 0.33 mg/dl) and G_3C (298.7 ± 10.91 mg/dl) in group of fish fed with *A.*

sativum , *C. odorata* and *T. triangulare* respectively and lowest values of 279.3±7.62 mg/dl in the control of group of fish fed with *A. sativum* and *C. odorata* and 275.3± 29.41 mg/dl for group of fish fed with *T. triangulare* as shown in figure 4.7. The Cholesterol level of fish fed with 30g/kg (G_1C) and 10g/kg (G_1B) of *A. sativum* feed additives increase significantly ($p<0.001$) when compared with control (no herbal supplementation) and also within the group G_1A decreased significantly ($P>0.01$) compared with G_1B and G_1C (Table 4.4). Decreased significant change ($P<0.001$) was detected in in the cholesterol level of fish fed G_2A, G_2B compared with the control and initial values and G_2B as well decreased significantly ($P<0.01$) when likened with G_2C (Table 4.5). The cholesterol level of fish fed *T. triangulare* also reduced meaningfully ($P<0.001$) when likened with Control group and initial value (Table 4.6). The cholesterol level of fish fed G_1A, G_1B and G_1C also increased meaningfully ($P<0.001$) when equated with G_2A, G_2B, G_2C, G_3A, G_3B, G_3C when compared across the groups (figure 4.7). The actions of serum enzymes (Alanine aminotransferase, ALT) in the group of fish fed with different feed additives were similar ($P>0.05$) within all treatment groups but among the groups G_1B improved meaningfully ($p<0.05$) equated with G_3A (figure 4.8). The activities of serum enzymes (Aspartate aminotransferase, AST) were similar in fish fed with *A. sativum* (Table 4.4) and in fish fed with *C. odorata* (Table 4.5) however an increased significant difference ($P<0.01$) was detected in G_3A fish fed with *T. triangulare* when likened with control and initial value (Table 4.6). Across the experimental groups, G_1A decreased significantly ($P<0.05$) when compared the actions of serum enzymes (Aspartate aminotransferase, AST) with G_3A group (figure 8). There was no significant difference ($P>0.05$) in the actions of serum enzymes (Alkaline phosphatase, ALP) in the group of fish fed with *A. sativum* when likened with control nevertheless G_1B showed an increased significant ($P<0.05$) when equated with initial value (Table 4.4). ALP activities increased significantly in the fish group nourished on G_2A and G_2C when compared with control and initial value (Table 4.5) and also G_2A increased significantly ($P<0.05$) when likened with G_2B within the cluster. There was a significant difference ($P<0.01$) in the activities of serum enzyme, Alkaline phosphatase (ALP) in the group of fish nourished with G_3A compared with control and G_3B and initial value in group of fish nourished with *T. triangulare* (Table 4.6). The activities of ALP when compared between the groups, G_1A, G_1B and G_1C decreased significantly ($P<0.01$)

when compared with G_2A, G_2C, G_3A and G_2A improved meaningfully ($P<0.01$) with G_2B, G_3B, G_3C and also G_2B increased meaningfully ($P<0.01$) with G_3B, G_3C and G_2C increased significantly ($P<0.01$) when likened with G_3B, G_3C and G_3A (figure 4.8).

Table 4.4. Comparative serum biochemical parameters of different African catfish fingerlings fed diets containing different levels of *Allium sativum* (mean ± SEM)

Biochemical Parameters	Initial value	Control	*Allium sativum*		
			G_1A	G_1B	G_1C
Total Protein	5.83 ± 0.24[a]	5.87 ± 0.27 [a]	6.07 ± 0.82 [a]	8.63 ± 0.09 [b]	5.23 ±0.48 [a]
Albumin	1.93 ± 0.24 [a]	2.10 ± 0.20[a]	1.83 ± 0.48 [a]	3.73 ± 0.09 [b]	1.13 ± 0.18 [a]
Globulin	3.90 ± 0.00 [a]	3.78 ± 0.46 [a]	4.23 ± 0.33 [a]	4.90 ± 0.00 [a]	4.47 ± 0.29 [a]
A-G Ratio	0.47 ± 0.7 [a]	0.50 ± 0.11 [a]	0.23 ± 0.07 [a]	0.70 ± 0.00 [a]	0.33 ± 0.13 [a]
AST	186.0 ± 6.56 [a]	197.00 ± 9.63 [a]	187.00 ±4.93 [a]	221.33 ± 1.45 [a]	232.33 ±25.46[a]
ALT	27.33 ± 1.86 [a]	21.67 ± 2.99[a]	25.67 ± 3.28 [a]	35.67 ± 2.91 [a]	20.67 ±0.33[a]
ALP	189.00 ± 2.89 [a]	219.70 ± 26.60 [a]	202.33 ± 4.49 [a]	203.33 ± 4.91[b]	237.7 ±55.34 [a]
Cholesterol	184.33 ± 2.91 [a]	165.33 ± 6.78 [a]	180.7 ± 4.49 [a]	209.70 ± 2.40 [b]	215.00 ± 3.06 [b]
Glucose	290.00 ± 3.22 [a]	279.33 ± 7.76 [a]	298.33 ± 0.33 [a]	309.33 ± 4.70 [a]	282.00 ± 16.56 [a]

Variables are Total protein, Albumin, Globulin, A-G Ratio (Albumin- Globulin Ratio), AST (Aspartate transeaminase), ALT (Alkaline transeaminase) ALP (Alkaline phosphatase). Mean±standard deviation of data for triplicate groups with different scripts in the same row differ significantly (One way Anova and Tukey's multiple range test, α0.05). G_1= Group of fish fed with *Allium sativum* additives (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively).

Table 4.5. Comparative serum biochemical parameters of different African catfish fingerlings fed diets containing different levels of *C. odorata* (mean ±SEM)

			Chromolaena odorata		
Biochemical Parameters	**Initial value**	**Control**	**G_2A**	**G_2B**	**G_2C**
Total Protein	5.83 ± 0.24[a]	5.87 ± 0.27 [a]	6.03 ± 0.27[a]	6.43 ± 0.07[a]	5.60 ± 0.58[a]
Albumin	1.93 ± 0.24 [a]	2.10 ± 0.20[a]	1.37 ± 0.09 [a]	1.57 ± 0.03 [a]	1.23 ± 0.22 [a]
Globulin	3.90 ± 0.00 [a]	3.78 ± 0.46 [a]	4.57 ± 0.15 [a]	4.77 ± 0.07 [a]	4.17 ± 0.20 [a]
A-G Ratio	0.47 ± 0.7 [a]	0.50 ± 0.11 [a]	0.27 ± 0.03 [a]	0.30 ± 0.00 [a]	0.27 ± 0.03 [a]
AST	186.0 ± 6.56 [a]	197.00 ± 9.63 [a]	258.7 ± 33.3 [a]	217.00 ± 16.26 [a]	241.33 ± 16.22 [a]
ALT	27.33 ± 1.86 [a]	21.67 ± 2.99[a]	19.33 ± 0.67 [a]	23.33 ± 1.76 [a]	20.33 ± 1.45 [a]
ALP	189.00 ± 2.89 [a]	219.70 ± 26.60 [a]	466.33 ±18.41[bc]	318.7 ± 4.41[b]	357.33 ± 25.86[bc]
Cholesterol	184.33 ± 2.91 [a]	165.33 ± 6.78 [a]	135.33 ± 0.67[bc]	112.33 ± 2.33 [b]	144.33 ± 0.33[bc]
Glucose	290.00 ± 3.22 [a]	279.33 ± 7.76 [a]	272.33 ± 23.07 [a]	256.00 ± 18.90[a]	322.7 ± 0.33 [a]

Variables are Total protein, Albumin, Globulin, A-G Ratio (Albumin- Globulin Ratio), AST (Aspartate transeaminase), ALT (Alkaline transeaminase) ALP (Alkaline phosphatase). Mean±standard deviation of data for triplicate groups with different scripts in the same row differ significantly (One way Anova and Tukey's multiple range test, α0.05). $G_{2=}$ Group of fish fed with *Chromolaena odorata* additives (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively).

Table 4.6: Comparative serum biochemical parameters of different African catfish fingerlings fed diets containing different levels of *Talinum triangulare* (mean ± SEM)

			Talinum triangulare		
Biochemical Parameters	**Initial value**	**Control**	**G_3A**	**G_3B**	**G_3C**
Total Protein	5.83 ± 0.24[a]	5.87 ± 0.27 [a]	6.37 ± 0.17 [a]	6.40 ± 0.12 [a]	6.23 ± 0.07[a]
Albumin	1.93 ± 0.24 [a]	2.10 ± 0.20[a]	1.50 ± 0.15 [a]	1.63 ± 0.08 [a]	1.47 ± 0.09 [a]
Globulin	3.90 ± 0.00 [a]	3.78 ± 0.46 [a]	4.87 ± 0.03 [a]	4.73 ± 0.03[a]	4.77 ± 0.03 [a]
A-G Ratio	0.47 ± 0.7 [a]	0.50 ± 0.11 [a]	0.27 ± 0.03 [a]	0.33 ± 0.03 [a]	0.27 ± 0.03 [a]
AST	186.0 ± 6.56 [a]	197.00 ± 9.63 [a]	286.33 ± 27.67[b]	225.00 ± 7.64 [a]	233.70 ± 6.84 [a]
ALT	27.33 ± 1.86 [a]	21.67 ± 2.99[a]	18.67 ± 2.33 [a]	27.00 ± 7.21 [a]	22.00 ± 3.06 [a]
ALP	189.00 ± 2.89 [a]	219.70 ± 26.60 [a]	362.7 ± 9.06[b]	190.33 ± 4.33[a]	160.00 ± 4.62 [a]
Cholesterol	184.33 ± 2.91 [a]	165.33 ± 6.78 [a]	134.33 ± 4.98 [b]	127.7 ± 1.45 [b]	125.7 ± 6.33 [b]
Glucose	290.00 ± 3.22 [a]	279.33 ± 7.76 [a]	295.00 ± 31.19[a]	275.33 ± 29.4 [a]	298.7 ± 10.91[a]

Variables are Total protein, Albumin, Globulin, A-G Ratio (Albumin- Globulin Ratio), AST (Aspartate transeaminase), ALT (Alkaline transeaminase) ALP (Alkaline phosphatase). Mean±standard deviation of data for triplicate groups with different scripts in the same row differ significantly (One way Anova and Tukey's multiple range test, α0.05). $G_{3=}$ Group of fish fed with *Talinum triangulare* additives (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively).

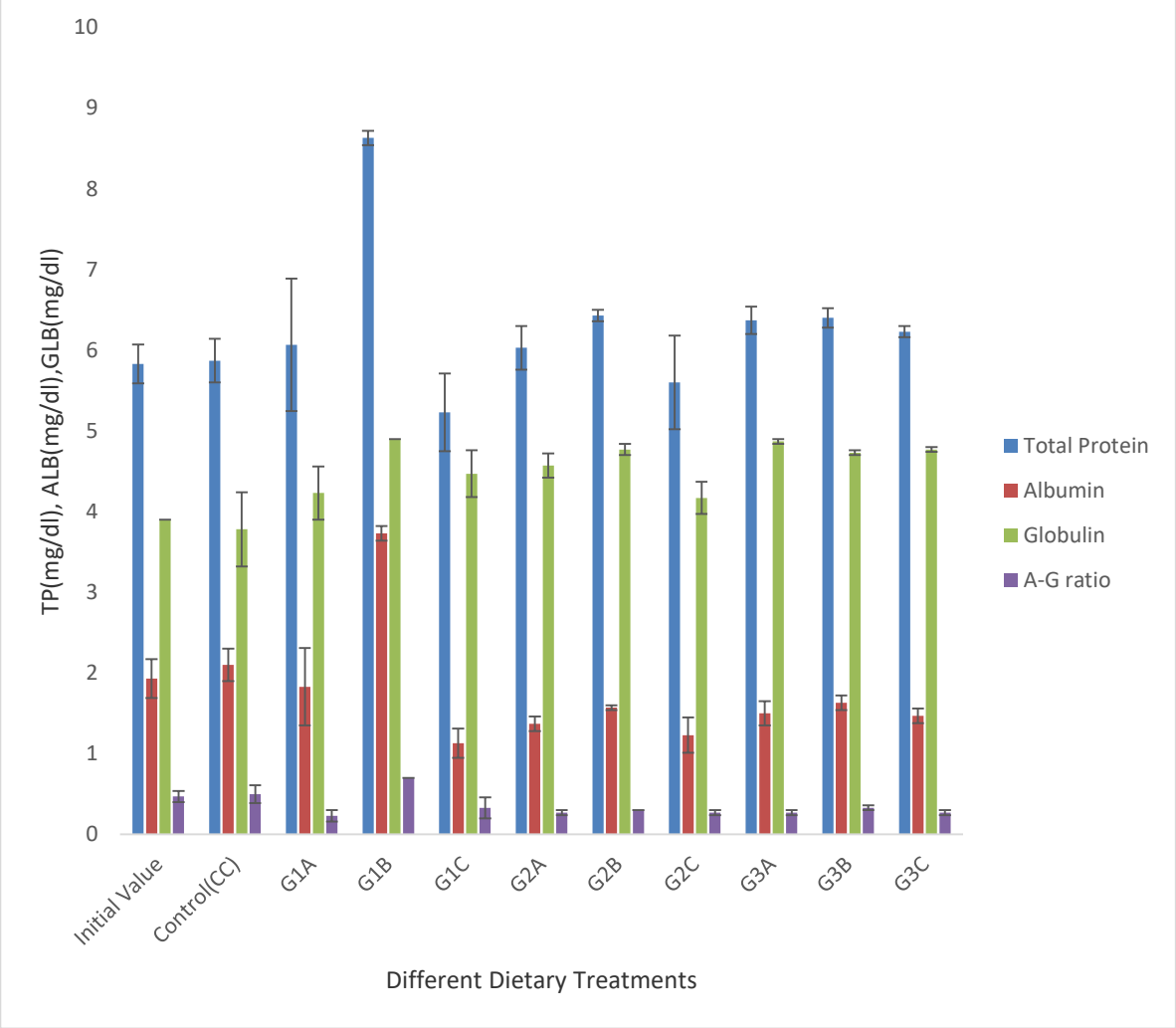


Figure 4.6. Different levels of total protein, albumin, globulin and albumin - globulin ratio (A-G) in initial, control and other treated fish. G1= Group of fish fed with *Allium sativum* additives; G2= Group of fish fed with *Chromolaena odorata* additives; G3= Group of fish fed with *Talinum triangulare* additives. (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively).

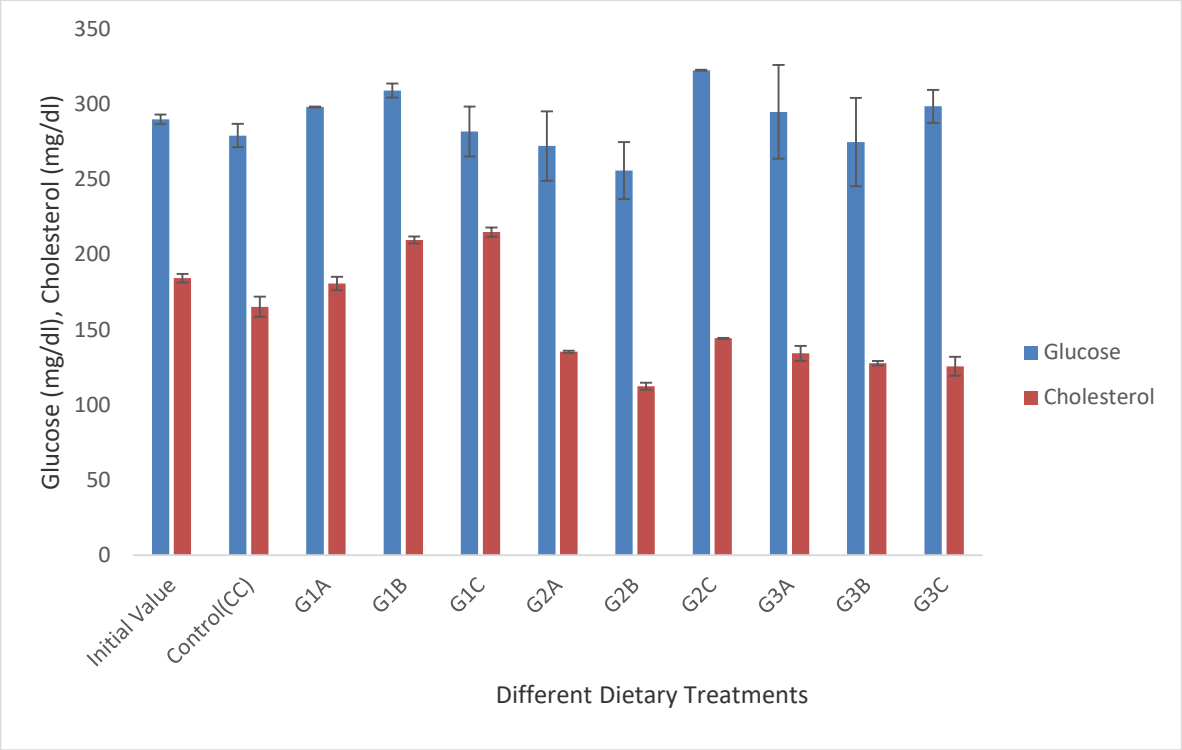


Figure 4.7. Different glucose and cholesterol levels in initial, control and other treated fish. G1= Group of fish fed with *Allium sativum* additives; G2= Group of fish fed with *Chromolaena odorata* additives; G3= Group of fish fed with *Talinum triangulare* additives. (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively).

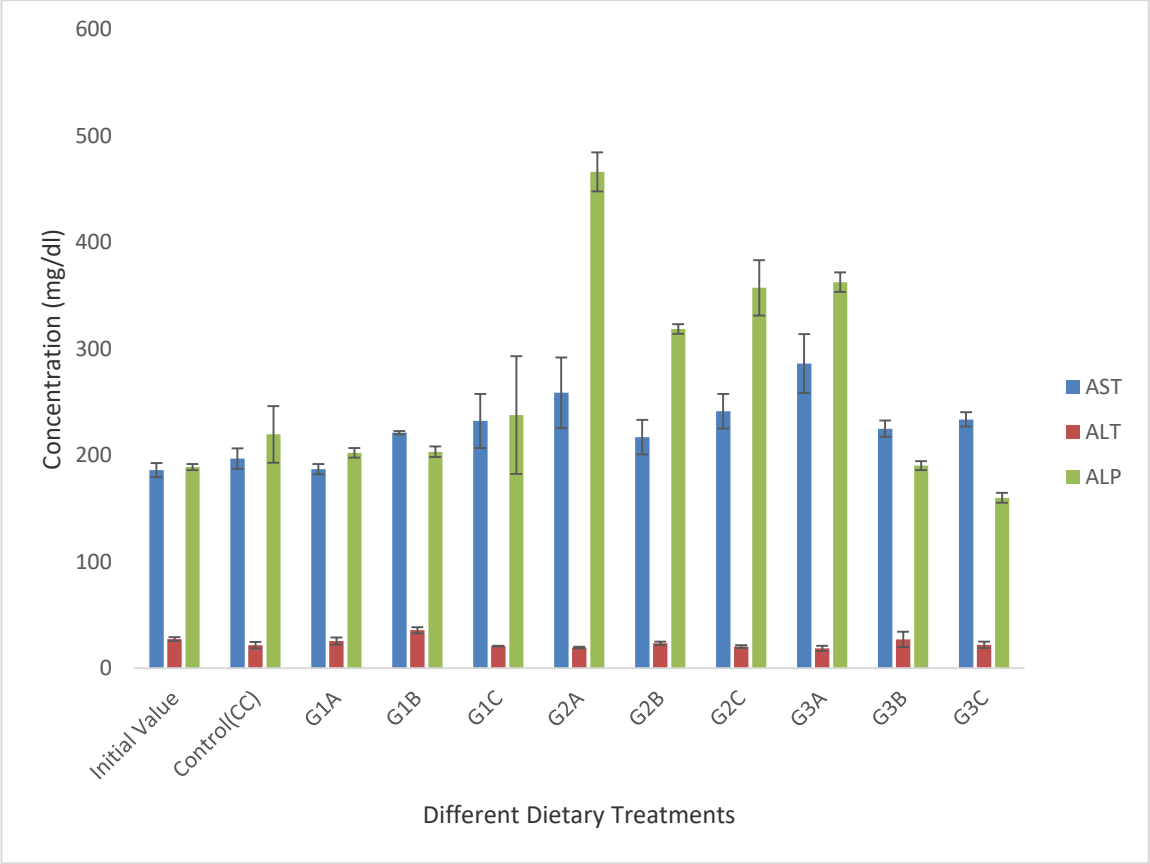


Figure 4.8. Different levels of serum enzymes activities in initial, control and other treated fish. G_1= Group of fish fed with *Allium sativum* additives; $G_{2=}$ Group of fish fed with *Chromolaena odorata* additives; $G_{3=}$ Group of fish fed with *Talinum triangulare* additives. (A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively).

4.3.3 Histopathological examination

Examination of different organs (gill, intestine, liver and kidney) of fish in control group that were fed with basic ration without herbal additives histologically showed no observable lesion (Plate 4.1). Gills, Liver, Intestine and Kidney of group of fish fed on 5g/kg of *A. sativum* (G_1A) showed no observable lesion (Plate 4.2). Histopathology of Intestine and Kidney of group fish fed on 10g/kg of *A. sativum* (G_1B) as shown in Plate 4.3 showed no observable lesion however the gill showed mild lamellae hyperplasia while random necrosis of hepatocytes was observed in the Liver. The intestine and gill of group of fish fed with 30g/kg of *Allium sativum* (G_1C) showed no observable lesion while diffuse swelling of hepatocytes was detected in the group and kidney revealed a patchy tubular epithelia necrosis (Plate 4.4). Intestine of the group of fish fed on 5g/kg of *C. odorata* (G_2A) showed vacuolar degeneration of enterocytes and marked lamellae hyperplasia of the gill. The kidney showed diffuse tubular epithelial necrosis and atrophy of tubules while diffuse swelling of hepatocytes was observed in the liver (Plate 4.5). No observable lesion in the intestine and kidney of group of fish fed on 10g/kg of *C. odorata* (G_2B), the gill showed moderate lamellae hyperplasia, however, diffuse degeneration and multifocal necrosis of hepatitis was observed in the liver (Plate 4.6). The group of fish fed on 30g/kg of *C. odorata* (G_2C) showed no observable lesion in intestine and kidney, moderate diffuse lamella hyperplasia in gill and liver showed diffuse hepatocellular degeneration and necrosis (Plate 4.7). Within the cluster of fish nourished on different levels of *T. triangulare* as feed additives, no lesion was detected in the intestine and kidney of fish fed with 5g/kg (G_3A) however, marked lamellae hyperplasia and diffuse swelling of hepatocytes were observed in the gill and the liver respectively (Plate 4.8). Intestine and Kidney of group of fish fed on 10g/kg of *T. triangulare* (G_3B) showed no observable lesion and a mild lamellae hyperplasia was observed in the gill while the liver showed diffuse vacuolation of hepatocytes (fat accumulation) which was regarded as normal (Plate 4.9). The group of fish fed on 30g/kg of *T. triangulare* (G_3C) showed no observable lesion in intestine, moderate diffuse lamella hyperplasia in gill and liver showed diffuse swelling of hepatocytes while diffuse tubular epithelia necrosis was observed in the group (Plate 4.10)

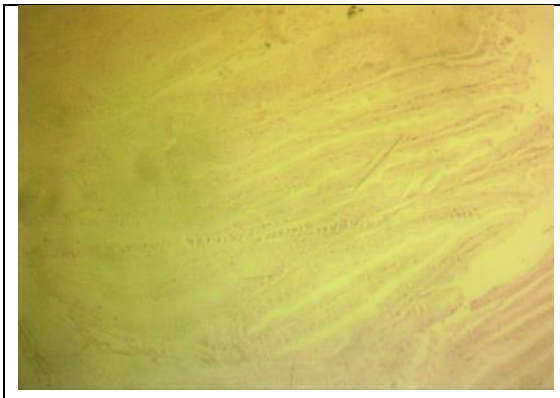
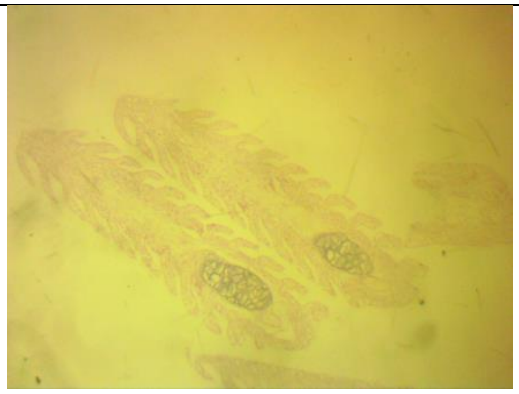
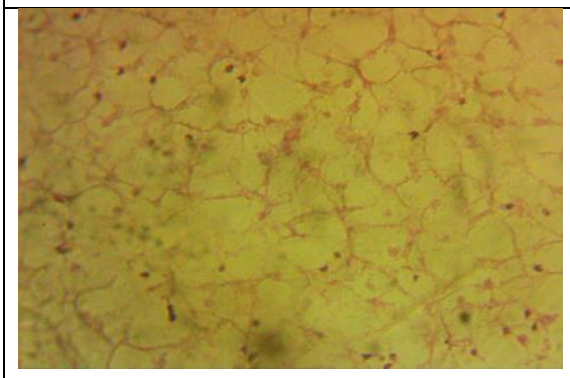
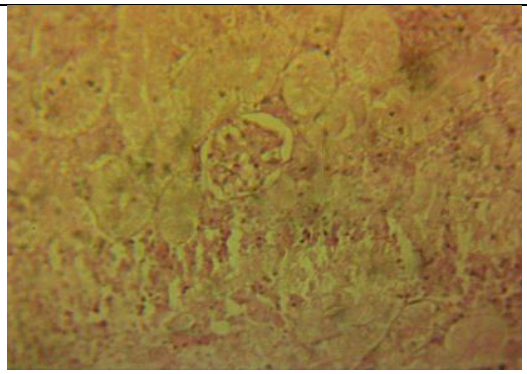
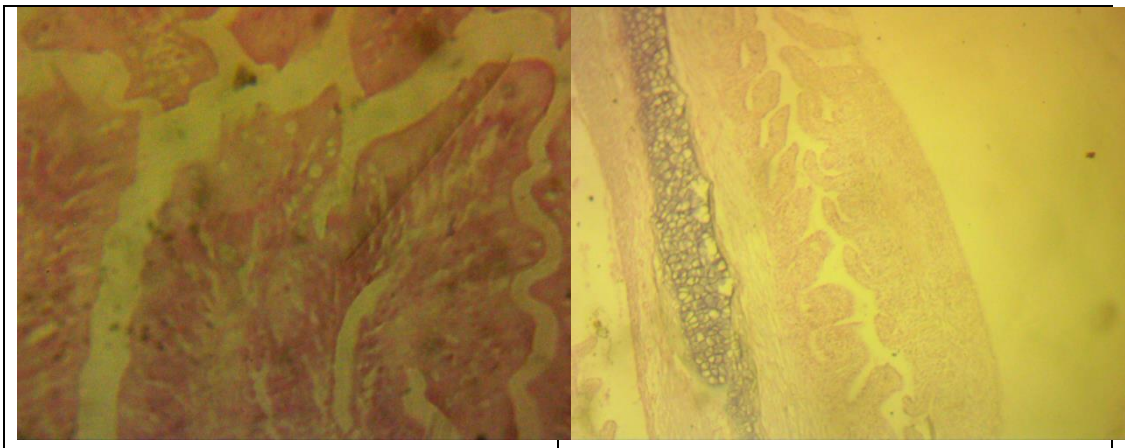
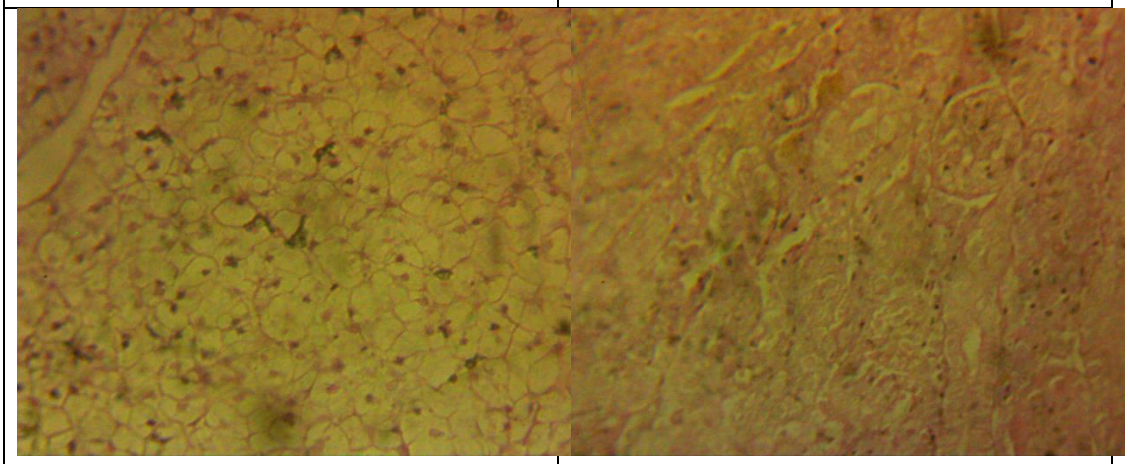
	
Intestine- no observable lesion	Gill- no observable lesion
	
Liver- no observable lesion (fat accumulation)	Kidney- no observable lesion

Plate 4.1. Histopathology of intestine, gill, liver and kidney of representatives of fish fed with 0.0g/kg feed additives (control). (H and E) 400 x



Intestine- no observable lesion

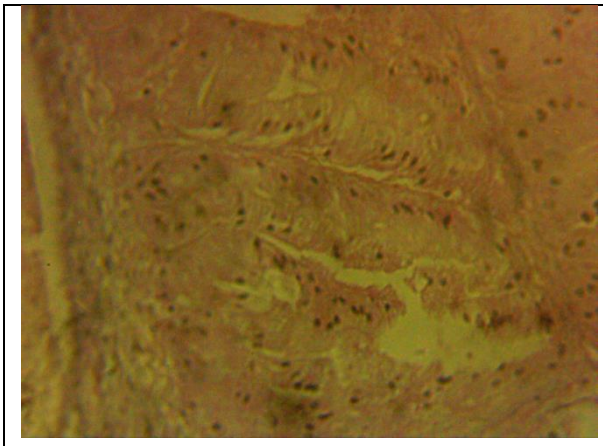
Gill- no observable lesion



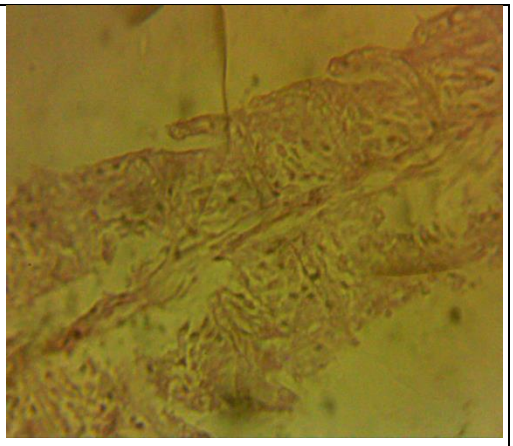
Liver- no observable lesion

Kidney- no observable lesion

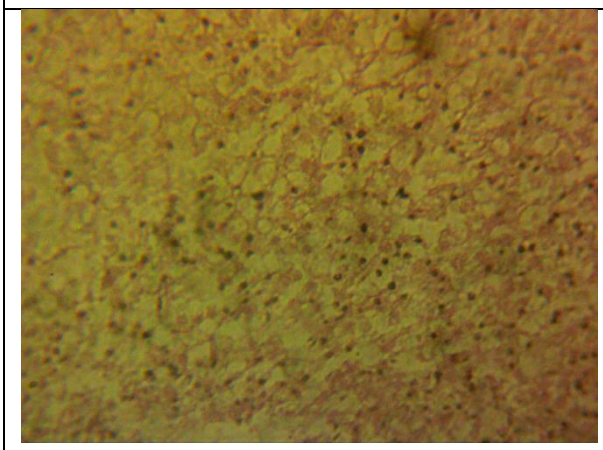
Plate 4.2. Histopathology of intestine, gill, liver and kidney of representatives of fish fed with 5g/kg (0.5%) of Allium sativum (H and E) 400 x



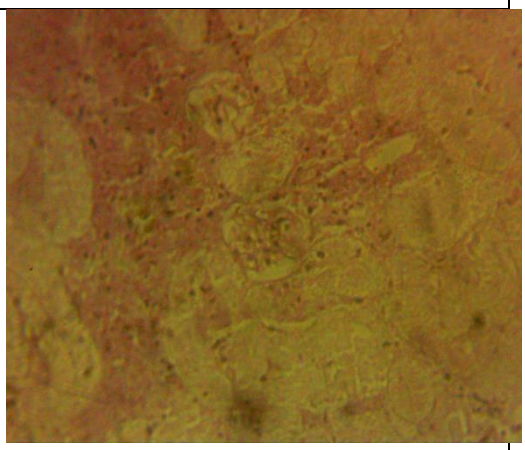
Intestine- no observable lesion



Gill- mild lamellae hyperplasia



Liver- random necrosis of hepatocytes



Kidney- no observable lesion

Plate 4.3. Histopathology of intestine, gill, liver and kidney of representatives of fish fed with 10g/kg (1%) of Allium sativum (H and E) 400 x

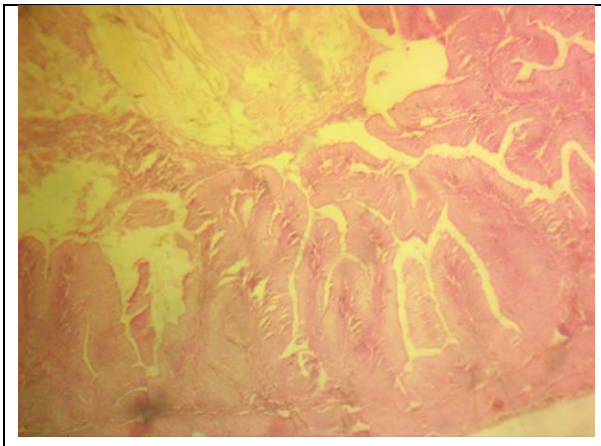
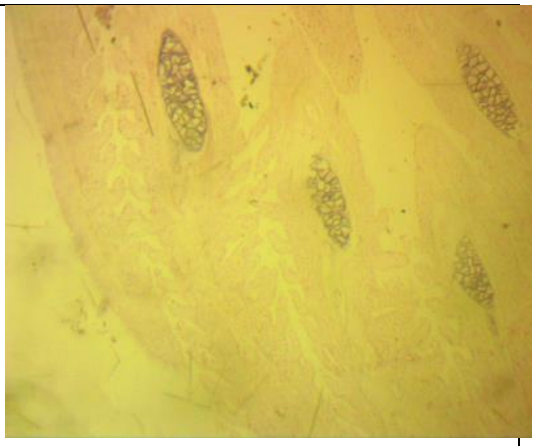
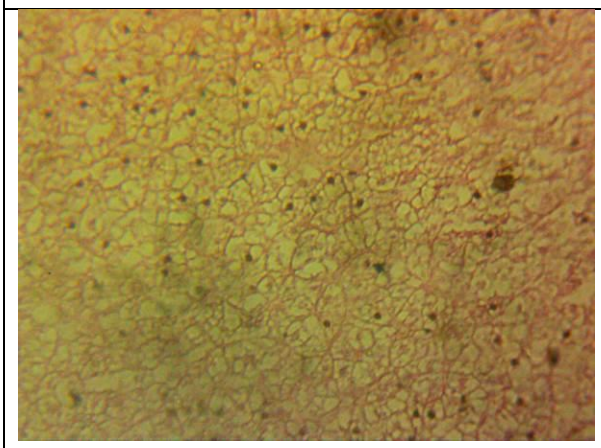
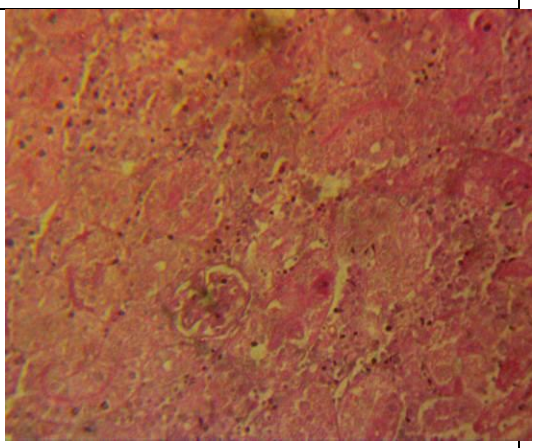
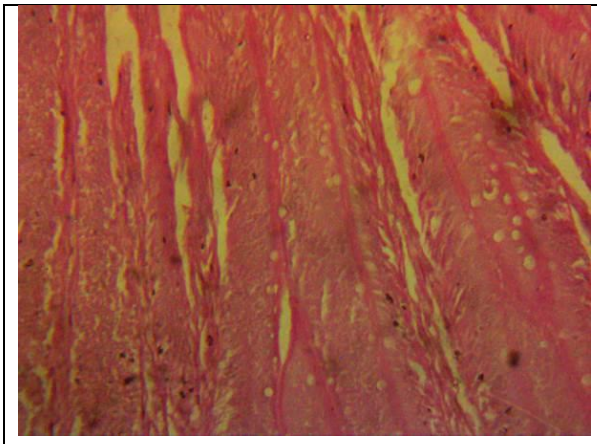
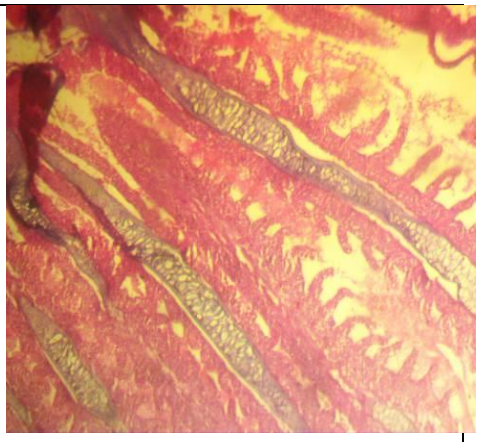
	
Intestine- no observable lesion	Gill- no observable lesion
	
Liver- diffuse swelling of hepatocytes	Kidney- patchy tubular epithelia necrosis

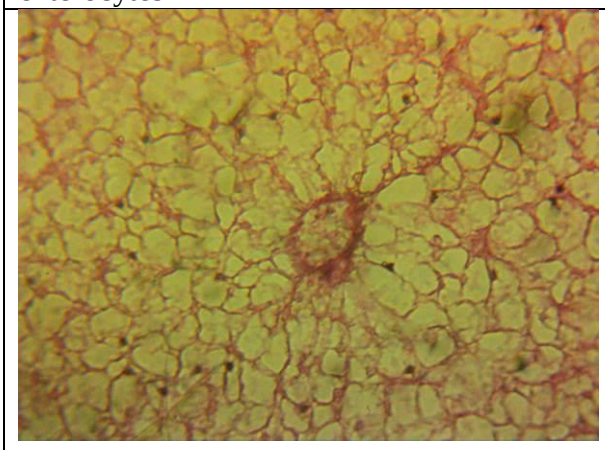
Plate 4.4. Histopathology of intestine, gill, liver and kidney of representatives of fish fed with 30g/kg (3%) of Allium sativum. (H and E) 400 x



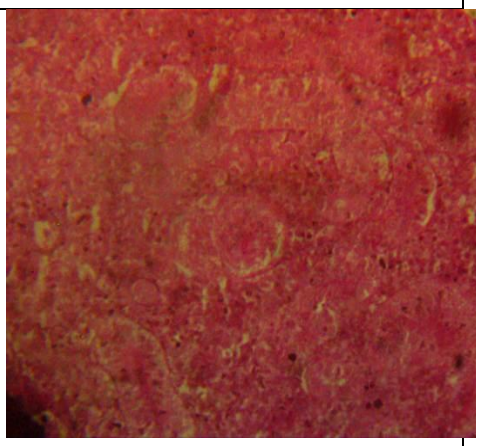
Intestine- vacuolar degeneration of enterocytes



Gill- marked lamellae hyperplasia

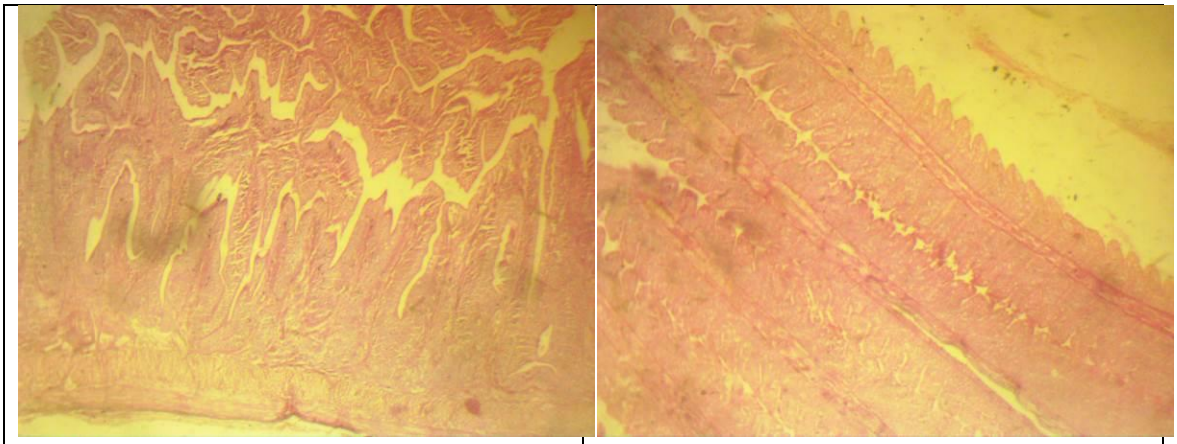


Liver- diffuse swelling of hepatocytes

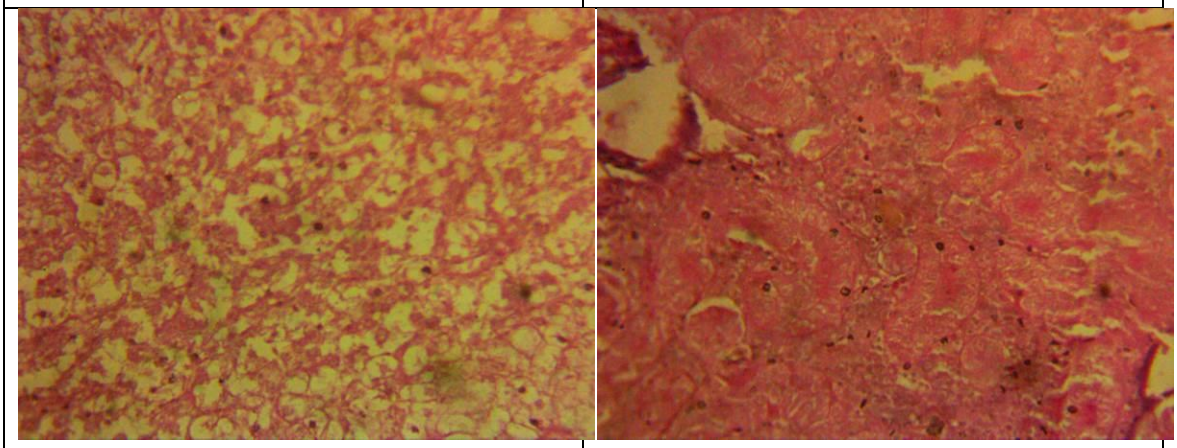


Kidney- diffuse tubular epithelial necrosis and atrophy of tubules

Plate 4.5. Histopathology of intestine, gill, liver and kidney of representatives of fish fed with 5g/kg (0.5%) of *Chromolaena odorata*. (H and E) 400 x

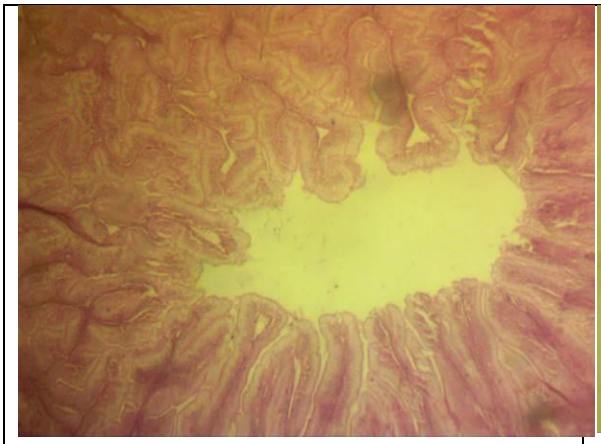


Intestine- no observable lesion	Gill- moderate lamellae hyperplasia

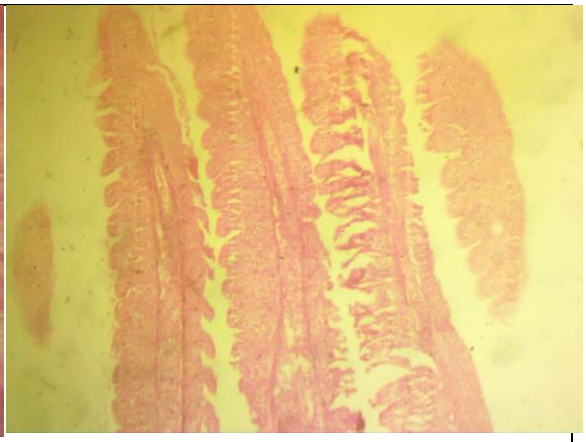


Liver- diffuse degeneration and multifocal necrosis of hepatocytes	Kidney- no observable lesion

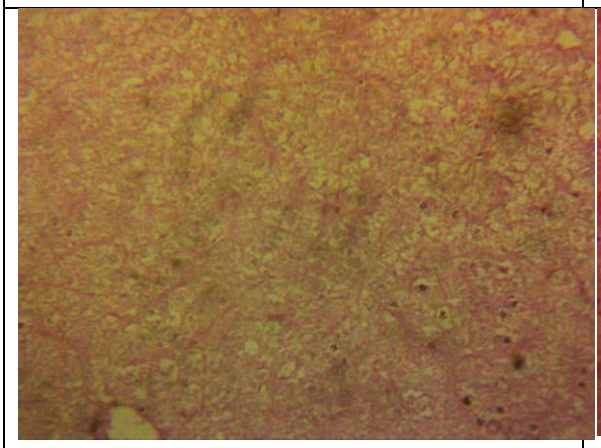
Plate 4.6. Histopathology of intestine, gill, liver and kidney of representatives of fish fed with 10g/kg (1.0%) of *Chromolaena odorata*. (H and E) 400 x



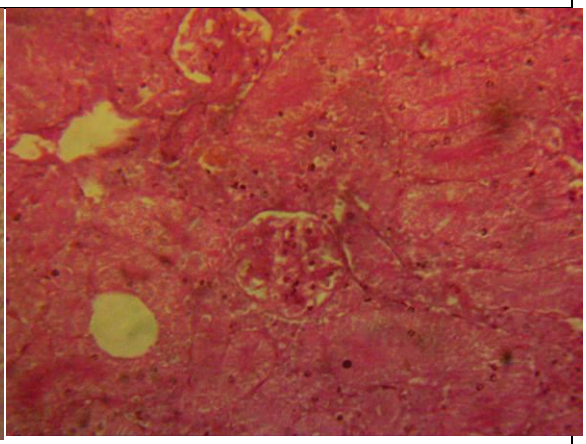
Intestine- no observable lesion



Gill- moderate diffuse lamella hyperplasia



Liver- diffuse hepatocellular degeneration and necrosis



Kidney- no observable lesion

Plate 4.7. Histopathology of intestine, gill, liver and kidney of representatives of fish Fed with 30g/kg (3.0 %) of *Chromolaena odorata*. (H and E) 400 x

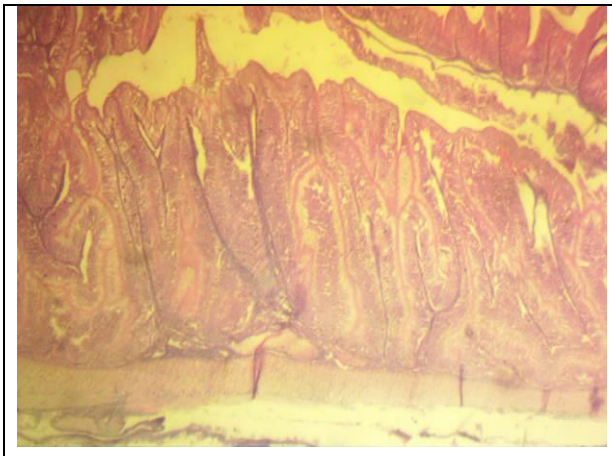
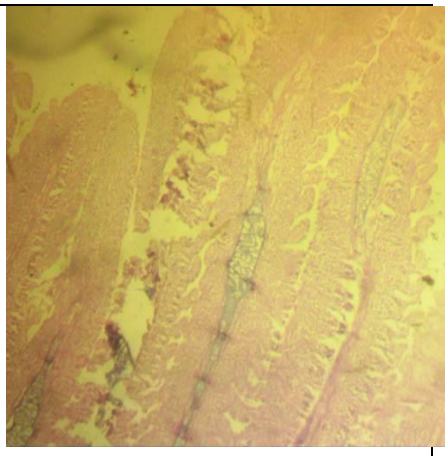
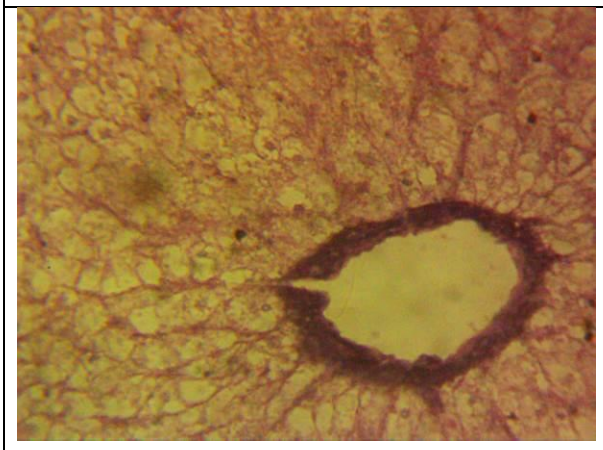
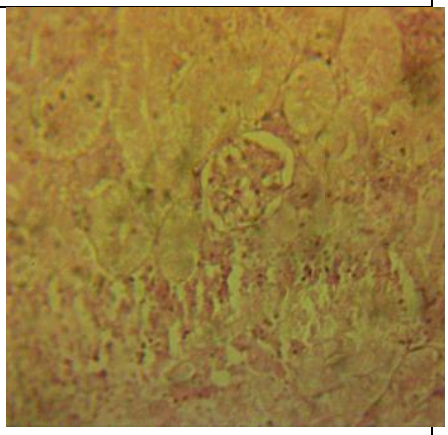
	
Intestine- no observable lesion	Gill- marked lamellae hyperplasia
	
Liver- diffuse swelling of hepatocytes	Kidney- no observable lesion

Plate 4.8. Histopathology of intestine, gill, liver and kidney of representatives of fish Fed with 5g/kg (0.5 %) of *Talinum triangulare*. (H and E) 400 x

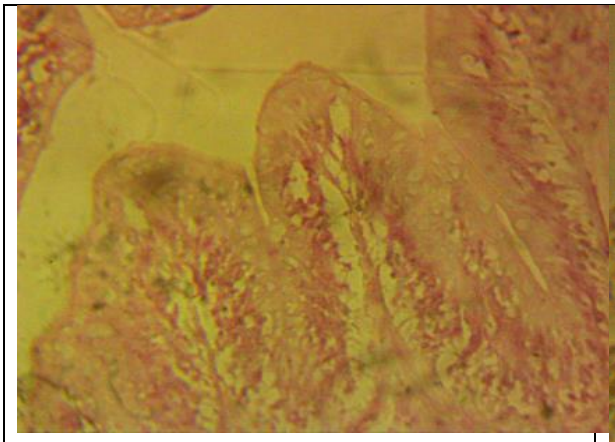
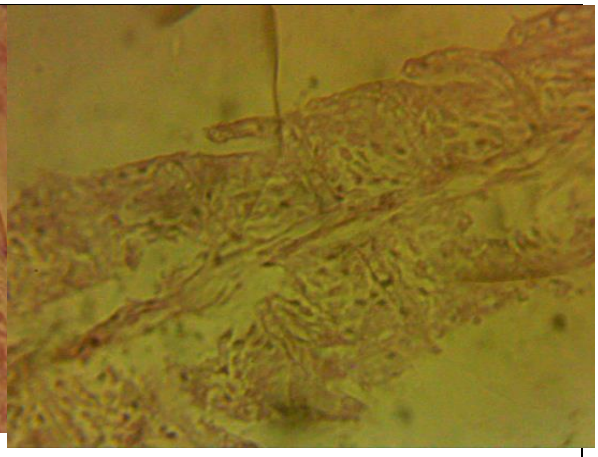
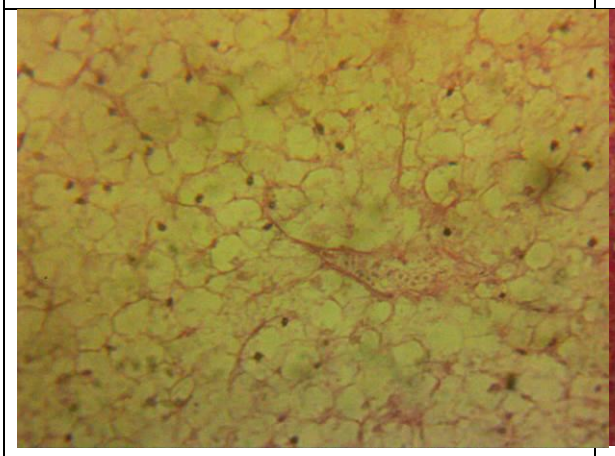
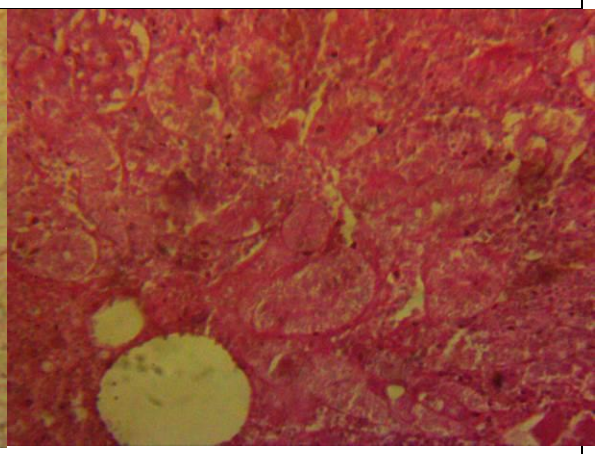
	
Intestines- no observable lesion	Gill- mild lamellae hyperplasia
	
Liver- diffuse vacuolation of hepatocytes (fat accumulation, normal)	Kidney- no observable lesion

Plate 4.9. Histopathology of intestine, gill, liver and kidney of representatives of fish Fed with 10g/kg (1.0 %) of *Talinum triangulare*. (H and E) 400 x

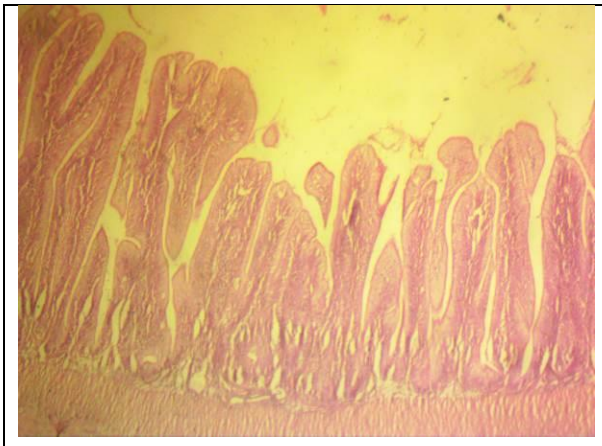
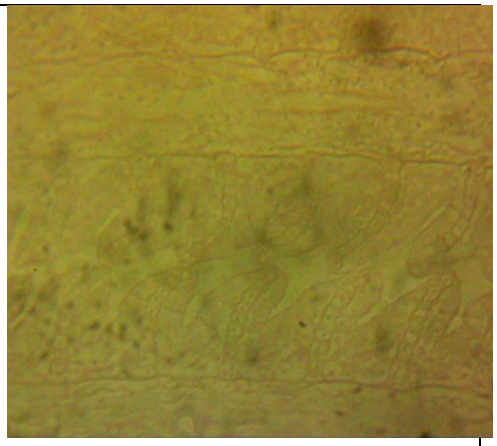
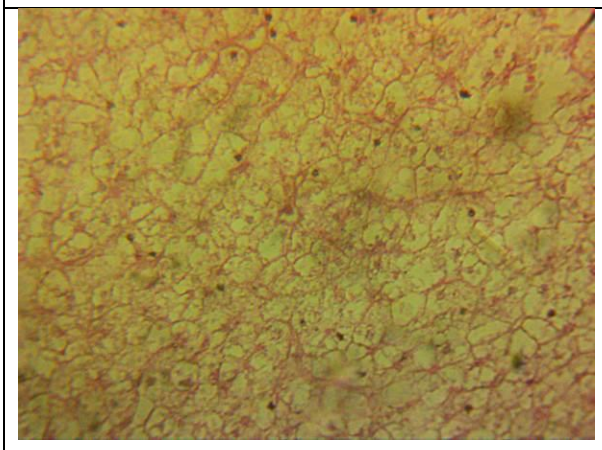
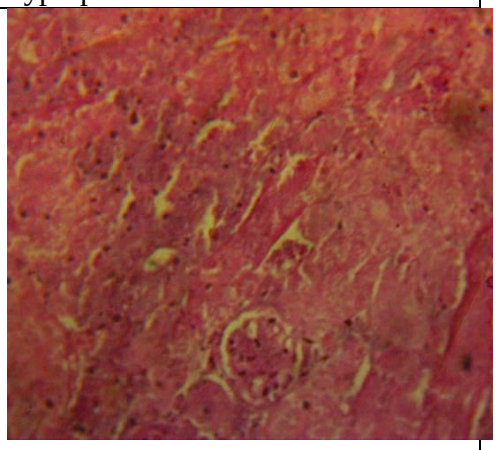
	
Intestine- no observable lesion	Gills- moderate diffuse lamellae hyperplasia
	
Liver- diffuse swelling of hepatocytes	Kidney- diffuse tubular epithelia necrosis

Plate 4.10. Histopathology of intestine, gill, liver and kidney of representatives of fish Fed with 30g/kg (3.0 %) of *Talinum triangulare*. (H and E) 400 x

4.4 Disscusion

Numerous research works regarding the blood characteristics as well as serum enzyme actions of diverse fish species had been carried out and they had been used among the limited means obtainable to screen health position in different species of fish to identify acute and chronic patho-physiologic deviations associated with toxicants, H_2O superiority, nutrition and infection. Study on fluctuations in serum component levels and hematological parameters in African Catfish as a result of feeding medicinal plants are scanty. According to Feist and Longshaw (2000) blood composition of the animal during malnutrition conditions or diseases usually altered. The composition of blood of any species of animal under normal conditions changes within narrow limits (Banergee *et al*., 2002).

Haematocrit (Ht or Hct) is the proportion (percentage) of erythrocyte in blood (Sowumi, 2003). In line with finding of Isaac *et al.* (2013) haematocrit is involved in the absorption of nutrients and oxygen transportation in the tissue. Aletor and Egberongbe (1998) stated that PCV and RBC counts are mostly influenced by nutritional management. Largely, fish PCV differs based on functioning, well-being and action of the fish and bulky variations specify stressful conditions or even chronic stress appearance (Cnaani *et al*., 2004). Regarding the PCV values in this present experiment there was no noteworthy variance ($P>0.05$) in the standards recorded within the sub-groups and across the sub-groups when equated with placebo treatment however group of fish fed on 10g/kg of *T. triangulare* (G_3B) exhibited a upper value and an increase noteworthy value ($P<0.05$) when equated with initial values (values obtained before fish were subjected to laboratory condition). The uppermost value (34.67±0.67%) was documented in group of fish fed on 10g/kg of *T. triangulare* (G_3B) while lowest value (23.67±1.45%) of hematocrit was obtained in group of fish fed on 10g/kg of *C. odorata* (G_2B). These values observed were in the standard range of haematocrit for African Catfish as described by Adeyemo *et al*. (2014) that normal standards were customarily range in the middle of 20% and 35% and barely larger than 50%. These values observed were also in the range described by Okorie-Kanu *et al*. (2014) for *Heterobranchus longifillis* young adults raised in Umudike, South East, Nigeria. Transportation of O_2 to tissues in the animal body for oxidation of consumed diet in order to release energy is the physiological function of haemoglobin and also function in transportation of CO_2 out of the body of animals (Isaac *et al.*, 2013). In this

experiment, no noteworthy variance in Hb concentration was detected within and across the treatment groups compared with control however there exist noteworthy dissimilarity in group of fish nourished with 10g/kg of *T. triangulare* (G_3B) feed additive equated with the early values. Fish nourished with food containing 10g/kg of *T. triangulare* (G_3B) had the highest mean of Hb concentration (12.03±0.26 g/dl) while the least mean of Hb concentration (7.40 ± 0.61 g/dl) was obtained in fish nourished with food comprising 10g/kg of *C. odorata* (G_2B). Haemoglobin concentration standards detected in this experiment were in the range documented by Omitoyin (2006) in *Clarias gariepinus* juvenile nourished with poultry waste and also within the range recounted by Osuigwe *et al*. (2007) who fed fenugreek *Clarias sp* with Jackbean meal formulated ration; low Hb value was connected with low energetic fishes, as observed by Satheeshkumar *et al.* (2011). Packed Cell Volume and haemoglobin are main pointers of different causes of pressure in fish (Rainza-Paiva *et a.l*, 2000) and Osuigwe *et al.* (2007) recounted that incidence of anti-nutritional factors in the feedstuff decrease these parameters.

The erythrocytes count ranged between 1.58 – 3.54 $x10^6$ µl (figure 2) recorded in this experiment is in the range of (2.3 - 2.9 x 10^6 µl) and (1.5 x 10^6 µl) established for catfish by Gabriel *et al*., 2004 and Adeyemo *et al*., 2003 correspondingly. However higher values of 3.0, 3.42, and 3.54 (x 10^6µl) were recorded for group of fish fed with5g/kg of *C. odorata*, 10g/kg of *C. odorata* and 10g/kg of *T. triangulare* respectively and similar observations were noted by Dada and Ikuerowo (2009) who recorded the value of 3.50 ± 0.35 x 10^6 µl after ethanoic extracts of *Garcinia kola* seeds were fed to African Catfish brood stock. In this present study, higher values recorded in these treatments could be due to higher activity displayed by the fish as explained by Svobodova *et al*. (2008) who recounted that energetic species showed great haematological parameters standards when compared to low active species. Life blood of the many fish species is dominated by red blood cells. Decreased significant differences in the count of RBC volumes observed in some group of the experimental fish when likened with fish in control group, suggesting low activity of those fish compared with control groups. Additionally to the actual amount of red cells existing in circulating blood, Packed Cell Volume (PCV), or hematocrit; certain comparative measurements are used to differentiate the various types of anaemia. These are the MCV, or the average volume of RBCs expressed in cubic microns or fl, the mean

corpuscular heamoglobin (MCH), or the Hb content of the erythrocytes expressed in microns or pg; and MCHC, or the concentration of Hb expressed in percent or g/dl and these were calculated from obtained hematological data. In this present study, when comparison was made across the groups, no noteworthy dissimilarity (P>0.05) was detected in MCHC however the uppermost value (34.71±0.34%) was documented in group of fish nourished with 10g/kg of *T. triangulare* additive and the least value (31.22±2.14%) recorded in group of fish fed 10g/kg of *C. odorata* additive. Increase significant different (P<0.01) were observed in MCV and MCH in group of fish fed on 10g/kg of *A. satium* additive and in group fish fed 30g/kg of *T. triangulare* additive whereas group of fish nourished on 10g/kg of *C. odorata* additive and group of fish fed on 30g/kg of *C. odorata* additive showed the least values for MCV and MCH respectively. The mean corpuscular volume (MCV) range from 87.82±1.20 fl to 149.5±1.21 fl documented in this trial was greater than 79.20 to 105.32 fl described for *Heteroclarias* (Anyanwu *et al*., 2011), however MCHC range from 31.22±1.13% to 34.71±0.34% recorded in this study for fish fed herbal supplemented food equated justly fine with 30.70% re-counted for African Catfish cropped from Asejire dam (Adedeji and Adegbile, 2011) and also within the range of 30 to 66% described by Mohan and Senthilkumar (2016**).** The MCH range from 28.12±1.71 pg to 48.41±0.50 pg achieved in this work was greater than the range 20.82 to 26.60 pg re-counted for *Heteroclarias* nourished with *Carica papaya* leaf meal integrated food (Anyanwu *et al*., 2011). Preceding blood characteristics studies of nutritional effects indicated that PCV, Hb and erythrocytes counts are key and dependable pointers of numerous bases of strain (Rainza-Paiva *et al.*, 2000) and presence of anti-nutritional factors in the feed decrease these parameters (Osuigwe *et al.,* 2007). All the values of erythrocytes, PCV and Hb detected herein were within the suggested biological values described for *C. gariepinus* as described by some workers cited, an indication that *C. gariepinus* are tolerant to medicinal plants (*A. satium, C. odorata* and *T. triangulare*) fed separately at 0.5%, 1.0% and 3.0% concentration for each of the medicinal plants. Minor variations observed could be attributable to effect of diverse bioactive compounds of the plant on the fish, environmental conditions or management techniques.

The leukocytes or white blood cells, include the granulocytes, lymphocytes and monocytes; in contrast to red cells which carry on their function of oxygen and carbon-

dioxide transport within the circulation, the functions of leucocytes are largely performed elsewhere in the body. The blood stream transports leucocytes from haemopoietic tissue to their site of action, and to certain extent, to the site of their disposal or eventual destruction. The main work of the WBC and its differentials are to combat contagions. The foreign organisms invaded the body is phagocytosed by leucocytes and they also yield or at least carrying and allocate immunoglobulins in immune reaction. Animals with great number of leucocytes have abilities to produce immunoglobulins in the procedure of phagocytosis therefore can resist infection while animals with little WBC count are highly at danger of contamination (Isaac *et al.,* 2013) and improve adaptableness to local ecological and infection rampant situations (Soetan *et al*., 2013). There is wide range in reported number of total leukocytes in apparently normal African Catfish. In this present study, leucocytes counts increased meaningfully in the group of fish nourished ith 5g/kg *A. satium* compared with group of fish fed without feed additives and group of fish nourished with diverse concentrations of *C. odorata* and *T. triangulare*. Microbial contamination or antigen present in the circulating usually resulted in high WBC count (Oyawoye and Ogunkunle, 1998). The values, range from 11.55×10^3µl to 22.87×10^3 µl documented in this study were greater than what were recounted by Dienye and Olumuji (2014) who reported range of 7.20×10^3 µl to 8.02×10^3 µl when Moringa Oleifera leaf meal was served to African Catfish and value obtained were not up to $41.1 \pm 11.05 \times 10^3$ µl referenced by Erhunmwunse and Ainerua (2013) when some blood parameters of African catfish were characterized. The difference observed could be due to different in age of experimental fish, outcome of the feed additives and/or ecological conditions.

The lymphocyte count recorded in this experiment was meaningfully decreased ($P<0.001$) in cluster of fish fed with 5g/kg of *T. triangulare* when likened with the control. The uppermost value of 70.33±2.19% were documented in the cluster of fish nourished with 30g/kg of *T. triangulare*. Omoniyi *et al*. (2002) had previously recorded highest value of 70.0% in healthy juvenile catfish. Modulation of the immune defense and production of immunoglobulin in several immunological functions involved Lymphocytes. The Neutrophils, Eosinophils and monocytes values were alike in all the clusters and within the normal range. Kelly (1979) stated that monocytes composition in WBCs count of all species of animal is less than 10%. In this study, lymphocytes were the highest quantity of the

WBCs estimated in the blood of the experimental fish. This observation was in agreement with Ighwela *et al*. (2011)'s observation when fed *Oreochromis niloticus* with varying dietary maltose levels. This observation according to Ighwela *et al*. (2011) is in disparity with the WBCs composition of most terrestrial animal with neutrophil being the utmost in horse, pig, dog and cat whereas the utmost in non-monogastrics is eosinophils.

Blood biochemistry parameters are another indicators that might be used to define the health of fish (Martins *et al*., 2008). Ferrari *et al.* (2007) reported that biochemical fluctuations hinge on the fish species, age and health situation; and analysis of serum biochemical constituent's level have provided valuable statistics in discovery and analysis of metabolic disorders and ailments in fishes (Francesco *et al*., 2012). The biochemical analyses showed advantages for different feed additives. In this study, the level of plasma protein observed in overall groups range from 5.23 – 8.0mg/dl. This finding fall within the range reported earlier that overall plasma protein in fish could fluctuate from 2-8gd^{-1} (Ravi and Jithender, 2005). The level of plasma protein observed improved meaningfully ($P<0.001$) in fish fed 10g/kg *A. satium* (garlic) when compared with control groups and initial values and decrease somewhat but not significant in fish nourished with 30g/kg *A. satium* supplemented diets. The values obtained for Albumin and Globulin in this present study were within the standard ranges for *C. gariepinus* as reported by Omitoyin (2007) who stated the standard range for overall protein to be between 3 g – 6 mg/100 ml but slightly above 3.8 mg ± 0.11 mg/100 ml as reported by Adeyemo *et al*. (2003). The glucose values were alike in all treated groups ($P>0.05$). The lowest value of 256 ± 18.9g/dl was obtained from catfish fed with 10g/kg *C. odorata* supplemented diet while the highest value of 322.7 ± 0.33mg/dl was obtained in fish fed 30g/kg *C. odorata* supplemented diet. Blood glucose level may fluctuate according to water temperature as well as season and also it was reported that glucose concentration in fish decreases with size and age (Coz-Rakovac *et al*., 2005). As glucose in serum is a main metabolite of carbohydrate breakdown as pointed out by Artacho *et al*. (2007), the greater glucose level in blood could be credited to physical stress when blood samples were collected or affected by hormone or to the higher glycogen reserves in the fish. The upsurge in the serum protein, albumin and globulin contents recorded in group of fish nourished with 5g/kg of *Allium sativum*, 10g/kg *C. odorata* and 10g/kg *T. triangulare* reflect strong innate immunity as described by Jha *et al*.

(2007). These findings are in line with the finding of Nya and Austin (2009) and this suggested that *A sativum, C. odorata* and *T. triangulare* included at rate of 0.5 – 1.0% improved the immune response of African Catfish. There was noteworthy increase ($P< 0.01$) in the actions of serum enzymes (Aspartate aminotransferase, AST) in cluster of fish nourished with 5g/kg *T. triangulare* compared with control but values documented in group of fish nourished with different concentrations of *C. odorata* and *T. triangulare* based diets were similar. The activities of enzyme, Alanine aminotransferase, ALT, were also similar in all the experimental groups. However activity of enzyme alkaline phosphatase, ALP, was significantly increased in group of fish fed 5g/kg of *C. odorata*, group of fish fed 30g/kg of *C. odorata* and group of fish fed 5g/kg of *T. triangulare* compared with control and initial values in their respective groups. Increased AST, ALT and ALP actions in fish are indicative of hepatic cellular impairment resulting in their leakage into circulation (Basten, 2010). The serum AST, ALT and ALP actions reduced in the fish group fed on 5g/kg of *Allium sativum* compared with control. These results could be attributed to *Allium sativum*, which effect protection of the liver and stabilization of cell membrane against harmful agents and free radical-mediated lethal harms to the hepatocytes (Fazlolahzadeh *et al*., 2011). This was revealed in the decline of liver enzymes. *Allium sativum* could help the liver to preserve its usual function by fast-tracking the reformative capability of its cells. The raised blood cholesterol levels might be as a result of mobilization of stored cholesterol from tissue.

Biochemical, physiological indicators, cellular biomarkers and histopathological changes are among diverse biomarkers that are in use currently. They are sensitive, fewer variable and often stress-free to quantify. Changes occur in a specific part of organ or in the entire organ, when the concentration of contaminants is plentiful. The usage of histopathology as an endpoint in diagnosis of diseases has gained ground and as it was observed by Vander-Oost *et al*. (2003), histopathological alterations are frequently the consequence of the integration of a number of shared physiological processes. Histopathological analyses provide a valued screening means before severe damage happens because ultrastructure of tissues is changed when concentration of the pollutant are still at low levels. Couch (1975) reported that tissues harvested from the gill, intestine, liver and kidney of fishes are finest for histopathological studies. The result of the

histopathological examination of different organs in this experiment after feeding for 42 days showed no observable lesion in the intestine, gills, liver and kidney of the fish fed with basic diets (control group). The result observed in the group of fish fed with 5g/kg *Allium sativum* was similar to what was observed in the control. In all the experimental diets, no lesion was detected in the intestine of the experimental fish except the group of fish fed 5g/kg of *Chromolaena odorata* which showed vacuolar degeneration of enterocytes. Different inclusion rates of *Allium sativum* showed no observable lesion on the gill of experimental fish except a mild lamellae hyperplasia that was detected in group of fish nourished with 10g/kg of *Allium sativum* as feed additive. However, effect range from mild lamellae hyperplasia to marked lamellae hyperplasia were observed in the gills of the fish nourished with different inclusion rates of *C. odorata* and *T. triangulare.* These lesions observed may impair respiratory function. The histopathological changes observed in the liver are random necrosis of hepatocytes, diffuse swelling of hepatocytes, and diffuse degeneration and multifocal necrosis of hepatocytes in some of the experimental fish. Fish liver histology can serve as a model for studying the connections between ecological parameters and hepatic structures and functions (Gernhofer *et al*., 2001) and nutritional factor as well. Kidney revealed patchy tubular epithelia necrosis in group of fish fed 30g/kg *Allium sativum,* diffuse tubular epithelia necrosis and atrophy of tubules in 5g/kg fed *C. odorata* and diffuse tubular epithelia necrosis was observed in group of fish dosed with 30g/kg *T. triangulare*. In this experiment, no mortality was recorded, the fish were active and showed no clinical signs throughout the experimental period. The observed histological effects in some of the experimental fish could implies that small quantity of these plant additives is required for normal metabolism and development and if the level surpasses the physiological requirements, they could act as a toxicant. Moreover, short- term exposure periods may be required, although long term exposure might provide a signal of the commencement of cellular injury and would then specify whether the injury recognized, increased or decreased in intensity or remained the same throughout the exposure period.

CHAPTER FIVE

PROTECTIVE POTENTIALS OF *Allium sativum, Chromoleana odorata* AND *Talinum triangulare* SUPLEMENTED DIET AGAINST *Pseudomonas aeruginosa* INFECTION IN THE AFRICAN CATFISH

5.1 Introduction

Aqua-farming, worldwide is a rising, exciting and vital segment for high protein diet. With intensive cultivation of fish and shellfish globally, fish are exposed to a wide-ranging of infections that led to death and reduced profitability in fish production. Investigations have shown that the absence of effective infectious disease management has the prospective of being the main restrictive factor for achieving very steady fish making (Phillip *et al*., 2000). Increasing fish growth and infection resistance of cultivated animals are main hindrance confronting aquaculturists, and of more important are bacterial infections which are one of the restrictive causes for fish cultivation including African Catfish production. Amongst all other microbes, *Aeromonad, Edwardsiella tarda*, and *Pseudomonad* are the main bacteria fish pathogenic organisms that are extensively spread in aquatic animals in the environment (Rahman *et al*., 2009). Generally, antimicrobial have been used in aqua-farming for the avoidance and management of bacterial infections (Stephen *et al.*, 2006). Nevertheless, the usage of antimicrobials in aqua-farming indicates threat such as emergence of bacterial strains that are unaffected by antibiotics action, and the incidence of antibiotics residue in fish available for buyers (Harikrishnan and Balasundaram, 2011). Majority of the chemotherapeutants are unsuccessful in treating an infectious culturing system also their uses are main expenditures that meaningfully decrease the fish productivity (Phillip *et al*., 2000), so prevention is better than cure. Vaccines which can be used for prevention are often specific against pathogens (Iruthayam *et al*., 2014), and widespread of pathogenic

organisms in fish farm also limit vaccine (Ardo *et al*., 2008). Consequently, numerous alternate tactics to use of chemicals and vaccine have been projected such as immune-therapy like probiotics and immune stimulating agents such as herbal plants, mammon oligosaccharides, beta- glucan and *Saccharomyces cerevi* (live yeast) which help as nutritional complements to advance fish growing and stimulate immune reactions (Irianto and Austin, 2002).

Immunostimulants appear to epitomize a valuable alternate to chemotherapy and vaccination in the management of fish infections since they can improve the innate immune response (Anderson, 1992) and it is well recognized that fish depend more deeply on non-specific protective mechanism than mammals (Anderson, 1992). Immune boosters provoke an active and deep immune reaction to those contagious agents for instance bacteria, viruses, fungi and parasites without dangers of harmfulness, carcinogenicity or residues in tissue (Harikrishnan *et al*., 2011). The immunostimulants also have extra advantages for instance growth improvement and upsurge the survival rates of the fish under pressure (Heo *et al*., 2004). Charkrabarti *et al*. (2012) reported that integration of therapeutic plants in the food of the fish enthused the immune system of fish and improved their resistance to disease. Therapeutic plants have prospective use as an immune stimulating agents in fish cultivation as several of them are better sources of volatile oils, saponins, phenolics, tannins, alkaloids, polysaccharides and polypeptides which accountable for various actions such as anti-stress, appetizers, tonics, antimicrobial, immunostimulants and act against many diseases (Pandey and Madhuri, 2010). The preventative effect of nutritional garlic application to rainbow trout, contaminated with *Aeromonas hydrophila* was established by Nya and Austin (2011). Therefore, phytobiotics in fish disease control are becoming popular, since they are cost effective, environment friendly and have negligible negative effects (Govind *et al*., 2012). Majority of the medicinal plants and their extracts can be administered orally, which is the most suitable process of drug administration.

Blood forms interrelated and unavoidable component in all immune system and the alterations in the blood characteristics can be associated with the reaction of the animal to the fluctuating ecological situation, nutritional factors and consequently useful to monitor the well-being of the fish subjected to dietary factor. In this present work the influence of dietary *Allium sativum, Chromoleana odorata, Talinum triangulare* on specific growth

performance, haematology, survival and immunostimulation of African catfish (*Clarias gariepinus*) challenged with *P. aeruginosa* was evaluated and compared.

5.2 Materials and Mmthods

5.2.1 Feed preparation

The basal food (Control diet - D1) was organized by blending Yellow corn 35g, Wheat fiber 9.5g, Soybean meal (44%) 28.5g, dry fish meal (65%) 17g, Vegetable oil 6.5g, Calcium carbonate 0.3g, Mineral mixture 1.7g and Vitamin mixture 1.0g made as a dough, pelletized and then dried it under the sun, smashed into reduced required sizes. The supplemented foods (D2, D3 and D4) were organised by means of the same ingredients for basic diet in which *Allium sativum* (0.5%), *Chromoleana odorata* (1.0%) *and Talinum triangulare* (1.0%) powder were added respectively after sterilization process.

5.2.2 Bacteria culture and inoculums

Tissue samples were harvested from gills, kidney, skin, liver and intestine of diseased and healthy fish, cultured on Nutrient agar (LAB. 008, Lancashire BL97111, UK) by standard methods and incubated overnight at 37°C. The colonies that gave bluish-green appearance were picked and sub-cultured on the Pseudomonas Selective agar (Centrimide agar) to observe the pigment production from the bacteria (Austin and Austin, 2007). The growing bacteria on the latter medium were sub-cultured on nutrient agar and MacConkey agar and incubated for 24h at 42^{o}C once more to differentiate *Pseudomonas aeruginosa* from other species (Ayad and Anssam, 2007). The organisms were subjected to various biochemical tests according to Koneman *et al*., 2006 as shown in Table 5.1. API-20E (Biomérieux) test kit was used to identify the isolate to species level. The Characteristics of the isolates were compared with reference strain *P. aeruginosa* ATCC® 27853(™).

Table 5.1. Morphology, biochemical characteristics and sugar fermentation of the isolate

Morphology Characteristics Observations	
Shape	rod
Pigment	bluish green
Arrangement	single
Biochemical test	**Results**
Gram reaction	-
Motility	+
Oxidase	+
Catalase	+
Citrate utilization	+
Indole	–
Urease	–
H_2S formation	–
Methyl red	-
Voges-proskauer (VP)	-
H_2S Production	-
Sugar Fermentation	
Glucose	+
Xylose	-
Manitor	-
Maltose	+
Sucrose	-
Lactose	-

Key. (+) positive reaction, (-) negative reaction.

5.2.3 Antibiotics sensitivity test

The vulnerability of the isolates to these antibiotics, Nalidixic acid, 30µg ; Ofloxacin, 30µg; Augmentin, 30µg; Tetracycline, 50µg; Amoxyclin, 30µg; Cotrimoxazole, 25µg; Nitrofurantoin, 30µg; Gentamicin, 10µg were performed using disc diffusion method. Fresh Nutrient Agar was organized in line with the manufacturer`s specification and it was pasteurized at 121^{o}C for 15 minutes. The antibiotic discs were positioned on nutrient agar plates formerly seeded with an 18-24 hr culture of the test organisms by means of cotton swab. The plates were incubated at 37^{o}C for 24 hours, after which zones of clearance were inspected and construed consequently (Chortyk *et al*., 1993). The width of the zone of clearance was calculated both horizontally and vertically and the result was shown in Table 5.2. The resistance pattern of each isolate was produced from the antibiogram. Previously, the efficacy of all the antibiotics used in the study were established by means of reference strain **(***Pseudomonas aeruginsa,* ATCC® 27853(™)). The isolates displaying highest resistance concerning the amount of antibiotics used, were carefully chosen for further study.

Table 5.2. The antibiogram of some strains of *P. aeruginosa* harvested from the samples

Antibiotics	Pa 1			Pa 2			Pa 3		
	Zone of inhibition (mm)		Susce-pitibility	Zone of inhibition (mm)		Susce-pitibility	Zone of inhibition (mm)		Susce-pitibility
	H	**V**		**H**	**V**		**H**	**V**	
NAL	25	17	S	21	15	S	22	17	S
OFL	16	11	S	20	17	S	19	15	S
AUG	N	N	R	N	N	R	N	N	R
TET	N	N	R	N	N	R	N	N	R
AMX	N	N	R	N	N	R	N	N	R
COT	N	N	R	N	N	R	N	N	R
NIT	16	22	S	20	14	S	18	20	S
GEN	16	17	S	16	14	S	19	17	S

Pa = *Pseudomonas aeruginosa* isolate, H = Horizontal, V = Vertical, N=No inhibition, S= Sensitive (≥ 8 mm), R= Resistance (≤ 8 mm). Nal, Nalidixic acid 30µg ; Ofl, Ofloxacin 30µg; Aug, Augmentin 30µg; Tet, Tetracycline 50µg; Amx, Amoxyclin 30µg; Cot, Cotrimoxazole 25µg; Nit, Nitrofurantoin 30µg; Gen, Gentamicin 10µg.

5.2.4 Determination of infectious dose (LD_{50}) of *Pseudomonas aeruginosa*

A preliminary LD_{50} assay of pure culture *P. aeruginosa* was piloted to estimate the appropriate concentration of colony forming units (CFU) required to cause death in 50.0% of the fish populace tested. Thirty six fish, mean weight 54.12 ± 0.26g and mean length, 18.95±0.05cm which were earlier acclimatized and fed with commercial fish feed pellet for two weeks were haphazardly distributed into 6 groups, each group containing 6 apparently healthy fish. The stock solution of the isolate which was serially diluted earlier were injected intraperitoneally [*i.e.*10^{-4}(PT_1), 10^{-5}(PT_2), 10^{-6}(PT_3), 10^{-7}(PT_4), 10^{-8}(PT_5)] into the fish to assure virulence except the control group (PT_0) which were injected with normal saline (Table 5.3). Bacterium innocula were estimated to flank the number of *P. aeruginosa* required to infect 50% of the fish in a single group. The fish were observed for 7 days for death. Number of infected fish and uninfected fish was used to compute the LD_{50} in line with the technique of Reed and Muench (1938) as shown in Table 5.4.

Table 5.3. Showing daily mortality of fish challenged with different concentration of *Pseudomonas aeruginosa*

Fish group	No of Fish	Type of innoculate	Route Of injection	No fish died during 7days after injection							No of Fish uninfected	No of Fish infected
				1	2	3	4	5	6	7		
PT_0(Control)	6	NS	i/p	0	0	0	0	0	0	0	6	0
$PT_1(10^{-4})$	6	*P.a*	i/p	5	1	0	0	0	0	0	0	6
$PT_2(10^{-5})$	6	*P.a*	i/p	3	0	0	0	0	0	0	3	3
$PT_3(10^{-6})$	6	*P.a*	i/p	3	0	0	0	0	0	0	3	3
$PT_4(10^{-7})$	6	*P.a*	i/p	2	0	0	0	0	0	0	4	2
$PT_5(10^{-8})$	6	*P.a*	i/p	0	0	0	0	0	0	0	6	0

*PT_0= Control, Others (PT_1-PT_5) represent groups injected with different concentration of viable *Pseudomonas aeruginosa* cells, NS = Normal Saline, *P. a = Pseudomonas aeruginosa*

Table 5.4. Determination of concentration of LD_{50} for *Pseudomonas aeruginosa*

Dose (Dilution factor)	Number of Fish uninfected	Number of Fish infected	Total Uninfected [a]	Total Infected [b]	Percent infected [c]
10^8	6	0	16	0	0
10^7	4	2	10	2	17
10^6	3	3	6	5	46
10^5	3	3	3	8	73
10^4	0	6	0	14	100

Key.

[a] Addition of all of the uninfected fish at that dose and higher.

[b] Addition of all of the infected fish at that dose and lower.

[c] The sum of infected divided by the addition of the total uninfected and total infected multiplied by 100.

By means of the figures in the table above the subsequent calculations was carried out according to Reed and Muench (1938)

50 - 46 (the percent infected below 50%)

73 (the percent infected above 50%) - 46 (the percent infected below 50%)

= 0.15

$\log 10^6$ (dose at which less than 50% of the fish become infected)

= 6

0.15 + 6 = 6.15

inverse log 6.15

$LD_{50} = 1.4 \times 10^6$

Replicate plate counts (RPC) was used to assess serial dilution correspond to 1.4×10^6 bacteria cell/ml.

5.2.5 Evaluation of antimicrobial action of the plants

The aqueous extract was prepared by kept 60g of each of plant materials in 500mL conical flask and 300mL of water was add-on as solvent. The mouth of the conical flask was sealed with aluminum foil and mounted on shaker for 72 h for uninterrupted shaking at 150.0 revolution per 60 seconds for comprehensive mixing. The extract was sieved thrice by means of a sterile muslin fabric and then extract was further filtered by means of Whatman no 1filter paper (Raja, 2012). The obtained crude extracts were poured into a sterile bottles at concentrations of 100% (200mg/ml), and two folds dilution was used to serially diluted extract further into 50% and 25% concentrations. They were labelled appropriately and kept in the refrigerator before use. Agar diffusion technique as modified by Osadebe and Ukwueze (2004) was accepted to evaluate the antimicrobial activity of the plants. Broth cultures of the *P. aeruginosa* containing 1.4×10^6 bacteria/ml (previously determined) was placed into a germ-free Petri dish and 15 ml melted Mueller Hilton Agar added. The content was meticulously mixed and permitted to harden. Four hovels were prepared in each of the plate (5.00 mm width) by means of a disinfected cork-borer and equivalent volume of leaf extracts were moved into the holes by means of a Pasteur's pipette. Equal volume of distilled water was used as negative control. The plates were permitted to settle for 60 minutes for the pre-diffusion of the extracts to happen and were incubated at 37°C for 24 h.

At the completion of the incubation period, the antimicrobial action was evaluated by calculating the width of zone of inhibition (ZOI) exhibited by the extract (Table 5.5).

Table 5.5. Antibacterial activity of aqeous extract of the plant leaves

Plants Tested	Concentrations		Diameter of zone of inhibition (mm)						
			Mean	SD	CV	Min	Max	Median	Sum
Allium Sativum	100	%	12.00	±1.41	23.57%	10.00	16.00	11.00	48
	50	%	11.50	±1.71	29.70%	8.00	16.00	11.00	46
	25	%	–	–	–	–	–	–	–
Chromolaena odorata	100	%	12.50	±1.26	20.13%	10.00	16.00	12.00	50
	50	%	9.50	±0.96	20.16%	8.00	12.00	9.00	38
	25	%	6.00	±0.82	27.22%	4.00	8.00	6.00	24
Talinum triangulare	100	%	–	–	–	–	–	–	–
	50	%	–	–	–	–	–	–	–
	25	%	–	–	–	–	–	–	–
Distilled water			–	–	–	–	–	–	–

SD= Standard Deviation, CV= Coefficient of Variations,–= No inhibition, mm= millimetres

5.2.6 Experimental design

The African Catfish (n=150), with initial weight (53.05±0.23g) and of initial length (18.79±0.03cm) were procured from a homegrown fish pool and permitted to adapt to research laboratory situations for 15 days. For the time of adaptation they were nourished with basal diet *ad libitum.* Throughout the trial period, the water variables: T^o, pH, DO and ammonia concentration were measured. The water in the aquarium was changed one time in two day. The fish were principally distributed into four trial groups (A – D). The group A (n=60) was reserved as control group and fish were nourished basic food (D1, no herbal additive). The group B (n=30) was nourished with 0.5% *Allium sativum* integrated ration (D2), group C (n=30) was fed with 1.0% *Chromolaena odorata* (D3) and group D (n=30) was fed with 1.0% *Talinum triangulare* incorporated diet (D4). Each group of the experimental fish were fed for 42 days with their respective diet variants. Growth parameters were evaluated post 42 days feeding. Hematological parameters and biochemical parameters were analyzed at end 42 days post feeding.

5.2.7 Haematological analysis

The blood characteristics were investigated at 21 and 42 days during the feeding trial**.** The blood samples were drawn by caudal vein rupture using 21 gauge hypodermic needle and transferred to the bottle containing EDTA as anticoagulant. Total leucocytes were count up by means of Haemocytometer with better-quality Neubaurer ruling chamber (Weber and sons, England) by means of Haem's fluid and Turks fluid as diluents correspondingly, Hb concentration was measured by Cyanmethamoglobin technique. May-Grunewald's Giemsa's stain was used to stain blood smears for differential leucocytes cells. The plasma indexes (glucose, cholesterol, triglyceride and total protein measured in milligram per decilitre) were evaluated with spectrophotometer by means of standard kits (RX MONZA CH200, AP 542, TP 245, AB 362, GL364, AS 101, AL 101, Randox Laboratories Limited, UK.).

5.2.8 Disease challenge

At end of 42 days feeding experimental period, 10 fish from groups B - D were randomly selected and then challenged with 0.2ml culture suspension containing 1.4×10^6 bacteria cell ml^{-1}, viable cells of *Pseudomonas aeruginosa* injected intraperitoneally. Ten fish in group

A injected with *P. aeruginosa* (Positive Control) and another ten fish were equally randomly picked from group A injected with Normal saline to serve as negative control. Deaths were checked for 1 week after the inoculation. The relative level of protection (RLP) and mortality (%) amid the tested fish were measured in line with Ruangroupan *et al*. (1986) technique.

$$\text{Mortality (\%)} = \frac{\text{No of Fish dead}}{\text{No of Fish injected}} \times 100$$

$$\text{RLP} = 100 - \frac{\text{\%age of fish died in the treated group}}{\text{\%age of fish died in the control group}} \times 100$$

5.2.9 Data analysis

Each variables values observed were reported as mean ± SEM. Growth perfomance, blood characteristics parameters and biochemical factors were verified by means of ANOVA (one- way) and the average standards were equated by means of Tukey Test at $\alpha_{0.05}$.

5.3 Result

5.3.1 Water quality

Average values of water superiority caculated on one occasion every week throughout the course of the trial period showed the levels of temperature to be 28.7 ± 0.4^{o}C, pH 7.1 ± 0.2, DO 5.58 ± 0.6 mg/dl and ammonia concentrations was less than 0.1mg/l in all treatment tanks.

5.3.2 Effect of feeding diet containing 0.5% *A. sativum*, 1.0% *C. odorata* and 1.0% *T. triangulare* as feed additives for 42 days on growth performance of *C. gariepinus* juvenile.

Growth performance of the *Clarias gariepinus* juvenile fed with 0.5 % *Allium sativum* (Group B), 1% *Chromolaena odorata* (Group C) and 1% *Talinum triangulare* (Group D)

as feed additves over a 42-day period is shown in Table 5.6. Fish nourished with 0.5 % *Allium sativum* additive gave the best mean weight gain of 6.0 ± 0.53g and is significant when likened with the control. Conversely fish fed basal diet (0 %) without herbal additives (Group A) gave the slowest growth performance with mean weight gain of 3.75± 0.34g. The final weight is significant higher ($P<0.0001$) in group of fish fed with 0.5 % *Allium sativum* (Group B) and a significant higher value ($P<0.05$) was observed in the group of fish fed with 1% *Talinum triangulare* (Group D) compared with control group. The specific growth rate showed growing trend in all trial groups with significant higher ($P<0.05$) value detected in the fish fed with 0.5 % *Allium sativum* (Group B). No death was documented within the first 42 days of the trial.

Table 5.6. Growth performance of African catfish juvenile (*C. gariepinus*) fed with feed supplemented with herbal additives for 42 days

Groups	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Weight Gain (%)	Specific growth rate (%)
A(control)	53.67±0.10[a]	57.42±0.28[a]	3.75±0.34[a]	6.99[a]	0.07±0.01[a]
B	54.04±0.46[a]	60.03±0.11[b]	5.99±0.53[b]	11.08[b]	0.11±0.01[b]
C	54.67±0.10[a]	58.82±0.26[b]	4.15±0.32[a]	7.59[a]	0.08±0.01[a]
D	53.67±0.40[a]	57.69±0.69[a]	4.69±0.51[a]	8.85[a]	0.09±0.01[a]

The same superscript alphabets in the same column are not significantly different (P>0.05)

Key.

A = Control Treatment without Herbal Feed Additive

B = Treatment With 0.5% *Allium sativum*

C = Treatment With 1% *Chromolaena odorata*

D = Treatment With 1% *Talinum triangulare*

5.3.3 Effect of feeding diet containing 0.5% *A. sativum*, 1.0% *C. odorata* and 1.0% *T. triangulare* as feed additives for 21 and 42 days on haematology and biochemical parameters of *C. gariepinus* juvenile.

The results of hematocrit value, hemoglobin concentration, erythrocytes count (RBCs), MCV, MCH, and MCHC (Table 5.7), total leukocytes and differential leukocytes (Table 5.8) and some biochemical serum parameters (Table 5.9 and Table 5.10) when evaluated at end of 21 and 42 days post exposure were summarized as follows. There were no significant difference in Haematocrit (PCV), RBC, Hb concentration, MCV, MCH, MCHC, Platelet counts, Eosinophil value when evaluated at end at end of 21 and 42 days post exposure. The hematocrit values were related ($P>0.05$) amongst dissimilar groups however a higher values of 23.67 ± 1.47% and 23.0 ±0.0 % were recorded in group D for 21 and 42 days respectively whereas a lower value of 17.15 ± 7.40 % was recorded in group A (control group) at end of 21 days and the lower value of 18.0±0.0 % was observed in group C at end of 42 days. Higher Hb of 8.50 ± 0.98g/dl was recorded in group C at end of 21 days, a decrease in trend of Hb concentration was observed at end of 42 days with higher value of 7.97 ± 0.49g/dl was observed in group C. The highest value of erythrocytes count (2.31± 0.54 x 10^6 µl) was recorded in group C and lowest value (1.34 ± 0.05 x 10^6 µl) recorded in group B at end of 21 days. More so, the values of erythrocytes after 42 days showed a similar values among the groups however a higher value of 2.23 ± 0.58 x 10^6 µl was recorded in group B and a lowest value of 1.2 ± 0 0.04 x 10^6 µl was observed in group C. The MCV, MCH and MCHC values of the experimental fish were not significantly different ($P<0.05$) when evaluated at end of 21 days however highest values of 129.8 ± 15.29fl for MCV, 53.76 ± 8.08pg for MCH and 33.64 ± 0.39 g/dl for MCHC were recorded in the group D, A and B respectively and lowest value of 106.4 ± 49.28 for MCV, 39.30 ± 5.39 pg for MCH, and 33.02 ± 0.50 g/dl for MCHC were recorded in groups A, C and C correspondingly. By 42 days post exposure, highest values of 182.7 ± 3.58 fl for MCV, 57.73 ± 1.39 pg for MCH and 33.64 ± 0.39 g/dl for MCHC were recorded in the group D, D and C respectively and lowest value of 121.0 ± 26.50fl for MCV, 40.41 ± 9.24 pg for MCH, and 31.59 ± 0.14 g/dl for MCHC were recorded in groups B, B and D respectively

Total leukocytes and differential leukocytes of African Catfish juveniles evaluated at 21 and 42 days were shown in Table 5.8. The value of 11.83 ± 1.05 x 10^3 µl observed in group D for the experimental fish at end of 21 days displayed a decrease significant value ($P<0.05$) when likened with group A (control) with value of 16.5 ± 1.33 x 10^3 µl. At end of 42 days post exposure, values observed in group C increased meaningfully ($P<0.001$) when likened with control group. Group C revealed a substantial increase when likened with group D. The overall lymphocytes of all the fish of each group were found to range from 56.00 ± 0.58 % for group D to 71.0 ± 1.16% for group C at end of 21 days. The total lymphocytes count (71.0 ± 1.16%) observed in group C was meaningfully higher ($P<0.001$) when likened with the total lymphocytes count (59.0 ± 0.58%) of the fish in group A (control). At 42 days, the total lymphocytes count observed in group B (57.67 ± 0.88) decrease meaningfully ($P<0.001$) when likened with the control group and groups C and D. Similarly in case of Heterophils the result observed at end of 21 days rearing were as follows: decrease significant values of 27.67 ± 2.60 % ($P<0.05$) and 22.67 ± 0.88 % ($P<0.001$) were detected in group B and group C respectively equated with group A (control, 35.67 ± 0.33%). More so group B decrease significantly when compared with group D and also group C (22.67 ± 0.88) decrease significantly when compared with group D. At end of 42 days rearing, significantly higher values ($P<0.001$) were observed in group B (38.00 ± 1.16%) compared with group A (control, 25.67 ± 1.45%). Group B also revealed significantly higher value ($P<0.01$) when likened with group C (24.0 ± 0.58%) and group D (30.0 ± 0.58%). The monocytes values were meaningfully higher ($P<0.05$) in group B (4.67 ± 0.33%) compared with control group. Also group B (4.67 ± 0.33%) showed significantly higher value ($P<0.01$) than groups D (2.67 ± 0.33) at end of 21 days feeding regime. While the following observations were made at end of 42 days rearing; there was no substantial difference ($P>0.05$) in the average values of the monocytes however a higher value of 3.00 ± 0.0 % and lower value of (2.67 ± 0.33 %) were observed in groups E and B respectively. The biochemical serum parameters were evaluated at end of 21 and 42 days post exposure and the following were observed (Table 5.9 and Table 5.10): At end of 21 days, there was no noteworthy change in the average values of the plasma protein ($P>0.05$) of preserved groups likened with control group however group B showed meaningfully lower ($P<0.05$) value when likened with groups D. Comparatively, the total plasma protein

values were similar among the treated groups and the control group after 42 days nevertheless a highest value of 5.57 ± 0.03mg/dl was recorded for group C while a lower value of 5.2±0.12 mg/dl was recorded in group A (control). The value of Albumin at end of 21 days showed a decrease significantly value ($P<0.01$) for group C and D compared with control group. More so group B decrease significantly ($P<0.001$) when compared with groups C and D. On the other hand there was no meaningful difference ($P>0.05$) in the average values of the treated groups likened with control group at end of 42 days however a higher value of 1.70 ± 0.12 mg/dl was recorded in group B and a lower value of 1.47 ± 0.09 mg/dl was recorded in group D. No noteworthy variance ($P>0.05$) in the standards of Globulin among the treated groups and the control group at end of 21 days but a higher value of 4.43 ± 0.260 mg/dl was recorded in group D and a lowest value of 3.97 ± 0.07 mg/dl was recorded in group B. However, at end of 42 days, a higher value of 4.2 ± 0.17 mg/dl was recorded in group C and a lowest value of 3.67±0.15 mg/dl was recorded in group A. An insignificant ($P>0.05$) effect was detected in the A-G ratio among the groups when compared with control at end of 21 days however groups D showed a significantly higher ($P<0.05$) value likened with groups A, control after 42 days.

Blood serum components analysis revealed that serum enzyme ALP and ALT significantly reduced in group B and C likened with control group however AST values remain similar among the groups when evaluated at end of 21 days. At end of 42 days, AST value in group B increased significantly likened with control group however the values of ALT and ALP were marginally different compared with control group. Glucose, Creatinine and the blood urea nitrogen (BUN) values observed were similar ($P<0.05$) amongst the groups when evaluated at end of 21 and 42 days (Table 5.10). However the Cholesterol levels observed in this experiment decreased significantly in group B while groups C and D values increased significantly likened with the control group at end of 21 days. At end of 42 days, Cholesterol level decreased meaningfully ($P<0.01$) in cluster C when likened with control cluster as shown in Table 5.10.

Table 5.7. Comparison of haematological parameters of *C. gariepinus* juvenile fed diets containing 0.5% *A. sativum,* 1.0%*C. odorata* 1.0%*T. triangulare* as feed additives for 21 and 42 days.

Parameters	**Days**	**A**	**B**	**C**	**D**
PCV (%)	21	17.15± 7.40[a]	19.00±1.16 [a]	25.67±2.60 [a]	23.67±1.45 [a]
	42	20.0±0.0 [a]	24.0±1.73 [a]	18.0±0.0 [a]	23.0±0.0 [a]
Hb (g/dl)	21	8.20± 0.40 [a]	6.40±0.46 [a]	8.50±0.98 [a]	7.97±0.38 [a]
	42	6.6±0.06 [a]	7.97±0.49 [a]	6.0±0.12 [a]	7.27± 0.03 [a]
RBC (x	21	1.63±0.34 [a]	1.34±0.05 [a]	2.31±0.54 [a]	1.90±0.33 [a]
10^6µ/L)	42	1.27±0.06 [a]	2.23±0.58 [a]	1.20±0.04 [a]	1.26±0.03 [a]
MCV (fl)	21	106.4±49.28 [a]	142.7±13.56 [a]	119.5±17.86 [a]	129.8±15.29 [a]
	42	158.1±7.21 [a]	121.0±26.50 [a]	150.8±5.21 [a]	182.7±3.58 [a]
MCH (pg)	21	53.76±8.08 [a]	48.11±5.11 [a]	39.30±5.39 [a]	43.94±5.87 [a]
	42	52.23±2.84 [a]	40.41±9.24 [a]	50.20±0.89 [a]	57.73±1.39 [a]
MCHC (g/dl)	21	33.44±0.73 [a]	33.64±0.39 [a]	33.02±0.50 [a]	33.72±0.52 [a]
	42	33.00±0.29 [a]	33.25±0.61 [a]	33.33±0.64 [a]	31.59±0.14 [a]

Data are represented as mean of three samples replicates ± standard error. Mean values with the same superscript letter in the same row are not significantly different. (P>0.05).

Key.

A = Control Treatment without Herbal Feed Additive

B = Treatment With 0.5% *Allium sativum*

C = Treatment With 1% Chromolaena odorata

D = Treatment With 1% *Talinum triangulare*

Table 5.8. Comparison of total and differential leukocytes of *C. gariepinus* juvenile fed diets containing 0.5% *A. sativum,* 1.0%*C. odorata* and 1.0%*T. triangulare* as feed additives for 21 and 42 days

Group	Days	TLC (x 10^3µl)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophil (%)
A	21	16.5 ±1.33 [a]	59.00 ±0.58 [a]	35.67±0.33 [a]	3.00±0.58 [a]	2.67±0.33 [a]
	42	13.5±0.043 [a]	67.67±0.88 [c]	25.67±1.45 [a]	3.0±0.58 [a]	4.00±0.0 [a]
B	21	13.7±0.88 [a]	65.00±2.31 [a]	27.67±2.60 [b]	4.67±0.33 [b]	2.67±0.33 [a]
	42	12.8 ±0.213 [a]	57.67±0.88 [a]	38.0±1.16 [c]	2.67±0.33 [a]	2.00±0.0 [a]
C	21	17.8±0.29 [a]	71.00±1.16 [b]	22.67±0.88 [c]	4.00±0.00 [a]	3.00±0.58 [a]
	42	18.9±1.516 [b]	70.0±0.58 [a]	24.0±0.58 [a]	3.00±0.0 [a]	2.67±0.33 [a]
D	21	11.8±1.05 [a]	56.00±0.58 [a]	38.00±0.58 [a]	2.67±0.33 [a]	3.67±0.33 [a]
	42	10.8±0.462 [a]	63.67±0.88 [b]	30.0±0.58 [a]	3.00±0.58 [a]	3.67±0.88 [a]

Data are represented as mean of three samples replicates ± standard error. Mean values with the same superscript letter in the same row are not significantly different. ($p>0.05$. TLC = Total Leukocytes Count.

Key.

A = Control Treatment without Herbal Feed Additive

B = Treatment With 0.5% *Allium sativum*

C = Treatment With 1% *Chromolaena odorata*

D = Treatment With 1% *Talinum triangulare*

Table 5.9. Comparison of some biochemical parameters of *C. gariepinus* juvenile fed diet Containing 0.5% *A. sativum*, 1.0%*C. odorata* 1.0%*T. triangulare* as feed additives for 21 and 42 days

Parameters	**Days**	**A**	**B**	**C**	**D**
Total Protein	21	5.43±0.03 [a]	5.20±0.21 [a]	5.87±0.23 [a]	6.93±0.57 [a]
	42	5.2±0.12 [a]	5.5±0.12 [a]	5.57±0.09 [a]	5.37±0.03 [a]
Albumin	21	1.30±0.06 [a]	1.07±0.09 [a]	1.93±0.13 [b]	2.33±0.23 [b]
	42	1.47±0.03 [a]	1.70±0.12 [a]	1.5±0.17 [a]	1.47±0.09 [a]
Globulin	21	4.17±0.03 [a]	3.97±0.07 [a]	4.0±0.17 [a]	4.43±0.260 [a]
	42	3.67±0.15 [a]	3.87±0.03 [a]	4.2±0.17 [a]	3.9±0.12 [a]
A-.G Ratio	21	0.33±0.07 [a]	0.20±0.00 [a]	0.47±0.03 [a]	0.50±0.0 [b]
	42	0.37±0.03 [a]	0.4±0.0 [a]	0.17±0.03 [a]	0.370.03 [a]
AST	21	172.3±1.76 [a]	174.0±1.16 [a]	191.7±0.88 [a]	188.0±1.16 [a]
	42	188.3±2.03 [a]	206.7±3.37 [b]	189.7±0.33 [a]	186.7±3.18 [a]
ALT	21	38.33±0.88 [a]	37.33±0.67 [a]	27.33±0.67 [b]	38.67±1.20 [a]
	42	32.0±1.0 [a]	40.67±4.33 [a]	42.67±2.60 [a]	35.6±4.93 [a]
ALP	21	301.3±4.37 [a]	185.0±2.52 [b]	327.0±2.52 [a]	253.7±0.88 [a]
	42	194.0±3.51 [a]	223.0±5.77 [a]	199.0±2.31 [a]	218.0±1.16 [a]

Data are represented as mean of three samples replicates ± standard error. Mean values with the same superscript letter in the same row are not significantly different. ($p>0.05$).

Key.

A = Control Treatment without Herbal Feed Additive

B = Treatment With 0.5% *Allium sativum*

C = Treatment With 1% *Chromolaena odorata*

D = Treatment With 1% *Talinum triangulare*

Table 5.10. Comparison of BUN, creatinine, glucose and cholesterol of *C. gariepinus* juvenile fed diets containing 0.5% *A. sativum,* 1.0%*C. odorata* and 1.0%*T. triangulare* as feed additives for 21 and 42 days

Parameters	Days	A	B	C	D
BUN	21	9.63 ±0.20 [a]	10.0±0.12 [a]	10.20 ±0.46 [a]	10.37±0.47 [a]
	42	10.43±0.26 [a]	11.83±0.27 [a]	9.53 ±0.09 [a]	9.60±0.06 [a]
Creatinine	21	0.70± 0.06 [a]	0.57±0.07 [a]	0.70±0.0 [a]	0.80±0.0 [a]
	42	0.77±0.03 [a]	0.70±0.0 [a]	0.77±0.0 [a]	0.7±0.0 [a]
Glucose	21	217.3±4.81 [a]	210.3± 0.88 [a]	256.3±25.12 [a]	252.3± 34.86 [a]
	42	264.7±18.19 [a]	269.7±0.37 [a]	236.0±0.58 [a]	291.0±27.14 [a]
Cholesterol	21	188.7± 1.20 [a]	125.7±2.6 [b]	235.3±0.33 [c]	235.7±0.88 [c]
	42	209.3±5.49 [a]	199.0±2.31 [a]	185.0±1.73 [b]	210.0±4.04 [a]

Data are represented as mean of three samples replicates ± standard error. Mean values with the same superscript letter in the same row are not significantly different. (p>0.05). BUN = Blood Urea Nitrogen

Key.

A = Control Treatment without Herbal Feed Additive

B = Treatment With 0.5% *Allium sativum*

C = Treatment With 1% *Chromolaena odorata*

D = Treatment With 1% *Talinum triangulare*

5.3.4 Disease resistance (challenge test)

Fish mortality after intraperitoneal (IP) injection of *P. aeruginosa* occurred post 21hr and increased in the 1st day after injection and then declined till 6th day when mortality stopped. The accumulative death was forty percent, twenty percent and thirty percent in fish nourished with 0.5% of *Allium sativum* (D2 diet), 1% *Chromoleana odorata* (D3 diet) and 1% *Talinum triangulare* (D4 diet) respectively for 42 days against *P. aeruginosa* and they conferred relative protection of 50, 75 and 62.5 respectively (Table 5.11). The highest mortality of 80% and relative level of protection of 0.0 were observed in the group of fish fed with control diet (D1) as shown in Table 5.10. Fish fed with basic diet (0%, negative control) and injected with normal saline showed percentage survivability of 100%. No death was documented in the treated animal during 42 days before challenge test.

Table 5.11. Survival test (disease resistance)

Fish Group	No of Fish	Type of innoculate	Days after challenge							No of Dead Fish	NS	M (%)	S (%)	RLP
			1	2	3	4	5	6	7					
A (-ve)	10	*NS	0	0	0	0	0	0	0	0	10	0	100	100.0
A (+ve)	10	*P a*	3	1	2	1	1	0	0	8	2	80	20	0.0
B	10	*P a*	1	2	1	0	0	0	0	4	6	40	60	50.0
C	10	*P a*	2	0	0	0	0	0	0	2	8	20	80	75.0
D	10	*P a*	1	1	1	0	0	0	0	3	7	30	70	62.5

*NS=Normal Saline, NS=Number of Survival, M (%) = Mortality, S (%) = Survival, *P a = Pseudomonas aeruginosa*, RLP= Relative Level of Protection.

Key.

A = Control Treatment without Herbal Feed Additive

B = Treatment With 0.5% *Allium sativum*

C = Treatment With 1% *Chromolaena odorata*

D = Treatment With 1% *Talinum triangulare*

5.4 Discussion

Immunostimulants increase resistance to diseases by improving nonspecific immune reaction (Sakai, 1999). Fish use a range of adaptive and non-specific protective strategies to counter attacking pathogenic organisms (Dugenci *et al.,* 2003). Since immunostimulants confer overall advantage in terms of survival and resistance to diseases, animals receiving them can be expected to perform better in terms of growth and thereby contribute to production. Sahoo and Majumdar (2002) have shown that immunomodulators increase specific immunity and reduce mortality in immunocompromised carp. The study of disease control in crustacean farming through use of immunostimulant (Smith *et al.,* 2003) and the demonstrated effect of medicinal plant excerpts on rainbow trout (Dugenci *et al.,* 2003) has been established. The trial was carried out to assess the antibacterial effect of locally available plants, *Allium sativum*, *Chromolaena odorata* and *Talinum triangulare* to assess their protective potential benefits in aquaculture. Previous studies have shown antibacterial and antifungal possessions of garlic and black seed (*Nigella sativa*) against bacterial and fungal agents isolated from *O. niloticus* (Diab *et al*., 2002). Studies to establish a link between immunostimulant potential of *Allium sativum*, *Chromolaena odorata* and *Talinum triangulare* on improved growth, disease resistance and survivability in *Clarias gariepinus* have been lacking. The immunostimulatory effect of these herbal plants used in this experiment was discussed with respect to the following headings:

5.4.1 Water quality

The levels of temperature (28.7 ± 0.4^{o}C), pH (7.1 ± 0.2), dissolved oxygen (DO) (5.58 ± 0.6 mg/dl) and ammonia equal or less than 0.1mg/l documented from the cultivated tanks in this current study were within the suitable range for catfish cultivation (Boyd, 1984) and have no apparent influences on catfish growth. Some studies found and demostrated that every growth rate was related to the water quality (Pichavant *et al.,* 2001). Results revealed that water qualities were not affected due to addition of herbal feed additives to the food of the experimental African catfish. Regular changing of water carried out in this experiment could have helped maintain good water quality. The interaction of poor water quality or environmental disorder, presence of harmful microbes and nutritional disorder cause disease in aquatic animals and this have been a key obstacle to aqua-farming globally

(Kumar and Anantharaja, 2007). Nevertheless, in *Clarias gariepinus*, undesirable effect of poor water superiority are infrequent as fish are adapting to a variety of ecological factors.

5.4.2 Growth performance of experimental fish (*Clarias gariepinus*)

In this current study, the incorporation of *Allium sativum*, *Chromolaena odorata* and *Talinum triangulare* as feed supplement improved feed consumption and growth. The outcomes agreed with the results of some workers such as Aly and Mohammed (2010) who used *Echinacea purpurea* and *Allium sativum* as immunostimulant in Nile Tilapia (*Oreochromis niloticus*), Abdel- Tawwab *et al*. (2010) who fortified diets of *Oreochromis niloticus* with Green Tea and Amla for growth and production and Sivagurunathan *et al.* (2012) who used *Phyllathus emblica* in growth and blood characteristics in *Tilapia mossambicus* tested with *P. aeruginosa*. Incorporation of *Allium sativum*, *Chromolaena odorata* and *Talinum triangulare* in the feed could have improved deliciousness, breakdown and absorption of nutrients.

5.4.3 Haemo- biochemical parameters of experimental fish

Blood characteristics parameters, including RBC count, Hb concentration, PCV and WBC count, have offered valued facts to aquaculturists in the valuation of fish wellbeing (Ramasamy *et al*., 2010). Observing these standards and assembling statistics on the status of WBC could reveal over-all immunity status of fish (Banaee *et al*., 2008). Hematocrit level is a pointer for fish health which provides sign on fish health and clarifies aberrations caused by immune booster (Dorucu *et al*., 2009). This studies indicated that dietary supplementation of *Allium sativum*, *Chromolaena odorata* and *Talinum triangulare* as feed additives for at least 42 days showed improved PCV and Hb concentration. The outcomes propose that feeding of herbal plants for at minimum of 21 days can upsurge the amount of RBC, haematocrit and haemoglobin standards. Therefore, feeding of *Allium sativum*, *Chromolaena odorata* and *Talinum triangulare* separately may affect the activity of haematopoietic tissues like spleen and head kidney which perform essential role in blood cell formation in fish. Though the outcomes in this trial revealed that feeding of 0.5% *Allium sativum* and 1.0% *Chromolaena odorata* and 1.0% *Talinum triangulare* as supplement for at least 42 days improved haematocrit and also improved values of hemoglobin content (Hb) when likened with control, no substantial changes were

discovered in the amount of RBC, MCV, MCH and MCHC values. It was therefore revealed that feeding of these herbal plants could concentrate Hb in RBCs of fish. This was in line with accounts of Ndong and Fall (2007) and Shalaby *et al*. (2006) who gave account of increase Hb concentration, PCV, and quantities of WBCs and thrombocyte in Nile tilapia and hybrid tilapia that were fed diet enriched with garlic. Ji *et al*. (2007) also reported an upsurge in hemoglobin levels of Nile tilapia nourished with herbal supplementary food and suffice to say that difference in species or different condition of rearing could lead to difference in observations.

Leukocytes values were meaningfully improved in group of fish nourished with 10g/kg of *Chromolaena odorata* when likened with the fish nourished without medicinal plant additive, this result proved that dietary supplementation of 1% *Chromolaena odorata* improved the complete immune response of *Clarias gareipinus* as was showed by the substantial upsurge of the White Blood Cell counts. Akinwale *et al*. (2004) recounted that a quantifiable upsurge in WBC of fish and any other animal is associated with immunity and ability to resist diseases. Overall and differential WBC counts are vital guides of innate defense actions in fish (De-Pedro *et al*., 2005), as WBC are fundamentally required in phagocytic and immune reactions to challenges of parasite, bacteria and virus (Houstan, 1990). WBC activities could be enhanced by normal immune boosters as was earlier recounted in gold fish which were fed with combinations of selected aromatic plants integrated in shrimp and fish food (Harikrishnan *et al*., 2010). The outcomes of this current research corroborated the work of Sahu *et al*. (2007) who recounted that leucocyte counts are advanced in *Labeo rohita* fingerlings treated with *Mangifera indica* when equated to control. The alterations of WBC, RBC and Hb concentration might be due to motivation of the immune system with herbal extracts, which in sequence might be specific reaction of the animal occasioning in a fortfied immune protection (Whyte, 2007).

There is substantial upsurge in the Total Leucocytes Counts in the group of fish nourished with 10g/kg of *Chromolaena odorata* be measured as a pointer for improvement in overall resistance of fish fed *Chromolaena odorata* and *Allium sativum*. The significant increase in neutrophils in the cluster of fish nourished with 5g per Kg of *Allium sativum* and higher values recorded in the cluster nourished with 10g/kg of *Talinum triangulare* compared with the control group might be innate immunity and significant upsurge in

lymphocyte counts in the cluster of fish nourished with 10g/kg of *Chromolaena odorata* and relative improvement of lymphocytes in other groups at end of 21 days could be ascribed to the specific immune response. The results of this research shown noteworthy increase ($P<0.001$) alternations in lymphocytes value of cluster of fish nourished with 10g/kg of *Chromolaena odorata* groups and improved value of cluster of fish nourished with 5g/kg of *Allium sativum* when equated with control group after 21 days. Lymphocytes are associated with several immunological reactions for instance the making of antibodies and variation of the immune defense. Neutrophil values increase significantly ($P<0.001$) in group of fish fed with 5g/kg of *Allium sativum* and eosinopphil value reduce considerably ($P<0.01$) when likened with control group while similar result were observed at end of 42 days in the experimental groups. The result of the serum biochemistry presented in Table 5.9 and 5.10 showed that the total protein was not meaningfully ($P>0.05$) affected by addition of herbal feed additives however the results showed improved total protein in fish nourished with herbal additives likened with control. Albumin level were enhanced by the feed supplemented with *Chromolaena odorata* (1.0%) and *Talinum triangulare* (1.0%). The upsurge of globulins in plasma of fish fed with *Allium sativum* (0.5%), *Chromolaena odorata* (1.0%) and *Talinum triangulare* (1.0%) fed separately enhanced immune system of the experimental fish.

The experimental infection by means of *P. aeruginosa* showed low death percentage in treated fish equated with the control. Relative level of protection (RLP) obtained in C (75%) > D (62.5%) > B (50%) > A (0.0%). These outcomes were in line with Amany-Diab *et al.* (2014) who documented that the death rate in Nile tilapia fed with one percent and two percent curcumin in the diet after injected with *Pseudomonas fluorescens* were reduced compared with control group.

CHAPTER SIX

APPLICATION OF *Allium sativum, Chromoleana odorata* AND *Talinum triangulare* IN THE MANAGEMENT OF WOUND IN THE AFRICAN CATFISH

6.1 Introduction

Bioactive compounds obtained from plants had been found to be useful as therapeutic agents against various animal, plant and human ailments, additionally to their nutritional and food values and this has made plants more valuable to animal and human lives (Ogbonnia *et al.*, 2008). Today, herbs have been used to treat several ailments (Oz, 2010). Herbal treatment is gaining success in diseases management and is eco-friendly, cost effective and side effects are minimal (Ogbonnia *et al*., 2010). Stimulation of immune is potentiated by medicinal plants. Medicinal plants which were reported to be safe for consumers are widely available in Africa and these medicinal plants similarly have important role in aqua-farming (Direkbusarakom, 1998). Internationally, therapeutic plants are used because of their activities against bacteria, fungi and virus (Aibinu, 2006). Rawling *et al*. (2009) reported that recent works had demonstrated the usefullness of herbal products in fish cultivation.

Garlic, (*Allium sativum*) *L*. belongs to Alliaceae family, has been commonly acknowledged as a valued medicinal plant, documented as spice and had been used for various ailments and physiological disorders. Garlic has been documented for controlling pathogens such as fungi and bacteria and generally improve the wellbeing of fish (Corzo-Martinez *et al.*, 2007). *A. sativum* has also been identified as herb that has potential for wound healing (Jalali *et al*., 2009).

Siam weed (*Chromolaena odorata*) in humid part of Africa has a reputation of being therapeutic plant for treatment of different diseases comprising fever, malaria, and toothache (Olajide, 2000). Traditionally, crude water leaf extract of the Siam weed has been used as an antiseptic for wound bandage (Zachariades *et al*., 2009). Fresh cuts bleeding and

nose bleeding had been arrested using fresh juice from the leaf of *C. odorata* as haemostatic agent and It has been documented to be beneficial in the management of wounds as well as haemorhoids (Phan, 2001). *Talinum triangulare* is a cosmopolitan wild plant ubiquitous in the tropics. It is a non- conventional vegetable crop which initiated from tropic and is extensively developed in Western part of Africa, Southern part of America and Asia (Schippers, 2009). *T. triangulare* is a source of vitamins, beta-carotene, minerals (for example K, Ca and Mg) proteins and pectin (Ezekwe *et al*., 2013). It has been proved to have valuable therapeutic capabilities for instance purgative, treatment of digestive tract illnesses and diarrhea (Mensor, 2001) and also proved to be medically useful in management of cardiac ailments such as stroke (Aja *et al.*, 2010).

Discovery of more medicinal plants has compelled scientific examination of their biological activities so as to offer information that will assist physicians, veterinarian as well as farmers in taken prudent decisions in using the plants (Oyewole and AKingbala, 2011). Hence, the objective of this work was to appraise the influence of various therapeutic herbs in management of diseases using practical field trials model. The dietary effect of *Allium sativum*, *Chromolaena odorata* and *Talinum triangulare* on rate of local wound healing in adult African Catfish was therefore evaluated.

6.2 Materials and methods

6.2.1 Experimental technique

In this trial, twenty four (24) healthy fish of average weight 146 ± 4.74g were used for the wound healing study. Fish were permitted to adapt for 7 days. The fish were randomized into eight groups (Q1, Q2, Q3, Q4, Q5, Q6, Q7 and Q8). Each group consist of three (3) fresh water African Catfish. Group Q1 and Q2 were fed basic diet (Control) containing no herbal extracts *ad libitum*. Group Q3 and Q4 were fed feed containing 5g/kg of *Allium sativum* as feed additives, Group Q5 and Q6 were fed feed containing 10g/kg of *Chromolaena odorata* as feed additives while Group Q7 and Q8 were fed feed containing 10g/kg of *Talinum triangulare* as feed additives. The herbal embedded feed was fed twice a day for 21 days. After rearing for 21 days, the fish were anesthetized by immersion in 1 litre of cold water containing 200mg of MS-222. The artificial wound was created for the fish by gently incised wound of 45.0 mm in length and 1.0 mm in depth on the dorso-lateral part

with help of sterile blade (Plate 6.1). Then, Groups Q2 (Positive control), Q4, Q6 and Q8 were injected with 0.1ml of already predetermined pathogenic *Pseudomonas aeruginosa* intraperitoneally. All the fish were stressed by skipping feeding for 24 hours and then all the groups were continued to be fed with the feed supplemented with medicinal plants except groups Q1 and Q2 (fed basic diet) which served as negative and positive control respectively. All the fish were left for observation. The wound closure was assessed using macroscopical parameters (measurements) at steady intermissions of time and calculated as per hundred of wound closing and the establishment of fresh epithelial tissue indicates epithelization time. The period of epithelization is number of days necessary for dropping of the scar without any remaining of the fresh wound (Kokane *et al*., 2009) and this was calculated in line with method previously used by Bazafkan *et al*. (2014).

$$\text{Wound closure \%} = \frac{\text{Wound area on day 0 - Wound area on day } n^{th}}{\text{Wound area on day 0}} \times 100$$

Where n = number of days.

In this experiment, the rate of wound healing and survival were evaluated to study the curative potential of each medicinal plant as therapeutic aid for diseases management in fish health. The assessment of the wound was based on macroscopic factors. Initially, the wound prime extent was measured and then the wound area was calculated on the day 1^{st} and subsequently evaluated on 3^{rd}, 6^{th}, 9^{th}, 12^{th} and 15^{th} days and the above formula was used to compute the curative per hundred.

Plate 6.1. (A) Showing process of wound creation

6.3 Result

Wound restorative progression was evaluated morphologically. The average length (mm) and percentage of wound closure in dissimilar treatment clusters on days 3, 6, 9, 12, and 15 after the sterile incision was created were assessed and likened with those of negative and positive control clusters as shown in Table 6.1. The pattern of rate of wound closure was shown in Table 6.2. On the third day, group of fish fed with 10g/kg *Chromolaena odorata* as feed additives and not challenged with bacterial infection (**Q5**) showed the highest percentage of wound closure (55.5%) and lowest percentage of wound closing (11.1%) was detected in cluster of fish fed without herbal feed additives and equally challenged with bacterial strain (**Q2**). The fish in the positive control group (**Q2**) started to shown abnormal signs post 18 hrs injection. On 3rd day the fish developed spots on lateral side, large brownish abrasions near tail, and hemorrhagic spots near pelvic region, wound got contaminated (Plate 6.2**)** and by fifth day, two of the fish in the group **Q2** died. At 6th day, the rate of healing was still fastest in *Chromolaena odorata* treated fish, **Q5** (68.9%) followed by *Allium sativum* treated fish, **Q3** (60.0%). Others wound improved as follows: **Q6** (57.8%), **Q4** (46.7%), **Q7** (37.8%), **Q8** (28.9%) and **Q1** (22.2%). By the eighth day post inflicted wound, the remaining one fish in group **Q2** died. On the 9th day, the rate of wound healing was observed to be fastest in group of fish fed with 10g/kg *Talinum triangulare* (**Q7**) (82.2%), followed by **Q5** (77.8%), **Q3** (71.1%), **Q6** (66.7%), **Q4** (55.6%), **Q8** (44.9%) in that order with lowest value observed in **Q1** (33.3%). By the 12th day, **Q7** (95.6%) still maintain highest rate of wound closure, followed by **Q5** (84.4%) and **Q3** (80.0%). By the 15th day, the scar has finally dropped on fish in group **Q7** while other groups still carried scar but with different degree of wound closure. Plate 6.3 showed epithelization of the wound in progress. It was observed that the more stressed fish (starvation and induced mild infection) showed less improvement on rate of wound closure than the less stressed (starvation only) fish as fish were aging as shown in the pattern of rate of wound healing (Table 6.2). The wound closure level up in all remaining fish by the end of 23rd day.

Plate 6.2. Showing contamination of the wound in positive control

Plate 6.3. Showing process of epithelization

Table 6.1. Comparison of average length (mm) and percentage of wound closure in different groups of the experimental fish on 3rd, 6th, 9th, 12th and 15th days

	Group of Fish							
Days	**#Q1**	***Q2**	***Q3**	****Q4**	***Q5**	****Q6**	***Q7**	****Q8**
3	38(15.6)	40(11.1)	24(46.7)	30(33.3)	20(55.6)	26(42.2)	35(22.2)	37(17.8)
6	35(22.2)	NA	18(60.0)	24(46.7)	14(68.9)	19(57.8)	28(37.8)	32(28.9)
9	30(33.3)	NA	13(71.1)	20(55.6)	10(77.8)	15(66.7)	8(82.2)	25(44.9)
12	23(48.9)	NA	9(80.0)	14(68.9)	7(84.4)	12(73.3)	2(95.6)	18(60.0)
15	16(64.4)	NA	4(91.1)	10(77.7)	2(95.6)	10(77.8)	0(100.0)	6(86.7)

*Group fed herbal diet without pathogenic *P.aeruginosa* injection; **Group fed herbal diet with *P aeruginosa* injection; *** Group fed without herbal diet with *P. aeruginosa* injection. # Group fed without herbal diet and without pathogenic *P.aeruginosa* injection. (%) in parenthesis.

Key.

Q1 = Negative Control Treatment without Herbal Feed Additive,

Q2 = Positive Control Treatment without Herbal Feed Additive

Q3 = Treatment With 0.5% Allium sativum,

Q4 = Treatment With 0.5% Allium sativum

Q5 = Treatment With 1% Chromolaena odorata

Q6 = Treatment With 1% Chromolaena odorata

Q7 = Treatment With 1% Talinum triangulare,

Q8 = Treatment With 1% Talinum triangulare,

Table 6.2. Pattern of rate of wound closure in the experimental fish

Days	Group of experimental fish
3	Q5 > Q3 > Q6 > Q4 > Q7 > Q8 > Q1 > Q2
6	Q5 > Q3 > Q6 > Q4 > Q7 > Q8 > Q1
9	Q7 > Q5 > Q3 > Q6 > Q4 > Q8 > Q1
12	Q7 > Q5 > Q3 > Q6 > Q4 > Q8 > Q1
15	Q7 > Q5 > Q3 > Q8 > Q6 > Q4 > Q1

Key.

Q1 = Negative Control Treatment without Herbal Feed Additive,

Q2 = Positive Control Treatment without Herbal Feed Additive

Q3 = Treatment With 1% *Allium sativum*,

Q4 = Treatment With 1% *Allium sativum*

Q5 = Treatment With 1% *Chromolaena odorata*

Q6 = Treatment With 1% *Chromolaena odorata*

Q7 = Treatment With 1% *Talinum triangulare*

Q8 = Treatment With 1% *Talinum triangulare*

6.4 Discussion

The medicinal plants as an essential therapeutic agents for management of illnesses in fish specifically as a substitute to antimicrobials in fish health was investigated in this study. Three medicinal plants were chosen and used to treat wound created deliberately on fish and those fish were stressed by starvation and some further stressed by infected with low dosage of pathogenic *Pseudomonas aeruginosa*. Wounds are likely happenings of life time. 0It could be caused by chemical, physical, microbial e.t.c affront to the skin (Raina *et al*., 2008). Wound healing machinery is a compound procedure, where the tissue or the skin undergoes healing or repair after the injury (Thomson, 2000). In typical skin, the peripheral layer epidermis and the inward or the profound layer subsist in unflattering equilibrium state, framing a defensive barricade against the outer surroundings. When the defensive barricade is damaged, the standard wound curative procedure begins instantly. The whole process of wound restorative beginning right after injury, may last from days to years (Nagori and Solanki, 2011). A condition of lack of healthy sustenance may give a deficient measure of protein which bring about the diminished level of collagen production or an expanded casual for contamination (Albritton, 1991).

Among the three medicinal plants used in this experiment, *Chromolaena odorata* showed immediate response for wound healing. This proposes that *C. odorata* might have hemolytic, immunostimulant, antioxidative, anti-inflammatory and antimicrobial activities (Lovkova *et al*., 1999). *C. odorata* extracts stopped hemorrhage from new wounds by decreasing whole blood clotting time which are essential guides of haemostatic action (Obadoni, 2002). Anyasor *et al*. (2011) reported the decreasing of clotting period of blood by the *C. odorata* leaf extracts and this has indicated that the leaf extracts could also retard the blood clotting pathways. Occurrence of tannins as well as saponins in the herbs is assumed to be accountable for its haemostatic action, in so doing, backup the old-style usage of the *C. odorata* foliage in wound restorative (Zachariades *et al*., 2009).

Garlic (*Allium sativum*) also showed high potential for wound healing in this study as feeding of the garlic as feed additives to some group of fish prevent degeneration of the wound. Londhe *et al*. (2011) stated that the pharmacological effect of *Allium sativum* which need more devotion of time by scientists consist of the anti-coagulant, anti- inflammatory and wound restorative activity. This study proved the efficacy of garlic in wound healing,

its anticoagulant activity, anti- inflammatory and immunomodulatory action in African Catfish.

Talinum triangulare (Water leaf) showed a marvelous improvement on wound healing compared with other two medicinal plants tested, though initially, its aid in healing processes was not as fast as other two, it healed faster than the other two plants investigated. Ezekwe *et al*., (2013) reported that *T. triangulare* significantly increased RBC concentration. This implies that *T. triangulare* foliage might be used for handling of ailment situations for instance anaemia and might also be used for animal to boost their blood level. In a research conductd by Aja *et al*. (2010), the result of quantitative analysis of *Talinum triangulare* revealed advanced levels of bioactive compounds in dry sample than wet sample. Analysis of the dried leaves of water leaf phytochemically carried out showed a considerable quantity of alkaloids, saponins, phenols amid others and moderate level of cardiac glycoside, flavonoid and tannins. The availabity of these bioactive compounds in abundance makes it useful medicinally and nutritionally (Oloyede, 2005). Water leaf is useful therapeutically in the handling of cardiovascular illnesses like stroke, obesity, e.t.c (Adewumi and Sofowora, 1980) and conservatively it is used as sweetener of other botanical species. Aja *et al*. (2010) reported that a *Talinum triangulare* was virtuous for the treatment of cardiovascular ailments and oxidative stress, as flavonoids were antioxidants.

Medicinal plants was used as a fresh raw material for making of synthetic drugs (Ji, 2007). Phytochemicals which are naturally present in plants use nutrient and dietetic fibres to safeguard animal and Man against infections (Soladoye *et al*., 2012). Plants derivative products have largely contribute to human healthiness (Karuppiah and Rajaram, 2012). Nigeria is richly endowed with precious medicinal plants and many have been documented (Soladoye *et al*., 2012) however, lack of adequate research is partially accountable for their under-utilization particularly in areas outside the traditional neighborhoods where they are originate and ingested. This work will arouse interest of scientists for further research on benefits of medicinal plant(s) and the ailments for which they are used for; with the purpose of developing potential drugs for some common diseases.

CHAPTER SEVEN

Conclusion and Recommendations

Contributions to Knowledge

7.1 Conclusion

The findings in this study have shown that *A. sativum, C. odorata, T. triangulare* dried powder could be incorporated up to 3.0% level as feed additives in *Clarias gariepinus* feed since all the trial feed were eaten by the fish demonstrating that the quantity of herbal plants incorporated into the diets did not disturb the sweetness of the foods. Nevertheless, study demonstrated the excellent addition level were those of the diets with lower inclusion rates of 0.5% -1.0% that would not cause negative physiological effects on the fish. These herbs are nearby accessible in the tropics and could be obtained all through the year. The inclusion of *A. sativum, C. odorata, T. triangulare* as feed additives separately have the prospective to make substantial contributions to development of the *Clarias gariepinus* fingerlings. They have prospective to replace antibiotics in aquaculture diets. This will be a potential alternative to antibiotics and prevent excessive use of antibiotics to improve growth and/or resistance to diseases in aquaculture systems. It is expected that these results will arouse a series of studies on the exploitation of these 3 medicinal plants as additives in foods for fishes. In the last decade, there is increased public awareness on detrimental effect of antibiotics to human consumers due to emergence of antibiotics resistant bacterial strains and antibiotics residue in food fish which led to the banning of the use of antibiotics as growth promoters in aqua-farming foods in the advanced countries of the world. Use of medicinal plants as feed additives could also decrease feed price to the aquaculturists, whose utmost main input cost originates from feed.

Characterisation and comparative blood characteristics are better pointers of the functional position of animals, valued in checking food poisonousness specifically with

feed components that disturb the blood together with the well-being of farm animals, a pathological indicator of the position of unprotected animals to toxicant and other circumstances and studies of haemtological parameters are also valuable in the diagnosis of various ailments in addition to exploration of the degree of impairment to blood. Animals with perfect blood conformation are expected to demonstrate good performance. In this current study, the values of blood characteristics obtained were within the range for *Claraias gariepinus* as they were reported by various researchers referenced in our discussions however dissimilarities in blood factors of fish in this study might be attributed to dissimilarities in the inclusion rates of different feed additives in the diets.

The plants additives used were tolerated by the experimental fish therefore it could be concluded that *A. sativum*, *C.odorata* and *T. triangulare* could be fed separately to *Claraias gariepinus* as feed additives so as to limit the use of chemicals. The significant increase in haematocrit and haemoglobulin concentration especially at 1.0% inclusion rates of *T. triangulare,* the substantial increase in lymphocyte counts, total protein level, globulin level and reduction in serum enzymes (ALT, AST) especially at 0.5% inclusion rates of *A. sativum,* 1.0% of *C. odorata* and 1.0% of *T. triangulare* are all indications of improvement in immunologic function of the blood, an indication of upgrading of both innate and specific immune reactions.

The non-toxic nature of these plants was also demonstrated by histopathological studies. Tissues harvested from the experimental fish were subjected to histopathological studies revealed nothing to mild cellular alterations especially at dose of 5g/kg (0.5%) *Allium sativum*, 10g/kg (1.0%) *C. odorata* and 10g/kg (1.0%) *T. triangulare.* Henceforth, the application of *Allium sativum, Chromolaena odorata* and *Talinum triangulare* at dose of 0.5-1.0% for each of these medicinal plants as phyto-therapy in aquaculture has enormous prospective and it has to be discovered more by unravel the machinery of action totally. The excessive usage of synthentic drugs have resulted into drug residue and emergence of hardy pathogens in cured fishes. Drug residue have harmful effect on the animals, pollutes the environments and threatens human consumed them. The present

study demonstrated that integration of herbal plants in fish food composition may not only act as growth promoter but also enhanced the resistance of challenged fish. This modulation of the fish immunity has really improved the resistance of tested fish to *Pseudomonas aeruginosa* as was specified by the substantial reduction in deaths of fish fed *Allium sativum, Chromolaena odorata* and *Talinum triangulare*. Immunostimulation in terms of enhanced survival in experimental groups equated to the control groups was detected, both during rearing season and after artificial bacterial infection.

The present study also provides some valuable statistics concerning the efficacy of therapeutic herbs for treatment of wound in farmed fish. It has demonstrated that dietary inclusion of *Allium sativum, Chromolaena odorata, Talinum triangulare* separately at lower inclusion rates of 0.5% -1.0% improve wound healing process of fish and prevent contamination of wound and this could reduce death that might occur as a result of physical attack among the cultured fish and also an indication of improving ability of the cultured organism against induced stress.

7.2 Recommendations

There is need for expansion and upkeeping of aqua-farming in Africa particularly in the countryside. The home-grown of fish food is very essential. Cultured fish needs excellent composed food for development and reaching market size in time. The unfavourable side effects of number of feed additives available in Nigeria market is of public health concern. It is recommended that 0.5% *Allium sativum* (garlic), 1.0 % *Chromolaena odorata* (Siam weed) and 1.0 %. *Talinum triangulare* (Water leaf) could be fed separately as feed additives for growth promotion in *Clarias gariepinus* fingerlings.

Administration of medicinal plants as feed additives appears to be a practical method and is recommended as they enhancing survival of African Catfish. They also have the potential to enhance survivability in case of bacterial infection. Modulation of the fish immunity has seriously improved the resistance of tested fish to *Pseudomonas aeruginosa* infection as was shown by the noteworthy reduction in deaths in fish fed 0.5% *Allium sativum*, 1.0 % *Chromolaena odorata* and 1.0 %. *Talinum triangulare* as feed additive.

The present study provides some useful information regarding the efficacy of medicinal herbs on managing and handling of wounds. Herbs documented may serve as

possible sources of new wound healing molecules. Coalescing the old-style and current understanding could lead to evolvement of superior medications for wound restorative with less negative effects. The outcome of the current work suggests pharmacological signal on the legends usage of fresh leaf of some plants for healing wounds. Exploring the injury recuperating action of these restorative plants awaits future research. The fish farmers should be encouraged to include these herbs in fish food as it will reduce the dependency on antibiotics as growing supporter however further study needs to be carried out with similar food composition in normal earthen pond conditions and in re-circulating schemes to reveal growth performances of the fish in normal situations. Garlic, Water leaf and Siam weed are nearby obtainable in the tropical and can be acquired all through the year.

Finally, the confirmatory reports of these works point to medicinal plants in growth promotion, fish well-being management and promoting wound healing prove to be quick substitute. Advance works could be carried out on these therapeutic herbs for dosage standardization. Fish farmers are therefore encouraged to supplement these medicinal plants in fish diet. It is recommended to carry out result demonstration to publicise the use of therapeutic herbs among the farmers. Authorities should make or review if there is any existing law concerning the use of therapeutic herbs in aqua-farming with the respect to the outcome of this and other similar studies

7.3 Contributions to Knowledge

1. This work had shown that *Allium sativum, Chromolaena odorata, Talinum triangulare* could be fed as additives in African catfish culturing and at low doses (0.5-1.0%) do not have negative physiological effect.
2. The work demonstrated that inclusion of 0.5% *Allium sativum* or 1.0% *Talinum triangulare* could enhance growth in *Clarias gariepinus* with no negative consequence on the wellbeing of the fish.
3. This work also demonstrated that 1.0% *Talinum triangulare* included as feed additive in fish could be useful for management of conditions such as severe anaemia and boosting of blood level in African Catfish.

4. This work also demonstrated that 1.0% *Chromolaena odorata* included as feed additive in African Catfish could prevent establishment of *Pseudomonas aeruginosa* infection.
5. This work also demonstrated that 1.0% *Talinum triangulare* included as feed additive in fish could promote wound healing in aquaculture.

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APPENDIXES

Appendix i. Phytochemical screening of *Allium sativum, Chromolaena odorata* and *Talinum triangulare* powder

(A) Test for Taninns

20ml of water was used to boil 1ml of plant extract in a test-tube and then filtered. 0.1% of ferric chloride was added to the filtrate. Blue-black or green colouration observed confirmed the presence of tannin.

(B) Test for Phlobatannins:

Aqueous hydrochloric acid (1%) was used to boil 2ml of plant extract. Presence of phlobatannins was confirmed by deposition of a red precipitate.

(C) Test for Saponin

Distilled water (20ml) was used to boil 5ml of the extract in a water bath and then filtered. Distilled water (5ml) was mixed with the filterate (10ml) and then shaked vigorously and a stable persistent froth was allowed. Drops (3) of olive oil was then mixed with the frothing and shaked vigorously. Formation of emulsion observed confirmed a positive presence of Saponin.

(D) Test of Flavonoids:

The plant extract (5ml) was added to 1% Alluminium chloride solution (3ml). The presence of flavonoids was confirmed by observing a yellow colouration. Dilute ammonia solution (5ml) added to the already prepared mixture and then addition of concentrated H_2SO_4, the mixture was allowed to stand. The disappearance of yellow colouration confirmed presence of flavonoids.

(E) Test for Steroids.

The plant extract (2ml) was added to acetic anhydride (2ml) and then H_2SO_4 (2ml) was added. The presence of sterioids was confirmed as colour changed from violet to green or blue.

(F) Test for Terpenoids (Salkowski test)

The plant extract (5ml) was mixed with chloroform (2ml) and then concentrated H_2SO_4 (3ml) was added carefully to form a layer. A reddish brown coloration at the interface indicated presence of terpenoids.

(G**) Test for Cardiac Glycosides and Cardenolides (Keller – Killani test)**.

The plant extract (5ml) was mixed with glacial acetic acid containing one drop of ferric chloride solution and then concentrated H_2SO_4 (1ml) was added. Presence of cardenolides was confirmed when a brown ring (deoxysugar characteristics of cardenolides) was formed at the interface. The presence of glycoside was also confirmed as a result of appearance of a violet-green ring below the brown ring.

(H) Test for Alkaloids

The plant extract (1ml) was stirred with 1% aqueous HCL (5ml) on a steam bath and filtered while hot. Distilled water was added to the residue. One mililitre of the filtrate was treated with few drops of Mayer's reagent (Potassium mercuric iodide- solution) or Dragendorff's reagent (solution of Potassium bismuth iodide) or Wagner's reagent (solution of iodine in Potassium iodide). Cream colour formation confirmed presence of alkaloids with Mayer's reagent. Reddish-brown precipitate with Dragendorff's reagent or Wagner's reagent also confirmed presence of alkaloids.

Appendix ii. Average values of water quality parameters monitored during the experiment

Groups	Temp(0C)	DO(mg/l)	PH	TDS(ppm)	NH_3	NO_2
C1	28.6	6.46	7.1	60	0	0.02
C2	27.8	6.3	7.1	74	0	0.02
C3	27.7	6.49	7.1	78	0	0
T1A1	28.3	6.46	7.2	75	0	0.03
T1A2	27.6	6.46	7.1	75	0.01	0.03
T1A3	28.7	6.44	7.1	74	0	0.04
T1B1	27.6	6.43	7.1	72	0	0
T1B2	28.3	6.41	7.1	74	0.01	0.02
T1B3	27.7	6.48	7.1	79	0	0.04
T1C1	28.7	6.47	7.1	76	0.01	0.02
T1C2	27.7	6.48	7.1	76	0	0
T1C3	28.9	6.39	7.3	75	0	0.01
T2A1	26.9	6.5	7.1	78	0	0
T2A2	28.9	6.38	7.1	75	0	0.01
T2A3	28.1	6.43	7	76	0	0.02
T2B1	27.7	6.58	7.1	77	0	0.02
T2B2	28.8	6.5	7.1	77	0	0.02
T2B3	27.7	6.49	7.1	76	0	0.03
T2C1	28.6	6.46	7.1	71	0	0.01
T2C2	27.7	6.4	7.3	74	0	0
T2C3	28.8	6.39	7.1	65	0	0.01
T3A1	27.7	6.58	7.1	74	0	0.01
T3A2	27.7	6.52	7.1	78	0	0.02
T3A3	28.6	6.43	7.1	75	0	0
T3B1	27.5	6.46	7.1	74	0	0
T3B2	28.6	6.48	7.1	72	0	0.02
T3B3	27.8	6.4	7.1	72	0	0.01
T3C1	27.7	6.46	7	74	0	0.03
T3C2	28.6	6.48	7.1	79	0	0.01
T3C3	27.7	6.4	7.1	76	0	0.01

Average of 10 weeks readings. CC = Control (0%), $T_{1=}$ (Group of fish fed with *Allium sativum* additives), $T_{2=}$ (Group of fish fed with *Chromolaena odorata* additives), $T_{3=}$ (Group of fish fed with *Talinum triangulare* additives). A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively

Appendix iii. Average weight (g) of African catfish fingerlings fed diets containing different levels of *Allium sativum, Chromolaena odorata Talinum triangulare* as feed additives.

Groups	0	1	2	3	4	5	6	7	8	9	10
C1	1.08	1.17	1.19	1.25	1.3	1.4	1.52	1.63	1.74	1.78	2.1
C2	1.02	1.08	1.23	1.25	1.34	1.36	1.38	1.45	1.48	1.53	1.67
C3	1.1	1.3	1.42	1.48	1.52	1.58	1.64	1.68	1.72	1.84	1.96
T1A1	1.08	1.22	1.33	1.44	1.56	1.67	1.87	2.01	2.13	2.25	2.35
T1A2	1.07	1.33	1.38	1.51	1.89	1.99	2.01	2.25	2.37	2.39	2.43
T1A3	1.08	1.19	1.27	1.34	1.47	1.6	1.75	1.9	2.06	2.27	2.36
T1B1	1.18	1.19	1.22	1.32	1.49	1.5	1.5	1.54	1.63	1.8	2
T1B2	1.17	1.22	1.34	1.45	1.48	1.89	2	2.14	2.29	2.43	2.71
T1B3	1.18	1.29	1.4	1.43	1.46	1.53	1.64	1.87	2.05	2.12	2.36
T1C1	1.18	1.36	1.44	1.48	1.54	1.75	1.83	2.16	2.33	2.4	2.5
T1C2	1	1.42	1.52	1.6	1.69	1.75	1.9	2.18	2.36	2.41	2.55
T1C3	1.09	1.39	1.48	1.58	1.6	1.63	1.79	2.03	2.26	2.38	2.53
T2A1	1.08	1.17	1.27	1.29	1.3	1.36	1.42	1.48	1.5	1.57	1.84
T2A2	1.08	1.18	1.28	1.44	1.56	1.67	1.75	2.28	2.45	2.5	2.56
T2A3	1.1	1.19	1.28	1.37	1.43	1.52	1.56	1.85	2	2.2	2.34
T2B1	1.12	1.2	1.27	1.38	1.4	1.63	1.71	1.78	1.95	1.99	2.31
T2B2	1.07	1.27	1.3	1.38	1.44	1.57	1.75	1.91	2	2.3	2.45
T2B3	1.21	1.24	1.29	1.41	1.39	1.52	1.73	1.88	2.3	2.17	2.4
T2C1	1.09	1.17	1.28	1.28	1.34	1.36	1.42	1.45	1.5	1.6	1.77
T2C2	1.15	1.17	1.22	1.22	1.24	1.31	1.33	1.5	1.8	2	2.27
T2C3	1.16	1.21	1.16	1.21	1.23	1.27	1.29	1.33	1.45	1.7	2.1
T3A1	1	1.05	1.12	1.13	1.42	1.45	1.5	1.6	1.56	1.75	2.29
T3A2	1.1	1.22	1.28	1.33	1.33	1.4	1.75	1.78	1.85	1.97	2
T3A3	1.05	1.08	1.13	1.23	1.32	1.43	1.63	1.69	1.71	1.86	2.14
T3B1	1.09	1.25	1.27	1.31	1.38	1.45	1.6	1.57	2.14	2.29	2.45
T3B2	1.08	1.17	1.19	1.33	1.44	1.49	1.75	1.75	1.86	2.14	2.19
T3B3	1.13	1.17	1.22	1.3	1.41	1.47	1.68	1.66	2.1	2.22	2.5
T3C1	1.17	1.2	1.24	1.27	1.33	1.44	1.75	1.78	2	2.14	2.43
T3C2	1	1.11	1.25	1.33	1.47	1.5	1.56	1.67	1.83	1.84	1. 88
T3C3	1.09	1.16	1.25	1.3	1.4	1.46	1.66	1.73	1.92	1.99	2.16

C = Control (0%), $T_{1=}$ (Group of fish fed with *Allium sativum* additives), $T_{2=}$ (Group of fish fed with *Chromolaena odorata* additives), $T_{3=}$ (Group of fish fed with *Talinum triangulare* additives). A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively

Appendix iv. Average length (cm) of African catfish fingerlings fed diets containing different levels of *Allium sativum, Chromolaena odorata Talinum triangulare* as feed additives

Groups	L0	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
C1	5.05	5	5.07	5.38	5.83	5.85	5.85	6.17	6.33	6.39	6.42
C2	4.56	4.63	4.83	5	5.33	5.39	5.67	5.7	5.75	5.79	5.79
C3	5	5.46	5.46	5.5	6	6.1	6.1	6.12	6.33	6.33	6.4
T1A1	4.67	4.8	4.85	4.85	4.9	4.9	5.2	5.4	5.55	5.68	5.8
T1A2	5	5	5.15	5.18	5.18	5.25	5.45	5.56	5.58	5.58	6
T1A3	4.87	5	5.29	5.29	5.35	5.38	5.5	5.5	5.6	5.8	6.2
T1B1	5	5.05	5.53	5.67	5.75	5.83	6	6.17	6.2	6.22	6.3
T1B2	4.9	5.2	5.4	5.56	5.58	6	6.17	6.23	6.4	6.5	6.6
T1B3	5	5.13	5.25	5.28	5.35	5.46	5.5	5.6	5.8	5.85	5.85
T1C1	5.2	5.23	6.17	6.3	6.35	6.4	6.45	6.45	6.5	6.5	6.55
T1C2	4.45	5.1	5.67	6	6.33	6.4	6.45	6.45	6.6	6.6	6.65
T1C3	4.85	5.15	5.3	5.45	5.6	5.6	5.8	5.85	5.9	6.2	6.4
T2A1	4.67	4.8	5	5.15	5.15	5.3	5.4	5.45	5.5	5.8	6.07
T2A2	4.73	4.85	5.17	5.33	5.33	5.67	6	6.2	6.2	6.45	6.5
T2A3	4.73	5.08	5.32	5.37	5.55	5.57	5.9	6.1	6.15	6.2	6.3
T2B1	5	5.15	5.3	5.35	5.42	5.45	5.55	5.65	5.65	5.7	5.8
T2B2	4.5	4.5	4.8	4.95	5.2	5.25	5.4	5.6	5.8	5.85	6
T2B3	4.7	4.83	5.1	5.3	5.4	5.5	5.55	5.75	5.85	5.9	6.2
T2C1	5.17	5.2	5.25	5.35	5.4	5.5	5.57	5.59	5.61	5.61	5.68
T2C2	5.15	5.33	5.33	5.6	5.67	5.93	6.02	6.18	6.25	6.4	6.5
T2C3	5.22	5.3	5.35	5.54	5.59	5.7	5.79	5.85	5.85	6	6.1
T3A1	4.5	4.6	4.6	4.67	4.67	5.07	5.67	5.69	5.89	6	6.34
T3A2	5.13	5.17	5.3	5.43	5.47	5.9	5.9	6	6.25	6.33	6.4
T3A3	4.8	4.85	4.95	5.05	5.09	5.38	5.6	5.6	5.83	5.98	6.07
T3B1	4.83	5	5.17	5.67	5.67	5.75	5.8	6	6.2	6.35	6.38
T3B2	4.9	5.17	5.3	5.35	5.48	5.69	5.75	5.8	6	6.1	6.25
T3B3	4.65	4.8	4.85	5.2	5.35	5.4	5.6	5.8	6.1	6.2	6.35
T3C1	4.6	4.7	5.3	5.37	5.47	5.75	5.83	5.97	5.97	6.03	6.13
T3C2	5	5.28	5.38	5.43	5.5	5.85	6.1	6.15	6.25	6.3	6.45
T3C3	4.6	4.7	4.75	4.75	4.84	4.9	5.2	5.7	5.8	6.1	6.35

$C_{=}$ Control (0%), $T_{1=}$ (Group of fish fed with *Allium sativum* additives), $T_{2=}$ (Group of fish fed with *Chromolaena odorata* additives), $T_{3=}$ (Group of fish fed with *Talinum triangulare* additives). A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively

Appendix v. Comparative haematological parameters of different African catfish fingerlings fed Diets containing different Levels of *Allium sativum, Chromolaena odorata* and *Talinum triangulare* as feed additives

Parameters	PCV	Hb	Rbc	wbc	Platelet	Lymphocytes	Het	Mono	Eos	Basophil
B1	27	9	3.41	20300	306000	58	35	5	2	0
B2	22	7	1.5	21000	206000	66	29	1	3	1
B3	20	6.6	1.46	20950	300000	55	38	3	4	0
C1	28	8.8	3.44	16900	364000	64	32	1	3	0
C2	25	8.7	2.55	20000	320000	75	20	2	3	0
C3	30	9.7	3.62	18200	348000	74	23	2	1	0
C4	29	9.4	3.71	15600	167000	71	21	5	3	0
C5	36	10	3.22	13600	98000	66	28	4	2	0
C6	32	9.6	3.55	14800	120000	68	26	3	3	0
T1A1	34	11.7	3.33	27500	244000	63	29	4	3	1
T1A2	33	10.7	3.29	20600	268000	61	31	4	4	0
T1A3	18	5.7	1.52	20500	213000	49	43	5	3	0
T1B1	25	8.3	1.53	17000	192000	57	37	3	3	0
T1B2	23	7.2	1.39	16300	282000	70	24	2	4	0
T1B3	25	8.4	2.37	12000	213000	65	29	3	2	1
T1C1	26	8.7	2.4	10500	192000	70	23	2	5	0
T1C2	23	7.2	1.7	13500	138000	69	23	4	4	0
T1C3	24	8	2	12500	145000	68	24	3	4	0
T2A1	29	9	3.35	12000	154000	55	39	3	3	0
T2A2	24	8.4	2.66	16400	144000	63	28	2	6	1
T2A3	26	8.6	3	14000	150000	62	32	3	4	1
T2B1	26	8.6	2.59	16750	132000	58	35	2	5	0
T2B2	21	6.6	1.7	11200	168000	62	32	4	2	0
T2B3	24	7	2.2	15200	148000	60	34	3	3	0
T2C1	31	10.3	3.27	12550	180000	63	30	2	5	0
T2C2	29	9.5	3.54	16050	308000	55	37	3	5	0
T2C3	31	9	3.46	14500	200000	60	36	3	5	0
T3A1	22	7	1.73	13750	322000	41	35	1	3	0
T3A2	26	8.8	2.43	10400	160000	59	34	3	4	0
T3A3	24	8	1.8	12600	180000	48	32	3	3	0
T3B1	36	12.5	3.49	12000	184000	66	32	4	4	0
T3B2	34	11.6	3.64	14000	142000	73	22	3	1	1
T3B3	34	12	3.5	12800	162000	72	24	3	1	1
T3C1	24	8	1.62	11000	139000	65	21	2	4	0
T3C2	23	7.4	1.55	11650	210000	67	24	5	3	1
T3C3	24	7.6	1.58	12000	168000	66	22	4	3	1

B= Initial value, CC = Control (0%), $T_{1=}$ (Group of fish fed with *Allium sativum* additives), $T_{2=}$ (Group of fish fed with *Chromolaena odorata* additives), $T_{3=}$ (Group of fish fed with *Talinum triangulare* additives). A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively.

Appendix vi. Red blood cell (RBC) constants of different African catfish fingerlings fed diets containing different levels of *Allium sativum, Chromolaena odorata Talinum triangulare* as feed additives

Groups	MCV	MCH	MCHC
B1	79.17889	26.39296	33.33333
B2	146.6667	46.66667	31.81818
B3	136.9863	45.20548	33
C1	81.39535	25.5814	31.42857
C2	98.03922	34.11765	34.8
C3	82.87293	26.79558	32.33333
C4	78.16712	25.33693	32.41379
C5	111.8012	31.0559	27.77778
C6	90.14085	27.04225	30
T1A1	102.1021	35.13514	34.41176
T1A2	100.304	32.5228	32.42424
T1A3	118.4211	37.5	31.66667
T1B1	163.3987	54.24837	33.2
T1B2	165.4676	51.79856	31.30435
T1B3	105.4852	35.44304	33.6
T1C1	108.3333	36.25	33.46154
T1C2	135.2941	42.35294	31.30435
T1C3	120	40	33.33333
T2A1	86.56716	26.86567	31.03448
T2A2	90.22556	31.57895	35
T2A3	86.66667	28.66667	33.07692
T2B1	100.3861	33.20463	33.07692
T2B2	123.5294	38.82353	31.42857
T2B3	109.0909	31.81818	29.16667
T2C1	94.80122	31.49847	33.22581
T2C2	81.9209	26.83616	32.75862
T2C3	89.59538	26.01156	29.03226
T3A1	127.1676	40.46243	31.81818
T3A2	106.9959	36.21399	33.84615
T3A3	133.3333	44.44444	33.33333
T3B1	103.1519	35.81662	34.72222
T3B2	93.40659	31.86813	34.11765
T3B3	97.14286	34.28571	35.29412
T3C1	148.1481	49.38272	33.33333
T3C2	148.3871	47.74194	32.17391
T3C3	151.8987	48.10127	31.66667

B= Initial value, C = Control (0%), $T_{1=}$ (Group of fish fed with *Allium sativum* additives), $T_{2=}$ (Group of fish fed with *Chromolaena odorata* additives), $T_{3=}$ (Group of fish fed with *Talinum triangulare* additives). A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively

Appendix vii. Comparative serum biochemical parameters of different African catfish fingerlings fed diets containing different levels of *Allium sativum, Chromolaena odorata* and *Talinum triangulare* as feed additives

Groups	Total Protein	Albumin	Globulin	A.G ratio	AST	ALT	ALP	Cholesterol	Glucose	BUN	Creatinine
B1	5.7	1.8	3.9	0.4	181	25	184	189	295	NA	NA
B2	5.5	1.6	3.9	0.4	178	26	189	179	284	NA	NA
B3	6.3	2.4	3.9	0.6	199	31	194	185	291	NA	NA
C1	5.5	2.7	2.8	0.3	179	27	182	184	296	NA	NA
C2	5	2.3	2.7	0.8	178	29	171	177	295	NA	NA
C3	5.4	2.6	2.8	0.9	182	29	198	180	297	NA	NA
C4	6.6	1.7	4.9	0.3	190	15	163	152	272	10.4	0.7
C5	6.3	1.6	4.7	0.3	233	15	312	149	256	11.2	0.7
C6	6.4	1.7	4.8	0.4	220	15	292	150	260	11.2	0.7
T1A1	5.3	1.4	3.9	0.3	186	24	211	187	298	NA	NA
T1A2	5.2	1.3	3.9	0.3	179	21	196	172	299	NA	NA
T1A3	7.7	2.8	4.9	0.1	196	32	200	183	298	NA	NA
T1B1	8.5	3.6	4.9	0.7	221	35	200	213	300	NA	NA
T1B2	8.8	3.9	4.9	0.7	224	41	213	205	315	NA	NA
T1B3	8.6	3.7	4.9	0.7	219	31	197	211	313	NA	NA
T1C1	6.2	1.4	4.8	0.2	193	20	127	213	301	10.8	0.8
T1C2	4.7	0.8	3.9	0.2	280	21	292	221	249	11.2	0.6
T1C3	4.8	1.2	4.7	0.6	224	21	294	211	296	11.2	0.7
T2A1	6.3	1.5	4.8	0.3	192	20	503	136	240	11.7	0.8
T2A2	5.5	1.2	4.3	0.2	292	18	451	134	317	11	0.6
T2A3	6.3	1.4	4.6	0.3	292	20	445	136	260	11.2	0.7
T2B1	6.5	1.6	4.9	0.3	186	20	327	110	220	10.6	0.7
T2B2	6.3	1.6	4.7	0.3	241	26	317	117	284	11.3	0.7
T2B3	6.5	1.5	4.7	0.3	224	24	312	110	264	11.2	0.6
T2C1	6.6	1.5	4.5	0.3	212	18	345	144	323	10.3	0.7
T2C2	4.6	0.8	3.8	0.2	268	23	407	145	322	10.8	0.6
T2C3	5.6	1.4	4.2	0.3	244	20	320	144	323	10.3	0.7
T3A1	6.2	1.3	4.9	0.2	316	23	375	125	332	11.4	0.7
T3A2	6.7	1.8	4.9	0.3	231	15	345	142	233	11	0.7
T3A3	6.2	1.4	4.8	0.3	312	18	368	136	320	11.2	0.6
T3B1	6.6	1.8	4.8	0.4	215	13	183	125	217	11.4	0.7
T3B2	6.2	1.5	4.7	0.3	240	37	198	130	311	11	0.7
T3B3	6.4	1.6	4.7	0.3	220	31	190	128	298	11.2	0.7
T3C1	6.1	1.3	4.8	0.2	220	16	152	138	320	10.6	0.6
T3C2	6.3	1.6	4.7	0.3	241	26	168	117	284	11.3	0.7
T3C3	6.3	1.5	4.8	0.3	240	24	160	122	292	11.2	0.7

B= Initial value, C = Control (0%) $T_{1=}$ (Group of fish fed with *Allium sativum* additives), $T_{2=}$ (Group of fish fed with *Chromolaena odorata* additives), $T_{3=}$ (Group of fish fed with *Talinum triangulare* additives). A, B, and C represent different inclusion rates of 0.5%, 1% and 3% respectively

Appendix viii. Comparison of haematological parameters of *C. gariepinus* juvenile fed diets containing 0.5% *Allium sativum,* 1.0%*Chromolaena odorata* and 1.0%*Talinum triangulare* as feed additives for 21 days.

Groups	PCV	HB	RBC	WBC	PLATELET	LYM	HET	MON	EOS	BASOPHIL	MCV	MCH	MCHC
CC	26	8.7	2.31	14200	115000	58	36	4	2	0	112.5541	37.66234	33.46154
CC	2.45	8.5	1.35	16500	123500	59	36	3	3	0	18.14815	62.96296	34.69388
CC	23	7.4	1.22	18800	132000	60	35	2	3	0	188.5246	60.65574	32.17391
AL	21	7.2	1.26	15250	102000	69	23	5	3	0	166.6667	57.14286	34.28571
AL	19	6.4	1.34	13725	109500	65	28	5	3	1	141.791	47.76119	33.68421
AL	17	5.6	1.42	12200	117000	61	32	4	2	I	119.7183	39.43662	32.94118
CH	30	10.2	3.24	18300	101000	73	21	4	2	0	92.59259	31.48148	34
CH	26	8.5	2.31	17800	112000	71	23	4	3	0	112.5541	36.79654	32.69231
CH	21	6.8	1.37	17300	123000	69	24	4	4	0	153.2847	49.63504	32.38095
TA	21	7.3	1.33	10000	128000	55	39	2	4	0	157.8947	54.88722	34.7619
TA	24	8	1.9	11825	130000	56	38	3	4	0	126.3158	42.10526	33.33333
TA	26	8.6	2.47	13650	132000	57	37	3	3	0	105.2632	34.81781	33.07692

CC = Control (0%), Al = Group of fish fed with 0.5%*Allium sativum* additives, CH= (Group of fish fed with 1.0% *Chromolaena odorata* additives), TA = Group of fish fed with 1.0% *Talinum triangulare* additives.

Appendix ix. Comparison of haematological parameters of *C. gariepinus* juvenile fed diets containing 0.5% *Allium sativum,* 1.0%*Chromolaena odorata* and 1.0%*Talinum triangulare* as feed additives for 42 days

GROUPS	PCV	HB	RBC	WBC	PLATELET	LYM	HET	MON	EOS	BASOPHIL	MCV	MCH	MCHC
CC	20	6.5	1.37	13450	320000	69	23	4	4	0	145.9854	47.44526	32.5
CC	20	6.6	1.27	13525	239500	68	26	3	4	0	157.4803	51.9685	33
CC	20	6.7	1.17	13600	159000	66	28	2	4	0	170.9402	57.26496	33.5
AL	27	8.8	3.24	12500	144000	56	40	2	2	0	83.33333	27.16049	32.59259
AL	24	8	2.23	12650	132000	58	38	3	2	0	107.6233	35.87444	33.33333
AL	21	7.1	1.22	13200	120000	59	36	3	2	0	172.1311	58.19672	33.80952
CH	18	6.2	1.28	21600	224000	71	23	3	2	1	140.625	48.4375	34.44444
CH	18	6	1.17	18975	217000	70	24	3	3	1	153.8462	51.28205	33.33333
CH	18	5.8	1.14	16350	210000	69	25	3	3	0	157.8947	50.87719	32.22222
TA	23	7.2	1.31	11600	189000	65	29	4	2	0	175.5725	54.96183	31.30435
TA	23	7.3	1.23	10800	154500	64	30	3	4	0	186.9919	59.34959	31.73913
TA	23	7.3	1.24	10000	120000	62	31	2	5	0	185.4839	58.87097	31.73913

CC = Control (0%), Al = Group of fish fed with 0.5%*Allium sativum* additives, CH= (Group of fish fed with 1.0% *Chromolaena odorata* additives), TA = Group of fish fed with 1.0% *Talinum triangulare* additives.

Appendix x. Comparison of Biochemical Parameters of *C. gariepinus* juvenile fed diets containing 0.5% *Allium sativum,* 1.0%*Chromolaena odorata* 1.0%*Talinum and triangulare* as feed additives for 21 days**.**

GROUPS	TOTAL PROTEIN	ALBUMIN	GLOBULIN	A.G RATIO	AST	ALT	ALP	BUN	CREATININE	GLUCOSE	CHOLESTEROL
CC	5.5	1.4	4.1	0.4	175	37	310	9.3	0.6	208	191
CC	5.4	1.3	4.2	0.4	169	38	298	9.6	0.8	220	188
CC	5.4	1.2	4.2	0.2	173	40	296	10	0.7	224	187
AL	4.8	0.9	3.9	0.2	176	38	183	9.8	0.5	210	121
AL	5.5	1.1	3.9	0.2	172	38	182	10	0.5	212	126
AL	5.3	1.2	4.1	0.2	174	36	190	10.2	0.7	209	130
CH	5.8	1.8	4	0.5	192	28	326	10.2	0.7	256	235
CH	5.5	1.8	3.7	0.4	193	28	345	9.4	0.7	213	235
CH	6.3	2.2	4.3	0.5	190	26	310	11	0.7	300	236
TA	7.4	2.4	4.4	0.5	188	38	254	9.8	0.8	220	236
TA	7.6	2.7	4.9	0.5	186	41	252	11.3	0.8	322	234
TA	5.8	1.9	4	0.5	190	37	255	10	0.8	215	237

CC = Control (0%), Al = Group of fish fed with 0.5%*Allium sativum* additives, CH= (Group of fish fed with 1.0% *Chromolaena odorata* additives), TA = Group of fish fed with 1.0% *Talinum triangulare* additives.

Appendix xi. Comparison of biochemical parameters of *C. gariepinus* juvenile fed diets containing 0.5% *Allium sativum,* 1.0% *Chromolaena odorata* and 1.0%*Talinum triangulare* as feed additives for 42 days**.**

GROUPS	TOTAL PROTEIN	ALBUMIN	GLOBULIN	A.G RATIO	AST	ALT	ALP	BUN	CREATININE	GLUCOSE	CHOLESTEROL
CC	5	1.4	3.4	0.4	192	33	187	10.9	0.7	296	219
CC	5.2	1.5	3.7	0.4	188	33	198	10.4	0.8	265	209
CC	5.4	1.5	3.9	0.3	185	30	197	10	0.8	233	200
AL	5.3	1.9	3.8	0.5	213	48	213	11.3	0.7	284	195
AL	5.5	1.7	3.9	0.4	207	41	223	12	0.7	270	199
AL	5.7	1.5	3.9	0.3	200	33	233	12.2	0.7	255	203
CH	5.4	1.2	3.9	0.2	189	38	195	9.4	0.7	237	188
CH	5.6	1.5	4.5	0.2	190	43	199	9.5	0.8	236	185
CH	5.7	1.8	4.2	0.1	190	47	203	9.7	0.8	235	182
TA	5.4	1.3	3.7	0.3	181	43	216	9.7	0.7	244	203
TA	5.4	1.5	3.9	0.4	187	36	218	9.6	0.7	291	210
TA	5.3	1.6	4.1	0.4	192	26	220	9.5	0.7	338	217

CC = Control (0%), Al = Group of fish fed with 0.5%*Allium sativum* additives, CH= (Group of fish fed with 1.0% *Chromolaena odorata* additives), TA = Group of fish fed with 1.0% *Talinum triangulare* additives.

Appendix xii. Statistical tables of weight Parameters of African catfish fingerlings fed diets containing different Levels of *Allium sativum, Chromolaena odorata* and *Talinum triangulare*

ANOVA table of Final Weight of the Experimental Fish used for Growth Parameters.

	Contol	0.5% A. s	1.0% A. s	3.0% A. s	0.5% C. o	1.0% C. o	3.0% C. o	0.5% T. t	1.0% T. t	3.0% T. t
Number of values	3	3	3	3	3	3	3	3	3	3
Minimum	1.670	2.350	2.000	2.500	1.840	2.310	1.770	2.000	2190	1.000
Median	1.960	2.360	2.360	2.530	2.340	2.400	2.100	2.140	2.450	2.160
75% Percentile	2.100	2.430	2.710	2.550	2.560	2.450	2.270	2.290	2.500	2.430
Maximum	2.100	2.430	2.710	2.550	2.560	2.450	2.270	2.290	2.500	2.430
Mean	1.910	2.380	2.357	2.527	2.247	2.387	2.047	2.143	2.380	1.863
Std. Deviation	0.2193	0.0435 9	0.3550	0.02517	0.3690	0.0709	0.2542	0.145 0	0.166 4	0.7598
Std. Error	0.1266	0.0251 7	0.2050	0.01453	0.2130	0.0409	0.1468	1.783	0.096 09	0.4386
Lower 95% CI of mean	1.365	2.272	1.475	2.464	1.330	2.210	1.415	1.783	1.967	0.0240
Upper 95% CI of mean	2.455	2.488	3.239	2.589	3.163	2.563	2.678	2.504	2.793	3.751
Coefficient of variation	11.48 %	1.83%	15.06%	1.00%	16.42%	2.97%	12.42%	6.77 %	6.99 %	40.77 %
Sum	5.730	7.140	7.070	7.580	6.740	7.160	6.140	6.430	7.140	5.590

Key: A. s = *Allium sativum*, C. o = *Chromolaena odorata*, T. t = *Talinum triangulare*

One-way analysis of variance (ANOVA) of Final Weight of the Experimental Fish Used for Growth Parameters.

P value	0.2175		
P value summary	ns		
Are means significantly different? (P < 0.05)	No		
Number of groups	10		
F	1.492		
R squared	0.4018		
ANOVA Table	SS	df	MS
Treatment (between columns)	1.354	9	0.1505
Residual (within columns)	2.017	20	0.1008
Total	3.371	29	

Tukey's Multiple Comparison Test of Final Weight of the Experimental Fish used for Growth Parameters.

Tukey's Multiple Comparison Test	Mean Diff.	Q	(P < 0.05)	Summary	95% CI of diff
Contol vs 0.5% A. sativum	-0.4700	2.564	No	ns	-1.388 to 0.4482
Contol vs 1.0% A. sativum	-0.4467	2.436	No	ns	-1.365 to 0.4715
Contol vs 3.0% A. sativum	-0.6167	3.363	No	ns	-1.535 to 0.3015
Contol vs 0.5% C. odorata	-0.3367	1.836	No	ns	-1.255 to 0.5815
Contol vs 1.0% C. odorata	-0.4767	2.600	No	ns	-1.395 to 0.4415
Contol vs 3.0% c. odorata	-0.1367	0.7454	No	ns	-1.055 to 0.7815
Contol vs 0.5% T. triangulare	-0.2333	1.273	No	ns	-1.152 to 0.6848
Contol vs 1.0% T. triangulare	-0.4700	2.564	No	ns	-1.388 to 0.4482
Contol vs 3.0% T. triangulare	0.04667	0.2545	No	ns	-0.8715 to 0.9648
0.5% A. sativum vs 1.0% A. sativum	0.02333	0.1273	No	ns	-0.8948 to 0.9415
0.5% A. sativum vs 3.0% A. sativum	-0.1467	0.8000	No	ns	-1.065 to 0.7715
0.5% A. sativum vs 0.5% C. odorata	0.1333	0.7272	No	ns	-0.7848 to 1.052
0.5% A. sativum vs 1.0% C. odorata	-0.006667	0.03636	No	ns	-0.9248 to 0.9115
0.5% A. sativum vs 3.0% c. odorata	0.3333	1.818	No	ns	-0.5848 to 1.252
0.5% A. sativum vs 0.5% T. triangulare	0.2367	1.291	No	ns	-0.6815 to 1.155
0.5% A. sativum vs 1.0% T. triangulare	-0.0000002384	0.000001300	No	ns	-0.9182 to 0.9182
0.5% A. sativum vs 3.0% T. triangulare	0.5167	2.818	No	ns	-0.4015 to 1.435
1.0% A. sativum vs 3.0% A. sativum	-0.1700	0.9272	No	ns	-1.088 to 0.7482
1.0% A. sativum vs 0.5% C. odorata	0.1100	0.6000	No	ns	-0.8082 to 1.028
1.0% A. sativum vs 1.0% C. odorata	-0.03000	0.1636	No	ns	-0.9482 to 0.8882
1.0% A. sativum vs 3.0% c. odorata	0.3100	1.691	No	ns	-0.6082 to 1.228
1.0% A. sativum vs 0.5% T. triangulare	0.2133	1.164	No	ns	-0.7048 to 1.132
1.0% A. sativum vs 1.0% T. triangulare	-0.02333	0.1273	No	ns	-0.9415 to 0.8948
1.0% A. sativum vs 3.0% T. triangulare	0.4933	2.691	No	ns	-0.4248 to 1.412
3.0% A. sativum vs 0.5% C. odorata	0.2800	1.527	No	ns	-0.6382 to 1.198
3.0% A. sativum vs 1.0% C. odorata	0.1400	0.7636	No	ns	-0.7782 to 1.058
3.0% A. sativum vs 3.0% c. odorata	0.4800	2.618	No	ns	-0.4382 to 1.398
3.0% A. sativum vs 0.5% T. triangulare	0.3833	2.091	No	ns	-0.5348 to 1.302
3.0% A. sativum vs 1.0% T. triangulare	0.1467	0.8000	No	ns	-0.7715 to 1.065
3.0% A. sativum vs 3.0% T. triangulare	0.6633	3.618	No	ns	-0.2548 to 1.582
0.5% C. odorata vs 1.0% C. odorata	-0.1400	0.7636	No	ns	-1.058 to 0.7782
0.5% C. odorata vs 3.0% c. odorata	0.2000	1.091	No	ns	-0.7182 to 1.118
0.5% C. odorata vs 0.5% T. triangulare	0.1033	0.5636	No	ns	-0.8148 to 1.022

0.5% C. odorata vs 1.0% T. triangulare	-0.1333	0.7272	No	ns	-1.052 to 0.7848
0.5% C. odorata vs 3.0% T. triangulare	0.3833	2.091	No	ns	-0.5348 to 1.302
1.0% C. odorata vs 3.0% c. odorata	0.3400	1.854	No	ns	-0.5782 to 1.258
1.0% C. odorata vs 0.5% T. triangulare	0.2433	1.327	No	ns	-0.6748 to 1.162
1.0% C. odorata vs 1.0% T. triangulare	0.006667	0.03636	No	ns	-0.9115 to 0.9248
1.0% C. odorata vs 3.0% T. triangulare	0.5233	2.854	No	ns	-0.3948 to 1.442
3.0% c. odorata vs 0.5% T. triangulare	-0.09667	0.5272	No	ns	-1.015 to 0.8215
3.0% c. odorata vs 1.0% T. triangulare	-0.3333	1.818	No	ns	-1.252 to 0.5848
3.0% c. odorata vs 3.0% T. triangulare	0.1833	1.000	No	ns	-0.7348 to 1.102
0.5% T. triangulare vs 1.0% T. triangulare	-0.2367	1.291	No	ns	-1.155 to 0.6815
0.5% T. triangulare vs 3.0% T. triangulare	0.2800	1.527	No	ns	-0.6382 to 1.198
1.0% T. triangulare vs 3.0% T. triangulare	0.5167	2.818	No	ns	-0.4015 to 1.435

Key: ns = not significant

One-way analysis of variance (ANOVA) of Initial Weight Vs Final Weight of the Experimental Fish

One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	20
F	20.68
R squared	0.9076

ANOVA Table	SS	df	MS
Treatment (between columns)	20.34	19	1.071
Residual (within columns)	2.071	40	0.05177
Total	22.41	59	

*** Significant at P<0.0001

Tukey's Multiple Comparison Test of Initial weight Vs Final Weight of the Experimental Fish

Tukey's Multiple Comparison Test	Mean Diff.	Q	(P < 0.05)	Summary	95% CI of diff
Initial Weight (Control) vs IW(05% A. sativum)	-0.01000	0.07613	No	ns	-0.7138 to 0.6938
Initial Weight (Control) vs IW(1.0% A. sativum)	-0.1100	0.8374	No	ns	-0.8138 to 0.5938
Initial Weight (Control) vs IW (3.0% A. sativum)	-0.02333	0.1776	No	ns	-0.7272 to 0.6805
Initial Weight (Control) vs IW(0.5% C. odorata)	-0.02000	0.1523	No	ns	-0.7238 to 0.6838
Initial Weight (Control) vs IW (1.0% C. odorata))	-0.06667	0.5075	No	ns	-0.7705 to 0.6372
Initial Weight (Control) vs IW (3.0% C. odorata)	-0.06667	0.5075	No	ns	-0.7705 to 0.6372
Initial Weight (Control) vs IW (0.5% T. triangulare)	0.01667	0.1269	No	ns	-0.6872 to 0.7205
Initial Weight (Control) vs IW (1.0% T. triangulare)	-0.03333	0.2538	No	ns	-0.7372 to 0.6705

Initial Weight (Control) vs IW (3.0% T. triangulare)	-0.02000	0.1523	No	ns	-0.7238 to 0.6838
Initial Weight (Control) vs Final Weight (Control)	-0.8433	6.420	Yes	**	-1.547 to -0.1395
Initial Weight (Control) vs FW(05% A. sativum)	-1.313	9.998	Yes	***	-2.017 to -0.6095
Initial Weight (Control) vs FW(1.0% A. sativum)	-1.290	9.820	Yes	***	-1.994 to -0.5862
Initial Weight (Control) vs FW (3.0% A. sativum)	-1.460	11.11	Yes	***	-2.164 to -0.7562
Initial Weight (Control) vs FW(0.5% C. odorata)	-1.180	8.983	Yes	***	-1.884 to -0.4762
Initial Weight (Control) vs FW (1.0% C. odorata))	-1.320	10.05	Yes	***	-2.024 to -0.6162
Initial Weight (Control) vs FW (3.0% C. odorata)	-0.9800	7.460	Yes	***	-1.684 to -0.2762
Initial Weight (Control) vs FW (0.5% T. triangulare)	-1.077	8.196	Yes	***	-1.781 to -0.3728
Initial Weight (Control) vs FW (1.0% T. triangulare)	-1.313	9.998	Yes	***	-2.017 to -0.6095
Initial Weight (Control) vs FW (3.0% T. triangulare)	-0.7967	6.065	Yes	*	-1.501 to -0.09283
IW(05% A. sativum) vs IW(1.0% A. sativum)	-0.1000	0.7613	No	ns	-0.8038 to 0.6038
IW(05% A. sativum) vs IW (3.0% A. sativum)	-0.01333	0.1015	No	ns	-0.7172 to 0.6905
IW(05% A. sativum) vs IW(0.5% C. odorata)	-0.01000	0.07613	No	ns	-0.7138 to 0.6938
IW(05% A. sativum) vs IW (1.0% C. odorata))	-0.05667	0.4314	No	ns	-0.7605 to 0.6472
IW(05% A. sativum) vs IW (3.0% C. odorata)	-0.05667	0.4314	No	ns	-0.7605 to 0.6472
IW(05% A. sativum) vs IW (0.5% T. triangulare)	0.02667	0.2030	No	ns	-0.6772 to 0.7305
IW(05% A. sativum) vs IW (1.0% T. triangulare)	-0.02333	0.1776	No	ns	-0.7272 to 0.6805
IW(05% A. sativum) vs IW (3.0% T. triangulare)	-0.01000	0.07613	No	ns	-0.7138 to 0.6938
IW(05% A. sativum) vs Final Weight (Control)	-0.8333	6.344	Yes	**	-1.537 to -0.1295
IW(05% A. sativum) vs FW(05% A. sativum)	-1.303	9.922	Yes	***	-2.007 to -0.5995
IW(05% A. sativum) vs FW(1.0% A. sativum)	-1.280	9.744	Yes	***	-1.984 to -0.5762

IW(05% A. sativum) vs FW (3.0% A. sativum)	-1.450	11.04	Yes	***	-2.154 to -0.7462
IW(05% A. sativum) vs FW(0.5% C. odorata)	-1.170	8.907	Yes	***	-1.874 to -0.4662
IW(05% A. sativum) vs FW (1.0% C. odorata))	-1.310	9.972	Yes	***	-2.014 to -0.6062
IW(05% A. sativum) vs FW (3.0% C. odorata)	-0.9700	7.384	Yes	***	-1.674 to -0.2662
IW(05% A. sativum) vs FW (0.5% T. triangulare)	-1.067	8.120	Yes	***	-1.771 to -0.3628
IW(05% A. sativum) vs FW (1.0% T. triangulare)	-1.303	9.922	Yes	***	-2.007 to -0.5995
IW(05% A. sativum) vs FW (3.0% T. triangulare)	-0.7867	5.989	Yes	*	-1.491 to -0.08283
IW(1.0% A. sativum) vs IW (3.0% A. sativum)	0.08667	0.6598	No	ns	-0.6172 to 0.7905
IW(1.0% A. sativum) vs IW(0.5% C. odorata)	0.09000	0.6851	No	ns	-0.6138 to 0.7938
IW(1.0% A. sativum) vs IW (1.0% C. odorata))	0.04333	0.3299	No	ns	-0.6605 to 0.7472
IW(1.0% A. sativum) vs IW (3.0% C. odorata)	0.04333	0.3299	No	ns	-0.6605 to 0.7472
IW(1.0% A. sativum) vs IW (0.5% T. triangulare)	0.1267	0.9643	No	ns	-0.5772 to 0.8305
IW(1.0% A. sativum) vs IW (1.0% T. triangulare)	0.07667	0.5836	No	ns	-0.6272 to 0.7805
IW(1.0% A. sativum) vs IW (3.0% T. triangulare)	0.09000	0.6851	No	ns	-0.6138 to 0.7938
IW(1.0% A. sativum) vs Final Weight (Control)	-0.7333	5.583	Yes	*	-1.437 to -0.02949
IW(1.0% A. sativum) vs FW(05% A. sativum)	-1.203	9.160	Yes	***	-1.907 to -0.4995
IW(1.0% A. sativum) vs FW(1.0% A. sativum)	-1.180	8.983	Yes	***	-1.884 to -0.4762
IW(1.0% A. sativum) vs FW (3.0% A. sativum)	-1.350	10.28	Yes	***	-2.054 to -0.6462
IW(1.0% A. sativum) vs FW(0.5% C. odorata)	-1.070	8.145	Yes	***	-1.774 to -0.3662
IW(1.0% A. sativum) vs FW (1.0% C. odorata))	-1.210	9.211	Yes	***	-1.914 to -0.5062
IW(1.0% A. sativum) vs FW (3.0% C. odorata)	-0.8700	6.623	Yes	**	-1.574 to -0.1662
IW(1.0% A. sativum) vs FW (0.5% T. triangulare)	-0.9667	7.359	Yes	***	-1.671 to -0.2628

IW(1.0% A. sativum) vs FW (1.0% T. triangulare)	-1.203	9.160	Yes	***	-1.907 to -0.4995
IW(1.0% A. sativum) vs FW (3.0% T. triangulare)	-0.6867	5.227	No	ns	-1.391 to 0.01717
IW (3.0% A. sativum) vs IW(0.5% C. odorata)	0.003333	0.02538	No	ns	-0.7005 to 0.7072
IW (3.0% A. sativum) vs IW (1.0% C. odorata))	-0.04333	0.3299	No	ns	-0.7472 to 0.6605
IW (3.0% A. sativum) vs IW (3.0% C. odorata)	-0.04333	0.3299	No	ns	-0.7472 to 0.6605
IW (3.0% A. sativum) vs IW (0.5% T. triangulare)	0.04000	0.3045	No	ns	-0.6638 to 0.7438
IW (3.0% A. sativum) vs IW (1.0% T. triangulare)	-0.01000	0.07613	No	ns	-0.7138 to 0.6938
IW (3.0% A. sativum) vs IW (3.0% T. triangulare)	0.003333	0.02538	No	ns	-0.7005 to 0.7072
IW (3.0% A. sativum) vs Final Weight (Control)	-0.8200	6.242	Yes	**	-1.524 to -0.1162
IW (3.0% A. sativum) vs FW(05% A. sativum)	-1.290	9.820	Yes	***	-1.994 to -0.5862
IW (3.0% A. sativum) vs FW(1.0% A. sativum)	-1.267	9.643	Yes	***	-1.971 to -0.5628
IW (3.0% A. sativum) vs FW (3.0% A. sativum)	-1.437	10.94	Yes	***	-2.141 to -0.7328
IW (3.0% A. sativum) vs FW(0.5% C. odorata)	-1.157	8.805	Yes	***	-1.861 to -0.4528
IW (3.0% A. sativum) vs FW (1.0% C. odorata))	-1.297	9.871	Yes	***	-2.001 to -0.5928
IW (3.0% A. sativum) vs FW (3.0% C. odorata)	-0.9567	7.283	Yes	**	-1.661 to -0.2528
IW (3.0% A. sativum) vs FW (0.5% T. triangulare)	-1.053	8.019	Yes	***	-1.757 to -0.3495
IW (3.0% A. sativum) vs FW (1.0% T. triangulare)	-1.290	9.820	Yes	***	-1.994 to -0.5862
IW (3.0% A. sativum) vs FW (3.0% T. triangulare)	-0.7733	5.887	Yes	*	-1.477 to -0.06949
IW(0.5% C. odorata) vs IW (1.0% C. odorata))	-0.04667	0.3553	No	ns	-0.7505 to 0.6572
IW(0.5% C. odorata) vs IW (3.0% C. odorata)	-0.04667	0.3553	No	ns	-0.7505 to 0.6572
IW(0.5% C. odorata) vs IW (0.5% T. triangulare)	0.03667	0.2791	No	ns	-0.6672 to 0.7405
IW(0.5% C. odorata) vs IW (1.0% T. triangulare)	-0.01333	0.1015	No	ns	-0.7172 to 0.6905

IW(0.5% C. odorata) vs IW (3.0% T. triangulare)	0.0000	0.0000	No	ns	-0.7038 to 0.7038
IW(0.5% C. odorata) vs Final Weight (Control)	-0.8233	6.268	Yes	**	-1.527 to -0.1195
IW(0.5% C. odorata) vs FW(05% A. sativum)	-1.293	9.846	Yes	***	-1.997 to -0.5895
IW(0.5% C. odorata) vs FW(1.0% A. sativum)	-1.270	9.668	Yes	***	-1.974 to -0.5662
IW(0.5% C. odorata) vs FW (3.0% A. sativum)	-1.440	10.96	Yes	***	-2.144 to -0.7362
IW(0.5% C. odorata) vs FW(0.5% C. odorata)	-1.160	8.831	Yes	***	-1.864 to -0.4562
IW(0.5% C. odorata) vs FW (1.0% C. odorata))	-1.300	9.896	Yes	***	-2.004 to -0.5962
IW(0.5% C. odorata) vs FW (3.0% C. odorata)	-0.9600	7.308	Yes	**	-1.664 to -0.2562
IW(0.5% C. odorata) vs FW (0.5% T. triangulare)	-1.057	8.044	Yes	***	-1.761 to -0.3528
IW(0.5% C. odorata) vs FW (1.0% T. triangulare)	-1.293	9.846	Yes	***	-1.997 to -0.5895
IW(0.5% C. odorata) vs FW (3.0% T. triangulare)	-0.7767	5.912	Yes	*	-1.481 to -0.07283
IW (1.0% C. odorata)) vs IW (3.0% C. odorata)	0.0000	0.0000	No	ns	-0.7038 to 0.7038
IW (1.0% C. odorata)) vs IW (0.5% T. triangulare)	0.08333	0.6344	No	ns	-0.6205 to 0.7872
IW (1.0% C. odorata)) vs IW (1.0% T. triangulare)	0.03333	0.2538	No	ns	-0.6705 to 0.7372
IW (1.0% C. odorata)) vs IW (3.0% T. triangulare)	0.04667	0.3553	No	ns	-0.6572 to 0.7505
IW (1.0% C. odorata)) vs Final Weight (Control)	-0.7767	5.912	Yes	*	-1.481 to -0.07283
IW (1.0% C. odorata)) vs FW(05% A. sativum)	-1.247	9.490	Yes	***	-1.951 to -0.5428
IW (1.0% C. odorata)) vs FW(1.0% A. sativum)	-1.223	9.313	Yes	***	-1.927 to -0.5195
IW (1.0% C. odorata)) vs FW (3.0% A. sativum)	-1.393	10.61	Yes	***	-2.097 to -0.6895
IW (1.0% C. odorata)) vs FW(0.5% C. odorata)	-1.113	8.475	Yes	***	-1.817 to -0.4095
IW (1.0% C. odorata)) vs FW (1.0% C. odorata))	-1.253	9.541	Yes	***	-1.957 to -0.5495
IW (1.0% C. odorata)) vs FW (3.0% C. odorata)	-0.9133	6.953	Yes	**	-1.617 to -0.2095

IW (1.0% C. odorata)) vs FW (0.5% T. triangulare)	-1.010	7.689	Yes	***	-1.714 to -0.3062
IW (1.0% C. odorata)) vs FW (1.0% T. triangulare)	-1.247	9.490	Yes	***	-1.951 to -0.5428
IW (1.0% C. odorata)) vs FW (3.0% T. triangulare)	-0.7300	5.557	Yes	*	-1.434 to -0.02616
IW (3.0% C. odorata) vs IW (0.5% T. triangulare)	0.08333	0.6344	No	ns	-0.6205 to 0.7872
IW (3.0% C. odorata) vs IW (1.0% T. triangulare)	0.03333	0.2538	No	ns	-0.6705 to 0.7372
IW (3.0% C. odorata) vs IW (3.0% T. triangulare)	0.04667	0.3553	No	ns	-0.6572 to 0.7505
IW (3.0% C. odorata) vs Final Weight (Control)	-0.7767	5.912	Yes	*	-1.481 to -0.07283
IW (3.0% C. odorata) vs FW(05% A. sativum)	-1.247	9.490	Yes	***	-1.951 to -0.5428
IW (3.0% C. odorata) vs FW(1.0% A. sativum)	-1.223	9.313	Yes	***	-1.927 to -0.5195
IW (3.0% C. odorata) vs FW (3.0% A. sativum)	-1.393	10.61	Yes	***	-2.097 to -0.6895
IW (3.0% C. odorata) vs FW(0.5% C. odorata)	-1.113	8.475	Yes	***	-1.817 to -0.4095
IW (3.0% C. odorata) vs FW (1.0% C. odorata))	-1.253	9.541	Yes	***	-1.957 to -0.5495
IW (3.0% C. odorata) vs FW (3.0% C. odorata)	-0.9133	6.953	Yes	**	-1.617 to -0.2095
IW (3.0% C. odorata) vs FW (0.5% T. triangulare)	-1.010	7.689	Yes	***	-1.714 to -0.3062
IW (3.0% C. odorata) vs FW (1.0% T. triangulare)	-1.247	9.490	Yes	***	-1.951 to -0.5428
IW (3.0% C. odorata) vs FW (3.0% T. triangulare)	-0.7300	5.557	Yes	*	-1.434 to -0.02616
IW (0.5% T. triangulare) vs IW (1.0% T. triangulare)	-0.05000	0.3806	No	ns	-0.7538 to 0.6538
IW (0.5% T. triangulare) vs IW (3.0% T. triangulare)	-0.03667	0.2791	No	ns	-0.7405 to 0.6672
IW (0.5% T. triangulare) vs Final Weight (Control)	-0.8600	6.547	Yes	**	-1.564 to -0.1562
IW (0.5% T. triangulare) vs FW(05% A. sativum)	-1.330	10.12	Yes	***	-2.034 to -0.6262
IW (0.5% T. triangulare) vs FW(1.0% A. sativum)	-1.307	9.947	Yes	***	-2.011 to -0.6028
IW (0.5% T. triangulare) vs FW (3.0% A. sativum)	-1.477	11.24	Yes	***	-2.181 to -0.7728

IW (0.5% T. triangulare) vs FW(0.5% C. odorata)	-1.197	9.110	Yes	***	-1.901 to -0.4928
IW (0.5% T. triangulare) vs FW (1.0% C. odorata))	-1.337	10.18	Yes	***	-2.041 to -0.6328
IW (0.5% T. triangulare) vs FW (3.0% C. odorata)	-0.9967	7.587	Yes	***	-1.701 to -0.2928
IW (0.5% T. triangulare) vs FW (0.5% T. triangulare)	-1.093	8.323	Yes	***	-1.797 to -0.3895
IW (0.5% T. triangulare) vs FW (1.0% T. triangulare)	-1.330	10.12	Yes	***	-2.034 to -0.6262
IW (0.5% T. triangulare) vs FW (3.0% T. triangulare)	-0.8133	6.192	Yes	*	-1.517 to -0.1095
IW (1.0% T. triangulare) vs IW (3.0% T. triangulare)	0.01333	0.1015	No	ns	-0.6905 to 0.7172
IW (1.0% T. triangulare) vs Final Weight (Control)	-0.8100	6.166	Yes	*	-1.514 to -0.1062
IW (1.0% T. triangulare) vs FW(05% A. sativum)	-1.280	9.744	Yes	***	-1.984 to -0.5762
IW (1.0% T. triangulare) vs FW(1.0% A. sativum)	-1.257	9.566	Yes	***	-1.961 to -0.5528
IW (1.0% T. triangulare) vs FW (3.0% A. sativum)	-1.427	10.86	Yes	***	-2.131 to -0.7228
IW (1.0% T. triangulare) vs FW(0.5% C. odorata)	-1.147	8.729	Yes	***	-1.851 to -0.4428
IW (1.0% T. triangulare) vs FW (1.0% C. odorata))	-1.287	9.795	Yes	***	-1.991 to -0.5828
IW (1.0% T. triangulare) vs FW (3.0% C. odorata)	-0.9467	7.207	Yes	**	-1.651 to -0.2428
IW (1.0% T. triangulare) vs FW (0.5% T. triangulare)	-1.043	7.942	Yes	***	-1.747 to -0.3395
IW (1.0% T. triangulare) vs FW (1.0% T. triangulare)	-1.280	9.744	Yes	***	-1.984 to -0.5762
IW (1.0% T. triangulare) vs FW (3.0% T. triangulare)	-0.7633	5.811	Yes	*	-1.467 to -0.05949
IW (3.0% T. triangulare) vs Final Weight (Control)	-0.8233	6.268	Yes	**	-1.527 to -0.1195
IW (3.0% T. triangulare) vs FW(05% A. sativum)	-1.293	9.846	Yes	***	-1.997 to -0.5895
IW (3.0% T. triangulare) vs FW(1.0% A. sativum)	-1.270	9.668	Yes	***	-1.974 to -0.5662
IW (3.0% T. triangulare) vs FW (3.0% A. sativum)	-1.440	10.96	Yes	***	-2.144 to -0.7362
IW (3.0% T. triangulare) vs FW(0.5% C. odorata)	-1.160	8.831	Yes	***	-1.864 to -0.4562

IW (3.0% T. triangulare) vs FW (1.0% C. odorata))	-1.300	9.896	Yes	***	-2.004 to -0.5962
IW (3.0% T. triangulare) vs FW (3.0% C. odorata)	-0.9600	7.308	Yes	**	-1.664 to -0.2562
IW (3.0% T. triangulare) vs FW (0.5% T. triangulare)	-1.057	8.044	Yes	***	-1.761 to -0.3528
IW (3.0% T. triangulare) vs FW (1.0% T. triangulare)	-1.293	9.846	Yes	***	-1.997 to -0.5895
IW (3.0% T. triangulare) vs FW (3.0% T. triangulare)	-0.7767	5.912	Yes	*	-1.481 to -0.07283
Final Weight (Control) vs FW(05% A. sativum)	-0.4700	3.578	No	ns	-1.174 to 0.2338
Final Weight (Control) vs FW(1.0% A. sativum)	-0.4467	3.400	No	ns	-1.151 to 0.2572
Final Weight (Control) vs FW (3.0% A. sativum)	-0.6167	4.694	No	ns	-1.321 to 0.08717
Final Weight (Control) vs FW(0.5% C. odorata)	-0.3367	2.563	No	ns	-1.041 to 0.3672
Final Weight (Control) vs FW (1.0% C. odorata))	-0.4767	3.629	No	ns	-1.181 to 0.2272
Final Weight (Control) vs FW (3.0% C. odorata)	-0.1367	1.040	No	ns	-0.8405 to 0.5672
Final Weight (Control) vs FW (0.5% T. triangulare)	-0.2333	1.776	No	ns	-0.9372 to 0.4705
Final Weight (Control) vs FW (1.0% T. triangulare)	-0.4700	3.578	No	ns	-1.174 to 0.2338
Final Weight (Control) vs FW (3.0% T. triangulare)	0.04667	0.3553	No	ns	-0.6572 to 0.7505
FW(05% A. sativum) vs FW(1.0% A. sativum)	0.02333	0.1776	No	ns	-0.6805 to 0.7272
FW(05% A. sativum) vs FW (3.0% A. sativum)	-0.1467	1.117	No	ns	-0.8505 to 0.5572
FW(05% A. sativum) vs FW(0.5% C. odorata)	0.1333	1.015	No	ns	-0.5705 to 0.8372
FW(05% A. sativum) vs FW (1.0% C. odorata))	-0.006667	0.05075	No	ns	-0.7105 to 0.6972
FW(05% A. sativum) vs FW (3.0% C. odorata)	0.3333	2.538	No	ns	-0.3705 to 1.037
FW(05% A. sativum) vs FW (0.5% T. triangulare)	0.2367	1.802	No	ns	-0.4672 to 0.9405
FW(05% A. sativum) vs FW (1.0% T. triangulare)	-0.0000002384	0.000001815	No	ns	-0.7038 to 0.7038
FW(05% A. sativum) vs FW (3.0% T. triangulare)	0.5167	3.933	No	ns	-0.1872 to 1.221

FW(1.0% A. sativum) vs FW (3.0% A. sativum)	-0.1700	1.294	No	ns	-0.8738 to 0.5338
FW(1.0% A. sativum) vs FW(0.5% C. odorata)	0.1100	0.8374	No	ns	-0.5938 to 0.8138
FW(1.0% A. sativum) vs FW (1.0% C. odorata))	-0.03000	0.2284	No	ns	-0.7338 to 0.6738
FW(1.0% A. sativum) vs FW (3.0% C. odorata)	0.3100	2.360	No	ns	-0.3938 to 1.014
FW(1.0% A. sativum) vs FW (0.5% T. triangulare)	0.2133	1.624	No	ns	-0.4905 to 0.9172
FW(1.0% A. sativum) vs FW (1.0% T. triangulare)	-0.02333	0.1776	No	ns	-0.7272 to 0.6805
FW(1.0% A. sativum) vs FW (3.0% T. triangulare)	0.4933	3.756	No	ns	-0.2105 to 1.197
FW (3.0% A. sativum) vs FW(0.5% C. odorata)	0.2800	2.132	No	ns	-0.4238 to 0.9838
FW (3.0% A. sativum) vs FW (1.0% C. odorata))	0.1400	1.066	No	ns	-0.5638 to 0.8438
FW (3.0% A. sativum) vs FW (3.0% C. odorata)	0.4800	3.654	No	ns	-0.2238 to 1.184
FW (3.0% A. sativum) vs FW (0.5% T. triangulare)	0.3833	2.918	No	ns	-0.3205 to 1.087
FW (3.0% A. sativum) vs FW (1.0% T. triangulare)	0.1467	1.117	No	ns	-0.5572 to 0.8505
FW (3.0% A. sativum) vs FW (3.0% T. triangulare)	0.6633	5.050	No	ns	-0.04051 to 1.367
FW(0.5% C. odorata) vs FW (1.0% C. odorata))	-0.1400	1.066	No	ns	-0.8438 to 0.5638
FW(0.5% C. odorata) vs FW (3.0% C. odorata)	0.2000	1.523	No	ns	-0.5038 to 0.9038
FW(0.5% C. odorata) vs FW (0.5% T. triangulare)	0.1033	0.7866	No	ns	-0.6005 to 0.8072
FW(0.5% C. odorata) vs FW (1.0% T. triangulare)	-0.1333	1.015	No	ns	-0.8372 to 0.5705
FW(0.5% C. odorata) vs FW (3.0% T. triangulare)	0.3833	2.918	No	ns	-0.3205 to 1.087
FW (1.0% C. odorata)) vs FW (3.0% C. odorata)	0.3400	2.588	No	ns	-0.3638 to 1.044
FW (1.0% C. odorata)) vs FW (0.5% T. triangulare)	0.2433	1.852	No	ns	-0.4605 to 0.9472
FW (1.0% C. odorata)) vs FW (1.0% T. triangulare)	0.006667	0.05075	No	ns	-0.6972 to 0.7105
FW (1.0% C. odorata)) vs FW (3.0% T. triangulare)	0.5233	3.984	No	ns	-0.1805 to 1.227

FW (3.0% C. odorata) vs FW (0.5% T. triangulare)	-0.09667	0.7359	No	ns	-0.8005 to 0.6072
FW (3.0% C. odorata) vs FW (1.0% T. triangulare)	-0.3333	2.538	No	ns	-1.037 to 0.3705
FW (3.0% C. odorata) vs FW (3.0% T. triangulare)	0.1833	1.396	No	ns	-0.5205 to 0.8872
FW (0.5% T. triangulare) vs FW (1.0% T. triangulare)	-0.2367	1.802	No	ns	-0.9405 to 0.4672
FW (0.5% T. triangulare) vs FW (3.0% T. triangulare)	0.2800	2.132	No	ns	-0.4238 to 0.9838
FW (1.0% T. triangulare) vs FW (3.0% T. triangulare)	0.5167	3.933	No	ns	-0.1872 to 1.221

Key: IW = Initial Weight, FW = Final Weight, ns = not significant, *Significant at P<0.05, ** Significant at P<0.001, ***Significant at P<0.0001

Appendix xiii. Statistical tables of some of haematological parameters of the African catfish juveniles fed diets containing different levels of *Allium sativum, Chromolaena odorata* and *Talinum triangulare*

One-way analysis of variance (ANOVA) of PCV of the Experimental Fish

One-way analysis of variance			
P value	0.0036		
P value summary	**		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	11		
F	3.744		
R squared	0.5996		
ANOVA Table	SS	df	MS
Treatment (between columns)	454.2	10	45.42
Residual (within columns)	303.3	25	12.13
Total	757.6	35	

** Significant at P<0.001

Tukey's Multiple Comparison Test of Packed Cell Volume (PCV) of the Experimental Fish.

Tukey's Multiple Comparison Test	Mean Diff.	Q	Significant? P < 0.05?	Summary	95% CI of diff
Initial Value vs 0.5% A. sativum	-5.333	2.652	No	ns	-15.38 to 4.714
Initial Value vs 1.0% A. sativum	-1.333	0.663 0	No	ns	-11.38 to 8.714
Initial Value vs 3.0% A. sativum	-1.333	0.663 0	No	ns	-11.38 to 8.714
Initial Value vs 0.5% C. odorata	-3.333	1.657	No	ns	-13.38 to 6.714
Initial Value vs 1.0% C. odorata	-0.6667	0.331 5	No	ns	-10.71 to 9.381
Initial Value vs 3.0% C. odorata	-7.333	3.646	No	ns	-17.38 to 2.714
Initial Value vs 0.5% T. triangulare	-1.000	0.497 2	No	ns	-11.05 to 9.048
Initial Value vs 1.0% T. triangulare	-11.67	5.801	Yes	*	-21.71 to -1.619
Initial Value vs 3.0% T. triangulare	-0.6667	0.331 5	No	ns	-10.71 to 9.381
Control vs 0.5% A. sativum	1.667	0.956 9	No	ns	-7.035 to 10.37
Control vs 1.0% A. sativum	5.667	3.254	No	ns	-3.035 to 14.37
Control vs 3.0% A. sativum	5.667	3.254	No	ns	-3.035 to 14.37
Control vs 0.5% C. odorata	3.667	2.105	No	ns	-5.035 to 12.37
Control vs 1.0% C. odorata	6.333	3.636	No	ns	-2.368 to 15.03
Control vs 3.0% C. odorata	-0.3333	0.191 4	No	ns	-9.035 to 8.368
Control vs 0.5% T. triangulare	6.000	3.445	No	ns	-2.702 to 14.70
Control vs 1.0% T. triangulare	-4.667	2.679	No	ns	-13.37 to 4.035
Control vs 3.0% T. triangulare	6.333	3.636	No	ns	-2.368 to 15.03
0.5% A. sativum vs 1.0% A. sativum	4.000	1.989	No	ns	-6.048 to 14.05
0.5% A. sativum vs 3.0% A. sativum	4.000	1.989	No	ns	-6.048 to 14.05

0.5% A. sativum vs 0.5% C. odorata	2.000	0.9945	No	ns	-8.048 to 12.05
0.5% A. sativum vs 1.0% C. odorata	4.667	2.320	No	ns	-5.381 to 14.71
0.5% A. sativum vs 3.0% C. odorata	-2.000	0.9945	No	ns	-12.05 to 8.048
0.5% A. sativum vs 0.5% T. triangulare	4.333	2.155	No	ns	-5.714 to 14.38
0.5% A. sativum vs 1.0% T. triangulare	-6.333	3.149	No	ns	-16.38 to 3.714
0.5% A. sativum vs 3.0% T. triangulare	4.667	2.320	No	ns	-5.381 to 14.71
1.0% A. sativum vs 3.0% A. sativum	0.0000	0.0000	No	ns	-10.05 to 10.05
1.0% A. sativum vs 0.5% C. odorata	-2.000	0.9945	No	ns	-12.05 to 8.048
1.0% A. sativum vs 1.0% C. odorata	0.6667	0.3315	No	ns	-9.381 to 10.71
1.0% A. sativum vs 3.0% C. odorata	-6.000	2.983	No	ns	-16.05 to 4.048
1.0% A. sativum vs 0.5% T. triangulare	0.3333	0.1657	No	ns	-9.714 to 10.38
1.0% A. sativum vs 1.0% T. triangulare	-10.33	5.138	Yes	*	-20.38 to -0.2856
1.0% A. sativum vs 3.0% T. triangulare	0.6667	0.3315	No	ns	-9.381 to 10.71
3.0% A. sativum vs 0.5% C. odorata	-2.000	0.9945	No	ns	-12.05 to 8.048
3.0% A. sativum vs 1.0% C. odorata	0.6667	0.3315	No	ns	-9.381 to 10.71
3.0% A. sativum vs 3.0% C. odorata	-6.000	2.983	No	ns	-16.05 to 4.048
3.0% A. sativum vs 0.5% T. triangulare	0.3333	0.1657	No	ns	-9.714 to 10.38
3.0% A. sativum vs 1.0% T. triangulare	-10.33	5.138	Yes	*	-20.38 to -0.2856
3.0% A. sativum vs 3.0% T. triangulare	0.6667	0.3315	No	ns	-9.381 to 10.71

0.5% C. odorata vs 1.0% C. odorata	2.667	1.326	No	ns	-7.381 to 12.71
0.5% C. odorata vs 3.0% C. odorata	-4.000	1.989	No	ns	-14.05 to 6.048
0.5% C. odorata vs 0.5% T. triangulare	2.333	1.160	No	ns	-7.714 to 12.38
0.5% C. odorata vs 1.0% T. triangulare	-8.333	4.144	No	ns	-18.38 to 1.714
0.5% C. odorata vs 3.0% T. triangulare	2.667	1.326	No	ns	-7.381 to 12.71
1.0% C. odorata vs 3.0% C. odorata	-6.667	3.315	No	ns	-16.71 to 3.381
1.0% C. odorata vs 0.5% T. triangulare	-0.3333	0.165 7	No	ns	-10.38 to 9.714
1.0% C. odorata vs 1.0% T. triangulare	-11.00	5.470	Yes	*	-21.05 to -0.9523
1.0% C. odorata vs 3.0% T. triangulare	0.0000	0.000 0	No	ns	-10.05 to 10.05
3.0% C. odorata vs 0.5% T. triangulare	6.333	3.149	No	ns	-3.714 to 16.38
3.0% C. odorata vs 1.0% T. triangulare	-4.333	2.155	No	ns	-14.38 to 5.714
3.0% C. odorata vs 3.0% T. triangulare	6.667	3.315	No	ns	-3.381 to 16.71
0.5% T. triangulare vs 1.0% T. triangulare	-10.67	5.304	Yes	*	-20.71 to -0.6190
0.5% T. triangulare vs 3.0% T. triangulare	0.3333	0.165 7	No	ns	-9.714 to 10.38
1.0% T. triangulare vs 3.0% T. triangulare	11.00	5.470	Yes	*	0.9523 to 21.05

Key: ns = not significant, *significant.

One-way analysis of variance (ANOVA) of Hb concentration the Experimental Fish

One-way analysis of variance			
P value	0.0014		
P value summary	**		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	11		
F	4.345		
R squared	0.6348		
ANOVA Table	SS	df	MS
Treatment (between columns)	57.06	10	5.706
Residual (within columns)	32.83	25	1.313
Total	89.89	35	

** Significant at P<0.001

Tukey's Multiple Comparison Test of Hb concentration of the Experimental Fish

Tukey's Multiple Comparison Test	Mean Diff.	Q	Significant? P < 0.05?	Summary	95% CI of diff
Initial Value vs control	-1.833	3.200	No	ns	-4.696 to 1.029
Initial Value vs 0.5% A. sativum	-1.833	2.771	No	ns	-5.139 to 1.472
Initial Value vs 1.0% A. sativum	-0.4333	0.6550	No	ns	-3.739 to 2.872
Initial Value vs 3.0% A. sativum	-0.4333	0.6550	No	ns	-3.739 to 2.872
Initial Value vs 0.5% C. odorata	-1.133	1.713	No	ns	-4.439 to 2.172
Initial Value vs 1.0% C. odorata	0.1333	0.2015	No	ns	-3.172 to 3.439
Initial Value vs 3.0% C. odorata	-2.067	3.124	No	ns	-5.372 to 1.239
Initial Value vs 0.5% T. triangulare	-0.4000	0.6046	No	ns	-3.705 to 2.905
Initial Value vs 1.0% T. triangulare	-4.500	6.802	Yes	**	-7.805 to -1.195
Initial Value vs 3.0% T. triangulare	-0.1333	0.2015	No	ns	-3.439 to 3.172
control vs 0.5% A. sativum	0.0000	0.0000	No	ns	-2.863 to 2.863
control vs 1.0% A. sativum	1.400	2.444	No	ns	-1.463 to 4.263
control vs 3.0% A. sativum	1.400	2.444	No	ns	-1.463 to 4.263
control vs 0.5% C. odorata	0.7000	1.222	No	ns	-2.163 to 3.563
control vs 1.0% C. odorata	1.967	3.433	No	ns	-0.8959 to 4.829
control vs 3.0% C. odorata	-0.2333	0.4073	No	ns	-3.096 to 2.629
control vs 0.5% T. triangulare	1.433	2.502	No	ns	-1.429 to 4.296
control vs 1.0% T. triangulare	-2.667	4.654	No	ns	-5.529 to 0.1959
control vs 3.0% T. triangulare	1.700	2.967	No	ns	-1.163 to 4.563

0.5% A. sativum vs 1.0% A. sativum	1.400	2.116	No	ns	-1.905 to 4.705
0.5% A. sativum vs 3.0% A. sativum	1.400	2.116	No	ns	-1.905 to 4.705
0.5% A. sativum vs 0.5% C. odorata	0.7000	1.058	No	ns	-2.605 to 4.005
0.5% A. sativum vs 1.0% C. odorata	1.967	2.973	No	ns	-1.339 to 5.272
0.5% A. sativum vs 3.0% C. odorata	-0.2333	0.3527	No	ns	-3.539 to 3.072
0.5% A. sativum vs 0.5% T. triangulare	1.433	2.167	No	ns	-1.872 to 4.739
0.5% A. sativum vs 1.0% T. triangulare	-2.667	4.031	No	ns	-5.972 to 0.6387
0.5% A. sativum vs 3.0% T. triangulare	1.700	2.570	No	ns	-1.605 to 5.005
1.0% A. sativum vs 3.0% A. sativum	0.0000	0.0000	No	ns	-3.305 to 3.305
1.0% A. sativum vs 0.5% C. odorata	-0.7000	1.058	No	ns	-4.005 to 2.605
1.0% A. sativum vs 1.0% C. odorata	0.5667	0.8565	No	ns	-2.739 to 3.872
1.0% A. sativum vs 3.0% C. odorata	-1.633	2.469	No	ns	-4.939 to 1.672
1.0% A. sativum vs 0.5% T. triangulare	0.03333	0.05038	No	ns	-3.272 to 3.339
1.0% A. sativum vs 1.0% T. triangulare	-4.067	6.147	Yes	**	-7.372 to -0.7613
1.0% A. sativum vs 3.0% T. triangulare	0.3000	0.4535	No	ns	-3.005 to 3.605
3.0% A. sativum vs 0.5% C. odorata	-0.7000	1.058	No	ns	-4.005 to 2.605
3.0% A. sativum vs 1.0% C. odorata	0.5667	0.8565	No	ns	-2.739 to 3.872
3.0% A. sativum vs 3.0% C. odorata	-1.633	2.469	No	ns	-4.939 to 1.672
3.0% A. sativum vs 0.5% T. triangulare	0.03333	0.05038	No	ns	-3.272 to 3.339
3.0% A. sativum vs 1.0% T. triangulare	-4.067	6.147	Yes	**	-7.372 to -0.7613
3.0% A. sativum vs 3.0% T. triangulare	0.3000	0.4535	No	ns	-3.005 to 3.605

0.5% C. odorata vs 1.0% C. odorata	1.267	1.915	No	ns	-2.039 to 4.572
0.5% C. odorata vs 3.0% C. odorata	-0.9333	1.411	No	ns	-4.239 to 2.372
0.5% C. odorata vs 0.5% T. triangulare	0.7333	1.108	No	ns	-2.572 to 4.039
0.5% C. odorata vs 1.0% T. triangulare	-3.367	5.089	Yes	*	-6.672 to -0.06130
0.5% C. odorata vs 3.0% T. triangulare	1.000	1.512	No	ns	-2.305 to 4.305
1.0% C. odorata vs 3.0% C. odorata	-2.200	3.325	No	ns	-5.505 to 1.105
1.0% C. odorata vs 0.5% T. triangulare	-0.5333	0.8062	No	ns	-3.839 to 2.772
1.0% C. odorata vs 1.0% T. triangulare	-4.633	7.003	Yes	**	-7.939 to -1.328
1.0% C. odorata vs 3.0% T. triangulare	-0.2667	0.4031	No	ns	-3.572 to 3.039
3.0% C. odorata vs 0.5% T. triangulare	1.667	2.519	No	ns	-1.639 to 4.972
3.0% C. odorata vs 1.0% T. triangulare	-2.433	3.678	No	ns	-5.739 to 0.8720
3.0% C. odorata vs 3.0% T. triangulare	1.933	2.922	No	ns	-1.372 to 5.239
0.5% T. triangulare vs 1.0% T. triangulare	-4.100	6.197	Yes	**	-7.405 to -0.7946
0.5% T. triangulare vs 3.0% T. triangulare	0.2667	0.4031	No	ns	-3.039 to 3.572
1.0% T. triangulare vs 3.0% T. triangulare	4.367	6.600	Yes	**	1.061 to 7.672

Key: ns = not significant, *Significant at P<0.05, ** Significant at P<0.001

One-way analysis of variance (ANOVA) of White Blood Cell (WBC) of the Experimental Fish.

One-way analysis of variance			
P value	< 0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	11		
F	8.249		
R squared	0.7674		

ANOVA Table	SS	df	MS
Treatment (between columns)	395400000	10	39540000
Residual (within columns)	119800000	25	4794000
Total	515300000	35	

*** Significant at P<0.0001

Tukey's Multiple Comparison Test of White Blood Cells (WBC) of the Experimental Fish

Tukey's Multiple Comparison Test	Mean Diff.	Q	Significant? P < 0.05?	Summary	95% CI of diff
Initial Value vs Control	4233	3.867	No	ns	-1236 to 9703
Initial Value vs 0.5% A. sativum	-2117	1.674	No	ns	-8432 to 4199
Initial Value vs 1.0% A. sativum	5650	4.470	No	ns	-665.6 to 11970
Initial Value vs 3.0% A. sativum	8583	6.790	Yes	**	2268 to 14900
Initial Value vs 0.5% C. odorata	6617	5.234	Yes	*	301.1 to 12930
Initial Value vs 1.0% C.odorata	6367	5.037	Yes	*	51.09 to 12680
Initial Value vs 3.0% C. odorata	6383	5.050	Yes	*	67.75 to 12700
Initial Value vs 0.5% T. triangulare	8500	6.724	Yes	**	2184 to 14820
Initial Value vs 1.0% T. triangulare	7817	6.184	Yes	**	1501 to 14130
Initial Value vs 3.0% T. triangulare	9200	7.278	Yes	**	2884 to 15520
Control vs 0.5% A. sativum	-6350	5.801	Yes	*	-11820 to -880.5
Control vs 1.0% A. sativum	1417	1.294	No	ns	-4053 to 6886
Control vs 3.0% A. sativum	4350	3.974	No	ns	-1119 to 9819
Control vs 0.5% C. odorata	2383	2.177	No	ns	-3086 to 7853
Control vs 1.0% C.odorata	2133	1.949	No	ns	-3336 to 7603
Control vs 3.0% C. odorata	2150	1.964	No	ns	-3319 to 7619
Control vs 0.5% T. triangulare	4267	3.897	No	ns	-1203 to 9736
Control vs 1.0% T. triangulare	3583	3.273	No	ns	-1886 to 9053
Control vs 3.0% T. triangulare	4967	4.537	No	ns	-502.8 to 10440
0.5% A. sativum vs 1.0% A. sativum	7767	6.144	Yes	**	1451 to 14080
0.5% A. sativum vs 3.0% A. sativum	10700	8.465	Yes	***	4384 to 17020
0.5% A. sativum vs 0.5% C. odorata	8733	6.909	Yes	**	2418 to 15050
0.5% A. sativum vs 1.0% C.odorata	8483	6.711	Yes	**	2168 to 14800
0.5% A. sativum vs 3.0% C. odorata	8500	6.724	Yes	**	2184 to 14820
0.5% A. sativum vs 0.5% T. triangulare	10620	8.399	Yes	***	4301 to 16930

0.5% A. sativum vs 1.0% T. triangulare	9933	7.858	Yes	***	3618 to 16250
0.5% A. sativum vs 3.0% T. triangulare	11320	8.952	Yes	***	5001 to 17630
1.0% A. sativum vs 3.0% A. sativum	2933	2.321	No	ns	-3382 to 9249
1.0% A. sativum vs 0.5% C. odorata	966.7	0.7647	No	ns	-5349 to 7282
1.0% A. sativum vs 1.0% C.odorata	716.7	0.5669	No	ns	-5599 to 7032
1.0% A. sativum vs 3.0% C. odorata	733.3	0.5801	No	ns	-5582 to 7049
1.0% A. sativum vs 0.5% T. triangulare	2850	2.255	No	ns	-3466 to 9166
1.0% A. sativum vs 1.0% T. triangulare	2167	1.714	No	ns	-4149 to 8482
1.0% A. sativum vs 3.0% T. triangulare	3550	2.808	No	ns	-2766 to 9866
3.0% A. sativum vs 0.5% C. odorata	-1967	1.556	No	ns	-8282 to 4349
3.0% A. sativum vs 1.0% C.odorata	-2217	1.754	No	ns	-8532 to 4099
3.0% A. sativum vs 3.0% C. odorata	-2200	1.740	No	ns	-8516 to 4116
3.0% A. sativum vs 0.5% T. triangulare	-83.33	0.06592	No	ns	-6399 to 6232
3.0% A. sativum vs 1.0% T. triangulare	-766.7	0.6065	No	ns	-7082 to 5549
3.0% A. sativum vs 3.0% T. triangulare	616.7	0.4878	No	ns	-5699 to 6932
0.5% C. odorata vs 1.0% C.odorata	-250.0	0.1978	No	ns	-6566 to 6066
0.5% C. odorata vs 3.0% C. odorata	-233.3	0.1846	No	ns	-6549 to 6082
0.5% C. odorata vs 0.5% T. triangulare	1883	1.490	No	ns	-4432 to 8199
0.5% C. odorata vs 1.0% T. triangulare	1200	0.9493	No	ns	-5116 to 7516
0.5% C. odorata vs 3.0% T. triangulare	2583	2.044	No	ns	-3732 to 8899
1.0% C.odorata vs 3.0% C. odorata	16.67	0.01318	No	ns	-6299 to 6332
1.0% C.odorata vs 0.5% T. triangulare	2133	1.688	No	ns	-4182 to 8449
1.0% C.odorata vs 1.0% T. triangulare	1450	1.147	No	ns	-4866 to 7766
1.0% C.odorata vs 3.0% T. triangulare	2833	2.241	No	ns	-3482 to 9149
3.0% C. odorata vs 0.5% T. triangulare	2117	1.674	No	ns	-4199 to 8432
3.0% C. odorata vs 1.0% T. triangulare	1433	1.134	No	ns	-4882 to 7749
3.0% C. odorata vs 3.0% T. triangulare	2817	2.228	No	ns	-3499 to 9132

0.5% T. triangulare vs 1.0% T. triangulare	-683.3	0.5406	No	ns	-6999 to 5632
0.5% T. triangulare vs 3.0% T. triangulare	700.0	0.5538	No	ns	-5616 to 7016
1.0% T. triangulare vs 3.0% T. triangulare	1383	1.094	No	ns	-4932 to 7699

Key: ns = not significant, *Significant at P<0.05, ** Significant at P<0.001, ***Significant at P<0.0001