Organosomatic indices and tissues bacteriological changes of *Clarias gariepinus* on feeds supplemented with neem (*Azadirachta indica*) leaves and turmeric (*Curcuma longa*) rhizome extracts

Olusola^{1**}, S. E., Olawoye¹, O. P. and Olaifa², F. E.

¹Department of Biological Sciences (Fisheries and Aquaculture Programme), Ondo State University of Science and Technology, Okitipupa.

²Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan.

**Corresponding author; belloolus@yahoo.com, se.olusola@osustech.edu.ng, 2348034110139

Abstract

The study evaluated the effect of dietary doses of neem leaves and turmeric rhizome extracts on tissue bacteriology and organosomatic indices of *Clarias gariepinus* juveniles. Nine experimental diets containing control (0%), NL 2 (1%), NL 3 (2%), NL 4 (3%), TR 5 (1%), TR 6 (2%), TR 7 (3%), CHRL 8 (15mg/kg diet) and CHRL 9 (30mg/kg diet) were formulated and replicated twice at 40% crude protein for 84 days. Microbial analyses of water, fish tissue and organ index of *C. gariepinus* were examined after 28, 56 and 84 days of feeding. Data were analyzed using ANOVA at $p = 0.05$. The results revealed that the fish fed treated experimental diets had decreased values of enterobacteriaceae and total viable counts of bacterial loads from the water and fish tissues than the control at 28, 56 and 84 days. Turmeric rhizome extracts performed better when compared to the neem leaves and control. Also, organ index showed that liver, kidney and heart were not significantly increased in all the treated groups and the control. Neem leaves and turmeric rhizome extracts could be used in organic aquaculture to stimulate immunity, reduce and prevent bacterial infections in fish farming.

Keywords: Turmeric rhizome, Neem leaves, *Clarias gariepinus*, Microbial loads, Immunity, Chloramphenicol

Introduction

Fish are considered one of the important food sources for human being because their flesh contains a high percentage of protein, calcium, phosphorus, and iodine that are vital to our health (FAO, 2014). Fish account for almost 17 percent of the global production intake of animal protein (FAO, 2014). Fish diseases are major constraint to aquaculture production. Fish disease is a rarely simple association between pathogen, a host and environmental problems such as poor water quality, overcrowding or other stressors often contribute to the disease outbreaks (Mehana *et al.*, 2015). The culture environment tends to produce poor physiological conditions for fish and increased susceptibility to

infection. However, the use of chemicals and drugs in the aquaculture systems caused residual problems and poor quality in fish and its products. Food safety has become a major issue of concern in the developed countries (Sakai, 1999 and Chinabut and Puttinawarat, 2005). Therefore, looking for less harmful approaches and more environmentally – friendly treatments become of premium importance.

Natural medicinal products origin from fungi and herbs have been used as feed additives for farm animals in developed countries and showed many bioactive such as anti – fungi, antimicrobial, antioxidant, antiviral, immune enhancement and stress reduction (Wang *et al.*, 1998; Odugbemi,

2006). A natural medicinal product is a substance produced by a living organism which is found in nature and which usually has a biological or pharmacological activity that can be used in drug discovery and drug design. Natural medicinal products are important in the treatment of life threatening conditions (Gomathinayagam *et al.*, 2014). Some medicinal plants/herbs had been evaluated experimentally in fish by various researchers, some with the use of specific part of the plant using various concentration/inclusion rate; *Allium cepa* (Bello *et al.*, 2012), *Tetraselmis chuii* (Cerezuela *et al.*, 2012), *Viscum album* (Park and Choi, 2012), *Ficus benghalensis* (Verma *et al.*, 2012), *Andrographis paniculata* (Prasad and Mukthira, 2011), *Euglena viridis* (Das *et al.*, (2009) and *Allium sativum* (Nya and Austin, 2009), but there is little or no information on utilization and mechanism of actions of *A. indica* and *C. longa* in fish nutrition. The present study aimed to evaluate the efficiency of using *A. indica* and *C. longa* as a potential antimicrobial agents in the organic aquaculture.

Materials and methods

Plant collection and identification

Azadirachta indica and *C. longa* were obtained in Igodan, Okitipupa, Ondo state and was identified by Dr. D. O. Aworinde in the Department of Biological Sciences, Ondo State University of Science and Technology, Okitipupa.

Preparation and extraction of plant materials

Turmeric extraction

The turmeric rhizome was washed with clean distilled water, scraped and allow to air dry at ambient temperature (25°C) for one hour. Three hundred grams (300g) of the air dried turmeric rhizome was blended

and mixed with 1800 mL methanol (95%) and was stirred every 3 hours to obtain homogeneous extracts in the laboratory of Fisheries and Aquaculture, Ondo State University of Science and Technology, Okitipupa for 24 hours. The pulp obtained was filtered using a sterile muslin cloth after which the extracts was obtained, air-dried and stored (4°C) until required.

Neem leaves extraction

The extraction was done as described by Ajaiyeoba and Fadare (2006). The leaves were air dried and finely chopped. The air-dried neem leaves were ground with a hammer mill and 300g of fine powered of neem leaves were soaked in 1800 mL of ethanol (95%) for 48 hours. Neem leaves were properly mixed with ethanol filtered using a sterile muslin cloth after which the extract was obtained, air-dried and stored at (25°C) until required.

Experimental system

The experiment was carried out in eighteen plastic experimental tanks (50×34×27cm) for twelve (12) weeks in the Fisheries Laboratory of Ondo State University of Science and Technology, Okitipupa. The water level in each tank was maintained at volume of 35 litres throughout the experimental period. Water in each tank was replaced every three (3) days throughout the period of experiment to maintain relatively uniform physiochemical parameters and also to prevent fouling that may result from food residues. The source of water was from Ondo State University of Science and Technology, Okitipupa water station and each experimental tank was well aerated using air stone and aerator pumps (Lawson, 1995). The water temperature of the experimental tanks was measured by mercury –in-glass thermometer. While the pH value was measured using pH meter (Jenway 2013 pH meter, 0.01 accuracy) after standardizing the meter.

Experimental procedure and feeding trials

Each treatment has two replicates, 20 fish per replicate with mean initial body weight of 6.33 ± 0.01 g uniform-sized fish was selected from 400 juveniles. Weighed and distributed in experimental tanks. The fish was acclimated for eight days in experimental tanks before the experiment. The experiment last for 12 weeks during which the fish was fed at 3% body weight daily. The diet per day was divided into two; half was given in the morning by 8.00-9.00am and other half in the evening by 5.00pm. Measurement of the weight changes was performed monthly on fish organ (heart, liver and kidney) as well as determination of microbial loads in fish tissues and water.

Preparation of experimental diets

Feed ingredients such as fish meal, soybean, yellow maize, wheat bran, starch, vegetable oil, vitamin premix and bone meal were added and feed ingredients was mixed thoroughly in a mixer. Each diet mixture treated separately was extruded through a 1/4mm die mincer of Hobart A-200T pelleting machine to form a noodle like strand which was mechanically broken into suitable sizes for the *C. gariepinus* juveniles. The pelleted diets were sun dried, packed in labelled polythene bags and stored in a cool dry place to prevent mycotoxin formation. The mean proximate composition of the experimental diets were 40.1% crude protein, 7.1% moisture, 6.5% ether extract, 11.6%, 34.7% nitrogen free extract. Nine experimental diets were prepared by incorporating turmeric rhizome, neem leaves extracts and chloramphenicol at the following inclusion levels; 0 (control), 1%, 2%, 3% and 15mg/kg, 30mg/kg of the diet, respectively.

Media preparation

All media used were prepared according to manufacturer's instruction. All these media

are allowed to cool after sterilization to about 45°C before pouring into petri dishes

Microbiological analysis

Water samples from the experimental bowls were collected monthly in sterile glass bottles. Distilled water was used for serial dilution; 1mL of water sample was added to 9mL sterile distilled water to 10^{-1} and then serially diluted to 10^{-4} . Each diluent (1mL) was poured in two Petri dishes; one received nutrient agar for total viable counts using the pure plate count method according to the standard methods for the examination of water and wastewater (APHA, 1985), the second Petri dish received MacConky agar for enterobacteriaceae counts according to Hitchins *et al.*, (1995). Petri dishes were gently tapped on the sides for a few times, petri dish for enterobacteriaceae counts and that of the dishes of total viable counts was incubated at 37°C for 24h.

Fish samples (skin, liver, gill and intestine) were collected monthly during the experimental period for bacteriological examination with through asepsis (medical examinations or procedures that prevent contamination or infection of microbes). 1g of fish sample was macerated in 9mL sterile peptone water in the mortar. 1mL of the suspension was diluted by peptone water to 10^{-4} . Each diluent (1mL) was poured in two Petri dishes; one received nutrient agar and the other received MacConky agar (APHA, 1985). The incubation period was 24h at 37°C. After incubation of water and fish sample dishes the colonies were counted using colony counter. Total viable counts and enterobacteriaceae counts were determined, the result were expressed in $\log_{10}$ CFU/mL and $\log_{10}$ CFU/g for water and fish, respectively.

Organ index

Three fish from each experimental treatment were killed by rapid cervical chopping and weighed. The liver, kidney

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and intestine were removed and weighed and the average was calculated. Moreover, organosomatic indices were calculated according to Fox *et al.* (1997).

Organ –somatic index = [organ weight (g)/body weight (g)] X 100.

Statistical analysis

Microbial loads of water and fish tissues and organosomatic indices resulting from the experiment were subjected to one-way analysis of variance (ANOVA) Using SPSS (Statistical Package for Social Sciences 2006 Version 15.0). Duncan's multiple range test was used to compare differences

among individual means.

Results

Microbial loads of fish tissues (skin, gill, liver and intestine)

The highest enterobacteriaceae and total viable counts were recorded in the control. The value decreased as the inclusion level increased and as the months increased in the treated groups (see Table 2A and 2B). The turmeric treated groups performed better when compared to neem and chloramphenicol treated groups.

Table 2A: The result of the enterobacteriaceae and total viable counts (log₁₀CFU/g) of *C. gariepinus* treated with turmeric rhizome and neem leaves extracts

Treatments	Fish sites	Week 4		Week 8		Week 12	
		Enterobacteriaceae counts	Total viable counts	Enterobacteriaceae counts	Total viable counts	Enterobacteriaceae counts	Total viable counts
Control	Intestine	3.75±0.01 ^c	3.80±0.00 ^d	3.65±0.00 ^d	3.72±0.02 ^d	3.58±0.03 ^d	3.68±0.04 ^f
	Skin	3.96±0.04 ^g	4.02±0.02 ^f	3.85±0.01 ^e	3.95±0.00 ^e	3.71±0.00 ^e	3.89±0.02 ^e
	Gill	3.88±0.02 ^f	3.92±0.03 ^d	3.79±0.00 ^f	3.83±0.02 ^e	3.65±0.00 ^f	3.77±0.03 ^g
	Liver	3.81±0.01 ^f	3.86±0.04 ^f	3.79±0.02 ^a	3.85±0.03 ^g	3.65±0.01 ^a	3.75±0.04 ^f
TR 2	Intestine	3.61±0.05 ^{cd}	3.75±0.02 ^d	3.59±0.05 ^{cd}	3.62±0.01 ^c	3.51±0.02 ^{cd}	3.54±0.02 ^e
	Skin	3.76±0.00 ^{de}	3.79±0.01 ^{de}	3.71±0.06 ^d	3.74±0.01 ^d	3.65±0.01 ^d	3.68±0.01 ^d
	Gill	3.67±0.03 ^d	3.74±0.00 ^e	3.58±0.02 ^e	3.63±0.03 ^d	3.41±0.03 ^e	3.45±0.02 ^e
	Liver	3.69±0.00 ^e	3.72±0.01 ^c	3.48±0.01 ^a	3.52±0.04 ^{de}	3.31±0.04 ^a	3.35±0.03 ^c
TR 3	Intestine	3.56±0.01 ^{bc}	3.66±0.03 ^{bc}	3.48±0.03 ^b	3.51±0.02 ^b	3.34±0.00 ^b	3.39±0.04 ^{bc}
	Skin	3.69±0.03 ^c	3.73±0.02 ^{cd}	3.52±0.02 ^b	3.55±0.03 ^{bc}	3.41±0.01 ^b	3.46±0.01 ^{bc}
	Gill	3.52±0.04 ^{bc}	3.61±0.01 ^b	3.39±0.01 ^c	3.45±0.04 ^{bc}	3.21±0.03 ^c	3.29±0.00 ^{bc}
	Liver	3.53±0.03 ^b	3.59±0.00 ^e	3.39±0.03 ^a	3.44±0.05 ^{bc}	3.19±0.05 ^a	3.26±0.03 ^b
TR 4	Intestine	3.42±0.02 ^a	3.48±0.03 ^a	3.31±0.00 ^a	3.35±0.02 ^a	3.19±0.04 ^a	3.21±0.05 ^a
	Skin	3.55±0.05 ^a	3.59±0.03 ^a	3.40±0.03 ^a	3.43±0.00 ^a	3.25±0.02 ^a	3.29±0.06 ^a
	Gill	3.41±0.06 ^a	3.48±0.04 ^a	2.24±0.02 ^a	3.29±0.01 ^a	3.16±0.01 ^a	3.20±0.01 ^a
	Liver	3.39±0.04 ^a	4.43±0.01 ^a	3.18±0.01 ^a	3.25±0.03 ^a	3.09±0.01 ^a	3.15±0.02 ^a
NL5	Intestine	3.72±0.01 ^c	3.75±0.01 ^d	3.61±0.01 ^{cd}	3.71±0.01 ^d	3.52±0.01 ^{cd}	3.55±0.04 ^e
	Skin	3.83±0.00 ^f	3.84±0.03 ^e	3.72±0.00 ^d	3.75±0.01 ^d	3.61±0.01 ^d	3.65±0.05 ^d
	Gill	3.75±0.03 ^c	3.78±0.06 ^e	3.62±0.00 ^e	3.63±0.09 ^d	3.53±0.03 ^c	3.55±0.06 ^f
	Liver	3.69±0.03 ^{de}	3.74±0.06 ^e	3.51±0.01 ^a	3.61±0.03 ^f	3.39±0.03 ^a	3.42±0.01 ^d
NL6	Intestine	6.63±0.00 ^f	3.68±0.01 ^c	3.51±0.02 ^c	3.52±0.03 ^b	3.38±0.02 ^c	3.43±0.03 ^{cd}
	Skin	3.71±0.02 ^{cd}	3.78±0.02 ^{de}	3.62±0.01 ^c	3.71±0.02 ^d	3.48±0.01 ^c	3.50±0.02 ^{bc}
	Gill	3.58±0.03 ^c	3.59±0.03 ^b	3.42±0.03 ^c	3.43±0.01 ^b	3.31±0.02 ^e	3.40±0.04 ^{de}
	Liver	3.56±0.03 ^{bc}	3.64±0.01 ^{cd}	3.44±0.03 ^a	3.57±0.01 ^{ef}	3.32±0.01 ^a	3.39±0.01 ^{cd}
NL7	Intestine	3.53±0.02 ^b	3.60±0.00 ^b	3.47±0.00 ^b	3.52±0.05 ^b	3.29±0.02 ^b	3.35±0.02 ^b
	Skin	3.61±0.02 ^{ab}	3.64±0.00 ^{ab}	3.54±0.01 ^b	3.51±0.02 ^b	3.41±0.01 ^b	3.44±0.02 ^b
	Gill	3.49±0.01 ^b	3.58±0.02 ^b	3.32±0.02 ^b	3.41±0.01 ^b	3.20±0.00 ^b	3.25±0.01 ^{ab}
	Liver	3.44±0.01 ^a	3.52±0.01 ^b	3.31±0.01 ^a	3.39±0.03 ^b	3.19±0.02 ^a	3.27±0.03 ^b

The mean values in each row with similar superscripts are not significantly different (p > 0.05), TR= Turmeric (rhizome) treatment, NL = Neem Leaves

Table 2B: Enterobacteriaceae and total viable counts (log₁₀CFU/g) of *C. gariepinus* treated with chloramphenicol

Treatments	Fish sites	Week 4		Week 8		Week 12	
		Enterobacteriaceae counts	Total viable counts	Enterobacteriaceae counts	Total viable counts	Enterobacteriaceae counts	Total viable counts
Control	Intestine	3.75±0.01 ^c	3.80±0.00 ^d	3.65±0.00 ^d	3.72±0.02 ^d	3.58±0.03 ^d	3.68±0.04 ^f
	Skin	3.96±0.00 ^g	4.02±0.02 ^f	3.85±0.01 ^e	3.95±0.00 ^e	3.71±0.00 ^e	3.89±0.02 ^e
	Gill	3.88±0.02 ^f	3.92±0.03 ^d	3.79±0.00 ^f	3.83±0.02 ^e	3.65±0.00 ^f	3.77±0.03 ^g
	Liver	3.81±0.01 ^f	3.86±0.04 ^f	3.79±0.02 ^a	3.83±0.03 ^g	3.67±0.01 ^a	3.75±0.04 ^f
CHR8	Intestine	3.70±0.00 ^e	3.79±0.01 ^d	3.58±0.01 ^c	3.62±0.02 ^c	3.40±0.01 ^c	3.44±0.00 ^{cd}
	Skin	3.78±0.01 ^{ef}	3.79±0.02 ^{de}	3.63±0.04 ^c	3.61±0.01 ^c	3.45±0.01 ^c	3.49±0.00 ^{bc}
	Gill	3.69±0.03 ^{de}	3.72±0.03 ^c	3.51±0.04 ^d	3.59±0.00 ^d	3.39±0.03 ^d	3.42±0.02 ^e
	Liver	3.70±0.04 ^e	3.74±0.04 ^e	3.63±0.01 ^a	3.60±0.00 ^f	3.43±0.00 ^a	3.48±0.01 ^c
CHR9	Intestine	3.62±0.03 ^d	3.64±0.04 ^{bc}	3.49±0.01 ^b	3.52±0.01 ^b	3.41±0.04 ^b	3.46±0.01 ^d
	Skin	3.67±0.02 ^{bc}	3.69±0.02 ^{bc}	3.53±0.02 ^b	3.61±0.02 ^c	3.50±0.04 ^b	3.52±0.02 ^c
	Gill	3.58±0.01 ^c	3.62±0.03 ^b	3.41±0.01 ^c	3.51±0.02 ^c	3.32±0.01 ^c	3.35±0.00 ^{cd}
	Liver	3.61±0.04 ^{cd}	3.68±0.04 ^{de}	3.52±0.01 ^a	3.50±0.01 ^{cd}	3.39±0.00 ^a	3.45±0.01 ^{de}

The mean values in each row with similar superscripts are not significantly different (p > 0.05), CHR= Chloramphenicol

Microbial analysis of water

Enterobacteriaceae counts and total viable counts were higher in control than the

treated groups. Enterobacteriaceae and total viable counts were significantly different ($P>0.05$) in all the treated groups of the experiment (Table 3).

Table 3: Enterobacteriaceae and total viable counts ($\log_{10}$ CFU/ml) of water

	Week 4		Week 8		Week 12	
	Enterobacteriaceae counts	Total viable counts	Enterobacteriaceae counts	Total viable counts	Enterobacteriaceae counts	Total viable counts
Control	2.15±0.09 ^{abc}	2.30±0.05 ^a	2.29±0.06 ^{ab}	2.43±0.15 ^a	2.17±0.02 ^{bc}	2.31±0.00 ^e
TR2	1.93±0.05 ^a	2.32±0.14 ^a	2.39±0.05 ^a	2.56±0.10 ^{ab}	2.48±0.01 ^c	2.69±0.01 ^h
TR3	2.15±0.24 ^a	2.48±0.02 ^{bc}	2.16±0.38 ^a	2.21±0.22 ^a	1.74±0.02 ^a	2.00±0.02 ^a
TR4	2.05±0.14 ^{ab}	2.36±0.15 ^a	2.24±0.20 ^{bc}	2.28±0.01 ^a	1.78±0.3 ^{ab}	1.82±0.03 ^b
NL5	2.25±0.10 ^a	2.59±0.01 ^c	2.34±0.00 ^a	2.46±0.06 ^{bc}	2.08±0.01 ^d	2.21±0.02 ^{bc}
NL6	2.26±0.00 ^a	2.35±0.14 ^{abc}	2.11±0.14 ^a	2.45±0.03 ^{ab}	2.26±0.01 ^{bc}	2.57±0.02 ^g
NL7	2.32±0.08 ^a	2.47±0.22 ^{bc}	2.20±0.00 ^a	2.94±0.08 ^{ab}	2.39±0.02 ^f	2.52±0.03 ^c
CHL8	2.44±0.04 ^a	2.56±0.03 ^{bc}	2.41±0.07 ^a	2.65±0.00 ^b	1.78±0.01 ^{ab}	1.88±0.04 ^{bc}
CHL9	2.22±0.31 ^{abc}	32.33±0.04 ^a	2.33±0.13 ^{ab}	2.48±0.15 ^a	1.85±0.02 ^{ab}	1.90±0.03 ^c

The mean values in each row with similar superscripts are not significantly different ($p > 0.05$), TR= Turmeric (rhizome) treatment NL=Neem leaf treatment, CHR=chloramphenicol treatment

Organ index of *C. gariepinus* fed experimental diets for 84 days

The result reveals that the treated groups had better organ index when compared to the control. The value obtained in liver,

kidney and heart were closely related and there were no significantly different ($p>0.05$) among the treatments (see table 4).

Table 4: Organ index of *C. gariepinus* before and after experiment treated with turmeric rhizome, neem leaves extracts and Chloramphenicol

Treatment	Liver	Kidney	Heart
Before	0.40± 0.01 ^{bc}	0.15± 0.02 ^a	0.12± 0.01 ^a
Control	0.35± 0.02 ^b	0.15±0.01 ^a	0.10±0.00 ^a
TR2	0.30±0.01 ^{ab}	0.10±0.00 ^a	0.10±0.01 ^a
TR3	0.30±0.00 ^{ab}	0.13±0.02 ^a	0.10±0.01 ^a
TR4	0.30±0.01 ^{ab}	0.11±0.00 ^a	0.10±0.00 ^a
NL5	0.25±0.00 ^{ab}	0.10±0.01 ^a	0.10±0.01 ^a
NL6	0.20±0.02 ^a	0.11±0.00 ^a	0.11±0.00 ^a
NL7	0.20±0.02 ^a	0.10±0.01 ^a	0.10±0.01 ^a
CHR8	0.25±0.01 ^{ab}	0.10±0.00 ^a	0.05±0.01 ^a
CHR9	0.20±0.00 ^a	0.10±0.01 ^a	0.10±0.00 ^a

The mean values in each column with similar superscripts are not significantly different ($p > 0.05$), TR= Turmeric rhizome, NL= Neem leaves, CHR= Chloramphenicol

Discussion

The result obtained in this study showed the presence of saponins, flavonoids, glucosinlates, amino acids and polysterols in neem leaves and turmeric rhizome extracts but tannin and phenol were not detected neem leaves and turmeric

rhizome. Environmental factors and the methods of preparation samples may influence the concentration of tannin and phenol. The concentrations of these metabolites in neem leaves and turmeric rhizome extracts were available in abundant and moderate quantities. This

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support the report of Njoku *et al.* (2009) that plants contain chemical compound such as saponins, tannins, flavonoids, glucosinlates, phenol, amino acids and polysterols known as metabolites which are biologically active. The phytochemical such as tannins, saponins and glucosinolates present in medicinal plants may have antimicrobial activity (Priya *et al.*, 2012).

In fish health managements, the most useful index of success is prevention of disease and systematic physicochemical analysis of the water and monitoring of the microorganism in aquaculture system (Krishna *et al.*, 2012). This study revealed that the microbial loads in intestine, skin, gill and liver of *C. gariepinus* varies with the skin and gill having highest value of total viable counts and enterobacteriaceae counts. The highest enterobacteriaceae and total viable counts were recorded in control. The value decreased as the inclusion level increased and as the month increased this support the report of Shalaby *et al.* (2006) and Bello *et al.* (2012). Investigation demonstrated that fresh water fishes were affected by infection with different types of bacteria (Khatun *et al.*, 2011). Bacteria are greater on skin than any part of the body as these parts are constantly exposed to challenges. However, gut contains more bacteria load than surrounding water (Khatun *et al.*, 2011). The antimicrobial effect of turmeric rhizome and neem leaves extracts in the diets lead to reduction in the microbial loads of fish tissues (skin, gill, liver and intestine) and inhibited the growth of microorganism that result in infection of fish. This might be attributed to the presence of antimicrobial properties in turmeric rhizome and neem leaves extracts (saponins, tannins and flavonoids).

Microbial analysis of water showed that the enterobacteriaceae populations in water were higher in control than the treated

groups containing turmeric rhizome, neem leaves and chloramphenicol at 4, 8 and 12 weeks. Observations revealed that the bacterial loads in the water of the experimental tanks were affected by the presence of *C. longa* and *A. indica* in the diets than control. This observation was supported by the findings of Shalaby *et al.* (2006) and Bello *et al.* (2012). The organosomatic indices are indicator of health which could be used to predict the health status of fish (Fox *et al.*, 1997). The results of organ indices showed that the liver, heart and kidney were not significantly ($p > 0.05$) increased in all the treated groups. Although, the result of the liver showed that there were significant differences ($p < 0.05$) among the treatments while the kidney and heart were not significantly different ($p > 0.05$). The finding agreed with the report of Abd-El-Rhman (2009) and Bello *et al.* (2012). The findings showed no traces of oedema and high variation of the intestinal organs, the inclusion of turmeric rhizome and neem leaves extracts in the diet of *C. gariepinus* could therefore be considered safe and non-toxic for consumption.

Conclusion and recommendation

Application of turmeric rhizome and neem leaves extracts in feed reduced the microbial populations in water and fish tissues. The relative abundance and availability of turmeric rhizome and neem leaves makes them cheap, natural, nutritional and antimicrobial natural product to be explored in aquaculture. Further research should be carried out on interaction /synergistic effects of turmeric rhizome and neem leaves extracts on *C. gariepinus* and other species of fish.

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References

- Abd El-Rhman, A. M. 2009.** Antagonism of *Aeromonas hydrophila* by propolis and its effect on the performance of Nile tilapia, *Oreochromis niloticus*. *Fish and Shellfish Immunology*, 27: 454 – 459.
- Adeoye, B. A. and Oyedapo, O. O. 2004.** Toxicity of erythrophleum stem-bark: role of alkaloids fraction. *African Journal of Traditional Complementary and Alternative Medicine (CAM)*, 1: 45 – 54
- Ajaiyeoba, E. O. and Fadare, D. A. 2006.** Antimicrobial potential of extracts and fractions of the African walnut – *Tetracarpidium conophorum*, *African Journal of Biotechnology*, 5(22): 2322-2325
- American Public Health Association, APHA 1985.** Standard methods for the examination of water and wastewater. 16th edition. Washington: American Public Health Association, American Water Works Association, Water Pollution Central Federation, 1268p.
- Bello, O. S., Emikpe, B. O. and Olaifa, F. E. 2012.** The body weight changes and gut morphometry of *Clarias gariepinus* juveniles on feeds supplemented with walnut (*Tetracarpidium conophorum*) leaf and onion (*Allium cepa*) bulb residue. *International Journal of Morphology*, 30(1): 253 – 257
- Cerezuela, R., Guardiola, F. A., Gonzalez, P., Meseguer, J. and Esteban, M. A. 2012.** Effects of dietary *Bacillus subtilis*, *Tetraselmis chuii*, and *Phaeodactylum tricornutum*, singularly or in combination, on the immune response and disease resistance of sea bream (*Sparus aurata* Linn). *Fish and Shellfish Immunology*, 33: 342 – 349. Doi: 10.1016/j.fsi.2012.05.004
- Chinabut, S. and Puttinawarat, S. 2005.** The choice of disease control strategies to secure international market access for aquaculture products. *Developmental Biology*, 121: 255 – 261
- Das, B. K., Pradhan, J. and Sahu, S. 2009.** The effect of *Euglena viridis* on immune response of rohu, *Labeo rohita* (Ham.). *Fish and Shellfish Immunology*, 26: 871–876.
- Food and Agriculture Organization, FAO 2014.** State of World Fisheries and Agriculture, Food and Agriculture Organization, Rome, Italy. www.fao/2/i3720e.
- Fox, H. E., White, S. A., Koa, M. F. and Fernald, R. D. 1997.** Stress and dominance in a social fish, *The Journal of Neuroscience*, 16(17): 6463–6469.
- Gomanthinayagam, S., Brij, B. T. and Rekha, G. 2014.** Studies of antimicrobial properties of different leaf extracts of Tulsi (*Ocimum tenuiflorum*) against human pathogens. *American International Journal of Contemporary Research*, 4(8): 149 – 157
- Hitchins, A. D., Feng, P., Watkins, W. D., Rippey, S. R. and Chandler, L. A. 1995.** *Escherichia coli* and the coliform bacteria. In: Watkins W. D. (Ed). Food and drug administration bacteriological analytical manual. 8th edition Arlington: Association of Official Analytical Chemists, 401-29.
- Khatun, H., Hossain, M. D., Jahan, S. N. and Khanom, D. A. 2011.** Bacterial

- infestation in different fish at Rajshahi. *Journal of Science Foundation*, 9(1-2):77-84.
- Krishna, R. H., Mohan, M. R. and Shiva, B. 2012.** Heterotrophic Bacterial Population in Two Fresh Water Fishes, *Glossogobius giuris* (Ham) and *Labeo rohita* (Hamilton). *International Journal of Fisheries and Aquaculture Sciences*, 2(2): 101-118.
- Lawson, T. B. 1995.** Fundamental of Aquaculture Engineering (Chapman and Hall 1995) International Thomson publishing Inc.: 28-39.
- Mehana, E. E., Rahmani, A. H. and Aly, S. M. 2015.** Immunostimulants and fish culture: An overview, *Annual Research and Review in Biology*, 5 (6): 1- 13
- Njoku, O. V. and Obi, M. 2009.** Phytochemical constituents of some selected medicinal plants. *African Journal of Pure and Apply Chemistry*, 3(11): 228-233.
- Nya, E. J. and Austin, B. 2009.** Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum), *Journal of Fish Diseases*, 32(11): 963-970.
- Odugbemi, T. 2006.** Medicinal plants as antimicrobial in outline and picture of medicinal plants from Nigeria, University of Lagos Press, 53 – 64
- Park, K. H. and Choi, S. H. 2012.** The effect of mistletoe, *Viscum album coloratum*, extract on innate immune response of Nile tilapia (*Oreochromis niloticus*). *Fish and Shellfish Immunology*, 32: 1016-1021
- Prasad, G. and Mukthiraj, S. 2011.** Effects of methanolic extract of *Andrographis paniculata* (Nees) on growth and haematology of *Oreochromis mossambicus* (Peters). *World Journal of Fish and Marine Sciences*, 3(6):473-479
- Priya, R., Prathapan, A., Raghu, K. G. and Nirmala M. A. 2012.** Chemical composition and *in vitro* antioxidative potential of essential oil isolated from *Curcuma longa* L. rhizome. *Asian Pacific Journal of Tropical Biomedicine*, 696-699.
- Sakai, M. 1999.** Current research status of fish immunostimulant. *Aquaculture*, 127(1): 63- 93
- Shalaby, A. M., Khattab, Y. A. and Abdel-Rahman, A. M. 2006.** Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia, *Journal of Venomous Animal Toxins including Tropical Diseases*, 12(2): 172 – 201
- Verma, V. K., Rani, K. V., Sehgal, N. and Prakash, O. 2012.** Immunostimulatory response induced by supplementation of *Ficus benghalensis* root powder, in the artificial feed the Indian freshwater murrel, *Channa punctatus*. *Fish and Shellfish Immunology*, 33: 590 - 596
- Wang, R., Li, D. and Bourne, S. 1998.** Can 2000 years of herbal medicine history help us solve problems in the year 2000? *Proceeding of Attech's 14th annual symposium (AAS'98)*, Kenturkey, USA, Pp 273 – 291

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