Dietary Application of *Chlorophytum borivilianum* and *Withania somnifera* Root Extracts Stimulates Growth, Immunity and Resistance Against *Aeromonas hydrophila* Infection in Nile Tilapia, *Oreochromis niloticus*

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Abstract

Using synthetic antibiotics in Nile tilapia culture poses health and environmental risks. Efforts are made to use phytochemicals as safer alternatives with optimum effect on fish yield. The present study aimed to determine the anabolic, immunomodulatory, anti-pathogenic effects of *Chlorophytum borivilianum* root ethanol (EC) and *Withania somnifera* root methanol (MW) extract in Nile tilapia during 120 d grow-out culture in cement cisterns. Juvenile Nile tilapia (0.020 ± 0.001 g; 1.02 ± 0.011 cm) were divided into three groups with three replicate cisterns each. One group was fed EC fortified diet, the second group was fed MW fortified diet (both at 0.75 g extract per kg feed) for 30 d followed by a basal diet for the next 90 d, while another group was fed a basal diet for 120 d. Fish fed plant extract-fortified diets showed significantly better ($P < 0.05$) weight gain (g), specific growth rate (%), immunostimulatory (phagocytic, lysozyme activities, respiratory burst), haematological and biochemical parameters than those fed the control diet. After 120 d, fish from each treatment group were challenged with the heterotrophic bacterium *Aeromonas hydrophila* [intraperitoneal injection with 0.1 mL (in saline solution) single sub-lethal dose (5×10^5 CFU.mL^{-1})]. Seven days post-infection, fish fed plant extract-fortified diets showed significantly better ($P < 0.05$) survival, immunological, haematological and biochemical parameters than control fish. The bioactive phytoconstituents in the plant extracts might be responsible for their growth-enhancing and anti-pathogenic effects. Dietary administration of MW yielded significantly better ($P < 0.05$) growth-promoting effects and resistance against *A. hydrophila* infection in Nile tilapia than EC extract.

Keywords: plant extracts, immuno-stimulation, bioactive phytoconstituents, antimicrobials, anabolic effect

Introduction

Farming of Nile tilapia has grown rapidly at the global scale for the last three decades compared with the aquaculture of other species (Debnath et al., 2023). However, such intensive farming practices for massive production have, in turn, induced stress and increased disease susceptibility for the species (Adikesavalu et al., 2017). Various bacterial infections including species of the genera *Vibrio*, *Aeromonas*, *Pseudomonas* and *Streptococcus* have been identified as the major diseases threatening intensively cultured tilapia farms (Haenen et al., 2023).

Aeromoniasis caused by *Aeromonas hydrophila* is one of the common bacterial diseases affecting tilapia culture (Basri et al., 2020). This motile aeromonad found ubiquitously in freshwater environments is reported as opportunistically pathogenic and may cause clinical disease outbreaks leading to high levels of morbidity and mortality in tilapia farming systems (Haenen et al., 2023). Therefore, developing protection against *A. hydrophila* infections is important to increase tilapia production during an intensive culture system.

Synthetic antimicrobials are often used as therapeutic, prophylactic and metaphylactic agents in aquaculture to control bacterial infections (Okocha et al., 2018). However, antimicrobial residues may occur in fish when the drugs are administered above their commended dosage (Okeke et al., 2022). Besides, consumption of such products may result in the development and

propagation of antibiotic resistance along the food chain (Okocha et al., 2018), and many ailments in humans (Lee et al., 2001; Canada-Canada et al., 2009). Hence, several works have been underway to use plant extracts containing various bioactive secondary metabolites as an alternative approach for improving fish health and mitigating *Aeromonas hydrophila* infection (Chakraborty and Hancz, 2011; Mawire et al., 2021). Immunostimulatory methods using biological substances not only provide environment-friendly prophylactic measures to fight diseases but also give protection against immunosuppressive stress conditions, effective both in juveniles and adults (Barman et al., 2013). In this context, natural plant chemicals with expected safer utilisation and handling issues, and possibly lower toxicity for both fish and the surrounding environment, are very attractive substitute to synthetic chemotherapeutics (Reverter et al., 2014).

Extracts from different medicinal herbs have been implicated as inducing growth and immuno-stimulation in Nile tilapia (Gabriel and Gonzàlez-Redondo, 2019). Our previous study has specified the androgenic efficacy of *Withania somnifera* (Ashwagandha) root methanol extract and *Chlorophytum borivilianum* (Safed Musli) root ethanol extract in Nile tilapia. Presence of different bioactive phytochemicals in both plant extracts with aromatase inhibitory activity was also detected (De et al., 2020). In another study, indications were that *W. somnifera* root powder has an immunostimulatory effect and increased disease resistance in *Labeo rohita* fingerlings against *A. hydrophila* infection (Sharma et al., 2010). Dietary supplementation with *W. somnifera* root also positively enhanced the innate immune system and survival rate in *Macrobrachium rosenbergii* against *A. hydrophila* infection (Harikrishnan et al., 2012). Ethanol extract of *W. somnifera* has been observed to boost immunity and growth in Nile tilapia (Mukherjee et al., 2019). Ethanolic extract of *C. borivilianum* roots as well as sapogenins isolated from the medicinal herb showed a pronounced anabolic effect in treated albino rats (Thakur and Dixit, 2006). Administration of *C. borivilianum* polysaccharide fraction also influenced immunity and promoted expression of immune related genes in rohu (Giri et al., 2015).

But no study has yet been conducted to comparatively analyse the growth, immune status and disease resistance against bacterial infection of monosex Nile tilapia fed diets supplemented with these two plant materials during cistern culture. Considering this, the present study was undertaken to evaluate the efficacy of ethanol extract of *C. borivilianum* root and methanol extract of *W. somnifera* root focusing on the growth potential, innate immunity and protection against *A. hydrophila* infection in Nile tilapia.

Materials and Methods

Ethical approval

All experiments in the present study were conducted following the guidelines of the Institutional Animal Ethics Committee, University of Calcutta (Registration #885/ac/05/CPCSEA), registered under the 'Committee for the Purpose of Control and Supervision of Experiments on Laboratory Animals' (CPCSEA), Ministry of Environment and Forests, Government of India.

Collection of fish seed

Three days old mixed sex hatchlings of Nile tilapia *Oreochromis niloticus* (Linnaeus 1758) (mean weight 0.020 ± 0.001 g; mean length 1.02 ± 0.011 cm) were collected from the hatchery of the Government of West Bengal, India, oxygen packed and transported to the experimental farm.

Preparations of plant extracts

Withania somnifera L. (Family: Solanaceae, Indian ginseng, local name ashwagandha) roots and *Chlorophytum borivilianum* L. (Family: Asparagaceae, local name safed musli) roots were purchased during spring (March, 2022) from Gariahat market, Kolkata, West Bengal (22.5198°N, 88.3663°E). After procurement, the identification and authentication of plant materials were done at the Department of Botany, University of Calcutta, West Bengal, India. Then the plant materials were washed in distilled water, air - dried in shade at 24–27 °C for 15 d and ground into powder form using an electric grinder. Powdered roots of *W. somnifera* and *C. borivilianum* were subject to solvent extraction by maceration under gentle agitation in a glass vessel for 48 h using methanol and ethanol as solvents (sample to solvent ratio 1:2 w/v), respectively. The filtered extracts were then concentrated under vacuum at 45 °C for dryness using rotary vacuum evaporator (Roteva ASP-8763.RD0.000, ASP-EQUITRON-ASP, India), dissolved in dimethyl sulfoxide (DMSO) and stored at –20 °C for future use. The concentration ($g.mL^{-1}$) of plant extract was measured by mantle heating (50 °C) of 1 mL sample of each extract dissolved in DMSO on petri plate (Hussain et al., 2009).

Determination of total phenol content, total flavonoid content, antioxidant property of plant extracts

Total phenol content (with gallic acid as standard) of *W. somnifera* root methanol extract and *C. borivilianum* root ethanol extract was determined following standard procedures using the Folin - Ciocalteu reagent (Maisuthisakul et al., 2007). Total flavonoid content (with rutin as standard) of both extracts was evaluated by a colorimetric assay following standard protocol (Maisuthisakul et al., 2007). Free radical scavenging activity of the plant extracts was determined with the stable radical DPPH (2, 2 – diphenyl 1 - picrylhydrazyl) by standard methods (Maisuthisakul et al., 2008).

Preparation of experimental diets with plant extracts

The basal diet was an artificial floating fish feed (Tokyu® Fish Food Spirulina, Tokyu, Japan) whose ingredients were white fish meal, wheat flour, shrimp meal, dried yeast, soybean meal, wheat germ meal, dehydrated alfalfa fish meal, soybean meal, cassava, rice bran, enzyme, vitamins [A, C, D, E, K, B_1, B_2, B_3, B_{12}, inositol, nicotinic acid, Ca - pantothenate choline, biotion, carotenoid, para - amino benzoic acid, folic acid], minerals [iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), cobalt (Co), phosphorous (P), magnesium (Mg), P - amino benzoic acid (Paba)] and special ingredients [carotenoids, NS garm, chlorophyll]; while the proximate compositions (%) were crude protein (32 %), crude fat (4 %), ash (10 %), moisture (9 %) and nitrogen - free extract (31 %) according to the manufacturer as printed on packets. Two plant extract fortified diets were prepared; one with methanol extract of *W. somnifera* root and another with ethanol extract of *C. borivilianum* root, both at the concentration of 0.75 g per kg feed, referred to as MW0.75 and EC0.75, respectively hereafter. The extraction solvents, dose of plant extracts and treatment duration were selected based on our previous study where dietary administration of *W. somnifera* root methanol extract and *C. borivilianum* root ethanol extract at 0.75 g per kg feed for 30 d produced ~90 % male tilapia (De et al., 2020). Plant extracts dissolved in DMSO were added to the finely ground (<500–1000 µm) basal diet at the desired concentration (Moundipa et al., 2005). The feed was then wetted with deionised water, mixed thoroughly, formed into pellets with a pelletiser (diameter 2 mm), and dried at room temperature. Pelleted feed was pulverised before feeding to the juvenile fish. The control diet was prepared by adding only DMSO without any plant extract.

Experimental design

A 120-d grow-out culture experiment was performed at the fish farm in Diamond Harbour, South 24 Parganas, West Bengal, India (22.1927°N, 88.1895°E) from June to September 2022 in nine aerated concrete cisterns (3.25 × 2.5 × 1.5 m). Each cistern or tank was filled with freshwater to maintain water level at 1 m depth keeping water volume of 8 m^3. The cisterns were stocked with two hundred mixed sex Nile tilapia hatchlings (mean weight 0.020 ± 0.001 g; mean length 1.02 ± 0.011 cm) in triplicate for each of the MW0.75, EC0.75 and control treatment groups. Nile tilapia hatchlings in MW0.75 and EC0.75 treatment groups were fed respective plant extract-fortified diets for the initial 30 d (De et al., 2020) followed by a basal diet for the next 90 d, while fish in the control group were fed a basal diet for the entire 120 d. Fish were fed at a rate of 20 % of body weight per day for the first 30 d and at a rate of 5 % of body weight per day for the next 90 d of culture. Organic matter was regularly removed from the bottom of the cistern adjusting the lost water. The wall and bottom of the cistern were brushed to control algal growth and the complete water of the cistern was exchanged every 15 d. Ten fish from each cistern were randomly collected and measured to record the mean body weight and mean length at every 15 d interval, and the feed amount was adjusted accordingly. All fish in each cistern were harvested, counted and the mean body weight and mean length were measured at the end of the four-month culture period.

Measurement of water quality parameters

During the entire culture period, water quality parameters viz. temperature (°C), DO_2 (ppm), free CO_2 (ppm), total ammonia (ppm), nitrite (ppm) and pH of the cisterns were measured every 15th day using the standard procedures of the American Public Health Association (APHA 1998).

Growout analysis

Growth parameters like body weight (g), length (cm), weight gain (g), daily weight gain (DWG; $g.fish^{-1}.d^{-1}$), specific growth rate (SGR %), feed conversion ratio (FCR), protein efficiency ratio (PER) were measured for the different experimental groups after one month and four months of dietary treatment with plant extracts following standard formulations (Ghosal et al., 2021).

Analysis of length-weight relationship

The length-weight relationship (LWR) of *O. niloticus* from different treatment groups was estimated at the end of 120 d culture period using the following power equation (Beyer, 1987): $W = aL^b$; where; W = Weight of fish (g), L = Total length of fish (cm), a = Intercept (initial growth constant), b = Slope (growth coefficient) (Getso et al., 2017).

Analysis of the sex ratio

At the end of the 120-d culture period, all fish from different treatment groups were initially sexed by visual observation of the external morphological features (Eccles, 1992). Fish were then sacrificed and dissected for confirmation of sex through visual observation of the testis in males and the ovary in females.

Study of immunostimulating properties

At the end of the 120-d culture period, fish were not fed for 24 h before blood sampling. Thirty fish per treatment group were randomly sampled, anesthetised with phenoxy-ethanol (1:20000 v/v) and blood was collected from the caudal vein using a sterile insulin syringe coated with heparin. Plasma and leukocytes were separated by density gradient centrifugation (700 ×g, 30 min, 4 °C) (TGL16M, Yingtai, China) of blood samples using Histopaque-1119 and Histopaque-1077 (Sigma-Aldrich). Isolated plasma was collected and stored at –20 °C, while leukocytes were

adjusted to 10^7 viable cells per millilitre using phenol red free Hank's balanced salt solution (HBSS) (Ardó et al., 2008). Phagocytotic activity of isolated leukocytes was determined spectrophotometrically (Type: 117, Systronics, India), from absorbance at 510 nm, after trypsin EDTA digestion of macrophages (Seeley et al., 1990). Respiratory burst activity of isolated leukocytes was determined spectrophotometrically by the nitro blue tetrazolium (NBT) assay, from the absorbance at 610 nm, measuring intracellular oxidative free radicals (Secombes, 1990). Plasma lysozyme activity was determined spectrophotometrically, from the absorbance at 530 nm (Sankaran and Gurnani, 1972). The total protein concentration of plasma was measured with Biuret reaction by colorimetric assay, using a protein diagnostic reagent kit (Sigma-Aldrich). Total immunoglobulin was calculated by incubating the plasma with polyethylene glycol (PEG) for 2 h at room temperature followed by centrifugation (1000 ×g, 15 min) and determination of the protein content of the supernatant.

Study of haematological parameters

Another 30 fish per treatment group were randomly sampled, anaesthetised with phenoxy-ethanol (1:20000 v/v) and blood was collected from the caudal vein using a sterile insulin syringe without anticoagulant. The collected blood sample was allowed to clot at room temperature for 10–20 min and was centrifuged at 1000 ×g for 20 min to isolate the serum. Key haematological parameters such as total count of RBCs (10^6.μL^{-1}) and WBCs (10^3.μL^{-1}), haemoglobin concentration (Hb g.dL^{-1}), haematocrit (Ht, %), mean corpuscular haemoglobin concentration (MCHC, g.dl^{-1}), mean corpuscular haemoglobin (MCH, pg), mean corpuscular volume (MCV, μm^3), lymphocytes & thrombocytes (%), neutrophils (%), monocytes (%), total serum glucose (mg.mL^{-1}) and albumin (mg.mL^{-1}) were measured using standard protocols (Ghosal et al., 2021). In brief, RBC and WBC were counted using a haemocytometer (Improved Neubauer, Germany) with respective diluting solutions (Sigma). Haemoglobin (Hb) level was determined colorimetrically at 400 nm using a diagnostic kit (Sigma-Aldrich) following the manufacturer's protocol. Three - quarters of micro - Ht capillaries were filled with blood, sealed at one end with a capillary sealer, and centrifuged at 13,000 rpm for five min in a micro - Ht centrifuge (RM - 12C BL, Remi, India) to determine haematocrit (Ht). The mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration (MCHC) were calculated using the following equations: MCV (μm^3) = {Ht (%) × 10} / RBC count (10^6.mm^{-1}); MCH (pg) = {Hb (g.dL^{-1}) × 10} / RBC count (10^6 .mm^{-1}); MCHC (%) = {Hb (g.dL^{-1}) / Ht (%)} × 100. The differential count of WBCs (lymphocyte and thrombocyte %, neutrophil % and monocyte %) was analysed using FACS (BD FACSCalibur, Becton Dickinson, USA) after staining with WBC diluting fluid (1:10). Forward scatter (FSC) and side scatter (SSC) of each cell were measured, and different types of WBC populations in a sample were identified by its typical location in a FSC versus SSC contour plot using the FACSCalibur sorting module. Serum sample was analysed with colorimetric kits to measure total serum glucose (Abcam, Cambridge, UK #ab65333) at 570 nm and serum albumin (MyBioSource, San Diego, CA, USA #MBS019237) at 450 nm following manufacturers' protocol.

Study of biochemical parameters

The serum samples from different treatment groups were analysed for enzymatic antioxidants [glutathione S - transferase (GST; #MBS031087), glutathione reductase activity (GRD; #MBS011817)], non - enzymatic antioxidant [reduced glutathione (GSH; #MBS1601755)], biomarker for lipid peroxidation [malondialdehyde (MDA; #MBS007853)], and liver enzymes [alkaline phosphatase (ALP; #MBS1601674), glutamic - oxaloacetic transaminase (GOT; #MBS1601734), glutamic - pyruvate transaminase (GPT; #MBS1601732), acid phosphatase (ACP; #MBS1601693)] using commercial ELISA kits (MyBioSource, San Diego, CA, USA) following the manufacturer's protocols.

Bacterial challenge experiment

Pathogenic bacterium *Aeromonas hydrophila* was obtained from the National Centre for Microbial Resource, National Centre for Cell Science [Accession Number: MCC 2052 (T)], and cultured overnight in a nutrient broth (Himedia) at 30 °C in a shaking incubator. A separate pilot 96 h toxicity study was conducted with four months old adult Nile tilapia to determine the lethal dose (LD_{50}) of *A. hydrophila*. Adult Nile tilapia (110.20 ± 0.8 g; 17.85 ± 0.6 cm) were intraperitoneally injected with 0.1 mL (in saline solution) of 24 h live cultured bacteria at different doses, and cumulative mortality till 96 h in each dose group was calculated. The 96 h LD_{50} was determined by Probit analysis using SPSS 22.0 statistical tool and was found to be 10×10^5 CFU.mL^{-1} ($y = 4.879x + 0.412$, $p = 4.99 \times 10^{-40}$; 95 % Fiducial limit: Lower – 6.804; Upper - 10.424, $P < 0.05$).

At the end of 120 d feeding trial, 60 fish (20 fish from each replicating cistern) per control, MW0.75 and EC0.75 treatment group were collected and randomly distributed into six 100 litre aquaria corresponding to each treatment group, every aquarium containing 10 fish. Those fish were then intraperitoneally injected with 0.1 mL (in saline solution) of 24 h cultured pathogenic *A. hydrophila* sub-lethal dose (5×10^5 CFU.mL^{-1}) (Abdel - Razek et al., 2019). Another 60 fish from the control group were distributed in six more aquaria (10 fish per aquarium) and those fishes were injected with only saline solution (0.1 mL). Fish in all treatment groups were observed for morbidity and mortality for seven days post-challenge. The aquaria were continuously aerated semi-static systems, and water was replaced daily. Fish in all treatment groups

were fed a normal basal diet (Tokyu® Fish Food Spirulina, Tokyu, Japan) at 5 % of body weight per day during the seven days of the experiment and were starved 24 h before the final sampling on the eighth day. At the end of the experiment, blood samples were collected from 30 randomly chosen live fish from each of the bacteria-challenged groups to measure different immunological, haematological and biochemical parameters following the methods described earlier. No morbid signs and mortality were observed in the fish injected with only saline solution, and blood was not collected from those fish.

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics Version 22 software. All data were first tested for normality of distribution using Shapiro - Wilk's test. Necessary angular transformations were conducted for the percent data. The homogeneity of variance among different treatments was tested using Levene's test. Descriptive statistics of different parameters summarise as mean values ± standard errors. Total phenol content, flavonoid content and antiradical activity of two plant extracts were compared by independent samples two - tailed t - test (at 5 % significance level). Effects of dietary supplementation with plant extracts on survival, different growth parameters and production were analysed by one-way analysis of variance (ANOVA), while data for immunological, haematological and biochemical parameters before and after bacterial challenge were subjected to two-way ANOVAs taking plant extract feeding and bacterial challenge as two independent variables. Tukey's HSD test at the 5 % probability level was performed to determine which means differed significantly.

Results

Total phenol content, total flavonoid content and antioxidant property of plant extracts

Withania somnifera root methanol extract showed significantly higher ($P < 0.05$) antiradical activity and total phenol content, but significantly lower ($P < 0.05$) total flavonoid content compared to *C. borivilianum* root ethanol extract (Table 1).

Water quality parameters

Water physicochemical parameters in different treatment cisterns did not show any significant difference ($P > 0.05$) during the culture period (Table 2).

Growth parameters

After one month's treatment, both MW0.75 and EC0.75 showed significantly higher ($P < 0.05$) weight gain (WG), daily weight gain (DWG), specific growth rate (SGR) and protein efficiency ration (PER) compared to the control group. Fish in the MW0.75 treatment group showed significantly higher ($P < 0.05$) WG and DWG than fish in EC0.75 treatment group. However, there was no significant difference in SGR and PER between MW0.75 and EC0.75 treatment groups. Both MW0.75 and EC0.75 treatment groups showed significantly lower ($P < 0.05$) food conversion ratio (FCR) values compared to the control, but there was no significant difference ($P > 0.05$) in FCR values between the two groups (Table 3).

At the end of 120 d culture in the cisterns, both MW0.75 and EC0.75 treatment groups showed significantly higher ($P < 0.05$) survival compared to the control (94.2 %). Treatment group MW0.75 showed the highest survival (97.5 %), which was significantly higher ($P < 0.05$) than EC0.75 treatment group (96.0 %) as well the control (Table 4).

At the completion of four months of culture, fish fed MW0.75 and EC0.75 fortified diets showed significantly higher ($P < 0.05$) final weight (MW0.75: 165.42 ± 0.07 g; EC0.75: 153.98 ± 0.08 g) compared to fish fed control diet (100.29 ± 0.1 g). Treatment group MW0.75 showed the highest final weight, which was significantly higher ($P < 0.05$) than EC0.75 (Fig. 1a).

Moreover, fish fed MW0.75 and EC0.75 fortified diets showed significantly higher ($P < 0.05$) final length (MW0.75: 20.10 ± 0.05 cm; EC0.75: 19.90 ± 0.08 cm) compared to fish fed control diet (17.35 ± 0.06 cm). However, there was no significant difference ($P > 0.05$) in final length between MW0.75 and EC0.75 treatment groups (Fig. 1b).

Fish fed plant extract fortified diets showed significantly higher ($P < 0.05$) WG, DWG, SGR and FCR compared to the fish fed control diet throughout the entire culture period of 120 d (Table 4). Treatment group MW0.75 showed the highest mean values for WG, DWG and FCR, which were significantly higher ($P < 0.05$) than the EC0.75 treatment group. However, MW0.75 and EC0.75 treatment groups showed no significant difference ($P > 0.05$) in SGR value. Both MW0.75 and EC0.75 treatment groups showed significantly lower ($P < 0.05$) PER values than the control; MW0.75 having the lowest PER value, which was significantly lower ($P < 0.05$) than EC0.75 (Table 4). Mean production ($g.m^{-3}$) at the end of the 120 d culture in cement cisterns was the highest in treatment group MW0.75 (4034.05 ± 3.27 $g.m^{-3}$), and it was significantly higher ($P < 0.05$) than both EC0.75 (3695.04 ± 11.96 $g.m^{-3}$) and the control (2360.71 ± 6.07 $g.m^{-3}$) (Fig. 2). The mean in EC0.75 was significantly higher ($P < 0.05$) than in the control group (Fig. 2).

Analysis of length-weight relationship (LWR)

The power equations of three treatment groups were obtained by plotting the length (cm) along the X-axis and weight (g) along the Y-axis. All three treatment

Table 1. Mean (± 1 standard deviation [SD]) for antiradical activity, total phenol and flavonoid contents and results of T-test for *Withania somnifera* root methanol compared with *Chlorophytum borivilianum* root ethanol extract.

Parameter	Plant extract	Mean	SD	t	df	*P*
Antiradical activity (%)	*W. somnifera* root - methanol	4.023*	0.001	- 1399.98	4	< 0.05
	C. borivilianum root - ethanol	2.550	0.002			
Total phenol (mg GAE.g^{-1} dry wt)	*W. somnifera* root - methanol	116.86*	0.045	11.364	4	< 0.05
	C. borivilianum root - ethanol	113.86	0.455			
Total flavonoid (mg RE.g^{-1} dry wt)	*W. somnifera* root - methanol	0.49	0.003	- 280.72	4	< 0.05
	C. borivilianum root - ethanol	1.15*	0.003			

Note: *defines statistically significant difference ($P < 0.05$) in means between two plant extracts. Abbreviations: RE – rutin equivalents; GAE – gallic acid equivalents.

Table 2. Mean (± 1 standard error, n = 3) for physicochemical parameters of water in the cement cisterns of different treatment groups during grow - out culture.

Physicochemical parameters	Control	MW0.75	EC0.75
Temperature (°C)	27.90 ± 0.06	27.91 ± 0.11	27.92 ± 0.11
Dissolved O_2 (ppm)	6.79 ± 0.32	6.78 ± 0.32	6.78 ± 0.31
Free CO_2 (ppm)	4.03 ± 0.35	4.00 ± 0.35	4.03 ± 0.35
NH_3 (ppm)	0.029 ± 0.003	0.031 ± 0.003	0.031 ± 0.003
NO_2 (ppm)	0.02 ± 0.002	0.03 ± 0.002	0.03 ± 0.003
pH	8.89 ± 0.09	8.87 ± 0.09	8.84 ± 0.10

Note: η^2 = 0.232 (Temperature), 0.170 (DO_2), 0.174 (Free CO_2), 0.169 (NH_3), 0.923 (NO_2), 0.134 (pH). Control - fish fed basal diet for 120 d; MW0.75 – fish fed diet fortified with *Withania somnifera* root methanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d; EC0.75 - fish fed diet fortified with *Chlorophytum borivilianum* root ethanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d.

Table 3. Mean (± 1 standard error, n = 3) of different growth parameters in three treatment groups after one month's culture in cement cisterns.

Growth parameters	Control	MW0.75	EC0.75
Weight gain (g)	10.496 ± 0.014^a	21.326 ± 0.007^c	18.902 ± 0.064^b
Daily weight gain (g.d^{-1})	0.087 ± 0.0001^a	0.178 ± 0.0001^c	0.158 ± 0.0005^b
Specific growth rate (%)	5.169 ± 0.033^a	5.797 ± 0.014^b	5.739 ± 0.017^b
Feed conversion ratio	1.500 ± 0.047^b	1.349 ± 0.004^a	1.292 ± 0.008^a
Protein efficiency ratio	2.087 ± 0.065^a	2.316 ± 0.008^b	2.418 ± 0.014^b

Note: Different superscripts mark significant difference ($P < 0.05$) in means within rows. η^2 = 1.00 (Weight Gain), 1.00 (Daily Weight Gain), 0.987 (Specific growth rate), 0.833 (Feed conversion ratio), 0.864 (Protein efficiency ratio). Control - fish fed basal diet for 120 d; MW0.75 – fish fed diet fortified with *Withania somnifera* root methanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d; EC0.75 - fish fed diet fortified with *Chlorophytum borivilianum* root ethanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d.

Table 4. Mean (± 1 standard error, n = 3) percentage survival and different growth parameters in three treatment groups at the end of four months' culture in cement cisterns.

Growth parameters	Control	MW0.75	EC0.75
Weight gain (g)	100.278 ± 0.114^a	165.5 ± 0.134^c	153.96 ± 0.109^b
Daily weight gain (g.d^{-1})	0.836 ± 0.002^a	1.379 ± 0.001^c	1.283 ± 0.001^b
Specific growth rate (%)	7.048 ± 0.034^a	7.504 ± 0.014^b	7.486 ± 0.014^b
Feed conversion ratio	3.260 ± 0.005^a	3.690 ± 0.003^c	3.525 ± 0.002^b
Protein efficiency ratio	0.959 ± 0.0016^c	0.847 ± 0.0007^a	0.887 ± 0.0004^b
Survival (%)	94.167 ± 0.167^a	97.500 ± 0.000^c	96.000 ± 0.289^b

Note: Different superscripts mark significant difference ($P < 0.05$) in means within rows. η^2 = 0.962 (Survival percentage), 1.00 (Weight gain), 1.00 (Daily weight gain), 0.978 (Specific growth rate), 0.999 (Feed conversion ratio), 0.999 (Protein efficiency ratio). Control – fish fed basal diet for 120 d; MW0.75 – fish fed diet fortified with *Withania somnifera* root methanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d; EC0.75 – fish fed diet fortified with *Chlorophytum borivilianum* root ethanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d.

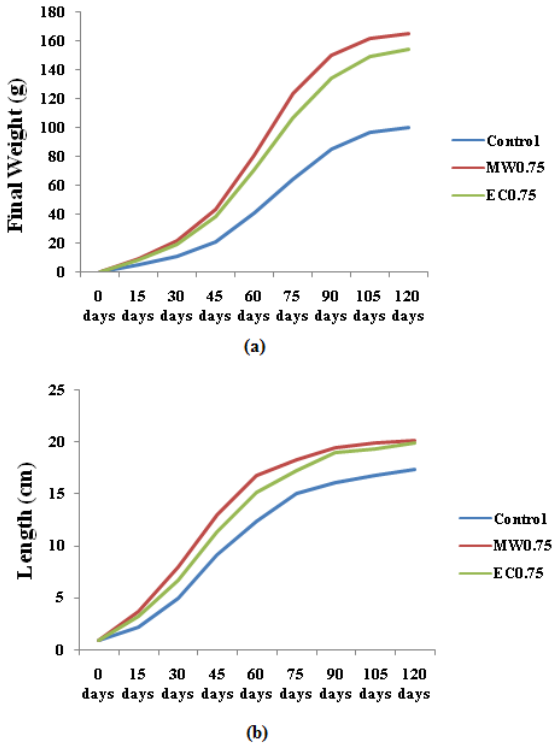

Fig. 1. Mean (a) Weight (g); and (b) Length (cm) of Nile tilapia at 15-d intervals in different treatment categories during four months of culture in cement cisterns. Control – fish fed basal diet for 120 d; MW0.75 – fish fed diet fortified with *Withania somnifera* root methanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d; EC0.75 - fish fed diet fortified with *Chlorophytum borivilianum* root ethanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d.

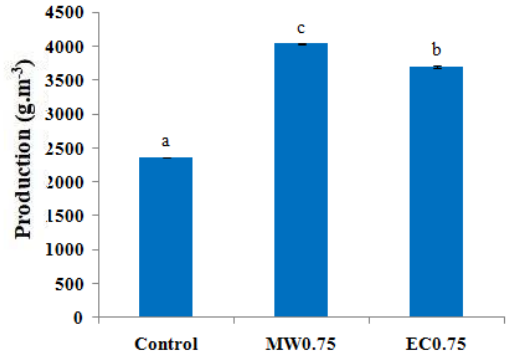

Fig. 2. Mean (± 1 standard error, n = 3) total production of monosex Nile tilapia in each of the three treatment categories at the end of four months culture in cement cisterns. Different superscripts mark significant differences ($P < 0.05$) in mean values after Tukey's HSD test. Control – fish fed basal diet for 120 d; MW0.75 – fish fed diet fortified with *Withania somnifera* root methanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d; EC0.75 - fish fed diet fortified with *Chlorophytum borivilianum* root ethanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d.

groups showed a strong correlation (R^2 ~ 0.9) between the two parameters. In the power equation curve of LWR, both MW0.75 and EC0.75 showed a higher value of 'b' and a lower value of 'a' compared to the control (Fig. 3).

Analysis of sex ratio

At the end of 120 d grow - out culture, the percentage of males in MW0.75 (80.83 ± 0.8%) and EC0.75 (87.50 ± 1.4%) treatment groups was significantly higher ($P < 0.05$) than in the control group (43.33 ± 0.8%), while the male percentage in EC0.75 treatment group was significantly higher ($P < 0.05$) than that of the MW0.75 treatment group [$\eta^2 = 0.994$ (male percentage)].

Survival and immunological parameters post bacterial challenge

At the end of 120 d culture in cisterns, fish in the MW0.75 treatment group showed the highest phagocytic activity, respiratory burst, sera lysozyme activity, total protein and total immunoglobulin, which were significantly higher ($P < 0.05$) compared to the fish in the control group (Table 5). Moreover, fish in EC0.75 treatment group showed significantly higher ($P < 0.05$) phagocytic activity, sera lysozyme activity and total immunoglobulin compared to the fish in control group.

On the other hand, 7 d post *A. hydrophila* challenge, control and EC0.75 treatment group showed a significant increase ($P < 0.05$), while MW0.75 treatment group showed a significant decrease ($P < 0.05$) in phagocytic activity and sera lysozyme activity compared to the corresponding treatment group pre-infection (Table 5). Sera lysozyme activity, total protein and total immunoglobulin levels in all treatment groups post bacterial infection were significantly higher ($P < 0.05$) compared to the respective treatment group pre-infection. Here, the MW0.75 treatment group showed the lowest phagocytic activity, respiratory burst and sera lysozyme activity, which were significantly lower ($P < 0.05$) than EC0.75 and control groups.

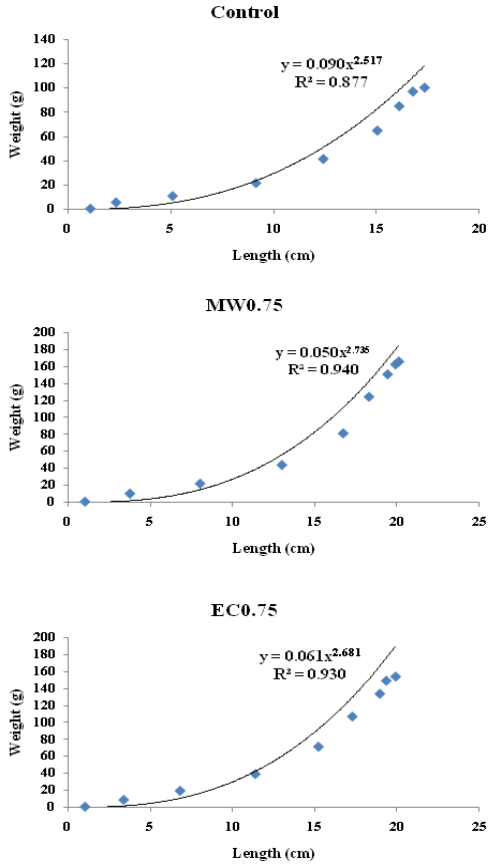

Fig. 3. Length-weight relationship curves of the different experimental categories of *O. niloticus* during 120 d culture. [Power equation is $y = a.x^b$; where, y = weight (g), x = length (cm), a = intercept, b = slope and R^2 = correlation coefficient]. Control – fish fed basal diet for 120 d (n = 9); MW0.75 – fish fed diet fortified with *Withania somnifera* root methanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d (n = 9); EC0.75 – fish fed diet fortified with *Chlorophytum borivilianum* root ethanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d (n = 9).

Table 5. Mean (± 1 standard error, n = 30) immunological parameters after four months culture in cement cisterns (Pre-Infection) and 7 d post *Aeromonas hydrophila* challenge (Post-Infection).

Treatment category	Phagocytic activity (OD 510 nm)	Respiratory burst (OD 620 nm)	Sera lysozyme activity (μg.mL^{-1})	Total protein (mg.mL^{-1})	Total Ig (mg.mL^{-1})
Pre-Infection					
Control	0.579 ± 0.0003^{a}	0.058 ± 0.001^{a}	21.17 ± 0.100^{a}	0.104 ± 0.002^{a}	0.076 ± 0.001^{a}
MW0.75	1.056 ± 0.0002^{e}	0.070 ± 0.001^{b}	33.89 ± 0.153^{e}	0.239 ± 0.003^{b}	0.123 ± 0.001^{c}
EC0.75	0.848 ± 0.0002^{c}	0.064 ± 0.001^{ab}	26.49 ± 0.088^{b}	0.118 ± 0.001^{a}	0.107 ± 0.000^{b}
Post-Infection					
Control-Inj.	1.205 ± 0.0002^{f}	0.835 ± 0.004^{e}	36.79 ± 0.063^{f}	1.099 ± 0.006^{c}	0.101 ± 0.004^{b}
MW0.75-Inj.	0.756 ± 0.0003^{b}	0.270 ± 0.001^{c}	27.71 ± 0.087^{c}	1.449 ± 0.012^{e}	0.322 ± 0.001^{e}
EC0.75-Inj	0.916 ± 0.0038^{d}	0.353 ± 0.002^{d}	29.69 ± 0.064^{d}	1.233 ± 0.008^{d}	0.255 ± 0.005^{d}
Pooled SE	0.015	0.021	0.381	0.042	0.007
Two-way ANOVA					
Dietary treatment	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Bacterial infection	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Dietary treatment × Bacterial infection	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

Note: Different superscripts mark significant difference ($P < 0.05$) in means within columns. η^2 = 0.998 (Phagocytic activity), 0.998 (Respiratory burst), 0.989 (Sera lysozyme activity), 0.996 (Total protein), 0.973 (Total Ig). Control – fish fed basal diet for 120 d; MW0.75 – fish fed diet fortified with *Withania somnifera* root methanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d; EC0.75 – fish fed diet fortified with *Chlorophytum borivilianum* root ethanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d. Control-Inj. – control fish (fed basal diet for 120 d) injected intraperitoneally with 24 h cultured pathogenic *A. hydrophila* (single sub-lethal dose, 5 × 10^5 CFU in 0.1 mL saline) at the end of 120 d culture; MW0.75-Inj. – fish fed *W. somnifera* root methanol extract fortified diet (0.75 g.kg^{-1} feed, initial 30 d) followed by basal diet (next 90 d) injected intraperitoneally with 24 h cultured pathogenic *A. hydrophila* (single sub-lethal dose, 5 × 10^5 CFU in 0.1 mL saline) at the end of 120 d culture; EC0.75-Inj. – fish fed *C. borivilianum* root ethanol extract fortified diet (0.75 g.kg^{-1} feed, initial 30 d) followed by basal diet (next 90 d) injected intraperitoneally with 24 h cultured pathogenic *A. hydrophila* (single sub-lethal dose, 5 × 10^5 CFU in 0.1 mL saline) at the end of 120 d culture.

However, total protein and total immunoglobulin levels were the highest in MW0.75 treatment group, and those were significantly higher ($P < 0.05$) than EC0.75 and control groups (Table 5). The two-way ANOVA revealed that all immunological parameters were significantly affected ($P < 0.05$) by dietary plant extract supplementation, bacterial infection and their interaction (Table 5). Furthermore, no mortality was observed in the control fish injected with only saline solution. However, 7 d post *A. hydrophila* challenge, fish in the control group showed the least survival (71.67 ± 1.7 %) followed by EC0.75 (73.33 ± 2.1 %) and MW0.75 (91.67 ± 1.7 %) (Fig. 4). Survival percentage in the MW0.75 treatment group was significantly higher ($P < 0.05$) than EC0.75 and control groups, which showed no significant difference ($P > 0.05$) in survival percentage between them (Fig. 4).

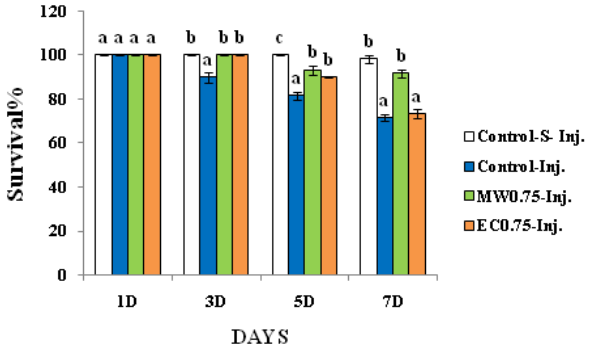

Fig. 4. Mean (± 1 standard error, n = 6) percentage survival for 7 d post bacterial challenge with *Aeromonas hydrophila*. Different superscripts mark significant differences ($P < 0.05$) in mean values after Tukey's HSD test. Control-S-Inj. – control fish (fed basal diet for 120 d) injected with saline (0.1 mL, single dose) at the end of 120 d culture; Control-Inj. – control fish (fed basal diet for 120 d) injected intraperitoneally with 24 h cultured pathogenic *A. hydrophila* (single sub - lethal dose, 5×10^5 CFU in 0.1 mL saline) at the end of 120 d culture; MW0.75-Inj. – fish fed *Withania somnifera* root methanol extract fortified diet (0.75 g.kg^{-1} feed, initial 30 d) followed by basal diet (next 90 d) injected intraperitoneally with 24 h cultured pathogenic *A. hydrophila* (single sub - lethal dose, 5×10^5 CFU in 0.1 mL saline) at the end of 120 d culture; EC0.75-Inj. – fish fed *Chlorophytum borivilianum* root ethanol extract fortified diet (0.75 g.kg^{-1} feed, initial 30 d) followed by basal diet (next 90 d) injected intraperitoneally with 24 h cultured pathogenic *A. hydrophila* (single sub - lethal dose, 5×10^5 CFU in 0.1 mL saline) at the end of 120 d culture.

Haematological parameters

At the end of 120 d culture in cement cisterns, total WBC count was the highest in MW0.75 treatment group, which was significantly higher ($P < 0.05$) than EC0.75 and control groups (Table 6a). Both MW0.75 and EC0.75 treatment groups showed significantly higher ($P < 0.05$) neutrophil percentage compared to control, EC0.75 treatment group showing the highest value. Monocyte percentage was the highest in EC0.75 treatment group, which was significantly higher ($P < 0.05$) compared to MW0.75 and control groups. Both MW0.75 and EC0.75 treatment groups showed significantly lower ($P < 0.05$) lymphocyte-thrombocyte percentage compared to the control but there was no significant ($P > 0.05$) difference in lymphocyte-thrombocyte percentage between MW0.75 and EC0.75. There was no significant difference ($P > 0.05$) in monocyte percentages between control and MW0.75 treatment group (Table 6a).

After 7 d *A. hydrophila* challenge, control and EC0.75 treatment group showed a significant decrease ($P < 0.05$), while MW0.75 treatment group showed a significant increase ($P < 0.05$) in total WBC count compared to the corresponding treatment group pre-infection (Table 6a). Post bacterial infection, control group showed significant increase ($P < 0.05$), while EC0.75 and MW0.75 groups showed significant decrease ($P < 0.05$) in neutrophil percentage compared to the corresponding treatment group pre-infection. Monocyte percentage in all three groups increased significantly ($P < 0.05$) post bacterial infection compared to the corresponding pre-infection percentage. However, the control group showed a significant decrease ($P < 0.05$), while EC0.75 and MW0.75 groups showed a significant increase ($P < 0.05$) in lymphocyte-thrombocyte percentage after 7 d *A. hydrophila* challenge compared to the corresponding treatment group pre-infection. Post bacterial infection, total WBC count was the highest in MW0.75 treatment group, which was also significantly higher ($P < 0.05$) than EC0.75 and control groups (Table 6a). Neutrophil percentage differed significantly ($P < 0.05$) between the treatment groups where MW0.75 showed the lowest, while control group showed the highest neutrophil percentage. Monocyte percentage was the highest in the control group which was significantly higher ($P < 0.05$) than MW0.75 and EC0.75 treatment groups. Moreover, MW0.75 treatment group showed significantly higher ($P < 0.05$) monocyte percentage compared to EC0.75 treatment group. Interestingly, fish fed plant extract fortified diets (MW0.75 and EC0.75) showed significant increase ($P < 0.05$) in lymphocyte-thrombocyte percentage compared to the control post bacterial infection. The two-way ANOVA revealed that all WBC indices were significantly ($P < 0.05$) affected by dietary plant extract supplementation, bacterial infection and their interaction (Table 6a).

There were no significant differences ($P > 0.05$) in any RBC indices between the treatment groups at the end of 120 d culture in cement cisterns (Table 6b). However, 7 d post *A. hydrophila* infection, mean total RBC count and haematocrit values in all three groups declined significantly ($P < 0.05$) compared to the corresponding pre-infection phase (Table 6b). Haemoglobin level decreased significantly ($P < 0.05$) in control and EC0.75 group post-infection compared to the respective pre-infection phase. After bacterial challenge, MCH value increased significantly ($P < 0.05$) in the control group compared to the pre-infection phase, while there was

Table 6. Mean (± 1 standard error, n = 30) haematological parameters: (a) WBC indices, (b) RBC indices and (c) serum indices at the end of four months culture in cement cisterns (Pre-Infection) and 7 d post *Aeromonas hydrophila* challenge (Post-Infection).

(a) WBC indices.

Treatment category	Total WBC ($10^3.\mu L^{-1}$)	Neutrophil (%)	Monocyte (%)	Lymphocyte and thrombocyte (%)
Pre-Infection				
Control	12.32 ± 0.03^{c}	14.24 ± 0.11^{c}	1.01 ± 0.06^{a}	84.63 ± 0.12^{c}
MW0.75	25.72 ± 0.02^{e}	20.90 ± 0.07^{d}	0.98 ± 0.06^{a}	76.59 ± 0.13^{b}
EC0.75	17.69 ± 0.02^{d}	23.49 ± 0.09^{e}	2.08 ± 0.09^{b}	74.14 ± 0.11^{b}
Post-Infection				
Control-Inj.	6.51 ± 0.01^{a}	60.26 ± 0.11^{f}	9.93 ± 0.12^{e}	30.11 ± 1.61^{a}
MW0.75-Inj.	56.96 ± 0.02^{f}	0.92 ± 0.08^{a}	5.39 ± 0.06^{d}	93.61 ± 0.05^{d}
EC0.75-Inj.	8.06 ± 0.02^{b}	2.60 ± 0.09^{b}	4.63 ± 0.11^{c}	93.01 ± 0.06^{d}
Pooled SE	1.287	1.486	0.244	1.631
Two-way ANOVA				
Dietary treatment	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Bacterial infection	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Dietary treatment × Bacterial infection	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

(b) RBC indices.

Treatment category	Total RBC ($10^6.\mu L^{-1}$)	Ht (%)	Hb ($g.dL^{-1}$)	MCH (pg)	MCV (μm^3)	MCHC ($g.dL^{-1}$)
Pre-Infection						
Control	2.06 ± 0.024^{c}	25.15 ± 0.06^{d}	8.28 ± 0.03^{c}	40.27 ± 0.45^{a}	122.35 ± 1.49^{b}	32.93 ± 0.13^{b}
MW0.75	2.08 ± 0.002^{c}	25.13 ± 0.05^{d}	8.29 ± 0.04^{c}	39.80 ± 0.20^{a}	120.60 ± 0.25^{b}	33.01 ± 0.19^{b}
EC0.75	2.09 ± 0.003^{c}	25.14 ± 0.06^{d}	8.33 ± 0.02^{c}	39.81 ± 0.14^{a}	120.15 ± 0.31^{b}	33.14 ± 0.14^{bc}
Post-Infection						
Control-Inj.	0.89 ± 0.002^{a}	24.87 ± 0.06^{c}	8.00 ± 0.04^{a}	90.35 ± 0.57^{b}	281.04 ± 1.07^{c}	32.15 ± 0.17^{a}
MW0.75-Inj.	2.02 ± 0.003^{b}	23.28 ± 0.06^{a}	8.22 ± 0.05^{bc}	40.75 ± 0.19^{a}	115.45 ± 0.28^{a}	35.30 ± 0.21^{d}
EC0.75-Inj.	1.98 ± 0.006^{b}	24.09 ± 0.03^{b}	8.12 ± 0.03^{ab}	41.13 ± 0.21^{a}	121.99 ± 0.33^{b}	33.72 ± 0.17^{c}
Pooled SE	0.033	0.057	0.018	1.399	4.497	0.086
Two-way ANOVA						
Dietary treatment	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Bacterial infection	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Dietary treatment × Bacterial infection	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

(c) Serum indices.

Treatment category	sGlu ($mg.mL^{-1}$)	sAlb ($mg.mL^{-1}$)
Pre-Infection		
Control	5.42 ± 0.012^{d}	1.14 ± 0.004^{c}
MW0.75	5.15 ± 0.010^{a}	1.24 ± 0.003^{f}
EC0.75	5.36 ± 0.007^{c}	1.19 ± 0.004^{e}
Post-Infection		
Control-Inj.	5.95 ± 0.012^{f}	0.81 ± 0.004^{a}
MW0.75-Inj.	5.19 ± 0.010^{b}	1.16 ± 0.003^{d}
EC0.75-Inj.	5.60 ± 0.003^{e}	1.08 ± 0.003^{b}
Pooled SE	0.020	0.011
Two-way ANOVA		
Dietary treatment	$P < 0.05$	$P < 0.05$
Bacterial infection	$P < 0.05$	$P < 0.05$
Dietary treatment × Bacterial infection	$P < 0.05$	$P < 0.05$

Note: Different superscripts mark significant difference ($P < 0.05$) in means within columns. η^2 = 1.00 (Total WBC count), 0.999 (Neutrophil percentage), 0.973 (Lymphocyte and Thrombocyte percentage), 0.978 (Monocyte percentage), 0.998 (Total RBC count), 0.860 (Ht), 0.235 (Hb), 0.991 (MCH), 0.995 (MCV), 0.536 (MCHC), 0.970 (sGlu), 0.982 (sAlb). Abbreviations: WBC - White blood cells,

Table 6. Continued.

RBC - Red blood cells, Ht - Hematocrit, Hb - Haemoglobin, MCH - Mean corpuscular haemoglobin, MCV - Mean corpuscular volume, MCHC - Mean corpuscular haemoglobin concentration, sGlu - Serum glucose, sAlb - Serum albumin. Control – fish fed basal diet for 120 d; MW0.75 – fish fed diet fortified with *Withania somnifera* root methanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d; EC0.75 – fish fed diet fortified with *Chlorophytum borivilianum* root ethanol extract (0.75 g.kg^{-1} feed) for initial 30 d followed by basal diet for next 90 d. Control-Inj. – control fish (fed basal diet for 120 d) injected intraperitoneally with 24 h cultured pathogenic *A. hydrophila* (single sub-lethal dose, 5 × 10^5 CFU in 0.1 mL saline) at the end of 120 d culture; MW0.75-Inj. – fish fed *W. somnifera* root methanol extract fortified diet (0.75 g.kg^{-1} feed, initial 30 d) followed by basal diet (next 90 d) injected intraperitoneally with 24 h cultured pathogenic *A. hydrophila* (single sub-lethal dose, 5 × 10^5 CFU in 0.1 mL saline) at the end of 120 d culture; EC0.75-Inj. – fish fed *C. borivilianum* root ethanol extract fortified diet (0.75 g.kg^{-1} feed, initial 30 d) followed by basal diet (next 90 d) injected intraperitoneally with 24 h cultured pathogenic *A. hydrophila* (single sub-lethal dose, 5 × 10^5 CFU in 0.1 mL saline) at the end of 120 d culture.

no significant difference ($P > 0.05$) between pre- and post-infection MCH values in the MW0.75 and EC0.75 treatment groups. MCV value increased significantly ($P < 0.05$) in control, decreased significantly ($P< 0.05$) in MW0.75, and showed no significant difference ($P > 0.05$) in EC0.75 treatment group post bacterial infection compared to the respective pre-infection phase. Moreover, 7 d post *A. hydrophila* infection, MCHC value decreased significantly ($P < 0.05$) in control, but increased significantly ($P < 0.05$) in MW0.75 treatment group compared to the respective pre-infection phase. MW0.75 treatment group showed the highest, while control group showed the lowest total RBC count, haemoglobin level and MCHC value seven days post bacterial infection. After *A. hydrophila* challenge, control group showed significantly higher ($P < 0.05$) haematocrit value, MCH value and MCV value, but significantly lower ($P < 0.05$) MCHC value compared to MW0.75 and EC0.75 treatment groups. The two-way ANOVA revealed that all RBC indices were also significantly ($P < 0.05$) affected by dietary plant extract supplementation, bacterial infection and their interaction (Table 6b).

After 120 d culture in the cement cisterns, both MW0.75 and EC0.75 treatment groups showed significantly lower ($P < 0.05$) serum glucose level, but significantly higher ($P < 0.05$) serum albumin level compared to the control (Table 6c). Fish in treatment group MW0.75 showed the lowest serum glucose level, and the highest serum albumin level, which were significantly different ($P < 0.05$) compared to those in EC0.75 treatment group as well (Table 6c). Serum glucose level increased significantly ($P < 0.05$), while serum albumin level decreased significantly ($P < 0.05$) in all groups post bacterial infection compared to the respective pre-infection phase. Seven days post bacterial challenge, serum glucose level was the highest in the control group, and the lowest in the MW0.75 treatment group (Table 6c). Serum glucose level in EC0.75 treatment group was significantly ($P < 0.05$) higher than MW0.75, but significantly lower ($P < 0.05$) than control group. On the other hand, serum albumin level was the highest in the MW0.75 treatment group, and the lowest in the control group. Serum albumin level in EC0.75 treatment group was significantly lower ($P < 0.05$) than MW0.75, but significantly higher ($P < 0.05$) than control group. The two-way ANOVA revealed that both serum glucose and albumin levels were significantly ($P < 0.05$) affected by dietary plant extract supplementation, bacterial infection and their interaction (Table 6c).

Biochemical parameters

At the end of 120 d of culture in cement cisterns, both MW0.75 and EC0.75 treatment groups showed significantly lower ($P < 0.05$) MDA levels compared to the control, MW0.75 showing the lowest value (Table 7a). Treatment group MW0.75 showed the highest GSH level, which was significantly higher ($P < 0.05$) compared to control and EC0.75 treatment groups. Besides, GSH level in EC0.75 treatment group was significantly higher ($P < 0.05$) compared to control. Both MW0.75 and EC0.75 showed significantly higher ($P < 0.05$) GST and GRD levels compared to control. EC0.75 showed the highest values for both parameters, which were significantly higher ($P < 0.05$) than MW0.75 (Table 7a).

Seven days post *A. hydrophila* challenge, MDA level increased in all three groups, albeit not significantly ($P > 0.05$) in MW0.75 compared to the respective pre-infection phase. Control group showed the highest MDA level, significantly higher ($P < 0.05$) than MW0.75 and EC0.75 treatment groups (Table 7a). On the other hand, MW0.75 treated group showed the lowest MDA level, which was significantly lower ($P < 0.05$) than EC0.75 and control. GSH levels in all three groups post-infection were significantly lower ($P < 0.05$) compared to the respective pre-infection phase. After 7 d bacterial infection, control group showed the lowest, while MW0.75 showed the highest GSH level. There was significant difference in GSH level between control, MW0.75 and EC0.75 treatment groups post *A. hydrophila* infection. GST levels in all three groups post bacterial infection increased significantly ($P < 0.05$) compared to the respective pre-infection phase. However, there was no significant difference ($P > 0.05$) in GRD levels of different treatment groups between pre- and post-infection phase. After 7 d *A. hydrophila* challenge, control showed the lowest, while EC0.75 showed the highest GST and GRD levels. The two-way ANOVA revealed that MDA, GSH, GST and GRD levels

Table 7: Mean (± 1 standard error, n = 30) biochemical parameters: (a) lipid peroxidation biomarker, non-enzymatic and enzymatic antioxidants, (b) serum levels of different liver enzymes at the end of four months culture in cement cisterns (Pre-Infection) and 7 d post *Aeromonas hydrophila* challenge (Post-Infection).

(a) Lipid peroxidation marker, non-enzymatic and enzymatic antioxidants.

Treatment category	MDA ($nmol.mL^{-1}$)	GSH ($nmol.mL^{-1}$)	GST ($ng.mL^{-1}$)	GRD ($U.L^{-1}$)
Pre-Infection				
Control	14.40 ± 0.03^{c}	20.60 ± 0.004^{b}	1.85 ± 0.002^{a}	253.53 ± 0.18^{a}
MW0.75	11.99 ± 0.05^{a}	26.62 ± 0.002^{f}	2.40 ± 0.004^{c}	272.64 ± 0.12^{b}
EC0.75	12.11 ± 0.05^{a}	25.72 ± 0.003^{e}	2.44 ± 0.003^{e}	272.92 ± 0.12^{bc}
Post-Infection				
Control-Inj.	15.14 ± 0.04^{d}	16.53 ± 0.002^{a}	1.87 ± 0.001^{b}	253.64 ± 0.02^{a}
MW0.75-Inj.	12.14 ± 0.01^{a}	21.91 ± 0.002^{d}	2.42 ± 0.002^{d}	273.12 ± 0.14^{bc}
EC0.75-Inj.	12.55 ± 0.03^{b}	21.74 ± 0.002^{c}	2.46 ± 0.002^{f}	273.40 ± 0.14^{c}
Pooled SE	0.094	0.249	0.020	0.687
Two-way ANOVA				
Dietary treatment	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Bacterial infection	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Dietary treatment × Bacterial infection	$P < 0.05$	$P < 0.05$	$P > 0.05$	$P < 0.05$

(b) Lipid peroxidation marker, non-enzymatic and enzymatic antioxidants.

Treatment category	ALP ($ng.L^{-1}$)	GOT ($ng.\ mL^{-1}$)	GPT ($U.L^{-1}$)	ACP ($ng.mL^{-1}$)
Pre-Infection				
Control	0.33 ± 0.002^{c}	22.99 ± 0.12^{c}	187.07 ± 0.05^{c}	6.75 ± 0.006^{a}
MW0.75	0.23 ± 0.003^{a}	15.49 ± 0.08^{a}	172.80 ± 0.08^{a}	8.35 ± 0.004^{e}
EC0.75	0.30 ± 0.003^{b}	18.78 ± 0.06^{b}	177.34 ± 0.12^{b}	8.17 ± 0.004^{c}
Post-Infection				
Control-Inj.	0.65 ± 0.002^{e}	31.86 ± 0.07^{e}	192.94 ± 0.05^{d}	9.75 ± 0.004^{f}
MW0.75-Inj.	0.23 ± 0.001^{a}	15.74 ± 0.05^{a}	172.88 ± 0.08^{a}	7.59 ± 0.024^{b}
EC0.75-Inj.	0.41 ± 0.002^{d}	28.87 ± 0.04^{d}	177.53 ± 0.08^{b}	8.29 ± 0.004^{d}
Pooled SE	0.011	0.471	0.558	0.068
Two-way ANOVA				
Dietary treatment	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Bacterial infection	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Dietary treatment × Bacterial infection	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

Note: Different superscripts mark significant difference ($P < 0.05$) in means within columns. η^2 = 0.974 (MDA), 1.00 (GSH), 0.998 (GST), 0.994 (GRD), 0.994 (ALP), 0.996 (GOT), 0.997 (GPT), 0.996 (ACP). Abbreviations: MDA - Malonaldehyde, GSH - Glutathione, GST - Glutathione S - transferase, GRD - Glutathione S - reductase, ALP - Alkaline phosphatase, GOT - Glutamic oxaloacetic transferase, GPT - Glutamic pyruvic transaminase, ACP - Acid phosphatase. Control – fish fed basal diet for 120 d; MW0.75 – fish fed diet fortified with *Withania somnifera* root methanol extract (0.75 $g.kg^{-1}$ feed) for initial 30 d followed by basal diet for next 90 d; EC0.75 – fish fed diet fortified with *Chlorophytum borivilianum* root ethanol extract (0.75 $g.kg^{-1}$ feed) for initial 30 d followed by basal diet for next 90 d. Control-Inj. – control fish (fed basal diet for 120 d) injected intraperitoneally with 24 h cultured pathogenic *A. hydrophila* (single sub-lethal dose, 5×10^5 CFU in 0.1 mL saline) at the end of 120 d culture; MW0.75-Inj. – fish fed *W. somnifera* root methanol extract fortified diet (0.75 $g.kg^{-1}$ feed, initial 30 d) followed by basal diet (next 90 d) injected intraperitoneally with 24 h cultured pathogenic *A. hydrophila* (single sub-lethal dose, 5×10^5 CFU in 0.1 mL saline) at the end of 120 d culture; EC0.75-Inj. – fish fed *C. borivilianum* root ethanol extract fortified diet (0.75 $g.kg^{-1}$ feed, initial 30 d) followed by basal diet (next 90 d) injected intraperitoneally with 24 h cultured pathogenic *A. hydrophila* (single sub-lethal dose, 5×10^5 CFU in 0.1 mL saline) at the end of 120 d culture.

were significantly ($P < 0.05$) affected by dietary plant extract supplementation, bacterial infection and their interaction (Table 7a).

At the end of 120 d culture in cement cisterns, both MW0.75 and EC0.75 showed significantly lower ($P < 0.05$) serum levels of hepatic enzymes GOT, GPT and ALP compared to control (Table 7b). However, serum ACP level in MW0.75 and EC0.75 was significantly higher ($P < 0.05$) compared to the control. Treatment

group MW0.75 showed the lowest serum GOT, GPT, ALP levels, and the highest ACP level, which were significantly different ($P < 0.05$) from the control and EC0.75. On the other hand, fish in the control group showed the highest serum GOT, GPT, ALP levels, and the lowest ACP level, which were significantly different ($P < 0.05$) from MW0.75 and EC0.75 (Table 7b).

After 7 d *A. hydrophila* challenge, control and EC0.75 showed significant increase ($P < 0.05$) in serum ALP, GOT and ACP levels compared to the respective pre-infection phase (Table 7b). GPT level in the control fish post bacterial infection was also significantly higher ($P < 0.05$) than that in pre-infection phase. Post *A. hydrophila* challenge, control fish showed the highest serum levels of all liver enzymes, and those were significantly higher ($P < 0.05$) than MW0.75 and EC0.75 treatment groups. On the other hand, fish in MW0.75 treatment group post bacterial infection showed the lowest serum levels of all liver enzymes, and those were significantly lower ($P < 0.05$) than EC0.75 treatment group as well. The two-way ANOVA revealed that serum levels of all liver enzymes were significantly ($P < 0.05$) affected by dietary plant extract supplementation, bacterial infection and their interaction (Table 7b).

Discussion

The present study aimed to evaluate whether dietary administration of *C. borivilianum* root ethanol extract and *W. somnifera* root methanol extract might provide growth enhancement, immuno-stimulation and protection against *A. hydrophila* infection in Nile tilapia. In India, monosex Nile tilapia is often harvested irregularly from low input culture systems once the fish reach marketable size (~100 g) for local consumers. A culture period of 4-6 months has been indicated with GIFT tilapia by the Central Institute of Freshwater Aquaculture (CIFA), India (NFDB, 2015). Thus, a 120-d grow-out culture was considered in the present study. However, our earlier study has demonstrated prominent masculinising effects of *C. borivilianum* root ethanol extract and *W. somnifera* root methanol extract in Nile tilapia after 30 d of dietary administration (De et al., 2020). Besides, treatment with plant extracts for 30 d is preferred for producing monosex all-male Nile tilapia population in most of the related literature (Mukherjee et al., 2018). Moreover, prolonged exposure to phytochemicals may pose the risk of accumulation and toxicity in fish (Alafiatayo et al., 2019). Considering these aspects, fish were fed diets fortified with the plant extracts only for the initial 30 d and a control diet for the subsequent 90 d of culture, while the immunity status of the fish was deduced at the time of final harvest after 120 d through measurements of immunological, haematological and biochemical parameters. Thus, the use of the plant extracts as potential replacements for synthetic antimicrobial chemicals during Nile tilapia culture might be validated with insight into the comparative efficacy of the two extracts.

The predominant secondary plant metabolites such as phenolics and flavonoids have been reported to act as antioxidants exerting antidiabetic, anti-inflammatory, antimicrobial and antiviral effects (Vodnar et al., 2017). The antiradical activity is directly proportional to the amount of phenols and flavonoids present in the plant extract (Carvalho et al., 2017). The present results also highlighted such observation as both the plant extracts had either high phenol or flavonoid content and showed high antiradical activity.

In an earlier experiment, the optimum temperature range for Nile tilapia survival was reported to be 24–30 °C, and the best growth and FCR in the fish was obtained at 28 °C (El - Sayed and Kawanna, 2008). In another study, the suitable range of water pH for rearing Nile tilapia was suggested as 5.5-9.0 (Rebouças et al., 2016). The recommended dissolved oxygen level for optimum growth of Nile tilapia was above 5 ppm (Makori et al., 2017), while free CO_2 in water below 20 ppm was advised for the fish (Danley et al., 2005). The optimum NH_3 concentration for Nile tilapia has been reported as less than 0.05 ppm by El-Sherif and El-Feky (2008). The safe concentration of nitrite in water in tilapia culture has been reported as 0.3 ppm (Rebouças et al., 2016). In the present grow-out culture experiment, water physico-chemical parameters in all treatment cisterns were within the optimal range for Nile tilapia culture without any significant difference ($P > 0.05$) between the groups. Thus, the notable variation in growth between fish fed control and plant extract-fortified diets might be due to the anabolic effects of the plant extracts, not because of any variation in the water parameters.

Many plant extracts have earlier been reported to act as appetisers and to enhance weight gain in cultured fish (Pavaraj et al., 2011; Takaoka et al., 2011; Chakraborty et al., 2014). Solvent extracts of four medicinal plants such as *Basella alba* leaves, *Tribulus terrestris* seeds, *Mucuna pruriens* seeds and *Asparagus racemosus* roots were found to show growth-inducing properties in Nile tilapia (Ghosal et al., 2021). *W. somnifera* extract has previously been reported to improve enzymatic activities in the tricarboxylic acid cycle and fatty acid metabolism (Mahdi et al., 2011; Kyathanahalli et al., 2014). It was also observed that near-normal body weight was maintained in *C. borivilianum* root extract-treated diabetic rats which might be attributed to the preservation of appetite or the increased availability of insulin exerting anabolic effects (Giribabu et al., 2014). Better growth in fish fed MW0.75 and EC0.75 diets in the present study might be attributed to the improved metabolic activity in those treatment groups.

Males of Nile tilapia grow faster due to sex-specific adaptive behaviour contributing to higher production in monosex all - male culture (Lind et al., 2015). Besides, several medicinal plants have also been reported to exert anabolic effects in fish (Putra et al., 2013). In the present experiment, enhanced growth of

the fish fed plant extract - fortified diets might be due to the higher proportion of males in those two groups as well as the anabolic effect of the extracts.

In the present experiment, a significant correlation was found between the final weight gain and food conversion ratio both after 30 d and 120 d culture period (Pearson correlation coefficient: after 30 d = 0.810, $P < 0.05$; after 120 d = 0.974, $P < 0.05$). After 30 d, fish fed plant extract - fortified diets showed significantly lower FCR compared to the fish fed control diet. Plant extracts have been reported to improve digestibility and availability of nutrients resulting in improved feed conversion and higher rate of protein synthesis (Putra et al., 2013; Talpur et al., 2013) and the present study is in accordance with the natural phenomenon in this regard. On the other hand, fish in the control group showed lower FCR after 120 d of grow - out culture compared to the plant extract - fed fish groups. Many earlier studies have indicated the effects of feeding rate on feed conversion ratio during fish culture (Barani et al., 2019; Heriansah et al., 2022). In the present study, however, fish in the control, MW0.75 and EC0.75 groups were fed at uniform rate without any adjustment based on the difference in their body weight during the culture period. Such optimisation of the feeding rate with the plant extract fortified diets during large - scale aquaculture practices will be warranted to decrease feed loss and improve FCR value.

The length-weight relationship is an index helpful to understanding growth profile, general fitness, and morphological differences between species and within the same species across different geographical regions (Santos et al., 2002). It was reported that the value of b may change during different periods illustrating the fullness of the stomach, general condition of appetite and gonads stages (Zaher et al., 2015). In an experiment with *Channa punctatus* higher slope (b) reflected faster growth (Datta et al., 2013). In the present study, all three experimental groups showed negative allometry ($b < 3.0$), but fish fed with MW0.75 and EC0.75 fortified diets showed higher 'b' value compared to the control indicating that the plant extract fortified diets might have propelled the growth of the fish towards isometry ($b = 3$), which in turn suggest ideal growth conditions (Jisr et al., 2018).

The innate immunity of fish is more efficient compared to that of mammals (Uribe et al., 2011). In fish culture, it was reported that the use of plant-based immuno-stimulants might enhance phagocytic activity, lymphocyte count, serum Ig and lysozyme (Chakraborty and Hancz, 2011). Rainbow trout (*Oncorhynchus mykiss*) fed a diet mixed with an aqueous extract of ginger (*Zingiber officinale*) showed significantly higher phagocytic and respiratory burst activity than the control group (Dügenci et al., 2003). Dietary treatment with 0.2 % and 0.4 % *Chlorophytum borivilianum* polysaccharide for four weeks upregulated phagocytic activity, respiratory burst, serum lysozyme activity, downregulated anti-inflammatory cytokines IL-10 and TGF-β, and showed resistance against *Aeromonas hydrophila* infection in *Labeo rohita* (Giri et al., 2015). In another experiment, dietary supplementation with combined plant extracts of *Solanum ferox* and *Zingiber zerumbet* resulted in a significant increase in phagocytic activity, respiratory burst, lysozyme activity and survival rate of Nile tilapia in the face of *A. hydrophila* infection (Hardi et al., 2019). Plant extracts such as *Basella alba* leaf ethanol extract and *Asparagus racemosus* root methanol extract have earlier been reported to induce immunity and promote growth in Nile tilapia (Ghosal et al., 2021). Immuno-stimulation has often been correlated with enhanced growth performance in aquaculture by several authors (Kord et al., 2021).

In our present experiment, immunological parameters after 120 d of Nile tilapia culture indicated that individual dietary administration of MW0.75 and EC0.75 induced immuno-stimulation, with MW0.75 treatment being more effective than EC0.75. The higher immuno-stimulating efficacy of methanol extract of *Withania somnifera* root may be a factor contributing to its better growth-promoting effect. Seven days post *A. hydrophila* challenge, however, immunity in Nile tilapia fed plant extract-fortified diets responded differently than in the control group. Such differential response pattern of immune parameters might indicate a more controlled inflammatory response and more efficient primary antibody response in plant extract-fed groups compared to control. WBC indices in Nile tilapia after 120 d of grow-out culture in cement cisterns suggested that dietary fortification with both the plant extracts might have stimulated innate immunity by increasing the percentage of cells related to nonspecific immune responses such as neutrophils and monocytes, simultaneously increasing the total count of WBCs. This may be advantageous in the prophylactic priming of the immune system of the animal, inducing a pro-inflammatory response (Kuttan, 2000; Park, 2020). Though the EC0.75 treatment group showed a significantly higher ($P < 0.05$) percentage of neutrophils and monocytes than the MW0.75 treatment group, the total WBC count was significantly lower ($P < 0.05$), rendering EC0.75 treatment less effective in immuno-stimulation than MW0.75 treatment in Nile tilapia. This observation may be explained by the fact that immuno-stimulation does not necessarily guarantee improved performance of the immune system. Chronic immuno-stimulation may result in immune exhaustion causing a loss of function of effector cells and their proliferative potential (Presti and Pantaleo, 2017), in turn leading to prolonged inflammation and organ damage (Verbsky and Routes, 2014).

White blood cell indices in Nile tilapia 7 d post *A. hydrophila* challenge indicated ineffective innate immune response in the control group which failed to fight the infection. In contrast, a significant increase in

total WBC count and lymphocyte percentage coupled with sharply reducing neutrophil percentage in the MW0.75 group post-infection compared to the pre-infection phase, suggested well-regulated inflammatory response and a probable clonal expansion of the lymphocytes. Decreased total WBC count and neutrophil percentage, and increased monocyte and lymphocyte percentage in EC0.75 post-infection compared to the pre-infection phase pointed to the onset of disease after bacterial challenge alongside an induced lymphocyte expansion.

After 120 d of culture, there was no significant change in the RBC indices in Nile tilapia across the categories indicating no adverse effect of plant extract supplementation in the present study. It has been reported that an increased total WBC count alongside essentially unchanged hematocrit indicates immuno-stimulation (Barman et al., 2013). Thus, the immuno-stimulatory effects of the dietary plant extract supplementation may be inferred from this result.

Moreover, significantly lower RBC count in control Nile tilapia compared to the plant extract-fed groups 7 d post *A. hydrophila* challenge suggested that the plant extracts might have protected the fish against the haemolytic activity of the bacteria. An increase in WBC count has previously been reported to cause a decrease in haematocrit (Eni - Yimini et al., 2015), which is also observed in the present study in the MW0.75 group 7 d post bacterial challenge. The decrease in haemoglobin levels in all the experimental groups after bacterial challenge might have been caused by the infection-induced hemolysis and the effect was more prominent in the control group. The increase in MCH value in the control Nile tilapia after bacterial challenge reflected an anaemic tendency but it was more likely to be caused by reduced food intake due to infection. Besides, an increase in MCV values after infection indicated an anaemic condition due to reduced food intake and/or hepatic disorder (Aslinia et al., 2006). Again, a decrease in the MCHC value in the control Nile tilapia after *A. hydrophila* infection in the present experiment indicated a reduced level of haemoglobin in bacteria-infected fish.

There has been a wide range of diverse effects that herbal immunostimulants exert on the RBC indices in fish. *Lates calcarifer* fed diet mixed with *Mentha piperita* showed a significant increase in RBC count, haemoglobin and WBC count compared to controls; but after challenge with *Vibrio harveyi*, both RBC count and haemoglobin decreased, though WBC count increased (Talpur, 2014). Dietary treatment with an aqueous extract of neem leaves produced lower RBC count, haemoglobin and PCV in catfish while increasing the number of circulating lymphocytes (Oniovosa et al., 2017). Dietary treatment with *Epibolium hirsutum* fortified feed had no significant effect on RBCs, haemoglobin and hematocrit levels in *Cyprinus carpio* fingerlings, both in infected and uninfected phases with *Aeromonas hydrophila*, though it increased WBC count (Pakravan et al., 2012). *Oncorhynchus mykiss* fed with a diet containing rhizome powder of *Zingiber officinale* showed a significant increase in phagocytic activity as well as in RBC count and hematocrit value compared to control (Haghighi and Rohani, 2013).

After 120 d of culture in cement cisterns, an increase in serum albumin level of Nile tilapia fed plant extract fortified diets compared to those fed control diet might have been due to physiological response since serum albumin works as a sink for the phytochemicals as well as for free fatty acids (Baker, 1998; Stillwell, 2016). More aggressive feeding in Nile tilapia of MW0.75 and EC0.75 treatment groups may also be a causative factor since high protein uptake has often been associated with increased serum albumin levels (Mutlu et al., 2006). On the other hand, hypoalbuminemia may be caused by hepatic and inflammatory disorders (Pupim et al., 2013) which corroborates with the results of the post-infection phase in this study.

Nile tilapia fed plant extract-supplemented diets showed better antioxidant properties compared to the fish fed the control diet. In an earlier study, it was said that a decrease in lipid peroxidation and an increase in levels of non-enzymatic and enzymatic antioxidants in fish fed plant extract - fortified diets depicted the ability of the plant bioactive phytoconstituents to scavenge free radicals and maintain normal cellular functioning (Yadav et al., 2015). Though MDA levels increased after bacterial infection in all the experimental groups, significantly lower MDA levels in Nile tilapia from MW0.75 and EC0.75 groups compared to the control group substantiated the antioxidant efficacy of the plant extracts. Moreover, an increase in GST level after bacterial challenge indicated augmentation of the detoxification process, while elevation in GRD level indicated its enhanced role in maintaining the GSH: GSSG ratio against bacteria-induced physiological stress. Significantly higher ($P < 0.05$) GST and GRD levels in MW0.75 and EC0.75 groups of Nile tilapia post *A. hydrophila* challenge, compared to those in the control group, validated the efficacy of the plant extracts to stimulate the activity of those antioxidant enzymes in the fish. A similar pattern of MDA and GST levels was observed in *Oncorhynchus mykiss* fed diets fortified with mint, sage and thyme (Sönmez et al., 2015).

Chlorophytum borivilianum root ethanol extract and *W. somnifera* root methanol extract seemed to have hepatoprotective effects as Nile tilapia in EC0.75 and MW0.75 treatment groups showed significantly lower ($P < 0.05$) levels of hepatic enzymes in serum compared to the control group at the end of 120 d culture period. Antioxidant phytochemicals in the plant extracts may scavenge reactive oxygen species and maintain the structural integrity of hepatocellular membrane and liver cell design to render such hepatoprotective effects at the cellular level (Monir et al., 2020). Withanolide-rich fraction of methanolic extract of *W.*

somnifera roots showed a hepatoprotective effect by significantly decreasing the elevated serum levels of bilirubin, AST, ALT and ALP due to hepatotoxicity induced in rats by overdose of acetaminophen (Devkar et al., 2016). The hepatoprotective efficacy of the root extract of *C. borivilianum* was also reported in a case of arsenic-induced hepatotoxicity (Sharma and Kumar, 2011).

On the other hand, acute or chronic injury to the liver may increase serum levels of aminotransferases such as GOT and GPT, with mild abnormalities in ALP (Giannini et al., 2005). Intraperitoneal injection of a known hepatotoxicant tetracycline was earlier observed to elevate serum levels of AST (GOT), ALT (GPT) and MDA in male SD rats (Deng et al., 2015). Rats treated with paracetamol showed significantly elevated levels of ALT and AST (Hamid et al., 2018). In another study, it was found that the administration of cyclosporine A increased levels of AST, ALT and bilirubin in male Wistar rats indicating liver damage (Korolczuk et al., 2016). In the present experiment, the increased levels of serum ALP, GOT and GPT in Nile tilapia after bacterial challenge compared to the pre-infection phase indicated the hepatotoxic effect of *A. hydrophila*. A similar hepatotoxic effect of *Aeromonas*, which elevated ALP, GOT and GPT levels in infected Nile tilapia, was reported in an earlier study (Neamat - Allah et al., 2021). ACP, an intracellular lysosomal enzyme and indicator of the ability of macrophages in the intracellular digestion of phagocytised pathogens (Attwood et al., 1996), shows increased serum levels in necrotic changes induced by chemicals or pathogens (Gill et al., 1990; Tilak and Mary, 2009). In the present study, increased serum ACP levels in EC0.75 and MW0.75 treatment groups after 120 d are indicative of immunoreactive effects of the treatment without adversely affecting the health of the fish, while that in the 7 d post *A. hydrophila* challenge appears to indicate potentiated immune response against bacterial infection.

Several fatty acids present in *W. somnifera* root methanol extract and *C. borivilianum* root ethanol extract (De et al., 2020) might contribute to the growth-promoting, immunostimulating and anti-microbial activities of the plant extracts observed in the present study. Palmitic acid present in *W. somnifera* root methanol extract, and stearic acid present in both *W. somnifera* root methanol extract and *C. borivilianum* root ethanol extract display antibacterial activity towards gram-negative bacteria (Casillas - Vargas et al., 2021). Lauric acid found in both *W. somnifera* root methanol extract and *C. borivilianum* root ethanol extract works as an agonist of free fatty acid receptor 1 (GPR40) improving glucose metabolism (Itoh et al., 2003; Anuar et al., 2023). It has been found to alleviate lipid peroxidation, show antioxidant and antimicrobial properties and protect the liver against ethanol-induced hepatotoxicity (Nitbani et al., 2022; Namachivayam and Gopalakrishnan, 2023). Moreover, its agonistic activity on the orphan receptor GPR84 enhances LPS - stimulated IL12 production in monocytes / macrophages, thereby affecting Th1 / Th2 balance to promote cell-mediated innate immunity and pathogen elimination (Wang et al., 2006). The linoleic acid present in both the plant extracts acts as an agonist for GPR40 and peroxisome proliferator-activated receptor - PPARα regulating glucose metabolism and beta oxidation of fatty acid, respectively (van Raalte et al., 2004). Alpha tocopherol found in *W. somnifera* root methanol extract, but not in *C. borivilianum* root ethanol extract (De et al., 2020), shows free radical scavenging activity (Yamauchi, 1997). Sucrose found in both plant extracts shows antihemolytic activity (Alghareeb et al., 2023). Besides, phenolic constituents and unsaturated fatty acids in the plant extracts are also reported to show antihemolytic activity at different doses and in different pathways (Bouhlali et al., 2015; Chauhan et al., 2018).

Previously, it was postulated that the bioactivity of plant extracts may result from synergistic effects of different phytocomponents (Junio et al., 2011). The two plant extracts in the present study, having different phytoconstituent milieus, could rationally end up showing variable degrees of growth-promoting and immunomodulating efficacy in Nile tilapia. The methanol extract of *W. somnifera* root has been observed to possess a greater variety and concentration of bioactive phytoconstituents having anabolic, immunomodulatory, hepatoprotective and antiradical properties than the ethanol extract of *C. borivilianum* root (De et al., 2020). This might be the reason for better growth, immunity and resistance against *A. hydrophila* infection in fish fed MW0.75 than fish fed EC0.75.

Conclusion

The better overall health of Nile tilapia fed plant extract-fortified diets in the current study helped improve the production during culture in cement cisterns. The bioactive phytocomponents present in the plant extracts are probably responsible for such enhanced production. Despite *C. borivilianum* root ethanol extract being more androgenic than the *W. somnifera* root methanol extract, the latter is a better candidate for Nile tilapia production because of its greater anabolic, antioxidant, antihemolytic and immunostimulatory effects. The findings of this study show that these plant extracts are promising, safer alternatives to the synthetic growth-promoting and antimicrobial chemicals used in Nile tilapia culture.

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References

Abdel-Razek, N., Awad, S.M., Abdel-Tawwab, M. 2019. Effect of dietary purslane (*Portulaca oleracea* L.) leaves powder on growth, immunostimulation, and protection of Nile tilapia, *Oreochromis niloticus* against *Aeromonas hydrophila* infection. Fish Physiology and Biochemistry 45:1907–1917. https://doi.org/10.1007/s10695-019-00685-8

Adikesavalu, H., Banerjee, S., Patra, A., Abraham, T. J. 2017. Meningoencephalitis in farmed monosex Nile tilapia (*Oreochromis niloticus* L.) caused by *Streptococcus agalactiae*. Archives of Polish Fisheries 25:187–200. https://doi.org/10.1515/aopf-2017-0018

Alafiatayo, A.A., Lai, K-S., Syahida, A., Mahmood, M., Shaharuddin, N.A. 2019. Phytochemical evaluation, embryotoxicity, and teratogenic effects of *Curcuma longa* extract on zebrafish (*Danio rerio*). Evidence-Based Complementary and Alternative Medicine 2019:3807207. https://doi.org/10.1155/2019/3807207

Alghareeb, S.A., Alfhili, M.A., Alsughayyir, J. 2023. Rosmarinic acid elicits calcium-dependent and sucrose-sensitive eryptosis and hemolysis through p38 MAPK, CK1α, and PKC. Molecules 28:8053. https://doi.org/10.3390/molecules28248053

Anuar, N.S., Shafie, S.A., Maznan, M.A.F., Zin, N.S.N.M., Azmi, N.A.S., Raoof, R.A., Myrzakozha, D., Samsulriza, N. 2023. Lauric acid improves hormonal profiles, antioxidant properties, sperm quality and histomorphometric changes in testis and epididymis of streptozotocin-induced diabetic infertility rats. Toxicology and Applied Pharmacology 470:116558. https://doi.org/10.1016/j.taap.2023.116558

Ardó, L., Yin, G., Xu, P., Váradi, L., Szigeti, G., Jeney, Z., Jeney, G. 2008. Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. Aquaculture 275:26–33. https://doi.org/10.1016/j.aquaculture.2007.12.022

Aslinia, F., Mazza, J.J., Yale, S.H. 2006. Megaloblastic anemia and other causes of macrocytosis. Clinical Medicine & Research 4:236–241. https://doi.org/10.3121/cmr.4.3.236

Attwood, E.M., Weich, D.J., Oosthuizen, J.M., 1996. The influence of carbon particles on the concentration of acid phosphatase and lysozyme enzymes within alveolar macrophages during the killing and degradation of *Mycobacterium bovis*. Tubercle and Lung Disease 77:341–347. https://doi.org/10.1016/s0962-8479(96)90099-4

Baker, M.E. 1998. Albumin's role in steroid hormone action and the origins of vertebrates: is albumin an essential protein? FEBS Letters 439:9–12. https://doi.org/10.1016/s0014-5793(98)01346-5

Barani, H.K., Dahmardeh, H., Miri, M., Rigi, M. 2019. The effects of feeding rates on growth performance, feed conversion efficiency and body composition of juvenile snow trout, *Schizothorax zarudnyi*. Iranian Journal of Fisheries Sciences 18:507–516. https://doi.org/10.22092/ijfs.2019.118285

Barman, D., Nen, P., Mandal, S.C., Kumar, V. 2013. Immunostimulants for aquaculture health management. Journal of Marine Science: Research & Development 3:134. https://doi.org/10.4172/2155-9910.1000134

Basri, L., Nor, R.M., Salleh, A., Yasin, I.S.M., Saad, M.Z., Rahaman, N.Y.A., Barkham, T., Amal, M.N.A. 2020. Co-Infections of tilapia lake virus, *Aeromonas hydrophila* and *Streptococcus agalactiae* in farmed red hybrid tilapia. Animals 10:2141. https://doi.org/10.3390/ani10112141

Beyer, J.E., 1987. On length-weight relationship. Computing the mean weight of the fish of a given length class. Fishbyte 5(1):11–13.

Bouhlali, E.T., Alem, C., Benlyas, M., Zegzouti, F. 2015. Antioxidant and anti-hemolytic activities of phenolic constituents of six Moroccan date fruit (*Phoenix dactylifera* L.) syrups. Journal of Global Innovations in Agricultural and Social Sciences 3:63–67. https://doi.org/10.17957/JGIASS/3.2-3.709

Canada-Canada, F., Munoz de la Pena, A., Espinosa-Mansilla, A. 2009. Analysis of antibiotics in fish sample. Analytical and Bioanalytical Chemistry 395:987–1008. https://doi.org/10.1007/s00216-009-2872-z

Carvalho, A.R., Costa, G., Figueirinha, A., Liberal, J., Prior, J.A., Lopes, M.C., Cruz, M.T., Batista, M.T. 2017. Urtica spp.: Phenolic composition, safety, antioxidant and anti - inflammatory activities. Food Research International 99:485–494. https://doi.org/10.1016/j.foodres.2017.06.008

Casillas-Vargas, G., Ocasio-Malavé, C., Medina, S., Morales-Guzmán, C., Del Valle, R.G., Carballeira, N.M., Sanabria-Ríos, D.J. 2021. Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next - generation of antibacterial agents. Progress in Lipid Research 82:101093. https://doi.org/10.1016/j.plipres.2021.101093

Chakraborty, S.B., Hancz, C. 2011. Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. Reviews in Aquaculture 3:103–119. https://doi.org/10.1111/j.1753-5131.2011.01048.x

Chakraborty, S.B., Horn, P., Hancz, C. 2014. Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. Reviews in Aquaculture 6:1–19. https://doi.org/10.1111/raq.12021

Chauhan, J.B., Kumar, A., Kapfo, W. 2018. Antioxidant and Antihemolytic Activity of *Averrhoa bilimbi* Extract. Innovare Journal of Life Science 6:5–7.

Danley, M.L., Kenney, P.B., Mazik, P.M., Kiser, R., Hankins, J.A. 2005. Effects of carbon dioxide exposure on intensively cultured rainbow trout *Oncorhyncus mykiss*: physiological responses and fillet attributes. Journal of the World Aquaculture Society 36:249–261. https://doi.org/10.1111/j.1749-7345.2005.tb00329.x

Datta, S.N., Kaur, V.I., Dhawan, A., Jassal, G. 2013. Estimation of length-weight relationship and condition factor of spotted snakehead *Channa punctata* (Bloch) under different feeding regimes. SpringerPlus 2:436. https://doi.org/10.1186/2193-1801-2-436

De, M., Ghosal, I., Mukherjee, D., Chakraborty, S.B. 2020. Identification of chemical constituents responsible for potential androgenic efficacy of *Withania somnifera* and *Chlorophytum borivilianum* root solvent extracts for production of monosex Nile tilapia, *Oreochromis niloticus*. Journal of Applied Aquaculture 34:247–265. https://doi.org/10.1080/10454438.2020.1829247

Debnath, S.C., McMurtrie, J., Temperton, B., Delamare-Deboutteville, J., Mohan, C.V., Tyler, C.R. 2023. Tilapia aquaculture, emerging diseases, and the roles of the skin microbiomes in health and disease. Aquaculture International 31:2945–2976. https://doi.org/10.1007/s10499-023-01117-4

Deng, Z., Yan, S., Hu, H., Duan, Z., Yin, L., Liao, S., Sun, Y., Yin, D., Li, G. 2015. Proteomic profile in carbonylated proteins in rat liver: Discovering possible mechanisms for tetracycline-induced steatosis. Proteomics 15:148–159. https://doi.org/10.1002/pmic.201400115

Devkar, S.T., Kandhare, A.D., Zanwar, A.A., Jagtap, S.D., Katyare, S.S., Bodhankar, S.L., Hegde, M.V. 2016. Hepatoprotective effect of withanolide-rich fraction in acetaminophen - intoxicated rat: decisive role of TNF - α, IL - 1β, COX - II and iNOS. Pharmaceutical Biology

54:2394–2403. https://doi.org/10.3109/13880209.2016.1157193

Drucker, D.J., Nauck, M.A. 2006. The incretin system: glucagon-like-1 receptor agonists and dipeptidylpeptidase-4 inhibitors in type 2 diabetes. The Lancet 368(9548):1696–1705. https://doi.org/10.1016/S0140-6736(06)69705-5

Dügenci, S.K., Arda, N., Candan, A. 2003. Some medicinal plants as immunostimulant for fish. Journal of Ethnopharmacology 88:99–106. https://doi.org/10.1016/s0378-8741(03)00182-x

Eccles, D.H. 1992. FAO species identification sheets for fishery purposes. Field guide to the freshwater fishes of Tanzania. Prepared and published with the support of the United Nations Development Programme (project URT/87/016). FAO, Rome. 145 pp.

El-Sayed, A-F.M., Kawanna, M. 2008. Optimum water temperature boosts the growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared in a recycling system. Aquaculture Research 39:670–672. https://doi.org/10.1111/j.1365-2109.2008.01915.x

El-Sherif, M.S., El-Feky, A. 2008. Effect of ammonia on Nile tilapia (*O. niloticus*) performance and some hematological and histological measures. In: 8th international symposium on tilapia in aquaculture, (eds. Elghobashy, H., Fitzsimmons, K., Diab, A.S.), pp. 513–530. Central Laboratory for Aquaculture Research, Abbasa, Ministry of Agriculture and Land Reclamation, Cairo, Egypt. https://www.researchgate.net/publication/334459535

Eni-Yimini, A., Benjamin, E., Obolo, D. 2015. Studies on the relationship between leukocytosis and haematocrit. Advances in Life Science and Technology 29:17–22.

Gabriel, N.N., Gonzàlez-Redondo, P. 2019. Review on the progress in the role of herbal extracts in tilapia culture. Cogent Food and Agriculture 5:1619651. https://doi.org/10.1080/23311932.2019.1619651

Getso, B.U., Abdullahi, J.M., Yola, I.A. 2017. Length-weight relationship and condition factor of *Clarias gariepinus* and *Oreochromis niloticus* of Wudil River, Kano, Nigeria. Agro-Science 16:1–4. https://doi.org/10.4314/as.v16i1.1

Ghosal, I., Mukherjee, D., Chakraborty, S.B. 2021. The effects of four plant extracts on growth, sex reversal, immunological and haemato-biochemical parameters in Nile tilapia, *Oreochmomis niloticus* (Linnaeus, 1758). Aquaculture Research 52:559–576. https://doi.org/10.1111/are.14914

Giannini, E.G., Testa, R., Savarino, V. 2005. Liver enzyme alteration: a guide for clinicians. Canadian Medical Association Journal 172(3):367–379. https://doi.org/10.1503/cmaj.1040752

Gill, T.S., Pande, J., Tewari, H. 1990. Enzyme modulation by sublethal concentrations of Aldicarb, Phosphamidon, and Endosulfan in fish tissues. Pesticide Biochemistry and Physiology 38:231–244. https://doi.org/10.1016/0048-3575(90)90095-J

Giri, S.S., Sen, S.S., Chi, C., Kim, H.J., Yun, S., Park, S.C., Sukumaran, V. 2015. *Chlorophytum borivilianum* polysaccharide fraction provokes the immune function and disease resistance of *Labeo rohita* against *Aeromonas hydrophila*. Journal of Immunology Research 2015:256510. https://doi.org/10.1155/2015/256510

Giribabu, N., Kumar, K.E., Rekha, S.S., Muniandy, S., Salleh, N. 2014. *Chlorophytum borivilianum* (Safed Musli) root extract prevents impairment in characteristics and elevation of oxidative stress in sperm of streptozotocin - induced adult male diabetic Wistar rats. BMC Complementary & Alternative Medicine 14:291. https://doi.org/10.1186/1472-6882-14-291

Haenen, O.L.M., Dong, H.T., Hoai, T.D., Crumlish, M., Karunasagar, I., Barkham, T., Chen, S.L., Zadoks, R., Kiermeier, A., Wang, B., Gamarro, E.G., Takeuchi, M., Azmai, M.N.A., Fouz, B., Pakingking, Jr. R., Wei, Z.W., Bondad-Reantaso, M.G. 2023. Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. Reviews in Aquaculture 15(S1):154–185. https://doi.org/10.1111/raq.12743

Haghighi, M., Rohani, M.S. 2013. The effect of powdered ginger (*Zingiber officinale*) on the haematological and immunological parameters of rainbow trout *Oncorhynchus mykiss*. Journal of Medicinal Plant and Herbal Therapy Research 1:8–12.

Hamid, A., Lee, L.S., Karim, S.R., Jufri, F. 2018. Hepatoprotective effects of Zerum bone against Paracetamol-induced acute hepatotoxicity in rats. Malaysian Journal of Medical Sciences 25:64–71. https://doi.org/10.21315/mjms2018.25.2.7

Hardi, E.H., Nugroho, R., Kusuma, I.W., Suwinarti, W., Sudaryono, A., Rostika, R. 2019. Borneo herbal plant extracts as a natural medication for prophylaxis and treatment of *Aeromonas hydrophila* and *Pseudomonas fluorescens* infection in tilapia (*Oreochromis niloticus*). F1000Research 7:1847. https://doi.org/10.12688/f1000research.16902.2

Harikrishnan, R., Balasundaram, C., Jawahar, S., Heo, M-S. 2012. Immunomodulatory effect of *Withania somnifera* supplementation diet in the giant freshwater prawn *Macrobrachium rosenbergii* (de Man) against *Aeromonas hydrophila*. Fish & Shellfish Immunology 32:94–100. https://doi.org/10.1016/j.fsi.2011.10.027

Heriansah, Syamsuddin, R., Najamuddin, Syafiuddin 2022. Effect of feeding rate on growth and feed conversion ratio in the cultivation recirculation systems of multi tropic model. IOP Conference Series: Earth and Environmental Science 1119:012066. https://doi.org/10.1088/1755-1315/1119/1/012066

Hussain, J., Khan, A.L., Rehman, N., Zainullah, K.F., Hussain, S.T., Shinwari, Z.K. 2009. Proximate and nutrient investigations of selected medicinal plants species of Pakistan. Pakistan Journal of Nutrition 8:620–24. https://doi.org/10.3923/pjn.2009.620.624

Itoh, Y., Kawamata, Y., Harada, M., Kobayashi, M., Fujii, R., Fukusumi, S., Ogi, K., Hosoya, M., Tanaka, Y., Uejima, H., Tanaka, H., Maruyama, M., Satoh, R., Okubo, S., Kizawa, H., Komatsu, H., Matsumura, F., Noguchi, Y., Shinohara, T., Hinuma, S., Fujisawa, Y., Fujino, M. 2003. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. Nature 422:173–176. https://doi.org/10.1038/nature01478

Jisr, N., Younes, G., Sukhn, C., El-Dakdouki, M. 2018. Length-weight relationships and relative condition factor of fish inhabiting the marine area of the Eastern Mediterranean city, Tripoli-Lebanon. The Egyptian Journal of Aquatic Research 44:299–305. https://doi.org/10.1016/j.ejar.2018.11.004

Junio, H.A., Sy-Cordero, A.A., Ettefagh, K.A., Burns, J.T., Micko, K.T., Graf, T.N., Richter, S.J., Cannon, R.E., Oberlies, N.H., Cech, N.B. 2011. Synergy-directed fractionation of botanical medicines: a case study with goldenseal (*Hydratis canadensis*). Journal of Natural Products 74:1621–1629. https://doi.org/10.1021/np200336g

Kord, M.I., Srour, T.M., Omar, E.A., Farag, A.A., Nour, A.A.M., Khalil, H.S. 2021. The immunostimulatory effects of commercial feed additives on growth performance, non-specific immune response, antioxidants assay, and intestinal morphometry of Nile tilapia, *Oreochromis niloticus*. Frontiers in Physiology 12:627499. https://doi.org/10.3389/fphys.2021.627499

Korolczuk, A., Carban, K., Amarowicz, M., Czechowska, G., Irla-Miduch, J. 2016. Oxidative stress and liver morphology in experimental cyclosporine A-induced hepatotoxicity. BioMed Research International 2016:5823271. https://doi.org/10.1155/2016/5823271

Kuttan, G. 2000. Immunomodulatory effect of some naturally occurring sulphur-containing compounds. Journal of Ethnopharmacology 72:93–99. https://doi.org/10.1016/s0378-8741(00)00211-7

Kyathanahalli, C.N., Manjunath, M.J., Muralidhara, M. 2014. Oral supplementation of standardized extract of *Withania somnifera* protects against diabetes-induced testicular oxidative impairments in prepubertal rats. Protoplasma 251:1021–1029. https://doi.org/10.1007/s00709-014-0612-5

Lee, H.J., Lee, M.H., Ruy, P.D. 2001. Public health risks: chemical and antibiotic residues - review. Asian-Australasian Journal of Animal Sciences 14:402–413. https://doi.org/10.5713/ajas.2001.402

Lind, C.E., Safari, A., Agyakwah, S.K., Attipoe, F.Y.K., El-Naggar, G.O., Hamzah, A., Hulata, G., Ibrahim, N.A., Khaw, H.L., Nguyen, N.H., Maluwa, A.O., Zaid, M., Zak, T., Ponzoni, R.W. 2015. Differences in sexual size dimorphism among farmed tilapia species and strains undergoing genetic improvement for body weight. Aquaculture Reports 1:20–27. https://doi.org/10.1016/j.aqrep.2015.03.003

Mahdi, A.A., Shukla, K.K., Ahmad, M.K., Singh, R., Shankhwar, S.N., Singh, V., Dalela, D. 2011. *Withania somnifera* improves semen quality in stress-related male fertility. Evidence-Based Complementary and Alternative Medicine 2011:576962. https://doi.org/10.1093/ecam/nep138

Maisuthisakul, P., Pasuk, S., Ritthiruangdej, P. 2008. Relationship between antioxidant properties and chemical composition of some Thai plants. Journal of Food Composition and Analysis 21:229–240. https://doi.org/10.1016/j.jfca.2007.11.005

Maisuthisakul, P., Suttajit, M., Pongsawatmanit, R. 2007. Assessment of phenolic content and free radical - scavenging capacity of some Thai indigenous plants. Food Chemistry 100:1409–1418. https://doi.org/10.1016/j.foodchem.2005.11.032

Makori, A., Abuom, P.O., Kapiyo, R., Anyona, D.N., Dida, G.O. 2017. Effects of water physico-chemical parameters on tilapia (*Oreochromis niloticus*) growth in earthen ponds in Teso North Sub-County, Busia County. Fisheries and Aquatic Sciences 20:30. https://doi.org/10.1186/s41240-017-0075-7

Mawire, P., Mozirandi, W., Heydenreich, M., Chi, G.F., Mukanganyama, S. 2021. Isolation and Antimicrobial Activities of Phytochemicals from *Parinari curatellifolia* (Chrysobalanceae). Advances in Pharmacological and Pharmaceutical Sciences 2021:8842629. https://doi.org/10.1155/2021/8842629

Monir, W., Abdel-Rahman, M.A., Hassan, S.E.D., Awad, S.M. 2020. Pomegranate peel and moringa-based diets enhanced biochemical and immune parameters of Nile tilapia against bacterial infection by *Aeromonas hydrophila*. Microbial Pathogenesis 145:104202. https://doi.org/10.1016/j.micpath.2020.104202

Moundipa, P.F., Beboy, N.S.E., Zelefack, F., Ngouela, S., Tsamo, E., Schill, W., Monsees, T. K. 2005. Effect of *Basella alba* and *Hibiscus macranthus* extracts on testosterone production of adult rat and bull Leydig cells. Asian Journal of Andrology 7:411–417. https://doi.org/10.1111/j.1745-7262.2005.00056.x

Mukherjee, D., Ghosal, I., Hancz, C., Chakraborty, S.B. 2018. Dietary administration of plant extracts for production of monosex tilapia: searching a suitable alternative to synthetic steroids in tilapia culture. Turkish Journal of Fisheries and Aquatic Sciences 18:267–275. https://doi.org/10.4194/1303-2712-v18_2_06

Mukherjee, D., Ghosal, I., Moniruzzaman, M., De, M., Chakraborty, S.B. 2019. Dietary administration of ethanol and methanol extracts of *Withania somnifera* root stimulates innate immunity, physiological parameters and growth in Nile tilapia *Oreochromis niloticus*. Croatian Journal of Fisheries 77:107–118. https://doi.org/10.2478/cjf-2019-0012

Mutlu, E.A., Keshavarzian, A., Mutlu, G.M. 2006. Hyperalbuminemia and elevated transaminases associated with high-protein diet. Scandinavian Journal of Gastroenterology 41:759–760. https://doi.org/10.1080/00365520500442625

Namachivayam, A., Gopalakrishnan, A.V. 2023. Effect of lauric acid against ethanol - induced hepatotoxicity by modulating oxidative stress/apoptosis signalling and HNF4α in Wistar albino rats. Heliyon 9:e21267. https://doi.org/10.1016/j.heliyon.2023.e21267

Neamat-Allah, A.N., Mahmoud, E.A., Mahsoub, Y. 2021. Effects of dietary white mulberry leaves on hemato-biochemical alterations, immunosuppression and oxidative stress induced by *Aeromonas hydrophila* in *Oreochromis niloticus*. Fish & Shellfish Immunology108:147–156. https://doi.org/10.1016/j.fsi.2020.11.028

NFDB. 2015. Guidelines for responsible farming of tilapia in India. National Fisheries Development Board, Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture and Farmers Welfare, Govt. of India. Hyderabad, India. November 2015. 16 pp. https://nfdb.gov.in/PDF/GUIDELINES/1 (Accessed 17 January 2025).

Nitbani, F.O., Tjitda, P.J.P., Nitti, F., Jumina, J., Detha, A.I.R. 2022. Antimicrobial properties of lauric acid and monolaurin in virgin coconut oil: A review. ChemBioEng Reviews 9:442–461. https://doi.org/10.1002/cben.202100050

Okeke, E.S., Chukwudozie, K.I., Nyaruaba, R., Ita, R.E., Oladipo, A., Ejeromedoghene, O., Atakpa, E.O., Agu, C.V., Agu, C.V., Okoye, C.O. 2022. Antibiotic resistance in aquaculture and aquatic organisms: a review of current nanotechnology applications for sustainable management. Environmental Science and Pollution Research 29:69241–69274. https://doi.org/10.1007/s11356-022-22319-y

Okocha, R.C., Olatoye, I.O., Adedeji, O.B. 2018. Food safety impacts of antimicrobial use and their residues in aquaculture. Public Health Reviews 39:21. https://doi.org/10.1186/s40985-018-0099-2

Oniovosa, U.E., Aina, O.O., Alarape, S.A., Babalola, O.E., Adeyemo, O.K. 2017. Effects of neem leaves aqueous extract on organ histology, haematological parameters and biochemical indices in catfish. Alexandria Journal of Veterinary Sciences 54:17–24. https://doi.org/10.5455/ajvs.256015

Pakravan, S., Hajimoradloo, A., Ghorbani, R. 2012. Effect of dietary willow herb, *Epilobium hirsutum* extract on growth performance, body composition, haematological parameters and *Aeromonas hydrophila* challenge on common carp, *Cyprinus carpio*. Aquaculture Research 43:861–869. https://doi.org/10.1111/j.1365-2109.2011.02901.x

Park, Y.M., Lee, H.Y., Shin, D.Y., Lee, Y.H., Yang, Y.J., Lee, H.S., Lee, J.O., Choi, K.S., Kang, J.H., Cho, Y.H., Kim, M.G., Yun, C.Y., Kim, M.J., Jang, D.J., Yang, H.J., Lee, Y-R. 2020. Immunostimulatory activity of black rice bran in cyclophosphamide-induced immunosuppressed rats. Natural Product Communications 2020:15. https://doi.org/10.1177/1934578X20934919

Pavaraj, M., Balasubram, V., Baskaran, S., Ramasamy, P. 2011. Development of immunity by extract of medicinal plant *Ocimum sanctum* on common carp *Cyprinus carpio (L.)*. Research Journal of Immunology 4:12–18. https://doi.org/10.3923/rji.2011.12.18

Presti, R., Pantaleo, G. 2017. The immunopathogenesis of HIV - 1 Infection. In: Infectious diseases. 4th Edition. Cohen, J., Powderly, W.G., Opal, S.M. (Eds.), Elsevier, pp. 837–845. https://doi.org/10.1016/B978-0-7020-6285-8.00092-7

Pupim, L.B., Martin, C.J., Ikizler, T.A. 2013. Assessment of protein and energy nutritional status. In: Nutritional management of renal disease. 3rd Edition. Kopple, J.D., Massry, S.G., Kalantar-Zadeh, K. (Eds.), Academic Press, pp. 137–158. https://doi.org/10.1016/B978-0-12-391934-2.00010-2

Putra, A.A.S., Santoso, U., Lee, M.C., Nan, F.H. 2013. Effects of dietary katuk leaf extract on growth performance, feeding behavior and water quality of grouper *Epinephelus coioides*. Aceh International Journal of Science and Technology 2:17–25. https://doi.org/10.13170/aijst.2.1.488

Rebouças, V.T., Lima, F.B.S., Cavalcante, D.H., Carmo e Sá, M.V. 2016. Reassessment of the suitable range of water pH for culture of Nile tilapia *Oreochromis niloticus* L. in eutrophic water. Acta Scientiarum.

Animal Sciences 38:361–368. https://doi.org/10.4025/actascianimsci.v38i4.32051

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., Sasal, P. 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture 433:50–61. https://doi.org/10.1016/j.aquaculture.2014.05.048

Sankaran, K., Gurnani, S. 1972. On the variation in the catalytic activity of lysozyme in fishes. Indian Journal of Biochemistry & Biophysics 9:162–165.

Santos, M.N., Gaspar, M.B., Vasconcelos, P., Monteiro, C.C. 2002. Weight–length relationships for 50 selected fish species of the Algarve coast (southern Portugal). Fisheries Research 59:289–295. https://doi.org/10.1016/S0165-7836(01)00401-5

Secombes, C.J. 1990. Isolation of salmonid macrophages and analysis of their killing activity. Techniques in Fish Immunology 1:137–154.

Seeley, K.R., Gillespie, P.D., Weeks, B.A. 1990. A simple technique for the rapid spectrophotometric determination of phagocytosis by fish macrophages. Marine Environmental Research 30:37–41. https://doi.org/10.1016/0141-1136(90)90009-D

Sharma, A., Deo, A.D., Riteshkumar, S.T., Chanu, T.I., Das, A. 2010. Effect of *Withania somnifera* (L. Dunal) root as a feed additive on immunological parameters and disease resistance to *Aeromonas hydrophila* in *Labeo rohita* (Hamilton) fingerlings. Fish and Shellfish Immunology 29:508–512. https://doi.org/10.1016/j.fsi.2010.05.005

Sharma, S.K., Kumar, M. 2011. Hepatoprotective effect of *Chlorophytum borivilianum* root extract against arsenic intoxication. Pharmacology Online 3:1021–1032.

Sönmez, A.Y., Bilen, S., Alak, G., Hisar, O., Yanik, T., Biswas, G. 2015. Growth performance and antioxidant enzyme activities in rainbow trout (*Oncorhynchus mykiss*) juveniles fed diets supplemented with sage, mint and thyme oils. Fish Physiology and Biochemistry 41:165–175. https://doi.org/10.1007/s10695-014-0014-9

Stillwell, W. 2016. Membrane biogenesis: fatty acids. In: An introduction to biological membranes. 2nd Edition. Stillwell, W. (Ed.), Elsevier, pp. 315–329. https://doi.org/10.1016/B978-0-444-63772-7.00014-2

Takaoka, O., Ji, S.C., Ishimaru, K., Lee, S-W., Jeong, G-S., Ito, J., Biswas, A., Takii, K. 2011. Effect of rotifer enrichment with herbal extracts on growth and resistance of red sea bream, *Pagrus major* (Temminck & Schelgel) larvae against *Vibrio anguilarum*. Aquaculture Research 42:1824–1829. https://doi.org/10.1111/j.1365-2109.2010.02783.x

Talpur, A.D. 2014. *Mentha piperita* (Peppermint) as feed additive enhanced growth performance, survival, immune response and disease resistance of Asian seabass, *Lates calcarifer* (Bloch) against *Vibrio harveyi* infection. Aquaculture 420:71–78. https://doi.org/10.1016/j.aquaculture.2013.10.039

Talpur, A.D., Ikhwanuddin, M., Bolong, A-M.A. 2013. Nutritional effects of ginger (*Zingiber officinale* Roscoe) on immuneresponse of Asian seabass, *Lates calcarifer* (Bloch) and disease resistance against *Vibrio harveyi*. Aquaculture 400–401:46–52. https://doi.org/10.1016/j.aquaculture.2013.02.043

Thakur, M., Dixit, V.K. 2006. Effect of *Chlorophytum borivilianum* on androgenic and sexual behavior of male rats. Indian Drugs 43:300–306.

Tilak, K.S., Mary, J. 2009. A study on enzymes ACP and ALP in the fish *Labeo rohita* (Hamilton) exposed to the toxicant dichlorvos, an organo phosphate. SSRN. https://dx.doi.org/10.2139/ssrn.1484554

Uribe, C., Folch, H., Enríquez, R., Moran, G. 2011. Innate and adaptive immunity in teleost fish: A review. Veterinarni Medicina 56:486–503. https://doi.org/10.17221/3294-VETMED

van Raalte, D.H., Li, M., Pritchard, P.H., Wasan, K.M. 2004. Peroxisome proliferator-activated receptor (PPAR)-alpha: a pharmacological target with a promising future. Pharmaceutical Research 21:1531–1538. https://doi.org/10.1023/B:PHAM.0000041444.06122.8d

Verbsky, W.J., Routes, M.J. 2014. Management of autoimmunity and inflammation. In: Stiehm's immune deficiencies. Sullivan, K.E., Stiehm, E.R. (Eds.), Academic Press, pp. 931–942. https://doi.org/10.1016/B978-0-12-405546-9.00052-2

Vodnar, D.C., Călinoiu, L.F., Dulf, F.V., Ştefănescu, B.E., Crişan, G., Socaciu, C. 2017. Identification of the bioactive compounds and antioxidants, antimutagenic and antimicrobial activities of thermally processed agro-industrial waste. Food Chemistry 231:131–140. https://doi.org/10.1016/j.foodchem.2017.03.131

Wang, J., Wu, X., Simonavicius, N., Tian, H., Ling, L. 2006. Medium-chain fatty acids as ligands for orphan G protein-coupled receptor GPR84. Journal of Biological Chemistry 281:34457–34464. https://doi.org/10.1074/jbc.M608019200

Yadav, S.S., Kumar, R., Khare, P., Tripathi, M. 2015. Oxidative stress biomarkers in the freshwater fish, *Heteropneustes fossilis* (Bloch) exposed to sodium fluoride: antioxidant defense and role of ascorbic acid. Toxicology International 22:71–76. https://doi.org/10.4103/0971-6580.172261

Yamauchi, R. 1997. Vitamin E: Mechanism of its antioxidant activity. Food Science and Technology International, Tokyo 3:301–309. https://doi.org/10.3136/fsti9596t9798.3.301

Zaher, F.M., Rahman, B.M.S., Rahman, A., Alam, M.A., Pramanik, M.H. 2015. Length-weight relationship and GSI of hilsa, *Tenualosa ilisha* (Hamilton, 1822) fishes in Meghna River, Bangladesh. International Journal of Natural and Social Sciences 2:82–88.