E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(1): 352-359

www.fisheriesjournal.com
Received: 22-11-2016
Accepted: 23-12-2016

C Sunitha
Dept. of Zoology, Holy Cross College, Nagercoil, India

S Mettilda
Dept. of Zoology, Holy Cross College, Nagercoil, India

J Vinoliya
Dept. of Zoology, Holy Cross College, Nagercoil, India

Correspondence
S Mettilda
Dept. of Zoology, Holy Cross College, Nagercoil, India

Effect of dietary intake of *Phyllanthus niruri* L. on fingerlings of freshwater fish, *Cyprinus carpio* L

C Sunitha, S Mettilda and J Vinoliya

Abstract
The aim of the present investigation was to elucidate the growth promoting effect and immunostimulatory role of the medicinal plant, *Phyllanthus niruri* on the freshwater fish, *Cyprinus carpio.* Bioenergetics, biochemical constituents (muscle, whole body, serum) and histological (intestine, liver) parameters of the fish *C. carpio* was evaluated to understand the effect of *P. niruri* supplemented diet. Incorporation of 1 and 2 % of *P. niruri* in the feed enhanced the consumption rate, assimilation and conversion efficiency, protein, carbohydrate and lipid content in muscle, whole body and serum of the fish, *C. carpio*. Histological analysis of the liver and intestine showed a normal architecture suggesting it as an effective dietary formulation. The results of challenge test suggest that the fishes fed with 2 % *P. niruri* diet had better immunostimulatory activity compared to the control group. Thus, our finding confirms our contention that *P. niruri* is a growth promoter and immunostimulator.

Keywords: bioenergetics, growth promoter, hepatoprotective, immunostimulator, medicinal plant

1. Introduction
Aquaculture has a promising role to play in the future where there would be a great demand for safe, nutritious and quality food [1]. With the emergence of large scale commercial fish culture, diseases of varied etiology are being increasingly recognized as a major problem to successful and sustainable farming [2]. The current disease treating protocols with antibiotics create environmental hazards such as residual effects, bio-magnification and resistant strain development [3]. Hence, the usage of heavy antibiotics in aquaculture field needs to be reduced and replaced with alternative practices for diseases management [4] and attention should be diverted to find novel products from plant resources. Herbal plants are a potential and promising source of pharmaceutical agent against fish pathogens in aquaculture [5] as they are an inexhaustible source of secondary metabolites [6] like volatile oils, saponins, phenolics, tannins, alkaloids, polysaccharides, polypeptides and are responsible for various medicinal activities like anti-stress, appetizer, tonic, antimicrobial and immunostimulant [3].
Some important herbal supplements evaluated in aquaculture are *Ocimum sanctum*, *Withania somnifera*, *Tinospora cordifolia* and *Emblica officinalis* [7, 8] *Nicotiana tobacum* [9, 10], *Solanum triblobatum, Andrographis paniculata and Psoralea corylifolia* [11, 12], *Asystasia vogeliana* [13], *Nymphoides cristatum* [14], *Croton tiglium* [15], *Moringa oleifera* [16], *Achyranthes aspera* [17], *Eclipta alba* [18], *Cynodon dactylon, Aegle marmelos*, *Zingiber officinale* [19], *Hibiscus sabdariffa* [20], *Melissa officinalis, Aloe vera* [21], *Azadirachta indica* [22], *Datura metel* [23], *Nerium indicum* [24], *Allium sativum* and *Vitex negundo* [25], *Allium cepa* and *Tetracarpidium conophorum* [26, 27]. These observations provide the aqua culturists with a promising management tool with the use of medicinal plants for control or treatment of fish diseases.
Phyllanthus niruri L. is a small herb with a wide range of medicinal properties and is used for treating jaundice, ulcers, skin diseases, diabetes, chest pain, constipation, kidney stones and urinary complications [28]. It also exhibit pharmacological activities like antibacterial, antiviral, hepatoprotective, antioxidant, anticancer, anti-inflammatory, anti-plasmodial, analgesic and diuretic property [29-31] as it has various compounds such as alkaloids, phenol, proteins, amino acids, tannin, carbohydrates, terpenoids, saponins, cardio glycosides, steroids, reducing sugar, resins, lignans, phyllanthin, hypophyllanthin, flavonoids, glycosinoids and anthraquinones [28, 32]. Based on its biomedical potential an effort was taken to assess the effect of *Phyllanthus niruri* L. supplemented diet on the survival, growth and healthy status of the freshwater fish, *Cyprinus carpio* L.

2. Materials and Methods

2.1 Collection and Processing of *Phyllanthus niruri* L.: *P. niruri* commonly known as keezhkhainelli or Keezhanelli in Tamil were collected from different regions of Kanyakumari district (77º15′ and 77º36′ east latitude and 8º03′ and 8º35′ north longitude). The plant was washed, dried in shade, powdered using mechanical grinder and stored in air tight container.

2.2 Preparation of control and experimental fish feed: The required quantities of rice bran, soya bean powder, fish meal, groundnut oil cake and tapioca powder were dried, powdered and made into soft dough. The dough was cooked for 1 hour, cooled and appropriate dose of vitamins and minerals were added. The prepared dough was divided into 3 parts (500 g each) and the first part served as control feed. To the 2nd and 3rd part, *P. niruri* powder was added in the order of 5 g/ 500 g (1%), 10 g/ 500 g (2%) and mixed thoroughly to prepare experimental feed I and experimental feed II respectively. Finally the dough was pressed through a hard pelletizer machine having a perforated disc and made in the form of noodles, dried and broken into pieces and then utilized for feeding.

2.3 Collection and Maintenance of *Cyprinus carpio* L.: Fingerlings of *C. carpio* were collected from Fish Seed Farm, Manimuthar, Tirunelveli, India (77º40995′ east latitude, 8º640507′ north longitude and 102 m altitude) and were maintained in 20 litre plastic tubs containing dechlorinated tap water. The fishes were acclimatized to laboratory conditions at room temperature. The water was changed every 24 hours and the fishes were fed *ad libitum* with control feed throughout the acclimatization period.

2.4 Experimental Setup: The fishes were grouped into three groups (control, experimental I and experimental II) of 75 fishes and each group of fish was then subdivided into three groups of 25 fishes to maintain replicates. Initial length and weight of fishes were taken and then introduced into plastic troughs of 20 litre capacity containing five litres of tap water. The fishes were fed daily morning with weighed quantity of different types of feed and the unfed was removed after five hours in the evening from each container and collected in separate dishes and were allowed to dry. The faecal pellets were collected from each trough before changing the water and then dried. The experiment was carried out for a period of 60 days. Growth, biochemical analysis and histology of intestine and liver were carried at thirty days interval.

2.5 Bioenergetics: The scheme of energy balance followed in the present study is the IBP formula [33]. Growth in terms of weight and length was assessed using the formula:

Growth in terms of body weight (weight gain) = Final weight – Initial weight.

Growth in terms of body length (length gain) = Final length – Initial length.

Specific growth rate (SGR) = $\frac{Final\ weight - Initial\ weight}{Duration\ (days)} \times 100$

2.6 Biochemical analysis: Blood was collected from sample fishes by cardiac puncture in non-heparinized tubes to estimate serum total protein, glucose and cholesterol. The blood sample was allowed to clot in an upright position for 30 - 45 minutes and then centrifuged for 15 minutes at 2500 rpm. Then serum was carefully separated and used for biochemical analysis. The analysis was carried out using diagnostic reagent kit supplied by Aspen Laboratories, India.

The muscle tissue for biochemical test was excised from the sacrificed fish at 30 days interval from each group. Sample fishes from each group was sacrificed, dried and powdered at 30 days interval for biochemical analysis. The biochemical constituents like protein, carbohydrate and lipid was estimated by Folin-Ciocalteu method [34], Anthrone method [35] and Phospho-vanillin method [36] respectively.

2.7 Histological analysis: Intestine and liver of the fish from sample fish of each group were excised at 30th and 60th day of exposure and fixed in 10% neutral buffered formalin and then given to Vivek Laboratory, Nagercoil for sectioning. The fixed tissue were dehydrated in graded series of alcohol, cleared in xylene and embedded in paraffin. Five micron sections were cut and stained with haematoxylin and eosin for the histological examination.

2.8 Immunostimulatory studies: After 60 days of experimental period the fishes of control and experimental group were challenged with *A. hydrophila*. Number of colony forming units (CFU) of *A. hydrophila* was found out by pour plate method. A definite concentration (10^{-3} CFU) of *A. hydrophila* suspension was prepared. 1 ml of this suspension was inoculated into each aquaria (control, experimental I and II). The mortality of the fishes was observed for understanding the immunostimulatory effect of *P. niruri.*

3. Results and Discussion

Fish is a rich source of animal protein and to achieve maximum yield it is necessary to provide artificial feed by which fish attain maximum weight in shortest possible time [37]. Several herbs such as garlic, onion, marjoram, caraway, basil, anise, fennel, licorice, black seed and fenugreek were analysed for the growth promoting activities [3, 38], feed conversion [39] and improvement of protein digestibility [40, 41] in aquatic animals. Present study focused to evaluate the potential of dietary *P. niruri* in the freshwater fish, *C. carpio*. Results on the effect of *P. niruri* supplemented diet on the bioenergetics, biochemical, histological changes and immune response of fish, *C. carpio* revealed dietary *P. niruri* as an excellent diet for freshwater fishes.

3.1 Bioenergetics

3.1.1 Growth rate: The growth of fish is directly dependent on food composition, quality and quantity [42, 43] and various types of feed formulation have been tried to substitute for natural food for both edible and ornamental fishes [44]. The present study demonstrated that diet supplemented with *P. niruri* enhanced growth in the experimental groups when compared to control. Among the tested diet, experimental feed II showed highest rate of growth in terms of both length and weight of fish (Table – 1 and 2), which may be due to the unstressful, more palatable and digestible nature i.e., the growth promoting effect of *P. niruri.* Similar findings were also reported in the fish, *Oreochromus mosssambicus* fed with *Andrographis paniculata* [45].

3.1.2 Feeding rate / Consumption rate: Food consumption in fish is regulated to maintain a constant energy intake or indeed, that energy requirement remains constant, when fish are fed on diets differing in ‘digestible energy’ and ‘digestible

protein' at different rates. The present experiment reveals that the amount of food consumed and the rate of consumption is more in experimental groups compared to the feeding rate of the control groups (Table - 3 and 4). This result indicated that incorporation of *P. niruri* in feeds produces positive effect on the feeding of the animals. As reported by Citarasu [46], active principles of plant in food may result in inducing the secretion of digestive enzymes that will result in stimulating the appetite and increasing food consumption. Similar improved feed intake and nutrient digestibility was also observed in *Spirulina* supplemented diet in blue gourami [47], red swordtail [48] and rainbow trout [49].

3.1.3 Assimilation rate and assimilation efficiency: Fishes fed with *P. niruri* incorporated diet exhibited increased food assimilation and assimilation efficiency when compared to control group (Table – 3, 4 and 5). This increased efficiency of food assimilation may be due to increase in digestibility which might also have contributed to the increase in food consumption [50, 51].

3.1.4 Metabolic Rate: In the present study, metabolization and metabolic rate was high for control group on 30th, 45th and 60th day (Table – 4 and 5) and was low in fishes fed with *P. niruri* incorporated diet. Metabolism is a measure of the rate at which animals transform materials and it is the sum of catabolism and maintenance function and anabolism which results in growth. The reduced rate of metabolism observed in the experimental groups might have been due to the stress free nature of the food and is related to the increased food intake and growth of fish. Francis *et al.* [52] working with Quillaja saponins reported that these saponins have the potential to increase growth in culture fish species and reduce their metabolic rate in tilapia. Therefore it can be concluded that presence of saponin in *P. niruri* [29, 32] may be the reason for reduced metabolism and increased growth [52].

3.1.5 Conversion efficiency: In the present experiment, though the quality and quantity of all the ingredients remained the same except for the quantity of plant powder added, slight difference in the conversion, conversion rate and conversion efficiencies have been observed. Marked differences were observed in the gross conversion efficiency and net conversion efficiency among various groups of fishes depending on the quality of the food provided. Both the gross and net conversion efficiencies were found to be higher in the fishes fed with experimental II diet (Table - 5) which may be due to the increased intake of feed and decreased cost of metabolism. The increase in food consumption and less demand for allocation of absorbed food energy for metabolic process (stress free) have possibly contributed to the increase in the food conversion ratio. Conversion efficiency is found to have been influenced by the quality of the food [53]. Increase in growth rate might also be due to the increase in gross food conversion efficiency as in rainbow trout supplemented with spirulina powder [49].

3.1.6 Growth Rate and feed conversion ratio: The fishes fed with *P. niruri* incorporated diet exhibited an increase in specific growth rate (Table - 1 and 2) and decrease in feed conversion ratio (Table - 3). Growth represents the net outcome of a series of biological process such as food intake, digestion, assimilation, metabolism and excretion [54]. Growth responses to dietary supplements result from the combined effects of both stimulation of food intake and improved metabolic efficiency [55]. The feed with comparatively more amount of *P. niruri* i.e. experimental II diet was consumed more and also assimilated better compared to control and experimental I diet. This shows that *P. niruri* supplemented diet did not create any stress to the animal [56, 57], increased the palatability and digestibility [46], resulting in improved growth and conversion rate [47, 50, 58]. Thus it is evident that dietary incorporation of *P. niruri* plays a significant role as growth promoter.

3.2 Biochemical parameters: Biochemical parameters can be helpful to identify the target organs of toxic effects and also the general health condition in animals. They also provide early warning of potentially harmful changes in stressed organisms [59]. Studies on the biochemical parameters revealed an increase in total protein, glucose and cholesterol of serum, muscle and whole body (Table - 6, 7 and 8) of the fishes fed with *P. niruri* supplemented diet. The quantitative determination of total serum protein reflects the capacity of protein synthesis of liver and denotes the osmolarity of the blood and the renal impairments. Thus serum protein may be considered as a biomarker to asses any toxic changes in fishes. Increase in protein content in fish may be due to the active principles of *P. niruri* [46], which supports anabolism and less wastage of energy. Carbohydrate is considered to be a sensitive biochemical indicator of environmental stress, stress induced by handling, feeding, forced activity, thermal shock and contact with chemical pollutants [60]. Dietary supplementation of *P. niruri* showed an increase in the carbohydrate content which may be due to the unstressful and good nutritive nature of the herb [28]. Dietary lipids are important nutrients affecting energy production and are essential for the growth and development in most of the fishes [61]. In the present study lipid content in the experimental II group was higher on 60th day similar to those observed in juvenile sterlet sturgeon fed diet with 0.5% garlic extract [61]. Fishes under stressful conditions secrete high amount of catecholamine and corticosteroids which produces an enhanced metabolic rate and in turn deplete metabolic reserves like proteins and lipids to provide the energy demand [62]. However, in the present investigation an increase in protein, carbohydrate and lipid content was observed which could be related to good food intake, no or low energy cost of homeostasis, tissue repair and detoxicating mechanism during stress. Thus our results strongly support the content that this dose (1% and 2%) of *P. niruri* is not toxic to the fish, *C. carpio* suggesting that administration of *P. niruri* (1% and 2%) may stimulate the consumption, improve the assimilation, enhance the growth and increase the protein, carbohydrate and lipid content of the fish, *C. carpio.* This highlights the unstressful and good nutritive nature of the *P. niruri* diet [63].

3.3 Histological analysis: Histological analysis of liver was carried out in this study as it plays an important part in many vital functions of the basic metabolism and is also the largest organ responsible for accumulation, biotransformation and contaminant excretion, including pollutant degradation and bio-activation [64]. In the present study, the liver of the experimental fishes exhibited normal compact hepatocytes, arteries and veins (Plate - 1) suggesting the hepatoprotective nature of *P. niruri.* Evaluation of histological structure of digestive organ in fish fed with new ingredients provides

valuable information about digestive capacity and potential health effects of new diets [65]. Fishes administered with *P. niruri* plant extract had positive effect on the histology of intestine (Plate - 2) and not much change was observed between the control and experimental group as the histology of intestine showed no leucocytes infiltration in the epithelium, mucous production or damage to the villi. The results indicated that the dietary supplementation of *P. niruri* did not affect the normal functionalities of the treated fish. Similar results were observed in the liver and intestine of the fish, *Labeo rohita* treated with *Mentha piperita* [66].

3.4 Immunostimulatory effect: To understand whether *P. niruri* added feed formulation could stimulate the immune system, the fishes were subjected to *A. hydrophila* treated water and the mortality was assessed. The results of challenge experiment (Table – 9) revealed that the experimental II diet fishes were able to survive till the 5th day without any mortality. The present finding is in agreement with previous studies in fishes fed with dietary supplementation of *Excoecaria agallocha* [67], *O. sanctum* [68], *Rosmarinus officinalis* [69], *A. aspera* [17], *Euphorbia hirta* [70] and *Camellia sinensis* [51].

4. Conclusion

Thus from the present work, it may be suggested that incorporation of *P. niruri* in the feed, makes the food more palatable, stimulates better consumption, improves assimilation, enhances growth and increases the protein content of the fish, *C. carpio.* Hence, this plant can be considered while formulating the feed for freshwater fishes. This investigation also proves the hepatoprotective nature of *P. niruri* as the architecture of the liver cells in the treated fish is intact in condition. Enhanced serum protein level and the results of *A. hydrophila* challenge test prove the immunostimulatory effect of *P. niruri* on the fish, *C. carpio.* Hence, it could be concluded that the inclusion of this herb as an additive in fish feed could possibly aid productivity in aquaculture.

Table 1: Growth in terms of body weight of fish, *C. carpio* fed on *P. niruri* supplemented diet.

Duration (Day)	Control			Experimental I			Experimental II		
	Weight (g)	Growth (g)	SGR%	Weight (g)	Growth (g)	SGR%	Weight (g)	Growth (g)	SGR%
1st	0.55± 0.05	-	-	0.55± 0.05	-	-	0.60± 0.11	-	-
30th	0.77± 0.04	0.21± 0.02	0.70± 0.05	0.80± 0.07	0.24± 0.02	0.80± 0.07	0.86± 0.09	0.26± 0.02	0.87± 0.08
60th	0.98± 0.09	0.42± 0.03	0.70± 0.05	1.05± 0.09	0.49± 0.03	0.82± 0.07	1.15± 0.16	0.55± 0.04	0.92± 0.07

Table 2: Growth in terms of body length of fish, *C. carpio* fed on *P. niruri* supplemented diet (values are expressed in cm).

Duration (Day)	Control		Experimental I		Experimental II	
	length	growth	Length	growth	length	growth
1st	2.53± 0.26	-	2.54± 0.26	-	2.68± 0.19	-
30th	2.98± 0.25	0.45± 0.03	3.01± 0.26	0.47± 0.03	3.16± 0.20	0.48± 0.03
60th	3.45± 0.25	0.92± 0.07	3.53± 0.25	0.99± 0.07	3.75± 0.24	1.08± 0.09

Table 3: Food consumed (C), assimilated (A), converted (P) and metabolized (M) by *C. carpio* fed on *P. niruri* incorporated diet.

Duration (Day)	Group	C (g)	A (g)	P (g)	M (g)	FCR
30th	Control	0.57± 0.02	0.52± 0.02	0.21± 0.26	0.31± 0.01	2.71± 0.05
	Exp. I	0.58± 0.02	0.54± 0.03	0.24± 0.01	0.30± 0.01	2.41± 0.03
	Exp. II	0.58± 0.02	0.54± 0.03	0.26± 0.01	0.28± 0.01	2.23± 0.01
60th	Control	1.82± 0.26	1.72± 0.24	0.42± 0.01	1.30± 0.04	4.33± 0.02
	Exp. I	1.84± 0.26	1.75± 0.30	0.49± 0.01	1.26± 0.04	3.76± 0.15
	Exp. II	1.85± 0.30	1.77± 0.27	0.55± 0.02	1.22± 0.04	3.36± 0.12

Table 4: Influence of different concentrations of *P. niruri* in the feed on the rate of consumption (Cr), assimilation (Ar), metabolism (Mr) and conversion (Pr) of the fish, *C. carpio* (Rates are expressed in mg/ g body weight / day).

Duration (Day)	Group	Cr	Ar	Mr	Pr
30th	Control	32.22± 3.56	30± 3.27	18.45± 0.50	12.50± 0.52
	Exp. I	33.92± 3.72	30.95± 2.17	17.85± 0.50	14.29± 0.46
	Exp. II	34.52± 3.72	32.14 ± 3.72	15.55± 0.52	14.44± 0.50
60th	Control	51.39± 4.13	49.16± 3.85	38.69± 4.71	12.50± 0.52
	Exp. I	54.16± 4.84	51.19± 4.90	37.50± 3.72	14.58± 0.50
	Exp. II	56.76± 4.90	52.08± 4.90	33.89± 3.72	15.28± 0.52

Table 5: Efficiencies of assimilation, gross conversion (K_1) and net conversion (K_2) shown by *C. carpio* fed on *P. niruri* incorporated feed (Values are expressed in %).

Duration (Day)	Group	Assimilation Efficiency	K_1	K_2
30th	Control	91.23 ± 1.702	36.84 ± 0.597	40.38 ± 0.337
	Exp. I	93.10 ± 0.942	41.37 ± 0.268	44.44 ± 0.208
	Exp. II	94.10 ± 0.942	44.82 ± 0.208	48.14 ± 0.212
60th	Control	94.51 ± 1.247	23.08 ± 0.736	24.41 ± 0.699
	Exp. I	95.10 ± 1.346	26.63 ± 0.699	28.00 ± 0.424
	Exp. II	96.68 ± 1.346	29.72 ± 0.424	31.07 ± 0.218

Table 6: Serum biochemistry of *C. carpio* fed on *P. niruri* supplemented diet.

Group	Protein (g/ dl)		Cholesterol (mg/ dl)		Glucose (mg/ dl)	
	Duration (days)					
	30	60	30	60	30	60
Control	6.02± 0.53	10.83± 1.02	52.64± 0.43	91.46± 0.75	71.75± 0.57	120.33± 2.75
Exp. I	7.66± 1.86	11.52± 0.85	54.30± 1.32	92.38± 0.83	73.57± 0.47	123.58± 2.84
Exp. II	8.30± 1.36	12.25± 0.91	56.29± 0.33	93.17± 0.40	74.45± 0.21	125.16± 2.98

Table 7: Changes in the muscle protein, carbohydrate and lipid level of fish, *C. carpio* fed on diet incorporated with different concentrations of *P. niruri*.

Group	Protein (mg %)		Carbohydrate (mg %)		Lipid (mg %)	
	Duration (days)					
	30	60	30	60	30	60
Control	15.33± 0.16	19.03± 1.46	10.18± 0.04	13.52± 0.26	7.23± 0.25	10.31± 0.26
Exp. I	16.41± 0.36	23.66± 2.19	10.92± 0.82	15.90± 0.46	7.12± 0.24	10.90± 0.45
Exp. II	18.68± 1.12	26.85± 2.66	12.02 ± 0.92	16.41± 0.36	7.84± 0.08	12.30 ± 0.57

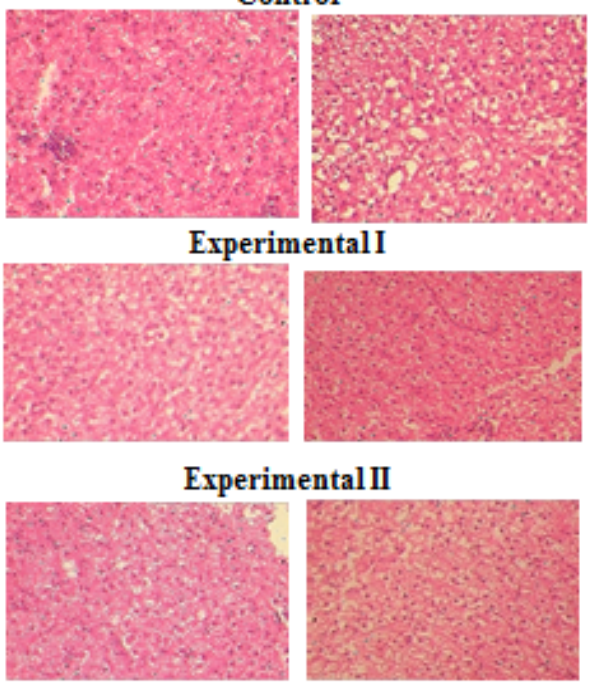
Table 8: Changes in the protein, carbohydrate and lipid level of whole (dried) body of *C. carpio* exposed to different concentrations of *P. niruri*.

Group	Protein (mg %)		Carbohydrate (mg %)		Lipid (mg %)	
	Duration (days)					
	30	60	30	60	30	60
Control	32.1 ± 1.23	40.75 ± 2.15	12.18 ± 0.04	21 ± 0.23	14.5 ± 0.98	18.26 ± 1.11
Exp. I	35.25 ± 1.61	44.45 ± 2.25	12.2 ± 0.92	24.95 ± 2.68	13.33 ± 0.68	15.5 ± 0.32
Exp. II	37.12 ± 1.65	48.9 ± 2.30	17.18 ± 1.04	29.5 ± 2.45	13.83 ± 0.77	20.26 ± 2.03

Table 9: % Mortality of fish *C. carpio* challenged with *A. hydrophila* after feeding *P. niruri* supplemented diet.

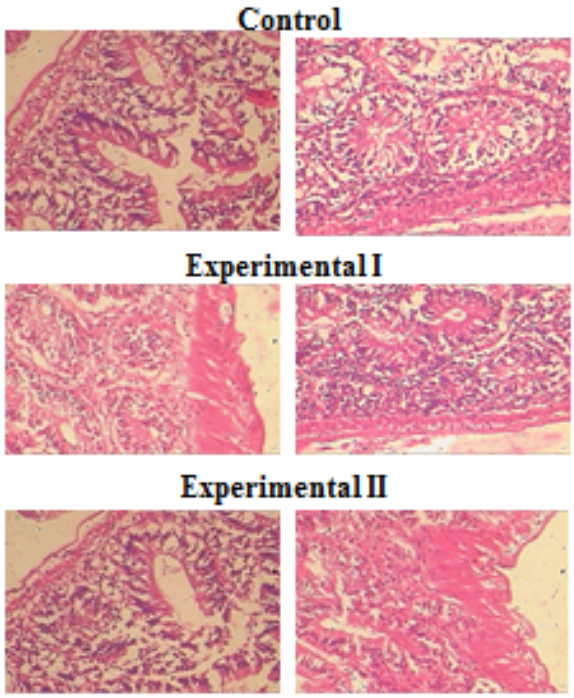
Group	% mortality of fishes on different days					
	1	2	3	4	5	6
Control	Nil	Nil	Nil	50	50	100
Exp. I	Nil	Nil	Nil	50	Nil	50
Exp. II	Nil	Nil	Nil	Nil	Nil	0

Plate - 1: Section of the liver of the fish, *C. carpio* fed on diet with *P. niruri* for 30 and 60 days



Photomicrographs of the liver of *C. carpio* treated with *P. niruri* diet showed normal hepatic tissue, showing hepatocytes with granular cytoplasm, central and round nucleus.

Plate - 2: Section of the intestine of the fish *C. carpio* fed on with *P. niruri* for 30 and 60 days.



Photomicrographs of the intestine of *C. carpio* treated with *P. niruri* diet showed normal histology.

5. References

1. Oliver J. Food, water, soil, oil: Peak everything – Almost. In: Abstracts from Aquaculture. Canada Conference, Canada, 2013.
2. Rao KG, Mohan CV, Seenappa D. The use of chemotherapeutic agents in fish culture in India. In: Diseases in Asian aquaculture, Shariff, IM. Subasinghe, R.P., Arthur, J.R., (eds.). Fish Health Section, Asian Fisheries Society, Manila, Philippines, 1992, 505.
3. Citarasu T, Babu MM, Sekar RJR, Marian PM. Developing Artemia enriched herbal diet for producing quality larvae in *Penaeus monodon* Fabricus. Asian Sci, 2002; 15:21-32.
4. Sorum HL, Abee-Lund TM. Antibiotic resistance in food-related bacteria - a result of interfering with the global web of bacterial genetics. Int. J Food. Microbiol. 2002; 78:43-56.
5. Sridevi M, Kondala Rao B, Sathiraju D. Sensitivity of Bacteria isolated from Champavathi estuary to some medicinal plants of Vizianagaramn dist., East coast of India. Drug Invent. Today. 2010; 2(7):366-368.
6. Istvan U. Seminatural products and related substances as alleged botanical pesticides. Pest Manage. Sci. 2000; 56(8):703-705.
7. Devasahayam TPA, Sainis SK. Immune system and antioxidants, especially those derived from Indian medicinal plants. Indian J Expt. Biol. 2002; 40:639.
8. Harikrishnan R, Nisha Rani M, Balasundaram C. Hematological and biochemical parameters in common carp, *Cyprinus carpio,* following herbal treatment for *Aeromonas hydrophila* infection. Aquaculture. 2003; 221 (1-4):41-50.
9. Agbon AO, Omoniyi IT, Teko AA. Acute toxicity of tobacco (*Nicotina tobaccum*) leaf dust on *Oreochromis niloticus.* J Aquat. Sci. 2002; 31:931-936.
10. Omoniyi I, Agbon AO, Sodunke SA. Effect of lethal and sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust extract on weight and haematological changes in *Clarius gariepinus* (Burchell). J. Appl. Sci. Environ. Mgt. (JASEM). 2002; 6(2):37-41.
11. Citarasu T, Venkataramalingam K, Babu MMR, Sekar RJ, Petermarian M. Influence of the antibacterial herbs, *Solanum trilobatum, Andrographis paniculata* and *Psoralea corylifolia* on the survival, growth and bacterial load of *Penaeus monodon* post larvae. Aquacult. Int. 2003; 11:581-595.
12. Roopavathy J, Krishnamoorthy P, Sukumaran M, Rajeswar K. Effect of *Andrographis paniculata* leaves extract on ovary of adult ornamental fish black molly (*Mollienisia latipinna*). Adv. Appl. Sci. Res. 2011; 2(4):314-319.
13. Ekanem AP, Meinelt T, Kloas W, Knopf K. Effects of the extracts of two African fish poison plants, *Asystasia vogeliana* and *Tephrosia vogeli* on embryos of zebra fish (*Danio rerio*). Bull. Environ. Contam. Toxicol. 2003; 71:551-556.
14. Patra BC, Kwon MG, Lee YH, Shin IS, Min SM. Effects of supplemental Undaria, Obosan and Wasabi in the experimental diets on growth, body composition, blood chemistry and non-specific immune response of juvenile flounder, *Paralichthys olivaceus.* J Aquacult. 2003; 16:210-215.
15. Ram P Yadav, Digvijay Singh, Singh SK, Ajay Singh. Metabolic changes in freshwater *Channa punctatus* due to stem bark extract of *Croton tiglium.* Pak. J Biol. Sci. 2003; 6(14):1223-1228.
16. Dongmeza E, Siddhuraju P, Francis G, Becker K. Effects of dehydrated methanolextracts of Moringa (*Moringa oleifera*) leaves and three of its fractions on growth performanceand feed nutrient assimilation in Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture. 2006; 261(1):407-422.
17. Rao YV, Das BK, Jyotyrmayee P, Chakrabarti R. Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila.* Fish and Shellfish Immunol. 2006; 20:263-273.
18. Christybapita DM, Divyagnaneswari M, Michael RD. Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus.* Fish Shellfish Immunol. 2007; 23(4):840-852.
19. Immanuel G, Uma RP, Iyapparaj P, Citarasu T, Punitha Peter SM, Michael Babu M. *et al*. Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia, *Oreochromis mossambicus.* J. Fish Biol. 2009; 74:1462-1475.
20. Yin G, Cao L, Xu P, Jency G, Nakao M. Hepatoprotective and antioxidant effects of *Hibiscus sabdariffa* extract against carbon tetrachloride - induced hepatocyte damage in *Cyprinus carpio. In vitro* Cell. Dev. Biol. Anim. 2011; 47(1):10-15.
21. Farahi A, Kasiri M, Sudagar M, SoleimaniIraei M, Zorriehzahra SMJ. Effect of dietary supplementation of *Melissa officinalis* and *Aloe vera* on haematological traits, lipid oxidation of carcass and performance in rainbow trout (*Oncorhynchus mykiss*). Online J. Anim. Feed Res. 2012; 2(1):1-5.
22. Saravanan M, Ramesh M, Malarvizhi A, Petkam R. Toxicity of neem leaf extracts (*Azadirachta indica* A. Juss) on some haematological, ionoregulatory, biochemical and enzymological parameters of Indian major carp, *Cirrhinus mrigala.* J Trop. Forestry and Environment. 2011; 1(1):14-26.
23. Ravikumar S, Palani Selvan G, Anitha Anandha Gracelin N. Antimicrobial activity of medicinal plants along Kanyakumari coast, Tamilnadu, India. Afr. J Basic Appl. Sci. 2010; 2(5-6):153-157.
24. Prasad, MR. Abhishek Kumar, Diwakar Mishra, Sunil K Srivastav, Ajai K Srivasta. Alterations in blood electrolytes of a freshwater catfish *Heteropneustes fossilis* in response to treatment with a botanical pesticide, *Nerium indicum* leaf extract. Fish Physiol. Biochem. 2011; 37:505-510.
25. Nargris A, Khatun M, Talukder D. Use of medicinal plants in the remedy of fish diseases. Bangladesh Res. Publ. J 2011; 5(3):192-195.
26. Bello OS, Olaifa FE, Emikpe BO, Ogunbanwo ST. The effect of Walnut (*Tetracarpidium conophorum)* leaf and onion (*Allium cepa*) bulb extracts as antimicrobial agents for fish. Afr. J Microbiol. Res. 2012a; 7(19):2027-2033.
27. Bello OS, Emilpe BO, Olaifa FE. The Body Weight Changes and Gut Morphometry of *Clarias gariepinus* juveniles on Feeds Supplemented with Walnut (*Tetracarpidium conophorum)* Leaf and Onion (*Allium cepa*) Bulb Residues. Int. J Morphol. 2012b; 30(1):253-257.
28. Narendra K, Swathi J, Sowjanya KM, Krishna Satya A. *Phyllanthus niruri:* A review on its Ethnobotanical,

Phytochemical and Pharmacological Profile. J Pharm. Res. 2012; 5(9):4681-4691.
29. Rajesh kumar NV, Joy KL, Girija K, Ramsewak RS, Nair MG, Ramadasan K. Antitumor and anticarcinogenic activity of *Phyllanthus amarus* extract. J Ethnopharmacol. 2002; 81:17-22.
30. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. Afr. J Biotechnol. 2006; 5(4):357-361.
31. Raka Kamal, Manas Mathur, Jaya Sharma, Ruchi Pareek. Studies on non- enzymatic antioxidants of *Phyllanthus niruri* L. Elixir Bio Tech. 2012; 48B:9700-9703.
32. Shanmugam B, Shanmugam KR, Ravi S, Subbiah GV, Mallikarjuna K, Reddy KS. Antibacterial activity and phytochemical screening of *P. niruri* in ethanolic, methanolic and aqueous extracts. Int. J Pharm. Sci. Rev. Res. 2014; 27(2):85-89.
33. Petrusewiez K, MacFadyen A. Productivity of terrestrial animals. IBP Hand-Book, Blackwell Scientific Publications, Oxford. 1970; 13:190.
34. Lowry OH, Rosebrough NJ, Farr AL, Randell RJ. Protein measurement with the folin phenol reagent. J Biol. Chem. 1951; 193:265-275.
35. Seiffer S, Dayton S, Novic B, Muntwyler E. The estimation of glycogen with the anthrone reagent. Arch. Biochem. 1950; 25:191-200.
36. Folch JM, Less GM, Stanley GH. A simple method for isolation and purification of total lipids from animal tissues. J Biol. Chem. 1957; 226:497-509.
37. Bhosale SV, Bhilave MP, Nadaf SB. Formulation of fish feed using ingredients from plant sources. Res. J Agric. Sci. 2010; 1:284-287.
38. Sivaram V, Babu MM, Immanuel G, Murugadass S, Citarasu T, Marian MP. Growth and immune response of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against *Vibrio harveyi* infections. Aquaculture. 2004; 237:9-20.
39. Shalaby SMM, Abd Elmonem AI, El-Dakar AY. Enhancement of growth performance, feed and nutrient utilization of Nile tilapia (*Oreochromis niloticus*) using licorice roots (Erksous) as a feed attractive. J Egypt. Acad. Soc. Environ. Develop. (B- Aquaculture), 2003; 4:119-142.
40. El-Dakar AY, Hassanien GDI, Gad SS, Sakr SE. Use of medical and aromatic plants in fish diet: 1. Effect of dried marjoram leaves on performance of hybrid tilapia *Oreochromis niloticus* x *Oreochromis aureus,* fingerlings. J Egypt. Acad. Soc. Environ. Develop. (B-Aquaculture). 2004a; 5:67-83.
41. El-Dakar AY, Hassanien GDI, Gad SS, Sakr SE. Use of medical and aromatic plants in fish diet: 2. Effect of dried basil leaves on performance of hybrid tilapia *Oreochromis niloticus* x *Oreochromis aureus,* fingerlings. 3rd Inter. Conf. on Animal Production and Health in Semi-Arid Areas, Suez Canal University. 2004b, 265-277.
42. Sampath K, Pandian TJ. Interaction of feeding frequency and density on feed utilization in air-breathing murrel, *Channa striatus*. Proc. Indian Acad. Sci. 1984; 93:445-453.
43. James R, Muthukrishan J, Sampath K. Effect of food quality on temporal and energetics cost of feeding in *Cyprinus carpio* (Cyprinidae). J Aqua. Trop. 1993; 8:47-53.
44. James R, Sampath K. Effect of different feeds on growth and fecundity in ornamental fish, *Betta splendens* (Regan) Indian J Fish. 2002; 49:279-285.
45. Prasad G, Mukthiraj S. Effect of methanolic extract of *Andrographis paniculata* (Nees) on growth and haematology of *Oreochromis mossambicus* (Peters). World J Fish Mar. Sci. 20011; 3(6):473-479.
46. Citarasu T. Herbal biomedicines: a new opportunity for aquaculture industry. Aquacult. Int. 2010; 18(3):403-414.
47. Rinna Hamlin S, Jansi M, Vasudhevan I. Effect of dietary *Spirulina plantensis* on feeding parameters of blue gourami, *Trichogaster trichopterus*. Int. J Res. Fish. Aquac. 2013; 3(3):103-106.
48. James R, Sampath K, Thagarathinam R, Vasudevan I. Effect of dietary Spirulina level on growth, fertility, coloration and leucocyte count in Red Swordtail, *Xiphophorus helleri*. Isr, J Aqua-Bamidgeh. 2006; 58(2):97-104.
49. Smith MAK. Estimation of growth potential by measurement of tissue protein synthetic rates in feeding and fasting rainbow trout, *Salmo gairdneri* Richardson. J Fish Biol. 1981; 19(2):213-220.
50. Arumugam Sivagurunathan A, Xavier Innocent NB, Guru Saraswathi S, Mariappan A. Immunostimulatory potential of dietary amla (*Phyllanthus emblica*) in growth and haematology of *Tilapia mossambicus* challenged with *Pseudomonas aeruginosa*. Int. Res. J Pharmacy. 2012; 3(7):165-168.
51. Mohsen Abdel-Tawwab, Mohammad H, Ahmad Medhar EA, Seden Saleh FM, Sakr. Use of Green Tea, *Camellia sinensis* L. in Practical Diet for growth and production of Nile Tilapia, *Oreochromis niloticus* (L.) against *Aeromonas hydrophila* Infection. J World Aquacult. Soc. 2010; 41:203-213.
52. Francis G, Makkar HPS, Becker K. Quillaja saponins - a natural growth promoter for fish. Animal Feed Sci. Tech. 2005; 121:147-157.
53. Padian T, Raghuraman R. Effects of feeding rates on conversion efficiency and chemical composition of the fish, *Tilapia mossambica* L Mar. Biol. 1972; 12:129-136.
54. Brett JR, Groves TDD. Physiological Energetics. In: Fish Physiology, WS. Hoar, DJ. Randall and JR. Brett (eds.), Academic Press, New York, 1979; VIII:279-352.
55. Baker DH. Equalized versus ad libitum feeding. Nutr. Rev. 1984; 42:269-273.
56. Ali SN, Al-Ogaily N, Al-Asgah, Gropp J. Effect of sublethal concentrations of copper on the growth performance of *Oreochromis niloticus*. J Appl. Ichthyol. 2003; 19:183-188.
57. Sarnowski P. The effect of metals on swim bladder inflation of common carp (*Cyprinus carpio* L.) larvae. Elect. J Polish Agric. Univ. Fish. 2004; 7:172-181.
58. Aly SM, Mohamed MF. *Echinacea purpurea* and *Allium sativum* as immunostimulants in fish culture using Nile tilapia (*Oreochromis niloticus*). J Anim. Physiol. Anim. Nutr. (Berl.). 2010; 94(5):e31-9.
59. Ferreira JG, Hawkins AJS, Bricker SB. Management of productivity, environmental effects and profitability of shellfish aquaculture - the Farm Aquaculture Resource Management (FARM) Model. Aquaculture. 2007; 264:160-174.
60. Nakano T, Tomlinson N. Catecholamines and carbohydrate concentration in rainbow trout, *Salmo*

gairdneri in physical disturbance. J Fishery Res. Board of Canada. 1967; 24:1701-1715.
61. Lee DH, Ra CS, Song YH, Sung KI, Kim JD. Effect of dietary garlic extracts on growth, feed utilization and whole body composition of juvenile sterlet sturgeon (*Acipenser ruthenus*). Asian Australas J Anim. Sci. 2012; 25:577-583.
62. Durmaz H, Sevgiler Y, Uner N. Tissue-specific antioxidative and neurotoxic responses to diazinon in *Oreochromis niloticus.* Pest. Biochem. Physiol. 2006; 84:251-226.
63. Sudhansuh Tiwari. Toxicological and biochemical effect of plant origin pesticides on freshwater teleost. DDU Gorakhpur University, UP, India, 2008.
64. Matos P, Fontainhas-Fernandes A, Peixoto F, Carrola J, Rocha E. Biochemical and histological hepatic changes of Nile tilapia, *Oreochromis niloticus* exposed to carbaryl Pesticide, Biochem. Physiol. 2007; 89:73-80.
65. Diaz AO, Escalante AH, Garcia AM, Goldenberg AL. Histology and histochemistry of the pharyngeal cavity and oesophagus of the silverside *Odontesthes bonariensis* (Cuvier and Valenciennes). Anat. Histol. Embryol. 2006; 35:42-46.
66. Nguyen. Thi Thanh Thuy. Effects of *Mentha piperita* (L.) extract mixed diet on hepatic histological alteration and disease resistance against *Aeromonas hydrophila* infection in rohu (*Labeo rohita* Ham.). J Mar. Sci. Tech. 2008; 8(1).
67. Dhayanithi NB, Ajith Kumar TT, Balasubramanian T. Effect of *Excoecaria agallocha* leaves against *Aeromonas hydrophila* in marine ornamental fish, *Amphiprion sebae.* Indian J Geo-Marine Sci. 2012; 41(1):76-82.
68. Logambal SM, Venkatalakshmi S, Micheal RD. Immunostimulatory effect of *Ocimum sanctum* Linn. in *Oreochromis mossambicus* (Peters). Hydrobiologia. 2000; 430(1):113-120.
69. Abutbul S, Golan-Goldhirsh A, Barazani O, Zilberg D. Use of *Rosmarinus officinalis* as a treatment against *Streptococcus iniae* in tilapia (*Oreochromis* Sp.). Aquaculture. 2004; 238:97-105.
70. Vijayakumari Pratheepa, Nataraja Pillai Sukumaran. Effect of *Euphorbia hirta* plant leaf extract on immunostimulant response of *Aeromonas hydrophila* infected *Cyprinus carpio.* Peer J 2014; 2:e671.