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Pseudomonad Infection in *Channa* Causes Changes in Behavior, Growth and Hematological Parameters with Reference to its Control by Plant Extract

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ABSTRACT

The present study was carried out to investigate the pathogenecity of *Pseudomonas* in freshwater fish, *Channa punctata* (Bloch 1793). Each fish excepting from control-I was intraperitoneally injected with 0.2 ml of the experimental bacterium (*Pseudomona aeruginosa*) (Treatment : T1, T2, T3, T4, T5 and T6) and control-II fish was injected with 0.2 ml of agar broth without bacterium, using 21/gauge sterile needle. Behavioral changes, growth parameters, blood parameters and histopathological changes have also been observed in the experimental fishes. Decrease in length and weight loss of infected fish is common features. Antagonistic activity of the plant extract mixture of *Allium sativum* and *Piper nigrum* against *P. aeruginosa* confirmed by restoration of normal behavior, growth rate ($P > 0.05$), normalization of physiological and biochemical changes of the fishes.

Keywords *Pseudomonas*, Fish behavior, *Allium sativum*, *Piper nigrum*.

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INTRODUCTION

An aquaculture practice in India has evolved as a viable commercial farming with diversified species and system. Intensive and semi-intensive fish farming are only the way to increase fish production, but it increases the risk of disease outbreaks (Nandi et al. 2016). Fishes are very much prone to a wide range of pathogenic bacterial strains (Banerjee et al. 2016). Bacterial diseases in fish are the major component that hamper the production of fish and affect the country economy.

The virulent bacteria secrete tissue degrading enzymes and toxins to escape from the immune defense of the host (Shayo et al. 2012). Among the etiological agents of bacterial fish diseases *Pseudomonas* is considered one of the most harmful fish pathogens. These bacteria are responsible for ulcer type diseases including ulcerative syndrome, bacteria haemorrhagic septicaemia, tail and fin rot, bacteria gill rot and dropsy (Paniagua et al. 1990).

Limited studies have been done on the effect of bacterial (Pseudomonads) infection on the behavior changes, growth parameter and on the hematological parameters in fresh water fishes in India.

Previously it was common practice to use antibiotics for avoidance and control of diseases in fish. However, the misuse of antibiotics leads to creation of antibiotic resistance among pathogenic bacteria (Romero et al. 2012). Many plant products such as

Aloe vera, onion, ginger, garlic, neem, peppermint and rosemary have been used as immunity stimulation and growth promoter in aquaculture (Hoseinifar et al. 2017). In aquaculture operations, garlic promotes growth, enhances immunity, stimulates appetite and strengthens the control of bacterial and fungal pathogens. These effects of garlic are due to the presence of various organosulfur compounds, including allicin which can activity kill a wide range of pathogens like fungi, bacteria and viruses (Nya and Austin 2009).

Medicinal plant such as *Piper nigrum* have been used in the treatment of bacterial infections in aquaculture. It contains an alkaloid called piperine which is also known for its significant pharmacological actions, including antioxidant, immunomodulatory, hepatoprotective, antifungal and antibacterial activities (Srinivasan 2009).

Here, the second objective is that use of plant extract to control the adverse of the pathogenic bacteria, *P. aeruginosa* on fresh water test fishes, *Channa* and reclamation of normal behavior, growth, physiological and biochemical parameters of the experimental fishes.

MATERIALS AND METHODS

Collection and acclimatization of fish

A 60 days experiment was setup for the study of the artificial bacterial infection of the fishes. Healthy (without external disease symptom) Fish, *Channa punctata* (weight: 32.50±0.8 g and length 11.5±0.40 cm) were collected from local fish market and acclimatized for 7 days in glass aquaria under controlled laboratory conditions. The tank/aquaria (50 L) were laid out in a completely Randomized Block Design (Gomez and Gomez 1984) with three replicates.

Artificial infection and LD50 study

The fish pathogenic strain *Pseudomonas aeruginosa PB112* (*JN996498*) was collected from the Parasitology Laboratory, Department of Zoology, University of Kalyani. The bacteria were then again cultured on nutrient agar and selective medium (Hi-Media). A direct suspension of the test organisms was prepared in broth following standard method of Ghosh et al. (2014).

LD50 dose of bacteria was determined by using of healthy, disease free test fish, *Channa*. The test fishes were inoculated intraperitoneally @ 0.2 ml $fish^{-1}$ with *Pseudomonas* bacterial cell suspension (0.4 % –TSA) consists of 2×10^2 to 2×10^6 CFU/ml of live pathogenic bacteria. The value of LD50 was determined using formula of Reed and Muench (1938). In each experimental fish, RI-0.2 ml of 2.0×10^2 CFU/ml, R2-02 ml of 2.0×10^3 CFU/ml, R3-0.2 ml of 2.0×10^4 CFU/ml, R4-0.2 ml of 2.0×10^5 CFU/ml and RS-0.2 ml of 2.0×10^6 CFU/ml of the isolated bacterial suspension was intraperitoneally injected using 21 gauge sterile needle ; and control-II fish received 0.2 ml of 0.4% (TSA) broth without bacterium. The observation time was 20 days. Challenged fish have been reserved in aquaria filled with fresh tap water.

Preparation of plant extract

Plant extract mixture of *Allium sativum* cloves and *Piper nigrum* seed have been prepared following the methods described below :

i. Each of the dried samples of *Allium sativum* cloves have been grinded to powder using a blender. A 10 g powder of garlic cloves were aked in 40% of methanol. The supernatant from extract (40% of methanol) were collected and labeled as supernatant extract of clove. These extracts were labeled as crude extract of clove and crude extract of peel (Natasya-Ain et al. 2018).

ii. The powdered dry sample of *Piper nigrum* seeds have been extracted by Soxlet equipment. 1g of seed extract was dissolved in 2ml dimethylsulphoxide (DMSO), and made up to 100 ml with deionized water. The stock solution containing 10 g/L of the extract was used then utilized for the different concentration (Ekanem et al. 2004).

The prepared plant mixture have been mixed in the 1 : 1 (volume/volume), in three different concentration.

Table 1. Formulation and proximate composition of 1 kg reference and experimental diet.

Ingredients (%)	Control-I	Control-II	T1 2.0×10^3 CFU/ml patho-genic bacteria/ fish	T2 2.0×10^4 CFU/ml patho-genic bacteria/ fish	T3 2.0×10^5 CFU/ml patho-genic bacteria/ fish	T4 2.0×10^3 CFU/ml patho-genic bacteria/ fish	T5 2.0×10^4 CFU/ml patho-genic bacteria/ fish	T6 2.0×10^5 CFU/ml patho-genic bacteria/ fish
Mustard oil cake (g)	388	388	388	388	388	388	388	388
Rice bran (g)	392	392	392	392	392	392	392	392
Fishmeal (g)	200	200	200	200	200	200	200	200
Vitamin premix[1] (g)	5	5	5	5	5	5	5	5
Mineral premix[2] (g)	5	5	5	5	5	5	5	5
Cr_2O_3 (g kg diet^{-1})	10	10	10	10	10	10	10	10
Extract of *Allium sativum* and *Piper nigrum* (*1 : 1, v/v*)	–	–	–	–	–	10 mg/ml+ 10 mg/ml	20 mg/ml+ 20 mg/ml	30 mg/ml+ 30 mg/ml
Binding of final experimental diet								
CMC (gkg diet^{-1})	5	5	5	5	5	5	5	5
Proximate composition (% dry matter basis)[3]								
Protein	30.88	30.88	30.88	30.88	30.88	30.60	30.60	30.60
Lipid	11.63	11.63	11.63	11.63	11.63	12.24	12.24	12.24
Crude fiber	3.80	3.80	3.80	3.80	3.80	4.60	4.60	4.60
Moisture	7.00	7.00	7.00	7.00	7.00	7.20	7.20	7.20
Ash	11.40	11.40	11.40	11.40	11.40	11.60	11.60	11.60
Dry matter	93.00	93.00	93.00	93.00	93.00	92.80	92.80	92.80
NFE	46.67	46.67	46.67	46.67	46.67	44.76	44.76	44.76
Gross energy (kJ g^{-1})[4]	18.64	18.64	18.64	18.64	18.64	18.61	18.61	18.61
P : E Ratio	16.58	16.58	16.58	16.58	16.58	16.76	16.76	16.76

Growth trial

In each experimental fish, T1-0.2 ml -of 2.0×10^3 CFU ml^{-1}, T2-0.2 ml of 2.0×10^4 CFU ml^{-1}, T3–0.2 ml of 2.0×10^5 CFU ml^{-1}, T4-0.2 ml of 2.0×10^3 CFU ml^{-1} and T5-0.2 ml of 2.0×10^4 CFU ml^{-1} and T6-0.2 ml of 2.0×10^5 CFU ml^{-1} of the isolated bacterial suspension was intramuscularly injected using 21 gauge sterile needle (Table 1). The control-II fish received 0.2 ml of 0.4% TSA broth without bacterium and another 20 fish were held untreated control-I.

The growth trial was conducted on living fishes of control (I and II), T1, T2, T3, T4, T5 and T6 fishes. Total voluntary diet intake rate by the fish and apparent protein digestibility (APD) of diet for each fish were determined from the trial using standard methods of Mondal et al. (2007). Increase in length and weight, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU) have been calculated using standard methods (Steffens 1989).

Behavioral study

The behavioral tests of the infected and uninfected fish have been conducted following the method of Gabagambi et al. (2019). In this study, the effects of *P. aeruginosa* on *Channa* have been investigated by examining three behavioral traits-swimming ; coughing, air-gulping and feeding.

Blood parameter analyses

Initial fish blood samples were collected before feeding trial by cutting the caudal peduncle of the experimental fishes (Keene et al. 1988).Subsequent blood samples were collected on 60th day. The hemoglobin, RBC, WBC and PCV were determined by the method of Blaxhall and Daisely (1973). The Mean corpuscular volume (MCV) was calculated assording to Seiverd (1964). Erythrocyte indices, such as MCHC and MCH, were calculated Dacie and Lewis (1984). Protein in liver tissue was determined by the method of Lowry et al. (1951), and the activities of the alkaline phosphatase (APL) was determined by the method of Walter and Schutt (1974). SGOT and SGPT activity were determined according to the method of Reitman and Frankel (1957).

Histopathological study

Fish tissue specimens of T3 infected experimental fish of the gills, liver and kidney have been removed and fixed in Bouin's fluid for 24 h, dehydrated. The fixed tissues were embedded in paraffin wax and sectioned into 7µm thick, stained with Hematoxylin-eosin and mounted in DPX. Then the sections have been examined under light microscope.

Monitoring of water qualities

Water sample were collected from the experimental tanks every week in the morning and physico-chemical parameters were analyzed following the standard methods of APHA 2005.

Statistical analyses

The nature of distribution of the observations of each response variable from the trials was verified by Kolmogorov–Smimov (K–S) and Shapiro–Wilks (S–W) tests to ensure a Gaussian distribution. Since all data were found mormally distributed they were subjected to Single factor ANOVA, without any further transformation, followed by least significant difference (LSD) test to compare mean between the treatments (Johnson and Wichern 1992).

RESULTS

Bacterial infection and LD 50 analysis

Five infective bacterial doses have been applied during the infection trial : R1–2.0×10^2 CFU/ml, R2–2.0×10^3 CFU/ml, R3–2.0×10^4 CFU/ml, R4–2.0×10^5 CFU/ml and R5–2.0×10^6 CFU/ml ; the doses were extremely virulent to the test fish of R3, R4 and R5. The nature of virulence of the bacterial strains was classified on the basis of formation of clinical signs and percentage mortalities. *P. aeruginosa* created disease symptoms in 20 h after inoculation, mortality in infected fishes was recorded after 45 h of infected bacterial inoculation. Infected fishes exhibited the same symptoms of naturally bacterial infected fishes like hemorrhages and red lesions on the body and high mucous secretion were observed in the infected fishes.

In *Channa*, R3 and R4 experimental fishes showed 70% and 90% mortality respectively during 5–6 days but R5 showed 100% mortality during 7–8 days after experiment. In this study 100% mortality have been recorded @ 0.2 ml suspension of 2.0×10^6 CFU/ml. The obtained LD50 values of *P. aeruginosa* for *Channa* was $2\times10^{4.63}$ CFU/ml. No mortality have been recorted in control-I and II experiment for both fishes.

Behavioral changes of the infected fishes

In the present study, uninfected control (I and II) fish behaved in natural manner and infected (T1, T2, T3 and T4) fishes differed. The effects of *P. aeruginosa* on *Channa* have been investigated by examining three different behavioral traits like swimming ; coughing, air-gulping and feeding behavior. Normal behavior have also been studied in T5 and T6.

Swimming behavior : Generally the host fish swims gently in aquarium. During slow speed pectoral and caudal fins played a vital role. The undulation of pectoral fins passes vertical waves down to the rear along the fins. During fast swimming lateral undulation of body have been noticed. Pectoral fins stood clamped to the side of the fish and undulated occasionally.

Table 2. Growth performance of *Channa* fed the reference and experimental diets. Values are mean ± SD (n=15). [abc]Means with dissimilar superscripts in the same column indicates significant difference (LSD) between the means at 5% level.

Diets	Diet intake (g/100g BW/d)	APD (%)	IBW (g)	FBW (g)	PIW	FCR	SGR (%/d)	PER	ANPU
Control-I	2.88	90.67	32.40[a]	57.30[a]	76.85[a]	2.60[a]	0.95[a]	1.22[a]	10.31[a]
	±0.07	± 0.62	± 0.10	±1.21	±3.74	± 0.13	± 0.03	± 0.05	± 0.16
Control-II	2.86	90.54	32.40[a]	56.73[a]	76.54[a]	2.66[a]	0.93[a]	1.19[a]	13.34[b]
	±0.06	± 0.68	± 0.10	±1.14	±2.60	± 0.12	± 0.03	± 0.05	±0.41
T1	2.52	89.29	32.40[a]	55.76[b]	72.11[b]	2.77[a]	0.90[a]	1.19[a]	15.21[c]
	±0.02	± 1.11	± 0.10	±0.96	±2.96	± 0.11	± 0.03	± 0.04	±0.14
T2	2.52	85.25	32.40[a]	52.16[c]	61.00[c]	3.27[b]	0.79[b]	1.00[b]	15.06[c]
	±0.02	± 0.25	± 0.10	±0.15	±0.40	± 0.09	± 0.01	± 0.01	±0.12
T3	2.41	75.96	32.40[a]	48.00[d]	48.14[d]	4.15[c]	0.65[c]	0.79[c]	15.22[c]
	±0.08	± 0.54	± 0.10	±0.60	±0.81	± 0.06	± 0.09	± 0.01	±0.14
T4	2.31	74.24	32.40[a]	46.86[e]	44.65[e]	4.47[d]	0.61[d]	0.73[c]	15.12[c]
	±0.08	± 0.26	± 0.10	±0.05	±0.17	±0.01	± 0.02	± 0.04	±0.13
T5	2.72	92.21	32.40[a]	57.40[a]	77.16[a]	2.59[a]	0.95[a]	1.22[a]	13.34[b]
	±0.04	± 0.70	± 0.10	±0.91	±2.82	±0.09	±0.03	±0.04	±0.38
T6	2.82[a]	92.05[a]	32.40[a]	59.00[f]	85.18[f]	2.43[a]	0.99[e]	1.30[a]	10.30[a]
	±0.04	± 0.62	± 0.10	±1.00	±3.08	±0.09	±0.02	±0.05	±0.16

Coughing, air-gulping : The operculae were clamped down and mouth was half-closed and opened again. Fresh air was taken into the buccal cavity and going out after use. During air gulping pelvic and dorsal fins are stretched slightly.

Feeding behavior : Changes in feeding behavior have also been noticed. If a small pellet of the feed was given from the top and the pellet settled down on the floor of the aquarium, then the fish comes close to the pellet and engulfed it. But when the size of the pellet of the feed was moderate the fish chewed it for a while following engulfed it and swallowed.

But the infected fishes (T1 to T4) exhibited irregular, erratic and darting swimming movements and loss of equilibrium. They slowly became lethargic, hyper excited, restless and secreted excess mucus all over the body of T1, T2, T3 and T4 fishes. Mucus secretion was also higher in T3 and T4 fishes. Initially opercular movements increased in infection periods but decreased further steadily as compared to unifected fish. Fishes gulped air from the surface frequently, swim at the surface and shoaling behavior has been disrupted in T3 and T4 fishes. Feeding appetite was also decreased in the infected fish in comparison the uninfected.

Table 3. Proximate composition of carcass (% wet weight) of *Channa*. Values are mean ±SD (n=5). [abc]Values in the same row with different supercripts are significantly different ($p<0.05$).

Components	Initial	Control-I	Control-II	T1	T2	Final T3	T4	T5	T6
Crude protein	12.40[a] ± 0.40	16.10[b] ± 0.26	16.24[b] ± 0.29	16.80[c] ±0.20	15.68[d] ± 0.04	14.90[e] ± 0.68	14.60[e] ±0.53	15.96[b] ±0.20	16.36[b] ±0.40
Crude lipid	4.16[a] ±0.20	4.48[a] ± 0.20	4.46[b] ± 0.10	3.42[c] ± 0.32	3.12[c] ± 0.32	3.18[d] ± 0.60	3.25[d] ± 0.60	3.20[d] ± 0.41	4.63[b] ±0.01
Ash	3.24[a] ±0.04	4.20[b] ± 0.04	4.60[b] ± 0.39	5.12[d] ± 0.22	4.80[c] ± 0.22	4.70[c] ± 0.02	4.10[b] ±0.02	3.45[d] ± 0.42	4.10[b] ±0.66

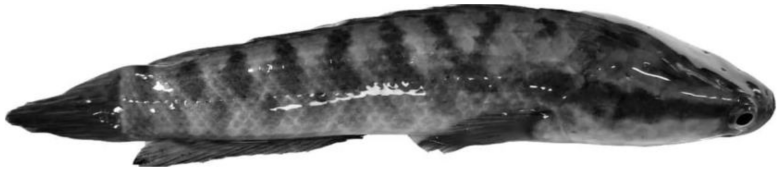

Plate 1. A normal uninfected fish ***Channa***

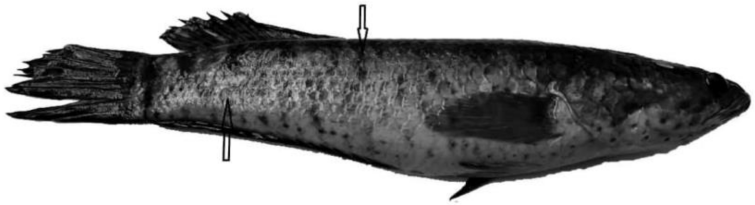

Plate 3. An infected (T2) fish ***Channa***

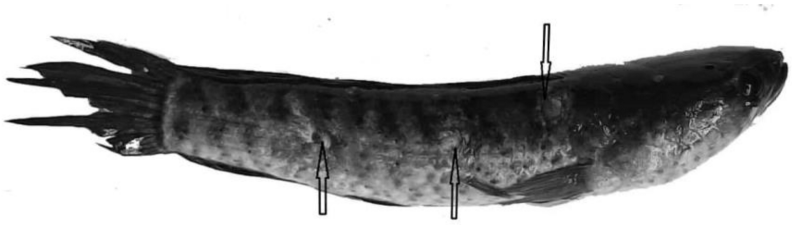

Plate 2. An infected (T3) fish ***Channa***

Plate 4. After recovery of the infected (T2) fish ***Channa***

Fig. 1. A normal uninfected fish *Channa*. **Fig. 2.** An infected (T3) fish *Channa*. **Fig. 3.** An infected (T2) fish *Channa*. **Fig. 4.** After recovery of the infected (T2) fish *Channa*.

Growth parameters

The weight of the healthy and bacterial infected fish have been recorded as (76.54–85.18) % and (44.65–71.11) % respectively. The length and weight were significantly decreased ($p < 0.05$) in the bacterial infected fishes (T1, T2, T3 and T4) than healthy control (I and II) and T5, T6 fishes (Table 2). The carcass

protein composition of was significantly decreased ($p < 0.05$) in the infected fishes (T1, T2, T3 and T4) than healthy control (I and II) and T5, T6 fishes (Table 3).

Clinical symptoms

Clinical abnormalities found in experimental fishes (T1, T2, T3 and T4) : clinical abnormalities including skin color darkness, loosening of scales, hemorrhages on the body surface, necrotizing ulcers on the skin, fin necrosis and mucous secretion (Figs. 2, 3). No clinical abnormalities or death were confirmed in the control (Fig. 1) and T5, T6 fishes. After treating with the plant extract mixture bacterial infected fish (T2) recovered to its normal physiological form (Fig. 4).

Changes in blood parameters, such as PCV, RBC, WBC, Hb, MCV, MCH, MCHC, ALP, SGPT and SGOT have been presented in Table 4. WBC and MCHC have been measured significantly higher values ($p < 0.05$) and RBC, Hb, PCV, MCV and MCH have been significantly lower ($p < 0.05$) in infected fishes than healthy fishes. The activity of liver enzymes, ALP, SGOT and SGPT in *Channa* during *P. aeruginosa* infection have been shown in Table 4. In the liver homogenate of *P. aeruginosa* infected test fishes, significant increase ($p < 0.05$) in ALP, SGPT and SGOT activities have been observed in comparison to control (I and II) T5, T6 fishes (Table 4).

Histopathological changes

The gills of *Channa* showed regular arrangement of filaments and the secondary gill in control (1) fish (Fig. 5a) where as the gills of infected fish (T3) showed degenerative changes in the gill filaments and necrosis in lamellar cells (Fig. 5b).

The normal kidney of control fish consists of a renal corpuscle, Bowman's capsule, glomerulus and different section of the renal tubules (Fig. 6a). But in bacterial infected fish (T3), the kidney showed decrease in number in renal cell, the proximal and distal collecting tubules. The kidney showed hyperplasia and degeneration leading to the total necrosis (Fig. 6b).

The healthy liver of control fish showed a typical compact cellular building with proper architecture of cells (Fig. 7a). The liver tissue of the *P. aeruginosa* infected fish (T3) showed loss of cellular architecture. Necrosis and inflammation of liver cells have also been observed in bacterial infected fishes (Fig. 7b).

Application of plant extract

Use of the plant extract mixture of *Allium sativum* and *Piper nigrum* in the diet, the mortality rate of fish decreased and the treated fish gradually recovered on day 14 and their ulcers completely healed on day 20 in the test fish, T4, T5 and T6.

Water quality parameters

Water quality parameters (temp 27.0–27.2°C, pH 7.2–7.6, dissolved oxygen 6.8–7.4 mg L^{-1}, free carbon dioxide 3.8–4.2 mg L^{-1}, total alkalinity 160–176 mg L^{-1} and total hardness 194–210 mg L^{-1} as $CaCO^3$) recorded during the experimental trial.

DISCUSSION

Bacterial diseases are the major obstruction in aquaculture industry throughout the world. *Pseudomonas* are considered as the most significant pathogenic bacteria for freshwater fishes (Kayis et al. 2009, El-Nagar 2010). Due to lack of knowledge useful control the disease often leads to high mortality causing heavy loss in the freshwater aquaculture.

The diet intake rate of the *Channa*, observed in the present investigation, was even higher in the experimental diets containing plant extract mixture. Uninfected and plant extract mixture treated experimental fishes, control (I and II), T5 and T6 of *Channa* showed maximum diet intake rate (2.72 to 2.86 g/100 g BW / day) which is higher than the infected fish, *Channa* (2.31 to 2.52 g / 100 g BW / day). Apparent protein digestibility (APD) of *Channa* was varying significantly between the experimental infected, plant extract treated infected and uninfected fishes. However, unlike APD the feed intake rate and the growth of *Channa* significantly varied between experimental infected and plant extract treated and uninfected fishes. Food intake rate of the diet was significantly higher in the uninfected and plant extract treated

Table 4. Hematological parameters of *Channa*. Values are mean ±SD (n=5). [abc]Values in the same row with different superscripts are significantly different ($p<0.05$)

	Control–I	Control-II	T1	T2	T3	T4	T5	T6
Hb (g/100 ml):	12.46[a]	12.86[a]	12.13[a]	12.66[a]	12.66[a]	12.67[a]	12.67[a]	12.68[a]
Initial value	± 0.04	± 0.39	± 0.22	± 0.04	± 0.22	± 0.02	± 0.42	± 0.41
Final value	13.30[a]	12.17[a]	9.54[b]	9.77[b]	9.46[b]	9.30[b]	12.37[a]	12.43[a]
(60th day)	± 0.04	± 0.39	± 0.29	± 0.39	± 0.30	± 0.39	± 0.39	± 0.39
RBC								
(×10^6 / ml):	3.41[a]	3.47[a]	3.44[a]	3.44[a]	3.50[a]	3.44[a]	3.50[a]	3.41[a]
Initial value	± 0.09	± 0.39	± 0.12	± 0.39	± 0.40	± 0.39	±0.20	± 0.12
Final value	3.51[a]	3.30[a]	2.44[b]	2.62[b]	2.51[b]	1.70[c]	3.44[a]	3.34[a]
(60th day)	± 0.21	± 0.39	± 0.04	± 0.39	± 0.20	± 0.15	±0.14	±0.11
WBC								
(×10^3/ml):	12.77[a]	12.77[a]	13.05[a]	12.92[a]	12.82[a]	12.84[a]	12.84[a]	13.07[a]
Initial value	± 0.04	± 0.05	± 0.04	± 0.39	± 0.39	± 0.21	±0.39	±0.39
Final value	12.84[a]	13.12[a]	14.10[b]	14.40[b]	14.63[b]	14.73[b]	12.77[a]	12.77[a]
(60th day)	± 0.04	± 0.04	± 0.04	± 0.15	± 0.39	± 0.14	± 0.12	±0.39
PCV (%)	29.06[a]	30.80[a]	31.86[b]	32.33[b]	30.73[b]	31.06[b]	31.46[b]	31.06[a]
Initial value	± 0.04	± 0.04	± 0.51	± 0.39	± 0.04	± 0.11	±0.12	± 0.39
Final value	31.36[a]	31.63[a]	29.06[b]	25.65[c]	24.99[c]	22.66[d]	31.20[a]	33.00[e]
(60th day)	± 0.30	± 0.11	± 0.15	± 0.14	± 0.39	± 0.12	± 0.39	±0.14
MCV (fl/cell):	143.00[a]	144.33[a]	144.67[a]	144.00[a]	144.33[a]	149.00[b]	144.67[a]	143.33[a]
Initial value	± 0.40	± 0.39	± 0.30	± 0.19	± 0.39	± 0.39	± 0.39	±0.39
Final value	147.33[a]	146.33[a]	105.00[a]	101.67[a]	104.00[a]	107.33[a]	145.33[a]	143.33[a]
(60th day)	± 0.39	± 0.11	± 0.14	± 0.16	± 0.39	± 0.20	±0.22	±0.39
MCH (pg):	44.60[a]	44.66[a]	45.00[b]	45.33[a]	44.00[a]	44.33[a]	44.33[a]	45.00[b]
Initial value	± 0.39	± 0.14	± 0.14	± 0.12	± 0.12	± 0.39	±0.20	±0.40
Final value	45.00[a]	44.33[a]	36.00[b]	34.33[b]	31.07[b]	34.67[b]	45.67[a]	46.00[b]
(60th day)	± 0.39	± 0.11	± 0.12	± 0.12	± 0.30	± 0.11	±0.12	±0.39
MCHC (g/dL):	25.90[a]	25.50[a]	25.17[a]	25.50[a]	24.50[a]	25.77[a]	25.47[a]	26.13[a]
Initial value	± 0.39	± 0.30	± 0.30	± 0.30	± 0.30	± 0.20	±0.39	±0.20
Final value	26.17[a]	25.83[a]	44.10[b]	44.43[b]	46.06[c]	49.20[d]	25.67[a]	26.33[a]
(60th day)	± 0.12	± 0.39	± 0.14	± 0.39	± 0.25	± 0.14	±0.39	±0.20
ALP (IU/L):	14.07[a]	13.87[a]	13.87[a]	14.13[a]	14.17[a]	14.03[a]	14.20[a]	13.80[a]
Initial value	± 0.20	± 0.14	± 0.12	± 0.12	± 0.14	± 0.39	±0.14	±0.39
Final value	13.83[a]	14.17[a]	40.67[b]	47.00[c]	49.20[d]	52.53[c]	14.43[a]	14.27[a]
(60th day)	± 0.20	± 0.15	± 0.39	± 0.22	± 0.20	± 0.39	±0.24	±0.22
SGPT (IU/L):	14.37[a]	14.60[a]	14.63[a]	14.60[a]	14.63[a]	14.43[a]	14.43[a]	14.43[a]
Initial value	± 0.14	± 0.10	± 0.12	± 0.10	± 0.12	± 0.14	±0.15	±0.39
Final value	15.30[a]	15.73[a]	41.00[b]	41.67[b]	49.33[c]	49.67[c]	14.43[d]	14.47[d]
(60th day)	± 0.10	± 0.05	± 0.08	± 0.09	± 0.10	± 0.20	±0.05	±0.08
SGOT (IU/L)	25.16[a]	24.63[a]	24.96[a]	24.63[a]	24.76[a]	24.97[a]	24.77[a]	24.70[a]
Initial value	± 0.05	± 0.04	± 0.08	± 0.10	± 0.20	± 0.14	±0.05	±0.08
Final value	24.63[a]	24.80[a]	37.67[b]	42.80[c]	47.00[d]	51.00[e]	24.10[a]	24.10
(60th day)	± 0.02	± 0.08	± 0.09	± 0.07	± 0.06	± 0.16	± 0.14	±0.24

infected fishes than the infected fishes. Higher APD of the fishes have clearly indicated the better growth due to the well balanced amino acids and unknown growth promoting factor of the plant extract treated infected fishes.

Growth of fish primarily depends on dietary protein and digestibility of the protein feed supplemented diets. In the present study the crude protein level in the diet was isonitrogenus, i.e., 30.90%. Interestingly, best growth was obtained in fish, *Channa* of fish fed the diets Control (I and II), T5, T6 as compared to T1, T2, T3 and T4. Protein digestibility correlated significantly to feed efficiency and growth as measured by FRC, SGR, PER and ANPU. The experimental fishes of Control. T5, T6 showed better performance

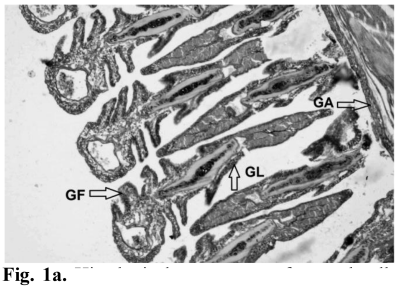

Fig. 1a.

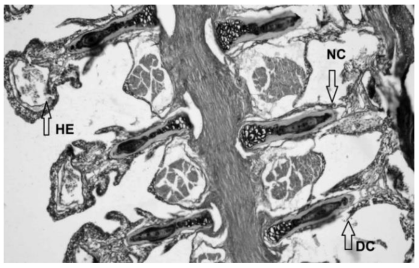

Fig. 1b.

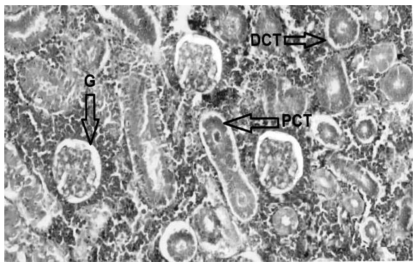

Fig. 1 c.

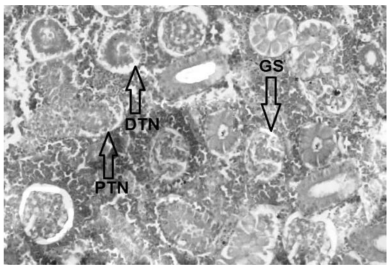

Fig. 1d.

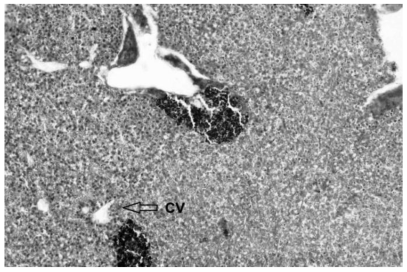

Fig. 1e.

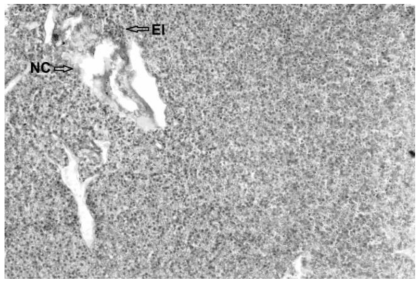

Fig. 1f.

Fig. 5a. Histological appearance of normal cells in the gill tissue of fish, *Channa punctata* from control-I group, GL (Gill lamellae), GF (Gill Filament), GA (Gill Arch) (H & E, -200). **Fig. 5b.** Histological appearance of hyperplasia, hemorrhage (HE), Degenerative cell (DC), necrosis of cell (NC) in primary and secondary gill lamellae of infected fish (T3), *Channa punctata*, (H & E, -200). **Fig. 6a.** Histological appearance of kidney tissue of control-I fish, *Channa punctata*, showing normal structure PT-Proximal tubule, G-Glomerulus and DT-Distal tubule (H & E, X-200). **Fig. 6b.** Histological appearance of kidney tissue of bacterial infected fish (T3), *Channa punctata*, showing necrotic structure of PTN–Necrotic Proximal tubule, GS–Glomerular shrinkage and necrotic structure of DTN–Necrotic Distal tubule (H & E, X-200). **Fig. 7a.** Histological appearance of liver tissue of control-I fish, *Channa punctata,* normal structure with central vein (CV) (H & E, X-100) (H & E, X-200). **Fig. 7b.** Histological appearance of liver tissue of infected fish (T3), *Channa punctata*, showing hyperplasia, hemorrhage (HE), necrosis of cell (NC) erythrocytes infiltration (EI) (H & E, X-200).

with regard to final body length, body weight and SGR. The FCR was the least in Control, T5, T6 ; and experimental fishes indicating highest efficiency. Both PER and ANPU also showed the highest value in Control, T5, T6 as compared to T1, T2, T3 and T4 experimental fishes.

Experimental fish, *Channa* in control (I and II), T5 and T6 swims in a usual teleost manner. Air gulping in the fish is a common behavioral character like that of other air breathing fishes. Feeding and air gulping behavior is similar to *Macropodus cupanus* (Pal 1969). We found that the fish infected with P. aeruginosa were more easily caught and positioned themselves higher up in the water column than fish which were uninfected and healthy, suggesting that this foreign organisms may affect the behavior of its fish as earlier suggested by Loot et al. (2002).

Infected fishes (T1–T4) shows higher swimming activity, air-gulping behavior and low appetite than the uninfected (control-I and II) healthy (T5 and T6) one.

The clinical symptoms shown by infected fishes are hemorrhages, loosening of scales, skin lesions and erosions at the fins and mucous secretion. Hemorrhagic symptoms have been observed in internal organs such as gill, liver and kidney. Similar symptoms have also been reported by Panda et al. (2012). Huge secretion of mucus over the gill lamellae has been observed in infected fishes. Secretion of mucus over the gill, reduces the diffusion of oxygen (David et al. 2002). Hence, changes in behavior and malfunction in respiration can be due to the bacterial infection in fish.

Normal range and numbers of leukocytes cells are generally use to determine the immune reactions and diseases (Chandra et al. 2015). In this experiment WBC count and MCHC increases gradually in the infected fish (T1, T2, T3 and T4) than control (I and II) and T5 and T6 but PCV, RBC count, Hb, MCV, MCH decreases in infected fishes (T1, T2, T3 and T4) gradually than control (I and II) and T5 and T6. Changes in WBC have an important character in the assessment of the health of *Clarias gariepinus* (Gabriel et al. 2004). Increases in the WBC and MCHC quantities in infected fish were accepted as a response of cellular immune system to fungal infection. RBC count in healthy fishes was 3.30–3.50 ($\times 10^6$ cells/ mm^3) and in bacterial infected fishes was 1.70–2.44 ($\times 10^6$ cells / mm^3). Gene et al. (2005) said that the erythrocytes count of fish infected with parasite was significantly lower in contrast to those in uninfected and healthy fish. Hematological parameters like Hb, PCV, MCV and MCH was significantly decreased in the infected fishes.

The histopathological examination showed that the changes take place in gill, liver and kidney due to *P. aeruginosa* infection than control (I and II), T5 and T6 as earlier suggested by Alagappan et al. (2009). Some degenerative and necrotic changes in gill, liver and kidney to malfunction and damage of hepatic metabolism (Camargo and Martinez 2007) and kidney function. This can be corroborated with increase the level of enzymes like SGOT and SGPT.

In the present experiment, the significant increase ($p < 0.05$) in the activity of ALP, SGPT and SGOT in liver in the infected fishes (T1, T2, T3 and T4) gradually than control (I and II), T5 and T6. The increase in the serum ALP, SGPT and SGOT activity may be an indication of considerable clinical damage and histopathological changes caused by the infection in the liver (Chandra et al. 2015).

It was reported that the various plant extracts can control numerous bacterial diseases of fish (Rajendiran et al. 2008). The present study showed that application of plant extract mixture of *Allium sativum* and *Piper nigrum* in the diet helped complete recovery from *P. aeruginosa* infection and restoration of normal growth, behavior, altered physiological and biochemical changes of *Channa*.

Present study indicates that the bacteria, *P. aeruginosa* infected air breathing fish, *Channa* was more pronounced in the growth rate, behavioral, hematological and response. However, significant changes in behavior and blood parameters like increase in WBC and other leukocytes counts and decrease in RBC and Hb in the cultured *Channa* were indicative of bacterial infection in exposed fishes. However, plant extract mixture of *Allium sativum* and *Piper nigrum* can be used as a potent alternative to control *P. aeruginosa* infection as evidenced by recovery from infection and restoration of different physiological and biochemical indices.

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