The Pharma Innovation Journal 2023; 12(2): 3096-3101

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 3096-3101

www.thepharmajournal.com
Received: 10-12-2022
Accepted: 20-01-2023

Abhishek Kumar
Department of aquaculture, College of Fisheries, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Meenakshi Kumari
Department of aquaculture, College of Fisheries, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Avdhesh Kumar
Department of Aquaculture, College of Fisheries, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Correspondence Author:
Abhishek Kumar
Department of aquaculture, College of Fisheries, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Survey, documentation and antibiotic susceptibility test of bacterial disease from the culture ponds of Tarai region, Uttarakhand, India

Abhishek Kumar, Meenakshi Kumari and Avdhesh Kumar

Abstract
Aquaculture is in a phase of rapid development and growth and itensification of fish farming often leads to the emergence of infectious disease. The present study was conducted to survey the farms for the occurrence of bacterial disease from October 2012 to March 2013 in eight fish farms of Dineshpur, Kalinagar and Pantnagar of Tarai region, Udham Singh Nagar, Uttarakhand. A total of 27 number of fishes were infected with disease. The occurrence of disease was highest in the month of November followed by October and was nil in the month of February and March. The bacteria were isolated from the lesions in fins, tail erosion and skin haemorrhages and were identified as *Aeromonas* and *Pseudomonas* based on biochemical characterization tests. The disease identified were Fin rot, Tail rot and Aeromonasis. The isolates exhibited sensitivity to antibiotics Offloxacin, Tetracyclin, Amoxycillin, Sulphadiazine, Neomycin and Streptomycin and therefore can be used for control and treatment of bacterial disease, on the other hand it was observed that isolates were completely resistant to Amphotericin and Fluconazole.

Keywords: Survey, Tarai region, biochemical test, bacterial disease, antibiotic susceptibility

Introduction
Fish is one of the most important source of animal protein and has been widely accepted as an excellent source of protein, rich in vitamins, minerals and other elements for the maintenance of healthy body [6]. Recently, traditional aquaculture has changed into a science based economy and is commercially active, therefore, diseases of all kinds occur on a large scale. However, fish mortality is not only the criteria to evaluate the effect of fish disease but also the morbidity, which leads to weight losses and poor growth in surviving fish resulting in substantial losses to the farmers. The single largest cause of economic losses in aquaculture is disease [21]. Infectious diseases caused by viruses, bacteria and parasites are continuing threats to consistent aquaculture industry. With increasing intensification, the incidence of diseases is also expected to increase proportionately. The importance of containing the threat of diseases in aquaculture is also a matter of global concern especially with increased trade and increased transboundary movements of goods, which include live fish and other aquatic organisms. Infectious diseases of cultured fish are among the most notable constraints on the expansion of aquaculture and realization of its full potential [31, 20].
Some bacteria cause only surface diseases as skin or gill infections, especially flexibacteria, but some inflict systemic disease [32]. There are basically two types of bacteria producing disease – obligate pathogens and facultative pathogens. Both these types of bacteria become pathogenic when the fish is immuno-compromised by some form of stressor [18]. Gram negative bacteria cause epizootics in nearly all cultured species. Many of these bacteria capable of causing disease are considered by some to be saprophytic in nature. Theses bacteria only become infectious when fishes are physiologically unbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, overstocking, which allow opportunistic bacterial infections to proceed.
In aquatic habitat, Bacteria are extremely diverse. Within pond environment bacteria inhabit the water phase, the bottom sediment and of course live on plants, animals and detritus. Aquatic animals such as fish are directly in contact with the microflora which is already present in the environment. Micro-organism living in mucus on the surface, gills and in the digestive tract of fish in different water bodies vary in terms of composition and abundance. Bacterial diseases are common in all fish and occur most often when aquatic environmental conditions are not favourable. They occur in nursery, rearing and grow out ponds causing serious concern to the fish farmers.

Some of them often wipe out the entire population of fish. Some of the important bacterial pathogens are *Aeromonas hydrophila, A. salmonicida, Pseudomonas fluorescens, Pseudomonas putrifaciens, Flexibacter columnaris, Edwardsiella tarda. Vibrio alginolyticus* and *V. parahaemolyticus*, which have been identified as most common disease causing agents in the fish.

Among all other bacteria, *Aeromonas and Pseudomonas* are the major bacterial fish pathogen, which are widely distributed in the aquatic organism in nature. *Aeromonas spp.* can be isolated from a wide range of environment and water samples. The most common pathogenic bacteria isolated from the environment is *Aeromonas hydrophila* [10].

In India, after a major epizootic disease, impact of Epizootic Ulcerative Syndrome (EUS), fish disease study has gained its importance. A variety of diseases has been identified, and their diagnosis and control measures have been developed. Fish disease like Tail rot, Gill rot, Fin rot, Bacterial gill disease, Cornea opacity, Dropsy and Aeromonasis have been reported from fish farming system of our country. The aquaculture sector is witnessing a massive economic loss due to fish disease. However, there is no availability of exact figures on loss due to diseases in fish farming till date. With this background, the present study was undertaken to survey the occurrence of bacterial diseases in eight fish farms of Tarai region, Udham Singh Nagar, Uttarakhand during the period October 2012 to March 2013.

The following objectives were set for the present study.

- To survey the farms of Tarai region
- To document the occurrence of different bacterial diseases in fish farming system.
- To isolate and identify the bacterial pathogen from diseased sample.
- To test the antibiotic susceptibility of different bacterial strains.

Materials and Methods

The present investigation was conducted in the carp culture ponds in the Tarai Region, Udham Singh Nagar of Uttarakhand during the period October 2012 to March 2013.

Farm site: Udham Singh Nagar District is the food bowl of Uttarakhand State. Prior to its formation, it was a part of District Nainital. It was separated out on the basis of Geo-physiographical conditions i.e. Tarai region. Udham Singh Nagar district falls in the Tarai region of Kumaon Division. The total geographical area of the district is 3055 Km^2. It is located between latitude 28° 53' N and 29° 23' N and laterally extends between longitudes 78° 45' E and 80° 08' E.

A total of eight culture ponds were selected from Dinespur (2 farms), Kalinagar (4 farms) and Pantnagar (2 farms) of U.S. Nagar, Uttarakhand. All eight are earthern ponds having area ranging from 0.20 ha -0.50 ha with the depth of 1.15-1.45 m. IMC and exotic Carps were stocked at the rate of 8000-12000 fingerlings/ha.

Sample collection: Each farm was surveyed once in a month for observation and documentation of occurrence of bacterial disease. A total of 2691 fishes were sampled, out of which 27 fishes were infected with disease.

Isolation of Bacterial pathogens: Tissue samples (fin and muscle tissue) from diseased fish were collected in phosphate buffer saline (PBS) solution and brought to the laboratory for isolation of bacterial pathogens.

The samples were taken to sterile mortar and homogenized with the help of sterile pestle. The physiological saline was added to the mixture. One part of extract was taken and 9 parts of physiological saline was added. This process was repeated six times to get sample with microbial concentration from 10^{-1} to 10^{-6}. From the different concentration, 100 microliter samples in duplicate plated on nutrient agar plate was spread. Plates were incubated at 28 °C for overnight. Then total plate count (TPC) was estimated (cfu/ml) with the help of bacterial colony counter.

The representative colonies from samples were picked up and purified by streak plating on nutrient agar. The plate was incubated overnight at 28 °C for proper growth of bacterial colony. The purified bacterial strains were preserved in freezing media.

Biochemical characterization: After proper growth in plates the bacterial isolates were subjected to several biochemical tests for proper identification. After getting results of biochemical analysis, it was compared with the key, based on Bergey's Manual of Systemic Bacteriology [9].

Antibiotics Sensitivity tests: Antibiotic sensitivity tests was performed to know whether the microorganisms were susceptible to particular market antibiotic or they were resistance against antibiotics. The test was performed by Bauer-Kirby method [8] and the guidelines of CLSI [11] were followed. The suspension of individual bacterial isolates were spread on the surface of Mueller-Hinton agar plate with the help of cotton swab. Plate was left for 5 min to dry with lid. Then antimicrobial discs were applied on the plate with the help of sterile forceps. Discs were kept at about 24 mm apart. Total 4 discs were applied per plate. Plates were incubated at 37 ºC for overnight. After overnight incubation, result was noted by measuring zone diameter of inhibition of growth around the antimicrobial disc, nearest to millimeter, with the help of a ruler. Results were interpreted as sensitive, intermediate and resistant after comparing with the interpretive chart provided by Hi-Media (values are based on test over standard strains).

Water quality parameters: The water samples were taken from experimental ponds for the estimation of pH, temperature, dissolved oxygen, free carbon dioxide and alkalinity [1].

Results

The results of present investigation has been described in three parts for the ease of presentation *viz*. (1) documentation of bacterial diseases in carp pond (2) biochemical identification of bacterial pathogen (3) susceptibility of bacteria to particular antibiotic. A total of 2691 fishes were sampled out of which 27 were infected with disease. After catching the fish from the water, live carp fishes were observed with naked eyes. The colour of the body was noticed. The external signs of the diseased fish were recorded. Haemorrhagic lesions in the skin were observed, tail and fin erosion and loss of scales were also observed. During the survey, tail erosion, fin erosion and skin haemorrhages were documented from these eight farms. Isolation and screening of microbes were carried out with the help of a number of biochemical tests. Microscopic identification of bacterial strain was done by Gram's staining and it was found that all are small rod shaped Gram negative bacteria.

Biochemical identification: The bacterial isolates were isolated from tissue samples (fin and muscle tissue) and designated as laboratory strain MP01, MP02, MP03, A-54, MP011, MP022 and P-01. MP01, MP02, MP03 and MP011 were isolated from muscle tissue whereas MP022, A-54, and P-01 was isolated from fin tissue. Selected isolates were subjected to the series of biochemical tests based on the Bergey's manual of systemic bacteriology volume one. The biochemical properties of these isolates are given in the table No. 1 and 2.

Based on the characteristics, these isolates were identified as member of genus *Aeromonas* sp. and *Pseudomonas* sp.

Table 1: Results of biochemical tests of bacteria

Sr. No.	Characteristics/Strains	MP01		MP02		MP03		A-54	
1	Gram	-ve		-ve		-ve		-ve	
2	Morphology	Small rods		Small rods		Small rods		Small rods	
3	Gelatinase	-ve		-ve		-ve		-ve	
4	Simmon citrate	+ve		+ve		+ve		+ve	
5(a)	Sulphide	-ve		-ve		-ve		-ve	
(b)	Indole	-ve		-ve		-ve		-ve	
(c)	Motility	+ve		+ve		+ve		+ve	
6	O/F (a) sealed	-ve		-ve		-ve		-ve	
	(b) Open	-ve		-ve		-ve		-ve	
7	Triple Sugar Iron	+ve		+ve		+ve		+ve	
8	Cytochrome	+ve		+ve		+ve		+ve	
9	Catalase	+ve		+ve		+ve		+ve	
10(a)	MR	-ve		-ve		-ve		-ve	
(b)	VP	+ve		+ve		+ve		+ve	
11	Starch	-ve		-ve		-ve		-ve	
12	Amino Acid test								
(a)	Blank	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
(b)	Arginine	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve
(c)	Lysine	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve
(d)	Ornithine	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve
13	Indole Nitrite	+ve		+ve		+ve		+ve	
14	Sugar fermentation								
(a)	Mannose	+ve		+ve		+ve		+ve	
(b)	Cellobiose	+ve		+ve		-ve		+ve	
(c)	Galactose	-ve		-ve		-ve		-ve	
(d)	Fructose	-ve		-ve		-ve		-ve	
(e)	Glucose	-ve		+ve		+ve		-ve	

Table 2: Results of biochemical tests of bacteria

Sr. No.	Characteristics/Strains	MP011	MP022	P-01
1	Gram	-ve	-ve	-ve
2	Morphology	Small rods	Small rods	Small rods
3	Gelatinase	-ve	-ve	-ve
4	Simmon citrate	+ve	+ve	+ve
5(a)	Sulphide	-ve	-ve	-ve
(b)	Indole	-ve	-ve	-ve
(c)	Motility	+ve	+ve	+ve
6	O/F (a) sealed	-ve	-ve	-ve
	(b) Open	+ve	+ve	+ve
7	Triple Sugar Iron	+ve	+ve	+ve
8	Cytochrome	+ve	+ve	-ve
9	Catalase	+ve	+ve	+ve
10(a)	MR	-ve	-ve	-ve
(b)	VP	-ve	+ve	-ve
11	Starch	-ve	-ve	-ve
12	Amino Acid test	-ve	-ve	-ve
(a)	Blank	-ve	+ve	-ve
(b)	Arginine	+ve	+ve	+ve
(c)	Lysine	+ve	-ve	+ve
(d)	Ornithine	+ve	+ve	+ve
13	Indole Nitrite	+ve	+ve	+ve
14	Sugar fermentation			
(a)	Mannose	+ve	+ve	+ve
(b)	Cellobiose	+ve	+ve	+ve
(c)	Galactose	+ve	-ve	-ve
(d)	Fructose	-ve	-ve	-ve
(e)	Glucose	-ve	+ve	-ve

Antibacterial Susceptibility Test: The antibacterial susceptibility tests of different bacterial strains were performed with antibiotics by disc diffusion method. It was found that A-54 and MP022 were resistant to Amphotericin and Fluconazole. MP022 was also resistant to Oxacillin.

Out of 12 antibiotics used, 9 were found to inhibit the growth of bacteria as depicted in table No. 3. All the bacterial isolates exhibited sensitivity to Vancomycin, Sulphadiazine, Neomycin, Offloxacin, Streptomycin, Amphotericin, Fluconazole, Amoxycillin, Rifampicin, Tetracycline and Penicillin. In my study I found that bacteria were more sensitive to Offloxacin and Tetracyclin. Therefore these antibiotics can be used for bacterial disease treatment.

Table 3: Showing tests of antibiotics against bacteria

Sr. No.	Bacterial culture/Antibiotics	Diameter of zone of inhibition(mm)	
		MP01	MP011
1	Vancomycin	15 S	16 S
2	Sulphadiazine	28 S	28 S
3	Neomycin	27 S	25 S
4	Offloxacin	34 S	35 S

Sr. No.	Bacterial culture/Antibiotics	Diameter of zone of inhibition(mm)	
		A -54	MP022
5	Oxacillin	10 S	R
6	Streptomycin	24 S	25 S
7	Amphotericin	R	R
8	Fluconazole	R	R

Sr. No.	Bacterial culture/Antibiotics	Diameter of zone of inhibition(mm)	
		MP02	P-01
9	Amoxycillin	29 S	27 S
10	Rifampicin	11 S	12 S
11	Tetracycline	30 S	33 S
12	Penicillin	14 S	16 S

R- resistant and S- sensitive

Water quality parameters: The temperature ranged from 12.8-30.7, pH ranged from 7.1-7.9, DO ranged from 6.9-8.1, total alkalinity ranged from 144-173 while Free CO_2 was nil or traceable.

Discussion

Aquaculture is playing an important role in boosting global fish production and in meeting the rising demand for animal protein. Fish diseases constitute one of the most important factor hindering the development and sustainability of aquaculture. Infectious diseases, in addition to causing mass mortalities among fishes, can reduce the value of fish as food for humans [22]. Bacterial fish diseases constitute one of the major challenges facing sustainable aquaculture production. Diseased fishes are the vehicles for human infection and deaths by septicemia [31].

Tail rot and fin rot disease is a bacterial disease of freshwater fish. It is widely distributed in tropical and temperate countries. Most of the species are susceptible to these diseases and it may cause large mortalities [19, 23]. In the present study the infection of tail rot and fin rot was also observed.

Tail rot and Fin rot disease observed in Indian Major Carps is similar to the findings of [26] investigation. They observed that affected fish showed lesion and erosion on the fins and tail in Indian Major Carps.

We found skin haemorrages in *Cyprinus carpio* and found the causal agent to be *Aeromonas*. The result is in favour to work by [12] who also observed that skin haemorrhage was common in this carp and also isolated *Aeromonas species.*

Fin rot and tail rot disease is found in different fish farms and the rate of incidence of this type of disease is assumed to have increased in recent years [16].

The results revealed that *Aeromonas sp.* is a Gram negative, straight, rods, motile by single polar flagella, catalase, cytochrome oxidase but not H_2S, and nitrates were reduced to nitrites. Voges-Proskauer reaction was positive, but the methyl red test was negative. Similar results were recorded by several authors as [15, 3, 24, 31, 13, 14, 7, 2].

MP011 and MP022 cultures showed gram negative, catalase and cytochrome oxidase positive, Simmons citrate positive.

Pseudomonas spp. infection is a serious and major economic problem all over the world. *Pseudomonas* species has been considered as a secondary invader of damaged fish tissue as well as a primary poor and weak pathogen [29]. *Pseudomonas spp.* has been reported to cause disease in a wide range of fish species.

In this study *Pseudomonas* was isolated from MP011, MP022, P-01. It was observed that it is Gram-negative rods that are oxidase and catalase positive, positive for citrate and arginine, the decarboxylases test were similar to [5] who reported *Pseudomonas spp.*

MP01, MP02, MP03, A-54 and MP011, MP022 P-01 showed the presence of *Aeromonas* and *Pseudomonas* in cultures respectively. The results are similar to the observations of [4]. He observed that *Aeromonas* was Gram-negative, straight, motile. Catalase, indole, cytochrome oxidase are produced, but not H_2S, nitrates are reduced to nitrites without the production of gas, The Voges-Proskauer reaction was positive, but not similar to the methyl red test. Sodium citrate was utilised. *Pseudomonas* was gram negative bacteria, motile, with short rods It gave positive reactions in oxidase and catalase tests.

The occurrence of disease was highest in the month of November followed by October and December. This result was similar to the study of [16] who found most of the diseases in winter season. There is no disease in the month of February and March.

Antibiotic susceptibility test

The antimicrobial susceptibility of test organism was performed by disc diffusion method. The inhibition zones of the pathogen pseudomonads and aeromonads showed that Tetracyclin and Offloxacin were particularly effective against both types of bacteria.

MP01 and MP011 were sensitive to neomycin which is in the favour to the work of [25, 28]. MP02 was sensitive to Tetracyclin. Susceptibility of *Aeromonas* to antimicrobial agents vary, but isolates are usually susceptible to Tetracycline [17]. In the present study, it was found that A-54 and MP022 were resistant to Amphotericin and Fluconazole. All the bacterial isolates exhibited sensitivity to Vancomycin, Sulphadiazine, Neomycin, Offloxacin, Streptomycin, Amphotericin, Fluconazole, Amoxycillin, Rifampicin, Tetracycline and Penicillin.

Water quality

Water quality parameters are indicators of living conditions of all fish. When pond conditions are poor they inhibit and reduce the suitability of environment for the growth and reproduction of fish. Good or optimum pond conditions

promote growth and reproduction for fish and reduce their susceptibility to diseases [27].

From the present investigation it was observed that the values of water quality parameters were in the optimum range except water temperature which was considerably low and fluctuated between 12.8 ºC to 30.7 ºC and it favours development of the disease, therefore should avoided.

Improper management practices like high stocking density, poor food and feeding schedule, microalgae infested water, high nutrient load, different sizes of fish were recorded in one pond. No protection from fish eating predators, frequently netting and handling the fish may be the possible reason for infection of bacterial disease in carp pond. Poor management practices and higher stocking rate often result into outbreaks which lead to mass mortality in fish [30]. Hence a proper management practice may be devised for effective control and prevention of bacterial infection in carp ponds.

Conclusion

From the present investigation it is concluded that fish diseases like Fin rot, Tail rot and Aeromonasis are commonly occurring disease in carp culture practices. So, an effective surveillance programme with best pond management practices may possibly prevent the occurrence of bacterial diseases in culture pond for better production as well as economic return.

Acknowledgement

We express our heartiest gratitude to Vice Chancellor and Dean, College of Fisheries, G.B.P.U.A.T. Pantnagar, Uttarakhand for providing necessary facilities during the work and to Mr. S.K. Mallik, Scientist, D.C.F.R. Bhimtal, Uttarakhand for immense guidance and sincere co-operation for conducting this investigation.

References

1. APHA. Standard methods for the examination of water and waste water, 16th edition. American Public Health Association, New York. 1985;119:25-40.
2. Abd El-Ghany N, El-Khatib N, El-Ashram AMM. Some studies on ulcerative fish syndrome in cultured *Oerochromis niloticus*. 1st Biotechnology Conference, National Research Center, Cairo, Egypt; c2009. p. 199-212.
3. Abd El-Rahman AMM. Studies on bacterial diseases among cultured Tilapia. M.V. Sc. Thesis (Fish diseases and Management) faculty of Vet. Med. Suez Canal University. p. 1996.
4. Ahmed Mohamed Essmat. Studies on Common Bacterial Pathogens Causing Mortality and Low Productivity of *Oreochromis niloticus* Zagazig University Faculty of Veterinary Medicine Department of Bacteriology, Mycology and Immunology; c2006.
5. Akinbowale OL, Peng H, Grant P, Barton MD. Antibiotic and heavy metal resistance in motile *Aeromonads* and *Pseudomonads* from rainbow trout (*Oncorhynchus mykiss*) farms in Australia. International Journal of Antimicrobial; c2007. p. 177-182.
6. Andrew AE. Fish Processing Technology, University of Ilorin press, Nigeria; c2001. p. 7-8,
7. Austin B, Austin DA. Bacterial fish pathogens, disease of farmed and wild fish (4th edition) Springer-Praxis; c2007. ISBN 978-1-4020-6068-7, Chichester, UK.
8. Bauer AW, Kirby WMM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disc method, American Journal of Clinical Pathology. 1966;45:493-496.
9. Bergey DH, John GH, Noel RK, Peter HAS. Bergey's Manual of Determinative Bacteriology, Balliere, 5th ed. Tindall and Cox, London; c1994.
10. Briede I, Medne R. Aeromonosis infection disease of fish. In: Riekstins N. (ed) Latvian Fish Year Book. Riga; c2004. p. 167-171.
11. CLSI (Clinical Laboratory Standard Institute). Performance standards for antimicrobial disk susceptibility tests; Approved standard-9th ed. CLSI document M2-A9. 26:1. Clinical Laboratory Standards Institute, Wayne, PA; c2006.
12. Daood N. Damascus University Journal for Basic Sciences. Isolation and Antibiotic Susceptibility of *Aeromonas* spp. From Freshwater Fish Farm and Farmed Carp (Dam of 16 Tishreen, Lattakia). 2012;28(1):27.
13. El-Ashram AMM. On *Aeromonas hydrophila* infection among cultured tilapia: A Biological, Histophathological and management study. Journal of Aquatic biology and Fisheries. 2002;6(3):181-202.
14. El-Ashram AMM, Abd El-Rahman AMM. A contribution on bacterial pathogens infecting mullet (*Mugil capito*) cultured in freshwater farms in sharkia Governorate, Egypt. Journal of Agricultural Research. 84(1B):461-471.
15. El-Gamal RM. Bacterial causes of fin rot in some fresh water fishes. M.V. Sc. Thesis, (Microbiology), Suez Canal University; c1995.
16. Faruk MAR, Alam MJ, Sarker MMR, Kabir MB. Status of Fish Disease and Health Management Practices in Rural Freshwater Aquaculture of Bangladesh. Pakistan Journal of Biological Sciences. 2004;7(12):2092-2098.
17. Fass RJ, Barnishan J. *In vitro* susceptibility of *Aeromonas hydrophila* to 32 antibiotics. Antimicrob. Agents Chemother. 1981;19:357-358.
18. Kirjusina M, Briede I, Bondad-Reantaso MG. Extension Manual on Some Important Viruses, Parasites and Bacteria of Aquatic Animals in Latvia. NDC/LZRA/FAO. Riga; c2007. p. 69.
19. Fririchs GN, Roberts RJ. The Bacteriology of Teleost. In: Fish Pathology, Roberts, R.J. (Ed). Bailliere Tindall, London; c1989. p. 289-291.
20. Klesius PH, Shoemaker CA, Evans JJ. A healthy management practice for preventing diseases in tilapia and other cultured fish. 5th International Symposium on tilapia aquacult. In the 21st Century. Brazil. 2000;2:545-552.
21. Meyer FP. Aquaculture disease and health management, Journal of Animal Science. 1991;69:4201-4208.
22. Nguyen TN. The utilization of soybean products in tilapia feed-A Review. 8th Int. Symposium on Tilapia in aquaculture, Cairo International Convention Center, Egypt. 2008;1:53-65.
23. Noga EJ. Fish Disease Diagnosis and Treatment. Iowa State University Press, Ames, Iowa; c2000. p. 367.
24. Plumb JA. Health maintenance and principal microbial diseases of cultured fish (Ames: Iowa State University Press); c1999.
25. Rahim Z, Sanyal SC, Aziz KMS, Huq MI, Chowdhury AA. Isolation of enterotoxigenic, hemolytic and antibiotic resistant *Aeromonas hydrophila* strains from

infected fish in Bangladesh. Journal of Applied and Environmental Microbiology. 1984;48:865-867.
26. Rahman MM, Ferdowsy H, Kashem MA, Foysal MJ. Tail and Fin Rot Disease of Indian Major Carps and Climbing Perch in Bangladesh. Journal of Biological Science. 2010;10(8):800-804.
27. Rakocy JE, McGinty AS. Pond culture of tilapia. SRAC Publication. 1989;280:4.
28. Ramazan Adanir DO, Turutoglu H. Isolation and Antibiotic Susceptibility of *Aeromonas hydrophila* in a Carp (*Cyprinus Carpio*) Hatchery Farm. Bulletin of the Veterinary Institute in Pulawy. 2007;51:361-364.
29. Schaperclaus W, Kulow H, Shreckenbach K. Fish Diseases. A. A. A. Balkema Rotterdam; c1992. p. 1.
30. Sharma P, Sihag RC, Bhardwaj A. Seasonal incidences of hemorrhagic septicemia and Epizootic Ulcerative Syndrome in mrigala (*Cirrhinus mrigala* L.) in different fish farms around Hisar, Haryana, Trends in Biosciences Journal. 2011;4:215-218.
31. Woo PTK, Bruno DW. Fish disease and disorders. Viral, bacterial and fungal infections. CABI publishing, London, U.K. 1999;3:874.
32. Inglis V, Roberts RJ, Bromage NR. Bacterial Diseases of Fish. Iowa State University, Ames; c2001. p. 1-59, 122-156.