International Research Journal of Natural and Applied Sciences
Vol. 3, Issue 8, August 2016 IF- 3.827 ISSN: (2349-4077)

Website: www.aarf.asia Email : editor@aarf.asia , editoraarf@gmail.com

EFFECT OF PROBIOTICS ON WATER QUALITY, SURVIVAL AND GROWTH OF *CATLA CATLA*, *LABEO ROHITA* AND *CENOPHYRGDON IDELLA*

K. Sunitha[1] and P.V. Krishna[2]

[1,2] Department of Zoology and Aquaculture

Acharya Nagarjuna University, Nagarjunanagar- 522510, A.P., INDIA

ABSTRACT

Aquaculture is the fastest-growing food-producing sector in the world and has exhibited continuous improving in total production throughout the recent decades. A carp polyculture experiment was carried out to evaluate the growth performance of major carp in semi-intensive fish culture. Three earthen fish ponds, were cultured Catla catla, Labeo rohita and Ctenopharyngodon idella with the stocking ratio of 4:6:1 located in Machilipatanam in Krishna District, Andhra Pradesh, India were selected and studied for a culture period during 2012 to 2013. For this study two types of commercial probiotics were used i.e., Aqua gut (fish) as feed probiotic and Nitro-PS+Micro+Pro (fish) as soil and water probiotic (manufactured by Asian Bio tech, Hyderabad. Andhra Pradesh. India). During the study period, water samples were assessed for the study of physico chemical parameters, plankton samples and fish yields were studied. The results indicates that the concentration of ammonia, nitrite and orthophosphates are low in the treated ponds than in the control pond. The present study revealed that the growth and good water quality are obtained higher in probiotic treated ponds than that of control pond.

Key words: Probiotics, water parameters, growth, fish, zooplankton

INTRODUCTION

India is an important country that produces fish through aquaculture in the world (FAO 2014). Fish diseases especially bacterial infections, are a major problem faced by the fish farming Industry, which is currently growing fast with an annual increase of approximately 12% (FAO, 2004). The use of antimicrobial in disease prevention and growth promotion can bring about the emergence of drug-resistant microorganisms and leave antibiotic residues in the fish in the environment (Weston, 1996; Esiobu *et al.,* 20002; FAO/WHO/OIF, 2006). The use of probiotics which control pathogens through a variety of mechanisms is viewed as an alternative to antibiotics and become a major field in the development of aquaculture keeping in view of the beneficial effects of probiotics, some enthusiastic farmers recently using commercially available probiotics in their fish ponds.

The quality of water during the culture period will deteriorate mainly due to the accumulation of metabolic wastes of living organisms, decomposition of unutilized feed and decay of biotic materials (Lakshmanan and Soundarapandian, 2008). In addition, many researchers have been demonstrated that the pathogens can be eliminated or minimized through the application of probiotics (bio-control) and hence can achieve good yield by maximizing both survival rate and growth rate and by minimizing the disease problems in aquaculture systems. In recent years, research on probiotic for aquatic animals is increasing with the demand for environment-friendly aquaculture practices. (Gomez-Gil *et al.,* 2000; Verschuere *et al.,* 2000 Rao, 2001; Ali, 2006; Sreedevi and Ramasubramanian, 2010; Dimitroglou *et al.,* 2011; Iribarren *et al.,* 2012 Badina *et al.,* 2013; Maryam *et al.,* 2013).

MATERIALS AND METHODS

Study area

The fish ponds located in a farm at Machilipatnam in Krishna district, Andhra Pradesh, India were chosen for the present study. The fishponds stocked with catla (*Catla catla*), rohu (*Labeo rohita)* and grass carp (*Ctenopharyngodon idella*) were selected and are designated as control pond (T1), Treatment-2 and Treatment-3. Treatment 2 and 3 were treated with probiotics control pond without probiotic. The study was carried out for a culture period August 2012 to July 2013. All the control and experimental ponds (triplicates) goes to rectangular in shape and size goes 2.5ha. In the present investigation, two types of commercial probiotics were used i.e., Aqua gut (fish) as feed probiotic-treatment-2 and Nitro-PS+ Micro-Pro (fish) as soil and water probiotics treatment-3 (manufactured by Asian Bio Tech, Hyderabad, Andhra Pradesh, India).

Pond preparation

All the ponds were dewatered and dried for fifteen days before stocking. Purpose of sundried is to disinfect the pond and also the stabilization of pH, liming with CaO was applied at the rate of 150 kg/ha with dusting method (Wahab *et al.*, 2002). Essential precautionary measures were taken to screen the water inlets to avoid the entry of exotic fishes and other unimportant material in to the fish ponds. After two weeks of pre stocking management methods, each pond was watered up to 1.5 to 2.0 m and this water level was maintained throughout the experimental period. All the ponds were fertilized with organic manure (cow dung, 2500 kg/ha) as started dose to stimulate the productivity of the ponds. Then the ponds were applied with inorganic fertilizers, poultry manure and triple super phosphate at the rate of 15 and 8 kg/ha respectively.

Stocking of fish species in experimental ponds

Two weeks after manuring, each pond was stocked with catla, rohu and grass carp in the ratio 4:6:1. The average body weight was recorded at the time of stocking.

Fertilization and supplementary feed

After stocking of fish species, daily the amount of organic manure, inorganic fertilizer and supplementary feed was calculated on N-equivalence of 0.2g N/100g body weight of fish. In this experiment, all the experimental ponds received the same quantity of Nitrogen, The supplementary feed having 27.9% crude protein including, ground nut oil cake, rice bran, coconut oil cake, dry fish, vitamin and minerals

Fish growth parameters

Fish were samples once in each month to record their gain in weight (g). Following growth parameters are studied during experiment

1. Increment of average body weight of fish (g)

2. Increment of average body length of fish (mm)

3. Survival rate:

Survival rate = No of fish harvested/ no of fish stocked x 100

4. Specific growth rate:

$$SGR = \frac{\text{In (Final wet body weight) - In (Initial wet body weight)}}{\text{Time duration (days)}} \times 100$$

Water samples

The present investigation, physico-chemical parameters of water, plankton and fish yield were studied at fortnight intervals by collecting water sample in 9 and 10 a.m. The physico-chemical parameters such as temperature, transparency, dissolved oxygen, pH, nitrite, nitrate, ammonia, phosphorus and iron of water were estimated by following the methods suggested in Wetzel and Likens (1979); APHA (1999). Quantitative samples of plankton were collected by towing the plankton net made of silk bolting cloth no.25 (mesh size 0.03-0.04mm) in the surface and sub-surface layers of pond water for about 15min.Quantitative samples were collected by filtering 100L of pond water through the plankton net. The plankton obtained was fixed in 5% formalin. Sample volumes were adjusted to 10mL and two sub-samples of 1.0mLcapacity were removed and counted in Sedgwick-rafter cell (Edmondson, 1959). The average of three counts was converted to number of individuals per liter (ind. /L) of water sampled

RESULTS

Physico-chemical parameters of water

The parameters of water studied and their Mean ± S.D values and ranges observed during the study period in three treatments are given in **Table 1.**

Physico-chemical parameters	Control (T1)		Treatment-2		Treatment-3	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Water temperature (^{0}C)	28.63±1.58	25.5-30.5	28.21±2.24	25.0-31.5	28.25±1.95	25.0-31.5
Transparency(cm)	35.25±5.33	29.0-45.0	33.17±3.27	28.0-40.0	32.08±3.26	28.0-40.0
Dissolved oxygen(mg/L)	5.48±0.47	4.8-6.2	5.30±0.72	4.2-6.2	5.88±0.45	5.0-6.4
pH	8.12±0.44	7.2-8.6	8.32±0.27	8.0-8.6	8.29±0.25	8.0-8.6
Ammonia (mg/L)	0.52±0.07	0.44-0.64	0.40±0.06	0.27-0.46	0.35±0.06	0.26-0.49
Nitrite(mg/L)	0.07±0.02	0.010-0.09	0.06±0.02	0.025-0.07	0.04±0.02	0.010-0.07
Nitrate(mg/L)	0.23±0.07	0.12-0.36	0.31±0.10	0.12-0.47	0.49±0.06	0.41-0.60
Orthophosphate(mg/L)	0.59±0.10	0.42-0.75	0.14±0.08	0.29-0.56	0.47±0.09	0.32-0.59
Iron(mg/L)	0.37±0.06	0.28-0.51	0.27±0.05	0.21-0.35	0.32±0.07	0.21-0.41

Zooplankton

Zooplankton was mainly represented by Rotifera, Copepoda and Cladocera in the order of dominance whereas the other zooplanktonic forms were almost negligible. Zooplankton was identified up to species level. The numerical (ind/l) and relative abundance (%) of different zooplankton communities are represented in Fig: 1-3

Fig. 1. Abundance of zooplankton communities of Treatment-1

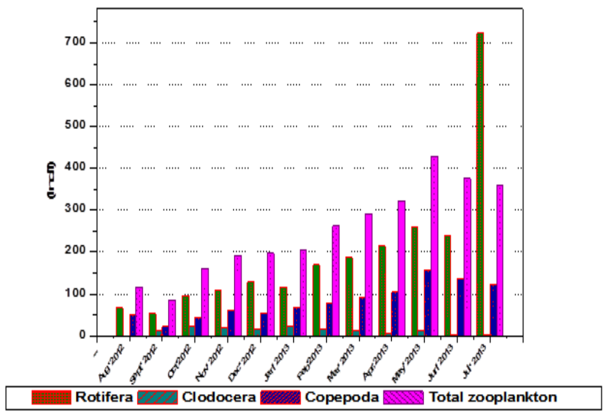


Fig.1a Numerical abundance (ind/l) of zooplankton groups

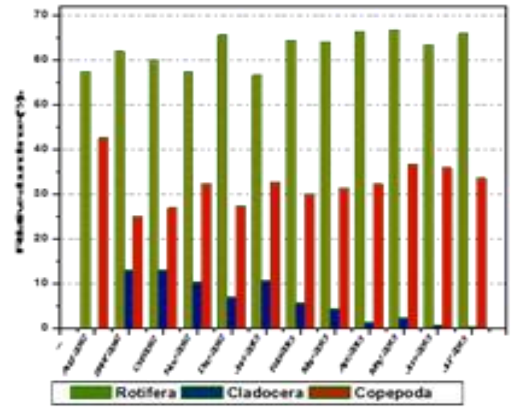


Fig. 2. Abundance of zooplankton communities of Treatment-2

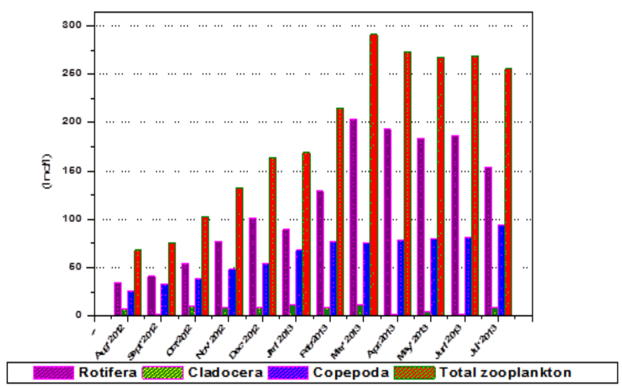


Fig.2a Numerical abundance (ind/l) of zooplankton groups

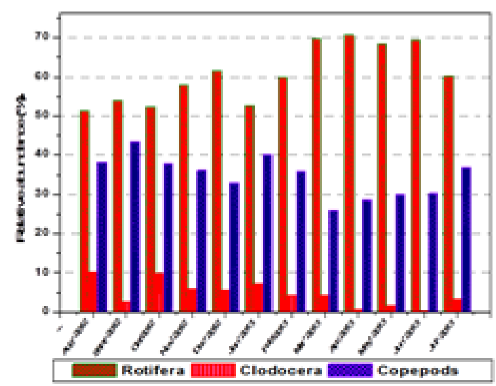


Fig.3 Abundance of zooplankton communities of Treatment-3

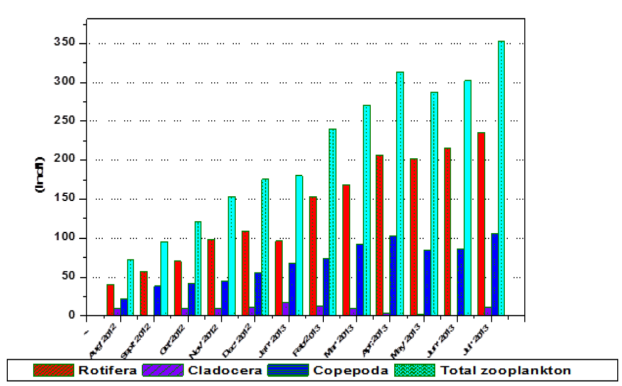
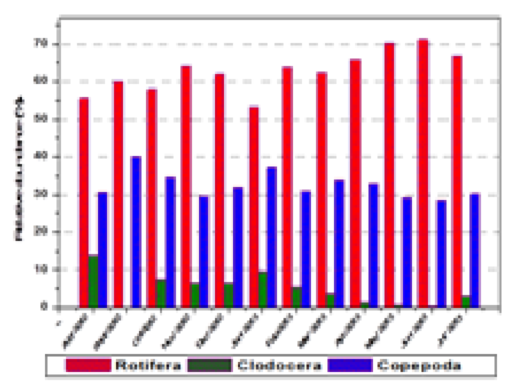


Fig.3a Numerical abundance (ind/l) of zooplankton groups



Fish

The number of fish Initial weight, final weight, specific growth rate, survival rate, gross yield and net yield shown in Table 3.

Table 2 Growth, survival and production of fishes in different probiotic treatments

Parameters	Species	Control pond T-1	Treatment pond	
			T-2	T-3
Initial Weight (g)	Catla	25.4	26.4	25.5
	Rohu	26.5	25.8	25.4
	Grass carp	25.3	25.1	24.5
Final Weight (g)	Catla	1280.4	1320.4	1410.2
	Rohu	1153.6	1254.0	1492.6
	Grass carp	1142.1	1299.2	1394.6
Specific growth rate (%)	Catla	2.6750	2.6899	2.7415
	Rohu	2.6256	2.6705	2.7667
	Grass carp	2.6259	2.6895	2.7417
Survival rate	Catla	76.4	79.1	88.6
	Rohu	78.3	85.7	89.3
	Grass carp	62.5	77.1	83.3
Grass yield/ tons/pond/year	Catla, Rohu and Grass carp	46.02	60.52	68.91
Net yield/ tons/pond/year	Catla, Rohu and Grass carp	26.49	41.51	52.01

DISSCUSSION

In semi-intensive and intensive culture practices, indiscriminate use of fertilizers and supplementary feeds in addition to high stocking densities of fish often lead to the deterioration of water quality. Water quality is an important indicator in appraising the eutrophic situation, primary production and fish yield potential. Quality of water for fish ponds depends upon the natural of soil, source of water and also on the location of ponds (Boyd, 1981). Water quality is determined by physico-chemical criteria of the water (Zakhia and Cuq, 1993). Probiotic are bio-friendly agents, they can be introduced in to the culture environment to control and compete with pathogenic bacteria as well as promote the growth of culture organisms. Several studies have attributed probiotic effect to the competition for energy sources (Farzanfar, 2006; Subhash *et al.*, 2007; Krishna *et al.,* 2009; Mohideen *et al.,* 2010; Cristia *et al.,* 2014; Divya *et al.,* 2014; Janardhan reddy *et al.,* 2015). The beneficial microorganisms (such as *Lactobacillus* sp. *Bacillus* sp. *Nitrosomonas* sp. and *Nitrobacter* sp.) would be very useful for controlling the pathogenic organisms and water quality (Prabhu *et al.,* 1999; Shariff *et al.,* 2001; Irianto and Austin, 2002). These bacteria are important to aqua farmers without them it is difficult to maintain healthy environment condition in the aquaculture ponds.

In the present study, water quality parameters of the ponds treated with probiotics were observed to be good which might be because of the various roles played by the microbes. Thus probiotics was found to use full in maintain the pond water pH a desired level (Sambasivam et al., 2003). Optimum levels of dissolved oxygen maintained in T2 and T3 might be due to the beneficial effect of probiotics which favored mineralization of organic matter. The application of probiotics, the fertilization of the pond by both organic and inorganic fertilizers, supplementary feed given in the pond and metabolites released by the fish might also be responsible for such variations. The nutrients, nitrate-N, nitrite-N, and ammonia-N in the pond water did not follow the same pattern of distribution and the variations may be due to biological or chemical reactions or combination of these two. The concentrations of ammonia and nitrites in T1 were slightly higher than that of the T2 and T3 (Table 1). This might be because of the use of nitrifying bacteria in the form of probiotics. As these bacteria are known to convert ammonia to nitrite and then to nitrate, low level of ammonia and nitrite observed in T2 and T3 compared to T1 can be supported. The oxidation of various forms of inorganic nitrogen in the well oxygenated surface water might have resulted in the increased concentration of nitrates. Feed probiotics and water probiotics also improved the health of fished to some extent but water quality parameters were much improved in T2 and T3 than control. Among the treatments T2 goes to better survival and high yield. Similar results observed by Abasali and Mohmad (2011).

Phosphorus occurs mainly in the form of phosphate and this element is recognized to be the most important critical factor in the maintenance of pond fertility (Boyd, 1982). As such phosphorus is absolutely necessary to sustain growth and reproduction of all biotic communities in aquatic ecosystem and in particular, this promotes the abundance of phytoplankton, zooplankton and fish in sequence. In the present study, orthophosphate ranged from 0.42 to 0.75 mg/l in T1; 0.29 to 0.56 mg/l in T2 and 0.32 to 0.59 mg/l T3. During the culture period, dissolved iron varied from 0.28 to 0.51 mg/l in T1; 0.21 to 0.35 mg/l in T2 and 0.21 to 0.41 mg/l in T3. It was observed that orthophosphate concentrations were maintained at low levels in T2 and T3 than in control pond. Probiotic bacteria utilize phosphate for their body metabolic activities and thus diminish this nutrient in pond waters (Rao, 2001).

The plankton community is comprised of primary producers (phytoplankton) and secondary producers (zooplankton). The phytoplankton population represents the biological wealth of a water body, constituting a vital link in the food chain. The zooplankton forms the principal source of food for fish within the water body (Prasad and Singh, 2003). In the present study, zooplankton was abundant in T2 and T3 compared to T1 (control pond). This might be because of the fact that probiotics favor the growth and development of zooplankton. Ludwig (1999) also stated that probiotics maximize zooplankton, as they form another nutrient for existing zooplankton in the pond medium and thus strengthen up the food supply to culture organisms. The abundance of different groups of zooplankton in aquatic ecosystems varies not only with the variations in environmental conditions but also on predatory pressure. In fish ponds, predation exerts perhaps the major influence on zooplankton abundance. In the present study, among zooplankters, rotifers were dominant followed by copepods and cladocerens throughout the culture period (Fig 1-3) in three ponds. In these ponds, initial sampling was done 3 to 5 days after stocking the fish. Generally after the release of fish into the pond, the number of cladocerans and copepods are much reduced and rotifers dominate the zooplankton. The low number of cladocerans and copepods might be due to the preferential predation by carp. In natural ponds, where the intensity of predation is less, the zooplankton was dominated by copepods followed by cladocerans and rotifers (Durga Prasad, 1981). The group could be minimum or absent in the natural habitats either because of predation or due to the other unfavorable environmental conditions. However, in these carp culture ponds, the low number of crustacean plankton and the abundant number of rotifers recorded during the study period is attributed to the preferential predation by the cultured fish. The weight attained by the fish at the time of harvesting in the three ponds indicates that the fish grow well in probiotic treated ponds T2 and T3 than control (Table 2). The grass and net yields obtained respectively were 60.52 and 41.51 t/ha/year in T2; 68.91 and 52.01 t/ha/year; 46.02 and 26.49 t/ha/year in control. The high yields obtained in these ponds might be due to high stocking densities of grass carp and carps and maintaining good water quality by the use of probiotics.

CONCLUSION

Hence, from the present study, it can be concluded that probiotics played a major role in maintaining optimum water quality parameters especially dissolved oxygen, ammonia, nitrite, nitrate and phosphates and zooplankton throughout the culture period which resulted in better growth, survival and disease resistance in the culture fish. Hence higher yields with better growth and survival can be achieved by using probiotics in aquaculture ponds.

ACKNOWLEDEMENTS

One of the authors, K.Sunitha is thankful to the UGC for providing financial assistance under the Rajiv Gandhi National Fellowship. Authors are grateful to the authorities of Acharya Nagarjuna University for providing necessary laboratory facilities.

REFERENCE

1. Ali, A., Bhatti, M.N., Khan, N. and Rehman, M.H. 2006. Role of soil and water chemistry in aquculture. Proceedings of International conference on "Solving problems of Freshwater *Fish Farming in Pakistan",* November 27-28, UVAS. 139-141pp.

2. Abasali, H and Mohmad, S. 2011. Effect of dietary probiotic level on the reproductive performance of female fry platy, *Xiphophorus maculates*, *Agricultural journal* 6(3):119-123

3. APHA, 1999. Standard methods for the examination of water and wastewater (c). 20th edition. Washington: American Public Health Association, American Water Works Association, and Water Pollution Control Federation. *Aquacult. Eng.,* 13: 115-128.

4. Balca'zar, E. 2003. Anti-infectious immune effectors in marine invertebrates: Potential tools for disease control in larviculture. *Aquaculture*, 227: 427-438.

5. Boyd, 1981. Water quality in Warmwater Fish Ponds. (2nd Ed). *Craftmaster Printers, Inc., Opclika, Alabama* 359pp.

6. Boyd, 1982. Water Quality Management in pond fish culture [Development in Aquaculture and Fisheries Sciences, 9]. *Elsevier Sciences Publishing Company, Inc., New York,* 318 pp.

7. Badina Abudurasaklge 2013. Probiotics use in intensive fish farming. *Afri. J. Micro. Res,* 7(22): 2701-2711.

8. Cristea, V. Patriche, N. Iulia Grecu, Sandita (Placinta) Ion, Mirela (Cretu) Mocanu, M.T. Coada. 2014. The effect on biochemical composition of different concentrations probiotic fed to juvenile carp (*Cyprinus carpio,* L. 1758) In A recirculating aquaculture system. Elena Bocioc Seientific papers –Animal science series: Lucrari Stiintifice-Seria Zootehnie, vol. 63

9. Dimitroglou A, Merrifield DL, Carnevali O, Picchietti S, Avella M, Daniels C, Güroy D, Davies SJ (2011). Microbial manipulations to improve fish health and production –a Mediterranean perspective. *Fish shellfish Immunol*. 30:1-16

10. Divya, K.R. Isamma, A. Arunjith. T.S, Sureshkumar. S and Krishnakumar. V. 2014. Effect of enriched artemia franciscana on production, survival, growth and biochemical composition of the freshwater fish *Catla catla* (Hamilton, 1992) *International journal of Recent biotechnology*, 2 (3):15-24

11. Durga Prasad, M.K. 1981. Studies on systematics and ecology of Branchiopods of Guntur district and its environs (Andhra Pradesh, India). Ph.D. Thesis (unpublished), Nagarjuna University, India.

12. Edmondson, W.T. 1966. Freshwater Biology. John Wiley and Sons Inc., New York, 1248pp.

13. Esiobu, N., Armenta, L. and Ike, J. 2002. Antibiotic resistance in soil and water environment. Int. J. Environment. Health Res., 12:133-44.

14. Farzanfar, A. 2006. The use of probiotics in shrimp culture. Federation of European Microbiological Society FEMS Immuno. *Med. Microbiol.* 48: 149-158.

15. FAO/WHO/OIE. 2006. Expert consultation on antimicrobial use in aquaculture and antimicrobial resistance. Seoul, Republic of South Korea, June 13-16.

16. FAO 2004. Fisheries Statistics, FAO, Rome, Italy.

17. FAO. 2014. Aquaculture production, 2014. Food and Agriculture Organization of the United Nations for a world with pot hunger, Fisheries and Aquaculture Department, 2014, food and Agriculture organization of the United nations, Rome, Italy

18. Gomez-Gil, B., Roque, A. and Turnbell, J.E. 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture*, 191: 259-270.

19. Iribarren D, Daga P, Moreira MT, Feijoog 2012. Potential environmental effcts of probiotics used in aquaculture. *Aquacult. Int.* 20(4):779-789.

20. Irianto, A. and Austin, B. 2002. Use of probiotics to control furunculous in rainbow trout, *Oncorhynchus myskiss* (Walbaum). *J. Fish Dis*., 25: 333-342.

21. Krishna, P. V., Madhusudhana Rao, K and Sharma, S. V. 2009. Effect of probiotics on the Growth and Survival of Tiger Prawn *Panaeus monodon* in Brackishwater ponds near Repalle, Guntur district, Andhra Pradesh. *ANU Jouranl of natural Sciences, December*, 123-127.

22. Janardana Reddy, S. 2015. Probiotics in aquaculture: Importance, Influence and Future Perspectives *Int. J. Bioassays*. 4 (03), 3710-3718

23. Lakshmana, R. and Soundarapandian, P. 2008. Effects of commercial probiotics on large scale culture of black tiger shrimp *Penaeues monodon* (Fabricius). *J. Microbiol*., 3 (3): 198-203.

24. Ludwig, G.M. 1999. Zooplankton succession and larval fish culture in freshwater pond (M). SRAC publication No.700. Stoneville: South Regional Aquaculture Center, Mississippi State University.

25. Maryam Kamgas, Rezapourghelam, M. Ghiasi and Masood Ghane 2013. Studies on *Bacillus subtiles*, as potential probiotics, on the bio-chemical parameters of rainbow trout, O*ncorhynchus mykiss* (Walbaum) to challenge infections. *Advanced Studies in Biology* 5 (1): 37-50.

26. Mohideen, M.M.A, T.S. Mohan, S.P. Mohmed and M.I. Zahir Hussain. 2010. Effect of probiotics on the growth rate of freshwater fish, Catla catla. International journal of Biological Technology 1(2):113-177.

27. Prasad, B.B. and Singh, R.B. 2003. Composition, abundance and distribution of phytoplankton and zoobenthos in a tropical water body. *Nat. Envin. Pollut. Technol.,* 2: 255-258.

28. Prabhu, N.M., Nazar, A.R., Rajagopal, S. and Khan, S.A. 1999. Use of probiotics in Water quality management during shrimp culture. *J. Aqua. Trop.* 3: 227-236.

29. Rao AV., 2001. Bioremediation technology to maintain healthy ecology in aquaculture ponds. *Aquacult. Technol*. pp. 80-84.

30. Shariff, M., Yusoff, F.M., Devaraja, T.N. and Srinivasa Rao, P.S. 2001. The effectiveness of a commercial microbial product in poorly prepared tiger shrimp, *Penaeus monodon* Fabricius ponds. *Aquaculture Res*., 32: 181-187.

31. Sreedevi PR, and Ramasubramanian, V. 2010. Biocontrol of ammonia pollution in the rearing water of fish by inducing a heterotrophic bacterial based food chain in the medium. *Aquacult. Int.* 19:789-796

32. Subhash, S.K., A.P. Lipton and R. Paul Raj. 2007. Influence of probiotics bacterium Lactobacillus acidphilus on the survival and growth of pearl oyster Pinctada fucata spat. *Ind.J.Fish.,* 54 (2):211-216.

33. Verschuere, L., Rombaut, G., Sorgeloos, P. and Verstraete, W. 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiol. Mol. Biol. Rev.,* 64: 655-671.

34. Wahab, M.A., Rahman, M.M. and Milstein, 2002. The effect of comman carp, *Cyprinus carpio* (L.) and mrigal, *Cirrhinus mrigala* (Hamiltion) as bottom feeders in major Indian carps polyculture. *Aquacult. Res.*, 26(9): 619-628.

35. Weston, D.P.1996. Environment considerations in the use of antibacterial drugs in aquaculture. In:Baird, D., Beveridge, M.V.M., Kelly, L.A and Muir, J.F. (Eds.), Aquaculture and water resource management, Oxford: Blackwell

36. Wetzel, R.G. and Likens, G.E. 1979. Limnological Analysis. Saunders: Philadelphia, PA, 357p

37. Zakhi, N. and Cuq, J.L. 1993. General survey on the quality of dried and commercialized tilapia in Mali.Proc. FAO expert Consultation on Fish Technology in Africa. ACCRA. Ghanna.No.467.Suppl.86-92 pp.