ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (342-348), Month: July - September 2018, Available at: www.researchpublish.com

Influences of Isolated Gut Probiotic *Bacillus subtilis* on Growth Performance of Freshwater fish *Labeo rohita*

[1*]S. Jayaprakash, [2]Dr. K. Parvathi, [3]C. Kalamani

[1*]Research Scholar in Zoology, Erode Arts and Science College (Autonomous), Erode, India.
[2]Assistant Professor of Zoology, Erode Arts and Science College (Autonomous), Erode, India.
[3]Research Scholar in Zoology, Erode Arts and Science College (Autonomous), Erode, India.
***Corresponding Author**: jprksh297@gmail.com

Abstract: **The study was carried out to find out the effect of isolated gut probiotics on the growth performance of freshwater fish fingerlings *Labeo rohita.* The beneficial effect of isolated gut probiotic *Bacillus subtilis* (SUB3845847 SeqJP2 MH128358) on weight, length, Weight gain percentage and Specific growth rate (SGR) of freshwater fish *Labeo rohita* for the period of 30 days. The two tub such as control tub (fed without probiotics) and experimental tub 1 (feed fed with *Bacillus subtilis* SUB3845847 SeqJP2 MH128358) were maintained and 30 fingerlings were introduced into each tub and continuous aeration was given. At the end of the evening 15 days, fish weight and length were measured up to 30 days. Results indicate that weight and length were significantly increased in the experimental tub than the control tub. Isolated gut probiotic diet improved digestive activity and stimulate the growth performance of freshwater fish *Labeo rohita*.**

Keywords: Bacillus subtilis (*SUB3845847 SeqJP2 MH128358), Growth performance, fingerlings *Labeo rohita.

I. INTRODUCTION

Aquaculture is one of the fast-growing systems in the world, which has emerged as an industry possible to supply protein-rich food throughout the world [1]. Fish is an important dietary animal protein source in human nutrition. Production of aquatic species through freshwater fisheries and aquaculture for protein supply is being encouraged throughout the world. According to nutritionists, fish is an excellent substitute of protein for red meat. The fish flesh contains all the essential amino acid and minerals *viz*., iodine, phosphorus, potassium, iron, copper and vitamin A and D in desirable concentrations [2]. Heavy production loss both in hatcheries and grows out systems due to a disease outbreak. In many land animals, growth stimulating microorganisms incorporated in the feed are reported to have beneficial effects. Since the microorganisms or probiotics are found to have the capability of improving the water quality, their application in aquaculture has gained momentum [3].

The term "probiotic" which literally means "for life" has since been employed to describe these health-promoting bacteria. Probiotic bacteria as "live microorganisms which when administrated in adequate amounts confer health benefits" [4].Probiotics are bio-friendly agents can be introduced into the cultured atmosphere to stimulate the growth of cultured aquatic organism and animal health by preventing the pathogenic bacteria in the same habitat [5]. Probiotics are beneficially affected by the host by improving its intestinal microbial balance, are quickly gaining interest as functional foods in the current era of self-care and complementary medicine. The use of probiotics in the culture of aquatic organisms is increasing with demand for more environment-friendly aquaculture practices [6]. Use of probiotics has been proposed as a measure to maintain a healthy environment in aquaculture and to prevent the occurrence of disease [7]. The microorganisms used as probiotics, including *Lactobacillus*, *Bacillus*, and yeasts, have been reported in fish [8]. The Indian major carps *Catla catla*, *Labeo rohita and Cirrhinus mrigala* are the most important commercial freshwater fish in

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (342-348), Month: July - September 2018, Available at: www.researchpublish.com

India with a maximum market demand and acceptability as food by the consumers due to their taste and flesh. They contribute about 67% of total freshwater fish production [9]. Among this, *Labeo rohita* contributes a major portion to the freshwater fish production in South India.

The aim of the present study was to evaluate the effects of probiotics the bacteria *Bacillus subtilis* growth performance of freshwater fish *Labeo rohita* for with the following objectives.

II. MATERIALS AND METHODS

A. Collection of Experimental Animals

Healthy, freshwater fish *Labeo rohita* (5.00 gm weight and 4.5 cm length) were purchased from Government fish farm, Bhavanisagar, Erode District, Tamil Nadu and acclimatized in laboratory conditions in a plastic tub (25^0 C/77^0 F, pH 7.0) with continuous aeration for three weeks prior to the commencement of the experiment. Stocked fish were fed with supplementary diet *ad libitum*.

B. Isolation of Probiotic Bacteria in fish Intestine

The freshwater fish *Labeo rohita* were washed with sterile distilled water and dissected to remove the fish intestine by the sterilization condition. The fish intestine was homogenized in the same sterile distilled water and centrifugation. After centrifugation, the supernatant was taken and serially diluted in sterile distilled water in the test tubes to 10^{-5} and 10^{-6} dilution and was pour plated on a nutrient agar plate and incubated for 24 h at room temperature [10].

C. Identification of Probiotic Bacteria in fish Intestine

Selective colonies were characterized and identified following Bergey's Manual of Systematic Bacteriology [10] for their colony and cell morphology, gram staining, biochemical and physiological tests [11] and 16S rRNA sequencing [12] identification of bacterial genera and species. Pure culture was maintained in MRS broth at -30^{o}C with 10% (v/v) glycerol [13].

D. Feed preparation

To prepare the diet, the following ingredients such as (Rice bran, Groundnut oil cake, Dry fish meal, Soya meal, Maize and vitamin & mineral mix) were purchased from local Erode market, Tamil Nadu, India. All the ingredients were mixed and powdered by a machine and were made into dough with the help of distilled water [14].

Table 1: Ingredients of Supplementary Feed

Ingredients	Control (%)	Experiment (%)
Rice bran	40	40
Groundnut	20	20
Fish meal	15	15
Soya Meal	15	15
Maize	9	8
Vitamin & Mineral mix	1	1
Bacillus subtilis	-	1

(-) The absence of *Bacillus subtilis*

E. Experimental Design

Experimental fish were divided into two groups such as control and experimental tub. Fish in the control tub were fed only with formulated feed (without any probiotic), fish in the experimental tub is fed with formulated feed mixed with isolated probiotic bacteria *Bacillus subtilis*. Each treatment had triplicates. The feed was given twice a day of 6 am and 6 pm. The experiment was conducted up to 30 days. At the end of 15 days, the weight and length of fish were noted.

Control - Supplementary Diet only

Treatment 1 - Supplementary Diet + Probiotics bacteria (*Bacillus subtilis*)

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (342-348), Month: July - September 2018, Available at: www.researchpublish.com

F. Growth Parameters

The growth parameters of the freshwater fish *Labeo rohita* fingerlings were measured by taking their body weight and length was measured at the end of 15 days up to 30 days. The growth performance was calculated using the following formulas:

$$\text{Length (cm)} = \text{Final length (cm)} - \text{Initial length (cm)}$$

$$\text{Length gain percentage (\%)} = \frac{\text{Final length(cm)} - \text{Initial length (cm)}}{\text{Initial length (cm)}} \times 100$$

$$\text{Live Weight Gain (g)} = \text{Final weight (gm)} - \text{Initial weight (gm)}$$

$$\text{Weight gain percentage (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight(g)}}{\text{Initial weight (g)}} \times 100$$

$$\text{Specific Growth Rate (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Days of experiment}} \times 100$$

I. Statistical Analysis

The data were subjected to statistical analysis the results were using Microsoft Excel (Version 2007) results were expressed in Mean ± SD.

III. RESULT

The freshwater fish *Labeo rohita* were fed with probiotic and the length, length gain percentage, weight gain, and weight gain percentage and specific growth rate of the fish were measured at the end of 15 days and 30 days of an experimental period. The growth parameters were calculated and results were presented in the (Tables 2 & Fig.1 and Fig.5). The weight of fish was observed as (6.0±0.47) gm in control fish and (7.9±0.26) gm in experimental fish at the end of 15 days of an experimental period (Table 2 & Figure 2), whereas at the end of 30 days, the length of fish was gradually increased as (8.7±0.30) cm in experimental fish than the control fish (6.4±0.26) cm (Table 2 & Figure 1). The total length was found to be increased as 2.3 cm in the experimental group than the control group 0.4 cm at the end of 30 days (Table2 & Figure 1).

The length of fish was observed as (7.3±0.18) cm in control fish and (8.1±0.36) cm in experimental fish at the end of 15 days of an experimental period (Table 2 & Figure 1), whereas at the end of 30 days, the length of fish was gradually increased as (8.5±0.29) cm in experimental fish than the control fish (7.4±0.25) cm (Table 2 & Figure 1). The total length was found to be increased as 2.3 cm in the experimental group than the control group 0.4 cm at the end of 30 days (Table 2 & Figure 2). The length gain was found to be increased as (4.28±0.22) in the experimental group than the control group (14.86±0.48) at the end of 30 days (Table 2 & Figure 3). Weight gain percentage was found to be maximum (35.93±1.32) in fish fed with probiotic feed than the control feed (20.0±1.03) after 30 days (Table 2 & Figure 4). The highest specific growth rate was found to be increased as (6.66 ±0.16) in the experimental group than the control group (7.66 ±0.28) at the end of 30 days (Table.1 and Fig.5). Thus suggesting that the high concentrations of probiotic-supplemented in diets may not further promote the growth of *Labeo rohita* in this study.

This result is in agreement with the report of [11]. Which showed that the use of higher concentration of the probiotic always leads to better performances of growth. The results of the growth parameters of *Labeo rohita* fingerlings with different feeding method are presented in (Table 2 and Fig.1 and Fig.5) Growth parameters of *Labeo rohita* fingerlings with different feed clearly showed that enhancement of the experiment when compared with control. *Labeo rohita* fingerlings showed that experiment diet is maximum increased in Length (8.5 ± 0.29), Length gain (14.86± 0.48) Weight (8.7 ± 0.30) and Weight gain % (35.93 ± 1.32) than compared to control diet.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (342-348), Month: July - September 2018, Available at: www.researchpublish.com

Table 2: Changes in the Weight, Weight gain, Length, Length gain and SGR on freshwater fish *Labeo rohita* of an experimental period.

Experiment	Control fish		Experiment fish	
	15 Days	30 Days	15 Days	30 Days
Weight	6.0±0.47	6.4±0.26	7.9±0.26	8.7±0.30
Weight Gain	20.0±1.03	6.66±0.16	31.66±0.96	35.93±1.32
Length	7.3±0.18	7.4±0.25	8.1±0.36	8.5±0.29
Length Gain	4.28±0.22	1.36±0.11	10.95±0.45	14.86±0.48
SGR	6.66±0.16	1.33±0.12	12.66±0.80	7.66±0.28

Values are in mean ±SD

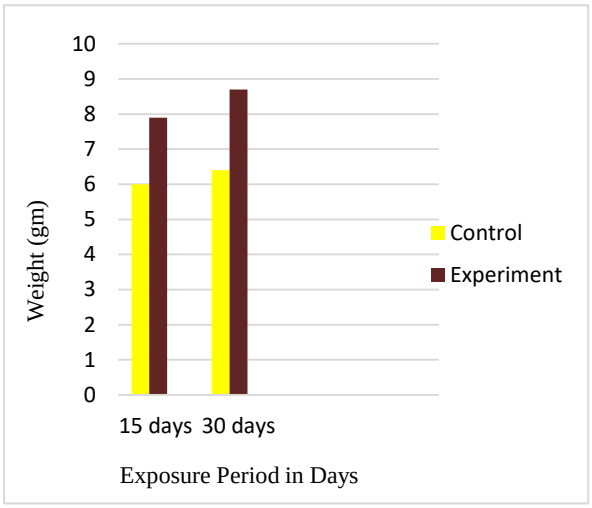

Fig 1: Changes in weight of *Labeo rohita* after 15 and 30 days of treatment with isolated probiotic *Bacillus subtilis*

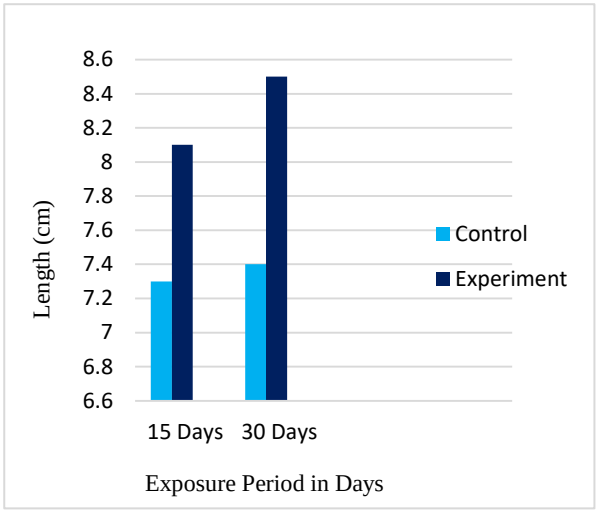

Fig 2: Changes in length of *Labeo rohita* after 15 and 30 days of treatment with isolated probiotic *Bacillus subtilis*.

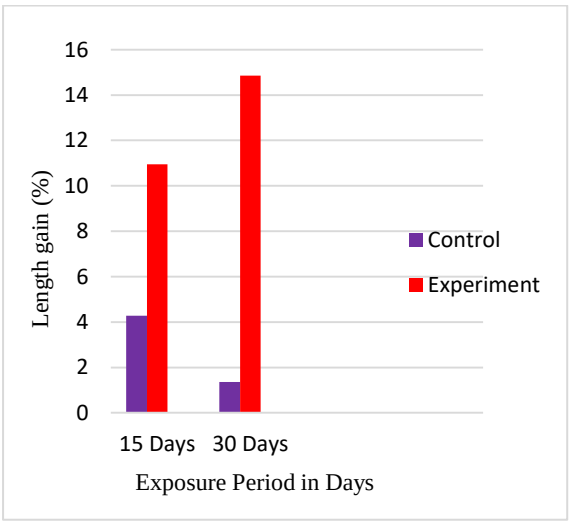

Fig 3: Changes in length gain of *Labeo rohita* after 15 and 30 days treatment with isolated probiotic *Bacillus subtilis*

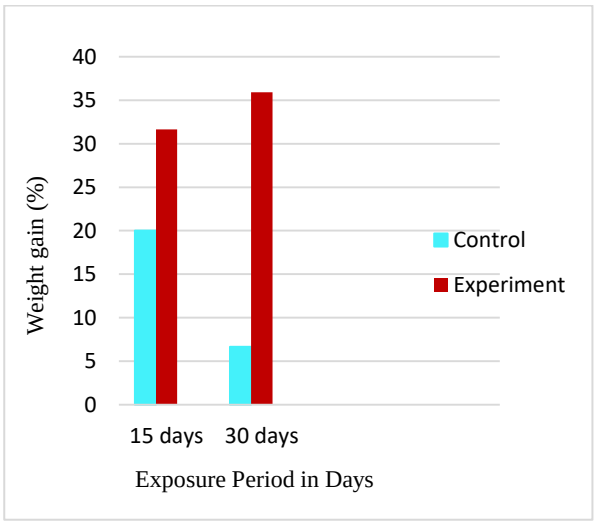

Fig 4: Changes in weight gain of *Labeo rohita* after 15 and 30 days treatment with isolated probiotic Bacillus subtilis.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (342-348), Month: July - September 2018, Available at: www.researchpublish.com

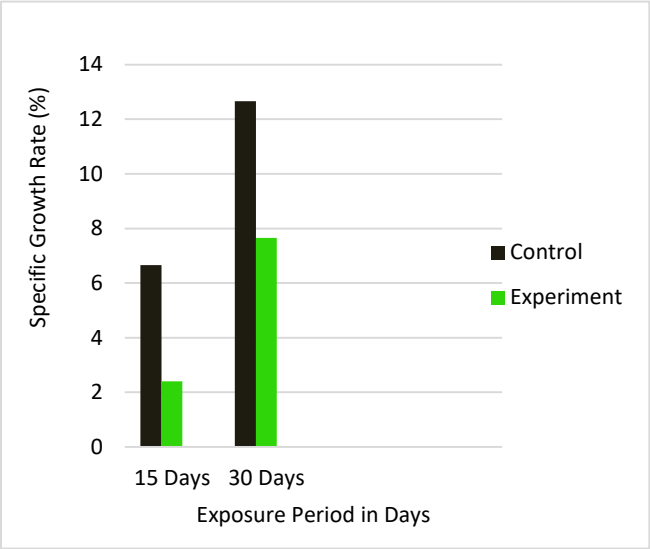

Fig 5: Changes in the specific growth rate of *Labeo rohita* after 15 and days treatment with isolated probiotic *Bacillus subtilis*

IV. DISCUSSION

The effects of probiotic diet have been studied in the many aquatic animals. The aquatic animal growth improving has been reported by feeding of *Bacillus sp*. in the *Labeo rohita* [15]. Carp farming has attained commercial culture status in India and many of its neighboring countries. With the intensification of fish culture, the feed has become the most important component of the culture system from the viewpoint of both fish production and cost. Fish-meal-based diets generally induce good growth. However, owing to the scarcity and increasing the cost of the fish meal, research on alternative sources is gaining importance [16]. Probiotics are the useful microorganisms promoting growth performance and protecting the host against pathogens. These microorganisms are included in the diets and have the beneficial effects on the host's gut microflora. Probiotics can be considered as a microbe to improve food nutritional value [17]. In the present study, the growth of *Labeo rohita* was significantly increased by probiotic diet. Such increase in the growth of aquatic animals fed with probiotic diets may be improved digestive activity by improvingan the synthesis of vitamins, enzymatic activity, with a consequent improvement of the digestibility and weight gain [18]. The ornamental fish *Puntius conchonius* were fed with commercial probiotic "Lact-act" histological section of skeletal muscle were observed in 15th days and 30th days of an experimental period [19]. Probiotics have been suggested as an option to improve the health and well-being of aquatic animals fish culture [20]. Some authors have reported an improvement of growth performance of organisms fed with diets added with probiotic cells [21 and 22]. Results of this study substantiate the fact that probiotics have direct growth-promoting effects on *Labeo rohita* which in accordance with the reports of [23]. As supplementary components in aquaculture feed, probiotics have strong adhesive and growth abilities [24]. In conclusion, the incorporation of probiotics in major carp diets improves water qualities and growth performance.

V. CONCLUSION

Fisheries in India is a very important economic activity in India. India is a second largest fish producer in the world. Rogue (*Labeo rohita*) is the most important Indian major carp species used in an aquaculture system. Fish is an important source of high-quality animal protein and are low in fat. Probiotics are live microbes, nowadays probiotics are widely used in aquaculture, its beneficial effect on host animals improving digestive activity and improve growth performance of aquatic animals. Probiotics are the useful microorganisms promoting growth performance and protecting the host against pathogens. My research work isolated probiotic *Bacillus subtilis* on improving growth of fingerlings freshwater fish *Labeo rohita*. The experimental tub (feed fed with *Bacillus subtilis* SUB3845847 SeqJP2 MH128358) results indicate that weight, length, weight gain percentage, and length gain percentage were significantly increased in the experimental tub than the control tub. Isolated probiotic diet improved digestive activity and stimulate the growth of freshwater fish *Labeo rohita*.

ACKNOWLEDGMENTS

Thankful to my Guide and Supervisor Dr. K. Parvathi, Assistant Professor of Zoology. This is my Ph.D. Research work, growth parameter is a part of my research work.

REFERENCES

[1] Prasad,G. (1996) The world aquaculture production: The present and future role of India. *Sea food Export Journal*, XXVII (7): 13 – 16.

[2] Sandhu, G.S. (2005). *A Textbook of Fish and Fisheries*, pp. 39-40. Dominant Publishers and Distributors, New Delhi, India.

[3] Krishnaveni, K., Palanivelu, K and Velavan, S. (2013). Spiritualizing effect of probiotic and spirulina on growth and biochemical performance in common carp. Int.Jour. of. Research in Zoology 3(3):27-31, 2278-1358.

[4] FAO/WHO. (2001). Report on Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. 1–4 October Cordoba, Argentina. Available at: ftp:// ftp.fao.org/ es/esn/food/probio_report_en.pdf.

[5] Parvathi K, Sivakumar P, Vinoth M. (2013): Influence of Selected Probiotics on Some Physico - Chemical Parameters of Water Bonfring. 394-396.

[6] Gatesoupe, F.J. (1999) The use of probiotics in aquaculture, Aquaculture, 180: 147-165.

[7] Lipton,A.P. (1998). Disease management in shrimp culture with special reference to probionts and additives. *Proceedings of the workshop, National Aquaculture week, Jan./Feb.* 1997. Aquaculture. Foundation of India, Chennai, India. p123.

[8] Boonthai, T., Vuthhiphandchai, V. and Nimrat, S. (2011). Probiotic bacteria effects on growth and bacterial composition of black tiger shrimp (*Penaus monodon*). Aquacult. Nutr., 17(6): 634-644.

[9] Parthasarathy R. and Ravi D. (2011) Probiotic bacteria as a growth promoter and biocontrol agent against Aeromonas hydrophila in Catla catla (Hamilton, 1822) Indian J. Fish., 58(3) : 87-93.

[10] Whitman, W.B., De Vos, P., Garrity, G.M., Jones, D., Noel, R., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.H. 2009. Bergey's manual of systematic bacteriology. 2nd edn, Vol. 3.

[11] Ghosh, S.; Sinha, A. and Sahu, C. (2008): Dietary probiotic supplementation in growth and health of live-bearing ornamental fishes. Aquacult. Nutri., 14: 289-299.

[12] Mandal, V.; Sen S. and Mandal N. (2008): Optimized culture conditions for bacteriocin production by *Pediococcus acidilactici* LAB 5 and its characterization. Indian J. Biochem. Biophys., 45: 106-110.

[13] Balcázar, J.L., Vendrell, D., de Blas, I., Ruiz-Zarzuela, I., Muzquiz, J.L., Girones, O. 2008. Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. *Aquaculture*, 278(1–4): 188–191.

[14] Sivakumar, P., Rajan, M.R., Ramachandran, P., 2014. Effects of probiotics on growth performance of common carp Cyprinus carpio var communis. Int J Pharm Bio Sci. 5(1), 835-839.

[15] Ghosh K, Sen SK, Ray AK. Supplementation of an isolated fish gut bacterium, *Bacillus circulans*, in formulated diets for rohu, *Labeo rohita*, fingerlings. Isr J Aquacult Bamidgeh 2003; 55:13–21.

[16] Shetty HPC, Nandeesha MC (1988). An overview of carp nutrition Research in India. In D Silva, SS (Ed.), Finfish Nutrition Research in Asia: Proceedings of the Second Asian Fish Nutrition Network Meeting. Heinemann Asia, Singapore, pp96-116.

[17] Ibrahim F, Quwehand A.C. and Salminen S.J. (2004). 18. Effect of temperature on invitro adhesion of fish probiotics. Microbial Ecology in Health and Disease.16: 222-227.

[18] Zhang Q, Ma HM, Mai KS, Zhang WB, Liufu ZG, Xu W. Interaction of dietary *Bacillus subtilis* and fructooligosaccharide on the growth performance, non-specific immunity of sea cucumber, *Apostichopusjaponicus*. Fish ShellfishImmunol 2010;29:204–211.

[19] Kalaimani, S and Parvathi, K. 2017. The Effect of Commercial Probiotics on Histological changes in the muscle of Ornamental fish *Puntius conchonius. Int.Jour.Advan.Research in Biological Science.* ISSN: 2348-8069.

[20] Verschuere, L., Rombaut, G., Sorgeloos, P., Verstraete, W., (2000). Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Review 64, 655 671.

[21] Ghosh S, Sinha A, Sahu C. Dietary probiotic supplementation in growth and health of live-bearing ornamental fishes. Aquac Nutr 2008; 14:289–299.

[22] Panigrahi, A. and Azad, I. (2007). Microbial intervention for better fish health in aquaculture: the Indian scenario. Fish Physiol. Biochem., 33:429-40, 2007

[23] Seenivasan C. Saravana Bhavan P, RadhakrishnanS., Muralisankar T. (2012) Effects of Probiotics on Survival, Growth and Biochemical Constituents of Freshwater Prawn Macrobrachium rosenbergii Post Larvae. Turkish Journal of Fisheries and Aquatic Sciences 12: 331-338.

[24] Mukhopadhyay P.K. and Paul B.N. (1996) Value addition components in aquaculture feeds. Fishing Chimes, 16:15-16.