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Efficacy of Soil Probiotic on Water Quality and Soil Quality Maintenance and Growth of Freshwater Fish Piaractus Brachypomus

Kiran Kumar Bazar[1], Naga Jyothi Pemmineti [1], * Subhan Ali Mohammad[2]

1. Department of Fishery Science & Aquaculture, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

2. Department of Biochemistry, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

*Correspondence: Dr.P.NAGA JYOTHI, Head&Bos Chairperson, p_n_jyothi@yahoo.com

Abstract

The accumulation of organic wastes deteriorates water quality and in turn decreases the growth of fingerlings of *Piaractus brachypomus* in fresh water ponds. To avoid this problem a supplementation trail was conducted for 90 days to determine the effect of commercial Soil probiotic 'Super-PS' in the water quality maintenance and growth of Piaractus *brachypomus* culture in fresh water pond. Probiotic bacteria are known to improve water quality in many ways. 'Super-PS' contains a combination of *Rhodobacter and Rhodococcus* species. Fresh water pond was treated with Soil probiotic *Rhodobacter and Rhodococcus* species during the culture period 1 to 30 days 15 lit/ha, 31 to 60 days 30 lit/ha, 61 to 90 days 40 lit/ha, 91 days continue (depending upon the pond condition, stocking density and days of culture) till harvest. The factors responsible for the improved water quality and significant growth up to harvest *Piaractus brachypomus* under the influence of Soil probiotics was analyzed and discussed.

Key words: *Rhodobacter*, *Rhodococcus*, probiotic, *Piaractus brachypomus*, water quality maintenance, growth performance.

1. Introduction

The *Piaractus brachypomus* (red pacu; Cuvier 1818), also identified as pirapitinga, red-bellied pacu, or white cachama, is a resident species of the Orinoco and Amazon basins, cultivated in Asia and South America with an annual production of 210 thousand tons in 2019, and is shows potential aquaculture species. Aquaculture may alleviate the overfishing tragedy, as well as advancement in food safety by enhancing fish supplies and several fish species of *Piaractus brachypomus* are increasingly being used for freshwater fish farming all over the world. *Piaractus brachypomus* has shown high tolerance to various growing systems and water quality conditions, including those of biofloc technology (BFT) with good productive results in the juvenile and growing stages [1].

Piaractus brachypomus (Red bellied pacu) has been introduced in to Indian aquaculture through ornamental fish trade. *Piaractus brachypomus* can be raised year round in warm or temperature controlled environments. *Piaractus brachypomus* are considered ideal for their low oxygen tolerance in the pond water and don't require a lot of costly protein in their diet. *Piaractus brachypomus* was introduced in India as an alien species during 2003 and 2004 from Bangladesh and Andhra Pradesh, West Bengal, Kerala, Maharashtra, Kolkata, Orissa, Tamilnadu, Bihar, Uttar Pradesh [2]. Several fish species have gained entry from Thailand to the North-Eastern States of India particularly to Tripura through Bangladesh and subsequently to the other parts of the country through West-Bengal. The basic purposes of introduction of these species to the culture ponds were their faster growth rate in a very short duration, immediate return and to meet the local market demand. The fish is laterally compressed, flat taking almost the shape of the *Piaractus brachypomus* commonly known as Red Pomfret or Rupchand or Pacu-the Silver Pomfret [3]. The present study was designed to use the commercial soil probiotic 'Super PS' to evaluate the growth and water quality parameters.

Water quality management using probiotic is an emerging technique. Probiotics added to the rearing water not only make the physico, chemical and biological properties of the water favourable but also increase the uptake of bacteria, probably acting as a (complementary) food source or contributing to the digestion of the food [4]. In water, it plays dual role such as bio-remediation and bio-control agent. Benefits such as improvement of water quality, supply of nutrients, growth factors and desirable alteration in the microbial ecology are also reported [5,6]. Previously it is reported that chronic exposure to high biofloc total suspended solids could affect growth, red blood cells, and generate gill alterations in *Piaractus brachypomus* [7]. Improved growth rate by the addition of Soil probiotic was demonstrated in cat fish, shrimp and carp respectively by Quieroz and Boyd (1998) [8]. Soil probiotic treatment may be considered as bio control method. Many published articles suggested that this may be the case, not just with fish, but also with shrimp and bivalves. Garriques and Arevalo (1995) [9] reported the successful manipulation of bacterial flora in hatchery production tanks by the addition of wild type *Vibrio alginolyticus*. Kennedy et al., (1998) [10] recorded the apparent elimination of *Vibrio* species from the common snook-centropomus undecimalis treated with *Bacillus subtilis*. In the previous study we are already reported the effect of soil probiotic 'Super PS' on water and soil quality maintenance and growth of fresh water fish *Pangasius hypophthalmus* [11]. Similarly, in the current study, we investigated the effect of soil probiotic 'Super PS' on water quality and soil quality maintenance and growth of fresh water fish *Piaractus Brachypomus.*

2. Materials and Methods

2.1 Source of fish and accumulation

Fingerlings of *Piaractus brachypomus* (Fig:1) collected from government fish farm at Kovur Mandal, Nellore District, Andhra Pradesh, were brought and acclimatized to the laboratory conditions. Fingerlings of fish were collected from the local fisheries department as per the standard pisci culture procedures and were kept in cement tanks for a week with sufficient aeration and declorinated water to acclimatize them to laboratory conditions.

Figure 1. *Piaractus brachypomus*

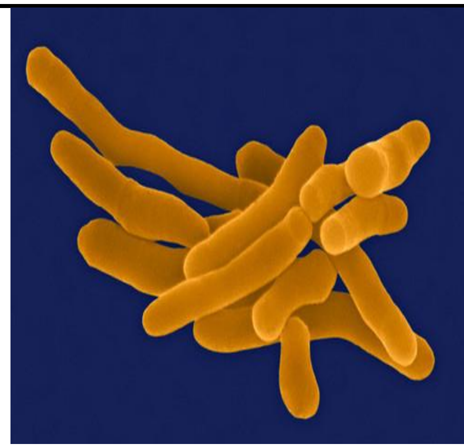
The *Piaractus brachypomus* fish body is deep, crossways with silvery sides and red coloration the belly, chin, pectoral fins, and the leading rays of the anal fins, left over rayed fins are uniformly dark colored. A small unraced adipose fins are present in the region of midway between the dorsal and caudal fins. A row of sharp serrate produced by modified scales are found on the abdomen.

2.2 Probiotic – 'Super PS'

'Super PS' is a soil probiotic made with beneficial bacteria which is used to improve pond bottom condition, reduces harmful bacteria and keep the environment friendly for aquaculture. It is composed mainly with *Rhodobacter* species and *Rhodococcus* species in the concentration of 10^9 CFU /ml. Benefits of 'Super PS' are it utilizes H_2S, maintaining optimum scale of water pH, biodegrade sludge, increases beneficial microorganism communities.

'Super PS' is sophisticated biotechnology product containing beneficial bacterial communities. These bacteria function in synergistic relationship to biodegrade organic pollutants and reduce hydrogen sulphide and toxic gases. 'Super PS' maintains optimum water quality and soil parameters for better growth of fish. It utilizes and reduces hydrogen sulphide in water column and promotes the non-pathogenic bacteria over pathogenic types. It oxidizes the organic matter and reduces black soil problems and promote stress free environment and increase the production.

Rhodobacter Species



Rhodococcus species

Figure 2. 'Super PS' Soil probiotic

2.3 Pond preparation and stocking

The experiment was carried out in one control and three experimental ponds. All the experimental ponds were dewatered and dried for 15 days before stocking. The purpose of the sun direct pond is to disinfect the pond and stabilize pH limiting the pond with $CaCO_3$ was applied at the rate of 125 kg/ha with the dusting method [11, 49]. Precautionary measures were taken to avoid any unimportant material in the pond. After removing the sediment, 50 to 100 ltr/ha of Super PS may be sprayed over the soil to utilize the organic matter and establish a beneficial bacterial community. After two weeks of pre-stocking, the ponds are watered up to 1.5 to 2.0 m, and this water level was maintained throughout the experimental period. After two weeks of pre-stocking and manuring the pond, each pond was stocked with *Piaractus brachypomus.* This study is a monoculture that includes only *Piaractus brachypomus*. The length and average body weight of fishes were noted at the time of stocking. At the time of stocking, fingerlings' size (length of the fish) was measured by centimeter-scale, and the weight of the fish was measured by electronic balance.

Figure 3. Length of the *Piaractus brachypomus* was measured by Centimeter Scale.

2.4. Experimental design and feeding method

The experiment was conducted in 3 ponds. One is kept as a control pond, and the other two are kept as experimental ponds. Initially, the 3 ponds were dewatered and allowed to dry in sunlight for a month to increase the hydrogen sulfide capacity and remove unnecessary weeds. The edges of the pond are repaired and constructed. After testing soil pH, liming was done at a dose of 250 kg/ha ($CaCO_3$) that helps to maintain good water quality. All 3 ponds are maintained with the same volume of water. All 3 ponds were fenced with fine-meshed nylon net so that any unwanted organisms could not enter the pond from outside. The control pond was cultured without any probiotic treatment and was expressed as T_1, and the other two ponds were cultured following a control diet and were expressed as T_2 and T_3. Initially, the probiotic was supplemented to the experimental ponds by spreading 40 liters Super PS/ha before stocking fishes to utilize the pond bottom's organic matter and to increase the beneficial bacteria on the pond bottom. Two supplementary diets were compared for the growth study we prepared for treatment 2 and 3 ponds (T_2-Experimental diet-1; T_3- Experimental diet-2).

- Diet 1: Control diet (T_1) was prepared by using the following ingredients (Rice bran (60g) Groundnut oil cake (100g) + Soybean cake (100g) + Fish meal (40g) + Vitamin C (2g)
- Diet 2: Experimental diet-1 (T_2) is prepared by using the following ingredients, (Control diet (10g) + Soil probiotic "Super PS" (2g) + Pellet feed (CP- 28) +Vitamin C (2g).
- Diet 3: Experimental diet-2 (T_3) is prepared by using the following ingredients, (Control diet (10g) + Soil probiotic "Super PS" (2g) +Pellet Feed CP-28) +Vitamin C (2g).
- Experimental duration: 90 days
- Fish size: Fingerlings (10 g)
- No. of Replicates: 6 No's
- Number of fish in each replicate: 10 No's

The ingredients of fish meal include soya meal, fish oil, rice bran, broken rice, rapeseed meal, vitamins and minerals. The standard body weight was documented at the time of stocking. After stocking of fish, 'Super PS' dose was 0.5 ppm/week at the starting stage, 1 ppm/week at the middle stage, and 1–2 ppm/week at the last stage of the experiment. This probiotic is given to fish through the feed.

2.5. Water quality parameters.

In the present study, physicochemical parameters of water and probiotic bacterial loads were studied at fortnight intervals by collecting water samples between 8 and 10 a.m. The physicochemical parameters were measured by the standard methods and field instruments. Water temperature was measured in the pond itself by using a standard centigrade thermometer. Field test instruments were used to examine water pH (Digital mini – pH meter, model 55) and dissolved oxygen (YSI-58). Transparency of water was measured in terms of light penetration method using Secchi disc [12]. Salinity was measured with a refractometer (Japan). Total ammonia [13], Nitrate – Nitrogen [14], and total alkalinity & Hardness [13] were analyzed by the standard protocols following the methods suggested by Golterman and Clymo (1970) [15]; Wetzel and Likens (1979) [16]; APHA (1999) [17].

Total ammonia concentration was measured by Hach comparison apparatus following the method reported by APHA, (1989) [13], and the deionized ammonia (NH_3) was calculated from total ammonia according to Boyd (1990) [12]. Nitrate-nitrogen was measured by phenolsulphonic acid method, according to Boyd (1984) [14]. Colour readings were measured with the spectrophotometer (Milton Roy 21D model) at 410 nm wavelength. Total alkalinity and total hardness were measured by titration, according to APHA (1985) [18].

2.6. Soil quality parameters.

Soil pH was determined electrochemically using an electrode pH meter Jackson (1973) [19], and the ratio of soil to water was 1:25. The electrical conductivity of soil was measured with the help of EC meter. Walkley and Black's wet oxidation method was used to determining the organic carbon within the samples. The levels of Ca and Mg were determined by ammonium acetate (pH-7.0) extract by the titrimetric method as described by Jackson [19].

2.7. Bacterial analysis.

Six fish as an initial sample before the commencement of the experiment and six fish from each trough at the termination of the experiment were starved for 24 hours in order to clean their intestinal tract before being sacrificed. The gut was aseptically dissected out both prior to commencement and at the termination of the experiment. Then the gut was homogenized with sterilized and chilled 0.1M, PBS, pH 7.4 (10:1 volume: weight). After five serial 1:10 dilutions, the homogenate was placed on a pseudomonas base medium (Hi-medium Mumbai). 0.1 mL of the sample was inoculated into the medium and incubated at 37^0C for 24 to 48 hours from the diluents. All the determinations were carried out in triplicates following incubation; plates containing viable colonies were used to calculate bacterial population results. The bacterial load of the fish gut was expressed as a number of colonies forming units per 'g' gut tissue CFU/g [20].

2.8. Growth performance analysis.

Growth performance was studied by different growth parameters. Average daily growth (ADG), Specific growth rate (SGR), Feed conversion ratio (FCR), and protein efficiency ratio (PER) was calculated and statistically analyzed (ANOVA). Final weight gain%, Survival rate (SR) %, total feed consumption, and total yield (Kg) were estimated. The following growth parameters were used for studying the growth performance of *Piaractus brachypomus* [20, 21].

Average daily gain (g) = Final weight (g) / Duration of culture (days)

Final Weight Gain % = Final Weight (g) – Initial Weight (g) / Initial Weight (g) ×100

Survival rate % = No of fish harvested / No of fish stocked ×100

Feed Conversion Ratio = Dry weight of feed consumed/ Increase in wet weight of fish;

Protein Efficiency Ratio = Wet weight gain of fish/ Protein consumed and

Specific Growth Rate = [(In final weight – In initial weight)/days on trial] × 100

2.9. Statistical analysis.

Statistical analysis was done by one-way analysis of variance (ANOVA) using MS-EXCEL and SPSS 16.0 software. The mean difference between different treatments was tested for significance at $P<0.05$, and Duncan's multiple range tests made comparisons to find out a significant difference between different treatments in respect of growth.

3. Results and Discussion

3.1. Water quality parameters.

The essential water quality parameters, like pH, alkalinity, hardness, temperature, dissolved oxygen (DO), nitrite, salinity, ammonia, were calculated and their values of Mean ± S.D (n=6) and ranges during the supplementation of supplementary diets along with a control diet were shown in Table 1. Every one of the water quality parameters were within the acceptable range for the freshwater culture of fish. In the current study, water quality parameters of the ponds treated with Probiotics were observed to have an excellent impact, which might be for the reason that of the microbes' diverse functions. Thus, Probiotics were functional in maintaining the pond water pH at the desired level [22]. The dissolved oxygen levels were maintained finest in the ponds treated with an experimental diet. This may be due to the effect of Probiotics, which are supported in the mineralization of organic matter. In the pond water the nutrients, Nitrate-N, Nitrite-N, and Ammonia-N, did not follow the equal distribution pattern, and the differences may be due to natural or elemental reactions or a combination of these two. The deliberations of ammonia and nitrites in control treated pond T1 were somewhat elevated than that of the experimental treated ponds T2 and T3 (Table 1).

3.2. Soil quality parameters.

In this study, varieties of soil quality parameters (pH, electrical conductivity, organic carbon, calcium, magnesium, phosphorus) were calculated and originate to be within the normal range presented in Table 2. More or less the soil pH was same during the study period in both control and experimental treated ponds. The pH of 7.0 – 7.6 ranges was observed. In a probiotic treated pond in contrast to the control pond the electrical conductivity was nearly 9 observed. Present study data shows that the normal concentrations of total phosphorus elevated in the probiotic treated pond compared to the control pond (2.33% - 3.36%). The Total Organic Carbon showed a significant difference between the control and probiotic treated pond (1.05% – 1.84%) ($P<0.05$). consequently, the Mg and Ca levels also showed considerable sediment changes after treated to commercial soil probiotic ‘Super PS’.

3.3. Bacterial analysis.

Very limited studies are reported on the composition of *Piaractus brachypomus* fish bacterial communities associated with the gastrointestinal tract. Characterization of the gastrointestinal bacterial micro biome of farmed juvenile and adult white Cachama (*Piaractus brachypomus*) was reported by culture-dependent methods, Denaturing Gradient Gel Electrophoresis (DGGE) and 16S rRNA gene survey methods [4]. Puello-caballero et al., 2018 [23], isolated bacteria from *Piaractus brachypomus* intestines and identified them using biochemical tests, whilst Sylvain et al., 2016 [24], using NGS approaches, described how gut associated micro biota of *Colossoma macropomum* is affected by pH changes in the surrounding water. However, a better knowledge of intestinal micro biota of *Piaractus brachypomus* could be helpful for the formulation of optimal live feed for the growth and development of fish.

Soil probiotic 'Super PS' contain *Rhodobacter species* and *Rhodococcus species* were added to the water of the ponds supplemented with experimental diet 1 and diet 2, whereas no probiotic was added to control ponds. The bacterial communities associated to the gastrointestinal tract in *Piaractus brachypomus* fish are involved in physiology, development and immune responses against pathogenic bacteria.

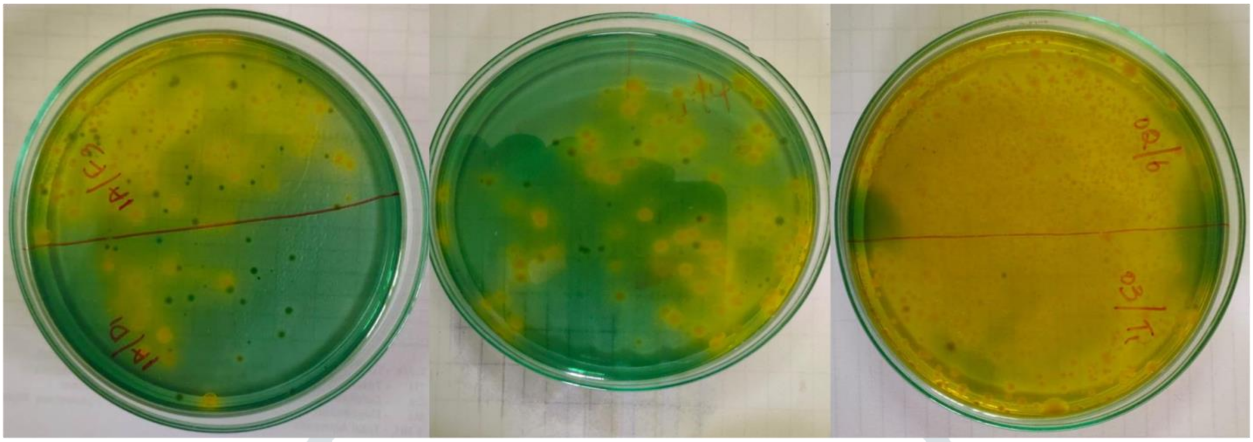


Figure 4. The relative bacterial loads during the culture period in different petri plates.

Heterotrophic bacterial count (CFU/g gut tissue) in *Piaractus brachypomus* fingerlings fed with a control diet and experimental diets were shown in Table 3. The total heterotrophic bacteria (THB) were high in experimental diets received fingerlings compared with control. The loads recorded were 4.2×10^3 cfu/mL in experimental diet 1; 4.6×10^3 cfu/mL in diet 2, and 1.26×10^3 cfu/mL in control diet received fingerlings. The relative bacterial loads during the culture period in different petri plates were shown in Figures 2 and 4. The differences in growth and survival of several bacterial colonies from the gut of fingerlings *Piaractus brachypomus* between control and treated groups could be attributed to the quality of diets.

3.4. Growth performance.

Dietary supplementation of 'Super PS' probiotic illustrated considerable growth performance and feed consumption efficiencies over the control diet fed fingerlings. After completion of 60 days of the culture period, the final average weight gain (%) was highest for the experimental diet 2 fingerlings, which differed significantly ($P<0.05$) from that of experimental diet 1 and control. There were considerable differences in the final standard weight of *Piaractus brachypomus* fingerlings among all the ponds ($P<0.05$). During the culture period, the highest weight gain was found in the pond fed with experimental diets. The soil probiotic 'Super PS' was used. The lowest weight gain was found in the control pond. SGR showed a similar tendency. The probiotic treated pond showed better SGR than the control ponds, which was significantly different ($P<0.05$). FCR and PER were most excellent for the experimental diet 2 fish group, which showed a significant difference ($P<0.05$) than that of experimental diet 1 and control. At the conclusion of the experiment, the survival rate of fingerlings was maximum in the ponds treated with experimental diets, whereas it was minimum in the control ponds. A however less considerable difference was found among the experimental diet treated ponds T2 and T3 ($P<0.05$). The results concerning growth performance and feed utilization by *Piaractus brachypomus* fingerlings fed experimental diets were presented in Table 4.

3.5. Discussion and conclusion.

The growth of aquaculture has increased speed, over the past decades; this is due to the use of Probiotics in aquaculture. Yet many factors, including pollution, infections and stress, may cause in major economic failures. The aquaculture production will not be totally successful without the therapeutic and preventive means to control all these factors. In long used in aquaculture practice antibiotics have tended to intensify the problem by increasing antibiotic resistance. Simultaneously, Probiotics have widely been recommended as eco-friendly alternatives to antibiotics. Nevertheless, the way in which Probiotics are functional in aquaculture is a key factor in their favorable performance [25].

Despite the technological development obtained in recent years, studies on stability and characterizations of fish pates are scarce and limited. The literature goes deep into the use of fat replacers mainly in pork and chicken liver pates. Recent study was reported on formulation from four lipid sources (pork back fat, canola oil, olive oil or sacha inchi oil), characterization and evaluation of the stability of a product based on white cachama (*Piaractus brachypomus*) [57]. Conversely, 'Probiotics applied in aquaculture are microbial cells (live or dead) or constituents of microbial cells that, when added to the water or feed, improve the general health of the host organism via improvements in the microbial balance in the environment'. Overall, Probiotics are considered bio-friendly agents that can be administered in aquatic culture environments to control pathogens and enhance feed utilization, survival, and growth rate of farmed species. Furthermore, they do not have any undesirable side effects on treated organisms [25, 26-28].

Probiotic use in the rearing water to improve water quality, and their administration to purify waste water from fish farms is helpful in areas with decreasing surface water, since the water can be reused for aqua cultural activities after treatment. In this study, a commercial soil probiotic, 'Super PS' was used as a feed supplement. 'Super PS' is a soil probiotic made with beneficial bacteria, which is used to improve pond bottom conditions, reduces harmful bacteria, and keep the environment friendly for aquaculture. The probiotic composed of mainly *Rhodobacter* species and *Rhodococcus species* not only significantly decreased ammonia levels, but also the starch and protein from underutilized feed in waste water [11].

Probiotics are nontoxic and non-pathogenic organisms without undesirable side effects when administered to aquatic organisms [29]. In the present study, soil probiotic 'Super PS' supplemented ponds gave a better production, and water parameters such as pH also are maintained at optimal range. Thus probiotic was found to use full in maintaining the pond water pH the desired level Probiotics are considered effective at strengthening innate immune responses, owing to the fact that they can interact with natural killer cells, monocytes, macrophages, and neutrophils. Their ability to enhance the number of macrophages, lymphocytes, erythrocytes, and granulocytes of a variety of fishes has been reported [30, 31–35]. Dead Probiotics are believed to have several advantages, as well. Such safe products can be applied to modify the biological responses [36]. Thus, Probiotics can defend the host against various infections caused by pathogenic microorganisms [37]. The use of soil probiotic 'Super PS' significantly reduced ammonia levels in experimental ponds than control ponds in which probiotic was not used. Among all the treatments, experimental diet 2 (T2) yielded improved results in survival rate, fish weight, growth and yield. These results are in agreement with earlier published similar results by Kiran kumar et al., 2022 [11]; Sreenivasulu et al., 2018 [38] and Abasali and Mohmad, 2011 [39].

The useful microorganisms hugely impacted the pathogenic organisms and developed water quality [39-42]. These bacteria are essential to aqua farmers; without them, it is difficult to maintain a healthy environment in aquaculture ponds. In view of the fact that the beginning of Probiotics in aquaculture, producing a number of studies have established their ability to control potential pathogens and enhance the growth rates and welfare of cultured aquatic animals (43-48).

In this study, the water quality parameters of the ponds were within the cultivable range. Microbes which are present in Probiotics played an important role in the maintenance of water quality parameters of the ponds. Water temperature was very narrow during the observation period (Table 1), the amplitude of difference in which is measured feature of tropical waters [49-52]. The temperature of water, in common follows the pattern of instability of air temperature. The low water temperature compromised the growth performance of juveniles Piaractus brachypomus. Juveniles Piaractus brachypomus adjusted their hematological and biochemical variables in response to low water temperature [53]. Water transparency showed an opposite correlation with temperature and with dissolved oxygen it is negatively correlated. For the well development of phytoplankton, the obtained interdependent relations can be elucidate using high temperatures. If the density of phytoplankton increases then there will be decrease in the Secchi disc transparency and increase in dissolved oxygen, which shows positive correlation with pH of water. Because of the Probiotics favorable outcome there is an urge for the mineralization of organic matter. From result it is evident that during hydrolysis pH of water shows more carbonates. The content of dissolved oxygen enhanced with the improvement of photosynthetic liberation of oxygen. A good deal of research has been reviewed to find out the connection between carbonates, bicarbonates, free carbon dioxide and pH [54, 55].

Decrease in the levels of ammonia and nitrite was observed in probiotic treated ponds when compared with control, and the minimum level was observed in T2. As a result of these favorable bacteria organic material was dissolved as a nutrient for rapid nitrification. A recent study in literature reveals that *HAMP1* mRNA level was up regulated in the liver and gills. *HAMP1* gene of *Piaractus brachypomus* may be involved in the inflammatory, antimicrobial, hypoxia and stress oxidative response [58]. For the maintenance of excellent quality of water which is beneficial for the fishes to overcome from the diseases, the amount of ammonia should be maintained in the pond. Recently, the large migratory fishes of the family Serrasalmidae (*Piaractus brachypomus* and *Piaractus orinoquensis*) were described as restricted to the Orinoco and Amazon basins. Both species provide important ecosystem services. They also are an important fisheries resource, which has caused that their populations have decreased in recent years. National fisheries policies still consider both species as one, which leads to inefficiencies in their management and conservation. There is a study in literature to genetically characterize these two species, using microsatellite and mitochondrial markers, and discuss the implication of these results for conservation and management [59]. Regular usage of Probiotics in a commercial manner will lowers the accumulation of organic matter, water quality improvement and increased environmental conditions (56). The data of present study exhibits the standard concentrations of TP (2.33-3.36%), organic carbon (OC) (1.055–1.8498%), Ca (0.11–0.20%), Mg (0.06-0.13%) in the sediment after the ponds are treated with commercial probiotic 'Super PS'. The physicochemical computed features agree with the biological functions of MBP, and its molecular characterization of myelin basic protein a (*mbpa*) gene from red-bellied pacu (*Piaractus brachypomus*) was well reported [60].

Due to increased probiotic supplement the final weight of the fish significantly increased throughout the culture period. Enhanced growth performance of *Piaractus brachypomus* is due to the supplementation of Probiotics. In probiotic supplemented experimental and control ponds, the standard body weight of the cultivated *Piaractus brachypomus* was 99.36 ± 4.55 g - 152.9 ± 5.63, and there is a significant difference among these two groups ($P<0.05$). In one of the recent studies digestibility was evaluated by a principle of additivity of enzymatic hydrolyzates from animal origin in White Cachama (Piaractus brachypomus) as a nutritional alternative [63]. In the probiotic pond extensive growth of *Piaractus brachypomus* was observed when compared to control pond. From the obtained data it is evident that probiotic supplemented ponds exhibit significant growth in *Piaractus brachypomus* compared to control diet. It is a very good inspiration in the fish farming, usage of Probiotics leads to the improvement in the size of the fish. The current research shows 93.98±1.69 % is an average survival rate in experimental probiotic ponds and 80.3%in control ponds. There is a significant difference among these two groups ($P<0.05$). Greater survival rate 13.68% was seen in probiotic supplemented ponds when compared to control ponds.

One of the recent studies it is concluded that the gills of *Piaractus brachypomus* have in vitro efficacy and tolerance of the essential oils of three species of the Lamiaceae family against monogeneans [61]. During the whole culture period probiotic shows a significant importance for the existence, growth, survival and resistance prone to diseases in aquatic animals was continued by favorable water and quality of soil was drawn from the conclusion. From literature it is evident that the short cycles of one day of feed restriction per week is a good strategy in *Piaractus brachypomus* production with initial average weight of 2.4 g. One day of feed restriction per week exhibited a 22% reduction in feed intake without altering the growth performance in *Piaractus brachypomus* juveniles [64]. A study in literature provided a detailed taxonomic description and the most useful discriminative features for the species, of five specimens of *Piaractus brachypomus* caught in water bodies [65]. For aquatic cultures supplementation of Probiotics was a strong substitute for antibiotics. The present research confined that Probiotics had an important role on the growth, survival and development of *Piaractus brachypomus* culture. Due to the nutritional potential of animal by-products, technologies have been adopted for their use, such as bioconversion techniques by enzymatic hydrolysis. Therefore, a study was published by using a hydrolyzed protein concentrate of animal waste in diets for *Piaractus brachypomus* [62].

To show the higher level growth production, group T2 was supplemented with soil and protein feed Probiotics. The current research findings were more favorable to the farmers for the good production of *Piaractus brachypomus.*

Table 1: Water quality parameters in control ponds and probiotic treated ponds that contain *Piaractus brachypomus* fingerlings

Physico-Chemical parameters	Feed 1: Control diet		Feed 2: Experimental diet-1		Feed 3: Experimental diet-2	
	Mean± S.D	Range	Mean± S.D	Range	Mean± S.D	Range
Water temperature (^0C)	26.33 ± 2.36	26.3-31.6	28.30 ± 3.16	28.0-31.60	29.66 ± 1.33	26.33-31.60
Secchi disc transparency (cm)	30.23±3.50	29.0-38.0	32.03±1.05	30.06-41.0	33.63±2.06	30.08-42.03
Dissolved Oxygen (mg/mL)	5.30 ± 0.86	5.0-6.0	5.33 ± 0.38	5.2-6.0	5.86 ± 0.33	5.3-6.2
pH	7.30 ± 0.16	7.3-8.0	7.13±0.33	7.09-8.3	7.26 ± 0.13	7.1-8.2
Total alkalinity (mg/L as $CaCO_3$)	120.0 ± 8.3	120-150	128.6± 6.05	125-180	139 ± 8.56	130-185
Total hardness (mg/L as $CaCO_3$)	222.6 ± 9.3	200-260	246.3 ± 12.3	200-300	252.6 ± 15.3	220-300
Nitrite-N (mg/L)	0.23 ± 0.02	0.15-0.30	0.08±0.03	0.06 ± 0.02	0.06 ± 0.06	0.05-0.23
Nitrate-N (mg/L)	0.19±0.05	0.12-0.40	0.22±0.08	0.11-0.36	0.39±0.07	0.20-0.40
Ammonia-N (mg/L)	1.09 ± 0.06	0.96-0.33	0.33 ± 0.05	0.23-0.38	0.16 ± 0.02	0.16-0.38

Table 2: Soil quality parameters in control ponds and probiotic treated ponds that contain *Piaractus brachypomus* fingerlings

Physico-chemical parameters	Feed 1: Control diet	Feed 2: Experimental diet-1	Feed 3: Experimental diet-2
pH (ranges)	7.0 -7.2^a	7.0 – 7.6^b	7.1 – 7.9^c
Electrical Conductivity (ds/m) (ranges)	2.5 – 6.96^a	6.9 – 8.50^b	8.6 – 9.0^c
P (meq/100gm) (%)	2.33^a	3.26^b	3.36^b
Organic Carbon (%)	1.0552^a	1.1356^b	1.8498^c
Ca (ranges) (%)	0.11 – 0.14^a	0.13 – 0.18^b	0.14 – 0.20^b
Mg (ranges) (%)	0.06 – 0.08^a	0.09 – 0.12^b	0.10 – 0.13^c

Data are Mean values ± S.D (n=3) Values in the same row with the same superscripts are not significantly different ($P<0.05$) (DMRT).

Table 3: Heterotrophic bacterial count (CFU/g gut tissue) in *Piaractus brachypomus* fingerlings fed with control and experimental diets for 60 days

[a]CFU/g gut	Control diet	Experimental diet 1	Experimental diet 2
Bacterial count on pseudomonas base medium (PBM)	$1.36×10^{3\,a}$ cfu/mL	$3.89×10^{3b}$ cfu/mL	$4.66×10^{3c}$ cfu/mL

Data are Mean values ± S.D (n=3) Values in the same row with the same superscripts are not significantly different (P<0.05) (DMRT).

Table 4: Growth and feed utilization efficiency in *Piaractus brachypomus* fingerlings fed with control and experimental diets for 60 days

Parameter	Control diet	Experimental diet 1	Experimental diet 2
Initial average weight	8.67 ± 0.03[a]	8.96 ± 0.02[a]	9.03± 0.01[a]
Final average weight	99.36 ± 4.55[a]	126.03 ± 3.60[b]	152.9 ± 5.63[c]
Weight gain (%)	675.20± 0.02[a]	1098.63±0.02[b]	1389.66±0.02 [c]
Survival (%)	80.3[a]	88.56±1.05[b]	93.98±1.69[c]
Food intake (g/kg body weight of fish/day)	12.03±0.10[a]	13.03±0.02 [a]	15.86±0.12 [a]
FCR	2.54 ± 0.03[a]	2.25 ± 0.02[ab]	2.11± 0.03[b]
PER	1.08±0.01[a]	1.39±0.02 [b]	1.62±0.02[c]
SGR (% day^{-1})	2.56 ± 0.01[a]	2.66 ± 0.01[ab]	2.79 ± 0.02 [b]

Data are Mean values ± S.D (n=3) Values in the same row with the same superscripts are not significantly different (P<0.05) (DMRT).

4.0 Conclusion

From the obtained results it was concluded that the commercial soil probiotic 'Super – PS', could be used as a feed supplement, which consists of *Rhodobacter* and *Rhodo coccus* species probiotic bacteria. These probiotic species show a great improvement in soil and water quality for the growth of fresh water fish *Piaractus brachypomus.*

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